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Morphological characterization of purple-grain maize (*zea mays* L.) diversity in Côte d'Ivoire

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Purple-grain maize is a marginal cereal grown exclusively and consumed in the Central-North of Côte d'Ivoire. However, few studies have been done on the diversity of this cultivar. Whereas, the use of local genetic resources available for breeding purposes requires a good knowledge of their agronomic and morphological characteristics. Thus, 70 accessions of purple-grain maize collected in the Hambol region were subject to an agro-morphological characterization study. The test was carried out in an alpha-lattice device (10 × 7) and repeated three times. The observations focus on 18 variables which have been selected in the maize descriptor proposed by Bioversity International. Descriptive analyses revealed a high inter-accession variability for quantitative traits. The multi varied analyses (principal component analysis (PCA) and a hierarchical cluster analysis (HCA)) made it possible to structure this diversity into two groups. The semi-flowering cycles (FlMa and FlFe), the heights of the plants (HaPl and HiEp) as well as the length of maize cobs (LoEp) were the main discriminating characters between the groups, because they could be used as estimators of phenotypic diversity for this plant. In addition, the accessions from each group could be brewed in order to develop different populations with broad genetic base. This will permit to enhance and to conserve diversity.

Key words: Côte d'Ivoire, agro-morphological diversity, purple-grain maize, marginal.

INTRODUCTION

Population growth and the pursuit of people well-being are leading today to the overexploitation of improved varieties to the detriment of local traditional varieties. This situation is particularly important for underutilized and neglected varieties. Such varieties are generally found in developing countries, where the introduction of high-yielding varieties that can improve farmers' incomes and living standards has led to their abandonment. Unfortunately, these abandonments often lead to significant genetic erosion of these so-called minor plants on farms. This is for example the case of purple-grain maize commonly called "Katiola'spurple" because of its location in this geographical area of Côte d'Ivoire (Kouakou et al., 2010). And yet, this variety that is an entire part of the culinary custom of the people who cultivate it is adopted by the producers and therefore, its improvement can be beneficial for the rural world. In fact, purple-grain maize, in addition to its good organoleptic

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quality (N'da et al., 2013), has therapeutic virtues (Kouakou et al., 2010). Studies by several authors have shown that purple-grain maize is a natural source of anthocyanins, a 100% natural antioxidant that helps prevent cardiovascular diseases, that combats cell aging, that reduces cholesterol (LDL) and also normalizes blood pressure (Herrera- Sotero et al., 2017; Lao et al., 2017). The purple matter present in the purple-grain maize is rich in polyphenols and the regular ingestion of this plant may be useful for people with hypertension. Aside from its nutritional value and its therapeutic virtues, purple-grain maize is used to make a delicious drink. This marginal culture therefore offers potentialities for agricultural diversification. And improving its production could help to ensure food security. However, it remains very little known. Compared to yellow and white grain maize varieties, very little research has been conducted on the genetic diversity of purple-grain maize at the national level. In Côte d'Ivoire, the genetic variability of this plant is still poorly known by breeders and it deserves special attention. Indeed, the research carried out on this plant focused only on the knowledge of agricultural practices related to the management of diversity (N'da et al., 2013). Whereas among the determining factors for a conservation strategy, the evaluation of the genetic diversity of the different cultivated local varieties is important. Morphological and agronomic characteristics are often used in the estimation of genetic variability. Despite criticisms of the use of these markers, which are usually polygenic and influenced by environmental conditions, they have been shown to be useful in many studies on maize (López-Morales et al., 2014; Obeng-Antwi et al., 2012; Aci et al., 2018; Öner, 2019). The present study is part of the framework of the efforts made to preserve this species. The purpose of the study was to characterize the genetic diversity of purple-grain maize accessions using the morphological and agronomic descriptors proposed by Bioversity International (formerly IPGRI). The results obtained from such a study could provide maize improvers with a solid database for the possible use of local genetic resources in the improvement programs of this plant.

MATERIALS AND METHODS

Plant material

The study was conducted on 70 accessions of purple-grain maize, prospected in 2013 in the Hambol region, located in the Central-North of Côte d'Ivoire. This plant material consists of 28 accessions from 11 villages in the department of Katiola, 26 accessions from 10 villages in the department of Niakaramadougou, 12 accessions from 3 villages in the department of Fronan and 4 accessions collected in 2 villages in the department of Dabakala (Figure 1).

Site and experimental device

The agro-morphological characterization of the material collected was carried out at the research station on food crops of the National Center for Agronomic Research of Bouaké (latitude: 7° 41'N, longitude: 5° 2'W) (Ouédraogo, 2017). An alpha-lattice device (10 blocks of 7 entries / repetition) with three repetitions was used (Oura, 2011). It was put in place after a plowing followed by an initial supply of NPK fertilizer of formula 15-15-15 at the rate of 150 kg/ha. For each entry, four lines of 5 m were sown in pockets on 28 August 2013, with a spacing of 0.75 m between the lines and 0.25 m between the pockets on the line. A thinning by plant per pocket was performed 15 days after the sprouting. This resulted in a density of about 53.333 plants per hectare. On the stem elongation (40 days after sowing), 100 kg/ha of urea (46% of nitrogen) was added. The test was conducted under satisfactory water supply conditions; a total rainfall was 1300 mm of which 655 mm was during corn season.

Crop management

Two types of maintenance were conducted. A pre-emergent herbicide treatment (Dynamic plus 500 EC) was performed. A series of weeding was subsequently conducted to control Adventists who escaped chemical weeding, followed by hilling. The first weeding took place on September 19th. The second weeding was done on October 20th, followed by hilling to bury the cover fertilizer. To control stem borer insects and defoliating insects a Carbofuran treatment at a rate of 3 kg per hectare was added.

Collection of data

A set of 18 maize traits was observed taking into account the maize descriptors proposed by Bioversity International (IPGRI, 1991). They relate to plant phenology, to vegetative development and panicle, to maize cob and grains. Phenological data were recorded every 2 or 3 days throughout the flowering period on the whole of the basic plot. Vegetative descriptors and the panicle descriptors were measured on 10 plants per accessions and per block, resulting in a total of 30 plants per accessions. Cobs descriptors and grains descriptors were measured on 20 cobs per accession, collected on plants whose vegetative and panicle descriptors were measured.

Statistical analysis

The normality of the measured variables was assessed by observing the frequency distribution curves. Subsequently, descriptive analyses were carried out with SPSS software version 16.0. The mean, the standard deviation, and the coefficient of variation were calculated for all the accessions in order to assess variability among the accessions.

The coefficient of variation was calculated using the following formula (Xiang et al., 2010):

$$CV\ (\%) = \frac{S}{\bar{x}} \times 100$$

where CV = coefficient of variation, S = standard deviation and $\bar{x}$= mean.

In order to examine the structure of the morphological variation and to identify the characters that make it possible to explain it better, a Principal Component Analysis (PCA) was carried out in several stages. At first, the data matrix constructed with the averages of individuals by accession has been standardized. To verify the degree of correlation between the variables, the determinant of the matrix was calculated and the sphericity tests of Bartlett (1954) and Kaiser (1974) were analyzed. The number of

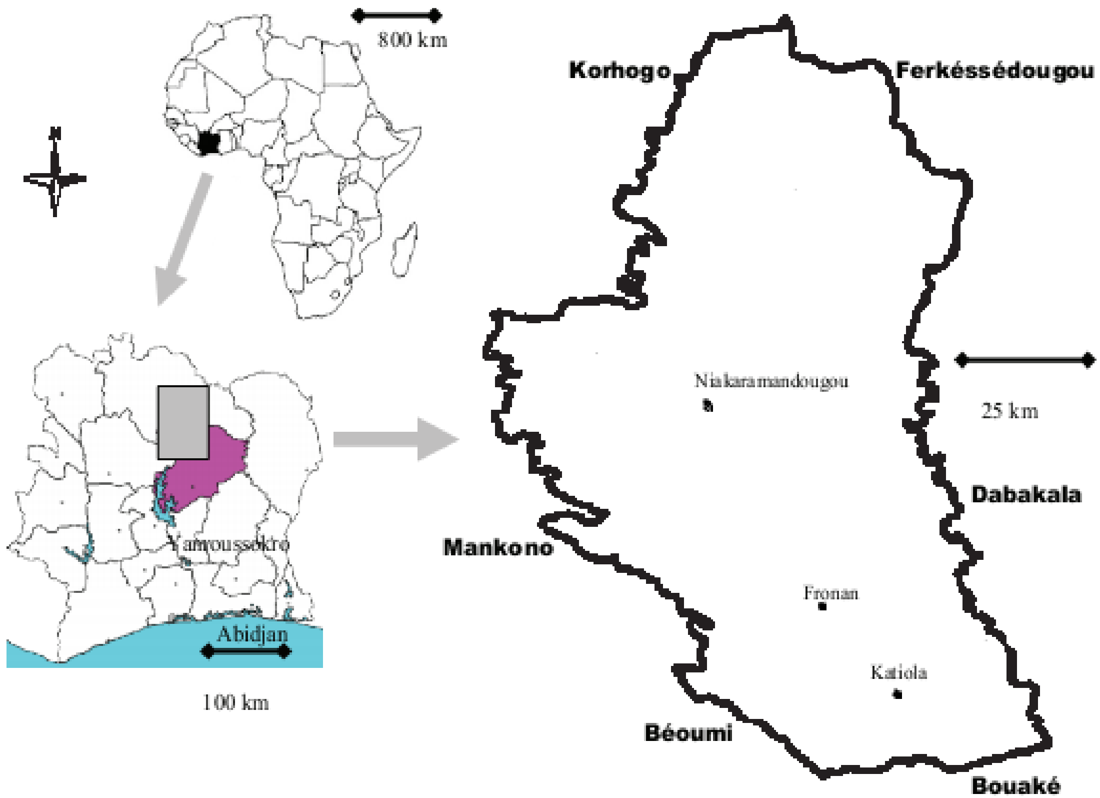

Figure 1. Location of the 3 sites prospected on the map of Côte d'Ivoire.
Source: Authors

axes has been determined according to their eigenvalue, i. e. an eigenvalue equal to or greater than the unit (the data being standardized; the inertia is equal to the number of variables; the axes must not be less than 1 to be taken into account. The Principal Component Analysis (PCA) has been supplemented by a cluster analysis to identify clusters of accessions sharing similar morphological characteristics. As part of this study, the hierarchical cluster analysis method was used for the grouping of accessions. Ward's method (Ward, 1963) was favored for aggregating accessions. This technique is suitable for grouping maize accessions. It minimizes intra-group inertia and maximizes inter-group inertia in order to obtain the most homogeneous groups possible. To determine the number of groups representing the optimal partition in the hierarchical tree, a method based on MANOVA (Mohammadi and Prasanna, 2003) was favored. It consists of cutting the dendrogram at several levels to generate different classifications. Then a MANOVA is carried out individually for each classification. The classification for which the MANOVA is significant ($P < 0.05$) was selected. Finally, to test the validity and optimize the groups obtained, an analysis of variance (ANOVA) had been performed. The ANOVAs were followed in case of significant differences, by comparison tests of mean. The Tukey HSD test, at the 0.05 significance level, was used for this purpose. Before ANOVA was performed, the normality of the data distribution and the equality of the variances (homoscedasticity) between the groups were verified. The variables were transformed using the log-based formula 10 {log (x + 1}} to stabilize variances and normalize distributions when they do not follow a normal distribution law. In the logarithmic transformation, x is the variable to be transformed. These analyses were performed with the Statistica 7.1 software (Statistica, 2005).

RESULTS

Of the 70 accessions initially sown in the field, 68 were subject to study because two accessions (Acv-29 and Acv-68) were unable to complete their vegetative cycle in time. The observation with the naked eye of the cobs of the different provenances made it possible to identify two morphotypes in all the accessions collected. These are the white-stemmed maize cob morphotypes and red-stemmed maize cob morphotypes (Figure 2).

Descriptive statistical analysis

The minimum values, the maximum values and the coefficients of variation (CV) of the quantitative traits measured on the 68 accessions (Table 1) give an idea of the variability of the germplasm characterized. Most characters have average coefficients of variation. The largest coefficients of variation were observed for the number of tertiary branching (CV = 55.35%), the length of grain (CV = 17.29%), the width of grain (CV = 15.34%) and the number of secondary branching (CV = 15.12 %). However, significant discrepancies were observed between the minimum values and the maximum values. This testifies to the existence of a diversity between the accessions. For example, the cycles seedling-flowering

(a) (b)

Figure 2. Variation in the color of the stem of maize cob in 70 accessions of purple-grain maize; (a) purple-grain maize with white-stemmed maize cob; (b) purple-grain maize with red-stemmed maize cob.
Source: Authors

Table 1. Values of the 18 quantitative traits used for the agro-morphological characterization of purple grain maize accessions.

Variable	N	Minimum value	Maximum value	Average	Error-standard	Variance	Coefficients of variation (%)
FlMa	70	55	68	61.18	2.74	7.515	4.48
FlFe	70	55	68	61.66	3.05	9.294	4.94
NoFe	70	12.6	16.6	13.90	0.59	0.348	4.25
HaPl	70	175.25	233.25	201. 04	11.37	129.468	5.66
HiEp	70	90.5	129.5	109.68	9.65	93.131	8.80
LoPa	70	37.55	46.4	41.44	1.78	3.203	4.32
LoPP	70	4.80	13.9	10.83	1.55	2.416	14.36
NRPr	70	9.65	15.4	12.54	1.41	1.992	11.25
NRSe	70	1.33	2.8	2.09	0.31	0.1	15.12
NRTe	70	0.10	1.2	0.44	0.24	0.06	55.35
PoEr	70	1.61	3.38	2.33	0.31	0.099	13.48
LoEp	70	10.85	15.9	13.64	0.90	0.811	6.60
NRGr	70	11	14.4	12.55	0.72	0.526	5.77
NGRa	70	15.9	36.8	30.45	2.65	7.041	8.71
DiEp	70	3.2	4	3.50	0.16	0.025	4.54
LoGr	70	2	4	2.96	0.52	0.262	17.29
LaGr	70	0.62	1.94	1.06	0.16	0.027	15.34
P100G	70	17.08	27.78	21.00	2.03	4.119	9.67

FlMa = Male flowering, FlFe = Female flowering, HaPl = Plant height, HiEp = cob insertion height, NoFe = Average number of leaves LoPP = Panicle pedicle length, LoPA = Panicle length, NRPR = Number of primary branches of the panicle, NRSe = Number of secondary branches; NBRTe = Number of tertiary branches; LoEp = Length of the cob, NRGr = number of rows of grains, NGRa = Average number of grains per row, DiEp = Diameter of the cob, LoGr = grain length, LaGr = grain width, P100G = weight of 100 grains.
Source: Analysis Result

Table 2. Condition of validity and removal of variables for each PCA.

	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6	PCA7
KMO	0.626	0.635	0.666	0.654	0.661	0.669	0.668
Bartlett	540.451	525.233	474.146	453.379	441.312	3371.933	363.246
ddl	153	136	91	78	66	33	28
(P)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Variables removed	NRSe	P100G, LoGr, LaGr	DiEp	NRGr	NRTe	LoPPet LoPa	

Source: Analysis Result

Table 3. Commonalities, eigenvectors, and percentage of variation expressed by the first three axes of the principal components analysis.

Variable	Commonalities	KMO	Axis1	Axis2	Axis3
FlMa	0.964	0.839	**0.969**	0.096	0.123
FlFe	0.950	0.835	**0.969**	0.008	0.105
NoFe	0.633	0.815	**0.620**	0.122	0.484
HaPl	0.802	0.810	0.123	0.161	**0.873**
HiEp	0.834	0.956	0.173	0.122	**0.888**
PoER	0.643	0.800	0.124	**0.695**	0.381
LoEp	0.716	0.546	0.218	**0.817**	0.033
NGRa	0.752	0.779	-0.131	**0.853**	0.079
Eigenvalues			3.387	1.763	1.143
% variance explained			42.338	22.046	14.290
% cumulated explained variance			42.338	64.384	78.675
Rotation method:		Varimax with Kaiser normalization			

Source: Analysis Result

male (FlMa) and seedling-flowering female (FlFe) ranged from 55 to 68 days with a mean of 61.18 and 61.66 days, respectively. Plants height ranged from 175.25 cm for the shortest accessions to 233.25 cm for the largest accessions with an average of 201.04 cm. The length of the cobs varied from 10.85 to 15.90 cm according to the accessions, with an average of 13.64 cm.

Multi-varied analyses

Relevance of quantitative traits in factorial axes

The principal component analysis (PCA) was performed on all 18 quantitative variables. According to Kaiser's rule, factors whose eigenvalue is greater than 1 have been retained. First of all, we made sure that the data was factorizable by realizing 7 successive PCAs where, at each step, we checked the relevance of a factorization of the data, as soon as we removed a variable from the analysis (Table 2). The 7 PCA led to the removal of 10 variables either because they were poorly explained by the factors retained because of a commonality score lower than 0.5, or because they were heavily loaded on several factors.

The PCA performed on the eight variables with a quality of representation greater than 0.5, highlights 3 factors whose eigenvalue is greater than 1. These factors have explained respectively 42.338, 22.046 and 14.290% of the variance of the total inertia of the cloud which constitutes a total acceptable of 78.675% (Table 3). The analysis of the composition of these three factors showed that the eight variables contributed significantly to at least one of the three axes. These eight variables are therefore most relevant for the explanation of variability. Three of the eight variables were positively correlated with the first factor. These are the male semi-flowering cycle (FlMa), the female semi-flowering cycle (FlFe) and the number of leaves. This axis can be described as the axis of precocity. These results highlight the importance of phenological traits in structuring the morphological variability of purple-grain maize. The second axis was positively correlated with variables such as Average number of grains per row (NGRa), Length of the cobs (LoEp) and weight of cobs (PoER). This axis can be considered as the axis of the yield. Two variables were strongly correlated with the third axis. These are the heights of the plants (HaPl) and the cob insertion heights (HiEp). This axis can be defined as the axis of vegetative development (Figure 3).

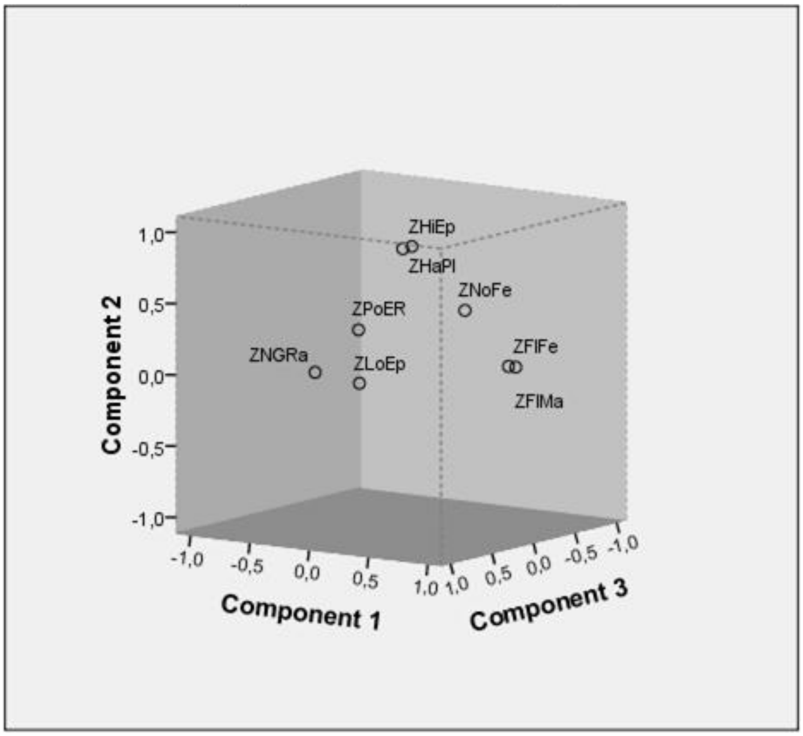

Figure 3. Projection of variables in plan 1-2 and 3 of the principal components analysis.
Source: Analysis Result

Structuring the agro-morphological diversity of purple-grain maize in the central-north of Côte d'Ivoire

The projection of individuals on the main plan formed by axes 1 and 2 (Figure 4) gave a more global picture of the diversity, but did not allow distinguishing a clear morphological structuration because of the high number of accessions analyzed. However, the examination of contributions and of square cosines indicated the accessions that contributed the most to the formation of the components. These accessions are located at the end of the plane. Thus, the first component has opposed early accessions with few leaves (acc46, acc16, acc56, acc4, acc17, acc11, acc31, acc19, acc26, acc60 and acc58), to later accessions with many leaves (acc68, acc57, acc69, acc55, acc63, acc49 and acc39). The second component has separated the accessions with shorter cobs (acc28, acc39 and acc32), from the accessions with longer cobs (acc20, acc54, acc70, acc25 and acc18).

Classification of purple-grain maize of the central-north of Côte d'Ivoire in homogeneous groups

In order to group accessions sharing similar morphological characteristics, an ascending hierarchical classification has been performed. The grouping method used was that of Ward with the distance, the so-called Euclidean. The analysis of multi varied variance allowed determining the number of optimal classes. It was after splitting into two groups that the value of F (F = 9.93, P <0.001) was the highest. The partition into two classes appears optimal, so it has been retained (Figure 5).

.An analysis of variance (ANOVAI) was performed on each variable separately. These analyzes revealed the specific characteristics that made it possible to differentiate the groups resulting from the classification. All characters, except the average number of grains per row (F = 0.89, P = 0.3494) and the weight of the cobs at harvest (F = 3.98, P = 0.0499) showed a highly significant difference (P <0.001) between the accessions of both groups (Table 4).

The first group consists of 26 accessions or 38.24% of the total. The accessions of this group had late flowering and were large in size. Moreover, it was in this group that we found the individuals with the best characteristics of yield (long cobs).

The second group was numerically the largest (61.76%). It was composed of early cycle accessions and smaller size. The cobs were of medium size.

DISCUSSION

Any varietal improvement program takes into account the

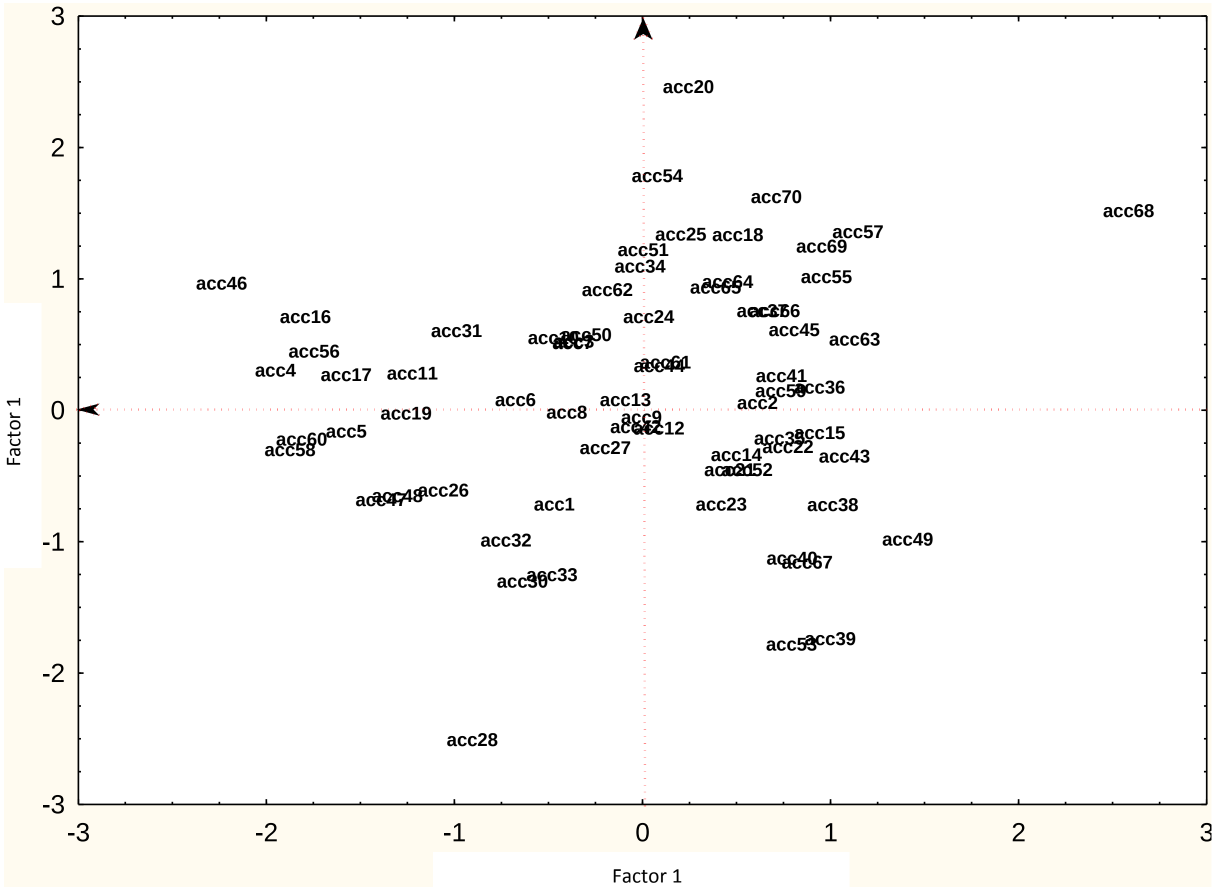

Figure 4. Projection of the 68 accessions of purple-grain maize in Plan 1 and 2 of the principal component analysis.
Source: Analysis Result

characterization of cultivated varieties and the cropping systems in which they are integrated. Such works should allow a judicious choice of genotypes to include in a varietal breeding program. The present study was an analysis of the structure of the agronomic and morphological diversity of 70 accessions of purple-grain maize from the central-north of Côte d'Ivoire. Its purpose was to identify within these accessions, the morphological characters that explain, at best, the varietal diversity reported by producers, and to see the degree of similarity or difference between them. Of the 70 accessions initially sown in the field, 68 were studied because two accessions could not complete their vegetative cycle in time. This situation is due to the fact that these accessions have a very long cycle. Indeed, until the rains stopped, they had not yet bloomed. As a result, they dried up without normally completing their vegetative cycle. The large differences between the minimum and maximum values and the high coefficients of variation for some traits suggested that there is a fairly large phenotypic heterogeneity within accessions of purple-grain maize. What constitutes an argument of choice for the conservation of the local varieties of purple-grain maize corn of the central-north of Côte d'Ivoire. The morphological variability observed between the studied accessions could be attributed to a variation in the genetic constitution of accessions, but also to reactions to environmental conditions. Indeed, producers cultivate several varieties of different characteristics, to respond to the variability of cultivation environments and thus remedy to climate risks. These results are consistent with those of several authors who have shown that local varieties of maize contain significant variability (Lucchin et al., 2003; Twumasi et al., 2017). In order to understand the organization of this diversity and to identify the characters that make it easier to explain it, we carried out a principal components analysis. The results of this analysis showed that the first three axes describe 78.675% of the total variance. The high representativeness of these axes is evidence of a strong phenotypic organization of the accessions studied. The morphological characters are strongly correlated (r> 0.6) to the axes of principal components 1, 2 and 3 (PC1, PC2 and PC3) and are dominated by phenological descriptors (number of days at 50% of male flowering, number of days at 50% of female flowering), vegetative (plant

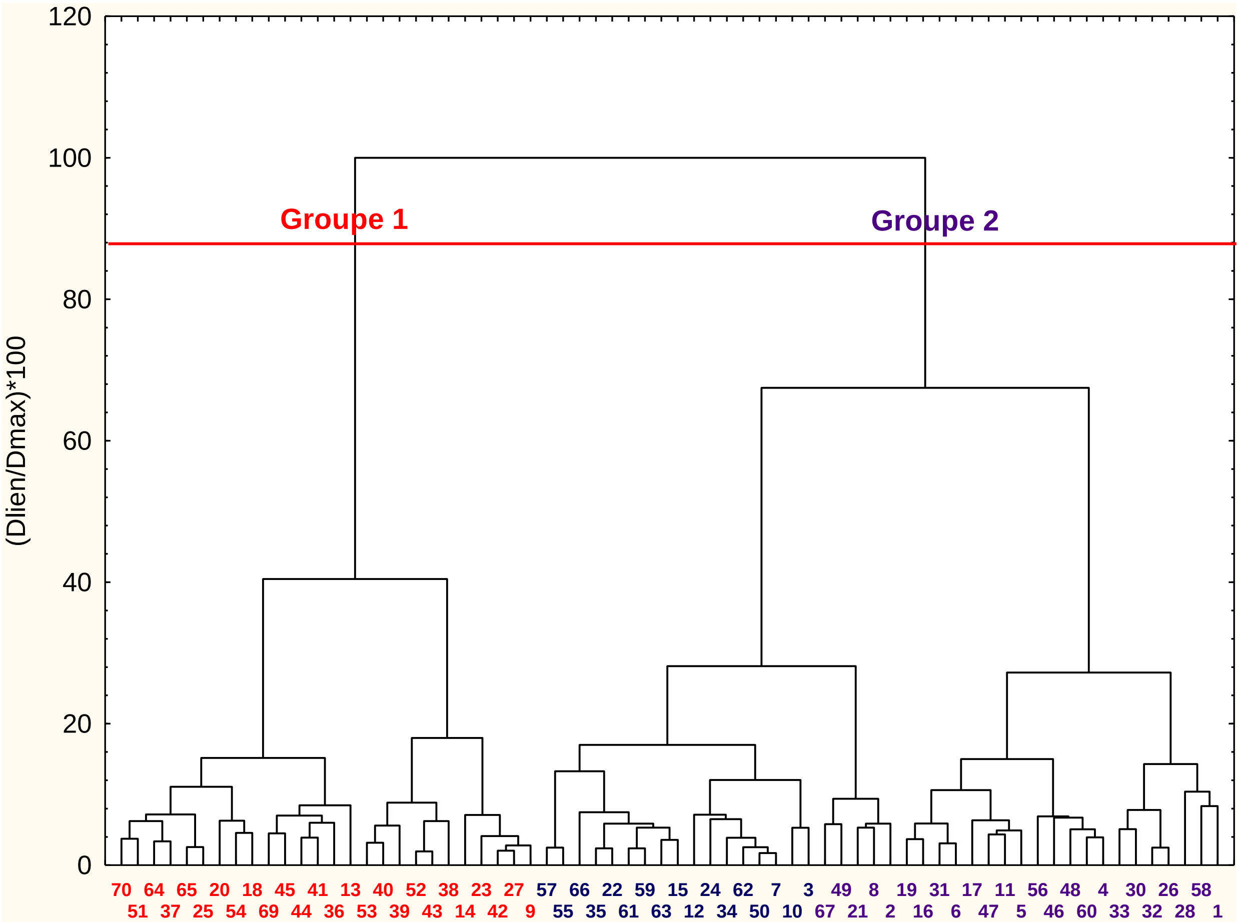

Figure 5. Ward hierarchical classification of the 68 accessions of purple-grain maize described by their agro-morphological characteristics
Source: Analysis Result

Table 4. Main characteristics of the different groups formed by hierarchical ascending classification and analysis of variance.

Variable	Groups		F	P (< 0.001)
	1	2		
FlMa	62.96 ± 0.42^{b}	59.84 ± 0.33^{a}	33.56	< 0.001
FlFe	63.51 ± 0.49^{b}	60.27±0.38^{a}	26.62	< 0.001
HaPl	210.49±1.59^{b}	195.30±1.25^{a}	55.72	< 0.001
HiEp	117.54 ± 1.33^{b}	104.80±1.05^{a}	56.29	< 0.001
LoEp	14.05±0.16^{b}	13.37 ± 0.12^{a}	10.53	0.0018
NGRa	30.93 ± 0.39^{a}	30.46±0.30^{a}	0.89	0.3494
PoER	2.41 ± 0.05^{a}	2.27±0.04^{a}	3.98	0.0499
Numbers	26	42	-	-
	38.24%	61.76%	-	-

Source: Analysis Result

heights and cob insertion height, number of leaves), and yield (weight of cobs, length of cobs, average number of grains per row). This indicates that these descriptors are the most relevant for explaining the variability between the accessions of purple-grain maize of Côte d'Ivoire's. They could therefore constitute basic criteria of comparison for subsequent investigations. The involvement of these variables in the differentiation of accessions could be closely related to the practices of producers in terms of selection, which was generally related to productivity criteria. Whereas the phenological, vegetative and yield characteristics were an integral part of these criteria. These results are in agreement with Gouesnard et al. (1997), Beyene et al. (2005) and Hartings et al. (2008), who indicated that phenological descriptors, vegetative descriptors and cob descriptors were very important for the classification of local maize varieties in France, Ethiopia and Italy, respectively. Our results also corroborate the observations made by Djè et al. (2007) on sorghum. The projection of the accessions in the plan defined by the first two principal components did not allow obtaining a clear morphological structuration. This could be explained by the fact that the number of accessions analyzed is high. On the other hand, the hierarchical analysis based on the significant components of the Principal Component Analysis made it possible to classify the accessions into two contrasting groups, each consisting of morphologically very close accessions. A first group was composed of late accessions, high size and with the best characteristics of yield and a second group was containing early accessions, small in size with poor yield characteristics. This structuring of the diversity is favorable to the use of association genetics based on candidate genes to identify genes related to agronomic traits. The selection pressure conditions to which the accessions are subjected could have favored this morphological and phenological dissimilarity observed inside this cultivar. These results are similar to those obtained by Lucchin et al. (2003) in Italy. According to these authors, the phenotypic selection carried out by each producer according to his own criteria would have led to a differentiation within the population of origin, to a differentiation of the variety "Nostrano di Storo" leading to the formation of very distinct sub-populations. Since the purple-grain maize had not yet been the subject of true varietal breeding, the groups obtained could serve as starting material for the improvement of this cultivar. The accessions from each group could be brewed to develop different populations with a broad genetic base. This will enhance and preserve the diversity. The populations from these groups of accessions may be subject to improvement within the respective population in order to propose short-term productive varieties to producers. Moreover, given that the two groups have complementary characteristics; inter-population crosses may be carried out in the long term to form a breeding pool from which crossover parents will be selected.

CONCLUSION AND PERSPECTIVES

The general objective of our study was to characterize accessions of purple-grain maize collected throughout the Hambol region. Our study showed that a strong phenotypic diversity of the purple-grain maize is maintained and managed by the populations of the Central-North of Côte d'Ivoire. Two groups of accessions were identified using eight morphological markers. Phenological descriptors (FlMa and FlFe), vegetative descriptors (HaPl and HiEp) and cobs descriptors (LoEp) seem to be the most discriminating characters. They allowed us to make a difference between early accessions and intermediate accessions. This observed diversity is an asset for the improvement of this variety. Due to the fact that local varieties play a central role in plant breeding, it would be wise to develop long-term conservation strategies to preserve this important source of variability. Since producers are the main actors in the management of varietal diversity in the traditional agrosystems, incentives toward them and their awareness raising could help maintain the local diversity of this cultivar. As a first step, we recommend promoting the use of purple-grain maize through public awareness campaigns about their market opportunities and on their agronomic benefits and nutritional benefits. Although morphological characterization is very useful for preselecting elite accessions, it remains insufficient for a description of the genetic structure of maize accessions. For this reason, exploitation of the polymorphism of neutral molecular markers vis-à-vis the environment (microsatellites for example), is a complementary and very useful approach for the conception of the management of plant genetic resources. This characterization while ensuring the choice of the material to be used in the improvement programs, will avoid the conservation of duplicates in the gene bank.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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