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Phenotypic diversity of farmer's traditional maize (Zea mays L) varieties in Cote d'Ivoire

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Abstract

Primitive cultivars and their wild relatives are an invaluable heritage for humanity. They must be preserved, studied and used to increase agricultural production. This study aims at assessing the phenotypic diversity of 118 maize (*Zea mays* L) accessions collected Côte d'Ivoire. The test was conducted in Ferkessédougou (north of Côte d'Ivoire) during the 2013/2014 and 2014/2015 rainy seasons. It was implanted in a square lattice design 11 x 11, repeated three times. Twenty-two quantitative variables were measured. From the results, it appeared that there was significant morphological variability among accessions. The multivariate analyses (Principal Components Analysis and Hierarchical Cluster Analysis) enabled to structure this diversity into five groups. Days to 50 % silking and to 50 % tasselling, ear insertion height, plant height, number of leaves below the uppermost ear, and ear parameters are those that best explain the agro-morphological diversity. The different groups obtained can serve as a starting point for the definition of a core collection. They offer a wide possibility of choice of breeding lines for the creation of improved maize varieties adapted to the different agro-climatic zones of Côte d'Ivoire.

Introduction

Food safety is a current issue in view of climate change which directly affects production plus the continuous increase of the population worldwide. According to the FAO statistics, the world population will reach 9.2 billion in 2050 (Food and Agriculture organization of the United Nation, 2012). In order to meet the needs of the population, it will be necessary to increase the global food-crop production by about 70 percent, and almost double the production in developing countries (Food and Agriculture organization of the United Nation, 2012). Cereals are an important part of food resources of humans and animals. Among all the cereals, maize (*Zea mays* L) is an excellent source of calories for many African populations because of the content of its seeds in starch. In Côte d'Ivoire, maize is the most consumed and cultivated cereal after rice (Oryza spp). However, productions are low and remain below the potential of this crop. These productions are estimated at 1.9441 t ha^{-1}, for a total planted area of 340,000 ha (FAOSTAT, 2014). The diseases, the almost total use of local varieties with low production potential, exacerbated by climate changes observed in recent years, are the main factors limiting maize production in Côte d'Ivoire. In order to help improve the productivity of this crop, it is thus necessary to remove those constraints by the development of improved varieties with high production potential. To get improved cultivars, both productive and stable, the breeder must have at his disposal the greatest genetic diversity of the species studied. The local varieties of cultivated plants or landraces, although less productive, are genetically diversified (Zeven, 1998). They show a better adaptation to climate and soil conditions of their region of origin (Zeven, 1998). These varieties are also valuable because they sometimes have very interesting genes, such as resistance to parasites. Local varieties constitute a huge reservoir of genetic resources for the improvement of allogamous species such as maize (Murariu et al, 2010; Murariu et al, 2011). They must therefore be preserved.

Field survey is the starting point to identify and map existing local varieties. It is in this context that the National Centre for Agronomic Research (CNRA) has initiated several prospecting missions in the three agro-climatic zones of Côte d'Ivoire. These surveys enabled to sample more than 600 maize accessions. However, for a better use and conservation of the collected plant material, it is important to know its genetic diversity. Indeed, the description and measurement of genetic diversity are essential prerequisites for the definition of genetic resources management strategy. Genetic diversity can be estimated on the basis of several criteria, including the phenotype, the pedigree and using molecular markers (Matus and Hayes, 2002).

Morphological characterization is the first step in the description and classification of genetic resources (Smith et al, 1991). Morphological characteristics have the advantage of being easily noticeable and constitute the level of diversity to which farmers and

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breeders have immediate access (Bellon and Berthaud, 2006). Morphological descriptors have been traditionally used for identification and management of maize cultivars in Italy (Camussi, 1979), West Africa (Alika et al, 1993), Canada (Azar et al, 1997), Spain (Bosch et al, 1997; Ruiz de Galarreta and Alvarez, 2001) and Mexico (Iouette and Smale, 2000). Today they are still used in the estimation of genetic variation of maize (Rakszegi et al, 2010; Sharma et al, 2010; Obeng-Antwi et al, 2012; Ndiso et al, 2013) and several crop species, such as rice (Sanni et al, 2008; Tripathi et al, 2013; Rabara et al, 2014; Thi Lang et al, 2014), sorghum (Bucheyeki et al, 2008; Ngugi and Maswili, 2010; Adugna, 2014), wheat (Dos Santos et al, 2009; Ateş Sönmezoğlu et al, 2012; Mondini et al, 2014), barley (Shakhatreh et al, 2010; Derbew et al, 2013) and tomato (Naz et al, 2013).

This study aims at: (i) describing and analyzing the agro-morphological diversity of maize accessions collected in Côte d'Ivoire; (ii) identifying the most discriminating variables and finally, (iii) providing an efficient management scheme for these plant breeding resources. It is supported by the assumptions according to which (i) local maize varieties in Côte d'Ivoire are genetically distinct; (ii) the genetic variability is structured according to the geographical origin.

Materials and Methods

Plant material

The gene bank of the National Centre for Agronomic Research houses an important collection of approximately 600 accessions of maize. This plant material resulted from various prospecting missions carried out in 2008, 2010 and 2013 in the three major agro-climatic zones of Côte d'Ivoire, along a north-south transect. The surveyed regions were located between longitudes 2°47'59" and 7 °42'32" W and latitudes 5°13'75" and 10°37'29" N, with altitudes ranging from 21 to 460 m. Annual rainfalls varied from 1080 to 2400 mm and average annual temperatures were between 27° and 30°C. A sample of 118 accessions was selected in order to cover all collection areas. The accessions included in this study were divided as follows: Zone 1: north (26), Zone 2: centre (37), Zone 3: south (55). Three improved varieties (Acr94TZEComp5-w, IWD-STR, Acr97TZLComp1-w) were used as controls. They came from the International Institute of Tropical Agriculture (IITA).

Experimental design

The accessions were characterized at the Research Station of Ferkessédougou (Latitude: 9°36' N; Longitude: 5°12' W; Altitude: 365 m) during the rainy seasons 2013/2014 and 2014/2015. The tests were conducted according to a Lattice design (randomized incomplete blocks) with three repetitions. This design is commonly used in morphological diversity studies, particularly when there are several numbers of accessions to be characterized. In this study, with the addition of three controls, each repetition consisted of 11 blocks of 11 inputs. The elementary plots were formed by two lines of 4 m in length, spaced at 0.75 m apart, the density being 53,333 plants ha^{-1}. Nitrogen fertilizer NPK (15-15-15) was spread during soil preparation at a dose of 300 kg ha^{-1}. Urea (46%) was spread in two parts: the first one during thinning and the second one during stem elongation (45 days after sowing) at 150 kg ha^{-1}. A selective herbicide treatment, consisting of a mixture of S-metolachlor 290 g/l and atrazine 370 g l^{-1}, was applied to the experimental plots at 3 l ha^{-1}, right after sowing before emergence of maize seedlings. The weed control was done later by hoeing as necessary. Concerning the control of defoliating insects, an insecticide, cypermethrin 50 g l^{-1} was spread at 0.8 to 1 litre per hectare as soon as the first attacks were noticed. Stem borers were controlled by spreading granular nematicide (carbofuran) at a dose of 3 kg per hectare in the leaf cone of young plants during the vegetative growth stage.

Parameters measured

Twenty two agro morphological descriptors, determined from the list of descriptors of the International Board for Plant Genetic Resources for maize (IBPGR, 1991), as well as the works published by other authors (Abu-Alrub et al, 2004; Mijangos -Cortes et al, 2007; Ortiz et al, 2008; Lopez-Morales et al, 2014), were used for phenotypic characterization.

The phenological traits evaluated were: days to 50% silk (DS) as an estimator of the life cycle, counted from the day of planting to the day when 50% of plants had emerged silks, and days to anthesis (DA) when 50% had shed pollen; anthesis–silking interval (ASI) was computed as the difference between days to 50% silking and 50% anthesis.

The vegetative characteristics measured were: plant height (PLHT) measured from the ground level to the tassel tip; ear insertion height (EHT) from the ground level to the insertion node of the ear; ratio ear height/plant height (EHT/PLHT); total number of leaves per plant (TNL) and number of leaves below (NLB) and above (NLA) the uppermost ear, determined at the time of anthesis.

The characteristics measured on the tassel were: tassel length (TL), measured from the point of origin of the lowermost branch to the tip of the central ear; tassel peduncle length (TPL), measured from the node to the first branch; and total number of branches (TNB) was counted.

The variables recorded on the ear were: ear length (EL) measured from tip to bottom; ear conicalness (EC); number of rows of kernel (NRK) and number of kernels per row (NKR) were counted; ear lower diameter (ELD); ear medium diameter (EMD), measured in the middle and ear upper diameter (EUD).

The kernel characteristics evaluated were: kernel length (KL), kernel width (KW), and 100 kernels weight

(HKWT) adjusted to 10% of humidity.

Phenological data were recorded every 2 or 3 days throughout the flowering period on the whole elementary plot. Vegetative and tassel descriptors were measured on 10 plants per accessions per block, that is, a total of 30 plants per accessions (Ruiz de Galarreta and Alvarez 2001; Jaric et al, 2010). The ear and kernel descriptors were measured on 20 ears per accession, harvested from plants whose vegetative and tassel descriptors were measured. Ten kernels taken from the central part from each of the 20 selected ears were measured in length and width, and the measures were divided by ten. The conicalness-ear-shape was determined according to the formula proposed by Ordas and Ron (1988): C = [(Di-Ds) / 2]*100 / [L/3], where Di is the lower diameter, Ds the upper diameter and L the ear length.

Statistical analysis

The collected data were organized in the form of a matrix crossing each observation (accessions) with the different parameters measured. This matrix was subjected to a descriptive analysis (average, minimum, maximum, standard deviation, coefficient of variation). In order to avoid bias related to the difference in scale between the variables, the data were standardized so that their average was zero and the standard deviation was equal to 1 (Mohammadi and prasanna, 2003). The data thus transformed were used to construct a correlation matrix. This matrix was used to perform a standardized principal components analysis (PCA) with "Varimax rotation" (Iezzoni and Pritts, 1991). Before doing so, the applicability of the method via the sampling precision test of Kaiser-Meyer-Olkin (KMO) and Bartlett's test of sphericity was checked. Following the standardized PCA, a hierarchical cluster analysis (HCA) (Peeters and Martinelli, 1989) was conducted, using the coordinates of individuals in key axes selected as classification variables. This way of working has a double advantage. It enables on one hand to dispense with the choice of the distance by imposing the Euclidean distance as a measure of similarity between accessions (Studnicki et al, 2013) and on the other hand to avoid difficulties due to correlations between variables as the factors obtained are independent. The unweighted pair group method using arithmetic averages (UPGMA) was used to aggregate the accessions (Légendre and Légendre, 1998). This technique is suitable for grouping maize accessions (Rinco et al, 1996) because it causes little distortion in comparison to the initial similarity matrix and provides more contrast in the classification (Legendre and Legendre, 1998). The quality of representation of the dendrogram provided relating to the original data was quantified using the cophenetic correlation coefficient, as described by Rincon et al (1996) and Legendre and Legendre (1998). This coefficient was estimated using the Mantel test (Mantel, 1967). To determine the number of groups representing the optimal partition in the hierarchical tree, a multivariate analysis of variance (MANOVA) was performed according to Mohammadi and Prasanna (2003). In order to test the validity and optimize the groups obtained, an analysis of variance (ANOVA) was conducted. Finally, a discriminant analysis (stepwise method) was performed to determine which variables differentiate best the groups identified in the hierarchical classification. The selection criteria used to estimate this discriminatory power is the multivariate Wilks' lambda. As a preliminary to this, the normality

Table 1 - Minimum, maximum and mean values, standard deviation (SD) and coefficients of variation (CV) for 22 quantitative characters in maize germplasm.

Traits	Minimum	Maximum	Mean	SD	CV (%)
DA (days)	50.000	74.330	63.164	5.428	8.593
DS (days)	52.000	76.667	65.894	6.017	9.131
ASI (days)	0.000	6.000	2.756	1.076	39.032
PLHT (cm)	174.967	305.667	252.072	30.469	12.087
EHT (cm)	76.000	197.333	142.289	26.629	18.715
EHT/PLHT	0.424	0.657	0.559	0.045	7.970
NLA (no)	5.767	8.300	7.228	0.382	5.284
NLB (no)	5.833	11.800	8.990	1.459	16.226
TNL (no)	11.867	19.333	16.217	1.677	10.342
TPL (cm)	17.000	25.283	21.630	1.478	6.835
TL (cm)	31.883	50.867	41.586	2.696	6.483
TNB (no)	11.000	23.467	18.806	2.240	11.913
EL (cm)	14.357	20.800	17.681	1.533	8.671
NRK (no)	11.200	16.000	14.096	0.940	6.671
NKR (no)	25.474	45.364	37.916	3.683	9.713
ELD (cm)	3.810	5.650	4.828	0.347	7.187
EMD (cm)	3.340	5.080	4.469	0.333	7.444
EUD (cm)	2.810	4.660	3.998	0.376	9.416
KL (mm)	6.100	10.600	8.719	1.022	11.722
KW (mm)	4.400	9.800	6.919	0.903	13.057
EC (%)	2.613	15.222	7.102	1.868	26.302
HKWT (g)	21.530	37.040	30.211	2.898	9.593

of variables and the equality of variance/covariance matrices of the groups were checked. The homogeneity of variance/covariance matrices was checked through the Box test. Descriptive analyses were performed with the SPSS 16.0 software. (Statistical Package for the Social Sciences software, 2007). The principal component analysis, the hierarchical cluster analysis, and the factorial and discriminant analyses were performed using the STATISTICA 7.1 (Statistica, 2005) and SPSS 16.0 softwares. The Mantel test was performed with the XLSTAT software (XLSTAT, 2007).

Results

Descriptive Analysis

Over the 118 ecotypes initially sown in the field, 116 were the subject of study as three ecotypes were unable to complete their vegetative cycle in time. The descriptive analysis of the 22 quantitative characters revealed significant differences between the minimum and maximum values (Table 1). This indicates the presence of fairly large morphological variability among accessions for these characters.

Principal Component Analysis

The PCA was performed on the correlation matrix. A first PCA carried out with all the 22 quantitative variables showed that the correlation matrix is singular (determinant = 0.0). Therefore, the ASI, EHT/PLHT, TNL variables, and EC from the linear combination of other variables were removed from the model. A second PCA showed that communalities were good for most variables (higher than 0.5), except for the TL (0.336), TNB (0.409), NLA (0.485), and HKWT (0.487) variables. A new PCA over 14 variables was performed excluding the four variables whose communality was lower than 0.5. It is clear from this analysis that all the variables are factorable (index of overall KMO = 0.848). The KMO indices per variable were higher than 0.50 except the TPL variable (0.243) which has been excluded. Moreover, the Bartlett's test of sphericity revealed significant results ($p = 0.000 < 0.05$; chi-square = 2,140.103), thus enabling to reject the H_0 hypothesis of no correlation. It is therefore possible to obtain an efficient summary in a reduced number of factors.

The principal component analysis performed on the remaining 13 variables enabled to select three factorial axes which have an intrinsic value higher than 1 (Table 2). These three principal components (PCs) restored 84.572% of the total variance. The first principal component (PC1) accounted for 60%, the second principal component (PC2) accounted for 15.625% and the third principal component (PC3) accounted for 8.946% of the variance. The first PC was positively and significantly correlated with the initial variables: DA, DS, PLHT, EHT, and NLB. This axis represents the precocity and vegetative development of accessions. The second PC was positively and significantly correlated with the variables EL, NKR, ELD, EMD, EUD, KL, and KW. It can be defined as the axis of yield. The third PC is positively correlated with the variable NRK. This axis provides additional information to PC2.

The projection of individuals and variables in the main plane (plane 1-2), did not reveal a typology of accessions given the high number of individuals analyzed (Figure 1). However, the analysis of contribu-

Table 2 - Eigenvectors, eigenvalues and accumulated variation of the first three principal components (PC) from the correlation matrix based on maize population means.

Traits	communalities	KMO	Eigenvectors		
			PC1	PC2	PC3
Zscore(DA)	0.920	0.839	0.925	0.202	0.156
Zscore(DS)	0.925	0.835	0.933	0.178	0.145
Zscore(PLHT)	0.905	0.815	0.894	0.319	0.045
Zscore(EHI)	0.927	0.810	0.932	0.227	0.077
Zscore(NLB)	0.879	0.956	0.879	0.323	0.030
Zscore(EL)	0.727	0.800	0.250	0.796	-0.175
Zscore(NRK)	0.872	0.546	0.173	0.135	0.907
Zscore(NKR)	0.708	0.779	0.192	0.794	-0.198
Zscore(ELD)	0.854	0.945	0.381	0.789	0.291
Zscore(EMD)	0.891	0.900	0.250	0.813	0.406
Zscore(EUD)	0.866	0.920	0.394	0.778	0.322
Zscore(KL)	0.826	0.854	0.203	0.863	0.198
Zscore(KW)	0.686	0.806	0.394	0.718	-0.153
Zscore(TPL)	0.674	0.243			
Zscore(TL)	0.336				
Zscore(TNB)	0.409				
Zscore(NLA)	0.485				
Zscore(HKWT)	0.487				
Eigenvalues			7.800	2.031	1.163
Proportion (%)			60.000	15.625	8.946
Cumulative (%)			60.000	75.625	84.572

Rotation Méthod: Varimax with Kaiser Normalisation.

PC - Principal component

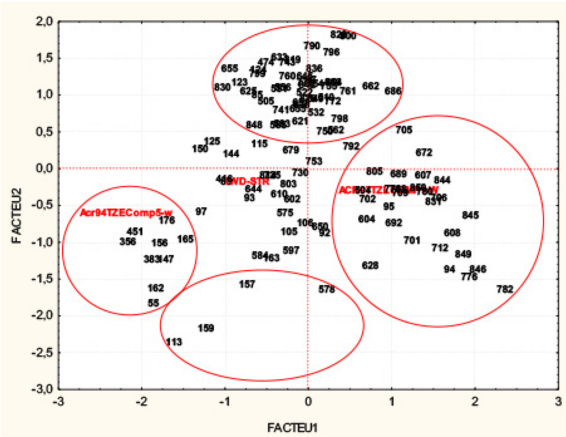

Figure 1 - Dispersion of 116 accessions evaluated in Côte d'Ivoire in the space defined by the first two main components.

tions and raised cosines indicated the individuals that most influenced the emergence of components. PC1 opposed precocious and short accessions (55, 147, 156, 165, 176, 356, 383, 451 and the precocious control Acr94TZEComp5-w) to the late and great accessions (94, 95, 608, 689, 701, 705, 706, 712, 776, 780, 782, 831, 844, 845, 846, 849, 850 and late control Acr97TZLComp1-w). Whereas PC2 enabled to differentiate the accessions having long ears, filled with kernels and large (123, 124, 474, 505, 621, 633, 645, 662, 686, 743, 760, 772, 790, 796, 798, 800, 819, 828, 830, 836), from the accessions having short ears with little kernels (113, 157, 159, 578).

Cluster analysis

A hierarchical cluster analysis (HCA) was carried out from three significant principal components of the PCA. In order to get the best possible classification, several analyses have been tested with different aggregation methods. It appeared that the aggregation method that provided the best result was the unweighted pair group method using arithmetic averages (UPGMA). This method provided the phenetic tree of Figure 2. The cophenetic correlation coefficient was 0.720; this indicates that the dendrogram is a reliable reflection of raw data. The multivariate analysis of variance enabled to determine the number of optimal classes. It was after the splitting in five groups that the value of F (F = 11.442; p = 0.000) was the highest. The partition into five classes appeared optimal, it was therefore selected.

Table 3 presents the morphological characteristics of the different groups obtained from the HCA. The first group consisted of 11 accessions and the Acr94TZEComp5-w control, that is, 10.17% of the total number. This group contained the most precocious and smallest accessions. It was in this group that were also found the accessions having the smallest ears with few kernels. This group was composed of accessions from northern Côte d'Ivoire only. Group 2 contained 22 intermediate cycle (18.64%) and large accessions. The ears of these accessions were average with an average number of kernels. It consisted of ten accessions from the southern area, two from the southwest, one from the centre, one from the centre-west, one from the centre-north, three from the north-centre and four from the north. Group 3 consisted of 60 accessions and the IWD-Str control. It contained the highest number of accessions (61), that is, 51.69%. This group also included intermediate cycle and large accessions. Individuals had long and big ears with many kernels per row. The kernels were long and wide. This group showed the accessions having the best yield characteristics. They came from all collection areas. Group 4 consisted of 21 accessions (17.79%) having late cycle and very large with ears inserted very high. The individuals in this group had more leaves than the individuals from other groups. The ears were long with many kernels. Eight accessions came from the South, seven from the southwest, four from the centre-west and one from the northwest. Group 5 consisted of two accessions (1.69%) precocious and medium sized. The ears were thin with a small diameter. The individuals in this group had purple and small kernels. These accessions were specific to the centre-north of Côte d'Ivoire, mainly Katiola department.

Factorial and Discriminant Analyses

Table 4 presents the results of the average group equality test. The Fisher's statistical F-test showed that 13 variables enables to discriminate between the five groups (p = 0.00 < 0.05). The flowering precocity (DS and DA) was the variable that most accounted for the difference between groups. Afterwards, there were EHT, PLHT, NLB, EUD, EMD, ELD, EL, KL,

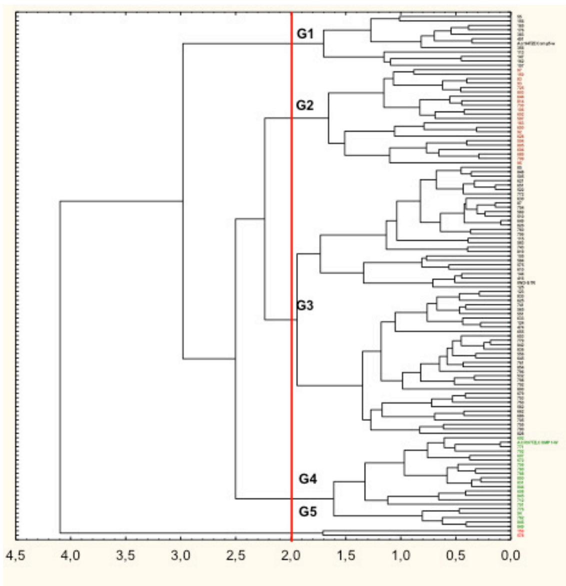

Figure 2 - Cluster analysis dendrogram (using Euclidean distances and aggregation criterion UPGMA) of the 116 accessions based on three main components higher than 1 PC.

KW, and NKR. The number of rows of kernel (NRK) seemed to be the least explanatory variable (lambda was closer to 1 and the value of F was lower).

Discussion

The field survey is the most important phase for the safeguard of plant breeding resources. Its purpose is to detect, locate the plant material still existing. It was in that context that surveys were conducted in the different agro-ecological zones of Côte d'Ivoire in order to collect genetic resources of maize. This study comes at the right time, on the one hand, to make an inventory of all the ecotypes of maize in Côte d'Ivoire and on the other hand, to establish a new database for the selection of improved varieties. Phenotypic studies showed that there was significant diversity among maize landraces cultivated by farmers. The characteristics related to precocity, vegetative growth and morphology of the ear were the most discriminating. The plant height, the ear insertion height and the ear length have also enabled to better identify maize accessions in Eastern Serbia (Jaric et al, 2010) and in the mountains of Michoacan, Mexico (Mijangos-Cortés et al, 2007). Similarly, precocity enabled to split into four groups the local maize varieties of Shikoku and Kyushu in Japan (Harada et al, 2009). Like most cultivated crops, the morphological diversity of maize observed in this study may result from voluntary and/or involuntary selection process from farmers. Indeed, in Côte d'Ivoire maize is grown primarily for its kernels used in human and animal consumption. The farmers would choose as seed to renew their crop, the biggest and most beautiful ears from the previous harvest. This mass selection might be the cause of a differentiation of varieties in several subgroups. Louette and Smale (2000), through the results of the study on seed selection practices by the farmers of Cuzalapa, Mexico, showed that phenotypic selection based on the characteristics of the ear helps maintain a phenotypic differentiation between the varieties of maize despite significant gene flow.

On the basis of the key components of PCA, a classification of ecotypes was performed. In the study on wheat, Khodadadi et al (2011) showed that the classification based on the components of the principal component analysis led to a better grouping of genotypes compared to a classification based on the original variables. The results obtained in this study help confirm this hypothesis. Indeed, the hierarchical cluster analysis clearly showed the presence of five distinct groups among the Ivorian ecotypes of maize studied. Each group contained accessions of different agro-ecological zones, spread unevenly. Accessions from high rainfall areas (annual rainfall: 1,521 mm; min = 1,500; max = 2,400 mm) were later. Those in zone 2, which is a transition zone between forest and savannah (average: 1,128 mm; min = 1,000 mm, max = 1,700 mm) were represented in groups 2 and 3 (intermediate). Whereas accessions of zone 1, which is a zone of less rainfall (average 1,092 mm; min = 1,000 mm and max = 1,600 mm) were precocious or intermediate. This confirms the previous observations and shows that precocity is the most important parameter in the structuring of phenotypic diversity. The involvement of other parameters in discriminating accessions is explained by the fact that these parameters were significantly correlated to the cycle. This is consistent with the work of Gouesnard et al (1997). According to these authors, the traits related to precocity are strongly correlated with height traits and are very important for the classification of maize populations. Our results also show that the climate zone has an influence on the cycle duration and the choice of varieties. Maize cultivation in Côte d'Ivoire being rain-fed, the best strategy for farmers in less rainfall zones (zone 1) consists in selecting precocious or intermediate varieties. These varieties complete their cycle earlier and thus evade the high temperatures and lack of water at the end of cycle. In addition to that, precocious varieties are more suitable in cropping systems that incorporate peanuts, cotton and maize, practiced in these areas. In these rotation systems, maize comes right after cotton. A precocious or extra precocious variety is thus advised. However, in areas where water supply conditions are satisfactory (zone 2) later varieties are

Table 3 - Mean values for five clusters formed by hierarchical clustering and analysis of variance.

	Clusters					F	P
Traits	1	2	3	4	5		
DA	52.305±2.346a	63.954±4.340b	63.136±2.843b	69.380±2.047c	55.166±7.306a	62.03	<0.001
DS	54±2.201a	66.863±4.969b	65.748±3.072b	72.976±2.354c	56.667±6.599a	64.32	<0.001
PLHT	191.441±12.318a	246.139±20.478b	255.685±19.903c	284.889±14.177d	226.309±26.883b	50.451	<0.001
EHI	91.592±10.809a	138.955±17.469bc	143.017±16.321c	174.853±14.493d	119.006±24.756b	54.009	<0.001
NLB	6.453±0.481a	8.492±1.158b	9.151±0.961c	10.680±0.696d	7.000±0.942a	44.419	<0.001
EL	15.322±0.612a	16.733±1.186b	18.628±1.118d	17.408±0.955c	16.250±0.989abc	32.436	<0.001
NRK	13.805±0.900a	14.857±0.617c	13.985±0.833a	14.055±0.855a	11.300±0.141b	11.64	<0.001
NKR	32.379±3.161a	36.245±2.418b	40.037±2.861c	36.977±2.258b	34.705±4.231ab	24.857	<0.001
ELD	4.272±0.162a	4.740±0.187b	5.017±0.282c	4.776±0.156b	3.920±0.155a	34.57	<0.001
EMD	3.975±0.167b	4.445±0.206c	4.645±0.248d	4.367±0.191c	3.405±0.091a	36.57	<0.001
EUD	3.318±0.224a	3.945±0.225b	4.187±0.273c	3.986±0.160b	2.975±0.233a	41.839	<0.001
KL	7.217±0.454a	8.514±0.643b	9.309±0.835c	8.312±0.467b	6.250±0.212a	32.025	<0.001
KW	5.567±0.541a	6.395±0.90b	7.383±0.673d	7.024±0.364c	5.550±0.494ab	25.295	<0.001
No. accessions	12	22	61	21	2		
	10.17%	18.64%	51.69%	17.79%	1.69%		

*Means followed by the same letter are not significantly different at the 5% level by tukey's multiple range test.

Table 4 - Tests of equality of group means.

Traits	Wilk's Lamda	F	df1	df2	Sig
Zscore (DS)	0.305	64.331	4	113	.000
Zscore (DA)	0.313	62.038	4	113	.000
Zscore (EHI)	0.343	54.008	4	113	.000
Zscore (PLHT)	0.359	50.447	4	113	.000
Zscore (NLB)	0.389	44.366	4	113	.000
Zscore (EUD)	0.403	41.823	4	113	.000
Zscore (EMD)	0.436	36.590	4	113	.000
Zscore (ELD)	0.450	34.571	4	113	.000
Zscore (EL)	0.465	32.441	4	113	.000
Zscore (KL)	0.469	32.027	4	113	.000
Zscore (KW)	0.528	25.303	4	113	.000
Zscore (NKR)	0.532	24.863	4	113	.000
Zscore (NRK)	0.708	11.638	4	113	.000

of special interest. They enable to extend the vegetative period of maize, which results in a higher yield potential. The influence of climatic zone noticed over the duration of the cycle could be also due to phenotypic plasticity. According to Haussmann et al (2012), thanks to phenotypic plasticity, genotypes grown under varied rainfall conditions adapt to the constraints of the environment through the reduction or increase certain characters such as the cycle, biomass, tillering, etc. These results are also in accordance with Manzelli et al (2005) who reported that the local environmental conditions affect the genotypic constitution of landraces, suggesting thus a close relationship between agro-ecological conditions and morphological variation of the genetic material. The importance of climatic conditions noticed in the structuring of crop diversity has been highlighted by several authors. Ntundu et al (2006) studied the morphological diversity of 100 accessions of Bambara groundnut [*Vigna subterranea* (L) Verdc] collected in Tanzania. They got three groups according to geographical origin. Accessions from southern Tanzania differed from the others. These authors concluded that weather conditions such as average rainfalls (1,000-1,600 mm per year) coupled with high humidity noticed in this agro ecological zone would be among the factors behind this distinction. Adugna (2014) studied the in situ diversity and the structure of local populations of sorghum in Ethiopia using phenotypic and molecular markers. He observed a wide variation in quantitative characters which are in part attributed to differences in rainfall and temperature in collection areas. Similarly, Brush and Perales (2007), comparing the number of maize varieties in villages in three areas representing an altitudinal gradient in Chiapas, Mexico, have shown that climate differences were the main determinant of diversity. Thus, for a future planning of maize genetic resource collection in Côte d'Ivoire, these factors should be taken into consideration.

Conclusion and implications for conservation

This study revealed that there was considerable morphological diversity among maize landraces cultivated in Côte d'Ivoire. Precocity was the most discriminating parameter. It helped structuring accessions into five groups. Late accessions were collected in forest areas with high rainfall (south, southwest, centre-west and northwest), while accessions from savannah areas with less rainfall (North-Centre, Northeast) were precocious or intermediate. It appears that climatic factors such as rainfall and temperature would influence the duration of cycle and must now be taken into account in the planning of maize collection missions in Côte d'Ivoire. It also appears that farmers' practices play a key role in the organization of the diversity of plants grown in traditional areas. In terms of in situ conservation actions must be taken to ensure the continuity of the mechanisms that are at the origin of this diversity and its maintenance, including the renewal of varieties from seeds extracted from previous harvests. Moreover, for ex situ conservation of genetic resources of maize, it is necessary to identify a small number of accessions that are representative of genetic variation in the collection. Indeed, given the allogamous mode of maize reproduction, it is mandatory to increase, preserve and store a large number of populations. Hence the idea of establishing a core collection. Then, for example, the five groups identified by hierarchical cluster analysis, can serve as starting point for a stratified sampling. A selection intensity (about 10 to 20% of the base collection) shall be adopted, then the number of accessions to be sampled in each stratum (group) shall be determined according to the logarithmic method. The use of molecular markers (SSR and SNP) will then enable to further analyze the genetic diversity of the core collection and define groups with combining ability or heterotic groups.

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References

Abu-Alrub I, Christiansen JL, Madsen S, Sevilla R, Ortiz R, 2004. Assessing tassel, kernel and ear variation in Peruvian highland maize. Plant Genet. Res. Newslett. 137: 34-41

Adugna A, 2014. Analysis of in situ diversity and population structure in Ethiopian cultivated *Sorghum bicolor* (L.) landraces using phenotypic traits and SSR markers. Springer Plus 3: 212

Alika JE, Aken'Ova ME, Fatokun CA, 1993. Variation among maize (*Zea mays* L.) accessions of Bendel State, Nigeria. Multivariate analysis of agronomic data. Euphytica 66: 65-71

Ateş Sönmezoğlu Ö, Bozmaz B, Yildirim A, Kandemir N, Aydin N, 2012. Genetic characterization of Turkish bread wheat landraces based on microsatellite markers and morphological characters. Turk J Biol 36: 589-597

Azar C, Mather DE, Hamilton RI, 1997. Maize landraces of the St.Lawrence–Great Lakes region of North America. Euphytica 98:141-148

Bellon MR, Berthaud J, 2006. Traditional Mexican agricultural systems and the potential impacts of transgenic varieties on maize diversity. Agr Hum. Values 23: 3–14

Bosch L, Casanas F, Sanchez E, Nuez F, 1997. Variability of maize landraces from Northwest Spain. Plant Genet Res Newslett 112: 90–92

Brush SB, Perales HR, 2007. A maize landscape: Ethnicity and agro-biodiversity in Chiapas Mexico. Agr Ecosyst Environ 121: 211–221

Bucheyeki TL, Gwanama C, Mgonja M, Chisi M, Folkertsma R, 2008. Morphological Characterization of Tanzanian Sorghum [*Sorghum bicolor* (L) Moench] Landraces. Kasetsart J (Nat Sci) 42: 579 – 588

Camussi A, 1979. Numerical taxonomy of Italian populations of maize based on quantitative traits. Maydica 24: 161-174

Derbew S, Mohammed H, Urage E, 2013. Phenotypic Diversity for Qualitative Characters of Barley (*Hordeum vulgare* (L.)) Landrace Collections from Southern Ethiopia. Inter J Sci Research 2: 34-40

Dos Santos Teresa MM, Gananca Filipe, Slaski Jan J, Pinheiro de Carvalho MAA, 2009. Morphological characterization of wheat genetic resources from the Island of Madeira, Portugal. Genet Resour Crop Ev 56: 363–375

FAOSTAT 2014. Statistical database of the food and agriculture of the United Nations. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor. Accessed 23 July 2014

Gouesnard B, Dallard J, Panouille A, Boyat A, 1997. Classification of French maize populations based on morphological traits. Agronomie 17: 491–498

IBPGR 1991. Descriptors for Maize. Mexico City (Mexique) / Rome (Italy), CIMMYT / IPGRI

Iezzoni AF, Pritts MP, 1991. Applications of principal component analysis to horticultural research. Hort Science 26: 334–338

Jaric JK, Prodanovic S, Iwarsson M, Minina A, 2010. Diversity of maize (*Zea mays* L.) landraces in eastern serbia: Morphological and storage protein characterization. Maydica 55: 231-238

Harada K, Huan NV, Ueno H, 2009. Classification of Maize Landraces from Shikoku and Kyushu, Japan, Based on Phenotypic Characteristics. JARQ 43(3): 213 – 220

Haussmann BIG, Rattunde HF, Weltzien-Rattunde E, Traoré PSC, vom Brocke K, Parzies HK, 2012. Breeding Strategies for Adaptation of Pearl Millet and Sorghum to Climate Variability and Change in West Africa. J Agron Crop Sci 198: 327-339

Khodadadi M, Fotokian MH, Miransari M, 2011. Genetic diversity of wheat (*Triticum aestivum* L.) genotypes based on cluster and principal component analyses for breeding strategies. Australian J Crop Sci 5(1): 17-24

Legendre P, Legendre L, 1998. Numerical Ecology. Second English Edition. Elsevier Science BV, Amsterdam

López-Morales F, Taboada-Gaytán OR, Gil-Muñoz A, López PA, Reyes-López D, 2014. Morphological diversity of native maize in the humid Tropics of Puebla, Mexico. Tropical and Subtropical Agroecosystems 17: 19 – 31

Louette D, Smale M, 2000. Farmers' seed selection practices and traditional maize varieties in Cuzalapa, Mexico. Euphytica 113: 25–41

Mantel N, 1967. The detection of disease clustering and a generalized regression approach Cancer Res 27: 209-220

Manzelli M, Benedettelli S, Vecchio V, 2005. Agricultural biodiversity in northwest Somalia an assessment among selected Somali sorghum (*Sorghum bicolor* (L.) Moench) germplasm. Biodivers Conserv 14: 3381-3392

Matus I, Hayes PM, 2002. Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome 45: 1095–1106

Mijangos-Cortés JO, Corona-Torres T, Espinosa-Victoria D, Muñoz-Orozco A, Romero-Peñaloza J, Santacruz-Varela A, 2007. Differentiation among maize (*Zea mays* L.) landraces from the Tarasca Mountain Chain, Michoacan, Mexico and the Chalqueno complex. Genet Resour Crop Ev 54: 309–325

Mohammadi SA, Prasanna BM, 2003. Analysis of Genetic Diversity in Crop Plants-Salient Statistical Tools and Considerations. Crop Sci 43: 1235–1248

Mondini L, Grausgruber H, Pagnotta MA, 2014. Evaluation of European emmer wheat germplasm for agro-morphological, grain quality traits and molecular traits Genet Resour Crop Ev 61: 69–87

Murariu M, Murariu D, Haş V, 2010. Maize local landraces used like prebreeding material for simultaneous improvement of main agronomic traits.

Lucrări științifice 53(2) seria Agronomie: 42-47
Murariu M, Murariu D, Placinta DD, Leonte C, Simioniuc D-P, Leahu A, Avramiuc M, 2011. Evaluation of romanian maize local landraces for Increasing the efficiency of their use in breeding Programs. Lucrări științifice 54(2) seria Agronomia. 77-83
Naz S, Zafrullah A, Shahzadhiand K, Munir N, 2013. Assessment of genetic diversity within germplasm accessions in tomato using morphological and molecular markers. J Anim Plant Sci 23(4): 1099-1106
Ndiso JB, Mugo S, Kibe AM, Pathaka RS, 2013. Phenotypic diversity in local coastal maize landraces in Kenya. Int J Agr Sci 3(10): 53-59
Ngugi K, Maswili R, 2010. Phenotypic diversity in sorghum landraces from Kenya. Afr Crop Sci J 18(4): 165-173
Ntundu WH, Shillah SA, Marandu WYF, Christian sen JL, 2006. Morphological diversity of bambara groundnut [*Vigna subterranea* (L.) Verdc.] landraces in Tanzania. Genet Resour Crop Ev 53: 367–378
Obeng-Antwi K, Craufurd PQ, Menkir A, Ellis RH, Sallah PYK, 2012. Phenotypic Diversity in Maize Landraces in Ghana. Int J Sci Ad Tech 2 (5): 39-70
Ordas A, de Ron AM, 1988. A method to measure comicalness in maize. Maydica 33: 261–267
Ortiz R, Crossa J, Franco J, Sevilla R, Burgueño J, 2008. Classification of Peruvian highland maize races using plant traits. Genet Resour Crop Ev 55: 151–162.
Peeters JP, Martinelli JA, 1989. Hierarchical cluster analysis as a tool to manage variation in germplasm collections. Theor Appl Genet 78: 42-48
Rabara RC, Ferrer MC, Diaz CL, Newingham MCV, Romero GO, 2014. Phenotypic Diversity of Farmers' Traditional Rice Varieties in the Philippines. Agronomy 4: 217-241
Rakszegi M, Kisgyörgy BN, Tearall K, Shewry PR, Lang L, Phillips A, Bedo Z, 2010. Diversity of agronomic and morphological traits in a mutant population of bread wheat studied in the Healthgrain program. Euphytica 174: 409–421
Rincon F, Johnson B, Crossa J, Taba S, 1996. Cluster analysis, and approach to sampling variability in maize accessions. Maydica 41: 307–316
Ruiz de Galarreta JI, Alvarez A, 2001. Morphological classification of maize landraces from northern Spain. Genet Resour Crop Ev 48: 391–400
Sanni KA, Fawole I, Guei RG, Ojo DK, Somado EA, Daniel TD, Ogunbayo SA, Sanchez I, 2008. Geographical patterns of phenotypic diversity in *Oryza sativa* landraces of Côte d'Ivoire. Euphytica 160: 389-400
Shakhatreh Y, Haddad N, Alrababah M, Grando S, Ceccarell S, 2010. Phenotypic diversity in wild barley (*Hordeum vulgare* L. ssp.Spontaneum (C. Koch) Thell.) accessions collected in Jordan. Genet Resour Crop Ev 57: 131–146
Sharma L, Prasanna BM, Ramesh B, 2010. Analysis of phenotypic and microsatellite-based diversity of maize landraces in India, especially from the North East Himalayan region. Genetica 138(6): 619-631
Studnicki M, Mądry W, Schmidt J, 2013. Comparing the Efficiency of Sampling Strategies to Establish a Representative in the Phenotypic-based Genetic Diversity Core Collection of Orchardgrass (*Dactylis glomerata* L.). Czech J Genet Plant Breed 49(1): 36–47
SPSS 2007. Statistical Package for the Social Sciences; version 16.0. Chicago: Polar Engineering and consulting
Statistica 2005. Statistica for windows; version 7.1.Tulsa: StatSoft Inc
Tripathi MP, Sthapit BR, Subedi LP, Sah SK, Gyawali S, 2013. Agro-morphological variation in ''Jhinuwa'' rice landraces (*Oryza sativa* L.) of Nepal. Genet Resour Crop Ev 60: 2261–2271
Zeven AC (1998). Landraces: A review of definitions and classifications. Euphytica 104: 127–139