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Agro-morphological variability of *Zea mays* (L.) accessions collected in Southern Benin

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A better approach to assess plant genetic diversity is the agro-morphological characterization. The main objective of this study was to investigate the morphological variability of 87 maize (*Zea mays* L.) accessions collected in different agro-ecological zones of southern Benin. Thus, 16 agro-morphological characters (seven quantitative and nine qualitative) were selected from the maize descriptors. The experimental design used is an incomplete randomized block with three replications. The mixed model analysis of two factors variance revealed a very highly significant difference for all accessions for each quantitative agro-morphological characteristic evaluated. The numerical classification of all corn accessions revealed five groups of accessions. The results of the stepwise discriminant analysis revealed five agro-morphological characteristics (germination days, female flowering, plant height, ear height and sensitivity to streak) most discriminating. The results of numerical classification supervised based on the most discriminating variables enable identification of the best accessions. Comparison of the two types of accessions (local and improved) revealed a significant difference among of them for agro-morphological characteristics with some exception. The groups obtained can constitute a database for breeders in a maize breeding program.

Key words: Cultivars, biodiversity, selection, *Zea mays* L., Benin

INTRODUCTION

Maize (*Zea mays* L) is one of the major cereal crops grown in the humid tropics and Sub-Saharan Africa. It is a versatile crop and ranks third following wheat and rice in world production as reported by FAO (2003). This cereal crop is a key source of food and livelihood for millions of people in many countries of the world (Enujeke, 2013). In Benin, maize is the most widely cultivated food crop with more than half of the production, coming from the south (ONASA, 2011). Benin has a wide range of corn varieties including both improved and local varieties. The local cultivars are recently reported to be largely (>67%) adopted all over the country (Salami et al., 2015a). The conservation and management methods of maize seed by farmers are potential source of substantial genetic diversity in cultivated varieties (Kouakou et al., 2010). This diversity can have an increase or reduce the yield. Indeed, in Southern Benin, the maize production yield has significantly decreased (Badu-Apraku and Yallou, 2009). Several reason, such as the poor quality of cultivated field and inappropriate use of seed can explained this reduction. Thus, in spite of the availability of improved varieties with better agricultural yields (N'da et al., 2014a), farmers prefer to use local lines with poor yield (Salami et al., 2015a). This situation was observed because the "improved" varieties seems not having the desired organoleptic properties. So, the development and introduction of new varieties must be based on local genetic resources (N'da et al., 2014a) not only for its large adoption but also for the preservation of genetic diversity (Koffi et al., 2011).

In traditional agriculture, local varieties constitute the bulk of the plant material used (Missihoun et al., 2012) and varieties with best growth characters, yield and components must be selected during breeding programs (Odeleye and Odeleye, 2001). Exploitation of maize genetic diversity is particularly important for maintaining and improving the productivity of this species in developing countries (Hoxha et al., 2004) such as Benin. Many procedures are often used for quantifying and analyzing biodiversity. Among them, we can mention techniques using morphological markers (Jaaska, 2001) for the study on agro-morphological characterization of many crop such as maize accessions (N'da et al., 2014b; Salami et al., 2015b). Therefore, the inventory and agro-morphological characterization of these genetic resources (improved varieties and local's accessions) are essential to provide a solid database on the characteristics of the maize in Southern Benin. The main objective of this study was to evaluate the genetic variability within the maize in the Southern Benin by using morphological descriptors.

MATERIALS AND METHODS

Collecting zone

Ninety one maize accessions (local and improved) varieties have been collected in the five departments (Atlantique, Ouémé Plateau, Mono and Couffo) of southern Benin (Figure 1) in 2013.

Study sites

The experimental fields (longitude 2°19' E, latitude 6°12' N) of the Center of Agricultural Research in southern Benin was used for the experimentation. The site is characterized by a maritime subequatorial climate made of two rainy and two dry seasons with 1200 mm average pluviometry, which spread over 8 months (May to November) with maximum precipitations in June and October and minimum precipitations in August. The average temperature is around 27°C (Adjanohoun et al., 2011). The soil is characterized by a deep reddish ferrous soil (Aïhou, 2003). The organic matter and organic carbon of the soil are respectively 1.03 and 0.60 % with an equivalent phosphorus assimimilable content of 9 ppm. The exchangeable bases such as potassium, calcium, magnesium and Sodium are 0.14 meq/100 g; 2.59 meq/100 g, 0.59 meq/100 g and 0.23 meq/100g of soil respectively, (Agbodjato et al., 2015).

Experimental plan

The experimental device is an incomplete randomized block design with three replications. Each collected accession was planted in 4 lines of 5 meters long representing a basic plot. The seeding rate is 80 cm × 40 cm with four seeds per hole, two weeks after sowing; the maize plants were separate to 2 plants per hole with a density of 62,500 plants per hectare. The useful plot was represented by the 2 central lines. Eighty seven of the 91 accessions collected germinated. The dose of fertilizer applied was 200 kg NPK whereas urea was supplied twice at 100 kg/ha each, first during the separate phase and the second 45 days after sowing. Regular weeding was done during the vegetative phase of the culture. The duration of the experience varied from 60 days to 120 days according to the evaluated varieties.

Data collection

Descriptors for Maize (CIMMYT/IBPGR, 1991) were used for agro-morphological description of accessions. A total of 16 agro-morphological descriptors including seven quantitative variables (days to germination, tasseling days, silking days, Days to ear leaf senescence, Plant height, Ear height and Tassel length) and nine qualitative variables (Foliage, Streak, Rust, Leaf blight, Sheath pubescence, Tassel colour, Silk colour, Stem colour and Root colour) were evaluated according to the method previously described by CIMMYT/IBPGR (1991) at each accession. On each elementary plot data were taken on 15 plants randomly selected and marked on the two center lines.

Data analysis

Quantitative variables were subjected to a descriptive analysis using the MINITAB software. The qualitative characteristics were made quantitative using Likert scale. The categorization of maize accessions was made through a digital based classification algorithm Ward made on adjusted means values of quantitative and qualitative agro-morphological characteristics by accessions from area from analysis of variance by R 3.0.3 software. Discrimination accessions groups obtained from the numerical classification was performed followed by a canonical discriminant analysis on groups of accessions using the most discriminant variables obtained in the

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Abbreviations: DAS, Days after seedling; CNS-Maïs, National Center of Specialization on corn; NPK, sodium phosphorus and potassium fertilizer.

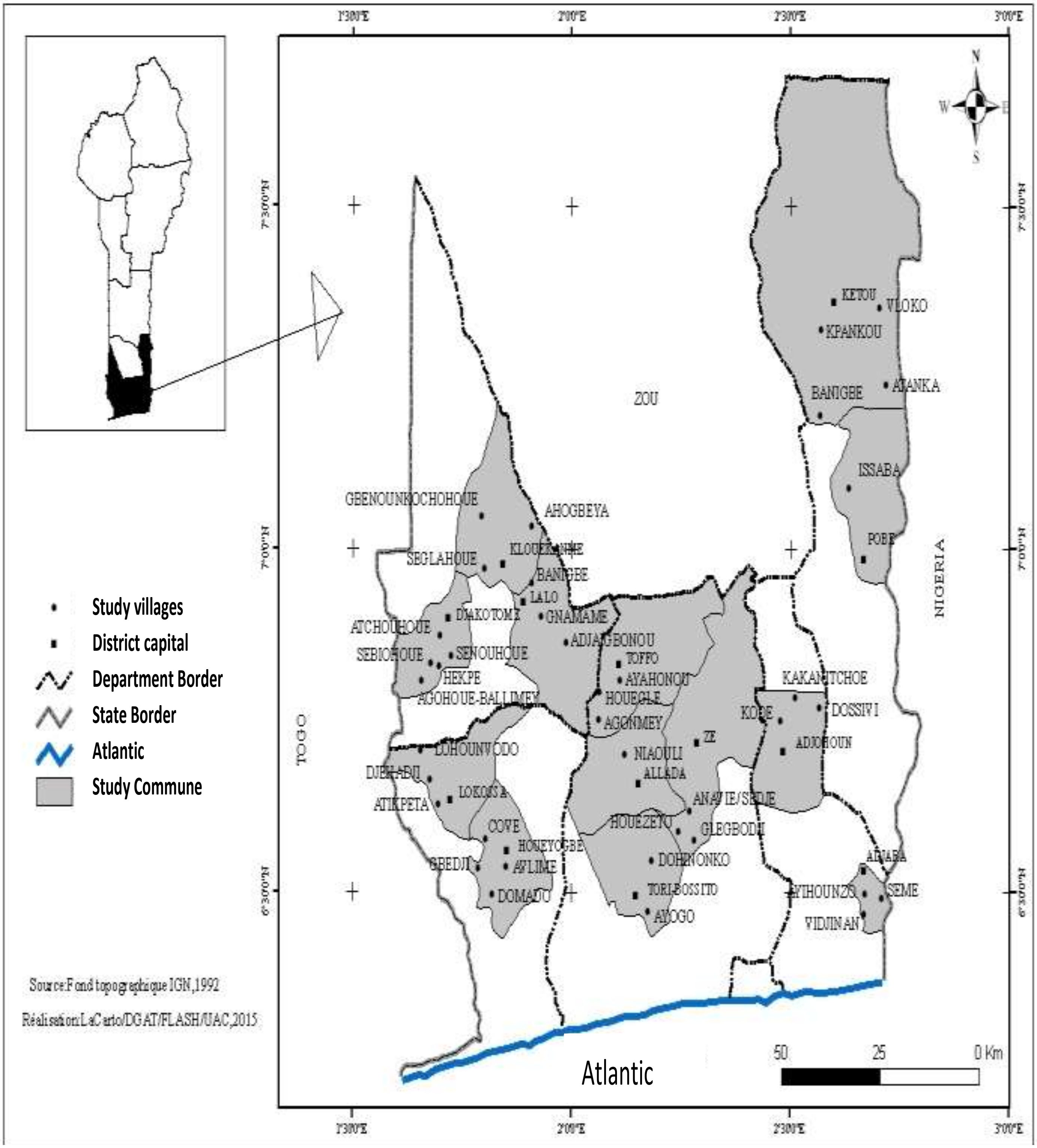

Figure 1. Map of southern of Benin showing communes and villages of samples collection.

previous analysis. Furthermore, an additional discrimination of groups of accessions has been made using a proportion multiple comparison test on colour of stem, silk, tassel and root that were occulted at the classification because it is difficult to make it quantitative. Regarding the comparison of the two categories of accessions (local and improved), all accessions were grouped into two classes (local and improved) and then subjected to analysis of variance of two factors (Block and category of accessions) on basis

Table 1. The minimum, maximum, mean value and variation coefficient of the studied quantitative variables.

Variable	Number of sample	Minimum	Maximum	Mean	Standard error	%Coefficient of variation
Plant height (cm	87	141.13	278.6	202.23	1.89	12.37
Ear height (cm)	87	60.86	158.13	110.25	1.64	19.65
Female flowering (day)	87	47	67	59.19	0.26	5.91
Male flowering (day)	87	43	63	57.18	0.24	5.70
Tassel length (cm)	87	28.06	45.53	36.50	0.21	7.70
Days to ear leaf senescence (day)	87	79	96	89.91	0.27	4.04
Days to Germination (day)	87	3	6	4.32	0.08	24.74

Figure 2. Dendrogram of numerical classification on maize accessions in South Benin.

of agro-morphological characteristics retained.

RESULTS

Descriptive statistical analysis of quantitative variables

The results of the mixed model analysis of variance of two factors revealed a very highly significant difference ($p < 0.001$) between all accessions for each evaluated quantitative agro-morphological characteristic. Variability induced by the blocks was observed only for the tassel length and plant height. Table 1 shows the statistics of the quantitative variables measured on accessions. The analysis of this table shows that the coefficient of variation varies between 4.04 and 24.74%. The results show that the variables such as plant height, ear height, and germination days have a high variation (> 10%) while female flowering, male flowering, tassel length and ear leaf senescence present low variation (< 10%).

Numerical classification of accessions and determination of the best accessions

The numerical classification of all the collected corn accessions revealed five groups of accessions with variable number of accessions (Figure 2). The high number of accession was recorded in the group G3 (20 accessions) followed by the G1 (19 accessions), the G2 (18 accessions), G4 (17 accessions) and G5 (13 accessions). On the other hand, three groups (G1, G2, and G4) were composed essentially of local accessions.
The other groups (G3 and G5) are a mixture of improved and local accessions. The investigated numerical

Table 2. Stepwise discriminant analysis on the different characteristics of corn accessions.

Variable	R-Square	F-value	Probability
Germination (day)	0.22	5.45	0.001
Female flowering (day)	0.13	2.79	0.032
Plant height (cm)	0.58	26.99	0.000
Ear height (cm)	0.36	10.97	0.000
Streak	0.12	2.68	0.037
Sheath pubescence	0.09	1.83	0.131
Male flowering (day)	0.02	0.38	0.825
Day to ear leaf senescence (day)	0.05	0.98	0.423
Tassel length (cm)	0.03	0.56	0.692
Foliage	0.04	0.88	0.478
Rust	0.08	1.64	0.172
Leaf blight	0.01	0.21	0.931

Table 3. Canonical correlation variables with the canonical axes: results of canonical discriminant analysis

Variable	Can1 (97.80%)	Can2 (1.90 %)
Germination (day)	0.15	-0.79
Female flowering (day)	-0.65	0.32
Plant height (cm)	-0.97	0.00
Ear height (cm)	-0.95	-0.08
Streak	-0.30	0.06

classification revealed that the plant height and ear height are the two variables with wide variation centroid from a group to another. Thus, the first five superior accessions based on these two variables are the following local cultivars: Adouatin, Hollikoun, Gbogboui, Atchivi and Soun Aton kouin.

Discrimination of accession groups obtained after the numerical classification

The results of the step by step discriminant analysis on the different agro-morphological characteristics of corn accessions (Table 2) revealed five discriminating variables ($p < 0.05$). These variables are germination days, female flowering, plant height, ear height, and sensitivity to streak. The canonical discriminant analysis on five groups of accession revealed two canonical axes representative of the studied characteristics. The first axis explains 97.80% of all the variability characteristics related to accessions and the second axis explain 1.90% of this variability. The results of Table 3 shows that the female flowering, plant height, ear height and sensitivity to streak are well represented on the first axis whereas the germination days and female flowering are represented on the second axis. The analysis of Figure 3 and Table 4 reveal a clear separation of the five accession's groups obtained. Accessions of group G5 are characterized by early female flowering (55 DAS), this character is late (61 DAS) for accessions of group G1 and G2 and average for accessions of G3 and G4 group. Accessions of G3 and G5 groups are characterized by small plant height and ear height while the accessions of group G2 have a tall height for these characters. Accessions of groups G2, G3 and G4 are characterized by a mean duration of germination days. Accessions G1 and G2 are characterized by accessions sensitive to streak. The discrimination of the five groups obtained according to the colors of stem, tassel, silk and root (Table 5) revealed overall for the two colors (green and anthocyanin) that there is no dominance of one of the colors to all groups with the exception of anthocyanin color of the tassel where accessions of group G5 represent 80% of the accessions, followed by accessions of the group G2 (20%). The tassel of accessions of group G1, G3 and G5 are mostly green.

Comparison of the two categories of accessions (local and improved)

The comparison the two types of accession (Table 6) revealed a significant difference between the two types of accession for agro-morphological characteristics with the

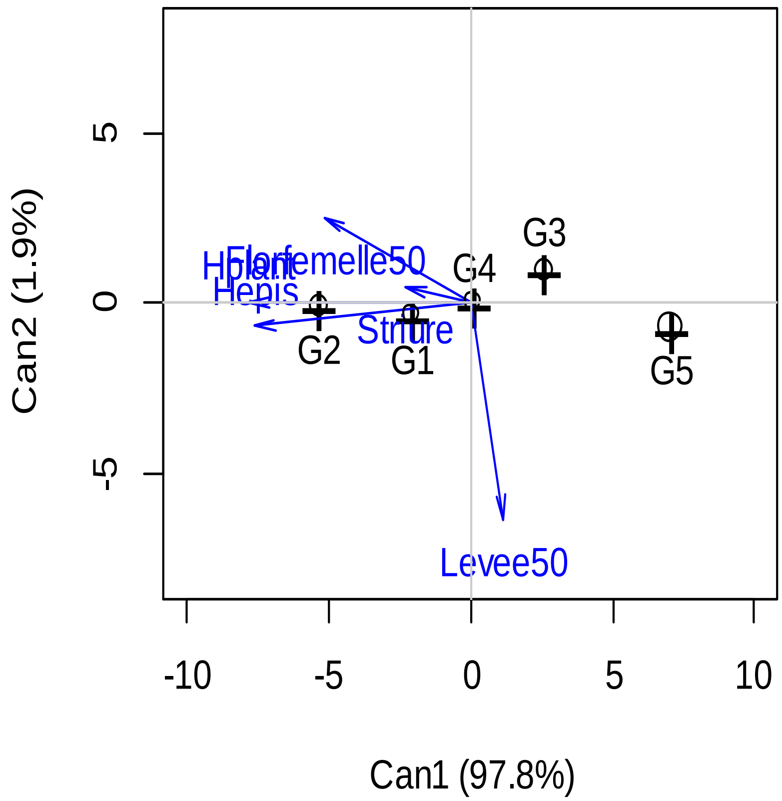

Figure 3. Graph of two canonical discriminant functions (Can1 and Can2) based on the most relevant variables revealed by discriminant analysis step by step. Legend: Hplant = Plant height, Florfemelle50 = female flowering, Hepis = Ear height, Levee50 = Germination day, Striure = Streak.

Table 4. Comparison of accession groups: ANOVA results.

Variables	G1		G2		G3		G4		G5		Prob.
	m	cv	m	cv	m	cv	m	cv	m	cv	
Days to germination (days)	4.61^{ba}	23.06	4.17^{bc}	27.61	3.71^{c}	20.82	4.20^{bc}	26.31	5.15^{a}	13.36	0.003
Plant height (cm)	213.23^{b}	2.29	229.59^{a}	3.98	189.18^{d}	4.30	201.77^{c}	2.55	166.12^{e}	6.07	0.000
Ear height (cm)	120.66^{b}	3.65	134.90^{a}	8.19	96.69^{d}	8.63	110.28^{c}	6.84	78.60^{e}	7.40	0.000
Days to ear leaf senescence (days)	92.08^{a}	2.04	92.33^{a}	1.65	89.02^{b}	4.27	89.48^{b}	3.20	85.27^{c}	3.92	0.000
Male flowering (day)	54.34^{ba}	2.70	59.61^{a}	2.51	57^{b}	4.86	56.83^{b}	4.59	52.96^{c}	6.60	0.000
Female flowering (day)	60.50^{ba}	2.71	61.72^{a}	2.74	58.97^{b}	5.56	58.75^{b}	5.07	54.74^{c}	5.26	0.000
Tassel length (cm)	37.14^{a}	6.49	37.70^{a}	5.44	36.16^{ba}	5.91	35.98	4.08	35.22^{b}	7.09	0.011

Proportions with the same letters on the same line are not significantly different. Legend: m = means; cv = coefficient variation.

exception of the tassel length, germination days, rust, leaf blight, and sheath pubescence. Variability associated with the blocks was observed for the tassel length and foliage. Local accessions showed higher values for each agro-morphological characteristic with significant differences (Table 7). For example, local accessions are larger (205.72 cm) than improved accessions (190.55 cm).

DISCUSSION

The morphological characterization is one of the most important steps in the description and classification of germplasm of crop plants (Radhouane, 2004) including maize. Thus, Beyene et al. (2005) reported that plant breeding program is based necessarily on the morpho-phenological variability of the species. Based on the

Table 5. Discrimination of groups according to the colors of the stem, tassel, silk and root: result of the comparison of proportions of the maize variety presenting a particular color

Organs	Color	G1	G2	G3	G4	G5	Probability
Stem	Anth	15.38[a]	17.95[a]	23.08[a]	20.51[a]	23.08[a]	0.895
	Green	27.08[a]	22.92[a]	20.83[a]	16.67[a]	12.5[a]	0.433
Tassel	Anth	0.00[c]	20.00[b]	0.00[c]	0.00[c]	80.00[a]	0.005
	Green	23.00[a]	23.00[a]	23.00[a]	18.00[a]	13.00[a]	0.510
Silk	Anth	18.42[a]	7.90[a]	23.68[a]	23.68[a]	26.32[a]	0.274
	Green	24.49[a]	30.62[a]	20.41[a]	14.28[a]	10.20[a]	0.091
Root	Anth	20.41[a]	10.20[a]	26.53[a]	22.45[a]	20.41[a]	0.380
	Green	23.68[a]	34.21[a]	15.79[a]	13.16[a]	13.16[a]	0.100

Proportions with the same letters on the same line are not significantly different.

Table 6. Comparison of types of accessions: Results of ANOVA

Variable	Source variation					
	Type of accession			Blok		
	DL	F-v	Pr.	DL	F-v	Pr.
Days to germination (days)	1	2.68	0.103	9	0.83	0.590
Plant height (cm)	1	10.58	0.001	9	1.75	0.082
Ear height (cm)	1	29.59	0.000	9	0.65	0.755
Female flowering (day)	1	15.64	0.000	9	0.83	0.585
Male flowering (day)	1	12.44	0.001	9	0.68	0.724
Tassel length (cm)	1	0.25	0.619	9	4.40	0.000
Days to ear leaf senescence (days)	1	6.54	0.011	9	0.59	0.801
Foliage	1	9.99	0.002	9	1.97	0.046
Streak	1	16.06	0.000	9	1.03	0.417
Rust	1	0.03	0.854	9	0.90	0.525
Leaf blight	1	0.13	0.719	9	1.37	0.204
Sheath pubescence	1	0.58	0.448	9	0.46	0.898

variables investigated in this study, a large morphological heterogeneity was found between the 87 evaluated accessions collected in the southern Benin. The mixed model analysis of variance of two factors revealed a very highly significant difference ($p < 0.001$) between all accessions for each quantitative agro-morphological characteristic evaluated. Significant differences were recorded between the minima and maxima for all quantitative traits. These results show that there is great diversity within accessions collected. The variables such as plant height, ear height, and germination days have a high variation (> 10%) while the female flowering, male flowering, tassel length and days to ear leaf senescence have low variation (< 10%). These results are in agreement with our previous report on the morphological diversity of maize accessions of the Central and Northern Benin (Salami et al., 2015b). Indeed, in this part of the country, it was observed that the plant height, ear height, tassel length showed a large variation (>10%) while female flowering, male flowering, days to ear leaf senescence and germination days showed small variation (<10%) (Salami et al., 2015b). This similarity can be explained by the fact that the two areas are neighbor and this proximity can be favorable to seeds exchange between farmers. In the same way, several authors Missihoun et al. (2012) showed that the seed management practices by peasant, including exchange of varieties among farmers are the source of a significant diversity among crop populations. Thus, the poly-varietal crop in the same field observed is the origin of gene flow that will produce and contribute to expand the genetic diversity (Koffi et al., 2011). The numerical classification of all accessions identified five phenotypic groups. These results are similar to the five groups obtained, for the *Z. mays*, after hierarchical ascending classification in Ivory Coast (N'da et al., 2014b). However, we obtained four groups of accessions in the central and northern Benin (Salami et al., 2015b). This difference observed between on one hand the South and on the other hand the Center-North confirm the wide diversity of varieties Benin.

Table 7. Comparison of two types of corn accessions: mean and coefficient of variation of the different agro-morphological characteristics

Variable	Local		Improved		Prob.
	m	cv	m	cv	
Germination (day)	4.43	24.84	4.00	22.65	0.103
Male flowering (day)	57.68	5.08	55.55	6.81	0.001
Female flowering (day)	59.76	5.43	57.30	6.47	0.000
Day to ear leaf senescence (day)	90.31	3.76	88.63	4.66	0.011
Plant height (cm)	205.72	11.25	190.55	14.55	0.001
Ear height (cm)	114.88	17.55	94.75	20.49	0.000
Tassel length (cm)	36.48	7.79	36.60	7.46	0.619
Foliage	3.09	35.82	2.60	46.74	0.002
Streak	0.99	44.85	0.60	82.69	0.000
Rust	1.58	36.60	1.60	46.51	0.854
Leaf blight	1.04	30.75	1.05	21.02	0.719
Sheath pubescence	1.03	16.59	1.05	21.02	0.448

m = mean, cv = coefficient of variation, Prob.= probability.

Indeed, maize crops were first introduce in the south (Ouémé, Mono, Atlantique), and later only the yellow maize was grown in the northern regions (especially in Borgou) (Yallou, 1994). The step by step discriminant analysis on the different agro-morphological characteristics of corn accessions revealed that 5 parameters (germination days, female flowering, plant height, ear height and sensitivity to streak) are the most discriminative. The same observation was made by N'da et al. (2014b) on the maize accessions in Côte d'Ivoire. Indeed, these authors reported that the early maturity, the plant heights, ear insertion, and the characters related to cob are the best variables to describe the variability of maize varieties grown. In northern Benin, numerical classification of accessions revealed four similar morphological groups. The four groups recorded in the northern Benin were discriminated by the germination parameters (precocity, cobs maturity) and variables related to the plants heights and ear insertion (Salami et al., 2015b). Thus, the quantitative traits should not be neglected by the conservatives when studying the diversity because these parameters are essential in peasant environments where they greatly influence the phenotypic selection criteria (Moreno et al., 2006; N'da et al., 2014a).

The discrimination of the five groups obtained according to the color of the stem, tassel, silk and root revealed globally for the two colors (green and anthocyanin) that there is no dominance of a color for all groups. Vegetative characters, phenotype, productivity, cob characteristics and flowering are the most remarkable characters in the fields and contribute to influence the choices of farmers (Djè et al., 2007; N'da et al., 2014a). The comparison of the two (local and improved) types of accessions revealed a significant difference for agro-morphological traits except tassel length, germination days, rust, leaf blight and sheath pubescence. For example, local accessions are larger than improved accessions according to plant height and the ear height. Likewise, local cultivars are later than improved accessions regarding the female and male flowering. These results corroborate those obtained by Abadassi (2013) when comparing traditional and improved accession in Benin. Similarly, the ear height was 150 cm for the traditional cultivar against 97 cm for the improved variety. In addition, our data shows that local cultivars had more foliage compared to improved varieties. They may serve as sources of begetters in a program to develop varieties that capture light energy and thus promote photosynthesis in a context of climate change. So, despite of the superior agronomic performance of improved varieties, we previously recorded a large adoption of local cultivars by farmers to the detriment of improved varieties (Salami et al., 2015a). This low-adoption of improved varieties is not only to maintain the family's traditional farming methods but also because the organoleptic qualities and socioeconomic preferences that do not fit the needs of consumers (Salami et al., 2015a).

Conclusion

The study of morphological variability of maize accessions grown in southern Benin shows a great morphological diversity. This diversity has been structured into five groups by the numerical classification based on the Ward algorithm. The most discriminating characters are revealed as precocity (germination days and female flowering), plant height, ear height and sensitivity to streak. The accessions studied present a variation for the all characters. Comparison of two types of accessions (local and improved) revealed a significant difference between the two types of accession for agro-morphological characteristics with the exception of some. Generally, local accessions showed higher values for each agro-morphological characteristic with significant differences. The study also identified an important number of morphological characters for the maize characterization. So, this varietal diversity underlined offers a wide potential for varietal improvement of maize in southern Benin. The use of molecular markers would increase knowledge of this maize collection for sustainable conservation and better use of genetic resources.

Conflict of Interest

The authors have not declared any conflict of interest.

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