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Genetic diversity in Algerian maize (*Zea mays* L) landraces using SSR markers

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Abstract

In the Sahara, maize (*Zea mays* L) has been adapted to extreme environmental conditions during the last five centuries; therefore, this germplasm has a potential value as source of tolerance to stress. No previous report of the genetic diversity of Saharan maize has been published so far. The objective of this study was to determine the genetic diversity of a collection of Saharan maize. Fifteen accessions representing the geographic diversity of Algeria were characterized with 18 SSR. Most loci (93%) were polymorphic; the total amount of alleles was 87 and the average of alleles per locus was 5.8. The total genetic diversity (He) was 0.57, being 69% intra-accessions and 31% inter-accession. Eight of the alleles were accession-specific and belonged to six populations. Genetic distance among the 15 accessions resulted in the definition of three main clusters related to the geographic origin. Maize germplasm from the Algerian Sahara can be classified at least in three groups and the most variable accessions are in the southern oasis. Some accessions were highly variable and can be sources of favorable alleles for breeding for tolerance to extreme stress conditions.

Keywords: Algeria, genetic diversity, maize, Sahara, SSR, *Zea mays*

Introduction

The origin of Algerian maize (*Zea mays* L) has not been determined yet. Maize was established in the North of Africa and the Sahara long time ago, apparently from Spain by Muslim pilgrims on their way to The Mecque (Laumont and Laby, 1950). This early introduction happened in the 16th Century, but later introductions were possibly carried out from neighboring African countries or from Europe. Maize was reported in several regions (Kabylie, Tell and Saharan oasis) by general Duval in 1856 (Laumont and Laby, 1950). This germplasm has been adapted to extreme climatic conditions because those regions have very cold winters and very dry and hot summers.

Given the large distances existing among oasis, scattered as islands in the vast desert, farmers maintain their own varieties, which are cultivated in two seasons (March-May and August-October) each year. This situation maintained and increased maize diversity, generating a wide phenotypic variability that needs to be characterized in order to be exploited in breeding programs (Hoxha et al, 2004; Wietholter et al, 2008), particularly to improve resistance to stresses.

Morphological characterization is important for breeding but is strongly affected by the environment. Conversely, molecular characterization is free from environmental effects and allows highly valuable complementary genetic information (Gauthier et al, 2002). Molecular markers such as microsatellites (SSR) have been very helpful for the characterization of genetic diversity in maize (Hoxha et al, 2004; Reif et al, 2006; Beyene et al, 2006; Yao et al, 2008; Liu et al, 2009; Eschholz et al, 2010). SSR are widely used for fingerprinting accessions because they are highly reproducible, polymorphic, co-dominant and generally abundant among crops (Powell et al, 1996).

The only report available on Saharan maize from Algeria, is based on the morphological characterization of 10 accessions cropped in temperate environments (Djemel et al, 2011). On the other hand, there are no published studies of genetic diversity from this germplasm. Given the potential value of these maize accessions for breeding programs, three genetic prospections were carried out between 2009 and 2011. Those prospections have collected 160 accessions from an area of about 500,000 km^2 in the Algerian Sahara. From that collection, a representative sample of 15 accessions was chosen for genetic characterization with 18 SSRs. The objective of this study was to determine the genetic diversity of this maize collection from the Algerian Saharan.

Materials and Methods

Plant material

Most of the accessions studied here come from the regions Timimoune, Adrar and Bechar (Southwest of Algeria) (Table 1). Accessions come from an area between 27°12' and 34°3'N (Supplementary Figure 1). Passport data were collected for each accession and a primary characterization was performed (data not shown). Generally, these accessions have conic ears

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Table 1 - Origin and brief description of the 15 accessions of maize from the Algerian Sahara included in the genetic characterization with SSR.

Region	Location[1]	Accession code	Ear shape	Ear type	Kernel color
Saida	Sidi Maamar, Ain El Hadjar,	SHH	conic	flint	yellow-orange
El Oued	El Mghaier,	MGH	cylindric	flint	yellow-black-brown-violet
Bechar	Beni Abbes,	BAB	conic	flint	yellow-orange
Bechar	Ouakouh,	BSA	cylindrical	flint	white-yellow
Bechar	Taghit,	BTM	conic	flint	yellow-orange
Bechar	Ouakda,	OBC	conic	flint	yellow
Ghardaia	El Goléa,	GOL	conic	flint	white-yellow-brown
Timimoune,	K'sar Tmana,	KST	cylindric	flint	yellow-orange
Timimoune,	K'sar M'sehel,	MST	conic	flint	yellow-violet
Timimoune,	Ouled Saïd,	OST	conic	flint	yellow-orange
Timimoune,	Kali,	KAL	conic	flint	yellow
Adrar	Ouled Arroussa,	AAS	conic	flint/dent	yellow
Adrar	Taourit,	TAO	conic	flint	yellow-orange
Adrar	Ouled Mahmoude, Lamtarfa,	LOM	cylindrical	flint	yellow
Tamanrasset	Ain Salah	IGS	conic	flint	yellow-orange

[1]Accessions are presented according to their geographic origins: from North to South

and flint kernels ranging in color from white to orange (Table 1). The general criterion used to choose the 15 accessions was the distance between their collection sites (> 50 km).

SSRs Markers and PCR

For each accession, DNA was extracted from 16 coleoptiles following a modified procedure from Liu and Whittier (1994). Eighteen SSRs distributed throughout the 10 chromosomes were used for characterizing the 15 accessions (Table 2). PCR was carried out as in Butrón et al (2003) with a thermocycler MyCycler (BIO-RAD Laboratories, Inc). The final reaction volume was 20 µl and the mixture included 50 ng μl^{-1} of genomic DNA (2 µl), 2 × PCR buffer with Taq DNA polymerase (Promega) and dNTPs (10 µl), 4 ng μl^{-1} primers F and R (2 × 0.8 µl) and 7.4 µl purified H_2O. The PCR program involved an initial phase at 95°C for five min, followed by 30 cycles (denaturation at 95°C - 30 s, hybridization at 56°C - 30 s and extension at 72°C - 30 s), then a final extension phase at 72°C for 10 min, and conservation of final products at 4°C until utilization.

Acrylamide gel electrophoresis

After amplification, SSR products (10 µl) were separated by electrophoresis along with a weight marker (HyperLadder V) using 1 x TBE on a 6% non-denaturing gel of acrylamide with 50 µl of ethidium bromide at 250 V for 3h in a vertical system (DASG-400-50, CBS Scientific co). The gels were placed on a UV table and photograhed.

Data Analysis

From the photographed gels, fragments were manually identified by their molecular weight. The fragments were numbered from low to high weight.

Table 2 - List of SSR markers used for characterization of 15 maize accessions.

Marker	Bin[1]	Motif	Forward	Reverse
umc1222	1.01	(AG)20	CTCAGAACAGAAGCCATCAAAAGC	CGTCTTCGTGAGAGACATCCTGT
umc1403	1.03	(GCA)4	GTACAACGGAGGCATTCTCAAGTT	TGTACATGGTGGTCTTGTTGAGGT
umc1335	1.06	(AG)24	ATGGCATGCATGTGTTTGTTTTAC	ACAGACGTCGCTAATTCCTGAAAG
umc1165	2.01	(TA)6	TATCTTCAGACCCAAACATCGTCC	GTCGATTGATTTCCCGATGTTAAA
umc1265	2.02	(TCAC)4	GCCTAGTCGCCTACCCTACCAAT	TGTGTTCTTGATTGGGTGAGACAT
phi127	2.08	AGAC	ATATGCATTGCCTGGAACTGGAAGGA	AATTCAAACACGCCTCCCGAGTGT
bnlg1520	2.09	(AG)22	TCCTCTTGCTCTCCATGTCC	ACAGCTGCGTAGCTTCTTCC
phi036	3.04	(AG)n	CCGTGGAGAGACGTTTGACGT	TCCATCACCACTCAGAATGTCAGTGA
umc1963	4.04	(AGC)3	CTCGTTCGAGGGGATGTACAAG	CTTGCACTGGCACAGAGACG
umc1329	4.06	(GCC)7	CCTCTCACATCTCCTCTCCCCT	GTGTCGGTGTAGGTCTCCGTCTT
umc1225	5.08	(AG)6	CTAGCTCCGTGTGAGTGAGTGAGT	TTCCTTCTTTCTTTCCTGTGCAAC
umc1424	6.06	(TCC)7	CCGGCTGCAGGGGTAGTAGTAG	ATGGTCAGGGGCTACGAGGAG
bnlg1740	6.07	(AG)21	TTTTCTCCTTGAGTTCGTTCG	ACAGGCAGAGCTCTCACACA
umc1545	7.00	(AAGA)4	GAAAACTGCATCAACAACAAGCTG	ATTGGTTGGTTCTTGCTTCCATTA
umc1327	8.01	(GCC)4	AGGGTTTTGCTCTTGGAATCTCTC	GAGGAAGGAGGAGGTCGTATCGT
umc1984	8.03	(CAG)3	CTCTGGCCTCTGATACCAGTTGAT	CATCCTCCTGCAGCTGTTAACTC
phi027	9.03	(GCGCT)n	GCGTACGTACGACGAAGACAC	CACAGCACGTTGCGGATTTCTCT
phi059	10.02	(ACC)n	AAGCTAATTAAGGCCGGTCATCCC	TCCGTGTACTCGGCGGACTC

[1]Position in the chromosome

Table 3 - Mathematic formulae corresponding to the parameters of genetic diversity used for genetic characterization of 15 maize accessions.

Genetic parameters	Formula[1]
Number of alleles per locus	$A = \sum_{i=1}^{n} Ai / n$
Expected hetorozygosity	$He = 1 - \sum (pi)^2$
Coefficient of consanguinity	$Fis = \frac{Hs - Hi}{Hs}$
Genetic differentiation	$Fst = \frac{Ht - Hs}{Ht}$
Genotypic deviation	$Fit = \frac{Ht - Hi}{Ht}$
Genetic distance	$GD = 1 - [2N_{11} / (2N_{11} + N_{10} + N_{01})]$

[1]*Ai*: Number of alleles at the i locus. p_i: frequency of the i allele for a locus. *Hi*: Mean observed hetorozygosity for all the loci of a genotype. It is also the probability of heterozygosis for a random locus. *Hs*: Expected hetorozygosity per genotype for each accession assuming Hardy-Weinberg equilibrium. *Ht*: Expected hetorozygosity for a genotype assuming Hardy-Weinberg equilibrium for the set of accessions, i.e. assuming that all accessions belong to a single panmicitc population. N_{11}: Number of alleles in both genotypes i and j. N_{10}: Number of alleles in the genotype i. N_{01}: Number of alleles in the genotype j.

Intra and inter-accession genetic diversity was determined by the number of alleles for each accession and locus by using the program GDA 1.1 (http://lewis.eeb.uconn.edu/lewishome/software.html), developed by Lewis and Zaykin (2002). This program calculated polymorphism rate, number of alleles per locus, number of alleles per polymorphic locus, expected heterozygosity (He), observed heterozygosity (Ho) and coefficient of consanguinity (f) (Table 3). This analysis allows the study of the genetic structure from the F statistics of Wright (1978): F_{is}, F_{it}, F_{st} and also the genetic distances between accessions following Nei (1978). The resulting matrix was used for cluster analysis by the unweighted pair group method based on arithmetic averages (UPGMA). The analysis of allele frequencies was obtained from the program GEN-POP 4.1 (Rousset, 2008) (data not shown).

Results

Amplification and polymorphism

Among the 18 SSR markers used for characterization of the 15 accessions, umc1984, bnlg1740 and umc1963 had large proportions of missing data and were removed from the analyses. Therefore, 15 SSR were used for the analyses, and one of them was monomorphic (umc1403); the polymorphism rate was 93%.

Genetic diversity intra-accession

The number of alleles per accession varied from 1.87 for MGH to 4.14 for GOL with a mean value of 2.82. Average mean genetic diversity within the set of accessions was 0.40, being highest for GOL (0.62) and lowest for MGH (0.27) (Table 4). The analyses of allelic frequencies showed that the mean frequency of rare alleles per accessions was 5.5, being maximum for BAB (12 alleles) and minimum for BTM and SHH (two alleles). MGH and KST had six and five fixed alleles, respectively, and KAL, MGH and KST had the largest number of alleles with frequency higher than 0.8. Altogether, eight specific alleles were detected; two for each of the accessions AAS and KST and one for accessions BSA, OBC, GOL and BAB (Table 4).

The 15 SSRs yielded 87 alleles, and the number of alleles per locus varied from one for umc1403 to nine for phi036 and umc1225. The mean number of alleles per locus was 5.8. The total genetic diversity (He) was 0.57; for those markers sowing some variability, genetic diversity varied from 0.29 for umc1329 to 0.79 for umc1327 (Table 5).

Genetic diversity inter-accession

The parameters F, F_{it}, F_{is}, and F_{st} revealed the genetic structure of the accessions (Table 5). Among polymorphic loci, F varied from 0.09 for umc1329 to 0.40 for umc1222, showing a slight deficit of heterozygotes among the set of accessions. Mean F_{is} was 0.026 and varied from -0.20 for bnlg1520 to 0.12 for umc1222, and mean F_{it} was 0.32 varying from 0.15 to 0.41 for the same SSRs, respectively. Mean F_{st} was 0.31, thus the inter-population genetic diversity was

Table 4 - Genetic parameters of the 15 maize accessions characterized with 15 SSR.

Accession	A	He	Ho	f	As	Ar	Af
AAS	3.00	0.40	0.41	-0.03	2	5	2
BSA	3.25	0.43	0.41	0.06	1	4	2
BAB	2.50	0.42	0.39	0.06	1	12	2
BTM	3.00	0.46	0.43	0.06	-	2	4
SHH	2.13	0.34	0.33	0.03	-	2	3
LOM	3.19	0.51	0.59	-0.15	-	5	2
OBC	2.38	0.35	0.33	0.05	1	5	2
MST	3.08	0.38	0.39	-0.03	-	8	1
TAO	2.93	0.35	0.47	-0.36	-	6	2
GOL	4.14	0.62	0.56	0.11	1	4	1
IGS	2.71	0.38	0.36	0.04	-	9	1
OST	2.71	0.42	0.45	-0.06	-	4	3
MGH	1.87	0.27	0.22	0.17	-	3	6
KAL	2.67	0.30	0.31	-0.04	-	8	3
KST	2.73	0.34	0.23	0.33	2	5	5
Mean	2.82	0.40	0.39	0.01		5.5	2.6

Number of alleles per accession (A), expected hetorozygosity (He), observed hetorozygosity (Ho) and coefficient of consanguinity (f), number of specific alleles per accession (As), number of rare alleles per accession (Ar), and number of fixed alleles per accessions (Af)

Table 5 - Genetic parameters for the 15 SSRs used for characterizing the 15 accessions.

SSR	A	He	Ho	Fis	Fit	Fst	F
umc1222	8	0.71	0.45	0.12	0.41	0.33	0.40
umc1403	1	0.00	0.00	-	-	-	0.00
umc1335	8	0.78	0.51	0.07	0.35	0.29	0.33
umc1165	5	0.63	0.42	0.08	0.34	0.29	0.33
umc1265	5	0.71	0.54	-0.07	0.25	0.30	0.23
phi127	3	0.30	0.20	0.08	0.33	0.27	0.32
bnlg1520	6	0.47	0.41	-0.20	0.15	0.28	0.12
phi036	9	0.71	0.44	0.10	0.39	0.32	0.37
umc1329	3	0.29	0.26	-0.07	0.09	0.15	0.09
umc1225	9	0.55	0.36	0.04	0.35	0.32	0.34
umc1424	7	0.65	0.45	0.11	0.31	0.23	0.30
umc1545	6	0.73	0.44	0.02	0.40	0.39	0.39
umc1327	7	0.79	0.51	0.01	0.36	0.35	0.35
phi027	4	0.38	0.31	-0.05	0.19	0.24	0.18
phi059	6	0.71	0.47	-0.006	0.35	0.35	0.33
Mean	5.8	0.57	0.39	0.026	0.32	0.31	0.31

Number of alleles per locus (A), expected heterozygoty (He) observed heterozygoty (Ho), F-statistics of Wright (1978) (F_{is}, F_{it} and F_{st}) and the index of consanguinity (F).

31% of the total genetic diversity.

A cluster based on genetic distances (Nei, 1978) was built with the method UPGMA (Figure 1). The accessions were clustered in three groups; the first had one single accession IGS, the second had nine accessions (SHH, LOM, MGH, OBC, MST, OST, KST, KAL and TAO) with two subgroups, and the third cluster comprised five accessions (BSA, BTM, BAB, GOL and AAS) (Figure 1).

Discussion

Local landraces are a potential source of favorable alleles for plant breeding because they have been selected for adaptation to environmental stresses by farmers for many years (Liu et al, 2009). In order to be exploited, the germplasm accessions must be characterized; molecular markers provide important information on genetic variability within and among accessions, and population structure. That information can be used for improving the management of genetic resources (Liu et al, 2009). Accordingly, the present study aims at characterizing a sample of 15 accessions, representative of a wider gene pool (160 accessions), as well as identifying the genetic relationships among them.

The results showed that 14 of the 15 SSRs analyzed were polymorphic. The only monomorphic marker was umc1403; this locus was not monomorphic among American (Noldin Almiron, 2008) or Spanish (Romay et al, 2011) germplasm, and could have been fixed in Saharan maize either by random drift or by selection during maize adaptation to desert conditions. The mean number of alleles (5.8) was higher that the value (2.7) reported by Wietholter et al (2008) in 37 Brazilian accessions characterized with 21 SSRs and the diversity (4.9) reported by Beyene et al (2006) for 62 accessions from Ethiopia characterized with 20 SSRs. Therefore, although the number of accessions here characterized was smaller than in those studies, the variability detected in the Algerian Sahara was higher.

The mean number of alleles per accession was 2.82, similar to the value (2.8) reported by Eschholz et al (2010) for maize from Switzerland, but lower than the figures reported by Ho et al (2005) for two groups of North American populations, which had 3.25 and 3.87 alleles per accession respectively. However, some of the Algerian accessions presented a mean number of alleles per locus similar or even higher than the mean value reported by Ho et al (2005): namely, GOL had 4.41 and BSA had 3.25 alleles per locus, which could be promising base populations for breeding programs. Several breeding programs have shown that populations with three or four alleles per locus respond efficiently to several cycles of selection (Rossini Pinto et al, 2003; Hinze et al, 2005; Romay et al, 2011).

Genetic diversity within the accessions (He = 0.396) was lower than that found in Paraguayan maize (He = 0.44) by Noldin Almiron (2008) and much lower than the values published by Reif et al (2006) for Mexican populations (He = 0.61) and Lia et al (2009) (He = 0.57). Romay et al (2011) also found lower He values for the synthetic varieties EPS6 (0.54) and EPS7 (0.51), which represent the variability from Northern and Southern Spain, respectively. Expected heterozygosity had similar values for tropical accessions BR-105 (0.56) and BR-106 (0.516) (Rossini Pinto et al, 2003). Two of the Saharan accessions, GOL (0.62) and LOM (0.51), had also high values of He; therefore, these two populations have genetic variability similar to accessions from the center of natural diversity of maize in Mexico (Reif et al, 2006) and higher than germplasm from Paraguay, Spain or the Tropics. Total genetic diversity reach 0.57, i.e. as the Paraguayan collection (0.57) studied by Noldin Almiron (2008), though lower than the genetic diversity reported by Vaz Patto et al (2004) for Portuguese

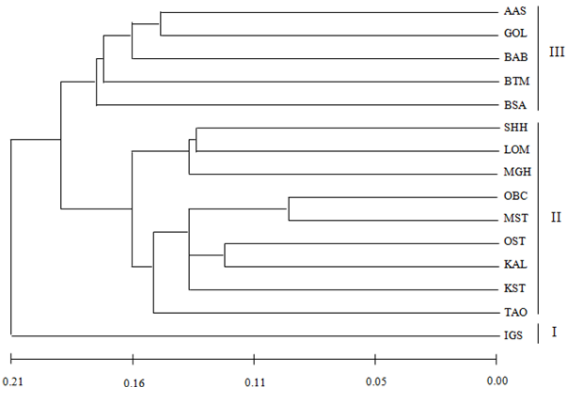

Figure 1 - Cluster of the 15 maize accessions characterized with 15 SSRs by using the Nei's genetic distance (Nei 1978) and the clustering method UPGMA.

varieties (0.62), Yao et al (2008) for a collection of Chinese maize (0.70) or Liu et al (2009) for two groups of Chinese varieties (0.61 and 0.63, respectively), Eschholz et al (2010) for maize accessions from Switzerland (0.78), or Sharma et al (2010) for Himalayan accessions (0.63).

The allelic frequencies showed that the accessions MGH and KST had the largest number of fixed alleles (six and five, respectively) and eight alleles with frequencies over 0.8. These accessions had the lowest genetic diversity probably due to high consanguinity produced in these two populations by farmers during multiplication. Actually, the coefficient of consanguinity was high (f = 0.16) for MGH and (f = 0.33) for KST. Furthermore, accessions from the north of the prospected area (MGH and SHH) had lower genetic diversity than those from the south (IGS, GOL, BSA). Moreover, the southern accessions had the largest number of rare alleles, as BAB, IGS, MST or TAO with 12, nine, eight, and six rare alleles, respectively. These results are consistent with the constant assertion by the farmers who provided the seed that their varieties are unique, maintained without seed exchange with other farmers. Furthermore, since maize was likely introduced in Africa from the coast, and natural selection for adaptation to desert conditions would have reduced the original variability, the larger variability detected among southern varieties indicates that the oasis could have behaved as a secondary center of diversification by isolation under extreme conditions.

Among the 87 alleles detected, eight alleles were specific of single accessions. Sharma et al (2010) found 163 specific alleles in a Himalayan collection and concluded that they were associated to both the mutation rate of SSRs and selection of those rare alleles within specific accessions. Such selection depends on the morphology and also on the environment wherein the accessions are adapted. Moreover, Behene et al (2006) identified 26 specific alleles in a maize collection from an Ethiopian region characterized by low rainfall and short growth cycle; they explained these alleles as a consequence of selection carried out for centuries by the farmers for adaptation to such conditions. This explanation could also apply to the Algerian Sahara maize. The genomic regions linked to these specific alleles could be candidate for improving stress tolerance, although in order to know this, further studies should be carried out including the same markers from the other reports.

The coefficient of consanguinity F = 0.297 was higher than those reported by Reif et al (2006) or Lia et al (2009): 0.24 and 0.22 respectively. This coefficient is in agreement with He (0.568) and Ho (0.389), confirming a deficit in heterozygotes in the total set of accessions. This is largely due to the loci umc1222, umc1545, phi036, and umc1327, which have F > 0.35. The Algerian genepool tested is not in Hardy-Weinberg equilibrium (HW) probably due to excessive consanguinity that modifies the genotypic frequencies and, thus, reduces genetic diversity along generations. On the other hand, the low value of the consanguinity index (F_{is} = 0.026) is synonymous of Hardy Weinberg equilibrium. This suggests that most of the accessions were in Hardy Weinberg equilibrium, while the total set of accessions was not. Maize is a cross-pollinated species and should be close to HW, but frequently this is not the case (Yao et al, 2008; Lia et al, 2009; Liu et al, 2009). In our study, nine of the 15 accessions were in HW equilibrium, and three of them had large excess of homozygotes. Fixation indexes and deviations from HW are normally explained by small samples for multiplication or by assortative mating (Labate et al, 1997; Hinze et al, 2005). Actually, within natural populations crosses are not at random and fixation indexes are usually higher than 0.5 (Rossini Pinto et al, 2003). According to F_{is}, there were on average 3% fewer heterozygotes within populations than expected under random mating, probably due to assortative mating. The F_{is} value found in the Algerian populations is similar to the values found by Romay et al (2012) in Spanish populations. Fst reflects the divergence in allele frequency between populations. The high genetic differentiation (F_{st} = 0.31) shows that inter-accession genetic diversity accounts for 31% of the total genetic diversity while intra-accession genetic diversity was 69% of the total genetic. Similar results were obtained by Yao et al (2008).

The 15 accessions were classified in three groups based on Nei's distance and the UPGMA method. The clusters were related to geographical origin. IGS is isolated in cluster I and comes from the most southern area (wilaya of Tamanrasset); representing a unique germplasm type. This is consistent with the observation that genetic distance is often associated with geographical distance (Beyene et al, 2006). Cluster II includes nine accessions coming from the center of the prospected area, mainly from the wilaya of Adrar. This second cluster consists of two subgroups with six and three accessions, respectively;

the main group is closer to Adrar while the smaller group includes the two accessions from the North of Algeria (MGH and SHH) along with a southern accession. Cluster III consists of five accessions; most of the populations of Bechar were included in Cluster III. The geographical distribution based on genetic distance is therefore quite consistent, although some populations from each group are not located in the expected area, e.g. GOL and LOM, possibly due to interchange of seed. The most variable populations considering the number of alleles per locus, heterozygosity, specific and rare alleles were located in the center of the sampled area, and mainly in cluster III, while northern and southern accessions had lower variability. These clusters suggest that there are at least three groups of maize germplasm in Algeria, in agreement with our hypothesized introductions from the original Spanish sources, other African neighbor countries and later introductions from Europe.

These results are a first approach to study genetic diversity and structure of maize from the Algerian Sahara. These open-pollinated populations have good variability and a high potential value for breeding as a result of adaptation to extreme conditions. Presumably, natural selection has concentrated favorable genes for tolerance to extreme stress in order to allow the adaptation to variable biotic and abiotic stressful and unpredictable environmental conditions. Maize from the desert could derive from early introductions that suffered selection for adaptation through their migration from the coast. Such selection should have reduced variability; however, most of the variability is located in the oasis of Central Algeria, suggesting that the oasis could have behaved as secondary centers of diversification.

The use of SSR has allowed the study of diversity within these accessions and the investigation of genetic relationships among them (Powell et al, 1996). The 15 SSRs have revealed a wide genetic diversity in this set of 15 accessions and also within each accession. GOL and LOM have the highest genetic diversity; they belong to different clusters and could be used as base populations for future breeding programs.

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