GENETIC STRUCTURE AND DIVERSITY IN SORGHUM BICOLOR (L.) MOENCH LANDRACES FROM MARGINAL SORGHUM PRODUCTION LANDS IN SENEGAL, BASED ON SSR MARKERS

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ABSTRACT

Data on sorghum genetic diversity in Senegal are missing despite its importance in the food and feed in the country. In order to contribute to the sustainable in situ management of sorghum germplasm, we investigated its genetic diversity and structure in its marginal production areas. Investigations were focused on Thiès, Diourbel and Kédougou regions where sorghum landraces have been less investigated and genetic information on landraces is unknown. A total of 148 sorghum accessions representative of landraces used in production systems have been sampled and analyzed with 30 microsatellite markers. A total of 138 alleles have been recorded. The number of alleles per locus varied from 3 (7 loci) to 8 (3 loci). The observed heterozygosity varied from 0 to 0.62. The low genetic distance (0.12) was recorded between Thiès and Diourbel populations and the highest distance (0.22) between Thiès and Kédougou populations. Dendrogram obtained according to Neighbour joining classification model allowed the classification of sorghum accessions into three main groups. The Genetic structure is not function to the regions indicating that landraces are not specific to a region. The results are a first step toward the sustainable in situ management of genetic resources. Data on the whole range of existing diversity of sorghum in Senegal is an important key for its germplasm management; so, the genotyping must be extended to accessions from the whole country.

Keywords: sorghum, landraces, diversity, microsatellite markers, Senegal.

INTRODUCTION

Sorghum (*Sorghum bicolor ssp bicolor* L. Moench.) is one of the major staple food and fodder crop in semi-tropical Africa and Asia (Doggett, 1988) maybe because of its unusual tolerance of hot and dry environments (Mullet *et al*., 2002). In Senegal, sorghum is one of the farmer's principal cereal crops (ANSD, 2010; Ba *et al*., 2010). The most important sorghum production areas according to ANSD (2018) are Kaffrine, Tambacounda, Sédhiou regions. Five major races or sorghum (bicolor, caudatum, dura, guinea and Kafir) have been identified in the World

according to panicle and spikelet morphology (Harlan and de Wet, 1976). *Sorghum bicolor subsp. bicolor* constitutes the cultivated form and exhibits great phenotypic variability. The cultivated form is mainly cross-pollinated. The frequency of outcrossing varied from 5 to 40 percent in the open field (Barnaud *et al.,* 2008).

Many studies in the world have investigated the patterns of genetic diversity among sorghum accessions. Some studies have demonstrated that genetic structuration is associated with geographic origin and racial classification (Deu *et al*., 2006). However, other studies showed a lack of

association between genetic structure and geographic origin or racial groups (Menkir *et al.*, 1997; Djé *et al.*, 2000). Unfortunately, such information is missing in Senegal.

Until now, the prospection and collect of sorghum cultivars in Senegal concerned mainly the Sine Saloum, Casamance, Tambacounda, Ferlo and Senegal River, areas known since a long time as zones of sorghum production. A total of 242 accessions have mainly been collected in these regions between 1974 and 2002 and brought in genes bank at ICRISAT/India. This sampling has been an important action in landraces conservation. However, in this previous collection, there is only 1 sample from Thies, 2 from Kédougou. So, it is expected that a thorough sampling of sorghum landraces in Thiès, Diourbel and Kédougou regions in Senegal could give additional information on this crop and allow extending the conservation action to landraces specifics to these zones. Indeed, the adoption of improved varieties is generally limited in the country. The largest part of sorghum production comes from farmer-selected landraces, especially in marginal production lands. These landraces are adapted to the local environments. It is also well known that contrasting sorghums with contrasting adaptation are often grown in the rainy season and post-rainy season within the same geographic region (Deu *et al.*, 2006). Moreover, ethnic traditions, social organizations and food preferences also probably could contribute to the extent and structure of crop diversity in these regions (Reenberg 2001; Deu *et al.*, 2008). The geographical distribution of landrace vernacular names, agromorphological types and genetic diversity on different spatial scales also provides valuable information to complement ex situ collections and establish relevant criteria to initiate and monitor in situ conservation programs (Brush, 2000). Genetic information on landraces prevalent in Senegal regions with low interest for sorghum cultivation will be an important input for further breeding programs and sorghum promoting projects interested in these areas. For these reasons, the study aims to characterize genetic diversity and spatial structure of sorghum landraces prevalent in Senegal zones with low interest for sorghum cultivation as the first step for future in situ conservation programs. The present study is part of global research activities at the national level since 2013 to improve major crops and to better manage germplasm resources in Senegal.

MATERIAL AND METHODS

An intensive collection of sorghum accessions was conducted in 16 villages belonging to Thiès, Diourbel and Kédougou regions in Senegal (figure 1). Villages were distant at least 20 Km and far from roads to avoid anthropic effects. In each village, 6 to 14 farmers have been interviewed individually and in focus groups. The survey included information on crop uses, seed origins, agricultural systems, desired varieties, etc. In each village, we sampled all local varieties listed by farmers. Sampling has been done either in farmers' fields, in panicle-drying zones or in-home granaries, depending on the advancement of harvest work.
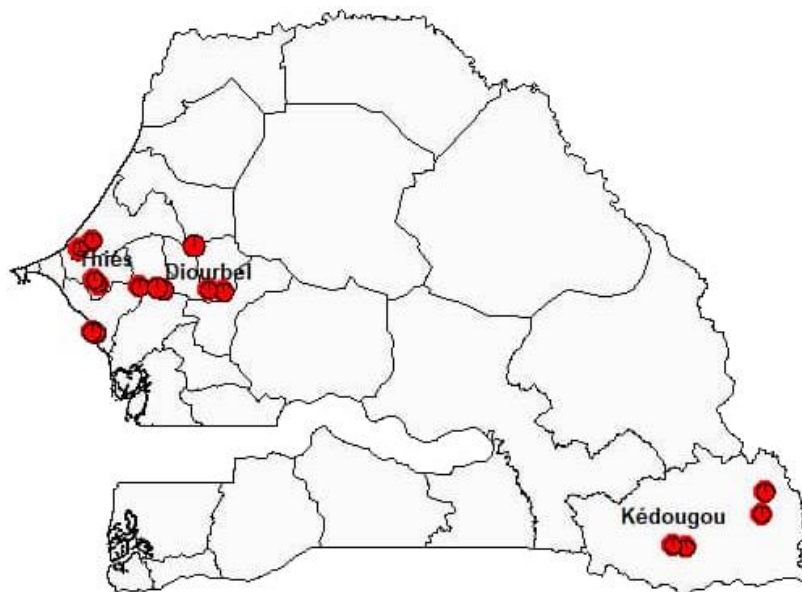

Figure 1. Sites of collect.

Table 1. List of regions, departments and villages where sorghum accessions have been sampled.

Regions	Departments	Villages	Accession number/village	Accession number/region
Thiès	Thiès	Keur Birane	6	50
		Taten Bambara	8	
	Tivaoune	Pambal Boye	8	
		Ndiaye Bope	6	
	Mbour	Keur Coly	10	
		Keur Cheikh	12	
Diourbel	Bambey	Touba Ndjemane	9	64
		Ndione	12	
	Mbacké	Koffa	8	
		SéoTienene	10	
	Diourbel	Mboule Mbapp	14	
		Gnignakh	11	
Kédougou	Saraya	Kondokhou	8	34
		Missirah Sirimana	10	
	Kédougou	Barafoute	8	
		Baraboye	8	

Table 2. Characteristics of the microsatellite primers used.

Marker names	Primer sequence F	Primer sequence R	Expected Size (bp)	Annealing Temperature (°C)
gpsb112	GTCTAACTCCTCGCTCC	TCCAACAAAATCCACC	209	50
gpsb121	GATAGCGAGGTGCCTA	TGCAGGAGTAAGAAATGA	157	50
gpsb127	CGCATCGTAGTTGTTG	GCAGCATTTCTGGAGT	189	50
gpsb128	CACGCGACCGTAAAG	GATAAGGTGATGGAGGAAA	264	50
gpsb129	CTCAACTCAACGCCTAC	ACTACTCCAATCCAATCC	257	50
gpsb015	CGTTCGTTCATTTCTCTTTC	GCTCTGGGCGTCTGAT	187	50.5
gpsb158	GAATTGAGTGGGATTTGT	GTCAGAGAATGGGTTCAT	283	50
gpsb170	ATAAATCCTTGCGTCCT	CCTAAACTTCCCTTTCCT	239	50
gpsb176	TAATTCTAGTCATCACCCC	TTGGCGTTCATAGTTTC	273	50
gpsb180	TCATTTGTAGTTGCGTTAG	GCTTTCACTTGGGGA	160	50
gpsb050	GGCTTCTTTCCTCTCC	GAGTTCTTTTATGTTTTGTGT	213	49
gpsb051	TGAAGGACTTTGATACCC	CCTAAGACTGCCAATGTT	177	49
gpsb063	CATCATGGTTTGTGGT	CTGCTCATTTTATCGTC	177	50
gpsb067	TAGTCCATACACCTTTCA	TCTCTCACACACATTCTTC	178	49.1
gpsb079	AGGACAGAACAGGAAGG	GTGAATTTGAGTTGGAAAG	151	50
gpsb095	TACTACAGGGGCTTGG	GTGTGGGAAGAGGGA	178	50
gpsb123	ATAGATGTTGACGAAGCA	GTGGTATGGGACTGGA	304	50
mSbCIR230	AACAATCGACCCTTCCTAAC	TCTTGTTCATGTGCCAGTAG	110	55.2
msbcir238	AGAAGAAAAGGGGTAAGAGC	CGAGAAACAATTACATGAACC	105	54.7
mSbCIR242	GGATGAATAAATCGGGTTG	GAAACAAAATACCCAGCAGT	101	54.9
mSbCIR248	GTTGGTCAGTGGTGGATAAA	ACTCCCATGTGCTGAATCT	101	55.7

mSbCIR286	GCTTCTATACTCCCCTCCAC	TTTATGGTAGGATGCTCTGC	112	55.5
mSbCIR311	GATCTCACATTGCTTGTTT	TGAGCTTCTCCACATTCT	112	51
mSbCIR313	AACGTACCACACGTATTTGA	TCTTGTGCTCGTAGAGTGTC	140	54
mSbCIR323	CTTGAACCCCACTCAAGAC	TACCTGCACTGCTGCTTC	284	55.8
msbcir347	GAACATCAGAGGGTTTACCA	GAACCAACTACGCTTGTGTC	118	55.4
SbAGB02	CTCTGATATGTCGTTGTGCT	ATAGAGAGGATAGCTTATAGCTCA	119	62
Xcup11	TACCGCCATGTCATCATCAG	CGTATCGCAAGCTGTGTTTG	204	60
Xcup14	TACATCACAGCAGGGACAGG	CTGGAAAGCCGAGCAGTATG	210	62
Xcup61	TTAGCATGTCCACCACAACC	AAAGCAACTCGTCTGATCCC	237	60
Xcup63	GTAAAGGGCAAGGCAACAAG	GCCCTACAAAATCTGCAAGC	185	60

DNA isolation

Genomic DNA was extracted using a modified MATAB (Mixed Alkyl Trimethyl Ammonium Bromide) extraction method (Risterucci *et al.*, 2000). Twenty (20) mg of dried leaves were ground (MM301, Retsch). The crushed material was transferred to a 2 ml Eppendorf tube containing 750 µl of 2% MATAB extraction buffer solution preheated to 65 °C. The mixture was homogenized for 20 seconds and then incubated in a water bath for 20 min with stirring every 5 min. After cooling to room temperature for 5 min, 750 µl of Chloroform Isoamyl Alcohol (CIAA) was added to the mixture, homogenized, then centrifuged at 13000 rpm for 20 min at 20 °C. The supernatant (600 µl) was removed and transferred to new 1.5 ml tubes containing an equivalent volume of cold isopropanol. The pellet of the DNA was precipitated at -20 °C for 2 h and then centrifuged at 13000 rpm for 20 min at 4 °C. After removing the supernatant, the pellet was cleaned with 500 µl of 70% ethanol, centrifuged at 13000 rpm for 20 min at 4 °C. This supernatant was removed, and the pellet dried for at least 1 h at room temperature before being dissolved in 150 µl of 1X TE during 2 H.

The DNA was quantified by migration in a 1% agarose (Lonza) gel containing 0.5 X TBE (Tris Borate EDTA); 2.5 µl of BET (Ethidium bromide) (0.5 mg / µl). The samples to be deposited were prepared by mixing 2 µl of DNA, 2 µl of bromophenol blue and 6 µl of ultrapure water. The gel was migrated at 100 volts for 30 min and then photographed under UV at 230 nm using a UV table (UVITEC). DNA yields were determined by concentration estimation, compared to the bands of a Smart Ladder (Eurogentec) of known concentration and diluted to a working concentration of 5 ng/ul.

Microsatellites amplification and electrophoresis

A PCR was carried out using 30 sorghum SSR markers (Table 2) previously used by Barnaud *et al.* (2008). PCR reactions were performed in a total reaction mixture of 10 µl containing: 25 ng template DNA; 1 µl of PCR buffer 10 X; 2.0 mM $MgCl_2$; 0.2 mM dNTP; 0.06 µM of the universal Cy5-labeled; 0.08 µM of M13-tailed forward primer at the 5´end (Schuelke, 2000), (F); 0.1 µM of reverse primer (R); 0.5 U of Taq DNA polymerase (Tina Taq). Amplifications were performed using an MJ Research PTC 200 thermal cycler (Bio-Rad Laboratories Inc.) with the following cycle profile: initial denaturation for 1 min at 95°C followed by 35 cycles with 30 sec at 94°C, 1 min at primer-specific annealing temperature (Table 2), 2 min at 72°C and a final extension step at 72°C for 8 min. All PCR products were separated on a 6,5% polyacrylamide denaturing gel using a LICOR 4300 sequencer by loading 2 µl of PCR product diluted 4 X in loading buffer.

SSR data analysis

The average number of alleles per locus, the expected heterozygosity (He) and the observed heterozygosity (Ho) per marker were calculated using R 3.6.0 software (R Core Team, 2019). R also allows computing the fixation index or F-statistic (Fst) to assess the differentiation of gene pool of the 3 regions investigated. The genetic distances and the analysis of molecular variance (AMOVA) were also calculated using GenAlex 6.501 (Peakall and Smouse, 2012) software. DARwin 6 (Perrier and Jacquemoud-Collet, 2006) software was used to make the dendrogram. Genetic population structure was inferred with the structure software (Pritchard *et al.*, 2000). The analysis was performed with K ancestral populations ranging from 1 to 10 with 500 000 iterations and a burn-in period of 100 000. Ten

(10) runs for each K-value were performed and outputs were summarized using Structure Harvester (Earl and vonHoldt, 2012). We evaluated the number of population K based on Evanno's method. For ancestry analysis (q), we used the simulation with the highest log probability. We classified individuals in groups based on an ancestry coefficient of 0.7 or higher.

RESULTS
Microsatellite amplification
The 30 microsatellite markers used, gave good amplification of DNA. Figure 2 shows an example of a polyacrylamide gel.

Allele number and percentage of polymorphic loci
All the loci were polymorphic and the number of alleles per locus varied from 3 (7 loci) to 8 (3 loci) as presented in table 3. A total of 138 alleles have been recorded corresponding to an average of 4.6 alleles per locus.
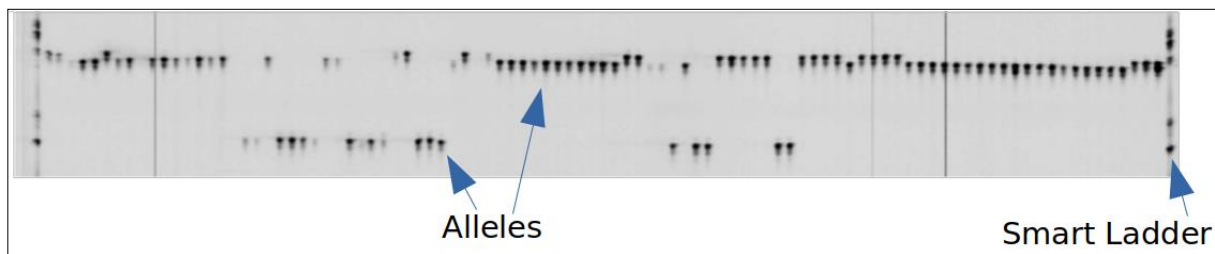
Figure 2. Revelation on polyacrylamide gel of the locus msbcir347 in sorghum population.

Table 3. Number of alleles per locus.

Locus	Nb allele	Ho	He	Locus	Nb allele	Ho	He
xcup 11	5	0.32	0.74	gpsb 127	4	0.11	0.71
xcup 63	6	0.03	0.78	gpsb 121	5	0.06	0.69
sgagb 02	6	0.14	0.74	msbcir 248	3	0.11	0.51
msbcir 242	5	0.14	0.62	msbcir 311	4	0	0.68
gpsb123	6	0.62	0.82	msbcir 313	4	0.02	0.56
gpsb 79	3	0.04	0.52	MSBCIR 323	4	0	0.72
gpsb 95	3	0.03	0.46	gpsb 67	4	0.14	0.64
msbcir 347	8	0.02	0.63	gpsb 15	6	0.16	0.82
gpsb 180	4	0.02	0.67	xcup 61	3	0.07	0.65
xcup 14	8	0.06	0.81	gpsb 50	8	0.03	0.8
gpsb 112	4	0.1	0.73	gpsb 63	4	0.01	0.63
gpsb 176	3	0.05	0.66	gpsb 51	4	0.09	0.69
gpsb 170	3	0.08	0.64	msbcir 286	5	0.08	0.69
gpsb 158	3	0	0.51	gpsb 129	5	0.09	0.58
gpsb 128	4	0.13	0.49	msbcir 230	4	0.04	0.74

Nb allele: number of alleles; Ho: observed heterogeneity; He: expected heterogeneity.

Observed and expected heterozygosity per locus and per population
Three loci (Gpsb158; Msbcir311; Msbcir323) have a null value of observed heterozygosity and the highest value was recorded with gpsb123 (Ho = 0.62). The expected heterozygosity varied from 0.46 (gpsb95) to 0.82 (gpsb123 ; gpsb15) as showed in table 3. The highest observed heterozygosity (Ho) was recorded in Kédougou population whereas the highest expected heterozygosity (He) was recorded in Diourbel population (Table 4). In all the populations, the He was at least six times higher compared to the corresponding Ho.

Genetic distances
The low Nei's genetic distance (0.12) was recorded

between Thiès and Diourbel populations and the highest distance (0.22) between Thiès and Kédougou populations.

Genetic differentiation index
Fst values are ranged from 0.03 to 0.06 (table 6). Low Fst values were recorded when comparing sorghum populations from Thiès and Diourbel, Diourbel and Kédougou. Kédougou and Thiès populations show moderate Fst.

Analysis of molecular variance
An analysis of the molecular variance (AMOVA) was performed using the matrix of distances for genetic differentiation. The overall genetic variation has been divided among pops (regions) (6%), among individuals within regions (84%), and within individuals (11%). The results obtained showed that the diversity within pops (intra-regional diversity) was greater than the diversity between pops (inter-regional diversity) (Table 7).

Table 4. Observed and expected heterozygosity per population.

Populations	Ho	He	F
Diourbel	0.094	0.655	0.859
Thiès	0.084	0.62	0.876
Kédougou	0.109	0.6	0.82

Ho: observed heterogeneity, He: expected heterogeneity; F: Wright's F-statistics.

Table 5. Nei's Genetic distances considering the region of sampling.

	Diourbel	Thiès
Thiès	0.12	
Kédougou	0.167	0.221

Table 6. Fst values according to regions.

	Diourbel	Thiès
Thiès	0.028 (p-value 0.0198)	
Kédougou	0.039 (p-value= 0.0099)	0.057 (p-value= 0.0099)

Table 7. Analysis of molecular variances.

Source	df	SS	MS	Est. Var.	%
Among Pops	2	161.781	80.89	0.636	6%
Among Indiv	145	2905.52	20.038	9.416	84%
Within Indiv	148	178.5	1.206	1.206	11%
Total	295	3245.801		11.258	100%

Df: degree of freedom; SS: sum of square; MS: mean of square; Est.Var: estimated variance.

Dendrogram
Neighbour joining cluster analysis of genomic SSR genetic diversity matrix resulted in the dendrogram represented in figure 3. The dendrogram allows distinguishing three main groups. The similarity dendrogram (Figure 3) showed 3 main groups (a, b and c). Fifty six (56) individuals constitute the group a, 70 for b and 22 individuals for the group c. Each group includes individuals from the three regions where sorghum was sampled. However, group c includes only 2 individuals from Thiès. In group a, we distinguish 2 subgroups; one subgroup includes only individuals from Diourbel.
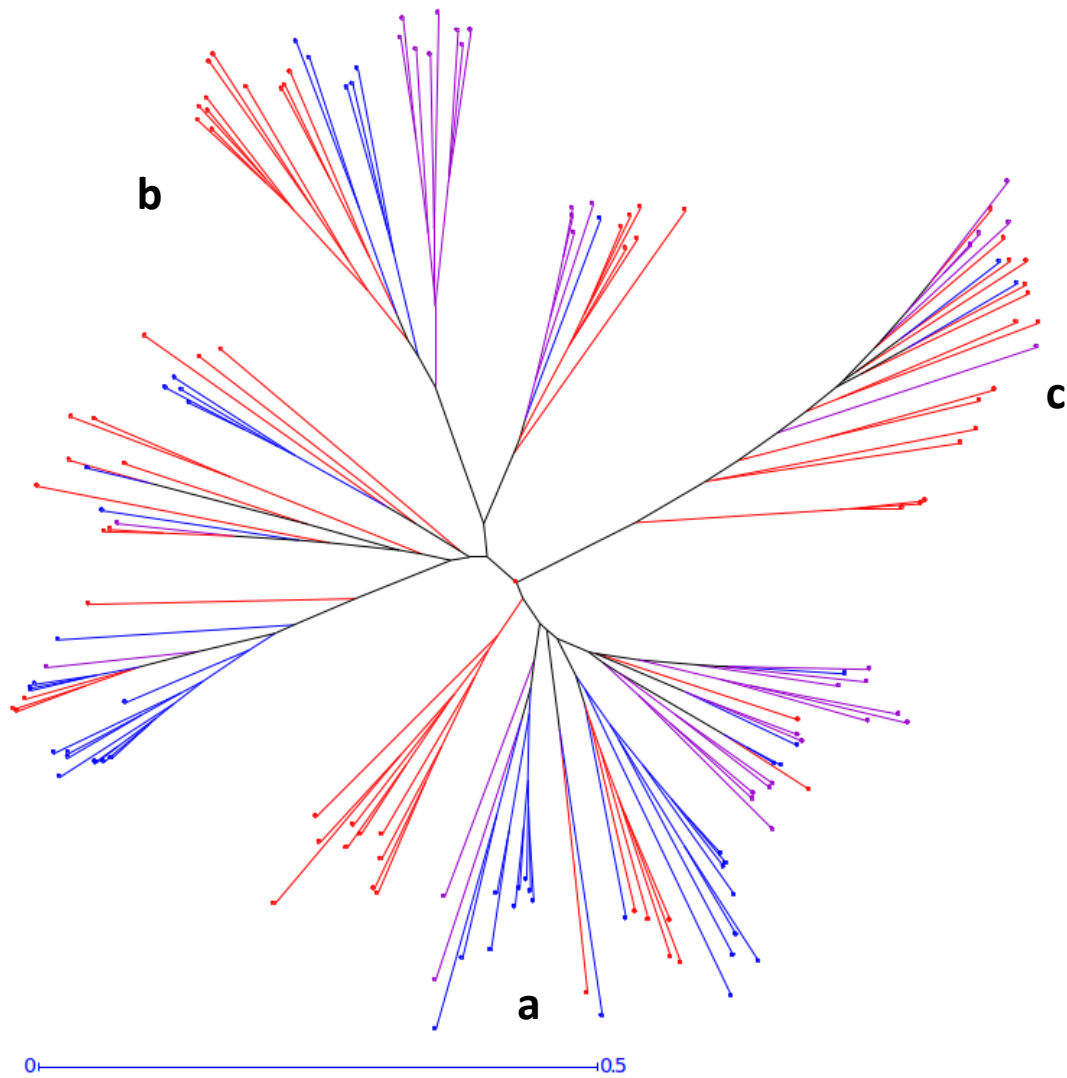
Figure 3. Dendrogram of similarity based on SSR markers.
legend:

- ▬▬▬ individuals sampled in Diourbel
- ▬▬▬ individuals sampled in Thiès
- ▬▬▬ individuals sampled in Kédougou

Structure analysis

STRUCTURE was able to correctly identify the number of subpopulations at Fst around 0.03 the most probable value of K was 4.

The structuring without a priori of the accessions was done with an ADMIXTURE analysis (Figure 4). This model based on the estimation of the membership coefficient was used to determine the optimal number of groups in the collection. Thus, the accessions were divided into four (4) groups. Group I is in the majority with 65 accessions

(59 from Diourbel and 6 from Thiès), followed by group II with 31 accessions, all from Thiès. Groups III and IV are in the minority with 20 and 27 accessions respectively. The first contained 13 accessions from Thiès and 7 from Kédougou, while the second consisted of samples from Kédougou. Groups II and IV are homogeneous with only accessions from one region, Thiès and Kédougou respectively. Five individuals are admixtures from Diourbel region.
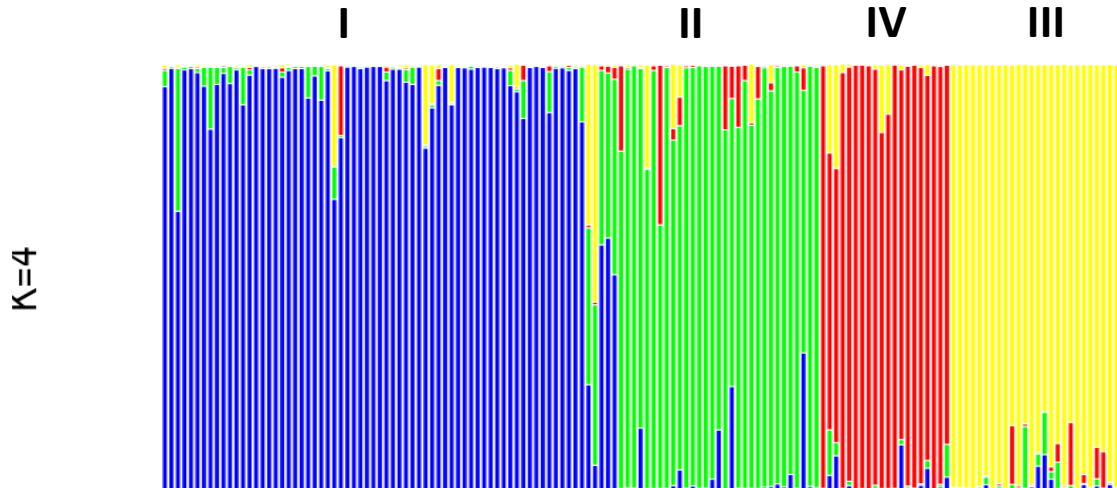
Figure 4. Genetic population structure predicted by STRUCTURE software.

DISCUSSION

All the markers used gave a net amplification of DNA fragments. The different makers have been properly chosen because of their specificity with sorghum. Moreover, they were polymorphic indicating that these markers are useful to assess the genetic structuration of sorghum.

The average of 4.6 alleles per locus recorded shows a relative diversity of sorghum in the regions investigated. The locus with 8 alleles has probably received low selection pressure compared to those with 3 alleles. The loci with the low number of allele are often quantitative traits loci which are suggested to breeding pressure.

The estimated diversity in this study is much smaller than the diversity observed in other sorghum populations. Indeed, Deu *et al.* (2008) used 28 SSRs markers to assess the genetic structure of 472 varieties from 76 villages in Niger. In their study, all the microsatellite loci were polymorphic and revealed 292 alleles, from 2 to 26 alleles per locus with an average of 10.43. Thirteen indigenous varieties were found in a single community in Zimbabwe (Oosterhout, 1997), 46 sorghum landraces identified by farmers in a village of North Cameroon (Barnaud *et al.*, 2007), 76 varieties grown by farmers from three communities in northern Benin (Kayode´ *et al.*, 2006). The relatively low diversity recorded in our study might reflect our collection protocol which concerned only some selected regions of Senegal.

So, comparisons of genetic diversity between the above studies are difficult, since estimates such as allelic richness or expected heterozygosity depend on the sampling schemes, the number of surveyed SSR, the size of the SSR repeats and the location of the SSR on the genome (between coding or non-coding DNA regions) as highlighted by Deu *et al.* (2008).

The genetic distances values suggest that population genetic in Thiès, Diourbel and Kédougou are genetically closed. Indeed, each population is a bulk of individuals belonging to the three genetic groups as shown by the dendrogram. This explains the low Fst recorded when we compare sorghum populations from Thiès, Diourbel and Kédougou. Deu *et al.* (2008) found a Fst of 0.07 between regions in Niger. A weak genetic differentiation between regions has been reported (Ollitrault *et al.*, 1997; Dje` *et al.*, 1999; Nkongolo and Nsapato, 2003; Kayode´ *et al.*, 2006).

In the present study, the population of each region is spread in the different genetic group identified. That confirms the fact that farmers maintain traditional crop diversity to manage risks and increase their resilience in the face of strong environmental heterogeneity in both space and time, and numerous pest and diseases (Teshome *et al.*, 1999; Deu *et al.*, 2008). Similar results have been revealed on maize in Mexico (Bellon, 1997).

The dendrogram showed 4 groups if we consider the homogeneous subpopulation from Diourbel. STRUCTURE also identify 4 subpopulations and is known to work extremely well for inferring the number of clusters when clusters were not well-differentiated (Emily *et al.*, 2006). In general, these populations and subpopulations include individuals from the three regions investigated designed with dendrogram and

structure analysis.

This indicates that cultivars are not specific to a region. This result also could be explained by an important informal seed exchange between populations in the three regions and high adaptability of the collected cultivars to a range of agroecological zones in Senegal.

Several studies based on local collections reveal a lack of correlation between genetic diversity parameters and environmental factors (Ayana *et al.*, 2000, 2001; Ghebru *et al.*, 2002; Zongo *et al.*, 2005) and a weak genetic differentiation between regions (Ollitrault *et al.*, 1997; Dje` *et al.*, 1999; Nkongolo and Nsapato, 2003; Kayode´ *et al.*, 2006). Deu et *al.* (2008) revealed differentiation between eastern and western Niger. Whereas the landraces collected in central Niger are intermixed in the two clusters.

Seed exchanges between farmers seem to explain a larger share of the observed genetic structure than climatic conditions. The fact that the farmers collectively maintain and rely on a large number of landraces to meet their production needs indicates that they must play a key role in future food security and conservation programs. We thus suggest, like others authors (Brush, 2000; Deu *et al.*, 2008), that ethnic and social factors are key information to implement in situ sorghum conservation programs and conduct further ex situ collections of sorghum in sub-Saharan Africa.

According to STRUCTURE analysis, Groups II and IV are homogeneous with only accessions from one region, Thiès and Kédougou respectively. This illustrates the presence of landraces with specific genetic background in these regions. Moreover, the dispersion of individuals belonging to Thiès in group I shows significative germplasm diversity of sorghum in this area.

To capture the whole range of existing diversity of sorghum in Senegal, a genotyping of accessions in ICRISAT/India together with the present collection is necessary. Further researches should integrate molecular markers to botanical races and agro-morphological traits for a better understanding and management of the genetic resources of sorghum landraces in Senegal.

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