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## Genetic diversity of sorghum (*Sorghum bicolor* (L.) Moench) accessions from thirteen regions of Burkina Faso

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### ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench) is a staple food crop for West African countries in general and Burkina Faso in particular. It is mainly grown by small holder farmers for their livelihoods. They grow their landraces which is a mixture of more than two varieties. Unfortunately, the yields of farmer varieties are low compared to improved ones bred by sorghum breeders with the potential up to 3t/ha. The objective of the study was to identify the genetic diversity between improved varieties released by research institutions and farmer accessions at the molecular level. DNA sample were collected from hundred and twenty-three accessions collected from thirteen regions of Burkina Faso. DNA samples were successfully genotyped using a multiplexed complex of 28 microsatellites DNA markers for 110 genotypes. The sorghum genotypes comprised of *Guinea*, *Caudatum* and *Guinea-Caudatum* races. Farmer varieties were defined mainly in *Guinea* and *Guinea-Caudatum* races while the improved varieties were mainly *Caudatum* races. The inbreeding level FIS (the inbreeding level within a given population) for each group improved, farmer varieties varied between  $-1 < FIS > 1$ . This shows the autogenous nature of sorghum varieties. Breeding schemes can therefore be designed for improvement of farmer preferred varieties.

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**Keywords:** Sorghum Microsatellites, *Guinea*, *Caudatum*, Yield, Landraces

### INTRODUCTION

Germplasm diversity is a key for successful genetic improvement of plants. There must be phenotypic and genetic variability among the material targeted to be improved. Several studies have been undertaken to assess the variability among the accessions of sorghum. In Ethiopia Adugna (2014) found that there's a huge variability

among farmers varieties. Shehzad and Okuno (2014) highlighted the necessity of diversity among sorghum varieties for the crop improvement. In Niger, the variability among sorghum accessions were revealed by (Deu et al., 2008). In a survey in Kenya Labeyrie et al. (2015) revealed the diversity among farmers varieties. Genetic diversity was found sorghum germplasm from Senegal (Tovignan et al.,

2015). In Burkina Faso, Barro-Kondombo (2010) found genetic diversity among sorghum accessions of three regions: Centre North, Centre West and Boucle du Mouhoun. The diversity among the farmers sorghum landraces was assessed by Ouedraogo (2015) for drought tolerant varieties. More recently, genetic diversity was found by Nerbewende et al. (2018) among sweet sorghum accessions collected in Burkina Faso. In fact, the germplasm collected was composed only of farmers' varieties. Most of the variability of sorghum comes from the open pollinated varieties possessed by farmers. Those differ from the improved varieties that have been purified and stabilized. There is a need to assess the genetic diversity to maintain and protect from genetic erosion. Several methods have been used to assess the genetic variability within sorghum populations around the world. This began with the crop's discovery and propagation. The phenotypic differences have been evaluated through several different methods. Molecular tools have been used to assess the diversity among the accessions with different agro-morphological traits at the genetic level. The assessment of the genetic variability could be useful to take into account the needs of the stakeholders involved in sorghum production improvement in Burkina Faso (Zongo et al., 2005). Several breeding programs on sorghum improvement around the world have characterised sorghum genetic diversity and several traits have been assessed. Varieties have been differentiated based on agronomical and physiological traits that are economically relevant including yield s, disease resistance, insect tolerance; weed tolerances, mineral deficiencies, toxicities tolerances, and extreme weather tolerances of wheat, maize, rice, sorghum, potatoes, tomatoes, eggplants, cotton, peanuts, soybeans, and beans.

The objective of this work was to determine the genetic differences among

improved varieties from researches centres and farmer accessions of Burkina Faso.

## **MATERIALS AND METHODS**

### **Germplasm Collection**

One hundred and twenty sorghum accessions obtained from farmers and Research centres were used in this study. Ninety-two accessions were collected during a participatory rural appraisal from farmers surveyed from different sites across the three agro-ecological zone of the country and from the main areas where sorghum is the major source of food (Figure 1). To avoid mixture and confounding of the farmer varieties, only panicles samples were collected from farmers and samples seeds were labelled according to their zones of provenance. Twenty-eight improved varieties were provided by Institute of Environment and Researches Agriculture (INERA) in Burkina Faso, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Mali, IER (Institute of Economy Rural) in Mali, and Texas A&M University.

### **Molecular characterisation of sorghum germplasm**

The modified extraction buffer of Gawel and Jarret (1991) Mixed alkyltrimethylammonium bromide (MATAB) was used to obtain purified DNA from one hundred and ten (110) sorghum seedlings from fifteen days old leaf samples. The DNA samples (110) were genotyped using a multiplexed complex of 28 microsatellites DNA markers. These markers have been used before to genotype a set of 3367 accessions of sorghum (Ramou et al., 2013; Billot et al., 2013). Various types of devices (laminar flow hood, centrifuges, thermo agitator and thermo blender) were used to obtain and homogenize the working solutions. Four types of thermocyclers were run for polymerization. The sequencer 24 capillaries ABI version 3500XL were used to sequence each allele that

was amplified during the polymerase chain reaction.

**Data collection and analysis**

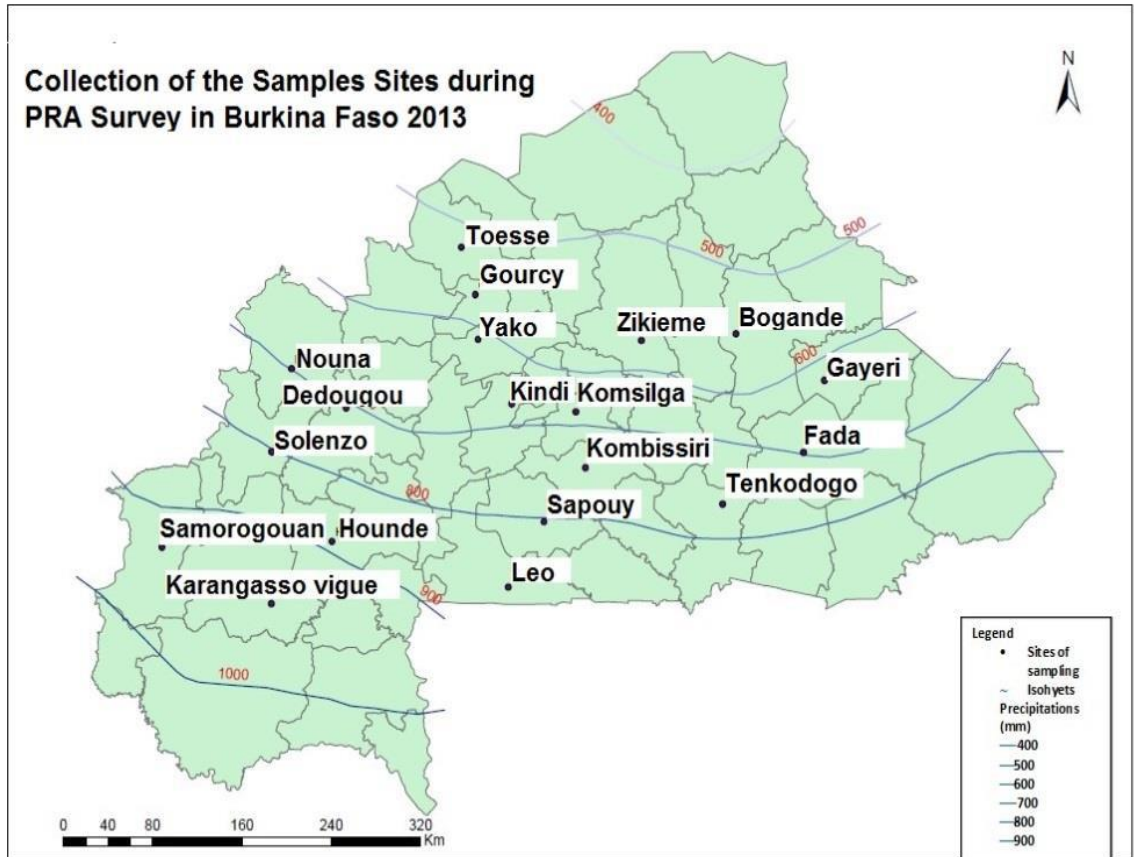
Molecular data were collected from the DNA of the accessions and an analysis of Genetic structure of the germplasm was obtained through the software Structure version (2.3.4). Genetic parameters were obtained through FSAT version (2.9.3.2) and Genetix version (4.0.5.2) that indicate the richness of the allelic and the genetic diversity index. PCA analysis and phylogenetic trees were done with DARWIN version (6). The genetic indices (observed and expected

heterozygosity) of Hardy Weinberg Ho, He, FST, FIS, RS A, FIT, (Cockerham and Weir 1984) were used to estimate the linkage among the markers. The PIC value for each locus was computed based on the formula of (Smith et al., 1997). This value provides the discrimination power of a locus on the basis of the expressed number of alleles and the frequencies of the alleles present at the locus.

$$PIC = 1 - \sum_i^n f_i^2$$

PIC= polymorphic information content in which:

$F_i$  = frequency of the  $i^{th}$  allele in the analyzed sorghum accessions.



**Figure 1:** Sites where sorghum samples were collected during PRA Survey in Burkina 2013.

## RESULTS

### Molecular characterization of the sorghum germplasm

#### *Genetic distance diversity parameters of the genotypes analysed*

From the diversity analysis, a total of 150 alleles were detected from the 28 SSR markers (Table 1). The allele size ranged from 96 SBAGB 02 (96-118) to 298 Gpsb 123 (286-298). The number of alleles per locus varied from 2 (Xtxp 136, XCup 62, XCup 63, XCup 61 Gpsb 148) to 19 (Xtxp 295). The values of heterozygosity  $H_o$   $0 < H_o > 0.18$  were very low while the expected heterozygosity value was  $0.02 < H_e > 0.82$ . Among the 28 loci analysed, 12 loci were highly polymorphic with a polymorphic information content  $PIC \geq 50\%$  (Xtxp 295, Gpsb 151, Xtxp 57, Xcup 07, Xcup 02, Xtxp 145, Xtxp 40, Gpsb 089, Xtxp 15, Gpsb 067, Sb5-206, and Gpsb 148). This value provides the discriminating power of a locus on the basis of the expressed number of alleles and the frequencies of generated alleles at each locus.

The genetic diversity parameters values were obtained by the software FSTAT version 2.9.3.2 and Genetix version 4.0.5.2. Among the 28 loci tested, a total 150 alleles were identified. Twenty-five of 28 markers scored alleles.

Loci analysis matrix from Darwin software version 6.012 shows that the accessions collected in different years and from different farming areas were genetically different. The time of cultivation of accessions by the farmers varies from five years to more than twenty years. The pink dots were illustrating the accessions that had been cultivated during the last five years the blue dots were for the varieties cultivated for about ten years, the brown dots were the varieties cultivated for about twenty years and the black illustrated the accessions cultivated more than twenty years of acquirement (Figure 2). The right side of the biplot showed more of the pink coloured dots; this was representing the improved varieties analysed while the left side of the biplot show a mixture of the varieties this was representing the farmers' varieties.

### Structure analysis of the sorghum accessions

The software STRUCTURE version 2.3.4 by Pritchard et al. (2000) and Falush et al. (2003) was used to study the genetic structure of the 110 accessions of sorghum with the run parameters of 100000 Burn-in periods, 500000 iterations, and the K values varying from 1 to 10. K represents the hypothetical groups of individuals that composed the full accessions. The Figure 3 shows the truncation of the k value of 3. That was the best estimates of the hypothetical number of the individuals genotyped to the three groups. The groupings were based on the groups of sorghum races previously described by the research stations' varieties.

The genetic diversity indices at K value equal 3 suggest a high index of differentiation among families (FST) value between the groups. The mean value of the FST for the group1 was 0.5133; the FST for the group2 was 0.3358 and the FST for the group3 was 0.785. The defined race groups were *Guinea*, *Caudaum* and *Guinea-Caudatum* (Figure 4. and Figure 5).

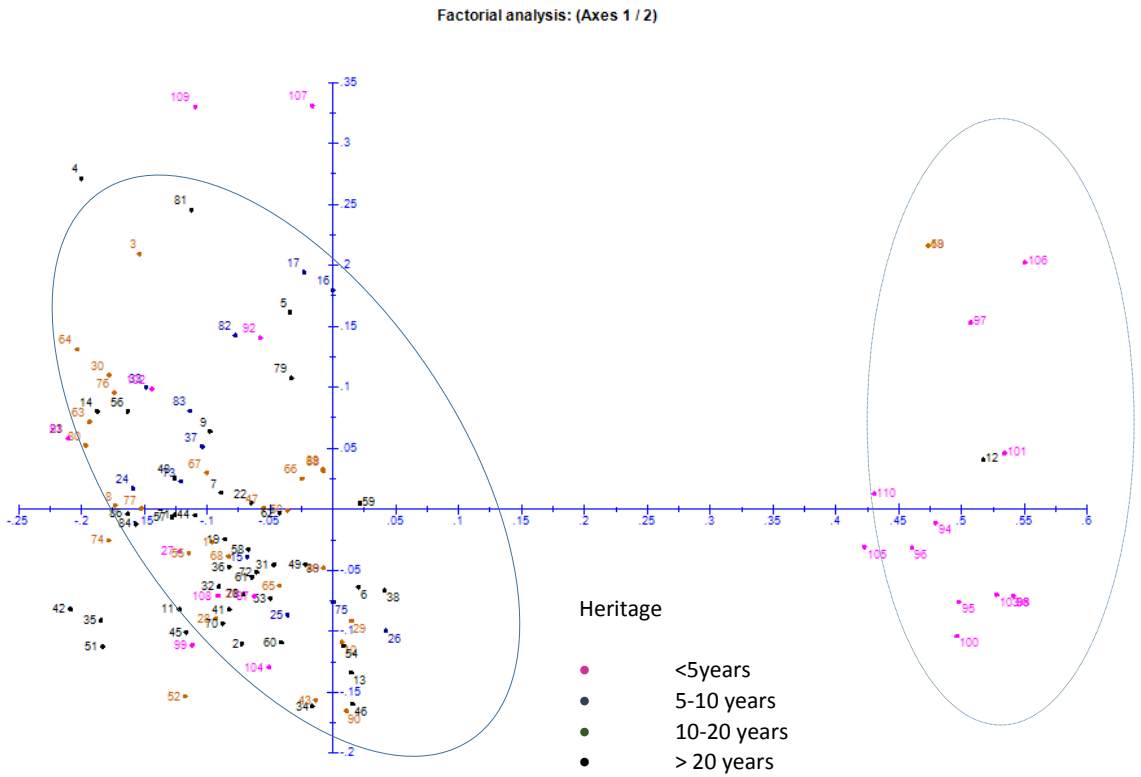
### Genetic indices based on structure of the groups of sorghum accessions

The data show high value of inbreeding for all the accessions (FIT) ( $0.80 < FIT > 0.83$ ) as well as for the value of the inbreeding level (FIS) within groups ( $0.80 < FIS > 0.83$ ) (Table 2). The genetic diversity index, FST value, varied between  $0 < FST > 1$  which shows the genetic diversity among the groups of populations was not high. The values of Fit vary from  $-1 < FIT > 1$  which shows the level of inbreeding within samples at the population level whereas the FIS that varied between  $-1 < FIS > 1$ , showing the level of inbreeding in each group of the accessions Only a few groups had significant FST values at  $P < 0.05$ . The groups based on region had an overall FST = 0.06. The groups based on Growth Cycle showed an FST = 0.02 and the group based on Year of collection had an FST = 0.08.

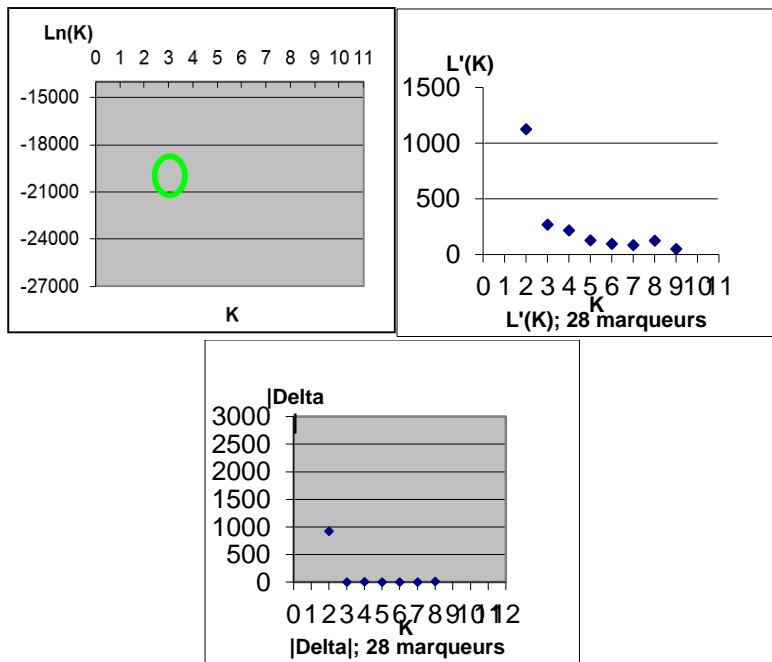
**Table 1:** Genetic index estimation of 110 genotypes analysed with 28 SSR markers.

Locus	Allele size	A	Rs	Ho	He	PIC
Gpsb067	170-186	8	7.91	0.06	0.48	0.47
Gpsb089	165-173	4	4.00	0.10	0.56	0.55
Gpsb123	286-298	5	4.95	0.02	0.29	0.29
Gpsb148	135-143	2	2.00	0.12	0.46	0.46
Gpsb151	104-130	8	8.00	0.15	0.76	0.76
sb4-72	181-195	5	5.00	0.08	0.39	0.39
Sb5-206	108-150	10	9.99	0.03	0.48	0.47
Sb6-84	181-195	4	4.00	0.05	0.39	0.39
SbAGB02	96-118	6	5.99	0.03	0.31	0.31
Xcup02	192-204	5	4.96	0.18	0.67	0.66
Xcup07	254-272	6	6.00	0.12	0.68	0.67
XCup11	165-172	2	2.00	0.00	0.25	0.25
XCup14	209-233	5	5.00	0.07	0.44	0.43
Xcup53	186-198	4	4.00	0.07	0.35	0.35
XCup61	198-201	2	2.00	0.12	0.37	0.37
XCup62	187-190	2	1.96	0.01	0.01	0.01
XCup63	139-145	2	2.00	0.06	0.33	0.33
Xtxp10	135-151	6	6.00	0.00	0.33	0.32
Xtxp114	211-217	3	3.00	0.00	0.39	0.38
Xtxp136	240-243	2	2.00	0.00	0.02	0.02
Xtxp145	208-244	7	7.00	0.14	0.61	0.60
Xtxp15	201-215	8	8.00	0.08	0.55	0.55
Xtxp278	243-252	3	3.00	0.06	0.29	0.29
Xtxp289	266-296	5	5.00	0.05	0.36	0.36
Xtxp295	161-201	19	18.86	0.15	0.82	0.81
Xtxp320	263-284	2	2.00	0.17	0.07	0.07
Xtxp40	135-138	7	6.96	0.00	0.60	0.59
Xtxp57	228-255	8	8.00	0.15	0.72	0.71

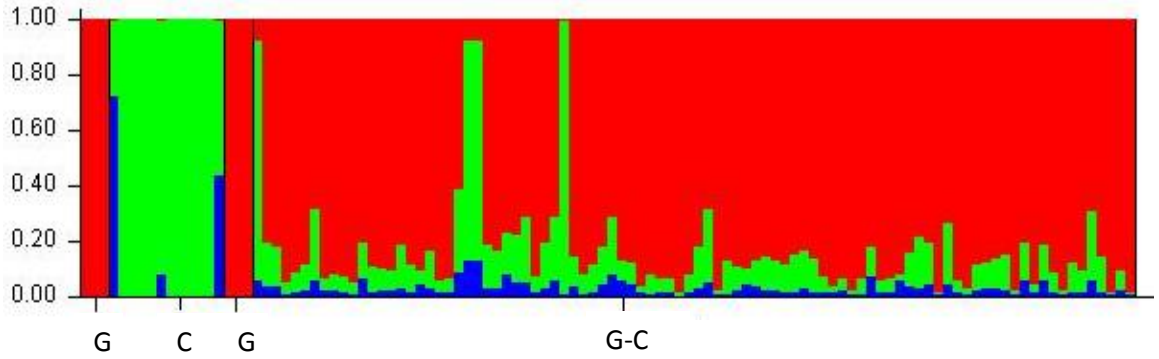
Ho: Observed heterozygosity He: Expected heterozygosity (Gene diversity) Rs allele richness A: number of allele PIC: Polymorphic information Content.



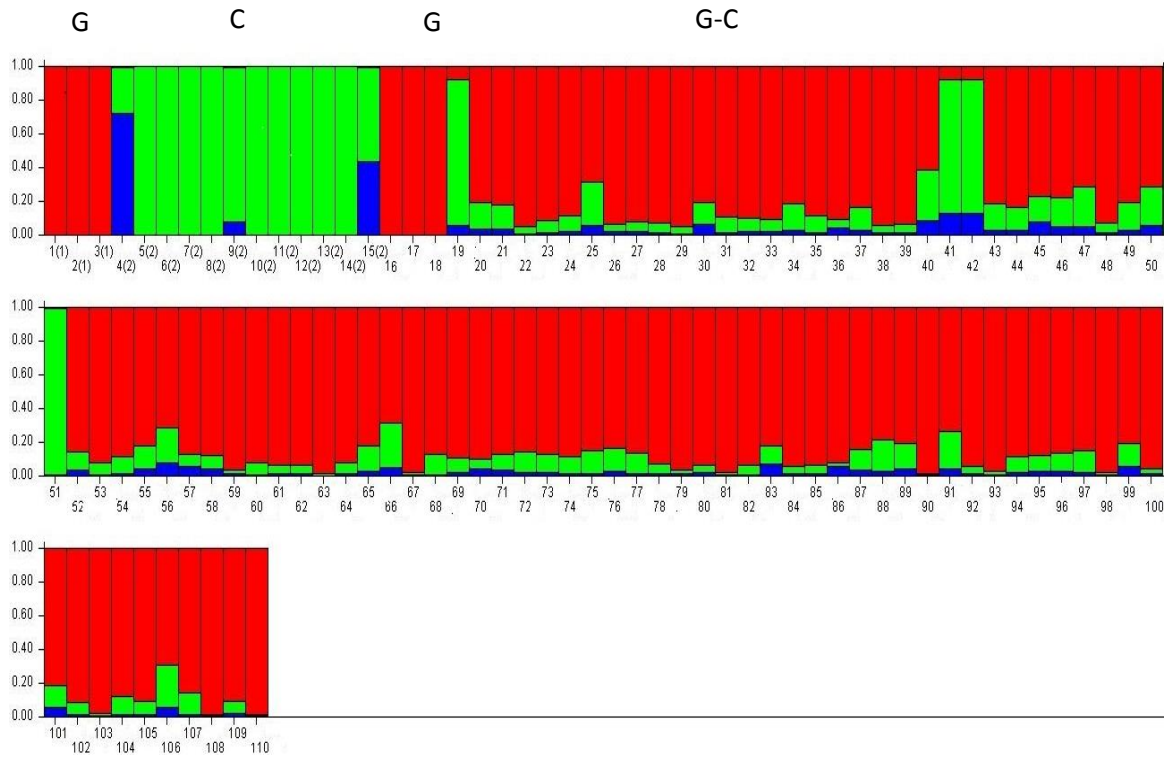
**Figure 2:** Principal coordinates analysis based on the dissimilarity index from the allelic data of the 28 microsatellites analyzed illustrating the years of acquirement of sorghum accessions.



**Figure 3:** Number of groups of accessions described by the K value from the analysis runs with STRUCTURE 2.3.4.



**Figure 4:** Diversity structure of a collection of sorghum accessions from Burkina Faso. The full red is (G) the group of Guinea, the full green is (C) the group of Caudatum and the mixture of the red, green and blue is (G-C) the group of Guinea Caudatum according to the STRUCTURE program version 2.3.4 (Pritchard et al., 2000).



**Figure 5:** Relationship between the groups described by the STRUCTURE program and the sorghum accessions analyzed.

The full red is (G) the group of Guinea, the full green is (C) the group of Caudatum and the mixture of the red, green and blue is (G-C) the group of Guinea Caudatum according to the STRUCTURE program version 2.3.4 (Pritchard et al., 2000).

**Table 2:** The genetic structure of the population of the accessions studied.

Types of populations	N	A	Rs	Ho	He	Fst	FIS	FIT
Overall	110					0	0.83	0.83
		5.34	5.36	0.07	0.43			
Years of cultivation	110					0.09*	0.82	0.83
<5 years	24	4.04	3.53	0.03	0.53			
5-10 years	12	3.86	2.82	0.12	0.37			
10-20 years	28	3.50	2.89	0.09	0.36			
>20 years	46	2.57	2.52	0.08	0.35			
Maturity cycle	110					0.02**	0.83	0.83
Average	71	4.57	89.73	3.20	0.42			
Long	28	3.68	81.91	2.93	0.36			
Short	11	3.21	89.82	3.21	0.51			
Sources of varieties of seed	110					0.16	0.81	0.84
Gift	7	1.96	1.95	0.03	0.32			
INERA	20	3.96	3.20	0.04	0.54			
Inherited	83	4.32	2.53	0.36	0.09			
Local and improved	110					0.20	0.81	0.85
Local accessions	90	4.39	3.37	0.08	0.36			
Improved accessions	20	3.96	3.92	0.04	0.54			
Populations Anthracnose Resistance	110					0.12	0.81	0.84
Middle Resistant	42	3.93	3.11	0.09	0.39			
Resistant	12	3.14	3.14	0.01	0.46			
Susceptible	56	4.43	3.12	0.08	0.37			
Populations Number of Internode	110					0.15	0.81	0.84
High number	64	3.82	2.92	0.07	0.33			
Middle number	29	3.71	3.42					
Short number	17	3.82	3.81					
Populations Sorghum race	110					0.31	0.79	0.85
Caudatum	16	3.11	3.10	0.03	0.40			
Guinea	22	2.21	2.16	0.04	0.27			
Guinea-Caudatum	72	3.82	3.02	0.09	0.35			
Populations Region	110					0.06*	0.82	0.83
Boucle du Mouhoun	19	3.21	2.40	0.08	0.38			
Centre	12	3.46	2.93	0.06	0.50			
Centre-Est	5	1.93	1.93	0.16	0.31			
Centre-Nord	10	2.14	1.97	0.11	0.31			
Centre-Ouest	10	2.00	1.83	0.03	0.25			
Est	10	2.46	2.15	0.08	0.34			
Haut-Bassin	29	3.96	2.79	0.05	0.50			
Nord	15	2.82	2.23	0.09	0.33			

\*Significance at  $p < 0.05$  N= Number of varieties A= Allele diversity / the mean of Allele per locus Rs= the richness of the alleles Ho= heterozygosity observed He= heterozygosity expected.



## DISCUSSION

The genomic diversity study of the Burkina Faso accessions with the 28 SSRs markers showed that the lines were genetically diverse and the total number of alleles found was 150 which is similar to the total number of alleles (143 alleles) found by Barro-Kondombo et al. (2010) with 29 loci used. The markers results showed a wide range of PIC -0.01 to 0.81. The low values reveal that some of the markers have a very low power of discrimination. The low polymorphic information content values were related to at least three loci, Xcup 62, Xcup11, Xtxp 40 and Xtxp 136. The low values of the Xcup loci are probably due to the fact that the markers were derived from expressed sequences tags (EST). The Xcup loci were found by sequences of genes that have been purified and sequenced. These markers do not vary much. Similar data were obtained by Barro-Kondombo et al. (2010), Mbeyagala et al. (2012) and Catherine et al. (2016). The hypothetical K value obtained showed the three clusters in which the lines were embedded. The three clusters were races *Caudatum*, *Guinea* and their intermediate, *Guinea-Caudatum*. The results are similar to the data obtained by Deu et al. (2008) on the diversity among Niger sorghum races. Barro-Kondombo et al. (2010) found similar results with landraces in Burkina Faso. In fact, the genetic structure distribution of the genotypes obtained by Barro-Kondombo et al. (2010) from farmers' landraces was three races. The highest race found was the Guinea race (75%) and its different sections. The *Bicolor* rate was very low, while *Caudatum* race was not found. The similarities in the data may indicate the distribution of the same sorghum varieties in Africa mainly in West sub-Saharan zones. That is also acknowledged by Huckabay (1967) and de Wet (1978) where by the countries that share similar climate may share the same varieties across the countries' boundaries.

Overall, the data showed a lower level of observed heterozygosity of  $H_o \leq 0.07$ . This value suggests that the population studied was fairly homogeneous. Similar data was observed elsewhere (Deu et al., 2008; Barro-Kondombo

et al., 2010). This reflects the inbreeding nature of the sorghum varieties in which the percentage of outcrossing is lower than 30% (Reddy et al., 2008). This is consistent with the mean value of the  $FIT=0.83$  and  $FIS=0.83$ . The FIT parameter represents the inbreeding level for a given population while the FIS represents the inbreeding level within the population (Cockerham and Weir, 1984). For sorghum varieties, the inbreeding indices values are generally high (Zongo et al., 2005). Similar results were also observed in other self-pollinated crops like *Phaseolus lunatus* L. or Lima beans (Ouedraogo et al., 2005). The overall mean value of the expected heterozygosity was low  $H_e \leq 0.45$ . This suggests that due to the fact that sorghum is a self-pollinated, one should not expect to see a high level of heterozygosity within groups of sorghum. Similar of low heterozygosity were found by (Deu et al., 2008; Barro-Kondombo et al. 2010; Mbeyagala, et al., 2012). The value of diversity parameters  $F_{ST}$  values of Cockerham and Weir (1984) represents the genetic differentiation among the families was significant and the coefficient varied from 0 to 1 for the families that were having high genetic differences. The  $F_{ST}$  value from the families analysed were generally low. However, for the families of populations based on the years of acquisition of the seeds by the farmers; the growth cycle lengths, as well as the family of the regions have shown significant  $F_{st}$  value.

## Conclusion

This study has identified several morphological groups that contain both farmers' accessions and the improved varieties. The imported germplasm has some similarities with some of the improved varieties released in the country. The evaluation of the agronomic traits showed that several sources of the germplasm collections overlap even though they were collected from different agro ecological zones. The varieties studied were generally tall, photoperiod sensitive, susceptible to disease and low yielding. Several groups of accessions (local and improved) have their origin among the basic races of sorghum, especially the *Guinea*, *Caudatum* and their

intermediate *Guinea-Caudatum* race. The allelic analysis results suggest that there are some similarities among sorghum lines from Burkina Faso and other West African countries, Niger and Mali. The computed genetic parameters FIT (the inbreeding level for an overall population) and FIS (the inbreeding level within a given population) were very high. This is consistent with the self-pollinating nature of the sorghum crop.

### COMPETING INTERESTS

The authors declare that they have no competing interests.

### AUTHORS' CONTRIBUTIONS

JS and BO suggested the title of the manuscript. ZSN, VG, PBT SOK designed and performed the research. JS, BO, VG, PBT and SOK reviewed the draft manuscript. All the authors read and approved the final manuscript.

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