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Assessment of genetic diversity of Burkina Faso sweet grain sorghum using microsatellite markers

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Sweet grain sorghum [*Sorghum bicolor* (L.) Moench] is an under-harvested crop produced mainly for its sweet grains in the pasty stage. Little is known of its genetic diversity remains. This study aims to determine the level and structure of the genetic diversity of sweet grain sorghum from Burkina Faso. Thus, 93 accessions were evaluated using 15 polymorphic microsatellite markers. The analysis revealed 49 alleles in total, 6 rare alleles, an average of 3 alleles per locus, a moderate Nei diversity of 0.474, a low level of heterozygosity (0.031) in the collection and very high Wright's fixation index (Fis) of 0.934. The accessions were organized into three genetic groups: A, B and C. Groups A and B were the farthest, with an Fst and a genetic distance of 0.37 and 0.22, respectively, whereas Groups B and C were the closest, with an Fst (genetic differentiation) of 0.279 and a genetic distance of 0.142. This diversity could be exploited in Burkina Faso sweet grain sorghum breeding programs.

Key words: Burkina Faso, neglected culture, sorghum, simple sequence repeats (SSR) markers, genetic variability.

INTRODUCTION

Traditional varieties are an important source of genetic diversity whose conservation contributes to the maintenance of genetic diversity (Ahmadi et al., 1988). Knowledge of the level and structure of their genetic diversity is an important asset in defining strategies for conservation and varietal improvement (Adoukonou-Sagbadja et al., 2007).

Previous studies on sweet grain sorghum with phenotypic markers have shown an important agro-morphological variability in the collection from Burkina Faso (Sawadogo et al., 2014). However, the observation of morphological characters can lead to a biased estimation of genetic diversity because they are influenced by the environment. Indeed, phenotypic diversity may be increased but genetic diversity remains stable (Lallemand, 2004). For a better estimate of the

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Table 1. Characteristics of microsatellite markers.

S/N	Locus	Number of nucleotide repeats	Chromosome	TM (°C)
1	gpsb123	(CA) 7 (GA) 5	8	50
2	gpsb151	(CT) 12	4	50
3	xtxp10	(CT) 14	9	50
4	xcup14	(AG) 10	3	54
5	xcup53	(TTTA) 5	1	54
6	xcup07	(CCA) 8	10	54
7	xtxp320	(AAG) 20	1	54
8	xtxp15	(TC) 16	5	55
9	xtxp145	(AG) 22	6	55
10	xtxp295	(TC) 19	7	55
11	xtxp136	(GCA) 5	5	55
12	sbagb02	(AG) 35	7	55
13	sb5-206	(AC) 13 (AG) 20	9	55
14	sb6-84	(AG) 14	2	55
15	sb4-72	(AG) 16	6	55

genetic diversity of sweet grain sorghum from Burkina Faso, it is imperative to evaluate it using molecular markers.

The development of genome mapping techniques and their associated attempts to link molecular variability with phenotypic variability are likely to become increasingly acute (Pham et al., 1992). Nuclear molecular markers, in particular microsatellites, otherwise called simple sequence repeats (SSR) are very polymorphic (Doldi et al., 1997; Schug et al., 1998), with Mendelian transmission (Saghai-Maroof et al., 1994). They are codominant and abundant in the genome, easy to use and highly effective in the studying of genetic diversity (Lagercrantz et al., 1993).

The present study was conducted to get a better knowledge of the genetic diversity of the Burkina Faso sweet grain sorghum. The objectives are to: (i) determine the level and (ii) structure of the molecular genetic diversity of sweet grain sorghum from Burkina Faso.

MATERIALS AND METHODS

Plant and molecular markers

The plant materials consisted of 93 accessions of sweet grain sorghum from Burkina Faso obtained by self-fertilization in 2010. Fifteen simple sequence repeat (SSR) markers were selected based on their polymorphism revealed in previous studies on sorghum (Barro-Kondombo, 2010; Nebié, 2014), and their distribution on all the chromosomes of the sorghum genome (Table 1) were used for characterization.

Extraction of total DNA

The total genomic DNA of the 93 accessions was extracted from young freshly picked leaves of 15 days. Flinders Technology Associates (FTA) card method was used for the extraction of DNA. The method works faster from DNA extraction to DNA amplification by polymerase chain reaction (PCR). It consists of taking the fresh young leaves and crushing them on the rough side (square) of the FTA card. The samples were dried in 30 min; then disks of 1 mm diameter were punched, which were placed in Eppendorf tubes. 200 µl of FTA buffer was then added to the punched disks. The whole was incubated at a room temperature for 5 min. This operation was repeated three times by renewing the buffer. At the end, the disks were rinsed with 200 µl of TE (Tris-EDTA) buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and then dried for 5 min before transferring directly each disk in an Eppendorf tube for polymerase chain reaction (PCR).

PCR amplification of genomic DNA

DNA amplification was performed with the thermocycler Eppendorf Master Cycler Gradient in a total volume of 25 µl containing 18 µl of ultrapure water for each sample, 5 µl of premix (with 1 U of Taq (Thermus aquaticus) DNA polymerase, 250 mM of dNTPs, 10 mM Tris-HCl, 30 mM KCl, 1.5 mM $MgCl_2$), 1 µl of each primer microsatellite F (Forward) and R (Reverse) and one disk of FTA card (0.25 to 0.5 ng/µl genomic DNA) for each sample. A marker of molecular weight of reference of 50 bp (base pairs) and a control without DNA were used. The PCR amplifications were performed in an Eppendorf Master cycler: it comprises an initial denaturation phase at 94°C for 4 min followed by 35 cycles of denaturation step at 94°C for 45 s, annealing at 50 to 55°C for 1 min and an extension at 72°C for 1 min 30 s and final extension at same temperature for 4 min.

The PCR products were separated for 1 h 30 min at 100 V on 2% agarose gel in TBE (Tris-Borate Ethidium) 0.5x and 15 µl of BET 5% (ethidium bromide), using fluorescent developer. The revelation of the amplification products was visualized on an UV transilluminator and photographed with a camera brand Canon Power Shot A620, 7.1 Mega Pixels. The size of DNA bands in base pairs was determined using the 1 kb DNA standard ladder (Invitrogen, Carlsbad, CA, USA).

Statistical analysis

Genetix 4.03 (Belkhir et al., 2002) was used to determine the

Table 2. Level of genetic diversity of each markers tested.

Locus	No d'alleles	He	Ho	PIC
gpsb123	5	0.653	0.425	0.648
gpsb151	3	0.562	0.000	0.555
sb4-72	3	0.366	0.000	0.362
sb5-206	3	0.598	0.000	0.592
sb6-84	3	0.493	0.000	0.486
sbagb02	2	0.044	0.000	0.043
xcup07	3	0.437	0.000	0.433
xcup14	4	0.485	0.011	0.48
xtxp136	2	0.498	0.00	0.493
xtxp10	3	0.533	0.000	0.528
xtxp145	5	0.668	0.000	0.661
xtxp15	3	0.579	0.000	0.573
xtxp295	2	0.087	0.000	0.086
xtxp320	5	0.653	0.013	0.646
xtxp57	3	0.484	0.000	0.478

No d'alleles: Number of alleles per locus, He: expected heterozygosity, Ho: observed heterozygosity, PIC: polymorphic information content.

observed heterozygosity (Ho). The total number of alleles (A^t), mean number of alleles per locus (A), number of rare alleles (A^r), the polymorphic information content (PIC) and the expected heterozygosity (He) were known using Fstat software V2.9.3.2 (Goudet, 2002).

The dissimilarity coefficients were used to generate an unweighted neighbor-joining tree with Jaccard's similarity coefficient and a bootstrapping value of 1,000 using the DARwin V5.0 software (Perrier et al*.,* 2006). Wright's fixation index (Fis), genetic differentiation between genetic groups based on Fst (Weir and Cockerham, 1984) and minimum distance of Nei between pairs of genetic groups were estimated using Fstat software V2.9.3.2. To verify the significance of the differences in these values, permutation (1000 to 3000 permutations) was carried out. From the Fis value, the allofecondation rate (t) was estimated using the formula Fis = (1-t) / (1 + t).

RESULTS

Genetic diversity of sweet grain sorghum

Results (Table 2) revealed that the number of alleles per locus varied from 2 for markers sbagb02, xtxp136 and xtxp295 to 5 for gpsb123, xtxp145, xtxp320 markers (Figure 1). Sbagb02 and xtxp145 markers have the extreme value of expected heterozygosity. The results indicate that only the locus: gpsb123, xtxp320 and xcup14 have observed heterozygosity values with an average of 0.03. The markers sb6-84 and xtxp145 showed extreme PIC values of 0.046 and 0.661, respectively.

A total of 49 alleles were detected in the collection with a low number of rare alleles (6), an average of 3 alleles per locus, and relatively low observed (0.031) and expected (0.474) heterozygosity. Results also show a very high value of Wright's fixation index (0.934) and a very low allofecondation rate (t) of 06.04%.

Organization of genetic diversity and description of genetic groups

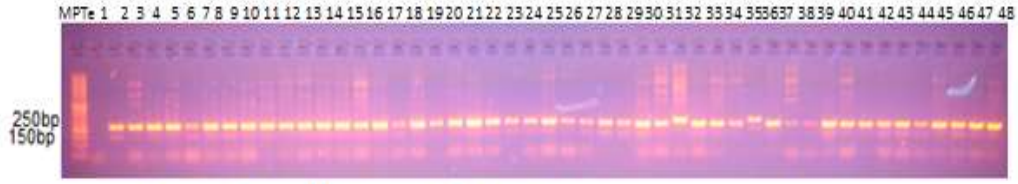
Sweet grain sorghum from Burkina Faso is divided into three genetic groups: A, B and C (Figure 2) consisting of 40, 43 and 09 accessions, respectively. The characteristics of the three genetic groups are shown in Table 3. Group A has the highest number of alleles (43) including 6 private alleles, highest expected heterozygosity (0.42), and lowest observed heterozygosity (0.0107). Group B has the highest value of rare alleles (18), observed heterozygosity (0.051), and lowest expected heterozygosity (0.30). Group C has low number of alleles (34) and mean values for other genetic diversity parameters.

Genetic distance between genetic groups

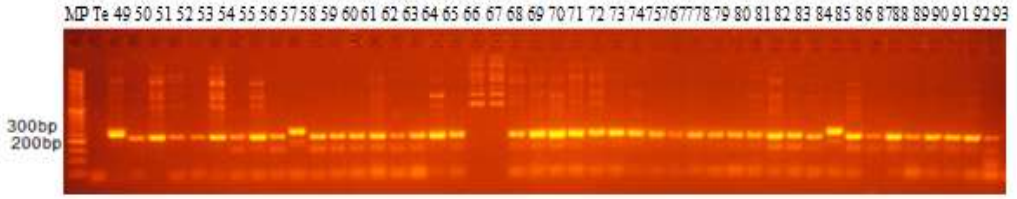
The genetic distances between the three genetic groups revealed by the minimum distance of Nei and the genetic differentiation by pair of genetic groups (Table 4) show that the three genetic groups differed very significantly from one another. Groups A and B are the most remote while groups B and C are the closest.

DISCUSSION

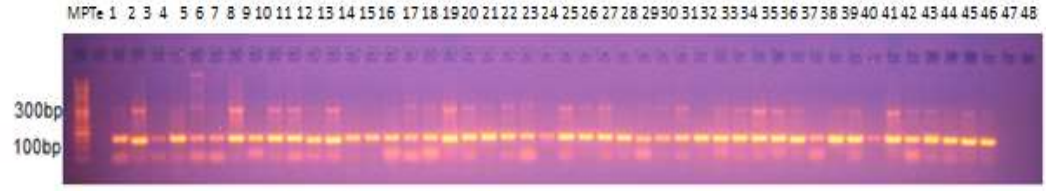
The markers xtxp145 and xtxp320 that showed 5 alleles were the most polymorphic. But, they revealed more



Marqueur xtxp145: accessions 1-48

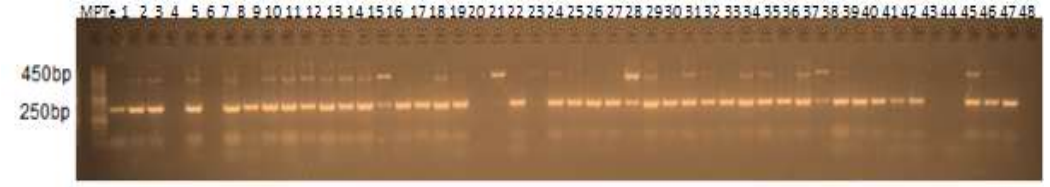


Marqueur xtxp145: accessions 49-93

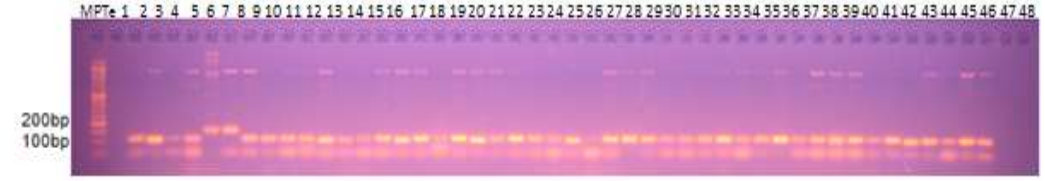


Marqueur xcup53: accessions 1-48

Marqueur gpsb151: accessions 1-48



Marqueur gpsb123: accessions 49-53



Marqueur sbag02: accessions 1-48

Figure 1. Picture of the migration profile of some polymorphic markers. MC, Molecular weight marker; Te, negative control without DNA.

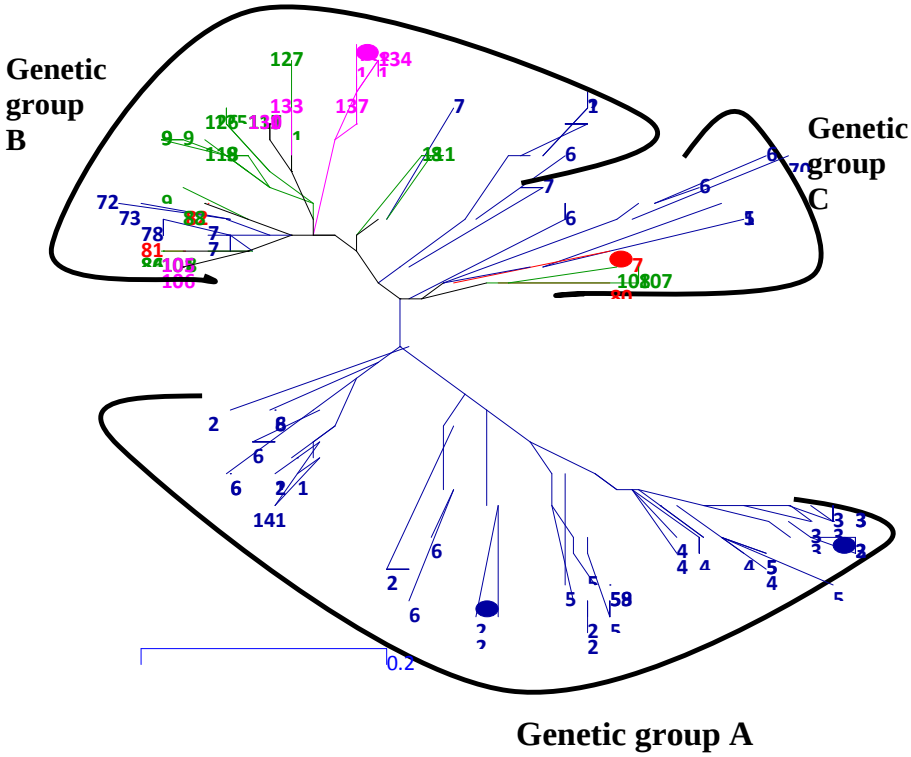


Figure 2. Radial representation of the dendrogram of the 93 accessions of sweet grain sorghum constructed from the dissimilarity matrix according to the Neighbor-Joining method.

Table 3. Characteristics of genetic groups.

Group	Genetic parameters							
	N	A^t	A^r	A^p	A	He	Ho	Fst
	92	**49**	**-**	**-**	**-**	**-**	**-**	**0.35**
A	40	43	12	6	2.87	0.42	0.01	-
B	43	39	18	3	2.6	0.30	0.05	-
C	09	34	15	0	2.27	0.38	0.02	-

N: Number of accessions, A^t: total number of alleles, A^r: number of rares alleles, A^p: number of private alleles, A: mean number of alleles per locus, He: expected heterozygosity, Ho: observed heterozygosity, Fst = genetic differentiation index.

Table 4. Genetic distance between pair of genetic groups of sweet grain sorghum.

Group	A	B	C
A	0	0.3702**	0.3093**
B	0.220	0	0.2794**
C	0.215	0.142	0

The upper diagonal is the comparison of Fst by pair of genetic group and the lower diagonal (bold) is the minimum distance of Nei. **Significant at 1%.

alleles in the sweet stalk sorghum of Burkina Faso, such as 12 for xtxp145 locus and 10 alleles for xtxp320 locus (Nebié, 2014). The PIC of SSR marker that takes into account the number of alleles detected and the relative frequencies of these alleles constitute an important estimate of the discriminating capacity of this marker (Smith et al., 2000). The relatively high value of the PIC of the SSR markers used shows that they have had a undifferentiated. These results indicate that the diversity observed was lower than those of several previous studies (Nebié, 2014; Missihoun et al., 2015; Al-Issawi, 2017). The SSR polymorphism, the level of diversity of accessions in the collection, and the sensitivity of DNA fragment separation systems could explain these strong discriminating capacity for these genetic studies. The markers xtxp145, xtxp320 and gpsb123 were therefore

the most discriminating with PIC greater than 0.6. But the maximum value of PIC per marker was lower than the value obtained by Amelework et al. (2015) and Galyuon et al. (2016): 0.88 and 0.89, respectively.

The 49 alleles detected show that the collection of sweet grain sorghum from Burkina Faso is relativelydifferences. In addition, the very low proportion of rare alleles (12%) in the collection may be related to the small sample size and the much smaller geographic origin of accessions or to a low microsatellite marker mutation rate. However, rare alleles may be of great interest if they are related only to a few particular genotypes. They can be used to identify particular genotypes or specific genome regions related to a particular type of sorghum (Agrahama and Tuinstra, 2003). Indeed, Casa et al. (2005) and Salih (2011) detected 64 and 59% of rare alleles in more extensive collections.

The collection is relatively poor in heterozygotes due to the mean observed heterozygosity rate and the very high Wright's fixation index (Fis). The Fis index was higher than the value of Barnaud et al. (2007), but similar value was obtained by Ouédraogo et al. (2017), which was 0.68 and 0.97, respectively. The observed heterozygosity rate was lower than the value of Salih (2011) but higher than the results obtained by Nebié (2014) (0.016) and Ouédraogo et al. 2017 (0.012). These results are probably related to the preferentially autogamous reproduction regime of species, the racial difference and the fact that accessions were obtained by self-pollination. The collection contains a large proportion of accessions close to the guinea whose glumes are open, which favors the allogamy whose rate can reach 24%, according to Barro-Kondombo (2010).

SSR markers are commonly used as a tool to examine the dynamics of differentiation in the population (Matsuoka et al., 2002) and for specific analyses (Liu et al., 2003; Barkley et al., 2006; Kwak and Gepts, 2009). The three genetic groups identified reveal a lower genetic diversity of sweet sorghum compared to sorghum in center and west of Burkina where Barro-Kondombo (2010) obtained 6 genetic groups. This low genetic diversity may be related to the more restricted range of these sorghum crops. The result is similar to those of Missihoun et al. (2015) who have obtained three groups with 61 accessions and 20 SSRs, while Muui et al. (2016) have obtained four groups with 20 SSRs and 44 accessions.

The study showed a weak genetic polymorphism in Burkina Faso sweet grain sorghum and a distribution of accessions into three genetic groups. The gpsb123, xtxp145, and xtxp320 markers were the most informative on the diversity of sweet grain sorghum and most discriminating. These results show that the molecular markers used were appropriate for the assessment of the genetic diversity of Burkina Faso sweet grain sorghum. A study of the phylogeny of these sorghums could allow to position them in relation to other sorghum and to complete the results of this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adoukonou-Sagbadja H, Wagner C, Dansi A, Ahlemeyer J, Daïnou O, Akpagana K, Ordon F, Friedt W (2007). Genetic diversity and population differentiation of traditional fonio millet (Digitaria spp.) landraces from different agro-ecological zones of West Africa. Theor. Appl. Genet. 115:917-931.

Agrahama HA, Tuinstra MR (2003). Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. Afr. J. Biotechnol. 2(10):334-340.

Ahmadi N, Becquer T, Larroque C, Arnaud M (1988). Variabilité génétique du riz (*Oryza sativa* L.) à Madagascar. Agron. Trop. 43:209-221.

Al-Issawi E HM (2017). Genetic diversity estimation using SSR markers and some yield components in seven forage sorghum [*Sorghum bicolor* (L.) Moench]. Pak. J. Biotechnol. 14(4):739-744.

Amelework B, Shimelis H, Tongoona P, Laing M, Mengistu F (2015). Genetic variation in lowland sorghum (Sorghum bicolor (L.) Moench) landraces assessed by simple sequence repeats. Plant Genetic Resources: Characterization and Utilization 13(2):131-141.

Barkley NA, Roose ML, Krueger RR, Federici CT (2006). Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Theor. Appl. Genet. pp. 1519-1531.

Barnaud A, Deu M, Garine E, McKey D, Joly HI (2007). Local genetic diversity of sorghum in a village in northern Cameroon: structure and dynamics of landraces. Theor. Appl. Genet. 114:237-248.

Barro-Kondombo CP (2010).Diversité agro-morphologique et génétique de variétés locales de sorgho *(Sorghum bicolor* [L.] Moench) au Burkina Faso. Eléments pour la valorisation des ressources génétiques locales. Thèse de doctorat, Université de Ouagadougou, 112p.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2002). Genetix 4.04, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Universite´ de Montpellier II, France. http://www.univ-montp2.fr/*genetix/genetix/genetix.htm.

Casa AM, Mitchell SE, Hamblin MT, Sun H, Bowers JE, Paterson AH, Aquadro CF, Kresovich S (2005). Diversity and selection in sorghum: simultaneous analyses using simple sequence repeats. Theor. Appl. Genet. 111:23-30.

Doldi ML, Vollmann J, Lelly T (1997). Genetic diversity in soybean as determined by RAPD and microsatellite analysis. Plant Breeding. 116:331-335

Frankel O, Brown AHD, Burdon JJ (1995).The conservation of plant biodiversity. New York, USA: Cambridge University Press, 299p.

Galyuon I.K, Madhusudhana R, BorrellAK, Hash TC, Howarth C (2016). Genetic diversity of stay-green sorghums and their derivatives revealed by microsatellites. Afr. J. Biotechnol. 15(25):1363-1374.

Goudet J (2002). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www.unil.ch/izea/softwares/fstat.html

Kwak M, Gepts P (2009). Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., Fabaceae). Theor. Appl. Genet. 118:979-992.

Lagercrantz U, Ellegran H, Anderson L (1993). The abundance of various polymorphic microsatellites motifs differs between plants and vertebrates. Nucl. Acids Res. 21:1111-1115.

Lallemand J (2004). Evaluation de la diversité génétique d'espèces cultivées. HDR, Université de Poitiers, 450p.

Liu K, Goodman M, Muse S, Smith JS, Buckler E, Doebley J (2003). Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics 165:2117-2128.

Matsuoka Y, Vigouroux Y, Goodman MM, Sanchez GJ, Buckler E, Doebley J (2002). A single domestication for maize shown by multilocus microsatellite genotyping. Proc. Nat. Acad. Sci. USA 99:6080-6084.

Missihoun AA, Adoukonou-Sagbadja H, Sedah P, Dagba AR, Ahanhanzo C, Agbangla c (2015). Genetic diversity of *Sorghum bicolor* (L.) Moench landraces from Northwestern Benin as revealed by microsatellite markers. Afr. J. Biotechnol. 14(16):1342-1353.

Muui CW, Muasya R M, Kirubi DT, Runo SM, Karugu A (2016). Genetic variability of sorghum landraces from lower Eastern Kenya based on simple sequence repeats (SSRs) markers. Afr. J. Biotechnol. 15(8):264-271.

Nebié B (2014). Diversité génétique d'une collection de sorgho a tige sucrée [*Sorghum bicolor* (L.) Moench] du Burkina Faso. Thèse Unique, Université de Ouagadougou, 118 p.

Perrier X, Jacquemoud-Collet JP (2006). DARwin software http://darwin.cirad.fr/darwin.

Pham JL, 1992. Evaluation des ressources génétiques du riz cultivé en Afrique par hybridation intra et interspécifique. Thèse doct. univ. Paris Sud Centre d'Orsay, 254p.

Ouédraogo N, Sanou J, Traoré H, Gracen V, Tongoona P, Danquah YE (2017). Genetic diversity among sorghum landraces and polymorphism assessment of local improved varieties for stay-green trait. Int. J. Biol. Chem. Sci. 11(1):1-14.

Saghai-Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994). Extraordinarily polymorphic microsatellites DNA in barley: Species diversity, chromosomal locations and population dynamics. Proc. Nat. Acad. Sci. 91:5466-5470.

Salih SA (2011). Genetic Diversity Analysis in Sorghum germplasm collection from eastern Africa as estimated by morpholo-agronomical and SSR markers. PhD, University of the Free State Bloemfontein, South Africa, 164p.

Sawadogo N, Nebié B, Kiébré M, Bationo-Kando P, Nanema KR, Traoré RE, Gapili N, Sawadogo M, Zongo JD (2014): Caractérisation agromorphologique des sorghos à grains sucrés (*Sorghum bicolor* (L.) Moench) du Burkina Faso. Int. J. Biol. Chem. Sci. 8(5):2183-2197.

Schug MD, Hutter CM, Wetterstrand KA, Gaudette MS, Mackay TFC, Aquadro CF (1998). The mutation rates of di-, tri-, and tetra-nucleotide repeats in *Drosophila melanogaster*. Mol. Biol. Evol. 15:1751-1760.

Smith JSC, Kresovich S, Hopkins MS, Mitchell SE, Dean RE, Woodman WL, Lee M, Porter K (2000). Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Crop Sci. 40:226-232.

Weir BS, Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.