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Genetic diversity of *Sorghum bicolor* (L.) Moench landraces from Northwestern Benin as revealed by microsatellite markers

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The understanding of genetic diversity within local crop varieties constitutes an important step in the preservation of their genetic potential. The objective of this study was to assess the genetic diversity of sorghum (*Sorghum bicolor* (L.) Moench) cultivated in the Northwest of Benin and to reveal certain fundamental evolutionary mechanisms. A total of 61 accessions of sorghum landraces belonging to the four identified races in Benin were estimated using 20 microsatellite markers. For all the loci analyzed, 140 polymorphic alleles were detected with a mean value of 7.00 per locus and polymorphic information content (PIC) average value was 0.33 for all the 20 simple sequence repeats (SSRs), suggesting an important genetic diversity within the cultivated sorghum germplasm used. An unweighted pair group method arithmetic average (UPGMA) clustering and principal coordinate analysis (PCoA) based on DICE coefficient revealed three major genetic groups supported by two main components: the botanical race and the morpho-physiological characteristics of the grains (colour and degree of bitterness). It was thus recommended that further research on genetic diversity of sorghum should integrate these genetic parameters for a better preservation of the genetic resources of this important crop in Benin.

Key words: Genetic diversity, simple sequence repeats (SSRs) markers, *Sorghum bicolor*, Benin.

INTRODUCTION

Knowledge of the level of genetic diversity and structure in crop plants constitutes a very important aspect in selection, conservation and/or genetic improvement programmes. It is necessary for the development of sustainable preservation programmes of plant genetic resources (Adoukonou-Sagbadja et al., 2007). Consequently, these last decades, an important basic activity in the management of genetic resources has been the assessment of genetic diversity and its structure within cultivated plants (Brown, 1989; Bhosale et al., 2011). For instance, these works largely focused on economically important cereals such as maize (*Zea mays*

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L.) (Perales et al., 2003; Bracco et al., 2009), rice (*Oryza sativa* L.) (Barry et al., 2007; Wang et al., 2014), wheat (*Triticum aestivum* L.) (Reif et al., 2005), barley (*Hordeum vulgare* L.) and sorghum (*Sorghum* spp.) (Bhosale et al., 2011).

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important staple cereal crops in the semi-arid regions around the world. In sub-Saharan Africa, it is the second most important cereal crop after maize. *S. bicolor* is a monocotyledon plant of tropical origin, belonging to *Poaceae* family and the genus of *Sorghum* which includes 20 to 30 species. It is an annual plant, preferentially autogamous (Ollitrault et al., 1997), diploid (2n = 20 chromosomes) (Harlan and de Wet, 1972). Using panicle, grain and spikelet morphology, Harlan and de Wet (1972) classified cultivated sorghum (*Sorghum bicolor* ssp. *bicolor*) into five basic races (*bicolor*, *caudatum*, *durra*, *kafir* and *guinea*) and 10 intermediate ones. In many African countries, the crop is dominated by sorghum landraces which were traditionally selected and maintained by indigenous farmers. In most producing countries including Benin, the great majority of the production is consumed locally. This is in accord with the strategic role of sorghum for local household in order to ensure food security (Kayode et al., 2006).

The genetic diversity of sorghum at global scale has been studied extensively by using different markers including nuclear DNA markers. Moreover, relatively few studies have been specifically conducted on genetic variation in some Beninese sorghum. For instance, amplified fragment length polymorphism (AFLP) markers were used on genetic variation of Beninese sorghum landraces (Kayode et al., 2006). This study showed that the genetic diversity of local varieties of the three studied regions was similar and no correlations could be established between the genetic features and the culinary characteristics of varieties. However, the factors shaping the genetic diversity of varieties (ecogeographic distribution, racial membership, farmers' agromorphological and organoleptic preferences, ethnic and other social factors, etc.) were not considered in the former investigations. But, the understanding of these factors is an important asset for the management, selection and genetic improvement of this crop. What is the state of the genetic diversity estimated with the molecular genetics tools (simple sequence repeats (SSRs) markers) of the sorghum landraces in Benin? What are the major factors which structure this diversity?

Microsatellite markers have been developed and largely used in sorghum during more than a decade (Brown et al., 1996; Taramino et al., 1997; Schloss et al., 2002; Barnaud et al., 2007; Barro-Kondombo et al., 2010). They are multi-allelic, codominantly inherited, locus specific, easily detected by polymerase chain reaction (PCR) and require only a small amount of starting DNA (Powell et al., 1996). In this study, microsatellite polymorphism was determined to estimate the genetic diversity in farmers' varieties or ''sorghum landraces'' grown in the north-west of Benin. Specifically, this work (1) assessed genetic diversity in collection using SSR markers and (2) determined the major factors which structure this diversity.

MATERIALS AND METHODS

A survey was conducted in Donga department (9°10'0" N and 1°49'60" E) (Figure 1) for the collection of plant materials in December, 2010 (Missihoun et al., 2012a). A total of 61 accessions of sorghum landraces (Table 1) was collected and then subjected to molecular analysis. The study was carried out at the Department of Genetics and Biotechnologies, University of Abomey-Calavi in Benin.

Genomic DNA extraction and SSRs genotyping

Young fresh leaves from bulked plant samples of each accession were harvested in the greenhouse and then brought to the laboratory for genotyping. DNA was extracted using the mixed alkyl tri-methylammonium bromide (MATAB) extraction protocol previously described for sorghum (Missihoun et al., 2012b). 0.2 g of leaf material was ground in a porcelain mortar with 2 mL of Tris-Sorbitol EDTA buffer. The mixture in Eppendorf tubes was centrifuged at 10.000 rpm for 10 min at 4°C. After centrifugation, the supernatant was taken out and discarded. 750 µL of 4% MATAB buffer preheated at 65°C were added to the pellet in the Eppendorf and placed in water bath at 65°C for 1 h 30 min while stirring up at each 10 min. Then, 750 µL of chloroform/Isoamyl alcohol (24:1) were added to the mixture cooled at ambient temperature and shaked by gently inverting for 2 min and then centrifuged at 10.000 rpm for 15 min at 4°C. The supernatant was taken out in a separate tube and then an equal volume of iso-propanol was added to it. The tube was carefully mixed to obtain DNA pellet. After centrifugation the supernatant was carefully discarded and the pellet was purified with 70% ethanol. The pellet was dried then sterile ultra-pure water was added to each tube containing the pellet.

To be sure of the success of the DNA extraction, 2 µL of genomic DNA extract were visualized on a 1% agarose gel. After this confirmation, DNA samples were kept in a freezer at -20°C for the genotyping.

Genomic sorghum SSR markers used in this study belonged to the linkage maps of sorghum published by Taramino et al. (1997), Bhattramakki et al. (2000) and Kong et al. (2000) which were recently used by Shehzad et al. (2009), Barro-Kondombo et al. (2010) and Bhosale et al. (2011). Thirty six (36) SSR primers were initially tested on four sorghum accessions and finally, 20 genomic sorghum SSR markers were selected based on their high polymorphic information content (PIC) value observed on the previous studies on African sorghum Landraces. These SSR markers are distributed across the sorghum genome with an average of two (2) SSRs per chromosome (Table 2).

Polymerase chain reaction (PCR) amplifications using the 20 selected SSRs were carried out in a Peltier-Effect Cycling's thermocycler in a volume of 25 µL containing 3 µL of genomic DNA (approximately 3 ng/uL), 0.2 µM of each primer (F and R), 2.5 µl of PCR buffer (10X), 200 µM dNTP, 1.25 µM $MgCl_2$, 0.1 U/uL Taq DNA-polymerase and sterile ultra-pure water (H_2O). The cycle of amplification included a pre-denaturation at 94°C for 4 min followed by 35 cycles, each cycle consisted of a denaturation at 94°C for 30 s, hybridization in the appropriate temperature (50°C or 60°C, Table 2) for 1 min and an elongation at 72°C for 1 min. A final incubation

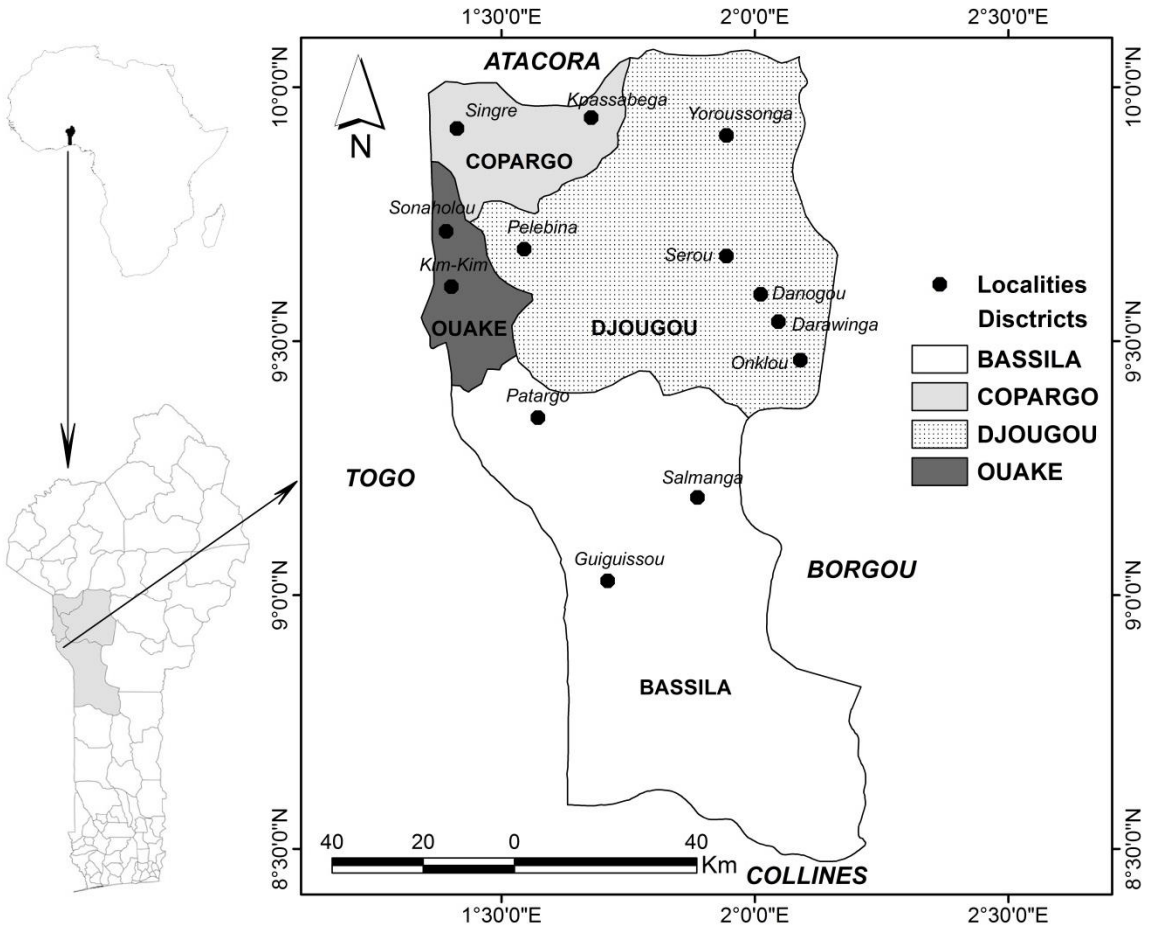

Figure 1. Maps showing the geographical locations of thirteen selected and surveyed villages.

at 72°C for 8 min ended the program. The effectiveness of the amplification was tested by electrophoresis on 2% agarose gel in 0.5X TBE buffer. Gels were run in horizontal gel system at 100 V for 30 min and later photographed under UV light. Afterwards, PCR amplification products were migrated by electrophoresis in 5% denaturing polyacrylamide gel of 305 × 385 mm (5% acrylamide-bisacrylamide (19:1), 8 mol urea in Tris-borate-EDTA/L (TBE) buffer, pH 8) at constant power of 60 W for 1 h 30 min to 2 h, depending on the expected product size. The detection of electrophoretic plates was carried out with silver nitrate according to Creste et al. (2001).

Data analysis

Fifty nine (59) accessions were considered for final analysis as 2 samples were eliminated for lack of amplification. NTSYS pc software 2.20q (Rohlf, 2000) was used for data analysis. Similarity coefficient of DICE based on the proportion of common alleles (Nei and Li, 1979) was calculated to measure genetic similarities between accessions. The diversity of each accession was analyzed based on three genetic diversity parameters: the rate of polymorphism (P), number of alleles per locus (Na) and polymorphism information content (PIC) which provided an estimation of the discriminatory power of a locus by taking into account not only the amount of alleles expressed but also the relative frequency of each allele (Botstein et al., 1980; Smith et al. 2000). The values of PIC were calculated according to the algorithm:

$$PIC = 1 - \Sigma f_i^2$$

Where, f_i was the frequency of the ith allele; PIC value ranged from 0 (monomorphic locus) to 1 (very highly discriminative, with many alleles of which each was in equal and low frequency).

Genetic structure was assessed by a dendrogram construction using unweighted pair group method arithmetic average (UPGMA) method following the Sequential agglomerative hierarchical nested method (SAHN) procedure of NTSYS software version 2.21f (Rohlf, 2000). Besides, to confirm the inferred groupings of the analyzed accessions and to better estimate the genetic differentiation between the groups, DCENTER and EIGEN procedures of this software were also used to conduct a principal coordinate analysis (PCoA) on the basis of the same matrix of genetic distances.

RESULTS

Genetic polymorphism and allele's distribution

The analysis of SSRs revealed a high allelic polymorphism. As a matter of fact, 100% of the loci investigated were polymorphic and each of them exhibited at least two alleles. Overall, the twenty SSR markers used in this study

Table 1. Code of the accession, vernacular name, site location, racial group, grain type and main characteristics to make distinction.

Code of the accession	Vernacular name	Location/Village	District	Racial group	Grain type		Main characteristics to make distinction
					Colour	Nature	
1a	Zokaram nini (Da) 1	Danogou	Djougou	G	Reddish	No bitter	High yield of grain, intermediate-maturing
3a	Zobomdjouha (Da) 1	Danogou	Djougou	G	white	No bitter	High yield of grain and easy to shell
4a	Zomoaha (Se) 2	Serou	Djougou	G	Red	No bitter	Early-maturing
5a	Agbani (On)	Onklou	Djougou	G	White	No bitter	Very high yield of grain but late-maturing
6a	Kèmnin piha	Kpassabega	Copargo	G	White	No bitter	High yield of grain
7a	Zomoaha (Se) 1	Serou	Djougou	G	Red	No bitter	Early-maturing
8a	Narabeunzo	Danogou	Djougou	B	blackish	No bitter	To be associated with‘’Wild Sorghum’’
9a	Agbani (Yo)	Youroussonga	Djougou	G	White	No bitter	Very high yield of grain but late-maturing
10a	Zoténtérém (Se)	Serou	Djougou	DK	Red	No bitter	Cultivated only for his red leaves which have used for traditional medicine
12a	Zomoora	Youroussonga	Djougou	D	Yellow	Bitter	Bitter yellow grain
13a	M'ssée	Youroussonga	Djougou	C	Yellowish	No bitter	Twin grain
14a	Zobomdjouha (Se)	Serou	Djougou	G	White	No bitter	High yield of grain
15a	Zokpénaï	Serou	Djougou	D	Yellowish	No bitter	Very early-maturing (two harvest by year) but cause itching during of harvest
16a	Zoniniléti	Serou	Djougou	G	Whitish	No bitter	High yield of grain
17a	Lamnéza	Kpassabega	Copargo	G	Reddish/ Whitish	No bitter	High yield of grain but late-maturing
18a	Zobomdjouha (Yo)	Youroussonga	Djougou	G	white	No bitter	High yield of grain and easy to shell
20a	Vèmah (Yo) 1	Youroussonga	Djougou	DG	White	No bitter	Very high yield of grain but poor quality of food
22a	Moussii	Pélébina	Djougou	D	Yellowish	No bitter	Early-maturing
23a	Zoumbouara	Onklou	Djougou	G	Red	No bitter	Pure red grain, Very early-maturing
24a	Zoumboua	Onklou	Djougou	G	Red	No bitter	Early-maturing
25a	Zooga	Pélébina	Djougou	G	White/Red	No bitter	High yield of grain but late-maturing
26a	Moukoulikouté	Sonaholou	Ouaké	C	Reddish/ Yellowish	No bitter	Low yield of grain, require a wet ground of sandbank, late-maturing
27a	Koussèm (So)	Sonaholou	Ouaké	G	Red	No bitter	Very early-maturing
29a	Koussèm (Da)	Darawinga	Djougou	G	Red	No bitter	Very early-maturing
30a	Moussema (So)	Sonaholou	Ouaké	G	Red	No bitter	Early-maturing
31a	Zobomdjouha (Da) 2	Danogou	Djougou	G	White/Red	No bitter	High yield of grain
32a	Kouhloumè (So)	Sonaholou	Ouaké	G	White	No bitter	Early-maturing
33a	Zomoaha (Yo) 1	Youroussonga	Djougou	G	Red	No bitter	Early-maturing
34a	Kouhloumè (K-K)	Kim-Kim	Ouaké	G	White	No bitter	Early-maturing
35a	Talèm'la (So)	Sonaholou	Ouaké	G	Whitish	No bitter	Very high yield of grain but late-maturing
36a	Zogawa	Danogou	Djougou	G	Whitish	No bitter	High yield of grain, intermediate-maturing

Table 1. Contd

37a	Zoténtérém (Da)	Danogou	Djougou	DK	Red	No bitter	Cultivated only for his red leaves which have used for traditional medicine
38a	Agbani (Da)	Danogou	Djougou	G	Whitish	No bitter	Very high yield of grain but late-maturing
39a	Talèm'la (K-K)	Kim-Kim	Ouaké	G	White	No bitter	Very high yield of grain but late-maturing
40a	Zopira B	Danogou	Djougou	G	White	No bitter	High yield of grain but late-maturing
41a	Zomouara	Danogou	Djougou	G	Red	No bitter	Early-maturing
42a	Zopéra	Pélébina	Djougou	G	White	No bitter	High yield of grain but late-maturing
43a	Zomoora	Pélébina	Djougou	D	Yellow	Bitter	Bitter yellow grain
44a	Koulom	Darawinga	Djougou	G	White	No bitter	High yield of grain but late-maturing
45a	Agbani (Se)	Serou	Djougou	G	White	No bitter	High yield of grain but late-maturing
46a	Sèmoutchè	Sonaholou	Ouaké	G	White	No bitter	Pure white grain, very loose panicle, cultural
47a	Zotihou	Danogou	Djougou	D	Yellow	Bitter	Bitter yellow grain
48a	Moussèma (Da)	Darawinga	Djougou	G	Red	No bitter	Early-maturing
49a	Zokaram nini (Da) 2	Danogou	Djougou	G	Reddish	No bitter	High yield of grain
51a	Zopiha 2	Kpassabega	Copargo	G	White	No bitter	High yield of grain but late-maturing
52a	Lam'za moaha	Kpassabega	Copargo	G	Whitish	No bitter	Medium yield of grain, intermediate-maturing
53a	Sèmgnin moaha	Kpassabega	Copargo	G	Whitish	No bitter	Medium yield of grain, intermediate-maturing
54a	Sèmgnin piha	Kpassabega	Copargo	G	Whitish	No bitter	High yield of grain, intermediate-maturing
55a	Zowémoaha 1A	Kpassabega	Copargo	G	Red	No bitter	Early-maturing
56a	Zowémoaha 1B	Kpassabega	Copargo	G	Red	No bitter	Early-maturing
57a	Zowémoaha 2B	Kpassabega	Copargo	G	Red	No bitter	Early-maturing
58a	Zopiha 1	Kpassabega	Copargo	G	White	No bitter	High yield of grain, late-maturing, demanding fertile soil and good pluviometry
59a	Zomoala	Kpassabega	Copargo	D	Yellow	No bitter	Bitter yellow grain
60a	Za (Si) 1	Singre	Copargo	G	Whitish	No bitter	High yield of grain, late-maturing, demanding fertile soil and good pluviometry
61a	Lamza	Kpassabega	Copargo	G	Whitish	No bitter	Medium yield of grain, intermediate-maturing
62a	Zomoaha	Singre	Copargo	G	Red	No bitter	Early-maturing
63a	Za (Si) 2	Singre	Copargo	G	Whitish	No bitter	High yield of grain, late-maturing, demanding fertile soil and good pluviometry
64a	Zomoila	Singre	Copargo	D	Yellow	Bitter	Bitter yellow grain
67a	Zowémoaha 2A	Kpassabega	Copargo	G	Red	No bitter	Early-maturing
69a	Aka foufou kékéré	Patargo	Bassila	G	White	No bitter	Medium yield of grain, intermediate-maturing
71a	Anoro kin'ka	Salmanga	Bassila	G	Reddish	No bitter	Medium yield of grain, intermediate-maturing

yielded 140 alleles which allowed the classification of all the 59 accessions of sorghum collected from different villages. The number of alleles per locus range from 2 to 14 with an average of seven alleles. The most polymorphic markers were Xtxp 295 and Xtxp 274 (14 alleles) and the least polymorphic ones were Xtxp 59, Xtxp 60 and Xtxp 65 (two alleles). The discriminant power of each SSR markers assessed on this study by the PIC

Table 2. List of SSRs used with their chromosome location, primer sequences (forward and reverse), repeat motif type, number of alleles recorded per locus and PIC values.

SSR locus and (chromosome location)	Forward (F) and reverse (R) primer sequences (5' to 3')	Type of SSRs	T°a	Na	PIC
Xtxp 149 (1)	F = AGCCTTGCATGATGTTCC R = GCTATGCTTGGTGTGGG	$(CT)_{10}$	60	12	0.34
Xtxp 284 (1)	F = CCAGATTGGCTGATGCATACACACT R =AAGGGTAATTTATGCACTCCAAGGTAGGAC	$(AAG)_{19}$	60	10	0.51
Xtxp 201 (2)	F = GCGTTTATGGAAGCAAAAT R = CTCATAAGGCAGGACCAAC	$(GA)_{36}$	60	5	0.08
Xtxp 197 (2)	F = GCGTCAATTAATCCAAACAGCCTC; R = GAGTTCCTATTCCCGTTCATGGTGAT	$(AC)_{10}$	60	4	0.45
Cba (3)	F = AAAGCTCGGCGTTAGAAATA; R = CGCTTAACAACTCCTACCATC	$(TA)_{18}$	60	3	0.25
Xtxp 59 (3)	F = GAAATCCACGATAGGGTAAGG; R = GACCCAGAATAGAAGAGAGG	$(GGA)_5$	60	2	0.47
Xtxp 51 (4)	F = TCTCGGACTCAAGAGCAGAGG;R = GGACAGCAGCGGCTTCAG	$(TG)_{11}$	60	6	0.25
Xtxp 60 (4)	F = GCTAGCTGACGCACGTCTCTG; R = TGCAACCGAGCGGTGACTA	$(GT)_4$ GC$(GT)_5$	60	2	0.45
Kaf2 (5)	F = TCGGCGAGCATCTTACA; R = TACGTAGGCGGTTGGATT	$(CAA)_9$	60	5	0.26
Xtxp 65 (5)	F = CACGTCGTCACCAACCAA; R = GTTAAACGAAAGGGAAATGGC	$(ACC)_4$+ $(CCA)_3$ CG$(CT)_8$	60	2	0.29
Xtxp 145 (6)	F = GTTCCTCCTGCCATTACT R = CTTCCGCACATCCAC	$(AG)_{22}$	60	13	0.14
Xtxp 274 (6)	F = GAAATTACAATGCTACCCCTAAAAGT R = ACTCTACTCCTTCCGTCCACAT	$(TTC)_{19}$	60	14	0.51
Xtxp 278 (7)	F = GGGTTTCAACTCTAGCCTACCGAACTTCCT; R = ATGCCTCATCATGGTTCGTTTTGCTT	$(TTG)_{12}$	60	5	0.21
Xtxp 295 (7)	F = AAATCATGCATCCATGTTCGTCTTC; R = CTCCCGCTACAAGAGTACATTCATAGCTTA	$(TC)_{19}$	60	14	0.21
Xtxp 47 (8)	F = CAATGGCTTGCACATGTCCTA; R = GGTGCGAGCTAGTTAAGTGGG	$(GT)_8(GC)_5$ + $(GT)_6$	60	3	0.19
Xtxp 273 (8)	F = GTACCCATTTAAATTGTTTGCAGTAG R = CAGAGGAGGAGGAAGAGAAGG		60	8	0.30
Xtxp 258 (9)	F = CACCAAGTGTCGCGAACTGAA; R = GCTTAGTGTGAGCGCTGACCAG	$(AAC)_{19}$	60	8	0.43
Xtxp 10 (9)	F = ATACTATCAAGAGGGGAGC R = AGTACTAGCCACACGTCAC	$(CT)_{14}$	50	4	0.23
Xtxp 217 (10)	F = GGCCTCGACTACGGAGTT R = TCGGCATATTGATTTGGTTT	$(GA)_{23}$	60	8	0.41
Xtxp 270 (10)	F = AGCAAGAAGAAGGCAAGAAGAAGG; R =GCGAAATTATTTTGAAATGGAGTTGA	$(GAA)_{12}(GAAA)_6$ + $(GAA)_{21}$ + $(GTA)_5$ + $(GTA)_3$ + $(GTA)_3$	60	12	0.65

SSRs polymorphism rate: 100%; Amount of alleles recorded in the population: 140 alleles; mean alleles/locus = 7.00.

value ranged from 0.08 to 0.65 with a mean value of 0.33 for all the 20 SSRs analyzed. Among the markers, Xtxp 270 was the most discriminant whereas Xtxp 201 and Xtxp 145 were poorly discriminant.

Among the alleles recorded in this study, 24 (17.14%) were "rare" (their frequency of occurrence range between 0.01 and 0.05), 50 (35.71%) had average frequencies ranging from 0.06 to 0.19; 41 (29.29%) were frequent(occurrence frequencies ranging from 0.20 to 0.50) and finally 25 (17.86%) were highly frequent with their occurrence frequencies ranging from 0.51 to 0.98 (Figure 2). With regards to their geographic distribution,

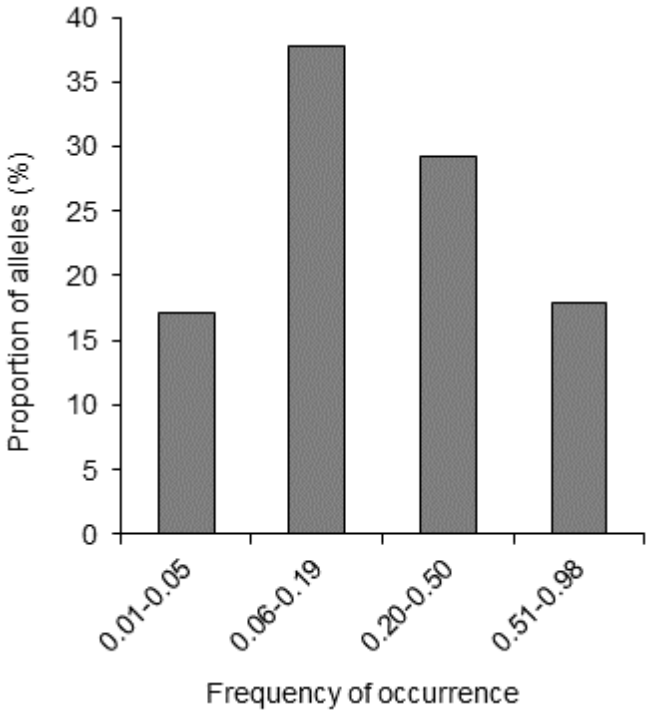

Figure 2. Amount of alleles SSRs in connection with their occurrence frequencies in the analyzed accession of sorghum.

97.17% of identified alleles in this study were present in the samples collected in Yom tribal area from Djougou district, 75.47% were present in the accessions sampled in Yom tribal from Copargo district and only 51.87% alleles were present in the accessions sampled in Lokpa tribal area living in Ouaké and Djougou districts.

Genetic relationship and samples' structure

Genetic distance estimated by dice genetic similarity (GS_D) for all accessions under investigation varied from 0.00 [landraces zooga (25a) vs. zogawa (36a)] to 0.93 [landraces Zokaram nini (1a) vs. Zoténtérém (37a)] with an overall mean of 0.47.

Genetic relationships between the different accessions of sorghum were analyzed by means of dendrogram using 140 alleles obtained from 20 SSR markers. This dendrogram obtained according to UPGMA hierarchical classification model using DICE coefficient allowed the classification of sorghum accessions into three main groups (Figure 3). Group I was subdivided into two sub-groups (Ia and Ib) containing sorghum accessions from guinea race and an accession from bicolor race. In terms of grain coloration, the sub-group Ia mainly consisted of accessions with red grains and subsidiary a few white grains accessions. In this sub-group, zooga (25a) and zogawa (36a), collected respectively at Pélébina and Danogou, two villages geographically distant in Djougou district were shown to be genetically identical, suggesting that the two accessions belong probably to the same variety although they were called differently by farmers. On the contrary, two accessions of same variety "Zowémoha'' (55a and 57a) were genetically identical, attesting their good identity as indicated by farmers. The sub-group Ib was mainly accessions with white grains and few red grains. The group II was made of accessions of durra race (12a, 47a, 59a, 64a, 15a and 22a) and one accession of *caudatum* race (13a). On the basis of grain bitterness and coloration, we could distinguish two sub-groups: the first consisted of sorghum accessions with yellow and bitter grains (12a, 47a, 59a and 64a) and sorghum with yellowish and twin grains (13a) and the second was composed of accessions with yellowish and no bitter grains. Finally, the group III contained two accessions of intermediate *durra-kafir* race (10a and 37a). These were staining sorghum landrace. The samples analyzed in this study seemed to be very well structured according to botanical race and morpho-physiological characteristics of the grains such as colour and degree of bitterness.

This classification of accessions into three major groups was confirmed by PCoA. The two major axes accounted for 24% of the total variation with axis 1 representing 13.99% and axis 2 accounting for 10.01% of the variation (Figure 4). The two sub-groups (Ia and Ib) in group I obtained from the genetic analysis correspond, with only few exceptions of the three morphotypes primarily identified in guinea race accessions in the same collection during the agro-morphological characterization (Missihoun, 2013).

The analysis of accessions from genetic groups revealed 34 accessions of group Ia collected from Yom farmers in Djougou and Copargo districts and 11 accessions collected from Lokpa farmers belonged to the group Ib. Eight accessions of group II (IIa and IIb) were collected from Yom farmers in Djougou and Copargo districts, whereas the 2 accessions of group III were collected from Yom in Djougou (Table 3).

DISCUSSION

Before the advent of GBS-based single nucleotide polymorphism (SNP) markers and additionally other whole-genome profiling markers (for example DArT), microsatellite markers are the most used worldwide in the characterization of sorghum genetic resources and particularly in genetic diversity analysis (Smith et al., 2000; Folkertsma et al., 2005; Barnaud et al., 2007; Deu et al., 2008; Barro-Kondombo et al., 2010; Bhosale et al., 2011). In Benin, although AFLP markers were used in genetic characterization of sorghum local varieties (Kayodé et al., 2006), it is the first time SSR markers were used for sorghum germplasm analysis. Microsatellite markers when compared with AFLP markers, they are specific, codominant and multi-allelic and well known to allow a good discrimination of closely related sorghum

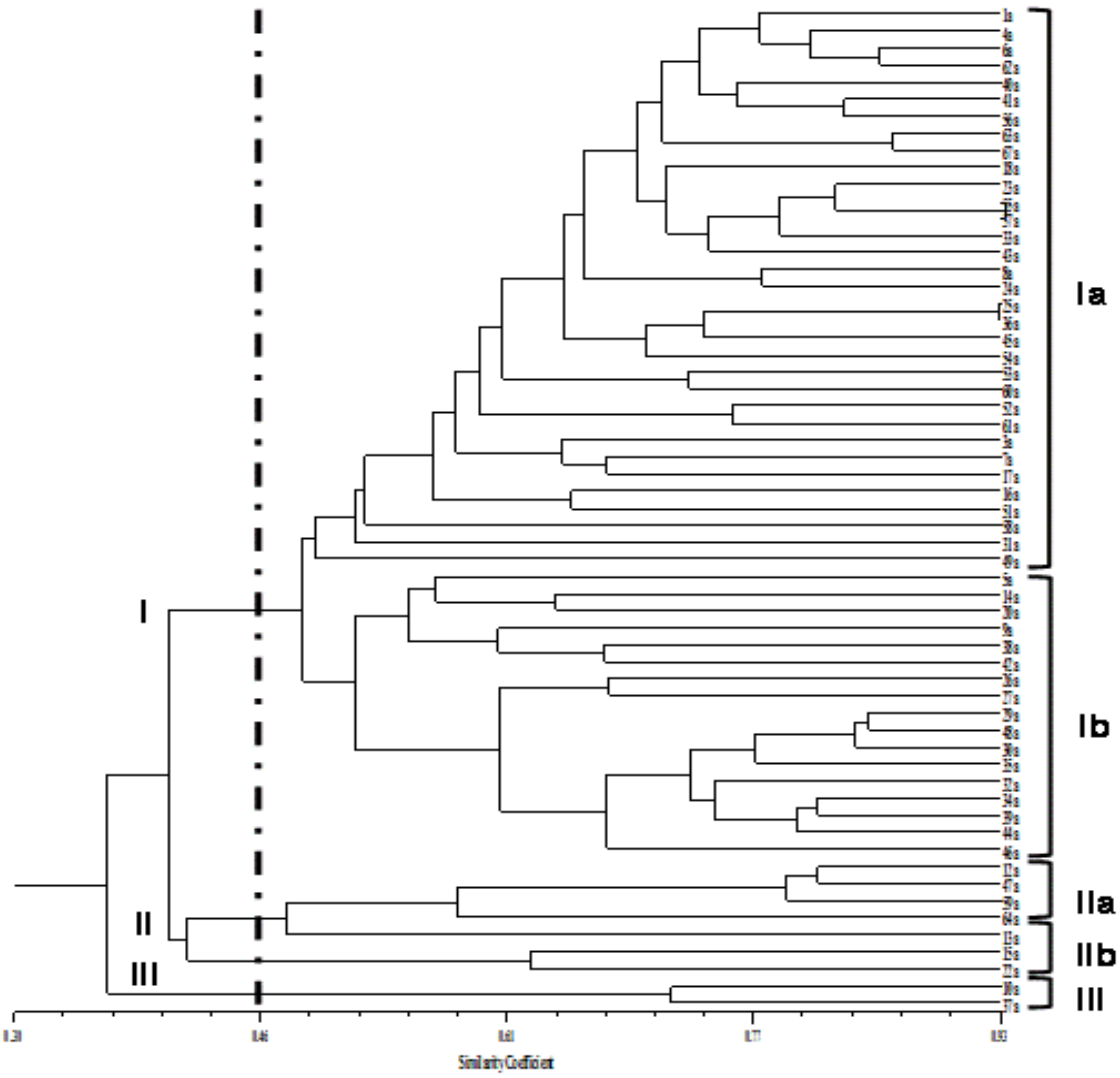

Figure 3. Dendrogram showing genetic connection between sorghum accessions by UPGMA analysis using DICE coefficient on SSRs data.

accessions (Djè et al., 1999; Smith et al., 2000; Ghebru et al., 2002).

Overall genetic diversity

The maintenance of crop genetic resources for evaluation and use in breeding programmes is crucial for the improvement of agricultural production (Sow et al., 2013). In this study, there was genetic diversity as revealed by different microsatellite markers. The average number of alleles recorded per locus (7.00 in average) in the present study was higher than that recorded by Agrama and Tuinstra (2003), Kudadjie (2006), Barro-Kondombo et al. (2010) which were 4.5 , 3.7 and 4.9 respectively on *Sorghum* ssp. and with the SSRs markers. Nonetheless, our results are similar to that of Sagnard et al. (2011) (7.48 alleles in average per locus) who worked on 455 sorghum accessions from Mali and Guinea using 15 SSR markers. However, the average number calculated in the present study was lower than that reported by Deu et al. (2008) and Bhosale et al. (2011), which were 10.43 and 19, respectively from 472 accessions of sorghum from Niger and 219 accessions of sorghum cultivated in West Africa and assessed by means of 28 and 27 SSR markers, respectively.

The comparison of the level of allelic diversity per locus from various studies seems different but there were reasons that could explain the discrepancies observed as compared to the present study. First, the size of the population used could be one of the reasons. The number of accessions (59) was higher than that of Agrama and Tuinstra (2003) (22 accessions), Kudadjie (2006) (42 accessions) but lower than that of Deu et al. (2008)

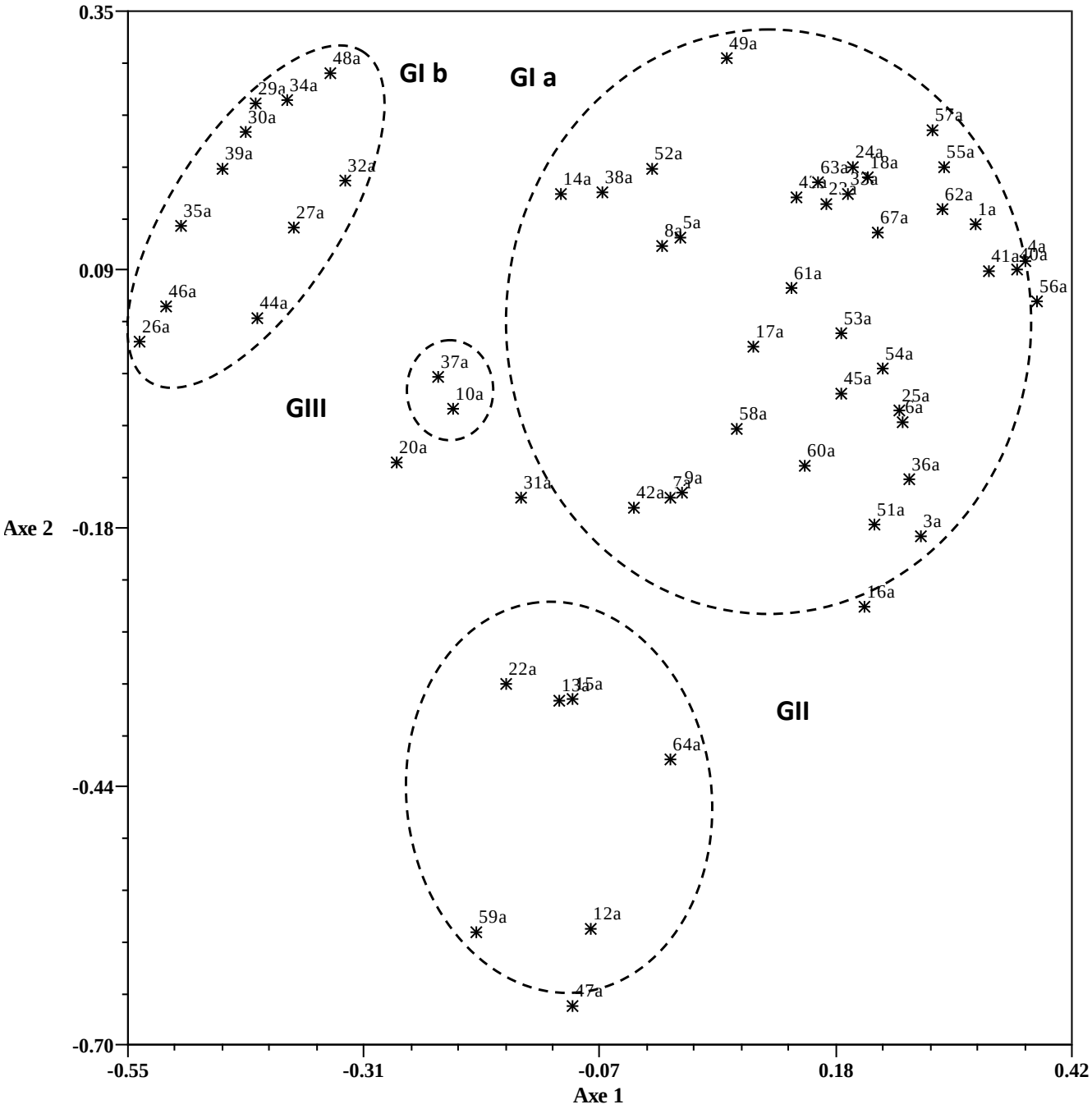

Figure 4. Principal coordinate analysis (PCoA) based on data of twenty SSRs polymorphic loci of 59 accessions of local varieties of sorghum.

(484 accessions), Barro-Kondombo et al. (2010) (124 accessions), Bhosale et al. (2011) (219 accessions) and Sagnard et al. (2011) (455 accessions). Moreover, the level of racial diversification of the samples analyzed could be taken into consideration. The samples used in the present study were more diversified in botanic terms (4 races on 5 represented in *Sorghum bicolor* ssp. *bicolor*) than that of Barro-Kondombo et al. (2010) (3 races on 5 are represented in *Sorghum bicolor* ssp. *bicolor*) who studied a larger population (120 accessions) but identified the lowest allelic diversity. Moreover, the analysis of the number and types of SSR markers used could be the reason. In this study, most of the markers used on samples from West African sub-region were polymorphic especially in Burkina-Faso (Barro-Kondombo et al., 2010), Niger (Deu et al., 2008) and Mali

Table 3. Distribution of accessions analyzed in the genetic groups individualized as compared to ethnic groups of producers.

Ethnical groups of farmers	Individualized genetic groups					Total of accessions
	Ia	Ib	IIa	IIb	III	
Lokpa		11				11
Yom/C*	14	1	2			17
Yom/D*	20	3	3	3	2	31
Total of accessions	34	15	5	3	2	59

*Yom/C: Yom de Copargo, Yom/D: Yom de Djougou.

(Sagnard et al., 2011). Furthermore, SSRs used belong to Xtxp series defined as non-coding regions and usually more polymorphic (Casa et al., 2005; Bhosale et al., 2011).

The proportion of "rare" alleles (17.14%) recorded in this study was lower as compared to that obtained in other studies (64% in Casa et al., 2005 and 64% in Deu et al., 2008). This could explain the fact that the samples used were from common ancestral origin with rare introduction relating to human migration. The case of Aka inodjo (*Ano manka*) and early-maturing varieties with red grains were not introduced (Missihoun et al., 2012a). Moreover, the restricted geographical origin of the samples as well the intense exchanges of materials between farmers among ethnical groups of populations belonging to the same geographic areas could justify the presence of shared alleles. Finally, the reduction of varietal pool during the evolution due to changes of pedological (poor and inadequate soils for cultivation) and agro-climatic (irregularity of rains and droughts), constraints reported by farmers (Kayodé et al., 2006; Missihoun et al., 2012a) could be a factor.

Another factor involved in allele's reduction during evolution is the method of selection of seeds by farmers, which preferred the best, attractive, long panicles and endowed with healthy grains (Missihoun et al., 2012a). Allelic discrimination of such a population appears to be difficult and showed by relatively low mean value of PIC (0.33). The structure of the population as observed in the present study, that is, the separation of the accessions according to the botanical race and morpho-physiological characteristics of the grains (colour, size and degree of bitterness of the grains) and particularly the separation of guinea race from others races (*caudatum* and *durra* precisely) was already reported in previous studies (Djè et al., 2000; Folkertsma et al., 2005; Barnaud et al., 2007; Deu et al., 2008; Barro-Kondombo et al., 2010; Bhosale et al., 2011).

Population structure and distribution

The main evolutionary forces shaping the genetic diversity in the populations of cultivated plants are among others gene flows, selection in connection with environmental heterogeneity and/or preference criteria of farmers-consumers and genetic variation due to randomness (genetic drift) (Neal, 2004; Mutegi et al., 2011). In the present work, the first factor of the structuration of sorghum genetic diversity identified was the racial parameter. The results are consistence with those of Deu et al. (2008), Kondombo et al. (2010) and Sagnard et al. (2011) respectively in Niger, Burkina-Faso and Mali on samples from world banks of genes (Deu et al., 2006).

The second factor identified was morpho-physiological characteristics of sorghum grains. This findings are similar to our results on agro-morphological characterization in which yellow grains of sorghum were different from bitter and no bitter accessions (Missihoun, 2013) and also farmers' classification using grain features as main criteria for varieties (Missihoun et al., 2012a). For instance, according to farmers from Koura ethnic group, all the local native varieties were named based on grain characteristics: *Aka kpankpan lako* (red sorghum with big grain), *Aka kpankpan kékéré* (red sorghum with small grain), *Aka foufou lako* (white sorghum with big grain) and *Aka foufou kékéré* (white sorghum with small grain). These results obtained in Benin are similar to those of Barro-Kondombo et al. (2010) recorded with SSRs in Burkina-Faso.

Finally, bicolor race was not supported by molecular analysis because the only one accession of this race was found in the group I of guinea race. Absence of differentiation of bicolor race from a genetic group according to molecular data has already been reported in previous studies (Deu et al., 1994; Perumal et al., 2007; Brown et al., 2011).

Implications for sorghum resources conservation and breeding programmes

Conservation genetics aim at identifying and understanding the evolutionary forces that have shaped the observed distribution of genetic diversity within a species on different scales, and identify populations or landraces that deserve priority conservation (Deu et al., 2008). In Benin, previous studies on genetic resources of

cultivated sorghum did not identify these evolutionary forces. In this study, two main factors determining the genetic structuring were identified: racial membership and morpho-physiological characteristics of the grain including grain color. This result is very important for genetic improvement of sorghum genetic resources in Benin. Beninese farmers characterized their local varieties based on grain colour (Missihoun et al., 2012a). In addition, racial classification of all accessions collected in Northwestern region were grouped into four races (*guinea*, *durra*, *caudatum* and *bicolor*) (Missihoun, 2013). At present, climatic fluctuations characterized by the reduction of raining period compel farmers to prefer red grains and low-yielding varieties to white grains and high yield potential varieties (Missihoun et al., 2012a). It is therefore important to conduct hybridization between varieties with white grains and long vegetative cycles, and varieties with red grains and short vegetative cycles to identify in the offspring of individuals adapted to the new growing conditions, that is, varieties with relatively short vegetative cycles and high yield potential. Moreover, it would also be interesting to conduct marker assisted selection exploiting natural hybridization that occurs in farmers' fields to identify hybrids that better meet current growing conditions. This hypothesis is supported by the very high rate (5-40%) of free hybridizations observed on-farm (Barnaud et al., 2008).

The results obtained in this study from the molecular genetics analysis are very important on the plant breeding programmes of varieties adapted to the current climatic fluctuations in Benin. Besides, the results are shown to be important to develop useful *in situ* conservation programs on sorghum in Benin.

Conclusion

The present study used microsatellite markers in estimating genetic diversity of Beninese sorghum landraces for the first time. The results reveal high genetic variability among the studied samples. This important genetic diversity was clearly structured following two important parameters: the racial group and morpho-physiological characteristics of grains (colour, bitterness degree).

The genetic partitioning of botanical races was obvious but guinea group included bicolor accession. It could be assumed that genetic proximity between the two races is due to domestication or lack of genetic support for differentiating the two races. Strategies for conservation and sustainable use of sorghum genetic resources in Benin should take into account the observed genetic components.

Conflict of interests

The authors did not declare any conflict of interest.

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