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# Evaluation of genetic diversity of okra accessions [*Abelmoschus esculentus* (L. Moench)] cultivated in Burkina Faso using microsatellite markers

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**Okra is a traditional vegetable grown throughout Burkina Faso. Despite a food and non-food valorization of all parts of the plant, its genetic diversity is still little known. Thus, 50 accessions of okra from Burkina Faso were characterized using 19 microsatellite markers in order to determine the level and structure of genetic diversity. The results reveal a total of 34 alleles including 3 rare alleles and a number of 2.58 effective alleles. A polymorphic information content (PIC) value between 0.11 and 0.86 and markers polymorphism rate of 42.10% were also obtained. Mean expected heterozygosity and Shannon diversity index were 0.46 and 0.77, respectively. In addition, a structuring of the 50 accessions in three genetic groups with indices of very similar accessions of 88 to 95% between climatic zones and 83 to 95% between ethnic groups were observed. The diversity obtained could be exploited in the program of selection and varietal improvement of okra.**

**Key words:** Genetic variability, simple sequence repeats (SSR) markers, varietal selection, valorization, genetic differentiation.

## INTRODUCTION

Okra [*Abelmoschus esculentus* (L. Moench)] is a fruit vegetable of the Malvaceae family. It is cultivated all over the world but especially in Africa and Asia (Koechlin, 1989). In Burkina Faso, okra is one of the main vegetables used in the preparation of sauces. It is especially popular for its fruits rich in trace elements, vitamins, fiber and mucilage (Hamon et al., 1997 ; Marius et al., 1997). Despite its potential, okra has long been neglected by government policies and research. There are practically no improved local varieties of okra in

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**Table 1.** Characteristics of 19 markers (Schafleitner et al., 2013).

| Marker | Sequence 5'- 3' | Sequence 3'- 5' | Repeated pattern |
|---|---|---|---|
| SSRs AVRDC-okra **1** | ATGGAGTGATTTTTGTGGAG | GACCCGAACTCACGTTACTA | (AAG)13 |
| SSRs AVRDC-okra 8 | TGCTGTGGAAGGTTTTTACT | ATGACGAAAGTGGTGAAAAG | (AAG)8 |
| SSRs AVRDC-okra 9 | ACCTTGAACACCAGGTACAG | TTGCTCTTATGAAGCAGTGA | (AAT)12 |
| SSRs AVRDC-okra 17 | ACGAGAGTGAAGTGGAACTG | CTCCTCTTTCCTTTTTCCAT | (AGA)7 |
| SSRs AVRDC-okra 21 | TCATGTCTTTCCACTCAACA | CCAAACAAAATATGCCTCTC | (AGA)9 |
| SSRs AVRDC-okra 28 | CCTCTTCATCCATCTTTTCA | GGAAGATGCTGTGAAGGTAG | (ATT)8 |
| SSRs AVRDC-okra 39 | TGAGGTGATGATGTGAGAGA | TTGTAGATGAGGTTTGAACG | (AG)16 |
| SSRs AVRDC-okra 52 | AACACATCCTCATCCTCATC | ACCGGAAGCTATTTACATGA | (CAT)8-(TCA)9 |
| SSRs AVRDC-okra 54 | CGAAAAGGAAACTCAACAAC | TGAACCTTATTTTCCTCGTG | (GAA)10 |
| SSRs AVRDC-okra 56 | GGCAACTTCGTAATTTCCTA | TGAGTAAAAGTGGGGTCTGT | (GAA)44 |
| SSRs AVRDC-okra 57 | CGAGGAGACCATGGAAGAAG | ATGAGGAGGACGAGCAAGAA | (GAA)9-GAG)7 |
| SSRs AVRDC-okra 63 | GTGTTTGAAAGGGACTGTGT | CTTCATCAAAACCATGCAG | (TCT)12 |
| SSRs AVRDC-okra 64 | AAGGAGGAGAAAGAGAAGGA | ATTTACTTGAGCAGCAGCAG | (TCT)22 |
| SSRs AVRDC-okra 66 | CACCAGAATTTCCCTTTTG | ACTGTTGTTTGGCTTATGCT | (TTC)12-(TTC)13 |
| SSRs AVRDC-okra 70 | GTAGCTGAACCCTTTGCTTA | CTATCATGGCGGATTCTTTA | (TC)11 |
| SSRs AVRDC-okra 77 | CTGTTTGTTCGTCGTAATCA | AAAGTTTCTTCCTTTCCACC | (GAAATA)4-(GAAACA)7 |
| SSRs AVRDC-okra 78 | CTCCGACAATTCAAGAAAAG | CACCCAATCAAGCTATGTTA | (TAT)11-(TATTGT)4-(TATCGT)4 |
| SSRs AVRDC-okra 86 | ATGCAAACAAGCTAGTGGAT | ATTCTCTTCAGGGTTTCCTC | (AGC)8 |
| SSRs AVRDC-okra 89 | TTTGAGTTCTTTCGTCCACT | GTATTTGGACATGGCGTTAT | (AGC)8 |

Burkina Faso (Balma et al., 2003 ; Sawadogo et al., 2009; Jiro et al., 2011). The genetic diversity of okra remains poorly known (Hamon, 1988). Evaluations of okra diversity performed are essentially based on phenotypic traits (Ariyo, 1993; Martinello et al., 2001; Akotkar et al., 2010; Bello et al., 2017). However, prior knowledge of the genetic diversity of a crop is essential for a better valuation of the species.

The present study on the genetic diversity of okra using specific microsatellite markers was conducted to better knowledge of the genetic diversity of okra cultivated in Burkina Faso. The objective was to determine the level and structuring of genetic diversity in order to contribute to a better management of the okra genetic resources and to establish an improvement program.

## MATERIALS AND METHODS

### Plant

Fifty okra accessions were characterized. These accessions were collected in the three climatic zones (Sahelian, Soudanese and Sudano-Sahelian) of Burkina Faso within four ethnic groups (Bissa, Bobo, Bwaba, and Mossi Gourounsi).

### Molecular markers

Nineteen specific microsatellite markers (Table 1) of *A. esculentus*, developed by Asian Vegetable Research and Development Center (AVRDC) nowadays called Word Vegetable Center were used. They are polymorphic, codominant, and neutral markers with a high polymorphic information content (PIC) (Schafleitner et al., 2013).

### Extraction of DNA with the FTA card method

Extraction of the total DNA of the 50 accessions was performed using FTA technology on young leaves of about 10 days. These are the first three leaves of the same plant that were picked, then crushed on an FTA map using a mortar and a parafilm. These cards were dried at room temperature and stored in a desiccator in the laboratory. For the recovery of the DNA, a disk 1 mm in diameter of the card prints was taken using a punch (Haris). Each disc was washed with Ethanol (70°) and incubated with TE 1X (Tris EDTA) according to the following steps: (i) two successive washes with 200 µl of ethanol 70° per disc, for 5 min each time to rid the samples of chlorophyll, leaf and cell debris and other impurities; (ii) two successive incubations with 200 µl of TE (Tris EDTA) per disc for 5 min each time during which the DNA molecule is solubilized.

The disk is then dried at ambient temperature and then directly transferred to the polymerase chain reaction (PCR) tube for amplification.

### PCR amplification and revelation

The PCR amplification was carried out with an Eppendorf brand thermocycler. During the different reactions, each tube contained 1 µl of each microsatellite primer, 5 µl of premix PCR (1 µl of Taq polymerase, 250 µM of the different dNTPs, 10 mM of KCl, and 1.5 mM of $MgCl_2$), 18 µl of ultra-pure water and finally the disk from the FTA card and carrying the DNA to amplify.

The PCR program used consisted of an initial denaturation at 95°C for 10 min followed by 35 cycles of denaturation at 94°C for 30 s, hybridization at 55°C in 45 s and a step of final extension at 72°C for 5 min. The PCR products were stored at 4°C after each amplification. The amplified products were revealed by 3% agarose gel electrophoresis in the presence of 5% Ethydium Bromide used as a fluorescent developer under ultraviolet light at a voltage of 100 V (1h). The deposits were made in the presence of a molecular weight marker consisting of two microsatellites of different sizes

**Table 2.** Genetic diversity parameters.

| Primer | A | Ae | I | He | PIC | $A^r$ |
|---|---|---|---|---|---|---|
| AVRDC-Okra 1 | 1 | 1.22 | 0.33 | 0.18 | 0.19 | 0 |
| AVRDC-Okra 8 | 1 | 1.32 | 0.44 | 0.25 | 0.26 | 0 |
| AVRDC-Okra 56 | 3 | 5.19 | 1.81 | 0.88 | 0.70 | 0 |
| AVRDC-Okra 28 | 2 | 2.97 | 0.83 | 0.56 | 0.46 | 0 |
| AVRDC-Okra 52 | 3 | 5.05 | 1.66 | 0.97 | 0.53 | 0 |
| AVRDC-Okra 63 | 3 | 3.97 | 1.13 | 0.69 | 0.54 | 0 |
| AVRDC-Okra 64 | 5 | 6.44 | 1.66 | 0.99 | 0.86 | 2 |
| AVRDC-Okra 70 | 2 | 3.03 | 0.97 | 0.63 | 0.54 | 0 |
| AVRDC-Okra 77 | 2 | 2.80 | 0.78 | 0.49 | 0.75 | 1 |
| AVRDC-Okra 9 | 3 | 3.94 | 0.91 | 0.56 | 0.85 | 0 |
| AVRDC-Okra 17 | 1 | 1.42 | 0.47 | 0.30 | 0.32 | 0 |
| AVRDC-Okra 21 | 1 | 1.17 | 0.28 | 0.15 | 0.15 | 0 |
| AVRDC-Okra 54 | 1 | 1.68 | 0.59 | 0.40 | 0.48 | 0 |
| AVRDC-Okra 57 | 1 | 1.13 | 0.23 | 0.11 | 0.11 | 0 |
| AVRDC-Okra 66 | 1 | 1.99 | 0.69 | 0.50 | 0.78 | 0 |
| AVRDC-Okra 78 | 1 | 1.22 | 0.33 | 0.18 | 0.19 | 0 |
| AVRDC-Okra 86 | 1 | 1.77 | 0.63 | 0.44 | 0.53 | 0 |
| AVRDC-Okra 89 | 1 | 1.17 | 0.28 | 0.15 | 0.15 | 0 |
| AVRDC-Okra 39 | 1 | 1.63 | 0.57 | 0.39 | 0.45 | 0 |
| Mean | 1.79 | 2.58 | 0.77 | 0.46 | 0.47 | - |
| Standard deviation | 1.13 | 1.62 | 0.49 | 0.28 | 0.25 | - |

A, Number of alleles/marker; Ae, number of effective alleles; I, Shannon diversity index; He, average expected heterozygosity; PIC, polymorphism information content; $A^r$, number of rare alleles.

ranging from 25 to 100 bp. A Canon PowerShot A620, 7.1 megapixel camera was used to photograph the migration gel.

#### Statistical analysis of molecular data

From the bands revealed by the markers, a binary coding 1 or 0 was made, respectively in case of presence and absence of bands. The GenALEx version 6.501 software (Nistelberg et al., 2013) was used to estimate genetic parameters such as total number of alleles ($A^t$), allelic richness or number of alleles per marker (A), effective number of alleles ($A_e$) [($A_e$ = 1/(1-h) = 1/Σpi2, where pi is the frequency of the allele, i is the locus under consideration and h = heterozygosity], number of rare alleles ($A^r$), polymorphic information content (PIC) (Smith et al., 2000), and polymorphism of markers (P). The Shannon genetic diversity index (I) (I = -1 [(p × ln (p) + q × ln (q))]) and the expected mean heterozygosity (He) or Nei gene diversity index (D) (He = 1/N [n/n-1(1-Σpi2)], where N is the number of loci, n is the number of accessions, pi is the frequency of the allele i, at the relevant locus were also performed with the same software. The genetic diversity structure was carried out using DARwin V5.0 software (Perrier et al., 2006) from the dissimilarity matrix of accessions according to the "simple matching" procedure according to the Neighbor-Joining method. The 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 too FSTAT software V2.9.3.2.

## RESULTS

### Level of diversity of markers

All 19 markers allowed amplification of the individuals tested, but only eight revealed more than one allele (Table 2). A total of 34 alleles including three rare alleles with a size between 25 and 500 bp (Figure 1) were observed. The mean number of effective alleles and the average expected heterozygosity were 2.58 and 0.46, respectively.

The AVRDC-Okra 64 marker (Figure 2) showed the highest number of alleles (5 alleles). The Shannon genetic diversity index (I) ranged from 0.23 to 0.97 with an average of 0.77. The Polymorphic Information Content ranged from 0.11 for the AVRDC-Okra 57 primer to 0.86 for the AVRDC-Okra 64 primer with an average of 0.47.

### Organization of the genetic diversity of okra accessions

#### *Structure of genetic diversity*

The analysis of genetic diversity using the "Neighbor-Joining" algorithm divided the 50 accessions of okra into three genetic groups (Figure 3). Indeed, genetic groups I and III consisted respectively of 62 and 30% of the accessions of the collection coming from the three climatic zones while the genetic group II contained 8% of the accessions coming from the Sudano-Sahelian.

Genetic group I contains all alleles (34) observed including the three rare alleles (Table 3). Genetic group II has the lowest genetic indices while genetic group III has

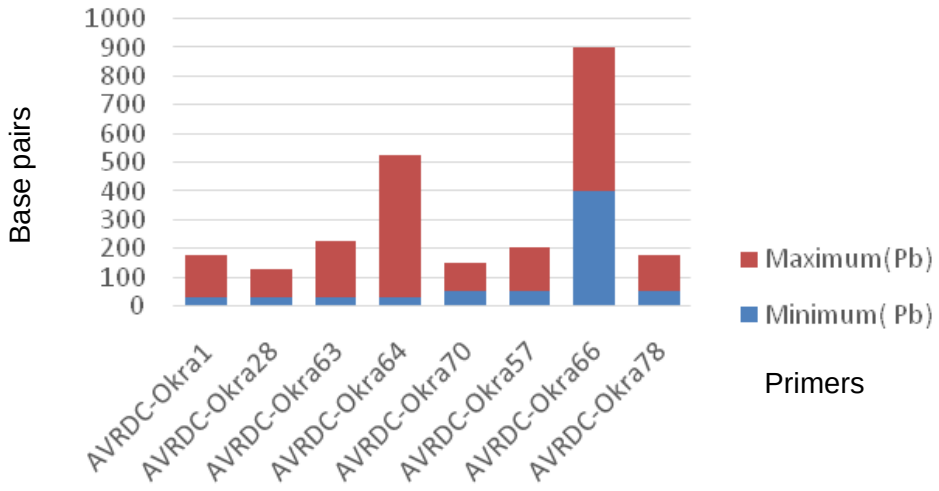


**Figure 1.** Microsatellite markers bands size.

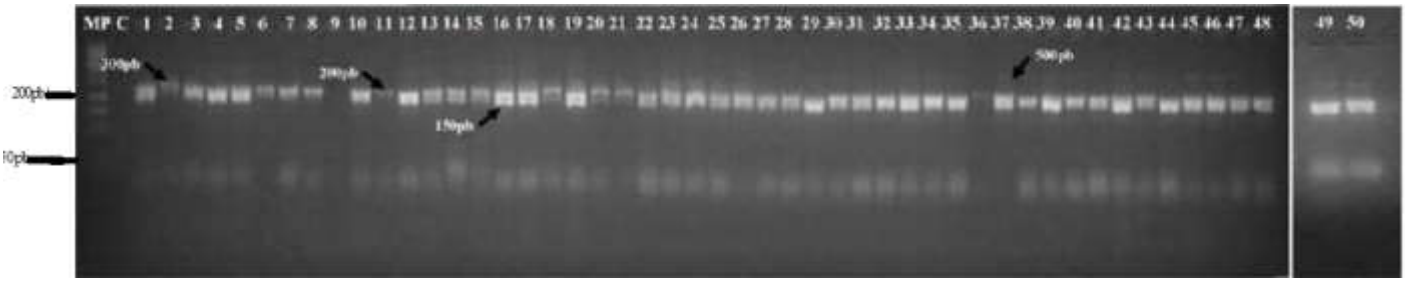

**Figure 2.** Migration profile of AVRDC-Okra marker 64.

the highest values of effective alleles number (2.94), Shannon genetic diversity index (0.93) and expected heterozygosity (0.66).

### *Differentiation between genetic groups*

Genetic differentiation (Fst) showed a significant difference only between groups I and III (Table 3). The highest Nei minimal genetic distance (0.087) was observed between genetic groups I and II (Table 4).

The structure of genetic diversity is very weakly influenced by climatic zone and ethnic group factors. Indeed, low genetic differentiation (Fst) and strong indices of similarity of accessions were observed between ethnic groups (Table 5) and between climatic zones (Table 6)

## DISCUSSION

The 19 primers used showed a genetic diversity of okra accessions. The average PIC of 0.47 shows that these markers are all informative because according to Smith et al. (2000), the PIC of simple sequence repeats (SSR) marker is an important estimate of the discriminating power of this marker. The value of PIC ranging from 0.11 to 0.86 confirms the reliability of these SSR markers. Indeed, Schafleitner et al. (2013) found a PIC value between 0.43 and 0.84 on okra with the same SSRs. Kumar et al. (2017) also reported a PIC ranging from 0.11 to 0.80 for 30 polymorphic SSRs used on okra genotypes. The AVRDC-Okra marker 64 and AVRDC-Okra marker 9 with respective PIC values of 0.86 and 0.85 are the most polymorphic.

The values of the effective alleles number (Ae = 2.58) and average number of alleles per marker (A = 1.78) indicate that this genetic diversity would be relatively small. These results confirm those of Hamon (1988) who showed a low genetic diversity of cultivated species of okra from West African origins. In fact, okra producers in Burkina preferentially select short-cycle and green-fruit okra cultivars (Ouédraogo et al., 2016), which could explain the low genetic diversity of the 50 accessions. However, greater diversity has been reported by Sawadogo et al. (2009) who achieved a higher mean

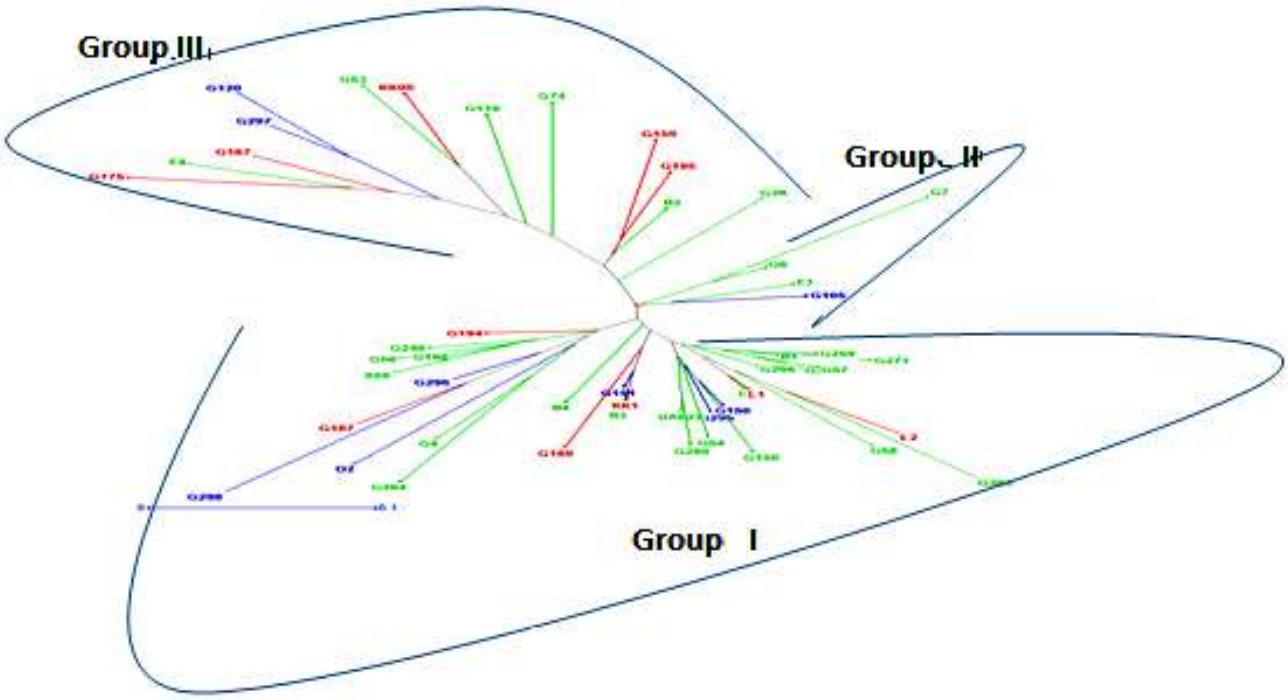


**Figure 3.** Radial representation of the dendrogram of the 50 accessions of okra cultivated in Burkina Faso constructed from the dissimilarity matrix according to the Neighbor-Joining method.

**Table 3.** Characteristics of genetic group diversity.

| Genetic group | Number of accessions | $A^t$ | A | $A_e$ | I | He | P(%) | $A^r$ |
|---|---|---|---|---|---|---|---|---|
| I | 31 | 34 | 1.78 | 2.83 | 0.89 | 0.61 | 100 | 3 |
| II | 4 | 27 | 1.42 | 2.39 | 0.47 | 0.36 | 44.12 | 0 |
| III | 15 | 33 | 1.73 | 2.94 | 0.93 | 0.66 | 94.12 | 0 |

$A^t$ , Total number of alleles; A, number of alleles per marker, Ae, number of effective alleles; I, Shannon genetic diversity index; He, average expected heterozygosity; P, percentage of polymorphism; $A^r$, number of rare alleles; AP, number of distinct or private alleles.

**Table 4.** Genetic differentiation (FST) and Nei genetic distance.

| Genetic group | I | II | III |
|---|---|---|---|
| I | 0 | 0.087 | 0.027 |
| II | $0.1737^{ns}$ | 0 | 0.087 |
| III | 0.236* | $0.0197^{ns}$ | 0 |

FST (below the diagonal), genetic distances of Nei (above the diagonal).

**Table 5.** Matrix Fst (below the diagonal) and index of average genetic similarities of accessions between ethnic groups (above the diagonal).

| Ethnic group ethnique | Bissa | Bobo | Bwaba | Mossi | Gourounsi |
|---|---|---|---|---|---|
| Bissa | 0 | 0.95** | 0.86** | 0.94** | 0.88** |
| Bobo | $0 .082^{ns}$ | 0 | 0.90 | 0.94** | 0.89** |
| Bwaba | $-0.063^{ns}$ | $0.006^{ns}$ | 0 | 0.92** | 0.83** |
| Mossi | $0.027^{ns}$ | $-0.027^{ns}$ | $-0.026^{ns}$ | 0 | 0.94* |
| Gourounsi | $0.036^{ns}$ | $0.013^{ns}$ | $0.045^{ns}$ | $0.066^{ns}$ | 0 |

**Table 6.** Genetic differentiation indices (FST) (Below Diagonal) and indices of mean genetic similarity of accessions between climatic zones (Above the diagonal).

| Climatic zone | Sahelian | Soudanese | Sudano-Sahelian |
|---|---|---|---|
| Sahelian | 0 | 0.89** | 0.93** |
| Soudanese | -0.0099$^{ns}$ | 0 | 0.96** |
| Sudano-Sahelian | 0.0105$^{ns}$ | 0.0277$^{ns}$ | 0 |

**Very significant.

number of allele per locus (A = 4.8) over 20 traditional okra varieties from Burkina Faso. This important diversity revealed by this author can be explained by the origin of these accessions which come from a selection of phenotypic diversity. Many previous studies (Schafleitner et al., 2013; Nasser, 2014; Kumar et al., 2017) report an influence of the origin of cultivars on the genetic diversity of okra. The high similarity indices of okra accessions show that climatic zone and ethnic group factors do not significantly influence their genetic variability. The genetic differentiation of okra subpopulations therefore depends on the genotype of accessions. These results are similar to those of Kiébré (2016) who found that the "genetic group" is the only factor that significantly influences the genetic differentiation of *Cleome gynandra* subpopulations.

The low genetic differentiation of accessions observed between climatic zones and between ethnic groups could be explained by the fact that producers use the same seeds. Nana (2010) reported also a low ecotypic differentiation of okra cultivated in Burkina Faso. In fact, the farmers exchange seeds with each other and also take with them the seeds during the exodus.

Genetic distance is a function of the level of genetic diversity in the population. The three genetic groups and the low genetic distance of Nei observed between them suggest a low genetic diversity of the okra collection. However, Sawadogo et al. (2009) obtained five genetic groups of okra ecotypes with a higher Nei genetic distance (0.51 to 0.77). The high values of the polymorphism rate of markers (91%), the Shannon genetic diversity index (0.77) and the presence of the three rare alleles explain the relatively higher level of diversity in the Sudano-Sahelian zone of Burkina. This zone, which is the largest of the three climatic zones, is characterized by ecological diversity that favors the differentiation of okra ecotypes. On the other hand, on *Cleome gynandra*, Kiébré (2016) observed a higher diversity in the Sudanian zone of Burkina as compared to the other two zones.The presence of rare alleles only in the Sudano-Sahelian zone could reflect an interaction between genotypes of cultivated accessions and the environment. According to Akinyele et al. (2011), the environment alone can affect the genotype of okra by stimulating permanent changes in the genome of the varieties. The rare and private alleles could be a potential genotype for the production and resistance to diseases of okra. Sawadogo (2015) reported that rare alleles would be of great interest if they are only related to some particular genotypes.

## Conclusion

Molecular markers revealed a genetic diversity of okra accessions cultivated in Burkina Faso. The 19 SSRs markers developed by AVRDC specifically for the gombo were all discriminating and informative. This study showed also a moderate value for expected heterozygotie, a high value of Shanon' indice and an organization of the 50 okra' accessions in three groups. The three genetic groups suggest the existence of several genotypes of cultivated okra accessions. Moreover, the genetic diversity of okra is more influenced by genotype than climatic zone or ethnic group factors. The results of this study provide a basis for implementing an *in situ* and *ex situ* conservation program and improving the genetic resources of okra cultivars. In the perspective of a better valorization of okra in Burkina Faso, a biochemical characterization and organoleptic tests of accessions is necessary. Also, the characterization of these accessions with SNP markers could allow to better appreciate the level of genetic diversity.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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