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Genetic Diversity of a Collection of *Solanum macrocarpon* from Burkina Faso Revealed by Microsatellite Markers

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Abstract: *Solanum macrocarpon* is an important vegetable native to Africa, with leaves and fruits. It is a potential source of income and nutrients that contribute to the nutritional balance of the local population's food intake. However, *S. macrocarpon* did not attract the interest of researchers in relation to other *Solanum* species. Indeed, knowledge of the genetic diversity of *S. macrocarpon* (Gboma Eggplant) in Africa is limited. Although agronomic variations and relationships generally reveal important traits of interest to breeders. This study attempts to estimate the genetic diversity of a collection of *S. macrocarpon* (32 accessions) from Burkina Faso using SSR markers. The results show that eleven (11) of the 22 SSR markers tested were informative for phylogenetic analysis and could serve as a perfect reference for diversity studies. The 11 polymorphic SSR primers selected amplified 22 alleles with an average of 2 per primer. The polymorphism information content (PIC) varied from 0.212 for emg01B17 to 0.437 for emd15D09. The genetic distances between the accessions studied varied from 0 to 0.68 and made it possible to distinguish three genetic groups. The expected Nei (He) heterozygosity for the genetic group ranged from 0.140 for group III to 0.261 for group I. The eleven pairs of SSR markers sufficiently discriminated against the accessions of *Solanum macrocarpon*. The results of this study will be useful for the conservation and genetic improvement of the *S. macrocarpon* species in Burkina Faso.

Keywords: Genetic diversity, Polymorphism, *Solanum macrocarpon*, SSR Markers, Burkina Faso

1. Introduction

Solanum macrocarpon L. (Gboma Eggplant) belongs to the genus *Solanum*, the subgenus *Leptostemonum* and the section *Melongena* (Miller) Dunal [1]. The cultivated *S. macrocarpon* was domesticated from *S. dasyphyllum* Schum. and Thonn., wild relatives [2]. It is cultivated throughout tropical Africa, especially in humid regions. The species is also cultivated in South America and Asia [3]. According to the [2], Gboma Eggplant is grown along the coasts of West Africa (Ghana, Nigeria, Togo, Ivory Coast and Benin), East and South (Malawi, Zambia, Zimbabwe and Mozambique). In Burkina Faso, *S. macrocarpon* is grown in the three border provinces (Nahouri, Sanguié and Sissili) with Ghana [4].

The fruits and leaves of *S. macrocarpon* are eaten fresh or simmered with other vegetables such as Amaranth and Cabbage. An excellent source of minerals, fibers and proteins [5-6], the marketing of *S. macrocarpon* leaves and fruits provides important income to many households [7]. It is also used in traditional medicine for the treatment of various conditions, ranging from weight loss, asthma, skin infections, rheumatic diseases, gastroesophageal reflux disease, constipation and diabetes [8].

Despite its nutritional and economic importance, knowledge about the genetic diversity of Gboma Eggplant in Africa is limited. The limited research on *S. macrocarpon* has been conducted on genetic variability among accessions in Ghana using various agronomic traits [9]. In Burkina Faso, agro-morphological characterization has shown the existence of diversity within accesses to *S. macrocarpon* despite the small-cultivated area [5]. Yet, the estimation of genetic

diversity is essential for the effective control, consumption and appropriate use of plant genetic resources [10]. According to the [11-12], the genetic diversity of cultivated species is the essential product of plant breeding. Indeed, the evaluation of genetic resources is crucial for breeders in order to establish variation between individuals on the one hand and on the other hand to select popular cultivars according to changes in consumer demand. In addition, biochemical traits, genealogical analysis and genetic fingerprinting using molecular markers [13-14] can assess the genetic diversity of breeding material. SSRs or microsatellites are the most commonly used markers for diversity studies. They are short, tandem, repeated nucleotide units of one to five nucleotides and their value for genetic analysis lies in their polyalignment, codominant inheritance, relative abundance, genotypic coverage and the relevance of high throughput PCR platforms [15]. A number of workers have developed SSRs for eggplant, including genomic SSRs by the [16-17] and genic SSRs by the [18] which they tested primarily on eggplant cultivars. This study was undertaken to use genomic SSR to determine the genetic diversity of thirty-two accessions *of Solanum macrocarpon.*

2. Materials and Methods

2.1. Plant Materials

Thirty-two (32) local accessions from three provinces of Burkina Faso were used in this study (Figure 1), including sixteen (16) from Sissili, thirteen (13) from Sanguié and three (3) from Nahouri (Table 1). These accessions were sown and cultivated directly on the ground. The nursery was watered regularly morning and evening to ensure good seedlings growth. The young leaves of each accession were collected, weighed and used to extract genomic DNA.

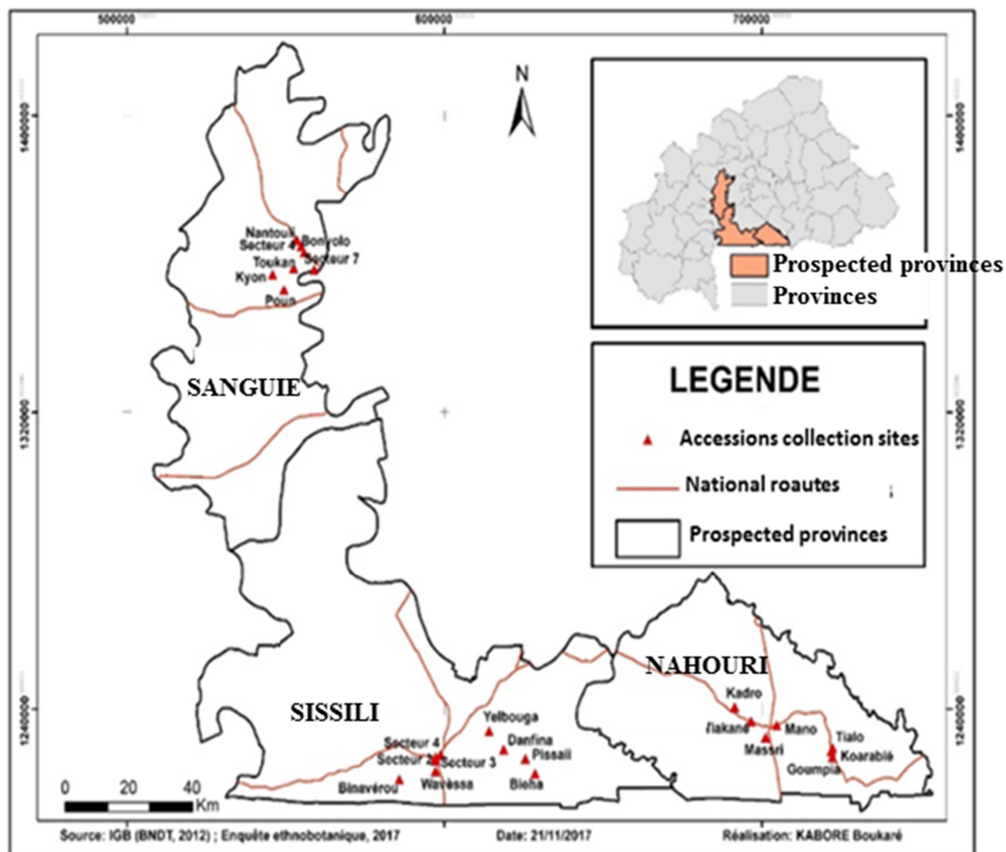


Figure 1. Investigated and collection sites of Solanum macrocarpon accessions cultivated in Burkina Faso.

Table 1. Characteristics of the studied Solanum macrocarpon accessions.

Number	Status	Provinces	Climatically zones
16	Cultivated	SISSILI	Sudanian
3	Cultivated	NAHOURI	Sudanian
13	Cultivated	SANGUIE	Sudano-sahelian

2.2. SSR DNA Marker Analysis

Twenty-two (22) Single Sequence Repeat (SSR) microsatellites markers were used in this study. They have been developed specifically for genetic diversity studies of *S. melongena* by the [16, 19]. These are markers already used by the [18, 20] (Table 2).

Table 2. *Characteristics of the twenty-two SSR markers used [20-21].*

Entry No.	Primer name	Forward sequence	Reverse sequence	Tm (°C)
1	emb01G19	AATTAAGGCTGAGAGGGGAAGACG	AAAGGAGGAAAGGGAAAGGGAAAG	51.5
2	emf01G17	ATGGCAACTGATAATGCAGACGTG	GTTTCTCACTCTTACATGTGGCTGGC	52.5
3	emh21J12	ACAGAACAATTCACCAGCAGTCAA	GTTTAGGAACAGGGAAAATCGTATCGGT	51.2
4	eme25D01	AGTCCCAACCAAAATCGTAGAGGC	GTTTCACTGAAGGATGTGGAGTGTGA	52.6
5	emg11A06	AGTGCTAATATGCAAGGGGAATGG	GTTTACGGTGATCTTTCCGTATTCCAAA	51. 3
6	emg01B17	ACAAGGCTCAAAGTCACAAGTCAA	GTTTGGCTCTGCCCCTAACATCTACAAA	51. 9
7	emf11H23	ATTCTGAAAACAAGAGCAGCCCTC	GTTTCTCAACACCTCTGTGTCTGGCAT	52. 6
8	emg11I03	ATTAGGCACAAGTGCCACCTGAAT	GTTTCAGCCGGGAGTCTGATAGGTAAAA	52. 7
9	emf11L21	ATAGCCTAGGTAACGTACCCCTCG	GTTTGGCTCTATTTCCTGGGCTTTTCAT	53. 7
10	emd12B05	ACGGAGTAGGCTCGGAGCGTGATATT	GTTTGAAAGGGCAAAAAGTCCAAACAAC	54. 0
11	emd05F05	ACGGGGGTGTCTCATTACACTACTGG	GTTTACCCGTTCCTCAGCTTATAGACCC	56. 2
12	emf01O01	AGGAATTGGATTTCCACTCATACG	GTTTGGAAGATGAGATTCCTTTCTTGA	49. 5
13	emf21K08	ATCAATGACACCCAAAACCCATTT	GTTTGAAAACCCAATACAAATCCGA	47. 5
14	emb01F16	AAAACAGAAGCAAAGTCGGCAGTC	GTCCACCAACACCTTACCATCCTC	52. 3
15	emh11G21	ATGTGTGAACTCAAATGGAAGGGA	GTTTCGAATTGCTTTTTGGTGCATGTAG	50. 4
16	emf21P02	ATGAAGCAGATCTTTCGACTGCAC	GTTTAGGCCAAGGATGTCAAACTGGT	51. 8
17	emh05B02	ATACCAAAGACACGTTGGGATCAT	GTTTCTAGGAGAGCATCTCCCTCCCT	52. 3
18	emd15D09	ATAATGGGCAAAGGGTCCATTAAC	GTTTGGAACCATGCAGTACCAGACATGA	51. 9
19	emj04E17	ACACGCTGCTGAAATAGTTTCTTAG	GTTTCGAGTTATGCTGAGAGCAGTGTGA	52. 1
20	emh11N11	ATTCAGTTCTTCGCTTTGGAGCTT	GTTTCCAAACCCGACCCATCCTAAATAA	51. 2
21	emj01G23	ATTAACTGGCCATGAACACCTGTC	GTTTGACCTCAATAAAGGGGGTTTGCAT	52. 0
22	emd18B04	ATTTCTGAGGTTTAACATCGCCGT	GTTTCGGAGGAGAGCAAGTTCTGCTTTA	51. 9

2.3. DNA Extraction and Quantification, PCR Amplification, and Gel Electrophoresis

The extraction of genomic DNA was carried out at the Molecular Biology Unit of the Plant Genetics and Breeding Team at Joseph KI-ZERBO University. Young leaves of fully developed and undamaged *Solanum macrocarpon* were collected from the genotypes of each cross. DNA was extracted using the DNAzol method [22] readapted to the species *S. macrocarpon*. As a result, 0.1 g of fresh young leaves from each sample were finely ground in a mortar in the presence of 750 µl of ultra-pure water. The resulting shred was centrifuged in a 2 ml eppendorf tube at 10,000 rpm at 4°C for 10 minutes. At the end of this first centrifugation, the supernatant was removed and then a volume of 200 µl of a DNAzol solution was added to the pellet. After homogenization, the tubes were placed in a water bath at 65°C for 2 hours. After the 2 hours, the tubes were centrifuged again at 10,000 rpm at 4°C for 15 minutes. This time, the supernatant was collected in new tubes and stored at a temperature of -20°C.

Polymerase chain reaction (PCR) was performed using the optimized SSR amplification conditions for *S. macrocarpon*. Each 20 µL PCR reaction consisted of 3 µl genomic DNA, 1 µl each of forward and reverse primer and 4 µl premix PCR (1U Taq DNA polymerase, 250 µM Tris-HCL, 10 mM KCl, 1.5 mM $MgCl_2$). Amplifications were carried out in a Thermal Cycler with the amplification conditions of initial denaturation at 94°C (30 s), 30 cycles of 94°C (30 s) denaturation, 47-58°C annealing (1 min) and 72°C (1 min) extension, followed by one cycle at 72°C (5 min) final extension.

Agarose gel electrophoresis was used to separate the amplified DNA fragments. PCR amplification products were electrophoresed in 2 % agarose gel using 1X Tris-borate EDTA (TBE) solution. Electrophoresis was carried out for 2 hours at 90 V. The gels were stained with BET (Bromide Ethidium), used by standard methods of [23] and statement under ultra-violet (UV) light. The DNA ladder (Bioline GmbH, Germany) was used in each gel as molecular size standard.

2.4. Data Analysis

A binary matrix based on the presence (1) or absence (0) of gel bands was performed for electrophoresis analysis profiles. The markers that gave clear bands were selected for statistical analysis. Three software programs were used (GenAlEx, Genetix 4.0.5.2 and Darwin 6.0.4). Genetic parameters were calculated using GenAlEx software to assess the level of diversity of all collections. These are the average number of alleles per locus (At), the number of effective alleles (Ae), the polymorphism rate (P), the Shannon diversity index (I), the expected heterozygosity (He) and the polymorphic information content (PIC). The diversity structure has been realized with Darwin software version 6.0. But the Genetix software was used to calculate the genetic distance between the genetic groups formed. Fstat software version 2. 9. 3. 2. for estimating the genetic differentiation index between populations (Fst).

3. Results

3.1. Level of SSR Marker Polymorphism

Of the 22 SSR primers selected for polymorphism, 11 SSR markers were found to be polymorphic for all accessions (Table 3). The 11 polymorphic SSR markers amplified 22 alleles in the eggplant access collection. 32 accessions of *S. macrocarpon* tested showed considerable polymorphism. The number of alleles per primer is between 2. The number of effective alleles (Ae) ranges from 1.258 (emg01B17) to 1.779 (emd15D09) with an average of 1.433 for all primers. The expected heterozygosity (He) of the collection is 0.372 and ranges from 0.219 to 0.451 respectively for the emg01B17 and

emd15D09 primers. As for the Shannon diversity index (I), with an average of 0.543 for all markers, it is included 0.628 for the emd15D09 marker and 0.361 for emg01B17. The polymorphism information content (PIC) ranged from 0.212 (emg01B17) to 0.437 (emd15D09) with an average of 0.359.

Table 3. *Level of diversity of markers tested.*

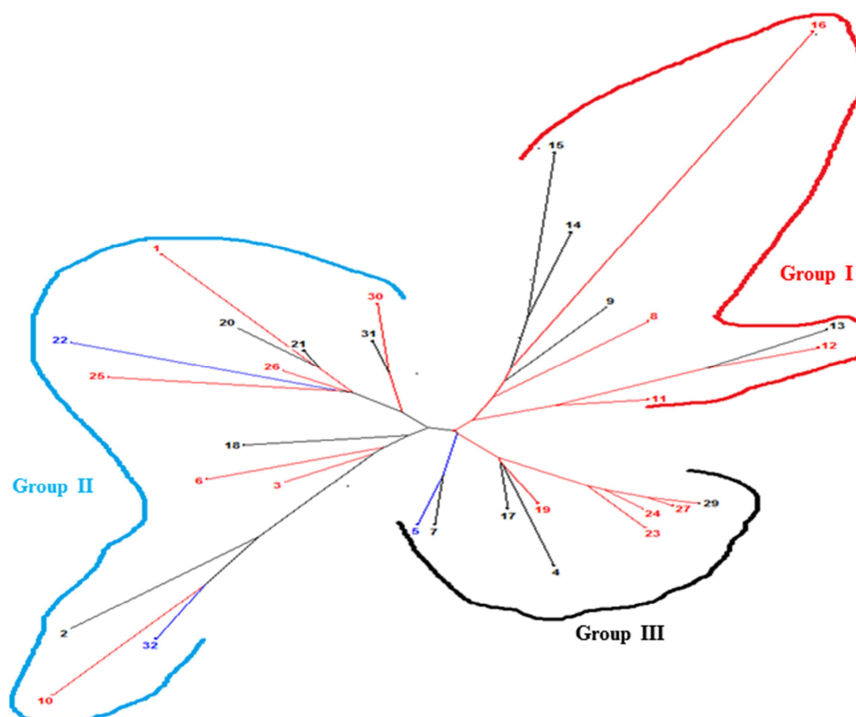
Primers	A^t	A_e	I	He	PIC	P (%)
emg11A06	2	1.777	0.626	0.450	0.435	
emg01B17	2	1.258	0.361	0.219	0.212	
emf11H23	2	1.599	0.543	0.379	0.358	
emf11L21	2	1.704	0.602	0.426	0.413	
emf21P02	2	1.562	0.544	0.371	0.360	
emd15D09	2	1.779	0.628	0.451	0.437	91.67
emj04E17	2	1.644	0.579	0.404	0.391	
emj01G23	2	1.470	0.500	0.330	0.320	
emb01G19	2	1.410	0.482	0.299	0.290	
emh11G21	2	1.742	0.616	0.440	0.426	
emd18B04	2	1.458	0.492	0.324	0.314	
Mean	2	1.433	0.543	0.372	0.359	

Legend: A^t: total number of alleles, A_e: number of effective alleles, He: expected heterozygosity, PIC: content of polymorphism information, I: Shannon diversity index, P: polymorphic percentage of loci.

3.2. Structuring the Genetic Diversity of the Collection of S. Macrocarpon from Burkina Faso

The estimated genetic distance for all accessions ranged from 0 to 0.68. On the one hand, the short genetic distance between two individuals (0) suggests a similarity with the loci studied. On the other hand, the highest distance (0.68) reflects the total divergence between these individuals. Examination of the dendrogram of the total population makes it possible to distinguish three distinct genetic groups (I, II, III) at the threshold of the 5% confidence interval independent of the collection area. Group I includes 8 accessions, Group II 14 accessions and Group III 10 accessions.

The estimation of the genetic diversity parameters of the three genetic groups identified by the dissimilarity matrix shows that the number of effective alleles varies from 1.251 for Group III to 1.442 for Group I. The expected heterozygosity ranged from 0.140 for Group III to 0.261 for Group I. The rate of polymorphism is 68.18% (Group I), 68.18% (Group II) and 36.36% (Group III). Finally, the Shannon diversity index and the polymorphic information content range from 0.205 to 0.387 and 0.156 to 0.299 for Groups III and I respectively. In general, diversity parameters are more important in Group I while Group III has the lowest values (Table 4).



Legend: Numbers 1 to 32 represent the individuals studied: (Red for Sissili, Black for Sanguié and Blue for Nahouri)

Figure 2. *Radial representation of the dendrogram of S. macrocarpon accessions, constructed from the dissimilarity matrix according to the Neighbour-Joining method.*

Table 4. *Distribution of genetic diversity of S. macrocarpon according to groups.*

Groups	N	A^t	A_e	I	He	PIC	P (%)
Group I	8	20	1.442	0.387	0.261	0.299	68.18
Group II	14	21	1.406	0.357	0.238	0.256	68.18
Group III	10	21	1.251	0.205	0.140	0.156	36.36

Legend: A^t: total number of alleles, A_e: number of effective alleles, He: expected heterozygosity, PIC: Polymorphic Information Content, I: Shannon Diversity Index, P: polymorphic loci rate, N: Number of individual.

3.3. Differentiation of Genetic Groups

The average value of the inter-population differentiation index (Fst) is 0.233; showing that intra-population variation and the rest of this variability (23.3%) explain a large part of the total genetic variability (76.67%) is attributed to inter-population variation. The matrix of genetic distances between populations shows a difference of 0.091 between populations II and III and 0.105 between populations I and III. These relatively low values indicate that populations are genetically similar and belong to the same genetic group (Table 5). Groups II and III are very close genetically to each other (the distance is 0.091, quite close to 0). In contrast, Group III is significantly further away from the other two I and II (distances of 0.103 and 0.105, respectively). The Principal Coordinate Analysis (PCoA) indicates axis 1 (Coord.1) explains 60.69% of the total genetic variation, and axis 2 (Coord.2) explains 39.31% and confirms the hypothesis that groups II and III are genetically close (Figure 3).

Table 5. *Inter-group genetic differentiation of S. macrocarpon.*

Genetic groups	Minimum distance from Nei			Differentiation index (Fst)		
	Group I	Group II	Group III	Group I	Group II	Group III
Group I	0			0		
Group II	0.103	0		0.199*	0	
Group III	0.105	0.091	0	0.257*	0.245*	0

* = Significant.

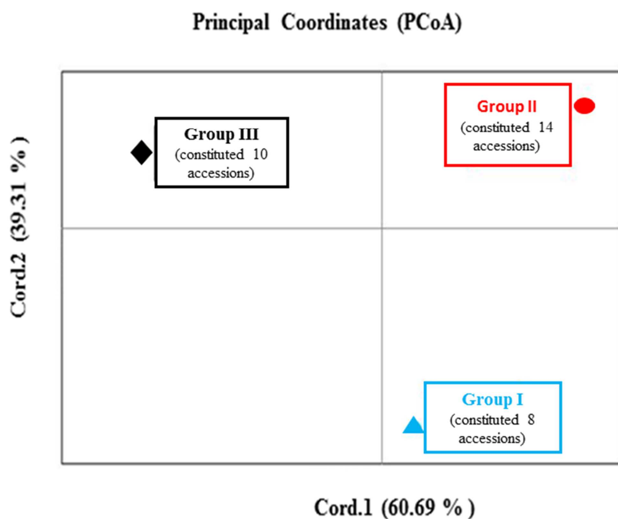


Figure 3. *Representation in the PCoA plan 1/2 of the three genetic groups.*

4. Discussion

With a polymorphism rate of 91.67%, the markers used were informative on the diversity of the accessions studied. Although significantly lower than the rate (97%) reported by the [20], this rate, which is higher than that reported (65.12%) by the [24], testifies to their effectiveness in analyzing the genetic diversity of the *Solanum* genus. On the basis of eleven identified polymorphic SSR markers, a diversity has been identified within the *S. macrocarpum* collection.

The average number of alleles per locus observed (2) is low compared to those obtained by the [18] (2.87) and by the [25] (2.34), respectively on eggplant varieties in India and Spain. This difference could be explained by a low diversity of Burkina Faso's accessions, on the one hand, the size of the sample and, on the other hand, the nature of the markers used. Indeed, the former used the same SSRs markers but on wild genotypes of Brinjal genotypes and the latter used SNPs markers. Although low, the average number of alleles per locus is however higher than that of [26] who reported an average number of alleles of 1.58 in *S. aethiopicum Kumba* group. These differences would be explained by genotypic diversity, sample size and the origin of the plant material. Indeed, the allelic richness of a population is known to depend on the sample size, since the chances of discovering a new allele increase each time a new individual is observed [27]. Similarly, according to the [28], the number of alleles per locus is affected by several factors such as genotype, primer sequences and minor variations in amplification protocols.

The polymorphism information content (PIC), which indicates not only the number of alleles detected but also the relative frequency of these alleles is an important means of estimating genetic diversity [29]. Indeed, the low values of the polymorphic information content (0.359), the average number of effective alleles (1.433) and the expected heterozygosity values ranging from 0.299 to 0.451 could also be due to the low level of diversity within the collection. The same is true of the small genetic distances between the accessions studied, which varied from 0 to 0.68, thus reflecting low genetic variability within the population. These low values observed would also be because the

primers used in the study were developed on the *S. melongena* species and are therefore not species-specific. According to the [30], the diversity revealed in a collection is all the lower as the markers used are developed on another species. The low values could also be explained by genetic erosion of the species caused by the sharp decline in the cultivation of the species in Burkina Faso. Although the area under cultivation of *S. macrocarpom* is reduced to three neighbouring provinces, the genetic basis of the species is comparable to that of *S. eathiopicum* grown throughout the country. Indeed, the value of 0.37 of the expected heterozygosity revealed by this study is significantly similar to that of [26, 31] who observed respectively an expected heterozygosity of 0.34 and 0.37 on a national collection of *S. aethiopicum* using EST-SSRs markers. The low diversity observed on these species can be attributed to the preferential self-pollinating reproduction mode of the genus *Solanum.* In general, in Burkina Faso, the diversity of a cultivated species, especially vegetable species, is revealed from a collection of a single climatic zone [26, 32-33]; this is due to the way seed is managed and the migratory flow of the population. In Burkina Faso, seed exchanges between farmers can lead to long-distance migration of plant material.

The study of the structuring of accessions showed three distinct groups I, II and III with intergroup Fst of 0.199 between II and I; 0.245 between II and III and 0.257 between III and I. According to the [34-35], Fst values between 0 and 0.05 reflect low genetic diversity between subpopulations, moderate when statistical differences are between 0.05 and 0.15; high when they are between 0.15 and 0.25 and very high when statistical differences between subpopulations are more than 0.25. Thus, these generally high values indicate a large discrepancy between the three groups observed.

5. Conclusion

Molecular characterization revealed the existence of genetic diversity throughout the entire collection of *S. macrocarpon* grown in Burkina Faso. It also revealed that diversity parameters remain relatively low due to the species' preferred self-pollinating reproductive mode. A three-group structure was obtained by the Neighbor-joining dissimilarity analysis. Indeed, the assessment of genetic dissimilarity or similarity is important not only for species improvement efforts, but also for the effective management and protection of genetic resources and for breeding purposes, in order to allow the possibility of rapid combination or verification of genetic material. The results of this study may be useful in the case of conservation and genetic improvement of *Solanum macrocarpon* in Burkina Faso.

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