

Original Research Article

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## Evaluation of Genetic Diversity of African Eggplant [*Solanum aethiopicum* (L.) sub sp *Kumba*] Using EST-SSR Molecular Markers

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### ABSTRACT

#### Keywords

*Kumba*, *Solanum aethiopicum*, Molecular diversity, Burkina Faso

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African eggplant (*Solanum aethiopicum* L.) is an important vegetable grown for its leaves and fruits in tropical regions. At Burkina Faso, *Kumba* group is widely used for food and traditional physic. It's also income source for majority population. Despite its socioeconomic importance, there is a few scientific knowledge on its genetic diversity. The objective of this study is to evaluate genetic diversity of *Kumba* group. For this, a molecular characterization of forty-nine (49) accessions collected in the three climatic zones was carried out. The analysis of diversity used EST-SSRs molecular markers revealed moderate genetic variability within the collection, structured into three molecular groups. Indeed, 19 of the 29 markers tested were polymorphic. Expected heterozygosity ( $H_e$ ) for the all collection ranged from 0.075 for smSSR41 marker to 0.507 for smSSR27 and smSSR35 markers. The Shannon diversity index (I), it ranged from 0.163 for smSSR41 marker to 1.307 for smSSR09 marker. The organization of this genetic diversity is weakly influenced by the climatic zone.

### Introduction

African eggplant (*Solanum aethiopicum* L.) has four cultivars groups (*Aculeatum*, *Shum*, *Gilo* and *Kumba*). At Burkina Faso, *Kumba* groups were mainly cultivated and occupied an important place in the food habits of the populations. It's the one cultivation grown widely for its leaves and fruit and is very spread in tropical regions. In fact, the leaves and fruits of *Kumba* are eaten under many forms raw, as ragout and used for flavor. (Daunay *et al.*, 1998; Adeniji and Aloyce,

2012 and Bationo-Kando *et al.*, 2015a) were reported that, it's also used in traditional physic for the treatment of several diseases (diarrhea, vomiting, cholera, hypertension, scorpion and bee stings). Its cultivation is income source for local populations. In view of its socio-economic importance, studies have been carried out in order to lay the foundations for its genetic improvement (Bationo-Kando *et al.*, 2015a, Sawadogo *et al.*, 2016). Unfortunately, most of these studies don't cover the whole territory or were conducted with phenotypic markers that are influenced

by environment factors. The single diversity study using molecular markers was performed by Bationo-Kando *et al.*, (2015b) but only concerns accessions from west region. The present study aims to evaluate the genetic diversity of *Kumba*'s accessions from the three climatic zones of Burkina Faso using molecular markers. Specifically, this involves determining the level and organization of *Kumba*'s genetic diversity using EST-SSR (Simple Sequence Repeat) markers.

## Materials and Methods

### Plant material

Forty-nine (49) local accessions from three climate zones of Burkina Faso were used in this study, which eighteen (18) from Sahelian zone, twenty-one (21) from Sudano-Sahelian zone and ten (10) from Sudanian zone. Three varieties (*Meketan*, *N'Goyo* and *KeurM'BirN'Dao*) were also used. These accessions were sown and grown in a greenhouse in some pots. Always, the pots were regularly watered on morning and evening finally to sure good growth of plant development of seedlings during the test (1 month). The young leaves of each accession were removed, weighed immediately and used for of genomic DNA extraction.

### Methods

#### Genomic DNA extraction

Genomic DNA extraction was conducted at Molecular Biology Unit of Plant Genetics and Improvement Team of University Ouaga I Pr Joseph KI-ZERBO. Young leaves were harvested from 20 days old plants. DNA was extracted from 0.4 g of fresh leaves following by "CTAB" method of Doyle and Doyle 1990 adapted to *Kumba*. Briefly, leaves were ground using a mortar and pestle and dissolved in Tris EDTA Sorbitol (TES). The

samples were centrifuged at 10,000 rpm and 4°C for 10 minutes. A volume of 1000 µl of CTAB at 60°C was added in each sample and all samples were placed during three hours to half in water bath at 60°C before centrifugation. Thus, a volume of 750 µl of CIAA (chloroform-isoamyl alcohol) was added in each sample and all samples were centrifuged during 15 minutes at 10,000 rpm and 4°C. After first centrifugation, the float was collected and put in some new tubes next a volume of isopropanol at -20 °C was added in each sample. Newly, the tubes were centrifuged again during 10 minutes at 10,000 rpm and 4 °C. The pellets of DNA were rinsed with 70% ethanol and centrifuged during 10 minutes at 10,000 rpm. After centrifugation, the floats were removed and cooled at room temperature. The DNA pellets of samples were dissolved in 150 µl of Tris EDTA and put in freezer at -20 °C.

#### EST-SSR marker

Twenty-nine (29) EST-SSRs markers used to study for genetic diversity of *Kumba*. These markers are the same used by Tumbilen *et al.*, in 2011 (Table 1).

#### PCR amplification

PCR amplification performed in 20 µl composed of 2 µl of genomic DNA diluted to 200 X (5 µg / µl), 1 µl of F and R primers, 0.5 µl of dNTP, 2.5 µl of buffer, 0.2 µL of Taq polymerase (1U), and 13.8 µL ultra-pure water. After homogenization, the tubes were placed in a thermocycler for amplification. The PCR program was as follows: Initial denaturation at 94 °C for 5 min, followed by 27 cycles of 94 °C for 30 s, hybridization at temperature (°C) of F primer for 45 s and extension at 72 °C for 45 s. After these cycles, secondly, 8 cycles were added of 94 °C for 30 s, hybridization at temperature (°C) of R primer for 45 s and an extension at 72 °C for

45 s. A final elongation step at 72°C, for 10 min. In fact, 35 cycles were carried in this PCR amplification at 4 °C.

### ***Electrophoresis migration and bands reading***

The results of PCR amplification were separated by electrophoresis (2% agarose gel with TBE) at 80 volts. The gels were stained with BET (Bromide Ethidium), used by standard methods of Sambrook *et al.*, (1989) and statement under ultra violet (UV) light. The DNA ladder (Bioline GmbH, Germany) was used in each gel as molecular size standard.

### **Data analysis**

A binary matrix based on the presence (1) or absence (0) of bands on the gel was performed for profiles of electrophoresis analysis. Markers gave white bands were retained for statistical analysis. Three software packages were used (GenAlEx 6.501, Genetix 4.0.5.2, and Darwin 6.0.4). The genetic parameters were calculated using GenAlEx software in order to evaluate the level of diversity of all collection. The structure of the diversity was carried out with Darwin software. But the Genetix software was used to calculate the genetic distance between the genetic groups formed.

## **Results and Discussion**

### **Kumba's collection diversity**

Molecular characterization revealed moderate genetic diversity in *Kumba's* collection of Burkina Faso. Among twenty-nine (29) markers, nineteen (19) were polymorphic (Table 2). Thirty (30) alleles were identified with mean of 1.582 alleles per marker. For the number of effective alleles ( $A_e$ ) values of 1.080 for smSSR41 marker and 1.988 for markers smSSR16, smSSR21, smSSR27 and

smSSR35 with mean of 1.699 were determined. The value of expected heterozygosity ( $H_e$ ) for whole collection varied from 0.075 for smSSR41 marker to 0.507 for the smSSR27 and smSSR35 markers with mean of 0.355. For all collection, the size of the alleles varies from 50 pb to 450 pb. The Shannon diversity index (I) varied from 0.163 (smSSR41) to 1.307 (smSSR09). The polymorphism information content (PIC) varied from 0.074 for smSSR41 to 0.497 for the smSSR16, smSSR21, smSSR27 and smSSR35 markers.

### **Kumba's diversity according to the "climate zone"**

The Analysis of Molecular Variance (AMOVA) according to Excoffier *et al.*, (1992) showed that the factor "climatic zone" have a significant role in molecular variability expression (10% of the total variance) in *Kumba's* collection (Table 3). The factor "accession" while came to 90% in variability expression. Significant differentiation indices ( $\Phi_{PT}$ ) were observed between accessions of different climatic zones. They varied from 0.087 (Sahelian and Sudano Sahelian zones) to 0.144 (Sudanian and Sudano-Sahelian zones). The genetic diversity parameters have varied the one zone to another zone and were decreasing according to the north-south climatic gradient. In Sahelian zone, expected heterozygosity (0.354) the genetic parameters recorded and Shannon index (0.510) are higher than those of Sudano-sahelian and Sudanian zones. They were also higher in Sudano-Sahelian zone than in Sudanian zone (Table 4).

### **Structuring of Kumba's diversity**

Genetic distances established for all accessions varied from 0.06 to 0.55. This result shown that, there were the lowest amounts of variation among the accessions.

**Table.1** EST-SSRs markers used in this study (Tumbilen *et al.*, 2011)

Marker name	Forward Sequence	Reverse Sequence	Tm (°C)
smSSR01	GTGACTACGGTTTCACTGGT	GATGACGACGACGATAATAGA	55.1
smSSR03	ATTGAAAGTTGCTCTGCTTC	GATCGAACCCACATCATC	54.5
smSSR04	CTCTGCTTCACCTCTGTGTT	CCATGAAAGAGAAGATCGAG	55.2
smSSR09	CACATGGGAACCTACTTACC	GACGACCATCAAACAAGAAT	54.7
smSSR11	AAACAAACTGAAACCCATGT	AAGTTTGCTGTTGCTGCT	54.5
smSSR12	AAACAGAAACCAGAGTACTTCA	CAGAAGAAGGTTTCAGTTTGC	54.3
smSSR14	ATACCACATCAATCCAAAGC	CATCATCATCTTACAGTGG	54.8
smSSR15	CTGTGGTTGCCTTATCAGTA	TAGTCCAAGGGTTTGATGAC	54.4
smSSR16	AAGAATTTGATGTTGAACCG	CTTTATCAGCCAATTTCTGG	55.1
smSSR17	TCTTGCCATTTAATTTCTC	CTATGTCCCTATTATGCCCA	54.8
smSSR18	TTAGGCATTTGATTAGCCT	TATGTCCCTAAGCATAACGG	54.9
smSSR19	GAACAATGATTATCGGATT	AGTTGATGTTGAATTTCCCA	55.2
smSSR21	AAGTTTACATGACAGCACCA	TGCCATCATCAATACCATA	54.4
smSSR22	CTCCGTCAAATTCCTATCAA	GGGAGTCCACATAGAGCATA	55.3
smSSR24	GATTTATGGCTTCTGATGGA	TCCTAACCCACTTGATGAAC	55.1
smSSR27	ATACATTTGAGCCGAGAGTG	TAAATCTGAGAAGGTCGCAT	55.2
smSSR28	CACACTCCTCAGAACTCCAT	CAGCAGTACCTCTGGTCAT	55.2
smSSR29	TCCACTTCAATTTCCAAGTC	GATCGCTTAGCAGAAGCC	55.7
smSSR31	CTTCTACCCACACTTCATC	TAGGCCGGAGATAGTTGTAA	54.8
smSSR35	CACCACAAAGAATTCCTAA	TTGCTAGAAATAGCAAAGGG	55.1
smSSR36	AGCACCAGGACAATGAATAC	CCATTTCTTCTCGACCTTA	54.8
smSSR37	AAAGAAGCTTCCGACGAA	CACTTGTTTCAGCACTTTGA	55.5
smSSR40	TTCTTTGATCTTCAATCCAA	ATGAAGCTGTTTCATGATTCC	55.1
smSSR41	CTCCTCCTGGTAAGGAGTCT	GCAGTATAGAGACGCGAAAT	54.9
smSSR42	ACAGTACACCAGAAACGGAA	GTTACAATGACGGTGGATCT	55.3
smSSR44	TGCATTTCATAACAGAAACCA	GCAAGGATATCACTGAGAGT	55.5
smSSR45	TTTCTCAACCCAAACTGAAC	GCAGCTCTCGCATAGATAGT	55.2
smSSR46	GGAAACCTTCATCACTTCA	AGGTCACCGTTACAATTACG	55.2
smSSR47	ACACGATGATCATAAGGGAG	ATCTAATCACTGTCGCTGCT	55.1

**Table.2** Diversity of parameters of the 19 EST-SSRs markers

Amorces	A <sup>t</sup>	A <sub>e</sub>	H <sub>e</sub>	I	PIC	P (%)
smSSR01	2	1.426	0.289	0.899	0.283	100
smSSR03	2	1.720	0.395	1.142	0.387	100
smSSR04	2	1.451	0.256	0.751	0.250	100
smSSR09	2	1.899	0.471	1.307	0.461	100
smSSR11	2	1.899	0.210	0.657	0.206	100
smSSR14	2	1.827	0.268	0.808	0.263	100
smSSR16	2	1.988	0.470	1.307	0.460	100
smSSR21	2	1.988	0.416	1.194	0.407	100
smSSR22	1	1.786	0.449	0.632	0.440	100
smSSR24	1	1.696	0.419	0.601	0.410	100
smSSR27	1	1.988	0.507	0.690	0.497	100
smSSR31	2	1.964	0.383	1.122	0.375	100
smSSR35	1	1.988	0.507	0.690	0.497	100
smSSR37	1	1.210	0.177	0.317	0.173	100
smSSR40	1	1.550	0.362	0.540	0.355	100
smSSR41	1	1.080	0.075	0.163	0.073	100
smSSR42	2	1.550	0.335	1.003	0.204	100
smSSR44	2	1.451	0.290	0.888	0.284	100
smSSR47	1	1.827	0.462	0.645	0.458	100
Means	<b>1.580</b>	<b>1.699</b>	<b>0.355</b>	<b>0.808</b>	<b>0.341</b>	<b>100</b>

A<sup>t</sup>: total number of alleles, A<sub>e</sub>: number of efficient alleles, H<sub>e</sub>: expected heterozygosity, PIC: Polymorphism Information Content, I: Shannon diversity index, P: loci polymorphic percentage

**Table.3** Analysis of Molecular Variance (AMOVA) of accessions of the three climatic zones of Burkina Faso, with a  $P_{value}$  obtained after 999 permutations

Source	df	SS	MS	Est. Var.	D%
Variance among climatic zones	2	32.385	10.795	0.538	10*
Variance within climatic zones	47	235.884	4.914	4.914	90
Total	49	268.269		5.452	100

Statistics include: df, degrees of freedom; SS, sum of squares; MS, mean squares; Est. Var., estimated variance; and %D, distribution of total variance.

\* $p = 0.0001$ .

**Table.4** Distribution of *Kumba's* genetic diversity following climatic zones

Climatic zones	Intra climatic zones Diversity		Index of differentiation ( $\Phi_{PT}$ )		
	$H_e$	I	Sahelian	S- Sahelian	Sudanian
Sahelian	0.354	0.510	0.000		
S- Sahelian	0.325	0.423	0.087*	0.000	
Sudanian	0.290	0.399	0.113**	0.144**	0.000

He: expected heterozygosity; I: Shannon diversity index; S-Sahelian: SudanoSahelian

**Table.5** Distribution of *Kumba* genetic diversity of genetic groups

Groups	$A_e$	$H_e$	I	PIC	P (%)
Group A	1.592	0.361	0.513	0.348	100
Group B	1.531	0.328	0.471	0.325	93.33
Group C	1.485	0.307	0.404	0.267	70.00
Means	1.536	0.332	0.462	0.313	87.78

$A_e$ : number of efficient alleles,  $H_e$ : expected heterozygosity, PIC: Polymorphism Information Content, I: Shannon diversity index, P: polymorphic loci rate

**Table.6** Nei's distance, index of differentiation for all genetic groups

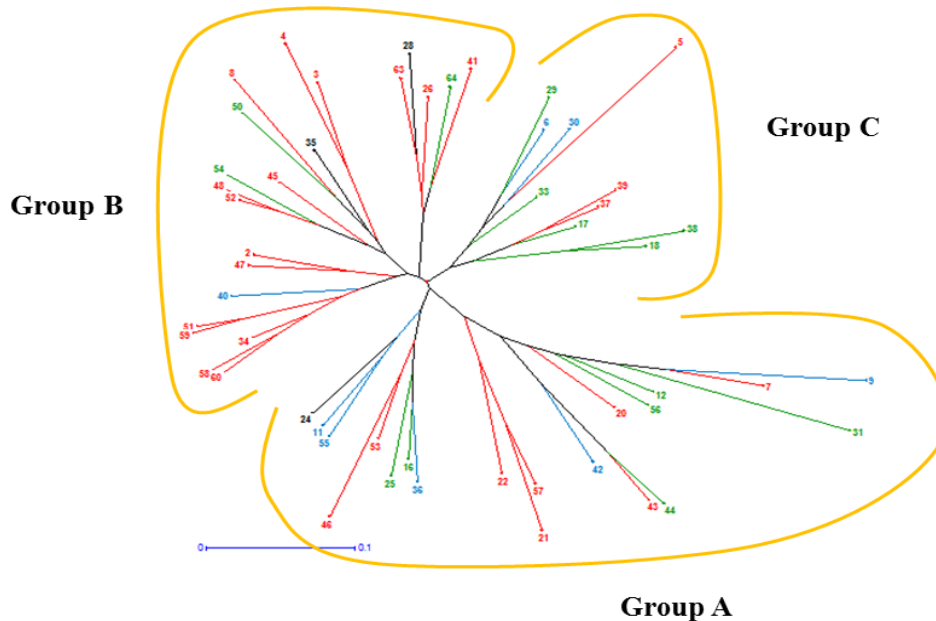
Genetic groups	Nei's distance			Index of differentiation ( $F_{st}$ )		
	Group A	Group B	Group C	Group A	Group B	Group C
Group A	0			0		
Group B	0.033	0		0.1321**	0	
Group C	0.058	0.009	0	0.1549**	0.1715**	0

\*\* : Significant at  $P < 0.01$

Fig.1 Nursery of *Solanum aethiopicum* sub sp *Kumba*



Fig.2 Dendrogram of all accessions constructed from the smSSR data using Neighbour-Joining method



Three groups were formed (A, B and C) for the unrooted neighbor joining tree. Group A has 20 accessions mainly comprises samples from the Sudano-sahelian zone and the improved variety *KeurM'BirN'Dao*. Group B was highly composite only of accessions from different climatic zones and improved varieties *Meketan* and *N'Goyo*. But the group C, composite 10 accessions, and was the most

balanced in terms of number of samples per climate zone (Figure 2).

#### Genetic parameters for genetic groups

The number of effective alleles varied from 1.592 (group A) to 1.485 (group C). The expected heterozygosity ( $H_e$ ) varied from 0.361 to 0.307 for groups A and C

respectively. Finally, the Shannon diversity index and the polymorphism information content varied respectively from 0.513 to 0.404 and 0.348 to 0.267 for groups A and C respectively. In general, the diversity parameters were the highest in group A, but in group C it's the lowest (Table 5).

### **Differentiation of genetic groups**

The minimal distance of Nei of genetic groups were the highest and varied from 0.058 (group A and group C) to 0.009 (group B and group C). Moreover, index of differentiation index ( $F_{st}$ ) revealed highly significant difference for genetic groups; it varied from 0.1391 (group A and group B) to 0.1715 (groups B and C). Therefore, group A was far to group C, but group B was near to group C (Table 6).

Moderate diversity has been observed in *Kumba*'s collection of Burkina Faso. In *Solanaceae*, low diversity parameters on cultivars and intraspecific lines have already been reported by Smulders *et al.*, (1997); Nunome *et al.*, (2003a), Stigel *et al.*, (2008) due to the preferential reproductive kind that is autogamous.

The rate of polymorphism of microsatellite markers tested was 65.5%, it was quasi to value (69.5%) reported by Nunome *et al.*, (2003a) on *S. melongena*. The mean number of alleles (1.58) was lower than that (4.2) reported by Tumbilen *et al.*, (2011), with the same markers used. Similarly, the mean number of effective alleles (1.699) and heterozygosity (0.355) are the lowest to those reported by Nunome and *al.* (2003b, 2009) with the values 3.1 and 0.585 on *S. melongena* respectively. According to Ben Naceur *et al.*, (2008), the number of alleles per locus was affected by several factors such as genotype, primer sequences as well as minor variations in amplification protocols. These differences were depended a both to the

species studied and the nature of the markers tested. Indeed, the collection studied by Tumbilen *et al.*, (2011) was diversified; there were revealed three culti groups of the species *S. aethiopicum*.

The EST-SSR markers used in this study were developed on *S. melongena* and amplified only the coding portions of the gene. According to Zhu *et al.*, (2012), the diversity of a studied collection is lower when the used markers were developed on another species. Also, same recent studies shown on intraspecific diversity studied, that EST-SSRs markers gave the lowest diversity parameters to results of genomic SSR markers reported by Demir *et al.*, 2010, Zhu *et al.*, 2012 Caguiat and Hautea, 2014. However, the polymorphism's rate of each allele (100%) shown that the used markers ability, revealed the genetic diversity of *Kumba*'s collection, but that is lowest. Same results were reported by Adeniji *et al.*, (2012) and Oppong *et al.*, (2015) on the same genus *Solanum*. The value of the PIC (0.347) studied was higher than those of Caguiat and Hautea (2014) with mean PIC = 0.111. The Shannon diversity index (0.808) recorded in this study is lower than that reported by Oppong *et al.*, in 2015 (1.058) on *S. aethiopicum*. The size of the alleles varied from 50 to 450 bp include those values reported by Adeniji *et al.*, (2013) and Ansari and Singh (2014), it was the same value similar to those reported by Nunome *et al.*, (2003b; 2009) and Bationo-Kando *et al.*, (2015b). The differences observed among the genotypes suggest the existence of sufficient inherent genetic variability among its. This variation can be exploited for further improvement of *Kumba*.

Despite the vast area of collection, the diversity revealed in this is the same value reported by Bationo-Kando *et al.*, (2015b). In fact, the result of heterozygosity (0.347) in this study was reported by Bationo-Kando *et*

*al.*, (2015b) on accessions from west of Burkina Faso. At Burkina Faso, the diversity of *Kumba* could thus be revealed from a collection of one climatic zone. This result could be explained by the method of seed management and the migratory flow of population. Indeed, in this country, seed exchange amongst farmers has been reported by previous studies on vegetables (Bationo-Kando *et al.*, 2015a, Kiébre, 2016, Ouédraogo, 2016). According to Diouf *et al.*, (2007), the exchange of seeds amongst farmers can lead to a migration of plant material over distances from 100 to 800 km. was due to migration of plant material that permitted from 100 to 800 km.

The random structuring of diversity, without reference to collection site (province or agro-climatic zone), reminded us of the existence of numerous duplicates in studied samples that is probably also in response to the management mode of the seed.

The massal selection carried out by the producers conducted to cultivate the same morphotypes in several localities. The improved control varieties used in this study are found in genetic Groups A and B. There are possibilities to select morphotypes that meet the criteria of consumer choice.

Molecular characterization using EST-SSR markers of African Eggplant of group *Kumba* grown in Burkina Faso shown moderate genetic variability within the collection. Although the collection included all the climatic zones and it presents a diversity similar to the collection of the western zone of Burkina Faso. The diversity of the *Kumba* of Burkina Faso can thus be highlighted by the one zone of collection. A structure of three genetic groups influenced lowly by the geographical origin of the accessions observed. Groups A and B with improved varieties could be used for the selection of

corresponding varieties of producers and consumers.

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