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GENETIC DIVERSITY OF CULTIVARS OF KUMBA GROUP (*SOLANUM AETHIOPICUM*) ASSESSED BY EST-SSRS

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ABSTRACT

To determine genetic diversity among 43 cultivars of Kumba group (*Solanum aethiopicum* L.), 17 simple sequence repeat (EST-SSR) markers were tested. Nine (9) EST-SSR markers used were polymorphic among the accessions studied. A total of 19 alleles were identified with an average of 2 alleles per marker. Furthermore, the accessions studied showed medium genetic diversity. Clustering analysis grouped the 43 accessions into 3 distinct clusters.

INTRODUCTION

Solanum aethiopicum cultivars of Kumba Group "bitter eggplant", mainly found in warm and semi-arid Sahel areas (Sunseri *et al.,* 2010), are mainly grown in Burkina Faso for their fruit and leaves. This is one of the most popular leafy vegetable. Both fruit and leaves are cooked as sauce and sometimes eaten raw. They are used as a medicine to treat diarrhoea, hypertension, etc., (Adeniji and Aloyce, 2012). The steady increase in demand leads to increasing its production as a cash crop. Fruit and leaves marketing provide significant incomes for the population. Today, eggplant ranks third after tomato and onion, and is followed by okra. In 2013, over 5510 tonnes of eggplants were harvested in Burkina Faso (FAO, 2013). Despite its socio-economic significance, local genetic varieties of this species are still less known, while the adopted enchanced varieties introduced constitute a serious threat to the local ones.

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Indeed, the earlier enhanced varieties yielding beautiful and large pieces of fruit have better agronomic behaviour with farmers. They are therefore preferred to local species which are adapted to a widerange of climatic and soil conditions, including drought and high Temperature. The evaluation of genetic resources is crucial for breeders to produce new cultivars or to further improve the existing ones, according to changing consumer demands or challenges during growth conditions such as resistance attributes.

The analysis of accessions derived from different geographical areas is important to study genetic diversity. In order to assess genetic diversity as well as to discriminate *S. aethiopicum* cultivars morphological approach was used (Bationo, Kando *et al.*, 2015). On the other hand, molecular markers have enormous potential to explore genetic diversity by detecting polymorphisms. They are useful tools for breeding, genotype identification, and the determination of genome organization and evolution in plants. However, despite its widespread cultivation and nutritional and economic importance, the eggplant genome has not yet been extensively evaluated as for the other solanaceous vegetables such as tomato, potato and

pepper. During recent decades, SSR also known as microsatellites became the most popular source of genetic markers owing to their high reproducibility, multi-allelic nature, co-dominant inheritance, abundance, and wide genome coverage. SSR markers have been successfully adopted to analyze genetic diversity of different plant species (He *et al.*, 2003; Frary *et al.*, 2005; Sarıkamış *et al.*, 2006, 2009, 2010). It was long assumed that SSRs were primarily associated with non-coding DNA, but it is now clear that they are also abundant in the single- and low-copy fraction of the genome commonly referred to as genic SSRs or EST-SSRs. A number of SSR markers have been identified in Solanaceae (Yi *et al.*, 2006; Bindler *et al.*, 2007), but not many are developed from eggplant.The aim of the present study was to characterize cultivars of Kumba group collected from different villages of west of Burkina Faso using EST-SSR markers and to assess the genetic diversity within this germplasm.

MATERIALS AND METHODS

Plant Material and DNA extraction

Solanumaethiopicum of Kumba group was represented by 43 different accessions from 24 villages of high production of Kumba in Burkina Faso. The collection site of each accession is shown in Table 1. DNA from each of the 43 accessions was extracted from young leaves using cetyltrimethylammonium bromide (CTAB) Method (Agbangla *etal*. (2002). The DNA concentration was given by direct reading by migration on a 1% agarose gel. DNA samples were then stored at -20°C for further investigations.

EST-SSR marker

Seventeen eggplant SSRs were selected to detect polymorphism and assess genetic diversity of the germplasm (Table 2). The selection of seventeen eggplant EST-SSRs was based on their high polymorphism information content and the quality scores reported (Tümbilen *et al*. 2009). The amplification protocol was the same used by Tümbilen *et al.* (2009), with minor modifications.

PCR reactions were 20 µL total for each sample and were composed of dH2O,1× PCR buff er, 0.2 mM dNTP, 0.2 U Taq Polymerase, 0.6 pmol F primer, 0.6 pmol of R primer, and 3 µL (~5 ng) sample DNA. The amplification program was: 94 °C for 5 min; 94 °C for 30 s, 56 °C for 45 s, 72 °C for 45 s for 27 cycles; 94 °C for 30 s,53 °C for 45 s, 72 °C for 45 s for 8 cycles; 72 °C for 10 min, hold at 4 °C. The amplification products were separated by electrophoresis on a 3% agarose gel with Tris-borate buffer at 80 Volts. The gels were stained with ethidium bromide using standard methods (Sambrook *et al*., 1989) and imaged under ultra violet (UV) light. The DNA ladder (Bioline GmbH, Germany) was used in each gel as molecular size standard.

Genetic Analysis

Nine primers that gave strong, reproducible and clearly detectable bands were used to study genetic diversity of the 43 accessions: smSSR03, smSSR11, smSSR16, smSSR19, smSSR27, smSSR28, smSSSR36, smSSR41. For analysis of smSSR data, each accession was genotyped for each smSSR based on the presence (1) and absence (0) of peaks (bands). Polymorphism (PIC) values provide an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at a locus, but also the relative frequencies of those alleles in the population under study. The PIC for each SSR locus was determined as described by Smith *et al*. (1997). Pair-wise genetic dissimilarities between individual genotypes were calculated using Jaccard's genetic dissimilarity coefficient, followed by cluster analysis with the SAHN subprogram using the unweighted pair group method with arithmetic mean (UPGMA) clustering method as implemented in the software package Power Marker V3.25 (Liu *et al*., 2005).

RESULTS

All 17 mSSRs used to study the diversity of the 43 local varieties of Kumba have been selected for their polymorphism. However, seven (7) did not amplified.

Table 1. Origin, Name and number of Kumba group cultivars used in the study

Climate area	Village	Name and code of accessions	Number of accessions
	Tomsere	TONSERE22 (TON22)	1
	Pâ	PÂ (PÂ30, PÂ33, PÂ 35, PÂ40, PÂ50, PÂ53)	6
	Mussakui	SUMAKUI (SUM20, MUSI6, MUS18)	3
	Dissomkui	DISSAKUI15 (DIS 15)	1
North-sudanian	Gadabou	GADABA2, 3 (GAD2, GAD3)	2
	Banou	BANOU2, 3, 6 (BAN2, 3, 6)	3
	Founa	FOUNA7 (FOU7)	1
	Petit-balle	PETIT BALE1 (PAB1)	1
	Lapara	LAPARA1 (LAP1)	1
	Diebougou	DIEBOUGOU1, 2 (DIE1, 2)	2
	Dolo	DOLO1, 3 (DOL1, 2)	1
	Bafla birifor	BAFLA BIRIFOR2 (BAB2)	1
	Bamako	BAMAKO1, 2 (BAM1, 2)	2
	Nicéo	NICEO1 (NIC1)	1
Sud-soudanian	Navelguen	NAVALGUEN1, 2, 3, 4 (NAV1, 2, 3, 4)	4
	Bafla	BAPLA3 (BAP3)	1
	Gongombiro	GONGOBIRO2 (GON2)	1
	Tomboni	TOMBONI1 (TOM1)	1
	Karba	KARBA1 (KAR1)	1
	Boutoni	BOUTONI2 (BOT2)	1
	Banhoun	BONHOUN4 (BAH4)	1
	Boni	BONI1 (BON1)	1
	Farakoba	FARKOBA1 (FAR1)	1
	Tarpila	TARFILA5 (TAR5)	1
	Simiema	SIEMERINA1, 2,4 (SIEM, 1, 2, 4)	3
	Boullon	BOULLON1 (BOL1)	1
Total	25		43

Table 2. Characteristic of 17 EST-SSR markers used in the study (smSSR = *S. melongena* SSR)

Marker Name	EST Identifier	Repeat motif and Number	Foward Sequence	Reverse Sequence	Left TM	Right Sequence	Product Size (bp)	≠alleles
smSSR01	sgn|E513845	(ATT)21	GTGACTACGGTTTCACTGGT	GATGACGACGACGATAATAGA	55.0	55.3	310	4
smSSR03	sgn|E51460	(TA)9 (GA)8	ATTGAAAGTTGCTCTGCTTC	GATCGAACCCACATCATC	54.8	54.3	145	11
smSSR11	sgn|E515884	(AGC)6	AAACAAACTGAAACCCATGT	AAGTTTGCTGTTGCTGCT	54.5	54.6	126	5
smSSR16	sgn|E518867	(AGA)7	AAGAATTTGATGTTGAACCG	CTTTATCAGCCAATTTCTGG	55.2	55.1	390	3
smSSR19	sgn|E520513	(GAA)6	GAACAATGATTCATCGGATT	AGTTGATGTTGAATTTCCCA	54.9	55.5	241	3
smSSR24	sgn|E515827	(TCA)5	GATTTATGGCTTCTGATGGA	TCCTAACCCACTTGATGAAC	55.2	55.0	229	3
smSSR27	sgn|E516784	(TGT)5	ATACATTTGAGCCGAGAGTG	TAAATCTGAGAAGGTCGCAT	55.4	55.0	184	3
smSSR28	sgn|E517072	(TCA)5	CACACTCCTCAGAACTCCAT	CAGCAGTACCTCTTGGTCAT	55.1	55.3	301	3
smSSR29	sgn|E517168	(CTT)5	TCCACTTCAATTTCCAAGTC	GATCGCTTAGCAGAAGCC	55.2	56.2	188	5
smSSR36	sgn|E517835	(CTG)5	AGCACCAGGACAATGAATAC	CCATTTCTTTCTCGACCTTA	55.1	54.6	231	3
smSSR37	sgn|E517892	(AAG)5	AAAGAAGCTTCCGACGAA	CACTTGTTTCAGCACTTTGA	56.1	55.0	115	3
smSSR40	sgn|E518161	(AAG)5	TTCTTTGATCTTCAATTCCAA	ATGAAGCTGTTCATGATTCC	55.0	55.1	283	3
smSSR41	sgn|E518430	(TCA)5	CTCCTCCTGGTAAGGAGTCT	GCAGTATAGAGACGCGAAAT	55.0	54.8	267	3
smSSR44	sgn|E519591	(CCA)5	TGCATTTCATACAGAAACCA	GCAAGGATATCACTGAGCTG	55.1	56.0	233	4
smSSR45	sgn|E519680	(TTC)5	TTTCTCAACCCAAACTGAAC	GCAGCTCTCGCATAGATAGT	55.3	55.0	172	3
smSSR46	sgn|E519853	(CAC)5	GGAAACCTTCATTCACTTCA	AGGTCACCGTTACAATTACG	55.2	55.2	272	4
smSSR47	sgn|E520160	(AGA)5	ACACGATGATCATAAGGGAG	ATCTAATCACTGTCGCTGCT	55.0	55.1	189	5

Table 3.Parameters of diversity of all accessions tested with 9 EST-SSR polymorphic

Marker	N	Na	He	Ho	PIC	I
SSR03	43	2	0,50	0,77	0,37	0,88
SSR11	43	2	0,48	0,28	0,37	1,34
SSR16	43	2	0,41	0,26	0,33	1,17
SSR19	43	2	0,42	0,35	0,34	1,1
SSR27	43	2	0,20	0,23	0,18	0,54
SSR28	43	2	0,49	0,05	0,37	1,38
SSR36	43	2	0,25	0,16	0,22	0,84
SSR41	43	2	0,18	0,16	0,17	0,59
SSR44	43	2	0,41	0,35	0,33	1,19
Mean	43	2	0,37	0,29	0,30	0,94

N= No of accessions, Na = No. of different alleles,
I= Shannon's Index, He = expected heterozygosity,
Ho = observed heterozygosity

Tableau 4.Nei's distance and genetic Differenciation Intex genetics groups

Genetics groups	Nei's distance			différenciationIndex (Fst)		
	Group 1	Group 2	Group3	Group 1	Group 2	Group 3
Group 1	0			0		
Group 2	0,167	0		0,2417*	0	
Group 3	0,124	0,099	0	0,1191*	0,1950*	0

*: significative

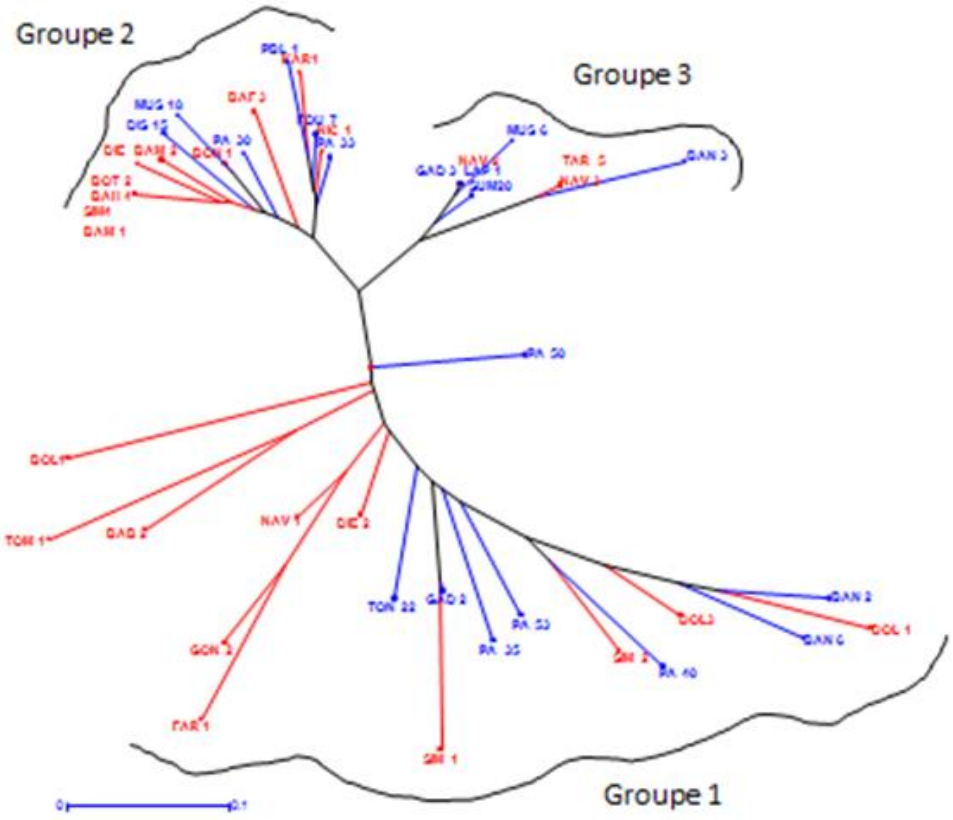

Figure 1. Radial representation of dendrogram constructed from the smSSR data using Neighbour-Joining method. Samples are labelled with accessions code (from Table 1)

These are mSSR01, smSSR24, smSSR29, smSSR37, smSSR44, smSSR45 and smSSR46. Of the 10 that have amplified marker (mSSR47) was monomorphic for all accessions. The other 9 primersrevealed a total of 18 polymorphic loci. The number of alleles per locus was 2 and the polymorphism information content (*PIC*) was ranged from 0.17 to 0.37 with an average of 0.30 (Table 3). Shannon's diversity parameter (I) for total population was equal to 0,432. Heterozygosity values among accessions was 0.35 and ranged from 0.18 (smSSR41) to 0.50 (smSSR03), with a mean value of 0.37. Except for smSSR03 locus, observed heterozygosity was lower than expected heterozygosity. Genetic distances values by pair of accessions vary from 0 to 0.72 (data not shown). This indicated that there was a low amount of variation among the accessions. The unrooted neighbour-joining tree obtained with whole accessions exhibited except PA50 three clusters distinct from geographical factors (Figure 1). The first group consisting of 18 accessions.

This group can be subdivided into two subgroups. It is very distant from the other groups. The second group, consisting of 16 accessions. It has the largest number of redundant genotypes. In most cases, these genotypes (identical) have different geographical origins. The third group formed by only 8 accessions. The genetics parameters among the three genetic groups based on the dissimilarity matrix according Neighbour-Joining method are shown in Table 4. Group 1 which displays a number of effective alleles of 1.89, a percentage of polymorphism of 94.4% an expected heterozygosity of 0.383, a Shannon diversity index 0.536 and 0.365 in a potential polymorphism information shows the parameters the highest genetic. Group 2, with a number of effective alleles of 1.55, a percentage of polymorphism of 77.8%, an expected heterozygosity of 0.196, a Shannon diversity index of 0.301 and 0.184 in a potential polymorphism information has the lowest genetic parameters while the group 3, excepted percentage of polymorphism and number of effective alleles, contains genetic parameters between those the two other groups. The genetic differentiation values between three genetic groups are high (Table 4). The largest Nei's distance minimum of 0.167 and largest index of differentiation (Fst) of 0.242 were observed between groups 1 and 2 while lowest values were observed between the groups 2 and 3.

DISCUSSION

The present study revealed the genetic diversity within a collection of Kumba group germplasm representing different villages of Burkina Faso, using molecular markers (smSSR) approach. In solanaceous plants, a low frequency of polymorphism among cultivars and intraspecific lines has been reported (Smulders *et al.*, 1997; Nunome *et al*., 2003; Stàgel *et al.*, 2008), probably due to its autogamous nature. Genic mSSRs selected on the basis of high polymorphism information content (Tümbilen *et al.*, 2009) successfully helped discriminate accessions in the present study. In this study, the mean number of alleles per smSSR locus (2) and18 loci detected in Kumba group were lower than that detected by Tümbilen *et al.* (2009) (4.2 alleles for 38 loci) with the same EST-SSRs on *S. aethiopicum* and Demir *et al.* (2010), Sunseri *et al.* (2010) on *Solanumelongina* and Benor *et al.* (2008) on

Solanumlycopersicum using SSR. However, He *et al.* (2003) found an average of 2.7 alleles per locus and 49.2% of these loci had two alleles on *Lycopersicum esculentus.* The high genetic diversity revealed by these other may be attributable to the origin and diversity of materiel vegetable. For example, the accessions used by Tümbilen *et al.* (2009) belong to three different cultigroup of *S. aethiopicum* (Kumba, Aculeatum, Gilo) as defined by Lester and Niakan (1986). Another factor that influenced the reported diversity level is the use of EST-SSRs.

A few studies have shown that EST-SSRs were less polymorphic than genome-SSRs in wheat (Eujayl *et al.*, 2002), rice (Cho *et al.*, 2000) and grape (Scott *et al.*, 2000). Our results from Kumba cultivars were similar to these studies indicating that EST-SSRs provided less genetic diversity information than genome-SSRs. The PIC value 0.17 to 0.37 with an average value of 0.30 is comparable to previously SSR reports on *Solanumlypersicum* collections (Benor *et al.*, 2008), while, He *et al.* (2003) reported 0.09 to 0.67 as PIC for *Lycopersicum esculentus*. Compared to data obtained for other species as *Pisum sativum* (Raman *et al*., 2005; Gong *et al.*, 2010), this PIC value indicating that Kumba represents a rather polymorphic autogamous species. The observed and expected heterozygosity values averaged 0.30 (0.17- 0.37) and 0.37 (0.18 – 0.50) respectly, indicate a moderate genetic diversity of cultivars of Kumba Group Burkina Faso also due to the mode of reproduction preferentially autogamous of the plant (Seka *et al.*, 2007; Oyelana et Ogunwenmo, 2012).

The random genetic diversity structure, without any reference to the collection site (Village, Province, Department, agro-climatic zone), the low dissimilarity between accessions (0-0,72) and the existence of many duplications within the groups in the sample studied probably in response to the seed management method. Indeed, Bationo Kando *et al.* (2015) had shown that, producers swap plant material either directly (donation) or indirectly in markets. Similarly, mass selection carried out by the majority of producers (62% of producers) can contribute to reduce variability within the same village/province. However, the various groups obtained provides for alternatives in selecting genitors for the creation of new varieties to meet the needs of producers. In conclusion, the cultivars reported here displayed medium genetic diversity. Both the genetic relationship information and heterozygosity information among the cultigroupe Kumba revealed by this study could be very helpful in future bitter eggplant breeding programs to improve fruit yield and maintain broad genetic diversity. Moreover, it is very important for bitter eggplant germplasm conservation that these accessions could be identified unequivocally using SSR markers.

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