

Full Length Research Paper

The use of cross species SSR primers to study genetic diversity of okra from Burkina Faso

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Sixteen pairs of primers designed to amplify SSR regions of *Medicago truncatula* were used to amplify genomic DNA samples of 20 different okra accessions collected from different regions Burkina Faso. These primers amplified a number of fragments that range from 1-16 with the sizes of 396-506 bp. Each accession was scored for the presence or absence of the bands and phylogenetic analysis of these data clustered the 20 accessions into five different groups. Two okra accessions were distinctly different from other 18, based on the molecular marker as well as on morphological features of their fruits. One of the primers, MT-27 amplified a unique 440 bp PCR product in these 2 okra accessions. This PCR product was sequenced and based on the sequence information, sequence specific primers were designed to PCR amplify the genomic DNA of all the okra accessions. This pair of primer amplified PCR products only in the two okra accessions where the amplification of the PCR products was seen with MT-27 primers. Our data indicate that cross species SSR primer developed for *Medicago truncatula* can also be used to analyze genetic diversity in unrelated species, like Okra.

Key words: Okra, SSR, genetic diversity.

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is one of the important vegetable crops in tropical, subtropical and Mediterranean regions of the world (Lamont, 1999; Hammon and Van Sloten, 1989; Duzyaman, 1997 and 2005). The centre of origin of okra is uncertain, but centre of diversity exists in West Africa, India and South East Asia (Charrier, 1984; Hammon and Van Sloten, 1989). Efforts have been made by breeders to select high yielding varieties such as seed yield, number of pods per plant, pod length and pod width. Considerable genetic variation exists in West African okra suggesting lot of out crossing among the taxon (Ariyo and Odulaja, 1991). Genetic characterization of any crop species is one of the important aspects of crop improvement. For this purpose, molecular biological techniques like AFLP, SSR, RFLP,

RAPD are mostly widely used (Santoni et al., 2000, Semagn et al., 2006; Ellis and Burke, 2007). Indeed considering their great number, their distribution in the genome, their accessibility and the cost of their analysis, these markers and more specifically the SSRs can be regarded as the most adapted for the characterization of accessions of a given crop plant species (Agrama and Tuinstra, 2003). There are reports of diversity study in okra that used morphological markers (Martinello et al., 2001; Sawadogo and Balma, 2003; Sawadogo et al., 2006). These markers are not stable, since they are affected by environmental conditions. Besides, their number is also limited. In contrast, molecular markers are stable and plenty, which could be used to identify unique genotypes or linked to specific agronomic traits. However, only two reports have used molecular biological techniques to study genetic diversity in okra (Martinello et al., 2001; Gulsen et al., 2007). Hence, it is important to study the genetic diversity in okra in general and more importantly, diversity in the burkinabé ecotypes of Burkina

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Table 1. Plant material collection from different agro ecological zones of Burkina Faso.

No.	Code/collection	Province of adaptation	Country
1	UAE40	Sahelian zone	BF
2	UAE38	Sahelian zone	BF
3	UAE39	Sahelian zone	BF
4	UAE37	Sahelian zone	BF
5	UAE41	Sahelian zone	BF
6	UAE42	Sahelian zone	BF
7	UAE43	Centre south	BF
8	UAE44	Centre south	BF
9	UAE45	Centre south	BF
10	UAE47	Centre south	BF
11	UAE48	Centre south	BF
12	UAE03	Mouhoun zone	BF
13	UAE01	Centre south	BF
14	UAE19	Centre south	BF
15	UAE22	Central region	BF
16	UAE33	Centre north	BF
17	UAE34	Centre north	BF
18	UAE35	Centre north	BF
19	UAM02	Centre north	BF
20	UAE31	Centre east	BF

kina Faso, The latter have not been previously subjected to molecular analysis using SSRs. This study could offer us better understanding of determining the genetic diversity of the okra varieties for our breeding programs. According to Sawadogo et al. (2006), okra is characterized by a diversity of the form and color of fruits and stems. It is a vegetable which one finds in a fresh state in all the markets of Burkina Faso during the rainy season and in a dehydrated form (sections, discs or powder) during the dry season due to its high mucilage content, its strong commercial value for poor women farmers and its vital importance as food diet of the populations of the cities and the campaigns. There are even varieties which are recommended to facilitate or enrich the nutrition for patients. Okra takes on considerable economic importance for women farmers in particular and plays an essential part in the nutritional balance of the rural populations in general. In addition, improved varieties of okra seem to be the last concern of breeders who are unaware of it in their research programs.

DNA sequence information of okra is limited. Gutierrez et al. (2005) reported that SSR primers from *Medicago truncatula* could amplify microsatellites from other legumes. The objective of this work is to explore the possibilities of using the SSR primers from *Medicago* to PCR amplify products from okra DNA samples. Our plan was to apply this technique to okra crop improvement programs with the aim to providing for the needs of rural

populations in term of varieties with higher yield.

MATERIALS AND METHODS

Plant material

Twenty accessions of okra cultivars collected by the breeding team of Malvacee at the University of Ouagadougou, that were maintained pure breeding for two years were used for this study. These accessions were collected from various origins (Table 1). Some of these ecotypes have been submitted to a participatory variety selection (Witcombe and Joshi, 1996; Weltzien et al., 1998) in three agro-ecological zones of Burkina Faso (Table 1). A total of sixteen microsatellite primers of *Medicago* used were used in out analysis and these are listed in Table 2. The table shows the sequence of microsatellite primer pairs for each locus, linkage group in *M. truncatula*, repetitive motif, and references (Gutierrez et al., 2005).

Genomic DNA isolation

Okra accessions (Table 1) were grown in the greenhouse at the Department of Biology, University of Virginia. Each pot had three seeds per pot. Fresh young leaves from two week old plants were collected and immediately frozen in liquid nitrogen and stored at -70°C until used for DNA isolation.

Total genomic DNA was extracted from leaves using DNAzol (MRC Inc, Cincinnati, Ohio) as described by the manufacturer with some modifications. After isopropanol precipitation of DNA, the pellet was washed with 70% ethanol, air dried and dissolved in 100 - 200 µl of TE (pH 8.0). DNA was stored at 4°C until desired. Quality of isolated DNA was checked by agarose gel electrophoresis (Sambrook et al., 1989). DNA was quantified spectrophotometrically and working solutions of DNA were prepared at the concentration of 100 ng/µL. 100 ng of DNA was used for each 25 µL PCR mixture (Gowda et al., 2002). PCR was performed as follows: initial denaturation at 94°C for 1 min followed by 35 cycles of 94°C for 1 min, 45°C for 1 min and 72°C for 2 min. Final extension was at 72°C for 10 min followed by incubation at 4°C. PCR products were resolved on 2% agarose gel.

Data analysis

The PCR amplified SSR fragments were visually scored as either present (1) or absent (0) for each accession with each primer combination. The binary matrix was then used to measure pair-wise genetic distance using Nei's (1978) unbiased genetic distance within XLSTAT version 6.5. A dendrogram, showing the genetic relationships between accessions, was constructed from the pair-wise genetic distance values.

Sequencing of specific fragments

DNA fragments unique to cultivars were isolated from the gel, sub-cloned and positive clones were sequenced according to Gowda et al. (1999, 2002). Different clones were sequenced on an ABI 310 automated sequencer (Applied Biosystems, Inc, Foster City, CA) using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems Inc, Foster City, CA) as recommended by the manufacturer.

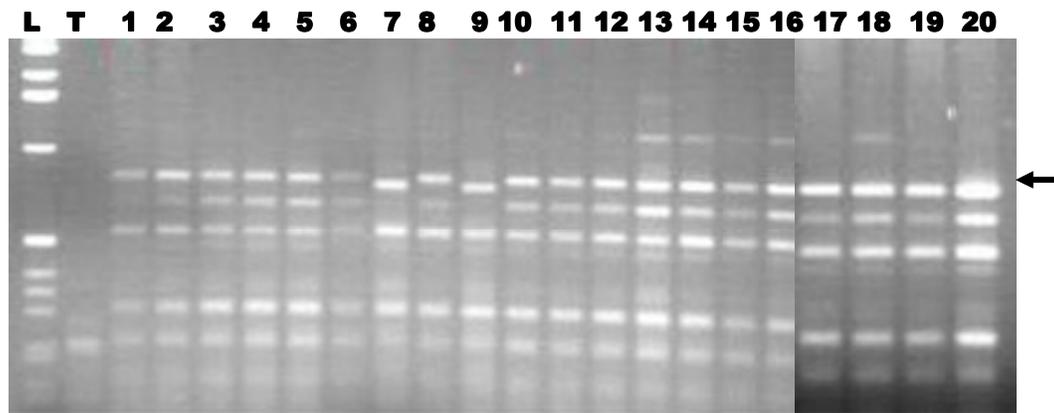
RESULTS AND DISCUSSION

Utility of microsatellites in okra diversity

Microsatellite related primers from *medicago* successfully

Table 2. List of *Medicago truncatula* EST and BAC microsatellite primers used in the present study.

No MTIC	Primers (5' - 3')		Linkage group	Repetitive motif	Reference
	Forward	Reverse			
3	TGGTGACGACATACAAGAAAAGA	CCCGGTGGTTTAGGAAGTTT	4	AAC]5	H
7	ACCACTTCTCCATCCATCCA	AGCTTGCTGCATGAGTGCT	/	[AAC]6	Julier et al.2003
8	CAAAGGCACTTCATCAGCAA	GTGAGCGTCAATGTTGGATG		[AAC]5+6	H
20	TGAAGGTCAAATTGCCAAGA	TCCTTGTTTTTGAAGGTCACG	/	[AAG]5	H
27	CGATCGGAACGAGACTTTA	CCCCGTTTTTCTTCTCTCCT	2	[AAG]6	Julier et al.2003
35	GAAGAAGAAAAGAGATAGATCTGTGG	GGCAGGAACAGATCCTTGAA	7	[AAG]8	Julier et al.2003
55	CAGTTCGGGAAGAGGACAAA	ATCCCAAACCAGGTTCTTCA	3	[AAG]6	H
62	TTCCGCCATAGTCTTTGAC	TGAAAGGGCTTAGAGGGTTTT	4	[AT]10	H
74	GGTGAAGGAACAACTCTGG	CCGGCATGATTAAGACACAC	2	[AT]16	H
82	CACCTTCCACTCAAACCA	GAGAGGATTCGGTGATGT	7	[TC]11	Julier et al.2003
95	AAAGGTGTTGGGTTTTGTGG	AGGAAGGAGAGGGACGAAAG	/	[TCC]6	Julier et al.2003
96	CCAGTGGCAGCTACGGTACTA	GAGACGGAGGAGAAGTTGCTT	5	[TCC]6	H
103	TGGGTTGTCCTTCTTTTTGG	GGGTGCAGAAGTTTGACCA	8	[TG]5	Julier et al. 2003
107	CAAACCATTTCCTCCATTGTG	TACGTAGCCCCTTGCTCATT	1	[AC]5	Julier et al. 2003
135	GCTGACTGGACGGATCTGAG	CCAAAGCATAAGCATTCA	8	[AG]10	Julier et al. 2003
136	TTTGTGTGCGAGAGATGCACA	CTTGAACTTCAACGGCATT	3	[AT]5	H

**Figure 1.** Diversity of the bands of 20 okra accessions with microsatellite MT35, L –1 Kb ladder, T – control –no DNA), lanes 1-20 are accessions numbered as per Table1. Arrow –group of specific bands to the varieties in position 13, 14, 15, 16 and 18.

PCR amplified genomic DNA of okra. The number of fragments amplified ranged from 1 to 10 and their size ranged from 396 to 506 bp (Figures 1 - 3). On an average, 4.88 bands were amplified per SSR primer (Table 3a). In addition, the diversity of bands varied according to the SSR primer used as well as according to the accessions used. Indeed Table 3b shows that varieties UAE48, UAE45, UAE42 and UAE39 have more than 70% of the identified bands while varieties UAE44, UAE03 add up less than 21%. Some of the bands were unique to certain varieties (Figures 1 and 3). One of the DNA fragments amplified by primer MT35, (B1 in Figure 1) is unique to only five varieties (13, 14, 15, 16 and 18), which makes it to distinguish them from remaining 16

varieties.

MT55 amplified three different bands, two of them (B2) are present only in 7 and 9 (UAE43 and UAE45 respectively) while other 18 have another band (B2) unique to them (Figure 2). In a somewhat similar fashion, MT27 amplified only one band unique to 7 and 9 (UAE43 and UAE45, respectively) as shown in Figure 3. Both varieties are unique according to the form of fruits, the presence of hairs on fruit, dark green fruit and their growth cycle very long.

So far UAE 45 is most distant by the analysis of the dendrogram. This analysis is supported by the observation of the polymorphic bands obtained with MT55 (2). Indeed, these bands show that the accession no. 7 (UAE

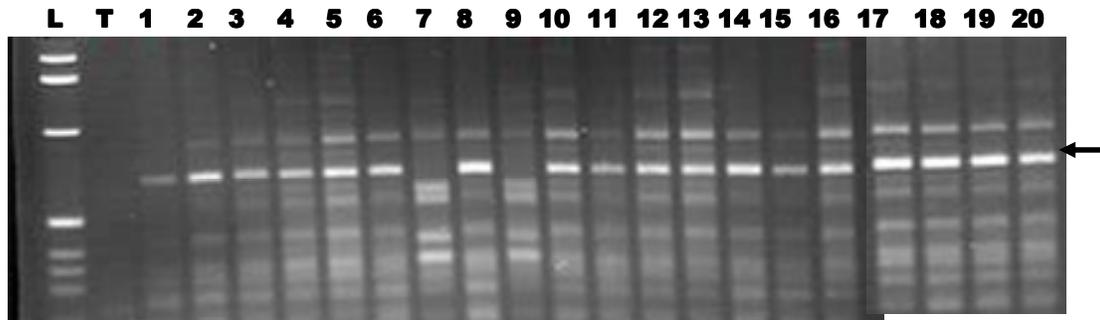


Figure 2. Diversity of bands of 20 okra accessions with microsatellite MT55. L – 1 Kb ladder, T – control no DNA), lanes 1-20 are different accessions numbered as in Table 1. Arrow – are band that distinguishes two cultivars from other 18.

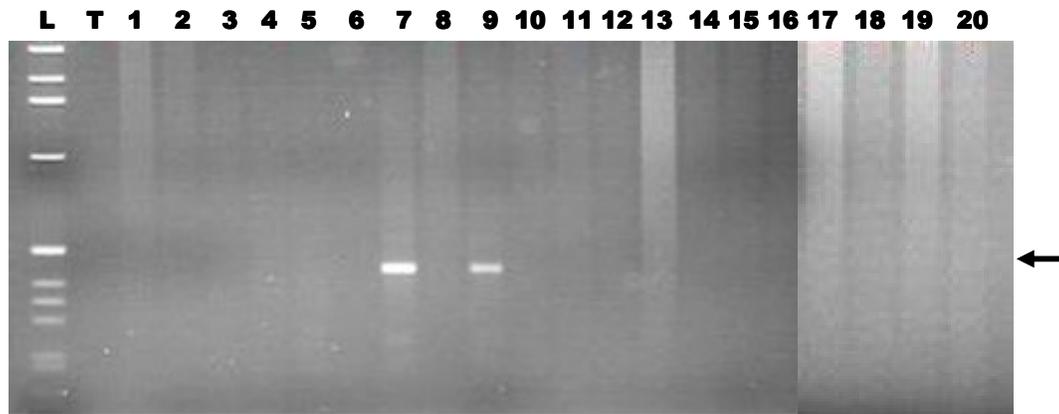


Figure 3. Diversity of the bands of 20 okra accessions with microsatellite MT27. L – 1 kb ladder, T – control- no DNA), lanes 1-20 – accession numbers. Specific bands in UAE43 and UAE45 is shown by arrow.

Table 3a. Distribution of the number of locus identified with the sixteen microsatellites.

	Bands (b)				
	NB/MT	396≤b<506	b=506	506<b<1010	b≥1010
Min	1	0	0	0	0
Max	10	5	3	4	1
Means	4.88	2.44	0.88	1.25	0.31
ET	2.16	1.46	0.81	1.44	0.60

43) and no. 9 (UAE 45) have a double band and these two bands were absent in 18 other accessions. Similarly, MT 27 amplified a DNA fragment each in accession number 7 and 9, but were absent in all other 18 accessions. The two fragments were considered for sequencing, since they appeared unique to the two accessions. Sequences of the bands from cultivar 7 and 9 were compared and found to be identical and were 440 bp long. There were differences in the SSR primer binding regions (Figure 5). There was an additional T in the

MT27F binding region and there was an additional C in the MT27R binding region (primer regions are shown above below the sequence). There were no SSRs in this 440 bp fragments.

The stratification and classification of the okra accessions

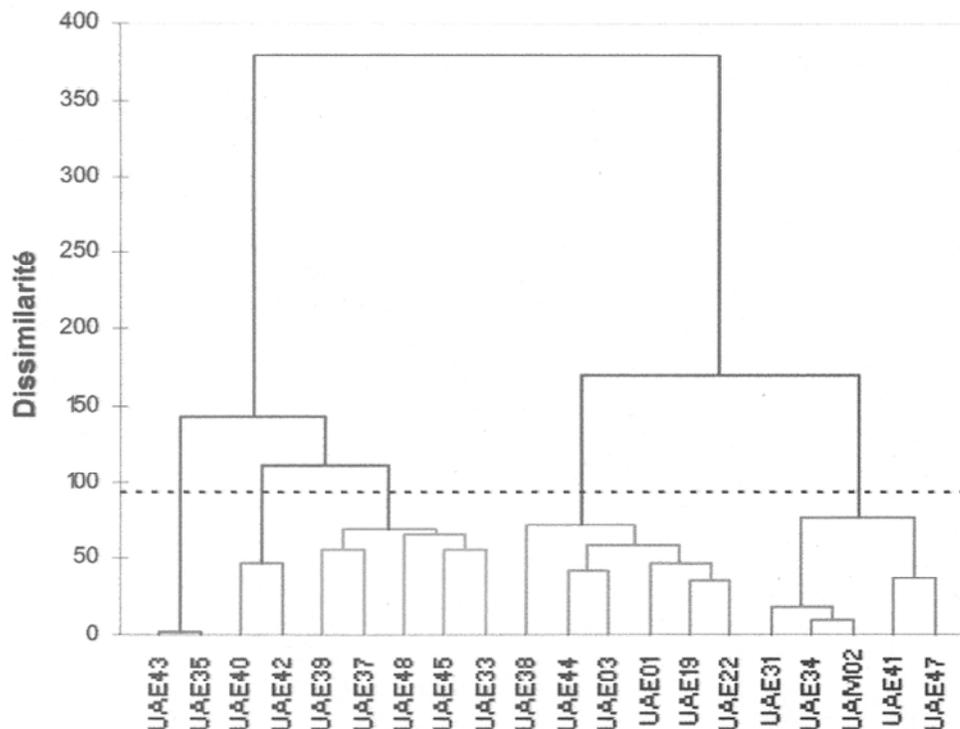
A phylogenetic tree constructed on the basis of dissimilarity to 95% between the varieties made it possible to

Table 3b. Percentage of bands presented by variety.

Accession	UAE40	UAE38	UAE39	UAE37	UAE41	UAE42	UAE43	UAE44	UAE45	UAE47
%Bands	62.03	31.65	70.89	62.03	35.44	72.15	48.10	12.66	73.42	26.58
Accession	UAE48	UAE03	UAE01	UAE19	UAE22	UAE33	UAE34	UAE35	UAM02	UAE31
%Bands	83.54	20.25	31.65	25.32	30.38	64.56	31.65	46.84	30.38	32.91

Table 4. Distances between the central varieties of the 5 groups of okra make up.

	1 (UAE40)	2 (UAE44)	3 (UAE45)	4 (UAM02)	5 (UAE43)
1 (UAE40)	0	6.245	5.745	6.083	5.916
2 (UAE44)	6.245	0	7.746	5.099	6.000
3 (UAE45)	5.745	7.746	0	6.782	5.657
4 (UAM02)	6.083	5.099	6.782	0	6.164
5 (UAE43)	5.916	6.000	5.657	6.164	0

**Figure 4.** Ascending hierarchical classification of the 20 okra accessions.

classify the 20 okra varieties into five (5) distinct classes namely 1, 2, 3, 4 and 5 with individual central objects UAE 40, UEA 44, UAE 45 and UAMO2 and UAE45, respectively (Table 4). Distances between the central objects show that variety UAE 44 (central object of class 2) and Var UAE 45 (central object of class 3) are most distant with 7.746 of distance. They are subjected by the couple of accessions UAE 45-UAMO2, 7.746, UAE 40-UAE 44 (6.245) and UAMO2-UAE 43 (6.164 ud).

This information gives us distance between these

accessions, shows a possibility for okra crop improvement in the case that a better recombination could be obtained by crossing UAE 45 with accessions UAE 44 and UAMO2 on the one hand, and on the other hand crossing UAE 44 with UAMO40, and UAMO2 with UAE43. Accession UAE45 seems most distant from all others and could constitute a source of genes for the improvement of all the other accessions. In addition, we note a perfect resemblance on the level of the polymorphism of the microsatellite markers between accessions

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1                               60
CCCCGTTTTTTCTTCTCTCCT
CCCCGTTTTTT . CTTCTCTCCTTAAACTTCTTCAATCTTCTTTATTGAGTCGAGTTTCATGA

61                               120
GCTATTAACACTACTGAGTTGTTATTCCAACAACCGGTGAAACAATGAGCTCAAAGGATC

121                              180
ATTCAAAGAATTAGGAGCACACAACGTATTTCTTACTTAGTGATTTTTTGGAGAATCGAAG

181                              240
GTATTCTTCATGCTTTTGTCTTATTTAATCACTTTTTGGTCATTTTTTTAGAGAATCGA

241                              300
AGGTGTTCTTCATCATTTTCGTTCTTCTTTAATCACTTTTCTGTTATACAATTTATTTTCA

301                              360
TTCGAAATTCCTTTCTAAAAATTATTATCTTTCATCCTTATTGTCTTCTTTCACTCCTTAT

361                              420
TTGATCTTTCTTTTTTGTGGGAGCAAGGCGGTTAAGATACAAATTCCTGATTTAATTTA
AT

421                              440
AAGTCCTC . GTTCCGATCG
TTCAGGACCCAAGGCTAGC

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Figure 5. Sequence of MT27 product from accession 7.

UAE43 and UAE35 though these two varieties have different origin (Table 1). That confirms the similarity of names given to these two accessions according to the shape of the fruit which could be translated into “okra with fruit like cheek of cat”. Analysis of the dendrogram also shows in class 4 the presence of UAE22 and UAE19 which are closely related confirming our previous report (Sawadogo et al., 2006).

Polymorphism of the bands

Of the 16 SSR primers used, the number of polymorphic bands is very variable depending upon the accessions. Indeed, MT35 and MT55 (Figures 1 and 3) showed much more bands than any other primers. On the other hand, MT27 amplified only one band (Figure 3) and it was present only in two accessions: UAE 43 and UAE 45 (no. 7 and 9). This constitutes an important distinction between these two accessions and the other 18 accessions. This analysis is also supported by the observation of the polymorphic bands obtained with MT 55 (Figure 2). In this case accessions no. 7 UAE 43 and no. 9 (UAE 45) have a double band and that these two bands are absent in the other 18 accessions.

The unique bands from accessions no. 7 and 9 amplified by primer MT27 had similar sequences. There were single nucleotide additions in the primer regions (Figure 5). Blast searches for homologs of this sequence in GenBank did not show high homology any known sequences. Based on these sequence information, specific primers were designed to amplify the genomic DNA of

different okra accessions. The primers amplified PCR products only in accession no. 7 and 9. This appears to be good marker for identifying these two accessions from others just by PCR of genomic DNA. In summary our data demonstrate that SSR primers from Medicago can be successfully applied for characterization of okra accessions and this diversity information used in our future breeding programs.

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