Indian Journal of Agricultural Sciences **85** (9): 1223–8, September 2015/Article

SSR based genetic diversity in *Abelmoschus* species

RANBIR S FOUGAT[1], ATUR R PUROHIT[2], SUSHIL KUMAR[3], MITHIL J PAREKH[4] and MUKESH KUMAR[5]

Anand Agricultural University, Anand, Gujarat 388 110

Received: 5 May 2015; Accepted: 18 June 2015

ABSTRACT

Genetic diversity analysis and germplasm characterization are essential steps in plant breeding and molecular markers are proved tool to accomplish. In spite of its high economic value of okra as fresh vegetable in tropical and subtropical regions of Asia and Africa, little attention has been paid to assess its genetic diversity at molecular level. In present study, genetic diversity analysis was performed on 24 *Abelmoschus* accessions using SSR markers. A total of 85 amplicons were detected from 18 SSR markers. Amplicon analysis revealed a very high level (93.72%) of polymorphism with a mean polymorphic information content (PIC) value of 0.53. Number of alleles scored per primer ranged from 2 to 15 with an average of 4.72 alleles per primer. The UPGMA cluster analysis grouped *Abelmoschus* genotypes into three main clusters at a cut off value of 0.62. In conclusion, SSR markers enabled discrimination among accessions and provide valuable information for future use in improvement of this genomic resource poor otherwise important vegetable crop.

Key words: Diversity, Marker, Okra, PCR, SSR

Okra [*Abelmoschus esculentus* (L.) Moench] is known to have originated in India but cultivated throughout tropical and subtropical low altitude regions of Asia and Africa with very poor productivity. *A. esculentus* is a cultigens of uncertain origin, but with pan (sub) tropical distribution. Cultivated okra and related wild species were originally included in the genus *Hibiscus*, section *Abelmoschus*, of the family Malvaceae. Most probably okra, with considerable variation in the chromosome numbers and ploidy levels, is an amphiploid (allotetraploid) derived from *A. tuberculatus* (2n=58) and *A. ficulneus* (2n=72) (Bisht and Bhat 2006). Involvement of *A. esculentus* (2n=72) race in the cultivar evolution cannot be completely discarded. Within *A. esculentus*, chromosome numbers 2n=72, 108, 120, 132 and 144 are derived as part of a regular series of polyploids with a basis chromosome number of 12 (Datta and Naug 1968).

The availability of genetic diversity and its successful collection, maintenance, utilization and conservation is pre-requisite for crop improvement program (Poehlman and Sleper 1995). The variability among germplasm can be assessed by using different morphological and molecular markers. In okra, morphological variability had been assessed by several researchers (Ariyo *et al.* 1993, Aladele 2009, Osawaru *et al.* 2013). Despite, broad genetic base within *A. esculentus* (Saifullah *et al.* 2010) it is still difficult to discriminate genotypes based on their phenotype. Moreover, phenotypic based selection and genetic analysis, which does not reliably reflect true genetic diversity, are highly affected by environment, quantitative inheritance of traits, partial and dominance trait expression (Saifullah *et al.* 2010, Akash *et al.* 2013). Many of these complications associated with phenotype based assay can be easily overcome through DNA based molecular markers.

With the advent of recent methods in molecular biology, different molecular markers have been applied for the study of molecular diversity in okra. Few of these are random amplified polymorphic marker (RAPD) markers (Martinello *et al.* 2001, Aladele *et al.* 2008), inter-simple sequence repeat (ISSR) markers (Yuan *et al.* 2014), amplified fragment length polymorphism (AFLP) markers (Akash *et al.* 2013) and sequence related amplified polymorphism (SRAP) markers (Gulsen *et al.* 2007). Simple sequence repeats (SSRs) markers which are abundant, multi-allelic, highly polymorphic and co-dominant have been seldom used in okra (Sawadogo *et al.* 2009). Hence, SSR markers may be expected to be very powerful in okra cultivar discrimination. Recently, Schafleitner *et al.* (2013) has reported 935 non-redundant SSR motifs identified in the unigene set through combined leaf and pod transcriptome analysis of okra. In the present study, SSRs developed in *A. esculentus* have been exploited for germplasm diversity analysis in *Abelmoschus* species.

MATERIALS AND METHODS

A set of 21 okra accessions and 3 accessions of wild

[1]Professor and Head (e mail: biotech.aau@gmail.com), [2]M Sc Scholar, [3]Assistant Professor (e mail: sushil254386@yahoo.com), [4]Ph D Scholar, [5]Junior Research Fellow, Department of Agricultural Biotechnology

species were evaluated in this study for genetic variation. The DNA was extracted using the protocol of Kochko and Hamon (1990). The main hindrance preventing the extraction of DNA from green leaves of okra is high concentration of polysaccharides and mucilage produced during photosynthesis which results in very sticky suspension. Moreover, this viscous and glue-like texture makes the DNA uncontrollable during pipetting and inhibits the Taq polymerase activity (Haq *et al.* 2013). In order to avoid this difficulty, seedling were raised in plastic cups under dark condition to minimize photosynthesis. The yellow and etiolated fresh leaves were collected from 10-14 day old seedlings for DNA extraction. After extraction, DNA was quantified on Nanodrop N.D.1000 (V.3.3.0, Thermo Scientific, USA) and integrity was assayed through ethidium bromide stained 0.8% agarose gel by visual comparison with λ DNA standard (Invitrogen, USA). This DNA was subsequently diluted to 10ng/µl for polymerase chain reactions (PCR).

PCR reactions for SSR were carried out in a final of volume of 10µl containing 20 ng template DNA, 1× PCR buffer, 0.2 mM dNTPs, 0.5pM of each primer, and 0.1 U Taq polymerase (Dream Taq, Thermo Scientific, USA). The PCR temperature profile was initial denaturation of 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, ΔT °C (primer specific) for 45 s, 72 °C for 60 s, and a final extension at 72°C for 7 min. SSR products were resolved on 6% non-denaturing polyacrylamide gels (PAGE) in 1X TBE buffer on the Sequi-gen GT sequencing cell electrophoresis (Bio-RAD, USA) with the gel size 38 × 30cm and 0.4 mm thickness, and then SSR products were visualized through

Table 1 Polymorphism and PIC of SSR markers used for diversity analysis of 24 *Abelmoschus* accessions

Marker name	Marker sequence (Forward/reverse; 5′→3′)	Product size (bp)	Number of amplicons	Number of polymorphic amplicons	Per cent polymorphism	Polymorphic information content (PIC)
Okra 108	AAGAAGGAGAAGAGGGAATG/ TAAACCGTCTAGGAACTCCA	230-240	2	2	100	0.29
Okra 111	GATGGAATTGAGAAACCAGA/ TGTGTTCTTCACTCTCGTCA	230-450	15	15	100	0.89
Okra 112	TGATCTGTCAAAGCTCCTCT/ GAAATGCTGGACAAGTTAGG	580-600	3	3	100	0.59
Okra 113	ACGAGAGTGAAGTGGAACTG/ CTCCTCTTTCCTTTTTCCAT	200-220	2	1	50	0.38
Okra 148	TGCTTATTCATGCTGACCTA/ AGCACTTGATATCCAAGGAA	250-300	3	2	66.3	0.58
Okra 151	AAAAGACTCATCGTGGACC/ GTGATTCCCTAATCCAAACA	320-330	2	2	100	0.14
Okra 152	GCTCTATTGATGGCGAGTAA/ AAAGTCATCCAAGGTGACAA	130-250	6	5	83.3	0.81
Okra 156	CTCTCTTTGATCAGGAGCTG/ TCAAGGACCGAATTATCATC	140-230	6	6	100	0.67
Okra 157	CAGAAACATCTCCAACATCA/ ACACTTTTCAAGGGAAACC	200-230	3	3	100	0.58
Okra 164	CAGAAGGTCCCTTTATTCCT/ TCACCCAACCATTTCTCTAC	170-180	2	2	100	0.37
Okra 165	GAGCTAAACCTTGCTTTTGA/ CTCTTATGACTTCGGTCCAG	150-230	4	4	100	0.70
Okra 166	TTCCAGTTGGAGAGGTAAGA/ CTTCCATTTCATCGACTTTC	200-400	15	15	100	0.82
Okra 167	CGGCACTCACATTTTACATA/ GCTGTGAAGCTCTCAAAAGT	110-200	5	5	100	0.76
Okra 169	CTGATTCAAAGCCTCAAGAC/ ATTATCTTTCCCAAGGCAAC	200	1	1	100	0.0
Okra 170	TGAAAGGAGAGGTACACTGG/ AGGTGACTGTGATAGATCCG	200-310	8	7	87.5	0.68
Okra 174	ATGAGCTGTTCTTTGCAGAT/ CTCCTTTAAGAACTGGGGAT	240-260	3	3	100	0.43
Okra 175	CCATTTACTACCCCTTCTCC/ CTCAGAATGTGTGATGATGC	110-130	3	3	100	0.57
Okra 183	TCCCACATCAAAGGTATTTC/ ATAGCAGAGGCAATCTTTCA	190-220	2	2	100	0.36
		Total	85	81	-	-
		Mean	4.72	4.5	93.72	0.53

silver staining (Mortz *et al.* 2001). A 100-bp DNA ladder was used as a size marker. PCR was repeated where number of amplicons per primer was more than 10.

The SSR loci were scored as dominant, due to the amplification of greater than two loci per SSR primer combination because of amphidiploidy. Thus, for diversity analysis, each SSR bands were scored as 0/1 (absence/ presence) and the genetic distances between cultivars were calculated with Jaccard's co-efficient and dendrogram was produced using the unweighted pair group method average (UPGMA) clustering using NTSYSpc version 2.02 (Rohlf 1998). To determine robustness of the dendrogram nodes, the marker data were bootstrapped with 1000 replications along with Jaccard's coefficient by the computer program WINBOOT (Yap and Nelson 1996). Multivariate principal coordinate analysis (PCoA) was carried out using Genalex 6.5 (Peakall and Smouse 2012). The polymorphic information content (PIC), a measure of the degree of polymorphism, was obtained with the PIC calculator (http://www.liv.ac.uk/ ~kempsj/pic.html).

RESULTS AND DISCUSSION

Though, okra is an important vegetable but few efforts have been made to characterize to the genus *Abelmoschus*, which hinders breeding programs (Kumar *et al.* 2010). In general, the primary objectives of okra characterization using morphological traits is to identify high yielding genotypes with resistance to YVMV, fruit borer, jassid, and higher vitamin C content particularly in the wild species which can be fruitfully utilized for the improvement of *A. esculentus*. Until recently, germplasm characterization and evaluation in okra were based on morphological traits, which are not understood at gene level and may differ under varying environmental conditions. To eliminate these errors and complications, protein (Torkpo *et al.* 2006) and random molecular markers (Martinello *et al.* 2001, Gulsen *et al.* 2007, Aladele *et al.* 2008) have been utilized to reveal genetic diversity in okra. However, to characterize the diversity in 24 accessions of *Abelmoschus* species with greater efficiency, SSR markers has been utilized in present study.

Performance of SSR marker system

Though, SSRs are potential marker system but have been not used for *Abelmoschus* breeding. Among 25 SSR primers used in this study, 18 (72%) primers could amplify successfully. High percent of amplification rate is in agreement with Schafleitner *et al.* (2013) where more than 95% SSR amplified reliably. In a set of 24 accessions, these 18 SSRs generated a total of 85 amplicons, of which 81 (95%) were polymorphic and this polymorphism is comparable with Schafleitner *et al.* (2013) (Table 1). Similarly, in okra, Ramneek *et al.* (2012) also observed high (72%) polymorphism rate of SSR transferred from cotton. High polymorphism is an indication of prevalence of good diversity among these 24 accessions studied in present investigation. Out of 18 primers, 13 primers were 100% polymorphic. The high polymorphism rate of the tested SSRs in comparison to randomly chosen SSRs in other plant species may due to the amphipolyploid nature of *Abelmoschus*, in which more mutations in gene sequences are expected than diploid plant (Schafleitner *et al.* 2013), thus ultimately leads to increased diversity and plasticity (Kyriakopoulou *et al.* 2014). Moreover, the Asian accessions of okra were previously reported to be more diverse than the accessions from other continents like USA or Africa (Salameh 2014). Number of total amplicons varied from 2 to 15 with an average of 4.72 loci per primer which is higher than SRAP markers (Gulsen *et al.* 2007) but fairly lower than AFLP (Akash *et al.* 2013). The amplicon size ranged from 110 (Okra-175 and Okra-157) to 450bp (Okra-15). The average of polymorphic loci was 4.5 loci per marker. The polymorphism information content was on average 0.53 and ranged from 0.00 to 0.89 (okra-111). The average PIC value was similar to Schafleitner *et al.* (2013) where mean PIC with 19 SSR markers was 0.49.

SSR based cluster analysis and principal coordinate analysis (PCoA)

Among the all pair-wise combinations of genotypes, Jaccard's similarity coefficients based on SSR markers ranged from 0.25 to 0.95 with an average value of 0.68. The highest genetic similarity was observed between GP220/ GP219 and GP234/GP215, while the lowest level was found between IC 470731/GP207 (Table 2). The UPGMA clustering algorithm based on SSR data grouped 24 accessions into three groups at an average cut-off value of 0.62 with a good support (48% bootstrap value, Fig 1). *Abelmoschus* accessions of different species, viz. *A. esculentus, A. moschatus* and *A. manihot* were located on distinct clusters and in accordance with Schafleitner *et al.* (2013). The cluster one comprised of 21 genotypes from *A. esculentus*. Despite narrow genetic base, molecular analysis further resolved cluster one clearly into five sub-clusters. The major sub-cluster in cluster one was sheltering the maximum (17) accessions. High similarity among the accessions studied was expected because okra has highly self-pollinated species (Hamon and Koechlin 1991) and similar results were also reported by Gulsen *et al.* (2007) with SRAP marker. However, four accessions namely GP205, GP207, GP208 and GAO5 were found quite distinct from the other cultivated accessions and revealed medium level of diversity. The genetic similarity within *A. esculentus* was fairly high, i.e. 0.79 and similarity coefficient was ranging from 0.52 (GP205/ GP207) to 0.95. Many previous studies with marker based cluster analysis showed a wide range of similarity ranging from 44 to 83% with RAPD marker (Haq *et al.* 2013) while 86 to 100% with SRAP marker (Gulsen *et al.* 2007) and 35 to 71% with ISSR marker (Yuan *et al.* 2014).

Second cluster consisted of two wild genotypes each belonging to *A. tuberculatus* and *A. manihot* with strong support (100% bootstrap value). Results are in agreement Ramneek *et al.* (2012) where cultivated and wild accessions of *Abelmoschus* were grouped separately with SSR markers

Table 2 Jaccard's similarity coefficient based on SSR markers used for diversity analysis of 24 *Abelmoschus* accessions

Germplasm(GP) number	GP 202	GP 204	GP 205	GP 206	GP 207	GP 208	GP 214	GP 215	GP 216	GP 218	GP 219	GP 220	GP 222	GP 223	GP 225	GP 234	GP 239	GP 245	GP 224	GA 05	GP 247	IC 555662	IC 470731	IC 470732
GP202	1																							
GP204	0.91	1																						
GP205	0.75	0.75	1																					
GP206	0.84	0.83	0.74	1																				
GP207	0.65	0.7	**0.52**	0.75	1																			
GP208	0.76	0.78	0.71	0.74	0.63	1																		
GP214	0.81	0.83	0.74	0.94	0.75	0.77	1																	
GP215	0.87	0.83	0.66	0.9	0.72	0.69	0.91	1																
GP216	0.84	0.83	0.66	0.88	0.77	0.71	0.91	0.94	1															
GP218	0.84	0.83	0.68	0.9	0.71	0.71	0.9	0.93	0.9	1														
GP219	0.89	0.88	0.75	0.84	0.65	0.76	0.84	0.84	0.81	0.9	1													
GP220	0.84	0.86	0.74	0.79	0.69	0.74	0.83	0.79	0.79	0.85	**0.95**	1												
GP222	0.73	0.75	0.71	0.83	0.69	0.72	0.89	0.83	0.85	0.88	0.82	0.8	1											
GP223	0.72	0.74	0.7	0.81	0.68	0.7	0.87	0.81	0.84	0.87	0.8	0.79	0.91	1										
GP225	0.76	0.75	0.66	0.85	0.75	0.72	0.86	0.85	0.89	0.88	0.82	0.77	0.86	0.85	1									
GP234	0.85	0.81	0.64	0.89	0.7	0.7	0.89	**0.95**	0.92	0.92	0.82	0.78	0.81	0.8	0.84	1								
GP239	0.74	0.73	0.63	0.78	0.62	0.59	0.78	0.87	0.81	0.83	0.74	0.69	0.81	0.77	0.75	0.85	1							
GP245	0.82	0.84	0.64	0.89	0.79	0.75	0.89	0.92	0.92	0.92	0.82	0.78	0.84	0.83	0.9	0.9	0.82	1						
GP224	0.69	0.69	0.59	0.76	0.66	0.6	0.77	0.82	0.79	0.85	0.75	0.76	0.8	0.88	0.77	0.8	0.81	0.8	1					
GAO5	0.65	0.62	0.54	0.66	0.58	0.6	0.69	0.71	0.74	0.71	0.62	0.58	0.69	0.76	0.75	0.7	0.67	0.75	0.71	1				
GP247	0.75	0.77	0.59	0.78	0.8	0.68	0.84	0.84	0.9	0.84	0.75	0.79	0.82	0.81	0.85	0.83	0.74	0.85	0.78	0.7	1			
IC555-662*	0.39	0.39	0.4	0.42	0.34	0.39	0.44	0.43	0.42	0.43	0.43	0.42	0.42	0.46	0.41	0.4	0.4	0.38	0.43	0.35	0.38	1		
IC470-731**	0.31	0.3	0.29	0.32	**0.25**	0.34	0.35	0.36	0.36	0.35	0.33	0.32	0.37	0.34	0.35	0.35	0.32	0.35	0.33	0.37	0.32	0.36	1	
IC470-732***	0.39	0.36	0.35	0.4	0.35	0.41	0.41	0.42	0.42	0.39	0.39	0.38	0.37	0.38	0.37	0.4	0.36	0.38	0.37	0.3	0.36	0.72	0.33	1

* *A. tuberculatus;* ***A. moschatus;* ****A. manihot*

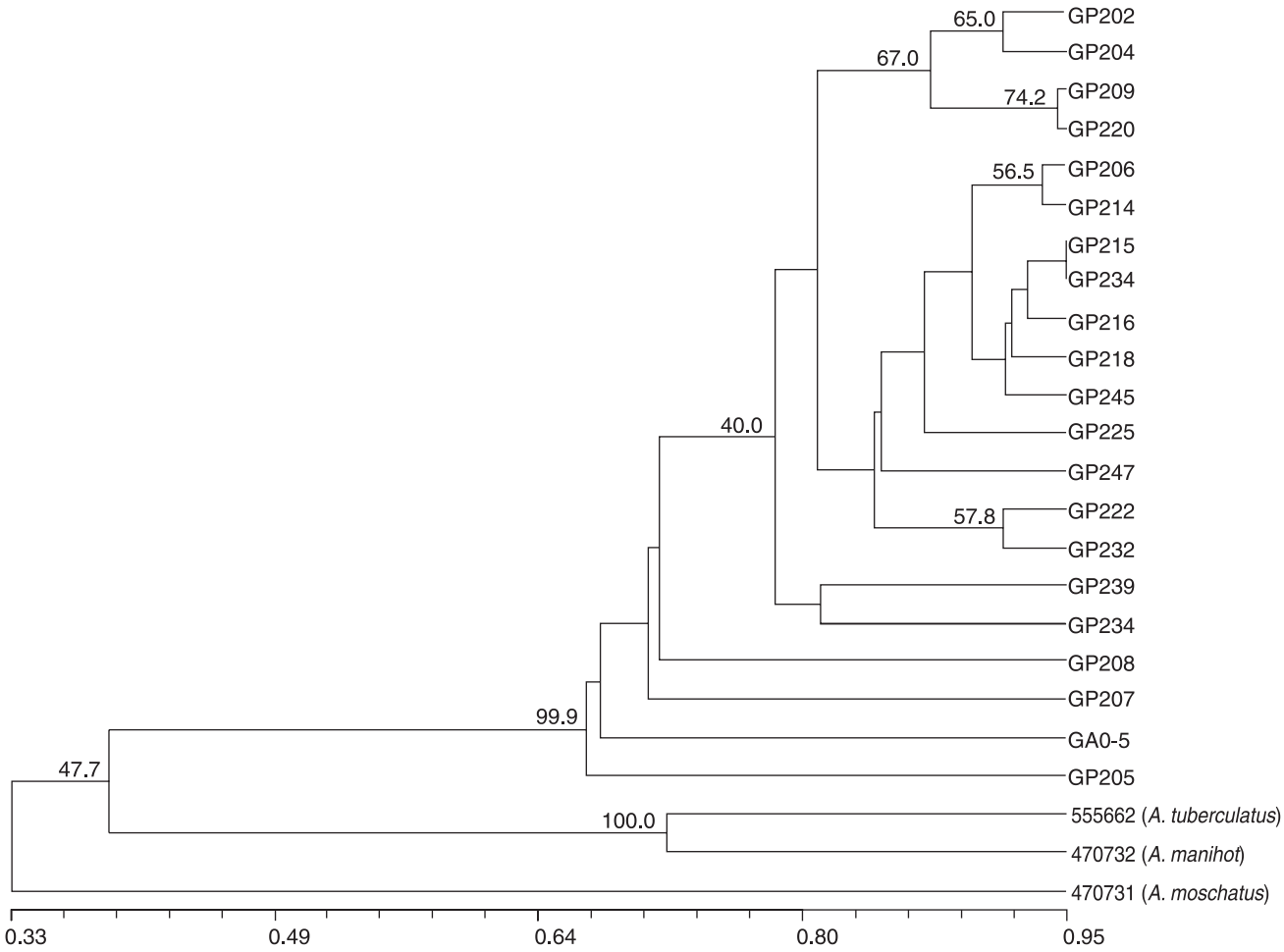

Fig 1 SSR based dendrogram of the genetic similarities among twenty five accessions of *Abelmoschus* species achieved by the UPGMA method. The bootstrap values which are higher than 40 are shown

as transferred from cotton. Molecular relationship of the three species revealed that *A. tuberculatus* and *A. manihot* formed a closely related group as both belongs to ploidy level 1 (2n =56–72; Bisht and Bhat 2006). The development of viable seeds in the cross between *A. manihot* and *A. tuberculatus* also support the molecular level intimacy of both species and validate the taxonomic relationship between them. Third cluster contained only a single accession from *A. moschatus*.

In order to visualize the relationships among genotypes, PCoA analysis based on Jaccard's similarity coefficient was performed. The first three coordinate axis accounted for 56% of the variation observed. Most variance (32%) was explained by the first component. PC2 and PC3 accounted for 13% and 10% of total variance, respectively. A high level of PCoA value was also observed by Akash *et al.* (2013) in landraces of okra. The PCoA revealed that the genotypes belonging to a particular cluster were grouped together in the PCoA plot. It showed that the *A. esculentus* accessions clustered together, whereas wild *Abelmoschs* accessions were scattered on plot; it is absolutely congruent with UPGMA dendrogram. Accessions GAO5 in the left lower part of the PCoA plot, differed considerably at a molecular level from the rest of *A. esculentus* accessions. This genotype is a high yielding variety released by Anand Agricultural University, Anand and under the Yellow Vein Mosaic Virus (YVMV) hot spot condition, GAO5 has merely 7.86% YVMV infection as compared to check (http://www.aau.in/sites/default/files/BOOKLET_2011.pdf). Probably, this may be the reason, why this genotype is located separately on PCoA plot.

It was concluded that studies with molecular markers have been reported in okra but it was limited to dominant and less reproducible marker system. The outcome of present study using SSR therefore has given valuable understandings of *Abelmoschus* species and the results proved that SSR analysis is useful tool for determining genetic diversity in okra genotypes. Moreover, better resolution of SSR amplicons on PAGE can be efficiently employed for unravelling the hidden variability of okra and its wild relatives which can be helpful to the plant breeders for planning breeding program, to understand species relationships, for germplasm collection, for linkage map construction and in establishment of core collections of *Abelmoschus* species.

ACKNOWLEDGEMENT

The authors are grateful to D P Gohil, Associate

Research Scientist, Main Vegetable Research Station, AAU, Anand for supplying the seeds of okra.

REFERENCES

Akash M W, Shiyab S M and Saleh M I. 2013. Yield and AFLP analyses of inter-landrace variability in okra (*Abelmoschus esculentus* L.). *Life Science Journal* **10**: 2 771–9.

Aladele S E. 2009. Morphological distinctiveness and metroglyph analysis of fifty accessions of West African okra (*Abelmoschus caillei*) (A. Chev.) Stevels. *Journal of Plant Breeding and Crop Science* **1**(7): 273–80.

Aladele S E, Ariyo O J andde La Pena R. 2008. Genetic relationships among West African okra (*Abelmoschus caillei*) and Asian genotypes (*Abelmoschus esculentus*) using RAPD. *African Journal of Biotechnology* **7**: 1 426–31.

Ariyo O J. 1990. Variation and heritability of fifteen characters on okra (*Abelmoschus esculentus* (L.) Moench).*Tropical Agriculture* **67**: 213–6.

Ariyo O J. 1993. Genetic diversity in West African okra [*Abelmoschus caillei* (A. Chev.) Stevels]-Multivariate analysis of morphological and agronomic characteristics. *Genetic Resources and Crop Evolution* **40**: 25–32.

Bisht I S and Bhat K V. 2006. Okra (*Abelmoschus* spp.). (*In*) *Genetic Resources, Chromosome Engineering, and Crop Improvement: Vegetable Crops*, 3, pp 148–62. Singh R J (Ed.). CRC Press.

Datta P C and Naug A. 1968. A few strains of *Abelmoschus esculentus* (L.) Moench- Their karyological study in relation to phylogeny and organ development. *Beiträgezur Biologie der Pflanzen* **45**: 113–26.

Gulsen O, Karagul S and Abak K. 2007. Diversity and relationships among Turkish okra germplasm by SRAP and phenotypic marker polymorphism. *Biologia Bratislava* **62**: 41–5.

Hamon S and Koechlin J. 1991. The reproductive biology of okra. 2. Self-fertilization kinetics in the cultivated okra (*Abelmoschus esculentus*), and consequences for breeding. *Euphytica* **53**: 49–55.

Haq I, Khan A A and Azmat M A. 2013. Assessment of genetic diversity in okra (*Abelmoschus esculentus* L.) using RAPD markers. *Pakistan Journal of Agricultural Sciences* **50**: 655–62.

Kochko A D and Hamon S. 1990. A rapid and efficient method for the isolation of restrictable total DNA from plants of the genus *Abelmoschus*. *Plant Molecular Biology Reporter* **8**: 3–7.

Kyriakopoulou O G, Arens P, Pelgrom K T B, Bebeli P and Passam H C. 2014. Genetic and morphological diversity of okra (*Abelmoschus esculentus* [L.] Moench.) genotypes and their possible relationships, with particular reference to Greek landraces. *Scientia Horticulturae* **171**: 58–70.

Martinello G E, Leal N R, Amaral Jr A T, Pereira M G and Daher R F. 2001. Comparison of morphological characteristics and RAPD for estimating genetic diversity in *Abelmoschus* spp. *Acta Horticulturae* **546**: 101–4.

Mortz E, Krogh T N, Vorum H and Gorg A. 2001. Improved silver staining protocols for high sensitivity protein identification using matrix-assisted laser desorption/ionization-time of flight analysis. *Proteomics* **1**: 1 359–63.

Osawaru M E, Ogwu M C and Dania-Ogbe F M. 2013. Morphological assessment of the genetic variability among 53 Accessions of West African okra [*Abelmoschus caillei* (A. Chev.) Stevels] from South Western Nigeria. *Nigerian Journal of Basic and Applied Sciences* **21**(3): 227–38.

Peakall R and Smouse P E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**: 2 537–9.

Poehlman J M and Sleper D A. 1995. *Breeding Field Crops,* 4th edn, pp 75–6. Iowa State Press.

Ramneek J, Pathak M and Pathak D. 2012. Preliminary investigation on the cross transferability of cotton SSR markers to *Abelmoschus* species.*Crop Improvement* **39**: 80–4.

Saifullah M, Rabbani M G and Garvey E J. 2010. Estimation of genetic diversity of okra (*Abelmoschus esculentus* L. Moench) using RAPD markers. *SAARC Journal of Agriculture* **8**: 19–28.

Salameh N M. 2014. Genetic diversity of okra (*Abelmoschus esculentus* l.) genotypes from different agroecological regions revealed by amplified fragment length polymorphism analysis. *American Journal of Applied Sciences* **11**: 1 157–63.

Sawadogo M, Ouedraogo J T, Balma D, Ouedraogo M, Gowda BS, Botanga C and Timko M P. 2009. The use of cross species SSR primers to study genetic diversity of okra from Burkina Faso. *African Journal of Biotechnology* **8**: 2 476–82.

Schafleitner R, Kumar S, Lin C, Hegde SG and Ebert A. 2013. The okra (*Abelmoschus esculentus*) transcriptome as a source for gene sequence information and molecular markers for diversity analysis. *Gene* **517**: 27–36.

Torkpo S K, Danquah E Y, Offei S K and Blay E T. 2006. Esterase, total protein and seed storage protein diversity in Okra (*Abelmoschus esculentus* L. Moench). *West African Journal of Applied Ecology* **9**: 1–7.

Yap I V and Nelson R J. 1996. Winboot: a program for performing bootstrap analysis of binary data to determine the confidence of UPGMA-based dendrograms. IRRI, Manilla, Phillipines.

Yuan C Y, Zhang C, Wang P, Hu S, Chang H P, Xiao W J, Lu X T, Jiang S B, Ye J Z and Guo X H. 2014. Genetic diversity analysis of okra (*Abelmoschus esculentus* L.) by inter-simple sequence repeat (ISSR) markers. *Genetics and Molecular Research* **13**: 3 165–75.