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GENETIC DIVERSITY AS ASSESSED BY MOLECULAR MARKERS AND MORPHOLOGICAL TRAITS IN EGYPTIAN OKRA GERMPLASM

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ABSTRACT

Twenty nine okra accessions (*Abelmoschus esculentus L.*) collected from different locations, were morphologically characterized. Significant differences were observed between some accessions for all quantitative characters studied while variations were detected and described for the qualitative characters. Forty two ISSR primers and five AFLP combinations were used to determine the level of polymorphism, molecular fingerprinting, identification of unique markers, and the estimation of genetic distances for the 29 okra accessions. The ISSR primers amplified 508 fragments of which 415 were polymorphic. The number of positive and negative unique markers were 103 and were useful in identifying 24 genotypes out of the 29 accessions. Moreover, five AFLP primer combinations yielded 449 amplicons, the total number of polymorphic amplicons was 439.

Genetic similarity matrices estimated from ISSR and AFLP data, depicted similarity coefficient ranged from 0.68 – 0.90 and 0.51-0.82, respectively. The highest similarity coefficient was between accessions collected from the same governorate, Generally, the constructed dendrograms based on ISSR and AFLP data, exhibited a tendency to cluster accessions in groups according to their geographical locations. In conclusion, additional okra germplasm needs to be collected from other locations and characterized to ensure the representation of most of the genetic diversity in Egyptian okra germplasm is conserved *ex situ*.

Key words: Okra, ISSR, AFLP, genetic distance.

INTRODUCTION

The cultivated okra (*Abelmoschus esculentus L.*) commonly known as 'Lady's fingers', 'Gumbo', and 'Bamia ', belongs to the family *Malvaceae*. The crop is a native of Africa and is still found growing wild around the River Nile as well as in Ethiopia, in tropical and sub-tropical areas (Kochhar, 1986). It can grow all year round, but in Egypt it's production is mainly during the summer season. Okra was previously included in the genus *Hibiscus*. Later, it was designated to *Abelmoschus*. Although about 50 species have been described, eight of them are most widely accepted (IBPGR, 1991).Contradicting evidence exists on the geographical origin of *A. esculentus*. One putative ancestor (*A. tuberculatus*) is native to Uttar Pradesh in North India, suggesting that *A. esculentus* originated in India. The other evidence is based on the plants cultivation in ancient times, and the presence of another putative ancestor (*A. ficulneus*) in East Africa, suggesting northern Egypt and Ethiopia as the geographical origin of *A.esculentus*. The major producers of okra are India, Costa Rica, Nigeria and Ghana (NARP, 1993).

Conservation and management of germplasm in gene banks require morphological characterization and descriptors are vital tools in that respect (Rubenstein and Heisey, 2003). Also, molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and between species (Omonhinmin and Osawaru, 2005; Chakravarthi and Naravaneni, 2006). In addition they can be used to identify unique genotypes and markers that are associated or linked to important agronomic traits. Therefore, the application of molecular marker techniques such as RAPD, ISSR, SSR, and AFLP and the proper documentation of useful polymorphism between accessions constitute an important role in enhancing germplasm utilization.

Since okra has been considered a minor crop, attention has not been paid to the collection and conservation of okra germplasm and likewise to its genetic improvement either at the national level or in the international research programs. Similarly studies using molecular markers in okra are lagging behind the other major crops (Martinello *et al.* 2001; Aladele *et al.* 2008; Saifullah and Rabbani, 2009; Kaur *et al.* 2013).

Therefore, the objectives of this study were to conduct morphological characterization of 29 okra accessions conserved at the National Gene Bank of Egypt, the estimation of the genetic variation and genetic relationships among these accessions using ISSR and AFLP marker systems techniques, and to develop genetic profiles for these accessions.

MATERIALS AND METHODS

Materials

This study was carried out on 29 okra accessions (Table 1) collected from different locations in Egypt during 2004 through 2006 and conserved in the National Gene Bank (NGB), Agricultural Research Centre (ARC).

Table (1): The NGB code number of 29 okra accessions, the governorate, location and year they were collected:

Serial No.	NGB code number	Governorate	Location	Collection year
1	11293	Sohag	Shandaweel	2005
2	11376	Sohag	Tahta	2005
3	11382	Sohag	El-Maragha	2005
4	18446	Sohag	Girga	2006
5	18406	Sohag	East Wanina village	2006
6	18380	Sohag	El-Mahamda village-Naga El-Omda	2006
7	13897	El-Dakahlia	Mit Ghamr	2006
8	18459	Sohag	Girga	2006
9	18524	Sohag	Shandaweel-Sheikh Youssef	2006
10	12316	Assuit	Assuit	2004
11	12125	Quena	Quena	2005
12	18475	Quena	Farshout	2006
13	18477	Quena	Farshout	2006
14	18499	Quena	Farshout	2006
15	18476	Quena	Abou Tesht	2006
16	18497	Quena	Abou Tesht	2006
17	14527	Quena	El Marashda	2006
18	18468	Quena	El Marashda	2006
19	18496	Quena	Dishna	2006
20	18500	Quena	Nagaa Hammad	2006
21	13635	Quena	Nagaa Hammad	2006
22	12000	Luxor	Awlad El-Shake	2004
23	14251	Aswan	Toushka	2006
24	11602	North Sinai	Arish-Sheikh Zewaid	2005
25	11607	North Sinai	Arish-Sheikh Zewaid	2005
26	13974	Ismailia	West Kantara	2006
27 and 28	14081	El Beheira	Etay El-Baroud *	2006
29	13645	Menoufia	Esna	2006

* samples 27 and 28 originated from one accession that segregated in two types of capsule colour, red (27) and green (28) during its multiplication.

Methods

Morphological Characterisation

The 29 accessions of okra were cultivated during the summer season (June - October) for two consecutive years (2009 and 2010). Each accession was represented by 20 individual plants. Data were recorded 80-100 days from sowing. The morphological characteristics were divided into quantitative and qualitative characters, 21 characters were recorded according to the "International Union for the Protection of New Varieties of Plants" (UPOV), GENEVA 1999, and 12 characters were based on field observations.

Molecular Characterization

The DNA was extracted with 5 Prime Kit (5 prime GmbH, Germany) according to its manual and purified using Qiagen DNeasy kit. DNA concentration was quantitatively measured on Bio photometer (Eppendorph, Germany) and adjusted to 100 ng / µl.

Inter Simple Sequence Repeats (ISSR)

In the present study, 63 ISSR primers were tested, 42 produced robust amplicons bands. These primers were all anchored in either 5' end or 3' end, and at both ends. Primers were synthesised by Bioron Corporation, Germany, (Table 2).

Table (2): ISSR primer name, sequence, and annealing temperature (Ta):

Serial number	Primer Name	Sequence	Ta (°C)
1	17899-B	$(CA)_6$ GG	42 ºC
2	ISSR-2	AGA $(TCC)_5$	50 ºC
3	ISSR-3	TGTA $(CA)_7$	46 ºC
4	888	TAC $(CA)_7$	46 ºC
5	890	ACG $(GT)_7$	50 ºC
6	891	TCT $(TG)_7$	46 ºC
7	811	$(GA)_8$ C	42 ºC
8	853	$(TC)_8$ GT	46 ºC
9	3	$(CA)_8$ AT	46 ºC
10	8	$(CA)_8$ GAC	50 ºC
11	16	CGTC $(AC)_7$	50 ºC
12	17	CAGC $(AC)_7$	50 ºC

Serial number	Primer Name	Sequence	Ta (°C)
13	ISSR-32	GAC $(CA)_7$ C	55 ºC
14	ISSR-34	$(AG)_8$ TG	53 ºC
15	835	$(AG)_8$ CC	55 ºC
16	841	$(GA)_8$ TC	53 ºC
17	842	$(GA)_8$ TG	53 ºC
18	889	AGG $(AC)_7$	53 ºC
19	BEC	(ca)7 tc	42 ºC
20	HAD	ct (cct)3 cac	42 ºC
21	BC-848	(CA)8RG	53 °C
22	BC-890	VHV (GT)7	53 °C
23	BC-891	HVH (TG)7	53 °C
24	814	$(CT)_8$ TG	49 ºC
25	ISSR-1	CAC $(TCC)_5$	50 ºC
26	851	$(GT)_8$ CG	55 ºC
27	8565	gt (cac)7	63 ºC
28	15	ggtc (ac)7	50 ºC
29	CHR	(ca)7 gg	46 ºC
30	809	$(AG)_8$ G	53 ºC
31	W814	(ct)8 tg	49 ºC
32	W7	(ct)8gg	49 ºC
33	5	(ca)8 gt	49 ºC
34	4	(ca)8 ac	49 ºC
35	HB-12	$(CAC)_3$ GC	45 ºC
36	HB-13	$(GAG)_3$ GC	45 ºC
37	844-A	$(CT)_8$ AC	53 ºC
38	BC-827	(AC)8 G	58 °C
39	BC-847	(CA)8 RC	52 °C
40	BC-888	BDB (CA)7	53 °C
41	BC-889	DBD (AC)7	58 °C
42	BC-857	(AC)8TG	52 °C

PCR was performed in 25 µl reaction volume containing 1µl Taq DNA polymerase (5 U/µl, Thermo Taq, USA), 1.5 mM $MgCl_2$, 20 mM dNTPs, 2.5 µl 10 X buffer, 100 pM oligonucleotide primer and 100 ng genomic DNA. The PCR reactions were placed in an Eppendorph Master Cycler programmed to 45 cycles as follows: an initial denaturation step at 95°C for 5 minutes, followed by 45 cycles of denaturation at 94°C for 1 minute, annealing temperature (Ta) for 1 minute, and an extension step at 72°C for 2 minutes, and a final extension step at 72°C for 10 minutes. The PCR products were separated on 2.5 % agarose gel in 1X TBE running buffer.

Amplified Fragment Length Polymorphisms (AFLP)

Five AFLP primer combinations were selected for this study as shown in (Table 3) using Invitrogen kit according to its manual. The products were separated on 6% acrylamide denaturing gel using BIO-RAD Sequi-Gen Sequencing gel system, and stained with silver staining kit (Promega Company) according to the manufacturer's manual.

Table (3): List of AFLP combinations used

No	EcoR1	Mse1
1	ACA	CTG
2	AAC	CAG
3	AAC	CTG
4	ACT	CTT
5	AAG	CAG

Data Analyses

The average means and the standard errors for the quantitative traits under study were calculated to determine the significant differences between the accessions at $P \leq 0.05$ according to Snedecor and Cochran (1980) using MSTAT-C program (1989).

From the banding patterns generated by ISSR and AFLP the clear and distinct amplified fragments were scored as (1) for present and (0) for absent bands. Genetic similarity matrices between genotypes were estimated according to Dice coefficient (Sneath and Sokal, 1973) based on Nei's (1972) genetic distances and the dendrogram was constructed using the unweigted paired group method of arithmetic averages(UPGMA) by the Non-Linear Dynamics Corporation, UK software.

RESULTS AND DISCUSSION

Morphologic Characteristics

Quantitative characters

Data for 16 quantitative traits were recorded on 20 individual plants per accession in each of the two seasons (2009

and 2010), 80 - 100 days from cultivation and the average means and their standard errors were calculated. The range between the lowest and highest value for each character are summarized in Table (4). There were significant differences in all the quantitative traits studied between some accessions.

Table (4): The range between the lowest and highest value recorded for the 16 quantitative characters:

No	Quantitative Characters	Range value		Mean
		Lowest ±SE	Highest ± SE	
1	Plant height (PH) / cm	50.90±12.12	134.09±36.96	85.25
2	Stem diameter (SD) / cm	1.75±0.27	3.19±0.41	2.49
3	number of secondary branches (NSB)	1.00±0.00	6.50±3.08	3.42
4	Leaf length (L) / cm	9.80±0.76	23.09±4.12	16.99
5	Leaf width (W) / cm	14.30±1.99	30.97±2.11	12.67
6	Petiole length (PL) / cm	9.36±0.69	33.23±0.72	22.27
7	Petiole diameter (PD) / cm	0.29±0.00	0.76±0.03	0.59
8	Number of petals (NOP)	5.00±0.00	8.00±0.00	6.26
9	Number of fruit ridges (NOFR)	5.00±1.00	10.00±0.82	7.52
10	Number of locules (NOL)	5.00±0.82	9.25±0.96	7.53
11	Thickness of carpels (TOC) / cm	0.17±0.06	0.40±0.10	0.30
12	Length of mature fruit (LMF) / cm	3.20±0.10	13.81±1.59	0.30
13	Diameter of mature fruit (DMF) / cm	2.35±0.82	3.96±0.82	3.32
14	Number of fruits on main stem (NFMS)	3.00±0.82	9.00±3.10	5.27
15	Number of total fruit per plant (NTFP)	3.83±0.75	25.00±4.64	7.91
16	Fruit weight (FW) / gm	27.77±4.74	41.87±1.25	37.11

It is important to study the extent of genetic variations and relationships between accessions for its utilization in breeding programs for further improvement. Results recorded for the quantitative traits in this study (Table 3) show a wide range of significant variations in accordance with those reported by Verma (1993), Mishra *et al*. (1996), Rithichai *et al*. (2004), Omonhimn and Osawaru (2005), Düzyaman (2005), Morakinyo and Adeymi (2007), Aktokar *et al*. (2010), and Ramanjinappa *et al*. (2011).

Qualitative Characters

The descriptive data recorded for the 29 okra accessions according to UPOV descriptor are summarized in Table (5) which gives the number of accessions recorded under each category of the descriptor. Variation of leaf depth of lobbing and the different fruit colour of okra accessions are shown in Fig1(A-C) and(D-F),respectively, shows the different fruit colour of okra accessions.

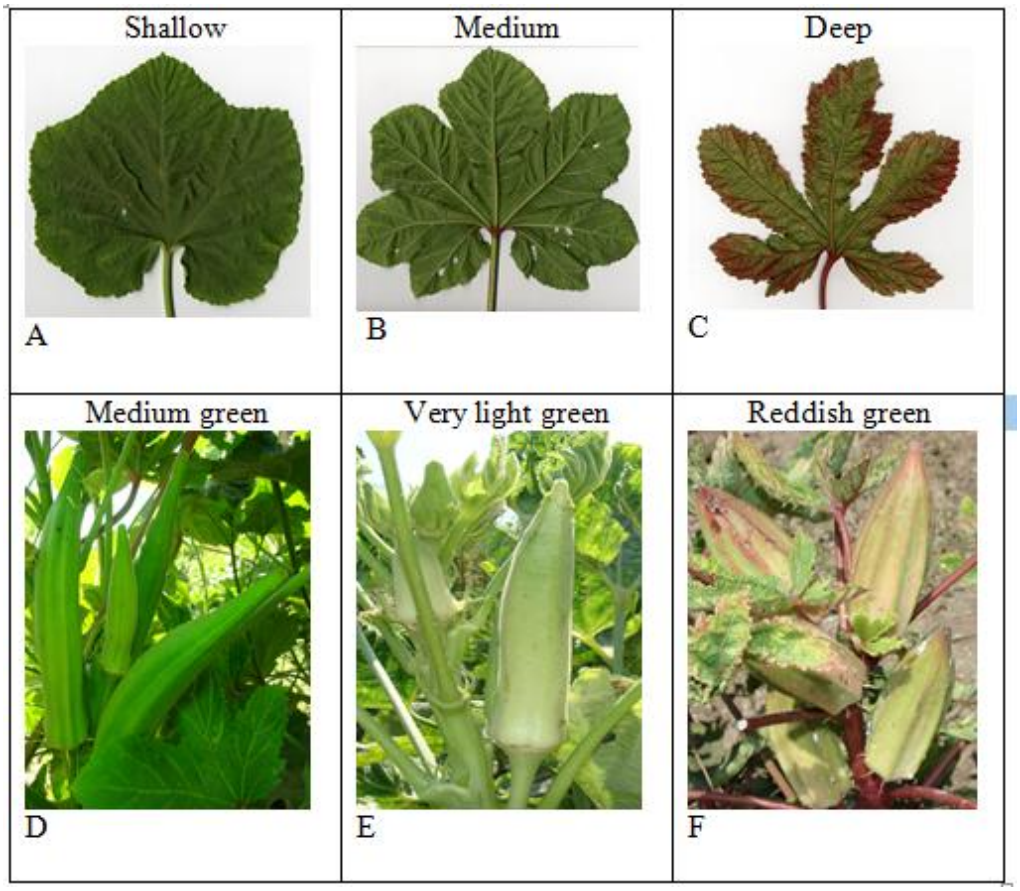

Fig1 (A-C): Leaf depth of lobing of the okra accessions.
(D-F): The different fruit colour of the okra accessions.

Table (5): The qualitative characters studied and the number of accessions recorded under each category of the descriptor:

No	Qualitative Characters	Description (accessions grouped into categories)
1	Plant degree of branching (DB)	Weak (6), Medium (17), Strong (6)
2	Plant growth habit (GH)	Erect (7), Semi erect (20), Spread (2)
3	Stem Colour (SC)	Green (25), Red (4)
4	Stem Colour Intensity (SCI)	Dark (8), Medium (20), very light (1)
5	Leaf Depth of Lobing (DL)	Deep (4), Medium (24), Shallow (1)
6	Leaf Margin Dentation (MD)	Deep (4), Medium (24), Shallow (1)
7	Leaf Colour between Veins (CV)	Green (29)
8	Leaf colour between veins Intensity (CVI)	Dark green (9), Medium Green (20)
9	Colour of main vein on leaf upper side (CVMU)	Green (18), Reddish green (2), Dark green (4), Red (1) , Dark red (4)
10	Colour Intensity of main vein on leaf under side (CVSU)	Green (18), reddish green (2), dark green (4), red (1), dark red (4)
11	Colour of main vein on leaf under side (CVMD)	Green (5), light green (18), reddish light green (2), red (4)
12	Colour of secondary vein on leaf under side (CVSD)	Green (5), light green (18), reddish light green (2), red (4)
13	Hairs on leaf under side (HB)	Normal (23), very rough (1), few (4), smooth (1)
14	Pigments on petiole upper side (PPU)	Green (10), red (6), dark red (13)
15	Petiole under side pigments (PPL)	Green (17), light green (5), reddish light green (2), dark red (5)
16	Flower size (FS)	Large (5), medium (18), small (6)
17	Petal Colour (PC)	Yellow (25), light yellow (4)
18	Red colour at the base of petals (CP)	Present (29)
19	Fruit Colour (FC),	Green (24), very light green (1), reddish green (4)
20	Fruit Colour Intensity (FCI)	Dark (9), medium (18), light (1), very light (1)
21	Colour of Fruit Peduncle (CoPe)	Green (14), reddish green (3), light green (6), very light green (1), red (5)
22	Surface between Ridges (SR)	Flat (28), convex (1)
23	Constriction of Basal part (CB)	Very weakly expressed (absent) (29)
24	Shape of Apex (SA)	Acute (26), broad acute (2), narrow acute (1)
25	Fruit Texture (FT)	medium (12), very rough (7), rough (4), smooth texture (3), very smooth (3)

Morphologic qualitative characters such as colour, shape and growth habit of the different plant parts revealed the scope of variation among the okra accessions (Table 5). Although variations were detected and described they did not discriminate between all accessions. Morphological qualitative characterization was studied in okra by several researchers, Hamon and Van Sloten (1989), Martinello *et al*. (2001), Ominhimin and Osawara (2005), Saifullah and Rabbani (2009), Oppong-Sekyere et al. (2011).

Molecular Characterization

ISSR Markers

In the present study, twenty nine accessions of okra were analysed using 42 ISSR primers which produce robust amplified bands. These primers were anchored at either 5´ and 3´ end and or at both ends. Results are shown in Table (6).

Table (6): Primer name, total number of amplicons, size of amplified fragments, number of monomorphic amplicons, number of polymorphic amplicons, and the percentage of polymorphism.

Primer name	Total number of amplicons	Size of amplified fragments	Number of monomorphic amplicons	Number of polymorhic amplicons	% of polymorphism
17899-B	13	281-988 bp	1	12	92%
814	20	100-628 bp	1	19	95%
844-A	22	162-939bp	---	22	100%
HB-12	11	472-933 bp	---	11	100%
HB-13	4	165-288bp	2	2	50%
ISSR-1	9	241-1001 bp	6	3	33%
ISSR-2	7	151-741 bp	7	---	0%
ISSR-3	10	280-962bp	4	6	60%
888	10	285-741bp	2	8	80%
890	12	329-1178 bp	4	8	67%
891	6	285-899 bp	4	2	67%

Primer name	Total number of amplicons	Size of amplified fragments	Number of monomorphic amplicons	Number of polymorhic amplicons	% of polymorphism
811	6	264-648 bp	1	5	83%
853	26	235-1626 bp	1	25	96%
3	26	292-1674 bp	1	25	96%
8	10	273-1300 bp	2	8	80%
16	12	239-1548 bp	6	6	50%
17	6	241-577 bp	3	3	50%
ISSR-32	9	241-529 bp	1	8	89%
ISSR-34	21	250-828 bp	1	20	95%
835	11	242-781 bp	1	10	91%
842	16	255-1050 bp	4	12	75%
841	6	226-967 bp	3	3	50%
851	12	247-860 bp	1	11	92%
ISSR-4	9	238-960 bp	3	6	67%
889	10	323-1001 bp	3	7	70%
8565	14	187-1254 bp	3	11	79%
W814	19	257-593 bp	---	19	100 %
W7	12	215-645 bp	1	11	92%
15	5	244-587 bp	2	3	60 %
5	25	245-1346 bp	1	24	96%
4	4	253-535 bp	3	1	25%
BEC	11	340-1201 bp	2	9	82%
CHR	10	299-1187 bp	1	9	90%
HAD	11	431-1782 bp	---	11	100 %
BC-827	12	256-676 bp	2	10	83%
BC -847	10	265-1000 bp	---	10	100 %
BC -848	20	225-970 bp	---	20	100 %
BC -857	24	158-1703 bp	---	24	100 %
BC -888	7	257-605 bp	3	4	57%
BC -889	5	248-581 bp	3	2	40%
BC -890	9	284-882 bp	1	8	89%
BC -891	6	339-866 bp	1	5	83%
Total	508		85	423	
Average	12.09		2.02	10.07	83.26 %

The forty two ISSR primers generated a total of 508 amplicons in the 29 okra accessions, ranging in size from 162 - 1782 bp, with an average of 12.09 amplicons per primer. Out of the total amplified bands 423 bands were observed to be polymorphic, with an average polymorphism of 83.26%. The highest polymorphic percentage (100%) was exhibited by seven primers (844-A, HB-12, W814, HAD, BC -847, BC -848, BC -857), and the lowest percentage of polymorphism (25 %) was generated by primer (4) primer namely ISSR-2 was monomorphic. The average number of monomorphic amplicons / primer was 2.02, and the average number of polymorphic amplicons per primer was 10.07.

Of the 508 total amplified bands 91 (17.9%) were observed to be unique. These markers were found to be useful as specific markers in identifying 24 out of the 29 accessions. The number of positive unique markers was 69, while 22 markers were found to be negative ones. The highest number of unique markers was found in accession 7 with a total of 13 specific markers, 12 of which were positive and 1 negative, ranging in size from 226-1178 bp and amplified by 11 of the ISSR primers under study.

Dendrogram based on ISSR markers

The dendrogram (Fig 2) was divided into two main clusters at 0.73 similarity coefficient. The first cluster included most of the accessions collected from Sohag governorate (5 accessions).While all the other accessions were grouped in the second cluster which was further sub divided discriminating the accessions collected from the Delta region (Menoufia and El Beheira governorates) in one group with the exception of El-Dakahlia genotype (accession no.7) which was grouped with accession number 12 collected from Quena (Upper Egypt) indicating that it might have originated in Quena and moved to Dakahlia (Delta region) by trade. Also, the dendrogram revealed the clustering of accession number 26 from Ismailia with those collected from North Sinai (24 and 25) which is to be expected as both governorates are located close to each other in the eastern region.

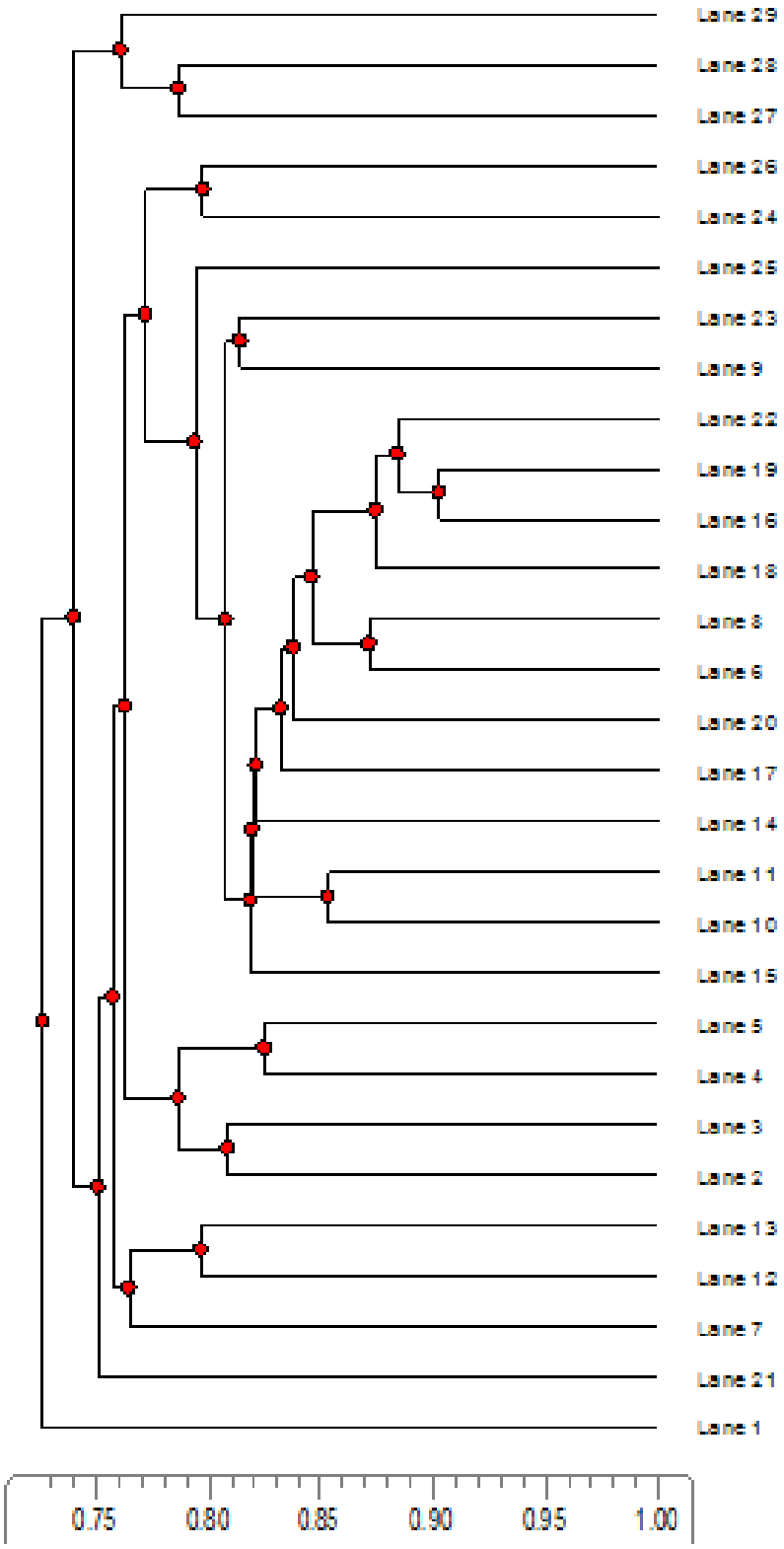

Fig (2): Dendrogram for the 29 okra accessions constructed from ISSR data using UPGMA and similarity matrix.

Knowledge of the levels and distribution of genetic diversity at the molecular level are important for designing conservation strategies of germplasm collections in gene banks which focuses on representative populations with the greatest genetic diversity.

Jie et al. (2008) investigated the polymorphism among 16 okra genetic resources of Abelmoschus esculentus (L.) using 14 ISSR primers which amplified 162 bands, among which 102 (62.96 %) were polymorphic. The average number of DNA bands amplified per primer was 11.6. The data analysis using UPGMA algorithm classified the genotypes into 4 groups. Our results showed a higher percentage of polymorphism (82 %) among the 29 Egyptian okra accessions using 42 ISSR primers which generated 508 amplicons and 12.09 amplicon per primer. These differences may be due to the large number of ISSR primers used that allowed a wide range of genome coverage in addition to the inherent diversity present in the okra accessions collected from the different eco- geographical locations.

Similarly, a high percentage of polymorphism was achieved by Hussein et al. (2003) in a study on 14 citrus accessions using five ISSR unanchored 5´ primers and three 5´ anchored primers which revealed 79.14% and 82.4% polymorphism, respectively. ISSR dendrogram has distinctively separated the okra accessions according to the geographical areas they were collected from. This is in agreement with the conclusion based on morphological

evaluations on okra genotypes reported by Düzyaman (2005) on 10 traditional Turkish cultivars, Morakinyo and Adeymi (2007) on 12 Nigerian genotypes, Saifullah and Rabbani (2009) on 121 genotypes in different parts of Bangladesh.

AFLP Markers

Five AFLP primer combinations out of 11 were selected for this study. Results are shown in table (7). All five AFLP primer combinations produced informative marker bands that differentiate between the okra accessions.

Table (7): Primer combinations, total number of amplicons, number of monomorphic amplicons, number of polymorphic amplicons, and the percentage of polymorphism as revealed by AFLP markers.

Primer combination	Total number of amplicons	Number of monomorphic amplicons	Number of polymorphic amplicons	% polymorphism
1-E-AAC/M-CAG	115	2	113	98.26 %
2-E-ACA/M-CTG	74	1	73	98.65 %
3-E-ACC/M-CTG	82	6	76	92.65 %
4-E-ACT/M-CTT	89	0	89	100 %
5-E-AAG/M-CAG	89	1	88	98.88 %
Total	449	10	439	97.77 %
Average	89.8	2	87.8	

The total number of amplicons generated was 449 with an average of 89.8 per primer combination. The total number of monomorphic amplicons was 10 with an average of 2 per primer combination, while the total number of polymorphic amplicons was found to be 439 with an average of 87.8 per primer combination, and the percentage of polymorphism was 97.77 %. All scored bands ranged in size from 100 bp to 700 bp.

The primer combination E-AAC / M-CAG amplified the highest number of amplicons (115 amplicons) with a percentage of polymorphism reaching 98.26%. While the lowest number of amplicons was achieved by primer combination (E-ACA/M-CTG) which generated 74 amplicons with polymorphism percentage of 98.65%. These results show that AFLP technique is an efficient tool in determining genetic variation among accessions as it produces multiple polymorphic markers from single selected primer combinations.

A total of 32 unique markers (7.29%) were identified to be useful as specific markers in discriminating between the okra accessions. Out of these unique markers 18 were positive and 14 were negative. The highest number of AFLP unique markers was scored for primer combination (E-AAG \ M-CAG) which produce 13 markers, followed by primer combination (E-AAC \ M-CAG) with 12 markers and primer combination (E-ACC \ M-CTG) gave 3 unique markers, while 2 markers were scored for both primer combinations (E-ACA \ M-CTG) and (E-ACT\ M-CTT). Genetic differentiation was achieved for all 29 accessions.

Dendrogram based on AFLP markers

The dendrogram (fig 3) was divided into 2 main clusters at 51% similarity, with 15 accessions falling in one cluster and the other 14 accessions in the second cluster. The first cluster was further sub divided at 62% similarity into two sub-groups discriminating the same five accessions from Sohag as was the case with the ISSR data, from the rest of the group. The second main cluster included the rest of the accessions collected from different locations, with similarity percentage ranging from 63% to about 82%. It was noted, that the same tendency of grouping accessions from the same region was observed, where accessions from the Delta region (Menoufia and El-Beheira) were grouped together. Similarily, accessions from Ismailia and North Sinai (Eastern region) were also close together. El-Dakahlia accession number7 from the Delta region exhibited genetic relatedness to Quena accessions (Upper Egypt) confirming the results of the ISSR data.

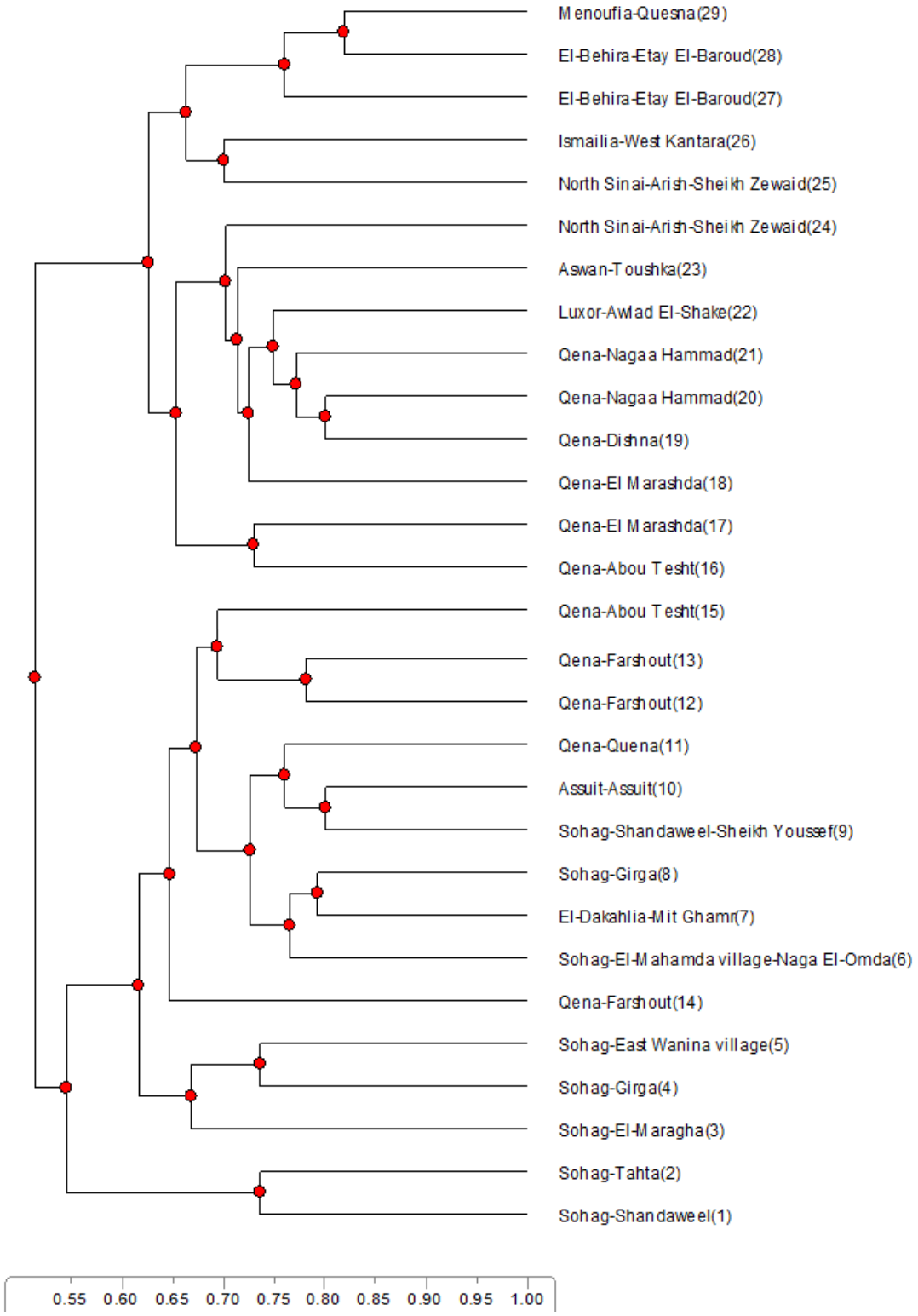

Fig (3): Dendrogram for the 29 okra accessions constructed from AFLP data.

Dendrogram based on combined ISSR and AFLP data

The ISSR and AFLP similarity matrices data showed that the range between the lowest and highest similarity coefficients was wider for AFLP (0.51-0.82) than ISSR (0.73-0.90) which indicates that the AFLP system was able to detect a higher level of polymorphism between pairwise combinations.

The dendrogram constructed from the combined ISSR and AFLP data (Fig 4) showed the same general trend in grouping accessions collected from the same region with the exception of El-Dakahlia (7) accession which showed a close genetic relationship with the Upper Egypt genotypes.

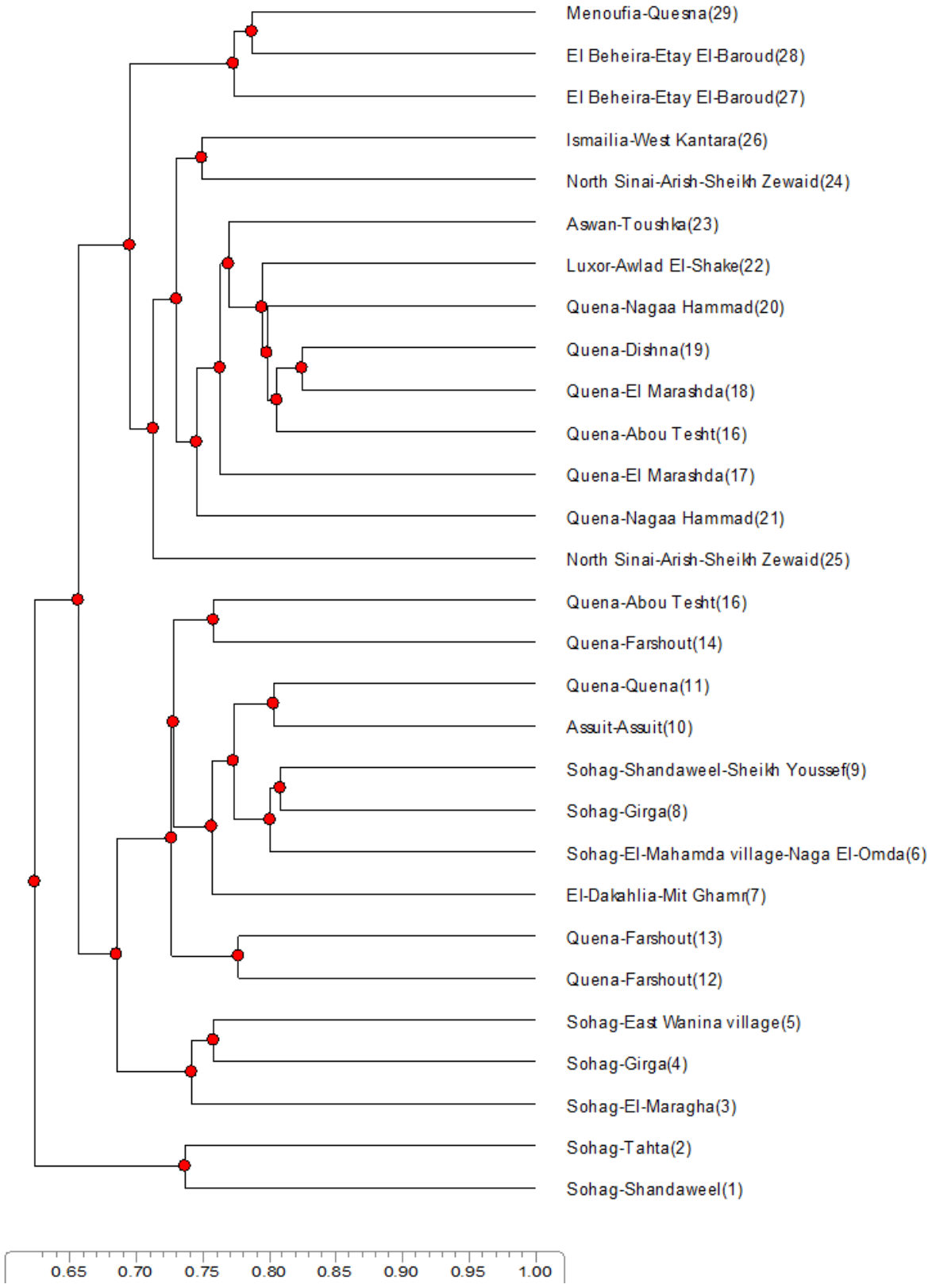

Fig (4): Dendrogram for the 29 okra accessions constructed from combined ISSR and AFLP data.

Our results are in agreement with Kim et al. (2010) who analyzed genetic diversity for seventeen Kenaf varieties (Hibiscus cannabinus L.) collected from several regions around Asia, Europe and Korea using morphological characters and AFLP marker technique. The AFLP analysis was conducted with 34 AFLP primer combinations which generated a total of 3193 polymorphic bands (out of 3914) with a polymorphic percentage of 82%. The clusters were divided into two major groups with a similarity coefficient of 0.63 by UPGMA analysis method, but each group did not show a common tendency. Therefore, based on the UPGMA results from the combined analysis of ISSR and AFLP data, the strategy for the management of okra genetic resources can be planned in constituting the core collection by selecting the accessions representing a maximum or wide range of diversity.

The ISSR and AFLP results were in agreement with those obtained by Hussein et al. (2003) in a study on 14 citrus accessions. The five AFLP combinations gave an average of 97.7 % polymorphism and 60.8 polymorphic amplicons per primer combination. Similarly, Cheng et al. (2004) studied the genetic relationships of 23 Kenaf (Hibiscus cannabis L.) accessions using morphological characterization and AFLP analysis. They found difficulty in identifying Kenaf accessions based merely on morphological characters, due to their limited variations. On the other hand, the AFLP

markers revealed 90.2% polymorphism with similarity coefficients ranging from 0.72 – 0.85 in the African and Asian subgroup while most of the Kenaf accessions currently grown in North and Central America and derived from Asian strain were included in another subgroup with a similarity coefficient ranging from 0.67 to 0.85 among accessions of this group.

Also, Murtaza (2006) used four AFLP primer combinations to assess the level of genetic variation among 20 cotton cultivars belonging to the old and new world species of cotton. The dendrogram resulting from the UPGMA cluster analysis separated the Gossypium hirsutum genotypes from G. Araboreum and assigned the G. hirsutum genotypes into groups corresponding with their origin and pedigree relationships.

In conclusion, additional okra genotypes need to be collected from other misrepresented locations to capture the genetic diversity present in the different ecogeographical regions.

REFERENCE

Akotkar DK. De. Pradip K. and AK Pal (2010). Genetic Variability and Diversity in Okra (*Abelmoschus esculentus L. Moench*). *Electronic Journal of Plant Breeding*, 1(4): 393-398.

Aladele, E. Sunday O. J. Ariyo and Robert de Lapena (2008). Genetic relationships among West African okra *(Abelmoschus caillei*) and Asian genotypes *(Abelmoschus esculentus)* using RAPD, *African Journal of Biotechnology* 7 (10) :1426-1431.

Chakravarthi, B.K. and Naravaneni, R. (2006). SSR marker based DNA fingerprinting and diversity study in rice *(Oryza sativa*. L.) *African journal of Biotechnology* 5(9): 684-688.

Cheng Zhou, Bao-Rong Lu, Kazuhiko Sameshima,, Da-Xu Fu and Jia-Kuan Chen (2004). Identification and genetic relationships of Kenaf (*Hibiscus cannabinus* L.) germplasm revealed by AFLP analysis. *Genetic Resources and Crop Evolution* 51: 393-401.

Düzyaman, Eftal (2005). Phenotypic Diversity within a Collection of Distinct Okra (*Abelmoschus esculentus*) Cultivars Derived from Turkish Land Races, *Genetic Resources and Crop Evolution* 52(8) : 1019-1030.

Hussein ,Ebtissam H.A, Samir, M.M. Abd-alla, Nahla, A. Awad and Mohamed S. Hussein (2003). Genetic analysis in some Citrus accessions using Microsatellites - and AFLP-based markers, *Arab J. Biotech* 6(2): 203-222.

Hamon, S. and van Sloten, D. H. (1989). Characterization and evaluation of okra. *In A. D. H. Brown and O. Frankel (eds.).* The use of crop genetic resources. Cambridge pp. 173-196.

IBPGR (1991). International Crop Network Series. 5. Report of an International Workshop on Okra Genetic Resources, *International Board for Plant Genetic Resources*, *Rome*, *Italy*.

Jie Huang, Chen Xiaobin, Ye Hualan and Liu Guodao (2008). The ISSR analysis of 16 Okra resources, *Chinese Agricultural Science Bulletin*.2008-05

Kim Wook Jin, Dong Sub Kim, Sang Hoon Kim, Jin-Baek Kim, Eun Jeong Goh, and Si-Yong Kang (2010). Analysis of Genetic Similarity Detected by AFLP and PCoA among Genotype of Kenaf (Hibiscus cannabinus L.) J, *Crop Sci.Biotech*13(4):243-249.

Kochhar, S.L. (1986) Tropical Crops. Macmillan Publishers Ltd. *London and Basigstoke*, pp 467.

Martinello, G.E., N.R. Leal, A.T. Amaral Jr, M.G. Pereira, R.F. Daher (2001). Comparison of morphological characteristics and RAPD for estimating genetic diversity in *Abelmoschus* spp. *acta horticulturae*, ISHS 546:101-104.

Morakinyo, J.A. and Adeymi (2007). Analysis of variation patterns in some accessions of okra (*Abelmoschus esculentus (L.) Moench*.), *Nigeria. Nigerian Journal of Botany* 20(1), 139-148.

MSTAT-C Statistical Software, (1989). Users guide: a microcomputer program for the design, management and analysis of agronomic research experiments. *Michigan University*, *East Lansing*, *MC*, *USA*.

Murtaza, N. (2006). Cotton genetic diversity study by AFLP markers, *Electronic Journal of Biotechnology* 9(4):456-460.

NARP (1993). National Agricultural Research Project, *Horticultural crops* 3 July. NARP, CSIR, Accra.

Nei, M. (1972). Genetic distance between populations. *Am. Nat*. 106:283-292.

Omonhinmin,, Conrad A. and Osawaru, M. Edwin (2005). Morphological characterization of two species of *Abelmoschus*: *Abelmoschus esculentus* and *Abelmoschus caillei*, *PGR Newsletter* 144: 51-55.

Oppong-Sekyere, D. R., Akromah, E. Y., Nyamah, E. Brenya and S. Yeboah (2011). Characterization of okra (*Abelmoschus spp*. L.) germplasm based on morphological characters in Ghana. *Journal of Plant Breeding and Crop Science* 3(13), pp. 368 – 379.

Ramanjinappa, V., K. H. Arunkumar, Ashok Hugar and M. S. Shashibhaskar (2011). Genetic variability in okra (*Abelmoschus esculentus l. moench*.). *Plant archives* 11 (1): 435-437.

Rithichai Panumart, Yukihiro Fujime, Sutevee Sukprakarn, Satoshi Terabayashi and Shuichi Date (2004). Morphological properties of flower and fruit in okra *Abelmoschus esculentus. Sci. Rep. Kyoto Pref. Uni.. Hum. Env. & Agr*. 56:67-74.

Rubenstein, K. and Heisey, P. (2003). Plant Genetic Resources: New Rules for International Exchange, Bioversity's Regional Office for the Americas), *IPGRI. Villa Gualino*, *Turin*, *Italy*, March 1-5: 63p.

Saifullah, M. and M. G. Rabbani (2009). Evaluation and characterization of okra (Abelmoschus esculentus L. Moench.) genotypes, *SAARC J. Agri*. 7 (1): 92-99.

Sneath, P.M.A. and Sokal, R.B. (1973). Numerical Taxonomy. The Principles and Practice of Numerical classification, *San Francisco*, *W H Freeman.*

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods, *6th Edn.*, *Iowa State university press*, *Ames*, *IA.*, *USA*.

Verma, V. D. (1993). Collecting eggplant and okra in Madhya Pradesh and Maharastra. *IBPGR Newsletter for Asia Pacific and Oceania* 13:14-15.