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ASSESSMENT OF GENETIC DIVERSITY IN OKRA (*Abelmoschus esculentus* L.) USING RAPD MARKERS

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Thirty nine okra genotypes were assessed for genetic variability using Random Amplified Polymorphic DNA (RAPD) markers. Twenty polymorphic RAPD primers amplified 111 DNA fragments, with an average of 5.5 fragments per primer. Among 39 okra genotypes, 107 fragments (96%) were found to be polymorphic. The UPGMA cluster analysis placed okra genotypes into seven main clusters. 'Sabzpari 2001' and 'Acc.No.019221' had shown maximum similarity (83%) while the minimum similarity (44.14%) was observed between the genotypes 'Punjab Selection' and 'Acc.No.019217'. Thus, by using RAPD primers a considerable polymorphism appeared to exist, which showed genetic variability in the okra genotypes.
Keywords: DNA markers, genetic distance, malvacae, salinity, vegetable.

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is an annual, often cross pollinated important vegetable of the tropical and subtropical areas. It was originated in India but now grown in many parts of the world. Okra seeds containing good quality edible oil and high protein are used to complement other protein sources. Okra flour is an effective food additive in wheat flour for baking bread with good technological and sensory characteristics.

The availability of genetic diversity is the pre-requisite for the success of any crop improvement program (Poehlman and Sleper, 1995). Genetic diversity either exists naturally in the gene pool or it is created artificially by using different methods (Poehlman and Sleper, 1995). The existing variability among germplasm can be assessed by using different morphological and molecular markers. The molecular analysis of the genome at the DNA level can provide a greater advantage because DNA sequences are the same in all of the living cells of a plant, regardless of physiological or developmental state of the tissue. The diversity based on phenotypic and morphological characters usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification (Vogel *et al*., 1996). Molecular markers have been proved to be powerful tool in the evaluation of genetic variation and finding genetic relationships within and among species (Chakravarthi and Naravaneni, 2006). Among the several DNA based techniques, Random Amplified Polymorphic DNA (RAPD) is simple, less technology intensive, cheap and does not require pre-sequencing for designing primers (Williams *et al.*, 1990; Aladele, 2008; Azmat and Khan, 2010). RAPD markers have been extensively used as a tool to estimate genetic diversity in a number of crop species like *Triticum* (Cao *et al*., 1998), cotton (Multani and Lyon, 1995; Rehman *et al*., 2002; Azmat and Khan, 2010) and okra (Aladele, 2008; Martinello *et al*., 2001). It has become very important to identify individual varieties using molecular markers as the number of genetically related varieties released by breeders has made morphological identification more difficult and the DNA fingerprints becomes the genetic identity of a genotype. Keeping in view the importance of okra as vegetable crop, its nutritional value, area and production in Pakistan and the wide spread of salinity the present studies were carried out. The aim of study was to provide information about genetic diversity at molecular level. The information gained would be useful in the breeding programs aimed at the development of salt tolerant okra in the country.

MATERIALS AND METHODS

***Plant material and DNA extraction*:** The 39 okra accessions/genotypes including salinity tolerant and susceptible genotypes (Ikram *et al*., 2012) were obtained from various sources in Pakistan and assessed for genetic variation. The genotypes were grown in 250 ml plastic cups in a glasshouse. The fresh leaves were collected from 10-14 day old seedlings for DNA extraction.

The DNA extraction was done by using QIAGEN DNeasy® Kit (QIAGEN Ltd., Crawley, UK) (Saunders *et al*., 2001). Okra has high polysaccharides and other secondary metabolites, which have viscous, glue-like texture, and makes the DNA uncontrollable in pipetting and also inhibit the Taq polymerase activity (Fang *et al*., 1992; Azmat *et al*., 2012). Therefore, QIAGEN DNeasy® Kit was used following the prescribed method to isolate clean and good quality genomic DNA suitable for PCR amplification. The quality of the extracted DNA was checked by running 7 µl

DNA mixed with 3 µl Bromophenol blue dye on 0.8% agarose gel prepared in 0.5X TBE buffer.

Three dilutions (15, 20 and 25 ng/µl) of DNA were prepared in deionized double distilled water (d_3H_20) to optimize the DNA concentration for best amplification. The concentration 15 ng/µl was found good for better amplification.

***Molecular analysis*:** In total 50 decamer RAPD primers of five series (A, B, C, J and K) obtained from Gene-Link Company, USA were used for PCR amplifications. Twenty of 50 RAPD primers were polymorphic which were used for further analysis. The PCR conditions were optimized for, DNA concentration (15ng/µl) , PCR buffer, Gelatin, $MgCl_2$ (50mM), dNTPs (0.2mM), primer (15ng/µl) and Taq DNA polymerase (0.2 unit/reaction) (MBI, Fermentas, Vilnius, Lithuania). The PCR were carried out in 25 µl reactions. The temperature profile for the reaction is given as: Hot start at 95°C (only at the start of reaction) for 5 min., denaturation at 95°C for 1min., primer annealing at 36°C for 1 min., extension at 72°C for 2 min. and final extension at 72°C (only at the end of reaction) for 10 min. The PCR products were resolved on 1.5% agarose gel by using ethidium bromide. The electrophoresed gels were examined under ultra violet Transilluminator and photographed using SyneGene Gel Documentation System (GDS). The amplification products were scored as present (1) and absent (0), and subjected to cluster analysis for the construction of similarity matrix and dendrogram by using Popgene32 software (Ver. 1.44) (Yeh *et al*., 2000)

RESULTS

Thirty nine okra genotypes were assessed for genetic diversity using 20 polymorphic RAPD primers. Considering all the primers and genotypes a total of 111 DNA fragments were amplified with an average of 5.5 fragments per primer. Of the total amplified fragments only 4 (3.6%) were monomorphic and the remaining 107 (96.4%) were polymorphic (Table 1). The maximum number of bands (11) was amplified by the primer GL (Gene Link) Decamer B-17 (AGGGAACGAG) while the minimum (2) bands were amplified by the primer GL Decamer J-15 (TGTAGCAGGG). The primers GL A-08 and GL J-19 amplified 5 DNA fragment each (Fig. 1a, b). The cluster analysis showed a wide range of similarity ranging from 44.14% to 82.88% (Table 2). The dendrogram placed okra genotypes into seven main clusters on the basis of similarity coefficients (Fig. 2). The cluster A was comprised of three genotypes i.e. Ikra III, Acc.No.015380-10934 and Clemson Spineless. Out of these three genotypes, Ikra III and Acc.No.015380-10934 were more similar to each other i.e. 73.87% and the third genotype Clemson Spineless also showed a close resemblance with these two. In the cluster B, there were 10 genotypes making four sub clusters. In the first sub cluster, there were three genotypes i.e. Pusa Green,

Table 1. Number of total and polymorphic bands produced by primers

Sr. No.	Name of primer	Primer sequence	Bands produced	Polymorphic bands
1	GL Decamer A-05	AGGGGTCTTG	3	3
2	GL Decamer A-07	GAAACGGGTG	7	6
3	GL Decamer A-08	GTGACGTAGG	5	3
4	GL Decamer A-09	GGGTAACGCC	4	4
5	GL Decamer A-11	CAATCGCCGT	5	5
6	GL Decamer B-15	GGAGGGTGTT	8	8
7	GL Decamer B-17	AGGGAACGAG	11	11
8	GL Decamer C-11	AAAGCTGCGG	4	4
9	GL Decamer J-01	CCCGGCATAA	4	4
10	GL Decamer J-07	CCTCTCGACA	4	4
11	GL Decamer J-08	CATACCGTGG	10	10
12	GL Decamer J-13	CCACACTACC	3	3
13	GL Decamer J-14	CACCCGGATG	7	7
14	GL Decamer J-15	TGTAGCAGGG	2	2
15	GL Decamer J-19	GGACACCACT	5	5
16	GL Decamer J-20	AAGCGGCCTC	6	6
17	GL Decamer K-02	GTCTCCGCAA	6	6
18	GL Decamer K-08	GAACACTGGG	4	4
19	GL Decamer K-17	CCCAGCTGTG	3	3
20	GL Decamer K-19	CACAGGCGGA	10	9
	Total		111	107
	Average		5.55	96.40

Table 2.Similarity matrix for Nei's and Li's coefficients of 39 okra genotypes obtained from RAPD markers

Sr	Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	**Ikra III**	*	0.7205	0.6667	0.6757	0.6577	0.6847	0.6396	0.6757	0.7387	0.6667	0.4955	0.7027	0.6757	0.7568	0.7027	0..6757	0.6486	0.6486	0.6577	0.5946
2	**Acc.No.019221**		*	0.7297	0.7568	0.6486	0.7658	0.6847	0.7387	0.6937	0.6396	0.5766	0.7477	0.6667	0.7477	0.7477	0.7027	0.7117	0.7657	0.6486	0.6396
3	**Parbhani Kranti**			*	0.6847	0.6486	0.7117	0.6667	0.6667	0.6626	0.6396	0.5586	0.6757	0.7027	0.7117	0.6577	0.6486	0.6577	0.6577	0.6126	0.5676
4	**IN-1048**				*	0.5856	0.7027	0.6757	0.6577	0.7027	0.6126	0.6396	0.6847	0.5856	0.6486	0.7387	0.6396	0.6847	0.6486	0.5676	0.7027
5	**Punjab Selection**					*	0.6306	0.5676	0.6577	0.5766	0.6847	0.4414	0.7387	0.6216	0.5586	0.5946	0.6036	0.5946	0.6667	0.6937	0.6306
6	**Acc.No.019236**						*	0.7568	0.7568	0.6577	0.7117	0.5225	0.6937	0.6847	0.7477	0.7117	0.7748	0.7477	0.6937	0.6486	0.6757
7	**Acc.No.019232**							*	0.7117	0.6667	0.6847	0.6036	0.7027	0.7297	0.6847	0.7027	0.7117	0.7928	0.5946	0.5676	0.6126
8	**Acc.No.019225**								*	0.6667	0.7387	0.5856	0.8108	0.7117	0.7207	0.6847	0.7477	0.6486	0.6486	0.6036	0.7387
9	**Acc.No.015380-10934**									*	0.6757	0.5586	0.7117	0.6126	0.7117	0.7477	0.6486	0.7297	0.6396	0.5946	0.6577
10	**Acc.No.015382**										*	0.4865	0.7297	0.6486	0.6757	0.6757	0.7207	0.6937	0.6396	0.5946	0.7477
11	**Acc.No.019217**											*	0.5946	0.5676	0.5225	0.5586	0.5315	0.5766	0.5225	0.5495	0.6306
12	**Acc.No.015371**												*	0.6847	0.6396	0.7297	0.7207	0.7117	0.7117	0.7027	0.6937
13	**Acc.No.019233**													*	0.7027	0.6847	0.6937	0.6847	0.6486	0.5676	0.5586
14	**Acc.No.019223**														*	0.8198	0.7387	0.6577	0.5315	0.5946	0.5856
15	**Acc.No.019231**															*	0.8108	0.7477	0.6216	0.6126	0.5856
16	**Acc.No.000010-10237**																*	0.7748	0.6306	0.6216	0.6847
17	**Chinese Red**																	*	0.6937	0.6126	0.6577
18	**Ikra 1**																		*	0.6847	0.6757
19	**Acc.No.015377-10811**																			*	0.6306
20	**Acc.No.019211**																				*
21	**Clemson Spineless**																				
22	**Acc.No.019235**																				
23	**Pusa Green**																				
24	**Sabzpari 2001**																				
25	**IN- 97**																				
26	**Acc.No.019228**																				
27	**Acc.No.019224**																				
28	**Ikra 4**																				
29	**Acc.No.000004**																				
30	**Pusa Swani**																				
31	**IN- 89**																				
32	**Acc.No.000026-10496**																				
33	**Acc.No.019234**																				
34	**Acc.No.019230**																				
35	**Acc.No.019215**																				
36	**Green Wonder**																				
37	**Acc.No.019216**																				
38	**University Okra**																				
39	**Acc.No.019218**																				

Continued…….

Sr	Genotypes	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1	**Ikra III**	0.7387	0.7297	0.7117	0.6216	0.5946	0.5676	0.6757	0.6216	0.7477	0.6036	0.5495	0.6306	0.6396	0.6937	0.7297	0.6396	0.6396	0.5946	0.6306
2	**Acc.No.019221**	0.7117	0.7928	0.7387	0.8288	0.6396	0.6486	0.7748	0.6667	0.7928	0.6126	0.7027	0.6577	0.7207	0.7027	0.7568	0.7027	0.6486	0.6757	0.5856
3	**Parbhani Kranti**	0.6757	0.7387	0.7387	0.7207	0.6757	0.5766	0.6847	0.6847	0.7207	0.6306	0.6486	0.6577	0.6667	0.7207	0.6126	0.6306	0.6126	0.6036	0.5495
4	**IN-1048**	0.6486	0.7658	0.7297	0.6937	0.5586	0.5495	0.6577	0.6216	0.6577	0.5856	0.5856	0.5586	0.6216	0.6577	0.6757	0.6577	0.6757	0.5946	0.5586
5	**Punjab Selection**	0.6126	0.6396	0.6577	0.6757	0.7027	0.6216	0.6036	0.6757	0.6937	0.6396	0.5495	0.7027	0.7117	0.6216	0.5676	0.5856	0.5315	0.5225	0.5225
6	**Acc.No.019236**	0.7117	0.7748	0.7928	0.7027	0.6937	0.6486	0.7207	0.6667	0.7568	0.5946	0.6486	0.6937	0.6847	0.6847	0.6847	0.7387	0.7027	0.6577	0.6216
7	**Acc.No.019232**	0.7027	0.7838	0.6937	0.6757	0.6306	0.5676	0.7477	0.7297	0.6937	0.7117	0.6577	0.7568	0.6216	0.6937	0.6396	0.6757	0.6577	0.6486	0.5766
8	**Acc.No.019225**	0.7027	0.7658	0.7297	0.7117	0.7027	0.6577	0.7117	0.7477	0.7297	0.6396	0.6577	0.7568	0.7297	0.6577	0.6577	0.6396	0.6757	0.6486	0.6216
9	**Acc.No.015380-10934**	0.7297	0.7027	0.6667	0.5766	0.6036	0.6306	0.7027	0.6306	0.6486	0.5586	0.5766	0.6036	0.7207	0.6486	0.6847	0.6126	0.6486	0.5495	0.5856
10	**Acc.No.015382**	0.6937	0.7027	0.7027	0.6306	0.7658	0.5586	0.7027	0.7387	0.6306	0.7027	0.5586	0.7117	0.6847	0.6667	0.6126	0.5405	0.7027	0.6396	0.5856
11	**Acc.No.019217**	0.6667	0.6216	0.6036	0.5315	0.4505	0.5676	0.6036	0.5315	0.5495	0.5135	0.6577	0.5766	0.5856	0.6036	0.4955	0.5856	0.6036	0.5405	0.6126
12	**Acc.No.015371**	0.7838	0.7387	0.7027	0.7027	0.6396	0.5946	0.7568	0.7207	0.8108	0.6306	0.6667	0.7838	0.7928	0.6486	0.6667	0.6306	0.7027	0.6577	0.6937
13	**Acc.No.019233**	0.6667	0.7117	0.6577	0.6577	0.6486	0.5676	0.6396	0.7297	0.6937	0.6757	0.5676	0.7748	0.6757	0.7297	0.6216	0.6396	0.6396	0.6486	0.5946
14	**Acc.No.019223**	0.7117	0.7568	0.7387	0.6306	0.6757	0.6126	0.7387	0.6486	0.7387	0.5766	0.5946	0.6757	0.6847	0.7387	0.7027	0.6847	0.7027	0.6216	0.5856
15	**Acc.No.019231**	0.6937	0.7387	0.7568	0.6847	0.5856	0.5946	0.7207	0.6667	0.7387	0.6126	0.6486	0.7297	0.7207	0.7027	0.7027	0.7027	0.7387	0.6036	0.6396
16	**Acc.No.000010-10237**	0.7207	0.7658	0.7477	0.7117	0.6306	0.6036	0.6757	0.6937	0.6937	0.6036	0.6036	0.7207	0.6577	0.7297	0.7117	0.7297	0.7658	0.6667	0.6847
17	**Chinese Red**	0.7658	0.7748	0.7387	0.7027	0.6036	0.5405	0.6846	0.6306	0.6847	0.6486	0.6306	0.7117	0.7027	0.7207	0.6847	0.7387	0.7387	0.7297	0.6577
18	**Ikra 1**	0.6937	0.5946	0.6306	0.7027	0.6577	0.5225	0.5766	0.6306	0.6667	0.6126	0.6126	0.6216	0.7027	0.6306	0.6126	0.6126	0.6486	0.7297	0.7117
19	**Acc.No.015377-10811**	0.6667	0.6216	0.6216	0.5856	0.5405	0.6036	0.7477	0.6396	0.7477	0.5856	0.6757	0.6486	0.7117	0.6757	0.6036	0.6036	0.6396	0.6667	0.7568
20	**Acc.No.019211**	0.6937	0.6486	0.6667	0.6126	0.6396	0.5946	0.6486	0.7206	0.5766	0.6306	0.6306	0.6036	0.6667	0.6486	0.5766	0.5766	0.6847	0.5856	0.5676
21	**Clemson Spineless**	*	0.7387	0.7027	0.6486	0.6396	0.6126	0.6847	0.6126	0.7387	0.6126	0.6306	0.6577	0.7387	0.6847	0.6126	0.6486	0.7027	0.6396	0.6757
22	**Acc.No.019235**		*	0.8018	0.7117	0.6036	0.6396	0.7477	0.6937	0.6937	0.6757	0.6036	0.6847	0.6216	0.7117	0.6937	0.7297	0.7117	0.6847	0.5766
23	**Pusa Green**			*	0.7117	0.5946	0.5856	0.7117	0.6757	0.7117	0.5856	0.6396	0.6306	0.6757	0.7117	0.7117	0.7297	0.6757	0.6126	0.6486
24	**Sabzpari 2001**				*	0.7027	0.6216	0.6757	0.6396	0.7117	0.6757	0.6937	0.6667	0.6937	0.7117	0.7297	0.6757	0.6396	0.6306	0.5946
25	**IN- 97**					*	0.6667	0.5946	0.6306	0.6306	0.7027	0.5766	0.6757	0.7027	0.6306	0.5405	0.5405	0.6126	0.6216	0.5135
26	**Acc.No.019228**						*	0.6577	0.5856	0.6036	0.5676	0.6757	0.6126	0.7117	0.6396	0.5676	0.6216	0.6036	0.5225	0.5225
27	**Acc.No.019224**							*	0.7658	0.8198	0.6577	0.7477	0.7568	0.7297	0.7117	0.6577	0.6396	0.6577	0.6306	0.6126
28	**Ikra 4**								*	0..7117	0.7117	0.5856	0.7568	0.6216	0.7477	0.6396	0.6396	0.5856	0.5946	0.5405
29	**Acc.No.000004**									*	0.6036	0.7117	0.7928	0.7838	0.7477	0.6577	0.6396	0.6577	0.6486	0.6667
30	**Pusa Swani**										*	0.6216	0.7207	0.6396	0.6396	0.5676	0.6036	0.5856	0.6667	0.4505
31	**IN- 89**											*	0.6667	0.7658	0.6577	0.6036	0.5676	0.6216	0.5766	0.6486
32	**Acc.No.000026-10496**												*	0.7568	0.7568	0.6306	0.6306	0.6486	0.6396	0.6036
33	**Acc.No.019234**													*	0.7117	0.6216	0.6036	0.6757	0.6126	0.6667
34	**Acc.No.019230**														*	0.7297	0.6577	0.6577	0.6486	0.6306
35	**Acc.No.019215**															*	0.8018	0.7297	0.6667	0.6486
36	**Green Wonder**																*	0.7297	0.6486	0.6486
37	**Acc.No.019216**																	*	0.7568	0.7387
38	**University Okra**																		*	0.6757
39	**Acc.No.019218**																			*

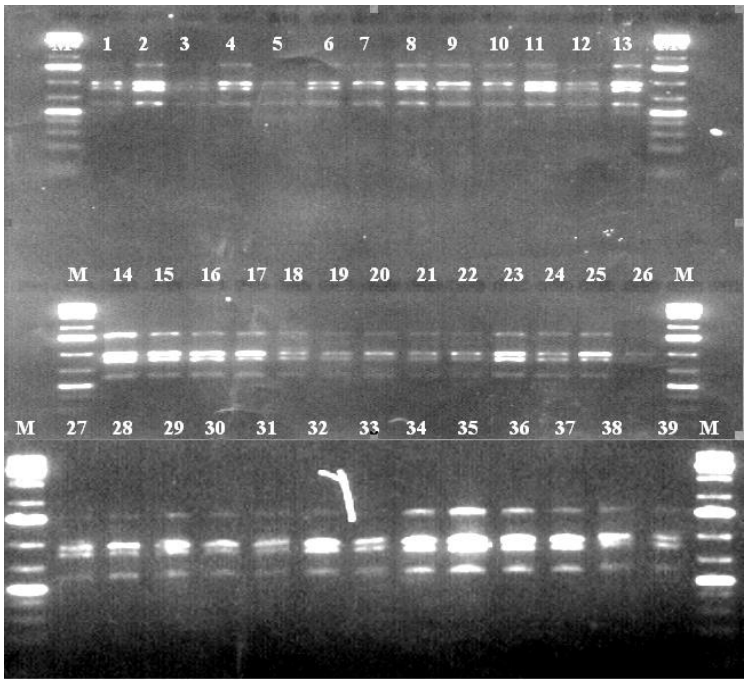

Figure 1a. The bands amplified by the Primer GL A-08 in 39 okra genotypes

The genotypes in lanes 1-39 are IkraIII, Acc.No.019221, Parbhani Kranti, IN-1048, Punjab Selection, Acc.No.019236, Acc.No.019232, Acc.No.019225, Acc.No.015380-10934, Acc.No.015382, Acc.No.019217, Acc.No.015371, Acc.No.019233, Acc.No.019223, Acc.No.019231, Acc.No.000010-10237, China Red, Ikra 1, Acc.No.015373-10811, Acc.No.019211, Clemson Spineless, Acc.No.019235, Pusa Green, Sabzpari 2001, IN-97, Acc.No.019228, Acc.No.019224, Ikra 4, Acc.No.000004, Pusa Sawani, IN- 89 Acc.No.000026-10496, Acc.No.019234, Acc.No.019230,

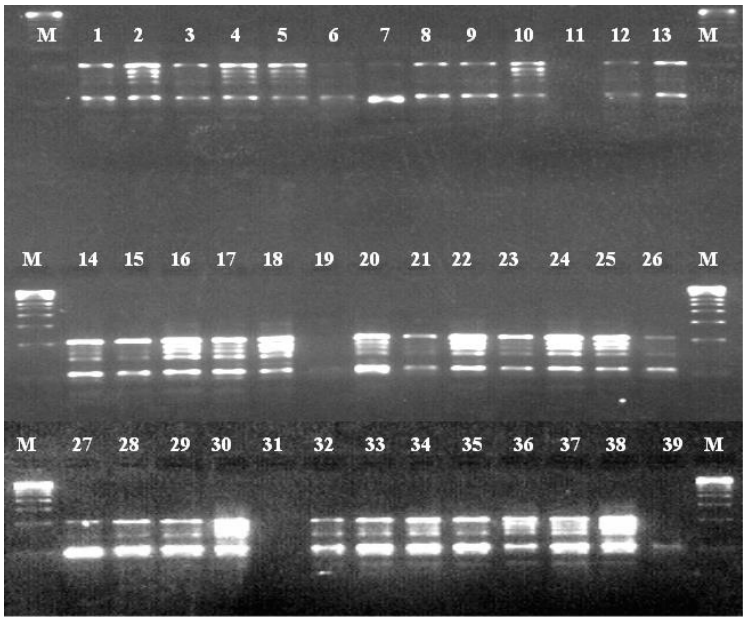

Figure 1b. The bands amplified by the Primer GL J-19 in 39 okra genotypes

The genotypes in lanes 1-39 are IkraIII, Acc.No.019221, Parbhani Kranti, IN-1048, Punjab Selection, Acc.No.019236, Acc.No.019232, Acc.No.019225, Acc.No.015380-10934, Acc.No.015382, Acc.No.019217, Acc.No.015371, Acc.No.019233, Acc.No.019223, Acc.No.019231, Acc.No.000010-10237, China Red, Ikra 1, Acc.No.015373-10811, Acc.No.019211, Clemson Spineless, Acc.No.019235, Pusa Green, Sabzpari 2001, IN-97, Acc.No.019228, Acc.No.019224, Ikra 4, Acc.No.000004, Pusa Sawani, IN- 89 Acc.No.000026-10496, Acc.No.019234, Acc.No.019230, Acc.No.019215, Green wonder, Acc.No.019216, University okra, Acc.No.019218, respectively

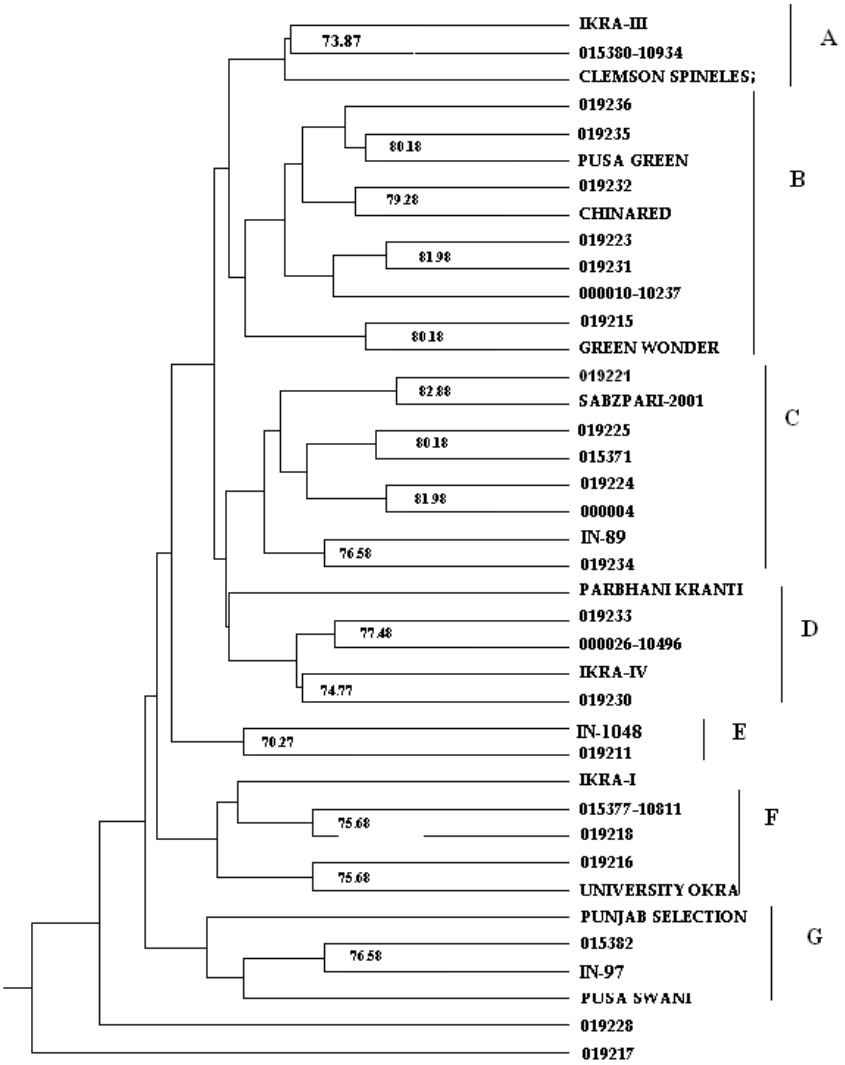

Figure 2. Dendrogram of 39 okra genotypes using RAPD primers

Acc.No.019235 and Acc.No.019236. The genotypes Pusa Green and Acc.No.019235 showed 80.18% similarity, while the third genotype (Acc.No.019236) showed a closer relationship with these two genotypes. In the second sub cluster, there were only two genotypes i.e. Chinese Red and Acc.No.019232, forming a sister group with 79.28 % similarity. The third sub cluster contained three genotypes i.e., Acc.No.019223, Acc.No.019231 and Acc.No.000010-10237. The Acc.No.019223 and Acc.No.019231 showed a similarity of 81.98%, whereas the third one was also in a close resemblance with these two. Similarly, the fourth sub cluster contained the genotypes, Green Wonder and Acc.No.019215 showing 80.18% similarity. All these four sub clusters were close with a distinct difference from other groups. Likewise, the third main cluster (C) had eight genotypes namely Sabzpari 2001, Acc.No.019221, Acc.No.019225, Acc.No.015371, Acc.No.019224, Acc.No. 000004, IN-89 and Acc.No.019234. On the basis of relatedness and differences, these eight genotypes further made four subgroups. The first sub cluster exhibited 82.88% similarity between the two genotypes (Sabzpari, 2001 and Acc.No.019221), Acc.No.019225 and Acc.No.015371 made the second sub cluster on the basis of their close resemblance (80.18%). The third subcluster was made by the Acc.No.019224 and Acc.No.000004, these two genotypes presented 81.98% similar banding pattern. The last, fourth sub cluster was produced by IN-89 with Acc.No.019234 showing genetic similarity of 76.58%. Also both these genotypes showed the minimum similarity to each other than the remaining three sub clusters that showed 82.88, 80.18, and 81.98% relatedness among the six genotypes respectively. Five genotypes comprised the fourth main cluster (D) namely Parbhani Kranti, Ikra IV, Acc.No.019230, Acc.No.019233 and Acc.No.000026-

10496. The genotypes, Ikra IV with Acc.No.019230 and Acc.No.019233 with Acc.No.000026-10496 formed sister groups with similarity values 74.77 and 77.48% respectively. The genotype Parbhani Kranti was also included in this cluster with similarity ranging from 65.77% to 72.07% with these genotypes. The fifth main cluster (E) was consisted of only two 2 genotypes IN-1048 and Acc.No.019211 with genetic relatedness of 70.27%. Any other genotype did not show close resemblance with this sub cluster. Similarly five genotypes, having close resemblance were grouped in the sixth cluster (F) i.e. Ikra I, Acc.No.015373-10811, Acc.No.019218, Acc.No.019216 and University Okra. In these five Acc.No.015373-10811 and Acc.No.019218 had 75.68% genetic relatedness and made a separate sister group, while another sub cluster was observed between the genotypes University Okra and Acc.No.019216. This sister group indicated 75.68% relatedness. The seventh cluster (G) had 4 genotypes, two of them, IN-97 and Acc.No.015382 made a sub cluster having 76.58% genetic similarity whereas the nearest relation with this subcluster was formed by Pusa Sawani. With this group of three genotypes (Pusa Sawani, IN-97 and Acc.No.015382) another genotype Punjab Selection showed a close resemblance and was also included in this main cluster having 63.96% genetic similarity with Pusa Sawani. The Acc.No.019228 and Acc.No.019217 showed the highest level of diversity with the remaining 37 genotypes. The Acc.No.019217 was least similar with other genotypes, with a genetic similarity ranging from 44.14 to 66.67%, while the Acc.No.019228 had 52.25 to 66.67% and showed genetic diversity with the remaining 37 genotypes. The Acc.No.019228 and Acc.No.019217 were 56.76% similar with each other. Thus, the survey of the 39 genotypes with RAPD markers indicated that considerable genetic diversity existed among the 39 okra genotypes.

DISCUSSION

Thirty nine genotypes of okra were assessed using 20 polymorphic RAPD primers. These twenty primers amplified 111 DNA fragments with an average of 5.5 fragments per primer. Out of the total amplified fragments, only 4 (3.6%) were monomorphic and the remaining 107 (96.4%) were polymorphic. This polymorphism was an indication of prevalence of moderate diversity among these 39 okra genotypes (Punitha and Raveendran, 2004). In lettuce 110 (65.9%) of the total 167 fragments produced by twenty ISSR (inter-simple sequence repeat) primers showed polymorphism with an average of 8.5 polymorphic bands per primer (Vicente *et al*., 2008), however, in eggplant nine RAPD primers showed 95.3% (Sadder *et al*., 2007). In okra 103 RAPD fragments were generated by 31 decamer primers in 43 genotypes of okra (Martinello *et al*., 2003). The cluster analysis showed a wide range of similarity raging from 44.14 to 82.88% while 86 to 100% genetic similarity was observed using Sequence-related amplified polymorphism (SRAP) markers in okra among 23 genotypes (Gulsen *et al*., 2007). The cluster analysis divided the 39 okra genotypes into seven main clusters and Acc.No.019217 and Acc.No.019228 were most diverse in dendrogram and were not grouped with any other genotype. Aladele *et al.* (2008) used RAPD markers in 93 okra genotypes, and only one genotype TOT7444 was found more diverse from the remaining genotypes.

The Acc.No.019217 (tolerant to NaCl salinity for germination percentage, relative germination percentage and absolute Na^+ accumulation) (Ikram *et al*., 2012) was found having medium distance from the three salinity susceptible accessions, Acc.No.015382, Acc.No.015380-10934 and Acc.No.019233 (Ikram *et al*., 2012) with similarity percentage of 48.65, 55.86 and 56.76% respectively. Similarly another tolerant genotype Ikra III (Ikram *et al*., 2012) showed a lesser percentage of similarity 66.67 and 67.57% with non tolerant accessions, Acc.No.015382 and Acc.No.019233 (Ikram *et al*., 2012) respectively. Genetic diversity was estimated among one susceptible and three salinity tolerant genotypes of rice and a similarity range of 31% to 53% was found (Xie *et al*., 2000).

Some tolerant and susceptible genotypes showed maximum similarity to each other such as Ikra III (tolerant) showed 73.87% similarity with Acc.No.015380-10934 (susceptible) and Acc.No.015371 (tolerant) showed 72.97% similarity with Acc.No.015382 (susceptible). Apparently the main reason of the close resemblance between tolerant and susceptible genotypes might be that the RAPD primers were unable to amplify the polymorphic sites between salinity tolerant and susceptible genotypes, or may be these primers did not have the sequence to produce polymorphism between these genotypes. The cause of polymorphism is the change of nucleotide sequence either in primer or template DNA (Williams *et al*., 1990). Roy *et al.* (1992) also found that RAPD markers were too sensitive to detect the variation among the individuals within and across the species.

Different methods for the purification of okra DNA were used, including CTAB (Doyle and Doyle, 1990) and a modified mini-prep method (Khan *et al*., 2004). But due to the presence of high range of polysaccharides, secondary metabolites and mucilage, it was very difficult to isolate good quality DNA from the okra genotypes. Bayer *et al*. (1999) also reported similar kind of difficulties during DNA extraction from the plants of Malvaceae family. Fang *et al*. (1992) also found that polysaccharides had viscous, glue like texture that makes it difficult to handle the DNA during pipetting as well as during amplification. To overcome this problem, QIAGEN DNeasy® Kit (QIAGEN Ltd., Crawley, UK) was used for the extraction of DNA from okra genotypes which was suitable for PCR amplification.

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