academicJournals

Vol. 12(21), pp. 3160-3170, 22 May, 2013
DOI: 10.5897/AJB12.2284
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Assessment of morphological and molecular diversity among okra [*Abelmoschus esculentus* (L.) Moench.] germplasm

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Accepted 3 May, 2013

Forty (40) RAPD primers and eight quantitative traits were used for the assessment of genetic diversity and establishing phenetic relationships in a set of 70 okra germplasm lines. Of the 40 RAPD primers, four namely, OPC-4, OPC-6, OPB-4 and OPB-5 did not show any amplification. A total of 170 RAPD scorable fragments were produced of which 92.94% alleles were observed to be polymorphic. Number of alleles scored per primer ranged from 2 to 7 with an average of 4.72 alleles per primer. PIC values ranged from 0 (OPC-5) to 0.89 (OPC-2) with an average value of 0.75. The genotypes were broadly divided into three clusters. Accession IC90171 appeared to be quite distinct as compared to other members of cluster I. Cluster III included five genotypes namely, IC117218, IC90165, IC117215, IC112496 and IC117217. Cophenetic r correlation value was found to be 0.94 suggesting that the cluster analysis strongly represents the similarity matrix. Genetic diversity as assessed by DARwin (based on quantitative morphological data) revealed that grouping of genotypes into different constellations did not follow any specific pattern suggesting independence of clustering pattern of the entries and their geographical origin.

Key words: Decamer primers, Randomly amplified polymorphic DNA (RAPD), genetic diversity, yellow vein mosaic virus, yield.

INTRODUCTION

Okra [*Abelmoschus esculentus* (L.) Moench] is one of the most important vegetable crops grown in different agro-ecological zone of India (Singh and Bhagchandani, 1967) and is cultivated in various tropical, subtropical and meditterean regions of the world. The centres of genetic diversity of okra include West Africa, India and Southeast Asia (Charrier, 1984; Hamon and Sloten, 1989). In various parts of the world, it is known as Okra, Ochro, Okoro, Quingumbo, Ladies finger, Gombo, Kopi Arab, Kacang Bendi, Bhindi (South Asia), Bamia, Bamya or Bamieh (middle east) or Gumbo (Southern USA). In Portugal and Angola, okra is known as Quiabo, and in Cuba as "quimbombo". In Japan, it is known as okura. Okra pods are a good source of vitamin A and flavo-noid, antioxidants like beta carotene, xanthin and lutein. Okra has been known to be beneficial to people suffering from leucorrhoea and general weakness. Due to its high iodine content, its fruits are considered useful to control goiter and have medicinal value in curing ulcers and relief from haemorrhoids. This vegetable provides an important input of vitamins and mineral salts, including calcium which are often deficient lacking in diet in deve-

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loping countries.

The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics (Semagn et al., 2006). Various types of molecular markers differing in their principles and methodologies are available such as AFLP, SSR, RFLP and RAPD.

Therefore, choice of a specific molecular marker type is one of the main challenges. Randomly amplified polymorphic DNA (RAPD), a PCR based marker uses a single arbitrary oligonucleotide primer to amplify template DNA without prior knowledge of target sequence (Williams et al., 1990). Earlier, molecular markers like RAPD have been used to assess genetic diversity in vegetable crops such as radish (Yamagishi et al., 1998; Matveeva et al., 2002; Huh and Ohnishi, 2003; Madhou et al., 2005; Kong et al., 2011), capsicum (Adetula, 2006), onion (D'ennequin et al., 1997; Friesen and Klaas; 1998; Tanikawa et al., 2002), ginger (Palai and Rout, 2007), watermelon (Levi et al., 2001; Solmaz et al., 2010), tomato (Bernardette et al., 2006; Juan et al., 2010), etc. Few reports of the use of RAPD markers for genetic diversity assessment of okra germplasm are available (Martinello et al., 2003; Aladele et al., 2008; Saifullah et al., 2010; Prakash et al., 2011).

The present study was carried out for the assessment of genetic diversity among okra germplasm based on RAPD markers and morphological characters.

MATERIALS AND METHODS

Genotypes

A total of 70 genotypes of okra [*Abelmoschus esculentus* (L.) Moench.] originating from different agroclimatic zones were procured (Table 1). The field and laboratory experiments were conducted at the Experimental Area and Biochemistry Laboratory of the Department of Vegetable Science, respectively during rainy season of 2010. The molecular analysis was carried out in the Molecular Biology Laboratory of School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana.

Field evaluation and data collection

The experiment was laid out in a Randomized Complete Block Design with three replications with spacing of 30 cm between the plants and a row to row distance of 45 cm for phenotypic evaluation. The recommended plant protection measures were followed to raise healthy crop. The data recorded on the 70 germplasm lines for eight agronomic characters were: Days to 50% flowering, fruit length, fruit width, marketable yield per plant, fruits per plant, average fruit weight, plant height at final harvest and field reaction to yellow vein mosaic virus using five plants.

DNA isolation and PCR amplification

Total genomic DNA was extracted from one week old seedlings as per method of Kochko and Hamon (1990) and purified. Quantity and quality of DNA isolated was determined by gel electrophoresis using a known quantity of ƛ DNA as standard. PCR reaction was performed on each DNA sample in a 20 µl reaction mixture containing 4 µl of 10 × Taq buffer (100 mM Tris- HCL, pH 8 with 50 mM EDTA, 500 mMNaCl, 10 mM 2- Merceptoethanol), 3 µl of 1 mM dNTPS, 0.2 µl of 5 unit of Taq DNA polymerase, 1.2 µl of 25 mM $MgCl_2$, 2 µl of template genomic DNA and 2 µl each of RAPD primers. The genomic DNA was subjected to PCR amplification using 40 random decamer primers. PCR amplification was performed in 96 wells microtiter plate in Thermo cycler of Applied Biosystems (Model EP Gradients). The reaction mixture was preheated at 94°C for 4 min followed by 45 cycles for 1 min denaturation at 94°C, 1 min annealing at 37°C and elongation or extension at 72°C for 2 min. After the last cycle, a final step of 8 min at 72°C was added to allow complete extension of all amplified fragments of DNA.

DNA gel electrophorosis

4 µl of 6X loading dye was added to each of the amplified products and mixed thoroughly. From this mixture, 10 µl of each sample was loaded in 1.5% agarose gel prepared in 1X TBE buffer. The PCR products were resolved by running gel at 5 V/cm for 3 h. The gels were visualized under UV light using photo documentation system (FOTODYNE).

Data analysis

The RAPD marker amplification profile of the genotypes was used to estimate the similarity based on number of shared amplified alleles. With the binary matrix (0, 1) compiled, pairwise relatedness between all accessions was estimated using Dice index of similarity (Dice, 1945). Using DARwin5 software package Version 5.0.158 (Perrier and Jacquemoud, 2006) and the unweighted Neighbor-joining method with 5000 bootstrap, a dendrogram was generated with the aim of analysing the relationships among the okra accessions.

RESULTS

Performance of genotypes based on quantitative traits

Mean squares due to genotypes were observed to be significant for all the traits studied viz., fruit length, marketable yield per plant, average fruit weight, plant height at final harvest, days to 50% flowering, fruit width, fruits per plant and field reaction to yellow vein mosaic virus. Mean performance of germplasm lines for various quantitative characters is presented in Table 2. Maximum fruit yield of 175 g per plant was recorded by accession IC 112451. The standard check Punjab 8 produced 108 g fruit yield per plant. Five entries namely, IC 112451 (4%), IC 117229 (7.3%), IC 128887 (8%), IC 128127 (8.3%) and IC 43587 (10%) recorded significantly less YVMV incidence than that of Punjab 8 (16.7%). Top five entries for higher iodine content were EC 169410 (16.33 ppm), IC 128124 (12.80), IC 43587 (12.19), EC 169341 (11.08), and IC 105457 (10.06).

Table 1. List of genotypes used in the present study.

S/N	Genotype	Source	S/N	Genotype	Source
1	Punjab 8	Punjab	36	IC 90170	Assam
2	Punjab Padmini	Punjab	37	IC 52299	Rajasthan
3	Punjab 7	Punjab	38	IC 52298	Rajasthan
4	Prabhani Kranti	Maharashtra	39	IC 90171	Rajasthan
5	Pusa Sawani	Delhi	40	IC 90172	Rajasthan
6	EC 169406	West Bengal	41	IC 117217	Andhra Pradesh
7	IC 169347	West Bengal	42	IC 112496	Andhra Pradesh
8	EC 169426	West Bengal	43	IC 117215	Andhra Pradesh
9	EC 169410	Brazil	44	IC 117214	Andhra Pradesh
10	EC 169341	Brazil	45	IC 128897	Andhra Pradesh
11	EC 169361	Brazil	46	IC 117218	Andhra Pradesh
12	IC 90168	Meghalaya	47	IC 108235	Himachal Pradesh
13	IC 90026	Meghalaya	48	IC 329370	Himachal Pradesh
14	IC 90073	Meghalaya	49	IC 329377	Himachal Pradesh
15	IC 45827	Tamil Nadu	50	IC 117299	Himachal Pradesh
16	IC 45888	Tamil Nadu	51	EC 329361	Himachal Pradesh
17	IC 45893	Tamil Nadu	52	IC 128123	Madhya Pradesh
18	IC 117223	Tamil Nadu	53	IC 128125	Madhya Pradesh
19	IC 117262	Tripura	54	IC 128127	Madhya Pradesh
20	IC 128131	Arunachal Pradesh	55	IC 128051	Madhya Pradesh
21	IC 116995	Karnataka	56	IC 128124	Madhya Pradesh
22	IC 43587	Karnataka	57	IC 117020	Madhya Pradesh
23	IC 39132	Delhi	58	IC 105457	Bihar
24	IC 43750	Kerala	59	EC 305634	Uttar Pradesh
25	IC 43737	Kerala	60	IC 16566	Uttar Pradesh
26	IC 43749	Kerala	61	IC 13917	Uttar Pradesh
27	IC 90743	Gujarat	62	IC 111544	Uttar Pradesh
28	IC 90176	Gujarat	63	IC 111546	Uttar Pradesh
29	IC 90185	Gujarat	64	IC 111484	Uttar Pradesh
30	IC 90212	Gujarat	65	IC 111499	Uttar Pradesh
31	IC 332451	Orissa	66	IC 57733	Maharashtra
32	IC 117229	Orissa	67	IC 112451	Maharashtra
33	IC 117078	Orissa	68	IC 128887	Maharashtra
34	IC 90134	Assam	69	KS442	Kalyanpur
35	IC 90165	Assam	70	EC 550848	Sri Lanka

Clustering based on quantitative traits

Cluster analysis of 70 genotypes based on eight quantitative traits was performed using DARwin software and a dendrogram was constructed as given in Figure 1. Genotypes under study were broadly divided into three major clusters. Cluster I was observed to be the largest one with 37 genotypes and was followed by cluster II (24 genotypes) and Cluster III (nine genotypes). Cophenetic r correlation value of 0.99 suggests that the cluster analysis strongly represents the similarity matrix. Bootstrap values above 30 are shown in dendrogram. Cluster I could be divided into two subclusters, each of which in turn could be divided into two groups and further subgroups.

RAPD polymorphism

A total of 40 RAPD primers were used to determine genetic diversity in a set of 70 genotypes of okra. RAPD primers that produced reproducible and clear scorable amplification products were selected in the present study for genetic diversity analysis. The details of the primers are given in Table 3. A total of 170 scorable alleles were detected, out of which 158 alleles were observed to be polymorphic. Number of alleles scored per primer ranged from 2 to 7 with an average

Table 2. Mean performance of genotypes of okra.

S/N	Genotype	Days to 50% flowering	Fruit length (cm)	Fruit width (cm)	Marketable yield per plant (g)	Fruits per plant	Average fruit weight (g)	Plant height at final harvest (cm)	Field reaction to yellow vein mosaic virus (%)	Iodine content (ppm)	Mucilage content (%)
1	Punjab 8	47.7	7.49	1.59	108	10.7	10.01	99	16.7	7.03	0.52
2	Punjab Padmini	47.3	9.57**	1.49	82**	8.7	9.58	100	26.7**	3.97*	0.45
3	Punjab7	48.3	8.49	1.57	80**	9.7	8.28	90	25.0**	6.43	0.48
4	Parbhani Kranti	47.3	9.87**	1.56	89*	8.7	11.11	107	27.7**	1.87*	0.38*
5	Pusa Sawani	46.0	9.09*	1.60	87*	8.7	9.91	103	18.7	2.78*	0.50
6	EC 169406	42.7**	9.97**	1.71	101	8.7	11.76*	99	25.3**	5.70	0.90*
7	IC 169347	46.3	8.89	1.98*	140**	14.3**	9.56	123**	15.0	1.47*	0.61
8	EC 169426	47.0	9.89**	1.57	100	9.3	10.14	109	20.3	9.17*	0.50
9	EC 169410	43.7**	8.50	2.01*	99	10.0	10.01	97	38.0**	16.33*	0.42
10	EC 169341	45.3	8.98*	1.54	101	10.0	10.14	120*	25.7**	11.08*	0.46
11	EC 169361	47.3	8.89	1.53	96	14.0**	6.82**	94	39.7**	2.72*	0.47
12	IC 90168	49.3	9.11*	1.58	48**	6.3**	8.18*	84	37.3**	3.67*	0.32**
13	IC 90026	46.7	9.30*	1.79	80**	9.7	8.07*	93	31.3**	6.11	0.35*
14	IC 90073	50.0	6.80	2.02*	57**	5.3**	10.37	84	32.0**	8.55*	0.54
15	IC 45827	50.0	8.63	1.52	60**	5.0**	12.33**	102	35.7**	8.18	0.30**
16	IC 45888	48.7	8.43	1.51	169**	16.3**	10.67	103	18.7	2.88*	0.38*
17	IC 45893	49.7	8.25	1.53	60**	7.3**	8.04*	91	32.0*	2.64*	0.63
18	IC 117223	48.7	8.60	1.53	80**	7.3**	11.53	97	28.3**	3.20*	0.57
19	IC 117262	49.3	7.97	1.53	103	9.7	11.09	101	40.0**	6.12	0.45
20	IC 128131	48.3	8.82	1.54	130*	14.0**	9.83	98	21.3	7.01	0.73**
21	IC 116995	50.0*	8.30	1.54	70**	7.7**	9.09	102	26.3*	4.14*	1.13*
22	IC 43587	50.0*	9.73**	1.52	110	11.0	10.39	116	10.0*	12.19*	0.46
23	IC 39132	48.3	7.87	1.89	90*	12.3	7.11**	93	42.0	4.23*	0.67*
24	IC 43750	48.7	7.87	1.98*	105	10.7	10.13	111	12.3	1.67*	0.73**
25	IC 43737	50.0*	7.37	2.25**	87*	12.3	7.27**	112	46.7**	6.96	0.25*
26	IC 43749	47.7	9.51**	1.49	73**	7.3**	9.93	102	21.3	1.87*	0.53
27	IC 90743	46.7	9.59**	1.47	82**	9.7	8.18*	99	27.7**	7.96	0.40
28	IC 90176	50.0*	9.15*	1.53	69**	6.3**	11.72	101	29.3**	3.50*	0.56
29	IC 90185	47.3	9.85**	1.55	76**	9.7	7.89*	100	19.0	2.62*	0.51
30	IC 90212	50.0*	7.65	1.37	39**	8.0	5.02**	98	27.0**	5.54*	0.55
31	IC 332451	47.6	9.04*	1.52	104	9.7	10.84	89	25.7**	3.34*	0.40
32	IC 117229	47.6	8.83	1.49	152**	14.7**	10.73	100	7.3**	7.30	0.53

Table 2. Contd.

33	IC 117078	46.7	7.23	2.11**	127*	16.3**	7.97*	69**	11.3	3.49**	0.48
34	IC 90134	48.3	9.15*	1.55	99	9.0	10.95	95	31.0**	7.76	0.42
35	IC 90165	49.0	8.39	1.6	65**	7.0	9.18	95	31.3**	6.25	0.60
36	IC 90170	46.0	9.04*	1.57	73**	8.0	9.39	103	42.0**	5.56	0.46
37	IC 52299	47.3	9.22*	1.57	81**	8.7	9.82	95	19.7	3.86**	0.51
38	IC 52298	45.7	8.55	1.63	72**	6.7**	10.81	94	26.7**	7.09	0.53
39	IC 90171	46.3	9.25*	1.45	99	9.7	11.09	97	20.3	3.70**	1.23**
40	IC 90172	50.0*	10.35**	2.03*	72**	7.7**	9.35	94	19.3	7.58	0.40
41	IC 117217	46.0	9.50**	1.67	67**	8.0	8.86	92	64.7**	4.41**	0.54
42	IC 112496	46.0	9.01*	1.53	74**	10.7	7.37**	91	56.7**	2.04**	0.43
43	IC 117215	49.7	8.83	1.55	56**	7.7**	8.26	97	21.0	3.00**	0.46
44	IC 117214	47.7	9.50**	1.55	76**	7.7**	9.62	88	36.7**	5.54	0.58
45	IC 128897	45.7	9.21*	1.54	87*	9.0	9.88	94	33.3**	3.41**	0.37*
46	IC 117218	45.7	9.83**	1.56	91	11.3	8.41*	92	27.0**	6.28	0.48
47	IC 108235	45.3*	9.30*	1.60	88*	11.7	7.36**	90	36.3**	5.69	1.20*
48	IC 329370	45.3*	8.87	2.03*	96	10.7	8.97	99	48.7**	6.03	0.93**
49	EC 329377	49.0	9.31*	1.53	157**	13.7**	11.59	77*	8.3**	1.41**	0.50
50	IC 117299	47.3	7.03	1.47	64**	7.3**	8.94	85	28.0**	2.53**	0.60
51	EC 329361	49.3	10.17**	1.57	46**	6.7**	8.1*	92	40.3**	1.80**	0.77**
52	IC 128123	47.7	10.04**	1.55	55**	5.3**	10.46	94	23.3*	5.15**	0.90*
53	IC 128125	50.0*	8.37	1.54	50**	5.7**	11.23	83	52.7**	7.17	0.63
54	IC 128127	49.7	7.53	1.51	158**	14.7**	11.03	73**	8.3**	3.18**	0.50
55	IC 128051	49.3	8.80	2.39**	80**	8.3**	9.66	99	36.3**	7.67	0.71**
56	IC 128124	49.7	9.11*	2.02*	51**	9.3	5.57**	82*	32.3**	12.80*	0.52
57	IC 117020	50.0*	6.83	1.54	24**	3.7**	7.12**	77*	24.3**	7.82	0.38*
58	IC 105457	48.3	9.97**	1.54	53**	5.0**	11.25	90	37.3**	10.16*	0.25**
59	EC 305634	48.3	9.30*	1.53	55**	7.0**	9.04	91	37.7**	2.01**	1.06**
60	IC 16566	49.3	9.10*	1.59	79**	8.0	9.94	104	37.7**	8.40*	0.27**
61	IC 13917	45.7	9.09*	1.51	64**	6.0**	10.87	95	35.0**	4.15**	0.60
62	IC 111544	45.7	8.83	1.56	85*	13.3**	6.32**	77*	38.7**	3.38**	0.80**
63	IC 111546	49.7	8.63	2.11**	76**	8.3*	9.1	78*	20.3**	1.49**	1.17**
64	IC 111484	49.3	8.78	1.87	69**	7.7**	9.57	90	18.3	3.87**	0.44
65	IC 111499	46.7	8.43	1.61	85*	7.7**	10.99	93	44.3**	2.37**	0.54
66	IC 57733	46.3	7.75	1.53	70**	7.3**	10.71	91	30.7**	3.27**	0.61
67	IC 112451	45.7	9.13*	1.73	175**	15.3**	11.81*	115	4.0**	5.64*	0.59
68	IC 128887	45.7	8.77	1.83	162**	15.0**	11.17	84	8.0**	6.52	0.63
69	KS442	45.3*	7.83	1.57	96	9.3	10.88	99	14.7	8.56*	0.78**

Table 2. Contd.

70	IC 550848	46.3	9.67**	1.77	84*	9.3	9.75	84	15.3	7.30	0.29**
	CD (0.0 5)	2.01	1.43	0.34	18.58	2.00	1.75	17.53	5.72	1.34	0.14
	CD (0.0 1)	2.64	1.88	0.44	24.45	2.63	2.30	23.07	7.53	1.77	0.18

*, ** Significant at 5 and 1%, respectively.

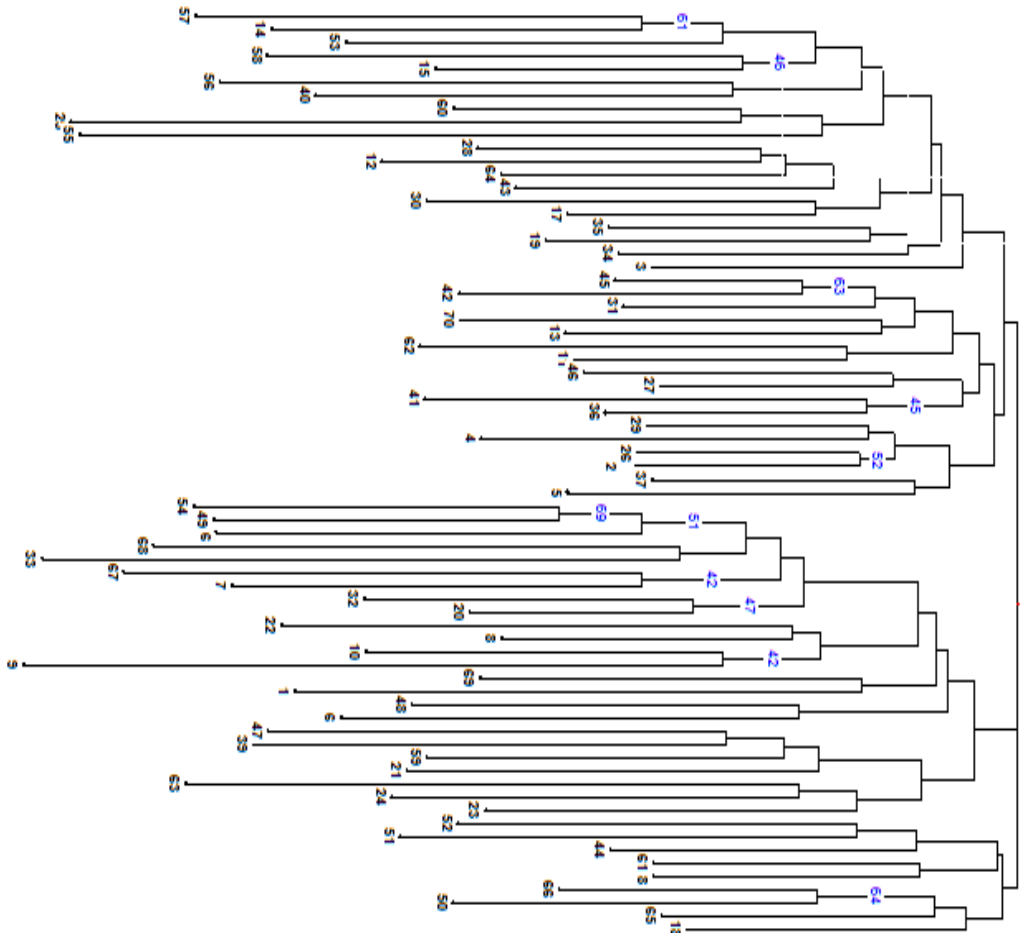

Figure 1. Dendrogram showing distribution of 70 okra genotype based upon 8 quantitative traits. The genotypes (1 to 70) are listed in Table 1.

Table 3. Level of polymorphism for RAPD primers in okra germplasm.

Primer	Primer sequence(5'-3')	Number of alleles	Amplification	polymorphic information content (PIC)
OPC1	TTCGAGCCAG	7	Amplified	0.82
OPC2	GTGAGGCGTC	4	Amplified	0.89
OPC3	GGGGGTCTTT	2	Amplified	0.58
OPC4	CCGCATCTAC	-	Not amplified	-
OPC5	GATGACCGCC	1	Amplified	0
OPC6	GAACGGACTC	-	Not amplified	-
OPC7	GTCCCGACGA	3	Amplified	0.79
OPC8	TGGACCGGTG	7	Amplified	0.8
OPC9	CTCACCGTCC	4	Amplified	0.87
OPC10	TGTCTGGGTG	4	Amplified	0.8
OPC11	AAAGCTGCGG	7	Amplified	0.78
OPC12	TGTCATCCCC	4	Amplified	0.79
OPC13	AAGCCTCGTC	4	Amplified	0.66
OPC14	TGCGTGCTTG	5	Amplified	0.81
OPC15	GACGGATCAG	4	Amplified	0.82
OPC16	CACACTCCAG	7	Amplified	0.51
OPC17	TTCCCCCCAG	4	Amplified	0.79
OPC18	TGAGTGGGTG	4	Amplified	0.86
OPC19	GTTGCCAGCC	7	Amplified	0.85
OPC20	ACTTCGCCAC	7	Amplified	0.85
OPB1	GTTTCGCTCC	4	Amplified	0.83
OPB2	TGATCCCTGG	4	Amplified	0.86
OPB3	CATCCCCCTG	5	Amplified	0.81
OPB4	GGACTGGAG	-	Not amplified	-
OPB5	TGCGCCCTTC	-	Not amplified	-
OPB6	TGCTCTGCCC	5	Amplified	0.86
OPB7	GGTGACGCAG	3	Amplified	0.8
OPB8	GTCCACACGG	4	Amplified	0.78
OPB9	TGGGGGACTC	4	Amplified	0.72
OPB10	CTGCTGGGAC	3	Amplified	0.86
OPB11	GTAGACCCGT	7	Amplified	0.79
OPB12	CCTTGACGCA	5	Amplified	0.64
OPB13	TTCCCCCGCT	3	Amplified	0.82
OPB14	TCCGCTCTGG	4	Amplified	0.78
OPB15	GGAGGGTGTT	4	Amplified	0.68
OPB16	TTTGCCCGGA	7	Amplified	0.78
OPB17	AGGGAACGAG	5	Amplified	0.81
OPB18	CCACAGCAGT	5	Amplified	0.52
OPB19	ACCCCCGAAG	7	Amplified	0.77
OPB20	GGACCCTTAC	6	Amplified	0.42
Average		4.72		0.75

of 4.72 alleles per primer. Reliable and reproducible DNA amplification was obtained for 36 primers. Four primers namely, OPC-4, OPC-6, OPB-4 and OPB-5 did not show any amplification. The PIC value ranged from as low as 0 (OPC-5) to as high as 0.89 (OPC-2) with an average of 0.75.

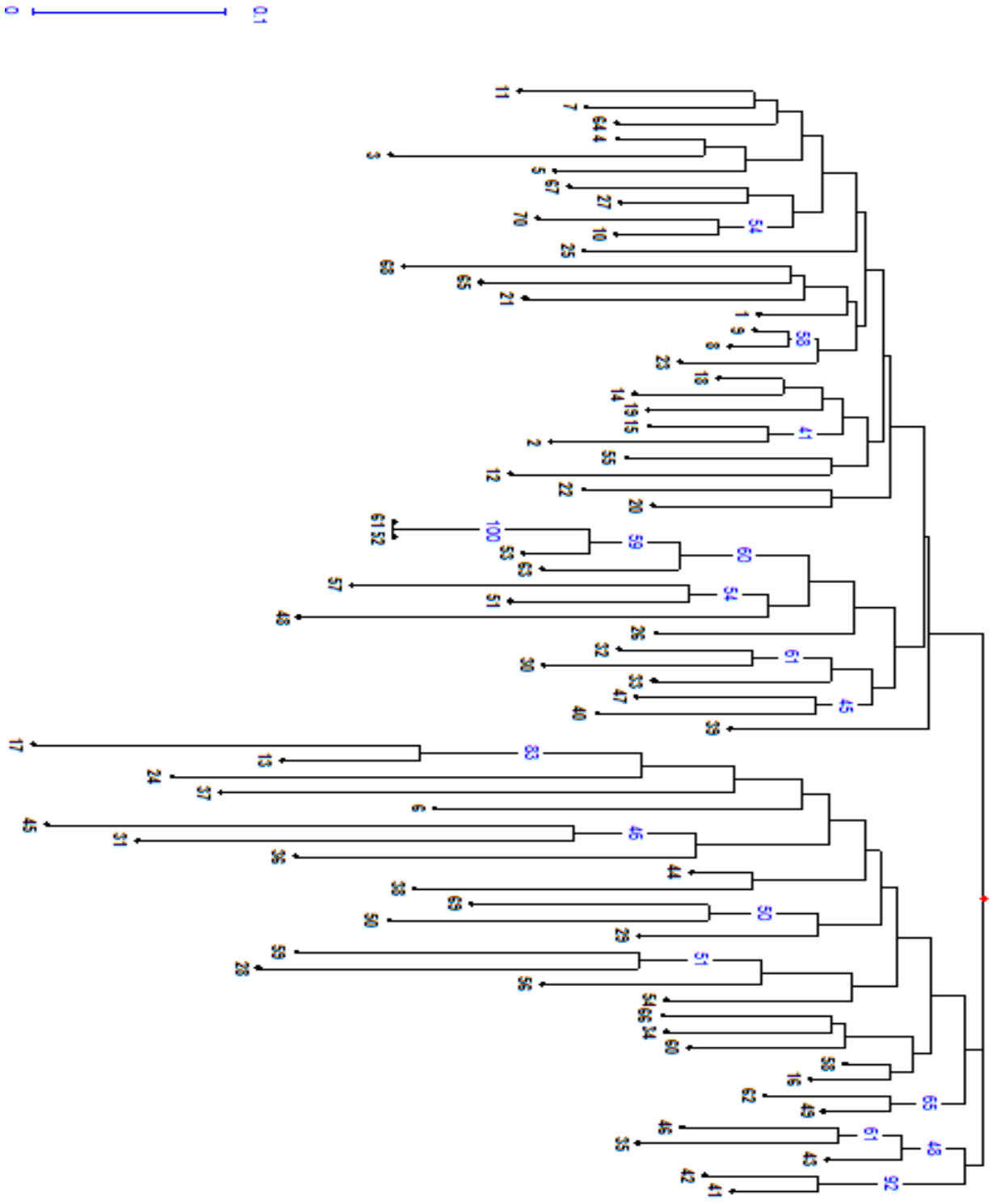

Figure 2. Dendrogram showing distribution of 70 okra genotype based upon RAPD primers. The genotypes (1 to 70) are listed in Table 1.

Cluster analysis and genetic distance among the 70 genotypes based on RAPD analysis

The dendrogram constructed based on RAPD analysis using DARwin is given in Figure 2. The 70 genotypes under study were broadly divided into three clusters. These clusters included 41, 24 and five genotypes, respectively. Cophenetic r correlation value was found to

Figure 3. Variation for fruit length and width observed in the material.

be 0.94 suggesting that the cluster analysis strongly represents the similarity matrix. Cluster I was further divided into two subgroups. One of the subgroups included only one genotype namely, IC 90171 which appears to be quite distinct as compared to other members of the cluster.

The five genotypes included in cluster III were: IC 117218, IC 90165, IC 117215, IC 112496 and IC 117217. The remaining group II could be further subdivided into two groups, one of which included two genotypes namely, IC 329377, IC 111544, whereas the other consisted of 22 accessions. The present investigation categorizes 70 okra accessions originating from diverse eco geographical regions into different clusters and gives a useful insight into their genetic relationships that may be of value in the okra breeding programme. Four of the six genotypes originating from Andhra Pradesh were grouped in cluster III, whereas, the other two accessions formed part of cluster II. All the three varieties namely, Punjab 8, Punjab 7, Punjab Padmini developed and commercialized by PAU Ludhiana were grouped together.

Similarly, entries EC 169410 and 169341 originating from Brazil were included in the same cluster. On the other hand, accessions from Meghalaya, Assam, Rajasthan, etc. were grouped in different clusters. The entries belonging to Brazil and Srilanka got clustered along with the Indian genotypes. Thus, no consistent relationship of genetic diversity and geographical origin of accessions could be established.

DISCUSSION

Okra genotypes showed large amount of variation for fruit size, and shape as shown in Figure 3. Significant differences were observed among the genotypes for marketable yield per plant. Accession IC 112451 recorded 175 g marketable yield per plant and was ranked first. The standard check Punjab 8 produced 108 g fruit yield per plant. Some of the studies reporting range for fruit yield per plant included Pradip et al. (2010) (29.2 to 95.7 g) Khan et al. (2005) (151.5 to 283.1 g) Hazra et al. (2002) (88.4 to 206.4 g) etc. Marked variation was also observed for YVMV disease incidence among the accessions studied. The genotype IC 117217 recorded maximum percent incidence of the disease (64.7), whereas, minimum disease incidence was exhibited by IC 112451 (4.0%). Joshi (2004) and Yadav et al. (2010) observed a range of 0 to 42.9%, 0 to 35%, respectively for this disease. Based on RAPD polymorphism (Figure 4), the primers namely, OPC1, OPC8, OPC11, OPC16, OPC19, OPC20, OPB11, OPB16 and OPB19 showed maximum number of bands (7). Number of alleles scored per primer ranged from two to seven with an average of 4.72 alleles per primer. In some other studies, number of alleles amplified per random primer have been reported to range from 2 to 6 (Gulsen et al., 2007), 8 to 12 (Aladele et al., 2008) and 7 to 9 (Saifullah et al., 2010).

Aladele et al. (2008) and Saifullah et al. (2010) reported 9.7 and 7.6 average number of bands per

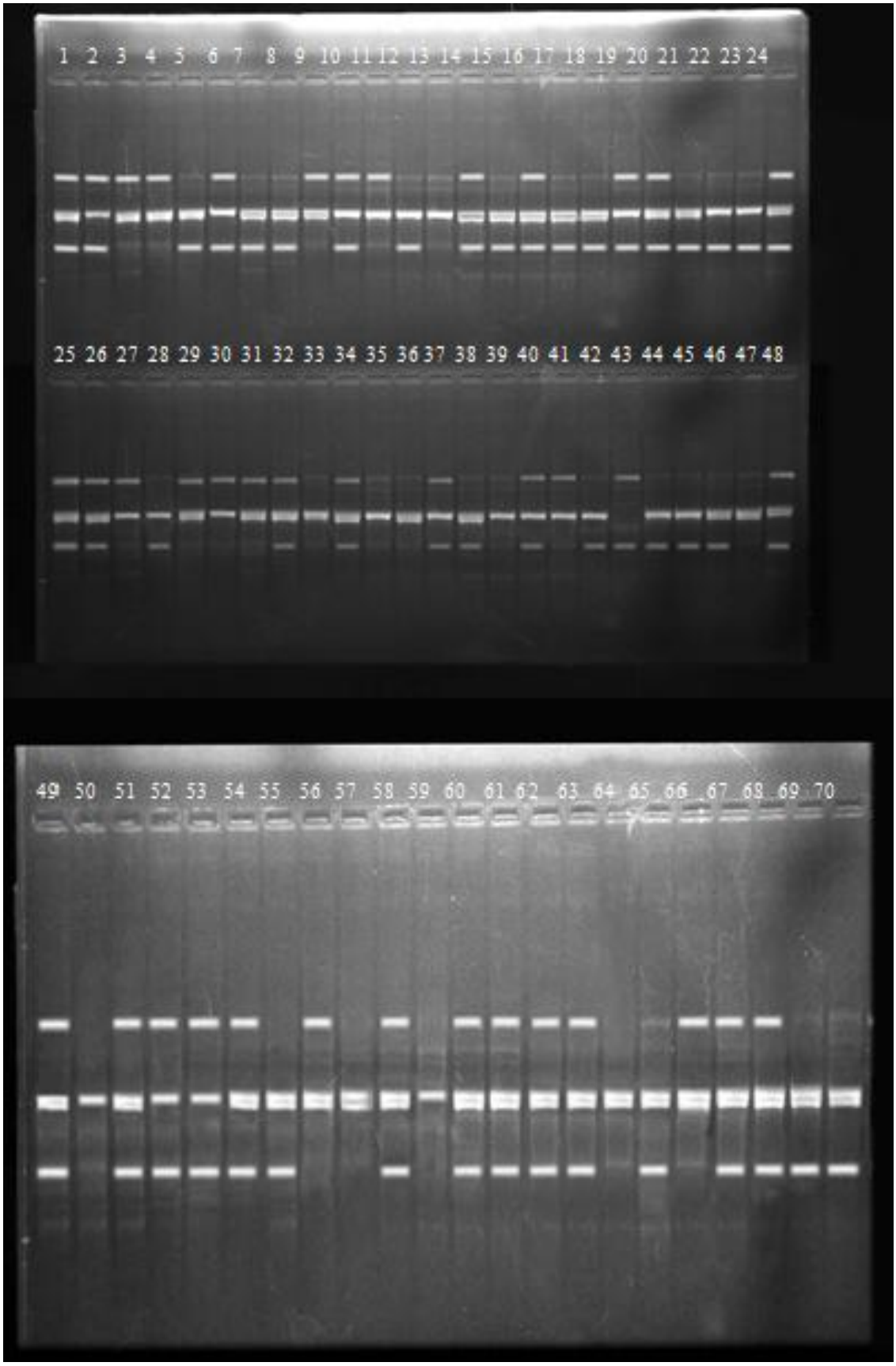

Figure 4. Banding pattern as revealed by primer OPC-18.

RAPD primer, respectively. Primer OPC-05 is observed to be monomorphic in the present investigation. Cross transferability of SSR primers derived from alfalfa and cotton has also been reported by Sawadogo et al. (2010) and Ramnek et al. (2012). Cophenetic r correlation value was found to be 0.94 suggesting that the cluster analysis strongly represents the similarity matrix. Similar Cophenetic r correlation value of 0.94 was reported by Gulsen et al. (2007). Cluster analysis studies revealed that similarity among the okra lines ranged from 100 to 15.41%. Gulsen et al. (2007) reported similarity value based on SRAP markers ranging from 100 to 86% among 23 okra genotypes. Aladele et al. (2008) observed relatively more molecular diversity in the Asian genotypes as compared to those of African origin. In another study by Saifullah et al. (2010), genetic distance values ranging from 0.00 to 0.66 were observed among okra accessions. The present investigation has provided a useful insight into the extent of genetic diversity in the okra germplasm and can be exploited in future breeding programs as well as for the development of mapping populations/linkage map.

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