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Evaluation of Genetic Diversity and Genome Fingerprinting of Bitter Gourd Genotypes (*Momordica charantia* L.) by Morphological and RAPD Markers

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ABSTRACT

In this study, morphological features along with Random Amplified Polymorphic DNA (RAPD) markers were used for assessing genetic diversity and relationships among 20 genotypes of bitter gourd (*Momordica charantia* L.) collected from different parts of India. The morphological data was recorded for their vegetative and reproductive characters along with the data on diseases infesting for two successive cycles. DNAs from 20 genotypes were isolated using the CTAB method and a total of 143 polymorphic amplified products were obtained from 14 decamer primers, which discriminated all the accessions with a mean of 10.2 amplified bands per primer, 48.3% (69 bands) of which were polymorphic bands. A dendrogram grouped the genotypes into two clusters 'A' and 'B' at 29 linkage distances. Cluster 'A' consisted of one variety 'Arka Harit' characterised by plants that are highly susceptible to fruit fly and downy mildew infestations. Cluster 'B' was sub-divided into 'B_1' and 'B_2' clusters at a linkage distance of 26 with one ('Nanjangood Local') and 18 genotypes, respectively. The accessions of sub-cluster 'B_2' were further divided into two minor clusters 'B_{2a}' and 'B_{2b}' at a 24 linkage distance with three small groups each. The genetic dissimilarity matrix based on Squared Euclidean Distance, showed a maximum dissimilarity (52%) between the genotypes 'Nanjangood Local' and 'IC-42261' and a minimum dissimilarity (9%) between the genotypes 'IC-42261' and 'VRBT-93', 'DARA-1', and 'IC-065782'. The analyses showed the potential of the commercial variety 'Panrushy' with respect to its capacity to resist fruit fly and downy mildew infestations, as well as high yield.

Keywords: cluster analysis, cultivar identification, dissimilarity matrix, RAPD-PCR, STATISTICA

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is an important Cucurbitaceous vegetable grown in the tropics. Among the cucurbits, it is considered a prized vegetable because of its higher nutrient values, besides its immense medicinal properties. The origin of the crop is probably India along with China as the secondary centre of diversity (Grubben 1977) and was domesticated in Asia, possibly in eastern India or southern China. The fruits have long been used in India, Indonesia and Thailand as a folk remedy for diabetes mellitus and a tea made of leaves is used to expel intestinal gas, to promote menstruation and as an antiviral for measles, hepatitis, and feverish conditions. It is used topically for sores, wounds and internally for worms and parasites.

A number of medicinal properties of all parts of the plants have been identified. The fruits are used as a tonic, purgative, stomachic carminative, anthelminthic, anti-inflammatory, febrifuge, stimulant, thermogenic, anti diabetic, etc. Bitter melon has been studied as a complementary drug in the treatment of diabetes both to reduce glucose levels and oxidative stress, as an antiviral therapy for HIV infection, and as a cytostatic in certain cancers (Lee-Huang *et al*. 1995). During the past decade the anti-diabetic properties of the crop have been studied extensively and a hypoglycemic principle called "charantian" has been isolated (Raman Lau 1996; Sarkar *et al*. 1996).

The choice of variety depends on market preference in the region, and is based on fruit shape and colour. Generally, there are three types: 1) small, 10–20 cm long, 100–300 g, usually dark green, very bitter; 2) long, 30–60 cm, 200–600 g, light green in colour and only slightly bitter and 3) triangular fruit type, cone-shaped, 9–12 cm long, 300-600 g, light to dark green with prominent tubercles, moderate to strongly bitter. Bitter gourd is monoecious and is cross-pollinated by insects, especially bees. The male flowers normally exceed the females by about 25-fold.

India is bestowed with a large amount of genetic diversity based on morphological characters (Robinson and Decker-Walters 1999). Indians cultivate primarily *M. charantia* (i) var. *charantia*, which produces large fusiform fruits and (ii) var. *muricata* (wild) with small and round fruits (Chakravarty 1990). In India, genetic analysis based on quantitative traits has been made in this crop by Mishra *et al*. (1998) and Ram *et al*. (2000). However, phenotypic characters have limitations as they are influenced by environmental factors and the developmental stages of the plant. In contrast, molecular markers, based on DNA sequence polymorphism are independent of environmental conditions and show a higher level of polymorphism (Simon *et al*. 2007).

Among the different types of molecular markers available, RAPDs are more useful for the assessment of genetic diversity due to their simplicity, speed and relatively low cost compared to other molecular markers (Williams *et al*. 1990; Rafalski and Tingey 1993). RAPD markers have been used extensively in cucumber to classify accessions (Horejsi and Staub 1999), to identify cultivars and hybrids of gourds (Meng *et al*. 1996), analysis of genetic diversity of watermelon and ash gourd (Lee *et al*. 1996; Sureja *et al*. 2006). In the present study, RAPD and morphological markers were used to estimate genetic diversity and assess relationships among twenty genotypes of bitter gourd maintained at the Department of Horticulture, University of Agricultural Sciences, Bangalore, India.

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MATERIALS AND METHODS

Plant materials

The plant materials used for the study comprised 20 genotypes of bitter gourd collected from Southern parts of India: 'Arka Harit', 'Panrushy', 'Nadushittu', 'CO-1', 'Chidambaram Small', 'CL Long', 'Indi Local', 'Green Long', 'White Long', 'Nanjangood Lo-cal', 'VRBT-28', 'VRBT-72', 'VRBT-41', 'VRBT-83', 'VRBT-93', 'VRBT-100', 'VRBT-103', 'IC-065782', 'IC-42261' and 'DARA-1'. Around 50 g of young and healthy leaves that were free from pests and disease damage were harvested from the 20 genotypes individually in an ice box from the field, wiped with 70% ethanol; air-dried and was stored at 4°C in sealed polythene covers for the isolation of DNA.

Morphological characteristics

The data were recorded on the morphological characters such as vine length (cm), total number of primary branches, productive length of vine (cm), number of days taken for opening of first female flower, number of days taken for opening of first male flower, node at which first female flower appear, number of days taken for 50 per cent flowering, fruit set per cent, sex ratio, number of fruits per plant, fruit weight (g), fruit length (cm), days taken to first harvest, number of seeds per fruit, crop duration (days), fruit fly infection (%), downy mildew infection (%), maximum yield per plant (kg) and yield (t/ha) for two successive cycles.

Table 1a Vegetative characters of 20 genotypes of *Momordica charantia* L.

Genotypes	Vine length (cm)	Total number of primary branches	Productive length of vine (cm)	Crop duration (days)	Percent of fruit fly infection	Percent of downy mildew infection
Arka Harit	186.5	8.13	158.93	136.0	71.5	71.49
Panrushy	488.5	9.50	386.43	230.0	21.0	18.60
Nadushittu	455.0	8.50	391.33	196.7	46.5	22.60
CO1	432.6	9.53	372.00	168.3	46.2	12.83
Chidambaram Small	153.4	9.60	114.80	153.3	54.9	19.93
CL Long	320.9	9.57	343.70	128.3	41.6	35.83
Indi Local	322.9	9.53	293.67	153.3	47.7	27.11
Green Long	335.1	7.19	289.27	169.3	39.7	33.32
White Long	390.4	9.20	308.17	179.8	39.6	21.33
Nanjangood Local	392.4	7.57	283.33	202.7	41.8	44.01
VRBT-28	314.6	6.53	203.23	127.7	50.0	61.72
VRBT-72	375.3	8.20	312.77	182.3	43.5	34.83
VRBT-103	310.2	10.4	218.40	198.3	42.5	35.21
VRBT-83	394.5	9.67	332.83	206.0	31.5	22.68
VRBT-100	393.9	8.34	316.57	212.7	27.2	22.03
IC-42261	357.7	8.37	273.83	195.0	42.0	31.78
VRBT-93	357.8	9.47	233.40	205.3	39.2	22.09
DARA-1	390.6	10.3	283.13	212.0	38.9	21.53
IC-065782	318.9	8.30	195.20	138.0	36.9	61.37
VRBT-41	350.6	7.90	231.80	165.0	42.2	46.71
Mean	352.0	8.79	277.1	178.0	42.2	33.30
SEM	17.5	0.23	16.6	6.92	2.29	3.61
T-test	0.001	0.001	0.001	0.001	0.001	0.001

Table 1b Reproductive characters of 20 genotypes of *Momordica charantia* L.

Genotypes	Days taken to open first female flower	Days taken to open first male flower	Node number at which first female flower appear	Number of days at 50% flowering	Fruit set percent	Sex ratio	Number of fruits per plant	Fruit weight (g)	Fruit length (cm)	Days taken to first harvest	Number of seeds per fruit	Maximum yield per plant (Kg)	Yield per hectare (tonnes)
Arka Harit	54.91	38.77	7.3	87.33	72.32	15.83	18.57	46.59	7.06	71.33	14.07	0.89	124.3
Panrushy	64.75	39.57	12.4	146.63	92.37	18.73	27.84	105.5	18.29	82.87	23.90	2.11	230.0
Nadushittu	64.84	40.62	10.1	139.67	87.03	14.77	16.41	84.53	28.59	72.07	28.43	1.35	196.6
CO1	61.44	37.65	17.5	148.53	90.52	18.53	16.29	102.6	20.53	77.80	17.23	1.79	168.3
Chidambaram Small	49.44	38.04	4.93	132.30	88.19	6.85	61.51	7.67	4.62	61.33	7.40	0.55	153.3
CL Long	60.40	38.93	13.7	135.23	86.42	16.39	24.66	60.96	17.06	68.67	19.40	1.25	128.3
Indi Local	55.47	38.25	10.2	132.60	82.42	17.34	17.36	65.27	13.12	64.60	22.08	1.16	153.3
Green Long	55.02	38.58	6.4	136.40	81.82	19.02	20.55	60.07	17.37	72.53	23.57	1.11	169.3
White Long	57.93	38.33	12.4	127.37	80.60	17.35	18.31	57.87	16.34	67.20	20.68	1.07	179.7
Nanjangood Local	70.13	41.84	8.6	127.00	81.98	27.29	17.89	61.00	12.40	81.33	19.48	1.11	202.7
VRBT-28	67.37	37.25	9.1	124.27	72.64	17.54	17.84	49.20	15.60	73.33	14.80	0.76	127.6
VRBT-72	53.90	39.03	7.8	135.67	78.72	18.94	20.59	56.04	17.56	65.33	17.84	1.18	182.3
VRBT-103	65.04	39.53	10.9	128.43	80.40	18.69	18.22	48.33	19.53	76.13	14.00	0.99	198.3
VRBT-83	52.47	40.00	11.8	137.23	83.82	16.21	25.17	56.67	18.40	63.67	14.00	1.22	206.0
VRBT-100	58.11	39.50	8.5	125.43	85.66	15.63	28.43	45.07	14.65	66.20	19.87	1.25	212.7
IC-42261	62.84	39.07	9.5	119.27	84.53	14.93	20.91	60.93	15.67	76.67	19.90	1.19	195.0
VRBT-93	60.87	39.80	14.3	133.10	78.89	19.32	17.62	54.53	13.91	72.00	16.47	0.97	205.3
DARA-1	53.20	38.93	8.4	129.77	79.16	21.06	21.02	55.00	13.60	64.07	16.56	1.10	212.0
IC-065782	59.34	39.69	7.5	125.40	64.85	22.77	14.78	45.20	12.20	66.00	21.51	0.71	138.0
VRBT-41	52.70	40.23	8.4	113.03	77.62	16.10	18.35	48.57	16.46	64.47	18.87	0.89	165.0
Mean	59.0	39.1	9.98	129.2	81.4	17.6	22.1	58.5	15.6	70.3	18.5	1.13	177.4
SEM	1.26	0.23	0.67	2.89	1.45	0.86	2.23	4.67	1.10	1.38	1.01	0.07	7.12
T-test	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

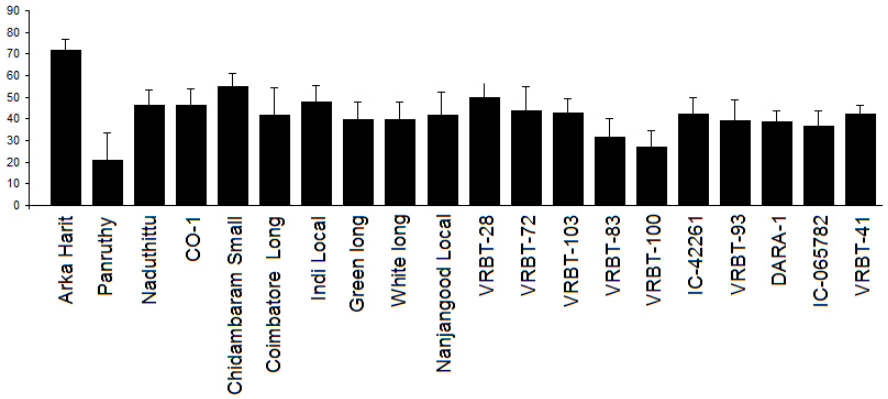

Fig. 1 Histogram showing the percentage of fruit fly infection among 20 genotypes of *M. charantia*.

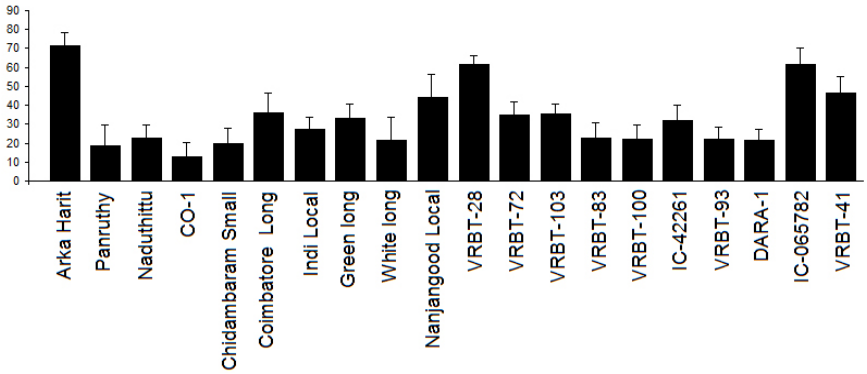

Fig. 2 Histogram showing the percentage of downy mildew infection among 20 genotypes of *M. charantia*.

The mean average of 30 individuals of each genotype was selected for the determination of morphological characteristics and the *t*-test was applied by using SPSS for Windows, v. 11.5.0 (SPSS 2002) to comparing the means (**Table 1a, 1b**). Based on the data on the incidence of fruit fly and downy mildew, histograms were constructed among the 20 genotypes of bitter gourd (**Figs. 1** and **2**, respectively). The phenotypic correlation among the morphological characters was carried out using SPAR and Indostat programmes following the simple lattice design suggested by Cochran and Cox (1959) **Table 2**. A one-way ANOVA and backward regression analysis were performed to analyse the correlation between the yield characters using SPSS for Windows, v. 11.5.0 (SPSS 2002).

DNA extraction and purification

DNA was extracted from the fresh leaf powder of bitter gourd by the Cetyl trimethyl ammonium bromide (CTAB) protocol as described by Simon *et al*. (2007). Leaf powder (0.5 g) was mixed with 10 ml extraction buffer, preheated to 65°C, containing 100 mM Tris-Base, pH 8.0, 20 mM EDTA, pH 8.0, 1.4 M NaCl, 3% (w/v) CTAB, 2% polyvinyl pyrrolidone and 1% β-mercaptoethanol, then incubated at 65°C for 30 min with gentle shaking. The mixture was cooled to room temperature, to which 10 ml cold 24:1 (v/v) chloroform: isoamylalcohol was added and the contents were mixed well. After centrifugation at 6,000 × *g* for 20 min at 4°C, the supernatant was transferred to a fresh tube and the chloroform: isoamylalcohol step was repeated until a clear supernatant was obtained. To the supernatant 5 M NaCl was added (0.5 v/v) and mixed gently followed by addition of 0.8 vol of cold isopropanol to precipitate the DNA. The mixture was incubated overnight at 4°C, and then centrifuged at 8,000 × *g* for 20 min. The resulting pellet was washed with 70% (v/v) ethanol, air dried, and dissolved in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). Two µg of RNase (Bovine pancreatic ribonuclease, Bangalore Genei, Bangalore, India) was added to each sample which was incubated for 3 h at 37°C, mixed with an equal volume of phenol and centrifuged at 6,000 × *g* for 20 min at room temperature. This step was followed by a wash with an equal volume of 1:1 (v/v) of phenol: chloroform then with chloroform alone. DNA was precipitated overnight at 4°C with 0.5 vol of 5 M NaCl and 1 vol of cold isopropanol and the resulting pellet obtained after centrifugation was dissolved in TE buffer, analysed on an agarose gel and quantified using a spectrophotometer (NanoDrop Technologies, Wilmington, USA).

PCR amplification

PCR amplification protocol was according to Williams *et al*. (1990) with minor modifications. Of the 30 primers screened using the bulk DNA, 14 showing clear and distinguishable bands were selected for RAPD-PCR analysis (**Table 3**). Reproducibility of the primers was tested by repeating the PCR amplification thrice under similar conditions. PCR reactions were carried out in a volume of 25 µl containing 25 ηg template DNA, 150 µM each of dNTP, 1.5 mM $MgCl_2$, 1 unit *Taq* DNA polymerase (Sigma Aldrich Chemicals, Bangalore, India), 5 pmol primer (OPA, OPB, OPC, OPD, OPE, OPF, OPG, OPH, OPI, OPJ and OPK series, Operon Technologies, Alameda, CA, US) in PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.05% (v/v) NP40 and 0.05% (v/v) Triton X-100). Amplifications were performed in a Corbett Research Thermocycler (Corbett Research Mortlake, New South Wales, Australia), programmed for an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, primer extension at 72°C for 2 min, and a final extension at 72°C for 5 min. PCR products were resolved on a 1.2% (w/v) agarose gel, visualised and documented using an Alpha Digidoc system (Alpha Innotech, San Leandro, CA, US).

RAPD profile analysis

Amplified fragments from each primer were manually scored for their presence (1) or absence (0) and a matrix of the different RAPD phenotypes of all fourteen primers were assembled for statistical analysis. The sizes of the fragments were estimated using 500 bp standard DNA markers, co-electrophoresed with the PCR products. A genetic dissimilarity matrix was developed using

Table 2 Phenotypic correlation coefficient among 14 quantitative characters in bitter gourd

Characters	Vine length (cm)	Number of primary branches	Productive length of vine (cm)	Number of days at 50% flowering	Fruit set percent	Sex ratio	Number of fruits per plant	Fruit weight (g)	Fruit length (cm)	Number of seeds per fruit	Crop duration (days)	Percent of fruit fly infestation	Percent of downy mildew infection	Maximum yield per plant (kg)
Vine length (cm)	1.00	0.077	0.838**	0.584**	0.359	0.435	0.458*	0.792**	0.713**	0.644**	0.641**	-0.663	-0.424	0.766**
Number of primary branches		1.00	0.157	0.323	0.373	-0.152	0.228	0.095	0.054	-0.189	0.321	-0.138	-0.531	0.25
Productive length of vine (cm)			1.00	0.592**	0.483*	0.203	0.316	0.785**	0.707**	0.617**	0.447*	-0.463	-0.456	0.815**
Number of days at 50% flowering				1.00	0.557*	0.072	-0.121	0.494*	0.492*	0.256	0.348	-0.544	-0.635	0.554*
Fruit set percent					1.00	-0.298	0.373	0.422	0.296	0.113	0.416	-0.326	-0.631	0.628**
Sex ratio						1.00	0.673	0.347	0.114	0.338	0.2	-0.279	0.186	0.177
Number of fruits per plant							1.00	0.494*	0.447*	-0.51*	-0.017	-0.055	-0.264	0.576
Fruit weight (g)								1.00	0.64	0.614	0.335	-0.332	-0.292	0.9**
Fruit length (cm)									1.00	0.562**	0.323	-0.334	-0.25	0.527*
Number of seeds per fruit										1.00	0.224	-0.334	-0.104	0.477*
Crop duration (days)											1.00	-0.572	-0.547	0.5*
Percent of fruit fly infestation												1.00	0.309	-0.463
Percent of downy mildew infection													1.00	-0.463
Maximum yield per plant (Kg)														1.00

* Significant at 5%; ** Significant at 1%

Table 3 RAPD-PCR primers. The sequence and level of polymorphism of selected polymorphic primers in bitter gourd.

Primer	Sequence (5′ to 3′)	G + C content (%)	Total № of bands	№ of polymorphic shared bands	№ of polymorphic unique bands	№ of monomorphic bands
OPA-04	AATCGGGCTG	60	10	04	1	5
OPA-11	CAATCGCCGT	60	11	05	2	4
OPA-13	CAGCACCCAC	70	09	03	0	6
OPB-08	GTCCACACGG	70	15	15	0	0
OPB-12	CCTTGACGCA	60	10	06	1	3
OPC-04	CCGCATCTAC	60	08	03	0	5
OPD-05	TGAGCGGACA	60	10	02	0	8
OPD-13	GGGGTGACGA	70	10	03	1	6
OPD-16	AGGGCGTAAG	60	11	03	1	7
OPE-08 OPF-01	TCACCACGGT	60	12	03	0	9
OPF-10	ACGGATCCTG	60	10	04	0	6
OPF-19	GGAAGCTTGG	60	09	04	0	5
OPG-02	CCTCTAGACC	60	09	04	1	4
	GGCACTGAGG	70	09	03	0	6

Squared Euclidean Distances, which estimates all pair-wise differences in the amplification products (Sokal and Sneath 1973) and a cluster analysis was based on Ward's method using a minimum variance algorithm (Ward 1963).

RESULTS AND DISCUSSION

Information on genetic diversity among plant species is important for efficient utilization of plant genetic resources. Geographical isolation of a population may cause its genome to drift away from other populations of the same species (Biron *et al*. 2002). Hence, authentic identification of taxa is necessary both for breeders to ensure protection of intellectual property right and also for propagators and consumers. The traditional method of identifying species by morphological characters is accompanied by DNA profiling that is more reliable because of several limitations of their morphological data (Nayak *et al*. 2003). Therefore, organisms with a high tendency for morphological differentiation, studies considering both molecular and morphological characters are highly relevant (Bagatini *et al*. 2005). Evidently, RAPD technology is a rapid and sensitive technique, which can be used to estimate relationships between closely, and more distantly related species of bitter gourd.

PCR amplification followed was according to the standard protocol (Williams *et al*. 1990) with minor modifications, which produced good amplifications with 25 ŋg of template DNA. The amplifications using 1 unit of *Taq* DNA polymerase and 1.5 mM $MgCl_2$ produced intense and clear banding patterns. The primer-screening step resulted in 14 decamer primers which detected good polymorphisms (**Table 3**) and 16 other random primers, which did not give any amplification products. Screening is essential to save time and cost, and to reject primers not informative for the analysis (Prakash *et al*. 2002). The amplification profiles of total genomic DNA from twenty bitter gourd genotypes with 14 random primers produced a total of 143 fragments ranging in size from 250 bp to 4.0 kbp with an average of 10.21 bands per primer. Of the 143 bands, 62 (43.36%) were polymorphic and shared between at least two individuals, 74 (51.75%) were monomorphic and common to all the individuals. Similarly, 36.5% polymorphism was obtained among 38 genotypes of bitter gourd collected from India (Behera *et al*. 2008). Only seven (4.89%) in our study

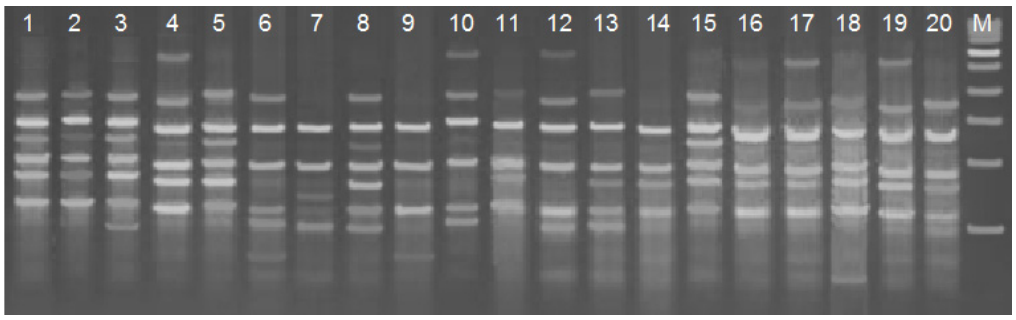

Fig. 3 Gel profile of bitter gourd according to OPB-08 primer. Genotypes (1 – 20): 'Arka Harit', 'Panrushy', 'Nadushittu', 'CO1', 'Chidambaram Small', 'CL Long', 'Indi Local', 'Green Long', 'White Long', 'Nanjangood Local', 'VRBT-28', 'VRBT-72', 'VRBT-103', 'VRBT-83', 'VRBT-100', 'IC-42261', 'VRBT-93', 'DARA-1', 'IC-065782' and 'VRBT-41'. M, 500 bp standard DNA markers.

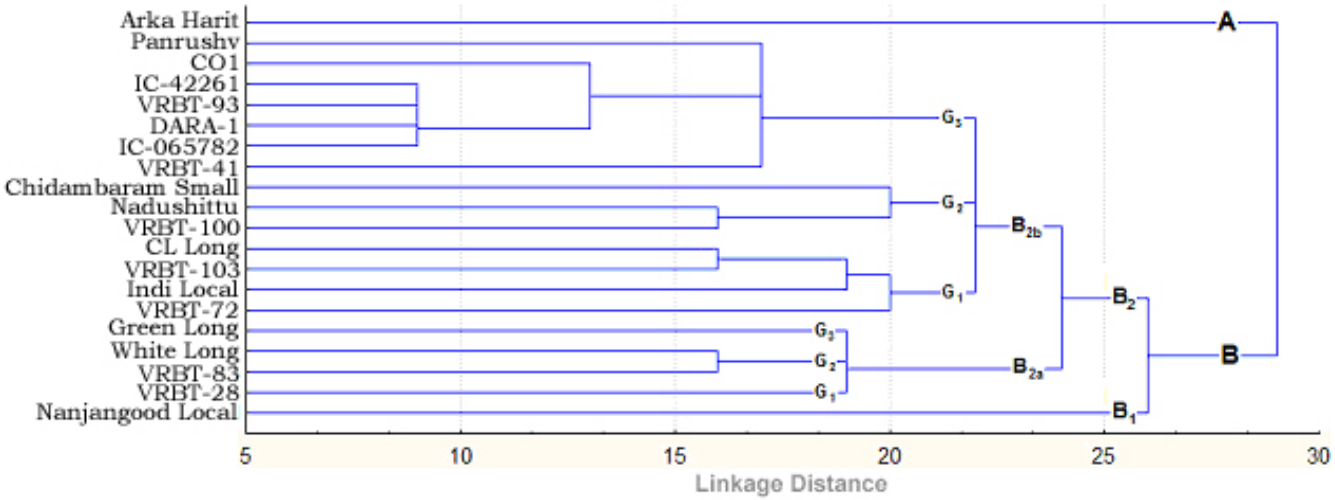

Fig. 4 Cluster analysis. Dendrogram showing RAPD-marker-based genetic relationships among 20 genotypes of *M. charantia*.

Table 4 Genetic dissimilarity matrix of 20 bitter gourd genotypes based on polymorphism of RAPD markers.

Arka Harit	0																			
Panrushy	41	0																		
Nadushittu	37	24	0																	
CO-1	48	19	27	0																
Chidambaram	43	22	20	23	0															
CL Long	39	24	26	29	24	0														
Indi Local	36	25	35	30	33	19	0													
Green Long	42	25	35	28	25	25	28	0												
White Long	31	34	40	31	36	40	33	19	0											
Nanjangood Local	29	46	32	51	43	36	41	41	36	0										
VRBT-28	30	33	37	28	37	35	28	28	19	33	0									
VRBT-72	33	28	28	29	26	26	25	31	34	26	27	0								
VRBT-103	35	24	30	29	32	16	19	29	32	40	29	20	0							
VRBT-83	39	28	32	25	34	36	33	23	16	38	21	32	30	0						
VRBT-100	41	22	20	23	16	22	31	29	36	36	35	22	26	28	0					
IC-42261	51	18	28	13	28	30	37	31	30	52	31	32	30	24	24	0				
VRBT-93	50	23	31	20	31	33	42	32	35	51	36	35	35	31	29	9	0			
DARA-1	48	21	31	20	31	31	38	24	27	47	30	33	31	25	25	9	14	0		
IC-065782	50	17	31	18	27	31	34	28	31	51	34	37	31	25	23	9	14	12	0	
VRBT-41	37	24	34	25	34	26	31	31	34	44	35	30	30	32	26	22	25	21	17	0

were polymorphic and unique. The number of fragments produced by a primer ranged from 9 (OPC 04) to 15 (OPB 08). Pattern of RAPD fragments produced by the random primer OPB-08 is shown in **Fig. 3**. The dissimilarity matrix obtained using Squared Euclidian Distance (Sokal and Sneath 1973) is shown in **Table 4**. The highest genetic dissimilarity (52%) was between genotypes 'Arka Harit' and 'Nanjangood Local', while the least genetic dissimilarity (9%) was noticed between the genotypes 'IC-42261' and 'VRBT-93', 'DARA-1', and 'IC-065782'.

In the dendrogram (**Fig. 4**), all 20 accessions were divided into two major clusters 'A' and 'B' at 29 linkage distance. The cluster 'A' consisted of 'Arka Harit' characterised by plants with lowest duration for 50% flowering (87.3 days), fruit set (72.32%) and yield (124.3 t/ha). It is highly susceptible to fruit fly (71.5%) and downy mildew infections (71.49%). Similar results were obtained by Bahera *et al.* (2008) and Dey *et al.* (2006) where the commercial variety showed less similarity compared with other genotypes. Cluster 'B' was divided into two sub-clusters 'B_1' and 'B_2' with 1 and 18 genotypes, respectively linked at 24 distance. The variety 'Nanjangood Local' of 'B_1' was characterised by plants with highest number of days taken for flowering (41.84 and 71.13 days for male and female flowers, respectively) and having highest sex ratio (27.29%) among all the genotypes. The sub-cluster 'B_2' consisted of two minor

clusters 'B_{2a}' and 'B_{2b}' linked at 24 linkage distance and segregated into three groups each. The cluster 'B_{2a}' consisted of four genotypes clustered at 19 linkage distance with groups 'G_1' and 'G_3' consisting 'VRBT-28' and 'Green Long', respectively. The variety 'VRBT-28' showed the fewest days for opening of male flowers (37.25 days) and crop duration (127.7 days). The genotypes 'White Long' and 'VRBT-83' clustered at 16 linkage distance in group 'G_2' of minor cluster 'B_{2a}' and shared a unique band of 780 bp produced by primer OPB-08.

The cluster 'B_{2b}' consisted of 14 genotypes linked at 22 linkage distance and segregated into three groups ('G_1', 'G_2' and 'G_3'). The group 'G_1' consisted of four genotypes ('CL Long', 'VRBT-103', 'Indi Local' and 'VRBT-72') clustered at 20 linkage distance. The genotypes 'CL Long' and 'VRBT-103' were closely related at 16 linkage distance and shared similar features like high number of primary branches, per cent of fruit fly and downy mildew infection but with a contrast in yield per hectare 128.3 and 198.3 t, respectively. Both these genotypes are closely related to 'Indi Local' at 19 linkage distance with similar morphological features however, contrast with respect to fruit length, number of seeds, high per cent of fruit fly infection and low per cent of downy mildew infection. Variety 'VRBT-72' stands as a separate entity in the group characterised by low number of primary branches (8.2), low of fruit set (74.72%) and high vine length (375.3 cm).

The group 'G_2' consisted of three genotypes clustered at 20 linkage distance with genotypes 'Nadushittu' and 'VRBT-100' closely related at 16 linkage distance. Both the genotypes shared similar morphological features but were contrast for percentage of fruit set, weight and length, and percentage of fruit fly infections. The variety 'Chidambaram Small' stands separate in the group with plants showing low sex ratio (6.85 %), fruit weight (7.67 g), small round fruits (4.62 cm), number of seeds (7.4) and highest number of fruits per plant (61.51) denoted as *M. charantia* var. *muricata* (wild variety) by Chakravarty (1990). Group 'G_3' consisted of seven genotypes clustered at 17 linkage distance. The genotypes 'IC-42261', 'VRBT-93', 'DARA-1' and 'IC-065782' were closely related at 9 linkage distance sharing similar morphological features but varying in sex ratio, downy mildew infection and yield per plant. All the four genotypes shared unique bands of 2,654 and 2,230 bp produced by primer OPA-11 and are closely related to genotype 'CO-1' at 13 linkage distance. The variety 'CO-1' is characterised by plants with longest duration at 50% flowering (148.53 days) and lowest incidence of downy mildew infection (12.83%). The genotypes 'VRBT-41' and 'Panrushy' stood separate in the group at 17 linkage distance. The variety 'IC-065782' was characterised by plants with the lowest number of fruits per plant (14.78). The variety 'VRBT-41' showed the lowest number of primary branches (7.9) and sex ratio (16.1) among the group. The variety 'Panrushy' showed the highest fruit set (92.37%), fruit weight (105.5 g), longest crop duration (230 days), lowest fruit fly incidence (21%) and highest yield of 230 t/ha. The comparison of morphological characters of the 'G_3' showed that all the accessions of the group showed increased vine length with more number of primary branches and higher yield. All the genotypes of the cluster 'B' shared unique band of 2,830, 740 bp produced by primer OPA-04 and 972 bp produced by primer OPA-11.

The results of this study indicated that RAPDs along with morphological markers offer reliable and effective means of assessing genetic variation in bitter gourd cultivars. Such an analysis, together with data from classical methods, could thus be used to make a more accurate reconstruction of evolution (Nayak *et al*. 2003). Cultivars showed low to moderate polymorphism, regardless of the locations from which they were obtained. The markers used to determine the genetic relationship among cultivars and diversity that could have a practical application in breeding hybrids (Jain *et al*. 1999). The study revealed that the variety 'Panrushy' characterised by high yielding with low disease incidence could be of commercially importance; 'Arka Harit' is the most unique genotype as suggested by Behera *et al*. (2008). Knowledge on genetic diversity will help in the efficient management of bitter gourd germplasm and hybridization programmes among breeders.

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