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Comparative analysis of genetic diversity in Indian bitter gourd (*Momordica charantia* L.) using RAPD and ISSR markers for developing crop improvement strategies

T.K. Behera [a,b,*], A.K. Singh [b], Jack.E. Staub [a]

[a] *USDA-Vegetable Crops Unit, Department of Horticulture, 1575 Linden Drive, University of Wisconsin, Madison, WI 53706, USA*
[b] *Indian Agricultural Research Institute, New Delhi 110012, India*

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Abstract

A genetic analysis of 38 diverse Indian bitter gourd (*Momordica charantia* var. *charantia*, and var. *muricata*) accessions was performed using 29 RAPD and 15 ISSR markers. RAPD primers yielded 208 amplicons of which 76 (36.5%) were polymorphic providing an average of 2.6 amplicons per primer. RAPD amplicons per primer ranged from 3 (OPE-19, OPW-09) to 15 (OPW-05), and varied in size from 200 bp to 3000 bp. Fifteen ISSR primers provided a total of 125 bands of which 94 (74.7%) were polymorphic. Polymorphic ISSR markers ranged from 0 (UBC-841) to 12 (UBC-890) providing a mean of 6.3 amplicons per primer that ranged in size from 150 bp to 2700 bp. Nevertheless, the concordance among bitter gourd accession groupings after cluster analysis was relatively high ($r = 0.77$), indicating that RAPD- and ISSR-based diversity assessments in this germplasm array were generally consistent. The *M. charantia* var. *charantia* (domesticated) and var. *muricata* (wild, free-living) accessions examined were genetically distinct, and these differences provided for the development of strategies for genetic analyses and crop improvement in this species.
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Keywords: Bitter melon; DNA markers; Breeding strategies

1. Introduction

Bitter gourd (syn. bitter melon; *Momordica charantia* L.) is an economically important member of the Cucurbitaceae that is widely cultivated in India, China, Malaysia, Africa, and South America (Raj et al., 1993; Singh, 1990). Although the general chemical composition of *M. charantia* immature fruit is similar to other cucurbits, bitter gourd possesses comparatively high concentrations of ascorbic acid and iron (Behera, 2004). White-fruited Indian varieties are, in fact, relatively high in polypeptide-p, phenolics, polyphenolic compounds, and natural oxidants and antioxidants (Horax et al., 2005; Khanna et al., 1981; Krawinkel and Keding, 2006). Bitter gourd has been used as a traditional medicine for diabetes (e.g., India, China, and Central America; Grover et al., 2002; Yeh et al., 2003) and other health-related ailments (e.g., health promoting substances such as charantin (Yeh et al., 2003) and vicine (Dutta et al., 1981)).

The diverse morphological characters (i.e., sex expression, growth habit, maturity, and fruit shape, size, colour and surface texture; Robinson and Decker-Walters, 1999; Behera et al., 2006b) of *M. charantia* in India provide for relatively broad phenotypic species variation. Genetic diversity assessments and linkage map construction can increase the effectiveness of breeding programs (Paterson et al., 1991; Fan et al., 2006). Among the various DNA marker-assisted techniques available, the randomly amplified polymorphic DNA (RAPD) technique (Williams et al., 1990) has been most popular because of speed, low cost and the use of only minute amounts of plant material for analysis. It is less restrictive than the restriction fragment length polymorphism (RFLP) technique (no hybridization and no use of radioisotope). Similarly, ISSR markers are useful in detecting genetic polymorphisms among accessions by generating a large number of markers that target multiple microsatellite loci distributed across the genome. They are simpler to use than the SSR technique as prior knowledge of the target sequences flanking of the repeat regions is not required

* Corresponding author at: Indian Agricultural Research Institute, New Delhi 110012, India. Tel.: +91 11 25840306.
E-mail address: tusar@rediffmail.com (T.K. Behera).

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(Reddy et al., 2002). Yang et al. (1996) found lower relative costs for ISSR, compared to RAPD, since RAPD present extra costs due to the relatively low frequency of reproducible polymorphisms.

Although DNA marker analysis can assist in such analyses (Staub et al., 1996), relatively few polymorphic markers have been identified in bitter melon (Dey et al., 2006). Inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers have, however, been successfully used in genetic diversity analysis of various cucurbits (Verma et al., 2007; Singh et al., 2007; Dey et al., 2006; Sureja et al., 2005; Djè et al., 2006; Levi et al., 2005; Staub et al., 2005; Levi et al., 2004; Ritschel et al., 2004; Chiba et al., 2003; Paris et al., 2003; Decker-Walters et al., 2001; Levi et al., 2001; Gwanama et al., 2000; Horejsi and Staub, 1999; Garcia et al., 1998; Meglic et al., 1996; Lee et al., 1996), and could have utility for genetic analysis of *M. charantia* germplasm. Genetic diversity analysis and map construction has, however, not been preformed in *M. charantia*. Therefore, a study was designed to assess the genetic diversity among 38 diverse bitter gourd accessions using RAPD and ISSR markers to identify potentially useful germplasm for crop improvement. This study represents the first step in determining the inheritance of economically important traits and their subsequent placement on a genetic map in this species.

2. Materials and methods

2.1. Plant materials and DNA extraction

Thirty-eight morphologically and geographically distinct *M. charantia* L. accessions (Table 1) were collected from different Indian states, and then grown in summer 2006 and maintained at the Research Farm of the Indian Agricultural Research Institute, New Delhi, India. All accessions examined herein were self-pollinated three times before evaluation.

The total genomic DNA was extracted by using CTAB-method (Doyle and Doyle, 1987) from young leaf tissue (i.e., terminal whorl) ground to a fine powder. DNA sample concentration was determined using a fluorometer employing a Hoechst dye (Hoefer Inc., San Francisco, CA, USA), and the DNA samples were diluted to 25 ng/μl prior to polymerase chain reaction (PCR) amplification.

2.2. RAPD analysis

PCR DNA amplification was performed using 29 random decamer primers (Operon Technologies, Almeda, CA, USA) selected based on a preliminary screen of eight randomly chosen bitter gourd accessions employing 116 RAPD primers (unpublished data). Amplifications were performed according to Williams et al. (1990) in a 25 μl reaction volume containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 20 pmol RAPD primer, 50 ng genomic DNA, and 0.5 U Taq DNA polymerase (Bangalore Genei, Bangalore, India). Amplification conditions (thermocycler Model-9700, Perkin-Elmer, Boston, MA, USA) were initial denaturation at 94 °C for 4 min and 45 cycles at 94 °C for 1 min and then 38 °C for 1 min, a ramp to 72 °C for 2 min, followed by 7 min at 72 °C and indefinite soak at 4 °C. Amplicons were separated on 1.5% agarose gel in 1× TBE buffer (100 mM Tris–HCl, pH 8.3, 83 mM boric acid, 1 mM EDTA) at 50 V. Gels were then stained with 0.5 μl/ml ethidium bromide solution, visualized by illumination under UV light, and then documented using a gel documentation and image analysis system (Syngene, UK).

2.3. ISSR analysis

Fifteen ISSR primers were obtained from the University of British Columbia (Vancouver, Canada), and PCR was carried out in 25-μl volume containing 50 ng template DNA, 0.5 U Taq DNA polymerase, 10 mM dNTP, 10 μM primer in 1× reaction buffer that contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, and 2.5 mM $MgCl_2$ (Bangalore Genei, Bangalore, India). ISSR primers were chosen based on their performance in a preliminary screen of the ISSRs that were successfully used in other cucurbits (Levi et al., 2005, 2004; Paris et al., 2003). Amplification protocols were those stated for RAPD analysis. Amplified products were loaded on 1.5% agarose gel and separated in 1× TBE buffer at 75 V, and then gels were stained with ethidium bromide, and visualized under UV by image analyses.

2.4. Data analysis

Only consistent, bright, reproducible (i.e., band absence was randomly verified) RAPD and ISSR bands were scored as present (1) or absent (0), where each character state was treated independently. Genetic similarity and cluster analyses were performed by subjecting character state data to empirical examination using the NTSYS-PC soft ware version 2.0 (Exeter Software, NY, USA; Rohlf, 1998), where the SIMQUAL program was used to calculate Jaccard's coefficients of similarity as follows:

$$\text{Jaccard's coefficient} = \frac{N_{AB}}{N_{AB} + N_A + N_B}$$

where N_{AB} is the number of bands shared by samples, N_A the amplified fragments in sample A, and N_B represents fragments in sample B. Coefficients values were then used to create similarity matrices. Correlation between each similarity matrix (i.e., RAPD and ISSR) was estimated using Mantel's matrix correspondence test which yields a product moment correlation (r) that defines the relatedness between the two matrices (Mantel, 1967). In addition, genetic relationships were compared by visual examination of dendrograms derived from clustering procedures using similarity matrices. Dendrograms were constructed using the UPGMA (unweighted pair group method with arithmetic average) procedure (Rohlf, 1998). In order to estimate the congruence among dendrograms (derived from RAPD, ISSR, and combined RAPD + ISSR marker information), cophenetic matrices for each marker and index type were computed and compared using the Mantle test (Mantel, 1967).

Table 1
Bitter gourd (*Momordica charantia* L.) accessions collected in different states of India

Number	Accession	Population type and origin[a]	Fruit character[b]
1	Jaynagar Sel-1	Landrace; West Bengal	Medium long,[c] dark green, continuous ridges
2	Pusa Do Mausami-white	A white mutant of 'Pusa Do Mausami-green' developed from landrace; Rajasthan	Medium long, white, continuous ridges
3	DBTG-1	Landrace; Orissa	Medium long, glossy green fruits with discontinuous ridges
4	DBTG-2	Landrace; Gujrat	Small,[d] light green, discontinuous ridges
5	Pusa Do Mausami-green	Released Indian variety developed from landrace; Rajasthan	Medium long, green, continuous ridges
6	Gayeshpur Sel-1	Landrace; West Bengal	Long,[e] dark green, discontinuous ridges
7	DBTG-3	Landrace; Orissa	Small ovate, light green, discontinuous ridges
8	DBTG–5-1	Landrace; Haryana	Medium long, glossy green, continuous ridges
9	CO-1	Released Indian variety developed from landrace; Tamil Nadu	Extra long,[f] dark green, continuous ridges
10	Priya	Released Indian variety developed from landrace; Kerala	Extra long, dark green, discontinuous ridges
11	DBTG-4	Landrace; Uttar Pradesh	Medium long, light green, discontinuous ridges
12	Preethi	Released Indian variety developed from landrace; Tamil Nadu	Long, white, discontinuous ridges
13	DBTG-6	Landrace; Pondicherry	Small round, dark green, discontinuous ridges
14	Gayeshpur Sel-29	Landrace; West Bengal	Medium long, light green, discontinuous ridges
15	DBTG-101	Landrace; Orissa	Small ovate, light green, continuous ridges
16	Dindigul local	Landrace; Tamil Nadu	Long, dark green fruits, discontinuous ridges
17	Mangalkudi local	Landrace; Tamil Nadu	Small round, dark green, continuous ridges
18	Arupokkatai local	Landrace; Tamil Nadu	Small, light green, discontinuous ridges
19	MDU-1	Released Indian mutant variety developed from landrace; Tamil Nadu	Extra long, light green, discontinuous ridges
20	DBTG-5-2	Landrace; Assam	Long, light green, continuous ridges
21	DBTG-7	Landrace; Madhya Pradesh	Medium long, greenish white, continuous ridges
22	Mohanpur Sel-215	Landrace; West Bengal	Long, dark green, continuous ridges
23	DBTG-8	Landrace; Meghalaya	Long, dark green, discontinuous ridges
24	DBTG-201	Landrace; Madhya Pradesh	Medium long, light green, continuous ridges
25	IC-2763	NBPGR, New Delhi	Long, dark green, continuous ridges
26	DBTG-9	Landrace; Orissa	Medium long, whitish green, continuous ridges
27	DBTG-202	Landrace; Jharkhand	Medium long, light green, discontinuous fruits
28	Arka Harit	Released Indian variety developed from landrace; Rajasthan	Spindle shaped, greyish green, discontinuous ridges
29	DBTG-10	Landrace; Meghalaya	Medium long, white, discontinuous ridges
30	DBTG-11	Landrace; Orissa	Long, dark green, continuous ridges
31	DBTG-12	Landrace; Tamil Nadu	Medium long, light green, continuous ridges
32	DBTG-5-3	Landrace; Assam	Long, light green, discontinuous ridges
33	DBTG-13	Landrace; West Bengal	Long, light green, continuous ridges
34	Nakhara	Landrace; Orissa	Medium long, dark green, continuous ridges
35	DBTG-102	Landrace; Orissa	Medium long, light green, continuous fruits
36	DBTG-103	Landrace; Uttar Pradesh	Medium long, dark green, discontinuous fruits
37	DBTG-14	Landrace; Orissa	Long, dark green, continuous ridges
38	WBK-1	Landrace; West Bengal	Medium long, dark green, continuous ridges

[a] State from which the accession is collected.
[b] Fruit size, colour and surface texture.
[c] Medium long (10–20 cm).
[d] Small (<10 cm).
[e] Long (20–30 cm).
[f] Extra long (>30 cm).

3. Results

3.1. RAPD morphotypes and cluster groupings

A total of 208 amplicons were produced by examining 38 *M. charantia* accessions with 29 RAPD primers. Amplicon numbers per primer ranged from 3 (OPE-19, OPW-09) to 15 (OPW-05) and varied in size between 200 bp and 3000 bp. Of the 208 amplified bands, 76 were polymorphic, with an average of 2.6 polymorphic fragments per primer (Table 2). The polymorphism percentage ranged from 14.3% (OPX-01) to a maximum of 60.0% (OPF-08), with an average of 36.5% polymorphisms when all primers were taken collectively. UPGMA-based cluster analysis grouped the 38 genotypes into two main cluster groups (Fig. 1, Panel A, Groups I and II, Nodes 1–2, similarity coefficient = 0.57), where Jaccard's similarity coefficient ranged between 0.57 ('Jaynagar Sel-1' vs. 'Mohanpur Sel-215') and 0.93 ('Arupokattai Local' vs. DBTG-5-2). Two genotypes (DBTG-101 and 'Mohanpur Sel-215') grouped uniquely into one cluster group (Group I, Node 1). Cluster Group II (36 genotypes; Nodes 2) contained two subgroups; Subgroup 1 consisted of one accession ('Jayanagar Sel-1'; Node 2) which was genetically distinct from the other 35 accessions (Subgroup 2) examined.

Table 2
The statistical data for 29 RAPD and 15 ISSR primers used for analysing 38 accessions of bitter gourd

	Sequence	TNB	NPB	PB (%)	PIC	MI	Rp
RAPD primer							
OPW-5	GGCGGATAAG	15	5	33.33	8.65	8.35	13.42
OPW-7	CTGGACGTCA'	11	3	27.70	2.75	1.93	16.42
OPW-9	GTGACCGAGT	3	1	33.33	0.72	0.24	5.05
OPW-16	CAGCCTACCA	3	1	33.33	0.62	0.42	5.32
OPW-20	TGTGGCAGCA	6	2	33.33	1.17	0.78	10.16
OPW-18	TTCAGGGCAC	7	3	42.85	1.19	0.85	12.63
OPW-6	AGGCCCGATG	8	4	50.00	2.83	2.12	11.68
OPW-8	GACTGCCTCT	9	4	44.44	5.29	4.71	8.47
OPW-13	CACAGCGACA	7	3	42.85	1.27	0.73	12.37
OPX-1	CTGGGCACGA	10	5	50.00	4.36	4.36	14.37
OPX-3	TGGCGCAGTG	7	1	14.28	1.97	0.84	10.89
OPW-1	CTCAGTGTCC	5	3	60.00	1.32	0.79	8.32
OPW-3	GTCCGGAGTG	11	5	45.45	5.20	5.20	15.68
OPX-5	CCTTTCCCTC	7	2	28.75	2.56	2.56	10.89
OPW-2	ACCCCGCCAA	11	3	27.30	3.19	3.19	17.32
OPW-11	CTGATGCGTG	7	3	42.85	1.74	1.24	11.79
OPW-19	CAAAGCGCTC	7	3	42.85	2.20	1.89	10.95
OPF-12	ACGGTACCAG	14	3	21.40	4.40	3.77	22.31
OPF-13	GGCTGCAGAA	8	3	37.50	2.37	2.07	13.26
OPC-13	AAGCCTCGTC	9	3	33.33	3.67	3.67	12.42
OPF-9	CCAAGCTTCC	6	2	33.33	0.42	0.07	11.53
OPC-16	CACACTCCAG	5	2	40.00	2.11	2.11	7.26
OPF-16	GGAGTACTGG	4	1	25.00	0.89	0.89	7.00
OPF-8	GGGATATCGG	5	3	60.00	1.35	1.08	8.37
OPC-17	TTCCCCCCAG	4	1	25.00	1.60	1.60	5.95
OPD-15	CATCCGTGCT	6	2	33.33	1.16	0.77	10.74
OPE-19	ACGGCGTATG	3	1	33.33	1.89	1.89	3.05
OPE-3	CCAGATGCAC	6	2	33.33	4.29	3.57	5.37
OPF-10	GGAAGCTTGG	4	2	50.00	0.71	0.35	7.21
Total		208	76	-	–	–	–
Average		7.17	2.62	36.50	2.47	2.15	10.69
ISSR Primer							
UBC808	AGAGAGAGAGAGAGAGC	10	9	90.00	1.01	0.91	18.94
UBC809	AGAGAGAGAGAGAGAGG	13	8	61.54	3.58	2.20	21.47
UBC825	ACACACACACACACACT	8	7	87.50	5.49	4.80	7.84
UBC840	GAGAGAGAGAGAGAGACTT	11	10	90.90	4.91	4.46	15.52
UBC841	GAGAGAGAGAGAGAGACTC	9	0	0.00	0.00	0.00	8.00
UBC848	CACACACACACACACAAGG	5	4	80.00	2.76	2.21	5.63
UBC850	GTGTGTGTGTGTGTGTCTC	8	3	37.50	0.48	0.18	15.47
UBC854	TCTCTCTCTCTCTCTCAGG	9	9	100.00	3.61	3.61	13.10
UBC855	ACACACACACACACACCTT	7	7	100.00	2.79	2.79	10.26
UBC856	ACACACACACACACACCTA	7	7	100.00	2.76	2.76	10.73
UBC861	ACCACCACCACCACCACC	7	7	100.00	2.47	2.47	11.10
UBC880	GGAGAGGAGAGGAGA	7	5	71.43	1.12	0.80	12.78
UBC888	CGTAGTCGTCACACACACACACA	5	3	60.00	1.15	0.69	8.57
UBC889	AGTCGTAGTACACACACACACAC	6	3	50.00	1.89	0.94	9.36
UBC890	ACGACTACGGTGTGTGTTTGTGT	13	12	92.31	5.68	5.24	17.94
Total		125	94	–	–	–	–
Average		8.33	6.3	74.7	2.65	2.27	13.12

TNB: total number of bands; NPB: number of polymorphic bands; PB: polymorphic bands; PIC: polymorphism information content; MI: marker index; and Rp: resolving power.

3.2. ISSR morphotypes and cluster groupings

Fifteen ISSR primers produced, on average, 125 bands in the accessions examined, of which 94 (74.7%) were polymorphic. The number of amplicons per accession varied from 0 (UBC-841) to 12 (UBC-890), where sizes ranged between 150 bp and 2700 bp. The percentage of polymorphism among accessions ranged from 0 to 100%, where the average number of bands observed per primer and those that were polymorphic were 8.3 and 6.3, respectively (Table 2). Jaccard's similarity coefficient ranged from 0.48 to 0.91. Similar to RAPD analysis, ISSR-based cluster analysis partitioned accessions into two major groups having a similarity of 0.56 (Fig. 1, Panel B, Node 1). These major

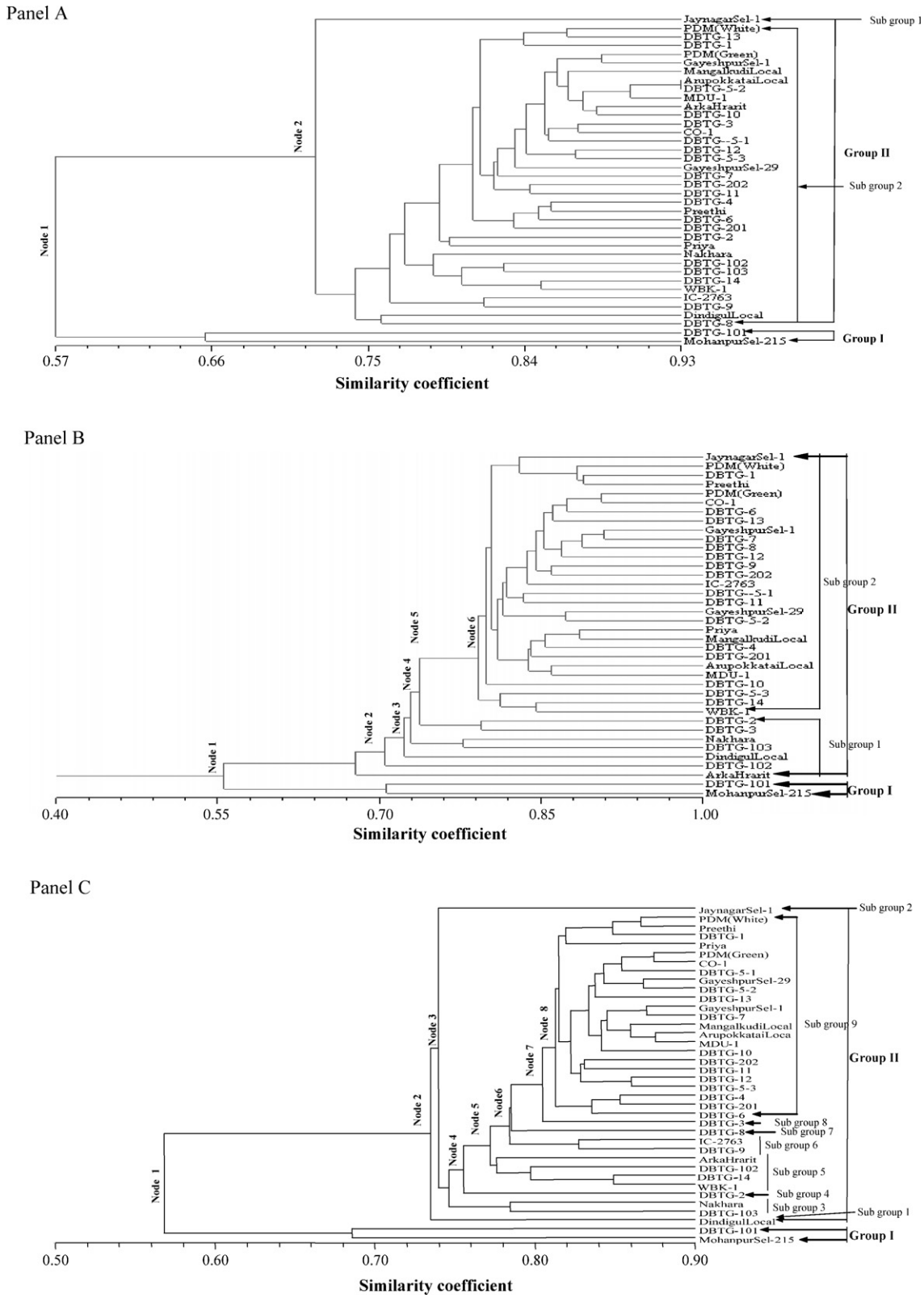

Fig. 1. Genetic similarity relationships based on Jaccard's coefficient of similarity (Jaccard, 1908) after cluster analysis of bitter gourd (*Momordica charantia* L.) accessions from India using RAPD (Panel A), ISSR (Panel B), and a combination of RAPD and ISSR (Panel C) markers.

cluster groupings contained the same accessions as after RAPD-UPGMA-based analysis (i.e., Groups I and II contain 2 and 36 accessions, respectively). Group II could be further partitioned into two relatively distinct subgroups [(Subgroup 1 (7; DBTG 2, DBTG 3, 'Nakhara', DBTG 103, 'Dindigul Local', DBTG 102 and 'Arka Harit'; Nodes 2–5) and Subgroup 2 (29; Node 6)] with subgroup similarities ranging between 0.68 and 0.78.

3.3. Cluster analysis using RAPD and ISSR profiles

A relatively high correlation ($r = 0.77$) was found between RAPD and ISSR marker matrices (cophenetic correlation values). Thus, when RAPD and ISSR data were combined for analysis, two major cluster groupings (Node 1; similarity = 0.56) were identified that contained accessions present in individual marker analyses (Fig. 1, Panels A–C). However, the combined marker analysis dendrogram was more comparable to that obtained by ISSR than RAPD analysis. Combined analysis revealed that accession similarities ranged from 0.57 to 0.87. While Group I consisted of DBTG-101 and 'Mohanpur Sel-215' (Node 1), Group II was composed of nine relatively distinct subgroups (Subgroups 1–9; Fig. 1, Panel C, Nodes 2–8). 'Dindigul Local' and 'Jayanagar Sel-1' constituted Subgroups 1 (Node 2) and 2 (Node 3), respectively, and the remaining 34 accessions formed seven subgroups [Subgroups 3–9; Nodes 4–8; genetic similarities (GS) ranging between 0.75 and 0.87]. Subgroup 3 comprised of two accessions (DBTG-103 and 'Nakhara'; GS = 0.75), Subgroup 4 contained one accession (DBTG-2; GS = 0.76), and Subgroup 5 was composed of four accessions ('Arka Harit', DBTG-102, DBTG-14 and WBK-1; GS = 0.78–0.85). Additional subgroups in this large cluster were Subgroup 6 (DBTG-9 and IC-2763; GS = 0.83), Subgroup 7 (DBTG-8), Subgroup 8 (DBTG-3), and Subgroup 9 (DBTG-6, DBTG-201, DBTG-4, DBTG-5-3, DBTG-12, DBTG-11, DBTG-202, DBTG-10, 'MDU-1', Arupkatai Local, Mangalkudi Local, DBTG-7, Gyeshpur Sel-1, DBTG-13, DBTG-5-2, Gyeshpur Sel-29, DBTG-5-1, 'CO-1', 'Pusa Do Mausami-green', 'Priya', DBTG-1, 'Preethi', and 'Pusa Do Mausami-white'; GS = 0.82–0.87).

Commercial cultivars 'Pusa Do Mausami-green' and 'CO-1' were genetically most similar (GS = 0.87), and 'Arka Harit' and 'Pusa Do Mausami-white' were most dissimilar (GS = 0.75) (Table 3). Similarities among the remaining accessions examined were >0.80, except 'Arka Harit' whose similarities to other accessions were somewhat lower (GS = 0.75–0.80).

4. Discussion

Plants respond and adapt differently to selection pressures imposed by distinctive agro-climatic zones (Singh et al., 1998). Since bitter gourd accessions from disparate ecosystems are often morphologically dissimilar (Table 1), it is not surprising that the accessions examined herein demonstrated dramatic genotypic differences [i.e., RAPD (36.5%) and ISSR (74.5%) polymorphisms]. The detection of polymorphisms herein is in large part due to the use of highly informative primers identified during primer-screening using a small set of diverse bitter gourd accessions (unpublished data). In our study, ISSR marker assessment was more effective (i.e., more banding morphotypes identified) than RAPD analysis. Similar results were obtained when RAPD and ISSR marker analysis was applied to relationship analyses in *Cucurbita pepo* (Katzir et al., 1996, 2000; Paris et al., 2003), and watermelon (*Cucumis lanatus* (Thunb.) Matsum and Nakai; Levi et al., 2004). The increased level of discrimination of ISSR markers witnessed herein may be due to comparatively higher values (Table 2) of average polymorphic information content (2.65 vs. 2.47), marker index (2.27 vs. 2.15), and resolving power (13.12 vs. 10.69) over RAPD markers as well as the diverse nature of the accessions examined.

The number of loci and their genome coverage are critical in obtaining reliable estimates of genetic relationships among cultivars (Loarce et al., 1996). Also, highly effective mixed-marker arrays (e.g., RAPD and ISSR) for genetic analysis require that they are informative and concordant with each other. The correlation between RAPD and ISSR Jaccard's similarity coefficient value was relatively high ($r = 0.77$) despite the comparatively low percentage of polymorphic bands detected by RAPD analysis. Correlation coefficients of similar magnitude were also found among the AFLP, ISSR and SSR markers evaluated by Paris et al. (2003) in *C. pepo*, and among AFLP, RAPD, and RFLP markers compared by Garcia-Mas et al. (2000) in *Cucumis melo*. High congruence ($r = 0.77$) was obtained among phenetic classification based on dendrograms using RAPD and ISSR data. The possible reasons may be that although these two marker systems detect polymorphisms, (both dominant markers), the primers anneal arbitrarily-and the fragments detected present almost the same size.

When compared to other arbitrary primers like RAPDs, ISSRs offer enormous potential for resolving intra- and inter-genomic relationships (Zietkiewicz et al., 1994). In watermelon, for instance, RAPD analysis was found to be less effective {288 RAPD markers [85 (29.5%) polymorphic]; Levi et al. (2001)} than ISSR analysis {111 ISSR markers [72 (65%) polymorphic]; Levi et al. (2004)} in assessing cultivar variation. Although this was also the case in our study, the combined RAPD and ISSR analysis [29 RAPD (76 markers) and 15 ISSR (94 markers) primers; Fig. 1] allowed for a

Table 3
Genetic similarities among bitter gourd (*Momordica charantia* L.) varieties based on 170 [RAPD (76) + ISSR (94)] molecular markers

Cultivars	Pusa Do Mausami—white	Pusa Do Mausami—green	CO 1	Priya	Preethi	MDU 1	Arka Harit
Pusa Do Mausami—white		0.85[a]	0.80	0.80	0.87	0.82	0.76
Pusa Do Mausami—green			0.87	0.85	0.83	0.86	0.80
CO 1				0.84	0.81	0.83	0.77
Priya					0.82	0.81	0.76
Preethi						0.81	0.76
MDU 1							0.79
Arka Harit							

[a] Genetic similarity estimates among seven released varieties of bitter gourd by using Jaccard's coefficient of similarity matrix (Jaccard, 1908).

somewhat extensive subgroup partitioning among *M. charantia* accessions which was often in concordance with known pedigree information. For instance, although the white-fruited mutant, 'Pusa Do Mausami-white' is phenotypically differentiated from its green-fruited counterpart 'Pusa Do Mausami-green', both are genetically similar (Fig. 1).

Even though there was relatively high correlation, among these two marker systems, the discrimination among genotypes within these groups was not increased, when RAPD, ISSR, and RAPD + ISSR derived dendrograms were compared. These differences may be attributed to marker sampling error and/or the level of polymorphism detected, reinforcing again the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars (Loarce et al., 1996). The putatively similar bands originating for RAPDs in different individuals are not necessarily homologous, although they may share the same size in base pairs. This situation may lead to spurious results when calculating genetic relationships (Fernandez et al., 2002).

Some *M. charantia* accession differences reflected differences in their geographic origin and botanic varietal nature. For instance, 'Mohanpur Sel-215' (*M. charantia* var. *charantia*), DBTG-101 (*M. charantia* var. *muricata*), 'Nakhara' (*M. charantia* var. *charantia*), and 'Jaynagar Sel-1' (*M. charantia* var. *charantia*) were genetically distinct from the other bitter gourd accessions examined, and originated from West Bengal and Orissa (i.e., East India). Both *M. charantia* var. *charantia* (large fusiform fruits) and *M. charantia* var. *muricata* (free-living producing small and round fruits) are cultivated in this growing region (Chakravarty, 1990). Given that Eastern India has been proposed as one of the centers for domestication of bitter gourd (Yang and Walters, 1992), these accessions are of considerable interest for genetic studies and plant improvement.

The genetic uniqueness of DBTG-8 (originating from the northeast India; *M. charantia* var. *charantia*) and DBTG-2 and 'Arka Harit' (both originating from western India; *M. charantia* var. *muricata*) (Fig. 1, Panel C) is likely due in large part to the adaptive selection imposed in unique agro-ecological zones. With rare exception (i.e. 'Arka Harit'), all cultivars ('Pusa Do Mausami'-green, 'Preethi', 'Priya', 'CO-1', 'MDU-1') possessed appreciable genetic affinities (long to extra long fruits, and prominent ridges; improved adaptation to differing Indian agro-climatic regions). These accessions develop relatively long fruit (~10 to 35 cm; >85 g), and likely share a common lineage through *M. charantia* var. *charantia* (domesticate) where ecotypes were likely derived by dispersion and further selection after initial domestication(s). In contrast, 'Arka Harit', a comparatively old cultivar (released by Indian Institute of Horticultural Research, Bangalore, India; 1974), develops small (60–70 g) and spindle-shaped fruits, and is likely descended from *M. charantia* var. *muricata* (wild, free-living form) without extensive refinement for horticultural type (e.g., selection for longer fruit).

In comparison to studies by Horejsi and Staub (1999; *C. sativus*), Garcia-Mas et al. (2000; *C. melo*), and Katzir et al. (2000; *C. melo*), the combined marker set used herein must be considered relatively large. However, this marker array was not able to uniquely discriminate all of the accessions examined, and therefore should be augmented by the addition of co-dominant polymorphic RFLPs, SSRs, and SNPs markers to enhance its discriminatory power. Given the success of such highly discriminatory standard marker arrays in other cucurbits (López-Sesé et al., 2003), an enhanced marker array in *M. charantia* would likely facilitate a more comprehensive analysis of genetic diversity.

The assessment of genetic variation among the bitter gourd genotypes examined herein defines a marker array (combined ISSR and RAPD) for improved germplasm curation, allows for the development of a standard accession reference array for further genetic analyses, and provides for the selection of potential parents for genome mapping and breeding. The establishment of a discriminatory marker array is the first step to broader assessments of bitter gourd germplasm for their genetic characterization, and the eventual development of a core collection in this species (Staub et al., 2002; López-Sesé et al., 2003). Based on genetic similarity estimates, a standard accession reference array for further analyses might include 'Pusa Do Mausami-green', 'Pusa Do Mausami-white', DBTG-2, Mohanpur Sel-215, DBTG-101, Nakhara, WBK-1, and Jaynagar Sel-1. As other collections of *M. charantia* are made, genetic diversity can be assessed in relationship to the standard accession reference array, and additional germplasm groupings can be made based on morphology and DNA polymorphisms such that a robust core collection can eventually be constructed.

Regardless of marker analyses type, Mohanpur Sel-125, DBTG-101, and Jaynagar Sel-1 were genetically distinct from the other accessions examined (GS = 0.57–0.72). Genetic differences between such *M. charantia* var. *charantia* and *M. charantia* var. *muricata* accessions are indicative of their use as potential parents for the establishment of narrow- and wide-based mapping populations. Such populations have been informative for the characterization of qualitative and quantitative traits in other cucurbit species (Serquen et al., 1997; Zalapa et al., 2007). Accessions possessing relatively high vitamin C [DBTG-3, DBTG-8, DBTG-6, and DBTG-9 (>100 mg/100 g vitamin C in edible portion vs. ~50 mg/100 g in standard cultivated types; Behera et al., 2006a)] and two horticulturally promising gynoecious lines (DBGy-201 and DBGy-202; Behera et al., 2006b) also should be considered as potential parents for use in map construction.

Since, long and dark green fruits with continuous ridges are suitable for both cooking and processing [i.e., using spices, preservatives (i.e., mustard oil, salt, potassium meta-bisulfite, etc.) and dried products], they have high demand in domestic and international markets. Since, white-fruited types are relatively rich in polypeptide-p, phenolics, polyphenolic compounds, and natural oxidants (Horax et al., 2005; Khanna et al., 1981), these might have pharmaceutical applications. Given this fact and the inherent genetic diversity among the bitter gourd accessions examined herein, Jayanagar Sel-1, Nakhara and WBK-1 (accessions with medium long, dark green fruits with continuous ridges) and 'Pusa Do Mausami-green' (medium long, green fruits with continuous ridges), 'Pusa Do

Mausami-white' (medium long, white fruits with continuous ridges) 'Priya' (extra long, dark green fruits with discontinuous ridges), 'CO-1' (extra long, dark green fruits with continuous ridges), 'MDU-1' (extra long, light green fruits with discontinuous ridges), DBTGy-201, and DBTGy-202 (accessions with gynoecious fruiting habit) would be suitable parents for use in constructing a narrow-based mapping population. Similarly, given their culinary attributes and potential medicinal applications, DBTG-101, DBTG-3, and DBTG-6 (high in vitamin C and iron contents; Behera et al., 2006a), DBTG-2, and 'Arka Harit' should be considered as potential parents for the development of a wide-based mapping population. Regardless of their potential, such prospective parental lines should be advanced by self-pollination and selection to fix unique, economically important attributes prior to their use as mapping parents (S_8 lines). The development of immortalized mapping populations (e.g., recombinant inbred lines) from such parents will allow for the detection and placement of quantitative trait loci (QTL) that control desirable traits (i.e., branching habit, fruit L:D ratio, flesh thickness, iron, and vitamin C). As in cucumber (Fazio et al., 2003; Fan et al., 2006), highly consistent, location independent marker/trait associations (LOD > 4) can then be evaluated for use in MAS for improvement of bitter gourd germplasm.

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