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Genetic diversity of Indian bitter gourd (*Momordica charantia*) by ISSR and morphological markers

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ABSTRACT

Genetic variation among 29 bitter gourd genotypes was assessed using morphological and ISSR markers during 2013–14. High genetic variability was observed for yield per plant (397–1990 g), number of fruits per plant (9.18–43), individual fruit weight (25.47–125.67 g), plant height (110–503 cm), fruit length (6.39–25.97 cm), fruit diameter (2.53–6.1 cm), number of seeds (5–22.33) and number of branches per plant (4.67–16.44). The pair-wise Jaccard's similarity coefficient ranged between 0.22–1.00 based on morphological traits. The size of fragments varied from 200 bp (by ISSR marker UBC-808, 811, and 835) to 1550 bp (by ISSR marker UBC-807). Maximum polymorphism was shown by primers UBC-825 and lowest level of polymorphism was shown by UBC-807, UBC-809 followed by UBC-812, UBC-810. On an average 63.16% polymorphism was obtained per primer. All genotypes were grouped into five main clusters. Jaccard's similarity coefficient ranged from 0.73–1.00. These data revealed that large amount of genetic variability exist among the examined genotypes of bitter gourd.

Key words: Bitter gourd, Dendrogram, Genetic diversity, Morphological traits, ISSR

Bitter gourd (*Momordica charantia* L., 2n=24) is one of the important cucurbitaceous vegetable crop having high amount of minerals, carbohydrates, proteins, vitamins, ascorbic acid and micronutrients; which is considered necessary for maintenance of health and prevention and cure of diseases. Fresh fruits possess anti-oxidant, anti-microbial, anti-viral, anti-diabetic activities (Dalamu *et al.* 2012). The crop originated probably in India with secondary centre of diversity in China but widely cultivated in India, China, Malaysia, Africa, and South America. India is bestowed with high genetic diversity in bitter gourd for several morphological characters (i.e. sex expression, growth habit, maturity, fruit shape, size, color and surface texture) (Behera *et al.* 2006). In comparison, molecular markers are more reliable in establishing genetic relationship in plants as they are based on DNA sequence polymorphism (Tiwari *et al.* 2009, Pandey *et al.* 2013, Pandey *et al.* 2017). ISSR markers are more powerful as they generate large number of markers that target microsatellite loci distributed across the genome, simpler than SSR as target flanking sequences of the repeat regions is not required (Tiwari *et al.* 2009) and also have low utility cost than RAPD (i.e. differences in identifying reproducible polymorphisms).

Although DNA marker analysis can assist in such analyses, relatively few polymorphic markers have been identified in bitter melon. 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), and could have utility for genetic analysis of *M. charantia* germplasm. Therefore, a study was designed to assess the genetic diversity among 29 diverse bitter gourd accessions using ISSR markers and morphological traits to identify potentially useful germplasm for crop improvement in India.

MATERIALS AND METHODS

Plant material: A collection of 29 bitter gourd accessions were used in this study. All the accessions were sown in the experimental field of ICAR-Indian Institute of Vegetable Research, Varanasi (Uttar Pradesh) during 2013–14.

Field evaluation and data collection: The experiment was laid out in a complete randomized block design (CRBD) having three replications with a row-to-row spacing of 100 cm and plant-to-plant spacing of 60 cm; each replication had 10 plants. Five plants were randomly chosen and tagged to record data on 08 quantitative traits, viz. plant height (cm), fruit length (cm), fruit diameter (cm), fruit weight (g), seeds per fruit (number), number of branches per plant, number of fruits per plant (number) and yield per plant (g). Fruits

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were harvested at edible maturity for recording the data. The 2-year pooled data for quantitative traits were analyzed. The effects of different scales of measurement for different quantitative traits were minimized by standardizing the data for each trait prior to cluster analysis; the STAND module of NTSYSpc (Rohlf 1998) software was used to achieve the same. Pair-wise distance matrix was used as an input for analysis of clusters. UPGMA-based clustering was done using SAHN module of NTSYSpc.

DNA extraction and PCR amplification: The total genomic DNA was extracted by a Cetyl-trimethylammonium bromide (CTAB) method (Doyle and Doyle 1990) from young leaves of bitter gourd. A set of 30 ISSR primers procured from Operon Life Technologies, USA, was initially screened and 18 primers with good, clear banding patterns were selected for analysis of genetic diversity. ISSR amplification was performed as follows. The reaction mixture consisted of 50 ng of template DNA, 10 mM primer, 10 mM dNTPs, 25 mM MgCl2, 10X reaction buffer, and 5 U Taq DNA polymerase in a total reaction volume of 25 µL. The reaction mixture was initially denatured at 94ºC for 5 min 2 cycles; followed by 41 cycles of denaturation 95ºC for 30 s, annealing temperature 50ºC for 45 sec, extension 72ºC for 90 sec; and a final extension at 72ºC for 5 min 2 cycles.

ISSR data analysis: Reproducible ISSR products were manually scored for band presence (1) or absence (0) for each accession and a binary qualitative data matrix was constructed. Primer banding characteristics such as number of total bands (TB), number of polymorphic bands (PB) and percentage of polymorphic bands (PPB) were obtained. To analyze the suitability of ISSR marker to evaluate genetic profiles of bitter gourd, the performance of the marker was measured using three parameters: polymorphic information content (PIC), resolving power (RP) and effective multiple ratio (EMR). Polymorphic information content for ISSR markers was calculated as (Roldan-Ruiz *et al.* 2000):

$$PICi = 1 - \sum Pij2$$

where PICi, Polymorphic information content of markers I; Pij, Frequency of the jth pattern for marker I and ∑, Extends over n patterns. The resolving power (RP) of each primer was calculated as (Prevost and Wilkinson 1999);

$$RP = \sum Ib$$

where Ib represents the informative fragments and can be represented on a scale of 0/1 as;

$$Ib = 1 - (2)|0.5 - pi|)$$

where pi is the proportion of accessions containing the ith band. Effective multiplex ratio (EMR) was calculated as;

$$EMR = (n*b)$$

where n is the average number of fragments amplified by accession to a specific system marker (multiplex ratio) and b is estimated from the number of polymorphic loci (PB) and the number of non-polymorphic loci (MB);

$$b = PB/ (PB + MB)$$

The data matrix was used as an input for clusters analysis. UPGMA-based clustering was done using NTSYS-PC 2.02j (Rohlf 1998), for dendrogram construction. Unweighted pair-group average (UPGMA) clusters were joined on the average distance between all members in the groups.

RESULTS AND DISCUSSION

Performance of different accessions based on morphological parameters: The mean value of selected vegetative and fruit characteristics of the accessions is given in Table 1. The plant height showed larger variation in Indian bitter gourd accessions as it varies from 110.2 cm (Gyno-333) to 503.11 cm (Phule Ujwala). A bigger yield gap per plant was observed in studied accessions ranges 396.3 g (HABG-22) to 1991.3 g (VDM-608). The number of fruits also varied from 9.14–42.8 in case of Meghna-2 and Punjab-14, respectively. Individual fruit weight was minimum in case of Preethi (25.30 g); however it was maximum in BRBTG-DC-1 (125.50 g). Fruit length and fruit diameter varied from 6.33 cm (Preethi) to 25.63 cm (DRAR-41), and 2.57 cm (DVBTG-5) to 6.08 cm (DVBTG-2), respectively. Number of seeds ranges from 4.92 (Punjab-14) to 22.31 (NR-1). At last the number of primary branching varied from 4.63 (NR-1) to 16.15 (Pusa Vishesh). Average mean of plant height, fruit length, fruit diameter, fruit weight, number of seeds, number of branching, number of fruits and yield per plant was 262.38 cm, 14.63 cm, 4.49 cm, 57.31 g, 14.15, 7.89, 16.88 and 1222.10 g, respectively.

Genotype cluster analysis based on quantitative traits: All the bitter gourd accessions grouped into three major clusters (Fig 1). The first cluster (cluster I) consisted of 8 accessions, viz. MC-84, DVBTG-7, VDM-608, DRAR-1, Punjab-14, Pant Karela, DVBTG-5 and Gyno-333. The second major cluster (cluster II) consisted of 19 accessions, this cluster could be further divided into two sub-clusters IIA and II-B, sub-cluster IIA (08 accessions), viz. CO-1, DVBTG-1, DRAR-41, BRBTG-DC-1, VNR-22, NDBT-7, DVBTG-2 and Kashi Urvashi, sub-cluster IIB (11 accession), viz. Preethi, Meghna-2, Sel-5, HABG-22, HABG-21, Sel-1, NDBT-9, BIGVAR-6, Phule Ujwala, Pusa Do Mausami and NR-1. Whereas, the third cluster (cluster III) had 2 accessions, viz. Pusa Vishesh, and Arka Harit.

Correlation among various morphological traits: PH was positively correlated with FL, FD, SF, and it was significantly positively correlated with FW, BP, however negative correlation observed with FP and YP (Table 2). FL was either significantly positive or positively correlated with FD, FW, SF, BP and YP except FP. Significantly positive correlation observed between FD and FW, SF, while positively correlated with FP and YP. FW showed negative correlation with BP and FP while it shows positive correlation with SF and YP. In spite of this SF showed negative correlation with BP, FP and YP. In continuation to this BP and FP was positively correlated with YP however, BP showed positive correlation with FP.

Characterization of ISSR loci: The size of the amplified products varied from approximately 200 –1550 bp. The all polymorphic primers yielded a total of 64 fragments

Table 1 Performance of 29 accessions of *Momordica charantia* for eight quantitative traits

Genotype	Plant height (cm)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	No. of seeds/fruit	No. of branch/ plant	No. of fruits/plant	Yield/ plant (g)
MC-84	144.67	10.26	3.12	35.68	14.55	9.31	15.33	1662.67
VDM-608	114.42	9.58	4.10	35.55	14.21	6.86	10.49	1991.33
DVBTG-5	153.63	18.41	2.57	30.31	12.17	15.31	12.52	1342.67
DVBTG-7	145.60	12.77	6.04	40.38	16.16	9.41	18.04	1187.00
Gyno-333	110.22	18.41	3.89	38.39	12.31	10.69	16.34	1879.00
Arka Harit	186.51	14.29	3.48	39.71	7.56	7.58	14.51	909.93
DRAR-1	115.17	16.29	5.66	36.54	16.15	7.20	14.43	1270.04
CO-1	220.84	20.52	5.51	77.54	17.19	9.57	16.29	1080.00
Punjab-14	150.45	8.00	3.75	32.74	4.92	8.61	42.80	1422.67
Pusa Vishesh	176.97	13.53	5.93	59.49	15.13	16.15	33.35	994.87
Sel-1	168.54	12.67	3.61	67.26	15.65	6.28	13.20	861.05
Pant Karela	188.12	10.92	3.25	35.44	5.49	11.66	37.86	1409.00
Preethi	290.94	6.33	3.56	25.30	11.12	8.93	13.26	1382.59
Meghna-2	292.21	13.87	5.52	65.44	13.93	9.62	9.14	1916.98
NDBT-7	217.75	16.87	3.72	68.04	12.82	7.26	15.53	920.32
NDBT-9	215.21	15.06	3.85	66.32	12.85	6.28	14.93	910.16
DVBTG-2	342.14	14.46	6.08	86.33	18.31	5.67	18.75	1610.52
DVBTG-1	357.41	15.32	5.89	84.27	17.62	5.95	18.33	1547.49
DRAR-41	401.23	25.63	4.94	104.75	12.33	6.92	16.90	1781.26
BIGVAR-6	251.29	15.32	5.31	59.90	15.87	6.64	11.71	722.03
Sel-5	369.27	10.33	3.83	41.80	13.99	5.33	14.36	592.00
HABG-22	346.18	12.01	3.73	40.45	14.31	4.65	9.69	393.32
HABG-21	401.48	13.31	3.83	39.45	11.42	5.58	11.93	476.98
Kashi Urvashi	342.71	16.99	4.41	78.10	9.31	8.05	22.55	1743.08
Phule Ujwala	503.11	18.94	4.52	57.51	14.67	5.66	11.86	690.25
BRBTG-DC-1	407.76	25.33	4.96	125.50	17.89	8.29	15.67	1968.18
VNR-22	363.39	17.42	5.91	87.65	17.98	5.31	18.30	1606.35
Pusa Do Mausami	265.11	11.16	4.94	60.31	22.29	5.62	11.98	724.27
NR-1	366.60	10.30	4.49	41.94	22.31	4.63	9.65	406.14
Mean	262.38	14.63	4.49	57.31	14.15	7.89	16.88	1222.1

Table 2 Correlation coefficients among various morphological parameters

Parameter	PH (cm)	FL (cm)	FD (cm)	FW (g)	SF	BP	FP	YP
PH (cm)	1							
FL (cm)	.311	1						
FD (cm)	.232	.281	1					
FW (g)	.503**	.718**	.532**	1				
SF	.267	.147	.573**	.367	1			
BP	.517**	.073	-.113	-.198	-.326	1		
FP	-.261	-.153	.010	-.035	-.521**	.448*	1	.
YP	-.199	.281	.168	.316	-.181	.325	.220	1

* Correlation is significant at the P≤ 0.05, ** Correlation is significant at the P≤ 0.01. PH, Plant height; FL, Fruit length; FD, Fruit diameter; FW, Fruit weight; SF, Seeds per fruit; BP, Number of branching per plant; FP, Number of fruit per plant; YP, Yield per plant.

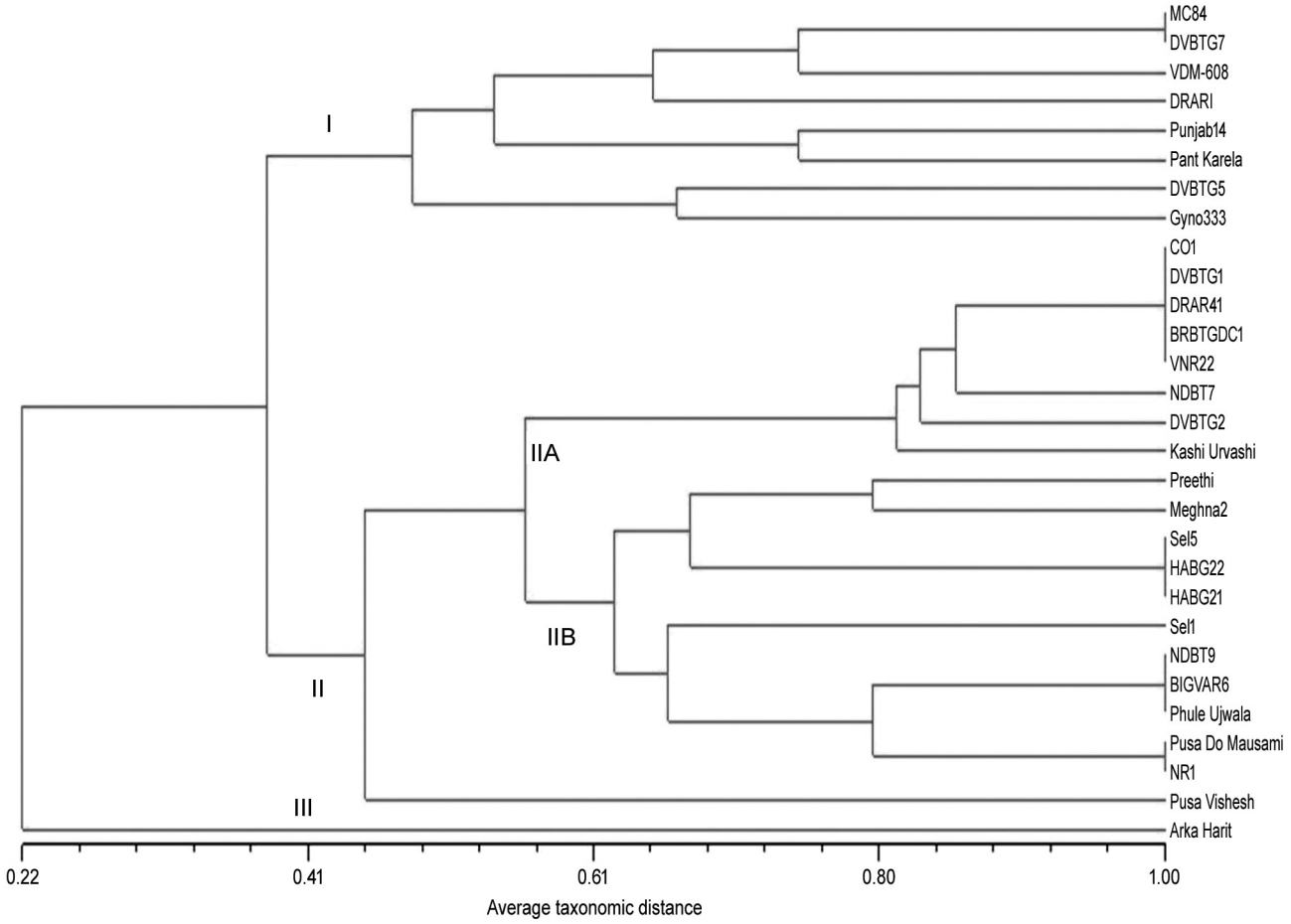

Fig 1 Genetic relationship among the 29 bitter gourd genotypes based on 8 quantitative traits using UPGMA cluster analysis of the distance matrix.

(amplified product). The number of amplicons per primer ranged from 1 (UBC-814 and UBC-823) to 5 (UBC-825), with an average of 3.56 amplicons per primer. The maximum number of polymorphic amplicons (5) was obtained with the primers UBC-825 and lowest level of polymorphism (1) was shown by UBC-807, UBC-809, UBC-810 followed by UBC-812, UBC-814 UBC-880. The percentage of polymorphism ranged from 25% (UBC-807, UBC-812) to 100% (UBC-814, UBC-815, UBC-825, UBC-856) with an average of 63.16% polymorphism obtained per primer. The average polymorphic information content (PIC) value was 0.278 and ranged from 0.102 (UBC-812) to 0.406 (UBC-856). The primers, UBC-815, UBC-866, UBC-825 and UBC-886 have the higher PIC values, respectively. Highest (EMR) effective multiplex ratio 28 was observed by primer UBC 814 and the lowest 5.75 was observed with the primer UBC 807 with an average EMR of 13.23 per primer. The resolving power ranged from 0.93 (UBC-866) to 1.75 (UBC-814) with an average of 1.36. In the present study, the reason of getting high polymorphism rate underneath the efficiency of the selected ISSR primers (Table 3).

Cluster analysis based on ISSR markers: In the dendrogram 29 bitter gourd genotypes were grouped into five main clusters. The maximum number of genotypes (12) was present in cluster I, the monoecious line (MC-84) was an outlier, other lines were (DVBTG-5, DVBTG-2, BIGVAR-6, DVBTG-7, HABG-22, NDBT-9, Preethi, Sel-1, Punjab-14, CO-1 and Sel-5). Cluster II had commercial varieties and 8 lines (DRAR-41, HABG-21, Meghna-2, Kashi Urvashi, NR-1, Phule Ujwala, NDBT-7 and Pusa Vishesh) mostly cultivated in India. Cluster III contained four lines (VDM-608, BRVTG-DC-1, Arka Harit, and Pant Karela). Cluster IV had 2 lines (DVBTG-1 and Gyno-333) whereas; cluster V enclosed three genotypes (DRAR-1, Pusa-Do-Mausami and VNR-22). The association amongst different genotypes is presented in the form of dendrogram prepared using rescaled distances (Fig 2).

Genetic variation is a pre-requisite for any crop improvement programme to be successful (Pandey *et al.* 2017). The morphological traits revealed a considerable variability in plant height, fruit length, fruit diameter, fruit weight, number of seeds, number of branching, number of fruits and yield per plant. The accession Preethi and Sel-1 was very close to each other based on genetic dendrogram as it grouped together at genetic distance 0.965 similarly CO-1 and Sel-5 was also very close to each other. Variation in growth habit based on plant length was of three types, viz. short, medium and tall. However, the maximum yield was not recorded in the tallest accession; it was observed in Punjab-14 with 150 cm height, in general plants with higher length gives

Table 3 List of ISSR Primers, TC, TB, PB, PPB%, PIC, RP and EMR

ISSR Primer	Primer Sequence	T ºC	TB	PB	PPB (%)	PIC	RP	EMR
UBC-807	AGAGAGAGAGAGAGAGT	55.0	4	1	25.0	0.263	1.43	5.75
UBC-809	AGAGAGAGAGAGAGAGG	58.0	2	1	50.0	0.169	1.72	13.75
UBC-810	GAGAGAGAGAGAGAGAT	55.0	3	1	33.3	0.304	1.69	9
UBC-811	GAGAGAGAGAGAGAGAC	52.0	4	2	50.0	0.408	1.34	10.75
UBC-812	GAGAGAGAGAGAGAGAA	50.9	4	1	25.0	0.251	1.70	6.81
UBC-814	CTCTCTCTCTCTCTCTA	55.0	1	1	100.0	0.218	1.75	28
UBC-815	CTCTCTCTCTCTCTCTG	55.0	4	4	100.0	0.402	1.34	21.5
UBC-825	ACACACACACACACACT	50.0	5	5	100.0	0.374	0.95	15.2
UBC-834	AGAGAGAGAGAGAGACYT	55.0	4	3	75.0	0.387	1.42	17.06
UBC-836	AGAGAGAGAGAGAGACYA	55.0	3	2	66.7	0.291	1.23	13.11
UBC-842	GAGAGAGAGAGAGAGAYG	59.1	5	2	40.0	0.329	1.55	9.92
UBC-843	CTCTCTCTCTCTCTCTRA	53.2	3	2	66.7	0.277	1.20	12.89
UBC-844	CTCTCTCTCTCTCTCTRC	50.9	3	2	66.7	0.331	1.50	16.22
UBC-856	ACACACACACACACACYA	56.0	4	4	100.0	0.414	0.79	12.75
UBC-866	CTCCTCCTCCTCCTCCTC	55.0	5	4	80.0	0.378	0.93	11.84
UBC-880	GGAGAGGAGAGGAGA	56.8	4	1	25.0	0.311	1.48	5.93
UBC-886	VDVCTCTCTCTCTCTCT	55.0	3	2	66.7	0.402	1.47	15.78
UBC-892	TAGATCTGATATCTGAATTCCC	53.2	3	2	66.7	0.326	1.10	11.78
Total	64	40		5.62	24.59	238.04		
Average/Primer	3.56	2.22	63.16	0.31	1.36	13.23		

T (ºC), annealing temperature; TB, total band; PB, polymorphic band; PPB (%), percentage polymorphic band (%); PIC, polymorphic information content; RP, resolving power of primer.

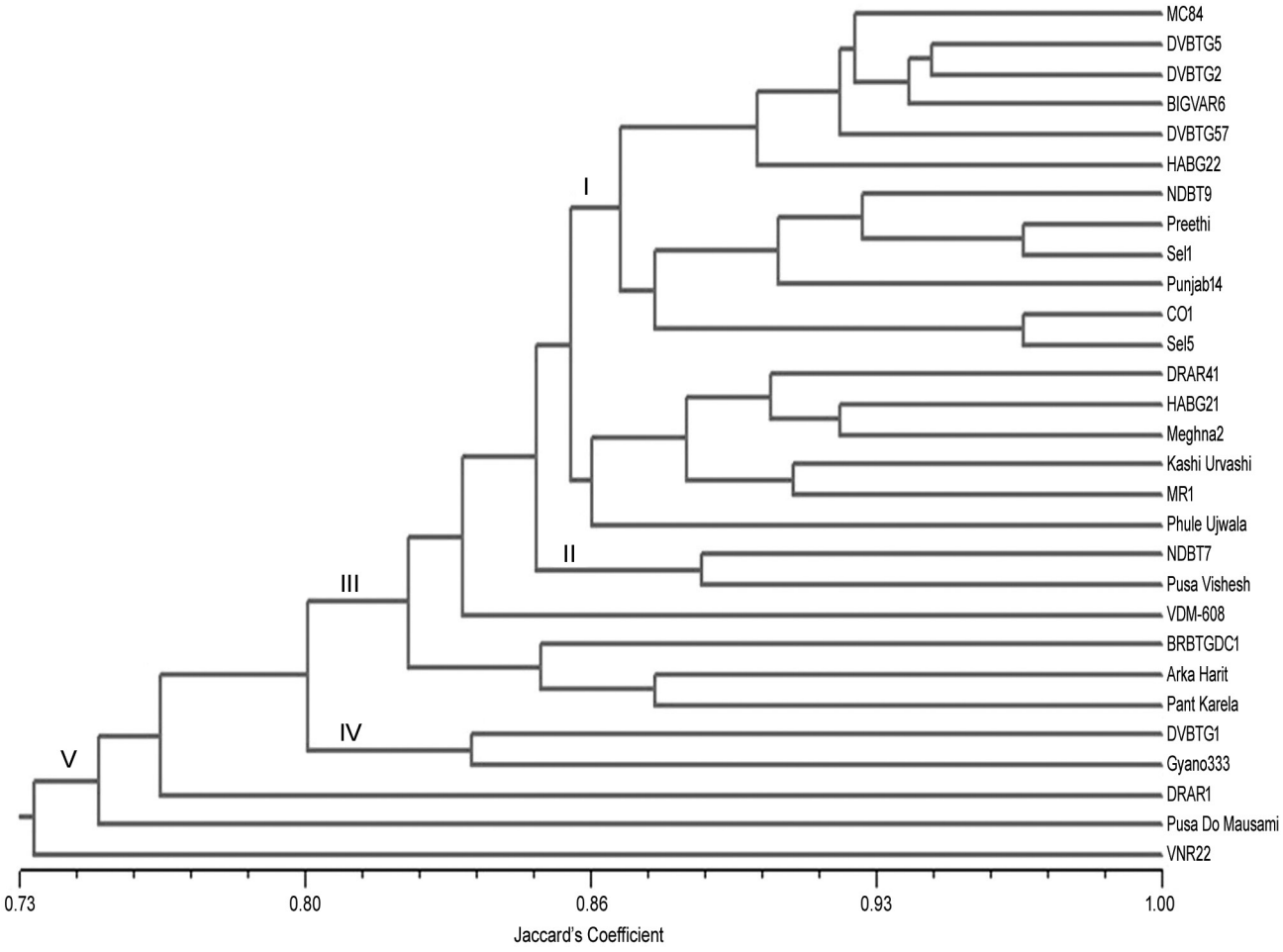

Fig 2 Genetic relationship of 29 accessions of bitter gourd based on ISSR and dendrogram constructed from cluster analysis according to Jaccard's similarity matrix and the UPGMA.

more number of plants, which may be due to more number of internodes bearing female flowers (Pandey *et al.* 2013). The variation in fruit traits was significant but comparatively low. The allelic diversity of the polymorphic markers ranged from 0.001 (UBC-812) to 0.366 (UBC-815) with an average of 0.278. The level of polymorphism observed in this study is similar to the report of Watcharawongpaiboon and Chunwongse (2008). Overall, this study revealed low genetic diversity (2.22 alleles per polymorphic locus) in 29 bitter gourd accessions, possibly, due to lack of polymorphism. Similar low levels of polymorphism were reported by (Staub *et al.* 1997) in collection of Indian bitter gourd accessions. The average taxonomic distance ranged from 0.22–1.00 with a mean of 0.39. But, non-significant correlation was observed between the Jaccard's similarity based on ISSR and average taxonomic distance based on quantitative characters. This is in agreement with the reports that morphological character based dissimilarity and Jaccard's similarity based on molecular markers usually showed non-significant correlation (Riday *et al.* 2003). In this study, no correspondence was observed between the clustering, based on morphological traits and molecular markers.

The Jaccard's similarity coefficient ranged from 0.73–1.00 with an average of 0.135. Among the genotypes Preethi and Sel-1 showed lowest diversity coefficient while VNR-22 showed highest diversity coefficient. All the genotypes showed diversity among themselves indicating considerable amount of variation in the material used for study. The dendrogram constructed from the data revealed five distinct clusters. Presently, array of DNA based molecular markers such as ISSR, SSR, RFLP, RAPD and AFLP etc. are available which detect polymorphism at the DNA level. Variation in the banding pattern of the amplification products occurs because of variation in the length of DNA sequences flanked by the primers. All the primers except UBC-807 gave highest polymorphism. Similarly, Levi and Thomas (2004) identified 80.2–97.8% genetic similarity among hair loom cultivars of watermelon using ISSR markers. They also concluded that ISSRs are highly effective in differentiating watermelon cultivars of elite lines with limited genetic diversity than RAPD marks.

It was observed that plant height, fruit length and fruit diameter was significantly positively correlated with fruit weight. Similarly plant height was significantly positively correlated with number of branching per plant and fruit diameter showed significantly positive correlation with number of seeds per fruit, similar results of various morphological traits were also reported in cucumber, ridge gourd and ash gourd by Pandey *et al.* (2017, 2013).

In general, the study revealed a low molecular diversity among the Indian bitter gourd accessions. Although, genetic erosion to biodiversity mainly due to cultivation of improved varieties has resulted in better productivity and quality, but it has resulted to alarming state of narrow genetic base. A diverse collection of bitter gourd accessions may provide an opportunity to broaden the genetic base and a boost to current breeding program.

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