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Multivariate analysis of the genetic diversity of bittergourd (*Momordica charantia* L.)

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Abstract : Genetic diversity of 33 genotypes of bittergourd of different geographical origin was assessed at the molecular level and compared to morphological traits for degree of divergence. The clustering pattern based on Mahalanobis D^2 statistic indicated that there was no association between geographical distribution of genotypes and genetic divergence. The cluster profile, based on quantitative data and RAPD markers revealed that morphologically distinct and superior lines were genetically differentiable. The clustering pattern based on yield related traits and molecular variation was different.

Key words: Genetic divergence, Bittergourd, RAPD, Polymorphism, Primer

Introduction

Bittergourd or balsam pear (*Momordica charantia* L.) belonging to the family Cucurbitaceae is a popular vegetable in India, having considerable nutritional, economic and medicinal importance. Being a monoecious and highly cross-pollinated crop, large variation is observed in fruit and vegetative characters. However, information on nature and magnitude of genetic diversity is meagre in this crop. Hence it is desirable to evaluate and characterize the genetic resources of bittergourd for sustainable utilization. Characterization of germplasm based on horticultural traits needs complementation with molecular markers as they can contribute greatly to the utilization of genetic diversity through descriptive information of structure of genotypes, analyses of relatedness, the study of identity and location of diversity. Hence, an effort was made to understand quantitative relationship and genetic relation using Mahalanobis D^2 statistical tool and RAPD markers.

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Materials and Methods

Plant material

Thirty-three genotypes of bittergourd collected from different agroclimatic regions of India were evaluated at the vegetable plot in the Department of Olericulture, College of Agriculture, Thiruvananthapuram. The details of the genotypes are furnished in Table 1. The crop was grown in a randomized block design with two replications at a spacing of 2.0 × 2.0 m. Biometrical observations on twenty characters were recorded on four randomly selected plants of each genotype in each replication. The genetic divergence was estimated using D^2 statistics of Mahalanobis (1928) and the populations were grouped into clusters by following the method suggested by Tocher (Rao, 1952).

DNA extraction and quantification

High-molecular weight genomic DNA was extracted from tender leaves of 15-20 days old seedlings as per Murray and Thompson (1980) protocol using CTAB. The quality of isolated DNA was tested by agarose gel electrophoresis and further quantitated by spectrophotometry (Spectronic Genesys 5).

RAPD assay

DNA amplification reactions were performed following the protocol of Staub *et al.* (2000) with minor modifications. Polymerase chain reactions of genomic DNA were carried out in 25 ml reaction volume containing 2.5 ml 10x PCR buffer, 1 ml $MgCl_2$, 2 ml each of dNTPs, 10 pM decamer primer (Operon Inc., CA, USA), 1 unit of Taq DNA polymerase (Invitrogen, USA) and 40 ng genomic DNA. Amplification was performed in a thermal cycler (PTC-100, MJ Research Inc.) for an initial denaturation at 94°C for 5 minutes, followed by 44 cycles of denaturation at 94°C for 15 seconds and annealing at 35°C for 15 seconds. An extension at 72°C for 75 seconds was included after the last cycle. The PCR product was

Table 1. Particulars of genotypes of *Momordica charantia* used in the study and their sources

Sl. No.	Genotype number	Source	Fruit morphology		
			Size	Skin colour	Skin texture
1	MC 1	Thiruvalla, Pathanamthitta, Kerala	Medium	Light green	With spines
2	MC 2	CO-1, Tamil Nadu Agricultural University, Coimbatore	Large	Green	Rough
3	MC 3	IC 68314, NBPGR, Thrissur	Medium	Green	Rough
4	MC 4	Preethi, Kerala Agricultural University, Thrissur	Large	Light green	With spines
5	MC 5	Kalpetta, Wayanad, Kerala	Small	White	With spines
6	MC 6	Pusa Do Mausami, IARI, New Delhi	Large	Green	Smooth
7	MC 7	Kuzhipalam, Thiruvananthapuram, Kerala	Large	Green	With spines
8	MC 8	IC 85632, NBPGR, Thrissur	Large	Light green	With spines
9	MC 9	Anchal, Kollam, Kerala	Medium	Light green	With spines
10	MC 10	MDU-1, Tamil Nadu Agricultural University, Madurai	Extra large	Green	Smooth
11	MC 11	Arka Harit, IIHR, Bangalore	Small	Dark green	Smooth
12	MC 12	Konkan Tara, Konkan Krishi Vidyapeeth, Dapoli	Large	Green	With spines
13	MC 13	IC 85650, NBPGR, Thrissur	Medium	Green	With spines
14	MC 14	IC 85603, NBPGR, Thrissur	Medium	Green	With spines
15	MC 15	Priya, Kerala Agricultural University, Thrissur	Extra large	Dark green	With spines
16	MC 16	Haripad, Alappuzha, Kerala	Medium	Green	With spines
17	MC 17	IC 85627, NBPGR, Thrissur	Small	Green	With spines
18	MC 18	IC 50523, NBPGR, Thrissur	Large	Green	Smooth
19	MC 19	Kattakada, Thiruvananthapuram, Kerala	Medium	Green	With spines
20	MC 20	Priyanka, KAU, Thiruvalla	Extra large	Light green	With spines
21	MC 21	Vellathuval, Idukki, Kerala	Large	Light green	With spines
22	MC 22	Chathamangalam, Kozhikode, Kerala	Large	Light green	With spines
23	MC 23	IC 113878, NBPGR, Thrissur	Medium	White	Smooth
24	MC 24	IC 85636, NBPGR, Thrissur	Large	Green	With spines
25	MC 25	IC 470569, NBPGR, Thrissur	Very small	Dark green	With spines
26	MC 26	Thripunithara, Ernakulam, Kerala	Large	Light green	With spines
27	MC 27	Charuplasseri, Palakkad, Kerala	Large	Light green	With spines
28	MC 28	Kadakkal, Thiruvananthapuram, Kerala	Medium	Green	With spines
29	MC 29	IC 68326, NBPGR, Thrissur	Medium	Dark green	With spines
30	MC 30	Chennai, Tamil Nadu	Medium	Green	With spines
31	MC 31	IC 85642, NBPGR , Thrissur	Medium	Green	With spines
32	MC 32	IC 85612, NBPGR, Thrissur	Medium	Light green	Rough
33	MC 33	Pala, Kottayam, Kerala	Very small	Green	Smooth

analyzed by electrophoresis on 1.2 per cent agarose gel prepared in 1x TAE buffer, visualized under UV-Vis transilluminator after ethidium bromide staining and photographed using gel documentation system (BIO RAD, USA).

Data analysis

Polymorphism was detected by scoring the presence (+) or absence (-) of the reproducible bands and further analyzed the data with NTSYSpc (Version 2.02i) software. The data from the three primers were used to estimate the similarity on the basis of the number of shared bands. A genetic similarity matrix was constructed using Jaccard's coefficient method (Jaccard, 1908) and was subjected to cluster analysis using UPGMA and dendrogram was generated.

Results and discussion

D^2 statistic

After computing D^2 values for all the possible pairs, thirty three genotypes were grouped into five gene constellations, which indicated a large genetic diversity (Table 2). The maximum number of genotypes (11) were included in Cluster I, followed by cluster III and V with 10 genotypes. Cluster II and IV had one genotype each. The commercially cultivated varieties like CO-1, Preethi, Konkan Tara and Priya grouped under cluster I, while Pusa Do Mousami and Arka Harit group into

Table 2. Clustering pattern of thirty three genotypes of *M. charantia*

Cluster No.	Number of genotypes	Genotypes
I	11	MC 1, MC 2, MC 4, MC 12, MC 15, MC 21, MC 22, MC 26, MC 27, MC 29, MC 32
II	1	MC 20
III	10	MC 3, MC 6, MC 7, MC 8, MC 9, MC 11, MC 14, MC 17, MC 28, MC 33
IV	1	MC 10
V	10	MC 5, MC 13, MC 16, MC 18, MC 19, MC 23, MC 24, MC 25, MC 30, MC 31

cluster III. It may be inferred from this result that almost all the commercially cultivated genotypes of our country may have originated from closely related sources. Other commercially released cultivars like Priyanka and MDU-1 grouped singly into cluster II and IV respectively, which indicate that these genotypes are quite distinct from rest of the germplasm.

The intra- and inter- cluster distance represent the index of genetic diversity among clusters (Table 3). The inter-cluster distances were greater than intra-cluster distances, revealing considerable amount of genetic diversity among the genotypes studied. The genetic distance (D) between clusters I, III and V were largest with cluster IV. The minimum intercluster distance was observed between clusters I and V (1022.33) indicating a close relationship among the genotypes included. The data in this table clearly showed that the genotypes usually did not cluster according to their geographical distribution. In general, the pattern of distribution of genotypes from different regions into different clusters was random. Similar observations were also reported by Devmore *et al.* (2007) and Dey *et al.* (2007) in bittergourd. The absence of relationship between genetic diversity and geographical distance indicates that forces other than geographical origin such as exchange of genetic stock, genetic drift, spontaneous variation, natural and artificial selection are responsible for genetic diversity. Therefore, selection of genotypes for hybridization should be based on genetic diversity other than geographic divergence.

Table 3. Average inter and intracluster distances in the thirty three genotypes of *M. charantia*

Cluster	I	II	III	IV	V
I	1197.78	1570.86	1566.15	1856.82	1022.33
II		0.00	2088.12	1545.21	1595.39
III			1149.66	2515.57	1167.00
IV				0.00	1822.31
V					903.03

Diagonal elements- intracluster values
Off diagonal elements- intercluster values

Considering the cluster means for various characters studied (Table 4), clusters II and IV were superior for most of the biometric characters, whereas clusters III was generally poor. Cluster I and V were found to be intermediate. It is also evident that except cluster III and V (represented by small fruited genotypes), all the clusters showed higher yield potential than cluster I, which was represented by most of the commercially cultivated varieties.

Cluster I consisted of genotypes with medium sized fruits with shortest internode, male and female flowers at lower nodes, earliness in fruit harvest and highest mosaic resistance. Cluster II (MC 20) had earliness in seedling germination, longest internode, lowest sex ratio as well as highest fruit length, fruit girth, average fruit weight, yield per plant and seeds per fruit. Cluster III comprised of genotypes with smallest fruits, shorter vine length and less number of branches with lower fruit yield. Cluster IV (MC 10) consisted of genotype with medium sized fruits with longest vine length, highest number of primary and secondary branches,

Table 4. Cluster means of twenty biometric characters in *M. charantia*

Cluster	Days to seedling emergence	Vine length (cm)	Internodal length (cm)	Number of primary branches	Number of secondary branches	Days to first male flower	Days to first female flower	Node to first male flower	Node to first female flower	Sex ratio
I	8.59	358.59	2.92	20.02	35.64	38.18	42.18	13.05	15.77	18.65
II	7.75	468.75	5.58	13.25	19.50	44.25	51.00	16.50	23.25	17.17
III	8.33	240.38	2.99	10.70	18.95	39.23	43.23	14.08	17.15	21.99
IV	11.75	572.50	3.28	21.00	26.50	51.00	54.50	17.75	20.00	17.19
V	10.08	348.25	2.99	19.00	32.50	41.88	30.38	19.05	24.20	22.18

Table 4. Continued

Cluster	Days to first fruit harvest	Fruit length (cm)	Fruit girth (cm)	Fruits per plant	Average fruit weight (g)	Yield per plant (kg)	Seeds per fruit	100-seed weight (g)	Fruit fly infestation	Mosaic incidence
I	52.06	24.56	16.90	22.16	189.95	2.97	20.45	20.15	5.84	21.68
II	59.55	38.83	25.53	14.75	578.75	5.89	33.00	21.60	8.75	41.00
III	55.03	15.82	14.23	14.33	116.01	1.33	15.65	15.00	4.62	46.45
IV	56.50	33.66	8.48	34.25	183.05	4.41	16.00	25.10	4.57	38.00
V	53.33	16.75	15.62	17.58	125.72	1.32	17.95	18.73	5.45	34.05

fruits per plant and 100-seed weight along with lowest fruit fly infestation. Cluster V comprised of small sized fruits with lowest fruit yield**.** The best cluster with respect to yield and other component characters is represented by cluster II followed by IV.

For crop improvement programmes, intercrossing among genotypes with outstanding mean performance for these characters would be effective. To develop early varieties with more yield, selection from cluster I will be effective as it showed higher yield with early maturity. It is clear that for getting maximum yield with largest and heaviest fruits from early crop, cluster II would be a good option. To breed good varieties from small fruited group, selection from cluster V will be highly useful and to breed long, slender fruited varieties having some demand in specific region of our country, selection from cluster IV will be useful.

RAPD polymorphism analysis

DNA amplification of 33 genotypes of *M. charantia* was studied using eighty decamer primers of kit A, B, E, J and UBC. Fifty six primers, out of the eighty decamer primers yielded amplification products indicating presence of sequence complementary to these primers in the DNA of bittergourd genotypes. A total of 158 RAPDs (average 1.98 bands per primer) were generated by the 56 primers, of which 86.08 per cent were polymorphic (136 bands) and twenty two were monomorphic.

For further amplification of DNA from thirty three bittergourd germplasm, the eight promising primers were identified for RAPD analysis based on performance in DNA amplification, production of highest number of polymorphic bands as well as intense bands and reproducibility. They were 0PA-02, 0PA-18, OPB-01, OPB-06, OPB-12, OPE-14, UBC-03 and UBC-05. This could be explained by the capability of individual primers to amplify the less conserved and highly repeated regions of the genomic DNA. There is high possibility for the amplified fragments to contain repeated sequences. In bittergourd, Pala (2001) identified six RAPD primers to show genetic relationship among the genotypes while Behera *et al*. (2007) used twenty nine RAPD primers for genetic diversity studies.

A total of 56 scorable bands (average of 7.00 bands per primer) were generated by the selected eight primers of which 23 were monomorphic and rest, 33 were polymorphic (58.93 per cent). The number of bands ranged from 1 to 9 with an average of 1.25 per primer. The primer OPA-02 was unique as it could distinguish maximum polymorphism among the genotypes tested. The highest number of scorable bands was given by OPA-18 of which seven of the bands produced were monomorphic. Among the OPB group, primer OPB-01 had three monomorphic bands, while OPB-06 and OPB-12 had 2 and 1 monomorphic bands respectively. The primer OPE-14 had only one band as monomorphic while the rest, 6 bands were highly polymorphic (fig 1). The primer UBC-03, which produced a total of six scorable bands had two bands as monomorphic. The primer UBC-05 produced eight scorable bands of which six bands were monomorphic for all the genotypes.

The estimation of Jaccard's similarity coefficients and construction of dendrogram by using UPGMA revealed the presence and extent of genetic similarities among the thirty three genotypes of *M. charantia* examined. The overall similarity coefficients ranged from 0.65 to 0.90 (fig. 2). Cluster analysis revealed that at about 0.65 similarity coefficient, the thirty three genotypes of *M. charantia* grouped into two clusters. The genotype with smallest fruit M 33 got differentiated from the rest of the bittergourd germplasm at 0.65 similarity coefficient. This substantiates the moderately broad distribution of genetic variability, which can be attributed to the broad genetic base in their origin.

In this study, RAPD marker analysis has revealed and grouped the *M. charantia* genotypes according to their genetic relationships reliably. The 33 genotypes formed 8 clusters in the UPGMA cluster analysis using eight primers depicting wide genetic variation among them

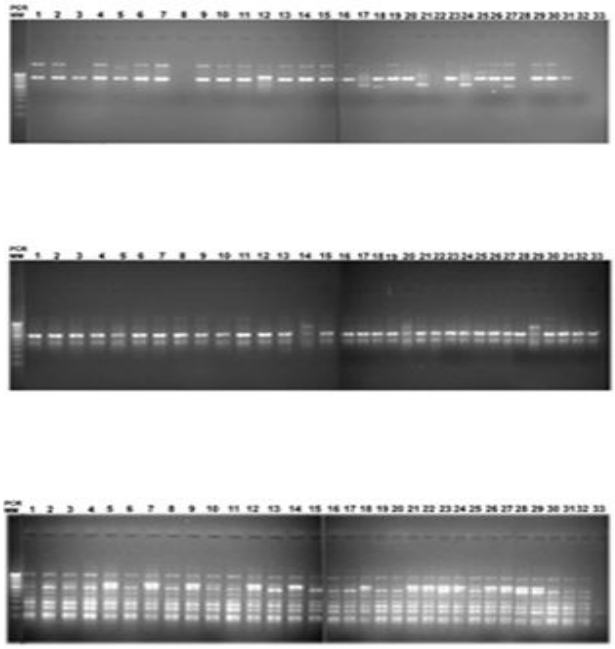

Figure 1. Amplification profiles of the DNA of thirty three genotypes of *M. charantia* L. using RAPD primers OPE-14 (Panel-A), UBC-03(Panel-B) and UBC-05 (Panel-C).

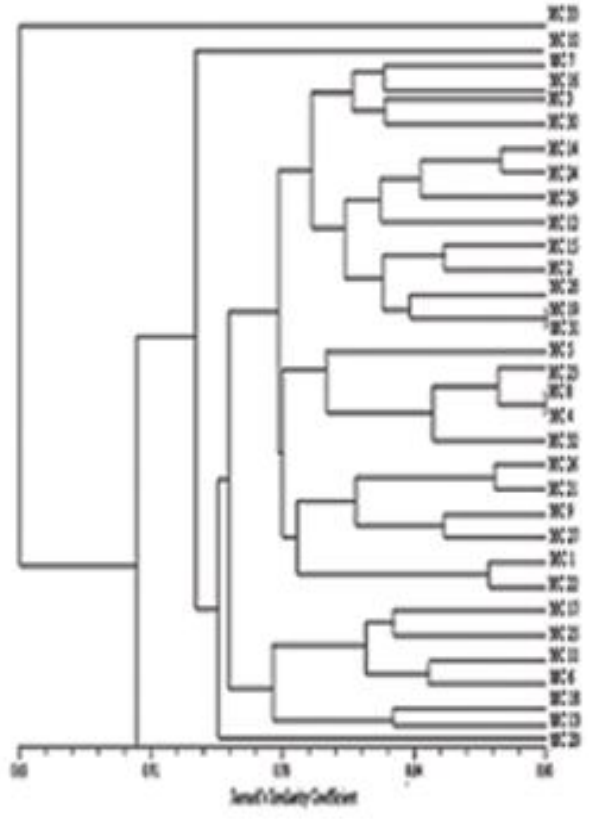

Figure 2. Dendrogram for thirty three genotypes of *M. charantia* based on data from RAPD primers

and provided varietal profiles. Quite distinct among these were two cluster formed at 0.65 similarity coefficient which clearly separates genotypes based on average fruit weight and fruit skin colour. Thus the study revealed that RAPD technique can be suggested as an objective and viable alternative or supplement to ampelography for bittergourd identification. It can easily differentiate *M. charantia* genotypes, even the closely related ones. Polymorphism obtained in the present study will be further useful in fingerprinting and in determining genetic diversity among bittergourd genotypes. For future studies on analysis of bittergourd genotypes, wider genetic base and greater number of RAPD primers are to be included for accurate results. Finally, the results support the idea that RAPD technique being relatively simpler, quicker, inexpensive, non-radioactive, versatile and universal can detect sufficient polymorphisms for germplasm characterization and genetic distance studies.

सारांश

विभिन्न भौगोलिक मूल के बीटरगार्ड के 33 जीनोटाइप के जेनेटिक विविधता का आणविक स्तर पर और विचलन डिग्री के लिए मारफोलाजिकल विशेषताओं की तुलना का मूल्यांकन किया गया था। क्लस्टरिंग महालनोबिस डी 2 आंकड़े पर आधारित पैटर्न का संकेत है कि जीनोटाइप और आनुवंशिक विचलन का भौगोलिक वितरण के बीच कोई संबंध नहीं था। क्लस्टर मात्रात्मक डेटा और आरएपीडी मार्कर पर आधारित प्रोफाइल से पता चला है कि मारफोलाजिकल अलग और बेहतर लाइनों आनुवंशिक अलग थे। क्लस्टरिंग उपज संबंधित लक्षण और आणविक परिवर्तन पर आधारित पैटर्न अलग था।

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