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Studies on Genetic Diversity of Twelve Accessions of *Momordica charantia* L. using Morphological, RAPD and SCAR Markers

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Abstract: The aim of present research was to study genetic diversity among twelve different accessions of *Momordica charantia* L., growing in eight different districts of West Bengal using morphological characters, RAPD and SCAR markers. Five plants for each of 12 accessions was selected and were examined for 13 morphological characters. RAPD analysis of 12 accessions of *M. charantia* was performed with 17 decamer primers from a total of 23 primers. A dendrogram was constructed by cluster analysis to establish the affinity and relationship between the 12 accessions of *Momordica charantia*, using average linkage between the groups. Accession specific Sequence Characterized Amplified Region (SCAR) marker was developed from accession 12 sequences, using primers designed from both the flanking ends of RAPD primer. When morphological characters were considered the 12 accessions of *M. charantia* could be clustered into 2 groups. Group I comprised of the accessions belonging to variety *muricata* and Group II comprised of the accessions belonging to variety *charantia*. When RAPD markers were considered, dendrogram constructed by cluster analysis with average linkage between groups produced two major clusters - Cluster I comprised of the accessions belonging to variety *muricata*. Cluster II comprised of the accessions belonging to variety *charantia* and variety *muricata*. Thus the clustering pattern based on RAPD markers was not in accordance with the grouping based on morphological characters. Development of accession specific SCAR marker (855 bp) in accession V12 was used to distinguish it from different accessions of *M. charantia* their by validating the RAPD marker. Molecular markers (RAPD, SCAR) were found to be more effective as compared to morphological marker in assessing the genetic diversity of twelve different accessions of *M. charantia*.

Key words: DNA, dendrogram, *Momordica charantia* L., morphological character, RAPD, SCAR

INTRODUCTION

Momordica charantia L. belonging to the family cucurbitaceae (Toyama *et al*., 2008) is a medicinally important vegetable crop plant. *M. charantia* has been widely used by orients as foodstuff (Tanaka *et al*., 2009). Two varieties of this plant are cultivated in India 1. *M. charantia* var. *charantia* with large fruits which are fusiform in shape and 2. *M. charantia* var. *muricata*, which are identified by small, round fruit (Chakravarty, 1990; Paul and Raychaudhuri, 2010). Genetic diversity among populations can be determined using morphological and molecular markers. Morphological characters have limitations since they are influenced by environmental factors, individual plant biology and the developmental stage of the plant. In contrast, molecular markers based on DNA sequence polymorphism are independent of environmental conditions and show higher levels of polymorphism. They can be used to detect variation at the DNA level and have proven to be effective tools for distinguishing between closely related genotypes. Among the different types of molecular markers available, random amplified polymorphic DNA (RAPD) are useful for the assessment of genetic diversity because of their simplicity, speed, relatively low cost compared to other molecular markers, small amount of plant material is required for DNA extraction and the method does not require prior knowledge of the sequence

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of the genome (Williams *et al*., 1990; Rafalski and Tingey, 1993). In a RAPD assay, a short, usually ten nucleotide long, arbitary primer is used, which generally anneals with multiple sites in different regions of the genome and amplifies several genetic loci simultaneously. RAPD markers have been used extensively in cucurbits to classify accessions (Horejsi and Staub, 1999), identify cultivars and hybrids (Meng *et al*., 1996; Changyuan *et al*., 2005; analyse genetic diversity (Lee *et al*., 1996; Garacia *et al*., 1998; Gwanama *et al*., 2000; Levi *et al*., 2001; Sureja *et al*., 2006; Dey *et al*., 2006; Rathod *et al*., 2008) etc.

RAPD technique is sensitive to PCR conditions, reproducibility of RAPD results is low, furthermore RAPD markers are dominant, i.e., it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). Co-dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely. To overcome this problem, (Paran and Michelmore, 1993) suggested a Sequence Characterized Amplified Region (SCAR) marker. RAPD marker can be converted into SCAR marker, by the development of primers from unique polymorphic RAPD sequences followed by amplification of genomic DNA (using SCAR primer) of any one particular genotype and not of other genotypes. In this way, SCAR markers can improve the reproducibility and reliability of PCR assays and therefore could justify their use as markers in studying the genetic affinity amongst intra and inter varietal and specific studies. Thus SCAR markers could be used in developing locus-specific, co-dominant markers from RAPDs. Reliable SCAR markers have already been successfully derived from RAPD fragments in *Lactuca*, *Triticum* and *Agrostis* (Paran and Michelmore, 1993; Hernandez *et al*., 1999; Elizabeth *et al*., 2003), for studying inter and intra specific genetic diversity.

The present investigation was aimed to study genetic diversity among 12 different accessions of *M. charantia* grown in different districts of West Bengal in India, through use of morphological and molecular markers- RAPD and SCAR.

MATERIALS AND METHODS

Seed collection: Seeds belonging to seven accessions of *M. charantia* var. *muricata* (V1, V2, V3, V4, V5, V6 and V7) and five accessions of *M. charantia* var. *charantia* (V8, V9, V10, V11 and V12) were collected from eight districts of West Bengal namely, South 24- Parganas (Jaynagar, Baraipur, Canning), East Midnapore (Panskura, Contai), Murshidabad, West Midnapore (Kharagpur, Daspur), Kolkata (Dhapa), Hoogly (Tarakeshwar), Bardhaman and North 24 Parganas (Bashirhat) (Table 1).

Table 1: Tabular representation of collection sites of seeds of *M. charantia*

Accession number	Variety	Collection Site
V 1	*M. charantia* var. *muricata*	JAYNAGAR
V 2	*M. charantia* var. *muricata*	BARAIPUR
V 3	*M. charantia* var. *muricata*	PANSKURA
V 4	*M. charantia* var. *muricata*	MURSHIDABAD
V 5	*M. charantia* var. *muricata*	DHAPA
V 6	*M. charantia* var. *muricata*	CONTAI
V 7	*M. charantia* var. *muricata*	KHARAGPUR
V 8	*M. charantia* var. *charantia*	TARAKESHWAR
V 9	*M. charantia* var. *charantia*	BARDHAMAN
V 10	*M. charantia* var. *charantia*	DASPUR
V 11	*M. charantia* var. *charantia*	CANNING
V 12	*M. charantia* var. *charantia*	BASHIRHAT

Evaluation of morphological characters: Five plants of each variety was selected and were examined for 13 morphological characters, 1. Vine length (cm) 2. Days to first male flower appearance, 3. Days to first female flower appearance 4. Node number to which first female flower appears 5. Male flowers per plant 6. Female flowers per plant 7. Sex ratio (total number of male flowers : total number of female flowers) 8. Days to edible maturity of the fruit 9. Average fruit length (cm) 10. Average fruit diameter (cm) 11. Number of fruits per vine 12. Average fruit weight (g) and 13. Yield per vine (g).

Extraction of genomic DNA: Seeds were germinated in petridishes containing moist filterpaper followed by transfer of seedlings in the green house. DNA was extracted from leaves of 20 days old plant using the method of Edward *et al*. (1991) Betal *et al*. (2003) and Pal and Raychaudhuri (2003). DNA isolated from 12 accessions of *M. charantia* were run in 1% agarose gel to check the quality and spectrophotometrically scanned between 220 to 320 nm. The OD values were noted at 260 and 280 nm. For RAPD analysis, DNA's were diluted in such a manner that 1 μL of the diluted solution contained 15 ng of genomic DNA.

Setting of RAPD reactions: RAPD was performed using the method of Williams *et al*. (1990). The amplification reactions were set at a final volume of 25 μL. Amplification reactions were carried out using DNA amplification Core Kit (Bangalore Genei, Bangalore, India). Amplification reactions (25 μL final volume) contained 15 ng genomic DNA, 0.2 mM dNTPs, 0.2 μM decamer random primer, 1x Taq DNA Polymerase buffer containing 50 mM KCl, 10 mM Tris-HCl and 1.5 mM $MgCl_2$ and 0.5 units of Taq DNA Polymerase. 17 random decamer custom oligonucleotide primers (60-70% G+C content) of arbitary sequences were used to perform RAPD reactions. Lyophilised oligonucleotide decamer primers were

purchased from Operon Technologies (USA). The arbitary sequences of Primers used in performing RAPD reactions.

DNA amplification was carried out in a DNA Thermal Cycler (Perkin Elmer, USA). DNA denaturation was done at 94°C for 4 min; followed by a 45-cycle amplification (94°C, 1 min; 35°C, 1 min; 72°C, 2 min) and a final extension step at 72°C for 7 min and finally the amplified product was brought down to 4°C temperature. The amplified products were run in 1.5% (w/v) agarose gel in TAE (pH 8.0) (Tris acetate EDTA buffer) buffer (2 M (w/v) Tris, 0.06 M (v/v) glacial acetic acid, 0.5 M (v/v) EDTA). Gel electrophoresis was performed at constant voltage of 72V for 3 h and 30 min and stained with ethidium bromide for 1 min followed by destaining for 10 min. The RAPD banding patterns were photographed using the gel documentation system of Biorad.

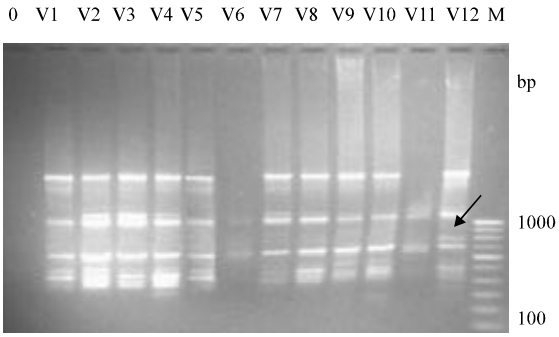

Fig. 1: RAPD profile from genomic DNA of twelve different accessions of *M. charantia* using primer P4 (5'CAAACGTCGG3'). 0-negative control (without template DNA), M-100 bp ladder. RAPD fragment specified by arrow was cloned and sequenced

RAPD banding pattern analysis: Positions of the clearly visible and scorable bands were transferred into binary matrix, with 1 for the presence and 0 for the absence of a band at a particular position. Pairwise Jaccards coefficient for similarities was calculated by the statistical package SPSS version 9.0 (Jaccard, 1908; Nei, 1972; Nei and Li, 1979; Pal and Raychaudhuri, 2003). A dendrogram was constructed by cluster analysis to establish the affinity and relationship between the 12 accessions of *Momordica charantia*, using average linkage between the groups.

Cloning and sequencing of RAPD marker: RAPD marker can be converted into SCAR, whereby a unique polymorphic band (855 bp) (Fig. 1) in accession No. 12 (V12), among the 12 different accessions of *M. charantia* was identified. The polymorphic band was extracted from gel using Gel extraction kit. The excised RAPD band was cloned in pUC (plasmid University of California) vector and sequenced using M13 forward and M13 reverse primers in a sequencing machine (ABI 3130 genetic analyzer, Los Angeles, USA). Gel extraction, cloning and sequencing of the RAPD fragment was performed by Chromous Biotech Private Limited, Bangalore, India. Sequence specific Homology searches were performed using the BLAST (Basic Local Alignment Search Tool) algorithm at http:www.ncbi.nlm.nih.gov of the National Centre for Biotechnology Information (NCBI).

Based on the sequences of RAPD fragment a pair of SCAR primers (5'AAACGTCGGGTCGACTGAT3' (forward primer) and 5'AAACGTCGGCCAATAGTAGC3' (reverse primer)) were designed using software-Web Primer (Fig. 2). The primers could amplify approximately 851 bp of the genomic DNA of accession 12. The SCAR primers were custom synthesized from Chromous Biotech Private Limited, Bangalore, India. The pair of SCAR

CAAACGTCGGGTCGACTGATGAAGTGGGCACTAGAGCTCAGTGAGCGTGACATCGCCTTCGAGTC
GAGAACAACATTGAAGGGTCAGGCAGCCGCAGACTTTGTTGCTGAGCTGACCCCACCCAGCCAAGGAAGCG
ATGTCCGTGACAATGGACACTCTACATGGATGGTTCATCCAATGAGAAAGGTTGTGGGGCAGGTCTGTGCT
CCTCGGCCCCGGTGGCTTCGGTTCAAGTACGCACTCCGGTTAGCTTCCGAGCTTCCAACAACGAAGCCGAG
TACAGGCATTGATAAATGGACTGAAGGTCGCTAGGGGGATGGGAGTAAAGCGACCCTGATTCTCAGCGATT
CCCAGCTGATCGTCAATCAGGTTACCAAGGAGTACCAAGTGAAGGAAGCCTTATGGAGAGGTATTTGGCCA
AGCCCGAGTGCTCCTCGCCCAGTTCAAGGACTATGTGATTGACGGGTGCCGAGGTCGAAACTCCAACGCGA
TGCACTGCCGCCTAGCCTCGGCATACGAGACTGCTTACCGAGAACAGTTCCAGTTGAATACTCGCTGAGTC
GTCCATCGACCAGCCGAGGTAATGGAGATCCAGTCAGCTCAGCCTAATGGTGATCCGATTAAGAACTTCCT
GGTCAGTGGCTCAGTCCCTGCTGATCCGAGCAGGCCAGAACTCGCGCCAAGCTGCTCACTACTTGATGCAA
GAAGGCAAGCTCTTTAAGAGGGAATATTCCCTACCATTACTGAGGGCCTCGACCTAGAGGAGGCGAGGTAC
GTTATCCGTGAGATTCACGAAGGGGTTTGCGGAAACCATGGAGGAGCTCGGTCCATGACCGCGAAGGTCGT
CCGCTAGGGCTACTATTGGCCGACGTTTG

Fig. 2: Nucleotide sequence of RAPD fragment (855 bp) obtained after PCR amplification of genomic DNA of accession V12, using primer P4. Nucleotides in bold represent the RAPD primer and those underlined indicate the designed SCAR primers

primers was used to amplify genomic DNA from 12 accessions of *M. charantia*. The amplification reactions were set at a final volume of 25 μL. Amplification reactions (25 μL final volume) contained 30 ng genomic DNA, 0.2 mM dNTPs, 0.5 μM each of forward and reverse primer, 1x Taq DNA Polymerase buffer containing 50 mM KCl, 10 mM Tris-Cl and 1.5 mM $MgCl_2$ and 1 unit of Taq DNA Polymerase. Thermal cycling conditions for PCR amplification using SCAR primers was an initial denaturation at 94°C (4 min), 35 cycles of 94°C for 1 min, 54.8°C for 1 min, 72°C for 2 min and final extension at 72°C for 7 min. PCR products were analysed by agarose gel electrophoresis. Presence and absence of the SCAR band was visually scored and compared with samples of each accession.

RESULTS

Evaluation of morphological characters: Plants belonginig to variety *muricata* and variety *charantia* showed differences in morphological characters such as vine length, weight of fruits and number of fruits per vine. Plants belonging to variety *muricata* showed lesser plant height (92.2±20.6-158.0±15.2) compared to plants belonging to variety *charantia* (236.0±13.2-361.4±26.3) (Table 2). Fruits belonging to variety *charantia* (5.0±1.3-8.8±1.1 cm) showed greater length as compared to the fruits belonging to variety *muricata* (2.3±0.1-3.4±0.3 cm) (Table 2). Fruits belonging to variety *muricata* (2.6±0.6-5.3±0.5 g) were lighter than the fruits belonging to variety *charantia* (13.3±1.6-23.1±2.2 g) (Table 3). Plants belonging to variety *muricata* (5.0-15.0) produced more number of fruits per vine than the plants belonging to variety *charantia* (3.0-5.0) (Table 4).

Accession V2 showed very early appearance of first female flower (56) and the appearance of female flower at lowest node (5th) indicated it's earliness and early harvest (Table 2). Individual fruit weight (23.1±2.2) and fruit length (8.8±1.1) were the highest in accession V11 and accession V8 respectively (Table 2). Accession V3 produced maximum number of fruits per plant (15) (Table 3). Accession V11 (115.2±7.3 g) showed maximum fruit yield per plant (Table 2).

Thus, when morphological characters such as vine length, number of fruits / vine, fruit length, fruit diameter, fruit weight, fruit yield/plant are considered (Table 4), the 12 accessions of *M. charantia* can be clustered into 2 groups. Group I comprising of accessions V1, V2, V3, V4, V5, V6 and V7 belonging to variety *muricata* and Group II comprising of accessions V8, V9, V10, V11 and V12 belonging to variety *charantia*.

Table 2: Few morphological characters of 12 accessions of *M. charantia*

Accession	A	B	C	D	E	F
V1	158.0±15.2	7.3±0.3	2.9±0.8	2.1±0.1	5.3±0.5	44.9±1.9
V2	158.0±6.8	8.5±0.4	2.6±0.2	2.0±0.1	3.9±1.3	41.2±3.7
V3	92.2±20.6	6.7±0.6	2.9±0.3	1.9±0.3	2.9±0.5	42.3±7.4
V4	108.0±13.3	10.2±1.1	3.4±0.3	2.3±0.2	3.9±0.6	16.0±3.4
V5	127.4±9.9	5.2±0.2	2.6±0.3	1.8±0.2	2.6±0.6	31.5±2.2
V6	106.0±9.7	8.8±0.7	2.3±0.1	1.9±0.1	2.7±0.4	13.3±1.9
V7	110.2±10.9	10.8±1.4	2.9±0.1	1.7±0.9	3.2±0.7	21.1±3.4
V8	236.0±13.2	8.9±0.2	8.8±1.1	5.6±1.2	16.9±1.4	84.9±2.3
V9	340.0±83.5	20.0±2.1	8.2±1.2	3.7±0.4	14.1±0.9	42.3±5.5
V10	312±45.05	11.9±2.1	6.8±0.7	3.0±0.2	13.3±1.6	53.4±9.4
V11	361.4±26.3	14.2±0.3	7.9±0.7	3.7±0.4	23.1±2.2	115.2±7.3
V12	281.4±47.0	11.1±0.9	5.0±1.3	3.2±0.2	17.1±1.3	56.0±9.6

A: Vine length (cm), B: Sex ratio (total no. of male flowers : total no. of female flowers), C: Average fruit length (cm), D: Average fruit diameter (cm), E: Average fruit weight (g), F: Yield per plant (g)

Table 3: Morphological characters (range) of 12 accessions of *M. charantia*

Morphological characters	Range
Days to first male flower appearance	41 (V2)-59 (V8)
Days to first female flower appearance	56 (V2)-77 (V12)
Node no. of first female flower	5 (V2)-23 (V12)
Male flowers per plant	48 (V6)-102 (V3)
Female flowers per plant	4 (V12)-15 (V5)
Days to edible maturity of fruit	82 (V4)-110 (V12)
Number of fruits per vine	3(V12)-15 (V3)

Table 4: A comparison of morphological characters of two varieties of *M. charantia*

	M. charantia var. *muricata*	
Morphological characters	Group I (V1-V7)	Group II (V8-V12)
Vine length (cm)	92.2±20.6-158±15.2	236.0±13.2-361.4±26.3
Number of fruits/vine	5.0-15.0	3.0-5.0
Fruit length (cm)	2.3±0.1-3.4±0.3	5.0±1.3-8.8±1.1
Fruit diameter (cm)	1.7± 0.9-2.3±0.2	3.0±0.2-5.6± 1.2
Fruit weight (g)	2.6±0.6-5.3±0.5	13.3±1.6-23.1±2.2
Fruit yield/plant (g)	13.3±1.9-44.9 ±1.9	42.3 ±5.5-115.2±7.3

RAPD data analysis: RAPD analysis of 12 accessions of *M. charantia* - V1, V2, V3, V4, V5, V6 and V7 (7 accessions of variety *muricata*) and V8, V9, V10, V11 and V12 (5 accessions of variety *charantia*) were performed using 23 selected RAPD primers. Amongst 23 primers used, 17 primers produced clear and reproducible bands. Among 17 primers, 16 primers produced polymorphic bands and only 1 primer produced monomorphic band. The details of the primers producing polymorphic bands are presented in Table 5. 16 primers produced 85 DNA fragments in total; among them 35 fragments were monomorphic and 50 DNA fragments showed polymorphism in one or more genotypes. The amplifications generated by primer P4 produced maximum number of DNA fragments (8). The size of the DNA fragments ranged from 320-1900 base pairs.

The total number of bands per primer ranged from 2-8, with an average of 5.31 bands per primer. The highest number of polymorphisms (6) was observed with primers

P4 (Fig. 1) and P5. The average number of polymorphic bands per primer was 3.125 and the percentage of polymorphic bands ranged from 20 to 100% with a mean value of 60.94%.

Pair wise Jaccard's coefficient (Jaccard, 1908; Nei, 1972; Nei and Li, 1979) for genetic similarities was calculated by the statistical software package SPSS version 9.0. The similarity coefficients based on 85 RAPD markers ranged from 0.562-0.881. Of the 66 pair wise combinations generated by 12 accessions of *M. charantia*, accessions V2 and V3; V6 and V9, showed highest similarity index (0.881). The result indicated that accessions V2 and V3; V6 and V9 are genetically closer than the other accessions. Accessions V1 and V11 showed lowest similarity index (0.562), which indicated maximum genetic diversity among the selected accessions. The mean similarity index was 0.74.

Dendrogram (Fig. 3) was constructed by cluster analysis with average linkage between groups. The dendrogram revealed that 12 accessions of *M. charantia* formed 2 major clusters. Among the 2 major clusters, first major cluster was further subdivided into two minor clusters. Of the 2 two minor clusters, first minor cluster comprised of a single accession, V5 and second minor cluster was again subdivided into two sub minor clusters. Among the two subminor clusters first subminor cluster comprised of a single accession, V1 and second subminor cluster comprised of two accessions namely, V2 and V3. Thus first major cluster comprised of 4 accessions (V1, V2, V3, V5) of *M. charantia* var. *muricata*.

Second major cluster was divided into two minor clusters, of which, first minor cluster comprised of a single accession V11 and second minor cluster was further subdivided into two sub minor clusters. Of the two subminor clusters, first one contained a single accession, V10 and second subminor cluster contained 6 accessions namely V4, V6, V7, V8, V9 and V12. Thus, second major cluster comprised of five accessions of *M. charantia* var. *charantia* (V8, V9, V10, V11, V12) and three accessions of *M. charantia* var. *muricata* (V4, V6, V7).

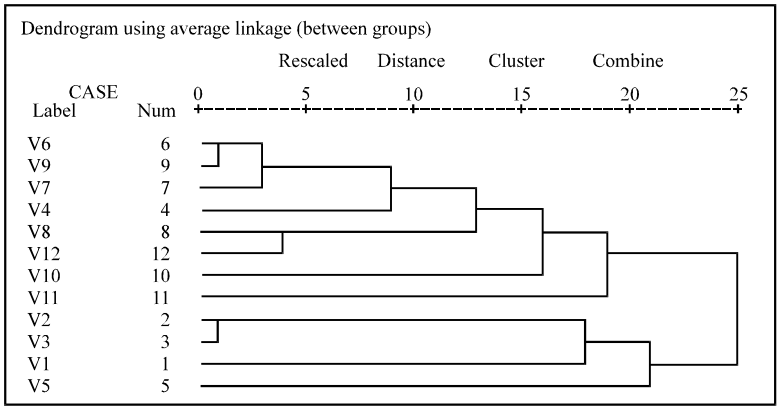

Fig. 3: Dendrogram showing relationship between 12 accessions of *M. charantia*

Table 5: Total number of bands and percentage of polymorphism of bands of 12 accessions of *M. charantia*

Primer	Primer sequence (5'-----3')	Total No. of bands	No. of polymorphic bands	No. of monomorphic bands	Polymorphism (%)
P1	GTAGCTGACG	5	4	1	80
P2	TGGCCAGTGA	6	3	3	50
P3	AGCCAGCGAA	7	2	5	28.57
P4	CAAACGTCGG	8	6	2	75
P5	GACCGCTTGT	6	6	0	100
P6	TGCGCCCTTC	5	1	4	20
P7	GTTTCGCTCC	5	1	4	20
P8	AGTCAGCCAC	5	3	2	60
P9	AATCGGGCTG	6	5	1	83.3
P10	AGGGGTCTTG	5	4	1	80
P11	CAGCACCCAC	7	5	2	71.42
P12	AGGTGACCGT	5	1	4	20
P13	CTTGCGATCC	2	1	1	50
P14	TGATCCCTGG	2	1	1	50
P15	GGACTGGAGT	5	3	2	60
P16	TGCTCTGCCC	6	4	2	66.66
Total		85	50	35	

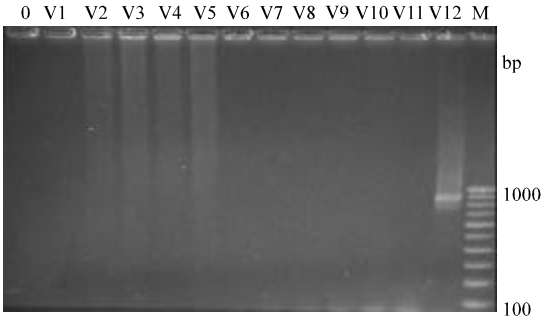

Fig. 4: PCR amplification of genomic DNA from accession 12 (V12) of *M. charantia* using designed SCAR primers-5'AAACGTCGGGTCG ACTGAT3' (forward primer) and 5'AAACGT CGGCCAATAGTAGC3' (reverse primer). 0-negative control (without template DNA), M-100 bp ladder

Development of SCAR marker: RAPD marker was converted into SCAR, whereby a unique polymorphic band (855 bp) was obtained in accession V12 (Fig. 1) after performing PCR with primer P4, among the 12 different accessions of *M. charantia*. The RAPD fragment was cloned in pUC (plasmid University of California) vector and sequenced in a sequencer. The sequence of 855bp has been given in Fig. 2. BLAST results revealed that the sequence did not have any homology with any known nucleotide sequence.

The first ten nucleotides of the sequence obtained matched completely with the corresponding RAPD primer P4. Sequence specific SCAR primers (Fig. 2) were designed using software-Web Primer. SCAR primer was tested using genomic DNA of twelve different accessions of *M. charantia* whereby, amplification by SCAR primers yielded single distinct band of 851 bp in accession V12 only (Fig. 4) but not in other accessions.

DISCUSSION

Genetic diversity existed between the two different varieties of *M. charantia*. When morphological characters such as vine length, number of fruits / vine, fruit length, fruit diameter, fruit weight, fruit yield per plant were considered, 12 different accessions of *M. charantia* could be clustered into 2 groups. Group I comprised of the accessions V1, V2, V3, V4, V5, V6 and V7 belonging to variety *muricata* and Group II comprised of the accessions V8, V9, V10, V11 and V12 belonging to variety *charantia.*

Morphological characters:

- V1, V2, V3, V4, V5, V6, V7 (*M. charantia* var. *muricata*) group 1
- V8, V9, V10, V11, V12 (*M. charantia* var. *charantia*) Group II

RAPD markers:

- V1, V2, V3, V5 (*M. charantia* var. *muricata*) CLUSTER I
- V8, V9, V10, V11, V12 (*M. charantia* var. *charantia*) CLUSTER II
- V4, V6, V7 (*M. charantia* var. *muricata)*

When RAPD markers were considered, there was high genetic variation among the accessions, with values of genetic diversity ranging from 0.562 to 0.881 using the Jaccard coefficient. Dendrogram constructed by cluster analysis with average linkage between groups produced two major clusters among 12 different accessions of *M. charantia.* Cluster I comprised of the accessions V1, V2, V3 and V5 belonging to variety *muricata*. Cluster II comprised of the accessions V8, V9, V10, V11 and V12 belonging to variety *charantia* along with accessions V4, V6, V7 belonging to variety *muricata.* Thus there was no significant correlation between the grouping obtained with RAPD markers and morphological characters.

Grouping obtained with RAPD markers did not match with the grouping obtained with morphological traits by (Youn and Chung, 1998) in pumpkin, (Chowdhury *et al*., 2001) in 47 soybean lines, (Ferriol *et al*., 2003) in strawberry and (Dey *et al*., 2006) in 38 bittergourd genotypes. According to (Dey *et al*., 2006), the main reason of mismatch between clustering based on RAPD and quantitative traits may be that most of the quantitative traits are controlled by a large number genes (polygenes) and these traits are highly influenced by the environment whereas the RAPD markers are not influenced by the environment.

Thus in the present study the clustering pattern based on RAPD markers was not in accordance with the grouping based on morphological characters an accessions V4, V6 and V7 probably were hybrids which arose as a result of cross between the two varieties.

In the present investigation RAPD analysis yielded significant genetic polymorphism among *M. charantia* accessions. Also the SCAR primers produced a single band of 851 bp only in accession V12, making the marker more specific and dependable compared to just a RAPD marker. The SCAR primer could thus be used for authentication of accession V12 and to distinguish it from other accessions.

SCAR markers have wide range of applications. It has been used for identification of varieties in olive (Hernandez *et al*., 2001), identification of species in *Phyllanthus* (Theerakulpiisut *et al*., 2008) and bamboo (Das *et al*., 2005), authentication of *Pueraia tuberosa* (Deviah and Venkatsubramanian, 2008) from it's commonly used substituents, adulterants and to screen *Hevea* seedling population for specific trait such as proline specific permease (Venkatachalam *et al*., 2006).

In the present study, SCAR marker has been developed to identify specific accession among morphologically slightly dissimilar accessions of *M. charantia*.

For the estimation of genetic diversity, different criteria, such as morphological, agronomic and physiological characters, pedigree records, molecular markers or a combination of criteria are used. Many workers such as Viana *et al*. (2010) in *Passiflora* species; Solouki *et al*. (2008) in *Matricaria chamomilla*; Tucak *et al*. (2008) in *Medicago* sp., Ogunbayo *et al*. (2007) in rice and Maric *et al*. (2004) in hexaploid wheat cultivars, have used both molecular (RAPD) and morphological characters to assess the genetic diversity amongst the different cultivars or accessions. All the five workers have found RAPD tool (molecular marker) to be more powerful tool in comparison to that of morphological marker in assessing the genetic diversity amongst the different germplasms.

In the present investigation although both genetic (RAPD and SCAR markers) and morphological characteristics reveals combinations of variation among *M. charantia* accessions but molecular markers (RAPD, SCAR) were found to be more effective as compared to morphological marker in assessing the genetic diversity. Thus RAPD could serve as an efficient tool for the selection of genetically diverse parents for inter and intravarietal crosses to produce hybrids.

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