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Genetic variability analysis within and between *Cucumis species* through RAPD markers

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ABSTRACT: *Genetic diversity in 30 of* Cucumis melo *and 10 each of* C. melo var. callosus *and* momordica *collected across Rajasthan and nearby area were evaluated through RAPD marker analysis. In RAPD analysis of* C. melo, *the highest discriminatory power (D) of 0.90 was shown by OPB 17 and in* C. melo *var.* callosus *and* momordica- *primer OPD 7 recorded the highest D value of 0.94. The dendrogram of* C. melo *depicted two separate groups, Group A with five accessions (KAZARI, Jhunjanu ,Madhuras ,K-22 and Khetri) and the 25 accessions into four sub groups. The two groups (A and B) separate from each other at 38 similarity showing 62 per cent diversity. An accession each of* C. melo *var.* momordica *of Bikaner and* C. melo *var.* callosus *of Balotra stood at 50 per cent diversity level.* C. melo *var.* callosus *showed marginally better diversity as compared to* C. melo var. momordica. *The similarity in annealing temperature of each of the seven SSR markers for any two of the three groups (*C. melo, C. melo var. momordica and C. melo var. callosus*) indicated a common genetic pool among these accessions.*

Key words*: Genetic diversity; RAPD, Molecular marker analysis*

INTRODUCTION

Melon (*Cucumis melo* L.; 2n=2x=24, family Cucurbitaeae), is a morphologically diverse outcrossing horticultural crop [1]. So many species of melon that has been developed into many cultivated varieties which include Snap melon (*C. melo* var. *momordica)* and Kachari (*C. melo* var. *callosus*). These large numbers of cultivars are the most important cucurbits of the hot arid region, representing the Thar Desert of Rajasthan, which is considered to be the secondary centre of origin of *C. melo*, which is native to Iran and adjacent areas on the west and east [2]. These are wildly grown in marginal and barren lands with minimal inputs. They are semi-domesticated crops grown as intercrops during summer and Kharif seasons. The edible fruits have long storage quality, good nutritive and medicinal value.

Cucumis melo includes a wide range of cultivars. Although crosses outside the species are sterile, intra-specific crosses are generally fertile, resulting in a confusing range of variation (Purseglove, 1968) [5]. It exhibits a wide range of morphological variation including fruits from a few grams to several kilograms, oblong to very elongated shape or high to low flesh and sugar content [8, 10].The diverse morphological variation within the species and its adaptation to the very harsh agro-ecological conditions of the region emphasizes that it harbor genes which are tolerant to drought, salinity, high temperature, solar irradiance etc [12].

In contrast to morphological markers, a variety of molecular markers based on protein, DNA, RNA etc. generate a characteristic banding pattern at molecular level. Apart from identification, they can also be used for testing parentage, genetic mapping and measurement of genetic diversity. Recent developments in DNA technologies have made it possible to uncover a large number of genetic polymorphism at the DNA sequence level and to use them as markers for evaluation and improvement of the genetic basis [11]. Molecular markers have been widely used in genetic analysis and breeding of plant species, with a multitude of applications. Among the various types of molecular markers available, microsatellites have received greater attention, especially for breeding purpose.

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Marker-assisted selection (MAS) or identification can be used to pyramid the major genes including resistance genes, with the ultimate goal of producing varieties with more desirable characters. Thus, with MAS it is now possible for the breeder to conduct many rounds of selection in a year. Molecular marker technology is now integrated into existing plant breeding programmes all over the world. Thus, the present investigation aimed at determining the diversity available at molecular level, among different cultivars of *Cucumis melo* of Rajasthan envisages the following objectives: To assess the genetic variability within and between *Cucumis species* through RAPD markers and analysis the hyper variable with discriminatory power in RAPD.

MATERIALS AND METHODS

Experimental site and layout

The present investigation was carried out at the Department of Biotechnology, College of Agriculture, Bikaner, located at Plant Biotechnology Centre, Swami Keshwanand Rajasthan Agricultural University, Bikaner.

Table 1
Location/variety specific details of *Cucumis species* accessions

Muskmelon (Cucumis melo L.)	*Kakari (Cucumis melo var. momordica)*	*Kachari (Cucumis melo var. callosus)*
Chandrapuri	AHK-82	AHK-199
Durgapura madhu	Athaiysan (Nagaur)	AHK-82
Durgapura Selection	Balotra (Barmer)	ARK-119
G-2	Barmer	Balotra (Barmer)
GP-1	Bikaner	Mundwa (Nagaur)
GP-115	Didwana (Nagaur)	Nawa (Nagaur)
GP-120	Jagatpura (Ajmer)	Pisangan (Ajmer)
GP-128	Kolyat (Bikaner)	Reechmalia (Ajmer)
GP-15	Nasirabad (Ajmer)	Sarvadi (Barmer)
GP-176	Sarvadi (Barmer)	Shergarh (Ajmer)
GP-182		
GP-187		
GP-199		
GP-20		
GP-7		
GP-81		
GP-9		
Husangabadi		
Jhunjhunu		
K-22		
KAZARI		
Khetri (Jhunjhunu)		
Kota		
Madhu		
Madhuras		
RM-43		
RM-50		
Sirsa		
Sunheri		

Plant material

The experimental material for the present investigation consisted 50 accessions of *Cucumis* species including 30 accession of muskmelon (*Cucumis melo* L.), and 10 each of kakari (*Cucumis melo var. momordica*) and kachari (*Cucumismelo var. callosus*) representing released varieties as well as land races collected from various districts of Rajasthan and ARS Durgapura.

High molecular weight genomic DNA was extracted from homogenized sample following the method described by by Doyle and Doyle [2]. The quantification of DNA was done by recording its absorbance at 260 nm and 280 nm using a UV- VIS Spectrophotometer (UNICAM) and gel electrophoresis. The quantified DNA was diluted to a final concentration of 25 ng μl^{-1} in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) for RAPD amplification and stored at –20°C. Random amplification of polymorphic DNA was done by using 6 primers of OPC, OPB and OPD series obtained from OPERON TECHNOLOGIES (Inc. Alameda, California) were used for PCR reactions. The PCR was performed in 'Biometra Thermocycler' using a program consisting of an initial denaturation step of 5 min at 94°C followed by 44 cycles of 1 min at 94°C, 1 min at 37°C and 2 min at 72°C; the program ended with a 7-min elongation step at 72°C. Following the amplification, the PCR products were electrophoresed for 2.5-3.5 hrs at 100 V with cooling. The gel was viewed under UV trans-illuminator and photographed.

The presence of each band was scored as '1' and its absence as '0'. Cluster analysis for the genetic distance was carried out using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method and constructed a dendrogram NTSYS pc version 2.02 (Rohlf, 1998) [7]. To compare the efficiency of the primers in accession differentiation, the discriminatory power (D) of each primer was calculated. A single numerical index of discrimination (D) based on the probability that two unrelated accessions from the primers tested will be placed into different groups and was calculated based on Simpson's index of diversity [9] as described by Hunter and Gaston [3]. The discriminatory power of each primer was calculated through on-line calculator available on the web, (*http://www.insilico.ehu.es/mini_tool/ discriminatory_power*).

RESULTS AND DISCUSSION

Out of the 10 primers used for RAPD screening six efficiently produced amplification, 4 did not amplify

any fragment in three genotypes, one each from three species taken for screening**.** In *C. melo* the number of polymorphic bands ranged from 2 to 6 with a range of polymorphisms *viz*., 42 per cent (OPD 18), 50 per cent (OPB 8), 55 per cent (OPB 17), 75 per cent (OPB5,OPC 7)and 100 per cent (OPD 13). The size of the amplicons generated varied from 200 bp (OPB 17) to 2500 bp (OPD 18). The total number of bands generated by 6 primers was 38 with an average of 6.3 bands per primer. The total number of polymorphic bands generated was 25 with an average polymorphic amplification of 4.2 bands per primer. The average polymorphism generated by these bands was 66.1 per cent. The average discriminatory power of all the primers was 0.66 per cent.

In *Cucumis melo* var. *callosus* and *momordica* species the number of polymorphic bands ranged from 1 to 7 with a range of polymorphisms *viz*., 50 per cent (OPD 13), 66 per cent (OPD 18), 75 per cent (OPB 8), 87 per cent (OPC7)and 100 per cent (OPB 5 and OPB 17). The size of the amplicons generated varied from 200 bp (OPB 17) to 2000 bp (OPC 7). The total number of bands generated by 6 primers was 26 with an average of 4.3 bands per primer. The total number of polymorphic bands generated was 22 with an average polymorphic amplification of 3.6 bands per primer. The average polymorphism generated by these bands was 79.6 per cent. The average discriminatory power of all the primers was 0.62 per cent.

It can be observed from the Table(s) 2 and 3 that all the 6 amplifying arbitrary primers produced polymorphic bands. The ability of a primer to distinguish between unrelated strains can be determined by the number of types (pattern types) defined by the primer and the relative frequencies of their types. The value of D is one when all the patterns are unique and 0 when patterns generated for all the varieties contain monomorphic bands. The value of D for *Cucumis melo* ranged from 0.19 (OPB 5) to 0.90 (OPB 17) and for (*Cucumis melo* var. *callosus* and *momordica)* ranged from 0.26 (OPD 13) to 0.94 (OPD 7) for single primer based RAPD patterns.

Genetic relationship among the accessions and cluster analysis

Genetic similarity estimates based on RAPD banding patterns were calculated using method of Jaccard's coefficient analysis for *C. melo* and for *C. melo var. callosus* and *C. melo* var. momordica. The similarity coefficient matrix generated was subjected to algorithm "Unweighted Pair Group Method for Arithmetic Average (UPGMA)" to generate clusters using NTSYS 2.02 pc program (Rohlf, 1998) [7,8]. The dendrogram showing relationship among various genotypes was constructed separately for *C melo* and for *C melo* var momordica and *C melo var callosus* (collective).

The dendrogram based on UPGMA clustering analysis depicted two separate groups of *Cucumis*

Table 2
List of primers showing total and polymorphic amplicons generated along with discriminatory power of each primer for 30 accessions of *Cucumis melo L.*

Primer	*Sequence (5''→3')*	*No. bands*	*No. of Polymorphic bands*	*Polymor-phism(%)*	*Discriminatory Power (D)*
opb-5	tgcgcccttc	4	3	75	0.19
OPB-8	GTCCACACGG	4	2	50	0.52
OPB-17	agggaacgag	9	5	55	0.90
opc-7	gaacggactc	8	6	75	0.81
OPd-13	ggggtgacga	6	6	100	0.88
OPd-18	gagagccaac	7	3	42	0.69
		38(TOTAL)	25(TOTAL)	66.1(Average)	0.66(Average)

Table 3
List of primers showing total and polymorphic amplicons generated along with discriminatory power of each primer for 20 accessions of *Cucumis melo* var.*callosus and momordica.*

Primer	*Sequence (5''→3')*	*No. bands*	*No. of Polymorphic bands*	*Polymor-phism(%)*	*Discriminatory Power (D)*
opb-5	tgcgcccttc	2	2	100	0.47
OPB-8	GTCCACACGG	4	3	75	0.43
OPB-17	agggaacgag	7	7	100	0.91
opc-7	gaacggactc	8	7	87	0.94
OPd-13	ggggtgacga	2	1	50	0.26
OPd-18	gagagccaac	3	2	66	0.68
		26 (TOTAL)	22(TOTAL)	79.6(Average)	0.61(Average)

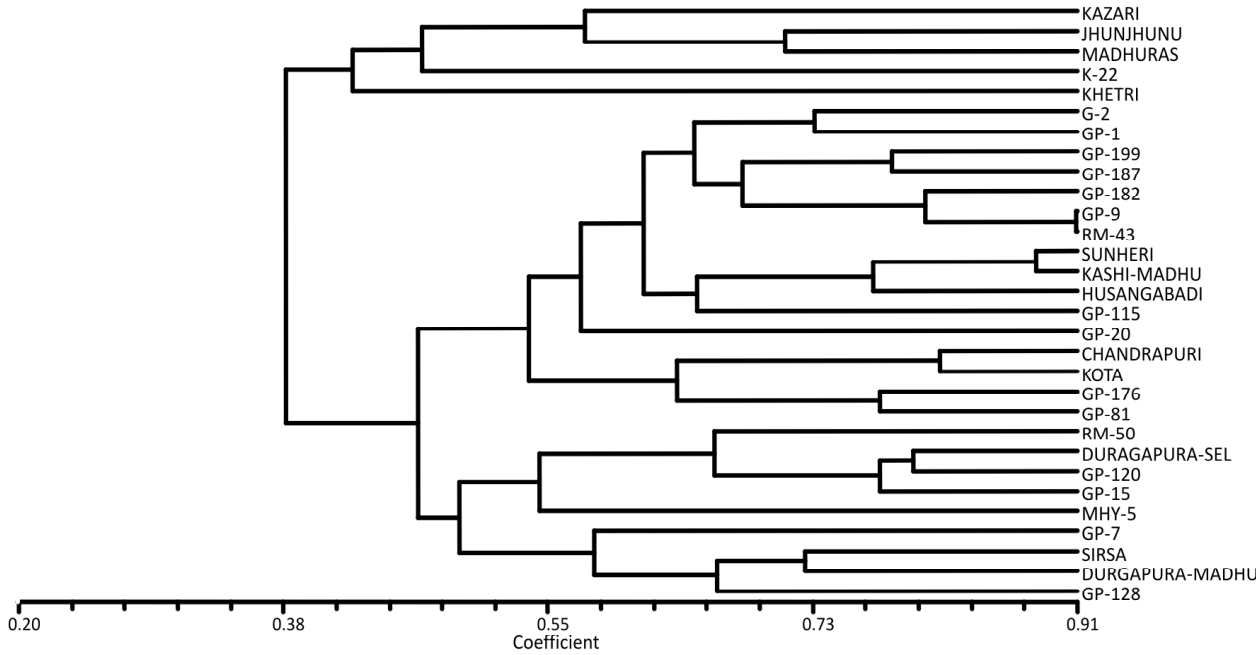

Figure 1: Dendrogram generated for thirty accessions of *Cucumis melo* L. using UPGMA cluster based on Jaccard's similarity coefficient for RAPD data

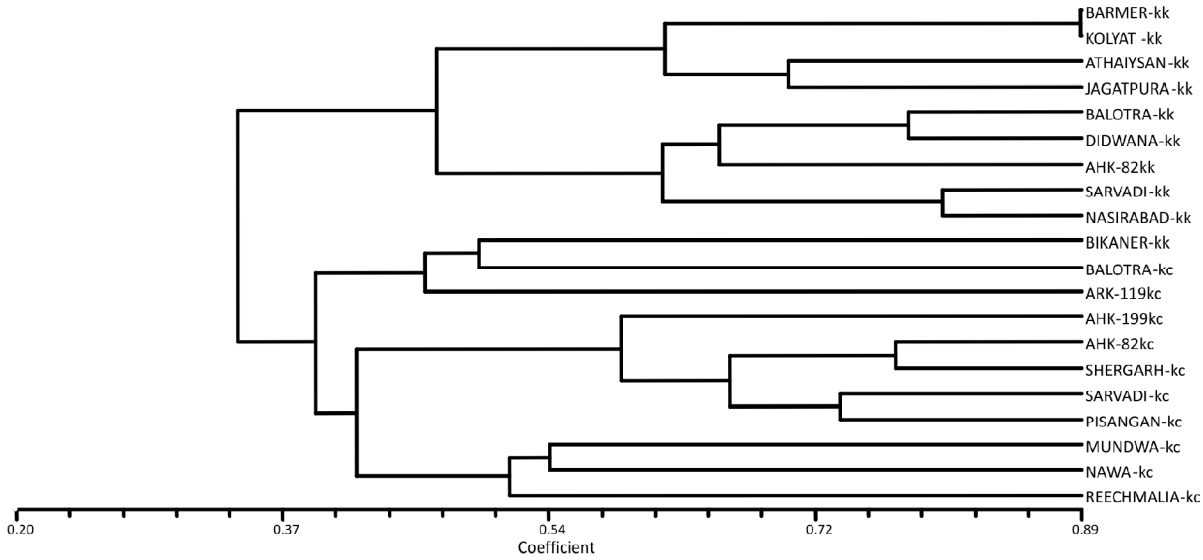

Figure 2: Dendrogram generated for twenty accessions of *Cucumis melo* var. *callosus* and *momordica* using UPGMA cluster based on Jaccard's similarity coefficient for RAPD data

melo. Group A consisted five accession mostly belong to Rajasthan *viz*. KAZARI, Jhunjanu, Madhuras ,K-22 and Khetri. The remaining 25 accessions can be further sub divided into four sub groups B1, B2, B3, B4. The two groups (A and B) separate from each other at 38 similarity showing 62 per cent diversity.

The clustering separated the *Cucumis melo* var. *callosus* and *momordica* group, as expected. However, one accession each of *Cucumis melo* var. *momordica* belonging to Bikaner and *Cucumis melo* var. *callosus* groups Balotra belong to same group at a higher diversity level (50 per cent). Thus these two accessions represent a 50:50 situation as in the case of a coin toss in both diversity as well as similarity. The accessions of *Cucumis melo* var. *callosus* and *momordica* groups showed high inter group diversity and joined each other at the level about 33 per cent similarity. Comparatively, *Cucumis melo* var. *callosus* showed

marginally better diversity among their accessions as compared to those of *C. melo var. momordica.* .

Markers which are used to study the polymorphism of DNA structures and sequences have become popular in order to compensate for the disadvantages of morphological markers. However, even isozyme marker analysis may be affected by environment and post translational modifications [4]. RAPD (DNA-Fingerprinting) analysis was used to assess the genetic relationship among the 50 accessions of three melon types- muskmelon (*Cucumis melo* L.), *kachari* and *kakari* (*Cucumis melo* var. *callosus* and *momordica,)* respectively. All the 50 accessions of melon collected from various districts of Rajasthan exhibited great diversity which may further be used for genetic improvement by associating the morphological characters, resistance to biotic and abiotic stresses fruit quality etc. with molecular markers for marker assisted selection in breeding programmes.

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