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Short Communication

Assessment of genetic diversity in *Cucumis melo* through RAPD analysis

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Abstract

Genetic diversity analysis of sixteen accessions of two *Cucumis melo* groups var. *callosus* (*kachari*) and *momordica* (*kakari*) collected from four districts *viz*., Ajmer, Bikaner, Churu and Nagaur of Rajasthan was carried out using RAPD primers. A total of 102 amplified fragments were generated, out of which 99 were polymorphic. Primers OPG17 could distinguish all the accessions recording the highest discriminatory power (1.00) followed by OPB 6 (0.991). The Jaccard's similarity coefficient recorded an average similarity of 0.40 indicating 60% diversity, ranging from 18 to 67% among all the 16 accessions. The SAHN clustering based on UPGMA algorithms divided the accessions into three groups, the I consisting nine (eight of *kakari* and one *kachari* (Nagaur 1k) accessions, the II included five (all *kachari* one Nagaur 2k and four from Ajmer and Bikaner) and the III had two *kachari* accessions of Churu. *Kachari* recorded 20% more genetic diversity as compared to *kakari*. Churu *Kachari* recorded maximum diversity (61 to 82%). The study indicated that the landraces possess wide genetic diversity, which can be used for improvement.

Key words: Genetic diversity; RAPD, molecular marker analysis

Melon (*Cucumis melo* L.; 2n = 2x = 24, family Cucurbitaceae) is a morphologically diverse, outcrossing horticultural crop of broad economic importance [1]. Although the origin of melon is in dispute, most authorities consider that it originated in Africa [2]. *C. melo* varies widely in plant habit, vine, leaf, and fruit characters. *Cucumis melo* var. *callosus* (*kachari*) and *C. melo* var. *momordica* (*kakari*) are the underutilized hot arid region plant species having high tolerance to drought, salinity and various insect pests. The genetic diversity of several commercially important melon groups, Cantalupensis and Inodorus has been characterized using molecular analyses [4, 5, 7].

Among the various molecular markers available for estimation of genetic diversity, RAPD is a multi locus marker, low cost and high reproducibility and possess the simplest and rapid technology. RAPD analysis has the utility and extensive applicability due to their assessment of genetic diversity, genetic relationship, identification of cultivar etc. Intra-specific classification of such variability has been quite difficult and confusing. Hence, the present study was undertaken to study the existing genetic diversity in these two groups through RAPD analysis. Most taxonomists rely on Naudin's work from 1859 [12-14]. In this study we analyzed a small set of two melon landraces distributed in Indian dessert and adjoining belts.

The experimental plant material consisted eight *kachari* and eight *kakari* accessions collected from four (two from each) districts of Rajasthan, India. The *kakari* accessions, were labeled as Ajm 1 and Ajm 2 (from Ajmer), Bkn 1 and Bkn 2 (from Bikaner), Chu1

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and Chu 2 (from Churu) and Nag 1 and Nag 2 (from Nagaur) and *kachari* accessions as Ajm 1k and Ajm 2k, Bkn 1k and Bkn 2k, Chu 1k and Chu 2k and Nag 1k and Nag 2k. A standard protocol was followed for DNA isolation. High molecular weight genomic DNA was extracted from homogenized sample following the method described by Doyle and Doyle [15]. DNA concentration was measured with a NanoDrop ND 100 spectrophotometer (NanoDrop Technologies, Inc.) and gel electrophoresis. DNA was diluted in water to a final concentration of 50 ng/îL and stored at –20°C. A total of 32 primers of OPA, OPB, OPC and OPG 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 42 cycles of 1 min at 94°C, 1 min at 37°C and 1 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 [16]. 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 [17] as described by Hunter and Gaston [18]. 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).

Out of 32 primers, only 15 produced amplicons. The total number of bands generated was 102 with an average of 6.8 bands per primer. The total number of polymorphic bands generated was 99 with an average polymorphic amplification of 6.6 bands per primer (Fig. 2) which is very higher than 1.5 bands per primer as reported in melon landraces by Yi *et al*. [20]. The average polymorphism generated by these bands was 97 per cent which is very similar to polymorphism obtained by SSR markers as reported by Kacar et al. [21]. The average discriminatory power of all the

Table 1. List of primers showing total and polymorphic amplicons generated along with discriminatory power of each primer for 16 (eight each) accessions of *Cucumis melo* var. *callosus and momordica*

Primer	Sequence (5' → 3')	Total no.of bands	No. of polymorphic bands	Polymorphisms (%)	Banding pattern type	Discriminatory power	PIC values
OPA-2	TGCCGAGCTG	5	5	100	8	0.841	0.328
OPA-4	AATCGGGCTG	4	4	100	8	0.833	0.325
OPA-16	AGCCAGCGAA	7	7	100	13	0.983	0.335
OPB-6	TGCTCTGCCC	7	6	85	15	0.991	0.340
OPB-8	GTCCACACGG	8	7	87	9	0.908	0.265
OPB-9	TGGGGGACTC	5	5	100	8	0.875	0.401
OPB-14	TCCGCTCTGG	4	4	100	6	0.775	0.332
OPB17	AGGGAACGAG	7	7	100	10	0.966	0.324
OPB-19	ACCCCCGAAG	8	8	100	7	0.941	0.410
OPC-2	GTGAGGCGTC	9	9	100	12	0.691	0.264
OPC-4	CCGCATCTAC	8	8	100	12	0.958	0.418
OPC-15	GACGGATCAG	8	8	100	13	0.975	0.332
OPC-16	CACACTCCAG	6	5	83	7	0.85	0.297
OPG-6	CTGAGACGGA	2	2	100	7	0.90	0.433
OPG-17	ACGACCGACA	14	14	100	16	1.00	0.249
Total/average		102	99	97	10.06	0.89	0.337

primers was 89.99 per cent. Out of the 15 amplifying arbitrary primers, one primer (OPG 17) produced unique patterns that can be used to categorize all the accessions. The other primers could distinguish all the accessions collectively and hence a combination of two or more primers can be selected to distinguish the accessions. The discriminatory power of the primers tested, ranged from 0.691 (OPC 2) to 1.00 (OPG 17) with an average of 0.89. Both the primers, which showed the minimum (OPC 2) and the maximum (OPG 17) discriminatory power had 100 per cent polymorphism level. The primer OPB 6 recorded 15 different types and stood the second best. *C. melo* is thought to contain the most diverse varieties in the genus *Cucumis* [2]. According to the previous works, genetic diversity of *C. melo* seems to be much bigger when comparing with *C. sativus* [22]. Elucidating the phylogenetic relationships in the genus *Cucumis* is of great importance, because the closest relatives and natural composition can provide valuable information to improve melon and cucumber breeding [23].

Inter and intra group genetic diversity of *Cucumis melo* var. *callosus* and *momoridica* estimates were calculated using method of Jaccard's coefficient analysis (Table 2). The Jaccard's pair wise similarity coefficient values ranged from 18% (between Chu 1k and Ajm 2) to 67% (between Chu 1 and Bik 1 & Bik 2) with an average of 40%. Thus, a great deal of diversity with an average of 60% was observed considering all the accessions studied. Among the kakari accessions, the average similarity was 56% (44% diversity) and the same for *kachari* accessions was 36 per cent (64% diversity). This shows that there is more diversity in *kachari* accessions as compared to kakari accessions. The intra-group average similarity recorded was 34% (66% diversity). Thus, there is more intra group diversity (6%) than the average diversity of all the accessions, which shows that *kachari* and *kakari* belong to different groups. The dendrogram showing relationship among the 16 accessions was constructed using these clusters (Fig. 1). The SAHN clustering based on UPGMA algorithms has grouped these 16 accessions into three groups. The first with nine accessions (eight of which are *kakari* and one *kachari* of Nagaur 1k), the second with five accessions (all *kachari*) with Nag 2k, Bik 1k and 2k, Ajm 1k and 2k and the third group with both the accessions of *kachari* of Churu. The fact that in the first group, the only *kachari* of Nagaur 1k is nearer to *kakari* of Nagaur 1, it can be surmised that the *kakari* and *kachari* from Nagaur are compatible with each other. The regional effect on genetic similarity was also evident from comparative similarity coefficient for within the district accessions and between the district accessions. Least similarity index (18%) was observed for Chu 1k (*Kachari*) collection, where as Chu 1, Bik 1 and Bik 2 (*Kakari*) collection showed highest similarity (67%). This shows that as far as *kakari* is concerned Nagaur has more diversity (44%), followed by Churu (40%), Ajmer (36 %) and Bikaner (35%). As far as kachari is concerned Churu has more diversity (67%), followed by Nagaur (65%), Bikaner (52%) and Ajmer (48%). Marked effect on regional influence on intra group diversity was also observed. Churu recorded maximum diversity (72%) followed by Bikaner and Nagaur (62% each) and the least intra group diversity was found in Ajmer (59%). *Cucumis melo* is a morphologically diverse species composed of tropical and subtropical wild and weedy populations as well as an abundance of domesticated types. Over the years, various germplasm evaluators and breeders have attempted to assess these collections to discard identical accessions. Markers aided by the polymorphisms in proteins and DNA structures 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 [24].

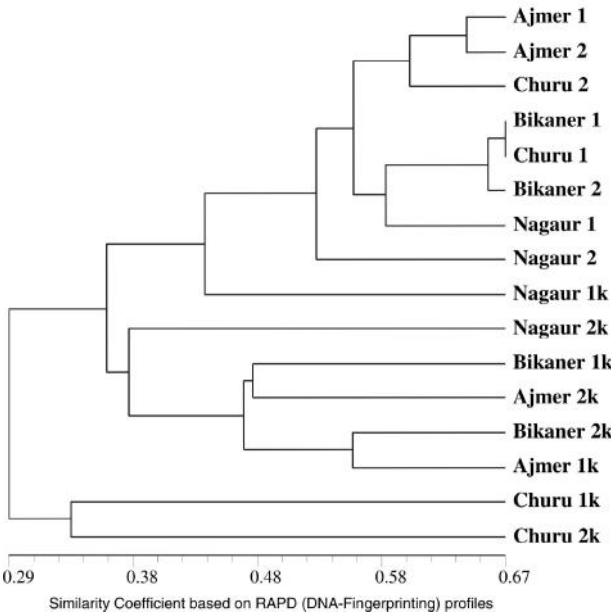

Fig. 1. Dendrogram generated for 16 accessions of *Cucumis melo* var. *callosus* and *momordica* using UPGMA cluster based on Jaccard's similarity coefficient for RAPD (DNA-Fingerprinting) data

Table 2. Jacard's similarity coefficients among 16 accessions (eight each) of *Cucumis melo* var. *callosus* and *momoridica*

Accessions	Ajm 1	Ajm 2	Bkn 1	Bkn 2	Chu 1	Chu 2	Nag 1	Nag 2	Nag 1k	Nag 2k	Chu 1k	Chu 2k	Bkn 1k	Bkn 2k	Ajm 1k	Ajm 2k
Ajm 1	1.00															
Ajm 2	0.64	1.00														
Bkn 1	0.55	0.59	1.00													
Bkn 2	0.48	0.59	0.65	1.00												
Chu 1	0.52	0.61	0.67	0.67	1.00											
Chu 2	0.60	0.59	0.53	0.57	0.60	1.00										
Nag 1	0.50	0.58	0.60	0.53	0.60	0.51	1.00									
Nag 2	0.50	0.53	0.50	0.52	0.55	0.51	0.56	1.00								
Nag 1k	0.45	0.42	0.43	0.43	0.45	0.44	0.44	0.45	1.00							
Nag 2k	0.32	0.29	0.34	0.32	0.30	0.30	0.33	0.30	0.35	1.00						
Chu 1k	0.19	0.18	0.28	0.28	0.22	0.26	0.24	0.21	0.20	0.34	1.00					
Chu 2k	0.25	0.29	0.34	0.36	0.34	0.31	0.30	0.32	0.22	0.28	0.33	1.00				
Bkn 1k	0.32	0.26	0.33	0.31	0.28	0.30	0.33	0.28	0.33	0.36	0.32	0.35	1.00			
Bkn 2k	0.40	0.38	0.45	0.43	0.40	0.39	0.37	0.39	0.36	0.43	0.33	0.39	0.48	1.00		
Ajm 1k	0.47	0.48	0.54	0.44	0.44	0.41	0.47	0.46	0.38	0.39	0.28	0.33	0.47	0.55	1.00	
Ajm 2k	0.33	0.34	0.33	0.35	0.29	0.37	0.38	0.29	0.31	0.33	0.27	0.30	0.48	0.41	0.52	1.00

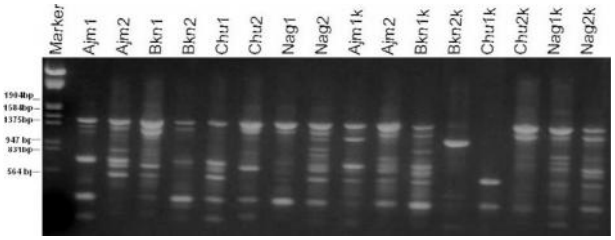

Fig. 2. RAPD profile generated by primer OPC-4 in 16 genotypes of *Kachri* and *Kakari* (*Cucumis melo* var. *callosus* and *momordica*) with marker (Lamda DNA Eco RI/Hind III double digest)

RAPD (DNA-Fingerprinting) analysis was used to assess the genetic relationship among the 16 accessions of two melon types- *kachari* and *kakari* (*Cucumis melo* var. *callosus* and *momordica,* respectively.

All the 16 accessions of melon collected from four different 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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