

Original Research Article

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RAPD Molecular Marker Based Genetic Diversity among Oriental Pickling Melon (*Cucumis melo* var. *conomon*) Genotypes in Karnataka, India

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ABSTRACT

Keywords

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Oriental pickling melon (*Cucumis melo* var. *conomon*) is one of the melon group vegetables of the Cucurbitaceae, with high potential as commercial short duration vegetable in South India. Twenty five RAPD primers were used which generated a total of 182 bands, out of which 107 bands were polymorphic (58%) revealing a substantial amount of genetic diversity within this species. Similarity values among all the accessions ranged from 0.948 to 0.619. According to the dendrogram formed, all the 15 pickling melon accessions were divided into three major clusters and four solitary clusters. Cluster 1 consisted of 2 genotypes, cluster 2 had four genotypes and cluster 3 had five genotypes. The genotype CMC GKVK12 appeared to be most diverse and genetically most similar genotypes were CMC GKVK14 and 15. The RAPD profile identified CMC GKVK12, CMC GKVK4, CMC GKVK11, and CMC GKVK13 genotypes as the most promising and also established their inter- relationship, which could be of great significance to choose them as parents in the future breeding programme.

Introduction

Oriental pickling melon (*Cucumis melo* var. *conomon*) is one among the melon group vegetables belongs to Cucurbitaceae family with a chromosomal number $2n=24$. It is also called as golden melon or culinary melon in English. Fruits are varying in size, small to medium and big fruits with smooth tender skin, white flesh usually with little sweetness and odour. The fruits which contain moderate amount of vitamins and minerals are used in the preparations of an array of traditional vegetarian dishes like chutney, curry, sambar and pickles. The fruits possess cooling properties

and are used as a skin moisturizer and as a digestive agent (George, 2008).

In Karnataka, the crop is cultivated under limited area using mostly local varieties which are low in productivity. There is a need for collection of local genotypes and evaluate them for their adoptability to particular locations especially to their nontraditional areas. A wide range of genetic diversity exists in Karnataka with respect to fruit morphology such as size, shape, fruit flesh thickness and exocarp colour. Molecular markers provide a

quick and reliable method for estimating genetic diversity among genotypes (Thormann *et al.*, 1994). In order to develop a reliable tool, efforts were therefore made to develop DNA finger print profile of cultivars to establish a relationship and also to understand the genetic distance which can be used for future breeding programme. The DNA finger print profile would supplement the existing DUS characterization till the molecular characterization is widely adopted.

Materials and Methods

Fifteen morphologically and geographically distinct pickling melon accessions (Table 1) were collected from different parts of South Karnataka and then grown in Summer, 2009 in the experimental plots of Vegetable Seed Production Unit (VSPU), Department of Horticulture, University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK), Bangalore. The genomic DNA was isolated from green and healthy leaves of twenty days old seedlings by cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990) with minor modifications. The extracted stock DNA was quantified on 0.8 per cent agarose using standard lambda uncut DNA and the DNA samples were diluted to 25 ng / μ l prior to polymerase chain reaction (PCR) amplification.

PCR DNA amplification was performed by using fifteen Operon decamer primers with 50-70 per cent GC content (Operon Technologies, USA) and ten Sigma decamer primers (Sigma Aldrich, USA). The polymerase chain reactions was performed in a 10 μ l mixture containing 25ng/ μ l DNA, 10 μ M / μ l Primer, 10X Taq assay Buffer, 1mM dNTPs, 1U Taq Polymerase and 25mM MgCl₂. Amplifications were performed in a thermal cycler (MJ Research Inc., USA). The standardized PCR include: initial denaturation

at 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds; primer annealing 38°C for 1 minute; primer extension at 72°C for 1 minute and a final primer extension at 72°C for 10 minutes. The amplicons were assessed by loading on to 1.5% Agarose along with bromophenol blue as loading dye. Amplicons were separated in an electrophoresis unit at 100 V for three hours using 1X TBE buffer and stained with ethidium bromide (50 mg/ 100ml agarose) (PALTE 1). The amplification products were scored across the lanes. The score '1' was assigned for the presence of band and '0' for absence of band at each loci. The variation in band intensity is not taken into consideration to avoid confusion in scoring. The binary data was used to estimate pair wise genetic distance based on Jaccard's similarity coefficients and a dendrogram was generated based on the unweighed pair group method for arithmetic mean (UPGMA) using software NTSYS-pc version 2.0.

Results and Discussion

In the RAPD marker analysis, a total of 182 bands were generated in fifteen accessions of pickling melon based on 25 RAPD primers. Of these 107 bands were polymorphic (58%). On an average 25 primers could amplify 58.79 per cent polymorphic loci (Table 2). Polymorphism within the pickling melon germplasm was found to be high, which reveals a substantial amount of genetic diversity within the species.

The range of amplified bands was between 4 and 12 with an average number of 7.28 bands per primer. OPD- 09 primer gave maximum number (12) of amplified bands, while lowest number (4) was generated by OPA-01, OPB-10 and OPC-05 primers. Average number of polymorphic bands per primer was 4.28, while highest polymorphism (100%) was revealed by the OPA-01, OPA-11, OPA-12

and OPD-05 primers. Contrasting to these, Primer-20 exhibited no polymorphism at all. The number of polymorphic loci per primer in the present study is comparable to that reported by Feyzian *et al.*, (2007) in Iranian melon and by Garcia *et al.*, (1998) in melon.

Genetic distance was measured using Jaccard's similarity coefficient and the values ranged from 0.948 to 0.619 (Table 3). Maximum similarity (0.948) was recorded between the genotypes CMC GKVK14 and CMC GKVK15. Both the genotypes were collected from same localities. However, minimum genetic similarity was found

between the genotypes CMC GKVK10 and CMC GKVK13 (0.619). Luan *et al.*, (2008) have reported an average dissimilarity of 0.53 ± 0.14 between any two pairs of accessions based on RAPD assay in Chinese melon, while (Matsui *et al.*, 2002) reported an average dissimilarity of 0.240 in oriental pickling melon.

In the UPGMA dendrogram (Fig. 1) all the 15 pickling melon accessions divided into three major clusters and four solitary clusters. Genotypes such as CMC GKVK4, CMC GKVK11, CMC GKVK12 and CMC GKVK13 fell into solitary clusters.

Table.1 Pickling melon working collections

S.No.	Accession Number	Source	Fruit characters
1.	CMC GKVK-1	UAS, GKVK, Bangalore	Fruit rind
2.	CMC GKVK-2	UAS, GKVK, Bangalore	Greenish yellow streaked
3.	CMC GKVK-3	UAS, GKVK, Bangalore	Greenish yellow striped
4.	CMC GKVK-4	UAS, GKVK, Bangalore	Greenish yellow striped
5.	CMC GKVK-5	Shiriyara	Greenish yellow striped
6.	CMC GKVK-6	Satkatu	Greenish yellow striped
7.	CMC GKVK-7	Satkatu	Greenish yellow striped
8.	CMC GKVK-8	Kollebylu	Greenish brown speckled
9.	CMC GKVK-9	Parampalli, Salegrama, Kundapura Taluk	Greenish yellow striped
10.	CMC GKVK-10	Udli, Barkuru, Brahmavara, Udupi (District)	Greenish brown speckled
11.	CMC GKVK-11	Barkuru, Brahmavara, Udupi (District)	Greenish yellow streaked
12.	CMC GKVK-12	Hanehalli, Barkuru, Brahmavara, Udupi (District)	Orange streaked
13.	CMC GKVK-13	Hanehalli, Barkuru, Brahmavara, Udupi (District)	Greenish yellow speckled
14.	CMC GKVK-14	Keraviske village, Karkala, Udupi (District)	Greenish yellow streaked
15.	CMC GKVK-15	Mannur Udupi (District)	Greenish brown speckled

Table.2 Polymorphism in pickling melon genotypes as influenced by RAPD primers

Sl. No	RAPD Primer	Sequence	Number of bands		Polymorphism %
			Total	Polymorphic	
1	OPF-08	GGG ATA TCG G	9	6	66.67
2	OPD-09	CTC TGG AGA C	12	8	66.67
3*	OPA-11	CAA TCG CCG T	6	6	100.00
4*	OPA-12	TCG GCG ATA G	5	5	100.00
5	OPC-06	GAA CGG ACT C	7	6	85.71
6*	OPD-05	TGA GCG GAC A	6	6	100.00
7*	OPA-01	CAG GCC CTT C	4	4	100.00
8	OPB-08	GTC CAC ACG G	5	4	80.00
9	OPB-10	CTG CTG TTA C	4	3	75.00
10	OPE-03	CCA GAT GCA C	10	8	80.00
11	OPE-06	AAG ACC CCT C	7	6	85.70
12	OPB-04	GGA CTG CTC C	8	7	85.70
13	OPE-11	GAG TCT CAG G	6	5	83.33
14	OPA-02	TGC CGA GCT G	7	4	57.14
15	OPC-05	GAT GAC CGC C	4	2	50.00
16	Primer-10	TAG CCC GCT T	9	7	77.78
17	Primer-4	GGG CAA TGA T	9	3	33.33
18	Primer-25	GCG GTT GAG G	11	4	36.36
19	Primer-18	CCC GTT TTG T	8	5	62.50
20	Primer-19	ATT GGG CGA T	8	2	25.00
21	Primer-20	GAA TTT CCC C	8	0	0
22	Primer-33	GGA AAC CTC T	9	1	11.11
23	Primer-7	CTG TCC CTT T	6	2	33.33
24	Primer-36	TAC GTC TTG C	8	2	25.00
25	Primer-5	TGC GCC CTT C	6	1	16.67
Total			182	107	
Average			7.28	4.28	58.79

Table.3 Similarity matrix of 15 pickling melon genotypes according to Jaccard's coefficient

	CMC GKVK1	CMC GKVK2	CMC GKVK3	CMC GKVK4	CMC GKVK5	CMC GKVK6	CMC GKVK7	CMC GKVK8	CMC GKVK9	CMC GKVK10	CMC GKVK11	CMC GKVK12	CMC GKVK13	CMC GKVK14	CMC GKVK15
CMC GKVK1	1.000														
CMC GKVK2	0.669	1.000													
CMC GKVK3	0.696	0.925	1.000												
CMC GKVK4	0.728	0.729	0.716	1.000											
CMC GKVK5	0.667	0.865	0.877	0.781	1.000										
CMC GKVK6	0.761	0.758	0.752	0.804	0.772	1.000									
CMC GKVK7	0.773	0.757	0.782	0.727	0.799	0.918	1.000								
CMC GKVK8	0.735	0.802	0.780	0.702	0.790	0.849	0.892	1.000							
CMC GKVK9	0.718	0.786	0.796	0.769	0.767	0.785	0.802	0.885	1.000						
CMC GKVK10	0.774	0.662	0.657	0.742	0.658	0.808	0.783	0.770	0.736	1.000					
CMC GKVK11	0.641	0.767	0.775	0.690	0.805	0.719	0.729	0.795	0.753	0.662	1.000				
CMC GKVK12	0.729	0.652	0.654	0.653	0.674	0.707	0.698	0.719	0.682	0.737	0.627	1.000			
CMC GKVK13	0.632	0.777	0.737	0.640	0.769	0.683	0.724	0.765	0.710	0.619	0.748	0.667	1.000		
CMC GKVK14	0.692	0.837	0.840	0.760	0.891	0.745	0.769	0.754	0.754	0.650	0.794	0.725	0.807	1.000	
CMC GKVK15	0.652	0.830	0.848	0.722	0.871	0.732	0.750	0.741	0.745	0.642	0.812	0.683	0.828	0.948	1.000

Fig.1 Dendrogram indicating diversity and relationship among 15 pickling melon genotypes

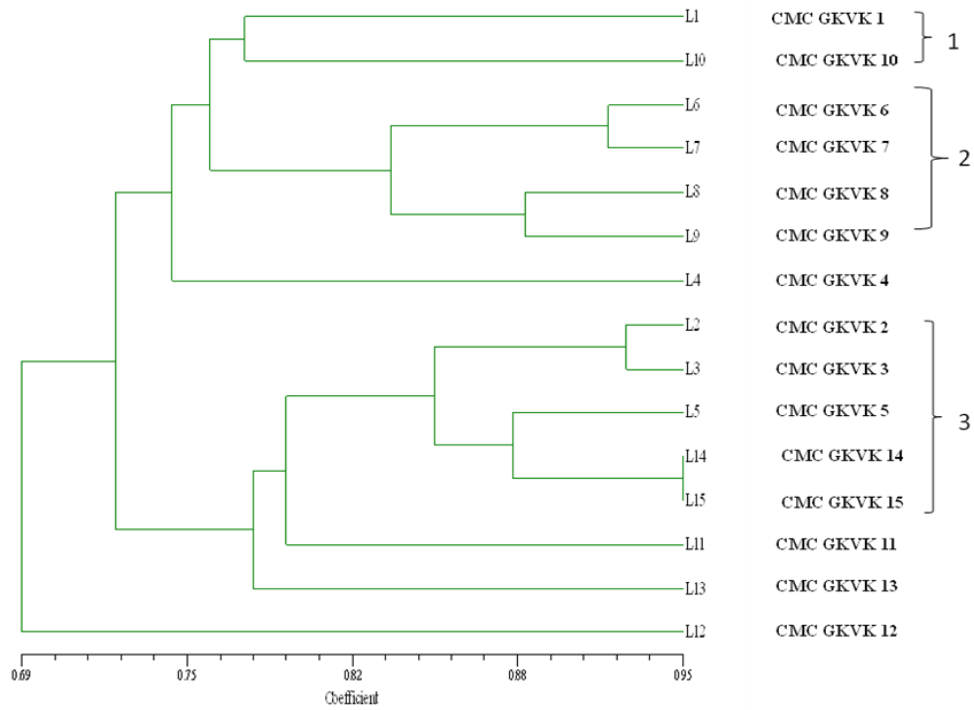
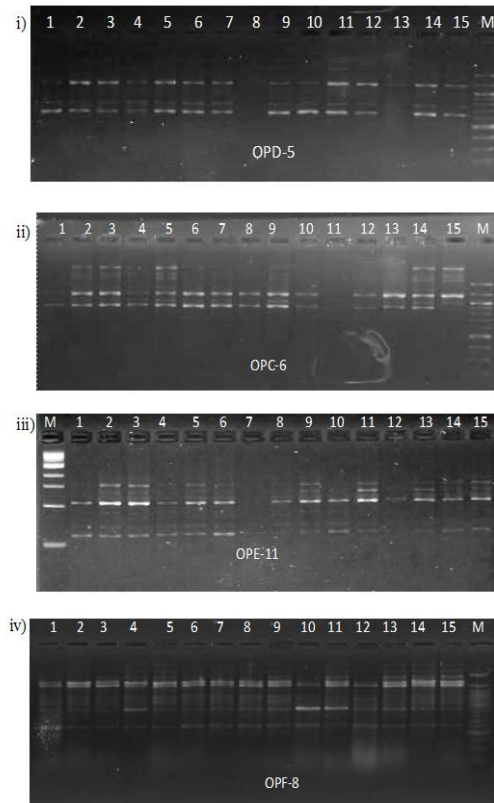


Plate.1 RAPD gel profile of 15 pickling melon accessions amplified by using OPD-5, OPC-6, OPE-11 and OPF-8 primers



Cluster 1 consisted of 2 genotypes (CMC GKVK1 and CMC GKVK 10), cluster 2 had four genotypes (CMC GKVK6, CMC GKVK7, CMC GKVK8 and CMC GKVK9) and cluster 3 had five genotypes (CMC GKVK2, CMC GKVK3, CMC GKVK5, CMC GKVK14 and CMC GKVK15), which were divided into two sub-clusters (sub cluster I and II). In sub cluster II, CMC GKVK14 and CMC GKVK15 accessions showed the highest similarity. Accession CMC GKVK12 was distinctly separated from all the accessions in the RAPD dendrogram with similarity value of 0.69 and it was the most distinct from other accessions. Previous studies on cluster analysis reported two major clusters among South and East Asian melon types (Tanaka *et al.*, 2007) and five distinct groups among African melon germplasm (Mliki *et al.*, 2001). The most promising accessions based on RAPD analysis were CMC GKVK12, CMC GKVK4, CMC GKVK11, and CMC GKVK13. These accessions need to be further evaluated and could be used either as direct selections or in the crop improvement programme. The information obtained from this study may be useful for identification of promising genotypes and understanding the genetic diversity present in this species.

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