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***RESEARCH PAPER***

# AFLP analysis of genetic relationships and diversity of some oriental pickling melon (*Cucumis melo* var. *conomon)* genotypes in Karnataka

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**Abstract :** Genetic diversity of indigenous plant population is the prerequisite for any research on plant breeding and improvement, making genetic diversity conservation of critical importance. The present study was conducted to assess the amplified fragment length polymorphism (AFLP) marker based diversity in 15 oriental pickling melon (*Cucumis melo* var. *conomon*) genotypes during 2009 at University of Agricultural Sciences (UAS), Bangalore. Thirteen primer combinations generated a total of 443 amplicons, of which 342 were polymorphic (76%) with an average of 26.31 bands. The primer combination E-CTT/M-GTG was found to amplify 45 scorable bands, with 91.11 per cent polymorphic bands. Cluster analysis based on Jaccard similarity index and UPGMA algorithm showed a high variation within the studied genotypes and a mean similarity index of 0.79 for AFLP markers. The genotypes CMC GKVK 1 and CMC GKVK 6 expressed the least similarity (0.627) and may serves as very good source in melon breeding programme. Genotypes CMC GKVK1, CMC GKVK8, CMC GKVK15, CMC GKVK11 and CMC GKVK6 fell into solitary clusters and these accessions could be used in future genetic, physiological and morphological studies.

**Key Words :** Oriental pickling melon, AFLP, Genetic diversity

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## INTRODUCTION

Oriental pickling melons (*Cucumis melo* var. *conomon*) are commonly grown in Far East Asia. It is a highly cross pollinated and usually andromonoecious in nature, preferring warm weather and bright sunlight for its better growth and development. Kerala, South Karnataka, Andhra Pradesh and Tamil Nadu are the major oriental pickling melon growing states in India. Collection and conservation of genetic resources with high level of diversity is necessary for fulfilling future demand of breeding programmes (Given *et al.*, 1987). Genetic diversity information will help breeders in

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selecting desirable parents in hybrid and new cultivar production and in maintaining population polymorphism. Increasing the genetic diversity is a major concern in species where inbreeding practices have resulted in the loss of genetic diversity (Ramanatha and Hodgkin, 2002), a process that could be responsible for the unsuccessful development of new combinations. On the other hand, because the performance of hybrids seems to be related to the genetic divergence of parental lines, information on the genetic similarity between genotypes may also facilitate the prediction of crosses that will produce hybrids with a higher performance (Luan *et al.,* 2010 and Sekhon and Gupta, 1995).

Different types of markers have been used to assess the genetic diversity in melon (*Cucumis melo*). Morphological characters can be useful for the evolution of diversity. But most often the desired phenotypic characters are phylogenetically inherited and are highly influenced by environmental conditions. In this regard the advent of molecular markers had revolutionized the entire scenario of plant sciences. In comparison with other marker types, these DNA-based marker techniques can detect the genetic diversity of a species in all tissues and at all stages of development, without being affected by the environmental conditions (Xu *et al.*, 2012). Among different molecular markers, amplified fragment length polymorphisms (AFLPs) generally reveal a higher number of polymorphisms and do not require DNA sequence information (Vos *et al.,* 1995). Moreover, studies comparing the RAPD, AFLP and RFLP markers on five melon genotypes with different origins (Spain, South Korea, India, Zimbabwe and Israel) suggest that while all three types of markers are equally informative, AFLPs show the highest efficiency in detecting polymorphism (Garcia *et al.*, 2000). AFLP finger prints are reliable and reproducible molecular markers, making them a more appropriate technique for detecting genetic variation among melon genotypes (Reddy *et al.*, 2005 and Yashiro *et al.*, 2005). Therefore, AFLP is considered as a reliable marker choice for genetic diversity studies in a large number of organisms and species (Park *et al.,* 2010).

The objective of this study was to assess AFLP marker based genetic divergence in pickling melon genotypes for the germplasm identification, breeding, protection and conservation.

## MATERIAL AND METHODS

### Plant material and DNA extraction :

Fifteen morphologically and geographically distinct pickling melon genotypes (Table A) were collected from different parts of South Karnataka and were raised during 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 cetyltrimethylammonium bromide (CTAB) method

**Table A: Pickling melon genotypes analyzed in this study**

| Sr. 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,Brahmavar, Udupi (district). | Greenish brown speckled |
| 11. | CMC GKVK-11 | Barkuru,Brahmavara, Udupi (district). | Greenish yellow streaked |
| 12. | CMC GKVK-12 | Brahmavara, Udupi (district) | Orange streaked |
| 13. | CMC GKVK-13 | ,Brahmavara, Udupi (district). | Greenish yellow speckled |
| 14. | CMC GKVK-14 | Karkala, Udupi (district). | Greenish yellow streaked |
| 15. | CMC GKVK-15 | Mannur Udupi (district). | Greenish brown speckled |

(Doyle and Doyle, 1987) with minor modifications. Each genotype leaf tissue (3.0g) was ground to a fine powder using mortar and pestle in liquid nitrogen and 10ml of extraction buffer (CTAB 6%, NaCl 5M, Tris HCl 1M, pH :8, EDTA 0.5M ) and 60 mg of PVP (6%) were added to fine leaf powder in 50ml tube.β-Mercaptoethanol (10 µl) was added to each tube and inverted gently several times. After shaking the tubes were incubated at 65°C for 1 hour and cooled to room temperature. Equal volume of 24:1 chloroform/ isoamyl alcohol was added and centrifuged at 5000 rpm for 6 minutes. The upper aqueous phase was transferred to a fresh centrifuge tube and added with equal volume of 24:1 chloroform/ isoamyl alcohol and centrifuged at 5000 rpm for 6 minutes. The upper aqueous phase was again taken and added with equal volume of 100 per cent chilled isopropanol to precipitate the nucleic acid. Spool out the nucleic acid into 1.5 ml eppendorf tube by keeping the tube at -20°C for half an hour and tubes were centrifuged at 10000 rpm for 8 minutes.The pellet was rinsed with 70 per cent ethanol and allowed to dry overnight (If necessary centrifuged at 6000 rpm for 4 minutes). Finally, 200µl of $T_{10}E_{0.1}$ Buffer (pH8) was added to the pellet and the dissolved pellet DNA was stored at -20°C for long use.

**AFLP analysis :**

According to the AFLP protocol, reaction was performed with some modifications (Vos *et al.*,1995). The genomic DNA was restricted by using the two restriction endonucleases, *Eco* RI and *Mse* I and double stranded adopters were ligated to the ends of the fragments, generating templet DNA for subsequent PCR amplifications. The 5 µL of NEB 4 buffer, 1.0 µL of *Eco*RI (10 U/µL) and 1.0 µL of *Mse* I (5 U/µL) were used for the restriction. The 0.4 µL of $T_4$ DNA ligase buffer with 10 mM ATP, 0.5 µL of *Eco* RI adapter (5 pmol/µ L), 0.5 µ L of *Mse* I adapter (50 pmol/µ L) and 2.4 µ L of $T_4$ DNA ligase (1U/µ L) were used for ligation. Ligation reaction was done at 37ºC for 1 h. The digested/ ligated AFLP templates were then diluted with 176 µ L of $T_{10}E_{0.1}$ at pH 8 and stored at-20ºC.

Pre-amplification is a polymerase chain reaction where each primer combination has a selection of one nucleotide base (*Eco* RI+A and *Mse* I+G). Pre-amplification mix involved 2 µ L of *Eco* RI + A (7.5 ng /µl), 2 µ L of *Mse*I+G (7.5 ng /µl), 2 µ L of 1 mM dNTP mix, 1µL of *Taq* buffer (10x) and 0.2 µL of *Taq* polymerase (5U/µ L). Pre-amplification was carried out with the following PCR programme: 94ºC/30 sec for anneling and 56ºC/1 min, 72ºC/1 min and 10ºC/30 min for 20 cycles. The pre-amplified products were diluted by adding 60 µL of $T_{10}E_{0.1}$, pH 8.

Re-amplication is a PCR where each primer has 3 nucleotide extensions. Re-amplication reaction mixture contained: 2 µL of *Eco*RI+A+N+N, 2 µL of *Mse*I+ G+N +N, 2 µL of dNTP mix (1 mM), 1 µL of *Taq* buffer (10x) and 0.2 µ L of *Taq* polymerase (5 U/µL) (Table 1). Re-amplication was carried out with the following PCR program: 94ºC/30 sec, 65ºC/30 sec reducing by 0.7ºC/ cycle to 56ºC and 72ºC/1min for 11 cycles and 94ºC/30 sec for anneling and 56ºC/30 sec, 72ºC/1 min and 10ºC/ 30 min for 24 cycles. 8 µL of stop loading dye was added to each sample and denaturation was carried out by heating to 94ºC for 5 min and then cooling to 10ºC for 5 min. Finally the product was stored at -20ºC and 4 µL of the mixture was loaded on a polyacrylamide gel. For each primer combination, samples of 15 genotypes were run on the same gel. The AFLP products were run out on 4.5 per cent poly acryl amide gel electrophoresis (PAGE) at 1550V for 1.5h and silver stained. After electrophoresis, gels were fixed and dried (Benbouza *et al*., 2006).

**Scoring and data analysis :**

For the genetic relationship studies, only distinct, reproducible, well-resolved AFLP fragments were scored as present (1) or absent (0) and a binary data matrix was constructed based on band scores. Different polymorphic fragments produced by each primer were treated as unit and numbered sequentially. Monomorphic fragments and those with low intensity were not taken into account.

Genetic diversity was assessed using NTSYS-pc version 2.0 software package (Rohlf, 2000). Genetic similarities (GS) between pairs of accessions were measured by the Jaccard similarity co-efficient (Jaccard, 1908). Dendrogram was constructed using Unweighed Paired Group Arithmatic Mean (UPGMA) algorithm based on these similarity co-efficients.

## RESULTS AND DISCUSSION

In the present study, AFLP fingerprinting was applied to assess the diversity in pickling melon. A total of 13 AFLP primer combinations (with 3 selective nucleotides) were used to amplify genomic DNA of 15 pickling melon genotypes. The 13 AFLP primer

combinations generated 443 amplicons, of which 342 were polymorphic with an average of 26.31 bands (Table 1). A number of recent studies have also shown the capacity of AFLP to be highly discriminating between the genotypes in a range of crops (Lombard *et al*., 2000; Behera *et al*., 2008 and Maras *et al*., 2008).

Among 13 primer combinations tested, E-CAA/M-GTG was found to amplify 52 scorable bands, followed by E-CTT/M-GTG, E-CAT/M-GTG and E-CAG/M-GCT generating 45, 42, 41 scorable bands, respectively. Out of the 13 promer combinations, two primer combinations E-CTT/M-GTG (91.11%) and E-CAG/M-GTG (88.24%) produced the highest percentage of poly morphic bands (Plate 1).Contrastingly, primer combination E-CAG/M-GCT could amplify 30 bands, of which only 18 were polymorphic (60 % polymorphism). On an average 13 primer combinations could amplify 76.03 per cent polymorphic bands with an average of 26.31 polymorphic loci per primer (Table 1). It fully indicated abundant polymorphism existed between pickling melon genotypes. The percentage of polymorphic fragments that Sheng *et al*. (2011) obtained using seven AFLP primers on eight genotypes was 57 per cent. Yashiro *et al.* (2005) found 27.8 average bands in 99 East and South Asian melon accessions. Garcis *et al.* (2000) studied genetic diversity among six Spanish melons and found 15.08 polymorphic bands with 12 AFLP primer combinations.

The UPGMA based dendrogram was obtained from the binary data deduced from the DNA profiles of the samples analyzed from 13 AFLP primer combinations. Jaccard's similarity co-efficients between genotypes

**Plate 1: AFLP gel profile of 15 pickling melon accessions amplified by using E-CAG+M-GTG and E-CAT+M-GTG primer combinations**

**Table 1 : AFLP primer combinations for pickling melon genotypes**

| Sr. No. | Primer combinations | Total bands | Polymorphic bands | Polymorphism (%) |
|---|---|---|---|---|
| 1. | E-CAC/M-GTG | 23 | 19 | 82.61 |
| 2. | E-CTT/M-GTG | 45 | 41 | 91.11 |
| 3. | E-CAA/M-GTG | 52 | 41 | 78.85 |
| 4. | E-CAG/M-GCG | 27 | 18 | 66.67 |
| 5. | E-CAG/M-GCT | 41 | 31 | 75.61 |
| 6. | E-CGA/M-GCA | 40 | 34 | 85.00 |
| 7. | E-CAA/M-GCT | 30 | 19 | 63.33 |
| 8. | E-CTA/M-GCT | 15 | 11 | 73.33 |
| 9. | E-CAG/M-GCT | 30 | 18 | 60.00 |
| 10. | E-CAT/M-GCA | 40 | 29 | 72.50 |
| 11. | E-CGA/M-GCG | 24 | 18 | 75.00 |
| 12. | E-CAG/M-GTG | 34 | 30 | 88.24 |
| 13. | E-CAT/M-GTG | 42 | 32 | 76.19 |
| Total | | 443 | 342 | |
| Average | | 34.08 | 26.31 | 76.03 |

**Table 2 : Genetic similarity values as determined by 10 AFLP markers using Jaccard similarity co-efficient**

| | 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 | 0.739 | 1.000 | | | | | | | | | | | | | |
| CMC GKVK3 | 0.735 | 0.944 | 1.000 | | | | | | | | | | | | |
| CMC GKVK4 | 0.740 | 0.907 | 0.904 | 1.000 | | | | | | | | | | | |
| CMC GKVK5 | 0.746 | 0.924 | 0.918 | 0.954 | 1.000 | | | | | | | | | | |
| CMC GKVK6 | 0.627 | 0.771 | 0.747 | 0.762 | 0.770 | 1.000 | | | | | | | | | |
| CMC GKVK7 | 0.743 | 0.937 | 0.927 | 0.918 | 0.939 | 0.785 | 1.000 | | | | | | | | |
| CMC GKVK8 | 0.712 | 0.875 | 0.866 | 0.889 | 0.895 | 0.734 | 0.879 | 1.000 | | | | | | | |
| CMC GKVK9 | 0.739 | 0.875 | 0.856 | 0.879 | 0.903 | 0.737 | 0.888 | 0.850 | 1.000 | | | | | | |
| CMC GKV10 | 0.753 | 0.907 | 0.898 | 0.900 | 0.921 | 0.751 | 0.927 | 0.867 | 0.935 | 1.000 | | | | | |
| CMC GKV11 | 0.687 | 0.836 | 0.824 | 0.815 | 0.832 | 0.693 | 0.834 | 0.778 | 0.817 | 0.866 | 1.000 | | | | |
| CMC GKV12 | 0.750 | 0.892 | 0.880 | 0.913 | 0.913 | 0.735 | 0.912 | 0.878 | 0.901 | 0.925 | 0.840 | 1.000 | | | |
| CMC GKV13 | 0.713 | 0.896 | 0.876 | 0.899 | 0.899 | 0.736 | 0.897 | 0.867 | 0.871 | 0.893 | 0.812 | 0.910 | 1.000 | | |
| CMC GKV14 | 0.716 | 0.872 | 0.859 | 0.879 | 0.878 | 0.735 | 0.876 | 0.839 | 0.857 | 0.879 | 0.798 | 0.886 | 0.897 | 1.000 | |
| CMC GKV15 | 0.685 | 0.873 | 0.867 | 0.872 | 0.871 | 0.709 | 0.874 | 0.823 | 0.840 | 0.880 | 0.826 | 0.872 | 8717.000 | 0.884 | 1.000 |

ranged from 0.954 to 0.626 (Table 2) with an average of 0.79, suggesting a higher genetic variation within pickling melon genotypes. The highest similarity co-efficient value of 0.954 was observed between genotypes CMC GKVK 4 and CMC GKVK 5 and the least value of 0.627 was between genotypes CMC GKVK 1 and CMC GKVK 6. Padmavathi *et al.* (2008) reported similarity co-efficient ranged from 0.70 to 1.00 among thirty eight melon accessions of Ukrain. However, our results showed that high similarity values were also observed among some pickling melon accessions, indicating lack of intercrossing between them or high levels of inbreeding. This result is similar to the results of Raghami *et al.* (2014), who assumed that, if out crossing occurred, the farmers had made efforts to maintain the genetic originality of the accessions, probably to keep the original fruit traits, because of regional consumer preferences. It was likely that the selection for agronomic characters had been practiced by farmers and this could partially explain the relatively high level of similarities within some accessions observed in this study.

The result of cluster analysis is shown on the dendrogram (Fig. 1) depicting the pattern of relationships among the studied genotypes. There was obvious difference among different genotypes. Cluster 1 consisted of five genotypes (CMC GKVK2, CMC GKVK3, CMC GKVK7, CMC GKVK4 and CMC GKVK 5), cluster 2 had three genotypes (CMC GKVK9, CMC GKVK10 and CMC GKVK12) and cluster 3 had only two genotypes (CMC GKVK13 and CMC GKVK14). Genotypes such as CMC GKVK1, CMC GKVK8, CMC GKVK15, CMC GKVK11

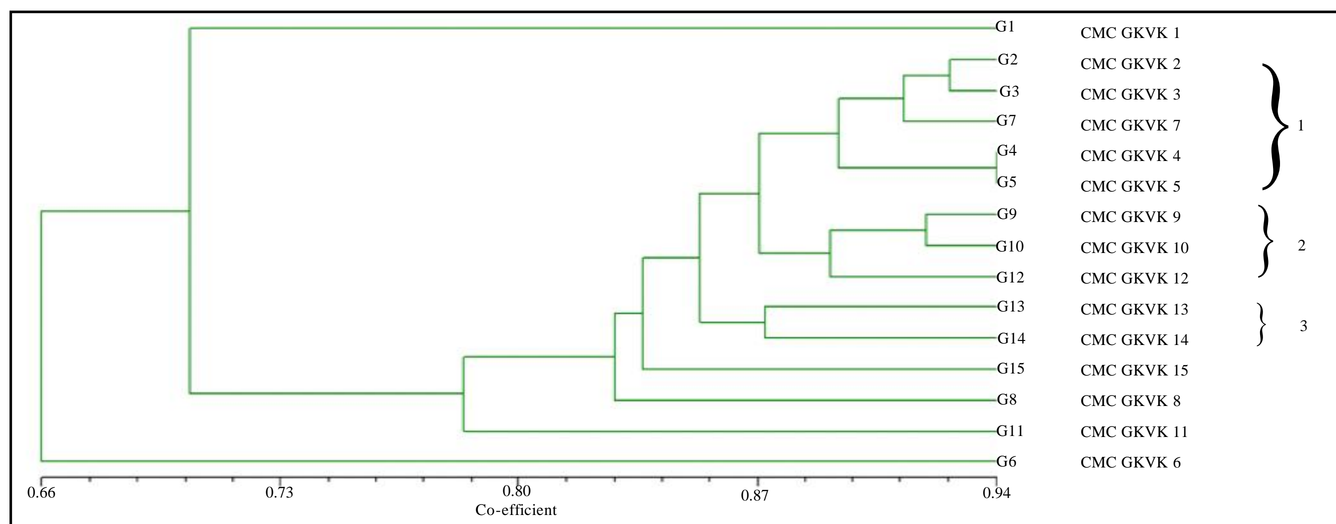


**Fig. 1 : Dendrogram based on Jaccard's similarity co-efficients using ten AFLP primer combinations**

and CMC GKVK6 fell into solitary clusters and cross-hybridizing between them might increase the genetic variation in the breeding population. Yashiro *et al.* (2005) revealed three major clusters among 99 East and South Asian melons based on their genetic similarity.

Cluster analysis also indicated that genotypes collected from same places were clustered into different groups. This difference was probably due to limited number of genotypes included in this experiment. A thorough understanding of the genetic diversity among some more collections of pickling melon genotypes is critical to future germplasm identification, breeding, protection and conservation. So, further studies may obtain more refined phylogenetic trees of pickling melon genotypes by combining genetic marker techniques and morphology/ physiology-base classification methods.

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