

Full Length Research Paper

Use of random amplified polymorphic DNA (RAPD) in differentiation of selected species of *Cucumis* grown in Southern India

Shiragambi Hanumantagouda Manohar and Hosakatte Niranjana Murthy*

Plant Biotechnology Laboratory, Department of Botany, Karnatak University, Dharwad, India.

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Cucumis sativus (Cucumber), *Cucumis melo* var. *acidulus* (Vellari) and *C. melo* var. *momordica* (Snapmelon) are three species which are widely cultivated in India as vegetable crops. These species are distinguished based on leaf, hypanthium and fruit characteristics; however, large variation in morphological and fruit traits has been observed in different populations. A combination of morphological and random amplified polymorphic DNA (RAPD) analysis were carried out in these species to have a better understanding of the taxonomy. RAPD analysis of genomic DNA revealed that polymorphism with OPBE, OPBF primer series and banding pattern of OPBE-18, OPBF-03 and OPBF-15 were able to distinguish between *C. sativus*, *C. melo* var. *acidulus* and *C. melo* var. *momordica*.

Key words: *Cucumis sativus*, *Cucumis melo* var. *acidulus*, *Cucumis melo* var. *momordica*, random amplified polymorphic DNA (RAPD).

INTRODUCTION

Cucumis belongs to the Cucurbitaceae family, which has 118 genera and 825 species (Jeffrey, 1990). The genus *Cucumis* contains 33 species, of which *Cucumis sativus* and *Cucumis melo* are the two most economically important (Ghebretinsae et al., 2007). Cucumber, *C. sativus* L. ($2n=2x=14$), are considered to be of Asiatic origin and are thought to have descended from the closely related, wild *C. sativus* var. *hardwickii* (Royle) Alef., found in the foothills of Nepal and Northern India (Whitaker and Davis, 1962; Harlan, 1975). Melon, *C. melo* L. ($2n=2x=24$), is a morphologically diverse, out crossing horticultural crop of broad economic importance. Africa has been generally regarded as the center of origin of *C. melo*, while India has been considered as an important center of diversification. Melon has been

divided into two subspecies according to the hypanthium hairiness: *C. melo* ssp. *melo* with long hairs and *C. melo* ssp. *agrestis* with short hairs (Jeffrey, 1990). Botanical groups belonging to the ssp. *agrestis* are found in Eastern Asia, Japan and India and the ssp. *melo* are found in India, Europe and the new world. Cultivation of melon dated back to 2000 BC in India, where various types of melons viz. var. *momordica*, var. *acidulus* and var. *flexuosus* (L.) Naudin are cultivated. *C. melo* var. *acidulus* Naudin is commonly known as 'Vellari' and is endemic to tropical humid Southern India (states of Kerala, Tamil Nadu and Karnataka). *C. melo* var. *momordica* (Roxb.) Duthie & Fuller is commonly known as 'Phut' or 'Snapmelon' which is widely cultivated in India. Cucumber (*C. sativus*) and melon (*C. melo* var. *acidulus* and *C. melo* var. *momordica*) land races exhibit tremendous variations in morphological and fruit traits in India. It is essential to distinguish species and varieties by morphological and molecular characteristics for further utilization of the diverse populations in crop improvement. In this study, the phenotypic and molecular markers were used to distinguish between the cucumbers and melons

*Corresponding author. E-mail: nmurthy60@yahoo.co.in. Tel: 9945162742.

Table 1. Correlation matrix of the *Cucumis* accessions used in the present study.

Parameter	1	2	3	4	5	6
<i>C. sativus</i> 1	1					
<i>C. sativus</i> 2	0.60	1				
<i>C. melo</i> var. <i>momordica</i> 3	0.08	-0.20	1			
<i>C. melo</i> var. <i>momordica</i> 4	0.02	-0.14	0.94	1		
<i>C. melo</i> var. <i>acidulus</i> 5	-0.14	-0.43	0.54	0.48	1	
<i>C. melo</i> var. <i>acidulus</i> 6	-0.14	-0.43	0.54	0.48	0.88	1

Correlations are significant at $p < 0.05$.

(var. *acidulus*, var. *momordica*) grown in Southern India.

MATERIALS AND METHODS

C. sativus, *C. melo* var. *acidulus* and *C. melo* var. *momordica* land races used in this study were collected in the state of Karnataka and were raised in the Botanical Garden, Karnatak University, Dharwad, India during March to June 2009. All landraces were evaluated for their phenotypic traits in a field level. For molecular studies, genomic DNA was isolated from fresh leaves using CTAB (cetyltrimethyl ammonium bromide) method (Murray and Thompson, 1980). The DNA samples were screened with 40 RAPD (OPBE₁₋₂₀ and OPBF₁₋₂₀ series, Operon, USA) primers for amplification products. The PCR reaction was performed with 20 ng template DNA; *Taq* buffer with 1.5 mM MgCl₂; dNTPs (1 mM/μl); *Taq* polymerase (2 units); primer (5 pm); spermidine (20 mM) and reaction volume of 20 μl. Amplifications were performed on a Eppendorf master cycler for 45 cycles. Amplification products were run on 1.5% agarose gel prepared in 0.5 X TAE buffer, stained with ethidium bromide and visualized under UV light (UVitec gel documentation). The presence of a particular band was scored as 1 and absence as 0. The binary data was analyzed using UPGMA (Statistica), for the construction of dendrogram and correlation matrix.

RESULTS

There were large variations in the phenotypic traits among the *Cucumis* collections. The leaves of *C. sativus* were broadly ovate, shallowly 5 lobed and acute, when compared with *C. melo* varieties which were generally orbicular-ovate to reniform, 5 lobed (Figure 1A and B). The hypanthium or the ovary of the *C. sativus* was generally spiked, when compared to *C. melo* varieties which have short hairy hypanthium (Figure 1C, D and E). The fruits were cylindrical in shape in *C. sativus*, while elliptical in *C. melo* var. *momordica* and oblate or ovate in *C. melo* var. *acidulus* (Figure 1 F, G and H). The fruits of cucumber were green to pale yellow, with or without spines and were non-sweet. The fruit skin of *C. melo* var. *acidulus* was smooth, orange or light yellow in colour, non sweet, had no aroma when ripe, fruit flesh was white or cream and it was non cracking type. The fruits of *C. melo* var. *mamordica* were with a smooth skin, orange or light yellow in color, little sweet and had little aroma and were cracking type at maturity.

RAPD analysis depicted polymorphism in DNA bands with the OPBE and OPBF primer series. Species specific polymorphic bands were observed with primer OPBE-18, OPBF-03 and OPBF-15. Primer OPBF-15 produced a band 825 bp which was specific to *C. sativus*, whereas, primer OPBE-18 produced bands of 1450, 235 and 190 bp which were specific to *C. melo*. (Figure 1I and J). Similarly, primer OPBE-18 and OPBF-15 produced *C. melo* var. *momordica* specific band of 428 and 410 bp respectively, and these bands were absent in *C. melo* var. *acidulus*. Based on the binary data of DNA polymorphism, a dendrogram was constructed which depicted differences between *C. sativus* and *C. melo* groups with linkage distance of 44. The constructed dendrogram formed two different clusters (cucumber and melon) with a linkage distance of 44 (Figure 1K). Correlation matrix was constructed using the binomial data of the RAPD analysis and the data suggest that there was negative correlation with *C. sativus* and *C. melo* varieties (Table 1). However, there was positive correlation between *C. melo* var. *momordica* and *C. melo* var. *acidulus*.

DISCUSSION

C. sativus and *C. melo* showed tremendous diversity in the Indian subcontinent (Staub et al., 1997; Fergany et al., 2010) and such a variation provides breeders with a rich genetic resource. However, it is essential to distinguish species and varieties by morphological and molecular characteristics for crop improvement. In this study, we have attempted to use the morphological and DNA markers to distinguish the cucumber and melon varieties. *C. sativus* collections had broadly ovate, shallowly five lobed, acute leaves, spiked hypanthium and cylindrical fruits. *C. melo* had orbicular-ovate to reniform leaves, short hairy hypanthium and elliptical or ovate fruits (Figure 1). *C. melo* varieties could be readily distinguished based fruit characteristics: *C. melo* var. *acidulus* were oval or elliptical, and were firm (non cracking) at maturity, whereas *C. melo* var. *momordica* were elliptical and cracking type at maturity. These descriptions were

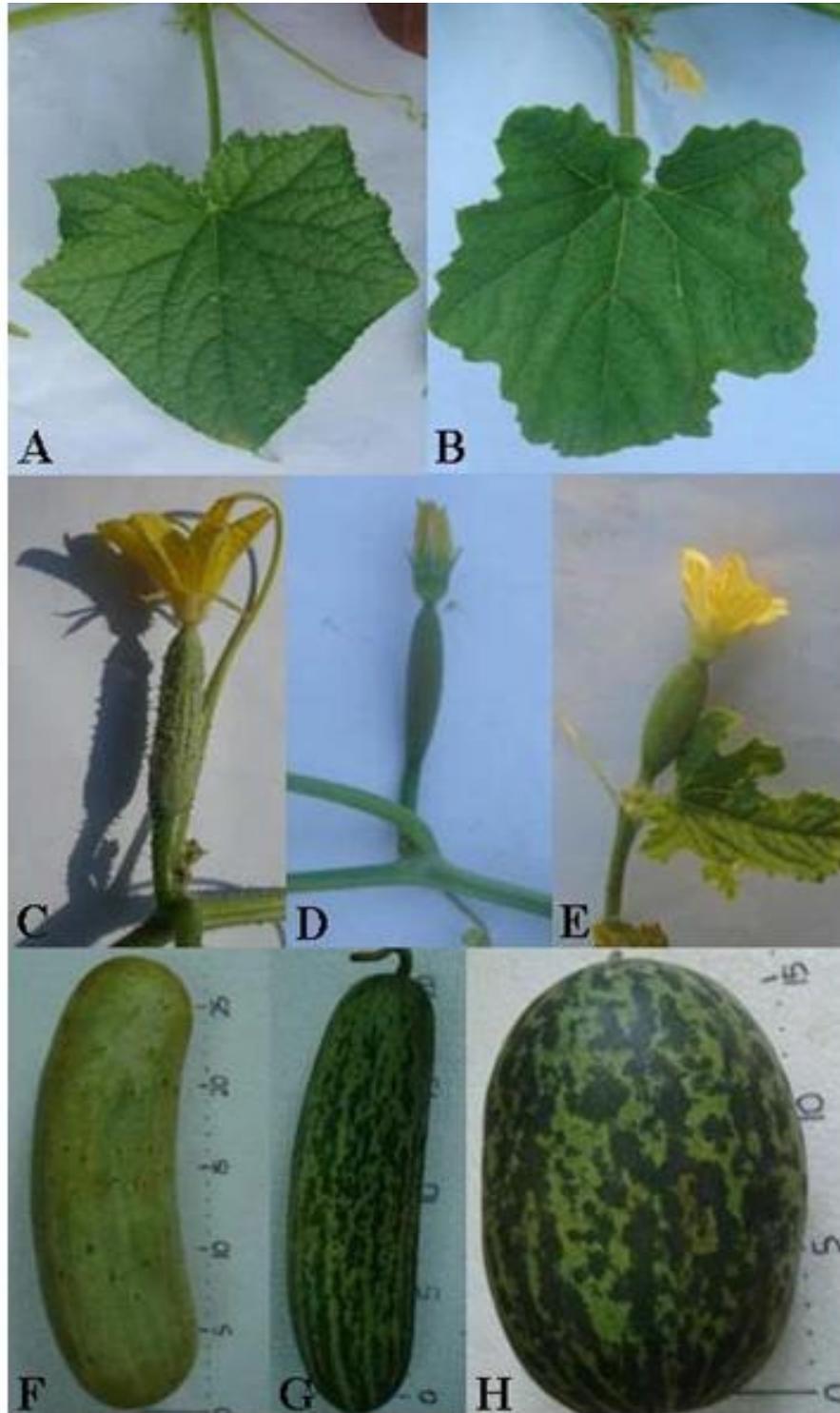


Figure 1. Variation in morphological and molecular markers in the genus *Cucumis* grown in Southern India. Leaf morphology of *C. sativus* (A) and *C. melo* (B); hypanthium of *C. sativus* (C), *C. melo* var. *momordica* (D) and *C. melo* var. *acidulus* (E); fruits of *C. sativus* (F), *C. melo* var. *momordica* (G) and *C. melo* var. *acidulus* (H); variety specific amplification products obtained using RAPD primer OPBE18 and OPBF15 primers, respectively. Lanes 1 to 2; *C. sativus*; lanes 3 to 4; *C. melo* var. *momordica*; lanes 5 to 6; *C. melo* var. *acidulus*; lane M: DNA marker (1 kb); K, Dendrogram showing genetic relationship among the *Cucumis* collection.

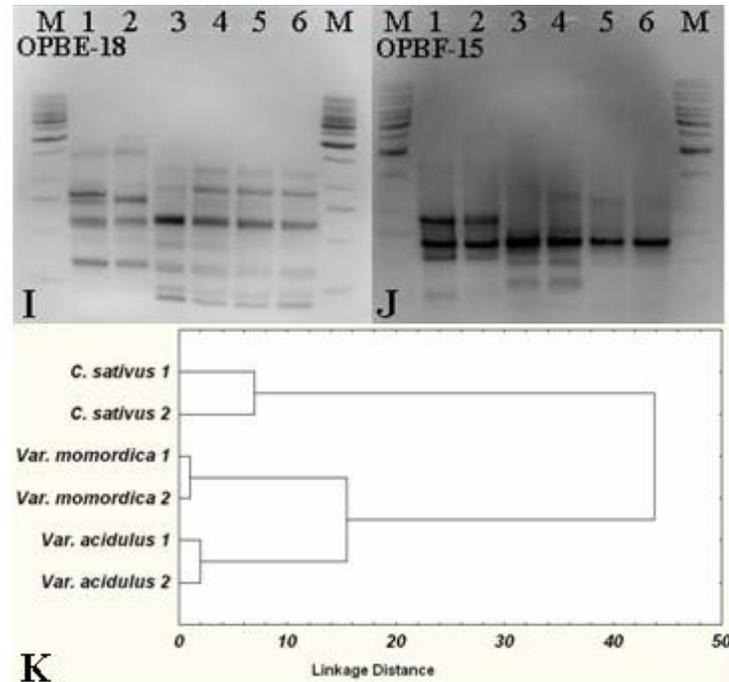


Figure 1. Contd.

in consensus with earlier reports (Pitrat, 2008).

Molecular marker data becomes handy during large scale germplasm analysis and RAPD markers have proved to be informative enough to allow for the evaluation of representative germplasm of *C. sativus* and *C. melo* (Staub et al., 1997; Garcia et al., 1998). RAPD analysis of this study provides species specific banding pattern to distinguish between *C. sativus* and *C. melo* var. *acidulus* and *C. melo* var. *momordica*. Each accession had a unique marker profile, indicating that RAPD was useful in differentiation among the species and varietal level. In both the dendrogram produced from UPGMA clustering (Figure 1K) and correlation matrix data (Table 1), *C. sativus* accessions could be distinguished from *C. melo* accessions. This grouping agrees with the botanical subdivisions made by Pitrat (2008).

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