Molecular diversity of Hungarian melon varieties revealed by RAPD markers

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Summary: RAPD markers were used to reveal genetic diversity between nine varieties of *Cucumis melo* L. and to identify the studied varieties. Of the 60 primers tested 12 primers produced polymorph patterns. A set of 4 primers was sufficient for distinction the nine investigated melon varieties.

Key words: *RAPD, melon, molecular marker*

Introduction

Melon (*Cucumis melo* L.) is a vegetable crop of great importance through the world. It is a member of the genus *Cucumis*, in the family *Cucurbitaceae*. *Neuhausen* (1992) determined that despite diversity of melon in horticultural traits such as fruit shape polymorphism at the DNA level among the groups is relatively low, using Restriction Fragment Length Polymorphism (RFLP). Development of DNA markers has greatly facilitated mapping. *Wang* et al. (1997) constructed a genetic map based primarily on Amplified Fragment Length Polymorphism (AFLP) markers using backcross population. *Baudracco-Arnas & Pitrat* (1996) constructed a molecular map of melon using RFLP, Random Amplification of Plymorphic RAPD, isozyme, disease resistance and morphological markers. *Katzir* et al. (1996) developed microsatellite markers for melon. *Garcia* et al (1998) determined genetic relationships among melon breeding lines by RAPD markers. Comparing the performance of AFLP, RAPD and RFLP markers for measuring diversity in different genotypes of melon *Garcia-Mas* et al. (2000) found that all three types of markers were equally informative although AFLP showed the highest efficiency for testing polymorphism. In these studies different estimates for the degree of genetic polymorphism were obtained, reflecting differences on the selected sets of genotypes.

The objective of this study was to find polymorph RAPD markers useful for differentiation of Hungarian melon varieties.

Material and method

Plant material

The nine melon cultivars, evaluated in this study are listed in *Table 1*. Seeds were obtained from the National Institute of Agricultural Quality Control and the Hungarian Melon Gene Bank, controlled by the Department of Genetics and Horticultural Plant breeding of SZIU.

DNA extraction

The seeds were germinated and maintained in vitro for leaf extraction. Total genomic DNA was isolated from 2 weeks old young leaves with QIAGEN DNEasy Plant System Mini Kit protocol (Qiagen, Gmbh., 2000).

Table 1 Description of melon cultivars examined

Cultivars	Growing season	Fruit	
		Shape	Flesh Color
1. Tétényi Csereshéjú	Mid-early maturing	Round	Orange
2. Javított Zentai	Very short growing season	Oblate	Yellow
3. Ezüst Ananász	Short growing season	Oblate	Orange
4. Hógolyó	Long growing season	Round	White-green
5. Muskotály	Mid-long growing season	Round -oval	Pale-green
6. Topáz	Short growing season	Oval	Light-green
7. Magyar Kincs	Mid-early maturing	Round	Pale-green
8. Fortuna	Midseason maturing	Round	Pale-green
9. Hale's Best	Early maturing	Oval	Salmon-orange

DNA amplification

Primers were purchased from Operon Technologies, Inc. Alameda, Calif. USA (Primer Kit series A, B and O). The protocol for RAPD analysis was adopted from *Williams* et al.(1990) with some modification. Reactions were performed in volumes of 25 µl containing 2.5 µl 1x reaction buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatine), 0.1 mM dNTP, 50 pmol primer, 25 ng genomic DNA, 1 unit of Red Taq polymerase (Sigma). After the initial denaturing step of 3 min at 94 °C amplifications were performed under the following conditions: 4 cycles of 20 s at 94 °C, 1 min at 33 °C and 1 min 72 °C; followed by 45 cycles of 20 s at 94 °C, 1 min at 36 °C and 1 min at 72 °C, a final extension step of 7 min at 72 °C (PTC-200 Gradient thermocycler, MJ Research Inc.). Amplification products were analysed by electrophoresis at 120V in 1% TAE agarose gel along with a 100 bp DNA ladder (Promega). DNA bands were visualized by ethidium bromide staining and were photographed under UV light using Polaroid camera. All PCR reactions were repeated three times and only reproducible DNA bands were retained for analyses.

Data analysis of RAPD

For data analysis of RAPD reactions, fragments were scored as present (1) or absent (0). For estimation of genetic distances between cultivars we used Nei's original measures of genetic identity and genetic distance *(Nei,* 1972) of the PopGene version 1.32 *(Yeh,* 1999) program.

Results and discussion

RAPD ananlysis

So far 60 primers were tested but only 12 were suitable to prove polymorphism *(Figure 1)*. The 12 polymorph primers showed 29 differential loci.

Genetic distance calculation were based from the data of the 12 selected, trustable primers suitable to show

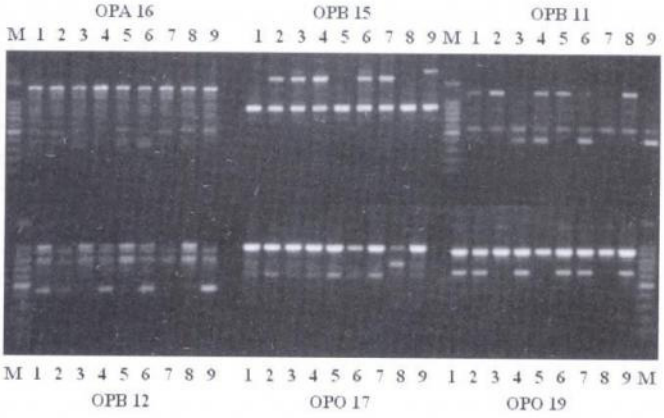

Figure 1 The polymorphisms of some selected primers of the 9 examined varieties

polymorphism. Average genetic distance was 0.38 (62% similarity) The largest genetic distance was observed between the *Javított Zentai* and the *Ezüst Ananász* (0.58). Based on the data of genetic distance matrix *(Table 2)* the *Muskotály* and *Tétényi Csereshéjú* as well as the *Fortuna* and *Hale's best* were the nearest variety pairs. The value of genetic distance was 1.15 in both case. However for real calculation of the genetic distances between varieties all data obtained by the different primers (polymorph and less polymorph) should be taken in consideration.

For distinction studies we found several primer combinations (set of 4 primers), which support the separation of varieties *(Figure 2)*. The use of limited primer set is a simple method, which could be utilized by plant variety protection and for examinations of the identity and the purity of the varieties.

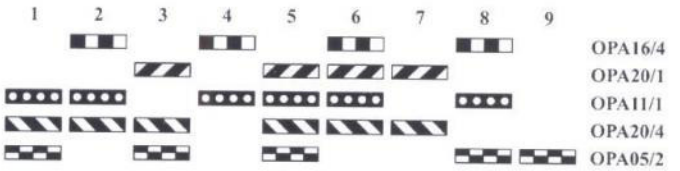

Figure 2 Separation of nine varieties using 4 primers refer to 5 locus

Table 2 Genetic distance (below diagonal) and similarity (above diagonal) between the examined varieties, computed with Nei's original distance measures *(Nei* 1972)

	1.Tétényi Csereshéjú	2.Javított Zentai	3.Ezüst Ananász	4.Hógolyó	5.Muskotály	6.Topáz	7.Magyar Kincs	8.Fortuna	9.Hale's Best
1. Tétényi Csereshéjú	****	0.7674	0.7442	0.7442	0.8605	0.6977	0.7907	0.7442	0.7907
2. Javított Zentai	0.2647	****	0.5581	0.7442	0.6744	0.7442	0.7442	0.8372	0.8372
3. Ezüst Ananász	0.2955	0.5831	****	0.6279	0.7907	0.6744	0.7674	0.5814	0.5814
4. Hógolyó	0.2955	0.2955	0.4654	****	0.6512	0.6744	0.7674	0.7209	0.7209
5. Muskotály	0.1503	0.3939	0.2348	0.4290	****	0.7442	0.8372	0.6977	0.6977
6. Topáz	0.3600	0.2955	0.3939	0.3939	0.2955	****	0.7674	0.6279	0.7209
7. Magyar Kincs	0.2348	0.2955	0.2647	0.2647	0.1777	0.2647	****	0.6279	0.6744
8. Fortuna	0.2955	0.1777	0.5423	0.3272	0.3600	0.4654	0.4654	****	0.8605
9. Hale's Best	0.2348	0.1777	0.5423	0.3272	0.3600	0.3272	0.3939	0.1503	****

Acknowledgement

This research was supported by the Hungarian Széchenyi Projekt (NKFP 4/036/2001) and the Ministry of Agriculture (FVM 211.a/2000). We thank for the seed samples to Szilád Szanyi and for the technical assistance to Bacskainé Papp Anna.

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