

Araştırma Makalesi/Research Article (Original Paper)

Molecular Genetic diversity in Lake Van Basin Melons (*Cucumis melo* L.) Based on RAPD and ISSR Markers

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Abstract: Lake Van Basin of Turkey is located in the secondary gene center for melon. The molecular genetic diversity among thirty-seven melon genotypes collected from Lake Van Basin was determined by RAPD and ISSR makers. Total 121 polymorphic molecular markers obtained from 8 RAPD and 10 ISSR primers were employed to characterize the genetic relationships among the melon genotypes. It was found that within-group genetic similarities ranged between 0.55 and 1.00; related genotypes or genotypes collected from similar regions were partitioned to similar clusters. The genetic diversity parameters among Lake Van Basin melon genotypes were found as $H = 0.175$ and $I = 0.231$, and 96.19 % of polymorphism.

Key words: *Cucumis melo* L., Genetic variation, Germplasm, RAPD, ISSR

Van Gölü Havzası Kavunlarındaki Moleküler Genetik Çeşitliliğin RAPD ve ISSR Belirteçleri İle Belirlenmesi

Özet: Türkiye Van Gölü Havzası kavunun ikincil gen merkezinde yer almaktadır. Van Gölü Havzası'ndan toplanan otuz yedi kavun genotipi arasındaki moleküler genetik çeşitlilik, RAPD ve ISSR belirteçleri yardımıyla belirlenmiştir. Sekiz adet RAPD ve 10 adet ISSR primerinden elde edilen toplam 121 polimorfik moleküler belirteç, kavun genotipleri arasındaki genetik ilişkilerin karakterizasyonunda kullanılmıştır. Grup içi genetik benzerlik katsayısının 0.55 ve 1.00 arasında değiştiği; benzer bölgelerden toplanan genotiplerin veya akraba genotiplerin benzer kümelerde yer aldığı gözlenmiştir. Van Gölü Havzası kavun genotipler arasında genetik çeşitlilik parametreleri olarak $H = 0.175$, $I = 0.231$ ve % 96.19 polimorfizm belirlenmiştir.

Anahtar kelimeler: *Cucumis melo* L., Genetik varyasyon, Gen kaynakları, RAPD, ISSR

Introduction

Melon (*Cucumis melo* L.) is one of the main cucurbit crops in the world and Turkey (Anonymous 2010). The secondary genetic diversity center of melon contains Turkey (Pitrat *et al.* 1999; Jeffrey 2001). Early domestication of melon most probably occurred in the Middle East (Robinson and Decker-Walters, 1997; Jeffrey 2001; Luan *et al.* 2008). It is detected that there has been a large diversity among melon genotypes (Pitrat *et al.*, 2000; Jeffrey, 2001; Sensoy *et al.* 2007a). The local melons of Turkey are also diverse (Zhukovsky1951; Günay 1993; Sensoy *et al.* 2007a; Sari *et al.* 2008; Szamosi *et al.* 2010; Yildiz *et al.* 2011). Turkish local melon genotypes have been collected at different institutions in Turkey (Kucuk *et al.* 2002; Demir *et al.* 2006; Sensoy *et al.* 2007a; Sari *et al.* 2008; Yildiz *et al.* 2011).

Morphological and isozyme markers are small in number and have some disadvantageous (Meglic and Staub 1996). However, DNA markers are not affected by the environment and large in number (Waugh and Powel 1992; Rafalski and Tinge 1993; Lee 1995; Winter and Kahl 1995; Sensoy *et al.* 2007a; Inan *et al.* 2012).

Molecular markers have been progressively more employed in melon diversity researches in recent years (Sensoy et al. 2007a,b; Luan et al. 2008; Nimmakayala et al. 2008; Sestili et al. 2008; Yi et al. 2009; Chen et al. 2010; Nhi et al. 2010; Soltani et al. 2010; Yildiz et al. 2011). Lake Van basin is located in the Eastern part of Turkey, has an altitude of 1720 m above sea level, and has a continental climate. In the present study, we employed ISSR and RAPD markers in order to characterize genetic similarity and diversity among melon genotypes collected in Lake Van Basin.

Materials and Methods

Plant material: The thirty-seven melon genotypes collected from Lake Van Basin by the Project TUBITAK-TOGTAG #2681 constituted the plant material of the present study.

DNA extraction: The CTAB procedure was employed to extract genomic DNA (Doyle and Doyle 1987); then Biotech UV 1101 photometer was used to qualify the DNA which was latter diluted in water to a final concentration of 25 ng/μl.

ISSR amplification: Ten ISSR primers were used (Table 1, Figure 1). PCR reaction mixture had 20 ng DNA, 1.5 mM MgCl₂, 0.2 μM Primer, 0.2 mM dNTP, 1X PCR buffer, 1 unit of Taq DNA polymerase (Promega, USA) in a total volume of 20 μL. The ISSR PCR reactions were performed as follow: 3 min at 94°C, 30 sec at 94°C, 45 sec at 50-60°C, 2 min at 72°C for 45 cycles and a final extension of 5 min at 72°C DNA Thermal Cycler (Sensoquest Progen Scientific Ltd. Mexborough, South Yorkshire, UK). The gels were prepared by adding 20 μL of ethidium bromide (10 mg/ml) in 500 ml of agarose. The amplified products were electrophoresed on 2% agarose gel in 1X TAE buffer at 115 V using Maxi-Plus Standard Horizontal Gel Unit (SCIE PLAS) for 3 h and visualized by Gel Logic 1500 (Kodak). A 100 bp ladder (Fermentas) was used as molecular weight.

Table 1. Polymorphisms determined at 121 loci by using ISSR and RAPD.

Primers	Nucleotids of primers	Total bands	Polymorphic bands	T _m (°C)
RAPD				
A04	AATCGGGCTG	8	8	36
A18	AGGTGACCGT	8	5	36
B06	TGCTCTGCCC	7	7	36
D02	GGACCCAACC	5	3	36
D13	GGGGTGACGA	9	9	36
E07	AGATGCAGCC	6	4	36
E14	TGCGGCTGAG	4	4	36
BC551	GGAAGTCCAC	6	6	36
ISSR				
B3	(GA)8A	11	11	50
B4	(AC)8YA	10	9	52
B5	(GA)8T	7	6	50
P2	DDC-(CAC)5	5	4	60
P4	(GT)8YC	7	6	56
Sola1	BDB-(ACA)5	4	4	50
Sola4	VHV-(GT)7G	5	5	56
Sola6	BDB-(CAC)5	5	4	60
Sola11	GAG-(CAA)5	8	6	50
CBCT5	(AC)8YT	6	4	52
TOTAL MARKERS		137	121	

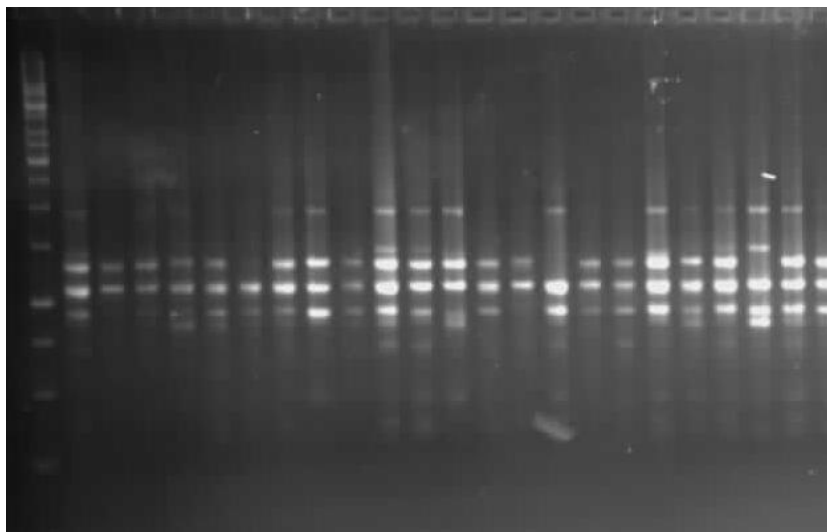


Figure 1. Image of Sola-4 primer (ISSR)

RAPD amplification: Eight 10-mer primers either from Operon Technologies or the University of British Columbia were employed in the study (Table 1, Figure 2). (Sensoy et al. 2007b). The reaction contained 30 ng DNA, 0.2 μ M primer, 100 μ M dNTPs, 1 U Taq DNA Polymerase (Fermentas), 100 mM TRIS-HCl, 1.5 mM $MgCl_2$, and 50 mM KCl, pH 8.8, in a 15- μ l final volume. DNA reactions were performed in a Model 212-1CE thermal cycler (Lab-Line Instruments Inc.). After 5 min of heating at 94 $^{\circ}C$, amplifications were performed under the following regime: 40 cycles of 60 s at 94 $^{\circ}C$, 63 s 36 $^{\circ}C$, 59 s ramps, 120 s 72 $^{\circ}C$, a final extension reaction of 10 min at 72 $^{\circ}C$. Reactions were replicated at least twice to control reproducibility of patterns. The PCR products were analyzed in 1.5 % agarose gels in 1x TAE at 90 V using a Model 192 horizontal gel electrophoresis system (BIO-RAD) for 3 h and stained with ethidium bromide and photographed by the gel documentation analysis system (Syngene UK).

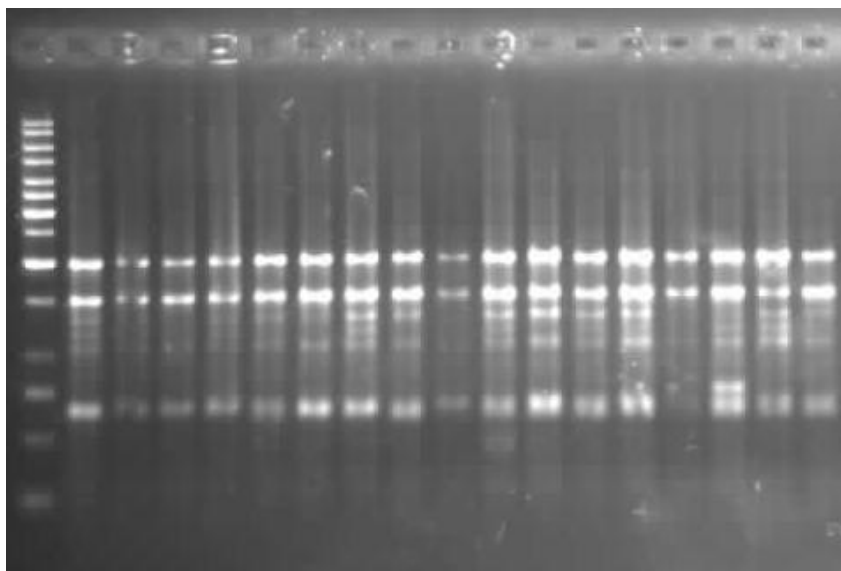


Figure 2. Image of A18 primer (RAPD)

Data analysis: Thirty-seven melon genotypes were examined and polymorphisms among them detected at 121 loci by using 8 RAPD and 10 ISSR primers were employed in the genetic assessment (Table 1). A binary data matrix (presence (1) / absence (0)) obtained from scoring polymorphic bands was used to determine Simple matching (Sokal and Sneath 1963) similarity coefficient to estimate the molecular genetic variation among melon genotypes. The unweighted pair-group method using arithmetic average (UPGMA) cluster analysis, the resulting dendrogram was performed on the genetic distance matrix using the computer program NTSYpc version 2.02k (Rohlf 1997). The computer program POPGENE (Yeh et

al, 1997) was employed to estimate the statistical measures of genetic diversity (i.e., Nei's gene diversity (Nei 1973), Shannon's information index (Shannon and Weaver 1949) and percentage of polymorphic loci) as measured by RAPD and ISSR markers for Lake can Basin melon genotypes.

Results and Discussion

To identify the genetic similarity and diversity among the melon genotypes, total 121 polymorphic markers from 8 RAPD and 10 ISSR primers were employed (Table 1). It was found that within-group genetic similarities varied from 0.55 to 1.00. Similar clusters had the related genotypes or genotypes collected from similar regions. Similarities among genotypes were determined by evaluating their clustering (Figure 3). Based on the molecular SM distance matrix, the most similar genotypes were (YYU53-YYU39); (YYU14-YYU20); (YYU3-YYU15); (YYU8-YYU28-YYU33-YYU34-YYU37-YYU49-YYU50-YYU51); (YYU22-YYU25-YYU38-YYU43); (YYU17-YYU19); and (YYU1 -YYU27). Of all evaluated genotypes, the most diverse one was YYU-31 followed by YYU-1 and YYU-24, while the least discrete ones were YYU-8 followed by YYU-33 and YYU51. According to the molecular dendrogram, YYU-31, YYU-1, YYU-24, and YYU-5 were the most distant genotypes. The other melon genotypes were divided into two main clusters (Figure 3).

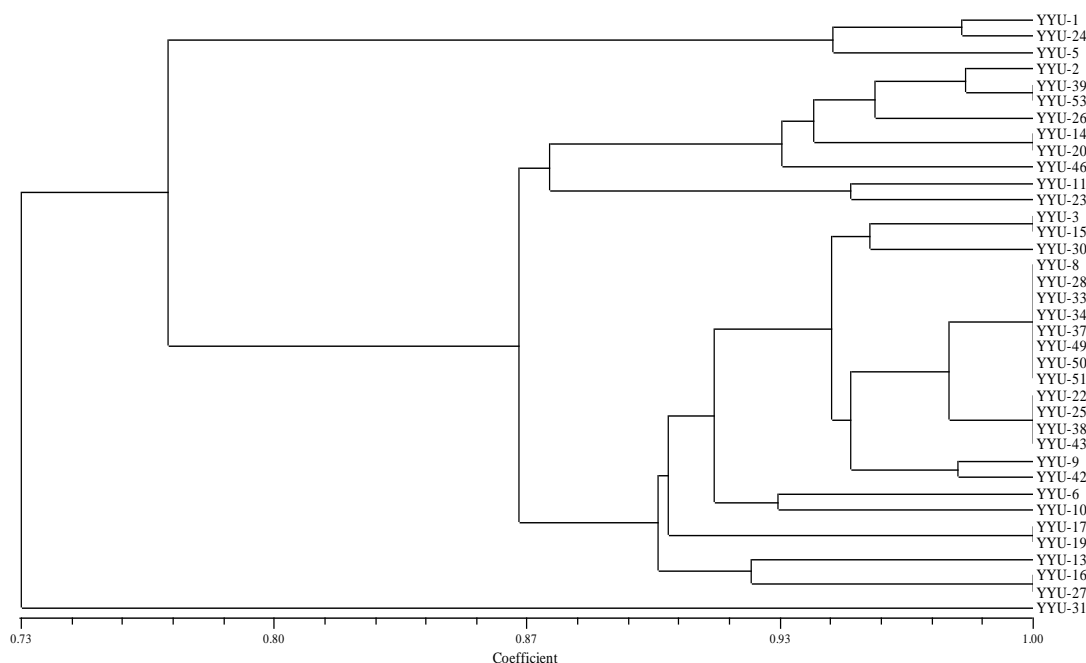


Figure 3. Associations among Lake Van Basin melon genotypes revealed by UPGMA clustering analysis on the basis of the molecular SM distance values.

Up to now, melon genotypes in Africa, America, Asia, and Europe (China, Greece, Hungary, India, Iran, Israel, Italy, Japan, Spain, Myanmar, Turkey, Ukraine, USA, and Vietnam) have been worked out. The RAPD markers were successful in the resolving of genetic resemblance among melon genotypes and were in agreement with the other molecular DNA markers (Garcia et al., 1998; Silberstein et al., 1999; Garcia-Mas et al. 2000; Mliki et al. 2001; Staub et al. 2004; Nakata et al. 2005; Sensoy et al. 2007a; Sheng et al. 2007; Luan et al. 2008; Yi et al. 2009; Nhi et al. 2010; Soltani et al. 2010; Yildiz et al. 2011). RAPD markers were also employed to describe the genetic resemblance among Turkish melon genotypes (Sensoy et al. 2007a). ISSR markers were also successfully employed to assess genetic variability in melon and other crops belonging to Cucurbitaceae family (Dje et al. 2006; Sestili et al. 2008; Dje et al. 2010; Yildiz et al. 2011; Inan et al. 2012)

Based on the statistical variation measures, the genetic diversity among the studied Lake Van Basin melon genotypes was significantly high ($H = 0.175$ and $I = 0.231$, and 96.19 % of polymorphism) (Table 2). Sensoy et al. (2007a) determined the genetic diversity among Turkish melon genotypes as $H = 0.29$, $I = 0.43$ and 90 % polymorphisms. Yildiz et al., (2011) studied ISSR, SRAP, and RAPD markers and determined high diversity among Turkish melon genotypes ($H = 0.28$, $I = 0.43$ and 90.7 % polym.) and

the reference accessions ($H = 0.30$, $I = 0.45$ and 87.6 % polym.). Lopez-Sese et al. (2002) studied the genetic diversity of Spanish genotypes as $H = 0.17$, $I = 0.25$ and 44 % polymorphism. The genetic variation of African melon genotypes were $H = 0.34$ and $I = 0.50$ and 85 % polymorphism (Mliki et al., 2001). Yi et al. (2009) analyzed molecularly (27 RAPD markers) the genetic diversity in Myanmar melon genotypes and determined the genetic diversity as 0.239. Luan et al. (2008) found that Chinese melon genotypes (66 accessions) had high genetic diversity values ($H = 0.33$, $I = 0.49$ and 90.6 % polym.) as Turkish ones. On the other hand Chen et al. (2010) had relatively lower genetic diversity values ($H = 0.22$, $I = 0.34$) in 61 Chinese melon accessions.

Table 2. Statistical measures of genetic variation as measured by ISSR and RAPD markers.

Genotypes	N ^a	H ^b	I ^c	% Polymorphism ^d
Lake Van Basin melon genotypes	37	0.175	0.231	96.19

^aN= Number of genotypes in each population; ^bH= Nei's gene diversity;

^cI= Shannon's information index; ^d: Percentage of polymorphic loci.

Evaluation of germplasm is essential in order to discard identical accessions. Molecular markers have been successfully employed in the determination of genetic similarity among plant genotypes (Gilbert et al. 1999). Local melon genotypes might possess valuable genotypes for different biotic and abiotic stress agents (Demir et al. 2006; Sensoy et al. 2007; Ekbic et al. 2010; Kusvuran et al. 2011). In the present study, relatively high genetic diversity was observed in Lake Van Basin, which might be effectively used in future improvement programs.

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