

# Analysis of Genetic Diversity Between *Eriobotrya japonica* (Thunb.) Lindl. (*Rosaceae*) Populations Growing in the Aegean Region of Turkey

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## Abstract

In this study, we performed a genetic diversity using RAPD (Random amplified polymorphic DNA) and ISSR (Inter-simple sequence repeats) markers for some *Eriobotrya japonica* populations grown in the Aegean region of Turkey. PCR was performed with all DNA samples and primers with ability of scoring band. Phylogenetic analysis and pairwise genetic distance between the populations were calculated by using the PAUP analysis program. According to the RAPD data, a total of 33 bands were obtained and 21 of them were polymorphic. The closest genetic distance (0.09677) was found between Aydın and Nazilli populations, while the most distant value was found 0.44000 between Salihli and Nazilli populations. According to the ISSR data, a total of 43 bands were obtained and 18 of them were polymorphic. The closest genetic distance (0.09302) was found between İzmir and Aydın populations, while the most distant values (0.48276) were found between Denizli and Nazilli populations. As a result of this study, the rate of polymorphism obtained in ISSR technique was found to be lower than the RAPD technique.

**Key Words:** *Eriobotrya japonica*, genetic diversity, ISSR, RAPD

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## Introduction

The *Rosaceae* family currently contains about 100 genera and 3000 species (Potter *et al.*, 2007a; Pang *et al.*, 2011). The family, which generally contains taxa in the form of tree and bush, has herbaceous taxa as well. Cosmopolitan family members mostly spread in the northern hemisphere. The taxa of the *Rosaceae* family spread in almost all kinds of habitat, on the other hand, they can live as a pioneer species in the early stages of forest succession or in forest-front habitat (Hummer and Janick, 2009; Serdar *et al.*, 2014). *Rosaceae* is one of the most economically important families of flowering plants that contain many species grown for their fruits and ornamental plants (Morgan *et al.*, 1994). *Eriobotrya japonica* (Thunb.) Lindl. belongs to the *Rosaceae* family

*Maloideae* subfamily and is a perennial plant. The origin of loquat was China, Japan and North India (Xinaghui *et al.*, 2012; Toker *et al.*, 2010). However, in Cyprus, Egypt, Greece, Israel, Italy, Spain, Tunisia and Turkey is grown loquat also.

According to 2003 data, there are 288,000 loquat trees in Turkey (Taskın and Erdal, 2011). Loquat leaves and fruits are traditionally used to treat coughs and phlegm; and its flower is a perfect source of honey (He *et al.*, 2011). Molecular markers, have a significant role in agricultural reproduction programs. Reaching significant information about the heredity of genetic and economically important plant species in a short period of time has been possible by means of the studies on molecular markers so far (Kalkışım *et al.*, 2016; Aksu and Sahin-Çelik, 2015). Molecular

characterization is conducted with DNA-based molecular markers such as RAPD and ISSR, which are not affected by environmental factors and are more polymorphic (Sharma *et al.*, 2017). RAPD first emerged as a technique which is based on Polymerase Chain Rectification (PCR) with the use of randomly selected primers in 1990 (Williams *et al.*, 1990). It is possible to use these markers to detect DNA polymorphism without the need for pre-determined genetic information (Sharma *et al.*, 2017). RAPD technique is used in population genetic, systematic and taxonomic classification studies (Coşkun and Parlak, 2013). The ISSR technique (Zietkiewicz *et al.*, 1994) is very similar to the RAPD technique, but ISSR primers are designed from microsatellite regions. However, the ISSR technique has more useful information than the RAPD technique. The biggest advantage of the ISSR technique is that it is not necessary to know the genome sequence for the design of primers (Khorshidi *et al.*, 2017). The aim of the study was to determine by use of RAPD and ISSR-PCR techniques for five *Eriobotrya japonica* populations genetic diversity grown in the Aegean region of Turkey.

## Material and Methods

### *Plant Materials, DNA Isolation and PCR*

Five *Eriobotrya japonica* population samples were collected from Denizli, Aydın, İzmir, Manisa-Salihli and Aydın-Nazilli hills and districts of the Aegean region and brought in laboratory silica jellies. Total genomic DNA isolation from the leaves of *Eriobotrya japonica* was performed using commercial kit (GeneMark). DNA samples were stored -20 °C. In order to visualize gDNA's 1.0% standard agarose gel electrophoresis procedure was performed. For RAPD-PCR amplification six RAPD primers were used (Tab. 1). RAPD amplification reactions were carried out in 25 µl volume containing 5 µL master mix [Cat. No: RP02-II-400, RP02-II-2000 (0.75U of Taq DNA polymerase, reaction buffer, 2mM MgCl<sub>2</sub>, 250µM dNTPs and enzyme stabilizer)], 1 µL RAPD primers, 2.0 µl of total genomic DNA (1/5 rates diluted), and 17µL of dH<sub>2</sub>O. The RAPD-PCR protocol is shown in Table 1. For ISSR-PCR amplification eight ISSR primers were used (Tab. 2). ISSR amplification reactions were carried out in 25 µl volume containing 5 µL master mix [Cat. No: RP02-II-400, RP02-II-2000 (0.75U of Taq

**Table 1.** Primers used in the RAPD-PCR reactions and PCR Amplification

Primer	DNA Sequences	PCR Amplification (35 Cycles)
OPA-15	5'-TTCCGAACCC-3'	
OPA-02	5'- TGCCGAGCTG -3'	94°C/2 min
OPE-08	5'-TCACCACGGT-3'	94°C/1 min
OPA-16	5'-AGCCAGCGAA-3'	32-34°C/1 min
OPA-18	5'-AGGTGACCGT-3'	72°C/1 min
OPA-13	5'- CAGCACCCAC-3'	72°C/10 min

**Table 2.** Primers used in the ISSR-PCR reactions and PCR Amplification

Primer	DNA Sequences	PCR Amplification (35 Cycles)
UBC-831	5'-CTCTCTCTCTCTCTT-3'	
UBC-830	5'-TGTGTGTGTGTGTGG-3'	
UBC-807	5'-AGAGAGAGAGAGAGT-3'	94°C/1 min
UBC-826	5'-ACACACACACACACC-3'	94°C/1 min
UBC-819	5'-GTGTGTGTGTGTGTA-3'	50-52°C/1 min
UBC-853	5' - TCTCTCTCTCTCRT-3'	72°C/1 min
UBC-855	5'-ACACACACACACACYT-3'	72°C/10 min.
UBC-856	5'-ACACACACACACACYA-3'	

DNA polymerase, reaction buffer, 2mM MgCl<sub>2</sub>, 250µM dNTPs and enzyme stabilizer], 1 µL ISSR primers, 2.0 µl of total genomic DNA (1/5 rates diluted), and 17µL of dH<sub>2</sub>O. The ISSR-PCR protocol is shown in Table 2. Amplification products were analyzed by electrophoresis on 1.0% agarose gel buffered with 0.5X TBE (Tris-Borate-EDTA).

#### Data Analysis

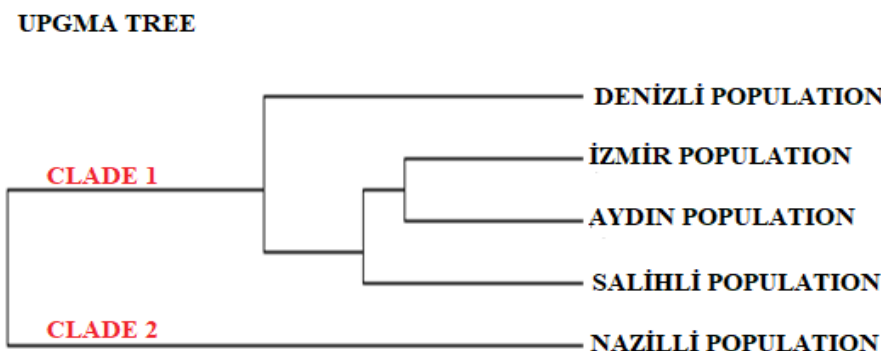
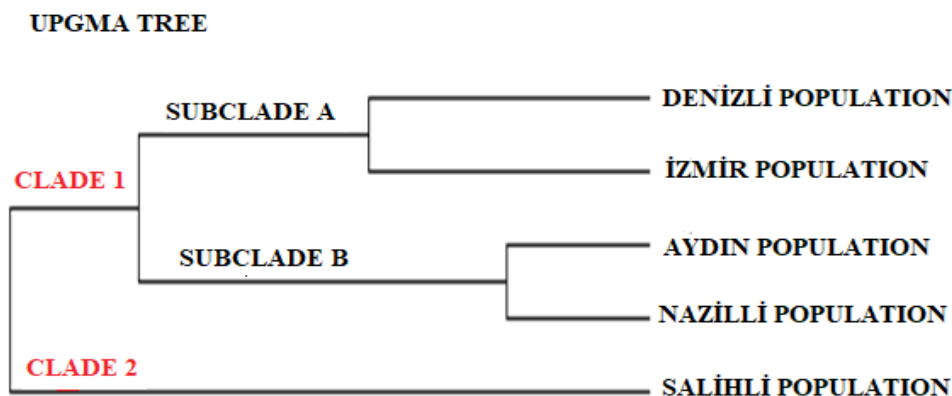
The images were used for the evaluation of the results in the analysis of RAPD and ISSR-PCR. In the analysis of the bands formed at the end of the amplification, only the strong bands were taken into consideration. Following the PCR analysis, DNA bands were scored as '1' in the presence of DNA, '0' in the absence of DNA and a '?' for missing data. PAUP 4.0b10 software was used to perform phylogenetic analyses using RAPD and ISSR data (Swofford, 2001).

### Results and Discussion

DNA-based markers have many advantages in evaluating genetic diversity (Alansi *et al.*, 2016). RAPD and ISSR markers are two polymerase chain reaction (PCR) based techniques commonly used

to predict genetic diversity in many plant species (Giachino, 2020). In the RAPD-PCR analysis, a total of 33 bands were obtained, 21 of them were polymorphic and the rate of polymorphism was approximately 63.63%. PAUP 4.0b10 analysis program was used to calculate the phylogenetic trees and genetic distances between populations. According to the PAUP analysis, the closest genetic distance was found 0.09677 between Aydın and Nazilli populations, while the most distant values was found 0.44000 between Salihli and Nazilli populations (Tab. 3). The phylogenetic tree was obtained using the UPGMA algorithm, and the tree consisted of two clades (Fig. 1).

The clade 1 consisted of two subclades. Subclade A consists of Denizli and İzmir populations, subclade B consists of Nazilli and Aydın populations. Clade 2 consisted only of Salihli population. In the ISSR PCR analysis, 43 bands were obtained, 18 of them were polymorphic and the rate of polymorphism was about 41.8%. PAUP 4.0b10 analysis program was used to calculate the phylogenetic trees and genetic distances between populations. According to the PAUP analysis,



the closest genetic distance was found 0.09302 between İzmir and Aydın populations, while the most distant values were found 0.48276 between Denizli and Nazilli populations (Tab. 4). The phylogenetic tree was obtained using the UPGMA algorithm, and the tree consisted of two clades (Fig. 2). The first clade consisted of Denizli, İzmir, Salihli and Aydın populations, and the second clade consisted only of Nazilli population.

In the past years there have been many genetic diversity and phylogenetic studies on loquat genotypes using different marker techniques. Vilanova *et al.* (2001) studied the genetic diversity of loquat cultivars in Spain using the RAPD markers. In the study, the researchers found that RAPD technology is useful in distinguishing cultivars obtained through hybridization. Hussain *et al.* (2009) detected the genetic diversity of 42 loquat genotypes spread in Pakistan using the RAPD marker technology. The study proved that RAPD markers are a reliable method to reveal the relationship between loquat genotypes. Xie *et al.* (2007) identified phylogenetic analyses of 17 species of *Eriobotrya* genus, which spreads in China, using ISSR marker technology. The study also revealed that it was closely related to *E. japonica* and *E. prinoides* var. *dadunensi*. Yang *et al.* (2009) investigated the genetic relationship of 18 species of *Eriobotrya* using AFLP marker technique.

The result of the cluster analysis was generally compatible with the known taxonomic grouping. In addition, the cluster of *Eriobotrya*'s 18 accessions is consistent with the systematic classification based on morphological features. Soriano *et al.* (2005) detected the genetic diversity of 40 cultivars of *Eriobotrya japonica* obtained from different countries using SSR marker technology. As a result of the study, the researchers showed that the SSRs developed for *Malus × domestica* (Borkh.) are suitable for the identification of the loquat variety, confirming intergenus transferability. Xinaghui *et al.* (2012) investigated phylogenetic relationships of *Eriobotrya* genus on the basis of nrDNA *Adh* sequences. The study also includes *E. prinoides* var. *dadunensis* and *E. japonica* were identified within the same group. It was concluded that some species cannot be separated although the *Adh* gene sequence has a high genetic diversity in *Eriobotrya*.

### Conclusion

As a result, the genetic diversity of the loquat populations spreading in the Aegean region was determined by RAPD and ISSR markers. As a result of this study, the rate of polymorphism obtained in ISSR technique was found to be lower than the RAPD technique. On the other hand, the obtained results will be a source for further genetic and

**Table 3.** Pairwise genetic distance matrix obtained from RAPD primers in five Turkish loquat populations

Population	1	2	3	4	5
Denizli	-	0.18182	0.33333	0.36364	0.38710
İzmir	6	-	0.40741	0.36364	0.32258
Salihli	9	11	-	0.40741	0.44000
Aydın	12	12	11	-	0.09677
Nazilli	12	10	11	3	-

**Table 4.** Pairwise genetic distance matrix obtained from ISSR primers in five Turkish loquat populations

Population	1	2	3	4	5
Denizli	-	0.10345	0.17241	0.24138	0.48276
İzmir	3	-	0.11628	0.09302	0.27907
Salihli	5	5	-	0.11628	0.30233
Aydın	7	4	5	-	0.18605
Nazilli	14	12	13	8	-

molecular analyses of *Eriobotrya japonica* populations.

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