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Genetic Diversity of *Elaeagnus angustifolia* L. (Elaeagnaceae) Populations in İzmir (Turkey)

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RESEARCH ARTICLE

Abstract

This study was performed out genetic diversity of some *Elaeagnus angustifolia* L. populations growing in İzmir province by using ISSR markers. In the study, PCR was performed using 15 ISSR primers. PCR products were run in agarose gel and visualized under UV light. Amplified products were scored as follows. A total of 46 bands were produced from 15 ISSR primers, of which 27 were polymorphic. The proportion of polymorphic bands was evaluated as approximately 58.7%. Genetic distances between phylogenetic trees and genotypes were calculated using the PAUP program. The phylogenetic tree consists of two large clades. The longest distance between populations was between Gümüldür-Özdere and Çeşme-Alaçatı population with a value of 0.50, while the closest distance was between Çeşme-Ayayorgi and Konak-Hatay populations with a value of 0.06. The results show that ISSR markers are useful tools for determining genetic relationships between *E. angustifolia* populations.

Keywords: *Elaeagnus angustifolia;* ISSR; genetic diversity; Turkey.

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INTRODUCTION

Turkey has a variety of climates ranging from subtropical to cold-temperate. Due to these different conditions, the country has significant plant genetic diversity and there are many fruit tree taxa in the country (Uzun et al., 2015). The family of Elaeagnaceae has three genus as being *Elaeagnus* L., *Hippophae* L. and *Shepherdia* Nutt, and has 77 species worldwide (Khadivi, 2018; Asadiar et al., 2013; Sun and Lin, 2010). Also known as oleaster or Russian olive, *Elaeagnus angustifolia* L. belongs to the *Elaeagnus* genus of the Elaeagnaceae family (Carradori et al., 2020; Hamidpour et al., 2017). *E. angustifolia* is a fast-growing tree growing up to 10 m in height and a trunk diameter of up to 30 cm (Uzun et al., 2018) This species, known as oleaster, is found in the central and western regions of the Asian continent, in Gobi Desert, in the Alps, and around the Mediterranean; and it is present in all of the Black Sea, Marmara, South Anatolia and South East Anatolia regions of our country (Göktürk et al., 2007). In Turkey, *E. angustifolia*, whose cultivated form is called oleaster and the natural form is called Russian olive, grows naturally (Gülcü and Uysal, 2010). Fruit of *E. angustifolia*, which is an ornamental tree, have been used as a source of food and pharmaceutical industry (Yagmur et al., 2020; Karami and Piri, 2009). In addition, its fruits are rich in vitamins and minerals (potassium, sodium and phosphorus) such as tocopherol, terpenoids, tannins, amino acids, flavonoid, saponins, vitamin C, B1, glucose, fructose, and α-carotene and are traditionally used in folk medicine as analgesic, in gastrointestinal system diseases, and for its antipyretic and diuretic activities

(Khadivi et al., 2020; Carradori et al., 2020; Hassanzadeh and Hassanpour, 2018; Abizov et al., 2008). However, it is known that substances such as tea, animal feed, pulp and seed pulp are produced from its leaves, and jams and drinks are produced from its fruits (Şahin and Altuntaş, 2018). It has also been used to treat a variety of ailments such as nausea, vomiting, bloating, stomach ailments, asthma, and hepatitis (Incilay, 2014). Molecular marker physically corresponds to a location in the genome and plays an important role in diversity studies, germplasm characterization, phylogenetic analysis, linkage mapping (Mehri et al., 2020; Mukherjee et al., 2013; Behera et al., 2008). With the advent of molecular techniques, DNA-based procedures have been proposed for variety identification (Hu and Quiros, 1991). ISSR (Inter-simple sequence repeats) technique, is applicable to organisms from diverse genera and because of the large number of primers available for analyses, it provides good overall genome coverage (Andiego et al., 2019). This technique, has been successfully used in plant genetics for genome fingerprinting, genetic diversity, phylogenetic analysis, and molecular breeding in marker assisted selection (Doğan et al., 2016; Tang et al., 2015; Son et al., 2012). The aim of this study was to perform a genetic diversity using ISSR markers for some *E. angustifolia* populations grown in the İzmir city of Turkey.

MATERIALS AND METHODS

Plant Materials, Genomic DNA Isolation and PCR

Leaf samples belonging to 11 *E. angustifolia* populations used in this study were collected from different districts of İzmir province of our country (Figure 1).

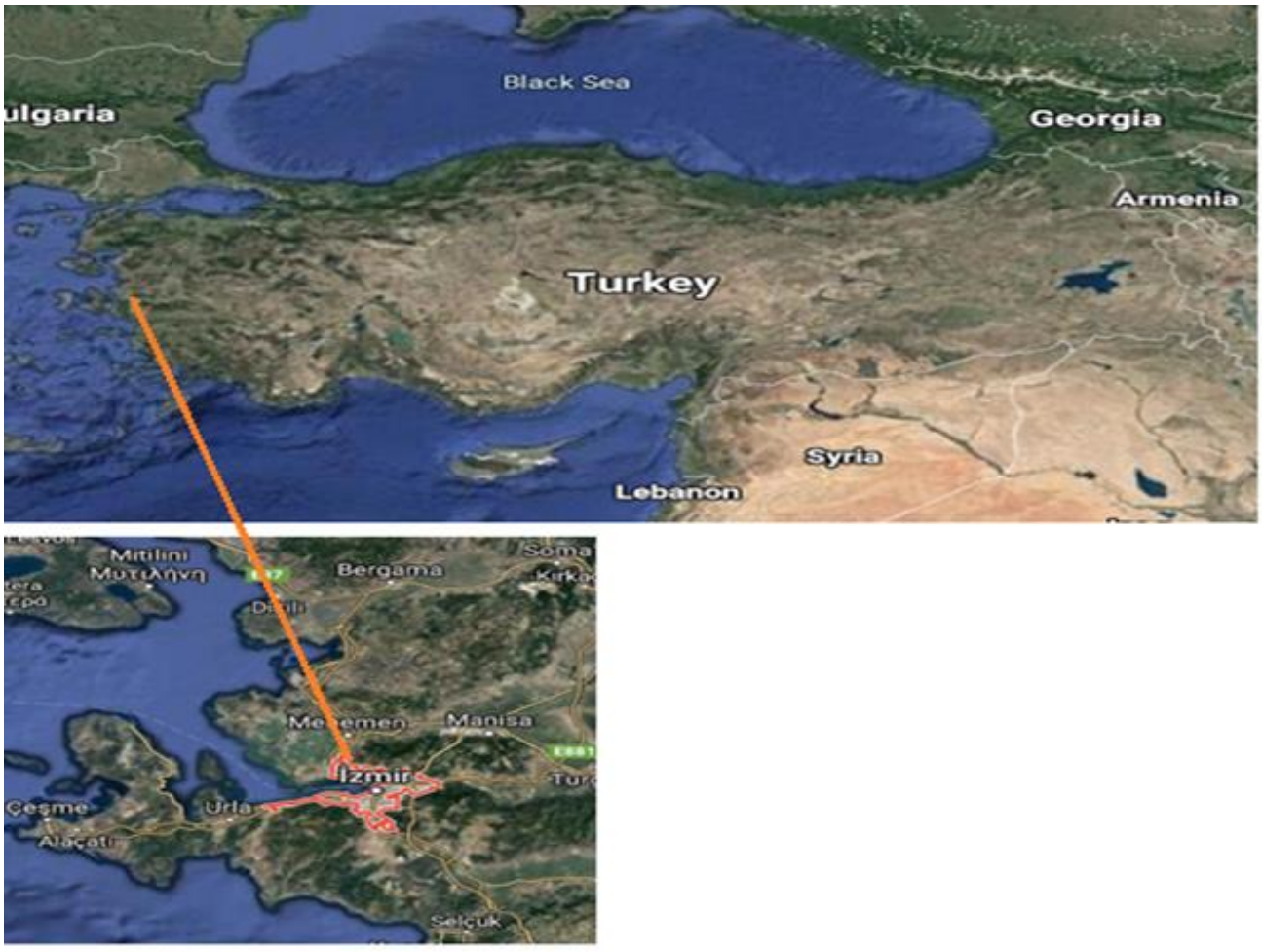

Figure 1. Location of the Izmir
(https.//www.google.com/maps_

Collected leaf samples were brought to the laboratory and prepared for genomic DNA isolation. Genomic DNA isolation was performed from plants using commercial kit (GeneMark, Cat No: DP022) as genomic DNA isolation method. The acquired gDNAs were stored at -20 degrees. PCR amplifications and selected ISSR primers for PCR amplifications are given in Table 1 and Table 2.

Table 1. Primers used in the ISSR-PCR reactions and their Tm degrees

Primers	DNA sequences (5'-3')	Tm°C
UBC-855	5'-ACACACACACACACYT-3'	52°C
UBC-810	5' -GAGAGAGAGAGAGAGAT-3'	50°C
UBC-826	5'-ACACACACACACACCACC-3'	52°C
UBC-856	5'–ACACACACACACACACYA-3'	52°C
UBC-807	5'-AGAGAGAGAGAGAGAGT-3'	50°C
UBC-830	5'-TGTGTGTGTGTGTGTGG-3'	52°C
UBC-808	5'-AGAGAGAGAGAGAGAGC-3'	52°C
UBC-836	5'-AGAGAGAGAGAGAGAGYA-3'	52°C
UBC-853	5'-TCTCTCTCTCTCTCT CRT-3'	52°C
UBC-892	5'-TAGATCTGATATCTGAAT-3'	52°C
UBC-834	5'-AGAGAGAGAGAGAGAYT-3'	52°C
UBC-831	5'-CTCTCTCTCTCTCTCTT-3'	50°C
UBC-818	5'-CACACACACACACACAG-3'	47°C
UBC-811	5'-GAGAGAGAGAGAGAGAC-3'	53°C
UBC-873	5'- GACAGACAGACAGACA-3'	48°C

Table 2. Cycles and conditions of ISSR-PCR reactions

Pre-heating	94 °C	4 min	1 cycle
1. step	94°C	1 min	
2. step	47-53°C	1 min	35 cycles
3. step	72°C	1 min	
4. step	72°C	10 min	1 cycle
5. step	4°C	20 min	

ISSR amplification reactions were carried out in 25 µL volumes containing 5 µL master mix (PCR buffer, MgCl2, dNTPs, Taq DNA polymerase), 1 µL ISSR primers, 2 µL of total genomic DNA, and 17 µL of sterilized dH2O. 1,5 % agarose gel TBE buffer was used for electrophoresis of PCR products. For the gel; 1,5 g agarose and 100 ml 1X TBE were combined and melted in microwave for 1 min. After the mixture was removed from the microwave, Ethidium Bromide (0.1 µg/ml) was added as dye and poured into the gel preparation plate of the horizontal type electrophoresis device. 5 µl of the reaction mixture in PCR tubes was added to 1 µl loading buffer (Loading Dye Solution) and mixed and 6 µl of this mixture was placed in the wells in the gel. After loading a 3 kb DNA marker into the first well, the device was subjected to electrophoresis at 90 V for 90 minutes. Then, the DNA bands were imaged under UV light and their photos were taken. Some gel picture of ISSR marker region shown in Figure 2.

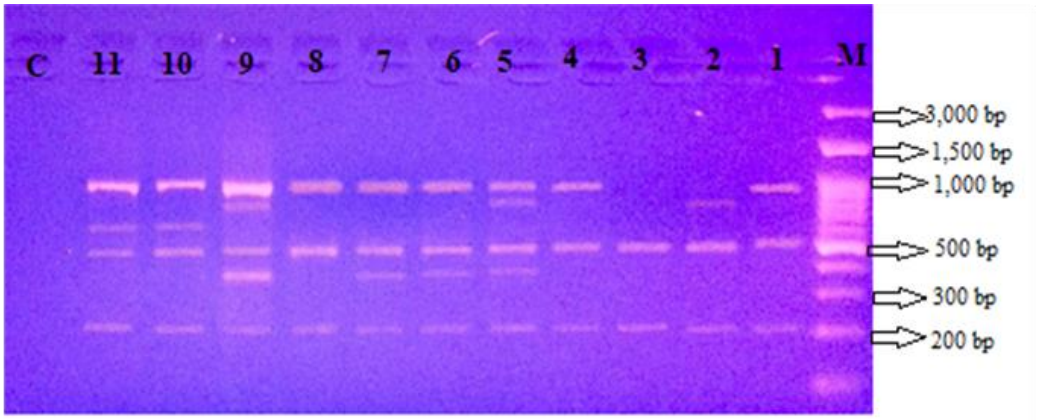

Figure 2. Gel image of ISSR-PCR band amplified with UBC-811

ISSR Analysis

After the PCR analyses, DNA bands were scored as follows: "1" was given if there is DNA in the DNA bands, "0" was given if there is no DNA, and "?" was given for missing data; and monomorphic bands were discarded and ISSR analyses were performed on polymorphic bands. Genetic relationship of *E. angustifolia* populations used in the study was analyzed using the PAUP 4.0b10 (Swofford, 2001) program, and genetic matrix between populations was revealed by drawing UPGMA phylogenetic tree of the same program according to the arithmetic means of the pedigree trees.

RESULTS AND DISCUSSIONS

Genetic diversity is the key to plant breeding programs, general measured by genetic distance or genetic similarity (Behera et a*l*., 2008; Fu et al., 2008). DNA markers provide an opportunity to measure genetic relationships between genotypes (Souframanien and Gopalakrishna, 2004). ISSR markers are a simple, efficient and repeatable marker system with high polymorphism, this marker is useful for fingerprinting, mapping, and taxonomic and phylogenetic analysis (Souza et al., 2017; Gürcan and Mehlenbacher, 2010).

In the study, 15 ISSR primers were used and a total of 46 bands were obtained. Of these bands, 19 were monomorphic and 27 were polymorphic. The rate of polymorphism is approximately 58.7%. The UPGMA phylogenetic tree, consists of two major clades. Clade 1 divide into two subclades. Subclades A, consists of Seferihisar- Sığacık, Urla Demircili village, Çeşme- Ayayorgi, Konak-Hatay, Urla-Zeytinalanı. Subclade B consists of Bornova-Çamdibi, Gümüldür-Özdere and Mordoğan populations. Clade 2, consists of Çeşme-Alaçatı, Urla-Kuşçular and Balçova populations (Figure 3).

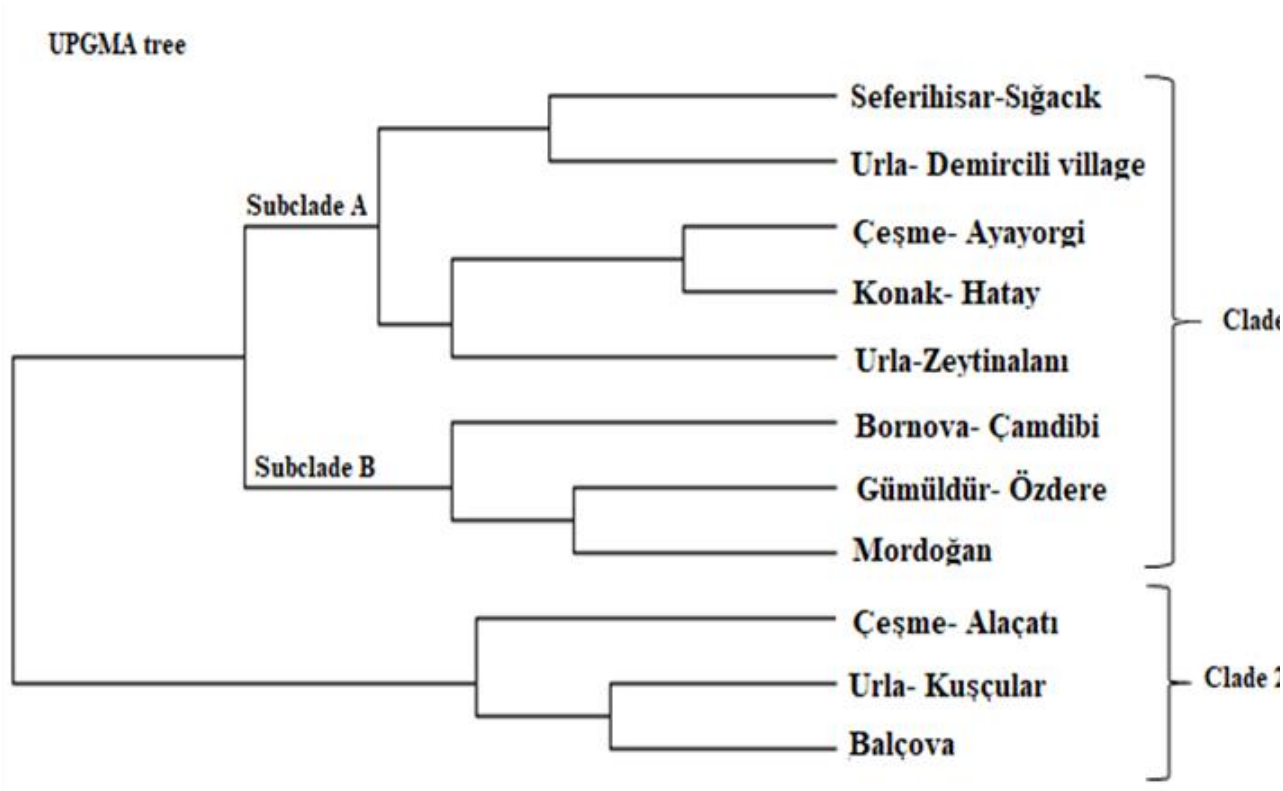

Figure 3. The UPGMA tree generated using ISSR data of *Elaeagnus angustifolia* populations

The longest distance between populations was between Gümüldür-Özdere and Çeşme-Alaçatı population with a value of 0.50, while the closest distance was between Çeşme-Ayayorgi and Konak-Hatay populations with a value of 0.06. (Table 3).

Table 3. Pairwise genetic distance matrix obtained from PCR with ISSR primers

Populations	1	2	3	4	5	6	7	8	9	10	11
Seferihisar-Sığacık (1)	-	0.17	0.13	0.12	0.27	0.22	0.32	0.32	0.45	0.41	0.27
Çeşme- Ayayorgi (2)	6	-	0.06	0.23	0.17	0.15	0.35	0.29	0.33	0.38	0.30
Konak- Hatay (3)	4	2	-	0.20	0.20	0.17	0.30	0.23	0.38	0.33	0.26
Urla- Demircili village (4)	5	8	6	-	0.18	0.25	0.27	0.20	0.46	0.35	0.27
Bornova- Çamdibi (5)	11	6	6	8	-	0.20	0.18	0.15	0.34	0.39	0.33
Urla-Zeytinalanı (6)	8	5	5	10	8	-	0.35	0.27	0.30	0.30	0.34
Gümüldür- Özdere (7)	13	12	9	12	8	14	-	0.11	0.50	0.46	0.42
Mordoğan (8)	13	10	7	9	7	11	5	-	0.46	0.35	0.33
Çeşme- Alaçatı (9)	10	6	7	12	9	8	13	12	-	0.14	0.17
Urla- Kuşçular (10)	10	7	6	10	11	8	13	10	4	-	0.10
Balçova (11)	8	7	6	9	11	10	14	11	5	3	-

In previous studies, Uzun et al. (2015) determined the genetic variation of 56 *E. angustifolia* populations from various districts and altitudes of Kayseri province, located in Central Anatolia region of Turkey, by using RAPD and ISSR markers. They used seven RAPD and fifteen ISSR primers in their study and obtained 74 bands for RAPD analysis and 135 bands for ISSR analysis. In the RAPD results, 67 polymorphic bands were obtained, the polymorphism rate was approximately 90%; and 115 polymorphic bands were obtained in ISSR results, and they determined the polymorphism rate as approximately 85%. By combining RAPD and ISSR data, they determined the genetic distance between the populations between 0.34 and 0.00. Uzun et al., (2018) determined the molecular characterization of 25 *E. angustifolia* populations, collected from Nevşehir, Aksaray, Konya, Sivas, Malatya, Adıyaman and Kahramanmaraş provinces of Turkey, by using the ISSR marker system. In their study, they produced a total of 92 fragments from 11 ISSR primers and revealed that 23 of them were polymorphic and the rate of polymorphism was 25%. They found through the UPGMA analysis that the similarity index was between 0.63 and 1.00. Talebi-Rad et al., (2015) determined the genetic diversity of *E. angustifolia* populations from five different points of the Eastern Azarbeycan state of Iran through RAPD markers. In their study, they identified a total of 29 polymorphic bands ranging from 100 to 1500 bp. They found the rate of polymorphism as approximately 97.22%. In their study results, they revealed that RAPD analysis showed that it is an appropriate method to examine genetic diversity and relationships between *E. angustifolia* populations. Asadiar et al., (2012) determined the genetic variation of *E. angustifolia* populations collected from nine different locations in the West Azarbeycan province of Iran through RAPD markers. In their study, they used nine RAPD primers and obtained a total of 122 bands. A total of 103 of these bands were polymorphic, and they determined the polymorphism rate as approximately 84.4%. Also, the estimated Jaccard's similarity index ranged from 0.36 to 0.62 for the RAPD marker. As a result of their studies, they revealed that the RAPD marker is a useful tool for evaluating genetic diversity within the *Elaeagnus* genus. Asadiar et al. (2013) determined the genetic relationship and polymorphism levels of nine *E. angustifolia* populations collected from nine different locations in the West Azarbeycan province of Iran through ISSR markers. In their study, they used 11 ISSR primers and obtained 116 bands. 92 of these bands were polymorphic and the rate of polymorphism was determined as approximately 79.3%. The estimated Jaccard's similarity index ranged from 0.44 to 0.76 for the ISSR marker. As a result of the study, they revealed that it can be concluded that ISSR markers are suitable tools for evaluating genetic diversity and relationships within the *Elaeagnus* genus. Yingthongchai et al., (2014) determined the genetic diversity and phylogenetic analysis of 88 *E. latifolia* populations collected from the North of Thailand through the ISSR marker system. 14 selected primers were used in the study, 278 bands were obtained in total and 264 of these bands were found to be polymorphic. The rate of polymorphism in their work was determined to be approximately 94.96%. They also found that Jaccard's similarity index or genetic similarity (G-Sj) ranged from 0.50-0.79. In their study, they revealed that the ISSR marker system can be used to distinguish the relationship between *E. latifolia* populations and to evaluate the genetic relationship.

CONCLUSIONS

This study has revealed genetic diversity of *E. angustifolia* populations distributed in İzmir city of Turkey by using ISSR technique. Approximately 58.7 % polymorphism was detected among *E. angustifolia* ISSR analysis. As a result, the outcomes of the research, which aimed to analyze the genetic diversity of *E. angustifolia* populations in different localities through ISSR molecular marker, will be used in the development of more efficient new varieties, particularly for breeding, through the association with many different specific characters of *E. angustifolia* populations

Author Contributions: E.S. Collecting plant samples. ES, E.S and H.U. experimental studies and data analysis. E.S. Wrote the paper and conceived and designed the analysis.

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Conflicts of Interest

The authors have no conflict of interest to declare

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