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Assessment of Genetic Relations among Cultivated Olives by ISSR Markers

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ABSTRACT

Olive grows in the mid-latitudes in the world and in areas with Mediterranean climate. Olive is one of the important agricultural products in Mediterranean Basin and Turkey. Its contribution to economy makes it valuable to determine the genetic relations among different olive varieties. In this study, genetic variations among 13 different olive cultivars were determined through 22 ISSR primers by obtaining valuable bands from 7 of them. The cultivars were mainly Turkish, namely, Edremit, Gemlik, Domat, Uslu, Çilli, Eşek, Kaba, Çekişte Nazilli, Memecik, Tavşan Yüreği, Halhalı, Manzanilla, and Çekişte Bozdoğan, grown in different regions. Totally, 92 bands were obtained and it was observed that all of these were polymorphic. Polymorphic Information Content (PIC), Marker Index (MI), and Resolving Power (RP) averaged 0.19, 2.36 and 2.73 per primer, respectively, showing the high efficiency and reliability of the markers used. In conclusion to ISSR markers analyses as the number of polymorphic loci, genetic diversity and olive relationships through UPGMA and Neighbor Joining dendrogram based on Nei's genetic distance and PCO, it was observed that Tavşan Yüreği and Halhalı varieties formed one set and Çekişte Nazilli and Manzanilla varieties formed another set together.

Keywords: Neighbor Joining, *Olea europaea sativa* L., Principal coordinate analysis, UPGMA.

INTRODUCTION

There is no exact information as to how many years have olive trees been grown on the earth, but it is known that wild olives (*Olea europaea oleaster* L.) have existed in Anatolia for thousands of years. *Olea europaea sativa* L., i.e. cultivated olives, are obtained by grafting the wild olives. Olive is a member of *Oleaceae* (Olive family). There are approximately 600 varieties in the world (Efe *et al*., 2013).

As it is known, cultivated olive trees are used for two products: table olives and olive oil production. Olive trees are also used for obtaining various products from their leaves, fruits, and seeds. The branches of trees are used to make spoons, ladles, plates, and toys, while woods are used for heating. Olive leaves are used in traditional treatment and also in carpets as dye material. Olive oil is exploited in the cosmetics sector with products such as olive oil shampoo, soap, care cream, mask, shower gel, etc. Olive cake (oil cake) that is obtained during olive oil production is used for heating (Anonymous, 2015).

Turkey ranks the second country in table olive production in the world following Spain, and it is the fourth in olive oil production (Karabulut, 2013). Olive cultivation is among high level agricultural activities in especially Aegean and Marmara Regions in Turkey. Olive is also grown in Mediterranean, Black Sea, and Southeast Anatolia regions. Turkey, being the homeland of olive, is quite rich of olive varieties (Varol *et al*., 2009).

It is also important to perform studies with regards to the genetic specifications of olive,

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providing quite high proceeds to the economies of countries. Many researchers have recently conducted studies for the purpose of determining genetic diversity among olive varieties by using ISSR (Inter-Simple Sequence Repeat) markers, determining clonal variations, and realizing cultivar identification through DNA markers. Martins-Lopes *et al*. (2007) scanned Portuguese and imported olive varieties through RAPD and ISSR primers and studied genetic diversity and performed identification of varieties. Gomes *et al*. (2008) used SSR, RAPD and ISSR markers in determining variation among the clones of Verdeal-Transmontana, which is a Portuguese olive variety, and identified diversity among the clones.

ISSR markers are used for evaluating genetic variation, molecular characterization, genetic diversity and relationship studies in wild and cultivated olives. El Saied *et al*. (2012) evaluated genetic variation among twenty-two olive varieties from Egypt by using ISSR markers (12 local and 10 imported varieties), and reported the usefulness of ISSR markers. They also noted that ISSR markers could be used for genetic characterization and cultivar identification. Wild olives are considered to be more resistant to environmental conditions and diseases with the high genetic diversity in their germplasm. Noormohammadi *et al*. (2012) determined genetic variation in wild olives grown in the west and south of Iran (*O. europaea subsp. cuspidata)* by using SSR and ISSR markers. Hegazi *et al*. (2012) also determined molecular characterization through ISSR primers in their study performed on local and imported olive varieties in Egypt. In evaluation of genetic relations and diversity between olives, Linos *et al*. (2014) used RAPD, ISSR, and SSR markers in Greek olive germ plasm, Brake *et al*. (2014) studied 13 olive varieties grown in Jordan through RAPD and ISSR markers using 15 RAPD and 14 ISSR primers, and Zhan *et al.* (2015) made research on genetic similarity in olives imported to China by using SSR and ISSR method. Kaya (2015) used ISSR method to determine genetic relations among some Turkish cultivars revealing high genetic variation. Souza *et al*. (2012) used SSR and ISSR markers to estimate cross-pollination rates of olive genotypes. Moreover, ISSR can be applied not only on leaves and similar plant material, but also on drupes, as sometimes needed at oil-mill level for checking the authenticity of high quality productions. In this regard, Pasqualone *et al*. (2001) demonstrated the possibility to identify lots of drupes from 10 different olive cultivars by means of 2 ISSR primers.

Genetic diversities do not show specific phenotypic effect; and they may result in the development of different forms of genetic expression product as well. Polymorphism means the existence of two or more forms of any characteristic in a population at levels that cannot be explained through mutations developed simultaneously. To consider a polymorphic locus as a genetic marker, it must show variation of a specific level within the population, it must be repeatable, and must show a hereditary feature (Elmacı and Asal, 1995; Düzgüneş *et al*., 1987). ISSR is one of these molecular markers, applied to olive species and its end-products together with several other classes of molecular markers, as recently reviewed by Pasqualone *et al*. (2016).

GA CT AG AC TC and CA repetitive primers used in ISSR show polymorphism higher than 3 or 4 repetitive primers among other 2 repetitive primers. The bands obtained through ISSR generally show a dominant characteristic and sometimes it is observed that they provided codominant markers. ISSR markers are used for an array of applications: genomic fingerprint, genetic expansion, phylogenic analyses, SSR motive frequency determination, gene marking and marker based selection, determination of genetic diversity in natural populations, discrimination of close relative types, and marking regions rich of genes (Altınkut Uncuoğlu, 2010).

The aim of this study was to reveal the genetic relationships based on Nei's genetic distance and clustering data among 13 olive cultivars, mainly Turkish, obtained from ISSR markers.

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MATERIALS AND METHODS

Plant Materials

The cultivated olive varieties selected for the determination of genetic relationship among them were supplied from the sources as follows: Edremit, Gemlik, Domat, Uslu, Manzanilla from Aegean Olive Cultivation Research Institute, Bornova; Çilli from Yamalak; Aydın, Eşek from Büyükbelen, Saruhanlı; Memecik from Yamalak, Aydın; Kaba from Yamalak, Aydın; Çekişte from Nazilli, Aydin; Çekişte from Bozdoğan, Aydın; Tavşan Yüreği from producers in Isparta; and Halhalı from Southeastern Anatolia Project Agricultural Research Institute, Şanlıurfa.

DNA Extraction

The leaf samples were collected and frozen by liquid nitrogen immediately before DNA isolation. The samples were ground with chilled mortar and pestle. Doyle and Doyle method modified according to the laboratory conditions were used in the extraction of olive DNA (Doyle and Doyle, 1987, 1990).

Following the isolation of olive DNAs, spectrophotometric methods were used in the determination of DNA quantity. Spectrophotometric analyses were performed at 260 nm in Gamma Helios spectrophotometer. Stock DNAs were diluted to contain 5 ng μL^{-1} DNA as preparation in PCR processes and placed in deep freezer (-20^{o}C).

PCR and ISSR procedure

In polymerase chain reaction, the primers of UBC (University of British Columbia) and IMA were used as ISSR primers. Lyophilized primers were diluted in the way suggested by the company. Among the PCR components, dNTP (deoxynucleotide triphosphate) stock solution was prepared in a way to have each dNTP (dATP, dTTP, dGTP, dCTP) as 0.2 mM. Olive DNA extracted as 25 ng in PCR was used as template. The components were added in 0.5 mL PCR tubes on ice while making preparations for the polymerase chain reaction and placed in thermal cycler (Eppendorf MasterCycler). ISSR primers were tested suggesting by Brake *et al*. (2014). The components and quantities used in polymerase chain reaction, the characteristics of UBC primers are shown in Table 1.

Annealing temperatures were adjusted and used based on the melting temperatures of ISSR primers if it's required. The amplifications were carried out in Eppendorf MasterCycler with following PCR conditions: 94°C, 5 minutes for initial denaturation; for 45 cycles, 94°C, 30 seconds; 52°C, 45 seconds; 72°C, 2 minutes; and final extension at 72°C, 5 minutes (Martins-Lopes *et al*., 2007).

The products reproduced as a result of polymerase chain reaction were subjected to agarose-gel electrophoresis to separate DNA, based on their sizes. In electrophoresis, 1.2% agarose-gel was used.

Data and Statistical Analysis

The gel image transferred to computer was evaluated and a data matrix was formed to show 1 in the presence of ISSR bands and 0 in the absence of ISRR bands. Each band was evaluated and processed as an independent locus in the samples. BioOneD^{++} program was used in the determination of molecular weights as base pair while evaluating the bands (Vilber Lourmat, France). UPGMA and Neighbor Joining trees using Nei's genetic distance were found out through MEGA6 program (Tamura *et al*., 2013) and Nei's genetic distance matrix (Nei, 1972) were calculated with POPGENE 1.32 (Yeh *et al*., 1997) program.

[DOR: 20.1001.1.16807073.2017.19.6.3.8]

Table 1. ISSR primers and characteristics.

Primer	Base sequence (5'-3')	Mol Weight (MW)	Melting temp (°C)
UBC 808	*AGA GAG AGA GAG AGA GC*	5366	52.8
UBC 809	*AGA GAG AGA GAG AGA GG*	5406	52.8
UBC 810	*GAG AGA GAG AGA GAG AT*	5381	50.4
UBC 811	*GAG AGA GAG AGA GAG AC*	5366	52.8
UBC 812	*GAG AGA GAG AGA GAG AA*	5390	50.4
UBC 817	*CAC ACA CAC ACA CAC AA*	5070	50.4
UBC 818	*CAC ACA CAC ACA CAC AG*	5086	52.8
UBC 823	*TCT CTC TCT CTC TCT CG*	4974	52.8
UBC 825	*ACA CAC ACA CAC ACA CT*	5061	50.4
UBC 826	*ACA CAC ACA CAC ACA CC*	5046	52.8
UBC 834	*AGA GAG AGA GAG AGA GYT*	5678	52.5
UBC 841	*GAG AGA GAG AGA GAG AYC*	5663	54.8
UBC 846	*CAC ACA CAC ACA CAC ART*	5383	52.5
UBC 849	*GTG TGT GTG TGT GTG TYA*	5615	52.5
UBC 850	*GTG TGT GTG TGT GTG TYC*	5591	54.8
UBC 855	*ACA CAC ACA CAC ACA CYT*	5358	52.5
UBC 874	*CCC TCC CTC CCT CCC T*	4625	59.4
UBC 889	*DBDACACAC ACA CAC AC*	4491	46.0
IMA 5	*CAC ACA CAC ACA CAC AGT*	5391	53.7
IMA 8	*GAG AGA GAG AGA GAG AGT*	5711	53.7
IMA 9	*GAG AGA GAG AGA GAG ACG*	5696	56.0
IMA 12	*CAC ACA CAC ACA CAC ATG*	5391	53.7

The performances of ISSR markers used in the study were determined by using three parameters, i.e. Polymorphic Information Content (PIC), Marker Index (MI), and Resolving Power (RP) (Gautam Murty *et al*., 2013; Kayış *et al*. 2010).

Polymorphism Information Content (PIC*i*) was computed as proposed by Anderson *et al*. (1993). *PIC* of each primer, Band Informativeness (BI) and the Resolving Power (RP) of each primer were calculated according to Prevost and Wilkinson (1999).

The Marker Index (MI) and the Effective Multiplex Ratio (EMR) were computed according to Milbourne *et al.* (1997). *EMR* is an important comparison scale. *MI* is used to evaluate the marker system (Powell *et al*., 1996); RP method is used to profile the genotypes or determine the profiling capacity of primers (Prevost and Wilkinson, 1999).

For Principal Coordinate Analysis, the matrix was calculated with the option of Minimum Similarity through FAMD 1.31 (Schlüter and Harris, 2006) program and Principal Coordinate Analysis was performed.

RESULTS

In this study, the highest *PIC* (Polymorphism Information Content) value was provided by ISSR primers UBC 849 (0.23) and the lowest *PIC* value was provided by 825 (0.14) primers with an average of 0.19. PIC value varied between 0.14 and 0.23. The UBC 826 primer showed the highest *EMR* (number of polymorphic locus in germplasm). This result already shows that the ISSR effective multiplex ratio is connected to fraction of polymorphic fragments. The marker index (MI= Polymorphic marker in each gel well) value was determined for each primer in order to identify which ISSR primer is generally more practical. Highest *MI* value was obtained from 850 primers (3.53) and the lowest *MI* value was obtained from UBC 849 (1.16). RP was used to reveal the profiling potential of the selected primers.

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Average *RP* value for each primer was found as 2.73. The highest *RP* value was provided by UBC 850 (4.15) and the lowest RP value was provided by UBC 849 (1.38). Table 2 shows the marker parameter values for ISSR primers providing valuable bands.

Most of bands were provided by UBC 826 primer with 20 bands among the ISSR primers. Least bands were observed in UBC 849 primer with 5 bands. The molecular sizes of totally 92 bands varied between 1,086 and 100 bp. The average band number per individual was 7.07 (Total Number of Bands / Number of Individuals). The number of bands per primer providing valuable bands was 13.14 (Total Number of Bands/Number of Primers providing valuable bands). No valuable bands were obtained from UBC 808, UBC 809, UBC 810, UBC 811, UBC 812, UBC 823, UBC 834, UBC 841, UBC 846, UBC 874, UBC 889, IMA 5, IMA 8, IMA 9, and IMA 12 primers.

Table 3 shows the number of bands of ISSR primers providing bands and their rates within total number of bands.

A data matrix was developed on the basis of these results. UPGMA and Neighbor Joining trees using Nei's genetic distance matrix were constructed by MEGA6 program (Tamura *et al*., 2013) calculated with Nei's genetic distance matrix (Nei, 1972) by POPGENE 1.32 (Yeh *et al*., 1997) program.

The highest number of bands was obtained in Edremit olives with UBC 826 primer, in Manzanilla olives with UBC 818 primer, in Çilli olives with UBC 850 primer as 4 bands. When the valuable bands obtained from seven ISSR primers were examined, it was observed that all bands had a polymorphic structure.

Results Based on Nei's Genetic Distance

The genetic distance values based on the matrix and dendrograms were found between**:** 0.12 (Tavşan Yüreği and Halhalı) (Uslu and Kaba) and 0.29 (Edremit and Çilli) (Edremit and Çekişte Nazilli) (Manzanilla and Çilli). Thus, the samples closest to each other were (Tavşan Yüreği

Table 2. Marker parameter values for each ISSR primer in cultivated olives.

ISSR PRIMERS	RP	No of polymorphic bands	*PIC* mean	*EMR*	*PIC*×*EMR*= *MI*
UBC 826	3.69	20	0.16	20	3.29
UBC 855	3.54	15	0.20	15	3.01
UBC 817	2.15	9	0.20	9	1.80
UBC 818	2.00	11	0.16	11	1.78
UBC 850	4.15	18	0.20	18	3.53
UBC 849	1.38	5	0.23	5	1.16
UBC 825	2.15	14	0.14	14	1.99

Table 3. Percentage of number of bands provided by ISSR primers among those providing bands in cultivated olives within the total number of bands (%).

ISSR Primer	No of bands	Base pair (bp)	% Within total number of bands
UBC 826	20	600-996	21.73
UBC 855	15	1031-673	16.30
UBC 817	9	877-681	9.78
UBC 818	11	600-100	11.95
UBC 850	18	1086-118	19.56
UBC 849	5	1048-900	5.43
UBC 825	14	900-615	15.21
Total number of bands	92		100

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and Halhalı) (Uslu and Kaba); and the samples most distant to each other were (Edremit and Çilli) (Edremit and Çekişte Nazilli) (Manzanilla and Çilli). Table 4 shows the genetic distance matrix.

When the UPGMA and Neighbor Joining dendrograms (Figures 1-2) developed based on Nei's (1972) genetic distance as well as the Principal Coordinate Analysis were examined, it was observed that Tavşan Yüreği and Halhalı varieties formed one set and Çekişte Nazilli and Manzanilla varieties formed another set in all three analyses.

The result of Principal Coordinate Analysis is shown in Figure 3. The eigenvalues were calculated as percentages of the sum of positive eigenvalues. The first three components were found as 13.61, 11.67 and 10.84, respectively. Those components (Co.1, Co.2 and Co.3) created 36.12% of the total variation. The other components were found less than 10%.

DISCUSSION

The genetic relation among cultivated varieties supplied from Manisa, Izmir, Muğla, Isparta and Şanlıurfa provinces were examined through ISSR method. ISSR-PCR method showing high polymorphism and performance allows it to be used frequently in studies such as genetic similarity, gene mapping, determination of genetic variation, etc. in plants (Gupta *et al*., 1994; Zietkiewicz *et al*., 1994).

The genetic similarity between Tavşan Yüreği and Halhalı, Uslu and Kaba varieties was high. This result is explained as the inability to know the fertilization of Tavşan Yüreği variety supplied from Isparta (Origin: Fethiye) and Halhalı brought from Şanlıurfa (Origin: Mardin-Derik) and Uslu supplied from Aegean Olive Cultivation Research Center (Origin: Manisa-Akhisar) and Kaba variety supplied from Aydın (Origin: Aydın Yamalak) (Canözer *et al*., 1991) and the fact that the varieties have been brought from some place to another

Table 4. Genetic distance matrix of cultivated olives obtained through the ISSR data.

	Edremit	Gemlik	Domat	Uslu	Çilli	Eşek	Kaba	Çekişte Nazilli	Memecik	Tavşan Yüreği	Halhalı	Manzanilla	Çekişte Bozdoğan
Edremit	*****												
Gemlik	0.2591	*****											
Domat	0.22	0.23	*****										
Uslu	0.23	0.22	0.15	*****									
Çilli	0.29	0.25	0.23	0.22	*****								
Eşek	0.26	0.27	0.15	0.14	0.27	*****							
Kaba	0.23	0.22	0.15	0.12	0.19	0.17	*****						
Çekişte Nazilli	0.29	0.27	0.23	0.17	0.25	0.25	0.17	*****					
Memecik	0.27	0.23	0.22	0.18	0.26	0.20	0.18	0.23	*****				
Tavşan Yüreği	0.26	0.22	0.20	0.17	0.25	0.22	0.17	0.22	0.18	*****			
Halhalı	0.26	0.22	0.20	0.17	0.22	0.22	0.17	0.22	0.20	0.12	*****		
Manzanilla	0.27	0.26	0.25	0.18	0.29	0.26	0.20	0.20	0.25	0.23	0.23	*****	
Çekişte	0.26	0.27	0.23	0.19	0.22	0.25	0.17	0.22	0.20	0.19	0.22	0.23	*****

[DOR: 20.1001.1.16807073.2017.19.6.3.8]

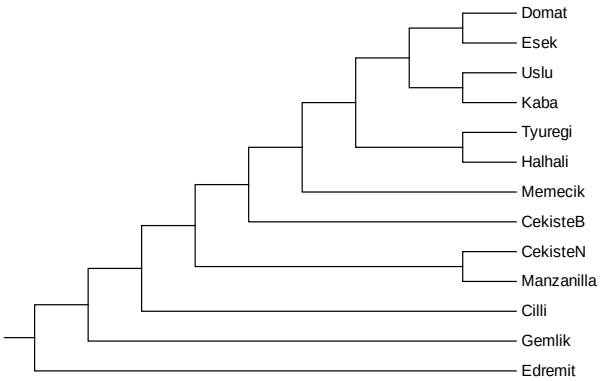

Figure 1. UPGMA dendrogram obtained from UBC 826; UBC 855; UBC 817; UBC 818; UBC 850; UBC 849, and UBC 825 primers in cultivated olives (MEGA6).

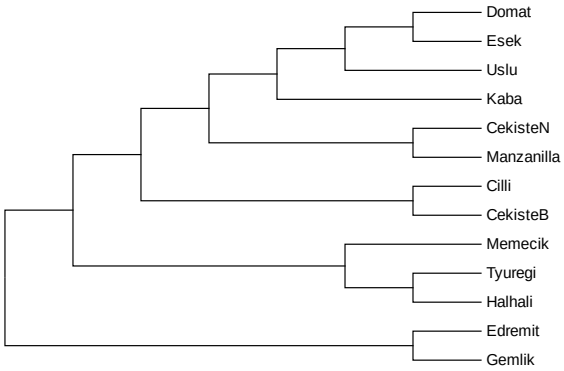

Figure 2. Neighbor Joining dendrogram formed by valuable bands obtained from UBC 826; UBC 855; UBC 817; UBC 818; UBC 850; UBC 849, and UBC 825 in cultivated olives (MEGA6).

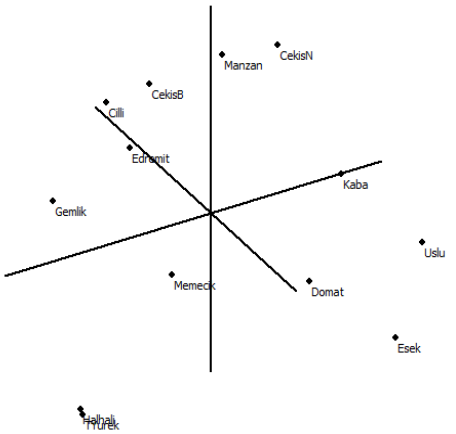

Figure 3. PCO plot of cultivated olives (FAMD 1.31).

through human factor, adapted and cultivated there in the recent years.

Cheghamirza *et al*. (2011) examined in their study 40 accessions, 5 Iranian olive varieties and 26 foreign origin varieties cultivated in Kermanshah province, through ISSR markers. As a result, they obtained 118 polymorphic bands from 8 ISSR primers. They reported as a result of their study, in which they used RAPD and ISSR markers together, that there was not any relation between genetic distances and different regions in olives cultivated in Kermanshah. They noted that this result indicated the fact that variety selection occurred in different genetic pools and different regions. They stated that their findings showed genetic differences in olive varieties and such difference might be used in breeding studies. In our study, it was determined that closest samples based on genetic distance values in cultivated olives were Tavşan Yüreği and Halhalı, Uslu and Kaba. This result supports the findings of Cheghamirza *et al*. (2011).

Martins-Lopes *et al*. (2009) studied polymorphism in the clones of Corbançosa, a Portuguese olive variety, through RAPD and ISSR markers and found polymorphism of similar level among the clones. They reported that the data they obtained showed a wide intra-varietal diversity. ISSR markers determine polymorphism not only among

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varieties of olives but also in intra-varietal clones successfully. Based on their results in which they Applied Molecular Variance Analysis (AMOVA), Martins-Lopes *et al*. (2009) determined that intra-clonal genetic variation was high rather than among different geographical regions. Although their samples came from a limited geographic area, they noted that there was significant genetic variation in Cobrançosa olive variety. One of the reasons why ISSR markers revealed polymorphism among clones is the multiplication of hyper-variable non-coding regions, because hyper-variable regions show quite polymorphic structure (Gemas *et al*., 2004). Obtaining high polymorphism from ISSR primers and Edremit and Çilli olives were determined to be father from expected, although they are both varieties of Aegean Region, correspond to the findings of researchers with regards to ISSR markers.

Kaya (2015) reported high genetic variation among the Turkish cultivars revealing their potential as genetic resources for clonal selection programs. The high polymorphism rate in the studied cultivars in this study may provide useful information for olive breeding programs in future.

When each band was evaluated as an independent locus, ISSR primers determined 13 loci per primer. High polymorphism as we determined is in compliance with the notes of other researchers that ISSR markers might be used in the determination of genetic diversity (Gemas *et al*., 2004; Terzopoulos *et al*., 2005; Martins-Lopes *et al*., 2009; Gomes *et al*., 2008).

Essadki *et al.* (2006) tested 13 ISSR primers and determined totally 26 polymorphic bands in four selected primers. Seven primers produced valuable bands. ISSR markers show dominant characteristic and, therefore, it has been reported that the number of ISSR markers, being studied over the profiling capacity in the profiling of varieties, might be increased and improved.

One of the reasons why ISSR markers are useful in gene mapping and population researches is that they find quite high polymorphism in populations formed by individuals of genotypes close to each other (Hegazi *et al*., 2014; Lanham and Brennan, 1998).

CONCLUSIONS

The genetic variation between thirteen cultivated olive samples was determined through ISSR method at DNA level. The genetic distance matrix, UPGMA, and NJ dendrograms, and PCO were performed.

The estimation of genetic relation among olive varieties cultivated in Turkey was ensured accurately through these markers. The parameters used were Polymorphic Information Content (PIC), Marker Index (MI) and Resolving Power (RP).

ISSR markers produced highly polymorphic bands to determine genetic relationships of *Olea europaea* L. cultivars in Turkey. Seven primers generated sufficient polymorphic bands to figure out the genetic diversity among the cultivars. Establishing genetic relationship of those cultivars would be suitable to use for future genetic improvement studies in olive cultivars.

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برآورد روابط ژنتیکی بین زیتون های کاشته شده با نشانگر های ISSR

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چکیده

زیتون در عرض های جغرافیایی میانی جهان و در مناطقی که آب وهوای مدیترانه ای دارند کاشت می شود. زیتون از محصولات مهم کشاورزی در دشت های مدیترانه ای و ترکیه است. با توجه به نقش این محصول در اقتصاد، تعیین روابط ژنتیکی بین ارقام مختلف آن ارزشمند است. در این پژوهش، تغییرات ژنتیکی بین ۱۳ کولتیوار زیتون با کاربرد ۲۲ نشانگر ISSR به دست آمده از باندهای ارزشمند ۷ کولتیوار از آن ها بررسی شد. کولتیوارهای مطالعه شده به نام های Edremit، Domat، Gemlik، Uslu، Çilli، Eşek، Kaba، Çekişte Nazilli ، Memecik ، Tavşan Yüreği، Halhalı، Manzanilla، و Çekişte Bozdoğan بیشتر ترکیه ای بودند که در مناطق مختلف کاشته می شوند. در بررسی های انجام شده، به طور کلی ۹۲ باند به دست آمد و مشاهده شد که همه آن ها چند شکلی(پلی مورفیک) بودند. میانگین محتوای اطلاعاتی چند شکلی (PIC)، نمایه نشانگر(MI)، و توان تمیز (RP) هر نشانگر به ترتیب ۰/۱۹، ۲/۳۶، و ۲/۷۳ بود که نشان از کارآیی بالا و قابل اعتماد بودن نشانگر های استفاده شده داشت. نتیجه تحلیل های ISSR برحسب تعداد جایگاه های چند شکلی (polymorphic loci) ، تنوع زیستی، و روابط بین کولتیوارهای زیتون از طریق UPGMA، دندروگرام های Neighbor Joining بر مبنای فاصله ژنتیکی Nei و PCO، چنین مشاهده شد که ارقام Tavşan Yüreği و Halhalı یک دسته را تشکیل می دادند و رقم های Çekişte Nazilli و Manzanilla با هم در دسته دیگری بودند.

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