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Original Article

Genetic Diversity Based on ISSR Markers of Apple Genotypes in Ardahan, Turkey

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Abstract

Within the present study, it was conducted a genetic diversity analysis using ISSR markers for some apple genotypes grown in Ardahan region, Turkey. Total genomic DNA (gDNA) isolation from apple leaves was performed using commercial kits. Five ISSR primers were used to determine the genetic diversity among the genotypes studied. Polymerase Chain Reaction (PCR) was performed with all gDNA samples to produce bands to score. PCR products were run in agarose gel and visualized under UV light. Bands on the gels were scored as "1", while no bands at the corresponding positions were scored as "0", to generate the matrix file. Five ISSR primers produced a total of 35 bands, and 21 of them were polymorphic. The polymorphic bands rated approximately 60%. Phylogenetic relationships and genetic distances between the genotypes were calculated by using the PAUP [Phylogenetic Analysis Using Parsimony (and Other Methods)] program. According to the PAUP data, the closest genetic distance was 0.03704 between 'Kaburga' and 'Japon Apple' genotypes, while the furthest genetic distance was 0.48148 between 'Karanfil Apple' and 'Sisli Uruset'. The phylogenetic analysis obtained using UPGMA algorithm produced a phylogenetic tree with two clades. The results suggest that ISSR markers are useful tools for determining genetic relationships among apple genotypes.

Keywords: apple; Ardahan; genetic diversity; ISSR

Introduction

Rosaceae is one of the most diversified and large plant families, including economically important fruit trees. This family consists of more than 100 genera and 3,000 species and it is the third most important plant family in terms of economic significance in mild climate regions (Zarei *et al*., 2017). The family, which generally includes taxa in tree and bush forms, also comprises herbaceous taxa. The family members, which have cosmopolite characteristics, are mostly spread in the Northern hemisphere (Serdar *et al*., 2014).

Apple (*Malus domestica*) of the Rosaceae family is one of the most important cultivated fruit trees in the mild climate regions of the world (Mahmood *et al*., 2016). Apple has been reported to be distributed to different gene centers in the world, primarily Europe, Anatolia, Himalaya, China, Japan, Korea and North America with its 48 species (Dziubiak, 2004; Ercişli, 2004). Turkey is one of the most important producers of apples, with an estimated production of 2,925,828 tons in 2016 (Fao, 2016). There are over 460 local apple genotypes in Turkey, all with different qualities (Ertürk and Akçay, 2010). Apple is one of the most important fruit species, commonly preferred by consumers due to its taste, nutritional content and economic value; its nutritive components include dietary fiber, as well as rich antioxidant active ingredients, carbohydrates and essential minerals (Wiseman, 2001; Sadik *et al*., 2003). It is a good source of phenolics and antioxidants for humans (Wolfe *et al*., 2003; Wolfe and Liu, 2003; Abaci and Sevindik, 2014).

Molecular marker methods are based on the principle of determining polymorphic regions in DNA molecules. Molecular markers are commonly used with the purpose of detecting genetic polymorphism, genetic identification, hybrid plant identification for hybridization development, genetic mapping and marker-assisted selection (Jiang, 2013; Sesli and Yegenoglu, 2017). One of the techniques is ISSR (Inter Simple Sequence Repeat) which offers excellent means to investigate genetic diversity of plants (Arslan and

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Tamkoç, 2011; Abou-Deif *et al*., 2013). In the present day, the ISSR technique is frequently used for determining the genetic diversity of apple genotypes (Goulao and Oliveira, 2001; Korbin *et al*., 2002; He *et al*., 2011; Uzun *et al*., 2016). Development of the ISSR technique has ensured the rapid use of the organisms in the studies of genetic variability. The ISSR is based on the PRC amplification of DNA fragments between two reversed, simple sequential recurrence regions of expandable distances (Zietkiewicz *et al*., 1994).

In the present study, it was investigated the genetic diversity using ISSR markers for some apple genotypes grown in the Ardahan region of Turkey.

Materials and Methods

Plant samples and genomic DNA isolation

Leaf samples of apple genotypes used in the study were collected from certain regions in Ardahan (Turkey) between July and August, 2015 (Fig. 1). Total genomic DNA (gDNA) samples were extracted using DNeasy Plant Mini Kit (GeneMark). The gDNA samples were stored at -20 °C when not at use.

PCR amplification

In order to visualize gDNA samples, 0.8% standard agarose gel electrophoresis procedure was performed. For ISSR-PCR amplification, five ISSR primers were used (Table 1). ISSR amplification reactions were carried out in 25 µL volume containing 5 µL master mix (PCR buffer, $MgCl_2$, dNTP, Taq DNA polymerase), 1 µL ISSR primers, 2.0 µL gDNA (around 10 ng/µL) and 17 µL of ddH_2O.

Table 2 shows the ISSR-PCR cycles with their respective conditions. Amplification products were analyzed by electrophoresis on 0.8% agarose gels buffered with 0.5X TBE (Tris-Borate-EDTA), stained with ethidium bromide and pictured under ultraviolet light (Figs. 2 and 3).

Data analysis

The pictures were used for the evaluation of the results in the analysis of ISSR-PCR. In the reading of the bands formed at the end of the amplification, only the bright bands were taken into consideration.

The presence (1) and absence (0) of bands were specified to construct the data matrix. PAUP [Phylogenetic Analysis Using Parsimony (and Other Methods)] Version 4.0b10 (Swofford, 2002) was used to perform phylogenetic analyses using ISSR data.

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

Primer	DNA Sequences	Tm
UBC-831	5'-CTCTCTCTCTCTCTCTT-3'	50
UBC-830	5'-TGTGTGTGTGTGTGTGG-3'	52
UBC-807	5'-AGAGAGAGAGAGAGAGT-3'	50
UBC-826	5'-ACACACACACACACACC-3'	52
UBC-808	5'-AGAGAGAGAGAGAGAGC-3'	52

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	50-52 °C	1 min	
3. step	72 °C	1 min	35 cycles
4. step	72 °C	10 min	1 cycle
5. step	4 °C	20 min	

Fig. 1. Location of Ardahan Province, Turkey

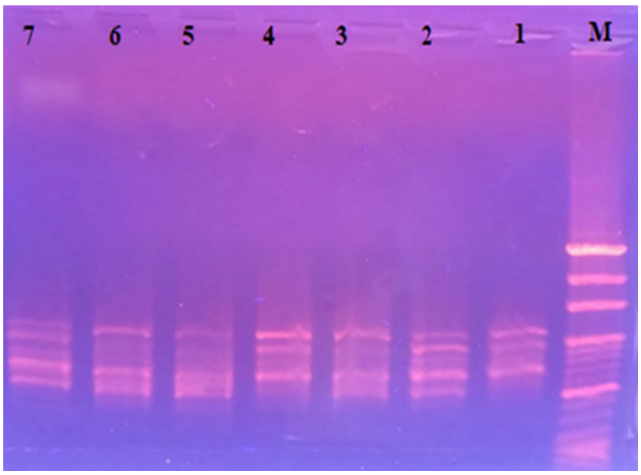

Fig. 2. ISSR-PCR gel photo amplified with UBC-826

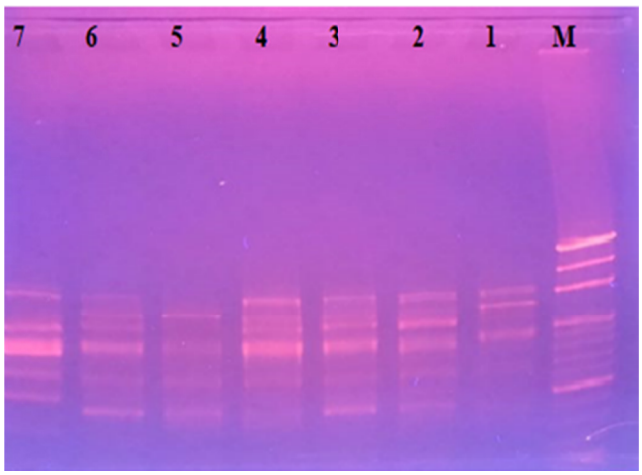

Fig. 3. ISSR-PCR gel photo amplified with UBC-830

Results and Discussion

In the ISSR-PCR analysis, a total of 35 bands were detected, among which 21 bands were polymorphic and the polymorphism rate was 60%. PAUP 4.0b10 analysis program was used to calculate the phylogenetic trees and genetic distances between populations. According to the PAUP data, the closest genetic distance was 0.03704 between 'Kaburga Apple' and 'Japon' genotypes, while the furthest genetic distance was 0.48148 between 'Karanfil Apple' and 'Sisli Uruset' (Table 3).

The phylogenetic tree was obtained using the UPGMA algorithm, and the tree was consisted of two clades (Fig. 4). Clade 1 was divided into five groups. Group A consisted of 'Sobe Apple', 'Kırmızı Safran', 'Kaba Apple' and 'Limon Apple', group B consists of 'Sarı Safran' and 'Yaz Apple', group C consist of 'Kaburga Apple' and' Japon Apple', group D consist of 'Uruset Apple;, 'Şah Apple' and 'Sisli Uruset' and group E consist of 'Paşa Apple' and 'Karpuz Apple'. Clade 2 consisted of only 'Karanfil Apple' (Fig. 4).

Uzun *et al*. (2016) used ISSR markers to detect Turkish apple genotypes and their relationships with some foreign genera and species. In their study, apple genotypes named 'Yaz', 'Karanfil', 'Paşa' and 'Şah' were found in the same group, while 'Kaba' apple was found in a different group. In the present study, the apple genotypes named 'Paşa', 'Yaz', 'Şah' and 'Kaba' were detected in Clade 1, while 'Karanfil Apple' was found in Clade 2. Osmanoğlu (2008) revealed genetic relationships of apple genotypes collected from Ardahan/Posof region using RAPD markers. In their study, apple genotypes named 'Kırmızı Safran', 'Sarı Safran', 'Kaburga' and 'Sobe' were found in the same group. These four genotypes took place in Clade 1 according to the UPGMA dendrogram obtained via ISSR data in the present study (Fig. 4). Osmanoğlu (2008) detected 'Paşa' and 'Uruset' apples in a group, while found 'Yaz' and 'Kaba' apples in another group. In the present study, on the other hand, 'Paşa' and 'Kaba', 'Uruset' and 'Yaz' apples were detected in Clade 1. The results were found partially similar. Daler *et al*. (2017) have studied the genetic relationships among six apple varieties cultivated in our country using RAPD markers. 10 RAPD primers produced 47 polymorphic bands. Kaya *et al*. (2015) carried out molecular analysis of apple genotypes collected from Van province, through using RAPD markers.

Table 3. Pairwise sequence distances among some apple genotypes for ISSR data using PAUP 4.0b10 distance matrix

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
'Sobe Apple'	-	0.07407	0.13636	0.18519	0.29630	0.18519	0.25926	0.22222	0.33333	0.37037	0.37037	0.25926	0.29630	0.25926
'Kırmızı Safran'	2	-	0.13636	0.18519	0.29630	0.11111	0.33333	0.14815	0.25926	0.37037	0.29630	0.33333	0.37037	0.18519
'Kaba Apple'	3	3	-	0.27273	0.40909	0.18182	0.31818	0.27273	0.40909	0.27273	0.36364	0.22727	0.36364	0.31818
'Sarı Safran'	5	5	6	-	0.11111	0.22222	0.29630	0.25926	0.29630	0.33333	0.25926	0.29630	0.33333	0.22222
'Yaz Apple'	8	8	9	3	-	0.28571	0.33333	0.29630	0.20000	0.28571	0.20000	0.25926	0.22857	0.28571
'Limon Apple'	5	3	4	6	10	-	0.37037	0.18519	0.31429	0.34286	0.25714	0.37037	0.34286	0.22857
'Karanfil Apple'	7	9	7	8	9	10	-	0.40741	0.44444	0.40741	0.48148	0.44444	0.33333	0.37037
'Kaburga Apple'	6	4	6	7	8	5	11	-	0.11111	0.22222	0.14815	0.25926	0.22222	0.03704
'Uruset Apple'	9	7	9	8	7	11	12	3	-	0.14286	0.11429	0.22222	0.14286	0.20000
'Şah Apple'	10	10	6	9	10	12	11	6	5	-	0.08571	0.11111	0.11429	0.22857
'Sisli Uruset'	10	8	6	7	7	9	13	4	4	3	-	0.18519	0.14286	0.14286
'Paşa Apple'	7	9	5	8	7	10	12	7	6	3	5	-	0.11111	0.29630
'Karpuz Apple'	8	10	8	9	8	12	9	6	5	4	5	3	-	0.22857
'Japon Apple'	7	5	7	6	10	8	10	1	7	8	5	8	8	-

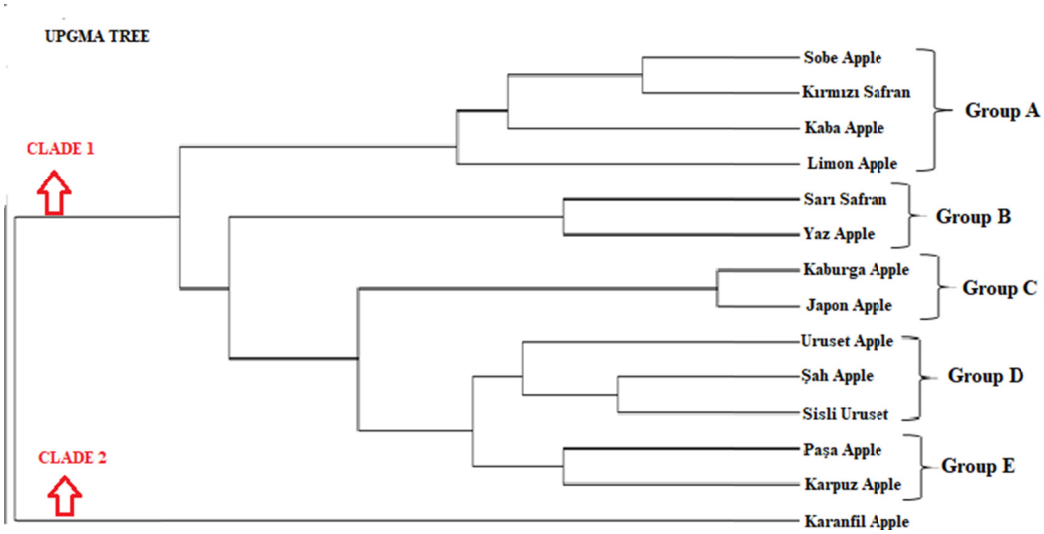

Fig. 4. The UPGMA tree generated using ISSR data of apple genotypes

Creating a dendrogram, the similarity index between genotypes was revealed. Molecular techniques including SSR (Hokanson *et al*., 1998; Kenis and Keulemans, 2005), AFLP (Kenis and Keulemans, 2005), nrDNA ITS, cpDNA *mat*K (Robinson *et al*., 2001), cpDNA *atp*B-*rbc*L (Savolainen *et al*., 1995), promoter region of *atp*B gene (Mahmood *et al*., 2016), have been used to determine taxonomic status, genetic diversity and phylogenetic analyses among apple species and genotypes.

Conclusions

The study reports the genetic relationships between 14 apple genotypes distributed in Ardahan province by using five ISSR primers. Genetic distance and phylogenetic relationship among the target populations were detected based on ISSR data, and the relationships were visualized on the phylogenetic tree constructed. The obtained results will be useful to serve plant breeding programs by means of revealing inter-species genetic relationships which have a great importance in breeding studies. Furthermore, it was also aimed to offer information about the relationships between different populations cultivated in the region. Undoubtedly, more data including more taxa (e.g. morphological and/or DNA sequence data) will improve this conclusion and yield more reliable results.

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