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MOLECULAR CHARACTERIZATION OF SERVICE TREE (*Sorbus domestica* L.) GENOTYPES SELECTED FROM TOKAT PROVINCE

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Abstract

This study was carried out to determine the genetic diversity of eleven service tree (*Sorbus domestica* L.) genotypes selected from Tokat province and taken under protection in the genetic resource plot. In the present study, ISSR-PCR studies were carried out on genotype DNAs obtained by Mini-CTAB method and the level of polymorphism between genotypes was determined using seven UBC-ISSR primers. A total of 65 bands were obtained in service tree genotypes with seven primers and 56 of these bands were determined to be polymorphic. The number of bands obtained per primer varied between 5 and 12, the mean band number was 5.90, and the mean polymorphic band number was 5.09. The data obtained as a result of the evaluation of ISSR-PCR bands were analyzed in Popgene32 and MEGA5.0 computer package program, and a dendrogram was obtained according to the UPGMA method. In the dendrogram obtained, it was determined that two groups were formed at the level of approximately 22% difference, and there was only one genotype in the small group while the other genotypes were in the large group.

Key words: genotype, ISSR, selection, service tree

Introduction. Service tree (*Sorbus domestica* L.) is a deciduous species in winter belonging to the Rosaceae family and can grow to 3–25 m in height. Flowers are hermaphrodite and in compound corymb inflorescence. Leaves have

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16–20 cm length, pinnate and 13–21 leaflets. Leaflets are 3–6 cm long, 1 cm wide and have serrate margins. Young shoots are green, and the bark of the tree turns into gray colour by ageing. Tips of buds are slightly hairy, significantly sticky, and in terminal shape [1,2].

Service tree (*S. domestica* L.) is a species that is more common in the Marmara Region, Central and Western Black Sea Region, northern Central Anatolia in Turkey, but also found in the Inner Aegean, Lakes region and Hatay region. Although it is not cultivated in the form of orchards, its consumption is quite common, especially in the provinces in the transitional regions (such as Tokat, Amasya, Kastamonu). It was reported that 12 species and 17 taxa are naturally found in Turkey [3]. In the world, it has been reported that it is a widespread species in Central Europe (France, Germany, Austria, Czech Republic, Slovakia, Slovenia, Switzerland), North Africa, and also a small distribution area in the British isles [4,5].

Knowing the definition and benefits of *S. domestica* fruits dates back to Ancient Rome and the Greeks. From here, it is known to spread all over Europe from the north to the centre. Starting from the 18th century, service tree cultivation began to decline, and until the middle of the 19th century, service tree fruit and tree were described as being carried from generation to generation as a family tree due to their rich value [6]. The service tree, which is used only as a border plant and for landscaping on roads today, has almost completely lost its importance.

In this way, fruit genetic resources are subject to genetic erosion by environmental and other pressures and are in danger of extinction. Service tree is among the species that is on the verge of extinction today. Identifying, collecting and conserving the diversity in such fruit genetic resources are also extremely important in terms of sustainability. It is very important to carry out selection studies in the regions where it is common, to determine the genetic diversity and to protect this species by developing new cultivars [7]. Studies on service tree in the world have generally been made on selection and there are very few studies in which genetic variation has been determined.

Service tree, which is among the rare and endangered species in many European countries and where genetic resource conservation is emphasized as a priority [8], is included in the Red Book of Switzerland [9]. Many selection studies have been carried out so far, especially in Central European countries [9–11] and some studies have identified morphological and genetic variation [5,8].

It is of great importance to determine the performance of service tree genotypes selected from the natural flora of Tokat province and taken under protection as promising, and to bring them into fruit growing as a cultivar-candidate. The aim of this study was to determine the diversity of genotypes selected from the flora of Tokat province with ISSR markers.

Material and methods. ***Plant material.*** The material of the study consisted of 11 (60TG12, 60PD08, 60PE23, 60NB01, 60NB02, 60PE70, 60TM14,

60NGB04, 60TM10, 60PC01 and 60PBE07) service tree genotypes obtained by selection from Tokat province and taken under ex-situ protection in the genetic resource parcel located in Tokat Gaziosmanpaşa University Application Center. Shoot buds from selected genotypes were grafted onto service tree seedlings and then taken under protection. Genotypes were selected from Tokat city centre and seven different regions connected to the centre.

DNA extraction. DNA isolation was performed using young leaf tissues according to the Cetyltrimethylammonium bromide (CTAB) method used by Doyle and Doyle [12]. The DNA samples obtained were measured in a spectrophotometer and the DNA samples were diluted to a final concentration of 10 ng/µL (1 mM EDTA, 10 mM Tris-HCl, pH 8.0).

ISSR amplification. To determine the polymorphism levels, seven UBC primers, which were previously used in the Rosacea family and service tree, were used (Table 1) [13,14]. ISSR PCR reaction was carried out by 2 µL of DNA (10 ng/µL) and 23 µL of reaction mix [5 µL of 10X PCR buffer solution, 2 µL of 25 mM Mg^{+2}, 1.25 µL of 2.5 mM dNTP, 0.1 µL of Taq DNA Polymerase, 1 µL of 0.5 µM primer and 13.65 µL of PCR water]. PCR products were separated using 1 X TBE buffer as gel and electrode buffer solution, and 2% agarose gel for electrophoresis at a constant voltage of 100 V for about 2 h.

Data analysis. The data used in the statistical analyses were obtained by evaluating the ISSR bands as one (1) in the presence and zero (0) in the absence of them. The similarities and differences between the genotypes were studied at the molecular level, Basic Coordinates Analysis was made using the similarity coefficient, and the UPGMA dendrogram was obtained according to the UPGMA (Unweighted Pair-Group Method with Arithmetic Average) method by analyzing them in Popgene32 version 1.32 (Population Genetic Analysis) and MEGA 5.0 (Molecular Evolutionary Genetic Analysis) computer package program (Fig. 2).

Table 1

Findings from ISSR primers used in the study and the results

Primer name	Sequence (5′-3′)	Degree of dissociation (°C)	Band number	Polymorphic band number	Polymorphic band ratio (%)
80	AGAGAGAGAGAGAGAGT	50	10	9	90
808	AGAGAGAGAGAGAGAGC	53	10	8	80
818	CACACACACACACACAG	52	11	11	100
835	AGAGAGAGAGAGAGAGYC	54	7	5	71
881	GGGTGGGTGGGTGGGT	54	10	6	60
888	BDBCACACACACACACA	52	5	5	100
889	DBDACACACACACACAC	52	12	12	100
Total			65	56	
Average			9.29	8.00	85.86

Furthermore, NTSYS-pc ver. 2.21b (Numerical Taxonomy and Multivariate Analysis System) package program developed by Rohlf [15] was used to create similarity/difference matrices between individuals and for Basic Coordinates Analysis.

Results and discussion. The ISSR technique was used because the bands produced in the study are reproducible, have high polymorphism, and make it possible to scan a large number of different loci [16,17].

Seven primers used created a total of 65 bands and 56 of these bands were determined as polymorphic (Table 1). Cao et al. [18] reported that 50 polymorphic bands were sufficient to successfully detect the similarities and differences between genotypes in the population. It was determined that the number of polymorphic bands obtained in the study exceeded this threshold and the number of primers used was sufficient.

Belletti et al. [19] reported that 53 polymorphic bands were obtained in 22 different *Sorbus torminalis* L. genotypes using six RAPD markers in Northwest Italy, and 54 polymorphic bands were obtained by using five ISSR primers in 26 *Sorbus torminalis* L. genotypes in Switzerland [20].

While the number of bands per primer in service tree genotypes varied between 5–12, the average number of bands was 9.29 and the mean number of polymorphic bands was 8.00. It was determined that the maximum number of bands per primer was obtained from primer 889 (12), and the smallest number of bands was obtained from primer 888 (5). Primers 818, 888 and 889 were found to be polymorphic among all genotypes (Table 1). The band images of primer 808 are given in Fig. 1.

While the average polymorphism rate of the bands obtained from the primers was found to be 85.86%, the lowest polymorphism rate per primer was determined as 60.00% in primer number 881 (Table 1).

The dendrogram obtained as a result of the evaluation of ISSR bands was divided into two main groups with a different level of approximately 22% (Fig. 2).

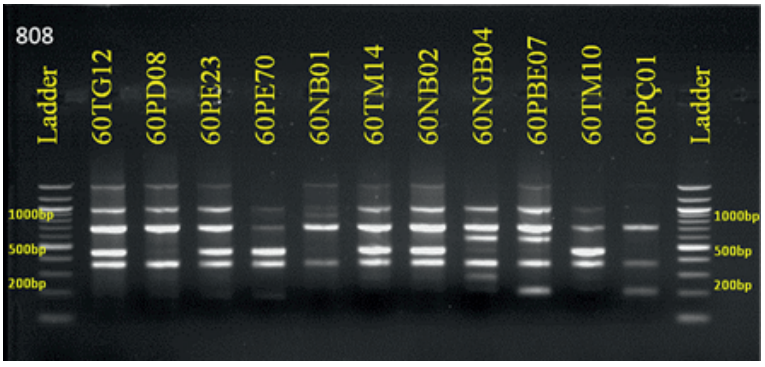

Fig. 1. Band images of primer 808

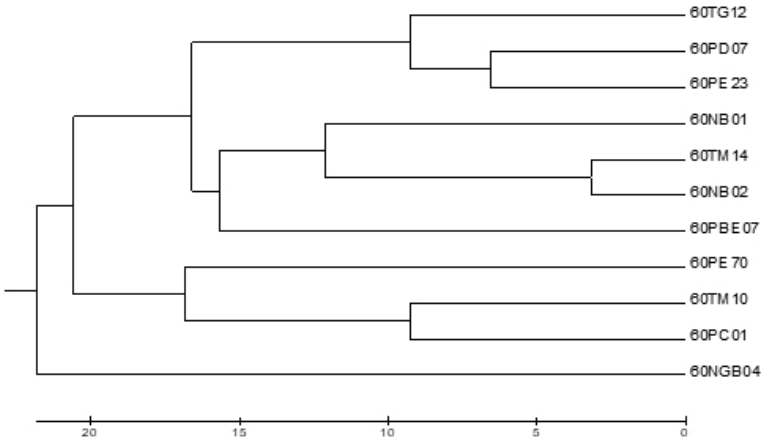

Fig. 2. Dendrogram obtained by cluster analysis

It was determined that there was only one genotype in the small group, the other genotypes were gathered in the other large group and the large group was divided into groups again at the level of 20%. According to the dendrogram, the two closest genotypes were 60TM14 and 60NB02.

It was determined that all genotypes in the large group were selected from Tokat centre and regions close to the centre. Service trees were mostly found on the edges of the vineyards in the region where the selection was made. In these areas, propagation was generally done vegetatively by grafting on bottom shoots. For this reason, it is considered that the genotypes had a high level of similarity and a low level of difference.

The genotype (60NGB04) in the small group was selected from a region where the population was not dense and different from other genotypes in terms of the place and altitude where it was selected. The genotype 60NGB04 was estimated to be older and generative, unlike the others. Although the place where the genotype was found is a farmer's garden, it is thought that the genotype has high self-fertility and is propagated by seed because the place is a forest village.

In our study, it was determined that the distribution of genotypes on the scatterplot (Fig. 3) obtained by Principal coordinate analysis showed a distribution similar to the dendrogram given in Fig. 2. It is seen in Fig. 3 that the genotypes (60PE70, 60TM10, 60PC01) that form a branch of the large group on the dendrogram and the groups formed in the other branch also form a group on the scatterplot.

Conclusion. Service tree is among the species that is on the verge of extinction today. Identifying, collecting and conserving the diversity in such fruit genetic resources are also extremely important in terms of sustainability. It is very important to carry out selection studies in regions where it is common, to determine genetic diversity and to protect this species by developing new culti-

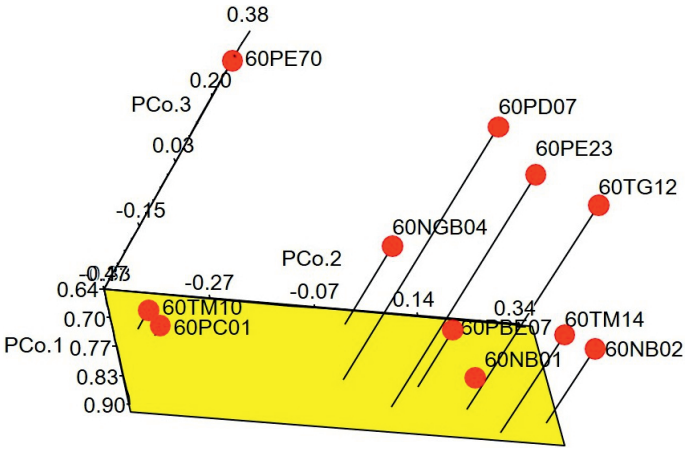

Fig. 3. Distribution of genotypes on the scatterplot created as a result of principal co-ordinate (PCO) analysis

vars [7,20]. In the world, studies on service tree are generally made on selection and there are very few studies in which genetic variation is determined. This study is the first in Turkey in terms of determining genetic variation, and it will shed light on future research.

As a result of the dendrogram and principal component analysis obtained as a result of the study, it was determined that the 60NGB04 genotype was different from the other genotypes. This may indicate that the genotypes collected from different regions are farther apart, the genetic similarities in the same region genotypes are higher, and the difference in the population in the region is gradually decreasing as a result of cross-pollination of the genotypes in the region.

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