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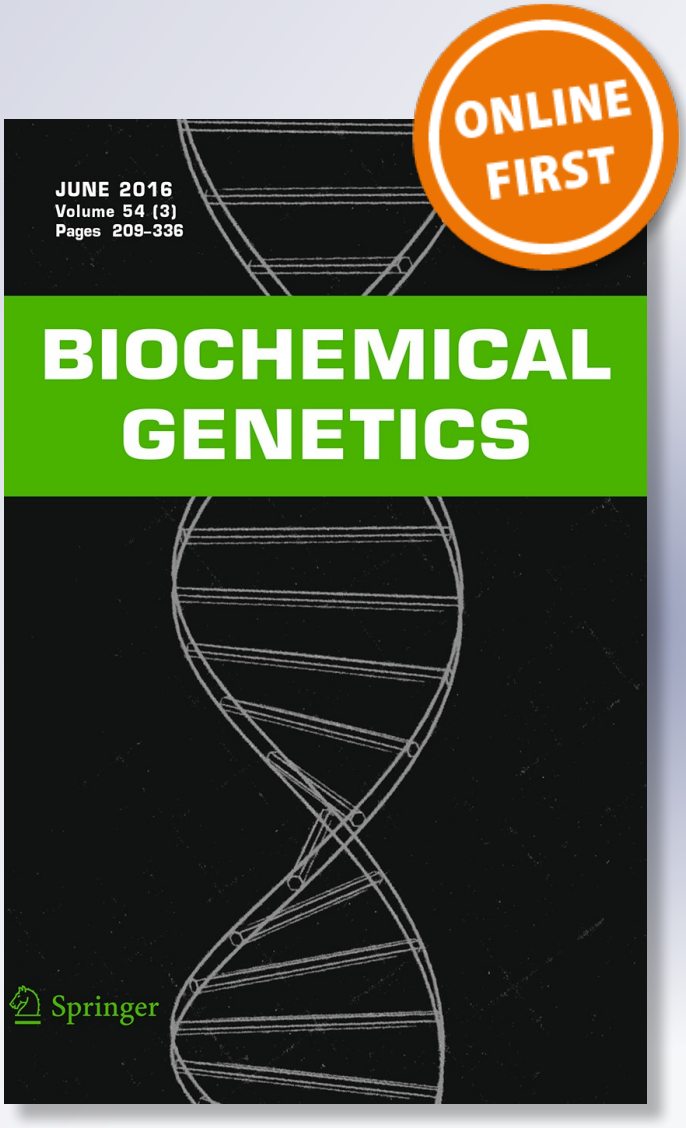


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ORIGINAL ARTICLE

# Molecular Evaluation of Genetic Diversity in Wild-Type Mastic Tree (*Pistacia lentiscus* L.)

**Alimu Abuduli[1] · Yıldız Aydin[2] · Muhammet Sakiroglu[3] · Ahmet Onay[4] · Sezai Ercisli[5] · Ahu Altinkut Uncuoglu[1]**

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**Abstract** In this study, the patterns of genetic variation and phylogenetic relationships of mastic tree (*Pistacia lentiscus* L.) genotypes including 12 males and 12 females were evaluated using SSR, RAPD, ISSR, and ITS markers yielding 40, 703, 929 alleles, and 260–292 base pairs for ITS1 region, respectively. The average number of alleles produced from SSR, RAPD, and ISSR primers were 5.7, 14, and 18, respectively. The grouping pattern obtained from Bayesian clustering method based on each marker dataset was produced. Principal component analyses (PCA) of molecular data was investigated and neighbor joining dendrograms were subsequently created. Overall, the results indicated that ISSR and RAPD markers were the most powerful to differentiate the genotypes in comparison with other types of molecular markers used in this study. The ISSR results indicated that male and female genotypes were distinctly separated from each other. In this frame, M9 (Alaçatı) and M10 (Mesta Sakız Adası-Chios) were the closest genotypes and while F11 (Seferihisar) and F12 (Bornova/Gökdere) genotypes fall into same cluster and showing closer genetic relation. The RAPD pattern indicated that M8 (Urla) and M10 (Mesta Sakız Adası-Chios), and F10 (Mesta Sakız Adası-Chios) and F11 (Seferihisar) genotypes were the closest male and female genotypes, respectively.

✉ Ahu Altinkut Uncuoglu
ahu.uncuoglu@marmara.edu.tr

[1] Department of Bioengineering, Faculty of Engineering, Marmara University, 34722 Istanbul, Turkey

[2] Department of Biology, Faculty of Arts and Sciences, Marmara University, 34722 Istanbul, Turkey

[3] Department of Bioengineering, Faculty of Engineering and Architecture, Kafkas University, 36100 Kars, Turkey

[4] Department of Biology, Faculty of Science, University of Dicle, Diyarbakir 21280, Turkey

[5] Department of Horticulture, Agricultural Faculty, Ataturk University, 25240 Erzurum, Turkey

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## Introduction

Mastic tree (*Pistacia lentiscus* L.), an evergreen dioecious shrub, is widely distributed along the Mediterranean basin shores. It is a member of a heterogeneous family of eleven species (Zohary 1952) and it is distributed throughout the Aegean and Mediterranean geographical regions in Turkey (Davis 1966). Mastic tree is well known in Mediterranean countries for its resin, mastic gum, used since antiquity for incense, as a chewing gum for pleasant breath, for spicing liqueurs and jam, and in the cosmetic industry (Browicz 1987).

There are only a few molecular studies that address genetic and taxonomic relationships of *Pistacia* species (Parfitt and Badenes 1997; Kafkas and Perl-Treves 2001, 2002; Kafkas et al. 2006a, b; Yi et al. 2008), *Pistacia vera* varieties (Hormaza et al. 1994a; Dollo et al. 1995; Al-Saghir and Porter 2006; Karimi et al. 2012), and on the screening of sex markers (Hormaza et al. 1994b; Verdú and Fayos 1998; Kafkas et al. 2001). Taxonomic classification and identification of mastic tree is extremely limited and not yet fully carried out. In Turkey, Kafkas and Perl-Treves (2001) addressed the taxonomic relationships and genetic variation of wild *Pistacia* germplasm including *P. atlantica*, *P. terebinthus*, and *P. eurycarpa* using morphological data and random amplified polymorphic DNA (RAPD). Although growth differences are evident among male and female trees, mastic trees are morphologically very similar. Therefore, it is not easy to distinguish these different genotypes (Yi et al. 2008). The pollen isozyme patterns of nine different enzymes in *P. lentiscus*, *P. terebinthus,* and *P. vera* were studied by Loukas and Pontikis (1979) in order to assay inter-specific relationships. Molecular classification of 10 *Pistacia* species including 2 *P. lentiscus* genotypes from USA and Israel was first reported by Parfitt and Badenes (1997) at the molecular level. Werner et al. (2002) investigated the genetic diversity of *P. lentiscus* in populations from southern Iberian Peninsula and North Africa using RAPD markers. Nahum et al. (2008) reported that study the genetic and phenotypic variability among and within the populations of *P. lentiscus* was correlated with the local environmental conditions and attempted to identify the relative roles of genetic, ecotypic differentiation and phenotypic plasticity in the adaptation of *P. lentiscus* to the wide range of habitats along the climatic gradient in Israel. Two different molecular marker techniques, RAPD and Inter Simple Sequence Repeat (ISSR), were studied to determine the genetic diversity among a female and the four major genotypes of the male mastic trees, Votomos, Maroulitis, Mavroskinos, and Siderakikos grown in the Greek island of Chios (Zografou et al. 2010). Based on these results, it was reported that the entries show genetic diversity among the genotypes and within the different individuals. Population structure of the *P. lentiscus* L. and a complete understanding of the genetic diversity is essential for its conservation and management. In genetic engineering programs, the major challenge for systematic use of *P. lentiscus* diversity is limited with germplasm characterization. Therefore, in this work, we report on the results of the genetic

variability and phylogenetic relationships between the different genotypes among the female and male mastic trees collected from different geographical locations around the Izmir region of Turkey and neighboring island of Chios using Simple Sequence Repeat (SSR), RAPD, ISSR, and Internal Transcribed Spacer (ITS) markers. Determination of genetic variation and genetic relationship among these genotypes is an important contribution for classification and utilization of mastic tree germplasm resources, and it also can be helpful for breeders in selection of diverse genotypes for their future breeding programs.

## Materials and Methods

### Plant Materials and DNA Extraction

The plant materials used in this experiment were 12 males (M1–M12) and 12 females (F1–F12), a total of 24 different genotypes of wild-type mastic tree leaf tissue collected from İzmir region of Turkey and in the neighboring island of Chios. The names and the origins of the samples are shown in Table 1. Young leaves from these samples were well washed in distilled water, frozen in liquid nitrogen, and stored at −80 °C for DNA extraction. Genomic DNA was extracted from leaf tissues by the CTAB method of Doyle and Doyle (1987) with minor modifications of Kafkas et al. (2001). The DNA was quantified by Qubit®2.0 (Invitrogen) Fluorometer using Qubit® dsDNA BR Assay Kit and diluted to the appropriate concentration. Qualities of extracted DNAs were examined by running on 0.8 % agarose gel.

**Table 1** Mastic tree samples and their geographical locations used in this study

| No | Male | No | Female | Location | Coordinates (N, E) |
|---|---|---|---|---|---|
| 1 | M1 | 13 | F1 | Domuzçukuru | 38°17′06″, 26°15′38″ |
| 2 | M2 | 14 | F2 | Karaabdullah | 38°16′26″, 26°26′22″ |
| 3 | M3 | 15 | F3 | Manzara Tepesi | 38°20′09″, 26°18′01″ |
| 4 | M4 | 16 | F4 | Paşalimanı | 38°20′19″, 26°26′09″ |
| 5 | M5 | 17 | F5 | Ildır | 38°23′55″, 26°28′58″ |
| 6 | M6 | 18 | F6 | Karaburun | 38°26′28″, 26°36′07″ |
| 7 | M7 | 19 | F7 | Gülbahçe | 38°22′25″, 26°38′34″ |
| 8 | M8 | 20 | F8 | Urla | 38°21′21″, 26°51′21″ |
| 9 | M9 | 21 | F9 | Alaçatı | 38°17′33″, 26°22′29″ |
| 10 | M10 | 22 | F10 | Mesta Sakız Adası (Chios) | 38°26′08″, 25°92′24″ |
| 11 | M11 | 23 | F11 | Seferihisar | 38°19′50″, 26°51′46″ |
| 12 | M12 | 24 | F12 | Bornova/Gökdere | 38°23′56″, 27°13′45″ |

### SSR, RAPD, and ISSR Amplification

We tested 7 SSR markers that were previously developed for mastic tree (Albaladejo et al. 2008). 50 RAPD markers and 50 ISSR markers were also used for Polymerase Chain Reaction (PCR) of DNA from 12 male and 12 female mastic tree genotypes. The reactions were conducted in volumes of 25 µl containing 1 × PCR buffer, 3.75 mM $MgCl_2$, 0.2 mM dNTPs, 0.9 µM forward primer and 0.9 µM reverse primer (1.8 µM of a given RAPD and ISSR primers), 0.16 ng of genomic DNA, and 0.025U of Taq DNA polymerase. The PCR amplifications for RAPD, ISSR, and SSR markers were carried out in ABI Veriti 96-well thermal cycler following the PCR protocol: initial denaturation at 94 °C for 3 min, 40 cycles at 94 °C for 1 min, X °C (X °C depending upon the primer pair) for 1 min, 72 °C for 1 min, and final extension step at 72 °C for 10 min and the reactions were kept at 4 °C. The PCR products derived from RAPD and ISSR markers were separated on a 1.5 % agarose gel. Amplification products derived from SSR markers were visualized by running on 2 % agarose gel in 0.5X TBE buffer system, followed by staining with Red Safe nucleic acid staining solution and digitally photographed under MiniBIS Pro Visualizing System (DNr Bio-Imaging Systems). Fragment sizes were estimated by using 50 bp and 100 bp molecular size DNA ladders.

### PCR Amplification and Sequence Analysis of ITS1 Region

The ITS1 region was amplified using primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS2 (5′-GCT GCG TTC TTC ATC GAT GC-′3) (White et al. 1990). ITS1 amplifications were performed in 25 µl reactions using 0.16 ng of genomic DNA, 1X PCR buffer, 0.2 mM dNTPs, 0.9 µM forward primers and 0.9 µM reverse primer, 3.75 mM MgCl2, and 0.025 U Taq DNA polymerase. PCR amplifications were performed using ABI Veriti 96-Well Thermal Cycler following the PCR protocol: initial denaturation at 94 °C for 3 min, 40 cycles at 94 °C for 1 min, 66 °C for 1 min, 72 °C for 1 min, and final extension step at 72 °C for 10 min and the reactions were kept at 4 °C. The PCR products were separated on a 1.5 % agarose gel stained with Red Safe Nucleic Acid Staining Solution (20,000x) and digitally photographed under MiniBIS Pro Visualizing System (DNr Bio-Imaging Systems). Amplifications were purified with a Charge-switch-Pro PCR Clean-up Purification Kit (Invitrogen-Cat. no. CS32250) by centrifugation protocol from the manufacturer. After purification, the ITS1 fragment will be used for the cycle sequencing reactions, and the reactions were carried out with AB BigDye Terminator v3.1 Cycle Sequencing Kit (Part no. 4376486). The cycle sequencing reaction mixture had a total reaction volume of 10 µL, and contained 2 µL of genomic DNA (4 ng/µL), 1µL 5X buffer, 2 µL Big Dye terminator, 1.8 µL ultra pure sterile water, and 3.2 µL primer (1 µM). Sequencing reactions were performed in the thermocycler with an initial denaturing step at 96 °C for 1 min, followed by 25 cycles of 10 s at 96 °C, 5 s at 50 °C, and a final extension step of 4 min at 60 °C. Samples were sequenced on an ABI PRISM 310 Genetic Analyzer. The products were aligned by BLAST (Basic Local Alignment Search Tool). Alignments were further adjusted by MEGA 6.06. All sequences have been deposited at GenBank

(http://www.ncbi.nlm.nih.gov/genbank) with accession numbers (KT792748, KT792749, KT792750, KT792751, KT792752, KT792753, KT792754, KT792755, KT792756, KT792757, KT792758, KT792759, KT792760, KT792761, KT792762, KT792763, KT792764, KT792765,KT792766, KT792767, KT792768,KT792769, KT792770, KT792771).

### Data Collection and Analysis

Band sizes resulted from 7 SSR, 50 RAPD, and 50 ISSR markers were counted and analyzed by Genosoft software version 3.8.2 from VWR (https://www.vwr.com). The analyzed fragments were scored as 1 and 0 based on their presence and absence. The statistics analysis including major allele frequency, gene diversity, and polymorphism information content (PIC) values were determined using a genetic analysis software POWER MARKER version 3.25 (Liu and Muse 2005). To infer the population structure, model-based software STRUCTURE 2.3.3 was used. To determine the number of the subpopulations (K), software was run using a burn-in of 10,000 and run length of 100,000. The number K was set from 1 to 10 and ten independent STRUCTURE runs were made for each K. In order to determine the genetic structure of the 24 accessions of mastic tree genotypes, the Principal Component Analyses (PCA) were performed by GenAlEx software 6.41 (Peakall and Smouse 2012). Cluster analysis and neighbor joining tree was constructed using software Phylip V 3.695 (Felsenstein 2005). Bootstrap procedure with 100 replicates was used to test the stability of the resulting dendrogram. NJ tree was visualized using the TREEVIEW program Page and Holmes (1998).

Alignments for ITS1 region were viewed in BLAST and formed as one single FASTA file for each sample. Determination of GC content for sequence data was calculated based on (G+C/A+T+G+C) $\times$ 100. Phylogenetic analyses and genetic distance matrix of ITS sequence data were performed in MEGA 6.06 (Tamura et al. 2013) and neighbor noining-based dendrograms were constructed.

## Results

### Diversity Analyses

In the frame of diversity analyses, number of alleles, major allele frequency (MAF), gene diversity value, and PIC value were calculated in mastic tree genotypes using each marker dataset.

As a result of screening of 24 mastic tree genotypes with 7 SSR markers, a total of 40 alleles were recorded across all the genotypes and average of 5.7 alleles per primer. According to diversity parameters the results indicated that the highest and lowest values of major allele frequency (MAF) were recorded as 0.458 and 0.208, respectively. The highest and lowest values of gene diversity were recorded as 0.843 and 0.714, respectively. The lowest PIC value was recorded as 0.672, while the highest PIC value was 0.823. The mean values of 7 SSR markers for MAF, gene diversity, and PIC values were 0.452, 0.656, and 0.628, respectively.

A total of 703 alleles were recorded with an average of 14 alleles per primer by 50 RAPD markers. The results of genetic diversity parameters indicated that the highest and lowest values of MAF were recorded as 0.75 and 0.083, respectively. The highest and lowest values of gene diversity were recorded as 0.955 and 0.424,

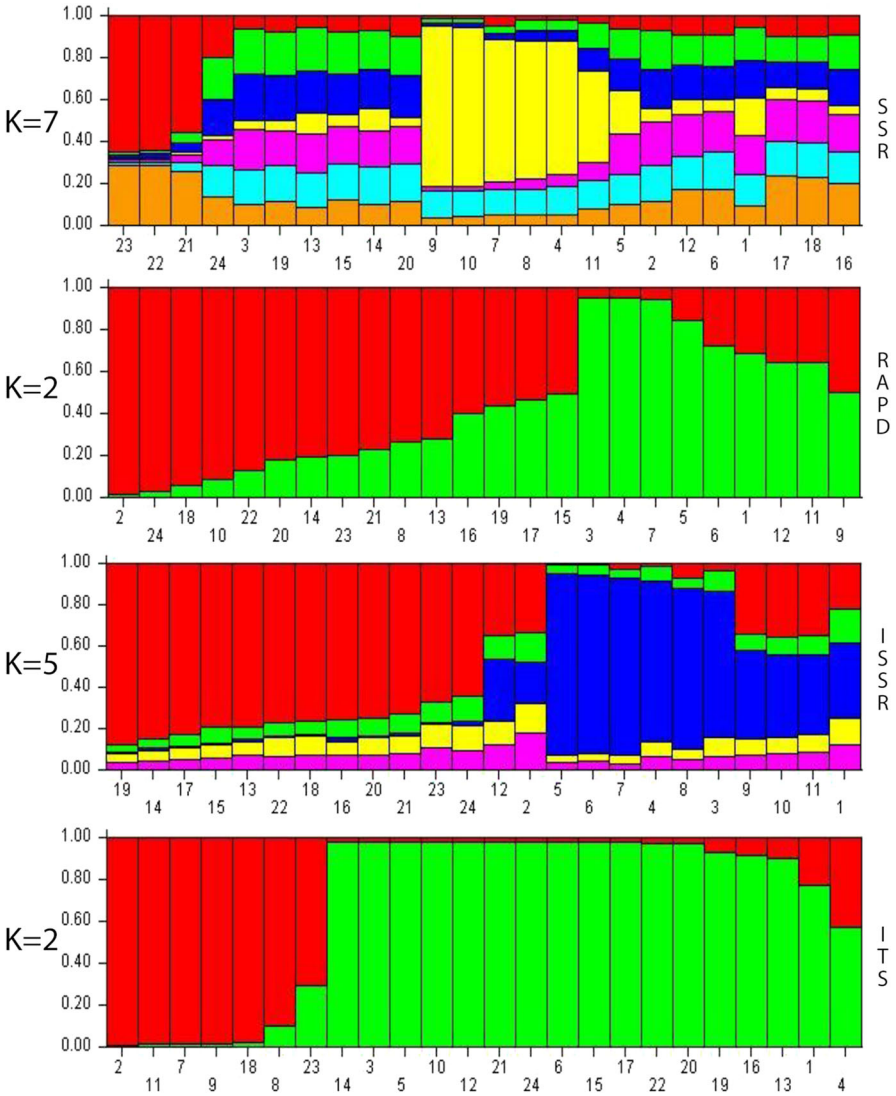


**Fig. 1** Comparative analysis of population structure in the male and female mastic tree genotypes for a priori defined number of clusters $K = 2$–7 inferred by the STRUCTURE software (Pritchard Lab, CA, USA) using data from SSR, RAPD, ISSR, and ITS markers. Each accession is represented by a vertical column divided into K colored segments that represent the individual's estimated membership fractions in $K$ clusters. *Black lines* separate the genotypes. The *numbers* at the bottom of the graph refer to the population codes mentioned as in Table 1

respectively. The lowest PIC value was recorded as 0.408, while the highest PIC value was 0.953. The mean values of 50 RAPD markers for MAF, gene diversity, and PIC values were 0.263, 0.847, and 0.835, respectively.

A total of 929 alleles were recorded with an average of 18 alleles per primer with 50 ISSR markers. Diversity analysis results indicated that the highest value of MAF was recorded as 0.75 and the lowest value was recorded as 0.042. The highest and lowest values of gene diversity were recorded as 0.958 and 0.427, respectively. The lowest PIC value recorded as 0.415, while the highest value of PIC values was 0.958. The mean values of 50 ISSR markers for major allele frequency, gene diversity, and PIC values were 0.173, 0.896, and 0.887, respectively.

The sequence length of ITS1 region in mastic tree genotypes was ranged from 260 bp to 292 bp. The percentage of G+C content for the ITS1 region varied from 57.2 to 58.8 % across genotypes. The values of ITS1 marker for MAF, gene diversity, and PIC values were 0.333, 0.847, and 0.837, respectively.

## Population Structure Analyses

Population structure of mastic tree genotypes based on each marker dataset was estimated using STRUCTURE version 2.3.3 software. The number of subpopulations ($K$) was identified based on maximum likelihood and delta $K$ ($\Delta K$) values. Delta $K$ value suggested that an optimal number of $K = 7$ implies the existence of seven subpopulations in the gene pool by SSR markers (Fig. 1). Details of seven identified subpopulations were indicated that the male mastic tree genotypes were well-clustered. Among these seven subpopulations, it may be identified two main clusters (yellow and red) and the other five subpopulations are mixed with these two main clusters. Yellow cluster was composed of 7 male genotypes (M4, M5, M7, M8, M9, M10, M11) and red cluster was composed of 4 genotypes (F21, F22, F23, F24) (Fig. 1). The structure result based on RAPD data implied the existence of two main subpopulations ($K = 2$) represented by different colors (red and green). In these two clusters, separation of female and male genotypes was distinctive. Red cluster was composed of 15 genotypes including 12 females and 3 males (F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12, M2, M8, M10) and the green cluster included only 9 male genotypes (M1, M3, M4, M5, M6, M7, M9, M11, M12) (Fig. 1). The Delta $K$ ($\Delta K$) value shows that optimal number of $K = 5$ imply the existence of five main subpopulations by ISSR markers. Among these five subpopulations, it may be identified two very distinct clusters (blue and red) and the other three subpopulations are mixed with these two main clusters. Red cluster was composed of 14 genotypes including 12 females and 2 males (F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12, M2, M12) and the blue cluster included only 10 male genotypes (M1, M3, M4, M5, M6, M7, M8, M9, M10, M11) (Fig. 1). The mastic tree germplasm was structured into two clusters ($K = 2$), represented by different colors (red, green) by ITS markers. Cluster I, represented by the red color, is composed of 7 (M2, M11, M7, M9, F6, M8, F11) genotypes; Cluster II, represented by the green color, is composed of 17 genotypes (F2, M3, M5, M10, M12, F9, F12, M6, F3, F5, F10, F8, F7, F4, F1, M1, M4) (Fig. 1).

## The PCA Analyses

The SSR data showed that 43.60 % of the variation is explained by the coordinates 1 and 2 with the first coordinates explaining 24.81 %, the second coordinates explaining 18.79 % by PCA (Fig. 2). The first three principle coordinates explained 60.91 % of the total variation. The PCA graph has indicated that 24 mastic tree genotypes did not separate as main groups and there was no similarity between the patterns constructed by both Structure and PCA regarding the distribution and sexual type of the genotypes.

The RAPD PCA data showed that 46.18 % of the variation is explained by the coordinates 1 and 2 with the first coordinates explaining 21.58 %, the second coordinates explaining 19.97 %. The first three principle coordinates explained 56.94 % of the total variation (Fig. 2). The PCA graph of the markers dataset has indicated that 24 mastic tree genotypes divided into four main groups. It can be inferred from the Structure and PCA graph that male and female genotypes are more distinctly separated from each other.

The ISSR PCA data showed that 42.89 % of the variation is explained by the coordinates 1 and 2 with the first coordinates explaining 26.52 % and the second coordinates explaining 16.37 % (Fig. 2). The first three principle coordinates explained 58.92 % of the total variation. The PCA graph has indicated that 24 mastic tree genotypes divided into five main groups which two of them included only female genotypes and the other three groups contained only male genotypes. From the results, it was observed that male and female genotypes distinctively separated to each other in five groups.

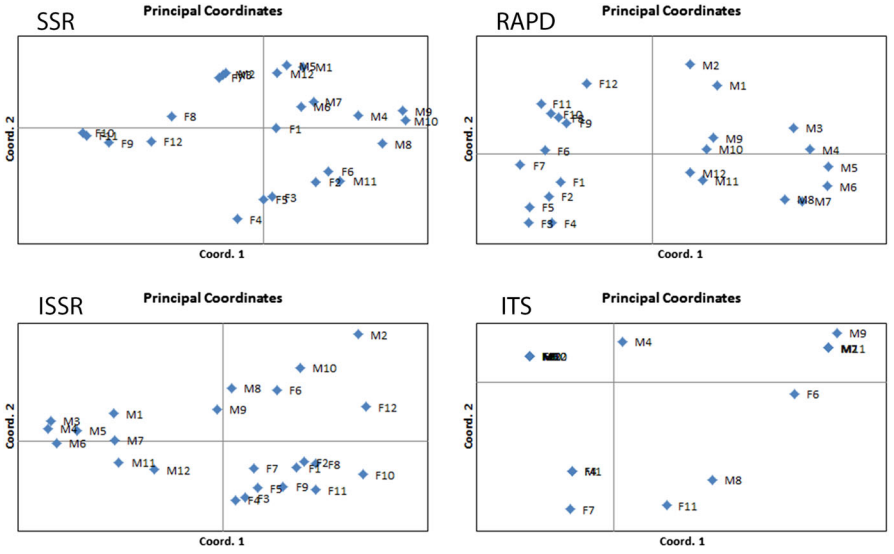


**Fig. 2** Principal components analysis (PCA) for 24 accessions as male and female mastic tree genotypes genotyped with from SSR, RAPD, ISSR, and ITS markers

The PCA results obtained by ITS markers showed that 67.16 % of the variation is explained by the coordinates 1 and 2 with the first coordinates explaining 47.99 % and the second coordinates explaining 19.18 %. The first three principle coordinates explained 78.46 % of the total variation. The PCA graph has indicated that 24 mastic tree genotypes divided into two main groups. It was observed that female mastic tree genotypes were separated well rather than male genotypes by the PCA (Fig. 2) and Structure analysis. The PCA graph has indicated that mastic tree genotypes did not separate as main groups.

## Phylogenetic Analysis

To investigate the relationships between the 24 mastic tree genotypes, neighbor joining arithmetical model was selected for construction of phylogenetic tree and the Seqboot, Restdist, Neighbor, and Consense sub programs of Phylip V.3.695 software was used.

Dendrogram constituted with 7 SSR markers data indicated that 24 accessions were separated as 5 groups and 3 outgroups (Fig. 3).

M10 (Mesta Sakız Adası-Chios) genotype appeared in 100 % of the bootstrap replicates in the first outgroup. The second and third outgroups included M9 (Alaçatı) and M11 (Seferihisar), respectively. M8 (Urla), M7 (Gülbahçe), and M4 (Paşalimanı) were included in the first group. The second group included M5 (Ildır), M2 (Karaabdullah), and M1 (Domuzçukuru) genotypes. The third group (F1, F2, F3, F4) and fourth group (F8, F9, F10, F11, F12) included a total of nine female genotypes and among them F10 (Mestasakız Adası-Chios) and F11 (Seferihisar) were closely related. The fifth group included both female (F5, F6, F7) and male (M3, M6, M12) genotypes. Roughly, male and female trees were grouped into the same clusters. Some geographically distant genotypes were found in the same cluster, for instance, M6 (Karaburun) and M12 (Bornova/Gökdere).

The Neighbor Joining (NJ) tree constituted based on 50 RAPD markers' data indicated that the dendrogram consisted of one outgroup (M7—Gülbahçe) and two groups (Fig. 4). The first group included 4 genotypes (M1—Domuzçukuru, M3—Manzara Tepesi, M4—Paşalimanı, M5—Ildır) and the other group included all female genotypes and male genotypes as follows: M2, M6, M8, M9, M10, M11, and M12. The observation indicated that M8 (Urla) and M10 (Mesta Sakız Adası-Chios) and F10 (Mesta Sakız Adası-Chios) and F11 (Seferihisar) genotypes were the closest male and female genotypes, respectively.

The phylogenetic tree constituted based on 50 ISSR markers data indicated that the dendrogram consisted of two outgroups (M1, M2) and two groups (Fig. 5). The first group included 3 genotypes (M3—Manzara Tepesi, M4—Paşalimanı, M5—Ildır) and the second group included all female genotypes and male genotypes (M6, M7, M8, M9, M10, M11, and M12). The results indicated that male and female genotypes were distinctly separated from each other. M9 (Alaçatı) and M10 (Mesta Sakız Adası-Chios) were the closest genotypes and F11 (Seferihisar) F12 (Bornova/Gökdere) genotypes fall into same cluster and showed closer genetic relation.

The Neighbor Joining (NJ) tree constituted based on ITS marker data indicated that the dendrogram consisted of two major groups (Fig. 6). The first major group

(1) includes 18 genotypes. Genotypes M6 (Karaburun), F5 (Ildır), M10 (Mesta Sakız Adası-Chios), M12 (Bornova/Gökdere), M5 (Ildır), F12 (Bornova/Gökdere), M3 (Manzara Tepesi), F1 (Domuzçukuru), F2 (Karaabdullah), F10 (Mesta Sakız

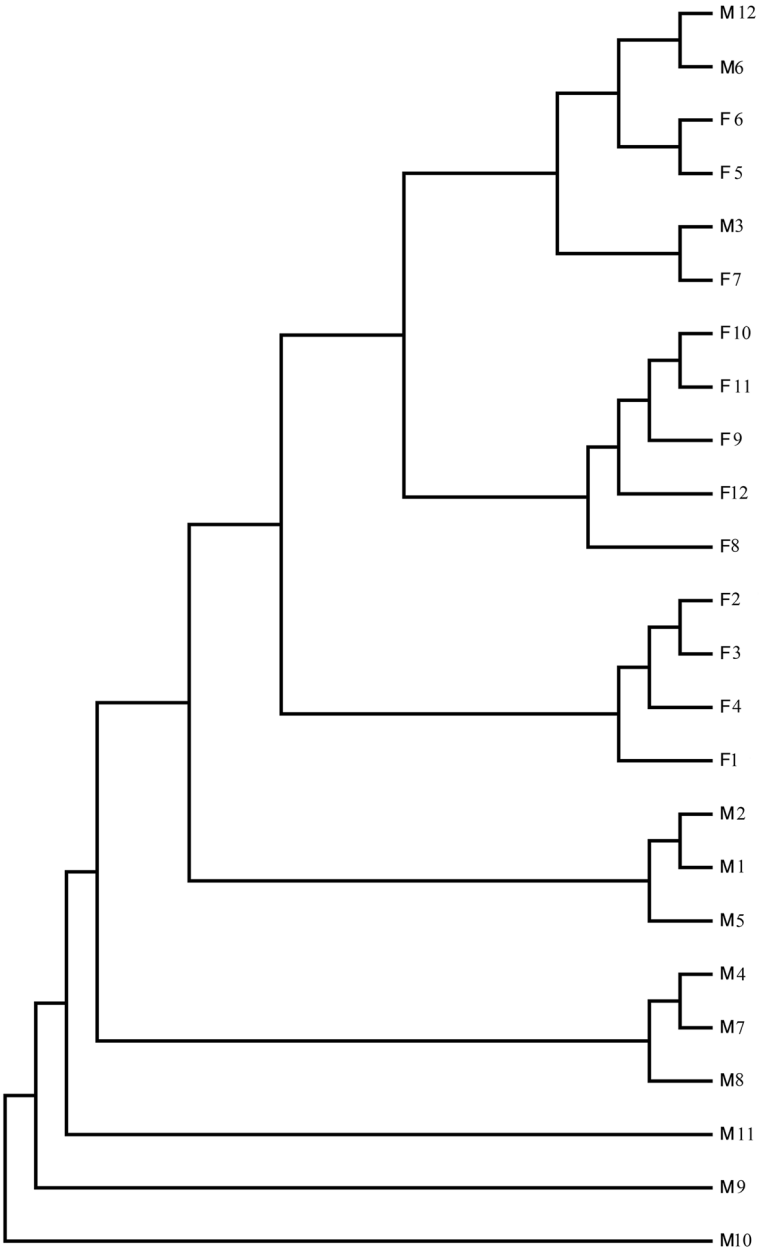


**Fig. 3** Neighbor Joining (NJ) tree of 24 mastic tree genotypes based on SSR data

Adası-Chios), F8 (Urla), F9 (Alaçatı), F3 (Manzara Tepesi), F4 (Paşalimanı), M1 (Domuzçukuru), F7 (Gülbahçe), M4 (Paşalimanı), and F11 (Seferihisar) are consisted of the first group. The second major group consisted of the following six genotypes: M8 (Urla), F6 (Karaburun), M7 (Gülbahçe), M2 (Karaabdullah), and M9 (Alaçatı) with M11 (Seferihisar). The result indicated that male and female genotypes were not separated to each other as they were distributed randomly.

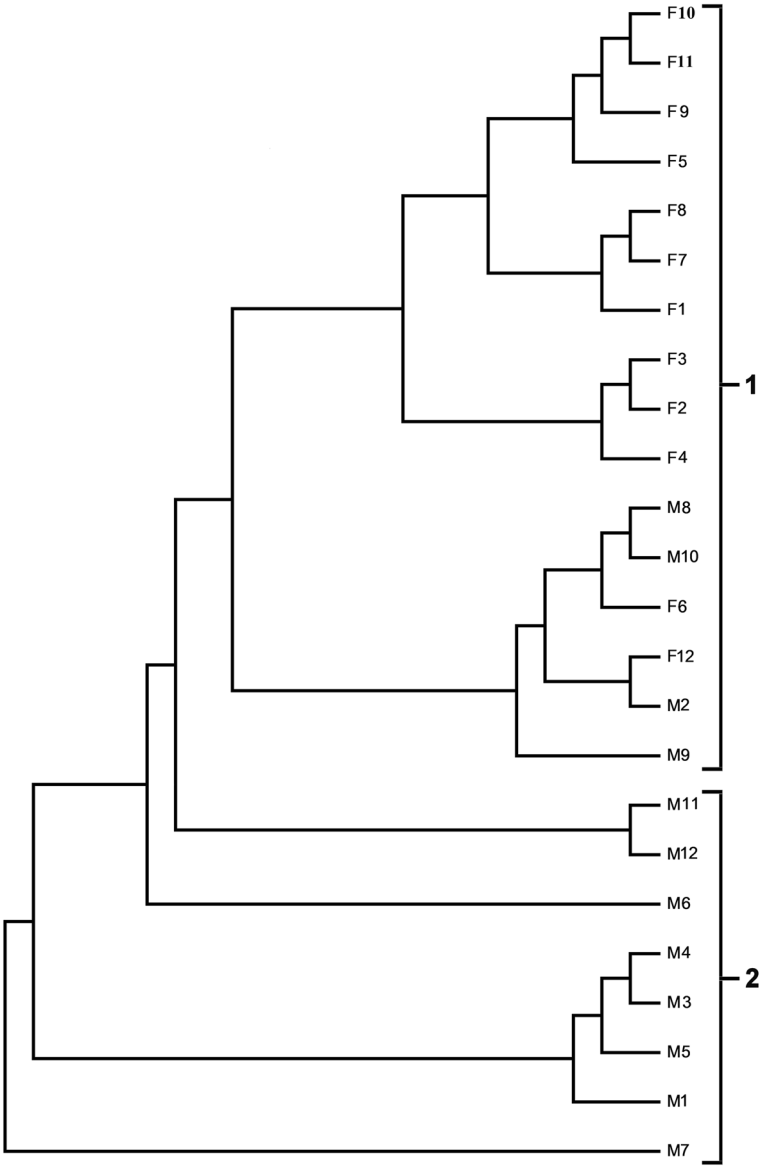


**Fig. 4** Phylogenetic tree based on RAPD data constructed for 24 mastic tree genotypes

Minimum distances were observed between M10 (Mesta Sakız Adası-Chios) and M12 (Bornova/Gökdere) with F2 (Karaabdullah) and F10 (Mesta Sakız Adası-Chios). On the contrary, the geographical locations of theses genotypes were not

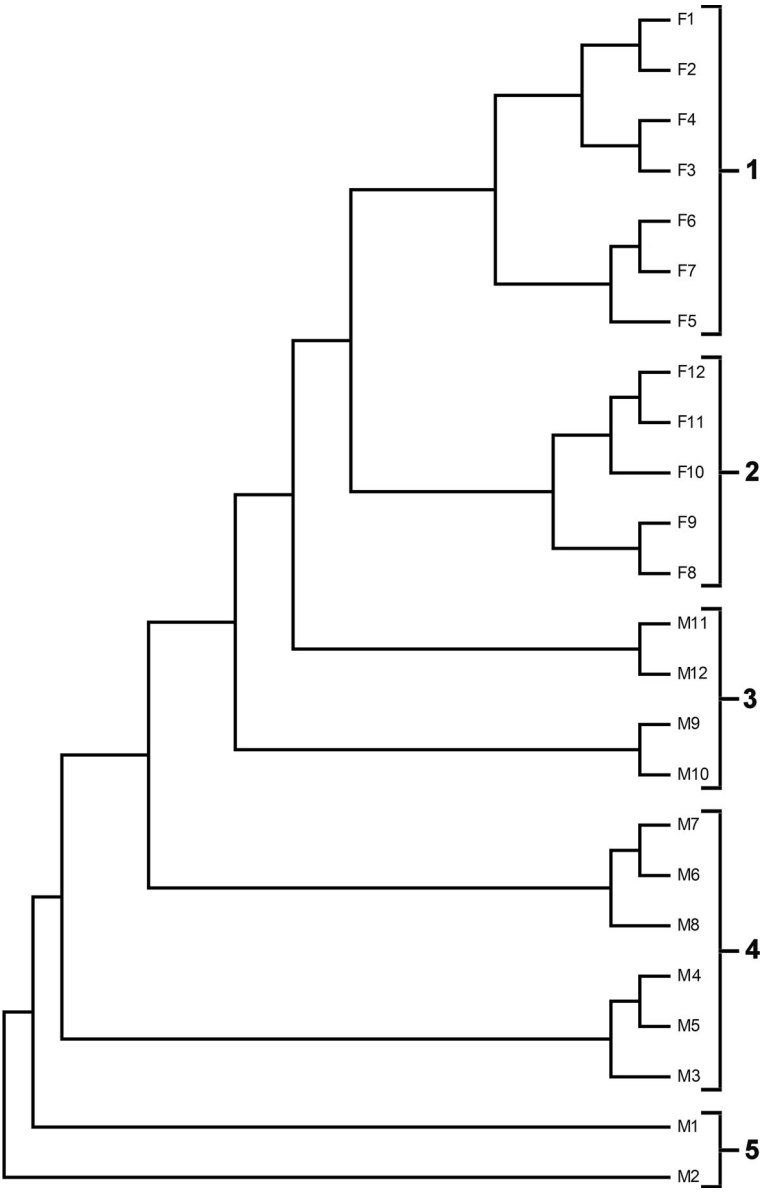


**Fig. 5** The Neighbor Joining (NJ) tree constructed for 24 mastic tree genotypes based on 50 ISSR markers data

very close. The most distinction was observed between M6 (Karaburun) with M11 (Seferihisar). These two genotypes are geographically distance from each other.

## Discussion

The most comprehensive studies concerning the taxonomic relationships between *Pistacia* species at the molecular level were performed by Zohary (1952) and Parfitt and Badenes (1997). This study comprises the first comparative study carried out to study the genetic diversity, phylogenetic relations and population structure among 24 wild-type mastic tree genotypes collected from different geographical localities,

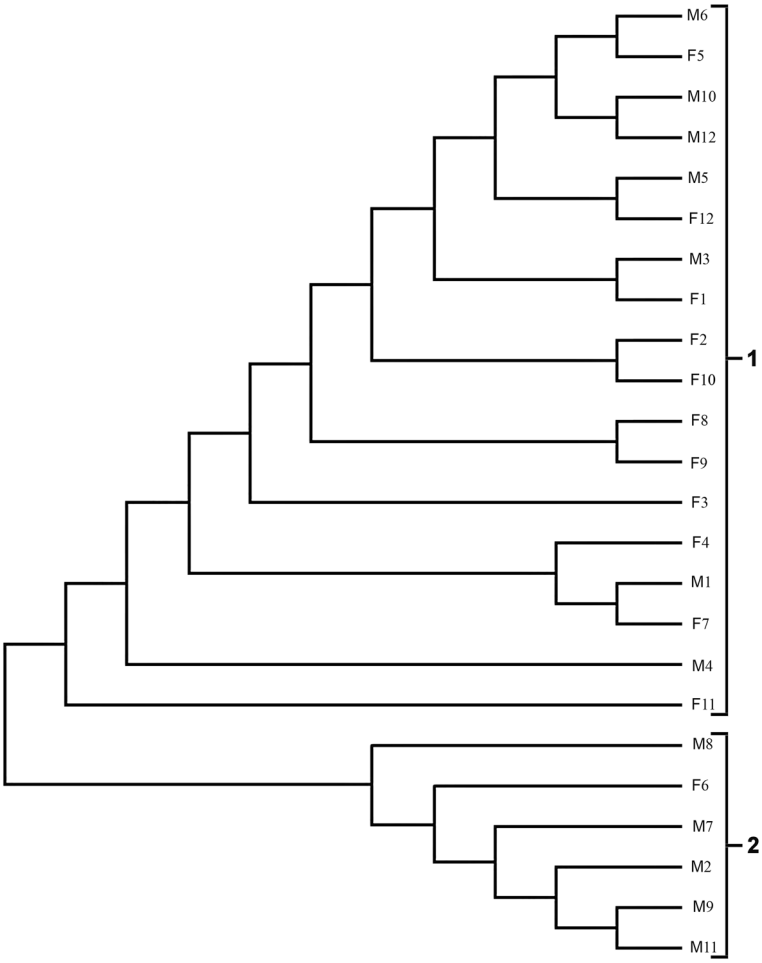


**Fig. 6** Neighbor joining (NJ) tree constructed for 24 mastic tree genotypes based on the ITS marker dataset

so that they can be utilized effectively by breeders in selection of diverse parents for future breeding programs. In the present study, SSR, RAPD, ISSR, and ITS markers were utilized for the evaluation of the genetic diversity and phylogenetic relationship between 24 mastic tree genotypes. According to these four molecular markers diversity parameters, we observed that all molecular markers appear to be efficient techniques to classify wild-type mastic tree genotypes and to determine the genetic diversity present in germplasm collections. Efficiency of molecular marker techniques depends on the amount of polymorphism generated by the primers. In the present study, SSR, RAPD, ISSR, and ITS techniques generated numerous polymorphic bands and the presence of a high percentage of polymorphism indicated high variability in these mastic tree genotypes. The ISSR primers generated greater polymorphism as compared to the RAPD primers and the ITS primer diversity parameters greater than the SSR markers. In comparison to the studies (Pazouki et al. 2010; Baghizadeh et al. 2010; Ahmad et al. 2003a), our SSR markers allele sizes were similar to the literature but genetic diversity parameters were higher than (0.452 for MAF, 0.656 for gene diversity, and 0.628 for PIC) these literature studies. This is maybe due to the stressful condition and environmental heterogeneity of our mastic tree genotypes as Izmir Çeşme region is characterized by both temperature and precipitation stresses. In addition, the number of alleles that we detected was relatively more than from those recorded previously for Syrian, Turkish, and American Pistchios (Ahmad et al. 2003b, 2005), as well as more than from those recorded (Pazouki et al. 2010) study. The allele numbers and genetic diversity parameters revealed by ISSR is higher than those revealed by RAPD. These results are in agreement with the findings of these two studies Parveen et al. (2013) and Baghizadeh et al. (2010).

According to the population structure analysis of mastic tree genotypes based on four different markers, it can be seen that the SSR, RAPD, and ISSR markers result were similar to each other. The results showed that M9 (Alaçatı) with M10 (Chios) and F10 (Chios) with F11 (Seferihisar) fall into the same subpopulations. Population structure analysis based on dominant markers combination data indicate that F10 (Chios) and F11 (Seferihisar) fall into the same subpopulation but M9 (Alaçatı) was separated with M10 (Chios) genotype. Population structure result based on ITS marker indicate that M9 (Alaçatı), F11 (Seferihisar), and M10 with F10 (Chios) genotypes fall into the same subpopulation group. The graphics of PCA result indicated that M9 (Alaçatı) with M10 (Chios) and F10 (Chios) with F11 (Seferihisar) genotypes were closely related genotypes based on SSR, RAPD, ISSR, and combined dominant markers dataset. The ITS marker-based PCA result showed us M9 (Alaçatı) with M10 (Chios) had closely related but F10 (Chios) with F11 (Seferihisar) genotypes were distant from each other. *P. lentiscus* is wind pollinated plant and its seeds are dispersed by birds, and thus, there may be an extensive gene flow even among distance populations. This may result in the seed dispersal within Izmir region of Turkey and neighboring island Chios. All these four different markers based on the PCA result suggest that male and female mastic tree genotypes roughly separated from each other. From phylogenetic relationship analysis based on RAPD and ISSR markers, we can observe that almost all the male and the female genotypes were separated from each other. These results are in

agreement with the findings of the literature studies—Kamiab et al. (2014), Kafkas et al. (2001), Esfandiyari et al. (2012), and Zografou et al. (2010). The ITS and SSR markers results were quite different from the other dendrograms. According to these results, the female and male genotypes were not separated strictly and distributed randomly.

Population structure analysis, PCA, and neighbor joining-based phylogenetic analysis were carried out four sets of molecular markers data; the results based on the entire four molecular marker profiles grouped the 24 mastic tree genotypes into different clusters. However, the grouping based on neighbor joining tree was found to be comparable to the Bayesian clusters obtained through STRUCTURE analysis and graphics obtained through the principle component analysis (PCA). The results also support no significant genetic differentiation in Izmir region wild-type mastic tree genotypes with neighboring island of Chios. The present study reports that phylogenetic relationship will thus be useful for planning future studies on mastic tree genetic resources. We also inferred the spatial genetic pattern by comparing the pattern of molecular distance with the geographic distance, and the Mantel test (Mantel 1967) revealed that there was no significant correlation between the genetic and geographic distance for any of the molecular marker type concluding no spatial genetic pattern for the distance sampled here (data not shown).

In the future studies, with larger samples collected from different and wider locations where wild *P. lentiscus* grown may be analyzed to clarify the genetic diversity of wild-type mastic trees by molecular markers based on single nucleotide changes. Continuous genetic diversity assessment will help maintain the diverse species for conservation and identify the genotypes producing the highest and best quality mastic resin (gum). The results can also help the breeders so that they can effectively select the parents leading to progenies with high differentiation among them. Thoroughness of this study will further help support the use of molecular markers for the successful development of a core collection of mastic tree germplasm for the Izmir region of Turkey and all over the world. In brief, this study provided insight into the genetic composition of the wild-type mastic tree genotypes.

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