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High genetic diversity within and low differentiation among *Juniperus excelsa* M. Bieb. populations: Molecular markers reveal their genetic structure patterns

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Abstract: *J. excelsa* M. Bieb forms about 82% of the total juniper forests in Turkey. A total of 456 plant samples belonging to 19 *J. excelsa* populations were collected to determine the genetic variation of *J. excelsa* and compare their genetic diversity among populations via simple-sequence repeat (SSR) and intron targeted amplified polymorphism (ITAP) markers. Seven SSR and 132 ITAP loci were polymorphic. The percentage of polymorphism for ITAP loci at the population level ranged from 31.34 to 55.97. Average values of expected heterozygosity, observed heterozygosity, Shannon's information index, Fis, Fst and Nm for SSR loci were 0.616, 0.512, 1.54, 0.124, 0.043, and 5.513, respectively. Genetic diversity values of ITAP loci were lower than those of SSR loci. Gst and Nm values for ITAP loci were 0.225 and 1.728, respectively. Pair-wise genetic distances varied between 0.023 and 0.292 for SSR loci, 0.010 and 0.110 for ITAP loci. The majority of the genetic variations originated from intra-population level (98% for SSRs, 80% for ITAPs). Mantel test results showed that there was no statistically significant correlation between pair-wise geographical and genetic distances. It was indicated that the populations had similiar structure pattern. It was seen that *J. excelsa* populations maintained their high genetic diversity. Additionally, genetic differentiations among the populations were low. No indication of genetic adverse effect related to habitat fragmentation was determined in the populations. In conclusion, it can be said that this situation is an advantage in terms of implementing conservation strategies for this species. The authorities in the Turkish Ministry of Forestry should plan some conservation studies in order to maintain the existing genetic diversity of this species.

Key words: Crimean juniper, genetic isolation, intron targeted amplified polymorphism (ITAP), molecular variance, simple-sequence repeat (SSR)

1. Introduction

Juniperus L. is a cosmopolitan and species-rich coniferous genus in Cupressaceae family across Northern Hemisphere. Seventy-five species have been described by Adams (2014) based on the newest molecular systematic revisions. Of these species, nine (*Juniperus drupacea* Labill., *J. communis* L., *J. deltoides* R.P. Adams, *J. macrocarpa* (Sibth. & Sm.) Ball, *J. sabina* L., *J. phoenicea* L., *J. foetidissima* Willd., *J. polycarpos* K. Koch and *J. excelsa* M. Bieb.) grow naturally in Turkey (Adams, 2014; Adams et al. 2016). *J. excelsa* (Crimean/Grecian juniper) populations that are naturally distributed in the Balkan Peninsula, Crimea, Syria-Lebanon, and Cyprus form pure or mixed forests in Turkey (Douaihy et al. 2011). Total juniper forests cover an area of 1.113.085 ha in Turkey, of which 82% are *J. excelsa* forests (OGM, 2014)[1]. *J. excelsa* populations, which are very important typical and natural elements of the landscape in Turkey grow frequently in semi-arid, nutrient poor, stony, rocky, and highly inclined-mountainous areas between altitudes of 300 and 2300 m (Coode and Cullen, 1965). Additionally, they are resistant against drought, poor soils, hot weather condition, and frost damages (Gültekin and Gültekin, 2006). Due to these ecological properties, *J. excelsa* plays a very important role with regard to the maintenance of ecosystems health on such areas.

The high level of genetic diversity level is crucial for the adaptation of living organisms against changing environments and loss of genetic diversity leads to lower fitness in living populations (Leonardi et. al, 2012; Govindaraj et al., 2015). By estimating population genetic parameters, populations can be analyzed and assessed in terms of fitness, inbreeding depression, genetic bottleneck,

[1] OGM (2014). Ardıç Ormanlarının Rehabilitasyonu Eylem Planı. Türkiye Cumhuriyeti Orman ve Su İşleri Bakanlığı Orman Genel Müdürlüğü Silvikültür Daire Başkanlığı Yayını (Action Plan of Rehabilitation of Juniper Forests. Republic of Turkey Ministry of Forestry and Water Affairs, Directorate General of Forestry Silviculture Department Publication). [online]. Website https://www.ogm.gov.tr/ekutuphane %20Rehabilitasyonu%20Eylem%20 Planı.pdf. [Accessed 13 October 2019] (in Turkish).

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and gene flow. Molecular markers used efficiently by different researchers since the 1990s are useful for determining level of genetic variation, biogeographical patterns, molecular phylogeny, and in conservation genetics of forest trees. Simple sequence repeats (SSRs, microsatellites) are DNA fragments in several base pair lengths found widely-homogeneously in genomes of organisms including chloroplast and mitochondria. SSRs have been employed since the 1990s to assess genetic diversity, detect genetic differentiation and structure patterns, estimate phylogeny, carry out QTL analysis, and mapping in addition to executing the breeding process and genetic fingerprinting works in living organisms (Vieira et al., 2016).

Nuclear SSR markers were developed for at least seven *Juniperus* species including *Juniperus communis* (Michalczyk et al., 2006), *J. przewalski* (Zhang et al., 2008), *J. tibetica* (Opgenoorth, 2009), *J. pingii* (Li et al., 2013), *J. cedrus* (Rumeu et al., 2013), *J. thurifera* (Teixeira et al., 2014) and *J. sabina* (Geng et al., 2016). Additionally, the SSR markers of *J. communis* have been used successfully for genetic analyses of *J. excelsa* (Douaihy et al., 2011; Yücedağ and Gailing, 2013), *J. procera* (Sertse et al., 2013), *J. deltoides*, *J. oxycedrus*, *J. navicularis*, *J. macrocarpa*, *J. brevifolia, J. cedrus* (Boratyński et al., 2014), and *J. brevifolia* (Bettencourt et al., 2015).

ITAPs (intron targeted amplified polymorphisms) were described and developed by Xiong et al. (2013). This technique was developed on the basis of sequence-related amplified polymorphisms (SRAP). While SRAP markers are obtained by amplifying both exons and introns in the genic regions of the genomes, ITAP markers target only the introns (Xiong et al., 2013). Thus, it can be possible with this marker type to efficiently analyze the polymorphisms originating from introns. Very few studies have been carried out recently using this marker system on plants (Xiong et al., 2013; Sheikh et al., 2018).

Habitat fragmentation occurs when a population loses continuity and is divided into smaller and isolated remnant patches of demes (Wilson et al., 2016). This phenomenon leads to the loss of biodiversity and degradation of ecosystems. Further, habitat degradation and fragmentations may cause more severe consequences such as loss of fitness, inbreeding depression, reduction in genetic diversity, and even extinction (Aguilar et al., 2008; Krauss et al., 2010). Gene flow may reduce among smaller patches giving rise to the heterozygosity deficit and greater genetic differentiation among patches of demes (Leonardi et al., 2012). The problem of habitat degradation and fragmentation was reported in literature for some juniper species, such as *J. communis* in Europe (Michalczyk, 2008), *J. polycarpos* and *J. indica* in India (Rawat and Everson, 2012) and populations of *J. communis, J. macrocarpa, J. navicularis* and *J. phoenicea* in coastal ecosystems in Europe (Picchi, 2008) and *J. excelsa* in Turkey (OGM, 2014). Except for Michalczyk (2008), other three studies did not include genetic analyses, but they emphasized that juniper populations are under anthropogenic threat and development, and maenagement conservation-rehabilitation strategies are required for the maintainence of juniper forests.

It is known that juniper forests have been destroyed and fragmented for centuries due to obtain wood for houses and buildings, to meet the energy requirements, manufacture goods, and turn forests into agricultural lands in Turkey (OGM, 2014). The primary objectives of the present study were 1) to analyze intra-species genetic diversity of *J. excelsa*, 2) to determine the genetic structure of the populations using SSR and ITAP markers, 3) to evaluate genetic effects of habitat fragmentation and degradation, and 4) to contribute to the development of new perspectives related to conservation and reforestation strategies of juniper forests in the future through the genetic data acquired in the present study.

2. Materials and methods

2.1. Sampling

Leaf samples were collected from 456 individuals (24 individuals per population) from 19 *J. excelsa* populations in Turkey. Additionally, 10 individual trees from *J. polycarpos* and six individual trees from *J. foetidissima* were sampled as outgroups (Figure 1, Supplementary Information 1). The distance among the sampled trees in each population was at least 20-30 m.

2.2. DNA isolation process

DNAs were isolated from dried leaf samples in accordance with the Dellaporta et al. (1983) protocol. The quality and quantity (ng/µL) of extracted DNAs were determined via Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific) following homogenization and isolation processes at the Plant Biotechnology Laboratory of Akdeniz University Biology Department.

2.3. SSR analysis

Eight SSR loci (Jc037, Jc166, JT02, JT33, JT34, JT37, JT38, and JT40) were analyzed in this study (Supplementary Information 2). Annealing temperatures and concentrations of the primer pairs were determined after PCR optimization process. 5' fluorescent labelled primers (FAM, VIC, NED, and PET) were used during analysis. Sample DNAs were amplified at the laboratory using the Qiagen multiplex-PCR kit. The reaction volume was 10 µL in total including 5 µL of 2X Qiagen Multiplex-PCR Kit, 2 µL of dH_2O of the kit, 2 µL of primer mixture (2.5-3.5 µM) and 1 µL of genomic DNA (10 ng/µL). Final concentrations of the primer pairs and genomic DNAs were 0.5-0.7 µM and 1 ng/µL, respectively. PCR steps were

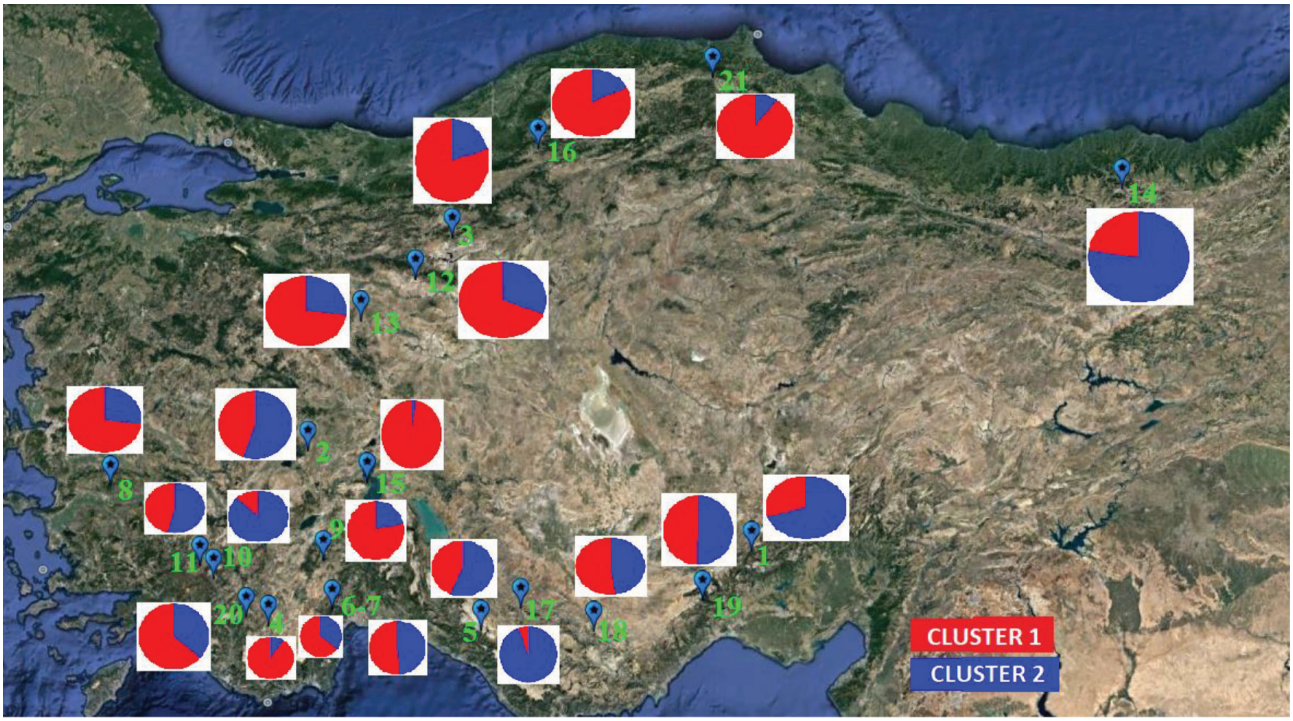

Figure 1. Placement of the populations on the map according to GPS data and distrubiton of the two genetic clusters in the populations according to ITAP-STRUCTURE results (1. Adana-Pozantı, 2. Afyon-Sandıklı, 3. Ankara-Beypazarı, 4. Antalya-Elmalı, 5. Antalya-Gündoğmuş, 6. Antalya-Termessos, 7. Antalya-Termessos (*J. foetidissima*), 8. Aydın-Efeler, 9. Burdur-Bucak, 10. Denizli-Acıpayam, 11. Denizli-Beyağaç, 12. Eskişehir-Mihalıççık, 13. Eskişehir-Seyitgazi, 14. Gümüşhane-Torul (*J. polycarpos*), 15. Isparta-Senirkent, 16. Karabük-Eskipazar, 17. Konya-Hadim, 18, Mersin-Mut, 19, Mersin-Tarsus, 20. Muğla-Seydikemer, 21. Sinop-Boyabat).

one cycle of predenaturation (95 °C, 15 min); 30 cycles of denaturation (95 °C, 30 s), annealing (52, 55 or 57 °C, 90 s) and extension (72 °C, 60 s); one cycle of final extension (60 °C, 30 min) and final hold (4 °C). The lengths of fragment PCR products were determined afterwards as the unit of base pairs (bp) using ABI PRISM 310 Genetic Analyzer. Genotype data of the individual samples were scored using GenMapper Software "Version 3.1.0" program.

2.4. ITAP analysis

Sequence information of 27 forward ("Em" series) and five reverse ("ITPR" series) primers were obtained from the studies by Yang et al. (2013) and Xiong et al. (2013), respectively. All potential primer pairs (27 × 5 = 135) were analyzed. The desired polymorphic, scorable and reproducible band profiles with good quality were generated with eight of all potential primer pairs (Em1-ITPR5, Em5-ITPR4, Em5-ITPR5, Em12-ITPR3, Em19-ITPR2, Em20-ITPR3, Em21-ITPR1, and Em26-ITPR3). Thus, these eight primer pairs were selected for PCR amplifications of all DNA specimens. PCR was performed in a 15 µL reaction volume including 1X Taq-buffer, 0.2 mM dNTP, 0.5 µM each forward and reverse primers, 5 mM $MgCl_2$, 50 ng genomic DNA, and 1.25 U Taq DNA polymerase (Fermentas) at final concentration. DNA amplifications were performed in a thermocycler (ABI 9600) using the following cycling program; pre-denaturation at 94 °C, 5 min. Afterwards, the first five cycles were run at 94 °C, 1 min as denaturation, 35 °C, 1 min as annealing, and 72 °C, 1 min as extension, respectively. Thereafter, the annealing temperature was raised to 50 °C for another 35 cycles. The final extension was then run at 72 °C for 10 min. Finally, PCR products were held at 4 °C until gel electrophoresis. PCR (polymerase chain reaction) products were resolved on 1.5% agarose gels in 1X TBE buffer. Gene Ruler 100 bp DNA ladder (Fermentas) was used as molecular size DNA standard. Visualizations of the gels were carried out under UV light using DNR Mini Bis Pro Bio-Imaging Systems, following the staining of the gels with ethidium bromide (1 µg/mL) for 15 min. The size of amplification products was estimated by comparing with DNA ladder fragments. All laboratory analyses (DNA isolation, DNA quantification, SSR, and ITAP analyses) were performed at the Akdeniz University Biology Department, Plant Biotechnology Laboratory.

2.5. Statistical analyses of SSR and ITAP data

Estimation of intra-population genetic diversity parameters and inter-populations genetic differentiation level was fulfilled by using the Popgene 1.32 software

(Yeh et al., 1999)[2]. Number of polymorphic loci (Npl), percentage of polymorphic loci (Ppl), observed number of alleles (na), effective number of alleles (ne), Shannon's information index (I) values were calculated for both SSR and ITAP data. Observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient within populations (Fis), genetic differentiation coefficient among populations (Fst), and gene flow among the populations (Nm; Nei, 1987) were computed only for SSR data. Furthermore, Hardy-Weinberg Equilibrium (HWE) test was implemented in order to establish any statistical difference between expected and observed heterozygosity according to Marcov Chain parameters (Dememorization: 1000, Batches: 100 and Iterations per batch: 1000) in the web-based program GENEPOP (Raymond and Rousset, 1995). Another HWE test was carried out in MS Excel based software GenAlEx 6.503 (Peakall and Smouse, 2006) for every locus in each population. The procedures for the analysis of Nei's gene diversity (h), total genetic diversity (Ht), genetic diversity within populations (Hs), genetic differentiation coefficient among populations estimated from Hs and Ht values (Gst) according to Nei (1987), and gene flow (Nm; McDermott and McDonald, 1993) were carried out only for ITAP data in Popgene.

Pair-wise genetic distances were computed according to Nei (1972) in Popgene and composed dendrograms according to unweighted pair group method with arithmetic mean (UPGMA) based on pair-wise genetic distances to visualize genetic similarities and differences among the sampled populations. Dendrograms were shaped harnessing the software Figtree v1.4.3 (Rambaut, 2016)[3]. AMOVA (analysis of molecular variance) and Mantel tests were carried out via GenAlEx 6.503. It was aimed through the use of AMOVA to distinguish the distribution of genetic variations between intra-population and inter-population. The aim of Mantel test was to determine the correlation between pair-wise geographical and genetic distance among the populations, respectively.

The computer program Structure v 2.3.4 (Pritchard et al., 2000) was used to define specific and similar structure patterns of the populations. The analysis was performed according to MCMC algorithm with 5000 burn-in period, 50,000 replications and 10 iterations. Results of the structure analysis was evaluated using the web-based program Structure Harvester (Earl and von Holdt 2012), and the number of the clusters (K) was obtained by employing Evanno et al. (2005) and Jakobsson and Rosenberg (2007) computations in Structure Harvester.

3. Results

3.1. Genetic diversity

Seven out of eight SSR loci (except for JT34) were polymorphic. A total of 88 alleles were observed in seven polymorphic SSR loci (Table 1). The lowest number of alleles was observed at loci JT02 and JT37 (3 alleles), whereas the highest was at locus JT33 (29 alleles). Six out of 88 alleles were unique. They were 153 and 155 bp (Karabük-Eskipazar) at locus Jc037, 144 bp (*J. foetidissima*) and 150 bp (Muğla-Seydikemer) at locus Jc166, 114 and 116 bp (Mersin-Mut) at locus JT38.

The number of ITAP loci ranged from 13 (for primer-pair Em1-ITPR5) to 21 (for primer-pair Em26-ITPR3). Average number of loci per each primer pair was 17. A total of 132 polymorphic loci were scored for ITAP data (totally 134 loci) except for two monomorphic loci of Em5-ITPR5-225 bp and Em19-ITPR2-150 bp (Supplementary Information 3).

Values of genetic diversity parameters with their range,

[2] Yeh FC, Yang RC, Boyle T (1999). PopGen32 computer program (ver. 1.31) microsoft windows based freeware for population genetic analysis [online]. Website https://sites.ualberta.ca/~fyeh/popgene.pdf [Accessed 13 October 2019

[3] Rambaut A (2016) FigTree, Tree Fig. Drawing Tool v. 1.4.3. Institute of Evolutionary Biology, University of Edinburgh. Avaliable at: http://tree.bio.ed.ac.uk/Accessed 13 October 2019.

Table 1. Genetic diversity statistics for SSR loci.

Loci	(na)	(ne)	(I)	(Ho)	(He)
Jc037	26	15.08	2.86	0.609	0.935
Jc166	11	3.63	1.47	0.572	0.725
JT02	3	1.03	0.10	0.034	0.034
JT33	29	19.58	3.12	0.717	0.950
JT37	3	1.85	0.69	0.483	0.459
JT38	10	3.36	1.46	0.725	0.703
JT40	6	2.03	1.05	0.445	0.508
Mean±SE	12.57 ± 4.04	6.65 ± 2.82	1.54 ± 0.42	0.512 ± 0.09	0.616 ± 0.12

Table 2. Summary of genetic diversity statistics of *J. excelsa* populations studied.

Genetic diversity parameters	SSR loci		ITAP loci	
	Mean ± SE	Range	Mean± SE	Range
na	6.428 ± 0.092	5.875-7.125	1.409 ± 0.016	1.313-1.560
ne	4.234 ± 0.112	3.275-5.133	1.179 ± 0.010	1.076-1.281
Npl	6.421 ± 0.116	6–7	54.74 ± 2.151	42-75
Ppl	80.26 ± 1.455	75-87.5	40.85 ± 1.605	31.34-55.97
I	1.166 ± 0.016	1.046-1.279	0.166 ± 0.008	0.088-0.252
h	Not computed		0.107 ± 0.006	0.051-0.166
Ho	0.449 ± 0.008	0.347-0.501	Not computed	
He	0.524 ± 0.006	0.472-0.568	Not computed	

mean, and standard error (SE) for SSR loci were also estimated (Table 1 and 2). The lowest genetic diversity among polymorphic SSR loci was detected at locus JT02 (na = 3, ne = 1.03, I = 0.1, Ho = 0.034, He = 0.034). Genetic diversity parameters of locus JT33 was the highest with respect to the same parameters (i.e na = 29, ne = 19.58, I = 3.12, He = 0.950) except observed heterozygosity. The highest observed heterozygosity was at locus JT38 (Ho = 0.725). No statistically significant difference (no Hardy-Weinberg disequilibrium) was found between the observed and expected heterozygosity for locus JT38 ($p \geq 0.05$). However, loci Jc037, Jc166, JT33, and JT40 displayed Hardy-Weinberg disequilibrium with significant statistical differences between the observed and expected heterozygosity ($p < 0.01$). While heterozygosity deficit was observed for loci Jc037, Jc166, JT33, and JT40, heterozygosity excess was determined for loci JT02 and JT37 ($p < 0.01$). There was heterozygosity excess at locus JT38, but it was statistically insignificant ($p = 0.068$). Moreover, JT02 locus was monomorphic at 11 populations according to HWE analysis. It was found that none of the populations was in Hardy-Weinberg equilibrium due to significant statistical differences between the observed and expected heterozygosity ($p < 0.05$).

Genetic diversity levels of the all studied populations were estimated with their range, mean, and standard error (SE) obtained for both SSR and ITAP loci (Table 2). The mean of the observed number of alleles (na) for all populations was 6.428 for the SSR loci. The lowest value was obtained 5.875 (Afyon-Sandıklı and Eskişehir-Seyitgazi), while the highest value was 7.125 (Eskişehir-Mihalıççık and Mersin-Mut). The effective number of alleles (ne) ranged from 3.275 (Antalya-Elmalı) to 5.133 (Aydın-Efeler), the mean of (ne) was 4.234. Numbers of polymorphic loci (Npl) were 6 or 7 (mean = 6.421) with percentages of polymorphic loci (Ppl) as 75 or 87.5 (mean = 80.26) per population. The lowest value of Shannon's information index (I) was 1.046 (Isparta-Senirkent), while the highest was 1.279 (Mersin-Mut) with a mean value of (I) 1.166. While the lowest observed heterozygosity (Ho) and expected heterozygosity (He) were computed as 0.347 and 0.472 in Isparta-Senirkent, the highest (Ho) was observed as 0.501 in Sinop-Boyabat and (He) was 0.568 in Antalya-Gündoğmuş. The mean values of (Ho) and (He) were calculated as 0.449 and 0.524, respectively. Based on the values of the genetic diversity parameters (Ho, He, and I) for 19 populations provided in Supplementary Information 4, Isparta-Senirkent was the population with the lowest genetic diversity. The highest value of (I) was calculated for Mersin-Mut with values of (Ho) and (He) as 0.482 and 0.563, respectively. Thus, Mersin-Mut had the second highest value of (He) and fourth highest value of (Ho). Moreover, the second highest value of (I = 1.265) and the third highest value of (He = 0.562) were calculated for Sinop-Boyabat. Although the highest value of (He = 0.568) and the fourth highest value of (I = 1.251) were obtained for Antalya-Gündoğmuş, the value of (Ho = 0.441) was not high for the population. In short, Mersin-Mut, Eskişehir-Mihalıççık, Sinop-Boyabat, and Denizli-Acıpayam are among the populations with the highest genetic diversity considering all parameters even though their rankings change according to the related parameter.

According to ITAP loci in population level, the mean values of (na), (ne), (I), (Npl), (Ppl), and (h) were determined as 1.409, 1.179, 0.166, 54.74, 40.85, and 0.107, respectively. The lowest values of (na), (Npl), and (Ppl) were estimated for Adana-Pozantı (na = 1.313, Npl = 42, Ppl = 31.34). Denizli-Acıpayam was the population with the lowest (ne = 1.076), (I = 0.088) and (h = 0.051) values. The population with the highest genetic diversity value was that of Ankara-Beypazarı (na = 1.560, ne = 1.281, I = 0.252, Npl = 75, Ppl = 55.97 and h = 0.166). The average values of (na), (ne), (I), and (h) with their standard errors were 1.985 (±0.005), 1.220 (±0.015), 0.228

(±0.011), and 0.138 (±0.007) based on all of 134 ITAP loci, respectively. All detailed data about genetic diversity level of the populations were presented in Supplementary Information 4.

3.2. Genetic differentiation and genetic structure

Nei's (1972) pair-wise genetic distances among populations were computed for both SSR and ITAP markers. The pair-wise genetic distances varied from 0.023 (between Denizli-Acıpayam and Eskişehir-Mihalıççık) to 0.292 (between *J. polycarpos* and Mersin-Tarsus) for SSR data (Supplementary Information 5). The pair-wise genetic distances ranged between 0.010 (between Antalya-Termessos and Muğla-Seydikemer) and 0.110 (between Isparta-Senirkent and Konya-Hadim) for ITAP data (Supplementary Information 6). The outgroup species, namely *J. polycarpos* and *J. foetidissima* were clustered together covering the outermost part in the SSR dendrogram (Figure 2). The same situation was observed for Antalya-Elmalı and Isparta-Senirkent in ITAP dendrogram (Figure 3). The outgroup species were clustered together, but they didn't take place the outermost part in the ITAP dendrogram. This finding suggested that *J. excelsa*, *J. foetidissima,* and *J. polycarpos* didn't differentiate in terms of intron-based loci profile. Averages of Fis, Fst, and Nm were calculated as 0.124, 0.043, and 5.513, respectively (Table 3). Furthermore, total genetic diversity (Ht), total intra-population genetic diversity (Hs), Gst and Nm values were calculated from ITAP data as follows: (Ht = 0.138 ± 0.001), (Hs = 0.107 ± 0.0008), (Gst = 0.225) and (Nm = 1.728).

Analysis of molecular variance (AMOVA) results showed that the genetic diversity originated mostly from the intra-population level for SSR (98%) and ITAPs (80%) markers. The variance of SSR (2%) among the populations was quite low, which is an indication that inter-population differentiation is very little (Table 4). However, this differentiation was higher in ITAP loci (20%) compared with SSR loci. We didn't observe statistically significant correlation between pair-wise genetic and geographical distances (km) according to the results of Mantel test (Rxy, R^2 and p values). Rxy, R^2 and p values were 0.233, 0.05, 0.08 for SSRs and 0.063, 0.004, 0.33 for ITAPs, respectively.

In the analysis of STRUCTURE, ΔK showed two peaks for K = 5 and K = 7 for SSRs, and K = 2 for ITAPs. The results of SSR-Structure analysis indicated no specific structure pattern according to K= 5 and K = 7 (Supplemenary Information 7). It was observed that all populations are admixtures of five and seven clusters. On the other hand, ITAP-Structure findings pointed out that a specific population structure according to K = 2 (Figure 1). Cluster 1 was seen predominantly in Isparta-Senirkent, Antalya-Elmalı, and Sinop-Boyabat (>87%), while Cluster 2 was observed mainly in Denizli-Acipayam and Konya-Hadim (>90%). Other populations and out-groups were more or less a mixture of Clusters 1 and 2.

4. Discussion

Fragmentation in terrestrial ecosystems at local, regional, national, and global levels has progressed rapidly over the last quarter century. Many species suffer from negative effects of habitat fragmentations. High genetic diversity within populations is important with regard to delaying the negative impact of habitat fragmentation on

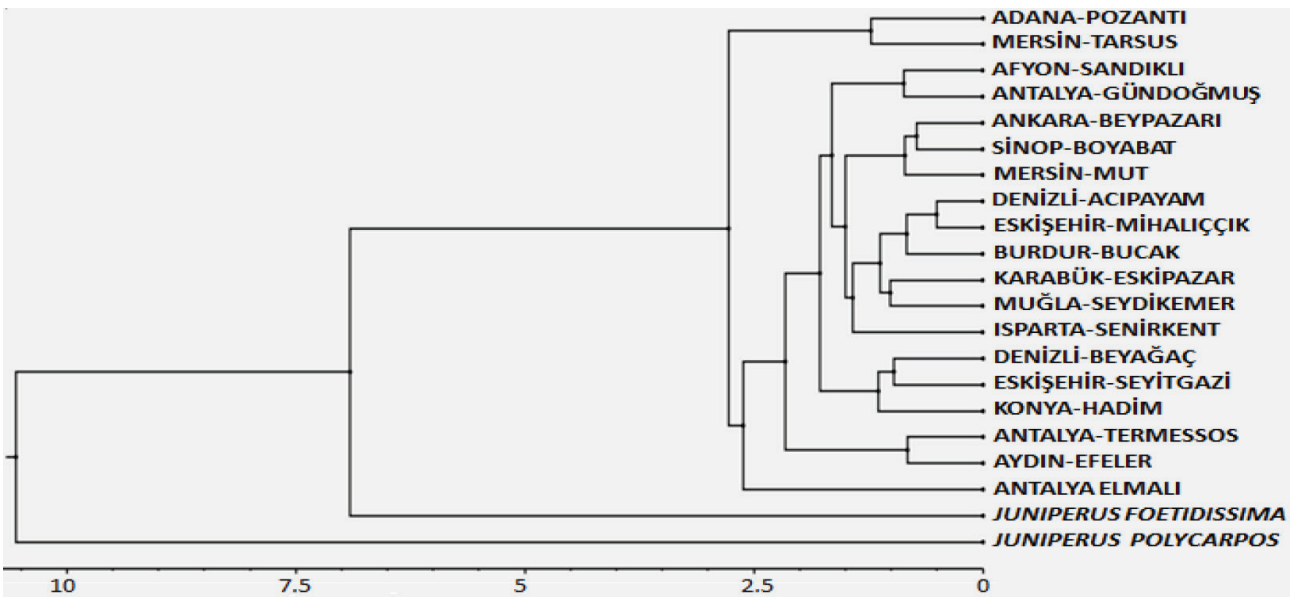

Figure 2. Dendrogram formed according to Nei (1972) pair-wise genetic distances among the populations based on SSR loci.

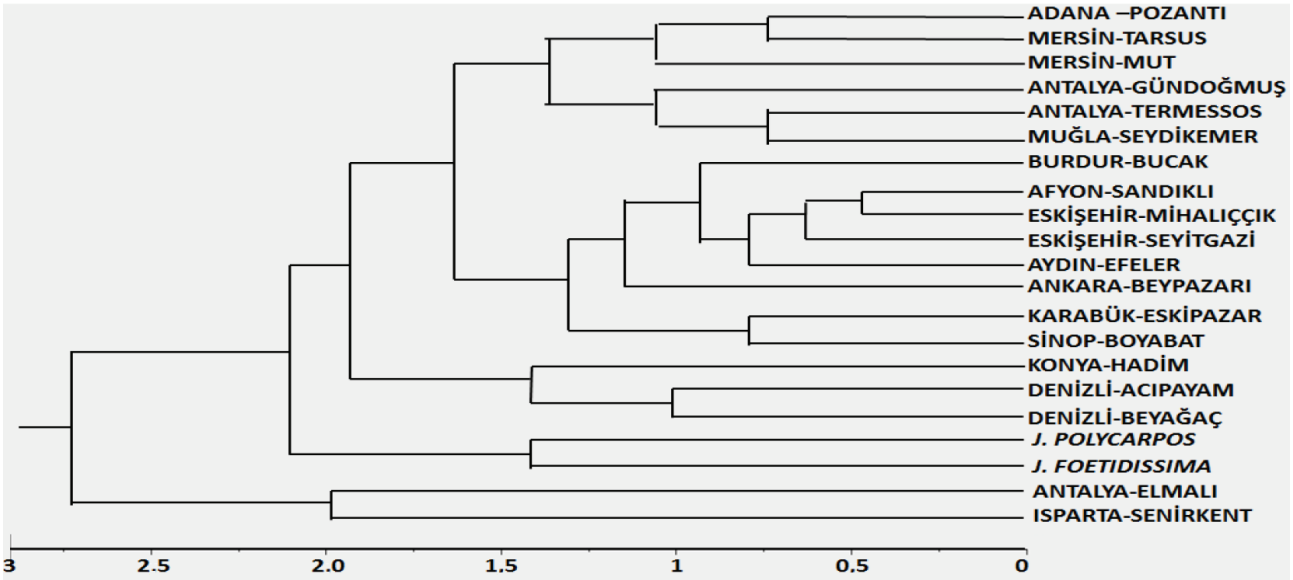

Figure 3. Dendrogram formed according to Nei (1972) pair-wise genetic distances among the populations based on ITAP loci

Table 3. Values of Fis, Fst and Nm-based on SSR loci.

Loci	Fis	Fst	Nm
Jc037	0.322	0.050	5.188
Jc166	0.161	0.051	4.691
JT02	-0.049	0.034	7.211
JT33	0.206	0.037	6.470
JT37	-0.131	0.060	3.918
JT38	-0.070	0.034	7.167
JT40	0.061	0.039	6.196
Average	0.124	0.043	5.513

the genetic structure of populations. The results of the present study clearly indicate that *J. excelsa* populations still maintain a high level of genetic diversity despite the discontinuous distribution of the populations. Although there is a positive relation between the Fst values and geographical distances among populations, it seems that, in *J. excelsa*, the high level of gene flow contributed to the maintenance of genetic diversity. As a matter of fact, high Nm values (especially for SSR loci) in this study support this statement.

Michalczyk (2008) evaluated the genetic effects of habitat degradation and fragmentation for *Juniperus communis* via SSR and AFLP markers. The results put forth that the studied populations have considerably high genetic diversity, an absence of genetic bottlenecks in all populations and no isolation by distance among *J. communis* populations (Michalczyk 2008). A conclusion was that the current habitat fragmentation has not genetically affected the genetic diversity level of the common juniper populations studied in Europe.

However, the values of observed heterozygsity per locus ranged from 0.292 to 0.692 for *J. communis,* whereas the average value of observed heterozygosity was 0.565 (Michalczyk et al., 2006). In this study, the average observed heterozygosity was 0.51. It is seen that *J. communis* populations in Europe have little more genetic diversity than *J. excelsa* in Turkey. Locus Jc037 was common for these two studies and the observed heterozygosity was 0.692 in the study by Michalczyk et al. (2006) and 0.609 in the present study. This difference may of course be due to the differences in the number and characteristics of the studied loci; however, cross-species transferred SSR loci from other juniper species might have lower levels of polymorphism, which indicates low transferability (Zhang et al., 2008; Sertse et al., 2013; Yücedağ and Gailing, 2013). The percentages of polymorphic AFLP loci in 23 *J. communis* populations (6-18 samples per population) were between 16.6 and 49.3 (Michalczyk et al., 2010). The percentages of polymorphic ITAP loci in 19 *J. excelsa* populations (24 samples per population) ranged from 31.34 to 55.97 in this study. It was assessed that genetic diversity level of AFLP loci of *J. communis* is substantially similar to ITAP loci of *J. excelsa.*

The genetic diversity parameters of *J. excelsa* populations in the current study have higher or slightly higher values than *J. brevifolia* and *J. thujifera*. The minimum and the maximum values of Shannon's Information index (I) for *J. excelsa* populations in this study were determined respectively as 1.046 and 1.279. *J. thurifera* distributed over

Table 4. Findings of AMOVA in the populations, based on SSR and ITAP markers.

Marker	Source of Variance	Value of Variances	Percentage of Variances
SSR	Inter-populations	0.050	2%
	Intra-populations/ Among Individuals	0.341	16%
	Intra-populations/ Intra-individuals	1.765	82%
ITAP	Inter-populations	2.553	20%
	Intra-populations	10.146	80%

Morocco and Spain is a tetraploid species with (I) values of the 11 *J. thurifera* populations varying between 0.181 and 0.225 (Teixeira et al., 2014). Similarly, the values for the same parameter ranged from 0.366 to 0.913 for 10 *J. brevifolia* populations in the Azores Islands (Bettencourt et al., 2015). It can be concluded that *J. excelsa* (24 samples per population) has higher genetic diversity than *J. brevifolia* (average 30 samples per population) and *J. thurifera* (average 23.7 samples per population) with respect to the (I) value of the SSR loci.

Genetic diversity and differentiations of some *J. excelsa* populations were previously evaluated by Douaihy et al. (2011) and Yücedağ and Gailing (2013). It was observed when the results of the present study were compared with the results of previous studies that the values of observed heterozygosity reported by Douaihy et al. (2011) (Ho = 0.46) and Yücedağ and Gailing (2013) (Ho = 0.46) were similar to the results of the present study (Ho = 0.51). It can be indicated that a similar genetic diversity level has been observed in these three studies with regard to the parameter of observed heterozygosity. The difference between the observed (Ho = 0.46) and expected heterozygosity (He = 0.76) values and inbreeding coefficient (Fis = 0.27-0.56) were highest in Douaihy et al. (2011). On the other hand, the mean value of (Fis) was 0.113 in the study by Yücedağ and Gailing (2013) and 0.124 in this study. The populations studied by Yücedağ and Gailing (2013) were limited with the Lakes District in Southern Turkey where Fst values were the lowest (0.028). This study covers a wider sampling area of the populations studied in Turkey, and the Fst value was intermediate (0.043). Douaihy et al. (2011) found the highest Fst value (0.069) because they analyzed not only populations from Turkey but also from Crimea, Greece, Cyprus, and Lebanon. It clearly appears that there is a positive relation between the Fst values and geographical distances among populations.

In the results of studies on different juniper species, including the results of present study, the values of observed heterozygosity were generally found lower than the values of expected heterozygosity (Michalczyk et al., 2006; Zhang et al., 2008; Douaihy et al., 2011; Yücedağ and Gailing, 2013; Rumeu et al., 2013; Bettencourt et al., 2015; Geng et al., 2016). The observed heterozygosity was lower in this study in comparison with the expected heterozygosity at loci Jc037, Jc166, JT33, and JT40 with statistically significant differences ($p < 0.01$). This is an indication that there is heterozygote deficiency at these loci. On the other hand, a statistically significant heterozygote excess was detected at loci JT02 and JT37 ($p < 0.01$) and a statistically nonsignificant heterozygote excess at locus JT38 ($p = 0.068$). Three alleles were observed at loci JT37 and JT02 with ten alleles at locus JT38. However, the remaining loci had more than ten alleles except for locus JT40 (six alleles). Based on the results of HWE for each population at each locus, similar numbers were obtained for the populations in HWE at loci JT37 (17 pops), JT38 (18 pops), and JT40 (16 pops). These results put forth that heterozygote deficiency is observed mostly at loci with a high number of alleles. These results were not interpreted as genetic degradation and low fitness in *J. excelsa* populations in this study. Heterozygote excess at loci JT02, JT37, and JT38, low Fis and Fst averages, high percentage of intra-population variance (98%), values of Shannon's information index indicated moderate-high genetic diversity in the populations. Long distance dispersal of pollen and seed, longevity and outcrossing make junipers and other conifers had high level of genetic diversity within populations and low level of inter-population differentiation (Hamrick et al., 1992; Sánchez-Gómez et al., 2018). It prevents habitat fragmentation effect on genetic structure of populations. Additionally, 10-100 generations are necessary in order to observe genetic signals of habitat fragmentation for such populations (Mona et al., 2014). The results of the present study clearly indicate that *J. excelsa* populations still maintain a high level of genetic diversity although the populations are distributed as discontinuous. However, this does not mean that genetic diversity in these populations will also remain high in the future, unless the genetic diversity of these populations is conserved.

The correlation between genetic diversity and population size was analyzed in an AFLP based study investigated the level of genetic diversity in an endangered

alpine plant *Eryngium alpinum* L. (Gaudeul et al. 2000). They found that population size was correlated positively with the percentage of polymorphic loci, Nei's expected heterozygosity, and Shannon's index. The results of Gaudeul et al. (2000) implicitly support that genetic diversity level is also positively correlated with sample size. The sample sizes per population and sum of all populations were respectively 24 and 456 in this study, 15-30 and 320 in Douaihy et al. (2011) and 30 and 180 in Yücedağ and Gailing (2013). Furthermore, the percentage of intra-individual variance within intra-populations was calculated as 82% in this study. This result was considered to be due to the variance component originating from heterozygous individuals, which supports the fact that *J. excelsa* populations potentially have higher genetic diversity (i.e lower heterozygote deficiency and lower inbreeding coefficient in this study).

A lower genetic diversity level has been estimated in many studies for the dominant markers in comparison with the codominant markers (Pecina-Quintero et al., 2012; Bettencourt et al., 2015; Elibol and Bilgen, 2017). The values of Shannon's information index (I) and Nei's gene diversity (h) employed as expected heterozygosity parameter for ITAP loci were lower than those of the SSRs loci in *J. excelsa* populations in this study (Table 2). This can be due to the fact that different marker types have different genetic architecture. Dominant markers do not distinguish between heterozygous and homozygous characters (Staub et al., 1996), and ITAPs are examples of dominant markers such as RAPD, AFLP, ISSR etc. Thus, genetic diversity originating from heterozygosity could not be detected with those dominant markers. In addition, intra-population genetic variance was 80% for ITAP loci, and it was lower than that of the SSR loci (98%) in this study. The same reason may also be valid for this difference. Gst value for ITAP loci was calculated as 0.225 in this study. This value is significantly higher than the value of Fst (0.043) calculated from SSR data. This can also be explained by the fact that the genetic diversity in ITAP loci was lower than that of the SSR loci.

The results of Structure analysis, AMOVA, and Mantel test for ITAP loci and SSR loci were quite similar with some minor differences. The results of structure analysis based on SSR loci indicated that the populations were similar complex structures in this study, which was consistent with the findings by Yücedağ and Gailing (2013). The results of Structure analysis, AMOVA, and Mantel test also indicated that inter-population differentiations are very low.

Seed dispersal in juniper species is highly via birds. Birds feed on juniper berry-like cones, so seeds disperse via their droppings travelled long distances from mother trees (Garcia 2001). According to the results of the present study, despite of the habitat fragmentation, the genetic differentiation among the populations is low, and the population structures revealed an admixture according to SSR loci. It seems that in *J. excelsa*, the gene flow has highly been ensured as a result of the effective spread of seeds and/or pollens. As a matter of fact, high Nm values (especially for SSR loci) in this study support this statement.

5. Conclusion

Unlike overall expectation in this study, analyses of the present study demonstrated that habitat fragmentation has noteworthy not negative effects on genetic diversity of *J. excelsa* populations. On the contrary, we concluded that the populations maintain a moderate to high level of intra-population genetic diversity and that genetic differentiation among populations is low. When it is considered that *J. excelsa* populations in Turkey usually show discontinuous distribution, it is difficult to predict how much longer they can protect their genetic diversity without any management program. This study revealed that the genetic potential of this species is still high. Even if the populations are almost fragmented, the loss of the genetic diversity of the species can be prevented by conserving the existing population structure. This study provides significant information for efficient conservation, management, utilization, and suitable conservation strategies for this species. We recommend to the practitioners in the Turkish Ministry of Forestry that conservation plans should be made to maintain the existing genetic diversity of this species.

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Supplementary Information 1. Detailed information about the populations studied.

Pop. No	Population Names	Latitude	Longitude	Altitude (m)
1	Adana-Pozantı	N37°31'55.4"	E34°59'31.2"	1188
2	Afyon-Sandıklı	N38°20'25.1"	E30°07'48.5"	1123
3	Ankara-Beypazarı	N40°12'48.4"	E31°40'02.2"	535
4	Antalya-Elmalı	N36°49'17.7"	E29°46'03.7"	1380
5	Antalya-Gündoğmuş	N36°51'02.5"	E32°05'11.0"	1405
6	Antalya-Termessos	N36°58'43.5"	E30°27'31.7"	1050
7	Antalya-Termessos (*J. foetidissima*)	N36°58'43.5"	E30°27'31.7"	1050
8	Aydın-Efeler	N37°56'43.9"	E27°53'51.0"	1470
9	Burdur-Bucak	N37°24'06.5"	E30°20'46.0"	1360
10	Denizli-Acıpayam	N37°11'41.6"	E29°08'02.7"	1460
11	Denizli-Beyağaç	N37°17'52.7"	E28°58'19.2"	1180
12	Eskişehir-Mihalıççık	N39°50'50.3"	E31°15'59.7"	980
13	Eskişiehir-Seyitgazi	N39°28'53.3"	E30°39'57.7"	1010
14	Gümüşhane-Torul (*J. polycarpos*)	N40°32'29.9"	E39°16'54.8"	1112
15	Isparta-Senirkent	N38°05'15.0"	E30°48'11.8"	1369
16	Karabük-Eskipazar	N41°00'01.2"	E32°37'18.5"	560
17	Konya-Hadim	N37°02'34.0"	E32°30'37.2"	1440
18	Mersin-Mut	N36°51'14.9"	E33°17'46.2"	1443
19	Mersin-Tarsus	N37°07'00.5"	E34°27'36.0"	1470
20	Muğla-Seydikemer	N36°52'41.5"	E29°31'03.7"	1388
21	Sinop-Boyabat	N41°37'22.1"	E34°37'18.1"	386

Supplementary Information 2. Details of SSR primer pairs used in the study.

Primer Name	Citation	Repeat Pattern	Annealing (°C)	Primer Concentrations (µm)	Size Range (bp) in Our Study
Jc037	Douaihy et al. (2011)	$(TG)_9$-$(AG)_{22}$	55	3.5	147-201
Jc166		$(TG)_{14}$	57	3.5	144-164
JT02	Teixeira et al. (2014)	$(GAA)_8$	52	3.5	160-167
JT33		$(CT)_{11}$	57	3.5	153-217
JT34		$(AG)_{12}$	55	2.5	89
JT37		$(GT)_{14}$	55	2.5	105-109
JT38		$(AC)_{14}$	55	2.5	98-116
JT40		$(CA)_{20}$	57	2.5	104-116

Supplementary Information 3. Numbers of loci and size ranges of ITAP primer pairs used in the study.

Primer Pairs	Numbers of loci	Size range (bp)
Em1-ITPR5	13	225-1050
Em5-ITPR4	14	150-850
Em5-ITPR5	16	150-1100
Em12-ITPR3	18	150-1100
Em19-ITPR2	17	150-1300
Em20-ITPR3	20	175-950

Em21-ITPR1	15	150-900
Em26-ITPR3	21	175-1300
TOTAL/AVERAGE	134/16.75	

Supplementary Information 4. Details of genetic diversity parameters in the populations studied for both SSR and ITAP loci.

For SSR loci								
Populations	**(na)***	**(ne)***	**(I)***	**(Npl)***	**(Ppl)***	**(Ho)***	**(He)***	**(h)***
Adana-Pozantı	6,125	4,170	1,160	7	87,5	0,462	0,534	Not computed
Afyon-Sandıklı	5,875	3,737	1,091	6	75,0	0,408	0,506	Not computed
Ankara-Beypazarı	6,625	4,179	1,190	6	75,0	0,458	0,536	Not computed
Antalya-Elmalı	6,125	3,275	1,095	7	87,5	0,458	0,501	Not computed
Antalya-Gündoğmuş	6,750	4,256	1,251	7	87,5	0,441	0,568	Not computed
Antalya-Termessos	6,375	4,033	1,138	6	75,0	0,446	0,512	Not computed
Aydın-Efeler	6,625	5,133	1,162	7	87,5	0,448	0,495	Not computed
Burdur-Bucak	6,500	4,451	1,188	6	75,0	0,471	0,537	Not computed
Denizli-Acıpayam	7,000	4,666	1,264	7	87,5	0,492	0,559	Not computed
Denizli-Beyağaç	6,125	3,784	1,090	6	75,0	0,443	0,500	Not computed
Eskişehir-Mihalıççık	7,125	4,630	1,215	6	75,0	0,392	0,529	Not computed
Eskişehir-Seyitgazi	5,875	4,302	1,148	7	87,5	0,446	0,530	Not computed
Isparta-Senirkent	6,000	4,008	1,046	6	75,0	0,347	0,472	Not computed
Karabük-Eskipazar	6,250	4,527	1,203	6	75,0	0,491	0,539	Not computed
Konya-Hadim	6,125	3,945	1,090	6	75,0	0,448	0,492	Not computed
Mersin-Mut	7,125	4,553	1,279	7	87,5	0,482	0,563	Not computed
Mersin-Tarsus	6,125	3,376	1,098	7	87,5	0,468	0,508	Not computed
Muğla-Seydikemer	6,750	4,414	1,175	6	75,0	0,438	0,516	Not computed
Sinop Boyabat	6,625	5,013	1,265	6	75,0	0,501	0,562	Not computed
MEAN	6,428	4,234	1,166	6,421	80,263	0,449	0,524	Not computed

STD DEVIATION	0,403	0,490	0,069	0,507	6,341	0,037	0,027	Not computed
STD ERROR	0,092	0,112	0,016	0,116	1,455	0,008	0,006	Not computed
For ITAP loci								
Populations	**(na)***	**(ne)***	**(I)***	**(Npl)***	**(Ppl)***	**(Ho)***	**(He)***	**(h)***
Adana-Pozantı	1,313	1,146	0,133	42	31,34	Not computed	Not computed	0,086
Afyon-Sandıklı	1,373	1,150	0,144	50	37,31	Not computed	Not computed	0,092
Ankara-Beypazarı	1,560	1,281	0,252	75	55,97	Not computed	Not computed	0,166
Antalya-Elmalı	1,381	1,178	0,164	51	38,06	Not computed	Not computed	0,107
Antalya-Gündoğmuş	1,343	1,168	0,150	46	34,33	Not computed	Not computed	0,098
Antalya-Termessos	1,381	1,189	0,171	51	38,06	Not computed	Not computed	0,112
Aydın-Efeler	1,321	1,156	0,140	43	32,09	Not computed	Not computed	0,092
Burdur-Bucak	1,448	1,214	0,191	60	44,78	Not computed	Not computed	0,125
Denizli-Acıpayam	1,351	1,076	0,088	47	35,07	Not computed	Not computed	0,051
Denizli-Beyağaç	1,463	1,173	0,173	62	46,27	Not computed	Not computed	0,109
Eskişehir-Mihalıççık	1,508	1,216	0,204	68	50,75	Not computed	Not computed	0,131
Eskişehir-Seyitgazi	1,485	1,227	0,203	65	48,51	Not computed	Not computed	0,132
Isparta-Senirkent	1,418	1,173	0,164	56	41,79	Not computed	Not computed	0,105
Karabük-Eskipazar	1,448	1,204	0,185	60	44,78	Not computed	Not computed	0,120
Konya-Hadim	1,328	1,121	0,118	44	32,84	Not computed	Not computed	0,074
Mersin-Mut	1,351	1,152	0,140	47	35,07	Not computed	Not computed	0,090
Mersin-Tarsus	1,448	1,189	0,177	60	44,78	Not computed	Not computed	0,114
Muğla-Seydikemer	1,373	1,201	0,176	50	37,31	Not computed	Not computed	0,117

Sinop Boyabat	1,470	1,195	0,185	63	47,01	Not computed	Not computed	0,119
MEAN	1,409	1,179	0,166	54,737	40,848	Not computed	Not computed	0,107
STD DEVIATION	0,070	0,044	0,036	9,374	6,997	Not computed	Not computed	0,025
STD ERROR	0,016	0,010	0,008	2,151	1,605	Not computed	Not computed	0,006

*(na): Observed number of alleles ,(ne): Effective number of alleles, (I): Shannon's Information Index, (Npl): Number of polymorphic loci, (Ppl): Percentage of polymorphic loci, (Ho): Observed heterozygosity, (He): Expected heterozygosity, (h): Nei's gene diversity

Supplementary Information 5. Nei's (1972) **Pair-wise** genetic distances among populations for SSR loci.

Adana-Pozantı	Afyon-Sandıklı	Ankara-Beypazarı	Antalya-Elmalı	Antalya-Gündoğmuş	Antalya-Termessos	*J. foetidissima*	Aydın-Efeler	Burdur-Bucak	Denizli-Acıpayam	Denizli-Beyağaç	Populations
****	****	****	****	****	****	****	****	****	****	****	**Adana-Pozantı**
0.056	****	****	****	****	****	****	****	****	****	****	**Afyon-Sandıklı**
0.048	0.044	****	****	****	****	****	****	****	****	****	**Ankara-Beypazarı**
0.090	0.062	0.060	****	****	****	****	****	****	****	****	**Antalya-Elmalı**
0.067	0.029	0.047	0.064	****	****	****	****	****	****	****	**Antalya-Gündoğmuş**
0.082	0.034	0.052	0.064	0.053	****	****	****	****	****	****	**Antalya-Termessos**
0.149	0.152	0.157	0.207	0.168	0.161	****	****	****	****	****	***J. foetidissima***
0.080	0.045	0.058	0.067	0.071	0.027	0.172	****	****	****	****	**Aydın-Efeler**
0.042	0.040	0.031	0.059	0.045	0.041	0.173	0.050	****	****	****	**Burdur-Bucak**
0.062	0.037	0.037	0.053	0.035	0.033	0.159	0.051	0.029	****	****	**Denizli-Acıpayam**
0.051	0.050	0.041	0.073	0.065	0.068	0.155	0.070	0.038	0.048	****	**Denizli-Beyağaç**
0.054	0.037	0.034	0.045	0.043	0.035	0.169	0.044	0.029	0.023	0.041	**Eskişehir-Mihalıççık**
0.036	0.039	0.044	0.074	0.048	0.055	0.145	0.074	0.043	0.035	0.031	**Eskişehir-Seyitgazi**
0.221	0.235	0.213	0.251	0.247	0.186	0.240	0.173	0.210	0.221	0.263	***J. polycarpos***
0.060	0.036	0.033	0.042	0.050	0.053	0.180	0.055	0.039	0.037	0.044	**Isparta-Senirkent**
0.045	0.045	0.043	0.061	0.054	0.040	0.175	0.037	0.035	0.035	0.060	**Karabük-Eskipazar**
0.045	0.043	0.037	0.085	0.059	0.061	0.131	0.073	0.050	0.049	0.036	**Konya-Hadim**
0.035	0.053	0.029	0.078	0.045	0.074	0.171	0.097	0.032	0.047	0.053	**Mersin-Mut**
0.036	0.093	0.051	0.090	0.082	0.126	0.197	0.129	0.054	0.079	0.068	**Mersin-Tarsus**
0.066	0.040	0.047	0.049	0.064	0.040	0.193	0.042	0.043	0.035	0.063	**Muğla-Seydikemer**
0.035	0.046	0.027	0.078	0.047	0.051	0.133	0.062	0.029	0.034	0.050	**Sinop-Boyabat**

Continued Supplementary Information 5

Eskişehir-Mihalıççık	Eskişehir-Seyitgazi	*J. polycarpos*	Isparta-Senirkent	Karabük-Eskipazar	Konya-Hadim	Mersin-Mut	Mersin-Tarsus	Muğla-Seydikemer	Sinop-Boyabat	**Populations**
****	****	****	****	****	****	****	****	****	****	**Adana-Pozantı**
****	****	****	****	****	****	****	****	****	****	**Afyon-Sandıklı**
****	****	****	****	****	****	****	****	****	****	**Ankara-Beypazarı**
****	****	****	****	****	****	****	****	****	****	**Antalya-Elmalı**
****	****	****	****	****	****	****	****	****	****	**Antalya-Gündoğmuş**
****	****	****	****	****	****	****	****	****	****	**Antalya-Termessos**
****	****	****	****	****	****	****	****	****	****	***J. foetidissima***
****	****	****	****	****	****	****	****	****	****	**Aydın-Efeler**
****	****	****	****	****	****	****	****	****	****	**Burdur-Bucak**
****	****	****	****	****	****	****	****	****	****	**Denizli-Acıpayam**
****	****	****	****	****	****	****	****	****	****	**Denizli-Beyağaç**
****	****	****	****	****	****	****	****	****	****	**Eskişehir-Mihalıççık**
0.035	****	****	****	****	****	****	****	****	****	**Eskişehir-Seyitgazi**
0.223	0.278	****	****	****	****	****	****	****	****	***J. polycarpos***
0.027	0.040	0.239	****	****	****	****	****	****	****	**Isparta-Senirkent**
0.031	0.054	0.193	0.050	****	****	****	****	****	****	**Karabük-Eskipazar**
0.047	0.031	0.246	0.042	0.054	****	****	****	****	****	**Konya-Hadim**
0.047	0.041	0.262	0.047	0.053	0.044	****	****	****	****	**Mersin-Mut**
0.077	0.057	0.292	0.058	0.082	0.067	0.039	****	****	****	**Mersin-Tarsus**
0.028	0.061	0.186	0.042	0.032	0.064	0.063	0.104	****	****	**Muğla-Seydikemer**
0.043	0.040	0.187	0.053	0.036	0.036	0.031	0.061	0.053	****	**Sinop-Boyabat**

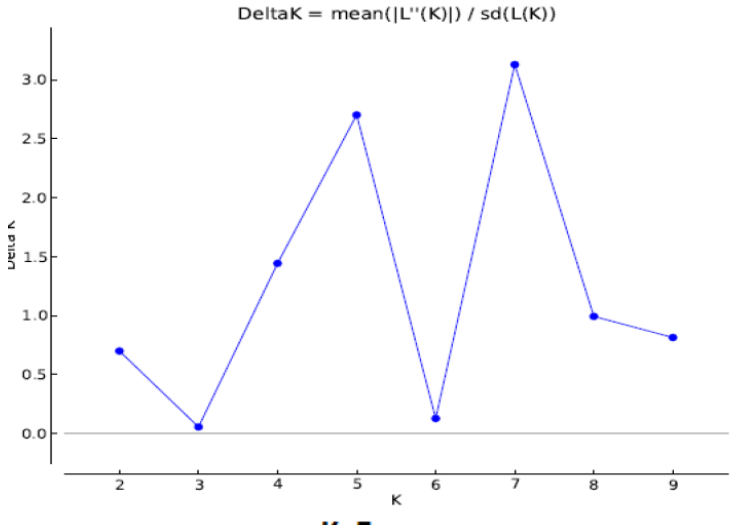
Supplementary Information 6. Nei's (1972) **Pair-wise** genetic distances among populations for ITAP loci

Adana-Pozantı	Afyon-Sandıklı	Ankara-Beypazarı	Antalya-Elmalı	Antalya-Gündoğmuş	Antalya-Termessos	*J. foetidissima*	Aydın-Efeler	Burdur-Bucak	Denizli-Acıpayam	Denizli-Beyağaç	Populations
****	****	****	****	****	****	****	****	****	****	****	**Adana-Pozantı**
0.028	****	****	****	****	****	****	****	****	****	****	**Afyon-Sandıklı**
0.059	0.030	****	****	****	****	****	****	****	****	****	**Ankara-Beypazarı**
0.057	0.061	0.054	****	****	****	****	****	****	****	****	**Antalya-Elmalı**
0.024	0.023	0.057	0.060	****	****	****	****	****	****	****	**Antalya-Gündoğmuş**
0.030	0.030	0.049	0.032	0.017	****	****	****	****	****	****	**Antalya-Termessos**
0.029	0.036	0.058	0.058	0.046	0.043	****	****	****	****	****	***J. foetidissima***
0.031	0.023	0.028	0.044	0.041	0.034	0.026	****	****	****	****	**Aydın-Efeler**
0.047	0.024	0.024	0.046	0.044	0.034	0.047	0.022	****	****	****	**Burdur-Bucak**
0.037	0.027	0.070	0.094	0.026	0.042	0.054	0.045	0.053	****	****	**Denizli-Acıpayam**
0.037	0.028	0.049	0.072	0.033	0.036	0.047	0.033	0.030	0.019	****	**Denizli-Beyağaç**
0.028	0.012	0.017	0.049	0.031	0.032	0.032	0.014	0.017	0.037	0.029	**Eskişehir-Mihalıççık**
0.031	0.014	0.028	0.041	0.021	0.018	0.034	0.015	0.020	0.031	0.027	**Eskişehir-Seyitgazi**
0.041	0.036	0.066	0.087	0.047	0.057	0.027	0.035	0.057	0.044	0.048	***J. polycarpos***
0.086	0.068	0.037	0.042	0.079	0.051	0.087	0.052	0.027	0.107	0.069	**Isparta-Senirkent**
0.032	0.025	0.023	0.034	0.035	0.025	0.039	0.018	0.022	0.059	0.038	**Karabük-Eskipazar**
0.023	0.026	0.066	0.085	0.033	0.042	0.049	0.045	0.059	0.022	0.036	**Konya-Hadim**
0.020	0.025	0.048	0.048	0.031	0.028	0.033	0.023	0.042	0.039	0.032	**Mersin-Mut**
0.011	0.026	0.053	0.052	0.021	0.024	0.032	0.029	0.040	0.041	0.038	**Mersin-Tarsus**
0.027	0.026	0.036	0.029	0.020	0.010	0.035	0.025	0.032	0.045	0.036	**Muğla-Seydikemer**
0.051	0.047	0.029	0.031	0.062	0.039	0.047	0.021	0.029	0.087	0.061	**Sinop-Boyabat**

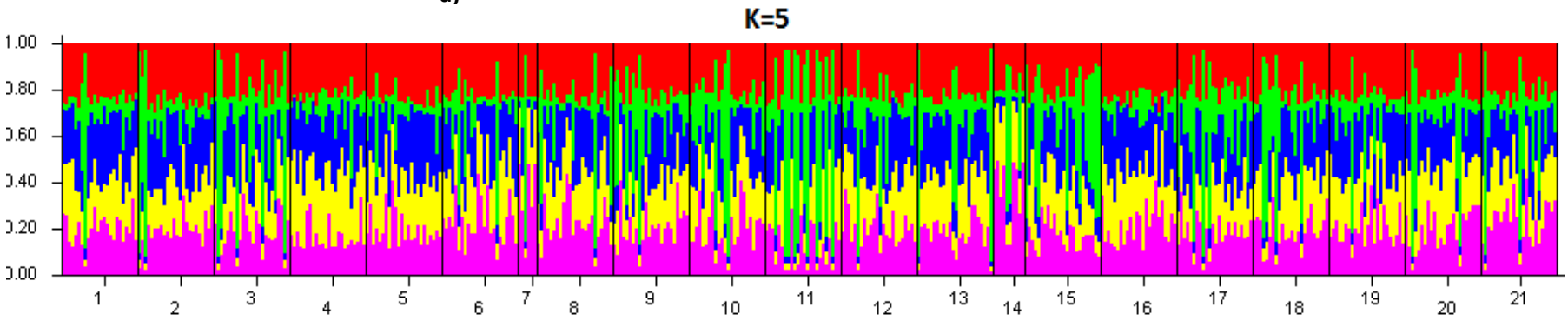
Continued Supplementary Information 6

Eskişehir-Mihalıççık	Eskişehir-Seyitgazi	*J. polycarpos*	Isparta-Senirkent	Karabük-Eskipazar	Konya-Hadim	Mersin-Mut	Mersin-Tarsus	Muğla-Seydikemer	Sinop-Boyabat	Populations
****	****	****	****	****	****	****	****	****	****	**Adana-Pozantı**
****	****	****	****	****	****	****	****	****	****	**Afyon-Sandıklı**
****	****	****	****	****	****	****	****	****	****	**Ankara-Beypazarı**
****	****	****	****	****	****	****	****	****	****	**Antalya-Elmalı**
****	****	****	****	****	****	****	****	****	****	**Antalya-Gündoğmuş**
****	****	****	****	****	****	****	****	****	****	**Antalya-Termessos**
****	****	****	****	****	****	****	****	****	****	***J. foetidissima***
****	****	****	****	****	****	****	****	****	****	**Aydın-Efeler**
****	****	****	****	****	****	****	****	****	****	**Burdur-Bucak**
****	****	****	****	****	****	****	****	****	****	**Denizli-Acıpayam**
****	****	****	****	****	****	****	****	****	****	**Denizli-Beyağaç**
****	****	****	****	****	****	****	****	****	****	**Eskişehir-Mihalıççık**
0.017	****	****	****	****	****	****	****	****	****	**Eskişehir-Seyitgazi**
0.035	0.042	****	****	****	****	****	****	****	****	***J. polycarpos***
0.050	0.048	0.109	****	****	****	****	****	****	****	**Isparta-Senirkent**
0.015	0.020	0.056	0.035	****	****	****	****	****	****	**Karabük-Eskipazar**
0.037	0.039	0.041	0.110	0.054	****	****	****	****	****	**Konya-Hadim**
0.028	0.023	0.043	0.072	0.026	0.030	****	****	****	****	**Mersin-Mut**
0.025	0.024	0.046	0.078	0.025	0.035	0.019	****	****	****	**Mersin-Tarsus**
0.022	0.019	0.049	0.049	0.018	0.040	0.028	0.027	****	****	**Muğla-Seydikemer**
0.030	0.030	0.021	0.030	0.013	0.081	0.039	0.044	0.032	****	**Sinop-Boyabat**

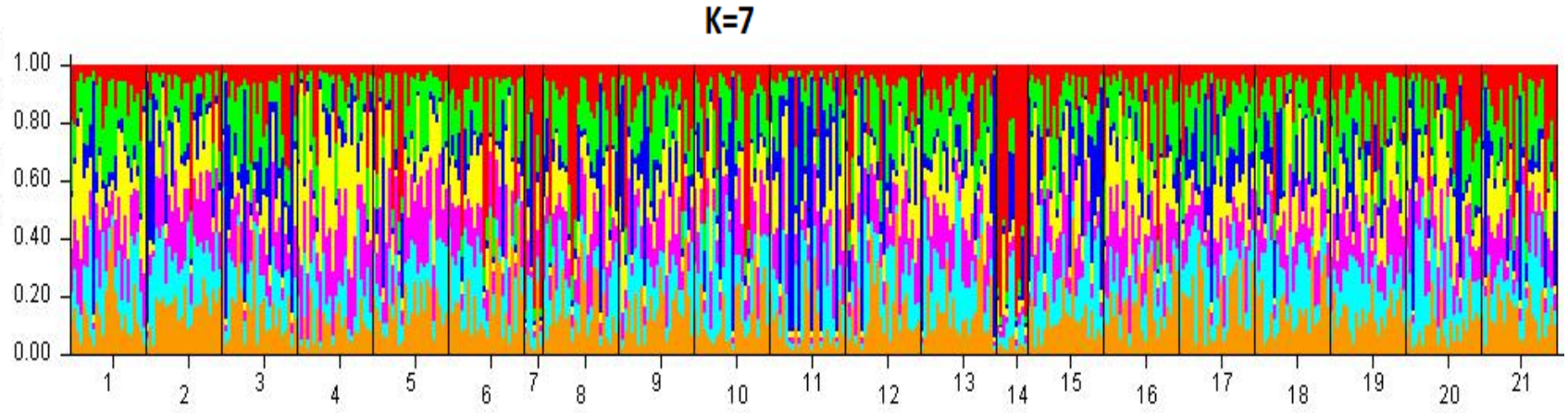
Supplementary Information 7. K-Delta graphics and Structure patterns of the populations for SSRs; (a) K=5 (b) K=7 (c)



a)



b)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0.00
1
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c)