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Genetic variation in white poplar (*Populus alba* L.) populations as characterized by SSR markers

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Abstract: The white poplar (*Populus alba* L.), which is tolerant to abiotic and biotic stresses, is a tree species that is resilient against changing climatic and environmental conditions, which makes it a suitable candidate for afforestation efforts. However, due to prolonged human intervention associated with the increasing population, the genetic resources of this species are at risk of loss. Additionally, while this species generally reproduces sexually, reproduction clonally via its root suckers has become widespread in some areas. Hence, the aim of this study was to investigate the genetic diversity of white poplar, which is increasingly experiencing genetic diversity loss. Herein, 15 different populations distributed overall in Türkiye were sampled, and the genetic structure and diversity of the species were examined using 10 simple sequence repeat molecular markers. The analyses revealed relatively high levels of genetic variation in the populations (observed heterozygosity: 0.50, expected heterozygosity: 0.46) and the molecular variance analysis determined 84% genetic variation within the populations. Overall, the distance between the populations decreased, the gene flow (N_m) increased, and the genetic differentiation decreased (N_m: 0.16–30.24, gene differentiation coefficient: 0.01–0.61). The principal component and phylogenetic analyses data also supported these results. The 3 populations at the highest altitude, Erzurum-Oltu, Kayseri-Akköy, and Konya-Meram, had a different genetic structure than the others. Furthermore, clonal reproduction was intensive in some populations together with the inclusion of human activities, and therefore, the genetic diversity of the species decreased. However, the white poplar populations in riverside and floodplain forests had higher genetic diversity and private alleles. For this reason, the Eskişehir-Porsuk Çayi and Samsun-Bafra populations could be recommended for in situ conservation of white poplar. This study provides information on the genetic diversity of white poplar populations in Türkiye, and thus contributes to more efficient and rational planning of breeding and conservation programs for the species.

Key words: Poplar, genetic differentiation, nuclear microsatellites, population genetics

1. Introduction

Populus alba (white poplar), a member of the Leuce section of the *Populus* genus, is an ecologically and economically important species, as highlighted by numerous studies (Caudullo and Rigo, 2016). Trees of the species primarily reproduce through vegetative means via the production of shoots from roots, and generative means via wind-dispersed seeds produced by mature trees (Palancean et al., 2018). White poplar is dioecious, meaning that male and female flowers are found on separate trees. Thus, in sexual reproduction, it is fertilized by individuals other than itself. *P. alba* is a pioneer species that is capable of colonizing areas due to its production of a large number of seeds and the rapid growth of its absorptive roots. It has an impressive lifespan of approximately 300–400 years and can reach a height of 30–40 m and a diameter of 1 m. The species can be managed with a rotation period of 20 years and a yearly growth of 20–38 m^3 (Richardson et al., 2014).

P. alba can be found in numerous regions across the world, including Türkiye, North Africa, Europe, North America, Central and Southern Russia, and Central Asia, thriving at elevations ranging from sea level up to 1100 m (Caudullo and Rigo, 2016). However, Turkish inventory studies have demonstrated that it can also be found at elevations of up to 1800 m in Giresun and Erzurum. The species is particularly widespread in Türkiye, specifically in forested areas and riverbeds (Tunçtaner, 1993).

P. alba is an important species for afforestation of saline soils and arid-land areas due to its tolerance to salty water. Additionally, it has been extensively utilized for its phy-

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toremediation ability as it is still utilized in the cleaning of polluted soil, water, and air in industrial areas throughout Europe (Palancean et al., 2018). Furthermore, white poplar is useful for natural flood control in riverine ecosystems through soil stabilization (Richardson et al., 2014). Moreover, white poplar has excellent resistance to insects and pathogens (Plett et al., 2010). When all of these features are combined with its rapid growth ability, this makes it a good candidate for resilient cultivation against future climatic and environmental changes (Muller-Starck et al., 1992). Due to these characteristics, it has been commercially produced through clonal selection in different countries (Richardson et al., 2014) and has been the subject of numerous studies (Castiglione et al., 2007; Imada and Tamai, 2009; Ciadamidaro et al., 2013). Furthermore, it has a silver appearance due to its 2-colored leaves, which makes it a popular choice for landscaping in parks and gardens (Tunçtaner, 1993). The species also has an ethnobatanical aspect, as its wood is used in the production of various products, such as spoons, buckets, sieves, cradles, shovel and hoe handles, construction timber, musical instruments, and fruit-vegetable crates (Palancean et al., 2018).

Due to the limited information available on white poplar's genetic structure from both its existing sources and wild populations, it was included in the European Forest Genetic Resources (EUFORGEN) program in 1999. As a result, in and ex situ conservation areas were subsequently registered in 4 countries, namely Spain, Italy, Slovakia, and Romania (Lefevre et al., 2001). Furthermore, various studies have been conducted in many countries using different molecular markers to reveal the genetic structure of white poplar (Brundu et al., 2008; Castiglione et al., 2010; Fussi et al., 2011; Dering et al., 2015; Liu et al., 2019; Guarino et al., 2020; Hou and Li, 2022). Although studies evaluating the molecular genetic diversity of *Populus euphratica* and *Populus nigra* in Türkiye have been conducted (Ciftci et al., 2017; Kansu and Kaya, 2020; Nebioğlu, 2021), to date, no studies have been conducted on the conservation, breeding, and genetic diversity of white poplar in Türkiye. Considering the importance of white poplar's functions and its potential for genetic resources, its genetic diversity should be evaluated as soon as possible in Türkiye, since the effective conservation of genetic resources and proper management of white poplar populations require knowledge of the genetic diversity of this species (Ledig and Conkle, 1983).

Hence, in this study, 15 different populations of *P. alba* species in Türkiye were sampled, and the genetic structure of the species was determined using 10 simple sequence repeat (SSR) molecular markers. The aim was to obtain information on the genetic structure of the species and contribute to the conservation and breeding programs.

2. Materials and methods

2.1. Plant materials

In this study, 285 white poplar (*P. alba*) trees from 15 populations located in Türkiye (Figure 1 and Table 1) were sampled. White poplar individuals were identified by experts at the Poplar and Fast-Growing Forest Trees Research Institute. The trees were selected at random, but in order to minimize the sampling of closely related individuals, young leaves were collected from trees that were at least 100 m apart from each other. The number of individuals per population ranged from 11 to 20.

Figure 1. Geographic locality of the 15 white poplar populations.

Table 1. Characteristics of the 15 white poplar populations.

No.	Population	Latitude	Longitude	Altitude (m)
1	Sakarya-Sakarya Nehri	40.7226	30.4244	34
2	Eskişehir-Porsuk Çayi	39.7438	30.4347	829
3	Edirne-Tunca Nehri	41.6616	26.5427	12
4	Tekirdağ-Malkara	40.9600	27.4050	243
5	İstanbul-Bahçeköy	41.1761	28.9850	127
6	Samsun-Bafra	41.4533	36.0425	5
7	Sakarya-Geyve	40.6247	30.3263	201
8	Bursa-Karacabey	40.2475	28.4700	28
9	Kayseri-Akköy	38.3475	35.0566	1178
10	Konya-Meram	37.8513	32.4316	1054
11	Kastamonu-Samatlar	41.2222	33.1425	582
12	Bursa-Iznik	40.4900	29.5780	118
13	Kocaeli-Izmit	40.7648	29.9394	3
14	Balikesır-Susurluk	39.8197	28.1633	101
15	Erzurum-Oltu	40.5213	41.9702	1320

2.2. Molecular analysis

Total DNA was isolated from frozen leaves (20 mg of tissue) using the i-genomic plant DNA Extraction Mini Kit (iNtRON Biotechnology Inc., Burlington, MA, USA), following the manufacturer's instructions. DNA quality and quantity were evaluated using both a NanoDrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and 1% agarose gel electrophoresis for 30 min at 80 V.

For the polymerase chain reaction (PCR) studies, 10 labeled microsatellite loci (Table 2) were selected according to the literature (Rahman et al., 2000; Schoot et al., 2000; Smulders et al., 2001; Tuskan et al., 2004). PCR reactions were performed in a final volume of 25 µL, containing 0.3 µM of fluorescent-labeled forward primer, 0.3 µM of reverse primer, 10–50 ng of total genomic DNA, and 5 µL of 5x HOT FIREPol Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia), which included 15 mM of $MgCl_2$. Forward primers were 5' labeled with phosphoramidite (HEX), fluorescein (FAM), and tetramethylrhodamine (TAMRA) dyes. The PCR products were genotyped using an automatic sequencer, Applied Biosystems 3730 XL DNA analyzer (Foster City, CA, USA), and the size of the fragments was determined using Peak Scanner Software v1.0 (https://peak-scanner-software.software.informer.com/1.0/).

Table 2. Details for the 10 nuclear microsatellite primers.

Locus	Repeat motif	Primer sequences forward, reverse (5', 3')	Annealing temperature
WPMS05	$(GT)_{27}$	F: TTCTTTTTCAACTGCCTAACTT R: TGATCCAATAACAGACAGAACA	50 °C
WPMS06	$(GT)_{24}$	F: GTATAACGATGACCCCACGAAGAC R: TATAAATAAAGGCATGACCAGACA	60 °C
WPMS14	$(CGT)_{28}$	F: CAGCCGCAGCCACTGAGAAATC R: GCCTGCTGAGAAGACTGCCTTGAC	60 °C
WPMS15	$(CCT)_{14}$	F: CAACAAACCATCAATGAAGAAGAC R: AGAGGGTGTTGGGGGTGACTA	60 °C
WPMS16	$(GTC)_{8}$	F: CTCGTACTATTTCCGATGATGACC R: AGATTATTAGGTGGGCCAAGGACT	55 °C

Table 2. Continued

WPMS17	$(CAC)_{15}$	F: ACATCCGCCAATGCTTCGGTGTTT R: GTGACGGTGGTGGCGGATTTTCTT	60 °C
WPMS18	$(GTG)_{13}$	F: CTTCACATAGGACATAGCAGCATC R: CACCAGAGTCATCACCAGTTATTG	60 °C
WPMS20	$(TTCTGG)_{8}$	F: GTGCGCACATCTATGACTATCG R: ATCTTGTAATTCTCCGGGCATCT	60 °C
PTR 8	$(A)_{11}(CT)_{8}$	F: TAGGCTAGCAGCTACTACAGTAACA R: TTAAGTGCGCGTATCCCAAAGA	60 °C
ORPM312	$(CCT)_{6}$	F: GTGGGGATCAATCCAAAAGA R: CCCATATCAAACCATTTGAAAAA	50 °C

2.3. Statistical analysis

Statistical analyses were performed to assess the genetic diversity parameters of the populations using R statistical programming language (3.3.2) (R Core Team, 2020). The following population genetic analysis libraries were used: ape (Paradis et al., 2004), hierfstat (Goudet, 2005), adegenet (Jombart, 2008), pegas (Paradis, 2010), diveRsity (Keenan et al., 2013), poppr (Kamvar et al., 2014), and PopGenReport (Adamack and Gruber, 2014).

Specifically, several genetic parameters were calculated for each microsatellite locus and population. The clonal reproduction, number of alleles, and private alleles in the populations were assessed using the poppr library. Moreover, expected heterozygosity (H_e) and observed heterozygosity (H_o) values were calculated based on Nei (1978) using the poppr library. The null allele frequency was estimated based on Brookfield (1996) using the PopGenReport library. Hardy-Weinberg equilibrium (HWE) analysis was performed using the pegas library. F-statistics values were calculated using the method described by Nei (1973) using the pegas and diveRsity libraries. Furthermore, the genetic divergence between the populations was also revealed using pairwise gene differentiation coefficient (F_{ST}) values, calculated using the method described by Nei (1973) and the hierfstat library. The gene flow (N_m) from the F_{ST} values was estimated using the formula described by Slatkin and Barton (1989). Analysis of molecular variance (AMOVA) with 1000 bootstrap replicates was also used to determine the hierarchical distribution of genetic variation among and within the populations. These tests were performed using the poppr library with the AMOVA function based on the method of Excofer et al. (2005). To estimate unbiased genetic distances among the populations, the poppr analysis library and the method described by Nei (1978), using the R statistical programming language (version 3.3.2), were utilized. Finally, discriminant analysis of the principal components was used to visualize the genetic structure of the sampled white poplar trees based on the SSR data set. This analysis was executed using the adegenet analysis library in R statistical programming language (version 3.3.2).

3. Results

3.1. Genetic diversity analysis of the SSR loci

The average number of alleles per locus, which is an indicator of genetic variation, was 7.1. Locus WPMS14 (11) exhibited the highest number of alleles, while locus WPMS20 (3) exhibited the lowest. The mean H_o and H_e values were calculated as 0.50 and 0.58, respectively, with ranges of 0.04–0.70 and 0.07–0.82 for the H_o and H_e values, respectively. The inbreeding coefficient (F_{IS}) value indicated an average inbreeding coefficient of –0.19 per locus, indicating an excess of heterozygosity within the populations. These data are presented in Table 3.

Upon examination of the F_{ST} values, which showed differentiation among the populations on a locus-by-locus basis, an average value of 0.27 was observed (Table 3).

The phenomenon of N_m is defined as the transmission of genetic material from one population to another through individual migration. The average N_m value, estimated on a locus-by-locus basis, was 0.89 (Table 3). Furthermore, examination of the HWE analysis revealed that the Eskişehir-Porsuk Çayi population was in HWE equilibrium for all of the loci examined. Conversely, significant deviations from the HWE equilibrium were observed in the Kayseri-Akköy (7), Konya-Meram (7), Kocaeli-Izmit (7), Erzurum-Oltu (6), and Bursa-Iznik (6) populations, as shown in Figure S1.

The null allele frequency calculation revealed that the frequency ranged from 0.03 (WPMS18) to 0.32 (WPMS20) across the loci (Table 3). One locus, WPMS20, exceeded the upper limit of 0.19 (Chapuis et al., 2008) among the estimated values, indicating the possibility of a null allele. Subsequently, statistical analyses were repeated without WPMS20, and the results did not differ; therefore, WPMS20 was included in the statistical analyses.

Table 3. Genetic parameters calculated on the basis of the loci.

Locus	Allelic range (bp)	n_a	Average H_o	Average H_e	F_{ST}	F_{IS}	N_m	Null allele frequency
WPMS05	298–315	9	0.70	0.78	0.19	–0.09	1.04	0.06
WPMS06	166–176	5	0.47	0.65	0.20	0.12	1.03	0.16
WPMS14	206–245	11	0.65	0.73	0.20	–0.10	1.02	0.06
WPMS15	184–211	9	0.68	0.75	0.15	–0.05	1.44	0.05
WPMS16	167–197	5	0.25	0.28	0.37	–0.37	0.42	0.06
WPMS17	108–163	8	0.58	0.62	0.22	–0.16	0.90	0.04
WPMS18	215–230	6	0.57	0.61	0.15	–0.10	1.38	0.03
WPMS20	221–229	3	0.04	0.07	0.76	–1.00	0.08	0.32
PTR8	132–142	6	0.45	0.51	0.28	–0.20	0.66	0.06
ORPM312	229–245	9	0.60	0.82	0.21	0.09	0.93	0.15
Mean		7.1	0.50	0.58	0.27	–0.19	0.89	0.10

n_a, number of alleles.

3.2. Genetic diversity analysis of the populations

In regard to genetic diversity, analysis of the populations indicated that the Tekirdağ-Malkara population possessed the highest average number of alleles (4.4), while the Erzurum-Oltu population had the lowest (1.6) among the 15 populations studied. The Samsun-Bafra population had the highest number of unique alleles (5). The H_o and H_e values were 0.50 and 0.46, respectively (Table 4).

Analysis of the clone data revealed a low number of genotype counts and hence, a high number of clone counts in some populations (Erzurum-Oltu, Kayseri-Akköy, Konya-Meram, Bursa-Iznik, Samsun-Bafra, and Kocaeli-Izmit populations) (Table 4).

The F_{ST} values between the populations ranged from 0.01 to 0.61, with the most significant differentiation observed between the Erzurum-Oltu and Kayseri-Akköy populations (Figure 2). The rate of N_m between the populations was estimated based on the F_{ST} value and was the lowest (0.16) between these 2 populations. In contrast, the highest N_m rate (30.24) was found between the Tekirdağ-Malkara and Edirne-Tunca Nehri populations (Figure 3).

AMOVA, which is a statistical approach used to partition genetic variation within and among populations, showed that 84.20% of the genetic variation was due to the differences within populations, while 21.23% of the variation was attributed to differences between populations (Table 5).

3.3. Phylogenetic analysis and principal component analysis (PCA) of populations

According to the phylogenetic analysis and PCA results, the 4 populations that had the highest degrees of differentiation were Erzurum-Oltu, Kayseri-Akköy, Konya-Meram, and Bursa-Iznik (Figure 4). The Kocaeli-Izmit and İstanbul-Bahçeköy populations were clustered together and separated from the remaining populations at a rate of 37.9%. Examination of the other populations revealed that the Marmara populations were grouped together, while the Western Black Sea populations and the Eskişehir-Porsuk Çayi population, which are geographically close to them, were also clustered together. Furthermore, the Samsun-Bafra population, located in the Central Black Sea region, was clustered close to these populations.

In addition, the PCA conducted in this study, as illustrated in Figure 5, revealed that the Erzurum-Oltu and Kayseri-Akköy populations were clearly separated from the other populations, forming distinct clusters. The rest of the populations were grouped together in a single cluster. Nevertheless, some individuals from the Samsun-Bafra population and one individual from the Sakarya-Sakarya Nehri population were slightly more distant from the other individuals.

Table 4. Genetic diversity values observed in terms of the populations.

Population	N	G	A	H_e	H_o	P_a
Sakarya-Sakarya Nehri	20	20	4	0.50	0.49	0
Eskişehir-Porsuk Çayi	20	19	4	0.50	0.54	1
Edirne-Tunca Nehri	20	20	4.3	0.56	0.47	2
Tekirdağ-Malkara	20	18	4.4	0.55	0.48	2
İstanbul-Bahçeköy	20	15	3.2	0.44	0.42	1
Samsun-Bafra	20	9	4.1	0.46	0.47	5
Sakarya-Geyve	20	20	3.7	0.46	0.48	0
Bursa-Karacabey	20	20	4	0.50	0.47	1
Kayseri-Akköy	19	2	2	0.38	0.68	0
Konya-Meram	20	2	2.2	0.32	0.49	0
Kastamonu-Samatlar	20	20	3.3	0.45	0.41	1
Bursa-Iznik	20	7	3.3	0.49	0.53	2
Kocaeli-Izmit	20	11	3.6	0.48	0.46	0
Balikesır-Susurluk	15	12	3.5	0.53	0.57	0
Erzurum-Oltu	11	1	1.6	0.31	0.60	2
Mean	19	13	3.41	0.46	0.50	1.13

N, sample size; G, number of genotypes; A, mean number of alleles per locus; P_a, number of private alleles.

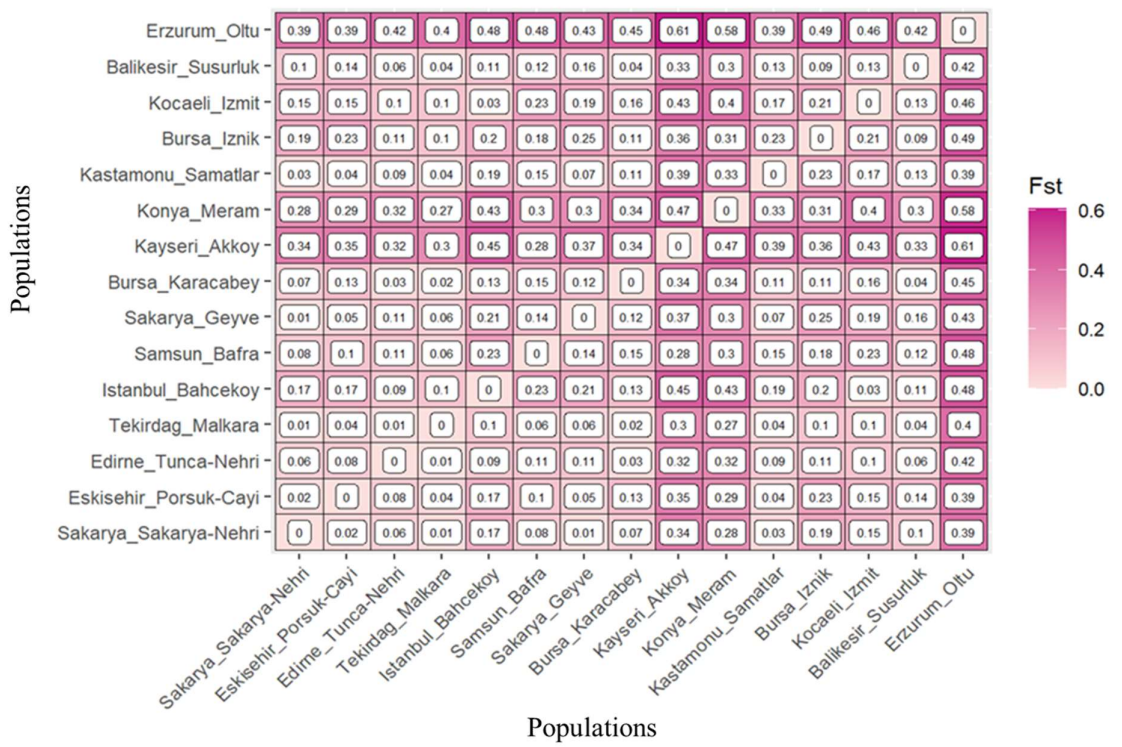

Figure 2. Pairwise F_{ST} values observed in the 15 white poplar populations.

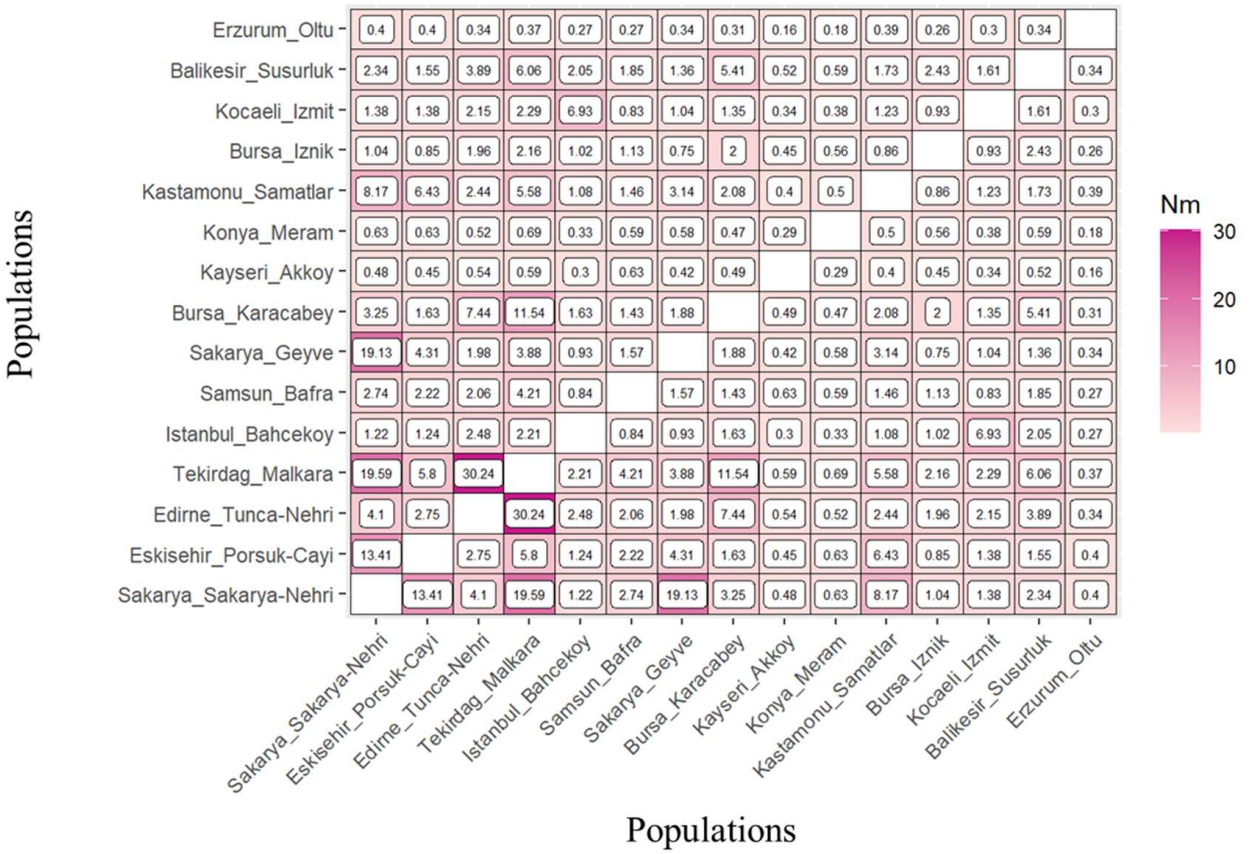

Figure 3. N_m rate among the 15 white poplar populations.

Table 5. AMOVA results for the 15 white poplar populations based on the 10 SSR markers.

Source of variation	df	SSD	MSD	Sigma	Total variance (%)	P
Between populations	14	728.3424	52.024457	1.2563881	21.23	<0.05
Between samples within populations	270	1171.7052	4.339649	–0.3215882	–5.43	<0.05
Within samples	285	1420.1052	4.982825	4.9828254	84.20	<0.05
Total	569	3320.1529	5.835067	5.9176252	100	

Df, degrees of freedom; SSD, sum of squared deviations; MSD, mean squared deviations; P, probability of obtaining a larger component estimate. Number of permutations = 1000.

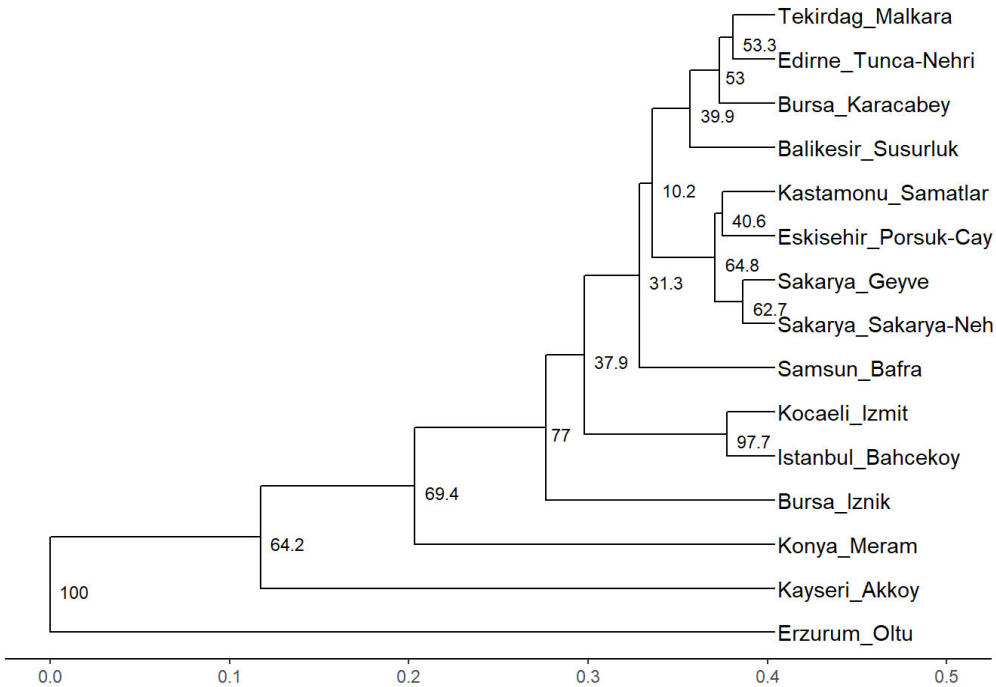

Figure 4. Phylogenetic dendrogram of white poplar populations obtained using the unweighted pair group method with arithmetic mean. Numbers above the branches indicate the bootstrap value of that branch based on 1000 permutations.

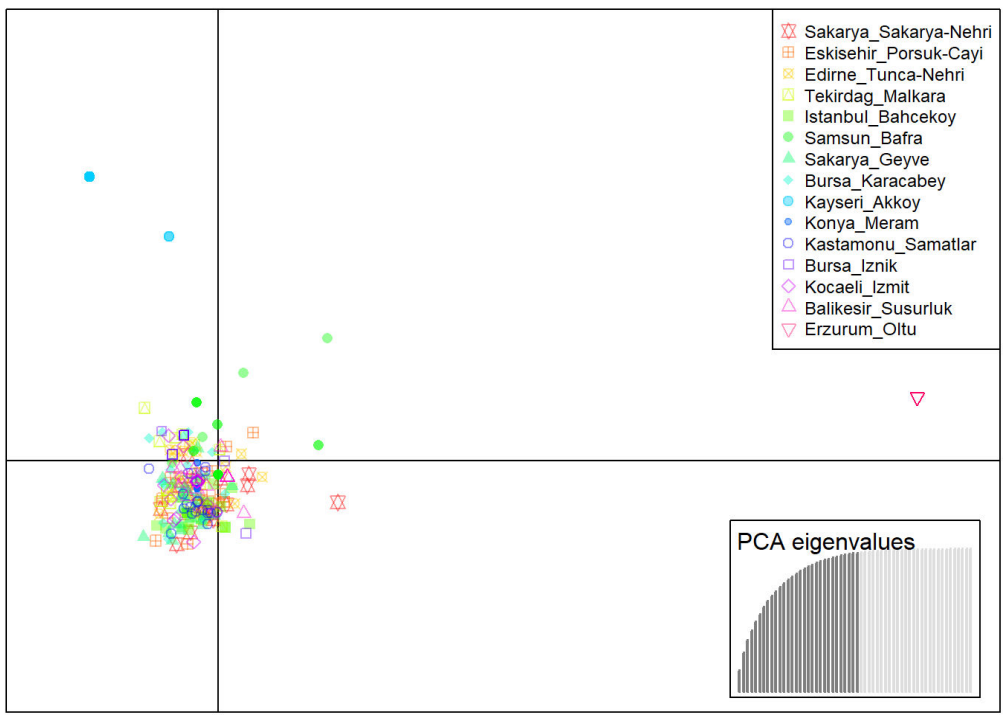

Figure 5. Groups obtained by the PCA analysis after analysis of the genetic data from the 10 microsatellite loci in the 15 white poplar populations.

4. Discussion

In this study, 10 microsatellite loci (WPMS05, WPMS06, WPMS14, WPMS15, WPMS16, WPMS17, WPMS18, WPMS20, PTR8, and ORPM312) were used to determine the genetic diversity of 15 populations of white poplar. All of the selected loci were polymorphic in the studied populations. The average number of alleles was 7.1 in the 285 individuals of *P. alba*. This value may be higher than that in other studies due to the large sample size (Lexer et al., 2005; Brundu et al., 2008; Castiglione et al., 2010; Fussi et al., 2011; Dering et al., 2015). Additionally, some populations had private alleles, but they were mostly found in the Samsun-Bafra population ($P_a = 5$). The presence of private alleles in populations is important for conservation and breeding programs because it allows for the selection of individuals with more adapted and resilient genes to ecological conditions.

For the WPMS20 primer, most individuals were homozygous for an allele size of 221 bp (exceptions: Erzurum-Oltu). The occurrence of only one allele for this primer in homozygosity was also reported in different genetic studies on white poplar (Brundu et al., 2008–219 bp; Fussi et al., 2011–235 bp). Furthermore, previous studies reported that the WPMS20 locus formed species-specific alleles for *P. deltoides* and *P. tremuloides* (Fossati et al., 2003; Liesebach et al., 2010). This suggests the possibility of WPMS20 locus forming species-specific alleles for *P. alba* and can be supported by further studies.

Heterozygosity is an important measure of genetic diversity (Slatkin and Barton, 1989). It was calculated in 2 ways, the H_o and H_e, and a relatively high level of genetic diversity was found. When the earlier studies on white poplar were examined, results similar to those in studies in Sardinia and Poland (Brundu et al., 2008; Dering et al., 2015), but lower levels of polymorphism were reported in studies in Austria, Slovakia, Italy, and China (Lexer et al., 2005; Van-Loo et al., 2008; Castiglione et al., 2010; Zeng et al., 2016).

The average F_{IS} value was calculated for the 15 populations and the result was negative, in contrast to those reported in previous studies done in Austria, Slovakia, Italy, Poland, and China ($F_{IS} = -0.19$; Van-Loo et al., 2008; Castiglione et al., 2010; Dering et al., 2015; Zeng et al., 2016). This value indicates a high degree of heterozygote excess and greater mating among trees with individuals other than themselves in the populations.

The AMOVA showed that 21% of the total molecular variation was between populations, while 84% was within populations. This indicates that the majority of the genetic variation occurs within populations rather than between them. The high level of genetic diversity observed within populations using microsatellite analysis in white the poplar populations was within the range of the values commonly observed and estimated in poplar species (Cole et al., 2005; Lexer et al., 2005; Guarino et al., 2015). This result could be due to species adaptation to alterations in environmental conditions and could indicate that the species is prone to tree breeding (Işık and Kaya, 1995).

After conducting genetic analyses using various methods, it was determined that the 3 populations at the highest altitude and the farthest distance, Erzurum-Oltu, Kayseri-Akköy, and Konya-Meram, exhibited a significantly different genetic structure compared to all of the other populations. High altitude plays an important role in the population structure and can accelerate population differentiation through selection pressure for adaptation, rather than maintaining the N_m (López-Gartner et al., 2009). It was found that these 3 populations had high levels of differentiation and low genetic exchange with other populations. Moreover, white poplar individuals in these populations were distributed in a narrower area. At the genetic level, N_m from other populations may decrease in such fragmented populations that have a low effective population size and spread in a narrow area. Thus, allelic richness and genetic diversity in populations decrease, which causes an increase in genetic differentiation (Lowe et al., 2005). In accordance, the critical N_m value (0.5) between these populations and others was below the threshold for genetic drift (Hamrick, 1989). The seeds of the white poplar in these populations are thought to have been transported randomly by wind or human activities, and the trees have colonized the area through vegetative propagation. The most likely explanation for the observed genetic structure in these 3 populations might be the human influence. It is estimated that the people living in the region selected and propagated the genotype that best adapted to the local conditions, resulting in the dominance of a single clone in the area. The high levels of clonal reproduction observed in these populations support this hypothesis.

The phylogenetic analysis of *P. alba* individuals from Bursa-Iznik, İstanbul-Bahçeköy, and Kocaeli-Izmit populations revealed that they were not grouped according to their geographic distribution, and relatively high levels of clonal reproduction were detected. These populations were located near urban centers with high levels of human activity, and it is believed that the trees were transported to these areas through human activities due to their popularity as landscape plants, owing to their 2-colored leaves (Tunçtaner, 1993). As a result, the number of genotypes has decreased, and they were not clustered according to geographic distance.

Clonal reproduction is a common trait in *Populus* species (Arens et al., 1998; Barsoum et al., 2004; Storme et al., 2004; Suvanto and Latva-Karjanmaa, 2005). For example, it was reported that a single *P. tremuloides* clone consisting of 47,000 ramets covered an area of 43 ha (Kemper-

man and Barnes, 1976). Genetic studies on *P. nigra* and *P. euphratica* in Türkiye have also shown that clonal reproduction is widespread (Ciftci et al., 2017; Kansu and Kaya, 2020). As in the current results, different molecular studies have also demonstrated that *P. alba* reproduces clonally via root suckers (Van-Loo et al., 2008; Santos del Blanco, 2013; Dering et al., 2015). Furthermore, despite being sampled over a wide area in Sardinia and Malta, *P. alba* formed huge monoclonal stands, which was attributed to human influence rather than autovegetative spread (Brundu et al., 2008; Fussi et. al., 2011).

On the contrary, the Edirne-Tunca Nehri, Tekirdağ-Malkara, Bursa-Karacabey and Balikesır-Susurluk populations were grouped together, while the Sakarya-Sakarya Nehri, Sakarya-Geyve, Eskişehir-Porsuk Çayi and Kastamonu-Samatlar populations were clustered together and the Samsun-Bafra population was grouped close to them, since these populations existed geographically close to each other. It is well-known that geographic proximity ensured high rates of N_m between populations resulting genetically closer populations. The pollen of the poplar tree can be carried to distances of 100 km by the wind, and its seeds can be dispersed by wind and water. In addition, plant materials such as pieces of roots and branches broken off of white poplar individuals on the river banks can be carried long distances away by the current (Remaley and Swearingen, 2014). Thus, N_m occurs over long distances, and this is frequently seen in poplar populations (Imbert and Lefevre, 2003; Dewoody et al., 2015). In addition, the genotype numbers of these populations were high, which indicates that the individuals fertilize between different genotypes rather than similar genotypes. This increases the allele diversity in the populations and contributes to the high rate of heterozygous individuals. In natural selection, heterozygous individuals have a higher chance of survival than homozygous individuals (Allendorf and Luikart, 2007). As a result, it was determined that N_m plays an important role in maintaining the genetic diversity of white poplar individuals and that clonal reproduction problems are not common in some populations.

White poplars generally grow on river banks and floodplain forests where water is abundant, and the mentioned populations were sampled from these ecosystems. However, these regions are among the most vulnerable ecosystems. The openness of riparian forests to agriculture and other land uses, and situations such as floodings, negatively affect the presence of tree species including white poplar. Thus, white poplar populations are decreasing in size and are increasingly exposed to habitat fragmentation. This poses a risk of loss of genetic diversity and associated adaptive potential. Moreover, since white poplar is used to restore riparian forest ecosystems, it poses an additional threat to genetic diversity (Palancean et al., 2018). Despite all of these threats, analyses have shown that the genetic structure of white poplar populations in riparian ecosystems in Türkiye (Sakarya-Sakarya Nehri, Sakarya-Geyve, Eskişehir-Porsuk Çayi, Edirne-Tunca Nehri, Balikesır-Susurluk) has not yet been disturbed. However, since white poplar individuals in these ecosystems are at risk, breeding and conservation efforts must be started before they lose their naturalness.

With the evaluation of the situation of white poplar in Türkiye in this study, it can be concluded that intense clonal reproduction was found in some populations in accordance with the above-mentioned previous studies. Moreover, the inclusion of human activities also decreased the level of genetic diversity in these populations. However, the total level of genetic diversity in populations was not as low as that reported in previous studies (Brundu et al., 2008; Fussi et al., 2011; Macaya-Sanz et al., 2012; Dering et al., 2015) and the genotype numbers and genetic diversity levels of the populations, especially in the riverside and floodplain forest ecosystems, were high. Furthermore, this study represents an important step for the protection, survival, and breeding of the white poplar species, whose numbers are decreasing due to various factors in the forests of Türkiye. As a result of this study, it is recommended that populations with high levels of genetic diversity (for instance, the Eskişehir-Porsuk Çayi population, where the species is distributed uninterruptedly in a wide area) should be protected in situ. The Eskişehir-Porsuk Stream is also very rich in terms of plant diversity (411 taxa) and one of the dominant species of the region is *P. alba* (Özdeniz, 2016). It is also a requirement to preserve the Samsun-Bafra population, with the highest number of private alleles in situ, since it is very important to preserve the private alleles of the species in order to maintain its potential to adapt to future environmental changes (Allendorf and Luikart, 2007). Those genotypes should also be protected ex situ in a clone bank since it is not possible to protect all populations with high genetic diversity and genotype numbers in situ. Furthermore, those populations are also very promising for white poplar breeding. In addition, the present study revealed that there is a large genetic base narrowing in trees that are the subject of landscape design in the urban ecosystem. Since trees planted in cities face many threats, it would be appropriate to conduct landscape studies with individuals obtained from saplings collected from as many trees as possible to preserve genetic diversity.

It is expected that the findings of this study will shed light on similar comprehensive studies on the genetics of forest trees in the present and future, since these studies are of great importance for understanding, protecting, and sustaining the genetic diversity of forest ecosystems.

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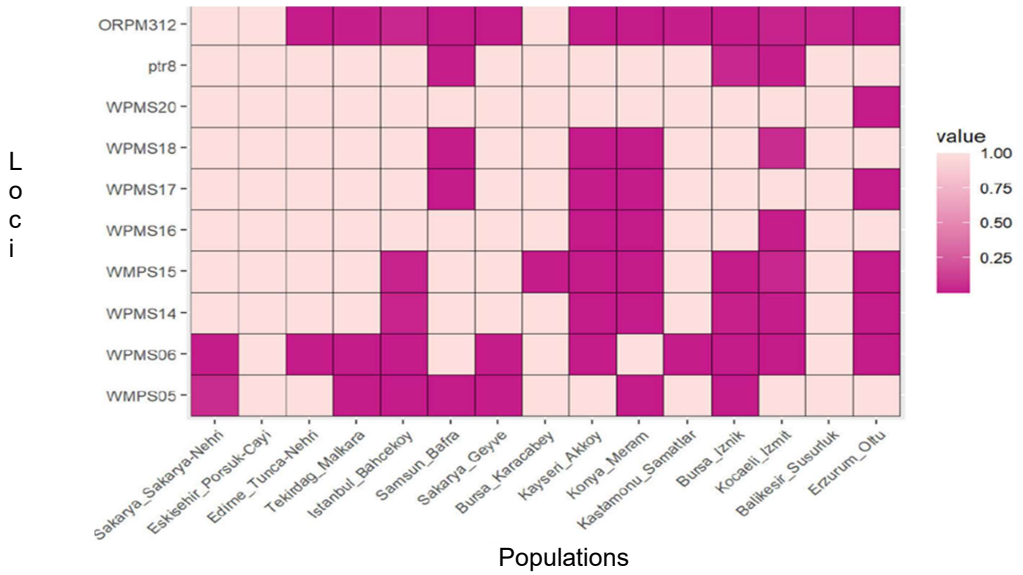

Figure S1. HWE analysis of the populations.