

Assessment of genetic diversity in natural European hophornbeam (*Ostrya carpinifolia* Scop.) populations in Turkey

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ABSTRACT

Genetic diversity is a crucial component for plant survivability and fitness in terms of adaptation, genetic stability and variability. In this study, a total of 160 genotypes were investigated using 12 random amplified polymorphic DNA (RAPD) primers to understand the genetic structure and diversity of nine naturally distributed *Ostrya carpinifolia* populations in Turkey. Twelve RAPD primers yielded 111 clearly identifiable DNA bands, of which 71 bands were found to be polymorphic (64%). Observed number of alleles (N_a), effective number of alleles (N_e) and Nei's gene diversity (h) were found as 2, 1.53 and 0.32, respectively. Total genetic variation (H_T), within-population genetic variation (H_S) and Nei's genetic differentiation coefficient (G_{ST}) were found as 0.32, 0.09 and 0.70, respectively. Genetic diversity analysis (AMOVA) revealed highly significant ($P < 0.001$) genetic variations among and within populations. 69.94% of total variation was observed among populations while 26.69% was within populations. Gene flow value was calculated as 0.21 ($N_m < 0.5$), which could homogenize the genetic structure of a population. Two geographically isolated populations demonstrated high gene diversity and polymorphic loci ratio, indicating a relationship between geographic distribution of populations and eco-geographic factors. The findings of this study will pave the way for understanding the genetic diversity between inter- and intra-populations of *O. carpinifolia* species, as well as they would provide valuable information for management, conservation and utilization of *in situ* and *ex situ* *Ostrya* germplasm.

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Introduction

Habitat destruction is a crucial problem in biodiversity preservation in most ecosystems.[1,2] Besides, to understand the genetic structure of populations is essential prerequisite in biodiversity conservation and management practices.[3,4] Many studies have reported the negative effects of fragmentations on population structure and genetic diversity in plants.[5–7] Population fragmentations reduce the genetic diversity and increase the genetic differentiation due to reduced gene flow, random inbreeding and genetic drift.[1,8,9] Anthropogenic activities and climatic changes have been mainly regarded as two major factors in population fragmentations, finally leading to the formation of barriers between populations and changing the genetic diversity.[10–12] Therefore, policy makers are required to assess the genetic structure and diversity of populations to develop more comprehensive and effective conservation policies in plant species.[2]

Betulaceae (birch) family includes about 120–150 species, comprising the trees and shrubs in six genera

and two subfamilies such as Betuloideae (*Alnus* sp. and *Betula* sp.) and Coryloideae (*Carpinus* sp., *Corylus* sp., *Ostrya* sp. and *Ostryopsis* sp.).[13] This family members are mainly distributed in northern temperate zone and usually characterized with their stipulate, doubly serrate leaves, catkins and small winged fruits or nuts with leafy bracts.[14,15] *Ostrya*, which is a genus in Betulaceae family, comprises the several species, including *O. carpinifolia* (European hophornbeam), *O. virginiana* (Eastern hophornbeam), *O. chiosensis* (Chios hophornbeam) and *O. knowltonii* (Knowlton hophornbeam),[16,17] which are native to Mexico, Eurasia, eastern Asia/Japan, USA and Canada.[18,19] *Ostrya carpinifolia*, or European hophornbeam, has a distribution from South France to Bulgaria, West Syria, Anatolia and to Transcaucasia. In Turkey, *O. carpinifolia* populations are distributed as small groups in angiosperm mixed forests in north and south Anatolia.[19–21] Turkey, which is located at crossroads of Europe and Asia, harbours a broad range of natural habitats, including from Mediterranean, Aegean and Black Sea beaches, to coastal and interior mountains,

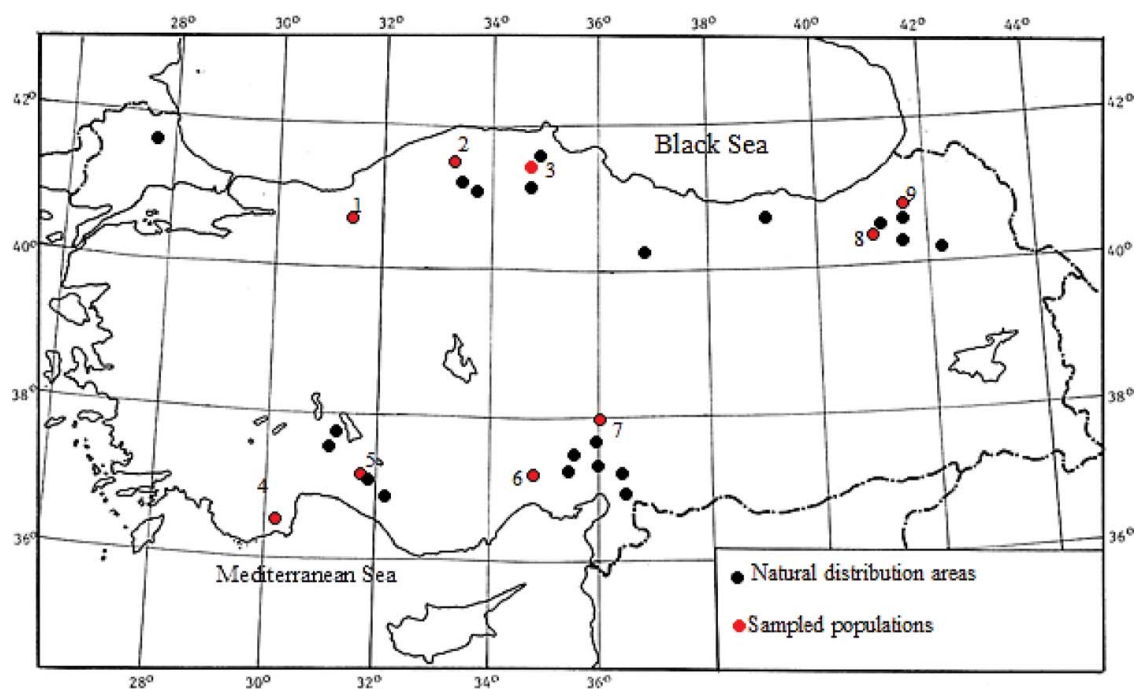


Figure 1. Natural distribution of *O. carpinifolia* populations in Turkey. Dots show the sampled populations (1–9) and natural distribution areas.

from fertile alluvial plains to arid, rocky hillslopes and valleys and to expansive steppes.[22] In addition, *O. carpinifolia* has also been used in various studies such as wood properties,[18,23] pathogenicity,[24] NO₂ pollution, pollen research and allergenicity [25] and seed germination. [26] However, the number of genetic studies about this species is still limited, although some of its relatives were reported in phylogenetic analyses.[13,15,27] Traditional assessments of plant systematics or phylogeny were mainly based on the analyses of anatomical and morphological characteristics of plants, which are usually affected by plant habitat and variability.[28] However, molecular markers are now commonly used in systematics studies, as well as in genetic diversity assessments in plants.[2,28] Random amplified polymorphic DNA (RAPD) is a simpler, cheaper and faster PCR-based marker system.[29] RAPD markers have been widely used in some wood species, including *Tilia tomentosa*, [30] *Dalbergia monticola*,[31] *Larix gmelinii* [32] and *Olea europaea*. [33] Despite of the ecological and commercial value of *O. carpinifolia*, little is known about the genetic aspects of this forest tree. Therefore, this study aimed to evaluate the genetic structure and diversity of natural *O. carpinifolia* populations distributed in Turkey using RAPD molecular markers. Study findings will pave the way for understanding of genetic diversity between inter- and intra-populations of *O. carpinifolia* species, as well as will provide valuable information for conservation initiatives.

Materials and methods

Sample collection and DNA extraction

A total of 160 plant leaf samples, average 20 individuals per population were collected from natural *O. carpinifolia* habitats in nine different regions of Turkey (Figure 1 and Table 1). Fresh leaf samples were carried to the laboratory using liquid nitrogen tank and stored at -80°C until DNA extraction. Total genomic DNA was extracted from 0.1g of powdered fresh leaves using E.Z.N.A. Plant DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. DNA concentration of each sample was measured by using BioSpecnano (Shimadzu, Kyoto, Japan) and then elutions were diluted with distilled water to a final concentration of $50\text{ ng }\mu\text{L}^{-1}$.

Table 1. Some geographical features of sampled *O. carpinifolia* populations.

Population no.	Population name	Population code	Latitude (N)	Longitude (E)	Altitude (m)
1	Duzce–Yigilca	D	40 55'	31 20'	550
2	Kastamonu–Sehdag	K	41 47'	33 07'	700
3	Sinop–Ayancik	S	41 47'	34 37'	450
4	Antalya–Finike	F	36 19'	30 05'	820
5	Antalya–Akseki	AK	37 05'	31 46'	1300
6	Nigde–Horoz	N	37 28'	34 47'	650
7	Adana–Saimbeyli	A	38 01'	36 06'	1225
8	Erzurum–Ispir	I	40 27'	41 00'	1947
9	Artvin–Hatilla	AR	41 11'	41 44'	650

Table 2. Details of 12 RAPD primers used in this study.

No.	Primer code	Sequence (5'-3')	Annealing temperature (°C)	Total no. of bands	No. of polymorphic bands	Polymorphic bands (%)
1	OPU02	CTGAGGTCTC	36 °C	12	7	58
2	OPI13	CTGGGGCTGA	36 °C	10	6	60
3	OPI16	TCTCCGCCCT	36 °C	12	7	58
4	OPI17	GGTGGTGATG	36 °C	9	5	55
5	OPI18	TGCCCAGCCT	36 °C	8	6	75
6	OPI19	AATGCGGGAG	36 °C	8	6	75
7	OPB01	GTTCGCCTCC	36 °C	10	7	70
8	OPB02	TGATCCCTGG	36 °C	7	4	57
9	OPB07	GGTGACGCAG	36 °C	10	7	70
10	OPB09	TGGGGGACTC	36 °C	8	4	50
11	OPA04	AATCGGGCTG	36 °C	10	7	70
12	OPA16	AGCCAGCGAA	36 °C	7	5	71
	Total			111	71	64

RAPD-PCR amplification

A total of 23 RAPD (Operon Technologies Inc., Alameda, CA, USA) primers were checked and 12 primers, which produced clear DNA bands, were selected for further analysis (Table 2). RAPD reactions were performed in a 20- μ L volume, containing 50 ng DNA, 2.5 mmol/L MgCl₂, 0.25 mmol/L dNTPs, 0.5 μ mmol/L primers, 1 U Taq DNA polymerase and 2.5 μ L of 10X Taq DNA polymerase buffer (Thermo Sci., Waltham, MA, USA). RAPD-PCR reactions were performed for a cycle of 3 min at 94 °C, followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C, and 1 min at 72 °C and a final cycle of 7 min at 72 °C. Amplification products were separated on 1.2% agarose gel with ethidium bromide in 1.2X TBE buffer, and digitally photographed under UV light. A 100-bp DNA ladder (Thermo Sci.) was used as a molecular ruler.

Data analysis

For statistical analysis, RAPD bands were scored as present (1) or absent (0) for each population and then band patterns were translated into binary data matrix according to statistical analysis platform. Subsequently, percentage of polymorphic loci (P), mean number of observed (N_a) and effective (N_e) alleles per locus, [34] Nei's gene diversity (*h*), [35–37] Shannon's information index (*I*), total genetic

variation (*H*_T), within-population genetic variation (*H*_s), Nei's genetic differentiation coefficient (*G*_{ST}) and gene flow (*N*m) [38] were estimated using POPGENE v. 1.32.[39] Inter- and intra-population analyses were performed by Arlequin 3.5.2 software [40] using AMOVA (Analysis of MOlecular VAriance) and population comparison tools with following settings; for population comparisons, Slatkin's and Reynold's distances using *F*_{ST} methods and pairwise differences (π) with Nei's method were selected for 100 permutations and 0.05 significance level; for AMOVA, standard AMOVA computations with 1000 permutations were adapted. A dendrogram was constructed based on Nei's [36] by using unweighted pair group method with calculating the arithmetic average (UPGMA) by POPGENE. Principal coordinate analysis was performed using MVSP 3.2 (MultiVariate Statistical Package).

Results and discussion

Genetic diversity is a fundamental component for plant survivability and fitness for adaptation, genetic stability and variability.[41] In this study, a total of 160 genotypes were evaluated using 12 RAPD primers to understand the genetic structure and diversity of nine naturally distributed *O. carpinifolia* populations in Turkey. From 160 individuals of 9 natural populations, 12 primers yielded 111 clearly identifiable DNA bands (Table 2), of which 71

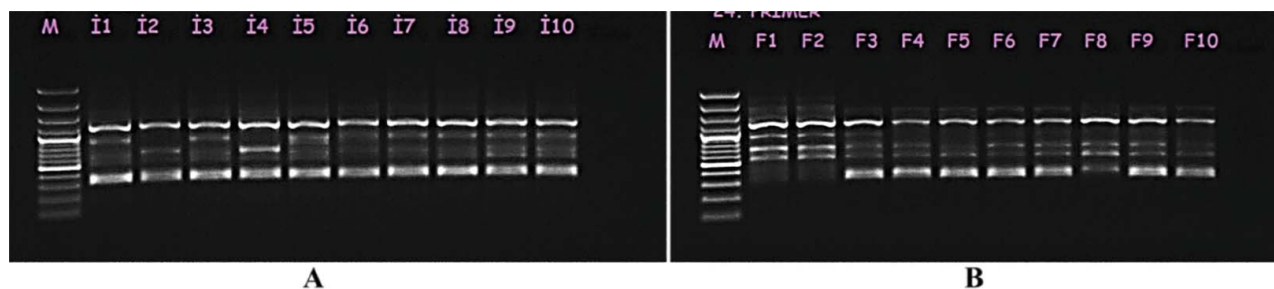


Figure 2. RAPD profiles of selected 10 genotypes in Erzurum–İspir (A) and Antalya–Finike (B) populations obtained by using OPA04 primer. (M: 100-bp standard marker)

bands were found to be polymorphic (64%). An average band number per primer was found as 9.25 while polymorphic band number was found as 5.9. Banding patterns and polymorphism of primer OPA04 are shown in Figure 2. Similar genetic diversity analyses were performed in some *O. carpinifolia* relatives using RAPD primers. For example, Zeng et al. [42] have identified 131 highly reproducible DNA bands in *Betula alnoides* using 16 RAPD primers with 64.1% (84 bands) polymorphism, which significantly shared the same polymorphic loci ratio with *O. carpinifolia* species. In a different study, a total of 83 DNA bands were reported in six populations of *B. pendula* subsp. *fontqueri* while 81 DNA bands were found in two *B. pubescens* populations using six RAPD primers. Besides, polymorphic DNA bands were reported as 32.2% and 44.6% in *B. pendula* subsp. *fontqueri* and *B. pubescens*, respectively,[43] which were considerably low compared to *O. carpinifolia* species. Moreover, in various studies of hazelnut (*Corylus avellana*), a total and mean polymorphic DNA bands were found as 216 and 9.4,[44] 96 and 3.84[45] and 241 and 5.7,[46] respectively. Compared to *O. carpinifolia* species, hazelnut genotypes demonstrated about 5.9 less yields of polymorphic DNA bands.

Within populations, percentage of polymorphic loci (P) ranged from 17.39% (Antalya–Akseki population) to 39.13% (Antalya–Finike) (Table 3). In addition, observed number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (*h*) and Shannon's information index (I) were found to vary between 1.17–1.30, 1.14–1.28, 0.07–0.16 and 0.09–0.23, respectively. For all loci, genetic variation parameters (Na, Ne, *h* and I) were found to be as 2, 1.53, 0.32 and 0.48, respectively. Zeng et al. [42] also reported mean number of alleles, effective number of alleles and gene diversity as 1.64, 1.36 and 0.214 for all loci, respectively. Thus, gene diversity in *O. carpinifolia* (0.32) species was found higher than that of *B. alnoides* (0.214).

Furthermore, gene diversity analyses in subdivided populations demonstrated Nei,[36] total genetic variation (H_T), within-population genetic variation (H_S), Nei's genetic differentiation coefficient (G_{ST}) and gene flow (Nm) as 0.32, 0.09, 0.70 and 0.21, respectively. Genetic

differentiation coefficient (G_{ST}) was found higher (0.70) than other parameters, suggesting that G_{ST} is affected by mutation and heterozygosity.[47] In addition, gene diversity or heterozygosity in entire population (H_T : 0.32) was higher than average gene diversity of subpopulations (H_S : 0.09). Gene flow was calculated as 0.21, indicating that genetic variations within populations could be related with low level of gene flow that shaped the genetic divergence among populations. Gene flow (Nm) is considered to be able to homogenize the genetic structure of a population.[10] It is the main determinant of population structure at $Nm > 0.5$, and important genetic differentiation could result from genetic drift when $Nm < 0.5$.[48] Besides, genetic variations formed by dynamic processes, including genetic drift can affect the survivability and adaptation of plant populations to changing environmental conditions.[49,50] Thus, genetic drift may become a major factor in genetic differentiation of gene pool in *O. carpinifolia* populations. Moreover, it is well known that breeding and mating system, floral morphology and reproduction mode could significantly contribute to genetic diversity.[51] Therefore, breeding systems and long perennial life history of *Ostrya* species may have become other major contributing factors to high level of genetic diversity in *O. carpinifolia* populations.

Moreover, genetic identity and distance data revealed that highest (0.8927) and lowest (0.5529) genetic identity values were between Artvin–Hatilla & Duzce–Yigilca and Nigde–Horoz & Sinop–Ayancik populations, respectively. Highest (0.5177) and lowest (0.1135) genetic distance values were between Antalya–Akseki & Nigde–Horoz and Artvin–Hatilla & Duzce–Yigilca populations, respectively (Table 4).

The comparative analysis of inter- and intra-population genetic variations was performed using Arlequin 3.5.2 software. For population comparison analysis (Figure 3), three different colours were used to scale the average number of pairwise differences (π) between/ among populations. Differences (π) within populations were diagonally scaled with orange, while differences (π_{xy}) between populations were scaled above the diagonal with green. In addition, net number of differences

Table 3. Genetic diversity within *O. carpinifolia* populations (mean \pm standard error).

Population name	Sample size	Polymorphic loci (P%)	Observed no. of alleles (Na)	Effective no. of alleles (Ne)	Nei's gene diversity (h)	Shannon's information index (I)
Duzce–Yigilca	15	28.26	1.28 \pm 0.45	1.21 \pm 0.37	0.12 \pm 0.20	0.17 \pm 0.28
Kastamonu–Sehdag	18	19.57	1.19 \pm 0.40	1.16 \pm 0.34	0.09 \pm 0.18	0.12 \pm 0.26
Sinop–Ayancik	20	19.57	1.19 \pm 0.40	1.14 \pm 0.31	0.08 \pm 0.17	0.11 \pm 0.24
Antalya–Finike	17	39.13	1.39 \pm 0.49	1.28 \pm 0.38	0.16 \pm 0.21	0.23 \pm 0.30
Antalya–Akseki	20	17.39	1.17 \pm 0.38	1.14 \pm 0.32	0.07 \pm 0.17	0.11 \pm 0.24
Nigde–Horoz	15	19.57	1.19 \pm 0.40	1.11 \pm 0.27	0.06 \pm 0.15	0.09 \pm 0.22
Adana–Saimbeyli	20	30.43	1.30 \pm 0.46	1.17 \pm 0.31	0.10 \pm 0.17	0.15 \pm 0.25
Erzurum–Ispir	15	23.91	1.24 \pm 0.43	1.12 \pm 0.28	0.07 \pm 0.15	0.11 \pm 0.22
Artvin–Hatilla	20	23.91	1.24 \pm 0.43	1.19 \pm 0.36	0.10 \pm 0.19	0.15 \pm 0.27

Table 4. Nei’s unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal).

Pop ID	1	2	3	4	5	6	7	8	9
1	****	0.6846	0.8197	0.7931	0.7238	0.6705	0.7624	0.6650	0.6893
2	0.3789	****	0.8184	0.7781	0.7613	0.7376	0.5959	0.7277	0.6665
3	0.1988	0.2004	****	0.8927	0.7621	0.7644	0.6586	0.7718	0.7583
4	0.2318	0.2509	0.1135	****	0.7588	0.7480	0.6361	0.7982	0.7380
5	0.3232	0.2727	0.2717	0.2760	****	0.7752	0.7290	0.6946	0.8270
6	0.3997	0.3044	0.2687	0.2904	0.2546	****	0.6975	0.6865	0.6680
7	0.2713	0.5177	0.4176	0.4523	0.3161	0.3603	****	0.5529	0.6439
8	0.4079	0.3179	0.2590	0.2254	0.3644	0.3762	0.5925	****	0.7663
9	0.3721	0.4057	0.2766	0.3038	0.1900	0.4035	0.4403	0.2662	****

Note: 1: Adana–Saimbeyli, 2: Antalya–Akseki, 3: Artvin–Hatilla, 4: Duzce–Yigilca, 5: Erzurum–Ispir, 6: Kastamonu–Sehdag, 7: Nigde–Horoz, 8: Sinop–Ayancik and 9: Antalya–Finike.

between populations using Nei’s distance was scaled below the diagonal with blue. Between populations (π_{xy}), Sinop–Ayancik & Nigde–Horoz, Antalya–Akseki & Adana–Saimbeyli, Antalya–Finike & Antalya–Akseki, Nigde–Horoz & Antalya–Akseki, Sinop–Ayancik & Adana–Saimbeyli and Nigde–Horoz & Duzce–Yigilca populations demonstrated high variations. Within population (π ; diagonal), Antalya–Finike and Adana–Saimbeyli showed higher variations. Based on Nei’s distance, Antalya–Akseki & Nigde–Horoz and Sinop–Ayancik &

Nigde–Horoz populations represented high genetic distance.

For molecular variation analysis (AMOVA; Table 5), nine populations were analysed under four main groups, namely Groups 1–4, based on their geographic distributions. Group 1 included Erzurum–Ispir and Artvin–Hatilla populations; Group 2 included Duzce–Yigilca, Kastamonu–Sehdag and Sinop–Ayancik populations; Group 3 included Nigde–Horoz and Adana–Saimbeyli populations and Group 4 included Antalya–Finike and Antalya–Akseki populations. Analysis showed highly significant ($P < 0.001$) genetic variations among and within populations. Estimation of total genetic variation demonstrated that 69.94% of total variation was observed among populations within the groups, while 26.69% was within populations. However, genetic variation was found as low as 3.37% among groups. Previous studies have reported that in seven *B. alnoides* populations, 8.60% of total variation was among populations while 91.40% was within populations.[42] In *B. pendula*, total variance was found as 64.22% among populations and 35.78% within populations.[43] Moreover, there has been also reported an important relationship between

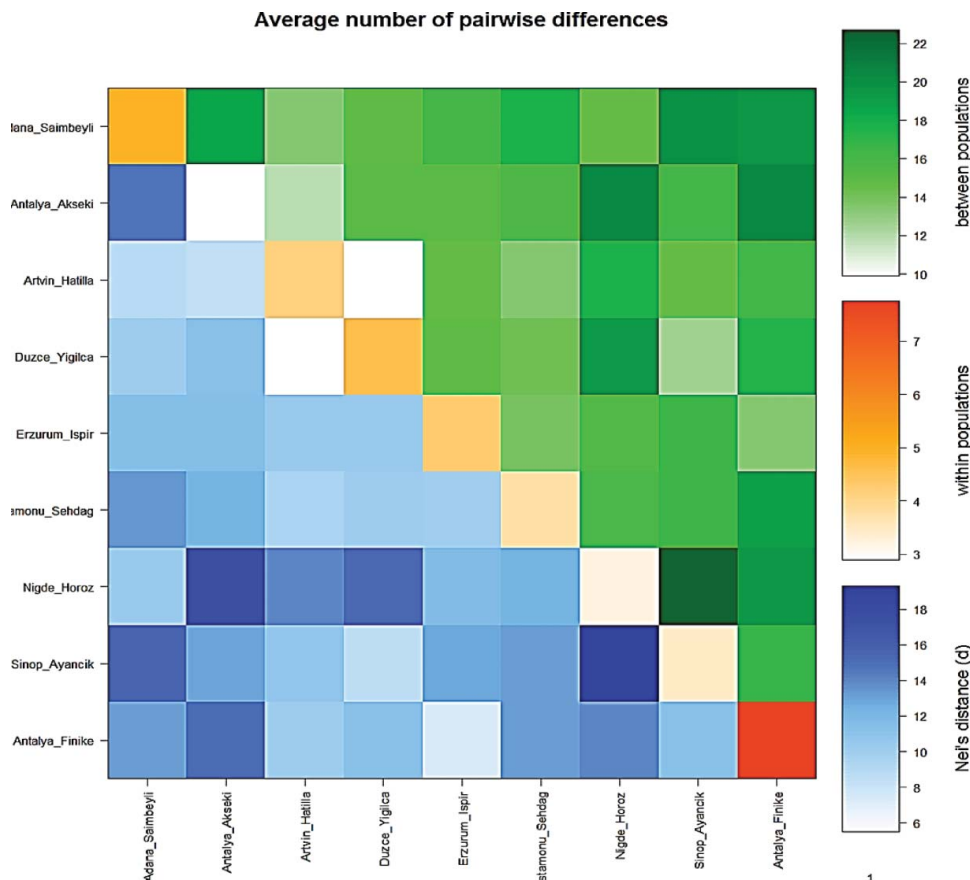


Figure 3. Inter- and intra-population comparisons by using average number of pairwise differences. Below diagonal, diagonal and above diagonal scales represent Nei’s distance, variation of within and between populations, respectively.

Table 5. Molecular variance (AMOVA) analysis of 160 individuals of *O. carpinifolia* genotypes from nine different populations using 12 primers with 111 identifiable DNA bands.

Source of variation	d. f.	Sum of squares (SSD)	Variance components	Percentage of variation	P-value
Among groups ^a	3	196.056	0.27473 Va	3.37	0.30792 (Va and FCT)
Among populations	5	296.233	5.70689 Vb	69.94	0.00000 (Vb and FSC)
Within populations	81	176.400	2.17778 Vc	26.69	0.00000 (Vc and FST)

^aGroup 1; Erzurum–İspir and Artvin–Hatilla, Group 2; Duzce–Yığılca, Kastamonu–Sehdag, and Sinop–Ayancık, Group 3; Nigde–Horoz and Adana–Saimbeyli, Group 4; Antalya–Finike and Antalya–Akseki.

geographic distribution of populations and eco-geographic factors. Some important eco-geographic factors such as latitude, altitude, temperature and moisture are also crucial determinants in genetic variability. So, morphological and physiological traits of plants are closely associated with their habitats.[41] Based on geographic location, Duzce–Yığılca and Antalya–Finike populations were isolated from other populations (as shown in Figure 1). In addition, these two populations also demonstrated high gene diversity and polymorphic loci ratio in analyses. Therefore, it seems that geographic

distributions of populations and eco-geographic factors may affect the gene flow, resulting in genetic variations.

Principal coordinate analysis of selected 90 individuals from nine populations demonstrated five major groups, namely as A–E (Figure 4). Highest number genotypes were detected in group A, including Antalya–Akseki, Artvin–Hatilla and Duzce–Yığılca populations. Nigde–Horoz population (N) in group C and Sinop–Ayancık (S) population in group D were clearly separated from other populations. In addition, Adana–Saimbeyli and Kastamonu–Sehdag populations were

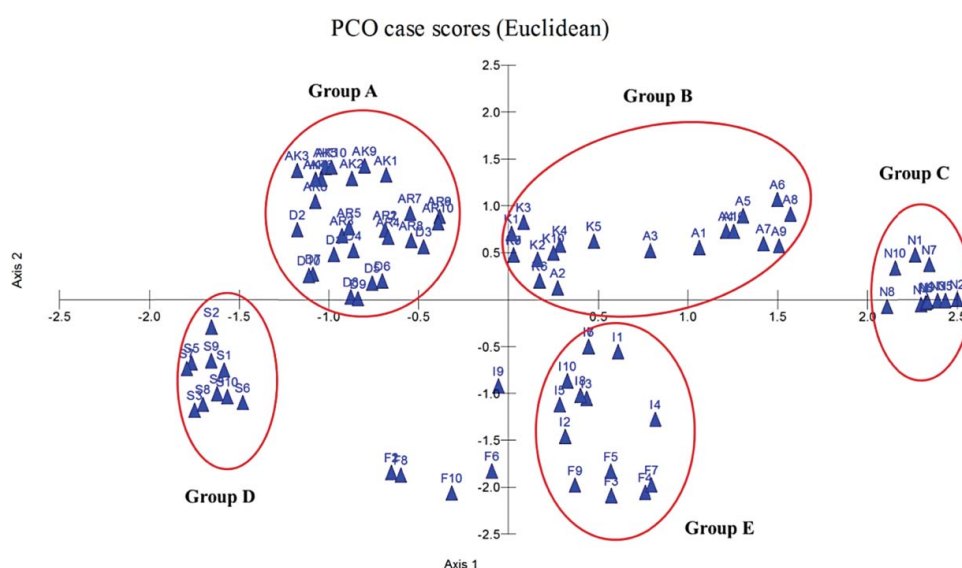


Figure 4. Principal coordinate analysis of RAPD profiles among 90 individuals of *O. carpinifolia* by using MVSP program. Each population was represented as 10 individuals. D: Duzce–Yığılca, K: Kastamonu–Sehdag, S: Sinop–Ayancık, F: Antalya–Finike, AK: Antalya–Akseki, N: Nigde–Horoz, A: Adana–Saimbeyli, I: Erzurum–İspir; AR: Artvin–Hatilla.

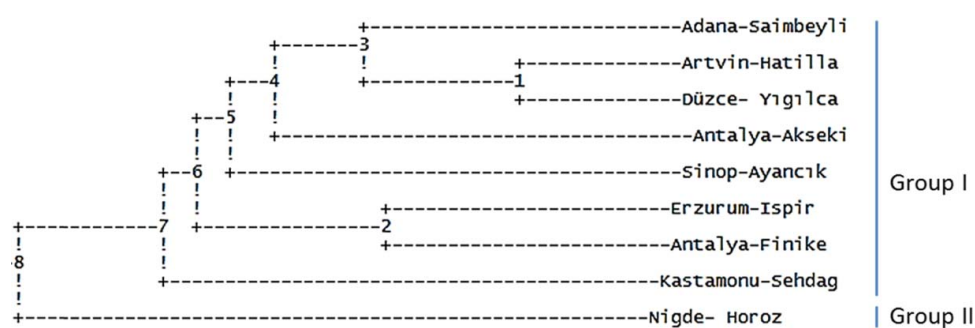


Figure 5. Genetic distance dendrogram of *O. carpinifolia* populations using UPGMA clustering method. The tree was constructed by using POPGENE v. 1.32.

grouped together in group B, while Antalya–Finike and Erzurum–Ispir populations were in group E. However, some genotypes of Antalya–Finike population (F2, 6, 8 and 10) and I9 genotype from Erzurum–Ispir population were isolated from all groups.

Genetic cluster analysis was performed using UPGMA method (Figure 5). Analysis demonstrated two major groups namely as group I and II. Group I included eight populations such as Adana–Saimbeyli, Artvin–Hatilla, Duzce–Yigilca, Antalya–Akseki, Sinop–Ayancik, Erzurum–Ispir, Antalya–Finike and Kastamonu–Sehdag, while group II only contained Nigde–Horoz population. This showed that genetic similarity data were in agreement with principal coordinate analysis. In addition, Artvin–Hatilla & Duzce–Yigilca and Erzurum–Ispir & Antalya–Finike populations were identified as most similar populations, while Nigde–Horoz was the most diverged population.

Conclusion

Distribution of genetic diversity within and among populations is a crucial factor to be taken into consideration in conservation efforts, particularly in case of *in situ* conservation. Molecular methods have become an important part of genetic diversity studies.[52] Knowledge obtained from genetic diversity analyses could be used in managing and conservation of both *in-situ* and *ex situ* germplasms.[41] Thus, genetic structure and diversity analyses in *O. carpinifolia* populations could provide valuable information for management, conservation and utilization of *in situ* and *ex situ* *Ostrya* germplasms. In this study, total variation among populations was found as 69.94%, however Duzce–Yigilca and Antalya–Finike populations showed high percentage of polymorphic loci (P%) and gene diversity (*h*). Therefore, these two populations should be subject to major conservation with special attention. However, all natural populations are recommended to be considered as a distinct management unit to provide germplasm resource for large-scale development of plantations in future. Also, *ex situ* conservation strategies such as seed collection, clonal multiplication, etc. should be separately developed.

Disclosure statement

No potential conflict of interest was reported by the authors.

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