Genetic diversity structure of Salix Caprea L. populations from fragmented riparian habitats

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Abstract

Goat willow (*Salix caprea* L.) is an ecologically important, cold-tolerant pioneer species that spread from central Anatolia to the Black Sea regions in Türkiye. Completed and ongoing construction of hydroelectric plants, especially in northeastern Turkey, threaten the genetic resources of goat willow in the region due to habitat loss and fragmentation. Therefore, it is important to asses genetic diversity structure and magnitude of goat willow populations in the northeastern Black Sea Region for effective conservation of genetic resources of the species. To characterize the genetic diversity of *Salix caprea*, 180 trees were sampled and screened by using ten microsatellite markers for genotyping, consequently analysis were carried out to estimate population genetic diversity parameters of populations. The results revealed that both observed (Ho = 0.50) and expected (He = 0.54) genetic diversities were low, but observed genetic diversity was less than expected due fragmentation and reduction in population sizes. Genetic structure analysis of populations suggested the presence of two major groups. One included the populations of Trabzon provinces and the other had the populations of the Artvin provinces. This structuring appeared to be caused by the restricted gene flow (polen, seed or vegetative material) through wind and water) due to geographical barrier (the east Black Sea mountain ranges) acting as geographic isolation mechanism between the populations of Trabzon and Artvin provinces. To prevent further deteroiation of genetic diversity due to habitat loss and fragmentation in the studied river systems in the region, it is suggested that effective gene conservation and management programs should be developed for the species by using the genetic information from the current study.

Introduction

Willows (*Salix* sp.) are important species of riparian ecosystems with the vital functions such as regulating water quality, controlling erosion and providing habitats for biodiversity. The species of *Salix* genus are also widely used for their economical functions which are land reclamation, phytoremediation, bioengineering, biomass production and agroforestry (Ball et al. 2005; Degirmenci et al. 2019). Among the willow species, *Salix caprea* (goat willow) is in the scope of researchers due to its great potential values for biomass production, reforestation, watershed protection, soil stabilization, phytoremediation and horticultural purposes (Enescu et al. 2016; Arıhan 2003; Norris et al. 2008; Varga et al. 2009; Neuner and Beiderbeck 1993).

Goat willow as a deciduous shrub (2–3 m in height) or tree (6–8 m in height) (Yücel et al. 1995) is mainly pollinated by insect and wind. Even though, vegetative reproduction is common for most willows species by hardwood cuttings, the species is not good rooters for vegetative reproduction (Liesebach and Naujocks 2004). These trees known as fast growing, and relatively short lived can grow in most soil types with a preference for damp soil. The species has a good ability to grow in forest understories in hedgerows, on rocky lake shores and variety of climatic conditions (Perdereau et al. 2014; Clinovschi 2005). However, it is susceptible to constant flooding and saturated soils with water (Neuner and Beiderbeck 1993). Goat willow has natural distribution ranging from Southern Norway to Eastern Spain in Europe and in temperate region of Asia and Syria (EUFORGEN 2023). It is a cold-tolerant pioneer species which is naturally distributed in the Black Sea Region of Türkiye, especially along with the Coruh river banks. Also, the species could be naturally found in suitable habitats in central Anatolia and Mediterranean Sea Regions of Türkiye (Avcı 2012).

Goat willow is highly suitable to produced biomass for bioenergy and biofuel industries (Hanley and Karp, 2013) and restore the disturbed riparian habitats, considering its high adaptive potential and its capacity to absorb and accumulate heavy metals (Varga et al., 2009). Furthermore, it is used as a soil retainer in places where there are steep drops with erosion risk due to its ability to root easily and spread very widely. Regarding the potential use of the species in both biomass production and riparian habitat restoration, determination of the magnitude and structure of genetic diversity in genetic resources of the species has gained importance in recent years. However, there are few studies carried out on goat willow to date to detect and mainly focused on phylogeography of the species with cpDNA data (Palme et al. 2003) and gene flow (Perdereau et al. 2014) in natural populations.

The habitats of goat willow in the Black sea regions of Türkiye have been fragmented or lost with constructions of dams and hydroelectrical plants in recent years (Küçükbaşol et al. 2016). It is known that there will be additional hydroelectric dams and river-type hydroelectric power plants constructions on the Coruh river system (Enerji Atlası 2023). The increased construction activities on the river systems of northeastern Türkiye will cause to further fragmentation and loss of the suitable habitats of goat willow as well as the river flow dynamics in the region. The impacts of habiat fragmentation and loss on magnitude genetic diversity and its patterns have been investigated in different *Salix* species. The implications of the results have been discussed with respect to conservation and restoration of natural populations in these studies (Kikuchi et al. 2011; Sochor et al. 2013; Singh et al. 2014; Degirmenci et al. 2019, Değirmenci et al. 2022). Even though, natural habitats of the species in its natural range have been degraded dramatically in Türkiye, there is no study to date, dealing with the genetic structure of goat willow populations in these highly disturbed habitats.

In the current study, the genetic diversity structure and extent of gene flow in fragmented goat willow populations from northeastern Türkiye were extensively studied by using nuclear SSR markers. The results of the study has provided invaluable information on population genetic structure of these populations which could be used to protect and restore the remaining genetic resources of the species in its fragmented habitats. The reported genetic data could be invaluable for the implementation of effective conservation, management and utilization of genetic resources of the species by responsible institutions.

Materials and Methods

Plant material and DNA extraction

One hundred and eighty-eight goat willow trees were sampled from ten populations in the eastern Black Sea Region of Türkiye with a restriction of at least 200 meters apart between trees. The sampled populations were spread over three provinces (Artvin, Giresun, Trabzon) and several rivers along the major Çoruh river system in the eastern Black Sea Region. In the Artvin province, sampled trees were around the tributaries of the Coruh River. In Trabzon and Giresun provinces, trees were sampled close to small rivers which originates from the Eastern Black Sea mountains and flow into the Black Sea. Considering the distribution range of the species in the country, sampling locations represent the major distribution area of goat willow. Detailed information about sampled populations was provided in Fig. 1 and Table 1. Fresh leaves of goat willow trees were collected and put in silica gels until they were brought to lab for DNA extraction. Dried leaves in the silica gels were crushed by using mortar and pestle with liquid nitrogen and stored at -80 ^{o}C till DNA extraction. A modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle 1990) was used to isolate nuclear DNA. After isolation, DNA pellets were dried by using a dessicator and dissolved in 75 mL TE buffer. The concentration of isolated DNA was determined with the Nanodrop Spectrophotometer (Thermo Scientific, Wilmington, USA). DNA quality was determined by checking the 260:280 OD ratios and by controlling whether it suits as a template in PCR (Polymerase Chain Reaction) with selected SSR primers.

Table 1
Information on the populations' location, code, and size

Location	Code of populations	Number of samples in populations	Latitude of population	Longitude of population	Altitude of population (average) (meter)
Eynesil (Giresun)	EYN	17	41.04562 N 40.98463 N	39.14716 E 39.11444 E	131–911 (679)
Kafkasor (Artvin/ Çoruh)	KAF	8	41.17306 N 41.16722 N	41.81250 E 41.80528 E	861–985 (917)
Surmene (Trabzon)	SU	18	40.92421 N 40.90783 N	40.22490 E 40.21444 E	66–352 (202)
Besikduzu (Trabzon)	BES	27	41.04498 N 41.00217 N	39.29028 E 39.22724 E	35–328 (207)
Koprubasi(Trabzon)	KPB	15	40.80633 N 40.8016 N	40.14268 E 40.12343 E	322–457 (374)
Borcka (Artvin/ Çoruh)	BOR	23	41.37300 N 41.32505 N	41.83321 E 41.73330 E	357–1391 (962)
Sacinka (Artvin/ Çoruh)	SAC	15	41.20182 N 41.18721 N	41.91773 E 41.89313 E	1187–1721 (1412)
Yusufeli (Artvin/ Çoruh)	YUS	16	41.02504 N 40.88095 N	41.43821 E 41.34472 E	1124–1506 (1348)
Iskenderli (Trabzon)	ISK	23	40.93923 N 40.92958 N	39.25489 E 39.24319 E	726–1005 (850)
Hatila (Artvin/ Çoruh)	HAT	26	41.14600 N 41.11942 N	41.68290 E 41.60625 E	840–1853 (1405)

Selection of microsatellite loci and amplification

Ten microsatellite DNA loci developed for different *Salix* and *Populus* species were identified and used to investigate the magnitude and structure of genetic diversity in goat willow populations. The selected primers named as Karp_W293, SB196 and SB24 from Barker et al. (2003), WPMS12 from Van Der Schoot et al. (2000), gSIMCT052, and gSIMCT24 from Stamati et al. (2003), WPMS18, WPMS15 and WPMS14 from Smulders et al. (2001), and Sare04 from Lian et al. (2001) were synthesized by the SACEM

Company with different fluorescent dyes (FAM, HEX, and TAMR) to recognize PCR products during fragment analysis. The volume of 20μL PCR amplification mixture included 10X PCR buffer, $MgCl_2$ (25 mM), dNTP mixture (10 mM), each primer pair (10 mM) and template DNA (10 ng). To amplify the microsatellite regions, the PCR cycle conditions were optimized according to Van Der Schoot et al. (2000), Smulders et al. (2001), Barker et al. (2003), and Lian et al. (2001), depending on the choice of SSR primers. During the PCR cyles, a hot start procedure was applied to avoid a non-specific amplification of DNA by inactivating the *Taq* polymerase at lower temperatures by using Eppendorf thermocycler (Eppendorf-Mastercycler, Eppendorf, Canada). The fragment analysis of the samples were performed by using the Applied Biosystems 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) with the internal standard size marker (The GeneScan 400HD ROX dye). For this analysis, collaboration with a biotech company (BM Laboratory, Çankaya, Ankara) was made. The allele determination after fragment analysis was carried out by using Peak Scanner Software v2.0 (Applied Biosystems Inc., Foster City, CA).

Analysis of microsatellite data

Firstly, due to vegetative propagation habits, the studied samples were checked with GenClone 2.0 software to detect the occurrence of duplicated genotypes (Arnaud-Haond et al. 2007). The presence of null allele was checked for ten loci by using GENEPOP 4.2 software (Raymond and Rousset 1995). The standard parameters of genetic variability; such as number of different alleles (Na), number of effective alleles (Ne), the proportion of polymorphic loci (P), observed heterozygosity (Ho), expected heterozygosity (He) were estimated by use of the GenAlEx 6.5 software (Peakall and Smause 2012). Moreover, F indices and number of migrant (Nm), which are also used to define the distribution of genetic variation were calculated by the GenAlEx software. To detect the possibility of deviation from Hardy-Weinberg (HW) equilibrium for each locus, exact tests were performed with the ARLEQUIN 3.5.1.2 using a Markov chain permutation (Excoffier and Lischer 2010). The genetic differentation across populations were determined by a Principal Coordinate Analysis (PCoA) based on the pairwise F_{ST} values in GENALEX (Supplementary Table 1).

The genetic structure of the ten *S.caprea* populations was investigated with the STRUCTURE 2.3.4 program (Pritchard et al. 2000), using an admixture model with correlated allele frequencies. Ten runs were performed for each K value set from one to ten with the 50000 burn-in period and the 150000 MCMC chains to determine subgroups that have distinctive allele frequencies. The optimal value of K was determined by examination of the ΔK statistic using the STRUCTURE HARVESTER software (Earl Dent and Vonholdt Bridgett 2012), calculated according to Evanno et al. (2005). The average cluster membership coefficient matrices were estimated by the CLUMPP software (Jakobsson and Rosenberg 2007). The output data of the CLUMPP was used by the POPHELPER software (Francis 2016) to display the population structure graphically. With the use of pairwise F_{ST} values of populations, a principal coordinate analysis was carried out by applying covariance and standardized option of the GenAlEx (Peakall and Smause 2012). Analyses of molecular variance (AMOVA) were carried out with the ARLEQUIN. In the partitioning of total genetic variation between river and within the river systems, the number of different alleles (F_{ST}) based on the infinite allele model was used by considering structure grouping of the studied populations. To examine a possible recent bottleneck event causing a reduction in effective population size, the Garza-Williamson Index (Garza and Williamson 2001) was estimated for each population by the ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010). To detect the presence or absence of genetic barriers among the studied populations, the Monmonier algorithm of the R-package *adegenet* (Jombart 2008) was applied with some point coordinates (from a point layer) and a matrix showing the genetic distance between points.

Results

The ten SSR markers used in the study were found to be useful for detecting polymorphism in 180 goat willow trees representin 10 populations with a total of 89 alleles. The results of clonal duplication analysis indicated that there were no clonally duplicated trees among the sampled trees. When goat willow populations were checked in terms of null alleles, the SARE04 locus was only found to have high null allele frequency without changing the result of genetic analysis.

The observed number of alleles for ten SSR loci was ranged from four in the loci WPMS12-WPMS15 to 18 in the locus Karp_W293 with an average of 8.9. Similarly, the highest and lowest effective allele number were found in Karp_W293 (6.00) and WPMS12 (1.15) loci, respectively with an average of 2.84 (Table 2).

Table 2
Descriptive statistics of ten loci for *S. caprea* populations in North-Eastern Türkiye

Loci	N	Na	Ne	Ho	He	F_{IS}	F_{IT}	F_{ST}	*Nm*
WPMS14	18.00 ± 1.83	5.20 ± 0.35	3.23 ± 0.20	0.60 ± 0.05	0.67 ± 0.02	0.116	0.175	0.07	3.53
WPMS15	18.00 ± 1.83	2.20 ± 0.20	2.01 ± 0.01	1.00 ± 0.00	0.50 ± 0.01	-0.984	-0.983	0.00	NE*
SB24	18.00 ± 1.83	5.70 ± 0.42	2.80 ± 0.23	0.68 ± 0.04	0.62 ± 0.03	-0.102	-0.065	0.03	7.11
SB196	17.80 ± 1.96	3.90 ± 0.27	1.77 ± 0.13	0.46 ± 0.06	0.40 ± 0.05	-0.157	-0.08	0.07	3.49
SARE04	18.00 ± 1.83	6.70 ± 0.42	4.19 ± 0.30	0.54 ± 0.04	0.75 ± 0.01	0.271	0.328	0.08	2.95
WPMS12	18.00 ± 1.83	2.00 ± 0.25	1.14 ± 0.03	0.08 ± 0.02	0.11 ± 0.02	0.315	0.346	0.05	5.28
WPMS18	17.80 ± 1.89	2.70 ± 0.26	1.57 ± 0.15	0.19 ± 0.03	0.32 ± 0.04	0.411	0.514	0.18	1.17
GSLMCT052	18.00 ± 1.83	4.20 ± 0.64	2.13 ± 0.36	0.25 ± 0.05	0.43 ± 0.06	0.407	0.489	0.14	1.57
GSIMCT24	18.00 ± 1.83	5.50 ± 0.34	3.57 ± 0.23	0.55 ± 0.03	0.70 ± 0.02	0.21	0.272	0.08	2.93
KARP_W293	17.90 ± 1.83	9.60 ± 0.65	5.98 ± 0.36	0.64 ± 0.03	0.82 ± 0.01	0.225	0.281	0.07	3.23
Total Mean	17.950 ± 0.55	4.77 ± 025	2.84 ± 0.15	0.50 ± 0.02	0.53 ± 0.02	0.07	0.12	0.08	3.13

N = Sample size, Na = Number of different alleles, Ne = Number of effective alleles, He = Expected heterozygosity, Ho = Observed heterozygosity, F_{IS} = Fixation index, $F_{IT=}$, $F_{ST=}$ Population differentiation, *Nm* = Number of migrant, * NE = not estimated due to formula Nm = $[(1/F_{ST}) - 1]/4$ since all genotypes for this locus were heterozygous (Peakall and Smause 2012)

The observed heterozygosity value for a single locus was ranged from 0.08 (WPMS12) to 1.00 (WPMS15) with an average of 0.50. Most of the studied loci indicated lower observed heterozygosity values than expected with positive inbreeding coefficient (F_{IS}) values. Only the WPMS15, SB24, and SB196 loci had negative F_{IS} values. The mean value of the F_{IS} was 0.07, indicating that heterozygotes were 7% lower than expected (Table 2). The SB24 and Karp-W293 loci made great contributions to the estimated genetic diversity (Ho: 0.68, He:0.62; Ho: 0.64, He: 0.82, respectively). When all samples were analyzed together, all loci were generally deviated from the HWE due to a significant heterozygous deficiency. The WPMS18 and GSIMCT052 loci with the highest F_{ST} values (0.18 and 0.14, respectively) had great contributions to the differentiation of goat willow populations (Table 2). All studied trees had the same heterozygote genotype for the WPMS15 locus so F_{ST} value was calculated as zero for this locus.

The mean number of observed alleles for the studied populations was 4.77 and ranged from 3.50 (Kafkasor) to 5.6 (Sacinka). Similarly, Kafkasor (2.50) had the lowest mean number of effective alleles while the Sacinka population was with the highest value (3.40) with an average of 2.84. For the studied populations of goat willow in North-Eastern Türkiye, various number of unique (private) alleles were detected. There were 3 unique allles in Eynesil and Yusufeli while 2 unique alleles were present in Surmene, Besikduzu, Sacinka, and Iskenderli populations (Table 3). The observed heterozygosities were generally low and ranged from 0.45 (Koprubasi) to 0.57 (Kafkasor) whereas expected heterozygosity ranged from 0.45 in Surmene to 0.61 in Sacinka population. The average fixation index value was positive (F_{IS}= 0.05) which indicates a sign of inbreeding and presence of excess of homozygote individuals. The mean F_{ST} values were found to be 0.08 showing a moderate level of differentiation among populations. The Garza-Williamson index values for all populations were found to be higher than the critical value of 0.68, indicating no past reduction in effective population sizes of the species (Supplementary Fig. 1).

Table 3
Descriptive population genetic diversity parameters for *S. caprea* populations in North-Eastern Türkiye

Pop	N	%P	Na	Ne	Pa	Ho	He	F_{IS}	F_{ST}
Eynesil	17	90.00%	4.50 ± 0.68	2.80 ± 0.33	3	0.48 ± 0.09	0.57 ± 0.07	0.12 ± 0.15	0.08
Kafkasor	8	90.00%	3.50 ± 0.60	2.50 ± 0.42		0.56 ± 0.11	0.49 ± 0.07	-0.09 ± 0.16	
Surmene	18	90.00%	4.400 ± 0.89	2.56 ± 0.52	2	0.46 ± 0.10	0.44 ± 0.09	-0.06 ± 0.12	
Besikduzu	27	100.00%	5.50 ± 0.82	2.87 ± 0.49	2	0.51 ± 0.08	0.55 ± 0.06	0.060 ± 0.13	
Koprubasi	13	100.00%	3.90 ± 0.60	2.68 ± 0.50		0.44 ± 0.09	0.49 ± 0.08	0.05 ± 0.15	
Borcka	21	100.00%	4.90 ± 0.99	2.95 ± 0.60		0.49 ± 0.10	0.53 ± 0.08	0.12 ± 0.18	
Sacinka	15	100.00%	5.60 ± 0.99	3.40 ± 0.60	2	0.54 ± 0.10	0.61 ± 0.06	0.14 ± 0.17	
Yusufeli	14	100.00%	5.20 ± 0.75	2.83 ± 0.43	3	0.52 ± 0.07	0.56 ± 0.06	0.04 ± 0.14	
Iskenderli	23	100.00%	5.50 ± 0.84	3.18 ± 0.66	2	0.52 ± 0.07	0.56 ± 0.07	0.01 ± 0.11	
Hatila	24	100.00%	4.70 ± 0.79	2.64 ± 0.42		0.47 ± 0.08	0.53 ± 0.06	0.11 ± 0.14	
Mean	17.950	97.00%	4.77 ± 0.25	2.84 ± 0.15		0.50 ± 0.02	0.53 ± 0.02	0.05 ± 0.04	

N = Sample size, %P = Proportion of polymorphic loci, Na = Number of different alleles, Ne = Number of effective alleles, Ho = Observed heterozygosity, He = Expected heterozygosity, F_{IS} = Fixation index, F_{ST} = Genetic differentation

The genetic structure analysis detected the presence of three main groups ($\Delta K = 3$, Supplementary Fig. 2). The populations were divided into three colored sections which correspond to the membership coefficients in the subgroups. The first and second clusters included populations from the east and west parts of the Trabzon province. The populations sampled from the Artvin province formed the distinct, the third cluster with a high admixture value (Fig. 2).

The results of PcoA were found to be very similar to the STRUCTURE results. The populations from the Artvin province were placed in the first cluster, while populations from Trabzon province formed the second cluster which included the Kafkasor population from the Artvin province. The Eynesil and Koprubasi populations were also located distantly from Besikduzu, Surmene and Iskenderli populations which were closely located in the study region (Fig. 3). Barrier detection analysis revealed that the existence of one possible genetic/geographic barrier between populations of the Trabzon and Artvin provinces (Fig. 4)

To understand the partition of total genetic diversity between and within river systems, an AMOVA was conducted. The variation attributed to among rivers systems was only 0.14% of the total variation (p = 0.000) while 5.41% of the total variation (p = 0.000) was attributed to differences among populations within the river systems. Of the total genetic variance, 94.45% was explained significantly by the differences among individual trees within populations (Table 4).

Table 4
Analyses of molecular variance (AMOVA) based on ten SSR loci

Source of variation	**Sum of squares**	**Variance components**	**Percentage of total variation**
Among rivers	18,336	0.00416 Va	0,14
Among populations within rivers	58,612	0.15901 Vb	5,41
Within Populations	972,119	2.77748 Vc	94,45
Total	1049,067	2,94066	100
Fixation index	F_{ST}=0.55		

Discussion

The determination of the level of genetic diversity in the fragmente populations of any species should aid to protect the genetic resources and to understand the population genetics of the species. In this study, 180 *S. caprea* trees were sampled from ten natural populations from the fragmented habitats to detect the genetic diversity and population structure of them in Türkiye for the first time.

Among the studied SSR markers, SARE04, SB24, gSIMCT052, gSIMCT24, and KARP_W293 markers which were developed for *S. alba/excelsa* species as well as WPMS14 locus developed for *P. nigra* species were found to be highly informative that could be used in future genetic diversity studies dealing with goat willow. A moderate level of genetic diversity was found in the studied goat willow populations of North Eastern Türkiye (Ho = 0.50, He = 0.53). Previous study by Perdereau et al. (2014) for Irish populations of goat willow reported the lower genetic diversity values (Ho = 0.41 and He = 0.49) than those found in the current study. However, both studies found lower observed heterozygosity than expected heterozygosity, indicating excess of homozygote trees in goat willow populations. In the current study, only Kafkasor and Surmene populations had higher observed heterozygosity values than the expected. The occurrence of a slight excess of homozygotes for many populations could result from inbreeding within local fragmented populations. Positive inbreeding coefficients found in goat willow could be attributed to small effective population size due to habitat fragmentation, in turn, fragmented populations. The fragmentation and isolation of populations are likely to limit genetic exchange among them. Due to small effective populaion sizes, genetic drift might also cause to an increment of homozygosity (Allendorf and Luikart 2007; Perdereau et al. 2014).

Studied populations in the current study showed a moderate level of differentiation (F_{ST} = 0.08). The differentiation among populations of goat willow indicate a permanent gene flow among populations with possibly cross-pollination, especially by the wind. Perdereau et al. (2014) reported F_{ST} value as 0.16 for 183 goat willow trees, while Puschenreiter et al. (2010) reported F_{ST} as 0.014 for the metallicolous and non-metallicolous goat willow populations based on phenotypic characteristics and nuclear microsatellite (SSR) markers. The difference in F_{ST} values for each study might be caused by the virtue of sampling locations and the use of microsatellite markers with different polymorphism levels.

Genetic structure analysis revealed the presence of three genetically separated groups according to their sampled locations. The first cluster included Eynesil, Besikduzu, and Iskenderli populations, sampled from east of the Trabzon province, while the second cluster included Surmene and Koprubasi populations, sampled from west of the Trabzon province.The Borcka, Yusufeli, Hatila, Sacinka, and Kafkasor populations which were sampled from the Çoruh river basin in the Artvin province were placed into the third cluster. A clear spatial separation was not observed among these three clusters since a moderate level of admixture was present. According to the Principal Coordinate Analysis, there appear to be two main groups; one group consisted of populations sampled from the the Çoruh river basin in the Artvin province and the other group consisted of populations sampled from the Trabzon province except for the Kafkasor population. Even though genetically close populations were also found geographically close, the Kafkasor population consisted of only eight samples was not properly grouped with other populations sampled from the Çoruh river basin. Another possibility is the human assited migration of goat willow vegetative materials to the Kafkasor population from the other populations of the Trabzon province because the Kafkasor is located where the Annual Kafkasor Bull Fighting takes place. From these results, it is clear that populations from the same river share a similar genetic structure as a result of extensive gene flow within the river system. Both the results from the genetic structure and PCoA suggest that there is a clear distinctions between the populations of the Artvin and Trabzon provinces due to restricted gene flow. The result was also supported by the barrier detection analysis indicated that there was one possible barrier, the East Black Sea mountain range, between populations of the Trabzon and Artvin provinces shaping the spatial genetic structure of goat willow populations by hindering gene flow. Even though gene flow was effective along the river banks, it was not to be so effective to overcome the East Black Sea mountain range between the Trabzon and Artvin provinces. This geographical barrier appears to be acting as geographic isolation mechanism by preventing gene flow (polen, seed or vegetative material) through wind and water.

Goat willow distribution in northeastern Türkiye is generally along the riverside. The studied populations were sampled from along the Coruh and Manahoz rivers and the valleys around those rivers. The natural habitats of goat willow were disturbed frequently by alpine tourism activities. In addition to constructed many hydroelectric plants on these two rivers. There are additional planned hydroelectric dams and river-type hydroelectric power plants constructions on the Coruh river system (Enerji Atlası 2023; Küçükbaşol et al. 2016). Therefore, goat willow genetic resources in the area are greatly threatened to lose present genetic diversity by additional habitat fragmentation and losses as well as local climate change imposed by the dam lakes (Gyau-Boakye, 2001). Although goat willow has not been included yet in the IUCN Red List of Threatened Species and defined as least concern in the “The Vascular Plant Red Data List for Great Britain” study (Cheffings & Farrell 2005), but it is obvious that genetic resources of the species are in danger in the northeastern Türkiye as a result of continued loss or fragmentation of the natural populations. Considering the level of habitat fragmentation and the results of the current study on genetic structure of goat willow populations, a conservation program should be planned to protect genetic resources of goat willow and to restore remaining natural populations in northeastern Türkiye.

Declarations

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Figures

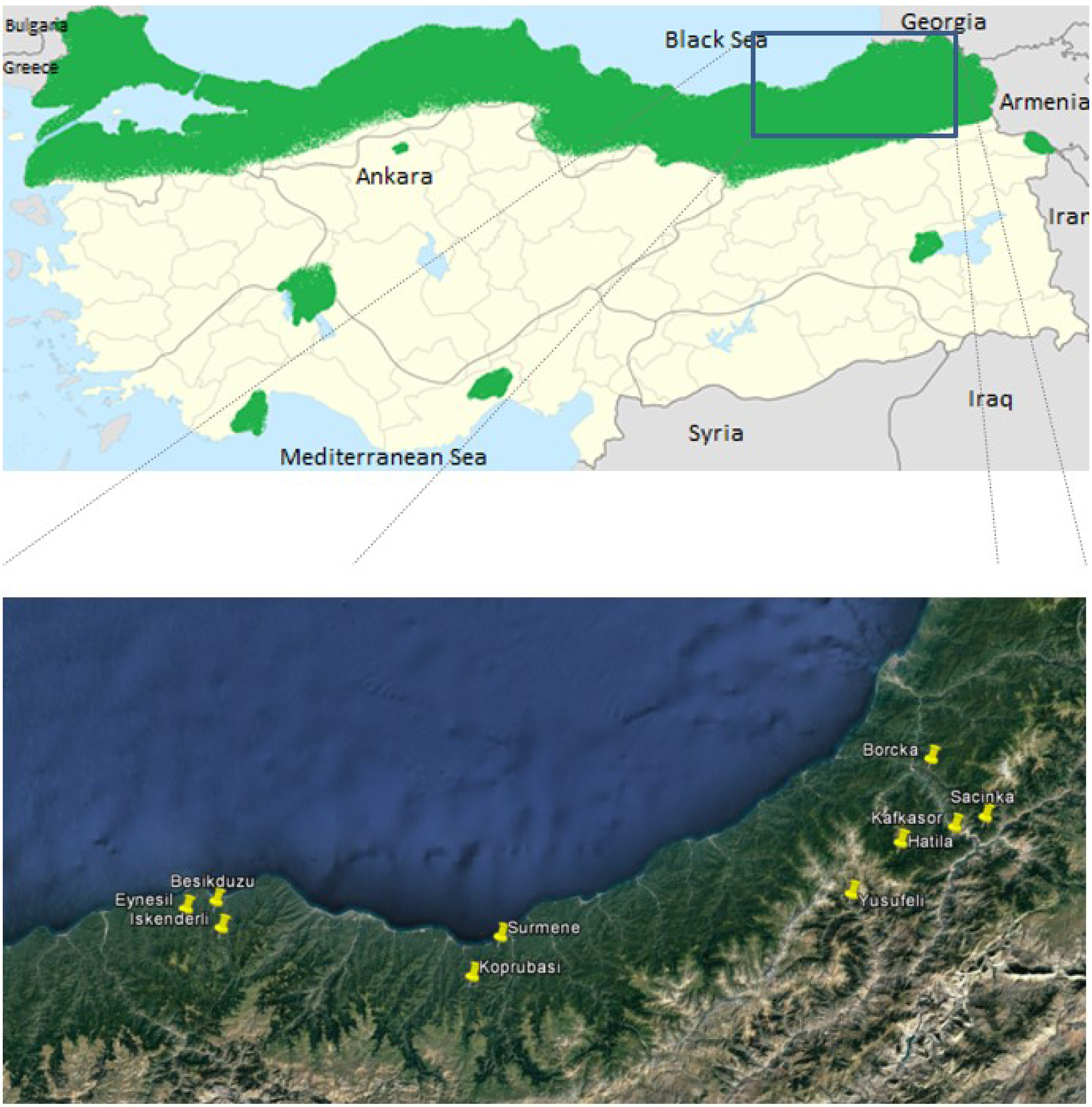

Figure 1

Natural distribution of *S. caprea*in Türkiye and locations of sampled populations

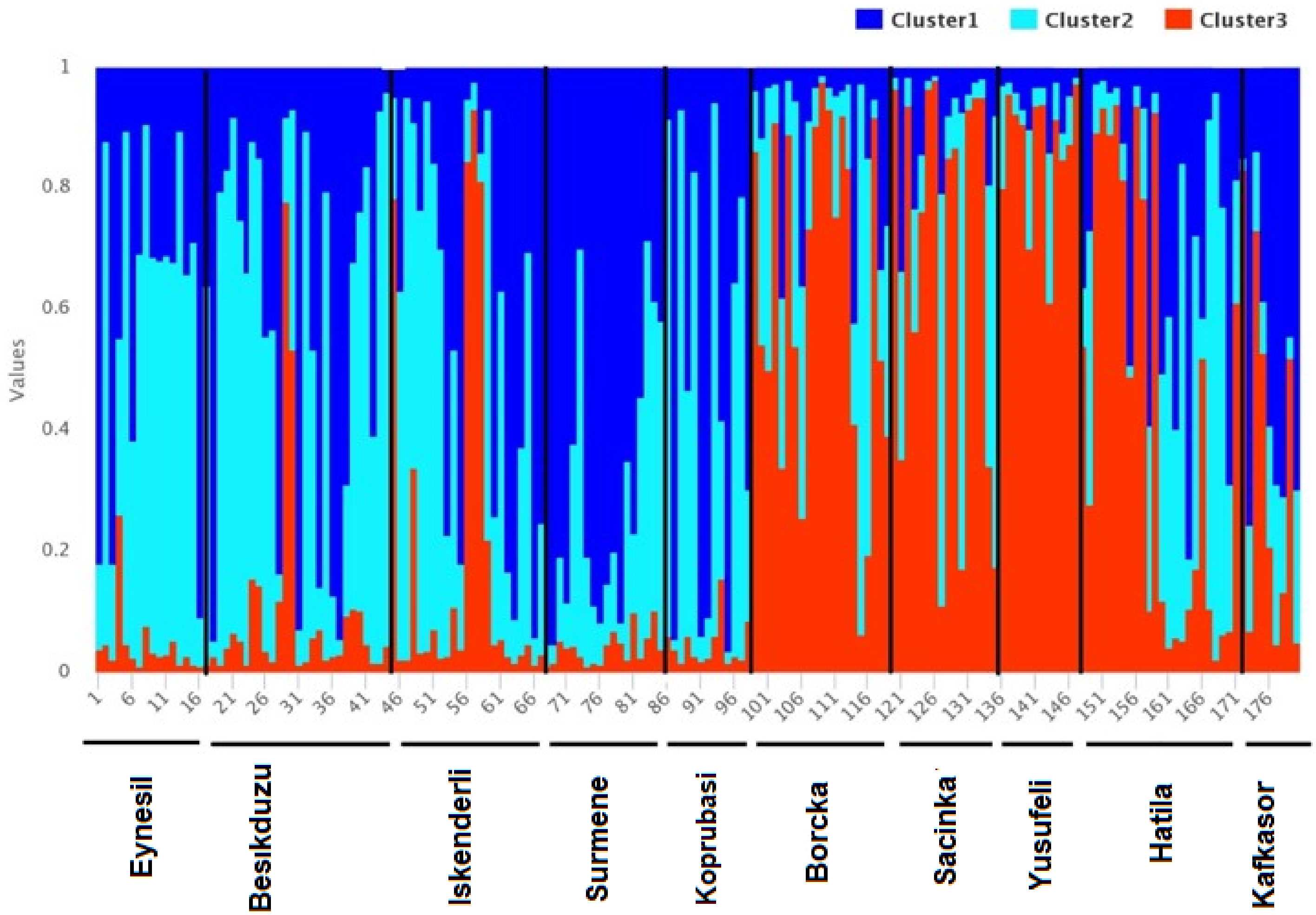

Figure 2

STRUCTURE grouping for 180 *S. caprea* genotypes from ten populations according to nuclear SSR markers in Eastern Black Sea Region. Blue, turquoise, and red colors present clusters 1, 2, 3, respectively

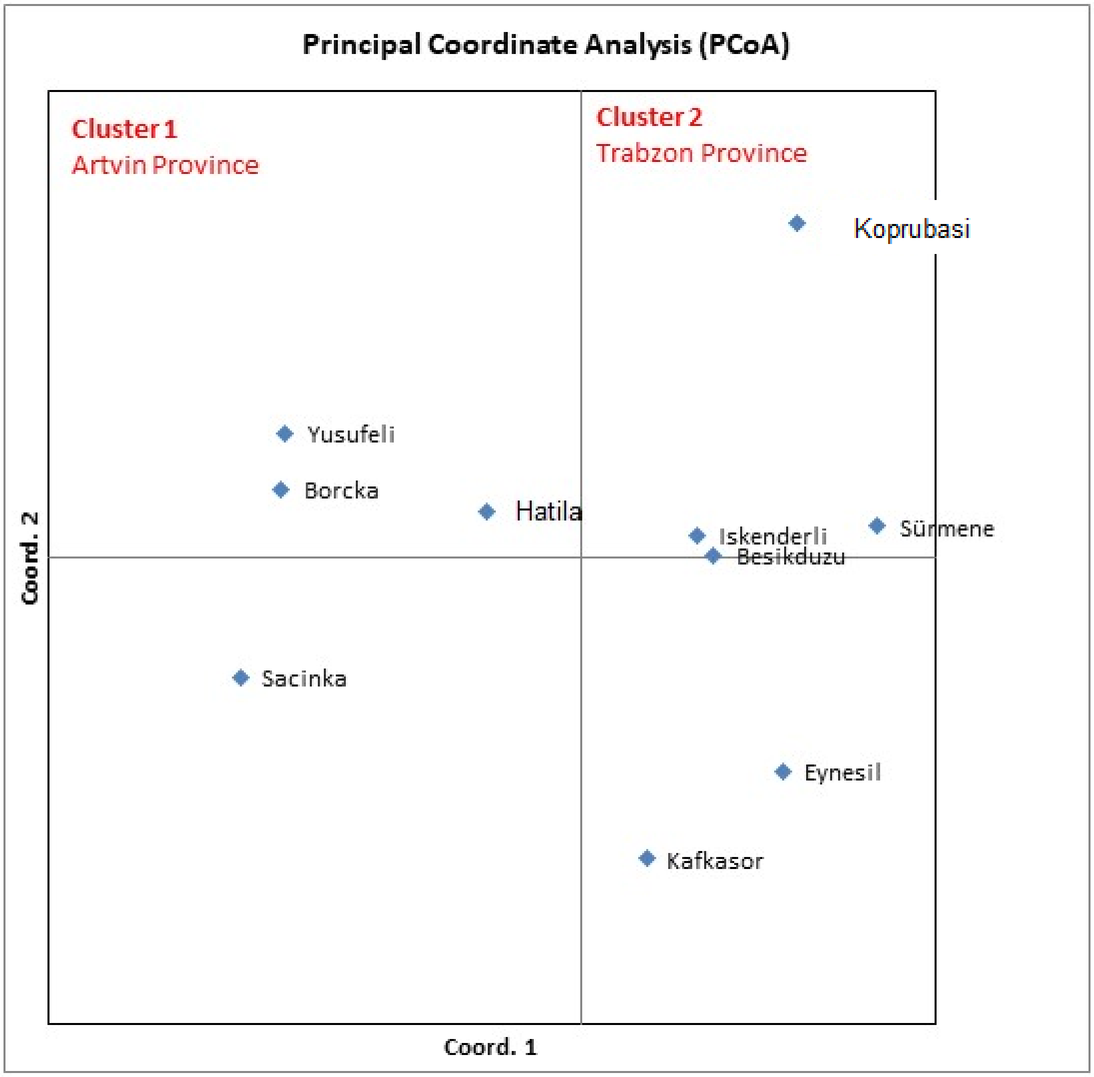

Figure 3

Principal coordinate Analysis (PcoA) for ten populations of *S. caprea*

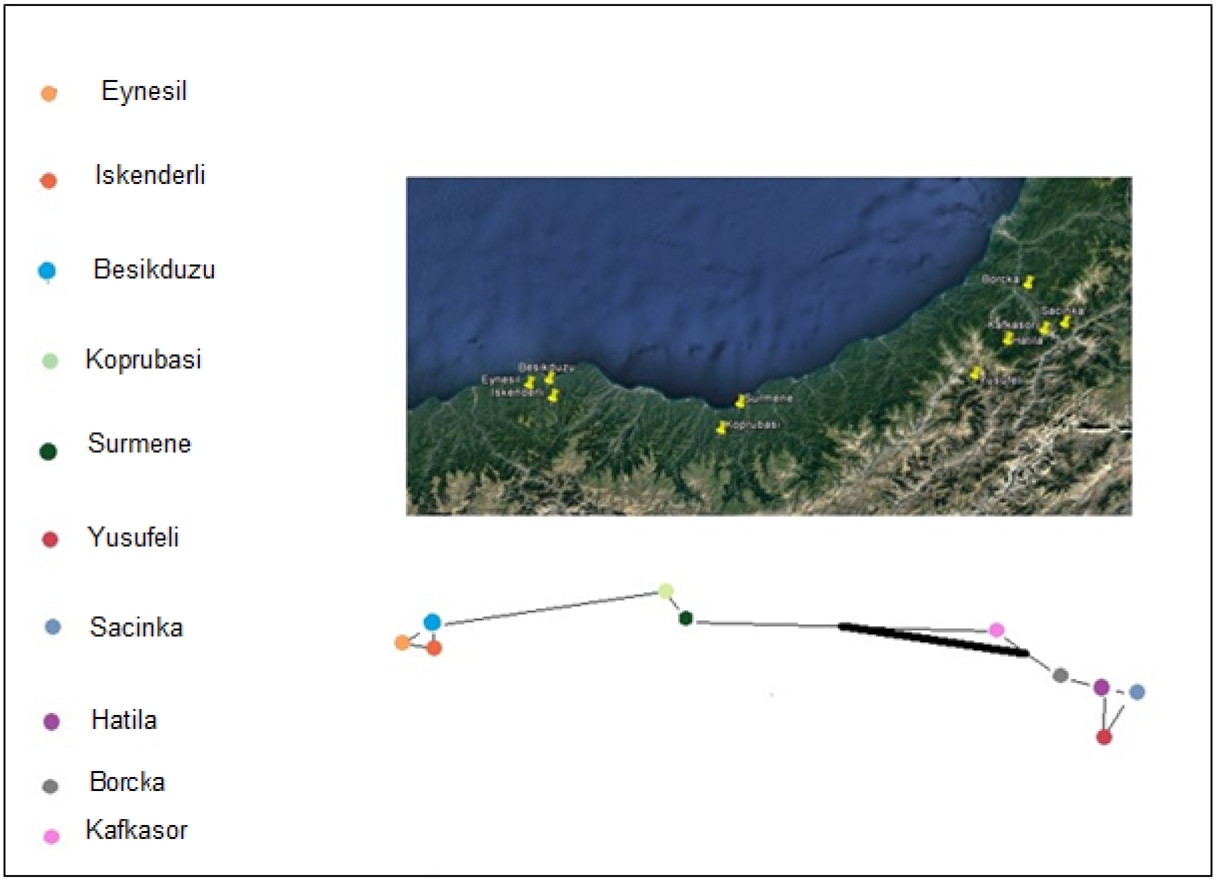

Figure 4

Barrier detection analysis indicated the occurrence of one possible barrier between Artvin and Trabzon provinces

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