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Original article

Assessment of the Genetic Structure and Diversity of *Orobanche cumana* populations from Turkey Using Simple Sequence Repeat Markers

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Abstract

Orobanche cumana Wallr. known as sunflower broomrape is a holoparasitic plant that causes huge yield losses in sunflower (*Helianthus annuus* L.) fields. Genetic characterization, genetic diversity, and race determination studies in O. cumana are very significant for preventing threats in sunflower fields. In this study, the broomrape populations sampled from Edirne, Kırklareli, Tekirdağ, and Adana provinces were used for genetic characterization. The sensitive Özdemirbey sunflower variety was used for growing O. cumana individuals. Eight simple sequence repeat (SSR) loci (Ocum52, Ocum70, Ocum81, Ocum87, Ocum108, Ocum141, Ocum160, and Ocum196) were used for the evaluation of genetic characterization and diversity of broomrape populations. All studied SSR loci were found to be polymorphic and yielded a total of 22 alleles in 143 samples analyzed. N_a = 2.089 (mean number of alleles per locus), N_e = 1.390 (mean effective alleles), I = 0.392 (mean Shannon's information index), H_o = 0.156 (mean observed heterozygosity), He = 0.239 (mean expected heterozygosity), and PIC = 0.228 (mean polymorphic information content) were calculated to assess genetic diversity of *O. cumana* populations.. As a result of molecular variance analysis, it was concluded that found that the genetic diversity of the populations was 38% among the population. The remaining 23% and 39% were due to among individuals and within individuals, respectively. The UPGMA method and STRUCTURE analysis divided the studied populations into 2 groups. Cluster I included LK2013, HT2016, T2018, and LE2013 populations, while group 2 included AE2003, AD2018, and MT2013 populations. The results we obtained have enabled us to reach important genetic diversity information about O. cumana, and the information obtained will provide important contributions for planned studies in the future.

Keywords: Genetic Diversity, *Helianthus annuus, Orobanche cumana*, SSR, STRUCTURE, UPGMA.

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INTRODUCTION

The angiosperm, obligate and non-photosynthetic root parasite Orobanche is one of the main threatening factors for economically significant crop production, especially from Europe to Asia. The genus Orobanche is the genus that includes the largest number of holoparasitic species in the Orobanchaceae family (Zare and Donmez, 2013). The most important agricultural pests belonging to Orobanche species in the world can be listed as; O. crenata, O. cernua, O. cumana, O. ramosa, and O. aegyptiaca (Parker, 2009). Asteraceae, Solanaceae, Fabaceae, Umbelliferae, and Cucurbitaceae family are particularly selected families as a host by these parasitic plants. The majority of broomrapes prefer the temperate and warm regions of the Northern Hemisphere, especially the Mediterranean region, as their habitat. Many countries such as Russia, Ukraine, Turkey, Spain, Serbia, and Romania have been reported severe crop yield and quality losses due to different Orobanche spp. (Kaya et al., 2009; Das et al., 2020).

Sunflower (Helianthus annuus L.) is an important oil plant grown in countries such as Ukraine, Russia, European countries, the USA, Argentina, India, and China (Prasad et al., 2017). Turkey is one of the major sunflower producers and more than 70% of our sunflower agricultural areas are located in the Thrace region. Infestation of sunflower plants is usually due to the cultivation of sunflowers at frequent intervals in the cultivation area, and to prevent this, resistant varieties are generally used (Evci et al., 2011). The main pest of sunflowers in Turkey is sunflower broomrape (O. cumana), which is common in all regions. O. cumana mainly parasitizes Artemisia spp. in the wild whereas it also parasitizes sunflower fields. The first record of sunflower parasitization by O. cumana was reported in Russia at the end of the 19th century (Skoric, 2012). Sunflower broomrape is an important parasitic plant that damages sunflower production from the past to the present. In the twentieth century, cultivars resistant to various broomrape species have been developed. From the beginning of the twenty-first century, sunflower cultivation areas began to expand. As a result, old crop practices were terminated. This has led to sudden changes in broomrape populations in countries such as Spain, Russia, Ukraine, and Turkey. Changes in broomrape populations in many countries over the past 15 years have resulted in the formation of new races (G and H) (Skoric et al., 2021). There are totally eight races of O. cumana and various countries commonly reported F, G, and H races (Kaya, 2014)

Although it has been reported that molecular marker studies on genetic diversity, population structure, racial identification, gene flow, and virulence genetic mechanisms for broomrape populations are not sufficient (Velasco et al., 2016); Recently, studies of molecular markers with highly informative markers have been increasing. Molecular markers are predominantly selected for genetic characterization studies due to having advantages like their abundance in number, unaffected characteristics by environmental factors, or the developmental stage of plants. Various types of DNA mutations such as deletions, insertions, point mutations, translocations, or errors in replication of tandem

repeat DNA motifs provided the development of diverse types of molecular markers (Gangapur et al., 2018). Molecular markers (dominant or codominant, single or multi-locus analysis) such as random amplified polymorphic DNA (RAPD) (Katzir et al., 1996; Paran et al, 1997; Gagne et al., 1998; Gagne et al., 2000; Roman et al., 2003; Atanasova et al., 2014; Molinero-Ruiz et al., 2014; Ivanovic et al., 2021), amplified fragment length polymorphism (AFLP) (Gagne et al., 2000), cpDNA diagnostic markers (Roman et al., 2007), inter simple sequence repeats (ISSR) (Benharrat et al, 2002), and simple sequence repeats (SSRs) (Pineda-Martos et al., 2013; Pineda-Martos et al., 2014a, 2014b; Guchetl et al., 2014a, 2014b; Martin-Sanz et al., 2016; Jebri et al., 2017a, 2017b; Duca et al., 2017; Ziadi et al., 2018; Bilgen et al., 2019; Duca et al., 2021) have been successfully used for genetic diversity, characterization, and race determination studies in O. cumana.

Simple Sequence Repeats (SSRs) as codominant DNA markers are widely distributed throughout eukaryotic genomes (Vieira et al., 2016; Bagshaw, 2017). They are widely preferred in plant genetic diversity, genetic characterization, or molecular phylogeny studies because of having advantageous characteristics like being highly informative, multi-allelic nature, high polymorphism level, reproducibility, and transferable between closely related species due to conserved flanking regions (Vieira et al., 2016; Kocaman et al., 2020). The aims of this study were to assess the genetic structure and diversity of seven O. cumana populations via eight SSR markers and to compare our results with SSR analysis of O. cumana populations from different countries.

MATERIALS and METHODS

Plant materials

Bulked seeds from seven *O. cumana* populations in the Edirne, Kırklareli, Tekirdağ, and Adana region were used in this study (Table 1). Bulked seeds were stored at +4ºC in our laboratory. *O. cumana* populations were propagated on the sensitive Özdemirbey sunflower variety. Multiplication and tissue collection of *O. cumana* populations were performed according to the study by Pineda-Martos et al. (2013). Adequate germination was observed in all populations included in the study. Fresh tissue samples belonging to each population (17 to 21 individuals/per population) were collected before flowering began and the sampled tissues were stored at - 20ºC till DNA isolation.

DNA Isolation

Firstly, each tissue sample was taken from - 20ºC, sliced into small pieces with a sterile lancet, and transferred to 2 ml centrifuge tubes. Then the ball mill (Retsch® MM400) was used for tissue grinding. The optimized Doyle and Doyle (1990) CTAB-based DNA extraction method was performed for genomic DNA isolation. The Nanodrop® LITE spectrophotometer was used to quantify and qualify the extracted DNA samples. The DNA samples were diluted as 50ng/µL and preserved at -20 °C for downstream SSR analysis.

Table 1. Information about studied *O. cumana* populations

Population Numbers	Abbreviation	Population Name	Collection Year	Number of Individuals
1	AE2003	Avarız/Edirne	2003	17
2	LK2013	Lüleburgaz/Kırklareli	2013	21
3	AD2018	Adana Region	2018	21
4	T2018	Tekirdağ Region	2018	21
5	MT2013	Muratlı/Tekirdağ	2013	21
6	LE2013	Sarıdanışment/Lalapaşa/Edirne	2013	21
7	HT2016	Hayrabolu/Tekirdağ	2016	21

SSR and Data analysis

PCR analysis of microsatellite primers was performed with the protocol given by Pineda-Martos et al. (2013) and the three-primer strategy of Schuelke (2000) (6-FAM™, NED™, PET™, or VIC™ fluorescent labeled M13 tail primer) was used. A total of 12 SSR loci were screened and eight polymorphic SSR loci (Ocum-52, Ocum-70, Ocum-81, Ocum-87, Ocum-108, Ocum-141, Ocum-160, and Ocum-196) were selected. Agarose gel electrophoresis (1.5% agarose, RedSafe Nucleic Acid Staining Solution, 1X TBE buffer, 90 V, 1 h 30 min) was used to control amplified PCR products for each SSR primer pair. 100 bp DNA ladder was used as a size standard and the amplified PCR bands were photographed in the UV transilluminator (Gel Imaging System Vilber Lourmat Quantum). Capillary electrophoresis system (3500 Genetic Analyzer, Applied Biosystems, Life Technologies, UK), The GeneScan™ 500 LIZ™ Size Standard and GeneMapper Software 5.0 (Applied Biosystems) were used to determine the amplified PCR product size.

In order to determine the genetic diversity and structure of the populations, percentage of polymorphic loci (P_{PL}), mean number of alleles per locus (N_a), effective number of alleles (N_e), the total number of private alleles observed (N_{pa}), Shannon's information index (I), expected (H_e) and observed (H_o) heterozygosity (Nei, 1987), Fixation index (F) were calculated with GenAlEx version 6.5 program (Peakall and Smouse, 2006). PIC (Polymorphic information contents) was calculated with the formula given in Botstein et al., (1980) for each SSR locus. The results of these statistical analyses were given in Tables 2, 3, and 4.

GenAlEx (Version 6.5) statistical program was used in the molecular analysis of variance (AMOVA) (Table 5). Nei's (1987) unbiased genetic distance and similarity coefficients were calculated to determine the level of genetic differentiation between populations (Nei, 1987) (Table 6). A dendrogram was created using Nei's unbiased genetic distance coefficient and UPGMA (Unweighted Pair-Group Method with Arithmetic Average) clustering method so that the results can be seen on a

visual graph (Sneath and Sokal, 1973). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0 program was used to create this dendrogram and the dendrogram was given in Figure 1 (Tamura et al., 2013). STRUCTURE Ver. 2.3.4 software (Pritchard et al., 2000) was used to perform the Bayesian-model-based cluster analysis for SSR data to determine gene pools of studied *O. cumana* populations (Figure 2) as described in the study by Elibol and Bilgen (2017).

RESULTS and DISCUSSION

The eight SSR markers generated 22 polymorphic alleles for the studied *O. cumana* populations (Table 2). The genetic diversity parameters of studied SSR loci, the observed alleles (N_o), Polymorphic information contents (PIC), expected (H_e) and observed (H_o) heterozygosity, Inbreeding coefficient (F_{IS}), the effect of subpopulations (S) compared to the total population (F_{ST}) were consequently used (Table 2). The number of alleles varied from 2 to 4 alleles per locus with a mean value of 3.14. The lowest number of allele was obtained from Ocum-108, Ocum-141 and Ocum-160 whereas the highest number of allele was obtained from the Ocum-87. Guchetl et al. (2014a) reported that 4 alleles for Ocum-87, 3 alleles for Ocum-70, and 2 alleles for other primers were observed in the 24 *O. cumana* population. Ziadi et al. (2018) observed 4 to 8 alleles in 4 SSR loci (Ocum-52, Ocum-81, Ocum-87, and Ocum-196) that are the same loci as our study. Considering the allele numbers and PIC values of the 24 SSR loci studied in total, it was stated that the studied populations would be divided into 3 groups in terms of genetic diversity. Belay et al. (2020) used 96 individuals of the *O. crenata* species, 7 alleles for Ocum-52 and 11 alleles for Ocum-81 were observed. In the literature, studies with different numbers of individuals collected from different geographical regions for *O. cumana* were found to be compatible with each other in terms of the number of SSR alleles observed. It has been determined that the same SSR loci in different species, such as *O. crenata*, have higher alleles. It can be said that the small differences determined as a result of the comparison are due to the number of studied populations, geographic origin of populations, and the number of individuals per population used in these studies (Table 3). Polymorphism information content (PIC) of the loci ranged from 0.058 (Ocum-70) to 0.257 (Ocum-52) for 8 SSR loci. It was observed that the results were compatible with each other in the comparison of PIC values using the same SSR markers as our study (Table 3).

Population-specific allele namely private allele is one of the most important criteria in determining genetic diversity. If such alleles are encountered for the first time in the population, they can be followed up and used to aid in race diagnosis in broomrape species. In this study, one private allele was determined for the Ocum-87 (128 bp allele) and Ocum-70 (101 bp allele) loci, respectively, in the T2018 (Tekirdağ-2018) and MT2013 (Muratlı/Tekirdağ-2013) populations. Bilgen et al. (2019) in Thrace populations, a total of 3 private alleles belonging to the Ocum-196 (193 bp), Ocum-52 (124 bp), and Ocum-81 (77 bp) loci was reported. 193 bp allele of Ocum-196 locus were reported in Avarız/Edirne2012 population in Bilgen et al. (2019), the same allele were observed at a relatively low

frequency in the population of the same region (AE2003) collected in a different year and another population in the nearby region (LE2013) in our study. Pineda-Martos et al. (2014b) reported only 1 private allele out of 14 populations.

Table 2. Estimated genetic diversity parameters of studied SSR loci used in the analysis of seven *O. cumana* populations

Locus	N_o	PIC	H_o	H_e	F_{IS}	F_{ST}	Nm
OCUM-52	3	0.257	0.434	0.308	-0.408	0.198	1.012
OCUM-70	3	0.058	0.066	0.074	0.106	0.040	6.021
OCUM-81	3	0.249	0.144	0.291	0.503	0.513	0.237
OCUM-87	4	0.225	0.262	0.268	0.022	0.277	0.653
OCUM-108	2	0.180	0.083	0.213	0.610	0.512	0.239
OCUM-141	2	0.230	0.092	0.276	0.667	0.447	0.309
OCUM-160	2	0.244	0.125	0.306	0.591	0.280	0.642
OCUM-196	3	0.155	0.042	0.180	0.764	0.133	1.623
Mean	3.14	0.228	0.156	0.239	0.357	0.300	1.342

*N_o= Observed alleles, PIC = Polymorphic information content, H_o = Observed Heterozygosity, H_e = Expected Heterozygosity (Nei 1987), F_{IS} = Inbreeding coefficient, F_{ST} = the effect of subpopulations (S) compared to the total population (T)

Table 3. Comparison of observed allele number and polymorphism information contents of studied *O. cumana*-specific SSR primers

	This study		Bilgen et al. 2019		Pineda-Martos et al. 2014a	
SSRs	**N_o**	**PIC**	**N_o**	**PIC**	**N_o**	**PIC**
Ocum-52	3	0.257	3	0.268	3	0.449
Ocum-70	3	0.058	2	0.217	5	0.672
Ocum-81	3	0.249	6	0.532	5	0.542
Ocum-87	4	0.225	3	0.263	5	0.642
Ocum-108	2	0.180	2	0.318	3	0.489
Ocum-141	2	0.230	2	0.157	4	0.625
Ocum-160	2	0.244	2	0.338	4	0.685
Ocum-196	3	0.155	3	0.172	3	0.535

In this study, Shannon's information index (I), which is one of the genetic diversity parameters to determine the variations within the populations, was calculated with the highest value in the T2018

population (0.555 ± 0.091), and the lowest value in the AD2018 population (0.243 ± 0.096). The mean Shannon's information index for the seven studied populations was found to be 0.392 ± 0.033 (Table 4). According to the mean value of Shannon's information index, it can be said that the diversity within the population is relatively low. Pineda-Martos et al. (2014b) using *O. cumana* individuals sampled from wild host plants, the mean Shannon's information index (I) was reported as 0.299. When Shannon's information index (I) of the studies is compared, consistent results are observed and it can be concluded that the gene pool of the species is narrow. Low observed allele number, presence of low-frequency alleles, and low intra-population diversity are possible due to the founder effect, which is effective in the formation of broomrape populations, and therefore, the species may be faced with the loss of alleles that will contribute to diversity (Pineda-Martos et al. 2014b). Guchetl et al. (2014a, 2014b) Shannon's information index (I) value in *O. cumana* populations sampled from Russia, Kazakhstan, and Romania was found to be relatively high when compared to other studies for Kazakhstan and Russia populations, and the reason for its high level was shown to be intense broomrape contamination in the region.

The mean observed heterozygosity value (Ho) was calculated as 0.156 and the mean expected heterozygosity value (He) was calculated as 0.239 (Table 4). In Bilgen et al. (2019), the mean Ho was 0.207 and the mean He was 0.349. The comparison of the genetic diversity parameters of the eight SSR loci used in our study and the values of the studies conducted with the same loci in the literature is performed. According to the comparison results, the highest Ho (mean observed heterozygosity) value was 0.434 in the Ocum-52 locus in our study, and Bilgen et al. (2019) calculated the same value as 0.408 for the Ocum-108 locus as 0.408, on the other hand, Pineda-Martos et al. (2014b) reported as 0.018 in the Ocum-160 locus. The lowest Ho values were found as 0.042 in Ocum-196 locus in our study, whereas Bilgen et al. (2019) calculated as 0.067 for the Ocum-196 locus. Pineda-Martos et al. (2014b), on the other hand, it was given as 0.000 in the Ocum-87 locus. The highest values of He (mean expected heterozygosity) value were calculated as 0.308 in Ocum-52 locus. Bilgen et al. (2019) and Pineda-Martos et al. (2014b) reported the highest values of He as 0.589 (Ocum-81) and 0.187 (Ocum-160 and Ocum 196), respectively. When the calculated Fst values were compared, the highest values were found to be 0.513 in Ocum-81 locus in our study. Bilgen et al. (2019) and Pineda-Martos et al. (2014b) reported the highest values of Fst as 0.551 (Ocum-141) and 0.910 (Ocum-108), respectively. The low rate of heterozygosity is indicative of severe heterozygosity deficiency at the SSR loci analyzed in the studies. It is reported that the biggest reason for this is the relatively high rate of self-pollination in these populations (Pineda-Martos et al., 2014b).

Table 4. Genetic diversity of *O. cumana* populations using eight SSR loci

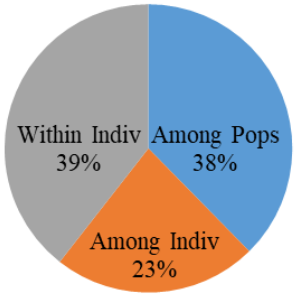
Abbreviation	P_{PL}*	N_a*	N_e*	N_{pa}*	I*	H_o*	H_e*	F*
AE2003	100	2.250	1.427	0	0.449	0.125	0.274	0.461
LK2013	75	1.750	1.244	0	0.254	0.155	0.156	0.132
AD2018	50	1.500	1.238	0	0.243	0.060	0.156	0.673
T2018	87.5	2.125	1.645	1	0.555	0.241	0.360	0.373
MT2013	100	2.375	1.322	1	0.356	0.077	0.205	0.473
LE2013	87.5	2.375	1.531	0	0.518	0.232	0.313	0.337
HT2016	100	2.250	1.323	0	0.366	0.202	0.211	0.300
Overall Mean	85.71	2.089	1.390	-	0.392	0.156	0.239	0.382

*P_{PL} = Polymorphic loci (%), N_a = Mean number of alleles per locus, N_e = Effective number of alleles, N_{pa} = Total number of private alleles observed, I = Shannon's information index, H_o = Observed Heterozygosity, H_e = Expected Heterozygosity (Nei 1987), F = Fixation index

According to the results of the molecular analysis of variance (AMOVA), 38% of the genetic diversity was calculated as among the population, 23% of the total variation was due to among individuals and 39% was within the individuals in the seven broomrape populations studied (Fst=0.300) (Table 5). Bilgen et al. (2019) reported that within-population diversity was 66% and inter-population diversity was 34%. Molinero-Ruiz et al. (2014) evaluated the diversity between and within groups. It has been reported that the inter-population diversity is 60% in the populations of Spain, Hungary, and Turkey, and the within-population diversity is 87% in the Turkish populations. Guchetl et al. (2014a, 2014b), on the other hand, it was found that the source of genetic diversity in populations of different countries was found to be within the population at a rate of 78%, and 22% was found to be diversity between populations (Fst=0.219). In Gagne et al. (1998), it was observed that genetic diversity in the population was low in *O. cumana* samples taken from Spain, Bulgaria, Romania, and Turkey. Belay et al. (2020) reported inter-population diversity as 2%, inter-individual diversity as 55%, and intra-individual diversity as 43% as a result of SSR analysis in *O. crenata* species. It was stated that there was a high rate of gene flow (low Fst) and no region-based differentiation in the studied *O. crenata* populations. In populations distributed in different geographical areas, genetic diversity levels and the distribution of genetic diversity within or between populations vary according to the rate of gene flow. The low rate of gene flow between populations affects the rate of genetic diversity between populations and may cause a decrease in population genetic diversity. In our study, when all populations were evaluated together using Fst values, the Nm value for each locus was calculated at a low rate of 1.342 in each generation. Bilgen et al. (2019) was stated the Nm value was 1.741.

Table 5. The Analysis of Molecular Variance (AMOVA) within and among populations

Source of Variation	df*	Sum of squares	Variance components	Percentage of variation
Among populations	6	154.461	0.597	38%
Among individuals	136	184.133	0.364	23%
Within individuals	143	89.500	0.626	39%



*df: degree of freedom

The genetic distance or similarity between the populations studied in the study was determined by calculating Nei's (1987) unbiased genetic similarity/difference coefficients. Genetic distance values calculated based on the eight SSR loci used in the study ranged between 0.006 and 0.541 (Table 6). The genetic distance was 0.006 with the lowest value among the LK2013-HT2016 populations and the highest value of 0.541 among the LK2013-MT2013 populations (Table 6). Seven populations were divided into 2 main groups (gene pool) in the dendrogram created according to genetic distance values and the UPGMA clustering method (Figure 1). While 4 populations (LK2013, HT2016, T2018, and LE2013) were sampled from Kırklareli, Edirne, and Tekirdağ in the 1st group, 3 populations (AE2003, AD2018, and MT2013) were sampled from Edirne, Tekirdağ, and Adana were observed in the 2nd group (Figure 1). Bilgen et al. (2019), a similar grouping was observed in the Thrace region populations divided into 2 groups. Ziadi et al. (2018), a phylogenetic tree was created according to the UPGMA clustering method, and Turkey *O. cumana populations* were divided into 6 clusters. In their study, the genetic similarity value was reported between 0.02 and 0.97. According to the dendrogram, Yeniköy, Sambro Havsa, and Adana populations were found to have low genetic similarity compared to the others. In addition, it has been reported that the populations of İskenderköy, Ürünlü, Budakdoğan, Ballıhoca Muratlı, Şahinköyü Malkara, and Gözsüz Village Malkara have high similarity. In Guchetl et al. (2014a, 2014b) was reported that Russia and Kazakhstan populations are in one group, and Romanian populations are in the other group. As a result of the study by Molinero-Ruiz et al. (2014), it was determined that the populations in the close geographical region of *O. cumana* populations were genetically separated from each other. In Pineda-Martos et al. (2014a), it was reported that in the genetic grouping of broomrape populations as a result of molecular marker analysis occurred according to the geographical origin and the host species type, and that the SSR marker is a strong marker in making these distinctions.

Table 6. Genetic distances matrix generated using Nei (1987) formula and SSR data

Abbreviation	AE2003	LK2013	AD2018	T2018	MT2013	LE2013	HT2016
AE2003	0.000						
LK2013	0.419	0.000					
AD2018	0.189	0.450	0.000				
T2018	0.180	0.120	0.126	0.000			
MT2013	0.143	**0.541**	0.137	0.143	0.000		
LE2013	0.161	0.170	0.212	0.084	0.280	0.000	
HT2016	0.373	**0.006**	0.453	0.111	0.521	0.138	0.000

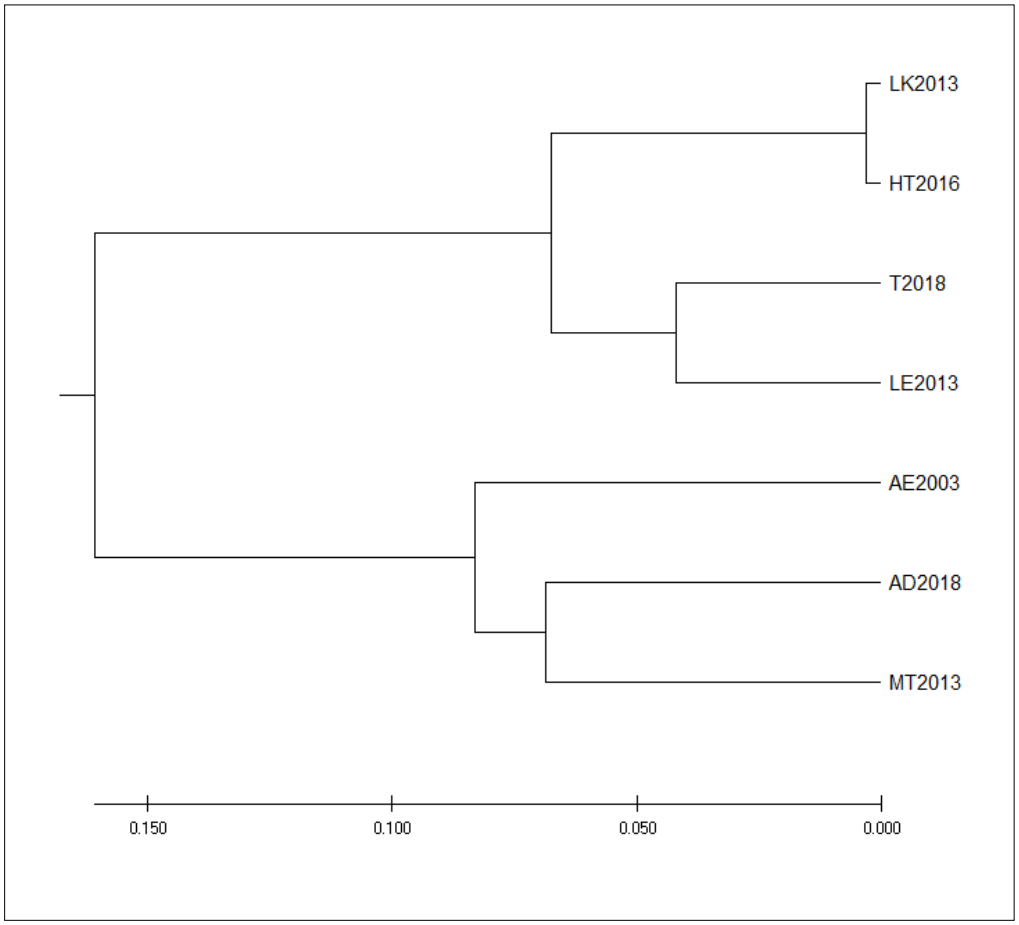
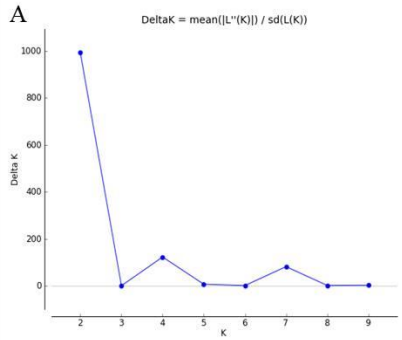


Figure 1. UPGMA dendrogram constructed using Nei's (1987) genetic distance values among seven studied *O. cumana* populations

As a result of Bayesian-based STRUCTURE analysis, different groups were determined in the studied broomrape populations using distinctive alleles and population similarity distributions were analyzed (Figure 2). The K value ranged from 1 to 10 with 10 replications and the most ideal value (K=2) was determined. As a result of the STRUCTURE analysis, 2 different groups were formed as in the UPGMA analysis. Group 1 includes LK2013, HT2016, T2018, and LE2013 populations, and group

2 includes AE2003, AD2018, and MT2013 populations (Figure 2). Bilgen et al. (2019), the most ideal K value for broomrape populations was found to be 2, Edirne and Kırklareli populations in the 1st group and Tekirdağ populations in the 2nd group. Pineda-Martos et al. (2014a), the K value was analyzed between 1 and 14, and the ideal K value was again found to be 2. In their study, Bulgarian populations and Spanish populations were in different groups.

A



B

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-1418.500000	0.221108	—	—	—
2	10	-1083.210000	0.251440	335.290000	249.580000	992.601442
3	10	-997.500000	2.796029	85.710000	0.160000	0.057224
4	10	-911.630000	0.743938	85.870000	90.930000	122.227851
5	10	-916.690000	4.804963	-5.060000	31.270000	6.507855
6	10	-890.480000	62.448322	26.210000	43.060000	0.689530
7	10	-821.210000	1.125907	69.270000	91.420000	81.196757
8	10	-843.360000	9.494115	-22.150000	10.800000	1.137547
9	10	-854.710000	11.394487	-11.350000	25.790000	2.263375
10	10	-891.850000	14.834962	-37.140000	—	—

C

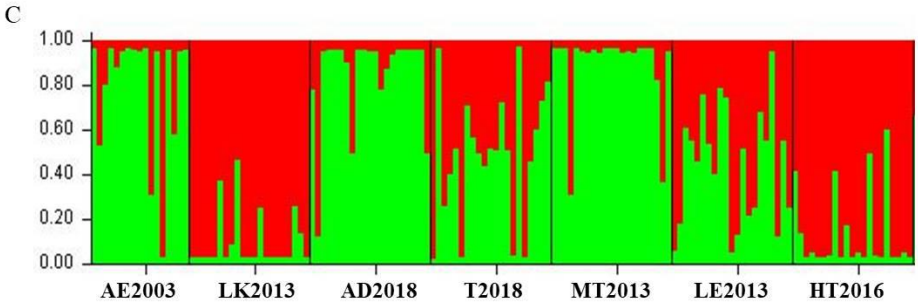


Figure 2. Bayesian clustering assignment based on the 8 SSR loci data. A) Delta K values obtained by the Evanno's method (Best peak at K=2); B) Table output of the Evanno method results (Yellow highlight shows the largest value in the Delta K column), and C) Population structure at K=2.

CONCLUSION

The genomic information obtained in recent years about the parasite and its host provides new perspectives for better understanding the species, determining the molecular basis of the interaction between them, and establishing applied breeding programs (Cuccurullo et al., 2022). The higher the genetic diversity of the parasitic plants, the more they have the ability to infect the plant by overcoming the resistance mechanism of the host plant. There is a need for sustainable and long-term breeding programs that aim to develop resistance mechanisms of each host plant, such as sunflower, and to obtain new resistant varieties. It is important to have varieties resistant to parasitic plant populations that spread in different ecological and geographical regions (Kaya 2003). In parasitic plant species such as *Orobanche* species, genetic characterization, genetic diversity and determining the distribution of genetic diversity and diversity within and between populations, and being able to identify new races occurring in a certain period of time are of great importance. Genetic studies are important to reveal the

evolutionary mechanisms that are effective in the transformation of parasitic plants, which were initially a threat to wild plants, into a species that infects agricultural plants over time. Studies on broomrape in our country are limited. As a result of the study, the information obtained about the *O. cumana* species will provide valuable scientific contributions to other studies on genetic structure, genetic diversity, and the evolution of the formation of new breeds by sampling all the broomrape regions in different hosts to be planned in the future. It should be noted that the overall efficiency and effectiveness of weed control programs, as well as the most appropriate means of weed control, will be enhanced by knowledge of the genetic relatedness or diversity available in local and regional *O. cumana* populations. For this reason, research studies aiming to reveal genetic diversity by choosing the most suitable type of molecular markers for the intended study make significant contributions to the literature.

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Declaration of Interest Statement

The authors declare no conflict of interest.

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