

Genetic characterization of *Orobanche cumana* populations from the Thrace region of Turkey using microsatellite markers

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Abstract: *Orobanche cumana* is a parasitic plant that can lead to advanced losses in yield of agricultural lands cultivating sunflower, depending on the sunflower varieties and the level of contamination. In our study, genetic diversity of six *O. cumana* populations from the Thrace region of Turkey was determined with the help of eight SSR (microsatellite) loci. All SSR loci were polymorphic. A total of 23 alleles were determined for the analyzed 120 samples. Allele number of each SSR locus ranged from two to six. Mean number of alleles ($N_a = 2.271$), effective allele number ($N_e = 1.667$), Shannon's information index ($I = 0.547$), and heterozygosity levels ($H_o = 0.207$ and $H_e = 0.340$) were calculated. A high proportion of the genetic variation (66%) was due to within-population variation and 34% of the diversity was due to among-population variations. Based on the UPGMA dendrogram and STRUCTURE analysis, there were two main clusters. Cluster I was classified into three groups containing four populations from the Kırklareli and Edirne regions. Two populations from Tekirdağ were in cluster II. The information obtained from this study is valuable to provide a significant contribution to studies on the genetic structure, diversity, and race evolution of *O. cumana* and to the developed crop breeding studies and management strategies for controlling *O. cumana* infestation.

Key words: Broomrape, *Helianthus annuus*, molecular marker analysis, simple sequence repeats

1. Introduction

Sunflower (*Helianthus annuus* L.) is one of the most significant oilseed crops in Europe, mainly grown for its edible oil and confectionery uses. Turkey takes part in the second group of sunflower producer countries (totally 27%) with China, Romania, Bulgaria, France, Hungary, and Spain (Molinero-Ruiz et al., 2015). *Orobanche cumana* Wallr. mainly parasitizes sunflowers (Pignone and Hammer, 2016). Sunflower broomrape (*O. cumana*) is a holoparasitic plant, devoid of leaves and chlorophyll, belonging to Orobanchaceae and causing a decrease in sunflower yield mainly in Europe and Asia (Parker, 2009; Pineda-Martos et al., 2014b; Pignone and Hammer, 2016). Although *O. cumana* was described in 1825, *O. cumana* and *O. cernua* L. were used as synonyms until the 1980s (Pujadas-Salva and Velasco, 2000). *Orobanche cumana* and *O. cernua* differ in morphology, physiology, phenology, chemical content, host range, and molecular characteristics (Joel, 1988; Joel et al., 2007). Sunflower cultivation affects the distribution of *O. cumana*, mainly distributed in

Southeast Europe, the Middle East, and Southwest Asia (Parker, 2013). This species has eight races (races A to H) breaking the tolerance mechanisms of sunflower hybrids tolerant to broomrape (Velasco et al., 2007; Timko and Scholes, 2013), and the most virulent races are F, G, and H (Kaya, 2014a, 2014b; Molinero-Ruiz et al., 2015). There are various mechanisms such as recombination, mutation, gene flow, genetic diversity increase, and selection within gene pools that might be helpful to determine the emergence of new races of broomrape (Pineda-Martos et al., 2013, 2014a). Significant reduction in sunflower yield from 50% to 90% was reported in broomrape-contaminated fields in several studies (Dominguez, 1996; Ciuca et al., 2004; Imerovski et al., 2013; Kaya, 2014a; Miladinovic et al., 2014).

Molecular marker studies about genetic diversity, population structure, gene flow, and virulence genetic mechanisms for broomrape populations are scarce (Velasco et al., 2016). The first molecular studies about genetic diversity of the genus *Orobanche* were done with

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isozymes (Verkleij et al., 1986; Castejón-Muñoz et al., 1991). Later, dominant markers such as RAPDs and ISSRs were used for estimating the genetic diversity of *O. cumana* populations (Katzir et al., 1996; Gagne et al., 1998, 2000; Benharrat et al., 2002; Roman et al., 2002; Ciuca et al., 2004; Atanasova et al., 2005). In recent years, SSRs have been used more frequently due to their significant characteristics such as codominant inheritance, multiple allele numbers, and high polymorphism levels (Pineda-Martos et al., 2013, 2014a, 2014b; Guchetl et al., 2014a, 2014b; Martin-Sanz et al., 2016). In the work of Pineda-Martos et al. (2013), 50 *O. cumana* populations from Spain were used for analysis of inter- and intrapopulation diversity with SSR analysis. The genetic recombination between distant populations was observed and it was reported to be an important driving force for race evolution. Pineda-Martos et al. (2014a) studied the genetic diversity and structure of *O. cumana* populations from Bulgaria and Spain with SSR analysis and reported bidirectional gene flow between studied populations. Jebri et al. (2017) studied genetic diversity of 9 populations from Tunisia with SSR and SNP analysis. Two distinct gene pools of sunflower broomrape populations in Tunisia were reported by Jebri et al. (2017). PCR-based molecular markers indicated that the intrapopulation diversity of studied broomrape populations was small and also these populations were discriminated according to their geographical origin (Pineda-Martos et al., 2013; Molinero-Ruiz et al., 2014).

Identification of broomrape races is useful for breeding studies and molecular marker analysis are effective possible ways of gaining knowledge of race types. According to the reviewed literature, there is no previous study about genetic diversity and characterization of

Turkey's *O. cumana* populations with SSR primers, except a study by Pineda-Martos et al. (2014b) that included two populations from Turkey. The main purpose of our study was to identify the genetic structure of six *O. cumana* populations from the Thrace region of Turkey via SSRs. The study is important in terms of testing the presence of findings that may indicate the formation of a new race in *O. cumana* populations of the Thrace region.

2. Materials and methods

2.1. Plant materials

Seeds from six different *O. cumana* populations were collected from 2011 to 2013 in sunflower fields of the Tekirdağ, Edirne, and Kırklareli provinces of the Thrace region. Geographic locations and coordinates of different sunflower fields in which *O. cumana* seeds were collected are given in Figure 1. Bulked seeds belonging to each population were stored at 4 °C. *Orobanche cumana* populations were propagated in 2016 on plants of the sunflower (*H. annuus*) cultivar Özdemirbey. Multiplication of *O. cumana* populations and tissue collection were performed as described by Pineda-Martos et al. (2013). Fresh tissue samples from 20 individuals belonging to each population were collected before flowering began and they were stored at -20 °C until DNA extraction.

2.2. DNA extraction from *O. cumana* individuals

DNA isolation was performed with the plant tissues from 20 individuals per population. Each tissue sample was ground using a ball mill (Retsch MM400). Doyle and Doyle's (1990) CTAB-based DNA extraction method was used with some modifications for genomic DNA isolation. The quantification and qualification of isolated DNAs was performed as described by Elibol and Bilgen (2017). The

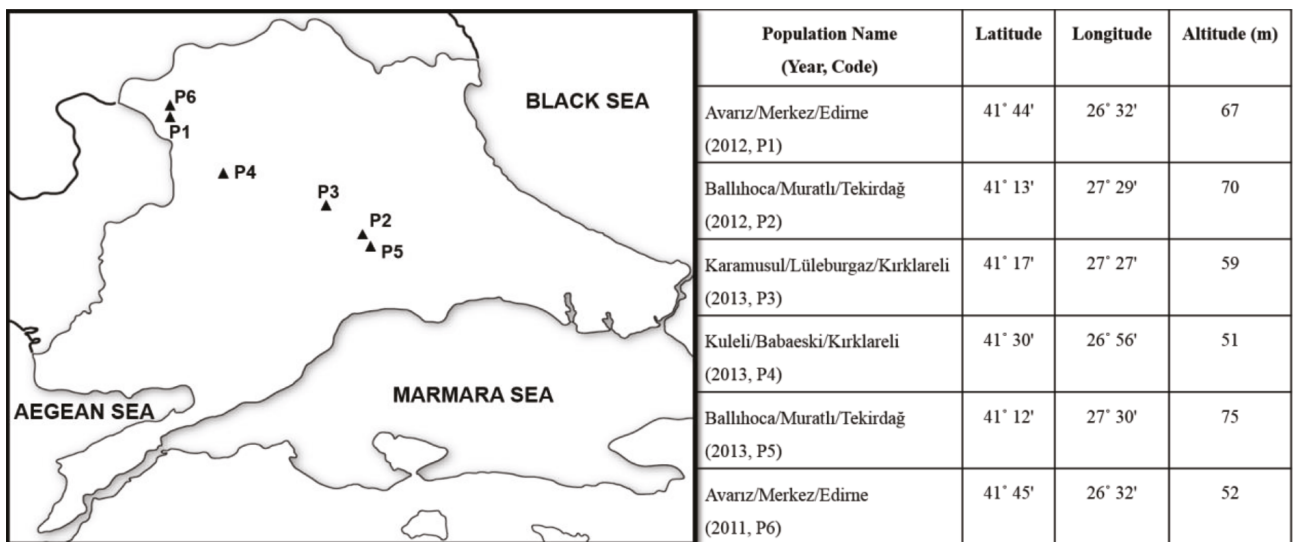


Figure 1. Geographic locations and coordinates of six studied *O. cumana* populations.

DNA samples were diluted to 10 ng/ μ L and preserved at -80°C until PCR analysis.

2.3. SSR analysis

Eight SSRs (Ocum-52, Ocum-70, Ocum-81, Ocum-87, Ocum-108, Ocum-141, Ocum-160, and Ocum-196) were chosen from 15 high-quality polymorphic SSR primers developed by Pineda-Martos et al. (2013) and these primers were used for the molecular characterization of *O. cumana* populations. The characteristics of the selected primers are indicated in Table 1. The PCR amplifications were performed as described by Pineda-Martos et al. (2013) using the Applied Biosystems ProFlex PCR System Thermal Cycler. PCR products were controlled by 2% agarose gel electrophoresis (1X TBE buffer, 100 V, 1 h). Gel Imaging System Vilber Lourmat Quantum ST5 was used to visualize the agarose gels. The DNA fragment analysis and determination of allele sizes were performed as described by Elibol and Bilgen (2017).

2.4. Data analysis

For each SSR locus, observed allele size range (bp) and observed allele number were determined. In statistical analysis of SSR data, allele and genotype frequencies, allele numbers (N_a), effective allele numbers (N_e), total private alleles observed (N_{pa}), Shannon's information index (I), heterozygosity levels (H_o and H_e), fixation index (F), and polymorphic information contents (PIC) were estimated via the software programs POPGENE Version 1.31 (Yeh et al., 1999) and GenAlEx Version 6.3 (Peakall and Smouse, 2006).

Analysis of molecular variance (AMOVA) was carried out with the help of GenAlEx Version 6.3 (Peakall and Smouse, 2006) to estimate the genetic diversity level within and among populations and the significance of AMOVA results was evaluated with 1000 permutations of the acquired SSR data. Nei's unbiased genetic distance measure (Nei, 1987) was used to estimate genetic relationships among populations. Cluster analysis according to Nei's genetic distance matrix was done with the unweighted pair group method (UPGMA). An UPGMA phenogram was constructed with Molecular Evolutionary Genetics Analysis Version 6.0 (MEGA6) software (Tamura et al., 2013). Bayesian model-based cluster analysis was performed for SSR data to determine gene pools of analyzed broomrape populations using STRUCTURE Ver. 2.3.4 software (Pritchard et al., 2000) as described by Elibol and Bilgen (2017).

3. Results

Polymorphism levels among six *O. cumana* populations collected from the Thrace region of Turkey were determined using eight SSRs (Table 1). A total of 23 alleles (mean value = 2.271 alleles/population and locus) were determined. Evaluating the six broomrape populations, Ocum-81 has the highest number of alleles (6 alleles), and the other SSR loci have 2 or 3 alleles (Table 1). Table 2 shows genetic diversity parameters estimated by eight SSR loci in the studied *O. cumana* populations. In order to determine how informative the selected SSR markers were, PIC values were estimated. All studied SSR markers were

Table 1. Characteristics of the SSR markers used to analyze the genetic diversity of *O. cumana* populations.

Locus	Primer sequences (5'-3')	Tm ($^{\circ}\text{C}$)	Observed allele size range (bp)	Observed number of alleles
OCUM-52	F: 5'-PET-CATGTCTAAGCTTTTGGCTCG-3' R: CAAGACTTGGAACAAGCAAATC	62	124-136	3
OCUM-70	F: 5'-NED-AAGCTGTAAACAATGCCTGAA-3' R: CCTCCTCCAGTACCACTAGGC	58	103-105	2
OCUM-81	F: 5'-6-FAM-TTACAAGGTGAAACCACCCA-3' R: CAGCTACTGTCCGCAAGAAA	58	73-99	6
OCUM-87	F: 5'-VIC-TTCTCGACAGCTTTGGGTAAA-3' R: ATGCCAACTTCGAGTGATCC	62	122-131	3
OCUM-108	F: 5'-VIC-TCGTTAATAAGTGGTTCACGAAAA-3' R: TGAATAAAAAATAAATGTACGGGTG	58	140-144	2
OCUM-141	F: 5'-6-FAM-CAGCAACTGTTTCTTCCATAGAG-3' R: TCCAAGAAGAGGAAAAGAAGTGA	62	188-191	2
OCUM-160	F: 5'-NED-TGAGGGTTTGTAAGTGGGC-3' R: CGTACCTTATCCCTCCGTCA	62	130-134	2
OCUM-196	F: 5'-PET-GTATGTGCGCCCGTCTTG-3' R: GGGGATGACTGTGTTTCGAT	58	193-201	3

Table 2. Genetic diversity estimated using eight SSR loci in *O. cumana* populations.

Population name (year, code)	N*	P _{PL} * (%)	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)	F*
Avarız/Merkez/Edirne (2012, P1)	20	87.5	2.125 (±0.227)	1.702 (±0.213)	1	0.559 (±0.121)	0.119 (±0.030)	0.350 (±0.075)	0.546 (±0.126)
Ballıhoca/Muratlı/Tekirdağ (2012, P2)	20	100	2.250 (±0.250)	1.752 (±0.286)	0	0.570 (±0.127)	0.219 (±0.042)	0.352 (±0.073)	0.222 (±0.146)
Karamusul/Lüleburgaz/ Kırklareli (2013, P3)	20	87.5	2.375 (±0.324)	1.600 (±0.193)	1	0.532 (±0.129)	0.169 (±0.055)	0.314 (±0.076)	0.310 (±0.152)
Kuleli/Babaeski/Kırklareli (2013, P4)	20	87.5	2.375 (±0.532)	1.779 (±0.179)	1	0.603 (±0.117)	0.213 (±0.082)	0.392 (±0.069)	0.426 (±0.182)
Ballıhoca/Muratlı/Tekirdağ (2013, P5)	20	100	2.250 (±0.164)	1.485 (±0.099)	0	0.497 (±0.065)	0.213 (±0.052)	0.305 (±0.046)	0.273 (±0.134)
Avarız/Merkez/Edirne (2011, P6)	20	87.5	2.250 (±0.412)	1.685 (±0.240)	0	0.519 (±0.139)	0.313 (±0.144)	0.325 (±0.085)	0.256 (±0.283)
Overall mean	20	91.67 (±2.64)	2.271 (±0.132)	1.667 (±0.082)	0.5 (±0.224)	0.547 (±0.046)	0.207 (±0.031)	0.340 (±0.028)	0.335 (±0.070)

*N = mean sample size, 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, ± standard errors in parentheses.

polymorphic, showing a PIC value of 0.289 (moderate). Ocum-81 (PIC = 0.544) was highly informative (PIC > 0.50). Four of the selected SSR markers (Ocum-52, Ocum-87, Ocum-108, and Ocum-160) were moderately informative markers having PIC values from 0.263 to 0.348 (0.25 < PIC < 0.50). The remaining SSR markers (Ocum-70, Ocum-141, and Ocum-196) had PIC values below 0.25, meaning that they were slightly informative markers. Based on SSR data, the mean number of alleles per locus (N_a) varied from 2.125 (P1-Avarız/Merkez/Edirne) to 2.375 (P3-Karamusul/Lüleburgaz/Kırklareli and P4-Kuleli/Babaeski/Kırklareli). The overall mean number of effective alleles per locus (N_e) was 1.667 ± 0.082 (varied from 1.485 to 1.779). Three private alleles (N_{pa}) were determined in the P1-Avarız/Merkez/Edirne, P3-Karamusul/Lüleburgaz/Kırklareli, and P4-Kuleli/Babaeski/Kırklareli populations. P1-Avarız/Merkez/Edirne has one population-specific (private) allele (193 bp) for Ocum-196 (f = 0.125), P3-Karamusul/Lüleburgaz/Kırklareli has one population-specific (private) allele (124 bp) for Ocum-52 (f = 0.075), and P4-Kuleli/Babaeski/Kırklareli has one private allele (77 bp) for Ocum-81 (f = 0.025).

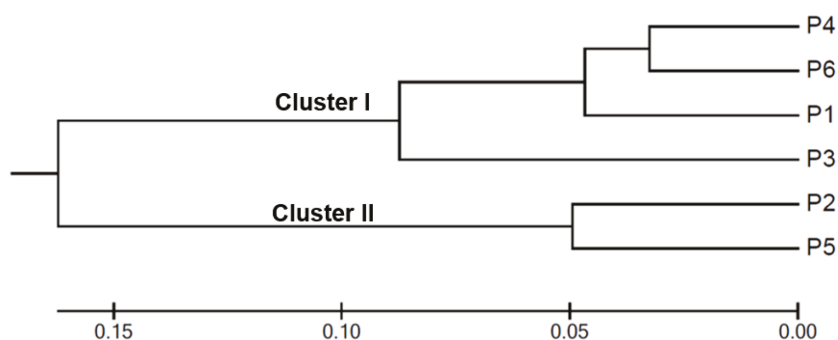
The overall average value of Shannon's information index (I) was calculated as 0.547, being highest in the P4-Kuleli/Babaeski/Kırklareli population (0.603) and lowest in P5-Ballıhoca/Muratlı/Tekirdağ (0.497). Estimated values of mean expected heterozygosity (H_e) and observed heterozygosity (H_o) were 0.340 and 0.207, respectively (Tables 2 and 3). The level of observed heterozygosity was

lower than the expected level, and this caused a positive mean inbreeding coefficient (F_{IS} = 0.368) (Table 3). The deficit of heterozygotes within populations (F_{IS}) across all the loci ranged from -0.019 to 0.675 and the fixation index of each locus (F_{ST} values) ranged from 0.151 to 0.551 (Table 3).

Nei's (1987) genetic distance coefficients were estimated [ranged from 0.065 (P4-P6) to 0.439 (P3-P5)] among all possible population pairs for SSR data (Figure 2). As a result of AMOVA, a high percentage (66%) of genetic diversity was due to differences within populations (Table 4). Thus, genetic diversity among populations accounted for 34% of the total diversity (Table 4). The UPGMA tree for SSRs according to Nei's genetic distance is shown in Figure 2. The phenogram indicated that there were two main clusters, where cluster I grouped four populations from Edirne (P1 and P6) and Kırklareli (P3 and P4) and cluster II grouped two Tekirdağ populations (P2 and P5). The determination of the ideal number of groups present in the studied *O. cumana* populations was done with the graph of delta K values (Figure 3). Bayesian-based analysis of the populations from the Thrace region of Turkey with STRUCTURE analysis performed for six populations using SSRs demonstrated two main genetic clusters for K value of 2 (optimum K) and K value of 3 (the closest to 2) (Figures 4 and 5), as in the UPGMA tree (Figure 2). In Figure 4, each column represents an individual belonging to specific population; two colors (red and green) denote a population cluster. STRUCTURE analysis has shown the

Table 3. Genetic parameters for each of the polymorphic SSR loci used in the analysis of six *O. cumana* populations.

Locus	H _o	H _e	F _{IS}	F _{ST}
OCUM-52	0.192	0.326	0.413	0.236
OCUM-70	0.392	0.267	-0.465	0.151
OCUM-81	0.192	0.589	0.675	0.207
OCUM-87	0.183	0.345	0.469	0.344
OCUM-108	0.408	0.401	-0.019	0.182
OCUM-141	0.083	0.185	0.551	0.551
OCUM-160	0.142	0.401	0.647	0.177
OCUM-196	0.067	0.203	0.672	0.201
Mean ± SE	0.207 (±0.052)	0.340 (±0.053)	0.368 (±0.144)	0.256 (±0.047)

**Figure 2.** UPGMA tree constructed using Nei's (1987) genetic distance values for six studied *O. cumana* populations based on SSR data.**Table 4.** The analysis of molecular variance (AMOVA) for eight SSR loci.

Source of variation	df*	Sum of squares	Variance components	Percentage of variation
Among populations	5	224.967	2.051	34%
Within populations	114	453.500	3.978	66%

*df: Degrees of freedom.

existence of two gene pools. The groups are represented by different colors (red, green, and blue) in the K = 3 grouping of populations (Figure 5).

4. Discussion

Understanding the genetic structure and genetic diversity of *O. cumana* populations, race evolution, and how different populations can interact with each other has significant value in gaining knowledge about its interaction with sunflower. There are limited studies about the genetic diversity of *O. cumana* populations with molecular markers

at a global scale. Determination of among- and within-population genetic diversity levels of studied populations and comparison of acquired information about genetic diversity with the other studies in the literature were achieved in this study. In our study, all studied SSRs were polymorphic. In comparison with other studies, Pineda-Martos et al. (2013) reported that 15 SSR loci (eight of these SSRs were the same as in our study) were polymorphic in 50 populations of *O. cumana* from Spain. Guchetl et al. (2014a, 2014b) studied populations from Romania, Russia, and Kazakhstan with 9 SSR loci (7 of these SSRs same with

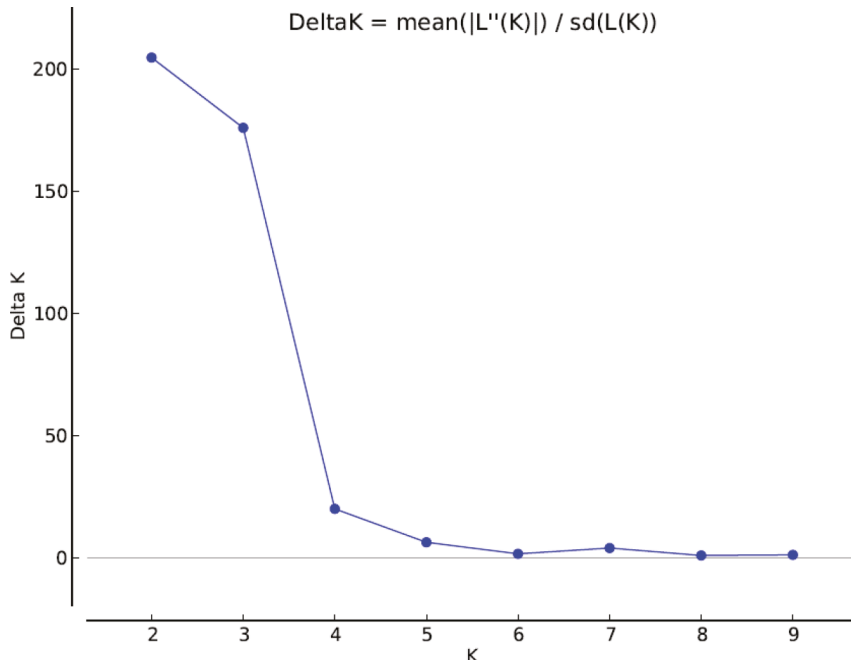


Figure 3. Delta K plot showing the best peak at K = 2 to determine the ideal number of groups present in the studied *O. cumana* populations using eight microsatellite loci.

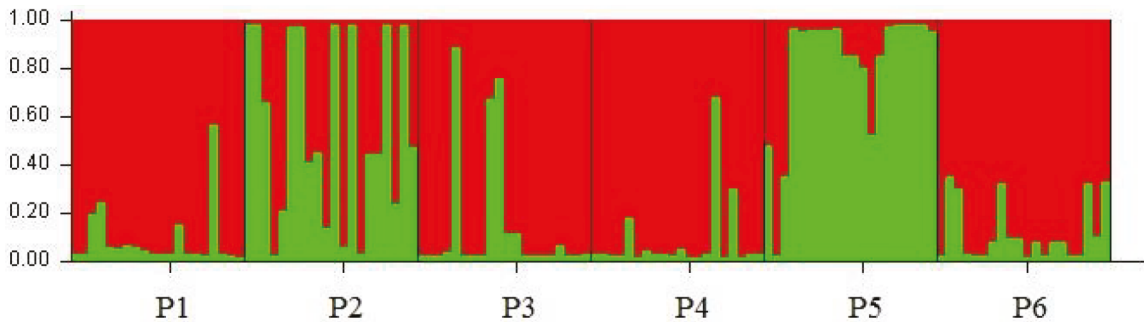


Figure 4. Genetic structure of six *O. cumana* populations as defined by STRUCTURE (K = 2, the highest value of K).

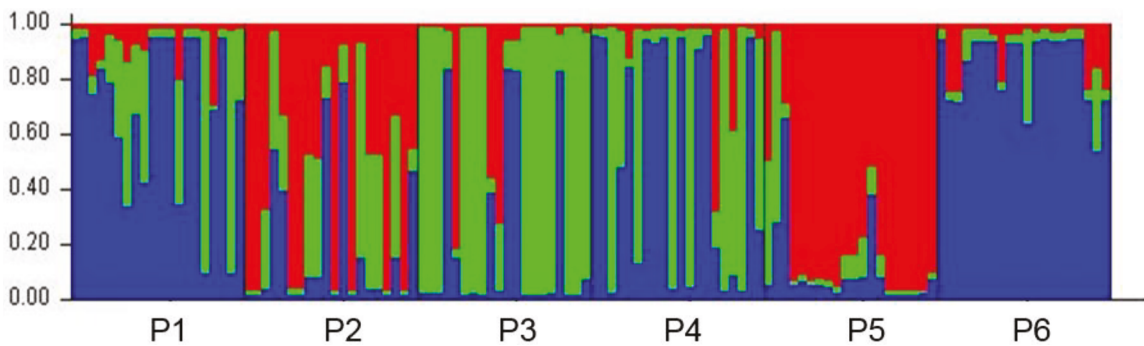


Figure 5. Genetic structure of six *O. cumana* populations as defined by STRUCTURE (K = 3, the closest value to K = 2).

our study) and reported that all the studied SSR loci were polymorphic. In the work of Molinero-Ruiz et al. (2014), genetic diversity of 11 populations from Romania, Spain, Hungary, and Turkey was assessed with 18 polymorphic

RAPD primers. Pineda-Martos et al. (2014b) stated that the percentage of polymorphic SSR markers belonging to *O. cumana* (50.3%) is higher than the percentage of polymorphic loci of other marker types. Previous studies

showed that these differences are due to both the used marker system and the different geographical origins of the studied populations.

A total of 23 alleles belonging to eight SSR loci (Ocum-52, Ocum-70, Ocum-81, Ocum-87, Ocum-108, Ocum-141, Ocum-160, Ocum-196) were determined in our study. Pineda-Martos et al. (2013) detected 19 alleles (2 to 3 alleles/locus) for the same SSR loci as in our study. In the work of Guchetl et al. (2014a, 2014b), 17 alleles (2 to 4 alleles per locus) were detected for the same SSR loci, except Ocum-160, with our study. The results of band sizes and allele numbers of SSRs in various studies conducted with different *O. cumana* populations in the literature and in this study are consistent with each other. One of the most basic measures of genetic diversity in the studied SSR loci is the identification of allelic richness and private alleles. Three private alleles belonging to three different populations (1 private allele per population) were determined in our study. Pineda-Martos et al. (2014a) also found 1 private allele in only one population from Bulgaria. The genetic diversity within a population is relatively high (mean $I = 0.547$) in this study. Pineda-Martos et al. (2014a) reported the I value for *O. cumana* populations from Bulgaria collected on wild hosts and on the sunflower as 0.299 and 0.099, respectively. Founder effect in small populations, low frequency of some alleles (i.e. rare alleles), or disappearance of some alleles in the population may be the main reasons for low genetic diversity and a narrower genetic pool. In the work of Guchetl et al. (2014a, 2014b), a high I value was determined for populations from Russia and Kazakhstan, whereas populations from Romania had a very low I value (0.05).

The natural dispersal range of *O. cumana* extends from Central Asia to southeast Europe and it especially infects *Artemisia* species. Towards the end of the 19th century, however, *O. cumana* infection began on sunflower in Russia after the increase of sunflower cultivation (Pineda-Martos et al., 2013). It is not yet known whether the infection on sunflower started by means of natural ability possessed by the broomrape or from a genotype of broomrape carrying mutations. Previous studies showed that populations from Russia and neighboring countries may have higher genetic diversity due to the intensive dispersal of seed from these regions.

Nei's (1987) overall average observed heterozygosity ($H_o = 0.207$) was relatively lower than the average expected heterozygosity level ($H_e = 0.349$) in the studied *O. cumana* populations. The observed heterozygosity value being lower than the expected heterozygosity shows a departure from Hardy-Weinberg equilibrium and a possibility of inbreeding in the populations. Pineda-Martos et al. (2013) reported a low level of H_o (0.00 to 0.09) in four populations from Spain, which are genetically distant from the main

genetic pools. They also reported higher H_e values in these populations (0.12 to 0.49). Pineda-Martos et al. (2014a) reported similar results in terms of heterozygosity levels in populations of *O. cumana* from Spain and Bulgaria. It has been reported that the analyzed SSR loci have a significant level of heterozygote deficiency, and the main reasons for this are a higher fixation index and selfing rate value. Guchetl et al. (2014a, 2014b) indicated higher heterozygosity in populations from Russia and Kazakhstan than populations from Romania. It is emphasized that geographic distance, different soil types, and climatic conditions might affect the level of heterozygosity in these populations. Gene flow (N_m) is one of the important factors affecting the distribution of genetic diversity both within and between populations. When all populations are evaluated together using F_{ST} values in this study, the N_m value is calculated as 1.741 in each generation. The low level of gene flow among populations is one of the reasons for the decrease in the genetic variation of the populations.

According to AMOVA results for SSR data, 34% of the total genetic diversity might be assigned to among-population differences and the remaining part to intrapopulation variation. Molinero-Ruiz et al. (2014) obtained 60% genetic variation among countries (populations from Spain, Hungary, and Turkey) and also indicated 87% within-population variation in five Turkish populations (from Tekirdağ and Kırklareli) by using RAPD analysis. Their pairwise AMOVA results showed that among-population variation increased with the increase of geographic distance. In a study by Pineda-Martos et al. (2013), populations in the main genetic pool had 100% of the genetic variation among populations, whereas other populations distant from the main genetic pool had 31.8% of the genetic variability among populations. Pineda-Martos et al. (2014a) also utilized AMOVA for populations from Bulgaria collected on wild hosts; they reported that 53.64% of the variation was due to among-population variations. They also evaluated all populations collected on wild hosts and on sunflower together and found that 59.54% of the genetic variability was due to among-population variations as well. Guchetl et al. (2014a, 2014b) reported that a rather high proportion of the genetic diversity (78%) was due to within-population variation and the remaining part was due to variation between populations. Jebri et al. (2017) reported greater differences within populations (76.65% of total variance) than differences between populations (25.35%). In another study performed with *O. cumana* populations from Spain, Romania, Bulgaria, and Turkey via RAPD analysis, a low level of within-population diversity due to a low level of gene flow from various geographic distances resulting from self-pollination was reported (Gagne et al., 1998).

According to the acquired UPGMA dendrogram and STRUCTURE analysis, our studied six populations can be divided into two main clusters. The Edirne and Kırklareli populations were in cluster I and the Tekirdağ populations were in cluster II. In the studies of Guchetl et al. (2014a, 2014b), two well-differentiated clusters were obtained: cluster I grouped populations from Russia and Kazakhstan, whereas cluster II combined populations from different regions of Romania. High levels of intrapopulation diversity and populations from Russia and Kazakhstan being in the same cluster were explained by genetic diversity and similarity of open-pollinated sunflower varieties that were cultivated since Soviet times. Molinero-Ruiz et al. (2014) reported that populations from close geographic regions grouped in the same cluster according to the geographic origin as in north and south Spanish *O. cumana* populations. Pineda-Martos et al. (2014b) indicated that cluster formation depends on the geographic origin and parasitized host types, and SSR markers had a high discriminating ability for *O. cumana* cluster analysis.

Parasitic plant populations with high genetic diversity have the ability to infect plants by overcoming the host resistance mechanism. For sustainable and long-term breeding programs aimed at improving resistance mechanisms of host plants such as sunflower against parasitic weeds such as *Orobancha* species, it is of great importance to determine the level of genetic diversity among and within the parasitic plant populations (Kaya, 2003), and also finding new resistance sources to diseases or parasites and increasing genetic variation of the cultivated sunflower is significant for sunflower

breeders (Atlagic and Terzic, 2016). In addition, studies to determine the genetic diversity of parasitic biotypes in natural or agricultural areas are significant to understand the evolutionary mechanism of how wild parasitic plants transform to an agricultural plant infecting crops. The information gained from this study will provide important contributions to new studies about the genetic structure and diversity, and the evolution of the formation of new races, which will be carried out in *O. cumana* and close relatives.

In conclusion, the determination of the genetic structure and also the presence of private alleles in some of the studied populations as a result of SSR analysis might indicate the formation of new races of broomrape; therefore, genetic marker analysis and phenotypic studies such as the determination of race in the populations in the Thrace region should be planned in the future. To understand and discover the genetic diversity, dispersal, and evolutionary mechanisms of race formation in *O. cumana* and close relative species as a global concept, further studies to be conducted to confirm race evolution in broomrape populations by sampling from all areas within the dispersal area will be useful for scientific researchers.

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