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ASSESSING THE PHYLOGENY AND GENETIC VARIABILITY OF *Orobanche cumana* Wallr. POPULATIONS USING ISSR MARKERS

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Abstract

This study investigates the genetic differentiation and phylogeny of the parasitic weed Orobanche cumana Wallr. using ISSR markers in 33 populations from Serbia, Moldova, Romania, Bulgaria, Turkey, and China, representing E, G, and H physiological races. Phylogenetic relationships were analysed using Nei's standard genetic distance and the Neighbour-Joining method in POPTREE2. The results of the phylogenetic analysis indicated the presence of three distinct groups. The first clade included populations from Serbia, Turkey, and China. The second clade comprised populations from Moldova and Romania, and the third clade included Bulgarian populations, forming a separate branch. Genetic variation analysis of O. cumana populations at both country and race levels showed that all physiological races and the Moldavian populations exhibited high interpopulation diversity. In contrast, the primary genetic differentiation exhibited by the Bulgarian, Serbian, Turkish, and Chinese populations occurred principally within their respective populations. This genetic diversity is influenced by factors such as the expansion of sunflower production, agricultural development, and climate change. Furthermore, distinct evolutionary adaptations among O. cumana races were evident. The results obtained demonstrate the complex evolution and dispersal mechanisms of this parasitic species and offer crucial insights into effective management strategies for the production of sunflowers.

Key words*: Orobanche cumana, sunflower, genetic differentiation, ISSR, phylogeny*.

INTRODUCTION

Orobanche cumana Wallr. is a non-photosynthetic, obligate, root-parasitic weed that has been identified as having a severe impact on the production of sunflower (*Helianthus annuus* L.). Yield losses can reach as high as 5% to 100% (Kaya, 2014; Miladinovic et al., 2014), depending on the sunflower genotype (Ciuca et al., 2004; Miladinovic et al., 2014), the aggressiveness of the parasite (Guchetl et al., 2014a), and regional environmental conditions (Kaya, 2014). Sunflower broomrape is a pervasive weed that poses a significant agricultural challenge on a global scale (Kaya, 2014; Miladinovic et al., 2014). The emergence of highly virulent races of *O. cumana*, which can effectively overcome the resistance of existing sunflower varieties and hybrids, is influenced by several factors. The intensive exploitation of agricultural land, particularly with large sunflower monocultures that return to the same fields every two to three years, neglects crop rotation, thereby facilitating the proliferation of this parasitic weed (Melero-Vara et al., 2000; Lukomets & Antonova, 2015). Additionally, the introduction of foreign sunflower germplasm has inadvertently heightened local susceptibility to broomrape infestations (Kaya, 2014). The parasite's high reproductive potential, rapid evolutionary dynamics, and climatic changes that favour its expansion further complicate management efforts (Kaya, 2014). Moreover, gene flow between wild and weedy populations has precipitated abrupt molecular alterations within *O. cumana*, thereby accelerating the emergence of novel, more aggressive races (Kaya, 2014; Melero-Vara et al., 2000). The eight races that have been identified are A through H, with the highest levels of virulence being demonstrated by races E, F, G, and H (Kaya, 2014; Duca & Bivol, 2023).

A comprehensive understanding of the evolutionary relationships and genetic differentiation among *O. cumana* populations is crucial to elucidate the adaptations and geographical distribution of its most aggressive races, with direct implications for agricultural management. Molecular phylogenetic approaches employing multilocus analyses facilitate the

reconstruction of species and population histories, elucidating the evolutionary relationships among different plant groups (Benharrat et al., 2002; Hristova et al., 2011). Microsatellite markers, of which a subset may be linked to genes under selection pressure whilst others evolve neutrally, are of particular value for the assessment of genetic variability (Bannikova, 2004). Early molecular studies utilising dominant markers, including Inter Simple Sequence Repeats (ISSRs), have played a crucial role in estimating the genetic diversity of *O. cumana* and related species (Román et al., 2002; Stoyanov et al., 2012; Duca et al., 2020). This has enhanced the understanding of taxonomic and phylogenetic relationships within the genus Orobanche. Furthermore, phylogeographic studies that examine the geographical distribution of genetic lineages provide additional insights into the evolutionary history of *O. cumana* (Avise, 2000). Notwithstanding the significance of *O. cumana*, the understanding of its evolutionary history and phylogeography across disparate geographical regions remains constrained.

This research addresses significant gaps in the field by examining the genetic variation and evolutionary relationships among *O. cumana* populations from different geographical regions and racial groups. Multilocus ISSR markers were utilised in the analysis to facilitate a comprehensive understanding of the population genetics and evolutionary history of *O. cumana.* The results should contribute to the development of improved agricultural management strategies to mitigate the impact of this highly adaptable parasitic weed.

MATERIALS AND METHODS

In the present study, a total of 336 accessions of *O. cumana* were analysed, representing 33 populations from six countries: Serbia (S), Moldova (M), Romania (R), Bulgaria (B), Turkey (T), and China (Ch), across 24 regions. In addition, the samples belonged to three physiological races, E, G and H (Figure 1).

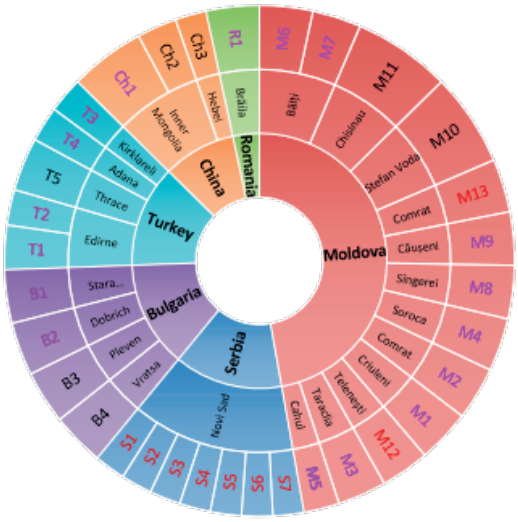

Figure 1. Geographic and racial distribution of *O. cumana* populations sampled for ISSR analysis (red: race E, black: race G, violet: race H)

Total genomic DNA was isolated from frozen samples using the Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit #K0791, following the manufacturer's protocol (Thermo Fisher Scientific, USA). The concentration and integrity of the isolated DNA were evaluated by agarose gel electrophoresis (1%) in 1× TAE buffer (40 mM Tris-acetate, pH 8.0; 1 mM EDTA) at a voltage of 2.5 V/cm, complemented by optical density readings obtained with a spectrophotometer (T60 UV-VIS, UK) (Sambrook & Russell, 2001).

Polymerase chain reaction was performed using a set of 13 di-, tri-, and tetranucleotide anchored and non-anchored ISSR primers, as previously reported by Benharrat (Benharrat et al., 2002) (Table 1).

Table 1. ISSR primers used in this study and their specifications

Code	ISSR primers Sequence (5′→ 3′)	NBN	GC, %
BC807	AGAGAGAGAGAGAGAGT	17	47
BC810	GAGAGAGAGAGAGAGAT	17	47
BC835	AGAGAGAGAGAGAGAGYC	18	56
BC841	GAGAGAGAGAGAGAGAYC	18	56
BC857	ACACACACACACACACYG	18	56
$(CAA)_5$	CAACAACAACAACAA	15	33
$(GACA)_4$	GACAGACAGACAGACA	16	50
$(CA)_6RG$	CACACACACACARG	14	57
$(CTC)_4RC$	CTCCTCCTCCTCRC	14	71
$(CAG)_5$	CAGCAGCAGCAGCAG	15	67
$(CT)_8TC$	CTCTCTCTCTCTCTCTTC	18	50
$(CA)_6AC$	CACACACACACAAC	14	50
$(AG)_8YA$	AGAGAGAGAGAGAGAGYA	18	50

Note: R-A, G; Y-C, T; NBN-nitrogenous bases number; GC, %-percentage content of cytosine (C) and guanine (G) nucleotides in primer

The PCR reactions were performed on a Genset 9700 thermocycler (Applied Biosystems) under the following conditions: an initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30 s, 45°C for 45 s, and 72°C for 2 min; followed by a final extension at 72°C for 5 min.

The ISSR amplification products were analysed by electrophoresis in a 2% agarose gel, which had been previously stained with ethidium bromide. The gels were then visualised under UV light (wavelength: λ = 305 nm). The molecular size of the amplicons was estimated using the GeneRuler Express DNA Ladder, ready-to-use SM1553 (Thermo Fisher Scientific, USA), and each gel image was captured with the Doc-Print VX2 gel documentation system (SXT-F20.M, France).

The size of the ISSR fragments was determined using Photo-Capt V. 15.02 software. Bands that exhibited weak staining were excluded from further analysis. The generated data were compiled into a binary matrix indicating each character or locus's presence (1) or absence (0). The allele frequency data was subjected to phylogenetic analysis, with Nei's standard genetic distance and the Neighbor-Joining (NJ) method implemented in the POPTREE2 software for Windows, including bootstrap analysis, employed for this purpose. Furthermore, genetic variation statistics for *O. cumana* populations were analysed using POPGENE v.1.32 software.

RESULTS AND DISCUSSIONS

Thirty-three populations of *O. cumana* from Serbia, Moldova, Romania, Bulgaria, Turkey, and China, comprising three physiological races (E, G, and H), were analysed using ISSR markers to assess genetic diversity and phylogenetic relationships. The results demonstrated that the samples were divided into two major clades based on their microsatellite loci. A notable finding was that the phylogenetic tree revealed that the Bulgarian populations of *O. cumana* occupied a separate branch, distinctly different from other populations (Figure 2A).

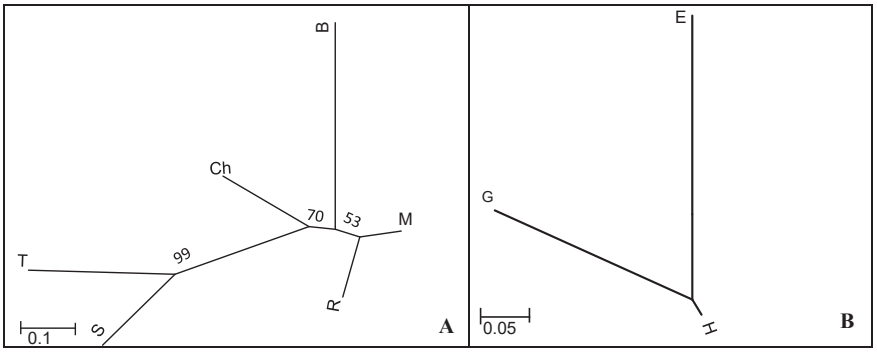

Figure 2. Phylogenetic relationships of *O. cumana* by geographic (A) and racial origins (B). Note: bootstrap values (expressed as percentages of 1000 replications) are given at the nodes

This suggests that Bulgarian populations may have unique genetic characteristics, possibly due to specific adaptive traits influenced by Bulgaria's unique agro-ecological conditions,

different crop management practices, and variations in environmental factors, including soil composition, climate patterns, and localised agricultural policies. Moreover, historical colonisation events may have resulted in genetic admixture, further contributing to the observed genetic distinctness of the Bulgarian populations. Understanding these differences is crucial as it provides insight into the potential for unique adaptations that may influence the parasitic behaviour of *O. cumana* and its impact on sunflower production. The knowledge thus acquired could inform targeted management strategies, taking the specific genetic profiles of local populations into account. The implementation of such management strategies is poised to yield more efficacious agricultural practices.

The analysis of genetic distance coefficients showed that the Bulgarian populations of *O. cumana* were more genetically similar to the Moldavian (0.07), Chinese (0.07), and Romanian (0.11) populations (Table 2). Conversely, the Bulgarian populations were more genetically distinct from the Turkish (0.18) and Serbian (0.19) populations, reflecting greater genetic distances (Table 2).

Table 2. Genetic identity and distance among diverse populations of *O. cumana* based on Nei's measures

Country	S	M	R	B	T	Ch
S	****	0.82	0.79	0.83	0.84	0.82
M	0.20	****	0.93	0.93	0.86	0.94
R	0.24	0.07	****	0.90	0.79	0.89
B	0.19	0.07	0.11	****	0.83	0.93
T	0.17	0.15	0.24	0.18	****	0.87
Ch	0.20	0.06	0.11	0.07	0.14	****

Race	E	G	H
E	****	0.91	0.93
G	0.09	****	0.97
H	0.07	0.03	****

Note: genetic identity is above the diagonal and genetic distance is below the diagonal

The results obtained suggest the presence of a closer genetic relationship between the Bulgarian population and its neighbouring populations. This relationship may be influenced by shared environmental conditions, historical interactions, or genetic exchange. Conversely, the greater genetic distances observed with the Turkish and Serbian populations may indicate more distinct evolutionary trajectories or adaptations to differing ecological conditions.

The total gene diversity (Ht) and the gene diversity within Bulgarian *O. cumana* populations (Hs) were found to be comparatively reduced, at 0.10 and 0.05, respectively (Table 3). These values indicate limited genetic variation within the Bulgarian populations. Additionally, the genetic differentiation (Gst) among these populations was notably elevated at 0.49, while the gene flow (Nm) was measured at 0.51 (Table 3).

Table 3. Population genetic variation statistics of *O. cumana*: country and race comparison

Country		Sample size	Ht	Hs	Gst	Nm (Gst)
S	Mean	49	0.18	0.11	0.36	0.88
	Sd		0.04	0.02		
M	Mean	168	0.17	0.08	0.52	0.47
	Sd		0.03	0.01		
B	Mean	48	0.10	0.05	0.49	0.51
	Sd		0.03	0.01		
T	Mean	41	0.20	0.13	0.36	0.90
	Sd		0.04	0.02		
Ch	Mean	18	0.10	0.07	0.37	0.85
	Sd		0.03	0.01		

Races		Sample size	Ht	Hs	Gst	Nm (Gst)
E	Mean	72	0.22	0.10	0.53	0.45
	Sd		0.04	0.01		
G	Mean	85	0.18	0.08	0.56	0.39
	Sd		0.03	0.01		
H	Mean	179	0.23	0.09	0.62	0.31
	Sd		0.03	0.01		

Note: Ht - total gene diversity; Hs - gene diversity within populations of the group; Gst = (Ht-Hs)/Ht, coefficient of gene differentiation among populations of the group; Nm - gene flow among populations from Gst (Nm = 0.5(1 - Gst)/Gst); Sd - standard deviation

This suggests that 51% of the genetic differentiation is attributable to variation within populations rather than among different populations. These patterns of genetic variability highlight a unique dynamic within the Bulgarian populations, likely shaped by localized environmental pressures and historical introgression events. Elevated Gst

values indicate significant genetic structuring, which can hinder gene flow and increase the potential for local adaptation. Conversely, the relatively elevated Nm value indicates ongoing gene exchange, which may facilitate the introduction of beneficial alleles, thereby enhancing resilience to environmental changes and agricultural practices.

Phylogenetic analysis of the data reveals that three populations from Serbia, Turkey, and China cluster within the first major clade of the tree. The populations from Serbia and Turkey formed one subclade within this clade, while the Chinese populations were grouped into a separate subclade (Figure 2A). Serbia and Turkey demonstrated a notably high bootstrap value of 99, indicating strong confidence in their close genetic relationship, which is further substantiated by a genetic distance value of 0.17 (Figure 2A, Table 2). This suggests a potential shared evolutionary origin influenced by similar geographic conditions, environmental factors, agronomic practices, or a common history of ecological adaptation. In contrast, the Chinese populations, with a bootstrap value of 70, also displayed genetic proximity to the Serbian (0.20) and Turkish (0.14) populations, albeit with less statistical confidence (Figure 2A, Table 2). This lower bootstrap value implies that the Chinese populations may possess distinct genetic characteristics or adaptations that differentiate them from those of Serbia and Turkey.

The analysis of population diversity indicated similar Gst and Nm coefficients among the populations from Serbia (0.36 and 0.88), Turkey (0.36 and 0.89), and China (0.37 and 0.85), respectively (Table 3). These values suggest that all three populations exhibit a high level of intrapopulation genetic differentiation. Additionally, the parameters of total gene diversity Ht for Turkey, Serbia, and China (0.20, 0.18, and 0.10, respectively) and within-population gene diversity Hs (0.13, 0.11, and 0.07, respectively) further support this finding (Table 3). Although genetic differences exist among these populations, they are not as pronounced as those observed in populations with higher Gst values. The analysis indicates that the majority of genetic differentiation occurs within these populations, with 64% for the Serbian and Turkish groups and 63% for the Chinese group. Furthermore, the elevated gene flow values observed among all populations suggest a considerable degree of gene migration, thereby highlighting a dynamic exchange of genetic material. This gene exchange is likely to enhance genetic diversity and reduce the risk of inbreeding among populations. High levels of gene flow suggest that these populations may be more adaptable to environmental change and less susceptible to the negative impacts of genetic isolation. This adaptability may provide greater resilience to external pressures by facilitating the exchange of beneficial genes among populations.

The second separate clade included the Moldavian and Romanian populations of *O. cumana*, indicating the presence of specific local adaptations and limited interpopulation genetic variability. These populations exhibited a close genetic distance of 0.07, accompanied by a relatively low bootstrap value of 53. This suggests a degree of genetic similarity and possibly historical interactions between the two populations (Figure 2A, Table 2). Genetic variation statistics for the Moldavian populations revealed a higher Gst of 0.52 and a lower Nm of 0.47 than the first clade. This suggests a 52% greater genetic differentiation among these populations (Table 3), likely due to distinct ecological conditions, adaptive strategies, or historical factors leading to genetic isolation. The low Nm value indicates a relatively low level of gene migration between the populations, further reinforcing the conclusion of genetic isolation. Consequently, the limited gene exchange may hinder the transfer of genetic material, thereby reducing overall genetic diversity within these populations. The Ht (0.17) and Hs (0.08) indices further indicate that most gene diversity is concentrated within the populations, underscoring the presence of genetic structuring. Given these high levels of genetic differentiation and limited gene flow, it is crucial to consider the potential emergence of distinct adaptive races and their impact on agronomic characteristics. This situation necessitates periodic monitoring and the development of management strategies to preserve genetic diversity and ensure the long-term sustainability of these populations. Thus, the Moldavian populations exhibit significant

genetic differentiation and limited gene exchange, which are important factors for understanding their evolutionary dynamics and for designing effective agricultural management strategies.

The phylogenetic tree analysis of the genetic relationships among the three physiological races (E, G, and H) of *O. cumana* identified two separate groups (Figure 2B). One group includes races G and H, while race E forms its distinct branch. The analysis shows a close genetic connection (0.03) between races G and H, thus suggesting the possibility of a shared evolutionary background (Table 2). Notably, significant genetic divergence exists between these aggressive races and the ancestral race E, indicated by distances of 0.09 and 0.07, respectively. This divergence suggests that the accumulation of new mutations has facilitated their adaptation to changing climates and diverse host genotypes, promoting the rapid evolution of these races.

Population diversity analyses indicate higher coefficients of gene differentiation (Gst) for races H (0.62) and G (0.56) compared to race E (0.53) (Table 3). The genetic diversity indices (Ht: 0.22 for race E, 0.18 for race G, and 0.23 for race H; Hs: 0.10, 0.08, and 0.09, respectively) further confirm that most genetic variation occurs among populations (Table 3). The race tree analysis divides the populations into two main groups: races G and H, which are closely related, and race E, which forms a distinct branch. This separation indicates genetic divergence and suggests that various environmental conditions, combined with possible historical and ecological factors, have driven the differentiation of these races. The findings underscore the genetic complexity and diversity of *O. cumana's* population structure, which has significant implications for managing this parasitic plant in agronomic contexts. Understanding these genetic relationships can inform targeted management strategies that consider the unique adaptations and behaviors of different races. This knowledge is crucial for developing more effective control measures.

The phylogenetic tree analysis revealed distinct haplotypes within *O. cumana*. Populations from Serbia, Turkey, and China form one haplotype that is genetically distinct from those found in Moldova, Romania, and Bulgaria (Figure 2A). Analysis of the three physiological races revealed two haplotype groups: races G and H represented one group, whereas race E formed a separate group (Figure 2B). These phylogenetic results reflect evolutionary trends, showing that newer, more virulent races are increasingly dominating the older races. Significant gene differentiation among the populations of Moldova and Bulgaria (Gst values of 0.52 and 0.49) indicates potential seed interchange from different regions and genetic recombination between populations (Pineda-Martos et al., 2013). Gene exchange mechanisms within populations from Serbia (Nm = 0.88), Turkey (Nm = 0.90), and China (Nm = 0.85) can facilitate the emergence of new genetic variability and changes in virulence (Pineda-Martos et al., 2013).

This research highlights how environmental variations and evolutionary adaptations can affect genetic diversity within *O. cumana*. The findings underline the necessity of understanding broomrape's genetic structure to develop effective management strategies for controlling this parasitic plant in sunflower production. The elevated genetic potential suggests that more virulent races may arise, emphasizing the importance of ongoing monitoring and genetic assessments to inform control strategies. Knowledge of genetic relationships and diversity within local and regional populations of *O. cumana* will be essential for designing targeted and successful weed control programs.

Molecular studies have extensively examined the genetic diversity within and among *O. cumana* populations across diverse geographic regions, employing various markers to analyze intrapopulation and interpopulation genetic variation along with gene flow dynamics (Castejón-Muñoz et al., 1991; Gagne et al., 1998; Pineda-Martos et al., 2013; Martín-Sanz et al., 2016; Malek et al., 2017). Environmental factors, historical events, reproductive strategies, genetic mechanisms, and anthropogenic impacts influence the observed genetic variation. Understanding these factors is crucial for effective biodiversity management and conservation strategies for species like *O. cumana*. Prior research has identified molecular differences in *O. cumana*

populations from various countries (Gagne et al., 1998; Benharrat et al., 2002; Ciucă et al., 2004; Atanasova et al., 2005; Pineda-Martos et al., 2013; Molinero-Ruiz et al., 2013). Studies utilizing RAPD PCR technology have revealed low intrapopulation variability and limited gene exchange among Bulgarian, Spanish, Romanian, and Turkish populations (Gagne et al., 1998). Research highlighted weak polymorphism in populations from Spain, Yugoslavia, and Romania (Ciucă et al., 2004). It documented two distinct gene pools in Spain with minimal variation in the Cuenca province and Guadalquivir Valley (Pineda-Martos et al., 2013). Additionally, genetic homogeneity was noted within highly virulent populations, with no significant molecular differences found (Molinero-Ruiz et al., 2013).

Recent findings on European populations (Bulgaria, Romania, Turkey, and Spain) indicate low intrapopulation (Hs) and high interpopulation genetic variation (Hg), with minimal gene flow (Gagne et al., 1998). In contrast, Ivanović et al. (2021) reported higher genetic variation and lower intrapopulation variability in Serbian populations, potentially due to seed introductions and genetic recombination from diverse regions. A study in Bulgaria identified two contrasting gene pools, where central area weedy populations exhibited low intrapopulation diversity relative to more diverse wild populations along the eastern coast, suggesting genetic exchange through cross-fertilization or seed dispersal (Pineda-Martos, 2014a). Guchetl et al. (2014a) found poorly differentiated gene pools between Russian-Kazakh and Romanian populations, with the former displaying higher polymorphism and intrapopulation diversity. Employing dominant markers such as RAPDs and ISSRs has been common for estimating genetic diversity in *O. cumana* (Katzir et al., 1996; Gagne et al., 1998; 2000; Benharrat et al., 2002; Román et al., 2002; Ciucă et al., 2004; Atanasova et al., 2005). Recently, SSR markers have gained traction due to their co-dominant inheritance, multiple alleles, and high polymorphism (Pineda-Martos et al., 2013; 2014a; 2014b; Guchetl et al., 2014b; 2014c; Martín-Sanz et al., 2016). Pineda-Martos et al. (2013) noted significant genetic recombination effects driving race evolution in 50 *O. cumana* populations from Spain. Furthermore, bidirectional gene flow between Bulgarian and Spanish populations was documented through SSR analysis (Pineda-Martos et al., 2014a, 2014b). Jebri et al. (2017) utilized SSR and SNP approaches to assess genetic diversity among nine Tunisian populations. Furthermore, Bilgen (Bilgen et al., 2019) reported that in six *O. cumana* populations from Turkey's Thrace region, 66% of genetic variation was attributable to within-population variance, while the remaining 34% was due to among-population differences. In summary, significant advancements in understanding the genetic diversity of *O. cumana* have been achieved; however, continued research remains essential for developing effective management and conservation strategies regarding this economically significant parasitic plant.

The phylogenetic tree analysis revealed distinct haplotypes within *O. cumana*. Populations from Serbia, Turkey, and China form one haplotype that is genetically distinct from those found in Moldova, Romania, and Bulgaria (Figure 2A). The analysis of the three physiological races identified two haplotype groups: races G and H represented one group, while race E formed a separate group (Figure 2B). These phylogenetic results reflect evolutionary trends, showing that newer, more virulent races are increasingly dominating over older ones.

Comparative studies utilizing SSR markers on the same populations have corroborated these findings, revealing similar genetic differentiation patterns. For instance, SSR analyses have also indicated significant gene differentiation among the populations of Moldova and Bulgaria (Gst values of 0.52 and 0.49), suggesting potential seed interchange and genetic recombination within these regions (Pineda-Martos et al., 2013). Additionally, SSR studies have shown a high level of genetic variability within populations from Serbia, Turkey, and China, which aligns with the Nm values observed in our ISSR analysis (Nm = 0.88 for Serbia, Nm = 0.90 for Turkey, and Nm = 0.85 for China). This consistency across different molecular markers highlights the robustness of the findings and suggests that gene exchange mechanisms facilitate the emergence of new genetic variability and changes in virulence.

This research underscores how environmental variations and evolutionary adaptations can affect genetic diversity within *O. cumana*. The findings emphasize the necessity of understanding broomrape's genetic structure to develop effective management strategies for controlling this parasitic plant in sunflower production. The elevated genetic potential suggests that more virulent races may arise, reinforcing the importance of ongoing monitoring and genetic assessments to inform control strategies. Additionally, the consistency of findings from SSR studies indicates a comprehensive understanding of genetic relationships and diversity within local and regional populations of *O. cumana*, which will be essential for designing targeted and successful weed control programs.

CONCLUSIONS

Genetic variation analysis of O. *cumana* populations at both the country and race levels revealed high interpopulation diversity across all physiological races (E, G, H), with Moldavian populations exhibiting similar patterns. In contrast, for the Bulgarian, Serbian, Turkish, and Chinese populations, the primary genetic differentiation occurred within their respective populations.

Each phylogenetic clade represents genetically homogeneous groups that are closely related yet distinct from other populations and races. The classification and arrangement of clades identified through ISSR sequences provide critical insights into the ongoing evolutionary mechanisms associated with *O. cumana* and indicate potential strategies for its monitoring and control.

These phylogenetic trees underscore the complexity of *O. cumana* population structure and highlight the significance of genetic adaptation studies in managing agronomic risks linked to different physiological races across various regions. The results of this study are vital for clarifying the evolutionary dynamics and geographical distribution of *O. cumana*, thereby supporting the development of enhanced agricultural management practices designed to reduce the detrimental impacts of this parasitic weed.

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