INTERPOPULATION RELATEDNESS OF *O. CUMANA* BELONGING TO DIFFERENT COUNTRIES

INTERRELAȚII DINTRE POPULAȚII DE *O. CUMANA* DIN DIFERITE ȚĂRI

Maria DUCA, ORCID: 0000-0002-5855-5194
Ana MUTU, ORCID: 0000-0001-8603-142X
Steliana CLAPCO, ORCID: 0000-0001-7147-2740
Moldova State University

UDC: 581.15:582.998.2
e-mail: mduca2000@yahoo.com

Orobanche cumana is an obligate root parasite that causes serious losses in sunflower production in many parts of the world. Host–parasite coevolution involves a continuous escalation of parasite virulence to overcome potential host resistance. Thus, eight races (races A to H) of broomrape were identified until now, the most virulent biotypes being spread especially in the Black sea region (Romania, Turkey, Ukraine and Bulgaria) [1]. A better understanding of population genetic mechanisms can provide useful insights into parasite evolution and enable the development of new control strategies of weeds. In this context, cluster analysis of interpopulation relatedness of *Orobanche cumana* from different sunflower growing countries was performed.

Thirty-three broomrape populations belonging to different sunflower producing countries (Moldova, Romania, Bulgaria, Serbia, China and Turkey) and races (E, G, H) were included in a comparative study of genetic diversity. The fresh tissue of 10-15 individual plants from each population, grown on sunflower genotypes in green house spot experiments, was collected and stored at –80°C until DNA extraction. The total genomic DNA was isolated from frozen material using *GeneJET Plant Genomic DNA Purification Mini Kit* #K0791 according to the manufacturer's protocol (*Thermo Fisher Scientific*, USA). SSR amplifications were carried out employing a total of 14 primers, in a thermocycler Veriti-96Well (*Applied Biosystems*) according to the standard procedure [2]. Ward's hierarchical agglomerative clustering method with squared Euclidean distance was performed using a program package (XLSTAT, version 2014).

Cluster analysis, with a high cophenetic correlation coefficients (0.86, $p < 0.01$), revealed three well differentiated groups (Figure 1). The largest cluster I grouped most of the populations from Eastern and Southeast Europe (Moldova, Romania and Bulgaria). The populations from Turkey and China formed two separate subcluster in second group and all Serbian broomrape populations were included in the 3rd cluster.

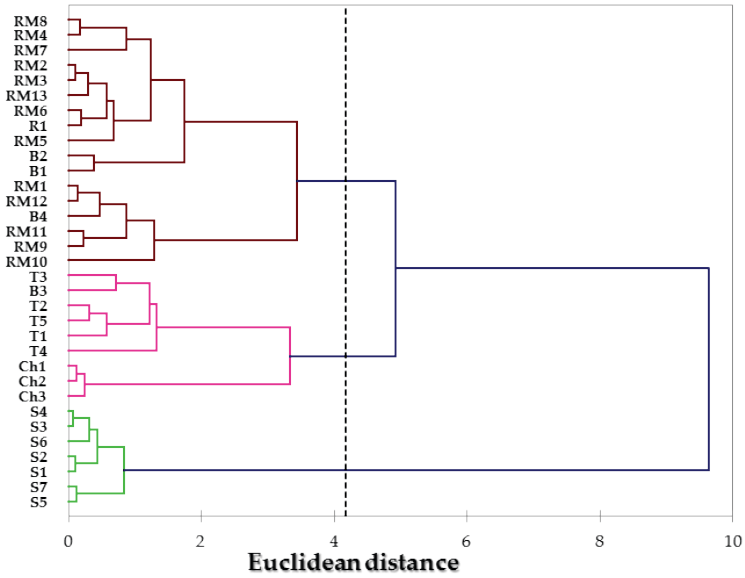


Fig. 1. Ward's method dendrogram based on Euclidean distances between 33 populations of *Orobanche cumana* belonging to the Republic of Moldova (RM1-RM13), Romania (R1), Turkey (T1-T5), Bulgaria (B1-B4), Serbia (S1-S7) and China (Ch1-Ch3) using SSR markers

The exception was second cluster resulting from the Ward analysis of the SSR data set, which supplementary included one population from Bulgaria (B3) clustering together with Turkish *O. cumana.* It has to be noted that this population, comparative to other Bulgarian samples collected especially from the regions closed to Romania, belonging to a location (Radnevo) situated in the proximity of Bulgarian-Turkish border. B3 is located in the same subcluster with T3 originated from Edirne (Turkey), which is placed at around 100 km distance.

A distribution of populations according to their geographical origin was observed inclusive in the case of Moldovan broomrape populations. Thus, *O. cumana* collected from the North (RM4 - Soroca, RM7 - Balti and RM7 – Prepelita) and South (RM2 – Svetlai, RM3 – Taraclia, RM13 – Comrat, RM5 – Alexanderfield) were clustered in two separate subclusters, while the populations from the Center and South-Est (RM1 – Izbiste, RM12 – Cazanesti, RM11 – Chisinau, RM9 – Grigorievca and RM9 – Popeasca) fell together into another subcluster. In this context, an unexpected result was the classification of geographically distant populations from China closer to Turkish broomrape.

Clustering was not influenced by the racial status of broomrape, the populations from different races (Tab. 1) being grouped together, similarly to the results reported by other researchers [3].

Table

Origin and racial status of *O. cumana* populations

Country	Collecting site	Population	Race	Country	Collecting site	Population	Race
Republic of Moldova	Izbiște	RM1	H	Serbia	ORSR 04	S1	≤E
	Svetlîi	RM2	H		ORSR 07	S2	≤E
	Taraclia	RM3	H		ORSR 11	S3	≤E
	Soroca	RM4	H		ORSR 14	S4	≤E
	Alexanderfield	RM5	H		ORSR 24	S5	≤E
	Balți	RM6	H		ORSR 25	S6	≤E
	Balți	RM7	H		ORSR 43	S7	≤E
	Prepelita	RM8	H	Bulgaria	Debovo	B1	G
	Grigorievca	RM9	H		Silanovtsi	B2	G
	Popeasca	RM10	G		Radnevo	B3	H
	Chisinau	RM11	G		Rosenova	B4	H
	Cazanesti	RM12	≤E	Turkey	Edirne, Keșan	T1	H
	Comrat	RM13	≤E		Adana	T2	H
China	Inner Mongolia, Bayanuur	Ch1	G		Edirne, Merker	T3	H
	Inner Mongolia, Hohhot	Ch2	H		Kirklareli, Luleburgaz	T4	H
	Hebei	Ch3	G		Trakia	T5	G

The cluster analysis of the SSR data set indicate the correspondence between clustering pattern and geographical origin of the populations, suggesting that the populations from closer regions may derive from the same introduction events. Clustering of populations was not associated with racial status of *O. cumana*.

References:

1. MOLINERO-RUIZ, L., DELAVAULT, Ph., PÉREZ-VICH, B. et al. History of the race structure of *Orobanche cumana* and the breeding of sunflower for resistance to this parasitic weed: A review.In: *Spanish Journal of Agricultural Research.* 13: e10R01.
2. SAMBROOK, J., RUSSELL, D. *Molecular cloning. A laboratory manual.* New York: Cold Spring Harbor Laboratory Press, 2001, vol. I-III. 1885 p.
3. PINEDA-MARTOS, R., VELASCO, L., FERNANDEZ-ESCOBAR, J. et al. Genetic diversity of *Orobanche cumana* populations from Spain assessed using SSR markers. In*: Weed Research*. 2013, no. 53, pp. 279–289

This study was supported by the research Project 20.80009.5107.01 „Genetico-molecular and biotechnological studies of the sunflower in the context of sustainable management of agricultural ecosystems”, funded by the NARD.