THE STUDY OF BROOMRAPE DIVERSITY IN DIFFERENT SUNFLOWER CULTIVATING COUNTRIES BASED ON MORPHOLOGICAL ARAMETERS OF PARASITE SEEDS

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Abstract

The root holoparasite *Orobanche cumana* Wallr. produces a very high number of extremely small seeds, which remain viable in the soil for decades and could be easily disseminated through the use of machinery or contaminated seeds. Due this fact and considering the global scale of sunflower seeds exchange, the control of parasite is extremely difficult. Currently broomrape is present in the majority of sunflower cultivation countries and spreads very quickly to new areas. In this context, it is of interest to analyze and highlight distinctive morphological features of *O. cumana* seeds collected from different European and Asian countries, such as the Republic of Moldova, Romania, Bulgaria, Ukraine, Serbia, Spain, Turkey and China. The morphometric analysis (seed length, width and length/width ratio) of broomrape samples did not show significant differences in their size. *O. cumana* seeds ranged between 0.316-0.393 mm x 0.148-0.176 mm, with a L/W ratio of 2.022-2,596. A moderate positive correlation (r=0.485) between length and width of broomrape seeds has been revealed. The mean value of L/W ratio in all investigated populations (38) was around 2.3, being in agreement with the results obtained by other authors and showing that *O. cumana* has preferentially elongated shape of seeds comparative to other broomrape species. Comparing to other studies, the mean value of L/W ratio was higher than 2.0 (2.18) even in Chinese populations. The coefficients of variation indicated low values, especially in the case of seed length (6.73-19.56%), which suggests a moderate level of intrapopulational variability, all studied populations being relative homogenous. The analysis of Euclidean distance showed small distances (0.001-0.577) between broomrape populations, the most distant being those collected from Seville (Spain), Tulcea (Romania), Xin Jiang (China), ORSR11 (Serbia), Popeasca and Sarata Mereseni (Republic of Moldova) and Edirne, Kesar (Turkey) in different combinations.

Key words: broomrape, *Orobanche cumana*, populations

The root, holoparasite *Orobanche cumana* Wallr. is one of the most important constraint for sunflower production in cultivating countries, which can cause from 20 to 100% yield decreasing (Domínguez J., 1996). The most affected traits are seed yield production, lipid and protein content in the seeds and oil content (Gisca I. *et al*, 2017; Duca M. *et al*, 2013).

Actually broomrape infests 16 million hectares of crop land worldwide and affects around 50% of the world's sunflower crops (*https://www3.nuseed.com*). The high price of oil, as well as the increasing market demand for sunflower seeds, contributes to the continuous growth of the areas cultivated with this culture. Excessive expansion of cultivated areas leads to irrational use of soils, failure of crop rotation, which causes the accumulation of pathogens in the soil.

Broomrape produces a huge number (around 500000 per plant) of extremely small seeds (between 200 and 300 µm), which remain viable in soil for decades (Plaza L. *et al*, 2004). Due to this fact, new infestations can easily occur through the use of machinery or contaminated seeds and the eradication/ prevention of parasitic weed is very difficult, especially considering the global scale of sunflower seeds exchange (Joel D. *et al*, 1987; Dongo A. *et al*, 2012). Breeding for crop resistance remains to be the most effective approach to control the parasite control. However, as response to continuous introduction of new resistant sunflower genotypes, broomrape evaluates, developing another virulent race, which overcomes the resistance (Pérez-Vich B. *et al*, 2013; Vrânceanu A. *et al*, 1980). Thus, during last two decades, highly virulent races of broomrape appeared and spread very quickly to new areas in Turkey (Kaya Y., 2014), Spain (Martin-Sanz A. *et al*, 2016), Bulgaria (Shindrova P., 2006), Romania (Pacureanu M. *et al*, 2009), Moldova (Duca M. *et*

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al, 2017b), Ukraine (Burlov B. *et al*, 2006), Russia (Antonova T. *et al*, 2013).

According to recent studies, high genetic diversity and frequency of mutation in relatively homogeneous populations of *O. cumana*, as well as genetic recombination, may be important driving forces of race evolution (Pineda-Martos R. *et al*, 2013; Martín-Sanz A. *et al*, 2016). Thus, in order to propose effective strategies for parasite control and reduction of productivity losses not only breeding efforts, but also better understanding of parasite biology and diversity are required.

Even genetic diversity within and between *O. cumana* populations has been successfully studied using different molecular tools, such as RAPD, ISSR and SSR (Benharrat *et al*, 2002; Duca M. *et al*, 2017c, 2019; Gagne *et al*, 1998; Bilgen *et al*, 2019; Guchetl *et al*, 2014; Pineda-Martos *et al*, 2013) the studies aimed to emphasis diversity within and among populations based on seed morphology, as well as to differentiate sunflower broomrape from different origins and races are still actual (Duca *et al*, 2017a; Krupp A. *et al*, 2014; Plaza L. *et al*, 2004). The usefulness of micromorphological traits of *Orobanche* seeds in differentiation of species or groups of species has been demonstrated by various authors (Abu Sbaih H. *et al*, 1994; Joel D., 1987; Plaza L. *et al*, 2004; Musselman L. *et al*, 1976). The high production of seeds per broomrape shoots, their size and shape, facilitates soil penetration and seed dissemination and are considered important factors in parasite adaptation and evolution (Piwowarczyk K., 2013; Plaza L. *et al*, 2004).

The main objective of this study was to evaluate the morphometric parameters (length, width and the ratio l/w) of broomrape seeds belonging to eight different European and Asian countries, such as the Republic of Moldova, Romania, Bulgaria, Ukraine, Serbia, Spain, Turkey and China.

MATERIAL AND METHODS

Seed morphology was studied in 38 populations of *Orobanche cumana* belonging to 8 different European and Asian countries, as follow **Serbia** (conventionally noted as – ***1**-ORSR 04; **2**-ORSR 07;**3**-ORSR 11; **4**-ORSR 14; **5**-ORSR 24; **6**-ORSR 25; **7**-ORSR 43*); **China** (***8**-Xin Jiang; **9**-He Bei; **10**-Inner Mongolia*); **Turkey** (***11**-Trakia; **12**-Adana; **13**-Tekirdag; **14**-Edirne, Merker; **15**-Kirklareli, Luleburgaz; **16**-Edirne, Keșar*); **Bulgaria** (***17**-Silanovici; **18**-Radnevo; **19**-Rosenova; **20**-Debovo*), **România** (***21**-Brăila; **22**-Tulcea*); **Republic of Moldova** (***23**-Chisinau; **24**-Sarata-Mereșeni; **25**-Popeasca; **26**-Gura-Galbenei; **27**-Prepelița; **28**-Izbiște; **29**-Svetlii; **30**-Alexanderfield; **31**- Balti; **32**-Taraclia; **33**-Egoreni; **34**-Singera; **35**-Congaz*), **Spain** (***36**-Sevilia*), **Ukraine** (***37**-Odessa; **38**-Izmail*).

The morphometric parameters (length, width and the ratio l/w) of broomrape seeds were evaluate by direct observation under light microscopy (Axio Zeiss Scope A1). Seed length was measured using the longest axis from end to end and width was measured using the longest axis at a 90° angle of the length.

Data on the width, length and the ratio l/w of 100 seeds per populations were subjected to elementary statistical procedures (the mean, the standard deviation and coefficient of variation). Statistical calculations of investigated morphological characters of broomrape seeds were performed using XLSTAT software (*https://www.xlstat.com/*).

Morphological characters were evaluated by one-way analysis of variance (ANOVA). The test checks whether there are significant differences in mean values of observed characters between populations. Values were considered significant if $p < 0.05$ in the Tukey test. The correlation between the morphometric parameters of broomrape seeds were tested by calculating the Pearson's correlation coefficient. The data were analyzed by computing, for each pairs, dissimilarity scores among the samples, using the Euclidean distance measure.

RESULTS AND DISCUTION

Broomrape seeds are smaller than 1.0 mm in length, with a wide variety of shapes (ellipsoid, oblongoid or ovoid) and seed coat (Plaza L. *et al*, 2004; Duca M. *et al*, 2017a). The morphometric analysis of broomrape seeds from eight different sunflower cultivating countries did not show significant differences in their size (*table 1*). Thus, mean seed length of investigated populations varying between 0.316-0.393 mm, with minimal and maximal values in the case of samples from Inner Mongolia (China) and Egoreni (Moldova), respectively. The most of the accession (79%) ranged between 0.341 mm to 0.375 mm.

For the seed length, the coefficients of variation ranged between 6.73-19.56%, which suggests a low level of intrapopulational variability. According to this parameter the most homogenous are populations from Tulcea, Romania (CV=6.73%), followed by Seville, Spain (CV=7.07%), Adana and Edirne, Merker from Turkey (CV=9.49% and 9.83%, respectively) and two populations (Egoreni and Balti) from the Republic of Moldova (CV=9.53% and 9.74%, respectively). Other populations are relative homogenous with the maximal values of CV in the samples belonging from Inner Mongolia, China (CV =19.56%) and Prepelita, Moldova (CV =17,22%).

Regarding the seed width, the lowest values were established in the population collected from Gura Galbenii (Republic of Moldova) followed by samples from Inner Mongolia (0.148 and 0.149 mm, respectively) and the largest ones (0.176 mm) were revealed in Chinese and Ukrainian populations Xin Jiang and Ismail. In the case of the majority of analysed populations (74%) the width was included in the interval of 0.151-0.167 mm. The seed width was more variable than length, the coefficient of variation ranging between 11.4% and 23.43%, with minimum observed in the samples from Turkey (Kirklareli, Luleburgaz and Tekirdag) and maximum in those belonging to Rosnevo (Bulgaria).

Table 1

Seed characteristics of *Orobanche cumana* belonging to different countries

Locality/ conventional notation	Seed length, mm	CV, %	Seed width, mm	CV, %	Length / Width ratio	CV, %
Serbia						
1-*ORSR 04*	0.343±0.044	12.76	0.154±0.023	14.85	2.265±0.413	18.25
2-*ORSR 07*	0.384±0.047	12.37	0.165±0.022	13.54	2.358±0.362	15.36
3-*ORSR 11*	0.335±0.045	13.48	0.160±0.023	14.42	2.133±0.372	17.44
4-*ORSR 14*	0.345±0.050	14.49	0.154±0.031	19.89	2.300±0.461	20.05
5-*ORSR 24*	0.346±0.045	12.87	0.158±0.023	14.65	2.236±0.452	20.20
6-*ORSR 25*	0.363±0.038	10.49	0.162±0.020	12.50	2.272±0.358	15.74
7-*ORSR 43*	0.359±0.037	10.40	0.153±0.021	13.44	2.387±0.415	17.39
China						
8-*Xin Jiang*	0.360±0.050	13.95	0.176±0.023	12.82	2.083±0.389	18.66
9-*He Bei*	0.382±0.043	11.29	0.171±0.023	13.67	2.275±0.407	17.87
10-*Inner Mongolia*	0.316±0.062	19.56	0.149±0.029	19.27	2.172±0.455	20.97
Turkey						
11-*Trakya*	0.358±0.051	14.25	0.158±0.024	15.40	2.314±0.460	19.90
12- *Adana*	0.337±0.032	9.49	0.155±0.020	13.25	2.208±0.302	13.68
13-*Tekirdag*	0.352±0.036	10.10	0.154±0.018	11.57	2.316±0.318	13.73
14-*Edirne, Merker*	0.355±0.035	9.83	0.164±0.022	13.28	2.207±0.354	16.04
15- *Kirklareli, Luleburgaz*	0.358±0.039	11.01	0.159±0.018	11.39	2.274±0.342	15.04
16-*Edirne, Kesar*	0.350±0.030	10.41	0.166±0.022	13.29	2.137±0.323	15.13
Bulgaria						
17-*Silanovici*	0.363±0.059	16.14	0.157±0.025	15.73	2.355±0.465	19.74
18-*Radnevo*	0.353±0.058	16.51	0.162±0.032	19.79	2.224±0.408	18.36
19-*Rosenova*	0.337±0.060	17.86	0.151±0.035	23.43	2.296±0.458	19.93
20-*Debovo*	0.388±0.041	10.45	0.163±0.024	14.95	2.423±0.429	17.69
Romania						
21-*Braila*	0.350±0.046	13.23	0.150±0.026	17.42	2.382±0.425	17.85
22-*Tulcea*	0.384±0.026	6.73	0.150±0.020	13.38	2.596±0.305	11.75
Republic of Moldova						
23-*Chisinau*	0.372±0.047	12.58	0.174±0.028	16.01	2.196±0.443	20.19
24-*Sarata-Mereseni*	0.325±0.048	14.86	0.164±0.030	18.59	2.022±0.336	16.63
25-*Popeasca*	0.327±0.049	14.93	0.157±0.026	16.61	2.116±0.382	18.05
26-*Gura-Galbenei*	0.341±0.050	14.58	0.148±0.027	18.25	2.356±0.441	18.71
27-*Prepelita*	0.353±0.061	17.22	0.158±0.025	16.07	2.259±0.411	18.18
28-*Izbiste*	0.344±0.046	13.52	0.157±0.025	16.07	2.241±0.445	19.87
29-*Svetlii*	0.344±0.050	14.68	0.157±0.026	16.41	2.226±0.350	15.71
30-*Alexanderfield*	0.361±0.050	13.88	0.165±0.028	17.19	2.228±0.394	17.67
31- *Balti*	0.358±0.035	9.74	0.161±0.022	13.61	2.258±0.352	15.57
32-*Taraclia*	0.386±0.046	11.82	0.163±0.028	16.94	2.434±0.464	19.07
33-*Egoreni*	0.393±0.037	9.53	0.174±0.025	14.17	2.312±0.420	18.18
34-*Singera*	0.353±0.035	9.92	0.167±0.021	12.56	2.155±0.356	16.54
35-*Congaz*	0.342±0.051	14.78	0.154±0.027	17.31	2.264±0.414	18.26
Spain						
36-*Sevilia*	0.388±0.027	7.07	0.154±0.023	14.88	2.560±0.360	14.05
Ukraine						
37-*Odessa*	0.375±0.040	10.81	0.174±0.023	13.14	2.179±0.312	14.30
38-*Izmail*	0.373±0.039	10.39	0.176±0.024	13.67	2.160±0.350	16.22

*Notes: Values represent the mean ± standard deviation of n=100 in mm. CV, % = (σ/μ)*100, where CV - Coefficient of Variation; σ - Standard Deviation; μ = Mean*

With the regard to length / width ratio, it varied between 2.022-2.596. Seeds collected from Tulcea (Romania) and Seville (Spain) had significantly higher values compared to other samples (2.560 and 2.596, respectively), while the seeds from Sarata Mereseni (Moldova) and Xin Jiang (China) indicating the smallest values (2.022 and 2.083, respectively). The mean value of L/W ratio in all investigated populations (38) was around 2.3, being in agreement with the results obtained by other authors and showing that *O. cumana* has preferentially elongated shape of seeds comparative to other broomrape species. Thus, in the case of *O. cernua, O. crenata, O. minor*, *O. caryophyllaceae* and some *Phelipanche* species L/W ratio is ≤ 2.0, which denotes a more spherical shape of seeds (Krupp A. *et al*, 2014; Plaza L. *et al*, 2004). According to Krupp et al (2014) a more spherical shape of seeds is also characteristic for Asian *O. cumana* samples (L/W=1.87-1.96) comparing to European. In contrast, in our study the mean value of L/W ratio was higher than 2.0 (2.18) even in Chinese populations.

Broomrape seeds reached a mean size of 0.363 x 0.160 mm, with a L/W ratio of 2.31 in the 38 investigated populations (*table 2*). The association analysis revealed a moderate positive correlation (r=0.485) between length and width of broomrape seeds.

Table 2

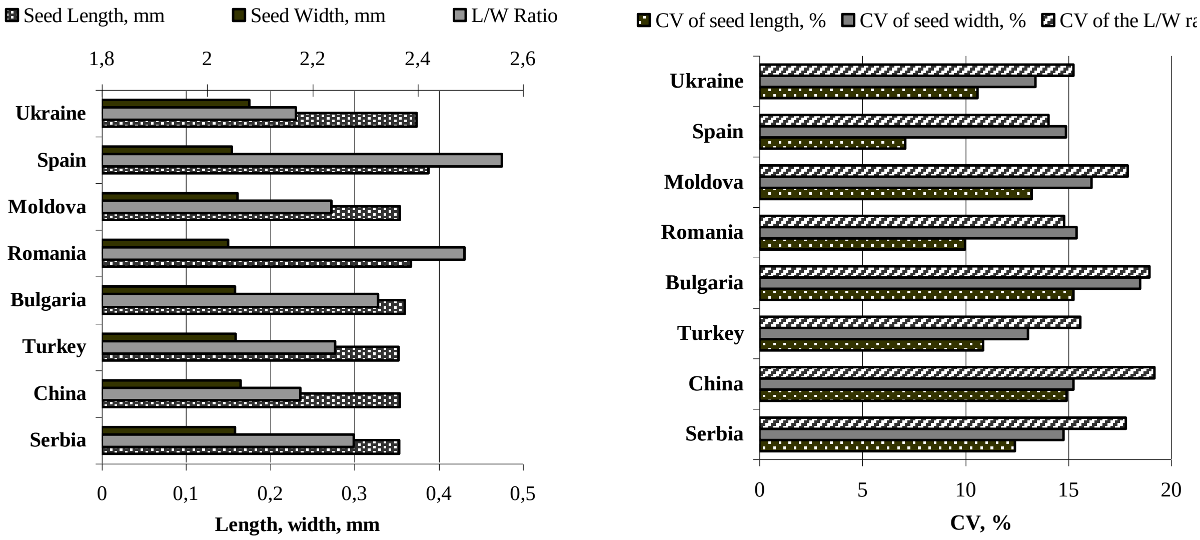
Mean values of morphometric parameters and the correlation coefficient

Number of populations	**Morphometric parameters** (***x*** ±$t_{05}Sx$) ***p<0,05***)			**Correlation coefficient *r* (*Pearson*, * *p-value < 0,05*)**
	L (mm)	**W(mm)**	**L/W**	**L:W**
38	0.363±0.004	0.160±0.003	2.310±0.048	0.485*

Notes: L - seed length; W - seed width; Tukey test of statistical significance (α=0.05) was used

The mean values of seed length, width, L/W ratio and coefficient variation (CV) per country are presented in *figure 1* (a, b). According to morphometric data, the most elongated seeds were observed in the case of Seville population, which indicated the highest values of seed length (0.388 x 0.154 mm) and L/W ratio (2.56). Previously, Plaza et al (2004) and Krupp et al (2014) reported seed size of 0.36-0.50 x 0.16-0.25 mm and 0.360 x 0.155 mm, respectively, for Spanish broomrape.

Similar, low divergences between the results of our measurements and those of Krupp have been revealed with the regard to Serbian (0.353 x 0.154 mm and 0.380 x 0.170 mm, respectively) and Romanian populations (0.367 x 0.150 mm and 0.357 x 0.159 mm, respectively).



a. **b.**

Figure 1. **The mean values of seed length, width, L/W ratio (a) and coefficient variation (b) per country**

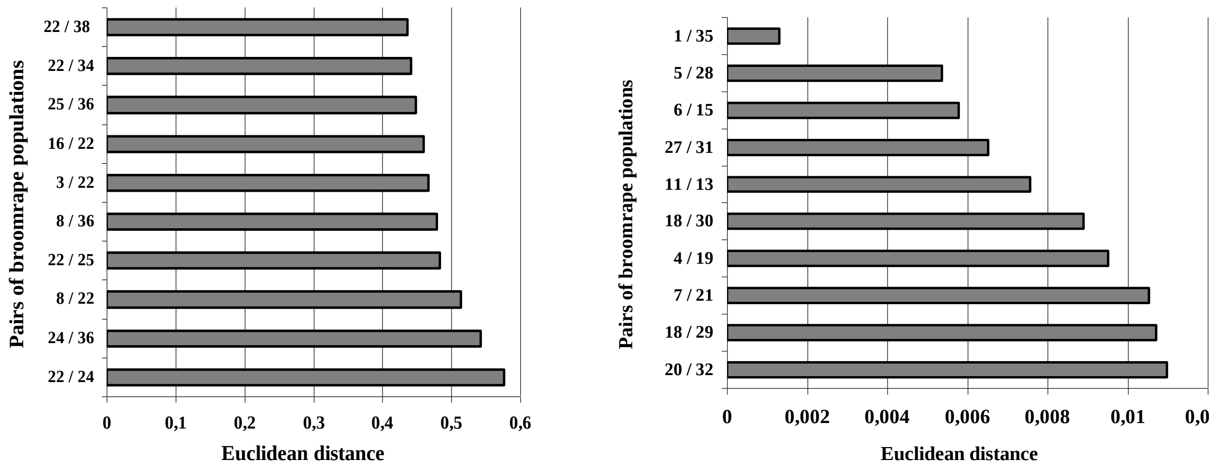
As for Moldavian samples, comparing obtained results with those of the analysis on 39 populations of sunflower broomrape collected in 2014 (Duca M. *et al*, 2017a) and 5 collected in 1921, 1938, 1948 and 1979 (unpublished data), no significant differences have been found. Thus, seed parameters have not changed substantially during *O. cumana* evolution.

A moderate magnitude of variability was recorded in the sunflower broomrape populations collected from different countries (*figure 1b*). Length/ width ratio showed the highest variability, especially in the samples collected from China and Bulgaria (CV=19.17% and 18.93%, respectively), followed by the Republic of Moldova (CV=17.89%) and Serbia (CV=17.78%).

Notably, Bulgarian samples indicated the highest values of CV inclusively for seed length (15.24%) and width (18.48%). On the other hand, the less variable trait was the seed length, the mean of CV ranging between 7.08-15.24%.

According to the measured seed parameters, the variability was higher within the individuals from the population (100 individual seeds) than among populations.

The analysis of Euclidean distance showed small distances between studied *O. cumana* populations, the shortest distance (0.001) being observed between the Serbian samples conventionally noted ORSR04 and Moldavian population Congaz and the longest one (0.577) between Tulcea (Romania) and Sarata Mereseni (Moldova) (*figure 2*). For almost 77.6% of the total combinations, the distance values were smaller than 0.200 and only in 19 cases (2.7%) the distance ranged between 0.400-0.577. The Serbian samples ORSR04, ORSR24 ORSR25 and populations Congaz; Izbiste (Moldova) and Kirklareli, Luleburgaz (Turkey), respectively, were the most similar, presenting the smallest values of Euclidean distances (0.001-0.006) (Figure 2a).



a. **b.**

Figure 2. **The minimal (a) and maximal (b) values of Euclidean distance between broomrape samples**

The most distant broomrape populations (0.448-0.577) were those collected from Seville (Spain), Tulcea (Romania), Xin Jiang (China), ORSR11 (Serbia), Popeasca and Sarata Mereseni (Republic of Moldova) and Edirne, Kesar (Turkey) in different combinations (Figure 2b). The differences in the morphometric characters of broomrape seeds collected from different regions, can result especially from the differences in environmental conditions (climatic and soil conditions), as well as nutrient supply from the host (Piwowarczyk K., 2013).

CONCLUSIONS

The current investigation reports the studies on morphometric parameters of the *O. cumana* seeds collected from 38 locations of the eight different European and Asian countries, as well as the Republic of Moldova, Romania, Bulgaria, Ukraine, Serbia, Spain, Turkey and China. Analysis of data (seed length, width and length/width ratio) did not show significant differences in broomrape seeds size. The coefficients of variation indicated low values, especially in the case of seed length (6.73-19.56%), which suggests a moderate level of intrapopulational variability, all studied populations being relative homogenous. More variable morphological trait were seed width and L/W ratio (11.39 - 23.43%). A moderate positive correlation (r=0.485) between length and width of broomrape seeds has been found. The present study provides some new data related the *O. cumana* phenotypical diversity and could be useful for understanding the complex parasite biology and development of the effective disease management strategies.

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