Maria Duca and Adriana Boicu* Morphological Diversity of Seeds in O. cumana Accessions from Republic of Moldova

https://doi.org/10.1515/helia-2017-0005 Received March 22, 2017; accepted October 19, 2017; previously published online October 28, 2017

Abstract: The seed morphology of 38 samples representing *O. cumana* populations from Republic of Moldova were examined to explore patterns of variation among the accessions. Features of seeds were assessed employing light microscopy and analyzed statistically. The morphological differences in length/ width ratio and seed shape in local broomrape populations have been established. The size of seeds ranged from 0.36 ~ 0.42 mm in length, and 0.14~0.18 mm in width. Seeds were generally small, ovoid to elongated in shape. Using one-way test (ANOVA), cluster and principal coordinate analysis, two groups were established by seed characteristics.

Keywords: broomrape, light microscopy, Orobanche cumana, populations, seed morphology, variability

Introduction

Broomrape (*Orobanche cumana* Wallr.) is an obligate chlorophyll-lacking holoparasite that lives attached to the roots of sunflower (*Helianthus annuus* L.), depleting the plant of nutrients and water. This parasitic plant has spread in Romania (Pacureanu-Joita *et al.*, 2012), Ukraine (Burlov and Burlov, 2010), Bulgaria (Shindrova, 2006; Shindrova and Penchev, 2012), Serbia (Škorić and Jocić, 2005; Maširević and Medić-Pap, 2009), Spain (Fernandez-Escobar *et al.*, 2009), Turkey (Kaya *et al.*, 2004; Bülbül *et al.*, 2009) et al. Actually, a considerable expansion has been noticed in the Republic of Moldova, especially in the drier and warmer areas of central and south-eastern regions with

^{*}Corresponding author: Adriana Boicu, Center of Functional Genetics, University of the Academy of Sciences of Moldova, 3/2 Academy str., Chisinau, MD-2028, Republic of Moldova, E-mail: aacciu@yahoo.com

Maria Duca, Center of Functional Genetics, University of the Academy of Sciences of Moldova, 3/2 Academy str., Chisinau, MD-2028, Republic of Moldova, E-mail: mduca2000@yahoo.com

increasingly occurrence during the last 5 years and considerable yield losses (Duca, 2015; Duca *et al.*, 2017; Glijin, 2014).

One of the major factors of wide geographical distribution of broomrape is the nature of their seed. A single broomrape plant produces thousands of tiny seeds, which can be easily dispersed by water, wind, animals, humans, machinery or attached to sunflower seeds (Castejon *et al.*, 1991).

The high production of seeds per capsule, the size and shape of seeds are important factors in the species' adaptation strategy to the environment aimed to increase the probability of finding new habitats (Plaza *et al.*, 2004; Piwowarczyk, 2013).

Shape of seeds facilitates soil penetration. Broomrape seeds survive and persist in soil for decades (Ungurean, 1986), forming a durable seed bank (Thompson *et al.*, 1997; Gevezova *et al.*, 2012) and thus maintain the parasite ability to germinate whenever a suitable host plant occasionally grows nearby (Škorić, 1988).

Based on the parasite morphology of seeds, using light microscopy (Ungurean, 1986), scanning electron microscopy (Jones and Safa, 1982; Abu Sbaih and Jury, 1994; Plaza *et al.*, 2004) and dark-field holographic microscopy (Duca *et al.*, 2014b) studies of diversity within and among populations were done. It was demonstrated that seed coat sculpturing can be used in the analysis of soil seed banks (Joel, 1987).

The aim of this study was to determine the micromorphological peculiarities of *O. cumana* populations seeds, sampled throughout Republic of Moldova, which would be of a great interest due to fast dispersion of infestations into new areas of cultivated sunflower.

Methods and materials

Plant material

The plant material studied here comes from several expeditions carried out in around forty locations of the Republic of Moldova, during 2006–2014 (Table 1). The samples of *O. cumana* were collected from naturally infected sunflower fields. The seeds were extracted from dried inflorescences and kept in the dark at 18 °C. On the basis of their ability to parasitize a set of differential lines and hybrids of sunflower these populations were classified in four physiological races $- \le E$, F, G, H (Duca *et al.*, 2017).

| Zone | Locality | Geographical coordinates | Year | Race |
|--------|---------------|--------------------------------------|------|------------|
| NORTH | Donduseni | Lat. N: 48.228892/Long. E: 27.613299 | 2011 | ≤ E |
| | Soroca | Lat. N: 48.104989/Long. E: 28.192428 | 2014 | G |
| | Balti | Lat. N: 47.780884/Long. E: 27.849793 | 2009 | н |
| | Prepelita | Lat. N: 47.579726/Long. E: 28.362788 | 2014 | F |
| CENTRE | Verejeni | Lat. N: 47.334185/Long. E: 28.261061 | 2014 | G |
| | Cazanesti | Lat. N: 47.381411/Long. E: 28.292311 | 2014 | ≤ E |
| | Brinzenii Noi | Lat. N: 47.373933/Long. E: 28.294544 | 2014 | ≤ E |
| | Ciocilteni | Lat. N: 47.303564/Long. E: 28.373435 | 2014 | н |
| | Rassvet | Lat. N: 47.204661/Long. E: 28.425010 | 2014 | F |
| | Frasinesti | Lat. N: 47.036199/Long. E: 28.034449 | 2014 | G |
| | Izbiste | Lat. N: 47.201635/Long. E: 29.078819 | 2014 | ≤ E |
| | Holercani | Lat. N: 47.323136/Long. E: 29.054021 | 2014 | ≤ E |
| | Chisinau | Lat. N: 46.575126/Long. E: 28.532648 | 2011 | ≤ E |
| | Singera | Lat. N: 46.888354/Long. E: 28.982607 | 2011 | ≤ E |
| | Bacioi | Lat. N: 46.915182/Long. E: 28.916058 | 2012 | ≤ E |
| | Floreni | Lat. N: 46.954296/Long. E: 28.962104 | 2014 | ≤ E |
| | Buteni | Lat. N: 46.852859/Long. E: 28.621420 | 2014 | ≤ E |
| | S. Mereseni | Lat. N: 46.774406/Long. E: 28.524220 | 2014 | н |
| | F. Galbenei | Lat. N: 46.881300/Long. E: 28.62378 | 2014 | ≤ E |
| SOUTH | Cazangic | Lat. N: 46.513600/Long. E: 28.417823 | 2014 | ≤ E |
| | Gura Galbenei | Lat. N: 46.713128/Long. E: 28.671418 | 2014 | G |
| | Cimislia | Lat. N: 46.530926/Long. E: 28.786454 | 2008 | ≤ E |
| | Grigorievca | Lat. N: 46.694832/Long. E: 29.342701 | 2014 | F |
| | Ermoclia | Lat. N: 46.333956/Long. E: 23.374065 | 2014 | н |
| | Talmaza | Lat. N: 46.627920/Long. E: 29.685250 | 2014 | G |
| | Stefan-Voda | Lat. N: 46.495555/Long. E: 29.684809 | 2006 | F |
| | Congaz | Lat. N: 46.126402/Long. E: 28.563278 | 2014 | н |
| | Chirsova | Lat. N: 46.152590/Long. E: 28.39645 | 2014 | G |
| | Besalma | Lat. N: 46.172859/Long. E: 28.648532 | 2014 | G |
| | Svetlii | Lat. N: 46.22800/Long. E: 28.334264 | 2014 | Н |
| | Carabetovca | Lat. N: 46.415016/Long. E: 28.887975 | 2014 | F |
| | Corten | Lat. N: 46.031638/Long. E: 28.718046 | 2014 | Н |
| | Ciadir-Lunga | Lat. N: 46.067189/Long. E: 28.790938 | 2006 | G |
| | Taraclia | Lat. N: 45.532478/Long. E: 28.351192 | 2009 | н |
| | Alexanderfeld | Lat. N: 45.806667/Long. E: 28.427778 | 2014 | н |
| | Manta | Lat. N: 45.792166/Long. E: 28.245798 | 2014 | G |
| | Slobozia-Mare | Lat. N: 45.622433/Long. E: 28.312738 | 2014 | F |
| | Crihana Veche | Lat. N: 45.834656/Long. E: 28.206904 | 2014 | ≤ E |

Table 1: Geographical origin of broomrape populations used in this study.

Morphological analysis of seeds

Morphological characteristics such as the length, width, ratio of L/W, shape and ornamentation of the broomrape seeds were evaluated. The size (length and width) of 100 seeds taken randomly from each accession, were investigated with 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.

Statistical analysis

Statistical calculations of investigated morphological characters of broomrape seeds were performed using InfoStat software (version 2016, InfoStat Group, University Córdoba, Argentina).

The collected data were subjected to elementary statistical procedures (the mean, the standard deviation, minimum and maximum value). 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 In order to classifying the studied accessions, cluster analysis was performed, and then a dendrogram was constructed.

Results and discussion

The seed morphometric characters of broomrape accessions examined by light microscopy are shown in Table 2. The micromorphological features of the seeds as seen in Table 3, including micrographs provided an additional source of characters for the separation of the examined populations.

Morphometric parameters of seeds

Mean **seed length** ranged from 0.36 to 0.41 mm and the majority (89%) of accessions, excepted samples from Donduseni, Izbiste, Alexanderfield and Crihana Veche, did not exceed 0.39 mm. The minimum individual seed length (0.216 mm) has been revealed in population from Stefan-Voda, while the maximum (0.54 mm) was established in Chisinau accession. It is notable that the standard deviation of seed length is low (0.031–0.056 mm), the coefficients of

| Locality | Seed Length [mm] | CV , % | Seed Width [mm] | CV , % | Ratio L/W | CV , % |
|---------------|------------------|---------------|-----------------|---------------|-------------|---------------|
| Donduseni | (0.284) 0.404 ± | 11.1 | (0.095) 0.155 ± | 16.1 | 2.682 ± | 19.2 |
| | 0.045 (0.473) | | 0.025 (0.216) | | 0.514 | |
| Soroca | (0.284) 0.393 ± | 12.7 | (0.095) 0.181 ± | 22.1 | $2.270 \pm$ | 22.7 |
| | 0.050 (0.486) | | 0.040 (0.324) | | 0.515 | |
| Balti | (0.270) 0.355 ± | 13.8 | (0.108) 0.145 ± | 15.2 | $2.507 \pm$ | 20.6 |
| | 0.049 (0.446) | | 0.022 (0.203) | | 0.516 | |
| Prepelita | (0.284) 0.385 ± | 11.7 | (0.135) 0.165 ± | 13.9 | $2.375 \pm$ | 17.2 |
| | 0.045 (0.473) | | 0.023 (0.216) | | 0.408 | |
| Verejeni | (0.270) 0.366 ± | 10.9 | (0.095) 0.170 ± | 21.8 | 2.258 ± | 24.4 |
| | 0.040 (0.473) | | 0.037 (0.270) | | 0.551 | |
| Cazanesti | (0.284) 0.386 ± | 10.9 | (0.108) 0.184 ± | 14.1 | 2.135 ± | 17.3 |
| | 0.042 (0.486) | | 0.026 (0.243) | | 0.369 | |
| Brinzenii Noi | (0.230) 0.380 ± | 13.2 | (0.108) 0.159 ± | 19.5 | 2.453 ± | 20.1 |
| | 0.050 (0.473) | | 0.031 (0.243) | | 0.494 | |
| Ciocilteni | (0.243) 0.356 ± | 11.2 | (0.095) 0.162 ± | 19.8 | 2.312 ± | 26.2 |
| | 0.040 (0.446) | | 0.032 (0.230) | | 0.606 | |
| Rasvet | (0.311) 0.371 ± | 9.4 | (0.122) 0.164 ± | 13.4 | 2.299 ± | 14.4 |
| | 0.035 (0.446) | | 0.022 (0.203) | | 0.332 | |
| Frasinesti | (0.270) 0.372 ± | 12.1 | (0.095) 0.150 ± | 18.7 | 2.558 ± | 18.6 |
| | 0.045 (0.486) | | 0.028 (0.203) | | 0.476 | |
| Izbiste | (0.270) 0.414 ± | 12.6 | (0.081) 0.153 ± | 19.6 | 2.843 ± | 27.5 |
| | 0.052 (0.513) | | 0.030 (0.203) | | 0.781 | |
| Holercani | (0.243) 0.383 ± | 14.1 | (0.095) 0.163 ± | 22.1 | 2.454 ± | 22.7 |
| | 0.054 (0.473) | | 0.036 (0.257) | | 0.557 | |
| Chisinau | (0.270) 0.379 ± | 12.4 | (0.108) 0.161 ± | 16.1 | 2.413 ± | 19.3 |
| | 0.047 (0.540) | | 0.026 (0.216) | | 0.465 | |
| Singera | (0.257) 0.381 ± | 13.1 | (0.108) 0.168 ± | 20.8 | 2.331 ± | 17.0 |
| - | 0.050 (0.473) | | 0.035 (0.257) | | 0.397 | |
| Bacioi | (0.297) 0.384 ± | 10.9 | (0.135) 0.170 ± | 11.2 | 2.277 ± | 12.6 |
| | 0.042 (0.473) | | 0.019 (0.216) | | 0.286 | |
| Floreni | (0.270) 0.363 ± | 10.5 | (0.081) 0.142 ± | 16.2 | 2.635 ± | 20.8 |
| | 0.038 (0.432) | | 0.023 (0.189) | | 0.549 | |
| Buteni | (0.270) 0.366 ± | 10.7 | (0.095) 0.143 ± | 20.3 | 2.659 ± | 22.3 |
| | 0.039 (0.446) | | 0.029 (0.216) | | 0.593 | |
| S.Mereseni | (0.297) 0.380 ± | 10.0 | (0.122) 0.161 ± | 15.5 | 2.404 ± | 16.4 |
| | 0.038 (0.459) | | 0.025 (0.216) | _ | 0.395 | |
| F.Galbenei | (0.270) 0.362 ± | 12.4 | (0.081) 0.148 ± | 21.6 | 2.546 ± | 20.1 |
| | 0.045 (0.432) | | 0.032 (0.230) | | 0.511 | |

 Table 2: Seed characteristics of Orobanche cumana accessions.

(continued)

| Table 2: (| continued) |
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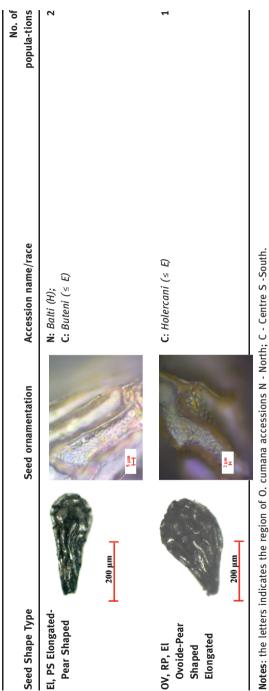
| Locality | Seed Length [mm] | CV , % | Seed Width [mm] | CV , % | Ratio L/W | CV , % |
|--------------|------------------|---------------|-----------------|---------------|----------------|---------------|
| Cazangic | (0.270) 0.363 ± | 9.1 | (0.122) 0.154 ± | 15.6 | 2.408 ± | 15.2 |
| | 0.033 (0.405) | | 0.024 (0.203) | | 0.366 | |
| G.Galbenei | (0.270) 0.360 ± | 10.6 | (0.122) 0.174 ± | 18.4 | $2.139 \pm$ | 20.7 |
| | 0.038 (0.405) | | 0.032 (0.243) | | 0.442 | |
| Cimislia | (0.284) 0.383 ± | 10.2 | (0.108) 0.164 ± | 14.0 | 2.392 ± | 17.0 |
| | 0.039 (0.459) | | 0.023 (0.216) | | 0.406 | |
| Grigorievca | (0.270) 0.368 ± | 12.2 | (0.081) 0.169 ± | 18.9 | $2.263 \pm$ | 22.1 |
| | 0.045 (0.446) | | 0.032 (0.230) | | 0.500 | |
| Ermoclia | (0.270) 0.368 ± | 12.8 | (0.095) 0.165 ± | 21.2 | $2.324 \pm$ | 24.5 |
| | 0.047 (0.473) | | 0.035 (0.243) | | 0.570 | |
| Talmaza | (0.284) 0.387 ± | 11.9 | (0.122) 0.161 ± | 14.3 | 2.445 ± | 18.7 |
| | 0.046 (0.486) | | 0.023 (0.216) | | 0.456 | |
| Stefan-Voda | (0.216) 0.377 ± | 14.9 | (0.108) 0.170 ± | 18.8 | 2.279 ± | 20.2 |
| | 0.056 (0.486) | | 0.032 (0.243) | | 0.461 | |
| Congaz | (0.257) 0.359 ± | 15.0 | (0.081) 0.148 ± | 17.6 | 2.479 ± | 21.2 |
| | 0.054 (0.473) | | 0.026 (0.203) | | 0.525 | |
| Chirsova | (0.297) 0.371 ± | 8.4 | (0.095) 0.162 ± | 19.1 | 2.378 ± | 21.8 |
| | 0.031 (0.432) | | 0.031 (0.216) | | 0.519 | |
| Besalma | (0.297) 0.387 ± | 10.3 | (0.095) 0.152 ± | 16.4 | 2.599 ± | 17.6 |
| | 0.040 (0.473) | | 0.025 (0.216) | | 0.457 | |
| Svetlii | (0.257) 0.371 ± | 11.9 | (0.095) 0.164 ± | 18.3 | 2.350 ± | 23.4 |
| | 0.044 (0.459) | | 0.030 (0.243) | | 0.550 | |
| Carabetovca | (0.257) 0.375 ± | 13.9 | (0.081) 0.171 ± | 18.1 | 2.270 ± | 23.3 |
| | 0.052 (0.486) | | 0.031 (0.243) | | 0.530 | |
| Corteni | (0.284) 0.379 ± | 11.9 | (0.108) 0.164 ± | 16.5 | 2.367 ± | 17.5 |
| | 0.045 (0.473) | | 0.027 (0.203) | | 0.415 | |
| Ciadir-Lunga | (0.284) 0.379 ± | 12.4 | (0.122) 0.165 ± | 12.7 | 2.331 ± | 17.2 |
| | 0.047 (0.486) | | 0.021 (0.203) | | 0.402 | |
| Taraclia | (0.311) 0.367 ± | 10.9 | (0.095) 0.148 ± | 19.6 | 2.557 ± | 18.0 |
| | 0.040 (0.459) | | 0.029 (0.216) | | 0.460 | |
| Alexanderfel | (0.324) 0.418 ± | 9.3 | (0.095) 0.173 ± | 17.3 | 2.504 ± | 23.4 |
| | 0.039 (0.486) | | 0.030 (0.243) | | 0.585 | |
| Manta | (0.338) 0.399 ± | 10.0 | (0.095) 0.158 ± | 19.0 | 2.635 ± | 23.5 |
| | 0.040 (0.500) | | 0.030 (0.230) | | 0.619 | |
| Slob. Mare | (0.270) 0.389 ± | 12.6 | (0.095) 0.156 ± | 19.2 | 2.585 ± | 22.3 |
| | 0.049 (0.486) | | 0.030 (0.230) | | 0.577 | |
| Crih. Veche | (0.238) 0.418 ± | 10.3 | (0.108) 0.164 ± | 14.0 | 2.601 ± | 17.5 |
| | 0.043 (0.540) | | 0.023 (0.216) | | 0.455 | |

Notes: Values represent the mean \pm standard deviation of n = 50 in mm. Number in brackets indicate minimum and maximum value of each sample. CV, $\% = (\sigma/\mu)*100$, where CV - Coefficient of Variation; σ - Standard Deviation; $\mu = Mean$

| broomrape accessions. |
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| OV OvoideN: Donduseni (\leq E): Sonoca (6): C: Vereieni (G): Cazanesti (\leq E): C E): Bacioi (\leq E): S. Mereseni (H): E): Bacioi (\leq E): S. Mereseni (H): Cazanesti (\leq E): Condused D): Do Im D)N: Donduseni (\leq E): S. Mereseni (H): Cazanesti (\leq E): Condused E): Frashesti (\leq E): Condused D): Cazanesti (\leq E): Condused E): Cazanesti (\leq E): Condused E): Cazanesti (\leq E): Condused (\leq E): Cond | Seed ornamentation Accession | No. of Accession name/race popula-tions |
|---|------------------------------|---|
| $\left(\begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$ | | N: Donduseni (≤ E); Soroca (G); Prepelita (F); C: Verejeni (G); Cazanesti (≤ E); Ciocilteni (H); Singera (≤ E); Bacioi (≤ E); S.Mereseni (H); S: Stefan-Voda (F); G.Galbenei (G); |
| line for the second sec | | C: Brinzenii Noi (≤ E); Frasinesti (G); Izbiste (≤ E); Floreni (≤ E); S: Grigorievca (F); Ermoclia (H); Talmaza (G); Congaz (H); Besalma (G); Svetlii (H); Carabetovca (F); Corteni (H). |
| | T | C: Rasvet (₱; Chisinau (≤ ₽); F.Galbenei (≤ Ė); S: Cazangic (≤ Ė); Cimislia (≤ Ė); Chirsova (G); C. Lunga (G); Taraclia (Ħ); Alexanderfeld (Ħ); Manta (G); Slobozia- Mare (₱; Crih. Veche (≤ Ė). |





variation ranging between 8.4% in Chirsova accession and 15% in Congaz, which suggests a low level of intrapopulational variability (Table 2).

The difference of seed length between populations is also very small indifferent of years of samples collecting, provenience and pathotype. For example, the mean values of the length of seeds collected from Prepelita – 0,385 \pm 0,045 mm, Cazanesti – 0,386 \pm 0,042 mm and Besalma – 0,387 \pm 0,040 mm are very closed even the samples have been belonged from different region (North, Centre and South part, respectively) and different physiological races – F, E or less virulent than E and G. A similar value of seed length (0,383 \pm 0,039 mm) has been established in Cimislia population which has been collected in 2008, comparative to above-mentioned populations collected in 2014.

From all 38 *Orobanche* populations, 30 (79%) of accessions have not showed statistically significant differences in seed length. Southern and Central populations

Crihana Veche, Alexanderfeld and Izbiste revealed the highest values of seed length significantly different, when are compared with the studied populations. According to obtained data this parameter has a low discriminant value within the taxon.

A higher level of intra- and interpopulational variability was demonstrated in evaluation of the *seed width*. The smallest value of this character has been attested to the population of Floreni (0,142 mm), while the accession Cazanesti had the widest (0,184 mm; Table 2). The minimum individual value of seed width was 0,081 mm in populations Frasinesti, Floreni, F. Galbenei, Grigorievca, St. Voda and Carabetovca, while the maximum individual value was 0,324 in population Soroca. The study revealed the existence of significant differences concerning the seed width within populations. The values of the variation coefficients ranged from 11,2% on Bacioi to 22,1% on Soroca accession (Table 2).

From investigated accessions, 20 (around 50%) did not show significant differences between any of the populations. The seed width of the central populations Floreni and Buteni, denotes the lowest average width in terms of statistical probability level 95%, compared to the values of the investigated populations.

Regarding the *length/width ratio* of seeds, the highest were recorded in individuals from the population Izbiste (2,843), followed by populations Donduseni (2,682) and Buteni (2,659), between them there is no statistical differences. Lowest average values were observed in the population of Cazanesti (2,135) and G.Galbenei (2,139). In all cases the length/width ratio is well above 2, which is a specific *O. cumana* trait (Krupp *et al.*, 2014). The values

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of the variation coefficients ranged from 14,4% on Rasvet to 27,5% on Izbiste accession (Table 2). There are no associations between this parameter and races of broomrape accessions (Figure 1).

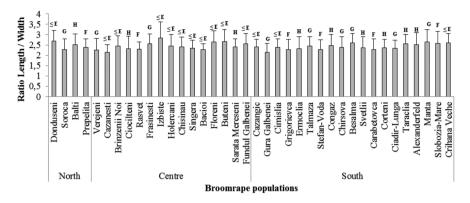


Figure 1: Graphical representation of mean values and standard deviations of the ratio between the length and width of *O. cumana* seeds.

According to the data, interpopulational variability was generally greater for seed width than for seed length. The seed of varying size within particular individuals, population or species is a very common phenomena. This polymorphism can depend on a variety of factors such as pollination and fertilization, nutrient supply from the host and climatic and soil conditions (Piwowarczyk, 2013).

Cluster analysis

Figure 2 illustrates the dendrogram based on cluster analysis of the morphological distance matrix of 38 broomrape accessions. Two major groups were formed (A and B). The first A cluster consists of three minor groups A1, A2 and A3. The A1 group includes the population Izbiste from central part of the country, the A2 – populations Manta, Floreni, Donduseni and Buteni, all from different region; the A3 group consists preferentially from southern accessions (Taraclia, Besalma, Slobozia Mare and Crihana Veche), an two central populations (F. Galbenei and Frasânesti), being according with their geographical origin.

The most numerous populations -27 (71%) formed the second cluster B of the dendrogram, which include broomrape from different races and different geographical regions. Thus, in this case it was not revealed some differentiation of groups according with their geographical origin.

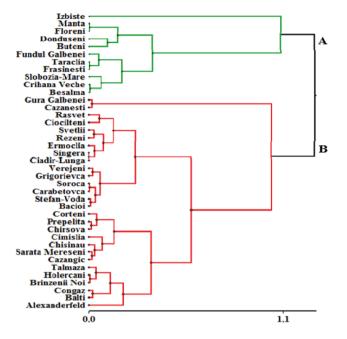


Figure 2: Hierarchical cluster analysis dendrogram of Euclidean similarity distance showing the relationship among *O. cumana* populations based on average values of L/W ratio.

Seed shape and ornamentation analysis

It is known that the micromorphology of seed shape in *Orobanche cumana* is highly diverse even amongst individuals of the same population, which can be due to the location in the ovary in relation to other seeds (Piwowarczyk, 2013). The evaluation of 38 Moldavian broomrape populations has demonstrated that seed shape and surface is variable. Though the seed shape of evaluated samples is highly diverse the most part of seeds from one accession had a similar shape. Thus, in the majority of cases seeds were ovoide (11 populations), elongated (12 populations) and ovoide - elongated (12 populations) with the micropylar region generally narrow, forming a neck (Table 3). The seeds from some individual populations were elongated-pear shaped (Balti and Buteni accesions) or ovoidepear shaped elongated (Holercani accesion). Such a variation of seed shape has been described for various species of *Orobanche* genus (Abu Sbaih and Jury, 1994; Plaza *et al.*, 2004).

Analyzing obtained results it has been established that the first group includes preferentially accessions from the central part of the country (6 from the total of 11), in the second and third groups were classified especially populations belongs to southern part of Moldova (8 and, respectively, 9 from the total of 12), that could be explained due to varied conditions of seed growth and maturation (Teryokhin, 1997). In the same time there are no correlations between seed shape and race of *O. cumana* populations, the accessions from different races being grouped together (Table 3).

Micrographs have illustrated that the *O. cumana* seed coat is dark and opaque with reticulated polygonal cells and very structured. These polygonal cells generally are pentagonal and hexagonal. The pattern of the polygonal cells range from cells with more or less isodiametric edges to longitudinally elongated cells. In all accessions, the seed coats are covered with two layers comprising the outer and inner periclinal walls (Table 3).

Data analysis showed that the external cellular layer of the majority populations presents pitted outer periclinal walls, belonging, according to Plaza et al. to morphological type III of seeds (Plaza *et al.*, 2004).

In our study seed coat sculpturing showed constancy among the majority of broomrape taxa, as previously pointed out by Musselman and Mann (1976), Teryokhin et al. (1993), Abu Sbaih and Jury (1994), Plaza *et al.* (2004) and Krupp *et al.* (2014).

Investigation of seed parameters using light microscopy showed a variability of seed size and shape. In some cases we could observe a clear trend of distribution according to geographical area.

Krupp *et al.* (2014) have determined that populations of *O. cumana* from five countries (Republic of Moldova, Romania, Russian Federation, Spain and Serbia) do not differ from each other in sizes of seeds and morphometric data did not provide a race correlation. Morphological investigations of *O. cumana* seeds performed by Duca *et al.*, (2014a), in order for an evaluation of broomrape variability of intraindividual seed size of variation, revealed that populations differentiation belong with their local origin. In general, the variability in case of broomrape populations is low reflecting a preserved stability of genome (Ciuca *et al.*, 2004).

Conclusions

In the presented study was explored the morphological parameters of Moldovan accession of *Orobanche cumana* for a first time. Morphometric results show that the size of broomrape seeds is a variable character between and within accessions. The size of broomrape seeds from the Republic of Moldova varied from $0,36 \times 0,14$ mm to $0,42 \times 0,18$ mm. The seed length indicated more stable values than width, indifferent of locations and year of samples collecting.

It is to be emphasized that morphometric data analysis revealed that length/ width ratio parameter is more suitable for *O. cumana* populations, which showed a high variability within local accessions. Seed shape is also a variable character, ranging from oblongoid and ellipsoid to ovoid in all investigated populations.

Clusterian analysis revealed that the ratio L/W correlates partly with the geographical location of populations used in this study, demonstrating the influence of different climatic conditions. No association with broomrape races was established and therefore it is necessary to perform investigations using modern molecular techniques, which provides more accurate data about the analyzed populations.

Acknowledgments: The authors express their gratitude to The Technical State University of Moldova, to the National Center for Material Study and Testing, principally to dr Monaico Eugen for his support and technical assistance on light microscopy measurements. This study was carried out with funding from the national research project 15.817.05.03F "Sunflower (*Helianthus annuus* L.) resistance to broomrape (*Orobanche cumana* Wallr.): genetic, molecular and physiological mechanisms".

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