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MICROMORPHOLOGICAL STUDY OF THE SEEDS IN BULGARIAN REPRESENTATIVES OF GENUS *PHELIPANCHE* (POMEL) SOJAK (OROBANCHACEAE)

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ABSTRACT. Seeds of six species of genus *Phelipanche* (Pomel) Sojak in Bulgaria as *Ph. arenaria* (Borkh.) Pomel, *Ph. purpurea* (Jacq.) Sojak, *Ph. mutelii* (Shultz) Pomel, *Ph. ramosa* (L.) Pomel, *Ph. nana* (Reut.) Sojak and *Ph. oxyloba* (Reut.) Sojak are investigated. Internal and external volumes of the seeds are calculated on the basis of measured seed parameters. The ornamentation of the internal tangential cell walls of the testa is described. Analytical key by the seed morphology is compiled.

KEY WORDS: broomrape, micromorphology, seed, testa, *Phelipanche*, *Orobanche*

INTRODUCTION

In the Bulgarian flora are known 7 species of genus *Phelipanche* (Pomel) Sojak. Diagnostic traits for all species of the familly *Orobanchaceae* are insufficient for classification and determination especially *in sicco*. Very often the species *Ph. arenaria* and *Ph. purpurea* are confused in the major Bulgarian herbaria. On the other side, the position of *Ph. mutelii, Ph. ramosa, Ph. nana* and *Ph. oxyloba* is not elucidated.

However there exists a clearly seen diversity in the seed morphology and the sculpting of the inner walls of the testa cells in *Orobanchaceae*. This variation could be an useful criteria for clarification of controversial species taxonomy and might suggest an alternate way of their determination.

The taxonomic rank of genus *Phelipanche* is disputable. According to the most popular concept the representatives of *Phelipanche* are situated in a separated section *Trionychon* of genus *Orobanche* (Beck-Mannagetta, 1890; Chater & Webb, 1972; Gilli, 1982; Musselman, 1991). The representatives of genus *Orobanche s.l.*

according to the specific heterocyclic substances, sculpting of the internal testa cell walls and morphological characters, are divided to the sections *Osproleon* (*Orobanche s.s.*) and *Trionychon* (*Phelipanche*). The last one is divided to the subsections *Arenariae* and *Ramosae* on the basis of the same criteria (Andary, 1994). Scanning electronmicroscopic investigations (Abu-Sbaih & Jury, 1994) of external wall of the testa cells in *Orobanche s.l.* suggests two groups in sect *Trionychon* (A and B). The first group unites all the species of *Phelipanche* except *Ph.arenaria.* According to the contemporary concepts *Phelipanche* (Pomel) Sojak is phyllogenetically outlying of *Orobanche* L. On the basis of carpology and seed morphology *Phelipanche* is divided in two sections (*Phelipanche* & Arenariae) as sect. *Arenariae* is divided in two series – *Arenariae* Teryokh. and *Purpureae* Teryokh (Teryokhin, 1997).

The delimitation in sect. *Phelipanche* is not clear. *Ph. oxyloba* is divided from *Ph. ramosa* by the shape of lower lip lobes (Chater&Webb, 1978). Tzvelev (1981) suggests that is no difference between *Ph. oxyloba* and *Ph. nana*. According to the correlation of bract to calyx and the shape of corolla lobes the species *Ph. oxyloba* can be divided from the group of *Ph. ramosa* presented by *Ph. mutelii, Ph. ramosa* and *Ph. nana* (Andreev et al., 1992). Teryokhin (1997) describes the seed morphlogy in the weed *Phelipanche* species (*Ph. aegyptiaca, Ph. mutelii, Ph. ramosa*) and accepts *Ph. oxyloba* and *Ph. nana* as subspecies of *Ph. ramosa*. In other side, *Ph. mutelii* and *Ph. nana* are often described as subspecies of *Ph. ramosa* but *Ph. oxyloba* is divided as a detached species (Chater & Webb, 1978; Musselman, 1991).

The *Phelipanche* representatives are first reviewed in Bulgarian "Floras" and Keys as *Phelipaea* Dsf. (Velenovsky, 1891; 1898). Later is accepted the concept of sect. *Trionychon* Wallr. in the genus *Orobanche* (Georgieff, 1937; Stoianov & Stefanov, 1925, 1948; Stoianov & al., 1964; Andreev, 1992; Delipavlov, 1995, Cheshmedziev, 2003). In this article is presented the concept of genus *Phelipanche* in relation of the contemporary viewpoint based on the seed morphology (Teryokhin, 1997).

MATERIAL AND METHODS

Seeds of totally 59 specimens from six proved Bulgarian species in personal and herbar collections are explored (table 1). The species *Ph. aegyptiaca* is excluded of the presented study because of missig authentical data about its distribution in Bulgaria. The own collected materials are deposed in the Herbarium of Agricultural University - Plovdiv (SOA). The seed surface is explored using reflected light microscope Carl Zeiss NU2, with magnification 50×12.5. The testa is paled with 5% KOH, then detached of the seed and mounted on glass slides for observation of the inner cell wall. The observations and measurements of the equatorial testa cells and internal sculpting of the cell walls are carried out using transverse light microscope Carl Zeiss Amplival, with magnification 10×8, 40×8, 100×8, 100×16, bright field. Digital images (3.2 Mpix) are taken with digital camera Daisy PhotoClip DM 334 using (Image-J), preliminary and measured software calibrated with objectivemicrometer (Lomo, scale 1 mm). The long axis and ecuatorial seed diameter

are measured in magnification 10×8 before and after the removal of testa. The seed volumes respectively external (*l*) and internal (*d*) are calculated using the formula below

$$V = \pi \frac{ld^2}{6}$$

The size of equatorial testa cells is measured in 40×8 magnification. In the inner tangential cell walls are measured the longer and shorter diameter of the thin wall zones in magnification 100×8 . The widths of the wall thickenings are measured as the nearest distances between the adjacent perforations. 50 standard measurement of all discussed descriptors are taken. Because of the high variation of the values the characters of the internal tangential cell wall are divided in classes and the rare values are excluded.

The classification of internal tangential cellular wall is according to Romanova et al. (1998).

RESULT AND DISCUSSION

1. Seed size and shape

The size and the shape of seeds vary in big space (fig.1-A-C). In spite of that after calculation of the seed volume (fig.1 – D-F) in sect. *Phelipanche* can be seen that *Ph. mutelii* have the largest seeds followed by *Ph. oxyloba. Phelipanche nana* and *Ph. ramosa* have relatively small seeds. The same subjection can be observed after and removal of the testa (fig.1-E). Not so clear difference can be seen in sect. *Arenariae*. According to fig.1-A,B,C,D *Ph. arenaria* have relatively smaller and more rounded seeds than *Ph. purpurea.* Regardless of the big variation of the seed size and shape, comparing the correlation between the long axis and equatorial diameter (fig.1-C) can be seen that in the seeds of *Ph. mutelii* and *Ph. ramosa* have predominate roundish shape. An comparative evaluation of the internal volume quota toward the external volume (fig.1 - D-F) appeared that the seeds of sect. *Arenariae* have a smaller relative internal volume than sect. *Phelipanche*. Folowing this criteria in sect. *Phelipanche* in spite of the high variation *Ph. mutelii* can be apparently distinguished by the other three species. Likewise *Ph. oxyloba* can be divided by the seed size from *Ph. nana* and *Ph. ramosa*.

2. Size of equatorial testa cells and ornamentation of the inner cell wall

Similar results are observed in comparison between the size of equatorial testa cells - in proportion to the seed size (fig.2). That is why *Ph. mutelii* and *Ph. oxyloba* are distinguished with bigger equatorial cells (fig. 2-A,B).

According to the ornamentation of the inner tangential cell walls in the testa (fig.3) and the proportion internal/external seed volume (fig. 1-F) a clear division to the sections *Arenariae* Teryokhin and *Phelipanche* can be seen.

Sect. *Arenariae* are known with reticulate or reticulate-labirynthlike sculpting of the internal cell wall. This sculpture is formed by irregular ribbon-shaped, sometimes almost indiscernible thickenings which are grouped in wider bundles. The thin wall areas in *Ph. arenaria* have a round to linear, much often trilateral-ovate shape (fig.3-a), as well in *Ph. purpurea* have an irregular shape (fig.3-b). The thin

wall zones in *Ph. arenaria* are arranged in streams where the streams are divided by wider ribbon-shaped thickenings. The thin wall zones of *Ph. purpurea* are large, often orientated across the long axis of the cell and longer than the half of the cell width. The ribbon-shaped thickenings are comparatively narrow. In the same species are found cells with large thin-wall zones which cover more than the half of the inner tangential cell wall. More stable size and shape of the thin wall zones can be observed in *Ph. arenaria* (fig.2-C,D). A comparison between the ratios of the average diameter of the thin zones to the width of the wall thickenings shows that the perforations in *Ph. arenaria* exceed the thickening width (2) - 3.5 - (4.5) times, while in *Ph. purpurea* the thin zones are (3) - 5 - (8) times wider than the thickened ribbons (fig.2-F-a,b).

Sect. *Phelipanche* have uniformly thickened cell walls with well-defined thin zones resembling to perforations. Two clear groups can be distinguished. *Phelipanche ramosa* and *Ph. nana* have a typical perforate inner tangential cell wall (fig.3-d,f) whereas *Ph. mutelii* and *Ph. oxyloba* have a reticulate-perforate (fig.3-c,e) ornamentation. Wide thickened zones with the perforation size can be seen in the cell wall of Ph. ramosa and Ph. nana while the perforation diameter Ph. mutelii and Ph. oxyloba always exceeds the width of the thickened zones. The distinguishing persist in the results of comparison by the average perforation diameters (fig.2-C-c,d,e,f) and in the proportions between long and short diameter of the perforations (fig.2-Dc,d,e,f). In Ph. nana and Ph. ramosa are observed predominate rounded perforations whereas the perforations in Ph. mutelii and Ph. oxyloba have polygonal-ovate or elongate shape. The average values of the thickened zones are not shown considerable differences (fig.2-E). The comparison between the proportions of the average perforation diameter and the width of the wall thickenings (fig.2-F,c,d,e,f) shows a clear distinguishing of Ph. mutelii, which perforations are 2-4 times wider than the thickenings. A big similarity is observed between Ph. ramosa and Ph. nana the perforations are 1.5-2.5 times wider than the thickened zones, and often could be found wide zones without perforations. Phelipanche oxyloba covers an intermediate position with perforations 2-3 times wider than the wall thickenings.

No significant differences are presented in the surface of the outer tangential cell walls using light microscopy.

Analytical key by the seed morphology of *Phelipanche* is compiled according to the presented data (table 2).

CONCLUSIONS

In the presented study is explored the testa of Bulgarian representatives of *Orobanchaceae* for a first time. According to the proportion between internal and external seed volume and in dependence of the ornamentation of the internal tangenial cell walls in the testa the genus can be divided in the sections *Phelipanche* and *Arenariae* Teryokh.. The representatives of sect. *Arenariae* (*Ph. arenaria* and *Ph. purpurea*) are distinguished one from another by the character of the thickening on the inner tangential cell walls.

Section Phelipanche is divided into two groups according to the ornamentation of the inner cell wall. Ph. mutelii is clearly divided by seed characters from the other three species. It confirms the taxonomic concept of Teryokhin (1997).

The relative values in the seed characters of Ph. ramosa and Ph. nana in conjunction with the other known facts support the concepts of Chater & Webb (1972) Tzvelev (1981), Musselman (1994) and Teryokhin (1997) for the status of Ph. nana as an internal taxon of Ph. ramosa.

Phelipanche oxyloba *is clearly distinguished by the seed characters from* Ph. ramosa *and* Ph. nana. *In spite of the morphological similarity to* Ph. ramosa, *the inner cell wall of this species shows ornamentation which stands nearer to* Ph. mutelii.

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		ab P.purpurea	ub <i>P.purpurea</i>	ub <i>P.purpurea</i>																															
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date	27.05.1984	23.07.1925	24.06.1926	24.06.1926	7.08.2003	2.07.2003	14.06.2003	ż		27.07.2003	5.09.2004	5.09.2004	2.07.2004	26.06.2004	4.07.2004	15.06.2003	16.06.1976	?.05.1910	17.06.2004		14.06.1930	1.09.2003	1.09.2003	14.10.2002	14.09.2004	22.09.2003	27.08.2002	26.06.2002	2.09.2003	12.08.1923	5.08.2004	5.08.2004	5.08.2004	22.07.1967	4.10.2003
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UTM f position re	a. Phelipan 35TLG15	34TGM45	34TGM45	34TGM45	35TKG85	35TKG85	35TLH12	35TLH17	b. Phelipan	34TGM35	34TGN07	34TGN07	35TKG85	35TLG24	35TLG25	35TLG25	35TLH03	35TLH80	35TNH63	c. Phelipan	34TFM73	35TLF89	35TLF89	35TLG06	35TLG15	35TLG15	35TLG15	35TLG16	35TLG42	35TLG61	35TLG83	35TLG83	35TLG83	35TLG92	35TMG32
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Table 1. Voucher specimens:

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00	35TLG15	172	300	Markovo	~	15.06.1988	SV	D.Delipavlov	SOA	47952
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38	35TPJ23	12	5	Durankulak	Medicago sp.	18.06.2004	SC	K Stoyanov	SOA	56910
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39	34TFL78	=	300	Samulilova Krepost	2	25.05.2004	SC	O.Todorov	SOA	56929
40	34TFM73	10.1	175	Krupnik	e.	14.06.1930	SV	B.Akhtarov	SOM	69688
41	34TFM73	10.1	175	Krupnik	. 6.	14.06.1930	SV	B.Akhtarov	SOM	69689
4	34TFN55	L-	710	Dragoman	. 6.	29.06.1930	SV	T.Georgieff	SOA	18535
1 9	34TGL29	14.1	1200	St.Elena Peak, chalky slope	e.	17.05.1996	SV	M.Nikolova	SO	98078 sub P.ramosa
÷ ÷	34TGM38	15	600	Gabrovetz	۰.	15.06.1889	SV	T.Georgieff	SO	68536 sub Pramosa
‡ !	35TLG25	172	300	Asenovgrad	۶.	18.05.1993	SV	D.Delipavlov	SOA	38272
3	35TLG25	172	300	Asenova Krepost	~	21.06.2004	SC	K.Stoyanov	SOA	56930
46	35TLG25	172	300	Asenovgrad	2	6.05.2004	SC	K Stoyanov	SOA	56911
47	35TLG42	172	620	Gabrovo, Kardjali distr.	۰.	1.09.2003	SC	K.Stoyanov	SOA	56931
49	35TMH60	19	200	Bakadiik hill near Yambol	· c.	30.05.1963	SV	I.Cheshmedziev	SOA	34442 sub O.cumana
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52	35TKG77	18	200	A le ko-K onstantinovo	Cannabis sativa L.	21.07.1967	SV	Kolev & Sedmakova	Sd	5689
53	35TLG25	172	300	Markovo	Nk otiana tabacum L.	3.09.1993	SV	 Èeschmedziev 	SOA	47718
54	35TLG26	18	140	Sadovo	Cannabis sativa L.	21.06.2002	SC	K Stoyanov	SOA	56533
55	35TLG26	18	140	Sadovo	Lycopersicon esculentam Mill.	27.08.2003	SC	K.Stoyanov	SOA	56528
56	35TLG26	18	140	Sadovo	Lycopersicon esculentam Mill.	27.08.2003	SC	K Stoyanov	SOA	56932
57	35TLG26	18	140	Sadovo	Lycopersicon esculentum Mill.	29.07.2004	SC	K.Stoyanov	SOA	56934
58	35TMJ25	0	9	Ruse	Lycopersicon esculentam Mill.	2.08.1991	SV	K.Antonova	SOM	150807
59	35TNJ53	2	100	Kara-Pelit		2.06.1902	SV	B.Davidov	SOM	69751
Ŭ,	pristic region	ts (accor	ding to F	lora R. Bulgaricae) 1 – Black S.	ea Coast (1.1 - southem, 1.2 -					
DU	wrthern), 2 -	Northcae	st Bulgar	ia, 3 - Danube Plain, 4 - Balkan	Foothill (4.1 - western, 4.2 -					
5	stem), 5-E	3alkan M	ountain	(5.1 – westesrn, 5.2 – central, 5.3	3 - eastern), 6 - Sofia region, 7-					
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ž).2 - norther	m), 11 – J	Belasitza	t, 12 - Slav yanka, 13 - Mesta Va	lley, 14 - Pirin (14.1 - southern,					
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4 ** *	crbar abbre	viations:	1) accor(ding to Index Herbariorum: SO-	- University of Sofia; SOA -					
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(table 1 - continuig)

Table 2. Analytical key of the representatives of genus *Phelipanche* (Pomel) Sojak in
Bulgaria by the seed characteristics.

- - 2. Inner tangential cellular walls with reticulate-labyrinth-like ornamentation. Thin wall zones roundish or fintly elongate, (2) - 3.5 - (4.5) times wider than the thickened zones, much shorter than the half of cell width *Ph. arenaria*
 - 2⁺. Inner tangential cellular walls with irregular ribbon-like thickenings much often orientated across the long axis of the cell. Thin wall zones with various shape, (3) 5 (8) times wider than the thickened ribbons, with usually bigger long diameter than the half of the cell width *Ph. purpurea*
- 1⁺. Inner tangential cellular walls with regular perforate or perforate-reticulate ornamentation. Thin wall zones well-defined as perforations, roundish to elongate, 1 4 times wider than the thickened parts. Internal seed volume usually more than 50% compared to the external sect. *Phelipanche* 3
 - - 4⁺. Seeds long much often $300 400 (480) \mu m$; with equatorial diameter $(150) 200 260 (350) \mu m$; lightly elongated seeds much often 1.3-1.7 times longer than the equatorial diameter. Perforations (2) 2.5 (3) times wider than the thickened parts...... *Ph. oxyloba*



Fig. 1. Seed size in the Bulgarian representatives of genus Phelipanche: $A - long axis (\mu m)$, $B - equatorial diameter (\mu m)$, $C - proportion between the axis and equatorial diameter; <math>D - external volume of the seed (\mu m^3 10^6)$; $E - internal volume of the seed (\mu m^3 10^6)$; F - proportion between internal and external volume of the seed (%);a - Ph. arenaria; b - Ph. purpurea;c - Ph. mutelii; d - Ph. nana; e - Ph. oxvloba; f - Ph. ramosa.



Fig.2. Size of equatorial testa cells: A – length (μm), B – width (μm); Internal tangential cellular walls: C – average diameter of the thin wall zones (μm), D – proportion between long and short diameter of the thin wall zones, E – width of the thickenings (μm), F – proportion between the average diameter of the perforations and width of the thickenings. a - Ph. arenaria; b - Ph. purpurea; c - Ph. mutelii; d - Ph. nana; e - Ph. oxyloba; f - Ph. ramosa



Fig 3. Inner tangential cell walls in the seed testa of: a - *Ph. arenaria*; b - *Ph. purpurea*; c - *Ph. mutelii*; d - *Ph. nana*; e - *Ph. oxyloba*; f - *Ph. ramosa*. (magnificaion 100×8, scale length 100 µm).