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CONTRIBUTIONS TO THE SEEDS' STUDY OF SOME SPECIES OF THE *PLANTAGO* L. GENUS

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Abstract: *Plantago* genus includes many species, some of them known to be used in traditional and modern medicine. The most numerous information about the *Plantago* species usage in our country refers to the leaves, while information about seeds usage is sporadically reminded. Lately, there was a particular interest in the consumption of psyllium, the trade name used for the product from seeds of *Plantago ovata*, *P. psyllium* (*P. afra*) or *P. arenaria*. A special economic interest presents the seeds of these species as they are a cheap source of gelling agent for micro-propagation techniques.
The morphological study of the seeds from populations of different areas has been focused on issues of biometrics, testa micro-morphology and myxospermy. Observations have shown differences between species, and also between different populations of the same species. The myxospermy phenomenon (formation of mucilage) emphasizes individual characteristics for several taxa which may have practical uses. The achieved results have both theoretical (in order to clarify some taxonomic issues) and practical value (by capitalization in pharmaceutical or other similar domain).

Key words: *Plantago*, seeds morphology, fructification, myxospermy, seed mucilage.

Introduction

The *Plantago* genus is represented in the Romanian flora by 16 species, among which we can mention *P. lanceolata* and *P. major*, which are known for a long time [WERYSZKO-CHMIELEWSKA & al. 2012] and used by the modern and traditional medicine, with major use of their leaves. The use of seeds from *Plantago lanceolata* and *P. major*, although less known, are numerous and diverse. Outside their common usage as laxative, due to their emollient and diuretically properties, they are also used in the temperate regions but also in some areas such as Africa, in the treatment of several diseases such as gastritis, gastro-enteritis and salmonellosis, as in the cases of different respiratory illnesses. The dry seed infusion is used in the treatment of intestinal parasites in children, against diarrhea and dysentery, or as eye soothing lotion.

The seeds' mucilage is an excellent thickening agent used in cosmetics (e.g. in different hair lotions and hair sprays) but also as a stabilizer in the ice-cream industry. It is also used for different chocolate products. The seeds can be used as a source of jellifying agent in the tissue cultures. Its quality is comparable with the one of the agar, but the cost is 10 times smaller [GURIB-FAKIM, 2008]. Recent studies [SAEEDI & al. 2013] have shown that the seeds' mucilage of *P. major* can be used as excipient which allows the controlled release of the active substance.

In the last two decades a specific interest was manifested on the Romanian market for the consumption of psyllium; under this name it is sold the product obtained from the seeds of the species *Plantago ovata*, *P. psyllium* (*P. afra*) or *P. arenaria*. The latest is a

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species which, in the studies for Romanian ethno-botanic was remembered as a laxative [BUTURĂ, 1979], week-known in the traditional medicine, although in some works published around the '50 it was mentioned to be the source of some active, yet unidentified, principles. The specie is widely used in the Mediterranean areas and in China is cultivated for its medicinal seeds.

The laxative action of the seeds is due to their capacity of forming mucilage when in contact with water. The phenomena called myxospermy, is characteristic to several botanical families among which we can mention *Plantaginaceae*. From an ecological point of view the seeds' mucilage is beneficial for their dispersion, for the germination, the plants' development and the seeds' protection, which is considered as an adaptation of the plants to the specific conditions of the deserts and the sandy areas.

Materials and method

The studied material is represented by seeds collected from 5 species of the genus *Plantago* [CHATER & CARTIER, 1976], each one taken from two different areas (Tab. 1).

Tab. 1. The Provenance of the collected material (GBI – Botanical Garden Iassy)

Species	**Years**	**Place**	**Distribution**
Plantago arenaria Waldst. & Kit. (a)	2011	GBI, Iaşi	Eurasia
Plantago arenaria Waldst. & Kit. (b)	2011	Letea Forest, Tulcea	
Plantago lanceolata L. (c)	2011	Poiana Stampei, Suceava	Eurasia
Plantago lanceolata L. (d)	2012	Natural Reserve Repedea, Iaşi	
Plantago major L. (e)	2011	Aluniş, Buzău	Eurasia
Plantago major L. (f)	2011	Gheorgheni, Harghita	
Plantago media L. (g)	2012	Natural Park Vânători Neamț, Neamț	Eurasia
Plantago media L. (h)	2011	GBI, Iaşi	
Plantago schwarzenbergiana Schur. (i)	2011	GBI, Iaşi	Hungary, Romania, Serbia, S Ukraine
Plantago schwarzenbergiana Schur. (j)	2011	Reserve Valea Ilenei, Iaşi	

The species *P. arenaria*, *P. lanceolata*, *P. major* and *P. media* are known as medicinal plants, used especially in the modern and traditional medicine. *P. schwarzenbergiana* is an endemic species in the alkaline sylvosteppe from the Eastern area of the Carpathian Basin, with a specific bio-geographical importance, present only in Hungary, Romania, Serbia and the South of Ukraine. In the Red List of the superior plants from Romania published in 1994 [OLTEAN & al. 1994], it is considered a rare sub-endemic taxon in the Romanian flora. Recent pharmacognostic studies [BEARA & al. 2011] have suggested that *P. schwarzenbergiana* is a natural source of antioxidants and anti-inflammatory agents which can be exploited in the future.

The characteristics parameters of the seeds, as shape, color, dimensions and aspect were observed under the binocular microscope type Optika. The photos which are illustrating the observations were done with a Canon A540 camera type.

For underlining the myxospermy phenomena, the nutlets were humidified in distilled water and maintained for a period of approx. 30 minutes, immersed in a solution of rhutenium red, and then analyzed by means of the binocular microscope type Optika. There

were followed some aspects concerning the presence or absence of the mucilage, its aspect and consistency, the proportion as compared to the seed's volume.

The study was conducted in the Laboratory of micropropagation and germplasm preservation of the Botanical Garden, University "Alexandru Ioan Cuza" Iasi.

Results and discussions

The descriptive information of the seeds of the studied species is not homogeneous in the specific literature [GLEASON & CRONQUIST, 1991; GRIGORIEV, 1958; ZHENYU, 2002; PAUCĂ & NYÁRÁDY, 1961], and in most cases is incomplete. Characteristics such as the number of seeds in the pyxis, the dimensions or the colour are different in the works published in different periods of time and with reference to species from very diverse areas (Tab. 2). Most probably these characteristics are mostly influenced by the ecological conditions, which is in connection to the very wide distribution area of the mentioned species (with the exception of *Plantago schwarzenbergiana*).

The taxa we have analyzed are presenting an obvious morphological diversity (dimensions, color), both between the individuals from different areas as well as within the collected material from the same area. The biometrical measurements have shown obvious differences to the species *P. lanceolata* and *P. media*, originated from areas where the ecological conditions are very different (mountain area compared with hilly area, with different pluviometric regimes). As well, all data obtained by us for the species *P. arenaria* and *P. lanceolata* are slightly different from those mentioned in the literature. We have observed that the width of the seed is a variable character within a much restrained interval as compared to the length (Tab. 3).

The morphological types of seeds (Fig. 1, 2) belonging to the *Plantago* genus mentioned by different authors are different. LIU & al. (1992) are mentioning four types: multi-angular, in species with 6-30 seeds in the fruit; navicular, in species with two seeds in the fruit; ovoid; recti-circular, in species with 1-2 or 4-5 seeds in the fruit, while SHEHATA & LOUTFY (2006) is underlining a wider morphological diversity (oblong, cymbiform, ovoid, fusiform, lenticular, circular, angular, ellipsoidal and reniform).

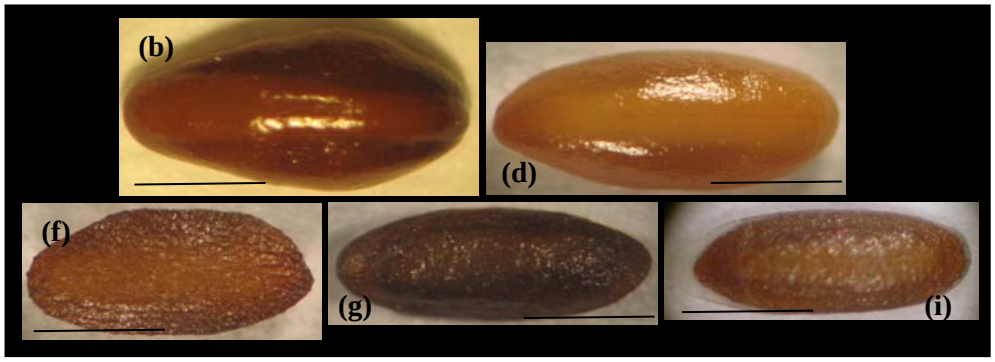

Fig. 1. Morphological aspects of the seeds of *Plantago* – dorsal (scale bar – 1 mm)

Tab. 2. Bibliographical information concerning the seeds of the *Plantago* species

Species	Flora RSR (Paucă, A., Nyárády E. I., 1961)	Flora URSS (Grigoriev Yu., 1958)	Flora of China (Zhenyu L., 2002)	Manual of Vascular … (Gleason H. A. & Cronquist A., 1991)	PROTA (Gurib-Fakim, A., 2008)
P. arenaria	Seeds 2, 2-2.5 mm, navicular shape, with a broad groove on a side	Seeds oblong- ellipsoid, dark brown, shiny, 2,5 mm long; one side convex, other canaliculate	Seeds 2, brown to blackish brown, ovoid-ellipsoid to ellipsoid, 2.5-2.8 mm, shiny, with a broad groove on ventral face	Seeds 1 or 2, brown, 2-3 mm	
P. lanceolata	Seeds 2, 2 mm long, blackish	Seeds oblong or oblong-ellipsoid, one side convex, other canaliculate	Seeds (1 or) 2, brown to dark brown, narrowly ellipsoid to oblong, 2-2.6 mm, shiny, with a broad groove on ventral face	Seeds (1) 2, shiny, blackish, 2 mm, deep concave on adaxial face	Seeds (1–)2–3, oblong-ellipsoid, 2.5–3 mm long, yellow-brown to dark brown, mucilaginous when wet
P. major	Seeds 6-30, dark brown, moderate verrucous	Seeds 1 mm long, horizontal, ± angulate	Seeds (8-)12-24(-34); yellowish brown, ovoid, ellipsoid, or rhomboid, 0.8-1.2 mm, angled, ventral face prominent to slightly flat	Seeds 6-30, 1 mm long, strongly reticulate	Seeds (4–)6–34, ellipsoid or ellipsoid-trigonous, 1–1.5 mm long, dark brown to dull black, mucilaginous when wet
P. media	Seeds 4 or more, moderate verrucous, black	Seeds 2-5(6)	Seeds 2-4, yellowish brown to brown, ellipsoid, 1.5-2 mm, shiny, ventral face prominent	Seeds 2-4, 2 mm	
P. schw.	Seeds 1, 2.5 mm long, irregular, ellipsoid or ± oblique truncate, moderate fleshy, convexe on dorsal face and minutely dotted				

Tab. 3. Comparison between personal biometrical data and the specific literature regarding the seeds of *Plantago* species

Species	Personal results (mm) min. – max.		Flora RSR (Paucă, A., Nyárády E. I., 1961)	Flora URSS (Grigoriev, Yu. 1958)	Flora of China (Zhenyu L., 2002)	Manual of Vascular … (Gleason H. A. & Cronquist A., 1991)	PROTA (2008) (Gurib-Fakim, A., 2008)
	Long	Width					
P. arenaria (a)	1.2 – 2.3	0.6 – 1.3	2-2.5 mm	2.5 mm	2.5 – 2.8 mm	2 – 3 mm	
P. arenaria (b)	1.7 – 2.5	0.5 – 1.2					
P. lanceolata (c)	2 – 2.8	0.9 – 1.1	2 mm		2 – 2.6 mm	2 mm	2.5 – 3 mm
P. lanceolata (d)	1.5 – 2.4	0.7 – 1.1					
P. major (e)	0.9 – 1.6	0.5 – 0.9		1 mm	0.8 – 1.2 mm	1 mm	1 – 1.5 mm
P. major (f)	0.7 – 1.6	0.4 – 0.9					
P. media (g)	1 – 1.9	0.4 – 1			1.5 – 2 mm	2 mm	
P. media (h)	1.2 – 2.1	1.1 – 1.8					
P. schw. (i)	1 – 1.6	0.4 – 0.6	1.25 mm				
P. schw. (j)	1 – 1.6	0.3 – 0.6					

According to the typology established by LIU (1992), the studied seeds are: multi-angular - *P. major*, the navicular type - *P. asiatica*, *P. lanceolata* and *P. media*, ovoid – P. *schwarzenbergiana*.

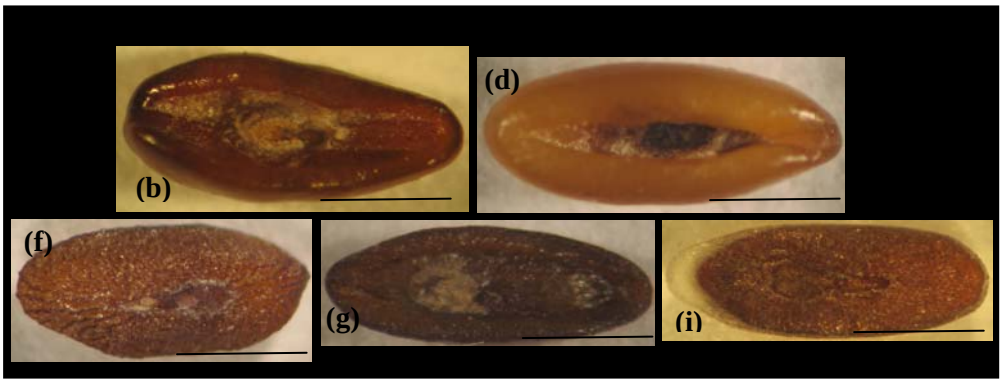

Fig. 2. Morphological aspects of the seeds of *Plantago* – ventral (scale bar – 1 mm)

The diversity of the seeds shape in *Plantago major*, which has a wide number of seeds in the pyxis belonging to the multi-angular type, is determined by their disposal in fructification (Fig. 3) and by the asymmetrical shape of the pyxis. Thus the seeds from the top of the pyxis are following its semi-spherical shape, and during the development the seeds are occupying all available spaces, which are shaping their angular, irregular characteristic. Another feature influenced by the simultaneous development of the seeds is the placement of the hilum on the median axes (Fig. 3), either in the central position, or in the lower third part.

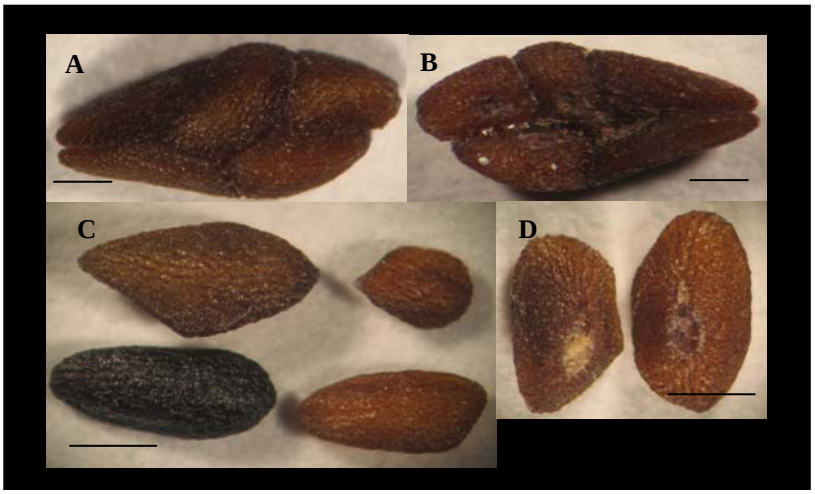

Fig. 3. Morphological aspects of the seeds of *Plantago major*. A. Fructification – dorsal view; B. Fructification – ventral view; C. Dorsal view of the seeds for highlighting the shape; D. Ventral view of the seeds for highlighting the hilum (scale bar – 1 mm)

At *Plantago arenaria* we can observe (Fig. 1) on the dorsal surface a light brown longitudinal area, an aspect mentioned only in the official monographs of the medicinal plants [WHO, 1999]; although here it is specified that the area is widening in the median area, and in the case of our studied material, the width of the band is quite variable, but always present.

Plantago schwarzenbergiana, due to its restrained area, is the species for which we can find less morphological descriptions. This taxa presents seeds of 1-1.6 mm long and 0.3-0.6 mm width, are brown-yellowish to black, dull, with an ellipsoidal shape or diagonally truncate, the surface has a reticular aspect, on the back they are convex, ventral almost flat, with a narrow and deep excavation in the center which is corresponding to the hilum (Fig. 2).

The myxospermy phenomenon is manifesting through the dispersion of the mucilaginous seeds, mainly with the help of the rain drops. The seeds are adhering through the mucilaginous layer to the moist surface of the soil together with the dry mother plant (atelechory) or they can adhere to the bird legs, being thus spread to large distances (zoochory). This feature can also have a defensive character, as it can annul or prevent the collection of the seeds by ants.

From a pharmacological point of view the presence of this phenomenon is valued by using seeds belonging to species like *Plantago* as laxative agents, but the information concerning the particularities of this phenomenon are very few. We know that the mucilage contains hydrophilic polysaccharides [KAR, 2003], present especially in the seeds testa, and in the case of *Plantago major* it has been recently shown [MAJID SAEEDI & al. 2013] that this mucilage is not essentially modifying the accompanied active substances, thus it can be used as excipient. But we do not have enough data upon the quantity and the composition of polysaccharides and it is very probable that these vary a lot, an aspect suggested by our results upon the studied species.

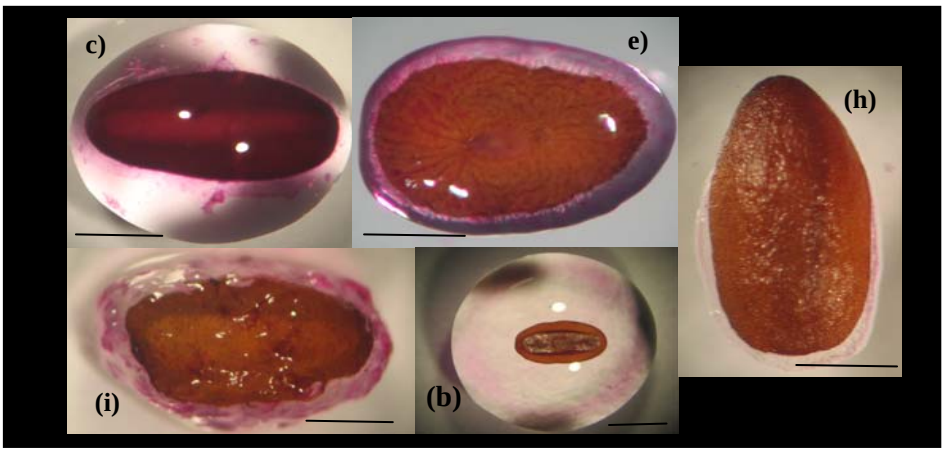

Fig. 4. Highlighting the myxospermy phenomenon to *Plantago* species (scale bar – 1 mm)

After soaking the seeds with water, we have observed the presence of the mucilage in all taxa, but the aspect and especially the resulted quantity are presenting obvious differences. Thus, the aspect (Fig. 4) is hyaline and even to all taxa, with the exception of *Plantago schwarzenbergiana* seeds; its form is circular to *P. arenaria* and *P. lanceolata* and it follows the same form of the seed to the others taxa. The mucilage quantity is smaller (less comparative with the volume of the seed) in *P. major*, *P. media* and *P. schwarzenbergiana*, while in *P. arenaria* and *P. lanceolata* is bigger (bigger or equal to the volume of the seed).

We can notice that there is no linear correlation between the xerofitism degree and the mucilage percentage emitted by seeds. Thus *P. arenaria* and *P. lanceolata*, where the quantity of mucilage is bigger are xerophilous species, respectively euryhygrous, with totally contrasting ecological requests. The quantitative differences between the species with similar ecological requests (*P. arenaria* – xerophilous and *P. media* – xeromesophilous) relating to the emitted mucilage are quite large.

Conclusions

The morphological study of the seeds is underlying their ecological variability, which explains the relatively wide range of descriptions from the classical textbooks. The data resulted from our observations can help completing the information of the specific literature and also to clarify some aspects relating to the genre taxonomy.

The study of the myxospermy phenomenon can supply useful data from a taxonomic, ecologic but also from a pharmacological point of view (aiming to obtain new pharmaceuticals). The diversity of the forms is that a more thorough study can provide more clear explanations upon the importance of this phenomenon in the process of plant adaptation to the environment.

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