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Pollen and seed morphology of *Zygophylum fabago* and *Peganum harmala* (Zygophyllaceae) from Bulgaria

Morfología del polen y las semillas de *Zygophylum fabago* y *Peganum harmala* (Zygophyllaceae) en Bulgaria

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Abstract. Scanning electron microscope (SEM) study of the pollen and seed morphology of *Peganum harmala* L. and *Zygophyllum fabago* L. (Zygophyllaceae), in native Bulgarian populations, was carried out. Additionally, a light microscope (LM) investigation on pollen grains was made in order to elucidate the peculiarities of the major parameters of pollen surface and size. It was established that pollen grains in *Z. fabago* are pantoporate. Exine ornamentation is reticulate. The pollen grains of *P. harmala* are oblate, spheroidal, elongated, oval in shape, colporate, exine striato-rugulate. The seeds of *P. harmala* are oblong-oval and slightly flattened at the side (incorrect tetrahedral to rhomboid), with a peaked edge, arc-curve laterally. The seed sculpture corresponds to the Tubular to Concave-type, which is characterized by polygonal cells and reticulate tectum. The seeds of *Z. fabago* are oval-rhomboid in shape. The seed sculpture corresponds to Concave to Convex-type which is characterized with polygonal cells, ranging in size, with cuticular wax folds.

Keywords: Exine; SEM; Spermoderm.

Resumen. Se llevó a cabo un estudio de la morfología del polen y las semillas de poblaciones búlgaras nativas de *Peganum harmala* L. y *Zygophyllum.fabago* L. (Zygophyllaceae) con microscopico electrónico de barrido (MEB). Se estableció que los granos de polen en *Z. fabago* son 3-zonocolporados. La ornamentación de la exina es reticulada. Los granos de polen en *P. harmala* son óvalo alargados, colporados, exina estriado-rugulada. Las semillas de *P. harmala* son tetraédricas de forma romboidal, con bordes puntiagudos, arco-curva lateralmente. La escultura de la semilla corresponde al tipo Tubular-cóncavo, que se caracteriza con células poligonales y tejado reticulado. Las semillas de *Z. fabago* son de forma oval-romboidales. La escultura de la semilla corresponde al tipo cóncavo-convexo que se caracteriza con células poligonales, variables en tamaño y con pliegues de cera cuticular.

Palabras clave: Exina; MEB; Cubierta seminal.

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INTRODUCTION

Zygophyllaceae R. Br. is a large and heterogeneous family that comprises 27 genera and 285 species subdivided into five subfamilies widespread in arid and semi-arid areas in the world (Sheahan & Chase 1996, 2000).

In Bulgaria this family is represented with three taxa: *Tribulus terrestris*, *Peganum harmala* and *Zygophyllum fabago* (Petrova, 1979). The three species are important medicinal plants used as compounds of pharmaceuticals products or as a popular remedy (Kuzmanov et al., 2007; Moloudizargari et al., 2013).

In a palynological aspect, *Zygophyllaceae* is an euripalynous family (Punt et al., 2007). Therefore, the study of pollen morphology in that family is an important step in the establishment of relationships and dependencies between the comprising taxa. The pollen morphological analysis has a proved taxonomical significance and is successfully used as an additional criterion for delimitation of the taxa (Terziysky & Atanasov, 1977).

The pollen morphology of the family has been examined by Erdman (1952), Sladkov (1954), Shimakura (1973) and Kuprianova & Alyoshina (1978). Erdman (1952) described tricolporate and reticulate pollen grains for *Zygophyllum album* L, pantoporate and metareticulate pollen grains for *T. terrestris,* and tricolporate and striato-rugulate pollen grains for *P. harmala* L. Yunus & Nair (1988) analyzed the pollen morphology of 3 genera of *Zygophyllaceae* from India. The exine ontogeny of *P. harmala*, *Z. album* and *T.terrestris* was studied by Ben Nasri-Ayachi & Nabi (2009). Perveen & Qaiser (2006) recognized 4 distinct pollen types in *Zygophyllaceae* family on the basis of tectum types, namely: *Nitraria retusa*-type, *Peganum harmala*-type, *Tribulus terrestris*-type, *Zygophyllum simplex*-type, and Abdel Khalik (2012) – six pollen groups on the basis of morphological characters of vegetative parts, pollen and seeds of 29 taxa from 7 genera of this family from Egipt, namely: *Fagonia* group, *Zygophyllum* and *Peganum* groups, *Tetradiclis* group, *Balantines* group, *Tribulus* group and *Seetzenia* group.

From the Bulgarian representatives of the family *Zygophyllaceae,* the pollen morphology was studied only on *T. terrestris* (Semerdjieva et al., 2011).

The importance of structural pattern analysis of the seed coat observed under the SEM as a reliable approach for identifying the species and assessing taxonomic relationships has been recognized well (Bartholtt, 1981; Koul et al., 2009; Gammarra et al., 2007). Until recently, the morphology of the seed coat sculpture under the SEM on the representatives of *Zygophyllaceae* family was studied in *Fagonia schweinfurthia*, *Peganum harmala* and *Zygophyllum simplex* L. from Saudi Arabia (Solman et al., 2010) and *T. terrestris* from Bulgaria (Semerdjieva et al., 2011).

The present study of the sculpture of pollen and seed of *P. harmala* and *Z. fabago* is a continuation of the undertaken investigation of pollen and seed morphology of the *Zygophyllaceae* family in Bulgaria (Semerdjieva et al., 2011). The present study aimed to reveal the peculiarities of spermoderm and sporoderm in *P. harmala* and *Z. fabago,* and provide important information that will complete the characteristic of the taxa included in the family *Zygophyllaceae* in Bulgaria.

MATERIALS AND METHODS

The material was collected from native Bulgarian populations of *Zygophyllum fabago* and *Peganum harmala* in the town Balchik, near to the Black sea. The specimens were preserved in Herbarium of Agricultural University, Plovdiv (SOA).

The scanning electron microscope (SEM) used in this investigation was a Jeol 5510, which belongs to the Faculty of Chemistry of Sofia University. The samples were covered with gold in a vacuum-evaporator for 60 seconds in an ionizing argon environment. The clamp holder for providing contact was made with silver paste. The method suggested by Terziyski (1981) was followed, namely: the objects were directly observed, without any preliminary physical or chemical treatments, the seeds were observed in air-dry conditions and the pollen grains were preserved in 95% ethanol. For the seed classification, the morphological classes of Thompson (1993) were used. The structure of spermoderm was determined accordingly to the terminology and classification given by Barthlott and Ehler (1977). For the pollen morphology, the terminology by Punt et al. (2007) was used. Additionally, a light microscope (LM) investigation on pollen grains was made in order to elucidate the peculiarities of the main parameters of the pollen surface and size. Measurements were made with an eyepiece micrometer (16x) and a microscope „Amplival", and the pictures were taken with a light digital camera Motic DMBA-210. The data were processed mathematically according to the descriptive statistics method (program Statistica for Windows - Statsofting, 2007). For each indication, 50 measurements were made.

RESULTS

Pollen morphology. The study of pollen morphology carried out revealed the characteristics of sporoderm (exine) in the two studied species (Table 1). The comparative analysis of values of main parameters of pollen surface showed that they differed between two studied species. Regardless of the established differences, concerning the P/E axis type, the pollen grains of *P. harmala* and *Z. fabago* fell into the same type – longiaxe (P/E>1.8) (the P/E was 2.15 in *P. harmala* and 2.30 in *Z. fabago*).

According to Erdman (1952) the pollen in two species can be defined as small (10-25 μm).

Table 1. General pollen characters of *Peganum harmala* and *Zygophyllum fabago*.
Table 1. Caracteres generales del polen de *Peganum harmala* y *Zygophyllum fabago*.

Parameters in μm	*Z. fabago*			*P. harmala*		
	Average	St. err.	St. Dev.	Average	St. err.	St. dev.
Thickness of exine	2.28	0.10	0.46	3.19	0.11	0.51
Length of pollen grain (P axis)	14.19	0.17	0.81	19.8	0.35	1.63
Width of pollen grain (E axis)	6.61	0.20	0.92	8.61	0.21	0.97
P/E	2.15	-	-	2.30	-	-
Aperture length	13.4	0.5	0.39	18.2	0.26	0.51
Lumina	0.2	-	-	0.3	-	-
Muri size	0.112	0.02	0.05	0.098	0.01	0.03
Brochi size	0.89	0.15	0.37	1.15	0.10	0.25

The pollen in *P. harmala* was oblate spheroidal in shape, colporate (Fig. 1a., Fig. 5a,b). Toward the apocolpium, the colpi formed an area with multilayered, cuticular deposits on the exine (subpilate) (Fig. 1b.). The colpi borders were thickened (crassimarginate), and the membrane had unequally compacted areas. The exine was reticulate and heterobrochate. The murus measures were from 0.06 μm to 0.15 μm, approximately. The brochi were irregular in shape and variable in size (from 0.89 μm to 1.60 μm) (Fig. 1c., Table 1). Singles pores were observed in separate areas of pollen grains (Fig. 1 a).

The pollen in *Z. fabago* was oblong-oval and slightly flattened in the side (incorrect tetrahedral to rhomboid) in shape (Fig. 2a., Fig 5c,d). The apertures were pori, which borders had a thickened walls and a smooth membrane (Fig. 2b.). The exine ornamentation was reticulate with clearly to slightly expressed wavy barriers with simple smooth texture. The brochus had a conically enlarged tip and ranging in size lumen (heterobrochate). The murus measures were from 0.05 μm to 0.19 μm approximately. The brochi were irregular in shape and variable in size (from 0.53 μm to 1.47 μm) (Fig. 2c.).

Seed coat. Using SEM allowed us a detailed study of the micro-morphology of the spermoderm in *P. harmala* and *Z. fabago.* The analyses of the results showed that: according to the classification of Thompson (1993), the seeds of *P. harmala* were irregular tetrahedral to rhomboid in shape, with a peaked edge (Fig. 3a,b,c). The seeds coat was multilayered, flat to slightly concave, the seeds were laterally arc-curved. On the upper side of the seed surface, the reticulum formed a triangular area of raised cell layer that continued towards the base and defined an area with an irregular polygonal shape. The seed surface was flat to slightly concave, with reticulate tectum. The cells of spermoderm were isodiametric. The anticlinal walls were smooth, with rounded edge and cuticular striations. On the upper surface of the seed, they were higher than the other seed surfaces and formed striations (Fig. 3d). The periclinal walls were wavy folded, with many striations on them.

The shape of the seeds in *Z. fabago* can be defined as oval-rhomboid according to the classification of Thompson (1993) (Fig. 4a.). The spermoderm cells were polygonal, ranging in size, with epicuticular wax folds (Fig. 4b,c.). On its surface, irregular granulate areas formed. The anticlinal walls were straight to arc-curved, with striation and rounded edge. The external periclinal walls were smooth to arc-curved, with striations and irregularly disposed of small papillae (Fig. 4b,c.).

DISCUSSION

Pollen morphology. The characteristics of pollen grains of *P. harmala* and *Z. fabago* followed the general pollen characters of the family *Zygophyllaceae* described by Perveen & Qaiser (2006), namely: radially symmetrical, 3-polycolporate-pantoporate, prolate-spheroidal to sub-prolate or prolate rarely, oblate-spheroidal pollen grains with reticulate tectum.

The disposition of the pores on the whole surface of pollen grains in *Z. fabago* has given us enough reasons to determine the pollen in this species as pantoporate. Pantoporate pollen was described also in *T. terrestris* (Yunus & Nair, 1988; Perveen & Qaiser, 2006; Ben Nasri-Ayachi & Nabli, 2009; Semerdjieva et al., 2011) that belongs to the *T. terrestris*-type of the pollen classification of Perveen & Qaiser (2006). These findings agree with the proposal of El-Atrush et al. (2015) that the *Peganum* species should be placed in the Peganiaceae family preceding the Zygophyllaceae regarding evolutionary trends, and *Tribulus* species should be placed in the Tribulaceae family following the Zygophyllaceae.

The pollen of *P. harmala* was defined as colporate (the pori are singles), as it was already described in "Results"

On the basis of exine ornamentation, Perveen & Qaiser (2006) distinguished four distinct pollen types in the family Zygophyllaceae: *Nitraria retusa*-type, *Peganum harmala*-type, *Tribulus terrestris* - type and *Zygophyllum simplex*-type. These authors determined the pollen of *Z. fabago* as *Zygophyllum simplex*-types, and pollen of *P. harmala* as *P. harmala*-type.

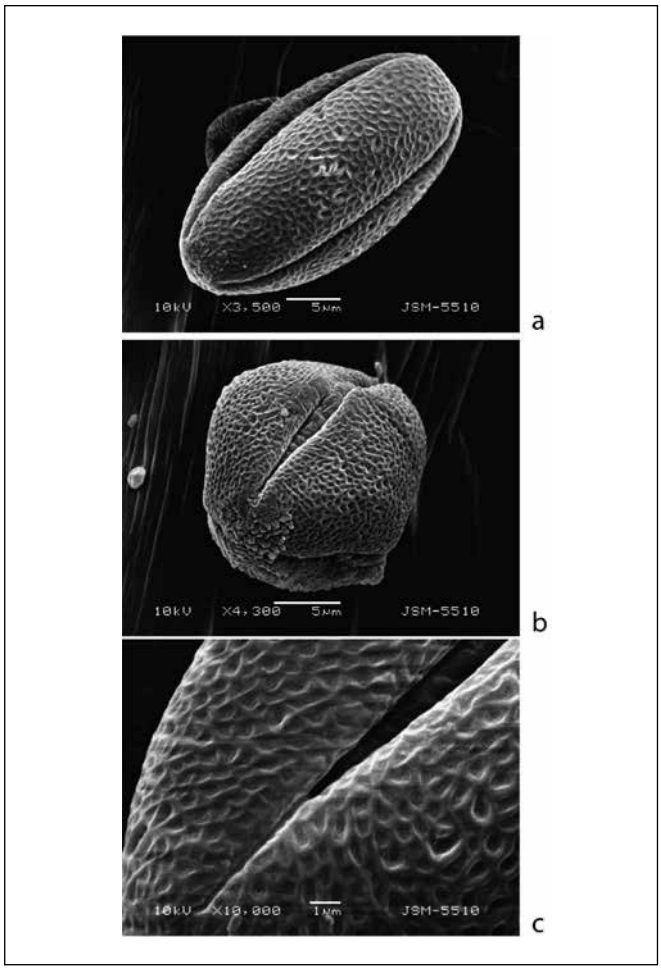

Fig. 1. Pollen of Peganum harmala (SEM). (a) General view of pollen grains; (b) Apocolpium, colpus forming an area with deposits on the exine; (c) Murus with irregular shape, colpi.
Fig. 1. Polen de Peganum harmala (MEB). (a) Vista general de los granos de polen; (b) Apocolpiumo, colpuso formando una zona con depósitos en la exina; (c) Muros con forma irregular, colpi.

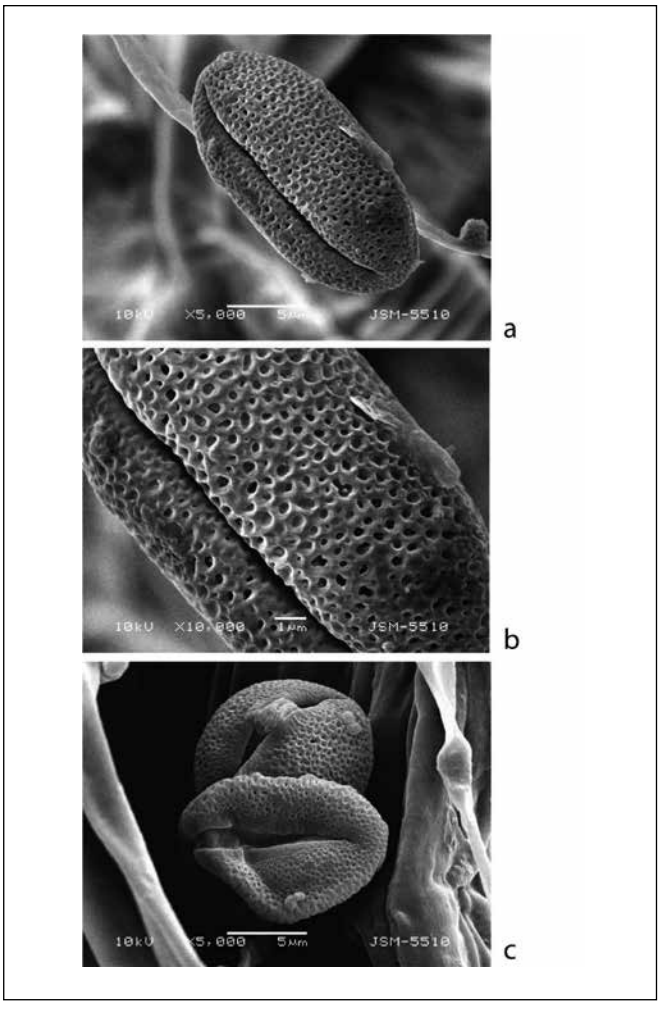

Fig. 2. Pollen of *Zygophyllum fabago* (SEM). (a) General view of pollen grains; (b) Colpus borders, thickened walls, smooth membrane; (c) Brochi, pores, colpi.
Fig. 2. Polen de *Zygophyllum fabago* (MEB). (a) Vista general de los granos de polen; (b) Paredes engrosadas en los bordes de los colpos, membrana apertural lisa; (c) Brochi, poros, colpi.

The former has the following characteristics: *Pollen class:* tri-colporate; *Shape:* sub-prolate to prolate; *Apertures:* ectocolpus long narrow with acute ends; *Exine:* sexine thicker than nexine; Ornamentation: reticulate rarely rugulate-reticulate or reticulate-foveolate. The latter has the following characteristics: *Pollen class*: tri-colporate; *Shape:* sub-prolate to prolate; *Apertures:* ectocolpus long narrow with acute ends; *Exine:* sexine thicker than nexine; *Ornamentation:* rugulate-reticulate. These characteristics of two cited pollen types given by Perveen & Qaiser (2006) show that the two types differ only in the ornamentation. However, in the present study, it was established that the two studied species (belonging to the two described types) differed also in the pollen class: tri-colporate for *P. harmala* and pantoporate for *Z. fabago*. The importance of pollen morphology in the identification and classification of plants (Doyle & Walker, 1975; Martens & Felz, 1980; Ray, 1983; Blackmore, 1984) has been widely recognized. We established differences in the characteristics of the pollen surface in the two studied species, which corresponded to two different pollen-types. This justifies that the two cited taxa can be assigned to different taxonomic groups. The same suggestion was made by Sheahan & Chase (1996, 2000) and El-Atrush et al. (2015), who recommended to separate *Peganum* species from Zygophyllaceae.

Seed coat. The shape of seeds is more or less genetically determined, but in some cases, even within the same type, there can exist differences (Werker, 1997). In the present study, we showed characteristics of the shape of seeds of *Z. fabago* and *P. harmala* which make them species-specific. These results agree with those established by Zhang et al. (2013): the characteristic of the seed surface in Zygophyllaceae species

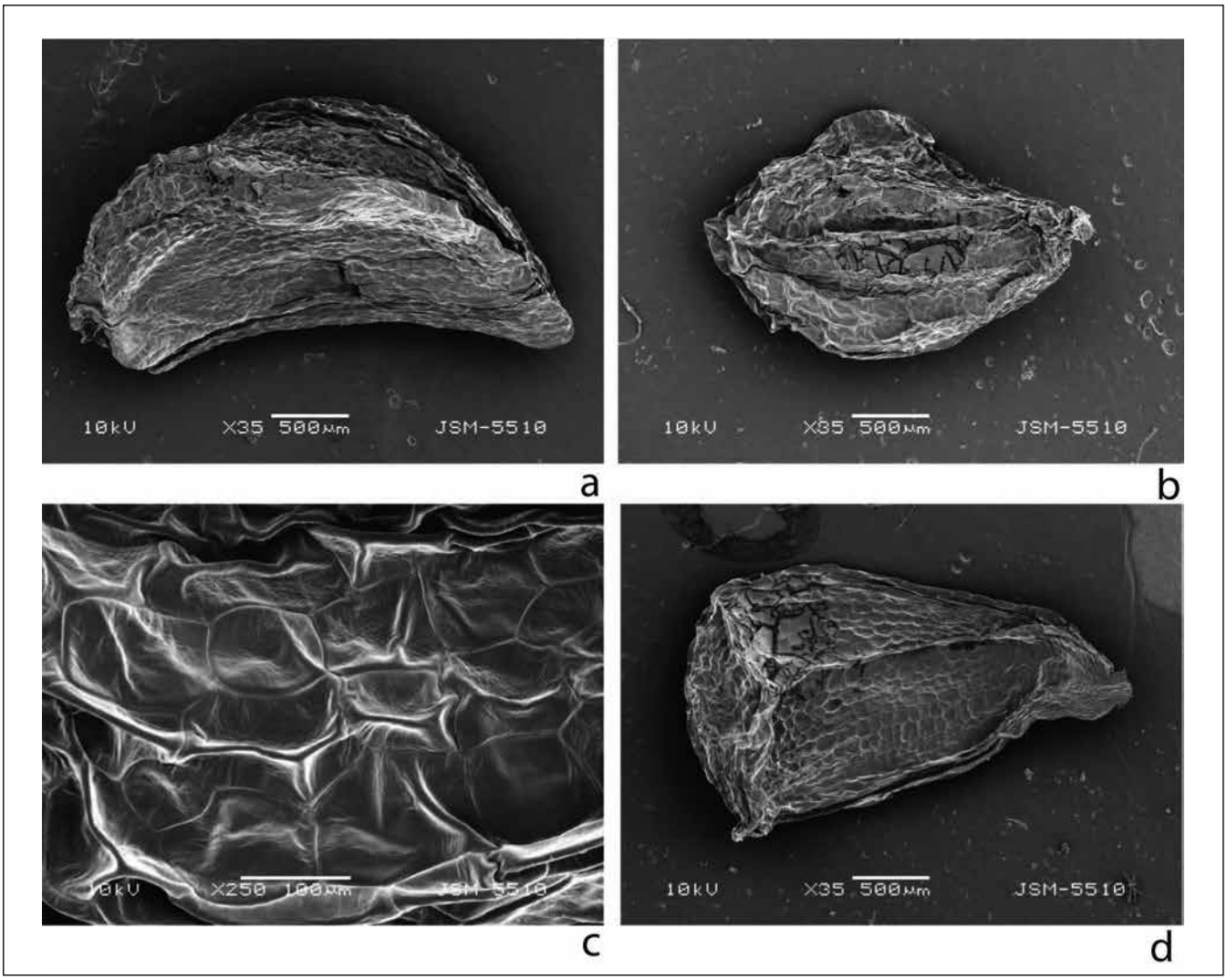

Fig. 3. Seeds of *Peganum harmala* (SEM). (a) Upper surface of the seed; (b) Lateral view of the seed; (c) Lower surface of the seed; (d) Spermoderal surface.

Fig. 3. Semillas de *Peganum harmala* (SEM). (a) Superficie superior de la semilla; (b) Vista lateral de la semilla; (c) Superficie de la cara ventral de la semilla; (d) Vista general de la semilla.

is different among the distinct species but is relatively stable within the species.

As noted by Axelius (1992) and Watanabe (1999), the seed surface ornamentation is an important diagnostic feature of seeds that can be used in taxonomic decisions in many families (Esau, 1953; Takhtajan, 1991). An important feature for the spermoderm specificity is the shape of cells of its surface layer, their dimensions, type of anticlinal and periclinal walls, and the presence of wax secretions (Deborah, 2005). The secondary sculpture of the periclinal walls can also be used for diagnosis of the seeds (Hufford, 1995; Koul et al., 2000). The present SEM study of micromorphology of spermoderm of *Z. fabago* and *P. harmala* showed that at the submicroscopic level, the seed surface of the two studied species differed considerably. It may be used as a criterion for distinguishing them. In *Z. fabago*, the surface can be defined as Concave to Convex-type, and in *P. harmala* as Tabular to lightly Concave-type, according to the classification of Bartholtt & Ehler (1977). The same type of sculpture was determined in *T. terrestris* seed (Semerdjieva et al., 2011).

This agrees with the consideration of Zhang et al. (2013): the species of Peganiaceae (in which are placed *Peganum* species from Dahlgren, 1989) could be separated from Zygophyllaceae, on the base of their seed morphological characteristics.

CONCLUSIONS

In the present and previous studies carried out, it was revealed the pollen morphology of the representatives of Zygophyllaceaee family from Bulgarian flora. Despite the isolated

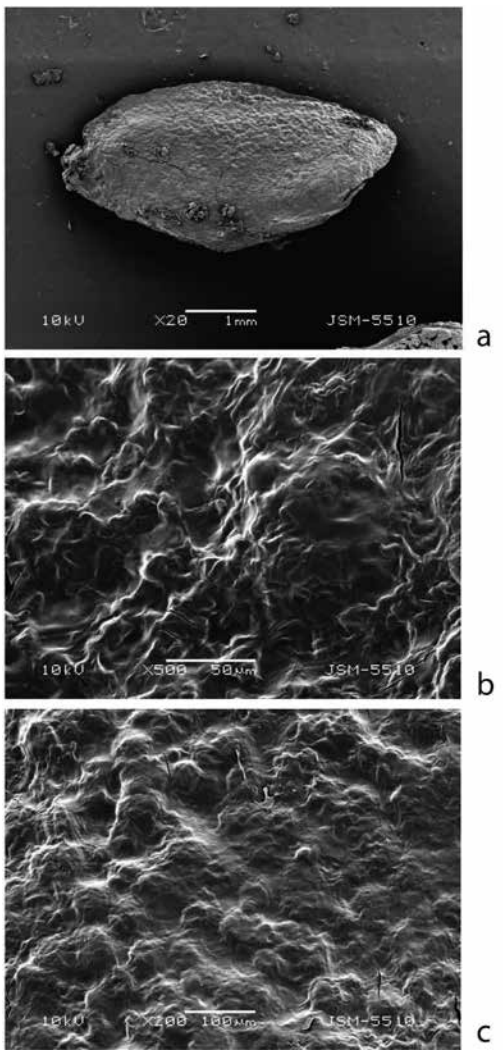

Fig. 4. Seeds of *Zygophyllum fabago* (SEM). (a) General view of the seed; (b) and (c) Spermodermal surface, papillae, epicuticular wax folds.
Fig. 4. Semillas de *Zygophyllum fabago* (SEM). (a) Vista general de la semilla; (b) y (c) Superficie spermodermal, papilas, pliegues de cera epicuticulares.

position of *T. terrestris* from *Z. fabago* and *P. harmala* regarding pollen grains sculpture, *T. terrestris* shows some similarities with the other two species, especially with *Z. fabago*. Thus, this latter species, typical of the Zygophyllum simplex pollen type, could be considered as a transitional one toward the *Tribulus terrestris*-type and *Peganum harmala*-type.

The results of the present study on pollen and seed morphology of the two *Zygophyllaceae* species support the proposed by others authors separation of *Peganum* species from *Zygophyllaceae* in a different family: Peganiaceae.

A comparative analysis of the seed surface studied in *Z. fabago* and *P. harmala*, and the previously studied *T. terrestris*, shows that the characteristics of the spermoderm of *Z. fabago* significantly differ from those in *P. harmala* and *T. terrestris*. The seed surface of the latter two species showed a similar character (Tabular to slightly Concave-type). Therefore, the submicroscopic characteristics of the seed coat can be used to solve biosystematic problems in the family *Zygophyllaceae*.

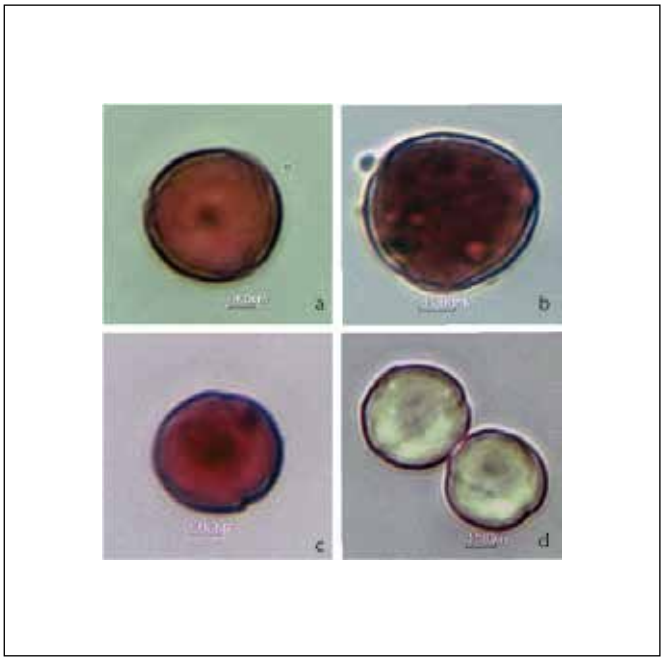

Fig. 5. General view of pollen of *Peganum harmala* and *Zygophyllum fabago* (LM). (a) (b) *Peganum harmala*; (c) (d) *Zygophyllum fabago*.
Fig. 5. Vista general del polen de *Peganum harmala* and *Zygophyllum fabago* (LM). (a) (b) *Peganum harmala*; (c) (d) *Zygophyllum fabago*.

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