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Seed morphology and surface microstructure of some *Euphorbia* (Euphorbiaceae) taxa distributed in Turkey-in-Europe

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Abstract: The taxa of the genus *Euphorbia* L. have seeds that mature in schizocarp fruits, called cocci, with three or more cells, each of which explosively splits open into separate parts at maturity. The *Euphorbia* seeds have taxonomically useful characters. We studied 13 taxa of the genus *Euphorbia* distributed in Turkey-in-Europe, with a focus on the seed characters; for morphological observations we used a stereomicroscope, and for surface microstructure we used a scanning electron microscope (SEM). The morphological observations showed that the seed characters of shape, size, color, surface features, and the features of the caruncle are uniquely different. SEM observations showed that the seed surface microstructures are also uniquely different, and detected globules that burst out of the surface between the cells. The function of lipid-rich globules is discussed.

Key words: Turkey, SEM, stereomicroscope, caruncle, globules, lipid globules

1. Introduction

The taxa of the genus *Euphorbia* L. have seeds that are (in most cases) taxonomically useful. If the samples are collected at the right time, the seeds pop out of the fruits while the samples are pressed and dried in order to be stored in herbaria. Sometimes the exemplars are covered with hundreds of seeds and the researcher can in most cases distinguish the taxon from the seed characters alone. As a matter of fact, Khan (1964) built up a key to Turkish *Euphorbia* based on seed characters alone at the end of his extensive revision of *Euphorbia* in Turkey.

The taxa of *Euphorbia* have a particular form of pseudanthium called a cyathium and the seeds maturate in schizocarp fruits, called cocci, with three or more cells, each of which explosively splits open into separate parts at maturity, functioning as a ballistic dispersal mechanism. The characteristic seeds have been a focus point in various studies with regard to anatomy (Mandl, 1926; Singh, 1969), morphology (Rössler, 1943; Bojňanský and Fargašová, 2007), micromorphology (Ehler, 1976; Park, 2000), and phylogeny (Pahlevani and Akhani, 2011; Salmaki et al., 2011). The shape, color, size, and especially microstructure (including ultrastructure) of seeds and fruits provide valuable systematic information. In practical terms, many important data gathered from epidermal surface analyses were obtained by scanning electron microscope (SEM). According to Barthlott (1981), these surface characters can be basically divided into four major groups: 1) cellular arrangement; 2) shape of cells; 3) relief of outer cell walls; and 4) epicuticular secretions. These characters, which show a great variety from the species to the family level, are not easily affected by environmental conditions.

After Khan's (1964) revision, several studies examined the distribution, taxonomy, and morphology of *Euphorbia* taxa in Turkey: Ertem completed a doctoral thesis (İstanbul University Faculty of Pharmacy) and later published the study with drawings of diagnostic characters (Baytop and Ertem, 1971). The genus *Euphorbia* in *Flora of Turkey* was written by Radcliffe-Smith (1982); Can et al. (2010) presented the pollen morphology of the taxa of the subgenus *Chamaesyce* in Turkey (poster presentation at the Biodiversity and Evolutionary Biology Symposium of DBG in Vienna); Can et al. (2012) published a research article regarding *E. amygdaloides* subsp. *robbiae*'s rediscovery; and Uruşak et al. (2013) published a floristic study of the Yıldız Mountains in Northwest Turkey with an update of the *Euphorbia* taxa, with new records of distribution. However, the examination of seed characters in this genus needs further investigation. Thus, the current study focused on the seed shape, color, surface properties, microstructure, caruncle properties, and periclinal walls of the following taxa distributed in Turkey-in-Europe: *Euphorbia falcata*

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L. subsp. *falcata*, *Euphorbia platyphyllos* L., *Euphorbia stricta* L., *Euphorbia taurinensis* All., *Euphorbia agraria* M.Bieb., *Euphorbia amygdaloides* L. subsp. *amygdaloides*, *Euphorbia characias* L. subsp. *wulfenii* (Hoppe ex W.Koch) Radcl.-Sm., *Euphorbia illirica* Lam., *Euphorbia myrsinites* L., *Euphorbia oblongata* Griseb., *Euphorbia pannonica* Host, *Euphorbia rigida* M.Bieb., and *Euphorbia niciciana* Borbás ex Novák.

2. Materials and methods

Examined specimens and their origins are presented in Table 1. The specimens were collected when fruits were fully developed and then pressed and dried as herbarium sheets. Macromorphological features of seeds were analyzed by an Olympus ZS51 stereomicroscope and Kameram imaging software. The colors were determined according to the Royal Horticultural Society (2007) Colour Charts (abbreviation: RHSCC) to avoid any subjectivity. Seed specimens were prepared for electron microscopy by mounting them to a table with silver adhesive. They were analyzed with a JEOL Neoscope-5000 scanning electron microscope (SEM). The lipid granules were analyzed with an Olympus CX41 light microscope and Olympus imaging software. Sudan III was used to dye the lipids.

3. Results

The results of the study are summarized in Table 2. The dorsal and ventral photomicrographs of the studied seeds are presented in Figure 1. The seed color varies from grayish white to brown, and the seed shape varies from round to tetragonal. Seed surfaces are different in each taxon. For instance, the sub-succulent *Euphorbia myrsinites* possesses seeds with a woody bark-like surface. Collected from the same locality, the annual herbaceous *E. taurinensis* possesses seeds with a velvety surface and unregular dark-colored cavities. The seed length also varies from c. 2 mm up to 4 mm.

Each studied seed possesses a caruncle of different shape, color, and size. Only in *Euphorbia falcata* subsp. *falcata* did we observe seeds with and without a caruncle. This is due to the fact that the caruncle of this taxon breaks away very easily with the slightest pressure. In fact, after a while it was hard to find seeds possessing a caruncle.

The SEM micrographs of seed surface microstructures are given in Figure 2. Surface microstructure was observed as mostly reticulate, except for *Euphorbia taurinensis* and *E. myrsinites*, whose seed surfaces differ by their rugous texture. The periclinal wall features in some taxa are protruding, while in others they are sunken or on the same level with the surface. The surface microstructure of the caruncle showed no particular taxonomic character in any of the taxa.

Table 1. List of the examined taxa with voucher number and province information.

Taxa		Voucher number	Province
1	*Euphorbia agraria* M.Bieb.	ISTF40792	Tekirdağ
2	*Euphorbia amygdaloides* L. subsp. *amygdaloides*	ISTF40786	Kırklareli
3	*Euphorbia characias* L. subsp. *wulfenii* (Hoppe ex W.Koch) Radcl.-Sm.	ISTF40794	Tekirdağ
4	*Euphorbia falcata* L. subsp. *falcata*	ISTF40798	Kırklareli
5	*Euphorbia illirica* Lam.	ISTF40797	Kırklareli
6	*Euphorbia myrsinites* L.	ISTF40796	Tekirdağ
7	*Euphorbia niciciana* Borbás ex Novák	ISTF40799	İstanbul
8	*Euphorbia oblongata* Griseb.	ISTF40803	Tekirdağ
9	*Euphorbia pannonica* Host	ISTF40801	İstanbul
10	*Euphorbia platyphyllos* L.	ISTF40789	Kırklareli
11	*Euphorbia rigida* M.Bieb.	ISTF40790	Çanakkale
12	*Euphorbia stricta* L.	ISTF40800	İstanbul
13	*Euphorbia taurinensis* All.	ISTF40795	Tekirdağ

Table 2. Features of the examined *Euphorbia* seeds. STM: Stereomicroscope. SEM: Scanning electron microscope. Seed colors were determined according to the Royal Horticultural Society (2007) Colour Charts.

	Taxon	Seed shape	Seed surface (STM)	Seed color	Caruncle	Seed surface microstructure (SEM)	Periclinal walls	Lipid granules
1	*E. agraria*	Ovoid-oblong	Smooth	Gray-deep gray (N187 B)	Compressed, sessile, white-gray	Reticulate	Smooth	Absent
2	*E. amygdaloides* subsp. *amygdaloides*	Ovoid-oblong	Smooth	Deep gray (N187 B)	Discrete, ±erect, lunate, orange	Reticulate	Smooth	Absent
3	*E. characias* subsp. *wulfenii*	Ovoid-spherical	Smooth	Deep gray (N92 B)	Erect, conical, peduncled, bilobate, yellow	Reticulate	Slightly protruding	Absent
4	*E. falcata* subsp. *falcata*	Compressed, ovoid-tetragonal	Smooth, transversal pits	Pale gray (N187 C)	Bulgy, conical, white, splits immediately	Reticulate	Slightly sunken	Present
5	*E. illirica*	Ovoid	Smooth	Pale pink-brown 6(N170 A)	Erect, fringy, yellow	Reticulate	Slightly sunken	Absent
6	*E. myrsinites*	Tetragonal	Tuberous, woody	Gray-brown (177 A)	Erect, ±conical, peduncled, white	Rugous, fissured	Protruding	Present
7	*E. niciciana*	Ovoid	Smooth	Pale gray (N187 B)	±Erect, reniform, sessile, yellow	Reticulate	Sunken	Present
8	*E. oblongata*	Ellipsoid	Smooth	Brown (187 A)	Compressed, sessile, small, crescent-shaped, brown-black	Reticulate	Slightly protruding	Absent
9	*E. pannonica*	Ovoid	Smooth	Pale gray, flecked (97 C)	±Erect, reniform, bilobate, orange	Reticulate	Slightly protruding	± Present
10	*E. platyphyllos*	Ovoid-oblong	Smooth	Gray-deep purple (N187 A)	Compressed, sessile, small, crescent-shaped, yellow	Reticulate	Smooth	Absent
11	*E. rigida*	Ellipsoid-cylindrical-tetragonal	± Smooth	Brownish white (N200 C)	Erect, conical, peduncled, brown-yellow	Reticulate	Protruding	Present
12	*E. stricta*	Ellipsoid	Smooth	Brown (187 A)	Compressed, sessile, small, crescent-shaped, brown-yellow	Reticulate	Smooth	Absent
13	*E. taurinensis*	Ovoid	Velvety, dark pits	Grayish white (N187 C)	Erect, conical, bilobate, white	Rugous	Protruding	Present

Figure 1. Stereomicroscope images of seeds belonging to the examined *Euphorbia* taxa. Left image dorsal, right image ventral. **A.** *E. falcata* subsp. *falcata*; **B.** *E. agraria* **C.** *E. amygdaloides* subsp. *amygdaloides*; **D.** *E. illirica*; **E.** *E. rigida*; **F.** *E. myrsinites*; **G.** *E. characias* subsp. *wulfenii*; **H.** *E. niciciana*; **I.** *E. platyphyllos*; **J.** *E. stricta*; **K.** *E. oblongata*; **L.** *E. pannonica*; **M.** *E. taurinensis* (white bar 1 mm).

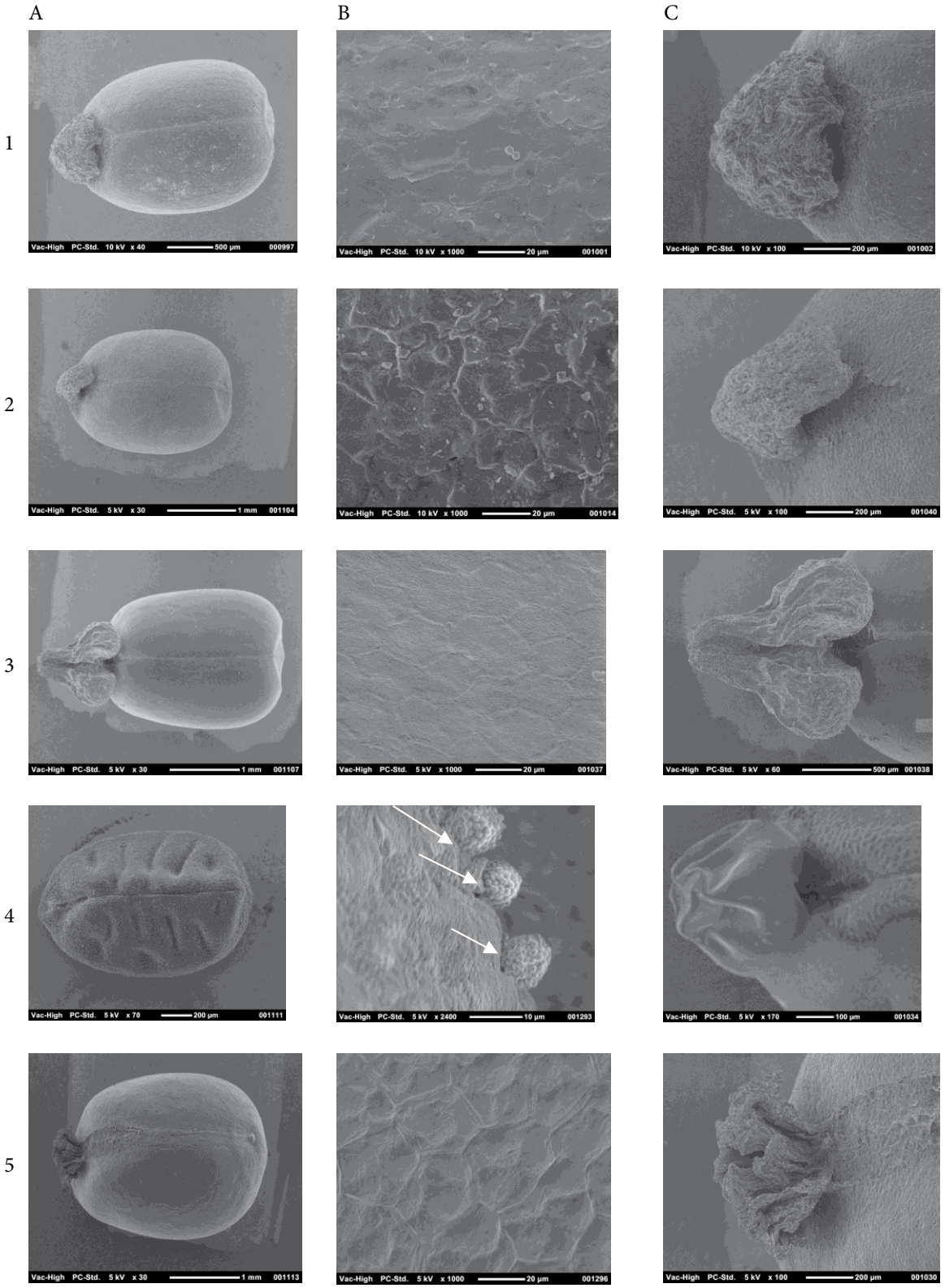


Figure 2. SEM micrographs of seeds belonging to the examined *Euphorbia* taxa. **A.** Ventral view; **B.** Surface from ventral (white arrow indicates lipid granules); **C.** General view of caruncle. **1.** *E. agraria*; **2.** *E. amygdaloides* subsp. *amygdaloides*; **3.** *E. characias* subsp. *wulfenii*; **4.** *E. falcata* subsp. *falcata*; **5.** *E. illirica*; **6.** *E. myrsinites*; **7.** *E. niciciana*; **8.** *E. oblongata*; **9.** *E. pannonica*; **10.** *E. platyphyllos*; **11.** *E. rigida*; **12.** *E. stricta*; **13.** *E. taurinensis*.

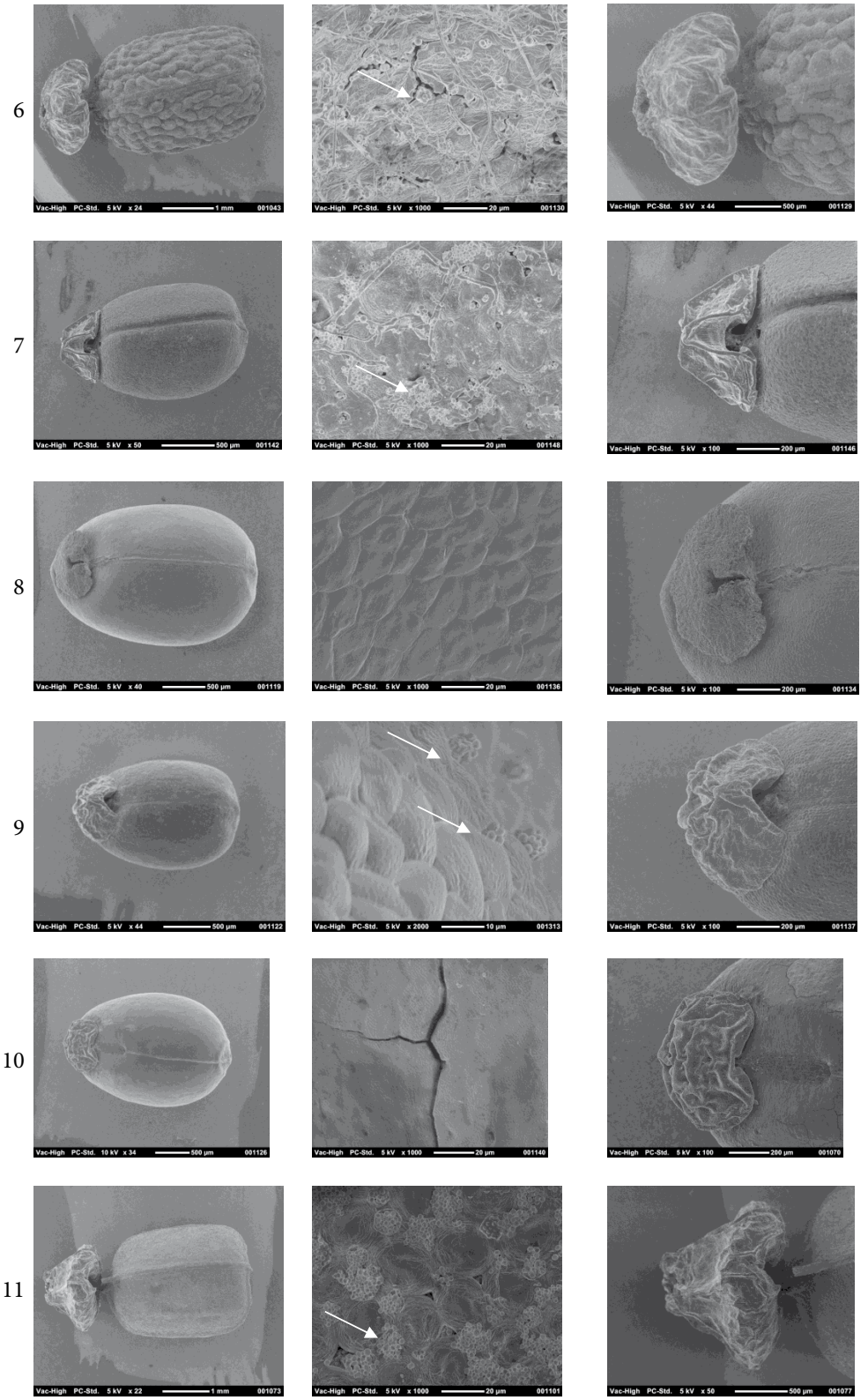


Figure 2. (Continued).

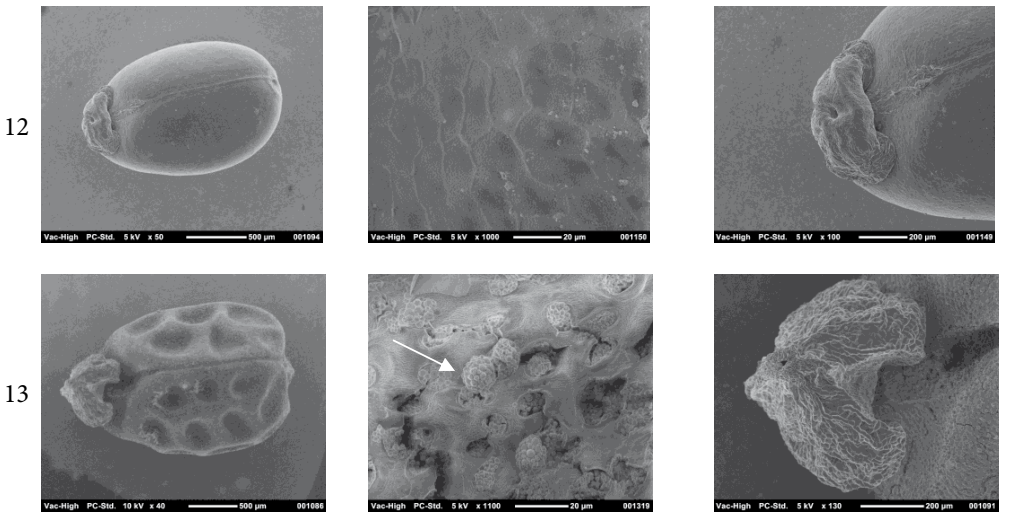
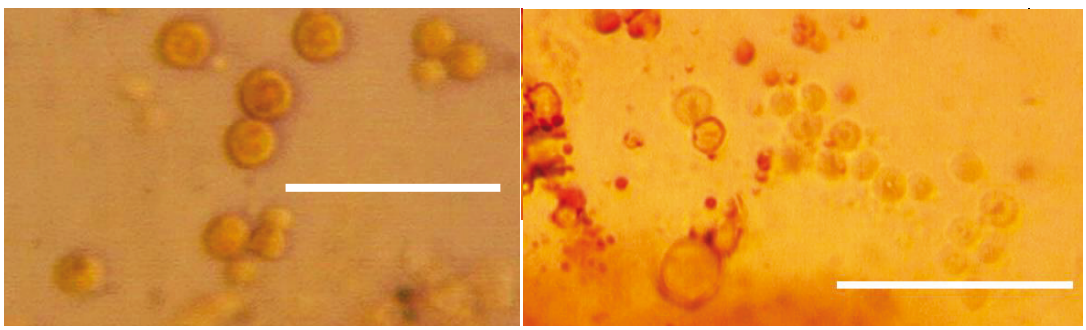


Figure 2. (Continued).

During the SEM studies we observed "globules" that were bursting out of the surface between the cells in six taxa (Figure 2, white arrows). It should be noted that the literature mentioned in the introduction makes no mention of such globules. Suspecting the presence of lipids, we scratched the seed surface of *Euphorbia myrsinites* and *E. rigida* with a razor and treated it with Sudan III. Figure 3 indicates the existence of lipids in the globules.

4. Discussion

Previous studies on seed morphology and surface micromorphology indicate that seed characters are important for the taxonomy of the genus *Euphorbia*. Our study also confirms their importance; it shows that seed features, such as ornamentations of the seed surface, seed shape and color, the presence or absence of a caruncle, and caruncle shape and color are useful characters for identification of Turkish *Euphorbia* taxa. In fact, with the help of a magnifying glass alone, one could possibly identify most of the Turkish *Euphorbia* taxa from only one seed. The examined seeds are variable in both shape and size. The size of the smallest seed is about 2 mm in length (*E. falcata* subsp. *falcata*, *E. niciciana*, *E. stricta*, *E. pannonica*, *E. taurinensis*) and the size of the largest seed is about 4 mm in length (*E. rigida*, *E. myrsinites*, *E. characias* subsp. *wulfenii*). Most of the examined seeds are ovoid or ovoid-oblong (*E. platyphyllos*, *E. taurinensis*, *E. agraria*, *E. amygdaloides* subsp. *amygdaloides*, *E. illirica*, *E. pannonica*, *E. niciciana*), whereas the three taxa with the largest seeds are differently shaped: *E. characias* subsp. *wulfenii* with ovoid to almost spherical seeds, *E. myrsinites* with tetragonal seeds, and *E. rigida* with ellipsoid to cylindrical-tetragonal seeds. The seeds of *E. stricta* and *E. oblongata* are also ellipsoid shaped, but the smaller seeds of *E. falcata* subsp. *falcata* are somewhat compressed. The seed colors also vary among the examined taxa, mostly in tones of brown and gray. There is more color variation in *E. illirica,* which is pale pink to brown (RHSCC: N170 A); in *E. platyphyllos* (RHSCC: N187 A), which is deep purple; and in *E. rigida* (RHSCC: N200 C), which is brownish



Euphorbia myrsinites *Euphorbia rigida*

Figure 3. Light microscope micrographs of lipid granules on the seed surfaces of two taxa (white bar = 10 µm).

white. The color and surface texture of *E. myrsinites* seeds is somewhat woody or bark-like, with a tuberous surface. The seed surface of *E. taurinensis* possesses irregularly shaped dark-colored pits and a velvety surface, whereas in *E. falcata* subsp. *falcata* the pits are transversally aligned.

The caruncle characters are unique as well. In most of the previous studies the caruncle was considered as present or absent. However, the caruncle also differs by its size, shape, and color. The caruncle of the examined taxa is conical (*E. falcata* subsp. *falcata*, *E. taurinensis*, *E. characias* subsp. *wulfenii*, *E. myrsinites*, *E. rigida*), crescent-shaped (*E. platyphyllos*, *E. stricta*, *E. oblongata*), or reniform (*E. pannonica*, *E. niciciana*), whereas the colors are mostly yellow to brown. However, *E. oblongata* seeds have a caruncle that is brown to blackish, so that at first sight the seeds seem to have no caruncle. There are also taxa that possess seeds with a white caruncle (*E. falcata* subsp. *falcata*, *E. taurinensis*, *E. agraria*, *E. myrsinites*). The caruncles of *E. taurinensis*, *E. pannonica*, and *E. characias* subsp. *wulfenii* are different with their bilobate shape. In some taxa the caruncle is easily breakable, possibly resulting in misleading observations of the study; in fact, Ertem indicated in her doctoral thesis (İstanbul University Faculty of Pharmacy) that *Euphorbia falcata* subsp. *falcata* seeds are ecarunculate and drew the seeds in the publication of Baytop and Ertem (1971) without a caruncle, which is not true, as can be seen from Figures 1 and 2. There is no correlation between the size of the seed or the caruncle and the breakability of the caruncle. The same-size seeds of *E. taurinensis* possess a caruncle that does not break away easily. It is important to observe an adequate amount of seeds before classifying a seed as ecarunculate.

The seed surface microstructure of the examined taxa shows that most of the seeds have reticulate testa surface. Only in two taxa (*E. taurinensis* and *E. myrsinites*) is the testa surface rugous or fissured. The seed surface microstructure and morphology of these two taxa are in correlation because *E. taurinensis* has a velvety seed surface and *E. myrsinites* has a woody or bark-like seed surface. The other taxa that have seeds with smooth surfaces also have reticulate testa structures and periclinal walls that are smooth, slightly sunken, or protruding. However, the periclinal walls of *E. taurinensis* and *E. myrsinites* seeds are protruding from the surface. Another observation on the testa structures of these two taxa and 4 other taxa (*E. falcata* subsp. *falcata*, *E. pannonica*, *E. niciciana*, and *E. rigida*) is that lipid globules are bursting out between the testa cells. Seeds of taxa belonging to Euphorbiaceae can have 36% lipid content of their dry mass (Levin, 1974), or even up to 59% in some cases such as in *E. heterophylla* (Suda and Giorgini, 2000). The caruncle is also a fat- and protein-rich fleshy appendage or protuberance that promotes dispersal by ants, functioning as an elaiosome (Pemberton and Irving, 1990; Baiges et al., 1991). However, the seed dispersal system in the taxa of *Euphorbia* is diplochorous: after a ballistic dispersal that scatters the seeds, some ant species find and retrieve the seeds to their nests (myrmecochorous dispersal) (Gomez and Espadaler, 1998; Narbona et al., 2005). Ecarunculate seeds have more restricted distributions than those with a caruncle (Khan, 1964; Baiges et al., 1991; Espadaler and Gomez, 1996; Gomez and Espadaler, 1998; Narbona et al., 2005). On the other hand, the ecarunculate seeds of some taxa in the subgenus *Chamaesyce* Raf. section *Anisophyllum* Roeper possess a seed coat that becomes sticky when wet (Yang and Berry, 2011), so that long-distance dispersal of seeds can occur by adhering to birds (epizoochory). Baiges et al. (1991) showed that seeds without a caruncle are rejected by ants, even if the seeds are artificially ecarunculate, and free caruncles are quickly removed by ants, which is in agreement with the attractiveness of elaiosome cells containing lipid droplets. However, whether the observed lipid globules function as elaiosomes or as a reserve mobilization in endosperm (Suda and Giorgini, 2000) is a complicated question. Further investigations are needed to clarify their function, evolution, and relationship with ants.

In conclusion, the results presented here are in agreement with previous studies and show that seed features of the studied taxa are important in identification and taxonomy (Khan, 1964; Baytop and Ertem, 1971; Radcliffe-Smith, 1982). More accurate illustrations of gross morphology of seeds are presented, and the detailed definitions of important characters in Table 2 will help identify the taxa in Turkey with the naked eye. To avoid subjectivity, the Royal Horticultural Society (2007) Colour Charts were used to determine seed color. More accurate definitions of caruncle characters are given, emphasizing that caruncle characters are stable and diverse and therefore relevant for the taxonomy of the genus. Detailed microphotographs of testa surfaces show the diversity of micromorphology in *Euphorbia* seeds. However, our micromorphology results also show that more studies on lipid globules are needed to understand the biology and ecology of *Euphorbia* seeds and their distribution strategies. Further studies on seed morphology and micromorphology in *Euphorbia* may also provide additional insights and clarifications of its taxonomy.

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