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Seed morphology of some taxa of the genus *Euphorbia* L. (Euphorbiaceae) in Turkey and its taxonomic significance

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Abstract: The morphological characteristics of seeds are not affected by environmental factors and can be used to identify the *Euphorbia* taxa. The present study aims to determine the morphological characteristics of seeds in 27 taxa of *Euphorbia* using scanning electron microscopy (SEM) and light microscope (LM). Findings revealed that the seeds of *Euphorbia* show variation in size, color, and seed surface ornamentations among different species. The seeds vary also in shape from ovoid, ellipsoidal, globose, quadrangular, compressed, to compressed-ellipsoidal with two exceptions in *E. petiolata* (oblong-quadrangular truncate), *E. macrocarpa* (subglobose), and *E. szovitsii* var. *kharputensis* (ellipsoidal quadrangular truncate). Furthermore, it was demonstrated that caruncle color, shape, and surface are different in the studied *Euphorbia* spp. Special attention was also paid to lipid granules indicating their shape variation among taxa from globose, broad ellipsoidal globose to reniform, although the granules were absent in some taxa. In addition, some taxa lack any caruncle. The unweighted pair group method with arithmetic mean and principal component analysis was used to assess the morphological differentiation of the seeds among the studied taxa. According to the dendrogram and plot obtained from cluster and PCA analyses, *Euphorbia* taxa are divided into four clusters corroborating the previous molecular phylogenetic results.

Key words: Caruncle, *Euphorbia*, LM, seed morphology, SEM

1. Introduction

Euphorbia is the largest genus of Euphorbiaceae and has over 2000 taxa that generally grow in the subtropics and temperate areas including the Middle East, the Mediterranean, South Africa, and the southern USA (Gilbert, 1987; Carter, 1994; Bruyns et al., 2006; Bolaji et al., 2014). The genus has a wide range of geophytes, herbs, shrubs, understory and canopy trees, as well as a variety of succulent and xerophytic forms and is distinguished by milky latex (Fayed et al., 2007; Dorsey et al., 2013). The presence of secondary metabolites in milky latex, including phenolics, terpenes, tannins, glucosinolates, alkaloids, enzymes, fatty acids, and proteins, is important in providing a defense mechanism in these species (Pintus et al., 2010; Benjamaa et al., 2022). Phylogenetic studies suggested that *Euphorbia* is divided into four subgroups: *Rhizanthium* (Boiss.) Wheeler (earlier *Athymalus*), *Chamaesyce* Raf., *Esula* Persoon, and *Euphorbia* (Bruyns et al., 2006; Horn et al., 2012; Riina et al., 2013; Dorsey et al., 2013). Also, the genus is represented in Turkey by 120 taxa which are distributed throughout the country (Can, 2012; Genc and Kultur, 2018).

Euphorbia has remarkable diversity both vegetativelyv and reproductively, and morphological characters associated with certain parts such as seeds, capsules, and cyathial glands are used to distinguish taxa in *Euphorbia* (Steinmann and Porter, 2002; Da Silva et al., 2016; Genc et al, 2018). Khan (1964) among others studied seed characters and geographic distribution to picture out the natural relationships of the Turkish *Euphorbia* species. Also, numerous studies on the systematics of *Euphorbia* spp. have highlighted the taxonomic significance of seed morphology (Krochmal, 1952; Park, 2000; Salmaki et al., 2011; Pahlevani et al., 2015; Da Silva et al., 2016; Genc and Kultur, 2018). Nonetheless, more detailed investigations are needed for understanding the morphological diversity of Turkish *Euphorbia* spp. (Genc et al., 2018). It is assumed that seed characters are not affected by the environment and display a high level of diversity from species to family in *Euphorbia* (Can and Kucuker, 2015; Pahlevani et al., 2015). The presence or absence of the caruncle and the shape, color, surface, and size of the seeds and caruncle are used in the systematics of *Euphorbia* (Khan, 1964;

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Simon et al., 1992; Park, 2000; Salmaki et al., 2011). The aim of the present study was to show the diversity of seed morphological characters, including seed size, shape, surface, seed color, caruncle, and lipid granules in 27 *Euphorbia* taxa grown in Turkey using scanning electron microscopy (SEM) and light microscope (LM).

2. Material and methods

2.1. Plant materials

Samples and specimens for the present study were collected by Dr. Murat KÜRŞAT, mainly during several field trips between 2019 and 2021 in different parts of Turkey (Figure 1). The original herbarium materials are deposited in the Herbarium of Bitlis Eren University (Table 1). The aim of this study was to characterize the seed morphology of species collected from various sites, mainly in eastern Anatolia. It is hoped that this research will aid in efforts to ascertain the seed morphology of taxa that will be collected from various regions of Turkey.

2.2. Seed morphological analysis

Seed measurements were carried out under a stereomicroscope (Leica S8APO) with an incorporated camera (Leica DFC295) and Leica application suite (LAS) imaging software. Seeds of 27 taxa of *Euphorbia* were measured and surveyed for color, length, width, shape, caruncle size, caruncle shape, and lipid granule shape based on three different specimens (when available). The colors were determined according to the Royal Horticultural Society (2007) Color Charts (RHSCC) to avoid any subjectivity. The terminology of seed coat surface sculpturing generally follows Ehler (1976), Heubl and Wanner (1996), Park (2000), and Salmaki et al. (2011). In order to examine the ornamentations of the seed surface, SEM micrographs were obtained under a ZEISS Supra 55 brand electron microscope at Van Yüzüncü Yıl University Science Application and Research Center (BUAM). Seed samples were placed directly on aluminum stubs with double-sided adhesive tapes and covered with gold to obtain images under the electron microscope. Microphotographs were obtained at different magnifications for each taxon studied.

2.3. Statistical analysis

The statistical analyses were based on the seed morphological characteristics obtained from 27 taxa (Table 3). Two quantitative characters (seed width and seed length) and five qualitative characters (seed shape, seed surface-LM, seed surface-SEM, caruncle shape, caruncle surface) were used for statistical analyses. Quantitative and qualitative characters were coded as in Table 2. In order to group the taxa with morphological similarities and to find taxa relationships, the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method using the Gower general similarity coefficient was carried out with the MVSP (Multi-Variate Statistical Package). Also, PCA (Principal Component Analysis) was used with MVSP software (Kovach, 1999).

Figure 1. Location map showing in yellow the regions where plant specimens were collected (modified from Ekinci et al. 2020).

Table 1. The collection data of investigated *Euphorbia* pecimens.

Taxa	Locality	Voucher and specimen code
E. chamaesyce L.	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 03.09.2019.	M. Kürşat 6114
E. petiolata Banks & Sol.	Malatya: Pütürge, Çevrimtaş Village, meadow lands, 11.08.2021.	M.Kürşat 6119
E. rhytidosperma Boiss. & Balansa	Osmaniye: Zorkun plateau, in the Forest, 1650 m, 22.06.2021.	M. Kürşat 6125
E. grisophylla M.L.S.Khan	Bitlis: Northern Hillside of the Mount Kambos, 1650m. 29.07.2019.	M. Kürşat 6113
E. macrocarpa Boiss. & Buhse	Van: Artos mountain, Northern slopes, 2200 m, 26.07.2020.	M. Kürşat 6112
E. orientalis L.	Van: 30 km of highway from Van to Hakkari, slopes, Zernek Irrigation Dam Lake, mountain steppe, 1960 m, 27.07.2019.	M. Kürşat 6101
E. altissima Boiss. var. *altissima*	Elazığ: Baskil, Nazaruşağı neighborhood surroundings, meadow lands, 28.07.2020.	M. Kürşat 6107
E. altissima var. *glabrescens* Boiss. ex M.S.Khan	Elazığ: Baskil, Nazaruşağı neighborhood surroundings, meadow lands, 08.08.2021.	M. Kürşat 6122
E. stricta L.	Artvin: Konaklı/Ardanuç- Lahşet plateau, 1900m, 30.06.2021.	M. Kürşat 6124
E. microsphaera Boiss.	Elazığ: Sindipik Village, 1800 m, 12.08.2021.	M.Kürşat 6120
E. gaillardotii Boiss. & Blanche	Elazığ: Freeway, Meryem Mountain, in the field, 08.08.2019.	M. Kürşat 6110
E. rhabdotosperma Radcl.-Sm.	Elazığ: Keban, Keban Dam, 1430-1450 m, 2021.	M. Kürşat 6118
E. helioscopia L.	Siirt: Tillo, Around Ismail Fakirullah Tomb, 1100 m, 09.04.2021.	M. Kürşat 6121
E. aleppica L.	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 04.08.2019.	M. Kürşat 6105
E. szovitsii var. *kharputensis* Azn. ex M.S.Khan	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 04.08.2019.	M. Kürşat 6115
E. falcata L.subsp. *falcat*a	Elazı: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 04.08.2019.	M. Kürşat 6111
E. denticulata Lam.	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 01.08.2019.	M. Kürşat 6102
E. craspedia Boiss.	Mardin: Savur, Pınardere neighborhood, Stony land,899 m, 08.04.2020.	M. Ayaz 070
E. macroclada Boiss.	Van,:Gevaş, Roadside, Slopes, 1750 m, 28.07.2019.	M. Kürşat 6103
E. cheiradenia Boiss. & Hohen.	Van: Kuzgun Kıran Pass, 2240 m, 22.07.2019.	M. Kürşat 6106
E. seguieriana Neck. subsp. *seguieriana*	Van: Gevaş to Edremit, Roadside, Slopes, 1750 m, 28.07.2019.	M. Kürşat 6109
E. heteradena Jaub. & Spach	Van: Gevaş to Edremit, in the field, 1750 m, 28.07.2019.	M. Kürşat 6108
E. esula subsp. *tommasiniana* (Bertol.) Kuzmanov	Van: Edremit, roadside, 1650 m, 28.07.2019.	M. Kürşat 6100
E.sanasunitensis Hand.-Mazz.	Bitlis: Northern Hillside of the Mount Kambos, 1650m. 29.07.2019.	M. Kürşat 6104
E. iberica Boiss.	Hakkari: Cilo plateau, Avaspi glaciers, 2540 m, 28.06.2021.	M. Kürşat 6117
E.oblongifolia (K.Koch) K.Koch	Artvin: Murgul-Damar, Kabaca plateau, Öküzyatağı location, 2200 m, 30.06.2021.	M. Kürşat 6123
E. erubescens Boiss.	Osmaniye: Zorkun plateau, in the Forest, 1650 m, 22.06.2021.	M. Kürşat 6126

3. Results and discussion

Seed and caruncle types in *Euphorbia* have been utilized to resolve taxonomic issues at the subgeneric, sectional, subsectional, or species levels since their traits are typically stable (Simon et al., 1992; Park, 2000; Salmaki et al., 2011). Table 3 and Figures 2–4 summarize selected seed features among the investigated taxa.

3.1. Seed size, shapes, and surfaces

The current study showed that *E. denticulata* and *E. craspedia* (subg. *Esula*, sect. *Myrsiniteae*), *E. petiolata* (subg. Chamaesyce), and *E. macrocarpa* had the largest seeds while *E. chamaesyce* (subg. *Chamaesyce*), *E. aleppica* (subg. *Esula*, sect. *Myrsiniteae*), and *E. szovitsii* var. *szovitsii* (subgen. *Esula*, sect. *Szovitsiae*) had the smallest seed size

Table 2. Morphological characters and character states used in the statistical analyses.

Characters		Characters states
Quantitative	Seed Width	Numerical
	Seed Length	Numerical
Qualitative	Seed Shape	Oblong- quadrangular truncate (0); Subglobose (1); Ellipsoidal quadrangular truncate (2); Compressed ovoid (3); Compressed ovoid-ellipsoidal, quadrangular (4); Compressed ovoid-ellipsoidal (5); Ovoid-quadrangular (6); Ovoid rounded quadrangular (7); Ovoid, subglobose, subtetragonous (8); Ovoid-ellipsoidal (9); Ovoid-globose (10); Ovoid (11)
	Seed Surface (LM)	Smooth-shinny (0); Smooth (1); Smooth brownish mottled (2); İrregularly deeply pitted and wrinkled (3); Transversely pitted, crenulate (4); Shallowly and irregularly pitted (5); Prominently reticulate-faveolate (6); Pustulate- rugose (7); Slightly roughened (8); Reticulate-rugose (9); Longitudinally striate rugose (10); Shallowly pustulate and strombuliforum (11); Minutely and regularly punctuate-tuberculate (12); Tuberculate (13); Tuberculate-rugose (14); Irregularly tuberculate-rugose (15); Smooth to granulate (16)
	Caruncle shape	Absent (0); Applanate transversely ovate (1); Appalanate conical (2); Prominently conical (3); Minutely reniform (4); Reniform (5); Fluted-conical (6); Conical (7); Globose (8); Applanate ochreous (9); Strongly fluted (10); Petasiform (11); Hemispherical (12); Lunate ochreous (13); Shallowly consicous small stipitate (14)
	Caruncle surface	Absent (0); Reticulate (1); Rugose (2); Rugose-reticulate (3); Rugose-verrucose (4); Verrucose (5); Aculate (6)
	Seed surface (SEM)	Smooth (0); Areolate-rugose (1); Striate-verrucose (2); Polygonal reticulate (3); Polygonal reticulate-rugose (4); Polygonal reticulate-verrucose (5); Polygonal reticulate-papillate (6); Tuberculate-vesiculate (7); Tuberculate-reticulate (8); Tuberculate-rugose (9); Aculate (10); İrregular-reticulate,verrucose (11)

(Table 3). According to Da Silva et al. (2016), different seed sizes can be a useful trait for identifying a species. Additionally, the current study showed that examined species mostly had ovoid seeds. However, it has been observed oblong-quadrangular truncate (*E. petiolata*), subglobose (*E. macrocarpa*), and ellipsoidal quadrangular truncate seeds (*E. szovitsii* var. *kharputensis*) (Table 3, Figure 2). Similarly, the studies by Salmaki et al. (2011) and Fayed and Hassan (2007) revealed that the majority of *Euphorbia* species have ovoid seed shapes. Also, it was determined that the seed shapes of the *Euphorbia* species were ovoid, oblong, quadrangular, and globose by Genc and Kultur (2018). Additionally, Da Silva et al. (2016) demonstrated that seeds of *Euphorbia* vary in size and were globose, ovoid, or prismatic in shape. Park (2000) found out that the seed shape of *Euphorbia* ranges from ovate to elliptic, and is pitted. Furthermore, as demonstrated by Simon et al. (1992), *Euphorbia* seeds can vary from oval to subglose to subcylindric. Moreover, Krochmal (1952) observed subglobose, or globose-ovoid shapes as well as ovoid. Also, the seed colors of studied *Euphorbia* taxa were determined as pale-grey-brownish, greyish, white, and various shades of brown, whitish, fawn, and yellow in this study (Table 3). Similarly, a study done by Can and Kucuker (2015) showed that the seed color varies from grayish white to brown, and the seed shape from round to tetragonal in *Euphorbia.* In another study by Genc and Kultur (2018) the seed color varies from gray to brown while Park (2000) observed the color of seeds vary from white to brown. In addition to the existence of yellow, brown, or white, Krochmal (1952) also demonstrated the presence of black, red, or silver in the *Euphorbia* seeds.

The present study showed that the most of studied *Euphorbia* species have smooth seed surfaces. Similar findings were made by Genc and Kultur (2018), who found that smooth seed surfaces are more common than pitted seed surfaces in Turkey. The current study further shows that the reticulate-faveolate, striate-rugose, and reticulate-rugose seed surfaces (LM) of *E. helioscopia*, *E. rhabdotosperma*, and *E. rhytidosperma* from sect. *Helioscopia*. SEM results of the current study also showed that *E. rhytidosperma* (polygonal reticulate-papillate), and *E. microsphaera* (smooth) have distinct seed surfaces in the sect. *Helioscopia*. In addition, this study demonstrated that *E. macrocarpa,* slightly roughened-LM, and aculate-SEM, has different seed surfaces from other studied taxa of sect. *Helioscopia*. Furthermore, sect. *Pithyusa* species under study have pitted seed surfaces, while *E. macroclada* (sect. *Pithyusa*) does not have pitted seeds (Table 3; Figures 2,3). However, Salmaki et al. (2011) stated that the sect. *Pithyusa*

Table 3. Morphological features of seeds of the examined *Euphorbia* specimens.

Taxon	Seed size	Seed shape	Seed surface (LM)	Seed color	Caruncle shape and color	Caruncle surface	Seed surface (SEM)	Lipid granules
E. chamaesyce	1.65x1.00	Ovoid-quadrangular	Irregularly tuberculate-rugose	Pale grey-brownish	Absent	------	Areolate-rugose	Globose
E. petiolata	5.24x3.33	Oblong-quadrangular truncate	Shallowly pustulate and strombuliforum	Greyish, white, dark brown	Shallowly consicous, small stipitate yellow	Aculate	Tuberculate-vesiculate	Absent
E. rhytidosperma	3.70x2.89	Ovoid	Reticulate-rugose	Brown, dark brown	Applanate transversely ovate, ochreous, sessile, yellow, white	Rugose	Polygonal reticulate-papillate	Absent
E. grisophylla	4.35x3.70	Ovoid	Smooth	Grey, yellowish, brown or mottled	Appalanate conical, sessile yellow	Verrucose	Polygonal reticulate-rugose	Absent
E. macrocarpa	5.07x5.02	Subglobose	Slightly roughened	Pale grey, brown, yellowish or mottled	Minutely reniform readily deciduous, ochreous, sessile, yellowish	Rugose	Aculate	Absent
E. orientalis	3.00x2.16	Ovoid-ellipsoidal	Smooth	Shinny brown	Reniform, sessile, yellow	Rugose-verrucose	Polygonal reticulate-rugose	Absent
E. altissima var. *altissima*	3.05x2.47	Ovoid-ellipsoidal	Smooth	Shinny brown	Reniform, deciduous, sessile, yellow	Rugose	Polygonal reticulate-verrucose	Absent
E. altissima var. *glabrescens*	2.81x2.05	Ovoid-ellipsoidal	Smooth	Shinny brown	Reniform, deciduous, sessile, yellow	Rugose	Polygonal reticulate-verrucose	Absent
E. stricta	2.06x1.38	Ovoid-ellipsoidal	Smooth	Shinny dark brown, shinny fawn	Reniform, sessile, yellowish	Reticulate	İrregular-reticulate,verrucose	Absent
E. microsphaera	3.29x2.28	Ovoid-ellipsoidal	Smooth-shinny	Brown, yellow	Reniform, sessile, yellowish	Rugose-reticulate	Smooth	Absent
E. gaillardotii	1.95x1.35	Ovoid-ellipsoidal	Shallowly and irregularly pitted	Pale grey, brownish	Absent	------	Polygonal reticulate-rugose	Broad ellipsoidal, globose
E. rhabdotosperma	2.35x1.85	Ovoid-globose	Longitudinally striate rugose	Dark brown	Applanate, transversely ovate, ochreous refringent, yellow	Aculate	Striate-verrucose	Absent
E. helioscopia	2.81x2.05	Ovoid-globose	Prominently reticulate-faveolate	Dark brown	Applanate, transversely ovate, straw, sessile, yellow	Aculate	Polygonal reticulate verrucose	Absent

Table 3. (Continued) Morphological features of seeds of the examined *Euphorbia* specimens.

Taxon	Seed size	Seed shape	Seed surface	Seed color	Caruncle shape	Caruncle surface	Seed surface (SEM)	Lipid granules
E. aleppica	1.74x1.30	Ovoid, subglobose, subtetragonous	Minutely and regularly punctuate-tuberculate	Pale grey, fawn, brownish	Absent	-----	Tuberculate-reticulate	Absent
E. szovitsii var. *kharputensis*	1.60x1.02	Ellipsoidal quadrangular truncate	Pustulate-rugose	Grey brownish	Globose, small stipitate, white	Rugose	Areolate-rugose	Globose
E. falcata subsp. *falcata*	2.86x1.41	Compressed ovoid-ellipsoidal, quadrangular	Transversely pitted, crenulate	Pale grey, brown	Prominently conical, readily caducous, sessile, base white, top yellow	Reticulate	Polygonal reticulate	Globose
E. denticulata	6.13x3.56	Ovoid, quadrangular	Tuberculate-rugose	Pale grey, pale brown	Prominently conical, undulate, stipitate white to yellow	Rugose-verrucose	Tuberculate-rugose	Globose
E. craspedia	6.03x3.56	Ovoid, quadrangular	Tuberculate	Pale grey, pale brown	Fluted-conical, stipitate, white to yellow	Rugose	Areolate-rugose	Globose
E. macroclada	4.38x2.56	Ovoid	Smooth	Pale grey, pale brown	Conical sessile, yellow, whitish	Rugose-verrucose	Polygonal reticulate-verrucose	Globose
E. cheiradenia	4.05x2.47	Compressed ovoid-ellipsoidal	Irregularly deeply pitted and wrinkled	Pale grey, brown	Conical stipitate base white, top yellowish	Rugose	Polygonal reticulate-rugose	Globose
E. seguieriana subsp. *seguieriana*	2.80x1.75	Ovoid-ellipsoidal	Smooth brownish mottled	Pale grey	Hemispherical sessile yellow	Rugose-verrucose	Polygonal reticulate-rugose	Broad ellipsoidal, globose
E. heteradena	4.63x3.75	Ovoid rounded quadrangular	Smooth	Pale grey, whitish, dark brown	Strongly fluted, ochreous, sessile, yellow to brown	Rugose-verrucose	Polygonal reticulate-rugose	Broad ellipsoidal, globose, reniform
E. esula subsp. *tommasiniana*	3.04x2.16	ovoid-ellipsoidal	Smooth	Grey to brown	Petasiform sessile, yellow	Rugose	Polygonal reticulate-rugose	Reniform
E. sanasunitensis	3.60x2.13	Ovoid	Smooth	Grey	Applanate ochreous sessile, yellow	Rugose-verrucose	Polygonal reticulate-rugose	Absent
E. iberica	2.70x2.19	Ovoid	Smooth	Grey to brown	Petasiform, sessile, yellow	Rugose	Polygonal reticulate-rugose	Absent
E. oblongifolia	2.78x2.24	Ovoid	Smooth to granulate	Brown	Patelliform sessile, yellow	Rugose	Polygonal reticulate-rugose	Absent
E. erubescens	3.16x2.44	Compressed ovoid	Smooth	Grey to brown	Lunate ochreous sessile yellow	Rugose-reticulate	Polygonal reticulate-rugose	Absent

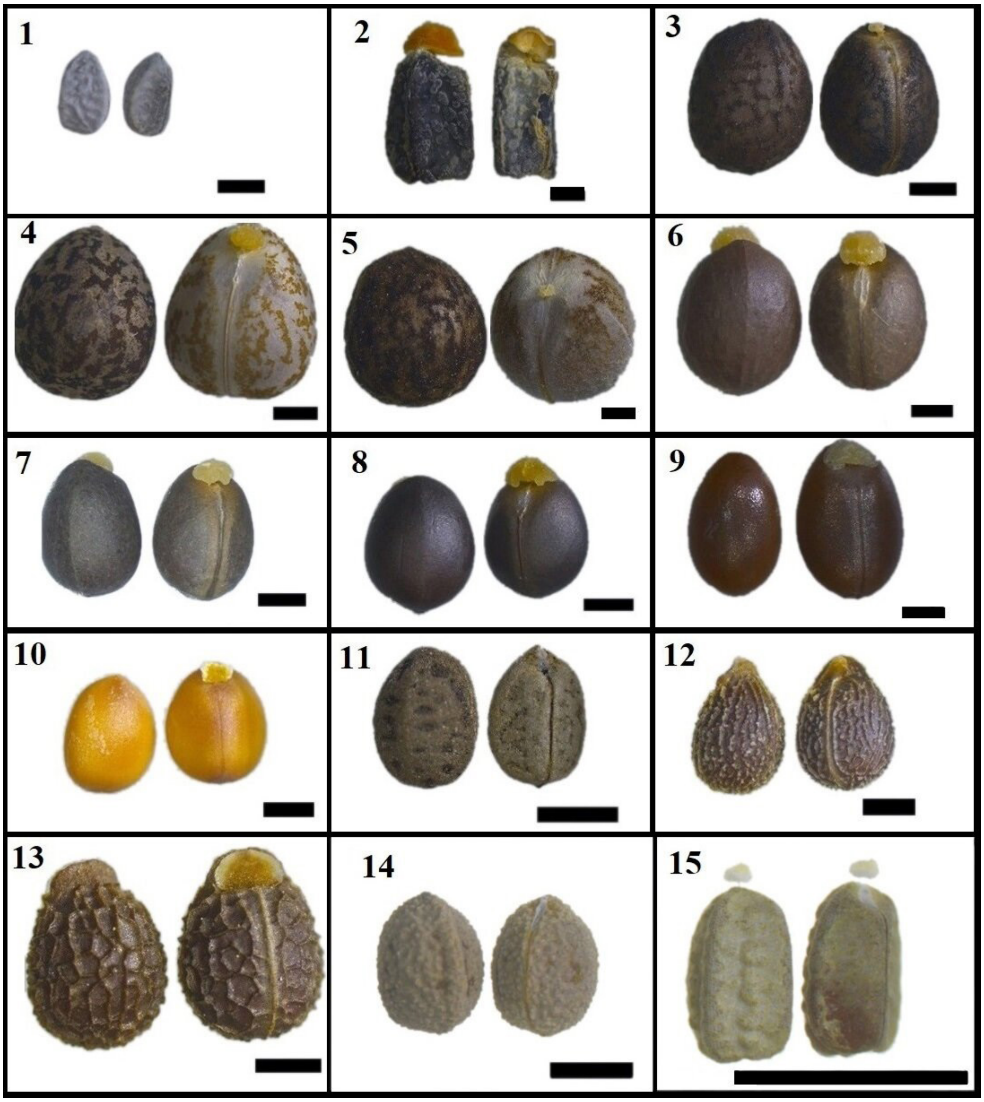

Figure 2. Stereomicroscope micrographs of seeds in *Euphorbia*: (1) *E. chamaesyce,* (2) *E. petiolata,* (3) *E. rhytidosperma,* (4) *E. grisophylla,* (5) *E. macrocarpa,* (6) *E. orientalis,* (7) *E. altissima* var. *altissima,* (8) *E. altissima* var. *glabrescens,* (9) *E. stricta,* (10) *E. microsphaera,* (11) *E. gaillardotii,* (12) *E. rhabdotosperma,* (13) *E. helioscopia,* (14) *E. aleppica,* (15) *E. szovitsii* var. *kharputensis,* scale bar = 1 mm.

is known for its irregularly pitted seeds. Additionally, tuberculate, punctuate, tuberculate- or pustulate-rugose, granulate, etc. shapes were found in the sections in this study (Table 3; Figures 2–4).

Similarly, SEM results done by Genc and Kultur (2018) demonstrated that *Euphorbia* species have different seed surfaces including reticulate-areolate, areolate, alveolate, colliculate, falsifoveate, pustulate, as well as smooth seed surfaces. Similarly, in his research on the taxonomy revision of *Euphorbia* in Turkey, Khan (1964), noted that they have varied seed surfaces and shapes. Additionally, several investigations revealed that different *Euphorbia* species had unique seed surfaces (Park, 2000; Fayed and Hassan, 2007; Can and Kucuker, 2015).

On the other hand, lipid granules were observed in some of the studied *Euphorbia* taxa in this study. Similar findings were found in Can and Kucuker's 2015 study, which showed that some of the *Euphorbia* species grown in Turkey have lipid granules. Their micromorphology findings also demonstrate the need for additional research on lipid globules to comprehend the biology, ecology, and dispersal mechanisms of *Euphorbia* seeds

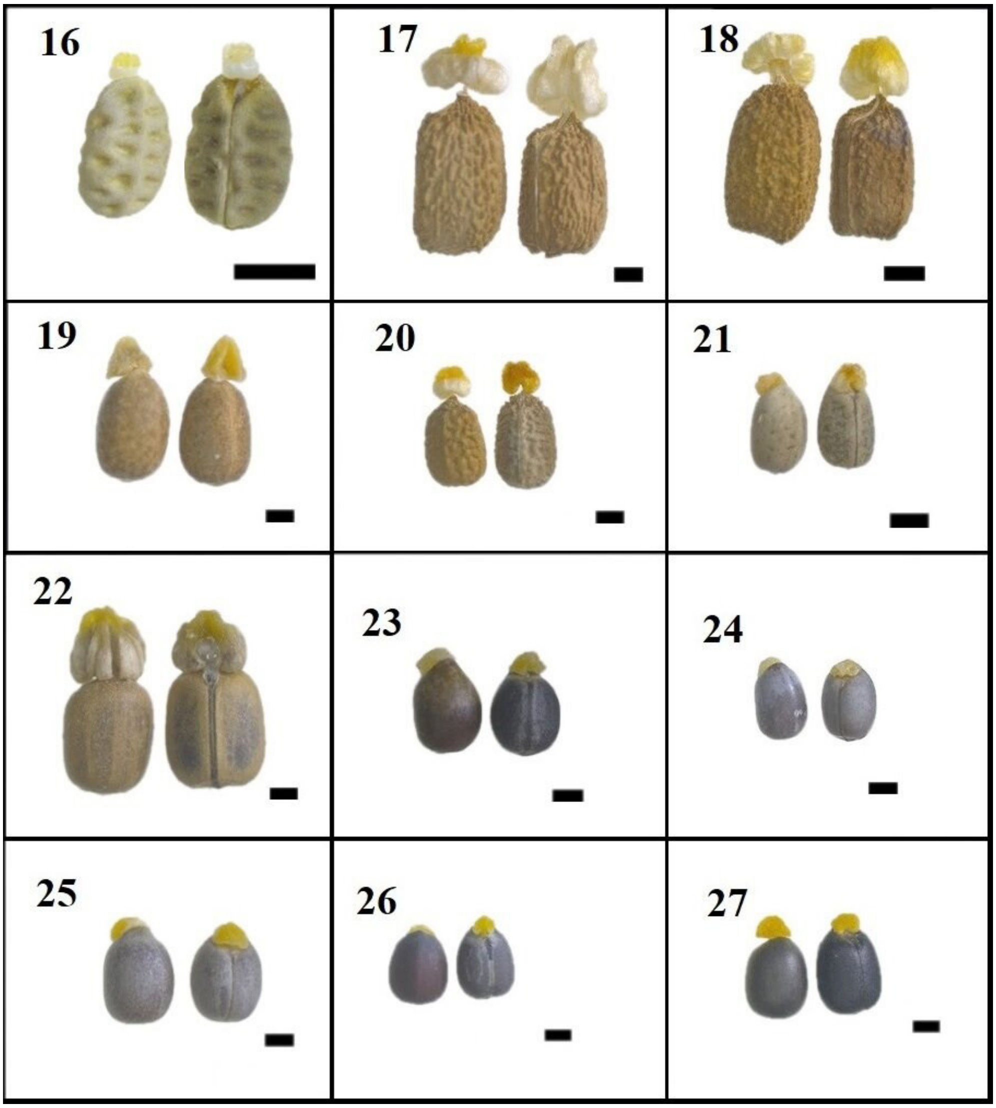

Figure 2. (Continued) Stereomicroscope micrographs of seeds in *Euphorbia*: (16) *E. falcata* subsp. *falcata,* (17) *E. denticulata,* (18) *E. craspedia,* (19) *E. macroclada,* (20) *E. cheiradenia,* (21) *E. seguieriana* subsp. *seguieriana,* (22) *E. heteradena,* (23) *E. esula* subsp. *tommasiniana,* (24) *E. sanasunitensis,* (25) *E. iberica,* (26) *E. oblongifolia,* (27) *E. erubescens,* scale bar = 1 mm.

(Can and Kucuker, 2015). The lipid granules mainly were globose apart from *E. esula* subsp. *tommasiniama* has reniform lipid granule in the present study. Our study showed that sect. *Helioscopia* species do not have lipid granules whilst studied sect. *Pithyusa* species have lipid granules. It could be suggested that lipid granules can be used for the delimitation of sections (Table 3; Figure 3). Similarly, Pahlevani and Akhani (2011) suggested that the arrangement and structure of the granulate components appear to be typical of some species.

3.2. Caruncle shape, color, and surface

The caruncle's shape, color, and surface of 27 *Euphorbia* were also determined in this study. The results showed that *E. chamaesyce* (sect. *Chamaesyce*), *E. gaillardotii* (sect. *Pithysua*), and *E. aleppica* (sect. *Myrsiniteae*) did not have a caruncle (Table 3; Figure 4). Similar to Simon et al. (1992)'s investigations, it was discovered in our study that species from the *Helioscopia* section exhibit caruncles. Also, Pahlevani et al. (2015) indicated that a caruncle is absent in the members of subgen. *Chamaesyce* and Pahlevani and Akhani (2011) suggested that the lack of a caruncle is significant characteristic in separating the subg. *Chamaesyce*. Furthermore, Krochmal (1952) and Khan (1964) noted the presence or lack of caruncles while Webster (1994) highlighted that some species of *Euphorbia* have caruncles. Simon et al. (1992) proposed using the

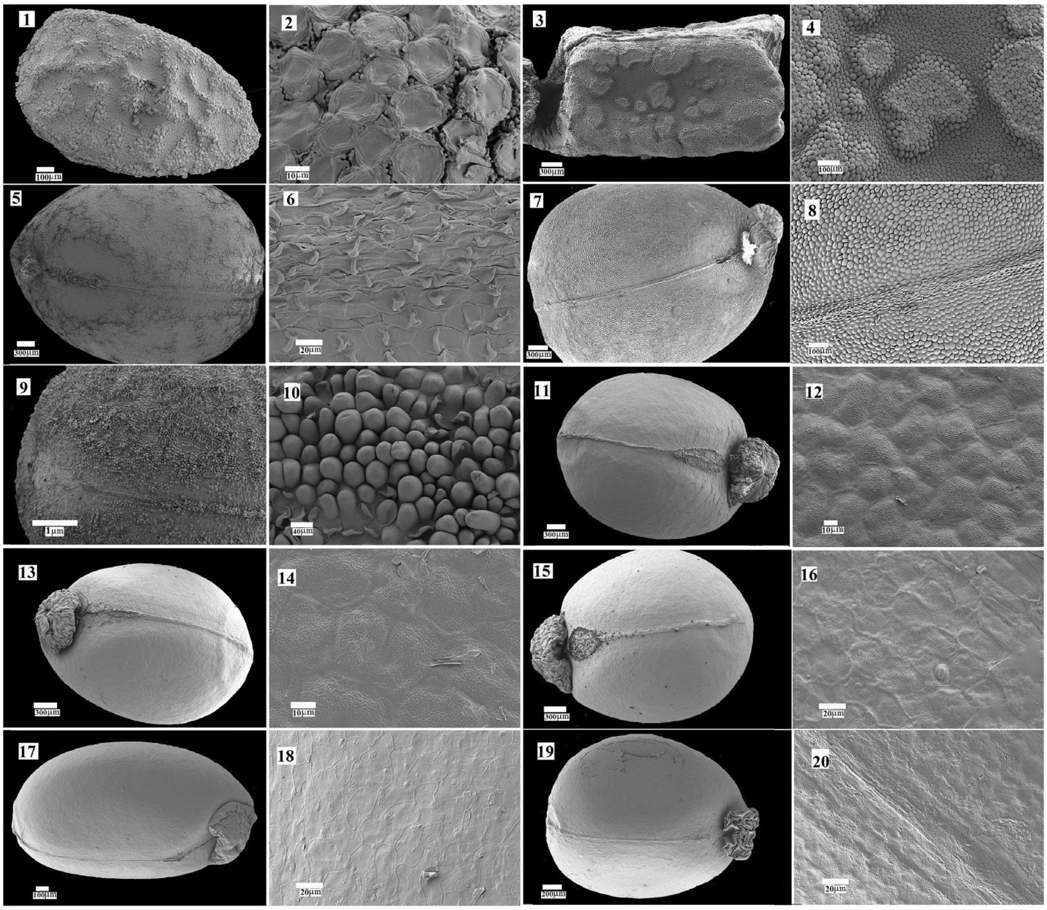

Figure 3. SEM micrographs of seeds: (1–2) *E. chamaesyce,* (3–4) *E. petiolata,* (5–6) *E. rhytidosperma,* (7–8) *E. grisophylla,* (9–10) *E. macrocarpa,* (11–12) *E. orientalis,* (13–14) *E. altissima* var. *altissima,* (15–16) *E. altissima* var. *glabrescens,* (17–18) *E. stricta,* (19–20) *E. microsphaera.*

caruncle form to identify species. The prominent caruncle surface was rugose or rugose-verrucose in our study and the color of the caruncles was shades of white or yellow except for *E. heteradena* has yellow to brown. Similarly, Fayed and Hassan (2007) reported that the color of caruncles varied from yellow(ish) to whit(ish). Additionally, the main caruncle shape was conical or reniform in the current study (Table 3; Figure 4). Genc and Kultur (2018) showed that in most of the investigated taxa, the conical caruncle is the fundamental type. Also, they indicated that there are reniform, petasiform, and hemispherical caruncles (Genc and Kultur, 2018). Our study also found petasiform, patelliform, and hemispherical caruncles. Similarly, Can and Kucuker (2015) demonstrated that the caruncles of the investigated species are conical, crescent-shaped, or reniform with the majority of the colors falling between yellow and brown. However, Can and Kucuker (2015) suggested that the microstructure of the caruncle surface demonstrated no taxonomical nature. On the contrary, other studies along with our study suggested that seed characteristics such as seed size, ornamentation, seed shape, color, and the presence or absence of caruncle are valuable types for distinguishing related species in *Euphorbia* (Pahlevani and Akhani, 2011; Da Silva et al., 2016; Genc and Kultur, 2018). Salmaki et al. (2011) further asserted that comparative study in this field is still lacking despite the fact that it is generally known that a caruncle's morphology is constant within a species and commonly distinguishes between different species.

3.3. Statistical analyses

The numerical taxonomy approach includes elements such as populations, species, genera, etc. from which data and features are acquired, and using various statistical formulas, similarity (and/or distance) between units is

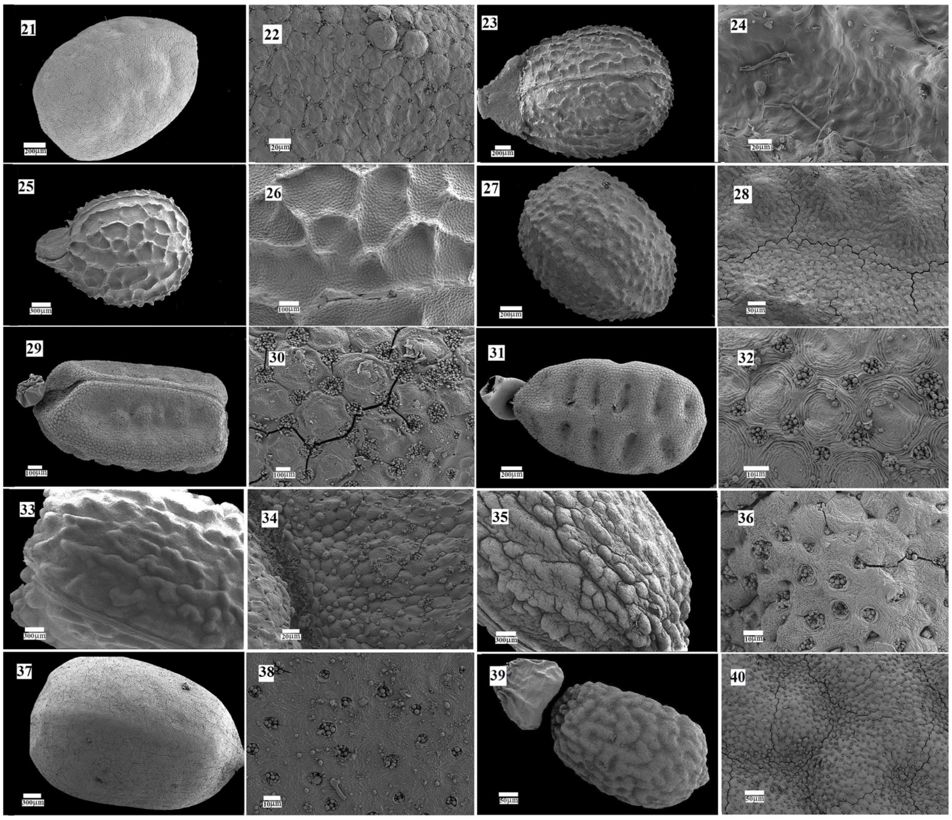

Figure 3. (Continued 1) SEM micrographs of seeds: (21–22) *E. gaillardotii,* (23–24) *E. rhabdotosperma,* (25–26) *E. helioscopia.* (27–28) *E. aleppica,* (29–30) *E. szovitsii* var. *kharputensis,* (31–32) *E. falcata* subsp. *falcata,* (33–34) *E. denticulata,* (35–36) *E. craspedia,* (37–38) *E. macroclada,* (39–40) *E. cheiradenia.*

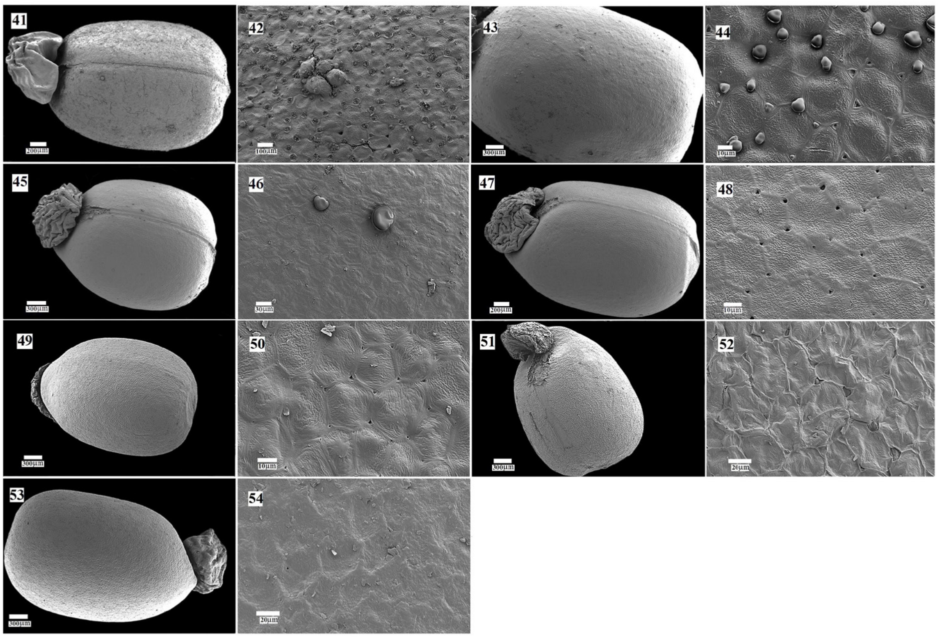

Figure 3. (Continued 2) SEM micrographs of seeds: (41–42) *E. seguieriana* subsp. *seguieriana,* (43–44) *E. heteradena,* (45–46) *E. esula* subsp. *tommasiniana,* (47–48) *E. sanasunitensis,* (49–50) *E. iberica,* (51–52) *E. oblongifolia,* (53–54) *E. erubescens.*

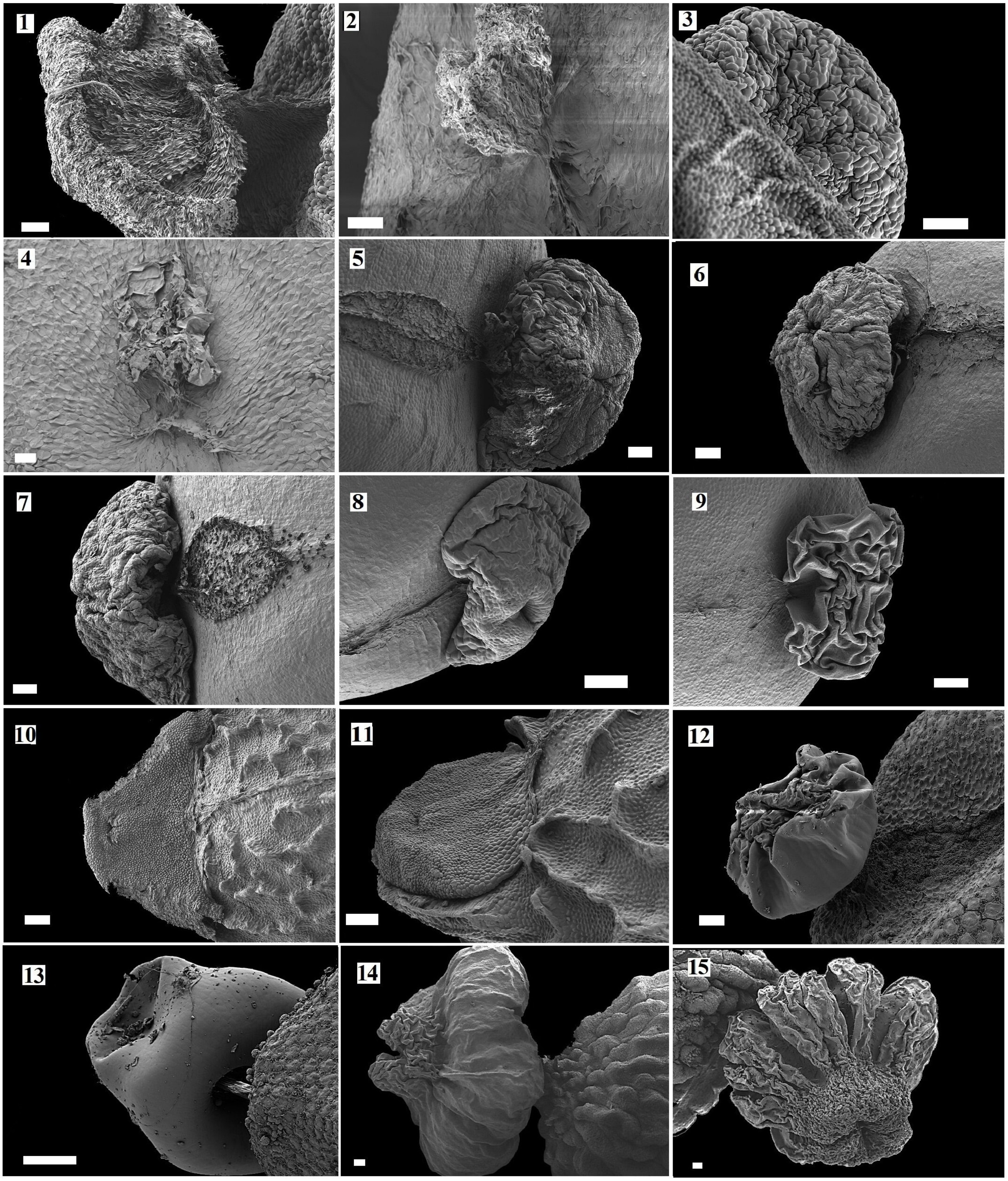

Figure 4. SEM micrographs of caruncle of the seeds: (1) *E. petiolata,* (2) *E. rhytidosperma* (3) *E. grisophylla,* (4) *E. macrocarpa,* (5) *E. orientalis,* (6) *E. altissima* var. *altissima,* (7) *E. altissima* var. *glabrescens,* (8) *E. stricta,* (9) *E. microsphaera,* (10) *E. rhabdotosperma,* (11) *E. helioscopia,* (12) *E. szovitsii* var. *kharputensis,* (13) *E. falcata* subsp. *falcata,*(14) *E. denticulata,* (15) *E. craspedia,* scale bar 100 µm.

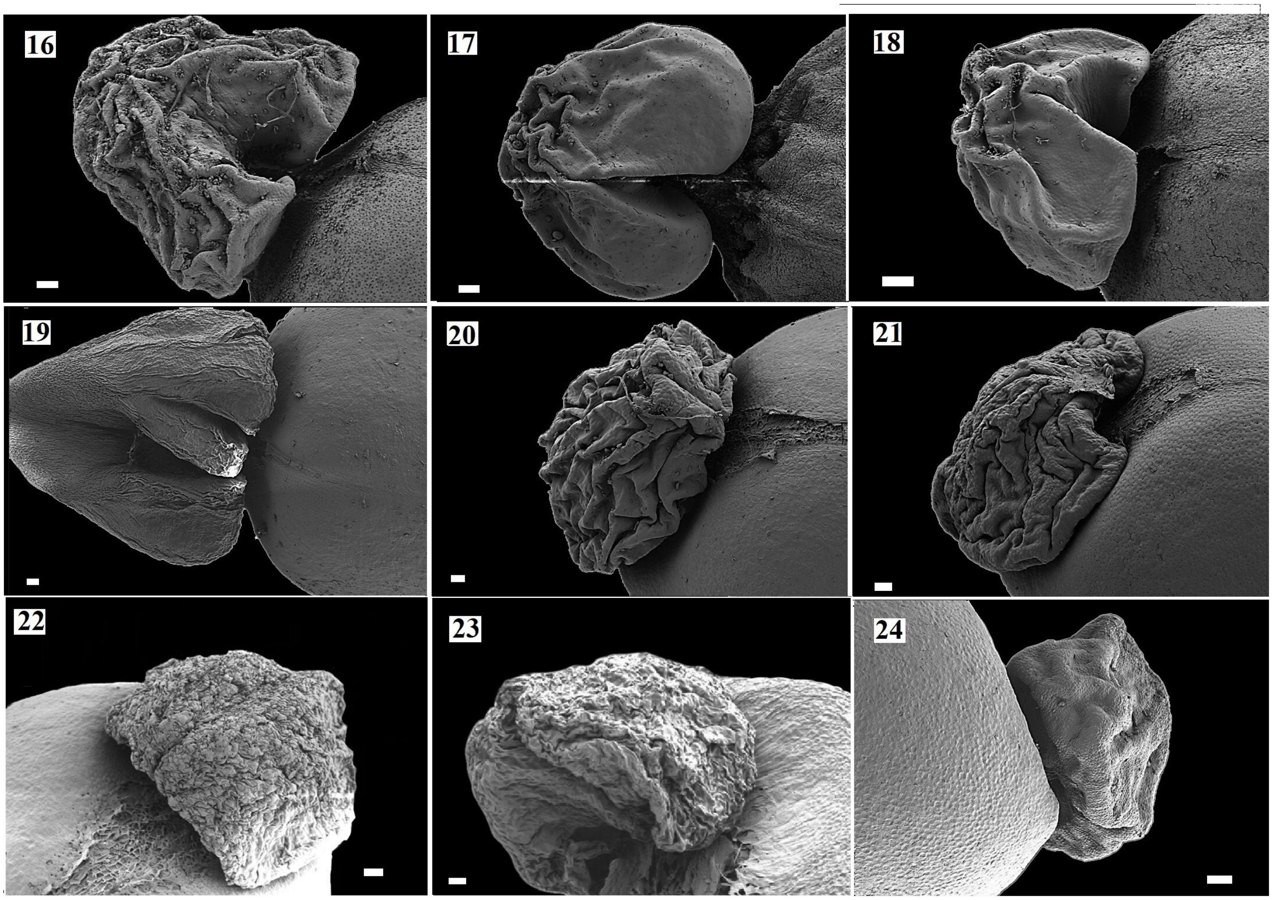

Figure 4. (Continued) SEM micrographs of caruncle of the seeds: (16) *E. macroclada,* (17) *E. cheiradenia,* (18) *E. seguieriana* subsp. *seguieriana,* (19) *E. heteradena,* (20) *E. esula* subsp. *tommasiniana,* (21) *E. sanasunitensis,* (22) *E. iberica,* (23) *E. oblongifolia,* (24) *E. erubescens,* scale bar 100 µm.

calculated to understand the relationships better (Singh, 2010). The cluster analysis based on UPGMA using the Gower similarity coefficient was performed on the seed morphological characters, obtained from 27 taxa in this study. There are four types of taxa according to the UPGMA dendrogram, defined as follows: type 1 includes three subtypes: *E. craspedia*, *E. denticulata*, and *E. macrocarpa*; type 2 includes only *E. petiolata*; type 3 which includes seventeen subtypes: *E. oblongifolia*, *E. erubescens*, *E. cheiradenia*, *E. sanasunitensis*, *E. macroclada*, *E. iberica*, *E. esula* subsp. *tommasiniana*, *E. seguieriana* subsp. *seguieriana*, *E. microsphaera*, *E. altissima* var. *glabrescens*, *E. altissima* var. *altissima*, *E. orientalis*, *E. heteradena*, *E. grisophylla*, *E. helioscopia*, *E. rhabdotosperma*) and *E. rhytidosperma*; type 4 includes other taxa (Figure 4). Similarly, molecular analysis done by Riina et al. (2013) showed that *E. craspedia* and *E. denticulata* were found to be closely related. Additionally, the findings of Riina et al. (2013), who discovered that *E. helioscopia* and *E. rhabdotosperma* are grouped together, concurred with those of the current study. In addition, in a research by Pahlevani et al. (2015) demonstrated that the species of sect. *Helioscopia* and *Pithyusa* were clustered close to each other based on seed and morphological characters. Furthermore, the results of our investigation supported those of Salmaki et al. (2011), who found that *E. esula* and *E. iberica* were closely grouped. On the other hand, Talebi et al. (2016) found that *E. orientalis* and *E. microsphaera* were clustered together as in our study. In addition, the results of Bruyns et al. (2006) showed that *E. helioscopia* and *E. stricta* were closely grouped, however, in the current investigation, the two species were clustered in distinct clades. Also, according to the similarity coefficients in Table 4, the most similar taxa are *E. altissima var. altissima* & *E. altissima* var. *glabrescens*. The taxa showing the second and third closest similarity are *E. esula* subsp. *tommasiniana* and *E. iberica*, *E. macroclada* and *E. sanasunitensis*, respectively (Table 4; Figures 5, 6). Moreover, the results obtained from the PCA including eigenvalues, percentages, and cumulative percentages for both axes are shown in Table 5. The results

Table 4. Gower general similarity coefficient for construction of dendrogram.

Node	Group 1	Group 2	Similarity	No. objects in group
1	*E. altissima var. altissima*	*E. altissima var. glabrescens*	0,978	2
2	*E. esula subsp. tommasiniana*	*E. iberica*	0,962	2
3	*E. orientalis*	Node 1	0,928	3
4	*E. macroclada*	*E. sanasunitensis*	0,927	2
5	*E. rhabdotosperma*	*E. helioscopia*	0,904	2
6	*E. seguieriana subsp. seguieriana*	Node 2	0,900	3
7	Node 3	*E. microsphaera*	0,890	4
8	*E. gaillardotii*	*E. aleppica*	0,864	2
9	Node 7	Node 6	0,864	7
10	Node 9	Node 4	0,856	9
11	*E. cheiradenia*	*E. erubescens*	0,842	2
12	*E. grisophylla*	*E. heteradena*	0,832	2
13	*E. chamaesyce*	Node 8	0,827	3
14	Node 10	Node 11	0,813	11
15	*E. denticulata*	*E. craspedia*	0,806	2
16	*E. szovitsii var. kharputensis*	*E. falcata subsp. falcata*	0,793	2
17	Node 12	Node 14	0,779	13
18	*E. rhytidosperma*	Node 5	0,773	3
19	Node 17	*E. oblongifolia*	0,731	14
20	Node 13	Node 16	0,729	5
21	Node 18	Node 19	0,715	17
22	Node 20	*E. stricta*	0,713	6
23	*E. macrocarpa*	Node 15	0,698	3
24	Node 22	Node 21	0,654	23
25	*E. petiolata*	Node 23	0,649	4
26	Node 24	Node 25	0,555	27

of the PCA analysis based on the seed morphological characters show that the seed width and length play a major role in the classification of type 2 (*E. petiolata*), and the seed surface (LM) and seed surface (SEM) morphological characteristics are more important than the others in determining the position of type 1 (*E. craspedia*, *E. denticulata,* and *E. macrocarpa*) (Figure 6). Also, seed shape, caruncle shape, and caruncle surface are important in positioning taxa in type 3. Seed width and length play an inverse role in positioning type 4 (*E. stricta*, *E. falcata* subsp. *falcata*, *E. szovitsii* var. *kharputensis*, *E. aleppica*, *E. gaillardotii,* and *E. chamaesyce*.) taxa; therefore, putting smaller seeds together is observed in Figure 6. According to the result of UPGMA analysis on the seed morphology of 27 taxa belonging to *Euphorbia*, morphologically close taxa are generally grouped under the same type. However, some seeds with different morphology were grouped in the same type. The PCA analysis indicates that the reason for this is that seed sizes are effective in the aggregation of these seeds (Figure 6).

4. Conclusions

The present study showed that the studied *Euphorbia* taxa have different seed sizes, and it was found that their seed colors, seed surfaces, and ornamentations are varied in the studied *Euphorbia* taxa. In addition, *Euphorbia* taxa mostly have ovoid seed shapes including ellipsoidal, globose, quadrangular, compressed, or compressed–ellipsoidal. Furthermore, this study found that the caruncles of the studied *Euphorbia* taxa have different colors, shapes, and surfaces. Moreover, the lipid granules were globose, broad ellipsoidal-globose, or reniform in the *Euphorbia* taxa. The results of the cluster analysis showed that *the Euphorbia* species are divided into four types based on the obtained

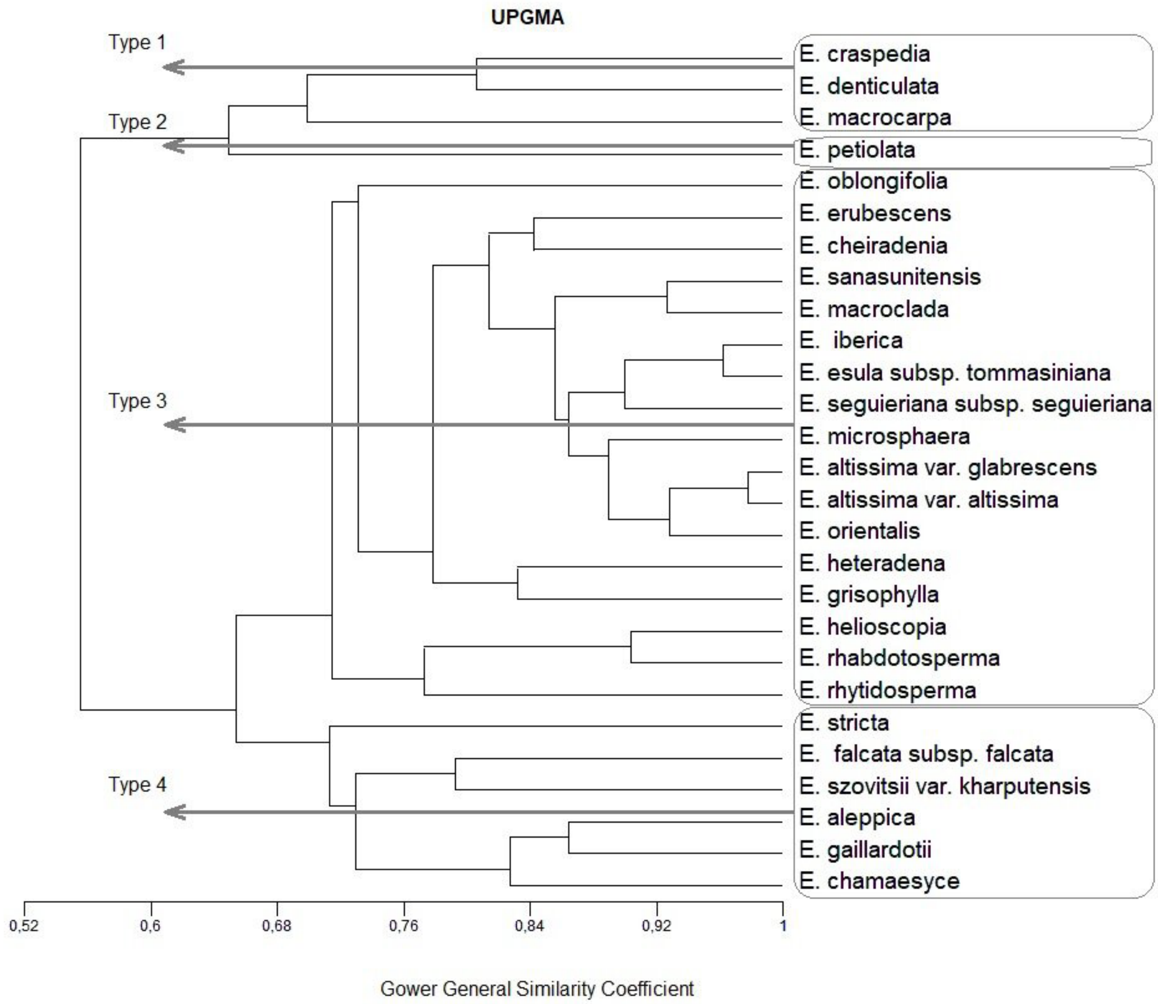

Figure 5. The cluster analysis of studied *Euphorbia* L. based on UPGMA using the Gower similarity coefficient.

similarity dendrogram. Also, principal component analysis supports these results. As a result, this study suggests that the seed morphologies of *Euphorbia* can be used as significant taxonomic characters.

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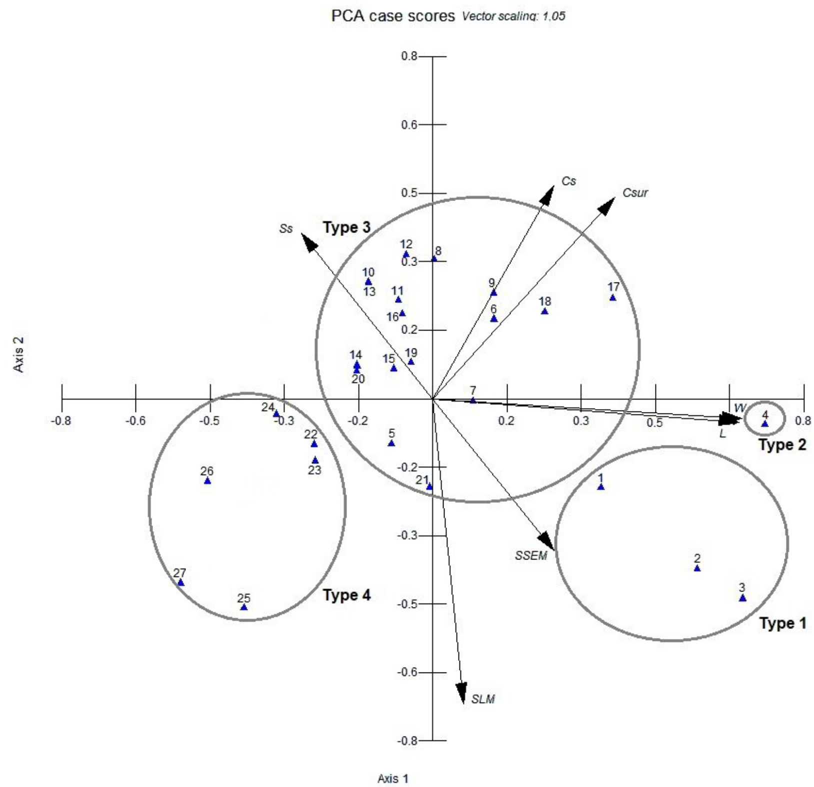

Figure 6. The results of the PCA analysis of studied *Euphorbia* L. taxa. 1: *E. craspedia*, 2: *E. denticulata*, 3: *E. macrocarpa*, 4: *E. petiolata*, 5: *E. oblongifolia*, 6: *E. erubescens*, 7: *E. cheiradenia*, 8: *E. sanasunitensis*, 9: *E. macroclada*, 10: *E. iberica*, 11: *E. esula* subsp. *tommasiniana*, 12: *E. seguieriana* subsp. *seguieriana*, 13: *E. microsphaera*, 14: *E. altissima* var. *glabrescens*, 15: *E. altissima* var. *altissima*, 16: *E. orientalis*, 17: *E. heteradena*, 18: *E. grisophylla*, 19: *E. helioscopia*, 20: *E. rhabdotosperma*, 21: *E. rhytidosperma*, 22: *E. stricta*, 23: *E. falcata* subsp. *falcata*, 24: *E. szovitsii* var. *kharputensis*, 25: *E. aleppica*, 26: *E. gaillardotii*, 27: *E. chamaesyce*.

Table 5. Both Axes values for principal component analysis.

PCA	Axis 1	Axis 2
Eigenvalues	2.410	1.455
Percentage	34.432	20.788
Cumulative Percentage	34.432	55.219

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