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Seed-coat microsculpturing of Turkish *Lepidium* (Brassicaceae) and its systematic application

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Abstract: This article investigates the seed exomorphic characteristics of 14 taxa of *Lepidium* L. in Brassicaceae by light microscope and scanning electron microscope (SEM). The exomorphic characteristics of the seed are size, seed shape, seed colour, seed wings, and seed surface pattern. All characteristics of the seeds are described, illustrated, and compared in this article. The results of SEM at higher magnification showed 6 types of seed surface patterns: tuberculate, reticulate, ruminate, reticulate-tuberculate, reticulate-areolate, and reticulate-fovate. In these seed surface patterns, there were diagnostic characteristics and differences at the specific level for *Lepidium.*

Key words: Brassicaceae, *Lepidium*, seed-coat, SEM, morphology

1. Introduction

The Brassicaceae is a large family that includes 338 genera and 3700 species. It has major scientific and economic importance (Koch & Mummenhoff, 2006). The tribal classification of the Brassicaceae is problematic because the characteristics traditionally used at this rank are few in number, usually only 1 or 2 morphological characteristics. These characteristics are also variable within genera, and they conflict with one another in their distribution patterns among genera and tribes; thus, they may not support natural groups (Khalik et al., 2002).

Seed morphology is frequently used for understanding and classifying Brassicaceae at the tribal level (Zohary, 1948; Appel & Al-Shehbaz, 2002; El Naggar, 2005). According to morphological studies, Lepidieae is a natural tribe of about 66 genera, of which 23 are monotypic, with some 680 species distributed throughout the world (Al-Shehbaz, 1986). However, according to molecular research, Lepidieae is not monophyletic (Al-Shehbaz et al., 2006; Beilstein et al., 2006).

Seed characteristics are also important at genus level. The genus *Lepidium* L. is one of the largest genera of the Brassicaceae, consisting of 175 species. It is distributed worldwide, primarily in temperate and subtropical regions. The genus is poorly represented in Arctic climates; in tropical areas, it grows in the mountains (Al-Shehbaz, 1986). In Turkey, 14 taxa are represented in which 1 endemic is placed in 4 sections: *Lepia* (Desv.) DC., *Lepiocardamon* Thell., *Dileptium* DC., and *Lepidium* L. (Juss, 1939; Hedge, 1965). In 1906, Thellung defined the genus *Cardaria* (Desv.) DC. as a section of *Lepidium*; later, however, *Cardaria* was accepted as its own genus based on its indehiscent fruits (Mulligan & Frankton, 1962; Al-Shehbaz, 1986). El Naggar (1993) accepted that *Lepidium*, *Cardaria,* and *Coronopus* Zinn are distinct genera in his numerical study. SEM studies show that *Cardaria* and *Coronopus* seed surface patterns are different from those of *Lepidium* (El Naggar, 2005). In 1995, Mummenhoff suggested that *Cardaria* should be classified within *Lepidium* according to his molecular research. Other molecular research suggested that not only *Cardaria* but also *Coronopus*, *Stroganowia* Kar. & Kir., *Winklera* Regel, and *Stubendorffia* Schrenk ex Fisch. should be classified within *Lepidium* (Al-Shehbaz et al., 2002, 2006; Koch et al., 2003; Bailey et al., 2006; Al-Shehbaz & Mummenhoff, 2011).

This study examined the exomorphic characteristics of Turkish *Lepidium* sensu stricto seeds by using SEM to highlight seed surface pattern and to detail ornamentation.

2. Materials and methods

The materials of this study were mature seeds of 14 taxa of the genus *Lepidium* collected from their natural habitats in Turkey in 2008 and 2009. The specimens collected were kept at the İstanbul University Department of Pharmaceutical Botany Herbarium (ISTE). The localities and voucher numbers of the taxa studied are given in Table 1, and seed exomorphic characteristics including seed size, seed shape, seed colour, seed wings, and seed surface pattern are presented in Table 2. Fourteen hundred

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Table 1. Studied *Lepidium* taxa, collection dates, and localities.

Taxa	Place of origin	Collection date	Herbarium number
L. campestre	Edirne	18.7.2009	ISTE 93396
L. spinosum	Muğla	28.4.2009	ISTE 93364
L. sativum subsp. *sativum*	İstanbul	10.5.2008	ISTE 93342
L. sativum subsp. *spinescens*	Şanlıurfa	08.6.2008	ISTE 93357
L. ruderale	Muş	02.6.2008	ISTE 93346
L. virginicum	Giresun	31.7.2008	ISTE 93360
L. perfoliatum	Van	03.6.2008	ISTE 93351
L. caespitosum	Ankara	20.7.2008	ISTE 93358
L. pumilum	Kayseri	30.6.2009	ISTE 93388
L. crassifolium	Van	04.6.2008	ISTE 93352
L. latifolium	Kayseri	30.6.2009	ISTE 93390
L. lyratum	Artvin	06.6.2009	ISTE 93384
L. graminifolium	Hatay	20.8.2009	ISTE 93361
L. vesicarium	Van	03.6.2008	ISTE 93349

seeds were used to examine morphological properties, which included seed size, general shape, colour, and wing. During scanning electron microscopy, mature seeds (2–3) from each of the taxa were selected and mounted onto stubs with double-sided adhesive tape and were then coated with gold. The seed surface pattern was examined on the lateral surfaces of the seeds. For each sample, photographs of testa were taken using a JEOL Neoscope 5000 at magnifications of 50×, 150×, 400×, and 1000×. The terminology of seed characteristics in this work was based on the descriptions used by Stearn (1992), Bartholtt (1981), and Koul et al. (2000).

This study showed 7 different types of seed surface patterns. These surface patterns are explained below.

Tuberculate: covered wart-like projections. Ruminate: penetrated by irregular channels giving an eroded appearance and running in different directions. Reticulate: having a raised network of narrow and sharply angled lines frequently presenting a geometric appearance, each area outlined by a reticulum being an interspace. Reticulate-

Table 2. Seed characteristics of studied *Lepidium* taxa.

Taxa	Shape	Wing	Color	Size	Ornamentation
L. campestre	Ovate	Absent	Blackish brown	2.2–2.5 × 1.2–1.6	Tuberculate
L. spinosum	Ovate	Present	Brown or blackish brown	2–2.5 × 1–1.4	Ruminate
L. sativum subsp. *sativum*	Ovate	Present	Brown	2.5–2.8 × 0.9–1.5	Reticulate-areolate
L. sativum subsp. *spinescens*	Ovate	Present	Brown	2.4–2.9 × 1.1–1.5	Ruminate
L. ruderale	Ovate	Absent	Brown	1.3–1.7 × 0.7–1	Reticulate
L. virginicum	Ovoid	Present	Brown	1.7–2 × 1–1.3	Reticulate-tuberculate
L. perfoliatum	Ovoid or ovate	Present	Brown	1.9–2.8 × 1.2–2	Reticulate-fovate
L. caespitosum	Ovate	Present	Brown	1.8–2.2 × 1–1.4	Tuberculate
L. pumilum	Ovate	Present	Brown	1.9–2.1 × 1.2–1.4	Tuberculate
L. crassifolium	Ovate	Present	Brown	2–2.4 × 1.2–1.5	Tuberculate
L. latifolium	Ovate	Present	Brown	0.8–1 × 0.5–0.7	Tuberculate
L. lyratum	Ovate	Absent	Brown	1.3–1.4 × 0.6–0.7	Reticulate
L. graminifolium	Elliptic	Absent	Brown	1.3–1.5 × 0.7–0.9	Tuberculate
L. vesicarium	Oblong-elliptic	Absent	Green	1.8–2.2 × 1–1.3	Reticulate

areolate: a type between reticulate and areolate (composed of circular or polygonal areas separated by grooves) types. Reticulate-tuberculate: a type between reticulate and tuberculate types. Reticulate-fovate: a type between reticulate and fovate (pitted or having depressions marked with little pits) types (Bartholtt, 1981; Stearn, 1992; Koul et al., 2000).

3. Results

The results of the study showed that the seed sizes of Turkish *Lepidium* were 0.8–2.9 × 0.5–1.6 mm. Among them, *Lepidium latifolium* L. had the smallest seed size with 0.8–1 × 0.5–0.7 mm. *L. campestre* (L.) R.Br., *L. ruderale* L., *L. lyratum* L., *L. graminifolium* L., and *L. vesicarium* L. had wingless seeds. In contrast, the other taxa had distinct or partial wings on seeds. The seed colour of Turkish *Lepidium* was brown except for 3: *L. campestre* and *L. sativum* L. subsp. *spinescens* (DC.) Thell. had blackish-brown seeds, and *L. vesicarium* had green seeds.

Research indicated that *Lepidium campestre, L. caespitosum* Desv., *L. pumilum* Boiss. & Bal., *L. crassifolium* Waldst. & Kit., *L. latifolium*, and *L. graminifolium* had tuberculate seed surface patterns (Table 2). Tuberculate patterns showed differentiation in detailed investigation with high magnification (Figures 1–3). Under this high magnification, the pattern was characterised by distinct hills, which had broad and flattened ends for *L. campestre*. There were distinct striae between the irregularly arranged hills, which were present on all seed surfaces (Figure 1). It was also observed that elevated patterns covered testa cells and their arrangement, and the pattern also had flattened-end hills and soft lower ridges with these hills in *L. caespitosum* (Figure 2). The pattern of *L. pumilum* showed soft broad lined hills on the high magnification SEM image. It was also seen that there were undulate structures between hills (Figure 2). Testa cells of *L. crassifolium* were observed using only high magnification SEM imaging. These cells had dull and thin boundaries, mostly covered with elevations. Structure and arrangement of elevations were unrelated to cells and cell boundaries. A slight undulate structure between elevations was noted (Figure 2). These elevations cover cell boundaries and arrangement in *L. latifolium* and had irregular structures and arrangement, and were defined by soft textured and undulate hills (Figure 2). The elevations in *L. graminifolium*, as related to pattern, covered cell boundaries and arrangement. Elevations were arranged like soft textured and not high hills and the tips of the hills were softly twisted; the structure between hills was smooth or undulate (Figure 3).

Lepidium spinosum Ard. and *L. sativum* subsp. *spinescens* had ruminate seed surface patterns consisting of irregular elevations (Table 2). When subjected to high magnification, the SEM image of *L. spinosum* showed that the cell boundary and arrangement were uncertain and the elevations had strong, but not sharp, features (Figure 1). In *L. sativum* subsp. S*pinescens*, testa cells were not distinguishable even under 1000× magnification because of soft textured, distinct hills covering all seed surfaces (Figure 1).

Lepidium sativum L. subsp. *sativum* had a reticulate-areolate seed surface pattern (Table 2). The high magnification SEM image showed isodiametric, irregularly arranged cells that were elongated and parallel with the seed surface. The cell boundaries had sharp lines, and testa cells were found to have highly raised anticlinal cell boundaries (Figure 1)

Lepidium ruderale, *L. lyratum*, and *L. vesicarium* had reticulate patterns (Table 2). Under high magnification, the SEM image showed isodiametric, irregularly arranged cells that were elongated in parallel with the seed surface. The soft lined cell boundaries were thin and higher than the cell centre in *L. ruderale* (Figure 1). The seed surface of *L. lyratum* had rounded testa cells arranged like a honeycomb. The cell boundaries were thin and had a soft structure, and seemed higher than the cell centre (Figure 2). The seed surface pattern of *L. vesicarium* consisted of isodiametric testa cells regularly arranged and elongated, and parallel with the seed surface. The cell boundaries were higher than the cell centre and sharp lines were nonexistent (Figure 3).

Lepidium virginicum had a reticulate-tuberculate pattern (Table 2). The testa cells were broad and isodiametric and the cell boundaries were thin and did not have very sharp lines. The elevations were located in the cell centres, which were flattened-end hills (Figure 1).

Lepidium perfoliatum L. had a reticulate-fovate pattern (Table 2). It had isodiametric and irregularly arranged cells. The cell boundaries were very thin and had soft lines, and the centres of the cells were higher than the boundaries. The testa cells of *L. perfoliatum* were found to have a reticulate structure (Figure 2).

4. Discussion

Scanning electron microscopy (SEM) studies show that fruit, seed, and leaf surface pattern characteristics are useful taxonomic characteristics for different families and genera (Kumar et al., 2012; Shahi Shavvon et al., 2012; Akçin et al., 2013). Seed surface pattern, seed colour, and seed size provide valuable characteristics in delimitation of taxa in Brassicaceae (Vaughan & Whitehouse, 1971; Barholtt, 1981; Brochmann, 1992; Koul et al., 2000). Fruit and seed characteristics should be critically evaluated in light of molecular and other morphological data (Moazzeni et al., 2010). Tuberculate seed surface pattern is the most common pattern among the studied taxa; however, it is not a defining characteristic for *Lepidium* because it

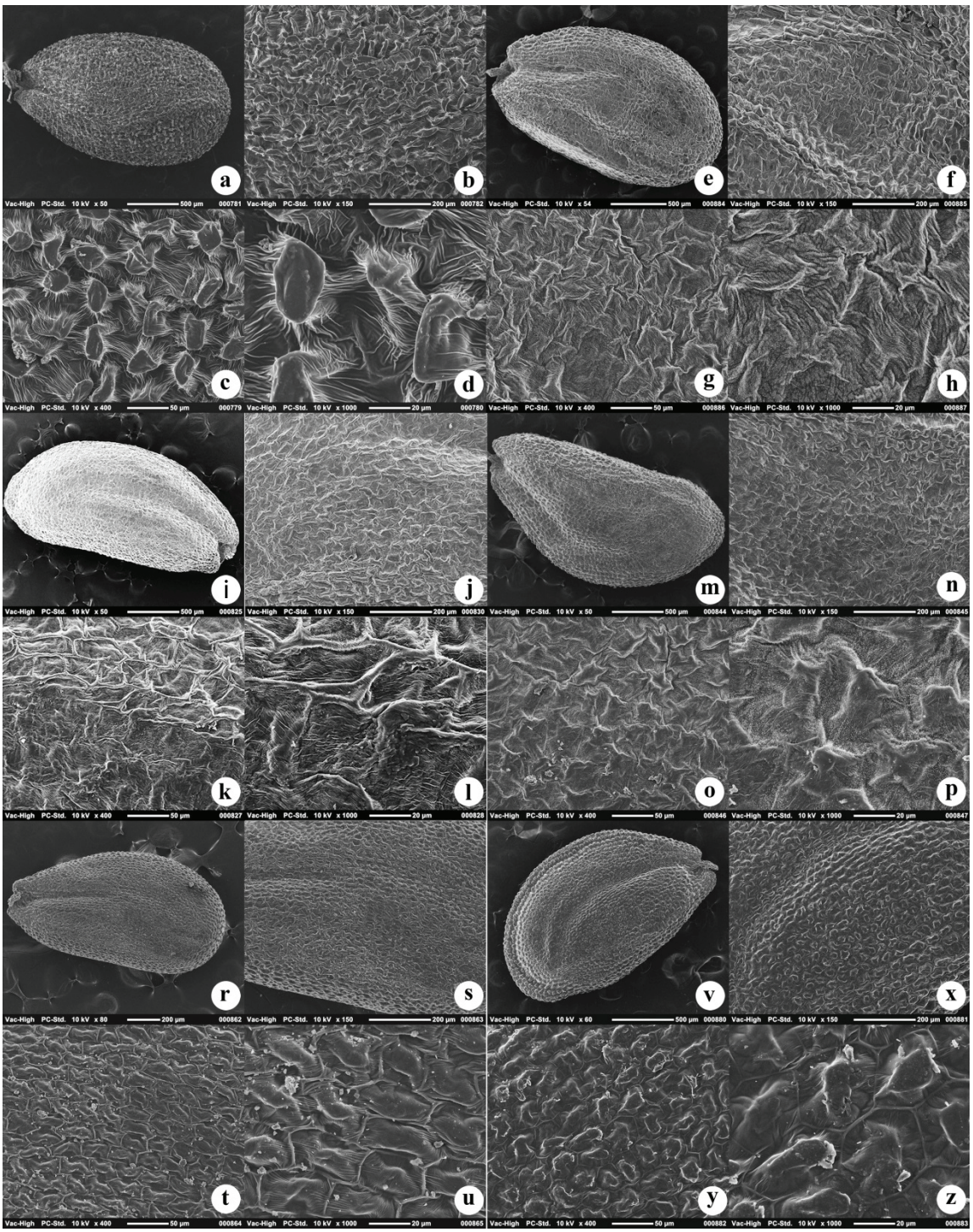

Figure 1. SEM micrographs of seeds of Turkish *Lepidium*. *L. campestre* (a–d), *L. spinosum* (e–h), *L. sativum* subsp. *sativum* (i–l), *L. sativum* subsp. *spinescens* (m–p), *L. ruderale* (r–u), *L. virginicum* (v–z).

also seems to occur in unrelated genera like *Erysimum* L., *Alyssum* L., *Lobularia* Desv., and *Camelina* Crantz (Murley, 1951). Other sculpturing types, like reticulate and reticulate-areolate, are also common in unrelated taxa in Brassicaceae (Tantawy et al., 2004; Moazzeni et al., 2007). Therefore, seed surface pattern is not useful for delimitation of *Lepidium*, nor is it useful for delimitation at the generic level in Brassicaceae. However, at the specific level there are diagnostic characteristics that might be useful (Brochman, 1992; Koul et al., 2000; Tantawy et al.,

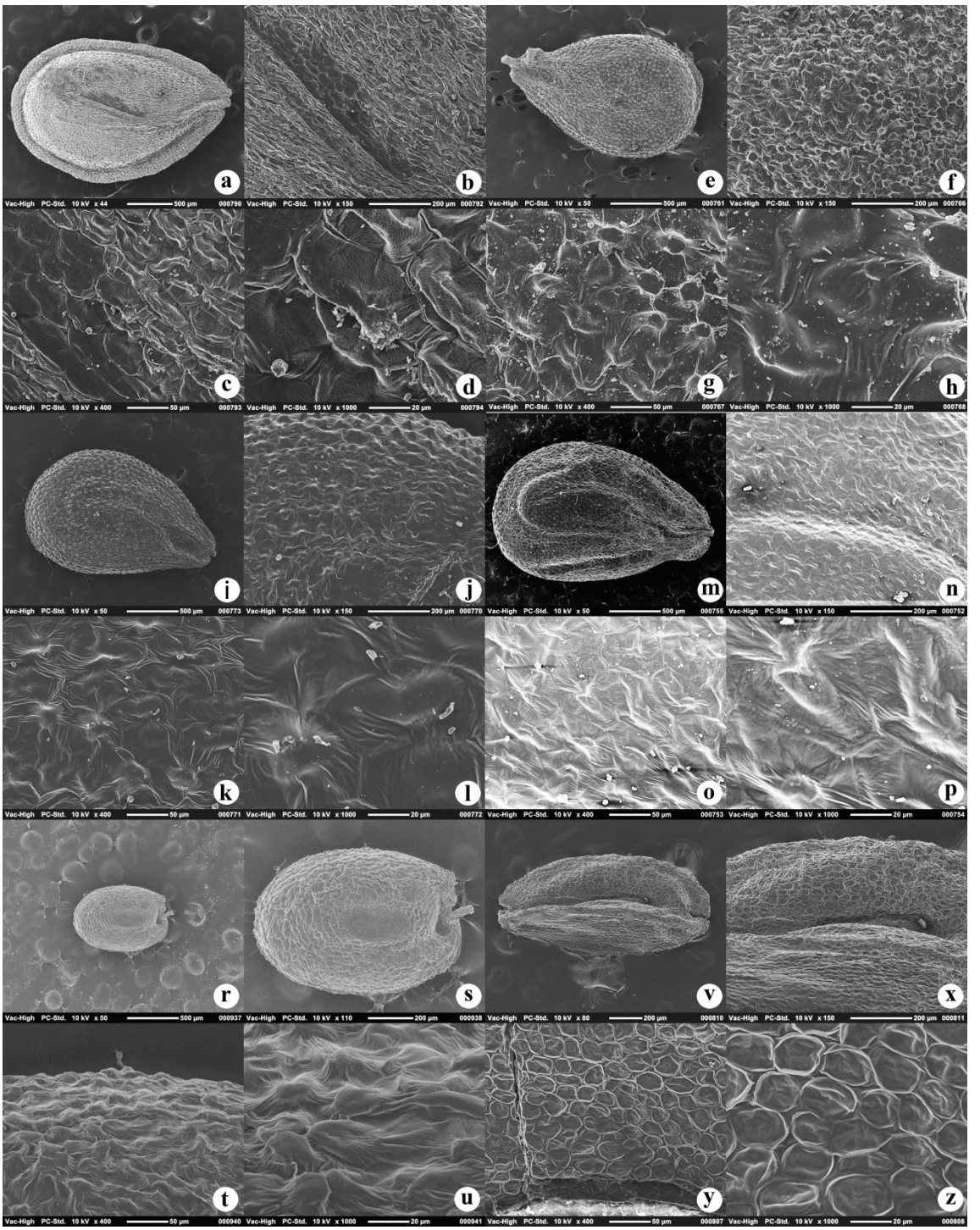

Figure 2. SEM micrographs of seeds of Turkish *Lepidium*. *L. perfoliatum* (a–d), *L. caespitosum* (e–h), *L. pumilum* (i–l), *L. crassifolium* (m–p), *L. latifolium* (r–u), *L. lyratum* (v–z).

2004; Moazzeni et al., 2007; Pınar et al., 2007; Kasem et al., 2011; Kaya et al., 2011).

This study confirms the previous finding that seed surfaces are tuberculate in *Lepidium campestre, L. latifolium*, and *L. graminifolium* (Murley, 1951). This is the first report to record the tuberculate seed surface patterns of *L. caespitosum, L. pumilum*, and *L. crassifolium*. These are related taxa distributed in salty and marshy places (Hedge, 1965). There are difficulties in separating these taxa in terms of seed surface pattern, even using high

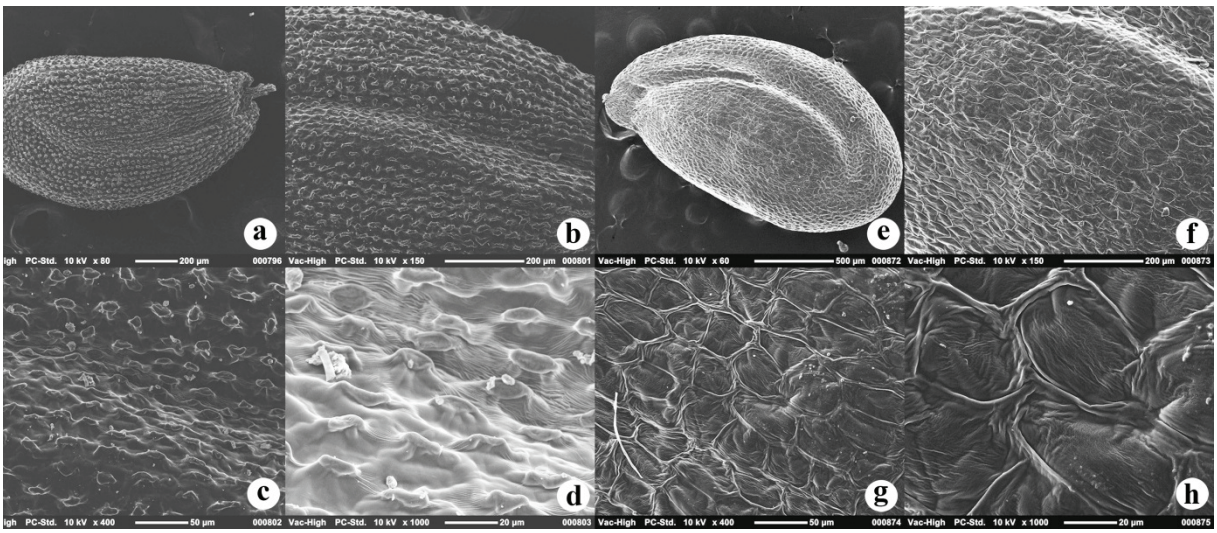

Figure 3. SEM micrographs of seeds of Turkish *Lepidium*. *L. graminifolium* (a–d), *L. vesicarium* (e–h).

magnification SEM photographs. This is also the first report of the reticulate seed surface pattern of *L. lyratum* (Figure 2) and *L. vesicarium* (Figure 3).

Lepidium spinosum has a ruminate seed surface pattern (Figure 1). It belongs to the section *Lepiocardamon*, together with *L. sativum* subsp. *sativum* and *L. sativum* subsp. *spinescens* (Hedge, 1965), and is easily recognised by its fruits, which are horny at the apex (Vasconellos, 1964; Hedge, 1965). *L. sativum* subsp. *sativum* and *L. sativum* subsp. *spinescens* are very close taxa. There are a few useful morphological characteristics to separate them from each other (Hedge, 1965; Jafri, 1973). This study shows that seed surface patterns are also useful in the separation of these taxa. *L. sativum* subsp. *sativum* has reticulate-areolate (Figure 1) and *L. sativum* subsp. *spinescens* has ruminate seed surface patterns (Figure 1). *L. spinosum* and *L. sativum* subsp. *spinescens* seed surface patterns are reported for the first time in this study. *L. sativum* subsp. *sativum* has previously been reported as reticulate-rugose (Tantawy et al., 2004), microreticulate (Kasem et al., 2011), and areolate (Murley, 1951).

Murley (1951) reported the seed surface of *Lepidium ruderale* as glebulate and puncticulate, *L. virginicum* as tuberculate, and *L. perfoliatum* as areolate. However, the present study does not confirm those results (Table 2). *L. ruderale* has reticulate (Figure 1), *L. virginicum* has reticulate-tuberculate (Figure 1), and *L. perfoliatum* has reticulate-fovate (Figure 2) seed surface patterns.

In conclusion, this study supports the use of seed surface patterns as a diagnostic characteristic for species level. There are other seed characteristics like seed size, seed shape, seed wing, and seed colour that might be helpful to distinguish some taxa (Tantawy et al., 2004; Pınar et al., 2007; Kaya et al., 2011). Furthermore, seed colour seems to be useful at the specific level, because of the green seeds of *Lepidium vesicarium* and the blackish brown seeds of *L. campestre* and *L. spinosum* (Table 2). Seed characteristics provide strong support for delimitation of studied taxa at the specific level, but understanding the importance of these characteristics at generic and subgeneric levels requires further studies.

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