


## Article

# Morphological Identification of Lepidii Seu Descurainiae Semen and Adulterant Seeds Using Microscopic Analysis

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**Abstract:** Lepidii seu Descurainiae Semen, the dried ripe seeds of *Descurainia sophia* (L.) Webb ex Prantl or *Lepidium apetalum* Willd., is used widely as a traditional herbal medicine in Northeast Asia. However, seeds of a number of other species have been misidentified as Lepidii seu Descurainiae Semen, and are therefore misused because of morphological similarities among the seeds of these species. To accurately identify Lepidii seu Descurainiae Semen, we investigated the morphology, mucilage reaction, and micromorphology of Lepidii seu Descurainiae Semen and the seeds of other plant species. We used a stereomicroscope and a scanning electron microscope to describe and compare various morphological and micromorphological characteristics of seeds, and a light microscope to determine the presence or absence of mucilage. We evaluated the potential usefulness of mucilage as a morphological marker for seed identification. The shape, outline, and size of seeds were useful characteristics for identification. The mucilage reaction and the three types of seed ornamentation that were identified in this study were particularly valuable for seed authentication. Based on these results, we created an identification key based on morphology and micromorphology of Lepidii seu Descurainiae Semen and adulterant seeds. The method of seed identification using microscopic examination, as described here, is easy and economical, making it a potential key tool for accurate identification of Lepidii seu Descurainiae Semen and seeds of other medicinal plants.

**Keywords:** *Descurainia sophia* (L.) Webb ex Prantl; Lepidii seu Descurainiae Semen; *Lepidium apetalum* Willd.; morphological identification key; mucilage; scanning electron microscope

## 1. Introduction

According to the Korean Herbal Pharmacopoeia [1,2], Lepidii seu Descurainiae Semen refers to the dried ripe seeds of *Descurainia sophia* (L.) Webb ex Prantl or *Lepidium apetalum* Willd., which belongs to the family Brassicaceae (Cruciferae). Lepidii seu Descurainiae Semen is used in Northeast Asian countries, including Korea, China, and Taiwan, as an herbal medicine, called “Jeong-Ryeok-Ja” in Korean and “Ting-Li-Zi” in Chinese.

Although the Pharmacopoeia of the People’s Republic of China and the Taiwan Herbal Pharmacopoeia have designated the seeds of *De. sophia* or *L. apetalum* as Descurainiae Semen (southern lepidium) or Lepidii Semen (northern lepidium), respectively, seeds of both plant species are sources of the same herbal medicine [1,3–5]. In Korea, prior to the revision of the Korean Herbal Pharmacopoeia (KHP) in 2013, the herbal medicine Jeong-Ryeok-Ja was believed to be derived from the seeds of *Draba nemorosa* L. and *L. apetalum* as Semen Drabae et Lepidii [2,6]. Seeds of *Dr. nemorosa* and *L. apetalum* are currently designated as sources of the same herbal medicine in the Pharmacopoeia of Democratic People’s Republic of Korea [1,7].

Traditionally, *Lepidii seu Descurainiae Semen* has been used as a demulcent, laxative, and an aperient drug [8]. Studies have shown that this herbal medicine has anti-inflammatory, analgesic, antipyretic, anticancer, and antioxidant properties. It is also used to treat functional constipation [9–12]. However, a number of misuse issues have emerged due to the inaccurate identification of seeds of other plant species as *Lepidii seu Descurainiae Semen* [2,13,14]. For example, seeds of *Dr. nemorosa*, defined in the unrevised KHP before 2013, are currently sold in herbal markets as *Lepidii seu Descurainiae Semen*. Similarly, seeds of *Erysimum cheiranthoides* L. and *Erysimum macilentum* Bunge (family Brassicaceae) are often found as adulterants in *Lepidii seu Descurainiae Semen* because of the morphological similarities among these species. The plant species *L. apetalum* is strictly distributed in the northern part of the Korean peninsula; however, *Lepidium virginicum* L., a close relative of *L. apetalum*, is found in all provinces of Korea. Therefore, it is likely to replace *L. apetalum*. The National Institute of Food and Drug Safety Evaluation has alerted consumers to discriminate between authentic and inauthentic *Lepidii seu Descurainiae Semen* [15]. Morphological analysis [2] and molecular studies [16] have been conducted to facilitate the accurate identification and authentication of original plant species with morphological similar species. However, the identification of minute seed medicines based on morphological characteristics has not been investigated.

It is important to accurately identify minute seed medicines. However, the seeds are difficult to distinguish by the naked eye. Therefore, studies using stereomicroscopes have been conducted to facilitate morphological identification of these seeds [17,18]. A macroscopic authentication study of Chinese materia medica seeds and fruits in the United Kingdom (U.K.) market was carried out to guide quality control of herbal medicine [19]. Production of mucilage by distinguished epidermal cells during hydration (myxocarpy) has been observed by light microscopy [20]. The presence or absence of a mucilage reaction is a useful diagnostic characteristic for the taxonomic identification of plant species [20–27]. Investigation of the micromorphological characteristics of seeds using a scanning electron microscope has been used to identify and distinguish species [28–34]. Thus, these techniques can be used to authenticate and distinguish genuine species from close relatives, inferior substitutes, adulterants, and counterfeits.

The present study conducted microscopic investigations and aimed to describe and illustrate the morphology, micromorphology, and mucilage reaction of *Lepidii seu Descurainiae Semen*, along with those of seeds of close relatives and adulterants. Based on the results, we created morphological and micromorphological identification keys that can be used to authenticate minute seeds.

## 2. Materials and Methods

### 2.1. Plant Materials

Two authentic plant species (*De. sophia* and *L. apetalum*) and four adulterant species (*Dr. nemorosa*, *E. cheiranthoides*, *E. macilentum*, and *L. virginicum*) were collected from natural populations in Korea and China during the fruiting season (April 2016 to May 2017). The morphological characteristics of mature seeds of these species were used for analysis. To confirm the consistency of the morphological characteristics, at least two samples of each species were collected from different sites and compared. All of the seed samples were deposited in the Korean Herbarium of Standard Herbal Resources (Index Herbariorum code KIAM) at the Korea Institute of Oriental Medicine, Daejeon, Korea. Detailed information about plant materials used in this study, including the collection sites, collection dates, and the voucher number of plant specimens deposited in KIAM, is summarized in Table 1.

**Table 1.** List of plant species used in this study along with the collection site, collection date, and voucher number.

Scientific Name	Collection Site	Collection Date	Voucher No.
<i>Descurainia sophia</i> (L.) Webb ex Prantl *	Pyeongtaek, Gyeonggi, Korea	11 May 2016	MBC_KIOM-2016-049
	Muju, Jeonbuk, Korea	31 May 2016	MBC_KIOM-2016-072
	Gurye, Jeonnam, Korea	2 June 2016	KWJ_KIOM-2016-025
	Danyang, Chungbuk, Korea	23 May 2017	MBC_KIOM-2017-029
<i>Lepidium apetalum</i> Willd. *	Antu, Yanbian, Jilin, China	30 June 2016	MBC_KIOM-2016-088
<i>Draba nemorosa</i> L. †	Anseong, Gyeonggi, Korea	12 May 2017	MBC_KIOM-2016-057
	Hapcheon, Gyeongnam, Korea	7 May 2017	MBC_KIOM-2016-029
	Yuseong, Daejeon, Korea	19 April 2016	CGY_KIOM-2016-2
<i>Erysimum cheiranthoides</i> L. †	Danyang, Chungbuk, Korea	15 July 2016	KIOM201601017294
	Danyang, Chungbuk, Korea	28 July 2016	MBC_KIOM-2016-228
<i>Erysimum macilentum</i> Bunge †	Gurye, Jeonnam, Korea	10 May 2016	MBC_KIOM-2016-040
	Andong, Gyeongbuk, Korea	25 May 2016	CGY_KIOM-2016-008
<i>Lepidium virginicum</i> L. †	Sancheong, Gyeongnam, Korea	6 May 2016	MBC_KIOM-2016-027
	Buan, Jeonbuk, Korea	1 June 2016	MBC_KIOM-2016-075
	Anseong, Gyeonggi, Korea	12 May 2016	MBC_KIOM-2016-059

\* Official plant species designated as sources of *Lepidii* seu *Descurainiae* Semen in the current Korean Herbal Pharmacopoeia and Pharmacopoeia of the People's Republic of China. † These plant species do not have an appropriate herbal name in the current Korean Herbal Pharmacopoeia and Pharmacopoeia of the People's Republic of China.

## 2.2. External Morphology

Measurements and optical observations of 20 seeds for each of the 15 collection sites (voucher) for a total of 300 seeds were recorded. A digital Vernier caliper (CD-15CP, Mitutoyo, Kawasaki, Japan) was used to measure the length, width, and length/width ratio. The general shape ( $\times 2$  magnification), cross-sectional shape ( $\times 8$  magnification), and color were recorded using a stereomicroscope (Olympus SZX16, Olympus, Tokyo, Japan) (Table 2). Cross-sections of seeds were prepared by cutting them down the middle part using a single-edge blade (DN-52, Dorco, Seoul, Korea). The hilum part was placed at the bottom, and images were captured while using a digital camera (Olympus DP21, Olympus, Tokyo, Japan). The weight of 1000 randomly selected seeds (1000-seed weight) was measured (in six replicates) using an electronic precision balance (PS 1000.R1, RADWAG Balances & Scales, Radom, Poland).

**Table 2.** Morphological characteristics of *Descurainia sophia* and *Lepidium apetalum* (authentic sources of *Lepidii* seu *Descurainiae* Semen) and adulterant species.

Scientific Name	Shape	Cross-Sectional Shape	WA <sup>a</sup>	Color <sup>b</sup>	Average Length (mm)	Average Width (mm)	Average 1000-Seed Weight (g)	MR <sup>c</sup>
<i>Descurainia sophia</i>	Oblong	Circular-to-elliptic	Absent	Br to RBr/S	1.14	0.60	0.155	+
<i>Lepidium apetalum</i>	Ovoid, obovate-to-oblong	Linear-elliptic to linear-triangular	Absent or narrow	Br to RBr/D	1.49	0.84	0.224	++
<i>Draba nemorosa</i>	Oblong-to-ovoid	Linear-elliptic	Absent	Br to RBr/D	0.71	0.44	0.035	-
<i>Erysimum cheiranthoides</i>	Obliquely oblong-to-obovate, pyriform	Circular or ovate	Absent	PBr to RBr/S	1.35	0.62	0.165	-
<i>Erysimum macilentum</i>	Oblong	Triangular-to-trapezoid	Absent	PBr to RBr/S	1.01	0.50	0.106	-
<i>Lepidium virginicum</i>	Ovoid, obovate-to-oblong	Linear-elliptic to linear-triangular	Wide	Br to RBr/D	1.98	1.19	0.413	++

<sup>a</sup> WA, wing-like appendage. <sup>b</sup> Br, brown; PBr, pale brown; RBr, reddish brown; D, dull; S, shiny. <sup>c</sup> MR, mucilage reaction; -, no reaction; +, weak reaction; ++, moderate reaction.

### 2.3. Statistical Analyses

To demonstrate variations in seed length and width among species, box plots were created using the ggplot2 library in R [35].

### 2.4. Mucilage Reaction

Five mature and healthy seeds of each collection site and a total of 15 samples, 75 seeds were treated with distilled water and examined under a light microscope (Olympus BX-53, Olympus, Tokyo, Japan). The degree of mucilage reaction was classified as absent, weak (0.1–0.5 mm), or moderate (>0.5 mm) [20], and images of the mucilage reaction were captured using a digital camera (Olympus DP21, Olympus, Tokyo, Japan).

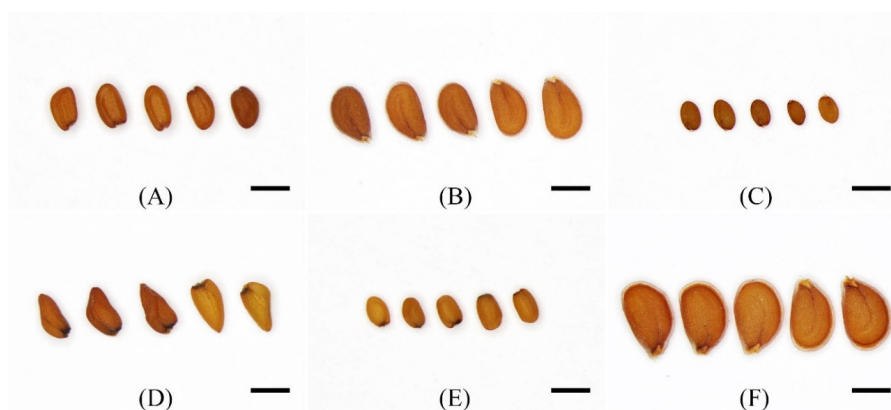
### 2.5. Micromorphology

To examine the seed coat or testa, selected seeds were mounted directly on aluminum stubs using double-side adhesive carbon tape. Stubs were coated with gold using a sputter coater (208HR, Cressington Scientific Instruments Ltd., Watford, UK), and testa were observed using a low voltage field emission scanning electron microscope (JSM-7000F, JEOL, Tokyo, Japan) at an accelerating voltage of 5 kV with a working distance of 9–10 mm. The micromorphological terminology used to describe the seeds that was used in this study followed that of Barthlott [29] and Song et al. [34].

## 3. Results

### 3.1. Morphological Characteristics of Seeds

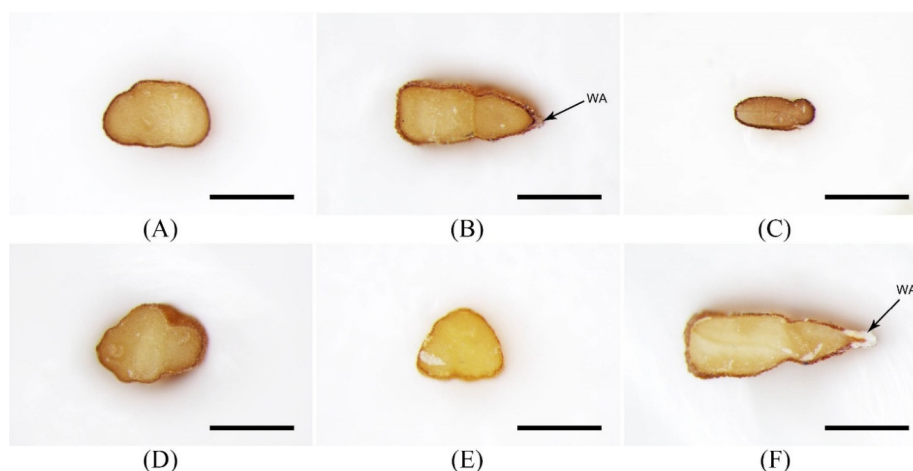
*Lepidii seu Descurainiae Semen* is the seeds of *De. sophia* or *L. apetalum*. Seeds of *De. sophia* were oblong in shape, circular-to-elliptic in cross-section, with one or two shallow ventral grooves and a concave hilum (Figures 1A and 2A). The testa was brown-to-reddish brown and slightly shiny, and the wing-like appendage was absent. Seeds of *De. sophia* ranged from 0.98–1.32 mm in length and 0.48–0.70 mm in width. The length:width ratio and 1000-seed weight varied from 1.56 to 2.71 and 0.140 to 0.170 g, respectively.



**Figure 1.** Stereomicroscope micrographs showing the seed morphology of six plant species. (A) *Descurainia sophia*; (B) *Lepidium apetalum*; (C) *Draba nemorosa*; (D) *Erysimum cheiranthoides*; (E) *Erysimum macilentum*; and (F) *Lepidium virginicum*. Scale bars = 1 mm.

In contrast, seeds of *L. apetalum* were ovoid, obovate-to-oblong in shape, and linear-elliptic to linear-triangular in cross-section (Figures 1B and 2B). Seeds possessed two grooves on both dorsal and ventral surfaces, although one groove was more prominent than the other. The hilum was concave. The testa was dull, brown-to-reddish brown, and the wing-like appendage was narrow or almost absent. Seeds of *L. apetalum* varied from 1.25 to 1.67 mm in length and 0.56 to 1.00 mm in

width. The length:width ratio of seeds ranged from 1.28 to 2.55 and the 1000-seed weight varied from 0.210–0.235 g.



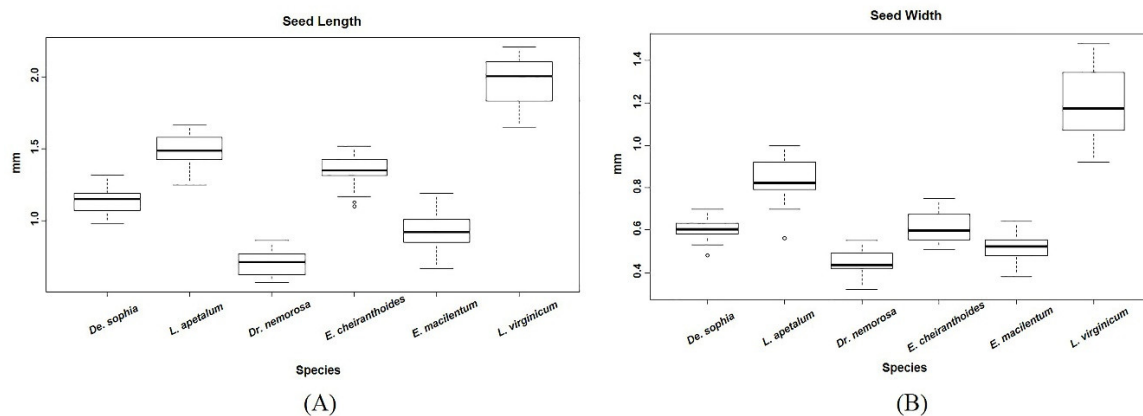
**Figure 2.** Stereomicroscope micrographs showing the cross-sectional shape of seeds. (A) *Descurainia Sophia*; (B) *Lepidium apetalum*; (C) *Draba nemorosa*; (D) *Erysimum cheiranthoides*; (E) *Erysimum macilentum*; and (F) *Lepidium virginicum*. WA: Wing-like appendage. Scale bars = 0.5 mm.

*Dr. nemorosa* is excluded from the current KHP. Seeds of *Dr. nemorosa*, Drabae Semen, are adulterants in Lepidii seu Descurainiae Semen. Drabae Semen was oblong-to-ovoid in shape, flat, and linear-elliptic in cross-section, with one shallow groove on the ventral surface (Figures 1C and 2C). The hilum was concave and globose. The testa was dull, brown-to-reddish brown, and it lacked the wing-like appendage. Seeds were 0.57–0.86 mm long and 0.33–0.55 mm wide, with a length:width ratio of 1.17–2.41 and 1000-seed weight of 0.030–0.040 g. Seeds of *Dr. nemorosa* were distinguishable from those of other species based on their relatively small size (average:  $0.71 \times 0.44$  mm; Table 2 and Figure 3) and cross-sectional in shape.

Seeds of *E. cheiranthoides* and *E. macilentum* are adulterants in Lepidii seu Descurainiae Semen because of their morphological similarities. Seeds of *E. cheiranthoides* were frequently twisted, obliquely oblong-to-obovate and pyriform in shape, and circular or ovate in cross-section, with a convex ventral surface (Figures 1D and 2D). The hilum was concave and almost black. The testa was slightly shiny and pale brown-to-reddish brown, without a wing-like appendage. Seeds were 1.13–1.52 mm long and 0.51–0.75 mm wide, with a length:width ratio ranging from 1.61 to 2.64 and 1000-seed weight ranging from 0.150 to 0.176 g. In contrast, seeds of *E. macilentum* were oblong. They were triangular-to-trapezoid in cross-section, with a slightly convex ventral surface. These seeds exhibited a concave hilum, with a slightly shiny and a pale brown-to-reddish brown testa; no wing-like appendage was visible (Figures 1E and 2E). Seeds measured 0.67–1.19 mm in length and 0.38–0.64 mm in width, with a length:width ratio of 1.21–2.53 and 1000-seed weight of 0.095–0.110 g. Seeds of *E. cheiranthoides* and *E. macilentum* were distinguishable from those of others that were based on their shape and cross-section, respectively.

*L. virginicum* is a close relative of *L. apetalum*. Seeds of *L. virginicum*, which are adulterants in Lepidii seu Descurainiae Semen, were ovoid, obovate-to-oblong in shape, and linear-elliptic to linear-triangular in cross-section, with one groove on both surfaces (Figures 1F and 2F). The hilum of *L. virginicum* seeds was concave, and the testa was dull and brown-to-reddish brown. These seeds possessed a clearly distinct wing-like appendage. The length and width of these seeds varied from 1.65 to 2.21 mm and 0.92 to 1.48 mm, respectively. Their length:width ratio and 1000-seed weight varied from 1.34 to 2.12 and 0.398 to 0.435 g, respectively. Although the seeds of *L. virginicum* were morphologically similar to those of *L. apetalum* in terms of shape, cross-sectional shape, and color, *L. virginicum* seeds were approximately 1.3-fold larger than *L. apetalum* seeds (Figure 3) and they exhibited a well-developed wing-like appendage, unlike *L. apetalum* seeds.

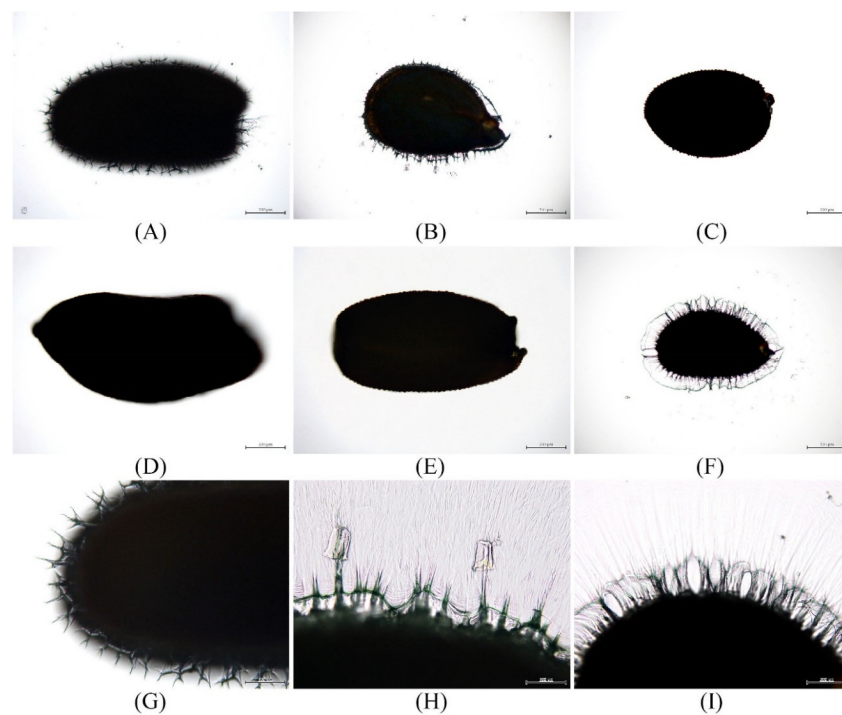




**Figure 3.** The size of *Lepidii* seu *Descurainiae* Semen and that of the seeds of related species: (A) seed length and (B) seed width. Box plots show the median, 25th, and 75th percentiles (box), 10th and 90th percentiles (whiskers), and outliers (open circle).

### 3.2. Mucilage Reaction

The mucilage present on the seed coat reacts with water and release. Thus, a positive mucilage reaction can be observed in seeds that produce mucilage. We treated the seeds of all six species with distilled water and characterized the mucilage reaction. A weak mucilage reaction was observed in the seeds of *De. sophia* (0.05–0.1 mm; Figure 4A,G), whereas a moderate reaction (0.2–0.4 mm) was observed in seeds of *L. apetalum* (Figure 4B,H) and *L. virginicum* (Figure 4F,I). In contrast, seeds of *Dr. nemorosa* (Figure 4C), *E. cheiranthoides* (Figure 4D), and *E. macilentum* (Figure 4E) showed no mucilage reaction. Moreover, these patterns were consistently observed at the species level; different sites of the same species had no variation.



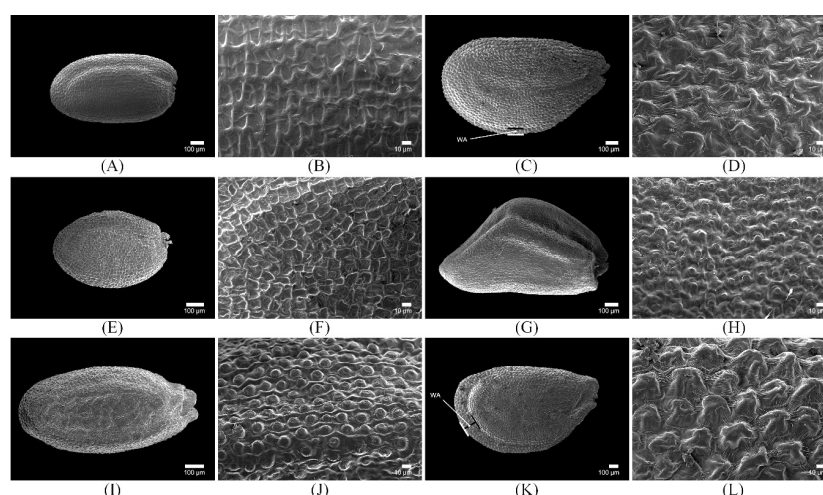
**Figure 4.** Light microscope micrographs showing release of mucilage from seeds upon hydration. (A,G) *Descurainia sophia*; weak mucilage reaction. (B,H) *Lepidium apetalum*; moderate mucilage reaction. (C) *Draba nemorosa*; no mucilage reaction. (D) *Erysimum cheiranthoides*; no mucilage reaction. (E) *Erysimum macilentum*; no mucilage reaction. (F,I) *Lepidium virginicum*; moderate mucilage reaction.

### 3.3. Micromorphological Characteristics

Examination of seeds using a scanning electron microscope revealed remarkable variation in surface patterns (Table 3 and Figure 5). We categorized seeds into three major types (Types I, II, and III) based on their ornamentation, epidermal cell pattern, and the shape of the anticlinal and periclinal cell walls. Each type was further categorized into two subtypes. The epidermal cell shape was irregular, rounded, isodiametric, or tetra-to-polygonal. The boundary of the anticlinal cell wall was almost indistinct in some seeds, but it was raised in others. Anticlinal cell walls were straight or slightly sinuous, thick or thin, and with a surface that was either smooth or possessed fine folds or coarse folded papillae. Periclinal cell walls showed a large variation among species, ranging from concave to strongly convex, with a surface that was either smooth with globular central tuberculae or coarse with folded central papillae.

**Table 3.** Micromorphological characteristics of *Descurainia sophia* and *Lepidium apetalum* (authentic sources of *Lepidii* seu *Descurainiae* Semen) and adulterant species.

Scientific Name	Ornamentation (Type)	Epidermal Cell Pattern	Anticlinal Cell Wall	Periclinal Cell Wall
<i>Descurainia sophia</i>	Simple reticulate (I-1)	Irregular, rounded-to-polygonal	Raised, straight to slightly sinuous, thick, smooth-to-fine folds	Concave, smooth
<i>Lepidium apetalum</i>	Reticulate-papillate (III-1)	Isodiametric, polygonal	Nearly indistinct, straight, thin, smooth-to-fine folds	Convex, coarse folded narrow central papillae, radiate-striate
<i>Draba nemorosa</i>	Simple reticulate (I-2)	Isodiametric, tetra-to-hexagonal	Raised, straight, thick, smooth or coarse folded papillae	Concave, smooth
<i>Erysimum cheiranthoides</i>	Reticulate-tuberculate (II-1)	Isodiametric, penta-to-hexagonal	Slightly raised, straight, thick, smooth-to-fine folds	Convex, globular narrow central tuberculae, radiate-striate
<i>Erysimum macilentum</i>	Reticulate-tuberculate (II-2)	Isodiametric, tetra-to-hexagonal	Raised, straight, thick, smooth-to-fine folds	Strongly convex, globular wide central tuberculae, radiate-striate
<i>Lepidium virginicum</i>	Reticulate-papillate (III-2)	Isodiametric, polygonal	Slightly raised, straight, thin, smooth-to-fine folds	Strongly convex, coarse folded wide central papillae, radiate-striate



**Figure 5.** Scanning electron microscope micrographs showing (A,C,E,G,I,K) an overview of the seed surface and (B,D,F,H,J,L) detailed seed ornamentation. (A,B) *Descurainia sophia* (Type I-1; simple reticulate); (C,D) *Lepidium apetalum* (Type III-1; reticulate-papillate); (E,F) *Draba nemorosa* (Type I-2; simple reticulate); (G,H) *Erysimum cheiranthoides* (Type II-1; reticulate-tuberculate); (I,J) *Erysimum macilentum* (Type II-2; reticulate-tuberculate); (K,L) *Lepidium virginicum* (Type III-1; reticulate-papillate). WA: wing-like appendage.

Type I seeds included those of *De. sophia* and *Dr. nemorosa*. These seeds showed simple reticulate ornamentation with a raised, thick anticlinal cell wall and a concave, smooth periclinal cell wall (Figure 5A,B,E,F). The reticulate ornamentation of Type I seeds was divided into two subtypes (Type I-1 and Type I-2), based on the epidermal cell pattern and anticlinal cell wall surface. Seeds of *De. sophia* were designated as Type I-1 and they showed an irregular, rounded, or polygonal epidermal cell pattern; the surface of the anticlinal wall was either smooth or had fine folds (Figure 5B). Seeds of *Dr. nemorosa* were categorized as Type I-2, with an isodiametric or tetra-to-hexagonal epidermal cell pattern; the surface of the anticlinal wall was either smooth or coarse with folded papillae (Figure 5F).

Type II seeds included those of *E. cheiranthoides* and *E. macilentum*. These seeds showed reticulate-tuberculate ornamentation, with a straight, thick, smooth-to-fine folded anticlinal cell wall and a convex, radiate-striate periclinal cell wall with globular central tuberculae (Figure 5G–J). The reticulate-tuberculate ornamentation of Type II seeds was further divided into two subtypes (Type II-1 and Type II-2), which were based on the degree of anticlinal cell wall and the diameter of the tuberculae. *E. cheiranthoides* seeds were designated as Type II-1; these possessed a slightly raised anticlinal cell wall and a convex periclinal cell wall with narrow tuberculae (5.0–9.2 µm in diameter; average = 6.9 µm) (Figure 5H). Seeds of *E. macilentum* were categorized as Type II-2; these seeds exhibited a raised anticlinal cell wall and a strongly convex periclinal cell wall with wide tuberculae (9.1–17.2 µm in diameter; average = 12.4 µm) (Figure 5J).

Type III seeds included those of *L. apetalum* and *L. virginicum*. These seeds showed reticulate-papillate ornamentation with an isodiametric or polygonal epidermal cell pattern. The anticlinal wall was straight and thin, with a smooth or finely folded surface, and the periclinal cell wall was radiate-striate with coarse folded central papillae (Figure 5C,D,K,L). The reticulate-papillate ornamentation of Type III seeds was divided into two subtypes (Type III-1 and Type III-2), based on the degree of anticlinal cell wall and the diameter of papillae. Type III-1 seeds were observed in *L. apetalum*; these showed a nearly indistinct anticlinal cell wall and a convex periclinal cell wall with narrow papillae (9.4–16.0 µm in diameter; average = 12.1 µm) (Figure 5D). Type III-1 seeds were observed in *L. virginicum*; these showed a slightly raised anticlinal cell wall and a strongly convex periclinal cell wall with wide papillae (24.6–36.5 µm in diameter; average = 30.3 µm) (Figure 5L).

### 3.4. Identification Key Based on Seed Morphology and Micromorphology

Based on our morphological and micromorphological analysis of Lepidii seu Descurainiae Semen and adulterant seeds, we designed a key to accurately identify seeds. According to this key, seeds are identified according to shape, cross-sectional shape, appearance, mucilage reaction, wing-like appendage, and combining micromorphological characteristics, such as ornamentation, epidermal cell pattern, and shape of the anticlinal and periclinal cell walls, as described below:

1. Seeds ovoid, obovate-to-oblong in shape; linear-elliptic to linear-triangular in cross-section; dull.
2. Seeds 0.57–0.86 × 0.32–0.55 mm; no mucilage reaction; simple reticulate ornamentation; concave, smooth periclinal cell wall —————  
*Draba nemorosa*
2. Seeds 1.25–2.21 × 0.56–1.48 mm; moderate mucilage reaction; reticulate-papillate ornamentation; coarse folded central papillae on periclinal cell wall.
3. Seeds 1.25–1.67 × 0.56–1.00 mm; wing-like appendage absent or narrow; nearly indistinct and convex anticlinal cell wall; narrow papillae (9.4–16.0 µm in diameter)  
—————  
*Lepidium apetalum*
3. Seeds 1.65–2.21 × 0.92–1.48 mm; wing-like appendage wide; slightly raised and strongly convex anticlinal cell wall; wide papillae (24.6–36.5 µm in diameter)  
—————  
*Lepidium virginicum*



1. Seeds oblong or obliquely oblong-to-obovate pyriform in shape; not linear-elliptic in cross-section; somewhat shiny.
4. Seeds circular-to-elliptic in cross-section; weak mucilage reaction; simple reticulate ornamentation; smooth periclinal cell wall —————  
*Descurainia sophia*
4. Seeds triangular-to-trapezoid in cross-section; no mucilage reaction; reticulate-tuberculate ornamentation; globular central tuberculae on periclinal cell wall.
5. Seeds obliquely oblong-to-obovate, pyriform in shape, circular or ovate in cross-section; slightly raised and convex anticlinal cell wall; narrow tuberculae (5.0–9.2  $\mu\text{m}$  in diameter)  
—————  
*Erysimum cheiranthoides*
5. Seeds oblong in shape, triangular-to-trapezoid in cross-section; raised and strongly convex anticlinal cell wall; wide tuberculae (9.1–17.2  $\mu\text{m}$  in diameter)  
—————  
*Erysimum macilentum*

#### 4. Discussion

We conducted a microscopic investigation of the morphology (shape, outline, color, size, wing-like appendage, and mucilage reaction) and micromorphology (ornamentation type, epidermal cell pattern, and anticlinal and periclinal cell walls) of the traditional herbal medicine *Lepidii seu Descurainiae Semen* and adulterant seeds. Using the results of microscopic analysis, we developed a morphological and micromorphological identification key that distinguishes authentic seed medicines from adulterants.

##### 4.1. Morphological Characteristics of Seeds

*Lepidii seu Descurainiae Semen* is minute seeds that are difficult to distinguish from adulterant seeds by the naked eye. In this study, we used a stereomicroscope to examine the shape, size, and color of these seeds to enable accurate identification. The cross-sectional shape and presence of grooves were particularly informative and enabled differentiation between authentic *Lepidii seu Descurainiae Semen* and adulterant seeds.

According to the dispensatory on the visual and organoleptic examination of herbal medicines, seeds of *Dr. nemorosa*, which are adulterants of *Lepidii seu Descurainiae Semen*, are flat and ovoid [15]. In this study, seeds of *Dr. nemorosa* were easily distinguished from those of other species based on their linear-elliptic cross-sectional shape alone (Figure 2C). Additionally, seeds of *Dr. nemorosa* were the smallest (Figure 3) and lightest among all six species studied (Table 2). The morphologically similar seeds of *E. cheiranthoides* and *E. macilentum* were distinguishable from *Lepidii seu Descurainiae Semen* based on their shape (obliquely oblong-to-obovate, pyriform; Figure 1D) and cross-sectional shape (triangular-to-trapezoid; Figure 2E), respectively. Although the seeds of *L. virginicum* and *L. apetalum*, which are close relatives, were highly similar, they could be distinguished by their size (Figure 1B vs. Figures 1F and 3), weight (Table 2), and the degree of development of the wing-like appendage (Figure 2B vs. Figure 2F). In the flora in China, Cheo et al. [36] characterized *L. apetalum* seeds as “wingless” and *L. virginicum* seeds as “narrowly winged, at least distally”. However, we observed *L. apetalum* seeds with a narrow wing-like appendage. Thus, further ontogenetic studies are necessary to evaluate the embryological origin and the definition of the wing-like appendage in these species.

##### 4.2. Mucilage Reaction

The mucilage reaction of fruits and/or seeds of higher plants upon hydration is used to identify plants at the species level [20–27]. Mucilage is reported frequently in the plant families Lamiaceae

(Labiatae) [20], Brassicaceae (Cruciferae) [21,23,25], and Asteraceae (Compositae) [24]. The mucilage reaction has been used to differentiate *Cuscuta chinensis* Lamark and *C. australis* R. Br. (Convolvulaceae), which are authentic sources of Cuscutae Semen [3,4] from *C. japonica* Choisy. Cuscutae Semen produces mucilage upon hydration, whereas *C. japonica* seeds show no mucilage reaction [17]. The degree of mucilage reaction has been used to distinguish between *Plantago asiatica* L. and *P. depressa* Willd. (Plantaginaceae) of Plantaginis Semen [18]. Here, mucilage was observed in the seeds of *De. sophia* and *L. apetalum*, which are authentic sources of Lepidii seu Descurainiae Semen; however, seeds of their adulterants showed no mucilage reaction. These data suggest that the mucilage reaction upon hydration is a convenient and useful method for the accurate identification of minute seed herbal medicines. Seeds of *L. virginicum*, which are morphologically similar to those of *L. apetalum*, also showed a mucilage reaction that was similar to that of *L. apetalum* seeds. Plant mucilage has received significant attention due to its pharmacological and therapeutic value [37–39]. Therefore, further analysis of the ingredients and pharmacological effects of mucilage in *L. virginicum* are needed to evaluate the potential of using *L. virginicum* as a medicinal substitute for *L. apetalum*.

#### 4.3. Micromorphological Characteristics

Analysis of the micromorphological features of seeds using a scanning electron microscope revealed that Lepidii seu Descurainiae Semen—*De. sophia* (type I-1) and *L. apetalum* (type III-1)—are easily distinguishable from the seeds of adulterant species. These micromorphological characteristics of the seed surface are informative and useful for the identification of plant species at inter- and/or intra-species level [28–34]. The shape of the anticlinal cell wall boundaries and the periclinal cell wall were particularly useful for distinguishing authentic seed medicines from closely related species.

## 5. Conclusions

This is the first comprehensive study of Lepidii seu Descurainiae Semen and adulterant seeds in order to accurately identify seeds. The shape, cross-sectional shape, size, mucilage reaction, and surface pattern of the seed are useful for identifying and authenticating the studied species. Overall, the data presented suggest that microscopic analysis of seed morphology is a convenient and economical method for identifying seeds. We demonstrated that the investigation of morphological and micromorphological characteristics using microscopic imaging techniques is instrumental for discrimination between authentic herbal medicines and their adulterants.

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**Conflicts of Interest:** The authors declare that there are no conflicts of interest.

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