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Seed-Coat microsculpturing of some Cardamine (Brassicaceae) taxa and its systematic importance

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Abstract

The seed exomorphic characteristics of four taxa (*Cardamine graeca*, *C. impatiens* subsp. *impatiens*, *C. raphanifolia* and *C. uliginosa*) belonging to *Cardamine* were investigated with scanning electron microscopy (SEM). This study presents exomorphic characteristics, including seed colour, shape, winged, measures, seed coat pattern. Four coat patterns were observed; regularly reticulate, irregularly-reticulate, blister and tuberculate-reticulate, and two shapes were distinguished; broadly oblong and oblong. The results showed that the morphological characteristics of seed could contribute as criteria to distinguish taxa. This is the first study in which the seed surface of any *Cardamine* taxa growing in Turkey is studied with SEM.

Key words: classification, Cruciferae, micromorphology, seed surface ornamentation, SEM, taxonomy

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Bazı Cardamine (Brassicaceae) taksonlarının tohum yüzey mikro şekilleri ve taksonomik önemi

Özet

Cardamine cinsine ait olan dört taksonun (*Cardamine graeca*, *C. impatiens* subsp. *impatiens*, *C. raphanifolia* and *C. uliginosa*) tohum dış morfolojileri, taramalı electron mikroskobu (SEM) ile incelendi. Bu çalışma tohum rengi, şekli, kanatı, ölçüsü ve tohum yüzey modelini içeren dış morfolojik karakterleri sunmaktadır. Dört tohum yüzeyi modeli; düzenli-retikulat, düzensiz-retikulat, kabarcıklı ve tüberküllü-retikulat gözlendi ve iki tane de tohum şekli; geniş oblong ve oblong tespit edildi. Sonuçlar tohumların morfolojik karakterlerinin taksonların ayrımına katkı sağlayabileceğini göstermiştir. Bu çalışma Türkiye'de yetişen herhangi bir *Cardamine* taksonunun tohum yüzeyinin SEM ile incelendiği ilk çalışmadır.

Anahtar kelimeler: sınıflandırma, Cruciferae, mikromorfoloji, SEM, taksonomi, tohum yüzeyi modellemesi

1. Introduction

Turkey is one of the richest countries in the world in terms of *Brassicaceae* with 571 species. At the first volume of the Flora of Turkey, the number of *Brassicaceae* genus and species was indicated to be 86 and 464, respectively [1]. These numbers have increased to 88 genus and 526 species in the 10th volume [2] and to 91 genus and 555 species in the 11th volume of Flora of Turkey [3]. In the 2nd supplement of Flora of Turkey, the total number of the species increased to 538 with the addition of one genus, 28 species, 15 subspecies, and two varieties [4].

Cardamine L. is the third largest genera of *Brassicaceae* family with approximately 200 species spreading all over the world except Antarctica [5, 6]. Taxonomic revision studies have been carried out on *Cardamine* species in different parts of the world [7, 8, 9]. In Turkey *Cardamine* has represented with 14 taxa and none of them is not endemic [10]. All studied taxa, except *C. raphanifolia* (Euro-Siberian element), are widely distributed [1].

Seed surfaces, color, and size are valuable characteristics for distinguishing taxa in Brassicaceae [11, 12, 13, 14]. The four taxa studied were closely positioned in the identification key of Flora of Turkey [1]. Therefore the main

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objective of this study is to evaluate the taxonomic significance of the seed coat pattern as seen by SEM in some taxa of the *Cardamine* and to contribute to the distinction of species.

2. Materials and methods

The study material comprised samples of ripe seeds from four taxa belonging to *Cardamine* collected from natural habitats in Turkey between 2015 and 2016. The samples were stored at Konya Selçuk University Faculty of Science Herbarium (KNYA). The locations and collector registration numbers of the taxa studied are provided in Table 1; the seed micromorphology characteristics are provided in Table 2. Twenty seeds from each taxon were examined in our study.

Samples examined in our analyses using scanning electron microscopy (SEM) were first passed through a series of 70, 80, 96, and 100% alcohol for 20 min each. The surfaces were then observed and photographed with the ZEISS EVO LS-10 scanning electron microscope at 30x, 1000x, and 2000x magnification in high vacuum mode.

The seed micromorphology terminology used was according to [13, 15, 16].

ТАХА	LOCALITY
Cardamine graeca L.	C4 Karaman: Sarıveliler, Çukurbağ Köyü, kurumuş dere kenarı, 800 m, 30.06.2015,
	E. Şirin 573 & M. Şirin (KNYA)
C. impatiens L. subsp.	A3 Bolu: Yedigöller Milli Parkı, Fagus orientalis ormanı, 1500 m, 04.05.2016, E.
impatiens	Şirin 615 & M. Şirin (KNYA)
C. raphanifolia Pourr.	A8 Erzurum: Aziziye, Ahırcık Yaylası, dere kenarı, 2400 m, 13.08.2015, E. Şirin
	538 & M. Şirin (KNYA)
C. uliginosa M. Bieb.	A9 Ardahan: Damal'dan Posof'a 20. km, çayırlık alan, 2450 m, 10.06.2016, E. Şirin
	639 & M. Şirin (KNYA)

Table 1. Localities of the studied taxa

3. Results

The micrographs from the seeds of the four taxa studied are shown in Fig. 1 and the macro- and micromorphological properties of the seeds are provided in Table 2.

C. graeca: Seed dark brown to black, oblong, winged, glabrous, 4.61–4.82 x 1.91–2.42 mm and the seed coat pattern is regularly reticulate (Table 2, Fig. 1).

C. impatiens: Seed brown, broadly oblong, wingless, glabrous, $1.11-1.02 \ge 0.71-0.83$ mm and the seed coat pattern is irregularly reticulate (Table 2, Fig. 1).

C. raphanifolia: Seed brown, oblong, wingless, glabrous, 0.82–1.12 x 0.42–0.71 mm and the seed coat pattern is blister (Table 2, Fig. 1).

C. uliginosa: Seed dark brown to black, broadly oblong, winged, glabrous, 4.52–4.92 x 2.06–3.28 mm and the seed coat pattern is tuberculate reticulate (Table 2, Fig. 1).

4. Conclusions and discussion

Seed surface patterns provide valuable characters for the identification of taxa in selected groups of Brassicaceae [11, 12, 13, 17]. In the previous study, seed surface patterns of the *C. graeca* and *C. impatiens* were evaluated as smooth [11, Table 3]. However, it would be more appropriate to evaluate *C.graeca* as regularly reticulate and *C. impatiens* as irregularly reticulate since the evaluation was not made using a SEM. Other seed surface patterns have been defined on *Cardamine flexuosa* (undulate) and *C. hirsuta* (microreticulate) [16]. Blister, which is the surface model of *C. raphanifolia*, was also observed in *Brassica nigra* and *B. oleracea*, which are not related to *Cardamine* [17].

In this study, a broadly oblong shape was observed in most of the seeds. A similar result was found in *Matthiola tricuspidata*, which is not related to *Cardamine* [19]. Although [11] interpreted the seed shapes of *C. graeca* as orbicular to oval and of *C. impatiens* as oval, according to Stearn's [15] system, it is more appropriate to interpret the seed shapes of *C. graeca* as oblong and *C. impatiens* as broadly oblong.

C. impatiens and *C. raphanifolia* differed from the others with wingless seed. Because all of the studied taxa had wingless seed, this character cannot be considered distinctive. *Bunias erucago, Euclidium syriacum, Hymenophysa pubescens* and *Neslia paniculata* are some other species in the same family with wingless seed structures [11]. *C. raphanifolia* had relatively smaller seeds compared to the others.

Because all of the studied taxa had dark brown to black seed color, this cannot be considered as a distinctive character. [20] observed a similar seed color in *Lepidium* taxa, which are not related with the *Cardamine*.

All of the taxa had a glabrous seed surface. [21] reported a similar situation in *Brassica juncea*, *B. tournefortii*, *Eruca sativa*, *Raphanus sativus*, *Cakile arabica*, and *Sisymbrium irio*, which are not related to *Cardamine*.

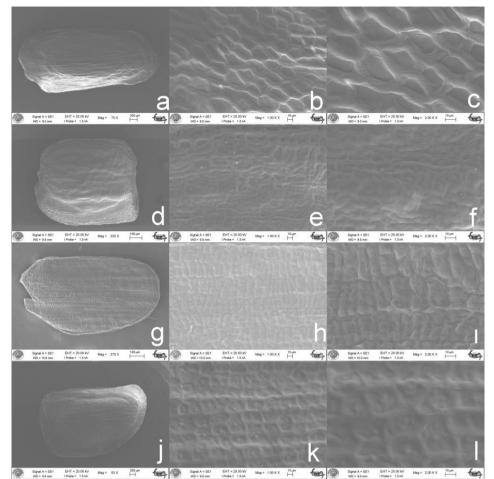


Figure 1. SEM micrographs of seeds of studied taxa: C. graeca (a-c), C. impatiens (d-f), C. raphanifolia (g-i) and C. uliginosa (j-l)

The studied characteristics were not distinctive for *Cardamine* in genus level but especially seed coat ornamentation can be used as distinctive characteristics among the studied taxa. In future seed morphology studies about the rest of the *Cardamine* species will contribute to the interpretation of relationships of the taxa.

Taxa	Colour	Shape	Length (Mm)	Width (Mm)	Ornamentation
Cardamine graeca	Dark brown to black	Oblong	4.61-4.82	1.91–2.42	Regularly reticulate
C. impatiens	Brown	Broadly oblong	1.11-1.02	0.71–0.83	Irregularly reticulate
C. raphanifolia	Brown	Oblong	0.82 - 1.12	0.42-0.71	Blister
C. uliginosa	Dark brown to black	Broadly oblong	4.52–4.92	2.06-3.28	Tuberculate reticulate

Table 2. Micro- and macromorphological seed features of studied taxa

Table 3. Comparison	with previous	studies
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Studied Taxa	Results	Results Of Previous Studies
Cardamine graeca	Regularly reticulate	Smooth [11]
C. impatiens	Irregularly reticulate	Smooth [11]
C. graeca	Oblong	Orbicular to oval [11]
C. impatiens	Broadly oblong	Oval [11]

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