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Article

Remarks Using the SEM method Species Seeds of *Gentiana* occurring in Turkey

Observações Utilizando o Método SEM de Sementes de Espécies de ***Gentiana*** *Ocorrentes na Turquia*

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ABSTRACT - Seeds of eleven perennial *Gentiana* collected from Turkey were analyzed using the SEM method. Other species excluding *G. septemfida, G. boissieri, G. gelida* were studied for the first time. They were identified and compared in terms of seed characteristics and surface ornamentations. Major characteristics including the outer periclinal walls of testa, sculpting of inner periclinal walls, seed shape, seed and testa cell, wing cell size, thickness of testa wall and seed shape have been proposed for Turkey *Gentiana*. They are divided into different types, such as no wing, chalazal wing, incomplete discoid wing, complete discoid wing according to the outer periclinal walls of testa. Considering primary sculpting of seeds, irregularly striate and shallowly reticulate type is observed. Anticlinal walls of *G. olivieri, G. boissieri* and *G. gelida* are curved, while others are straight. *G. lutea* has the largest mean seed (4.20 x 4.40 mm), while *G. cruciata* (0.67 x 0.60 mm) and *G. olivieri* have the smallest mean seeds (0.67 x 0.67 mm). Seed micromorphology can be used together with morphological character to form classifications in studied specimens for *Gentiana* genus.

Keywords: *Gentiana*, seed-coat, scanning electron microscope, Turkey.

RESUMO - Sementes de 11 espécies perenes ***Gentiana*** *coletadas na Turquia foram analisadas utilizando métodos de microscopia eletrônica de varredura (SEM). Foram estudadas outras espécies, excluindo* ***G. septemfida****,* ***G. boissieri*** *e* ***G. gelida****, por exemplo, pela primeira vez. Elas foram identificadas e comparadas em termos de características físicas das sementes e ornamentações de superfície. Características principais, incluindo as paredes periclinais externas da testa, escultura de paredes periclinais internas, a forma de sementes, sementes e células testa, tamanho de célula asa, espessura da parede testa e forma da semente ter sido corte para a* ***Gentiana*** *turca. Elas são divididas em diferentes tipos, como não asa, Ala chalazal, asa discoide incompleto, asa discóide completa periclinal De acordo com as paredes exteriores da testa. Considerando escultura primária de sementes, de forma irregular e rasa tipo reticulada é observada striatum. Paredes anticlinais de* ***G. olivieri****,* ***G. boissieri*** *e* ***G. gelida*** *desenvolvimento na área curvada, enquanto outros estão em linha reta.* ***G. lutea*** *tem a maior significar semente (4.20 x 4.40 mm), enquanto* ***G. cruciata*** *(0.67 x 0.60 mm) e* ***G. olivieri*** *alcaçuz sementes têm a menor (0.67 x 0.67 mm). Micromorfologia de sementes pode ser usado junto com caráter morfológico para formar classificações em espécimes estudados para* ***Gentiana*** *gênero.*

Palavras-chave: *Gentiana*, tegumento, microscópio eletrônico de varredura, Turquia.

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INTRODUCTION

The Gentianaceae family is represented by 7 genera in Turkey's Flora: *Cicendia*, *Blackstonia*, *Centaurium*, *Gentiana*, *Gentianella*, *Lomatogonium*, *Swertiana*. The genus *Gentiana* includes 14 taxa, two of which are annual, twelve of which are perennial. Only *G. boissieri* is an endemic species with a narrow distribution (Pritchard, 1978; Davis et al., 1988). Examinated species: *G. lutea* subsp. *symphyandra*, *G. asclepidae*, *G. cruciata*, *G. olivieri*, *G. pyreniaca*, *G. septemfida*, *G. boissieri*, *G. gelida*, *G. verna* subsp. *balcanica* and subsp. *pontica*, *G. brachyphylla* subsp. *favrati*. There is a large body of research on seed coat morphology. The introduction of SEM has boosted the tendency to use it in taxonomy. In a study carried out with SEM, Barthlott (1990) reported that seeds and small fruits that are complex, provide important taxonomic information and show micromorphologic diversity, can be used for diagnostic purposes due to their inner structures of detailed shape, different color, size and, partially, their inner structures.

Seed morphology has been used by many authors together with other characteristics to form classifications in other genera of the Gentianaceae family, especially *Gentiana*, *Gentianella* (Guérin, 1904; Ho and Liu, 1990; Kusnezow, 1894; Miége and Wüest, 1984). It is also a preferred method in identification of *Gentianopsis* (Gillette, 1957; Whitlock et al., 2010). The literature contains studies suggesting that the morphologic difference in genus *Gentiana* is also observed in seed micromorphology and that it should be supported in terms of taxonomy. Yuan (1993) reported that seed morphology is important when evaluating the relationships in sectional classification in the genus *Gentiana*. However, one cannot entirely act based on that, and the mentioned situation can be explained by similar seed coat sculpture of the genera *Swertiana* and *Gentianopsis* with different origins. Studies conducted in Taiwan (Chen and Wang, 1999) and Pakistan, Kashmir (Ömer and Quaser, 1995) have revealed that the micromorphologic structure in seeds clearly supports other data in identifying small and homogenic groups in the genus *Gentiana*. Davitashvili and Karrer (2006, 2010) also made evaluations using it with flower and leaf morphology. In Anatolia, roots of *G. lutea* and shoots of *G. asclepiadea*, *G. cruciata* and *G. olivieri* are used as antipyretic and appetite enhancers (Baytop, 1999).

Since there is a lack of comprehensive studies in the literature on the seeds of *Gentiana* species in Turkey, this paper aims to compare eleven perennial *Gentiana* species by evaluating seed micromorphology, which is considered as a taxonomic characteristic, and to determine similarities and differences between the species.

MATERIALS AND METHODS

Analyzed species of the genus *Gentiana* were collected from different localities in Turkey. The list of the collected species is presented below (Table 1). Examinations were conducted in ANK, GAZI and HUB herbariums. Mature seeds collected from the samples were analyzed both under light microscopy and SEM. Measurements were performed on at least 15 seeds of each species. Means and standard deviations were calculated. The seeds collected for SEM were washed

Table 1 - *Gentiana* species collected from different areas in Turkey

Species	Localities - Time of seed
G. lutea subsp. *symphyandra*	A2 Bursa, Uludağ, 1900 m, 05.07.2013, Euro-Sib. El.
G. asclepidae	A8 Trabzon: humidity places, 1800-2156 m, 02.09.2013. Euro-Sib. El.
G. cruciata	A9 Artvin: Yusufeli, meadow areas, 1872-1967 m, 09.07.2013, Euro-Sib. El.
G. olivieri	C6 Gaziantep: Nizip, dry creek edges, calcareous ground, 535-551 m, 06.06.2013, Ir.-Tur. El.
G. pyreniaca	A9 Ardahan: Posof, northern slopes, alpine, 2706 m, 11.06.2013, Euro-Sib. El.
G. septemfida	A7 Gümüşhane: Zigana Mountain, meadow areas, 2140 m,30.07.2013, Hyrcano- Euxine El.
G. boissieri	C5 Niğde: Bolkar Mountain, alpine, 2650 m., 16.08.2013, E. Med. El. End.
G. gelida	A8 Erzurum: Tortum, meadow areas, 2297-2408 m, 30.07.2013, Euxine El.
G. verna subsp. *balcanica*	A2 Bursa: Uludağ, stony fields, 2230-2330 m., 05.07.2013, Hyrcano- Euxine El.
G. verna subsp. *pontica*	A8 Bayburt: Soğanlı Pass, meadow areas, 2416 m, 14.06.2013, Euro-Sib. El.
G. brachyphylla subsp. *favrati*	C5 Niğde: Bolkar Mountain, alpine, 2700 m, 12.09.2013, Euro-Sib. El.

El: Element; End: Endemic for Turkey.

with 70% ethyl alcohol and were placed on stabs. The seeds were then prepared for the analysis by covering them with gold. A JSM 5600 microscope was used to take images at Selçuk University Advanced Technology Research and Application Center. Stearn (1983) and Barthlott (1981) were used for descriptive terminology.

RESULTS AND DISCUSSION

While studying seed morphology of *Gentiana*, we attempted to determine differences by applying 5 principle characteristics including the outer periclinal walls of testa, sculpting of inner periclinal walls, seed shape, seed and testa cell, wing cell size, thickness of testa wall and seed shape.

The outer periclinal walls of testa: the study of Davitashvili and Karrer (2010) was taken as a basis for classifications according to this characteristic. Accordingly, the *Gentiana* species we had were divided into four groups:

- Species Having No Wings: *G. cruciata*, *G. olivieri*, *G. pyreniaca*, *G. verna* subsp. *balcanica* and subsp. *pontica* (Figure 1E; Figure 2A, C; Figure 3E; Figure 4A).
- Species Having Chalazal Wing: they develop in the form of a small wing at the bottom of kalaza in testa: *G. septemfida* (Figure 2E).
- Incomplete Discoid Wing: presence of wings surrounding the entire seed excluding the micropyllar end: *G. lutea* subsp. *symphyandra*, *G. asclepidae* and *G. brachyphylla* subsp. *favrati* (Figure 1A, C; Figure 4C).
- Complete Discoid Wing: entire seed surrounded by a wing including the microphyllar end: *G. boissieri*, *G. gelida* (Figure 3A, C).

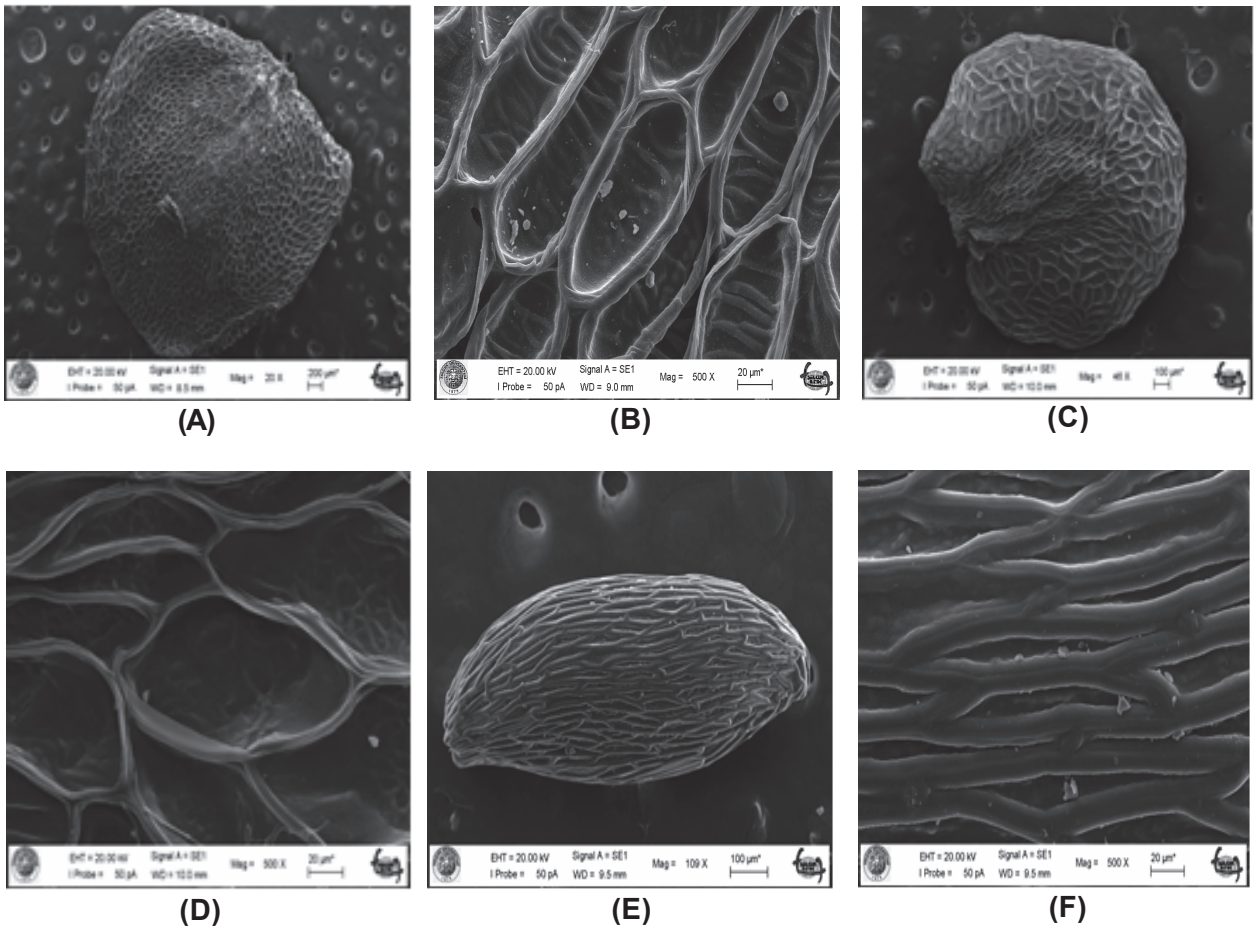

Figure 1 - SEM of seed surface of *Gentiana* at two different magnifications. (A-B): *G. lutea*; (C-D): *G. asclepidae*; (E-F): *G. cruciata*.

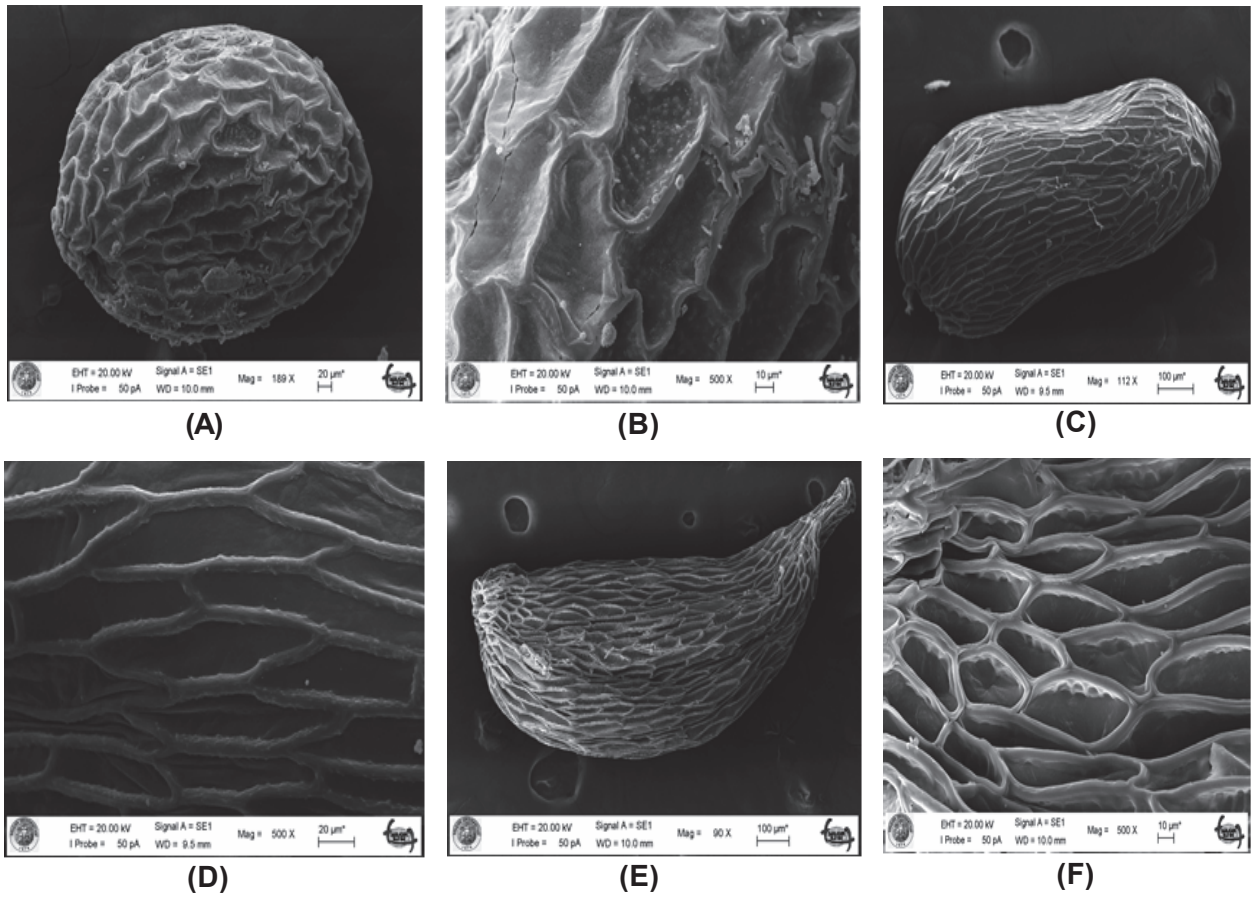

Figure 2 - SEM of seed surface of *Gentiana* at two different magnifications. (A-B): *G. olivieri*; C-D: *G. pyreniaca*; e (E-F): *G. septemfida*.

Primary sculpturing of seeds (sculpting of inner periclinal walls) is divided into two groups. In fact, both of them result from differences in reticulated seed coat.

- Irregularly Striate Type: surface of testa is in the form of striations which are covered with longitudinal lines with excavations. Lumen voids are irregular and narrow. Like in *G. cruciata* and *G. pyreniaca* samples, the inner part of the cell is covered with a secondary sculpture having a smooth structure (Figure 1E, F; Figures 2C, D).
- Shallowly Reticulate Type: when compared to the surface of testa mentioned above, it is isodiametric or reticulate due to polygonal cells with thickened walls: *G. lutea*, *G. asclepidae*, *G. olivieri*, *G. septemfida*, *G. boissieri*, *G. gelida*, *G. verna* subsp. *balcanica* and subsp. *pontica*, *G. brachyphylla* subsp. *favrati* (Figure 1A, B, C, D; Figure 2A, B, E, F; Figure 3A, B, C, D, E, F; Figure 4C, D).

In *G. boissieri*, *G. gelida* and *G. olivieri*, secondary sculpture is also covered with structures that are called pits (Figures 3B, D; Figure 2B). *G. olivieri* and *G. gelida* species are covered with a denser pits than *G. boissieri.* Among those with straight outer periclinal wall, *G. brachyphylla* subsp. *favrati*, *G. septemfida* and *G. verna* species lack secondary decorations (Figures 4D, B; Figure 2F; Figure 3F). Only *G. lutea* subsp. *symphyandra* has transverse lines inside the cell, while *G. asclepidae* is reticulate (Figure 1B, D).

Characters of testa cells of borders

- Species With Straight Anticlinal Walls: *G. lutea* subsp. symphyandra, *G. asclepidae*, *G. cruciata*, *G. pyreniaca*, *G. septemfida*, *G. verna* subsp. *balcanica* and subsp. *pontica*, *G. brachyphylla* subsp. *favrati* (Figure 1B, D, F; Figure 2D, F; Figure 3F; Figure 4B, D).

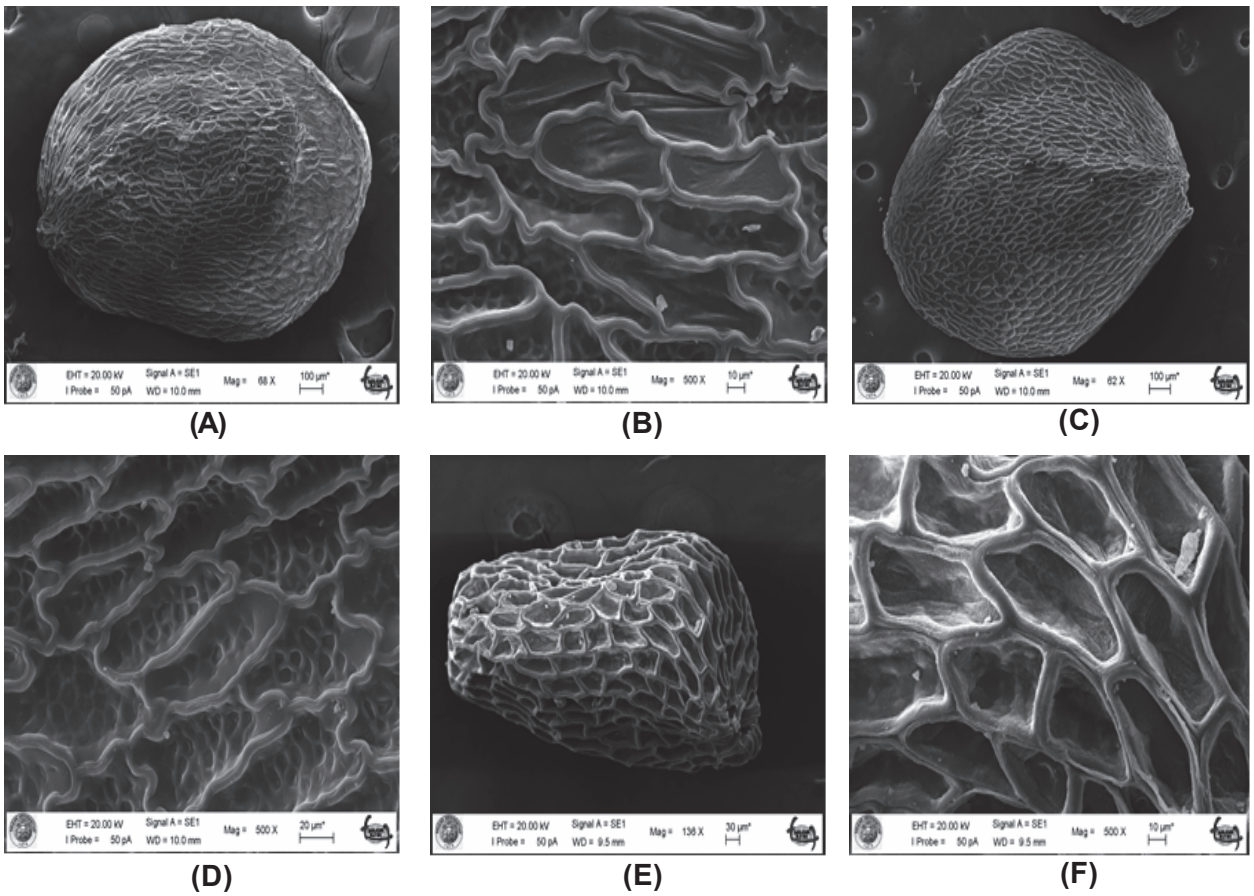

Figure 3 - SEM of seed surface of *Gentiana* at two different magnifications. (A-B): *G. boissieri*; (C D): *G. gelida*; (E-F): *G. verna* subsp. *balcanica*.

- Species With Curved Anticlinal Walls: *G. olivieri*, *G. boissieri*, *G. gelida* (Figure 2B; Figures 3B, D).

Seed and testa cell size, wing cell size, thickness of testa wall: seed size and shape is presented in Table 2. Measurements of testa cell size, wing cell size, thickness of testa wall are presented in Table 3.

Seed shape: *G. lutea* subsp. *symphyandra*, *G. boissieri*, *G. gelida*: obovate flattened seed, *G. asclepidae*: broadly obovate seed, *G. septemfida*, *G. verna* subsp. *balcanica* and subsp. *pontica*, *G. pyreniaca*: narrowly obovate seed, *G. cruciata*: oblong seed, *G. olivieri*: globose seed, *G. brachyphylla* subsp. *favrati*: obovate seed (Table 2, Figures 1-4).

Detailed information has been presented about seed type, seed surface morphology, seed shape and size and in species with wings, information has been presented about testa cell including the cells in wings and wall thicknesses of some perennial Gentianas collected from Turkey.

Analyses conducted under light microscopy showed that seed size differed in all *Gentiana* species. *G. lutea* subsp. *symphyandra* had the largest seeds 4.20±0.21(3.5-4.9) mm, X4.40±0.24(3.4-4.8) mm, *G. cruciata* and *G. olivieri* had the smallest seeds 0.67±0.12(0.5-1.25) mm, X0.60±0.07(0.5-0.75) mm and 0.67±0.12(0.5-0.9) mm, X0.67±0.12(0.5-0.9) mm respectively.

Seed colour changes from light brown to dark brown in *G. pyreniaca*, *G. septemfida*, *G. brachphylla* subsp. *favrati*. It is brown with pale margin in *G. lutea* subsp. *symphyandra*, *G. asclepiadea*, *G. boissieri* and *G. gelida*. *G. cruciata*, *G. olivieri*, *G. verna* subsp. *balcanica* and subsp. *pontica* are reddish brown in colour (Table 2). Researchers have made different classifications about seeds. Some authors questioned phylogenetic relationships using

Table 2 - Comparison of seed size, shape and color of *Gentiana* species in Turkey

Species	Length (mm)			Width (mm)			Shape	Colour
	M	±S	V	M	±S	V		
G. lutea subsp. *symphyandra*	4.20	±0.21	3.5-4.9	4.40	±0.24	3.4- 4.8	obovate flattened	brown with pale margin
G. asclepidae	1.29	±0.25	0.9-1.8	1.45	±0.31	1.0-2.2	broadly obovate	brown with pale margin
G. cruciata	0.67	±0.12	0.5-1.25	0.60	±0.07	0.5-0.75	oblong	reddish brown
G. olivieri	0.67	±0.12	0.5-0.9	0.67	±0.12	0.5-0.9	spheroid	reddish brown
G. pyreniaca	1.80	±0.21	1.5-2.2	0.53	±0.14	0.35-0.8	narrowly obovate	dark-to light brown
G. septemfida	1.61	±0.33	1.2-2.1	0.70	±0.12	0.5-0.9	narrowly obovate	dark to light brown
G. boissieri	1.60	±0.20	1.3-2.1	1.8	±0.21	1.5-2.2	obovate flattened	brown with pale margin
G. gelida	1.05	±0.11	1.0-1.4	1.54	±0.29	1.1-1.0	obovate flattened	brown with pale margin
G. verna subsp. *balcanica*	1.0	±0.11	0.8-1.2	0.69	±0.09	0.5-0.8	narrowly obovate	reddish brown
G. verna subsp. *pontica*	0.98	±0.09	0.8-1.1	0.56	±0.07	0.45-0.65	narrowly obovate	reddish brown
G.brachyphylla subsp. *favrati*	1.04	±0.09	0.9-1.2	0.62	±0.08	0.5-0.7	obovate spheroid	dark brown

M: Mean; S: Standard Deviation; V: Variation.

characteristics such as shape and curvature of the cell borders of testa cells in addition to inner and outer periclinal walls of testa while dividing the groups (Bouman et al., 2002; Davitashvili and Karrer, 2006; Whitlock et al., 2010). While evaluating relationships in genus *Gentiana*, seed size and presence of wings began to be used as a useful taxonomic characteristic in addition to this rich structure. In some *Gentiana* testa, the edges were observed to flatten and have a wing-like structure (Bouman et al., 2002) or contain protrusions that are called membranous lamellar (Davitashvili and Karrer, 2006). In this study, although this type of papillae seed-coat structure is not observed, some seeds flattened at the edges and developed wing-like structures, while some of them carried no wings.

In the discussion section, each characteristic will be separately analyzed. According to the outer periclinal walls of testa; Davitashvili and Karrer (2006) studied *G. boissieri* from the isotype collected from Bulgar Mountain-Turkey; *G. gelida* and *G. septemfida* from samples collected from Georgia. In a previous study, Kusnezow (1895) and Ho and Liu (2001) described *G. boissieri* as winged in *G. gelida* and as with side wing in *G. septemfida*. Davitashvili and Karrer (2006, 2010) used the term semi-winged seed for *G. gelida* and the term incomplete discoid wing for *G. asclepidae* subsp. *schistocalyx* while making the same identification for other two species. In addition, based on the borders of testa cells, they observed that *G. boissieri*, *G. gelida* and *G. asclepidae* subsp. *schistocalyx* were curved, while *G. septemfida* was straight. In light of these studies, *G. boissieri*, *G. gelida* species were evaluated as complete discoid wing; *G. septemfida* species were evaluated as chalazal wing, *G. asclepidae* seeds were evaluated as incomplete discoid wing.

According to sculpting of inner periclinal walls, the species was classified into two groups (irregularly striate and shallowly reticulate type), which results from the difference in reticulated seed coat. In *G. cruciata* and *G. pyreniaca*, surface of testa appears as striations, which are covered with longitudinal lines with excavations. Lumen voids are irregular and narrow. The inner of the cell is covered with a secondary sculpture having a smooth structure. Comparison of *G. lutea* subsp. *symphyandra*, *G. asclepidae*, *G. olivieri*, *G. septemfida*, *G. boissieri*, *G. gelida*, *G. verna* subsp. *balcanica* and subsp. *pontica*, *G. brachyphylla* subsp. *favrati* with the type mentioned above reveals that it seems reticulate due to isodiametric or polygonal cells with thickened walls (Figure 1B, D; Figure 2B, F; Figure 3B, D, F; Figure 4B, D). In *G. boissieri*, *G. gelida* and *G. olivieri* secondary

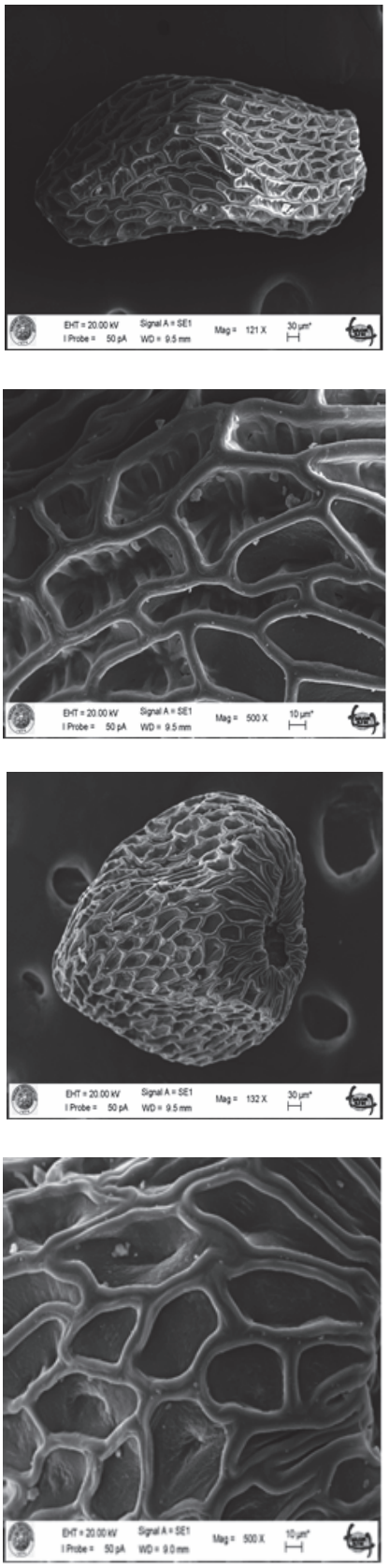

Figure 4 - SEM of seed surface of *Gentiana* at two different magnifications. (A-B): *G. verna* subsp. *pontica*; (C-D): *G. brachyphylla* subsp. *favrati.*

sculpture is covered with structures that are called pits (Figures 3B, D; Figures 2B). *G. olivieri* and *G. gelida* species are more densely covered with pits when compared to *G. boissieri*. Among the species with straight outer precilinal wall, *G. brachyphylla* subsp. *favrati*, *G. septemfida* and *G. verna* species do not have secondary decorations (Figures 4D, B; Figure 2F; Figure 3F). Only the inside of *G. lutea* has transversal lines, while *G. asclepidae* is reticulate (Figure 1B, D).

According to testa cells of borders; *G. lutea* subsp. symphyandra, *G. asclepidae*, *G. cruciata*, *G. pyreniaca*, *G. septemfida*, *G. verna* subsp. *balcanica* and subsp. *pontica*, *G. brachyphylla* subsp. *favrati* have straight anticlinal walls (Figures 1B, D, F; Figure 2D, F; Figure 3F; Figures 4B, F).

G. olivieri, *G. boissieri*, *G. gelida* have curved anticlinal walls (Figure 2B; Figures 3B, D). According to Davitashvili and Karrer (2010), the shape of testa cells and straight or curved structure of borders are of taxonomic importance that will be used in phylogenetic analysis. The findings of Davitashvili and Karrer (2006) revealing that *G. boissieri*, *G. gelida* has a curved structure, while *G. septemfida* has a straight structure, is consistent with our study. Testa cell has an elongated shape as reported by Davitashvili and Karrer (2010) in all of our species.

As to seed and testa cell size: while Flora of Turkey (Pritchard, 1978; Davis et al., 1988) provides information on capsule characteristics of some species of genus *Gentiana*, it provides no information on seed size or shape of any species. In our study, mean seed length varied between 0.67-4.20 mm, and seed width varied between 0.53-4.40 mm, respectively. *G. lutea* had the largest seeds (mean 4.20X4.40 mm), while *G. cruciata* and *G. olivieri* had the smallest seeds (0.5-1.25X0.5-0.75 mm and 0.5-0.9X0.5-0.9 mm, respectively) (Table 2). Mean cell size varied between 60X40 µm-150X23.3 µm in *G. septemfida* and *G. cruciata*. Testa cell size in wings in all species was larger than that in testa body. While mean wing cell was 180X96 µm in *G. Asclepiadea*, *G. brachphylla* subsp. *favrati* had the smallest wing cell (76.8X40.6 µm). *G. cruciata* had the largest testa wall thickness (20.5 µm) while *G. asclepiadea* had the smallest wall thickness (6.3 µm) (Table 3). Testa cell size was measured and used in comparisons in *Gentianopsis* seeds (Whitlock et al., 2010). The first comprehensive

Table 3 - Comparison of testa cell and wing size, thickness of testa wall of *Gentiana* species

Species	Testa cell (µm)						Testa cell of wing (µm)						Thickness of testa wall (µm)		
	Length			Widht			Length			Widht					
	M	±S	V	M	±S	V	M	±S	V	M	±S	V	M	±S	V
G. lutea	109	±0.99	130-160	33.5	±5.30	26-40	170.0	±1.4	120-320	92	±0.4	80-120	10.0	±1.73	8-12
G. asclepidae	126	±3.80	70-170	40.5	±1.00	30-60	180.0	±1.6	100-240	96	±1.2	50-140	6.3	±1.49	5-80
G.cruciata	150	±4.30	90-200	23.3	±0.80	15-40	-	-	-	-	-	-	20.5	±1.50	16-28
G.olivieri	97.6	±5.00	76-128	56.0	±1.10	32-80	-	-	-	-	-	-	10.3	±0.31	4-12
G. septemfida	60	±3.50	50-110	40.0	±0.83	30-50	-	-	-	-	-	-	8.4	±0.84	6-10
G.boissieri	67.5	±0.80	70-130	42.0	±0.57	30-50	106.0	±2.9	60-170	43.5	±0.08	30-60	7.1	±0.77	5-10
G.gelida	88	±1.81	60-120	23.0	±0.43	20-30	111.0	±2.4	70-150	30.8	±0.7	30-40	7.2	±0.71	6-10
G. verna subsp. *balcanica*	70	±1.20	50-90	35.0	±1.10	25-100	-	-	-	-	-	-	11.3	±0.07	6-14
G .verna subsp. *pontica*	66.6	±4.12	42-102	43.0	±1.90	18-60	-	-	-	-	-	-	12.1	±0.9	6-16
G. brachy-phylla subsp. favrati	70.8	±3.30	42-102	57.0	±2.10	42-78	76.8	±2.7	54-96	40.6	±1.6	30-60	12.4	±0.89	5-16

M: Mean; S: Standard Deviation; V: Variation.

measurements on *Gentiana* (test cell, wing cell length and width, testa wall thickness) were carried out by the authors of this paper.

As to seed shape: Seed shape and size have also been used as a distinctive characteristic by all authors. In our study, *G. lutea* subsp. *symphyandra*, *G. asclepidae*, *G. boissieri*, *G. gelida* species, which we term as obovate flattened and broadly obovate seeds, have also been termed as discoid wing by some authors. Werker (1997) explains this as wrapping of the embryo by testa cells, which have the form of a thin and straight membrane. Whitlock et al. (2010) studied *Gentianopsis* and unlike the structure mentioned above, they reported that sometimes the image of elongated inflated testa cell like wing can also cause confusion in classification. Davitashvili and Karrer (2010) termed the wrapping of single thin, flattened bicellular testa cell of the seed as "wing" and the other different structures as "membranous lamellae". The author termed *G. asclepidae* subsp. *schistocalyx* as broadly obovate; *G. septemfida* and *G. gelida* seeds as obovate flattened and *G. septemfida* as narrowly obovate. These terms were accepted by the authors of this study.

According to Flora of Turkey (1978), "*G. boissieri* may well be a local derivative of *G. septemfida* restricted to the Tauros and isolated geographically". In this respect, it can be seen that seed size and outer periclinal walls are much different from each other. Primary sculpture of seeds is shallowly reticulate in both of them. However, secondary sculpture is pitted in *G. boissieri* and is reticulate in *G. septemfida*. Testa cell of border have no similarity.

Both sub-species of *G. verna* do not contain wings. Seed shape is narrowly obovate in both of them. They are similar in terms of primary and secondary sculpture. Seed measurements are very similar to each other. 1.0±0.11(0.8-1.2) mmX0.69±0.09(0.5-0.8) mm in subsp. *balcanica* and 0.98±0.09(0.8-1.1) mmX0.56± 0.07(0.45-0.65) mm in subsp. *pontica* (Table 2). Testa cell measurement is higher in subsp. *pontica* with a border thickness of 12.1±0.9(6-16) µm (Table 3).

G. brachyphylla subsp. *favrati* is in fact like a small form of *G. verna* in terms of morphology. However, seed shape is totally different: it is obovate-spheroid. Although *G. verna* has no wings, the other one has incomplete discoid wing. Primary and secondary sculptures are quite different between the two species.

Seed characters had been neglected in flora of Turkey and even in taxononomic research. However, the potential taxonomic value of seed coat microsculpture has been demonstrated in this study. Hence, seed morphology (outer periclinal walls of testa, sculpting of inner periclinal walls, seed shape, seed and testa cell, wing cell size, thickness of testa wall and seed shape) can be used together with morphological character to form classifications in studied specimens for *Gentiana* genus.

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