Australian Journal of Crop Science AJCS
AJCS 5(6):660-667 (2011) ISSN:1835-2707

Scanning electron microscope (SEM) and light microscope (LM) studies on the seed morphology of *Verbascum* taxa (Scrophulariaceae) and their systematic implications

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Abstract

Verbascum L. is the largest genus of the family Scrophulariceae and includes several species are of medicinal importance. In this study, external seed morphologies of 30 taxa included in Group A belonging to genus *Verbascum* L. (Scrophulariaceae) in Turkey, have been investigated using SEM (Scanning Electron Microscopy) and LM (Light Microscopy). Light microscope observations showed that seed size ranges from 0.3 mm to 1.5 mm in length and 0.1 to 0.6 in width. Seeds vary from oblong, oblong prismatic, trigonious prismatic, prismatic, ovoid to eliptic in shape among the studied species. The seeds end to an acute, obtuse, truncate or rounded beak. The seed coat is transversal ridged or alveolate. The results of this study showed that considerable taxonomic insight can be gained from study of seed characters of *Verbascum*, especially at the species level. The variation in seed morphology is manifested mainly in shape, size, colour, seed coat ornamentation. To make a conclusion about the delimitation under the genus *Verbascum* based on the seed characteristics, all taxa included in the genus should be studied comparatively.

Keywords: Micromorphology, Seed morphologies, Turkey, *Verbascum,* SEM., LM., Scrophulariceae.
Abbreviations: SEM, Scanning Electron Microscope; LM, Light Microscopy; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Sect., section; subsp., subspecies; var., variety.

Introduction

The genus *Verbascum* L. belonging to tribe *Verbasceae* (Scrophulariaceae), comprises about 360 species worldwide. (Heywood, 1993). In Turkey, it is represented with 243 species which is divided into 13 artificial groups, with 129 additional hybrids (Huber-Morath, 1978; Davis et al., 1988; Vural and Aydoğdu, 1993; Sutorý, 2001, 2004; Karavelioğulları et al., 2004, 2006, 2008, 2009; Karavelioğulları and Aytaç, 2008; Kaynak et al., 2006; Özhatay, 2006; Parolly and Tan, 2007; Parolly and Eren, 2008; Yılmaz and Dane, 2008). Some members of this genus have been commonly used for their medicinal effects in traditional medicine. It is known that flowers of some species have mucolytic and expectorant effects. Leaves have been used as diuretic, sudorific, expectorant, sedative and constipate and seeds of *Verbascum* species (i.e *V. cheiranthifolium* Boiss.) are used for fishing because of their saponin contents which are toxic to fish (Baytop 1999; Akdemir et al., 2003; Khoshnoud et al., 2008). Many species in the genus i.e. *V. thapsus* L., *V. fruticulosum* Post. and *V. undulatum* Lam., *V. cheiranthifolium, V. georgicum* Benth., *Verbascum chionophyllum Hub.-Mor., V. cilicicum Boiss., V. pterocalycinum var. mutense* Hub.-Mor., *V. pycnostachyum* Boiss. & Helder., *V. splendidum* Boiss., *and V. sinuatum* L. have been investigated for their antibacterial, antifungal, antiviral, antimalarial and insectidal activities through in-vitro and in-vivo tests (Dülger et al., 2002; Akdemir et al., 2003; Şengül et al., 2005; Khoshnoud et al., 2008; Şener and Dülger, 2009). Huber-Morath (1978) divided the genus into two main groups based on stamen numbers. Species having 4 stamens and stalked bifit placentation are diverged from the others and placed in Group A. The group A comprises 45 taxa worldwide. (Huber-Morath, 1981; Meikle, 1985). The genus *Verbascum* is represented with two sections namely sect. *Aulacospermae* Murb. and sect. *Bothrospermae* Murb. world wide. The main differences among these sections are related to the seed morphology of the members. In sect. *Aulacospermae,* seeds are longitudinal ridged and sect. *Bothrospermae* has members which have transversal ridged alveolate seeds. All taxa distributed in Turkey belong to section *Bothrospermae* Murb. (Murbeck, 1925, 1933; Huber-Morath, 1971). Hartl (1959) classified the seed surface among the genera in Scrophulariaceae family into two types based on detailed seed anatomies,"*Scrophularia* type" including genera *Scrophularia*, *Verbascum*, *Celsia* L. and some *Sutera* Roth, and "*Torenia* type" including *Torenia* L., *Vandellia* P. Brown ex. L., *Tetranema* Benth. and several *Russelia* Jacq. species. The wide variation in seed morphology of the family Scrophulariaceae provides the information about the taxonomic status of the members of this family. There are many SEM studies based on micromorphological features of seeds, capsules and their indumentum characteristics in various genera of Scrophulariaceae (Canne, 1979, 1980; Chuang and Heckard, 1972; Elisens and Tomb, 1983; Sutton, 1988; Juan et al.,

Table 1. List of studied taxa and some important features of their seeds

	Species	Seed size (mm)	Seed colour	Seed shape	Seed surface
1	*V. natolicum* (Fisch. & Mey.) Hub.-Mor	0.7 x 0.3	Dark brown	Prismatic, with numerous linear and deep ridges, apiculate beak	Irregular polygonal cells, with densely and distinct vesicles
2	*V. spodiotricum* (Hub.-Mor.) Hub.-Mor.	0.3 x 0.1	Dark brown	Prismatic, with ±shallow alveolae, apiculate beak	Irregular polygonal cells, with densely and distinct vesicles
3a	*V. orientale* (L.) All. subsp. *orientale*	1.3 x 0.5	Black	Elliptic-oblong, with numerous linear and deep and wide ridges	Irregular polygonal cells, with densely and distinct vesicles
3b	*V. orientale* (L.) All. subsp. *brachysepalum* Karavel. & Aytaç	1.5 x 0.7	Black	Trigonious-prismatic, with numerous linear and deep ridges, acute to obtuse beak	Irregular, exserted polygonal cells, with densely and distinct vesicles
4	*V. cilicium* (Boiss. &Heldr.) O. Kuntze	0.6 x 0.4	Dark brown	Trigonius-prismatic with apiculate beak	Irregular polygonal cells, with densely and distinct vesicles
5a	*V. trapifolium* (Stapf) Hub.-Mor. var. *trapifolium*	0.7 x 0.3	Dark brown	Oblong-prismatic, with wide and deep alveolae and rounded beak	Irregular polygonal cells, with indistinct vesicles
5b	*V. trapifolium* (Stapf) Hub.-Mor. var. *flabellifolium* (Hub.-Mor.) Karavel. & Aytaç	0.7 x 0.3	Dark brown	Oblong-prismatic with linear deep and wide ridges and obtuse beak	Regular elongated polygonal cells, with densely and distinct vesicles
6a	*V. pyroliforme* (Boiss.& Heldr.) O. Kuntze subsp. *pyroliforme*	0.7 x 0.3	Dark brown	Oblong, with ± shallow alveolae and apiculate beak	Large regular polygonal cells, with densely and distinct vesicles
6b	*V. pyroliforme* (Boiss. & Heldr.) O. Kuntze subsp. *dudleyanum* (Hub.-Mor.) Karavel. &Aytaç	0.6 x 0.3	Dark brown	Oblong prismatic, with ±shallow alveolae and apiculate beak	Large regular polygonal cells, with densely and distinct vesicles
7	*V. coronopifolium* (Boiss.&Bal.) O. Kuntze	0.6 x 0.4	Dark brown	Trigonious-prismatic, with alveolae and ridges, apiculate beak	Irregular polygonal cells, with densely and distinct vesicles
8	*V. serratifolium* (Hub.-Mor.) Hub.-Mor	0.7 x 0.3	Dark brown	Prismatic, with ±shallow alveolae, apiculate beak	Small rectangular cells, with regular vesicles
9	*V. basivelatum* Hub.-Mor.	0.8 x 0.3	Black	Oblong, with ±shallow alveolae, rounded beak	Irregular polygonal cells, with densely and distinct vesicles
10	*V. bourgeauanum* Hub.-Mor.	0.6 x 0.4	Dark brown	Prismatic with numerous linear and deep and wide ridges	Irregular polygonal cells, with densely and distinct vesicles
11	*V. serpenticola* Hub.-Mor.	0.6 x 0.4	Dark brown	Prismatic, with numerous linear and deep ridges, apiculate beak	Irregular polygonal cells, with densely and distinct vesicles
12	*V. sorgerae* (Hub.-Mor.) Hub.-Mor.	0.7 x 0.4	Black	Prismatic, with ± shallow alveolae, apiculate beak	Irregular polygonal cells, with densely and distinct vesicles
13	*V. nudicaule* (Wydler) Takht.	0.9 x 0.5	Black	Broadly elliptic, with numerous linear and moderately deep ridges, rounded beak	Irregular polygonal cells, with densely and distinct vesicles
14a	*V. suworowianum* (C.Koch) O. Kuntze var. *suworowianum*	0.5 x 0.3	Black	Prismatic, with numerous linear and deep ridges, apiculate beak	Polygonal cells, with indistinct vesicles
14b	*V. suworowianum* (C.Koch) O. Kuntze var. *papillosum* (Murb.) Hub.-Mor.	0.5 x 0.3	Black	Oblong, with indistinct ±shallow alveolae, rounded beak	Partially reticulate

1994, 1997, 2000). Juan et al. (1997) studied the micromorphology of seed and fruits of ten *Verbascum* species growing in Spain and reported that capsule indumentum is the most useful character grouping the species under the genus. Attar et al (2007) investigated the micromorphological features of seeds and capsule surface of more than 22 species distributed in Iran. He emphasized that while the micromorphological features of the seeds of the genus *Verbascum* possess value for discriminating the taxa especially species level, they are not useful for infrageneric classification in the genus. The most recent research study on the micromorphology and anatomy of eight species of *Verbascum* found in West Azerbaijan was conducted by Kheiri et al. (2009). They concluded that some characters related to the seeds such as seed shape and structures of alveoli and ridges shows variability and do not possess value for delimitating the taxa. With respect to species richness of the genus in the region, Turkey seems to be a major center for *Verbascum*. The aim of the present study is to assess the potential systematic value of seed morphology of *Verbascum* species. The seed morphologies of 25 taxa have been reported for the first time with this study.

Materials and methods

Seeds samples of 30 taxa used in this study were gathered from their natural habitats in Turkey during the revision of *Verbascum* Group A (Karavelioğulları and Aytaç, 2008). Voucher specimens were deposited at different main herbaria such as GAZI, ANK, EGE, HUB, ISTE, G, HbYILDIRIMLI, (herbarium abbreviations were given according to Holmgren et al., 1990) in Turkey. Only mature and dry seeds were examined with a low power stereo microscope and the most

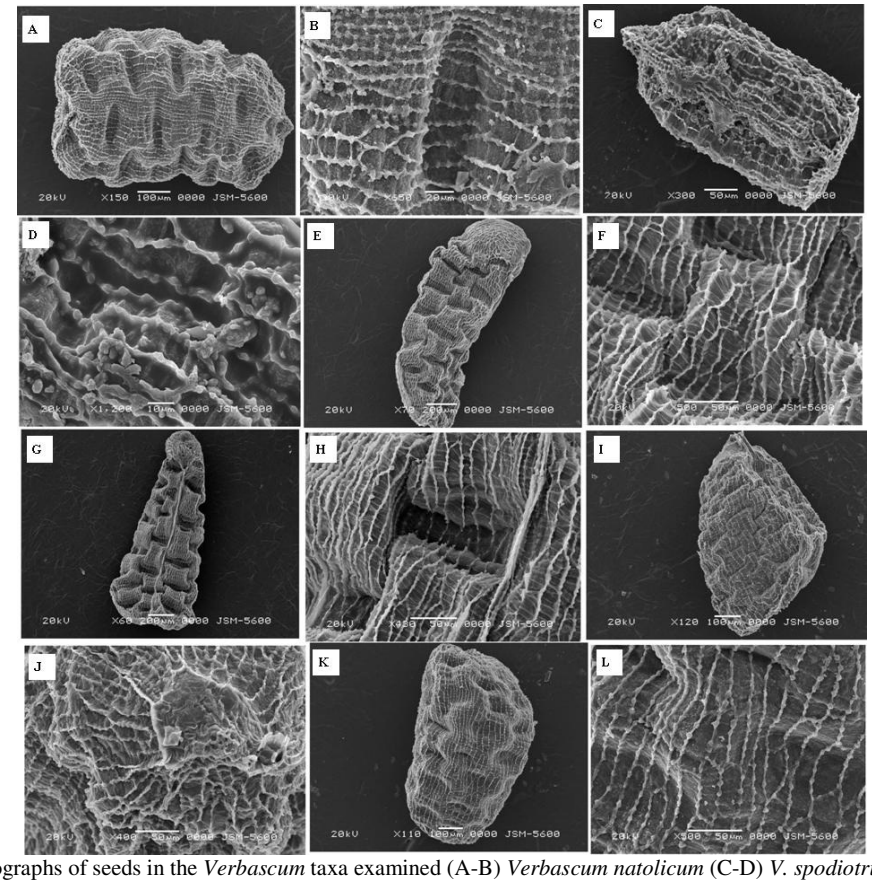

Fig 1. SEM micrographs of seeds in the *Verbascum* taxa examined (A-B) *Verbascum natolicum* (C-D) *V. spodiotricum* (E-F) *V. orientale* subsp. *orientale* (G-H) *V. orientale* subsp. *brachysepalum* (I-J) *V. cilicium* (K-L) *V. trapifolium* var. *flabellifolium.* (A, C, E, G, I, K) General appearance of seeds, (B, D, F, H, J, L) Seed surface of seeds.

important characters described for each taxon. SEM studies were conducted by direct mounting of seed samples on stubs attached with sticky tape. The specimens were coated in a sputter coater with Gold-Palladium using Polaron SC 502 trade gold coater. The specimens were then studied and photographed by a Jeol JSM-840. The terminology used for describing the seed coat features follows Juan et al. (1997) and Attar et al. (2007).

Results and discussion

In the following, we describe various aspects of seed structure that we found in our study. Morphological characters including seed sizes, shapes, colour and surface characteristics are summarized in Table 1. All the species studied were illustrated.

Seed size

Results show that the dimensions of seeds are variable among the species. Seed size ranges from 0.3 to 1.5 mm in length and 0.1 to 0.6 mm in width. Seeds can be divided into three groups on the basis of seed sizes (Table 1): (1) longer than 1 mm in length, (2) between 0.5 to 1 mm in length and (3) small ones, up to 0.5 mm in length. Among the studied species *V. orientale* subsp. *brachysepalum* (1.5 x 0.7), *V. orientale* subsp. *orientale* (1.3 x 0.5), *V. sinuatum* subsp. *gaillardotii* (1.3 x 0.5) and *V. bornmuellerianum* (1.1 x 0.6) have the biggest seeds, while species such as *V. spodiotricum* (0.3 x 0.1), *V. luciliae* (0.3 x 0.1), *V. rupicola* (0.3x0.1), *V. agrimoniifolium* subsp. *agrimoniifolium* (0.3 x 0.1), have the smallest ones. The remaining taxa possess intermediate seeds. Although Attar et al. (2007) indicated that the size of the seeds show variations among the different populations of the same species even different seeds of the same capsule, the observed variation pattern in sizes is limited among the species. Our measurements are congruent with those of the common species in the studies of Attar et al. (2007) and Kheiri et al. (2009). In our study, *V. orientale* has the longest seeds while *V. agrimonifolium* have very small ones as the same as in the study of Attar et al. (2007).

Seed shape

The shape of seeds varies sufficiently in the genus *Verbascum* to allow a distinction to be made among the species as well as subtaxa under the species. Prismatic, prismatic oblong, trigonious prismatic, oblong, elliptic oblong, ovate are the shapes of seeds observed among the studied species (Table 1). Typically most of the taxa such as *Verbascum natolicum* (Fig. 1A), *V. spodiotrichum* (Fig. 1C), *V.trapifolium* var. *trapifolium* (Fig. 2A), *V. serratifolium* (Fig. 2I), *V. bourgeauanum, V. sorgerae* (Fig. 3E), *V. suworowianum*var. *suworowianum* (Fig. 3I), *V. luciliae* (Fig. 4A), *V. agrimoniifolium* subsp. *agrimoniifolium* (Fig. 4E), *V. bugulifolium* (Fig. 4I) have prismatic seed. Trigonious-prismatic or pyramidal seeds are observed in only three taxa: *V. orientale* subsp. *brachysepalum* (Fig. 1G), *V. cilicium* (Fig. 1I), *V. coronopifolium* (Fig. 2G). Oblong seeds are seen in *V. sinuatum* subsp. *gaillardotii* (Fig. 5G), *V. oreophyllum*

Table 2. Comparative seed shape information of common species studied until now

Taxa	Attar et al. 2007	Kheiri et al 2009	Our study
V. agrimonifolium	Prismatic with apiculate beak	Prismatic with obtuse beak	Prismatic with apiculate beak
V. cherianthifolium	Ovate prismatic with obtuse beak	Oblong with rounded beak	----------
V. macrocarpum	Prismatic oblong with rounded truncate beak	Prismatic oblong with truncate beak	----------
V. nudicaule	Prismatic with rounded beak	----------	Broadly elliptic with rounded beak
V. oreophilum	----------	Trigonous with obtuse beak	Oblong prismatic with rounded beak
V. orientale	Trigonous-prismatic with acute beak	----------	Elliptic oblong or trigonious-prismatic with acute to obtuse beak
V. sinuatum	Prismatic with rounded beak	Prismatic with rounded beak	----------
V. speciosum	Ovate with rounded beak	Trigonious with obtuse beak	----------
V. suworowianum	Prismatic with apiculate beak	----------	Prismatic or oblong with apiculate or rounded beak
V. szowitsianum	Oblong-prismatic with obtuse beak	Oblong- prismatic with apiculate beak	----------

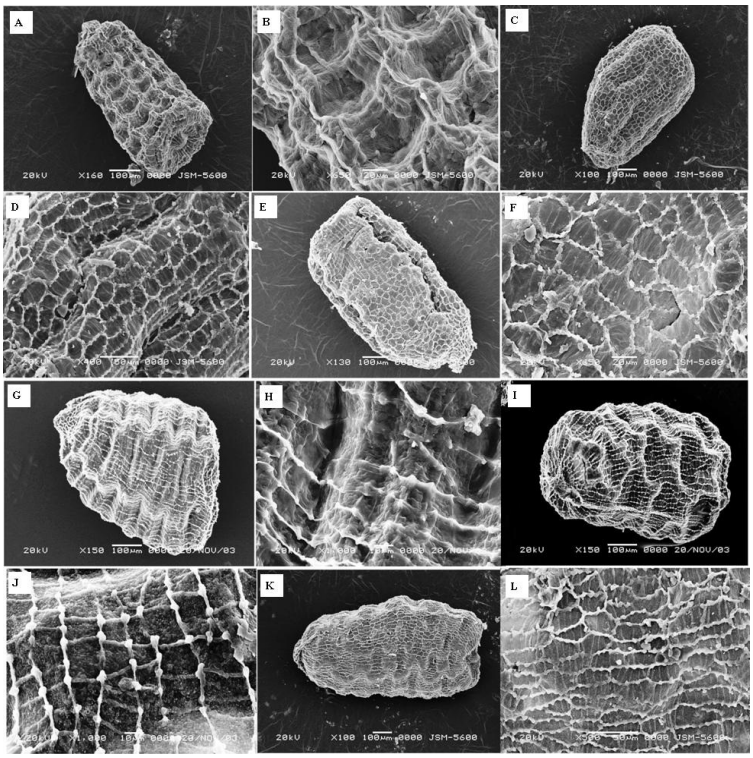

Fig 2. SEM micrographs of seeds in the *Verbascum* taxa examined (A-B) *V. trapifolium* var. *trapifolium* (C-D) *V. pyroliforme* subsp. *pyroliforme* (E-F). *V. pyroliforme* subsp. *dudleyanum* (G-H) *V. coronopifolium* (I-J) *V. serratifolium* (K-L) *V. basivelatum.* (A, C, E, G, I, K) General appearance of seeds, (B, D, F, H, J, L) Seed surface of seeds.

var. *oreophyllum* (Fig. 5C), *V.oreophyllum*var. *joannis* (Fig. 5E), *V. suworowianum* var. *papillosum* (Fig. 3K), *V. serpenticola* (Fig. 3C), *V. pyroliforme* subsp. *pyroliforme* (Fig. 2C), *V. trapifolium* var. *flabellifolium* (Fig. 1K). Eliptic oblong seeds are seen in *V. orientale* subsp. *orientale* (Fig. 1E) and *V. basivelatum* (Fig. 2K). *V. nudicaule* (Fig. 3G) is the only species which has ovate seeds and *V. rupicola* (Fig. 4C) has the cube shape seeds. Our results regarding seed shape are more or less consistent with the results of Attar et al. (2007) and Kheiri et al. (2009). The results showed that the seed shape seems to be specific to species as well as subspecies or variety. In the study of Attar et al. (2007), the specimens were indicated at the species level. Interestingly, the seeds of *V. orientale* differ at the subspecies level or two varieties of *V. suworowianum* have different seed shape. Attar et al. (2007) claimed that the overall shape and size of seeds among different specimens of one species and even different seeds of the same capsule is variable and cannot be used for separation of the species. Ellisens and Tomb (1983) resulted that this variation is influenced by packing in the capsule as other Scrophulariceae seeds. The results of our study confirmed that seed shape is one of the important characteristics which can be used to delimitate the taxa if the sampling is enough to show the variability. When we compare our results with the published studies, we found that seed shape characteristics may vary more or less among the taxa (Table 2).

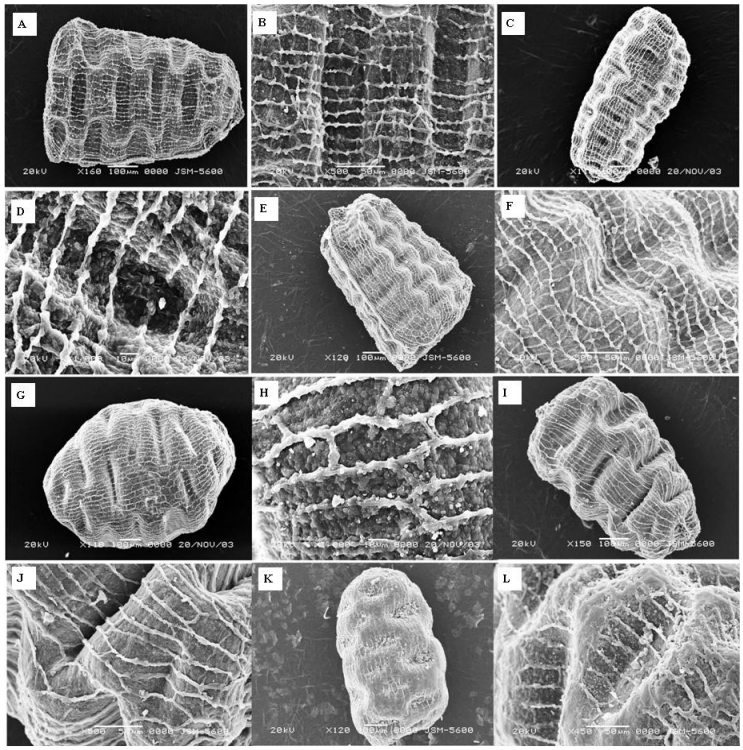

Fig 3. SEM micrographs of seeds in the *Verbascum* taxa examined (A-B) *V. bourgeauanum* (C-D) *V. serpenticola* (E-F) *V. sorgerae* (G-H) *V. nudicaule* (I-J) *V. suworowianum* var. *suworowianum* (K-L) *V. suworowianum* var. *papillosum* (A, C, E, G, I, K) General appearance of seeds, (B, D, F, H, J, L) Seed surface of seeds.

Apex of the seeds

The seeds end to an acute (such as *V. agrimoniifolium* subsp. *agrimoniifolium, V. sorgerae*, *V. coronopifolium, V. trapifolium* var. *trapifolium, V. natolicum*) obtuse (*V. pyroliforme* subsp. *dudleyanum*), truncate (such as *V. bugulifolium*, *V. levanticum*) or rounded beak (*V. orientale* subsp. *orientale, V. orientale* subsp. *brachysepalum, V. trapifolium* var. *flabellifolium, V. basivelatum, V. nudicaule*).

Seed colour

In our study two different colours are observed among the studied species; dark brown or black. Juan et al. (1997) also indicated that seeds of ten *Verbascum* species growing in Spain are dark brown and black in colours.

Seed ornamentation

The seed coat is longitudinally ridged or alveolate. In some species such as *Verbascum natolicum, V. orientale* subsp. o*rientale, V. orientale* subsp. *brachysepalum, V. cilicium, V. coronopifolium, V. nudicaule, V. suworowianum* var. s*uworowianum,* the seed coat is ridged and in *V. luciliae, V. agrimoniifolium* subsp. *agrimoniifolium, V. levanticum, V. ponticum V. sorgerae, V.trapifolium* is alveolate. In some species, such as *V. trapifolium* and *V. serratifolium* the alveoli are relatively wide and deep, while they are shallow in *V. basivelatum*, *V. agrimonifolium* and *V. luciliae*. The number of alveoli and ridges is also variable among the species. For example the ridges are densely arranged in the *V. orientale, V. suworowianum* and *V. songaricum.* Surface of seed coat is reticulate because of the net like appearance which is formed by polygonal cells with irregular or regular radial walls. The size of polygonal cells varies among the studied species, but it is almost constant in different populations of a certain species. Regular polygonal cells are present in *V. orientale*, *V. agrimonifolium*, *V. rupicola* while irregular cells are found in *V. suworowianum*, *V. bugulifolium, V. ponticum* The cells are small in size, like *V. orientale*, *V. agrimonifolium*, or large, like *V. bornmuellerianum, V. oreophyllum* var. *oreophyllum, V. oreophyllum* var. *joannis.* In most cases the surface is a net of irregular hexagonal chambers, but in *V. orientale* and *V. agrimonifolium* the chambers are rectangular in shape. The surface of seeds in *V. pyroliforme* subsp. *pyroliforme* is furnished with regular hexagonal chambers. The walls surrounding each cell vary in arrangement of the vesicles on top. The vesicles are arranged densely, e.g. in *Verbascum natolicum, V. trapifolium* var. *flabellifolium*. They are arranged sparsely and regular, e.g. in *V. agrimonifolium*. Morphological classification of the taxa reveal some difficulties as far as their status are concerned. For example, *V. pyroliforme* and *V. dudleyanum* are regarded as different species due to the differences in their habitat features and the number of flowers. Karavelioğulları and Aytaç (2008) re-evaluated the taxonomic status of *V. dudleyanum* as subspecies under *V. pyroliforme* because of the poor morphological differences. Çelebi et al. (2009) studied the relationships of some Turkish *Verbascum* taxa in the group A on the basis of seed proteins and stated that both *V. dudleyanum* and *V. pyroliforme* have the same banding pattern with small genetic distance at 11 %. The seed characteristics of two taxa also support the above mentioned treatments. They both have oblong seeds with ± shallow alveolae and apiculate beak. The surface of seeds is characterized with large regular polygonal cells with prominent vesicles distributed densely on the edges of walls (Fig. 2 C-F) (Table 1). *V. flabellifolium* and *V. trapifolium* are

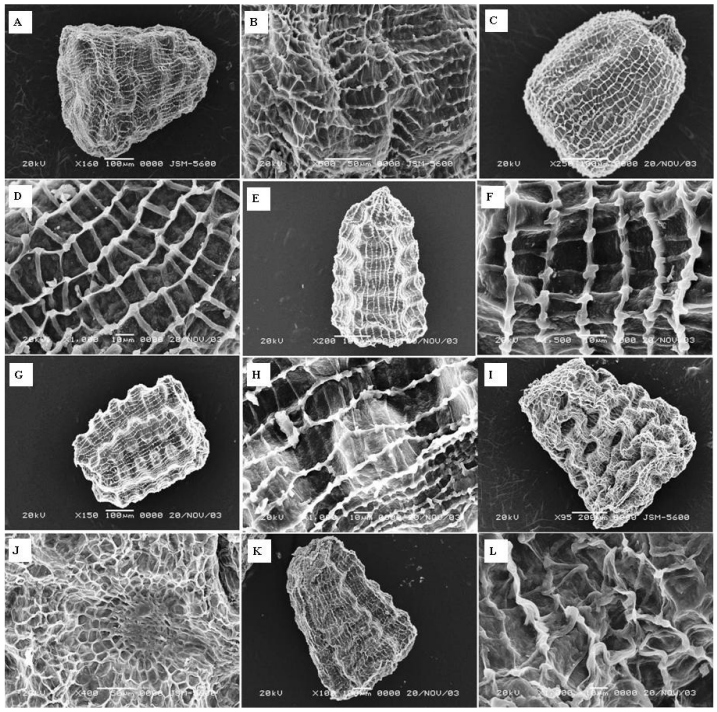

Fig 4. SEM micrographs of seeds in the *Verbascum* taxa examined (A-B) *V. luciliae* (C-D) *V. rupicola* (E-F).*V. agrimoniifolium subsp. agrimoniifolium* (G-H). *V. levanticum* (I-J).*V. bugulifolium* (K-L). *V.ponticum.* (A, C, E, G, I, K) General appearance of seeds, (B, D, F, H, J, L) Seed surface of seeds.

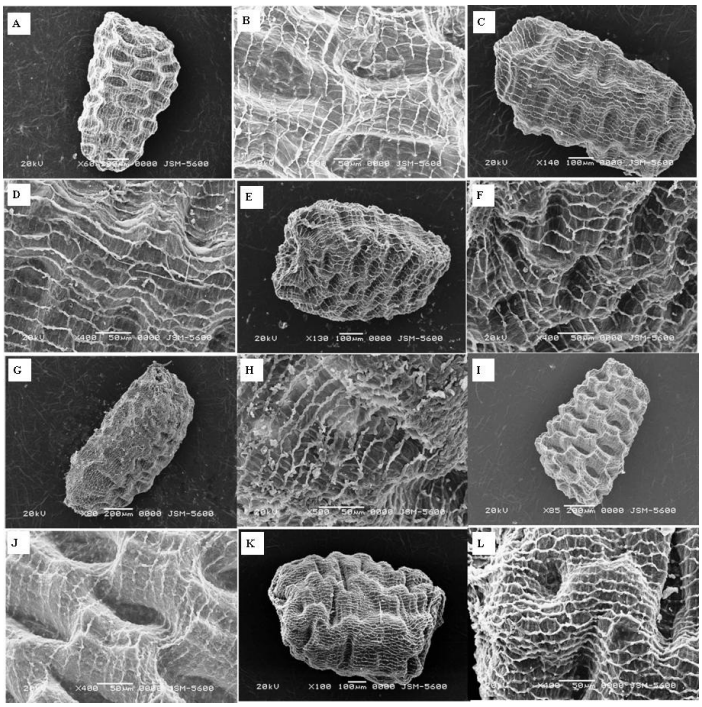

Fig 5. SEM micrographs of seeds in the *Verbascum* taxa examined (A-B) *V. bornmuellerianum* (C-D) *V.oreophilum* var.*oreophilum* (E-F) *V.oreophilum* var. *joannis* (G-H) *V. sinuatum subsp. gaillardotii* (I-J) *V. freynii* (K-L) *V.transcaucasicum.* (A, C, E, G, I, K) General appearance of seeds, (B, D, F, H, J, L) Seed surface of seeds.

morphologically very close to each other. They differ from each other by having glandular hairy basal leaves or not. *V. flabellifolium* was reduced to a variety under *V. trapifolium* by Karavelioğulları and Aytaç (2008). SDS-PAGE of seed proteins of two taxa showed that both taxa have similar banding patterns with 11 % genetic distance between each other (Çelebi et al 2009). In our study we observed that the seeds of these taxa have different features. In *V. trapifolium* the seed coat is alveolate (Fig. 2A) and surface is formed by irregular polygonal cells with indistinct vesicles (Fig. 2B) whereas *V. flabellifolium* have linear deep and wide ridges (Fig. 1K) and seed surface is characterized by regular elongated polygonal cells with dense and distinct vesicles (Fig. 1L; Table 1). *V. orientale* and *V. brachysephalum* are regarded as different species due to the differences in their pedicel lengths. *V. orientale* is one of the commonest species in the genus and has a wide range of distribution from Balkans to Fertile Crescent. On the contrary *V. brachysephalum* described from Anatolia is endemic to Turkey. *V. brachysephalum* was reduced to a subspecies under *V.orientale* by Karavelioğulları and Aytaç (2008) taxonomically and SDS-PAGE of seed proteins of two taxa support the conclusion and showed that both taxa have similar banding patterns with 11 % genetic distance between each other (Çelebi et al, 2009). The seeds of two taxa have similar seed characteristics. Both have ridged seed coats (Fig. 1E, G) and same pattern on the surface of seeds (Fig. 1F, H) but they differ only by their shapes. *V. orientale* has elliptic oblong seeds whereas *V. brachysephalum* have trigonious-prismatic seed (Table 1). Attar et al. (2007) described the seed shape of *V. orientale* as trigonious-prismatic. From the above findings, data on seed shape of some commonly distributed species may show variability and cannot be used alone to make a delimitation between the taxa.

Acknowledgements

We wish to thank the curators of following herbaria AEF, ANK, EGE, GAZI, HUB, ISTF, ISTE, KNYA, VANF, G, GB, P, K, B, E and W for allowing us to study their *Verbascum* collections and Karel Sutory for his help for providing literature. We also thank Gazi University (Project Number: 05/2001-19) and TUBITAK (Project Number: TBAG-1959), for the financial support of the mentioned projects.

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