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# ANATOMY, POLLEN AND SEED MORPHOLOGY OF ENDEMIC SPECIES VERBASCUM GLOBIFERUM HUB.-MOR. AND V. LYSIOSEPALUM HUB.-MOR. (SCROPHULARIACEAE) IN DIYARBAKIR, TURKEY AND THEIR TAXONOMIC IMPORTANCE

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## Abstract

*Verbascum globiferum* Hub.-Mor. and *V. lysiosepalum* Hub.-Mor. are endemic to Turkey which is a center of endemism for *Verbascum* species. This study gives the anatomical, palynological and seed micromorphological features of *V. globiferum* and *V. lysiosepalum* which grow in Diyarbakır. The xylem elements occupy a large area in the root cross-sections of the species. In stem cross-sections, the upper part of the epidermis cells is surrounded by a separate cuticle layer and the pith region covers a large area. In the leaves, the main vein is shapped as collateral bundle. Pollen grains are tricolporate-tricolpate, oblate-spheroidal, and exine ornamentation is reticulate. Seeds are brown and oblong-ovate to prismatic and alveolate. The seed coat ornamentation is irregular with polygonal cells, with densely, distinct vesicles.The capsules are pubescent, covered with stellate and branched hairy or glandular hairs.The seed displayed that substantial taxonomic understanding can be acquired from examining the seed characteristics of *Verbascum*, particularly at the species level.

## Intrudiction

*Verbascum* L. genus, which is also known as "Sığırkuyruğu" in Anatolia, is one of the largest genera of the Scrophulariaceae family (Heywood, 1993). The genus *Verbascum* with 360 species (Judd *et al.*, 1999) worldwide and 257 species and 132 supplement hybrids in Turkey, and has been divided into 13 artificial groups in Turkey (Huber-Morath, 1978; Davis *et al.*, 1988; Karavelioğulları, 2012). The endemism ratio of the genus is very high, with 202 endemic species (80%). (Huber-Morath, 1978; Davis *et al.*, 1988; Karavelioğulları, 2012; Karaveliogulları *et al.*, 2014; Firat, 2022).

The genus *Verbascum* is one of the largest genera in terms of the number of species it contains, which is known to have problems in diagnosis and taxonomy because it shows a lot of hybridization in general. There are few studies on the morphological and anatomical features of the genus (Çakır and Bağcı, 2006). There are many SEM studies based on pollen morphology of the some *Verbascum* species taxa (Juan *et al.*, 1997; Dane and Yilmaz, 2002; Kheyri, 2009; Asmat *et al.*, 2011; Al-Hadeethy *et al.*, 2014; Öztürk *et al.*, 2018; Aktas, 2019; Aktas *et al.*, 2020; Baser, 2021). Seed micromorphology of the genus *Verbascum* has been examined by several researchers including Juan *et al.* (1997), Petkovié *et al.* (2020. Anatomical studies of this genus were made by few researchers (Özdemir and Altan, 2007; Kheiri *et al.*, 2009; Yılmaz and Dane, 2011; Alan and Gökman, 2015; Küçük, 2017; Tekin and Yılmaz, 2018; Aktas, 2019; Aktas*et al.*, 2020; Küçük *et al.*, 2021). There are still deficiencies in information and many taxa are not studied yet in Turkey.

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*Verbascum globiferum* and *V. lysiosepalum* are species that grow in Diyarbakır in the Irano-Turanian phytogeographic region and are endemic to Turkey (Huber-Morath, 1978). In this study, the root, stem and leaf anatomy, pollen, and seed micromorphology of the endemic *V. globiferum* and *V. lysiosepalum* species have been investigated for the first time to asses their taxonomic values.

# **Material and Methods**

The specimens belonging to *Verbascum globiferum* and *V. lysiosepalum* were collected from Ergani and between Siverek and Diyarbakır (within the borders of Diyarbakır Province) localities in Diyarbakır (Fig. 1). We have deposited the voucher samples in the herbarium of the Department of Plants and Animal Production of Kızıltepe Vocational School, Mardin Artuklu University (Collector numbers; M.Kılıç 351, M.Kılıç 395, M.Kılıç 353, M.Kılıç 355, M.Kılıç 356, M.Kılıç 357-1, M.Kılıç 394). The taxonomic description of the plant was prepared according to Davis *et al.* (1988) and Karavelioğulları (2012).



Fig. 1. Distribution map of Verbascum globiferum (\*) and V. lysiosepalum (°).

Collected specimens were preserved in falcon tubes in 70% alcohol for use in anatomical studies. Sections taken from the root, stem and leaf parts of the plants with the help of a razor were prepared by staining with safranin-fast green and examined under the light microscope (4x and 10x) and photographed (Bozdağ *et al.*, 2016).

For palynological examinations, light (LM) and (SEM) values of all pollen grains were determined by standard methods described by Erdtman (1952). Pollen grains for LM examination were prepared following the standard procedure of Wodehouse (1935). Thirty pollen grains per specimen were regarded as sufficient for the palynological analysis (Wodehouse, 1935; Kheiri *et al.*, 2006; Cabi *et al.*, 2011). For SEM, pollen were removed by distilled water treatment, the air-dried, pollens were directly mounted on stubs using double-sided adhesive tape and it was covered with gold. The photomicrographs were taken with a ZEISS EVO 50 scanning electron microscope. The values of P (polar axis length), E (equatorial diameter), Clg (Colpus longitude), Clt (Colpus latitude), Plg (Polar longitude), Plt (Polar latitude), Ex (Exine thickness), and In (Intine thickness) were measured, and the P/E ratio was calculated, Apt (Aperture type), and Or (Ornamentation) for 30 pollen grains were measured under light microscope. The terminology of the pollen follows that of Punt *et al.* (2007). The values are presented as minimum, maximum and mean, that is represented in Table 2.

#### ANATOMY, POLLEN AND SEED MORPHOLOGY OF ENDEMIC SPECIES

Seeds were first examined using a Isolab stereomicroscope to ensure that they were of normal size and mature. In order to determine the average seed sizes, 30 mature seeds were measured. For SEM, seed debris were removed by distilled water treatment, the air-dried seeds were then mounted on stubs and it was covered. The photomicrographs were taken with ZEISS EVO 50 scanning electron microscope. Terminology for descriptions of morphological characteristics of the mericarps were followd by Sutton (1988), Juan *et al.* (1997), Attar *et al.* (2007) and Cabi *et al.* (2011).

## **Results and Discussion**

In this study, various features of root, stem and leaf anatomical structures, pollen, and seed surface structures of endemic *Verbascum globiferum* and *V. lysiosepalum* species are stated. Biometric measurements of the root, stem, and leaf tissues and cells are given in Table 1 and shown in Figs 2, 3, 4. The characteristics of pollen grains are summarized in Table 2 and shown in Fig. 5. The morphological characteristics of the seed grains, including their size, shape, color, and surface characteristics, are summarized in Table 3 and shown in Fig. 6. The morphological characteristics, are summarized in Table 3 and shown in Fig. 6. The morphological characteristics, are summarized in Table 4 and shown in Fig. 7.

#### Anatomy

*Root anatomy*: Cross-sections taken from the root of *Verbascum globiferum* have revealed that the periderm layer on the outermost surface of the root is thin and its cells are irregular. A multilayered parenchyma is present under the periderm. Below the parenchyma are 3-5 layered phloem cells. The cambium is indeterminate. The xylem covers a larger area and fills the middle of the root. Trachea cells are irregularly located, larger than the tracheit cells, and their length is greater than their width. Phloem occupies a narrower area than the xylem. Pith rays comprise 2-6 rowed rectangular cells. The pith consists of polygonal or orbicular parenchymatous cells (Fig. 2, Table 1).



Fig. 2. Cross-section of the root of A: *V. globiferum*, B: *V. lysiosepalum*. Pe: Periderm, P: Parenchyma, Ph: Phloem, X: Xylem, Pr: Pith ray, Tr: Trachea, Pt: Pith region.

|                      | ,     | Length( | Length(µ)         |        |        |                  |
|----------------------|-------|---------|-------------------|--------|--------|------------------|
| Species / Tissues    | Min.  | Mak.    | Mean±S.           | Min.   | Mak.   | Mean±S.          |
| V. globiferum        |       |         | Root              |        |        |                  |
| Peridermis cell      | 12.20 | 51.16   | 27.53±10.53       | 9.75   | 31.93  | 17.83±6.53       |
| Parenchyma cell      | 10.93 | 63.18   | 36.15±17.82       | 6.34   | 30.82  | 17.87±7.99       |
| Phloem cell          | 6.63  | 24.27   | $15.53 \pm 5.20$  | 3.21   | 14.15  | 7.02±2.69        |
| Trachea cell         | 20.04 | 52.17   | 35.14±8.17        | 16.35  | 59.08  | 36.44±12.81      |
| V. lysiosepalum      |       |         |                   |        |        |                  |
| Peridermis cell      | 7.76  | 38.50   | 19.58±7.61        | 7.74   | 24.16  | $14.48 \pm 4.40$ |
| Parenchyma cell      | 13.13 | 75.65   | $36.08 \pm 17.64$ | 7.20   | 27.40  | 17.07±6.36       |
| Phloem cell          | 9.44  | 19.78   | $12.90 \pm 2.85$  | 6.03   | 15.66  | $10.76 \pm 2.72$ |
| Trachea cell         | 29.41 | 96.77   | 58.41±22.72       | 27.46  | 81.70  | 55.26±16.88      |
| V. globiferum        |       |         | Stem              | 1      |        |                  |
| Cuticle              | -     | -       | -                 | 6.61   | 14.00  | 9.91±1.95        |
| Epidermis cell       | 10.15 | 32.49   | $18.68 \pm 5.86$  | 9.25   | 24.64  | $15.18 \pm 4.97$ |
| Collenchyma cell     | 6.82  | 43.91   | 18.36±11.68       | 7.41   | 48.06  | 17.92±11.25      |
| Parenchyma cell      | 15.21 | 48.36   | $27.46 \pm 8.99$  | 9.14   | 24.00  | $17.04 \pm 4.90$ |
| Phloem cell          | 4.50  | 18.17   | $9.58 \pm 3.20$   | 3.26   | 6.60   | $4.92 \pm 0.96$  |
| Trachea cell         | 10.57 | 26.91   | $18.08 \pm 4.81$  | 11.54  | 27.42  | 20.41±3.98       |
| Pith cell            | 28.60 | 159.25  | 96.98±42.62       | 24.08  | 189.62 | 93.30±51.01      |
| V. lysiosepalum      |       |         |                   |        |        |                  |
| Cuticle              | -     | -       | -                 | 3.55   | 10.40  | 7.09±1.95        |
| Epidermis cell       | 11.17 | 28,28   | 17.77±3.97        | 8.89   | 20.62  | 13.33±3.17       |
| Collenchyma cell     | 6.51  | 20.13   | $12.04 \pm 4.00$  | 7.64   | 18.21  | 12.41±3.02       |
| Parenchyma cell      | 14.27 | 35.27   | 24.21±5.51        | 9.87   | 27.67  | $18.52 \pm 4.40$ |
| Phloem cell          | 3.15  | 11.47   | $7.26 \pm 2.27$   | 2.60   | 9.68   | $5.79 \pm 2.24$  |
| Trachea cell         | 13.72 | 32.38   | 23.30±6.33        | 8.63   | 45.20  | 27.50±10.33      |
| Pith cell            | 31.10 | 106.74  | 64.65±23.83       | 29.39  | 103.32 | 64.49±24.51      |
| V. globiferum        |       |         | Leat              | f      |        |                  |
| Cuticle              | -     | -       | -                 | 5.15   | 13.90  | 8.57±2.36        |
| Upper epidermis cell | 11.20 | 70.26   | 34.40±12.71       | 8.24   | 27.30  | $18.41 \pm 5.41$ |
| Palisade parenchyma  | 13.09 | 22.29   | $16.83 \pm 2.61$  | 26.00  | 48.52  | 33.51±5.89       |
| Spongy parenchyma    | 11.73 | 22.78   | $16.92 \pm 2.82$  | 12.33  | 27.00  | 20.73±5.00       |
| Mesophyll layer      | -     | -       | -                 | 174.55 | 332.09 | 238.22±36.68     |
| Lower epidermis cell | 7.40  | 22.90   | $14.96 \pm 4.97$  | 9.25   | 20.39  | 13.52±2.71       |
| V. lysiosepalum      |       |         |                   |        |        |                  |
| Cuticle              | -     | -       | -                 | 4.65   | 13.32  | 8.28±2.09        |
| Upper epidermis cell | 11.39 | 25.34   | 18.21±4.24        | 8.76   | 30.04  | 16.79±5.00       |
| Palisade parenchyma  | 7.77  | 17.36   | $11.06 \pm 2.41$  | 22.22  | 44.03  | 31.21±5.00       |
| Spongy parenchyma    | 8.85  | 22.49   | 14.88±3.91        | 8.28   | 30.86  | 19.10±6.93       |
| Mesophyll layer      | -     | -       | -                 | 185.64 | 237.59 | 210.58±15.21     |
| Lower epidermis cell | 8 99  | 21.84   | 14.08+3.56        | 7.24   | 14.10  | 11 08+1 75       |

Table 1. The anatomical mesurements of Verbascum globiferum and V. lysiosepalum.

#### ANATOMY, POLLEN AND SEED MORPHOLOGY OF ENDEMIC SPECIES

Cross-sections taken from the root of *V. lysiosepalum* have revealed that the periderm layer on the outermost surface of the root is thin and its cells are irregular. A multilayered parenchyma is present under the periderm. Below the parenchyma are 2-4 layered phloem cells. The cambium is indeterminate. The xylem covers a larger area and fills the middle of the root. Trachea cells are irregularly located, larger than the tracheit cells, and their width is greater than their length. Phloem occupies a narrower area than the xylem. Pith rays comprise 2-6-rowed rectangular cells. The pith consists of polygonal or orbicular parenchymatous cells (Fig. 2, Table 1).

Stem anatomy: Cross-sections taken from the stem of *V. globiferum* have exhibited a monolayer epidermis covered by an undulate cuticle. The epidermis is composed of oval or rectangular cells. There are glandular and eglandular hairs on the epidermis. Underneath the epidermis, there are 3-5 layers of collenchyma cells. The parenchyma tissue consists of 7-10 layers of oval and orbicular parenchymatous cells. Under the parenchyma, there are 4-6 layers of sclerenchyma. Cambium is indistinguishable. The xylem occupies a larger area than phloem. The pith comprises hexagonal or orbicular parenchymatous cells with intercellular spaces (Fig. 3, Table 1).



Fig. 3. Cross-section of the stem of A: V. globiferum, B: V. lysiosepalum. Eg: Eglandular hair, Ch: Compound hair, Cu: Cuticle, Ep: Epidermis, Co: Collenchyma, P: Parenchyma, Sc: Sclerenchyma, Ph: Phloem, X: Xylem, Tr: Trachea, Pt: Pith region.

Cross-sections taken from the stem of *V. lysiosepalum* have exhibited a monolayer epidermis covered by an undulate cuticle. The epidermis is composed of oval, ovate or rectangular cells. There are compound, glandular, and eglandular hairs on the epidermis. Underneath the epidermis, there are 3-5 layers of collenchyma cells. The parenchyma tissue consists of 7-10 layers of oval and orbicular parenchymatous cells. Under the parenchyma, there are 5-7 layers of sclerenchyma. Cambium is indistinguishable. The xylem occupies a larger area than phloem. The pith comprises hexagonal or orbicular parenchymatous cells with intercellular spaces (Fig. 3, Table 1).

*Leaf anatomy*: Cross-sections of the lamina sections of adaxial and abaxial epidermis of *V. globiferum* have showed that both epidermis are covered with compound, glandular, and eglandular hairs and they consist of uniseriate rectangular or oval cells of cuticles. The vascular bundles are collateral. The curved vascular bundle is surrounded by parenchymal cells. The xylem

elements are arranged radially and form a single layer. The midrib is well developed. The parenchyma layer around the vascular bundle covers a large area. Cells of parenchymal tissue are polygonal and tightly arranged. Cells of the upper epidermis are clearly larger than the lower. Under the upper and lower epidermis is the hypodermis. The mesophyll tissue is divided into palisade and sponge parenchyma, with 3-5 layers below the upper epidermis and 1-2 layers of palisade parenchyma above the lower epidermis. Palisade parenchyma cells are elongated, cylindrical or quadrangular, and irregularly. Between the lower and upper palisade parenchyma cells are oval or polygonal and there is more space between the cells. Also, idioblasts have observed in the mesophyll tissue of leaves (Fig. 4, Table 1).



Fig. 4. Cross-section of the leaves of A: V. globiferum, B: V. lysiosepalum. G: Glandular hair, Ch: Compound hair, M: Mesophyll layer, ad: Adaxial surface, Co: Collenchyma, P: Parenchyma, X: Xylem, Ph: Phloem, ab: Abaxial surface, Ue: Upper epidermis, Le: Lower epidermis, Pp: Palisade parenchyma, Sp: Spongy parenchyma, Hp: Hypodermis, i: idioblast.

Cross-sections of the lamina sections of adaxial and abaxial epidermis of *V. lysiosepalum* have showed that both epidermis are covered with compound, glandular, and eglandular hairs and they consist of uniseriate rectangular or oval cells of cuticles. The vascular bundles are collateral. The curved vascular bundle is surrounded by parenchymal cells. The xylem elements are arranged radially and form a single layer. The midrib is well developed. The parenchyma layer around the vascular bundle covers a large area. Cells of parenchymal tissue are polygonal and tightly arranged. Cells of the upper epidermis are clearly larger than the lower. Under the upper and lower epidermis is the hypodermis. The mesophyll tissue is divided into palisade and sponge parenchyma, with 2-3 layers below the upper epidermis and 1-2 layers of palisade parenchyma above the lower epidermis. Palisade parenchyma cells are elongated, cylindrical or quadrangular, and irregularly. Between the lower and upper palisade parenchyma cells are sponge parenchyma (site consisting of 2-3 rows of cells. Sponge parenchyma cells are oval or polygonal and there is more space between the cells. Also, idioblasts have observed in the mesophyll tissue of leaves (Fig. 4, Table 1).

## Pollen morphology

*Verbascum globiferum* Hub.-Mor.: The pollen shape was oblate-spheroidal (P/E: 0.95) with a polar axis of 12.88  $\mu$ m and an equatorial axis of 13.53  $\mu$ m. The aperture type of pollen was found as 87 % tricolporate and 13 % tricolpate. The colpus was in long-acute ended with a colpus length of 9.48  $\mu$ m and width of 3.28  $\mu$ m; porus length of 3.71  $\mu$ m and porus width 3.51  $\mu$ m. Exine thickness was 1.01  $\mu$ m and intine thickness was 0.52  $\mu$ m. Ornamentation was reticulate and reticulum was shallow (Fig. 5, Table 2).

*Verbascum lysiosepalum* Hub.-Mor.: The pollen shape was oblate-spheroidal (P/E: 0.94) with a polar axis of 12.95  $\mu$ m and an equatorial axis of 13.70  $\mu$ m. The aperture type of pollen was found as 90 % tricolporate and 10 % tricolpate. The colpus was in long-acute ended with a colpus length of 10.27  $\mu$ m and width of 3.25  $\mu$ m; porus length of 4.07  $\mu$ m and porus width 3.61  $\mu$ m. Exine thickness was 1.09  $\mu$ m and intine thickness was 0.61  $\mu$ m. Ornamentation was reticulate and reticulum was distinct (Fig. 5, Table 2).



Fig. 5. Scanning electron micrographs of pollen in genus *Verbascum. V. globiferum* (A- Equatorial view, B-Exine sculpturing), *V. lysiosepalum* (C- Equatorial view, D- Exine sculpturing).

## Seed micromorphology

According to the measurements made, the dimensions *Verbascum globiferum* from 0.62 to 1.11 mm in length and 0.37 to 0.78 mm in width. The shape of the seeds in the genus *Verbascum* allows a distinction to be made between species and subspecies subtaxa. Prismatic-ovate, oblong, with  $\pm$ shallow alveolate, multiple linear, deep and broad backs are the shapes of seeds. The seed truncated and obtuse beak. The seed is color brown. Because of the irregular, exserted polygonal, and small rectangular cells, with densely and distinct vesicles, a networklike appearance is seen and the seed surface coat is longitudinally alveolate. Inside the cells are 3-4 transverse lines (Fig. 6, Table 3).

| Species         | Ρ(μ)    | Ε(μ)    | P/E   | Sh  | Clg (µ) | Clt (µ) | Plg (µ) | Plt (µ) | Ex (µ) | In (µ) | Apt    | Or |
|-----------------|---------|---------|-------|-----|---------|---------|---------|---------|--------|--------|--------|----|
|                 |         |         | ratio |     |         |         |         |         |        |        |        |    |
| V. globiferum   | 11.56   | 12.18   | 0.95  | Obs | 7.35    | 2.08    | 2.23    | 1.87    | 0.80   | 0.26   | 87% Tr | R  |
|                 | (12.88) | (13.53) |       |     | (9.48)  | (3.28)  | (3.71)  | (3.51)  | (1.01) | (0.52) | 13% T  |    |
|                 | 14.35   | 14.83   |       |     | 11.20   | 4.56    | 5.57    | 5.25    | 1.58   | 0.82   |        |    |
| V. lysiosepalum | 11.58   | 11.78   | 0.94  | Obs | 8.59    | 2.04    | 2.91    | 2.40    | 0.84   | 0.35   | 90% Tr | R  |
|                 | (12.95) | (13.70) |       |     | (10.27) | (3.25)  | (4.07)  | (3.61)  | (1.09) | (0.61) | 10% T  |    |
|                 | 14.75   | 15.76   |       |     | 11.89   | 4.12    | 5.55    | 5.74    | 1.66   | 0.98   |        |    |

Table 2. Pollen morphological characters in genus of Verbascum (min (mean) max).

R: Reticulate, T: Tricolpate, Tr: Tricolporate, Obs: Oblate-spheroidal, Sh: Shape.

Table 3. Seed morphological characters in genus of Verbascum.

| Species         | Grou    | Length      | Width    | Colour | Shape                       | Seed surface                      |
|-----------------|---------|-------------|----------|--------|-----------------------------|-----------------------------------|
|                 | $p^{a}$ | (mm) min    | (mm) min |        |                             |                                   |
|                 |         | (mean)      | (mean)   |        |                             |                                   |
|                 |         | max         | max      |        |                             |                                   |
| V. globiferum   | Κ       | 0.62        | 0.37     | Brown  | Prismatic-ovate, oblong,    | Irregular, exserted polygonal     |
|                 |         | (0.91) 1.11 | (0.56)   |        | with ±shallow alveolate,    | and small rectangular cells       |
|                 |         |             | 0.78     |        | multiple linear, deep and   | with densely and distinct         |
|                 |         |             |          |        | broad backs, often with     | vesicles. Inside the cells are 3- |
|                 |         |             |          |        | truncated beaks, and some   | 4 transverse lines                |
|                 |         |             |          |        | with obtuse beaks           |                                   |
| V. lysiosepalum | Κ       | 0.70        | 0.31     | Brown  | Prismatic, prismatic-ovate, | Irregular, exserted small         |
|                 |         | (0.94) 1.20 | (0.56)   |        | oblong, with shallow        | rectangular cells with densely    |
|                 |         |             | 0.68     |        | alveolate, multiple linear, | and distinct vesicles             |
|                 |         |             |          |        | slightly deep and slightly  |                                   |
|                 |         |             |          |        | broad backed, truncated     |                                   |
|                 |         |             |          |        | beaks                       |                                   |

|  | Т | abl | e 4 | . ( | Capsu | les moi | phol | logical | l characters i | in genus | of | Verl | bascun |
|--|---|-----|-----|-----|-------|---------|------|---------|----------------|----------|----|------|--------|
|--|---|-----|-----|-----|-------|---------|------|---------|----------------|----------|----|------|--------|

| Species       | Group <sup>a</sup> | Length (mm)<br>min | Width (mm)<br>min | Color | Shape      | Hair                           |
|---------------|--------------------|--------------------|-------------------|-------|------------|--------------------------------|
|               |                    | (mean) max         | (mean) max        |       |            |                                |
| V. globiferum | K                  | 3.89 (5.43)        | 3.10 (4.26)       | Brown | Ovate,     | Stellate, tomentose, branched, |
|               |                    | 6.49               | 4.87              |       | oblong     | glandular                      |
| <i>V</i> .    | K                  | 4.11 (5.44)        | 4.28 (5.47)       | Brown | Spherical, | Densely stellate, branched,    |
| lysiosepalum  |                    | 6.11               | 4.85              |       | ovate      | glandular                      |

<sup>a</sup> According to Huber-Morath (1978)

According to the measurements made, the dimensions *V. lysiosepalum* from 0.70 to 1.20 mm in length and 0.31 to 0.68 mm in width. The shape of the seeds in the genus *Verbascum* allows a distinction to be made between species and subspecies subtaxa. Prismatic, prismatic-ovate, oblong, with shallow alveolate, multiple linear, slightly deep and slightly broad backed are the shapes of seeds. The seed truncated beak. The seed is color brown. Because of the irregular, exserted small rectangular cells, with densely and distinct vesicles, a networklike appearance is seen and the seed surface coat is longitudinally alveolate (Fig. 6, Table 3).

#### Capsule morphology

According to the measurements made, the dimensions vary *Verbascum globiferum* from 3.89 to 6.49 mm in length and 3.10 to 4.87 mm in width and *V. lysiosepalum* from 4.11 to 6.11 mm in length and 4.28 to 5.47 mm in width. The shape of *V. globiferum* capsules is ovate, oblong, and the *V. lysiosepalum* is spherical and ovate. *V. globiferum* capsules is brown, while *V. lysiosepalum* 

is dark brown. *V. globiferum* is covered with stellate, tomentose, branched, and glandular hairs, while *V. lysiosepalum* is covered with densely stellate, branched, and glandular hairs (Fig. 7, Table 4).



Fig. 6. Scanning electron micrographs of seed in genus *Verbascum*. *V. globiferum* (A- General appearance, B- Surface ornamentation), *V. lysiosepalum* (C- General appearance, D- Surface ornamentation).



Fig. 7. Stereo microscopy photographs of capsules of *Verbascum globiferum* (A) and *V. lysiosepalum* (B) species.

Analyzes of anatomy, pollen, and seeds of the endemic *Verbascum globiferum* and *V*. *lysiosepalum* species studied for the first time in this study make them comparable to some of the other *Verbascum* members investigated.

The present study indicated that *V. globiferum* and *V. lysiosepalum* have a very large xylem area at the root and a thick cuticle layer on the stem. In addition, the same features were also noted by researchers (Özdemir and Altan, 2007; Alan and Gökmen, 2015; Küçük, 2017; Tekin and Yılmaz, 2018; Aktas *et al.*, 2020; Küçük *et al.*, 2021). In some studies (Alan and Gökmen, 2015; Aktas *et al.*, 2020), it was stated that the cambium was indeterminate in the vascular bundle in the root, and in this study, it was found that the root had similar characteristics for the species. In the leaf, cross-section analyses of the species, densely glandular, eglandular, and multicellular

branched hairs were observed on the epidermal cells. Similar results have been reported for other investigated *Verbascum* species (Alan and Gökmen, 2015; Küçük, 2017; Aktas *et al.*, 2020). Idioblasts seen in the mesophyll tissue of the leaf can be considered a factor for identification within the genus *Verbascum* (Lersten and Curtis, 2001). In this study, the presence of idioblasts in leaf mesophyll was determined and this feature was also noted in other studies (Kheiri *et al.*, 2009; Yılmaz and Dane, 2011; Tekin and Yılmaz, 2018).

The pollen of endemic V. globiferum and V. lysiosepalum species are isopolar and radially symmetric, oblate-spheroidal, tricolporate (there is tricolpate), the exine exhibits a tectate structure and reticulate ornamentation. Baser (2021), in his study on Verbascum (8 species), established the pollen grains as tricolporate and tricolpate. Aktas *et al.* (2020) determined the pollen grain as tricolpate in their study on the endemic Verbascum species. Aktas (2019) noted the pollen grain as tricolpate in his study on Verbascum species. Özturk *et al.* (2018) reported that there were tricolporate and tricolpate aperture types in their pollen study on V. pycnostachyum (K groups). Al-Hadeethy *et al.* (2014) stated that the aperture types of Verbascum species (20 species) were tricolporate. Aperture type of Verbascum reported to have tricolporate aperture type (Kheiri *et al.*, 2006; Asmat *et al.*, 2011).

The pollen of the examined species were determined as oblate-spheroidal shape. However, Aktas *et al.* (2020) recorded the prolate pollen shape in the endemic *Verbascum* species examined. Nevertheless, Baser (2021) recorded the prolate pollen shape in the *Verbascum* (8 species) taxa in the studied. Öztürk *et al.* (2018) stated that pollen shape was oblate-spheroidal on *V. pycnostachyum* (K groups). In addition, Al-Hadeethy *et al.* (2014) observed the presence of prolate-spheroidal and oblate-spheroidal pollen in *Verbascum* (20 species). Morphological pollen traits observed through LM proved not very important in their taxonomic use, but sculpting examined by SEM was found to be more significant in the classification of taxa (Pehlivan *et al.*, 2008; Baser, 2021).

The overlapping of exine thickness was found among the taxa studied, thus this character was of little taxonomic value (Al-Hadeethy *et al.*, 2014). Results obtained from the present study agree with previous studies on some species of *Verbascum* (Asmat *et al.*, 2011; Al-Hadeethy *et al.*, 2014; Öztürk *et al.*, 2018; Aktas *et al.*, 2020; Baser, 2021). However, the studied species showed a neat reticulate exine sculpture pattern. But *V. globiferum* reticulum was shallow and *V. lysiosepalum* reticulum was distinct.

The seeds were brown when mature. The size of the seeds usually ranged between 0.62 to 1.20 mm in length and 0.31 to 0.78 mm in wide. The measurements taken in the present study were compatible with those of the common species in the studies of Attar *et al.* (2007) and Kheiri *et al.* (2009). Yet, the measurements taken in this study were larger than the mean measurements of the species common in Cabi *et al.* (2011).

Seeds in shape from prismatic-ovate and oblong in the species studied, and they ended in an truncated, obtuse beak. The seed coat was longitudinally alveolate. Usually, most seeds of the species are prismatic-ovate (Table 3). The result regarding seed shape is more or less consistent with the results of Attar *et al.* (2007), Kheiri *et al.* (2009), Cabi *et al.* (2011) and Baser (2021).

The capsule of *V. globiferum* is covered with stellate, tomentose, branched, and glandular hairs, while *V. lysiosepalum* is covered with densely stellate, branched, and glandular hairs. In some *Verbascum* species distributed in Iran (Attar *et al.*, 2007), the capsules have a similar hair indumentum. Based on the available findings, one of the reliable characteristics for grouping in *Verbascum* is the indumentum of the capsule.

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