

POLLEN MORPHOLOGY OF *VERBASCUM* L. (SCROPHULARIACEAE) IN NORTHERN AND CENTRAL IRAQ

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Abstract

The present study highlights the taxonomic values of the pollen features of 20 species of *Verbascum* L. in Iraq. The pollen grains were acetolysed and observed under Light and Electron microscopes. Our results showed that the pollen grains of these species are usually radially symmetrical, isopolar, tricolporate and display reticulate sculpturing. The variations were found in shape, size, and exine thickness. Most of the species are subprolate, prolate spheroidal, and prolate, whereas oblate spheroidal pollens were found only in *V. thapsus*. The pollen size among the species studied ranges from 16.2-32.5 μm . The majority of species are medium sized except *V. agrimoniifolium* and *V. oreophilum* that are small sized, and four species i.e. *V. palmyrense*, *V. sinuatum*, *V. songaricum*, and *V. thapsus* having both small and medium sized pollen grains. Moreover, the result showed that the exine thickness ranges from 0.87-4.75 μm , but this value can overlap between some species. Therefore, only the shape of pollen grains could be used to classify these species into groups.

Introduction

The genus *Verbascum* L. (Scrophulariaceae) is mostly herbaceous with few shrubs and climbers, comprises about 350 species, and it is distributed all over the world (Zohary, 1974). *Verbascum* is widely spread in the Arabian Peninsula, especially in Saudi Arabia, Oman and Yemen (Huber-Morath, 1978; Ghadanfar, 1992; Wood, 1997). In Iraq, *Verbascum* is represented by 26 species (AL-Rawi, 1964; AL-Bermani, 1981).

The taxonomy of *Verbascum* species in Iraq is still uncertain, especially that as related to the geographical distribution of the species, the morphological variation and the condition of herbarium samples (AL-Bermani, 1981). Therefore, study of different characteristics would help in resolving the relationships and delimitation of these species. Pollen morphology is proved to be a useful character for improving the accuracy in the identification and classification of several plant groups (Erdtman, 1952; Rajbhandary *et al.*, 2012; Özler *et al.*, 2013; Sawar and Takahashi, 2013). Despite pollen morphology of *Verbascum* species was studied by several authors (Erdtman, 1952; Filippini *et al.*, 1990; Perveen, 1993; Lobin and Pormbski, 1994; Bukhari and Alfarhan, 2006; Kheiri *et al.*, 2006; Pehlivan *et al.*, 2008), the pollens of *Verbascum* found in Iraq has never been investigated. Therefore, this study was designed to investigate constant and diagnostic pollen morphological characteristics to be used as a tool for improving the accuracy in the identification and classification of *Verbascum*, and further authentication of the taxa.

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Materials and Methods

Pollen sample of 20 species of *Verbascum* L. grown in the north and central areas of Iraq, were collected between April 2012 and May 2013 (Table 1). The voucher specimens have been deposited in the herbarium of College of Education, Ibn Al-Haitham, University of Baghdad, Iraq. The pollen was prepared using the acetolysis method as described by Erdtman (1960). The measurements and observations were carried out using both Light microscope (LM) and Scanning Electron microscope (SEM). For LM analysis, the acetolysed pollens were placed in a small vial, and 5-7 drops of silicone oil were added, and then the samples were mounted on a glass slide sealed with paraffin and observed under light microscope. The measurements of polar axis (P), equatorial diameter (E) and exine thickness of pollen were done using 10 reading samples for each specimen. For SEM investigation, the acetolysed pollen was suspended in 100% ethanol. Then the suspension was dried on an aluminum stub, coated with gold and observed using LEO 1450VP Electron Microscopy. The terminology and pollen size classes follow Walker and Doyle (1975) and Punt *et al.* (1994).

Table 1. List of *Verbascum* L. species in Northern and Central of Iraq employed in the present study.

Species	Locations	Date of collections	Collection numbers
1. <i>Verbascum agrimoniifolium</i> (C. Koch) Hub. - Mor.	Sulaymaniyah Province	14 Jun 2012	M. AL-Hadeethy 166A
2. <i>V. alceoides</i> Boiss. & Hausskn. ex Boiss.	Sulaymaniyah Province	6 May 2012	M. AL-Hadeethy 235
3. <i>V. alepense</i> Benth.	Nineveh Province	11 May 2013	M. AL-Hadeethy 245
4. <i>V. andrusii</i> Post	Nineveh Province	27 Jun 2012	M. AL-Hadeethy 129
5. <i>V. assureense</i> (Bornm. & Hand. - Mazz.) Hub. - Mor.	Diyala Province	4 Apr 2012	M. AL-Hadeethy 172
6. <i>V. calvum</i> Boiss. & Kotschy ex Boiss.	Sulaymaniyah Province	14 Jun 2012	M. AL-Hadeethy 250
7. <i>V. carduchorum</i> Bornm.	Sulaymaniyah Province	21 Apr 2012	M. AL-Hadeethy 168
8. <i>V. cheiranthifolium</i> Boiss.	Sulaymaniyah Province	4 May 2012	M. AL-Hadeethy 225
9. <i>V. damascenum</i> Boiss.	Anbar Province	5 Apr 2013	M. AL-Hadeethy 152
10. <i>V. geminiflorum</i> Hochst.	Sulaymaniyah Province	6 May 2012	M. AL-Hadeethy 230
11. <i>V. laetum</i> Boiss. & Hausskn. ex Boiss.	Sulaymaniyah Province	20 Apr 2012	M. AL-Hadeethy 144
12. <i>V. macrocarpum</i> Boiss.	Sulaymaniyah Province	4 May 2012	M. AL-Hadeethy 200A
13. <i>V. oreophilum</i> C. Koch	Sulaymaniyah Province	5 May 2012	M. AL-Hadeethy 223
14. <i>V. palmyrense</i> Post	Nineveh Province	27 Jun 2012	M. AL-Hadeethy 220
15. <i>V. pseudodigitalis</i> Nábělek	Sulaymaniyah Province	20 Apr 2012	M. AL-Hadeethy 123
16. <i>V. sinaiticum</i> Benth.	Nineveh Province	27 Jun 2012	M. AL-Hadeethy 112
17. <i>V. sinuatum</i> L.	Kirkuk Province	7 Aug 2012	M. AL-Hadeethy 166B
18. <i>V. songaricum</i> Schrenk	Sulaymaniyah Province	4 May 2012	M. AL-Hadeethy 200B
19. <i>V. speciosum</i> Schrad.	Sulaymaniyah Province	21 Apr 2012	M. AL-Hadeethy 182
20. <i>V. thapsus</i> L.	Sulaymaniyah Province	20 Apr 2012	M. AL-Hadeethy 145

Results and Discussion

The results of the pollen morphology of *Verbascum* are summarized in Table 2. It was found that the pollen morphology of the species studied is uniform. The general characteristic is monad, radially symmetrical, isopolar, tricolporate and reticulate in exine sculpturing (Figs 1 & 2).

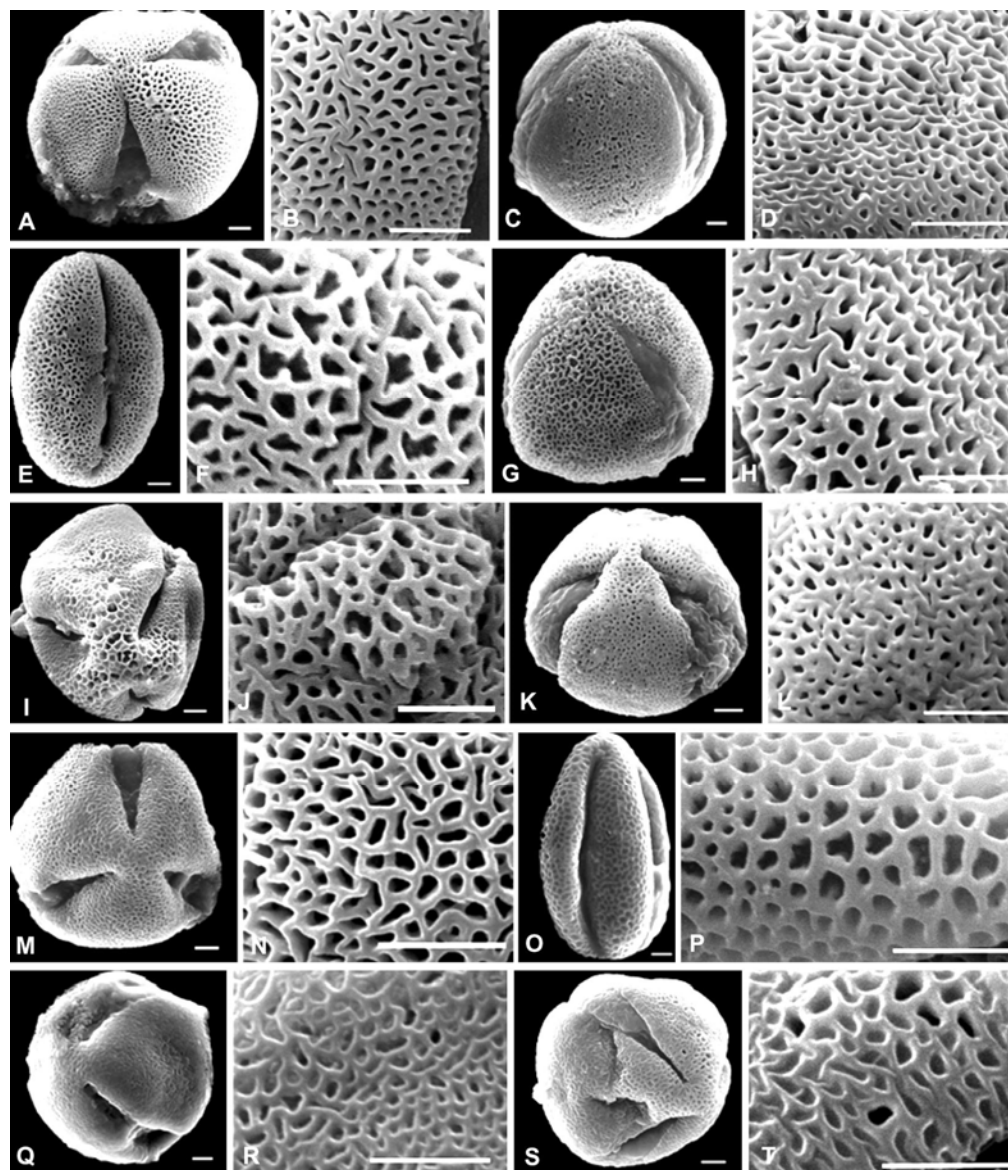


Fig. 1. SEM micrographs of the pollen grains of *Verbascum*. A, B. *V. agrimoniifolium*; C, D. *V. alceoides*; E, F. *V. alepense*; G, H. *V. andrusii*; I, J. *V. assureense*; K, L. *V. calvum*; M, N. *V. carduchorum*; O, P. *V. cheiranthifolium*; Q, R. *V. damascenum*; S, T. *V. geminiflorum*; A, G, I, K, M and Q. polar view; B, D, F, H, J, L, N, P, R and T. exine sculpturing; C, E, O, S. equatorial view (Scale bar = 2 μ m).

Table 2. Pollen morphological characteristics of *Verbascum* in Northern and Central Iraq.

Species	Polar axis (µm)	Equatorial axis (µm)	P/E Ratio	Shape	Size	Exine thickness (µm)	Exine sculpturing
1. <i>V. agrimoniifolium</i>	16.5-20.5 (17.54±1.39)	15.5-21.0 (17.39±1.65)	1.01	Prolate spheroidal	small	0.87-1.05 (0.93±0.10)	Reticulate
2. <i>V. alceoides</i>	27.5-32.5 (28.05±1.78)	22.5-25.0 (24.13±1.13)	1.16	Subprolate	medium	2.75-3.00 (2.65±0.41)	Reticulate
3. <i>V. alepense</i>	25.0-27.5 (26.38±1.04)	21.3-22.5 (21.88±0.63)	1.21	Subprolate	medium	3.00-3.72 (3.22±0.39)	Reticulate
4. <i>V. andrusii</i>	26.25-27.5 (26.88±0.63)	18.8-20.0 (19.25±0.61)	1.40	Prolate	medium	1.25-2.5 0 (2.15±0.48)	Reticulate
5. <i>V. assurense</i>	27.5-32.5 (30.88±1.68)	25.0-27.5 (26.50±1.22)	1.17	Subprolate	medium	3.75-4.25 (4.18±0.44)	Reticulate
6. <i>V. calvum</i>	27.5-30.0 (29.00±1.09)	22.5-25.0 (24.13±1.13)	1.20	Subprolate	medium	1.25-2.5 0 (2.08±0.51)	Reticulate
7. <i>V. carduchorum</i>	25.0-28.8 (27.13±1.38)	22.5-25.0 (24.00±1.22)	1.13	Prolate spheroidal	medium	3.25-4.00 (3.13±0.76)	Reticulate
8. <i>V. cheiranthifolium</i>	26.3-27.5 (26.88±0.63)	20.0-22.5 (21.38±1.18)	1.26	Subprolate	medium	1.25-2.5 0 (2.00±0.58)	Reticulate
9. <i>V. damascenum</i>	27.5-30.0 (29.25±1.00)	20.0-22.5 (21.6±0.98)	1.35	Prolate	medium	3.75-4.75 (4.00±0.58)	Reticulate
10. <i>V. geminiflorum</i>	26.25-27.5 (26.50±0.93)	20.0-22.5 (21.13±1.04)	1.25	Subprolate	medium	3.00-4.75 (3.58±1.28)	Reticulate
11. <i>V. laetum</i>	30.0-32.5 (31.23±0.97)	23.8-25.0 (24.50±0.61)	1.27	Subprolate	medium	2.50-3.75 (3.05±0.46)	Reticulate
12. <i>V. macrocarpum</i>	27.5-28.8 (28.13±0.63)	20.0-22.5 (21.75±1.00)	1.29	Subprolate	medium	2.50-3.75 (3.23±0.52)	Reticulate
13. <i>V. oreophilum</i>	19.1-22.5 (20.71±0.61)	18.8-20.0 (19.25±0.61)	1.08	Prolate spheroidal	small	1.75-2.50 (1.90±0.53)	Reticulate
14. <i>V. palmyrense</i>	22.5-25.0 (24.00±1.09)	20.0-22.5 (21.25±1.12)	1.13	Prolate spheroidal	small-medium	1.25-2.5 0 (2.05±0.53)	Reticulate
15. <i>V. pseudodigitalis</i>	27.5-30.0 (28.75±0.97)	20.0-21.3 (20.88±0.57)	1.38	Prolate	medium	2.25-2.50 (2.53±0.21)	Reticulate
16. <i>V. sinaiticum</i>	27.5-30.0 (29.13±0.98)	20.0-21.3 (20.88±0.57)	1.40	Prolate	medium	1.50-2.5 0 (2.00±0.49)	Reticulate
17. <i>V. sinuatum</i>	19.6-25.2 (23.86±1.09)	20.6-23.2 (23.55±0.61)	1.01	Prolate spheroidal	small - medium	2.00-2.5 0 (2.30±0.22)	Reticulate
18. <i>V. songaricum</i>	23.8-26.3 (25.38±0.98)	20.0-21.3 (20.38±0.57)	1.25	Subprolate	small - medium	1.25-3.00 (2.13±0.75)	Reticulate
19. <i>V. speciosum</i>	25.0-27.5 (26.50±1.09)	20.0-22.5 (21.25±1.25)	1.25	Subprolate	medium	3.50-4.00 (3.70±0.29)	Reticulate
20. <i>V. thapsus</i>	22.5-25.0 (23.75±0.77)	24.0-27.0 (25.00±0.50)	0.95	Oblate-spheroidal	small - medium	1.24-2.16 (1.91±0.35)	Reticulate

In *Verbascum*, pollen grains vary in shape, size and exine thickness. The shape can be prolate spheroidal, subprolate, prolate and oblate-spheroidal. The pollens are small to medium-sized. Most of the species have medium-sized pollens (25.0-32.5 µm) except in *V. agrimoniifolium* and *V. oreophilum*, where small-sized pollens (16.5-24.0 µm) are observed. *Verbascum palmyrense*, *V. sinuatum*, *V. songaricum*, and *V. thapsus* have both small and medium sized pollen grains. The polar axis (P) ranged from 16.5-32.5 µm. The largest polar axis was recorded in *V. alceoides*, *V. assurense* and *V. laetum* with 32.5 µm long and the smallest was found in *V. agrimoniifolium* with

16.5 μm long. The equatorial diameter (E) ranged from 15.5-27.5 μm and the largest one was present in *V. assurense* with 27.5 μm , while the smallest was found in *V. agrimoniifolium*. In addition, the exine thickness of the pollen of species studied ranged from 0.87 to 4.75 μm .

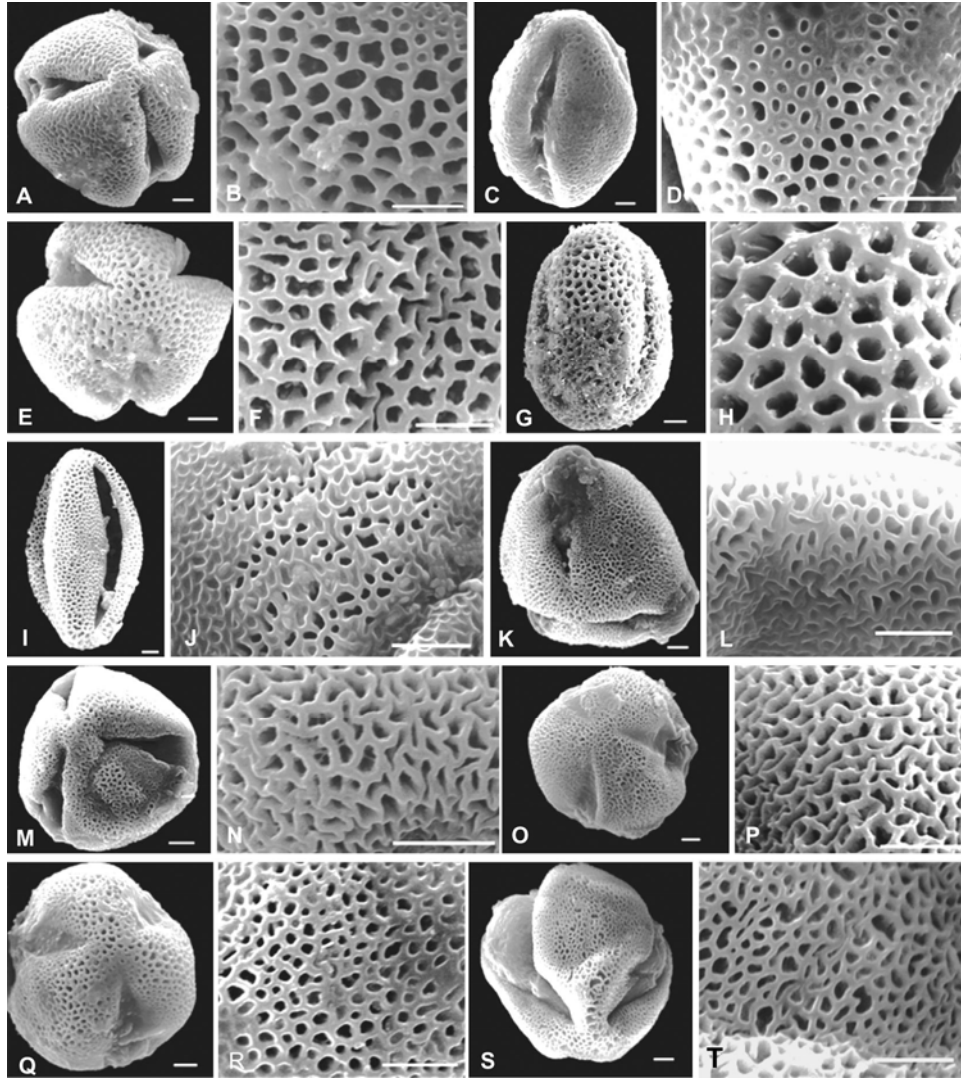


Fig. 2. SEM micrographs of the pollen grains of *Verbascum*. A, B. *V. laetum*; C, D. *V. macrocarpum*; E, F. *V. oreophilum*; G, H. *V. palmyrense*; I, J. *V. pseudodigitalis*; K, L. *V. sinaiticum*; M, N. *V. sinuatum*; O, P. *V. songaricum*; Q, R. *V. speciosum*; S, T. *V. Thapsus*; A, E, K, M, O, Q and S. polar view; B, D, F, H, J, L, N, P, R and T. exine sculpturing; C, G and I. equatorial view (Scale bar = 2 μm).

The overlapping of exine thickness is found among the taxa studied, thus this character is of little taxonomic value. Results obtained from the present study agree with previous studies on some species of *Verbascum* (Erdtman, 1952; Filippini *et al.*, 1990; Lobin and Pormbski, 1994;

Bokhri and Alfarhan, 2006; Asmat *et al.*, 2011). Several authors reported two types of exine sculpturing for *Verbascum* i.e. microreticulate and macroreticulate (Kheiri *et al.*, 2006; Pehlivan *et al.*, 2008; Karavelioğullari *et al.*, 2010). Pehlivan *et al.* (2008) concluded that the pollen morphological characteristics observed under the light microscope seems to have little value for taxonomic study but the sculpturing investigated under SEM provides a better result for dividing the *Verbascum* taxa into two groups with reticulate and coarsely reticulate. However, all taxa included in our study showed a uniform reticulate exine sculpturing pattern.

Our results showed that only the shape of pollen grains can be used for grouping the *Verbascum* into four groups viz. the Subprolate group including: *V. alceoides*, *V. alepense*, *V. assureense*, *V. calvum*, *V. cheiranthifolium*, *V. geminiflorum*, *V. laetum*, *V. macrocarpum*, *V. songaricum*, *V. speciosum*, the Prolate spheroidal group including: *V. agrimoniifolium*, *V. carduchorum*, *V. oreophilum*, *V. palmyrense*, and *V. sinuatum*; the Prolate group includes: *V. andrusii*, *V. damascenum*, *V. pseudodigitalis*, *V. sinaiticum* and the Oblate spheroidal with only one species *V. thapsus*. Even though the pollen characters alone cannot be used for species identification, they provide some information for the classification of these plants. Moreover, using this character in combination with other characters may help in clarifying some problematic taxa.

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