MICROMORPHOLOGICAL AND ANATOMICAL STUDIES OF CERTAIN SPECIES OF VERBASCUM (SCROPHULARIACEAE) IN WEST AZERBAIJAN, IRAN

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The high morphological diversity among the species of the genus *Verbascum* causes problems in the delimitation of the species. Therefore, there is a difficulty in offering a natural and well organized classification for the species of the genus. This research was based on the leaf, seed coat and fruit anatomy, and seed micromorphology of the species growing in Iran. The shape and density rate of epicarpic idioblasts can be a determining character for *Verbascum* species.

Two types of mesophyll were found within the species: Dorsiventral and Isobilateral. Also two types of thickness ratio of endocarp to pericarp (2/3, 1/2) were distinguished. However, a slight difference has been observed in the shape of epidermal cells of seeds and the presence of vesicle on the lateral walls of the epidermal cells of seeds.

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Key words. Verbascum, Iran, seed, micromorphology, leaf, fruit, anatomy, *Scrophulariaceae*.

مطالعه مورفولوژیکی و ساختار تشریحی بعضی از گونه‌های جنس گل ماهور Verbascum (Scrophulariaceae) در آذربایجان غربی، ایران

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تنوع مورفولوژیک با لا در جنس گل ماهور مشکل عمده ای در تعیین حدود گونه ها است. بنابراین در ارائه یک طبقه‌بندی سازمان یافته و طبیعی در جنس گل ماهور مشکل وجود دارد. تحقیق حاضرساختار تشریحی برگ، پوسته دانه، میوه و **ریزساختاری** دانه گونه‌های گل ماهور در ایران را مورد بررسی قرار می‌دهد. شکل و تراکم ایدیو بلاستهای اپی‌کارپ می‌تواند به عنوان صفات متمایز کننده گونه‌ای به شمار رود. دو نوع مزوفیل نردبانی–اسفنجی و نردبانی و دو نوع نسبت قطر اندوکارپ به اپی کارپ (۱/۲ و۲/۳) در بین گونه‌ها مشاهده شد. تزئینات پوسته نیز در همه گونه‌ها مشبک است، لیکن تفاوت جزئی در شکل سلولها و وجود حباب در دیواره‌های جانبی سلولهای اپیدرمی دانه‌ها دیده می‌شود.

INTRODUCTION

The genus *Verbascum* belonging to the tribe *Verbasceae* (*Scrophulariaceae*) (Valdes 1987) with 42 species in Iran (Sharifnia 2007) and 360 species worldwide (Judd et al. 2002) is the largest genus within the large family *Scrophulariaceae*. The main centers of its diversity are Turkey, Iran and Pakistan (Zohary 1973; Huber-Morath 1978). Its species are adapted to the various habitats and different regions of Iran including rocky mountains, open forests, road sides and the bank of the rivers. Among the species distributed in Iran, 15 are endemic (Sharifnia 2007). As hybridization is a very frequent phenomenon within *Verbascum* (Huber-Morath 1978, Karaveliogullari et al. 2004), a high frequency of morphological variations has been an intricate in delimiting the species.

In morphology, we still rely on the classification organized by Murbeck (1993), who divided the genus

into two sections: *Aulocusperma* (one species) and *Bothrosperma* (25 species). The latter section includes two subsections *Fasciculata* (with clustered flowers) and *Singuliflora* (with single flowers). Huber-Morath (1981) provided a key for the species based on the stem indumentum (branched or glandular hairs).

The palynology of the species was fulfilled by Karaveliogulari (2004); Dane & Yilmaz (2006); Olgun & Dane (1995) and Kheiri et al. (2006). All the species showed reticulate sculpture. Therefore, no species-specific observations were made within the genus.

Juan et al. (1997) conducted a research on the micromorphological characters of seed and fruits of 10 *Verbascum* species growing in Spain and found the capsule indumentum useful for grouping the species. They also provided a key for the species on the basis of the thickness of endocarp, the number of seeds per capsule and the type of the hairs on capsules. Attar et al. (2006) examined some more than 22 species growing in Iran for seed micromorphology and capsule indumentum and concluded that the micromorphological characters are not useful for grouping in *Verbascum*. However, capsule indumentum is the only reliable character for grouping *Verbascum* species.

The importance of anatomical studies was emphasized by Lersten & Curtis (2001) examining the leaves of 39 species of *Verbascum* and reporting the foliar idioblasts in the leaves of 13 species. Based on capsule anatomy, thickness of pericarp and the type of mesocarpic cells, Juan et al. (1997) distinguished between some Spanish *Verbascum* species.

The aim of this study is to examine the genus based on the leaf, seed coat and fruit anatomy, along with seed micromorphology to find taxonomic characters for species delimitation.

MATERIALS AND METHODS

A total of 18 populations belong to eight species of *Verbascum* including *V. szovitsianum* Boiss., *V. agrimonifolium* (C. Koch) Hub-Mor., *V. mucronatoum* Lam., *V. sinuatum* L., *V. oreophilum* C. Koch var. *oreophilum V. cheiranthifolium* Boiss., *V. macrocarpum* Boiss. and *V. speciosum* Schrader. were collected from mountains and plains of different regions of West Azerbaijan, Iran. A complete list of voucher specimens is given in Table 1. The herbarium specimens were deposited in the herbarium of Natural Resources and Agricultural Research Center of West Azerbaijan, Iran. Fresh leaf samples, mature fruits and seeds were collected and fixed in FAA solution for 48 hours. The samples were cleared with sodium hypochlorite 30% for 30 min., cut with double edged razor blade and stained with Carmine solution for 15 min. and Methyl Green for a few seconds, then fixed in Glycerin and studied by Light microscope model BX40 Olympus (LM). Leaves and seeds of some species were rinsed in 70% ethanol prior to dehydration. Dehydration and embedding were carried out by the tertiary butyl alcohol method (Johansen, 1940). Morphological observations were made and photographed by SEM, model Philips LX30 Autoscan.

RESULTS

Leaf anatomy

In this research, the presence of idioblasts in the mesophyll of leaves, the rate of density and shape of parenchymatous cells and the type of mesophyll were surveyed. The single celled idioblasts were observed in the mesophyll of *V. oreophilum* and *V. szovitsianum* from subsection *Fasiculata* and *V. agrimonifolium* & *V. macrocarpum* from subsection *Singuliflora* (Fig. 1. A & B). Idioblasts were round to elliptic in *V. oreophilum* and *V. agrimonifolium* and elliptic in *V. szovitsianum* and *Verbascum macrocarpum* (Fig. 1. A, B). Mesophyll was categorized in two types: dorsiventral and isobilateral. The type isobilateral was seen in *V. agrimonifolium* and *V. oreophilum*, in which adaxial and abaxial mesophyllous layers were made of palisadic parenchyma and there was a row of spongy cells between two layers (Fig. 1. A. & B). The type dorsiventral was seen in the mesophyll of five species, *V. szovitsianum, V. cheiranthifolium, V. mucronatum*, *V. sinuatum* and *V. macrocarpum* studied here and two different types of parenchymatous cells were detected in the leaves: adaxial palisadic cells and abaxial spongy cells (Fig. 1. C & D). Two different types of parenchymatous cells differing in density and form were detected within the species, type I: dentate palisadic parenchyma with less density and some intracellular spaces seen in *V. sinuatum* (Fig. 1. D), type Π: smooth palisadic parenchyma with cells attached together in a row observed in *V. agrimonifolium, V. mucronatum, V. macrocarpum, V. szovitsianum* and *V. cheiranthifolium* (Fig. 1. A, B, C).

Fruit anatomy

Two distinctive cellulosic epicarpic-mesocarpic and lignified endocarpic layers were distinguished in anatomical cross sections of pericarp. Moreover, there was a significant difference observed in the thicknesss ratio of endocarp to pericarp, the shape of idioblasts and the density rate of idioblasts in epicarp, among the species (Table 2) (Fig. 2. A-D).

Table1. *Verbasum* specimens used in the anatomical studies.

Species	Voucher specimen(s)
V. szovitsianum	Azerbaijan: Urmia to Oshnavieh road, 3 kilometers to Gausemlo valley,Kheiri 7518.
V. szovitsianum	Azerbaijan: Urmia to Oshnavieh road Gausemlo valley, Khaun valley, Kheiri 7522.
V. szovitsianum	Azerbaijan: Urmia to Oshnavieh road, Gausemlo valley, Nej valley, Kheiri 7525.
V. szovitsianum	Azerbaijan:Urmia, Noushinshahr 5Km, Asgaraubad, Sa'atloo station, Abedi 7525.
V. agrimonifolium	Azerbaijan: Urmia, Rajan , Kheiri, Rabat village, Alt:1700, Kheiri 7522.
V. agrimonifolium	Azerbaijan: Urmia to Oshnavieh road, 3 kilometers to Gausemlo valley, Kheiri 7520.
V. agrimonifolium	Azerbaijan: Urmia, Silvana road, Bardesour , Souluk village, Kheiri 7521.
V. agrimonifolium	Azerbaijan: Urmia to Oshnavieh road, Barandouz, Kheiri 7538.
V. speciosum	Azerbaijan: Urmia, Silvana road, Bardesour, Kheiri 7528.
V. speciosum	Azerbaijan: Urmia, Silvana road, Bardesour, Souluk, Kheiri 7531.
V. cheiranthifolium	Azerbaijan: Urmia, Band road, Kheiri 7529.
V. cheiranthifolium	Azerbaijan: Urmia, Silvana road, Kheiri 7530.
V. cheiranthifolium	Azerbaijan: Urmia, Rajan, Rabat valley, Kheiri 7532.
V. mucronatum	Azerbaijan: Urmia, Slivana road, Bardesour, Soulok village, Kheiri 7533.
V. mucronatum	Azerbaijan: Urmia, Rajan, Rabat valley, Kheiri 7534.
V. oreophilum	Azerbaijan: Urmia, Slivana road, Bardesour, Soulok village, Kheiri 7535.
V. macrocarpum	Azerbaijan: Urmia, Goutolu village, Kheiri 7536.
V. sinuatum	Azerbaijan: Urmia to Salmas road, Gouschi pass, Kheiri 7537.

Table 2. Anatomical fruit characters of *Verbascum* species.

Species	Density of idioblasts in epricarp	Shape of idioblast	Thickness ratio of endocarp to pericarp
Verbascum sinuatum	High	Huge elliptic longitudinally arranged in a row	2/3
Verbascum mucronatum	High (all the epicarp)	Huge elliptic perpendicularly arranged in a row	1/2
Verbascum szovitsianum	Low	Huge elliptic longitudinally arranged in a row	1/2
Verbascum oreophilum	Medium	Big & round	1/2
Verbascum speciosum	High (all the epicarp)	Huge elliptic perpendicularly arranged in a row	1/2
Verbascum macrocarpum	Low	Medium sized round to elliptic longitudinally arranged	1/2

Seed micromorphology

Seed morphology showed a slight difference among the species. So, it could be grouped in four categories as follows:

Group I: reticulate-shallow pitted in *V. agrimonifolium*, *V. mucronatum and V. szovitsianum* (Fig. 3: A, B, G & H) (Fig. 4. O); Group II: reticulate-pitted in *V. macrocarpum* , (Fig. 3: E, F); Group III: reticulate-wrinkled in *V. oreophilum, V. sinuatum and V. cheiranthifolium* (Fig. 4: I, J, K & L and Fig. 3:C & D); Group IV: reticulate- gemmate in *V. speciosum* (Fig. 4: M & N).

Also seed coat micromorphology showed longitudinal alveoli in *V. mucronatum, V. macrocarpum, V. agrimonifolium, V. szovitsianum* and *V. speciosum* or ridges in *V. oreophilum*, *V. cheiranthifolium* and *V. sinuatum*.

The shape of the seeds is variable: prismatic, prismatic-oblong, oblong, trigonous, ovate-trigonous. The cells are irregular or rectangular with or without vesicles on the walls.

The details on seed micromorphology are given in Table 3.

Seed anatomy

Anatomical observations on seed coat showed that the species are composed of a row of epidermal cells and a fibrous endothelium (Fig. 5. P & Q).

Table 3. Seed microcharacters of *Verbascum* species.

Species	Seed surface	Seed shape
Verbascum agrimonifolium	Rectangular cells with vesicles only on the angles of the walls	Prismatic, shallow alveolate with obtuse beak
Verbascum macrocarpum	Rectangular cells with vesicles only on the angles of the walls	Prismatic-oblong, wide & deep alveolate with truncate beak
Verbascum cheiranthifolium	Small polygonal or rectangular cells without vesicles	Oblong, deep & narrow alveolate with round beak
Verbascum oreophilum	Irregular small cells with indistinct vesicles	Trigonous, deep & narrow ridged with obtuse beak
Verbascum sinuatum	Irregular small cells with indistinct vesicles	Prismatic, narrow & deep ridged with apiculate beak
Verbascum mucronatum	Irregular large cells with dense vesicles covering all parts of the wall	Ovate, shallow and narrow ridged with round beak
Verbascum mucronatum	Irregular cells with gemmas	Ovate-trigonous, deep & narrow ridged with round beak

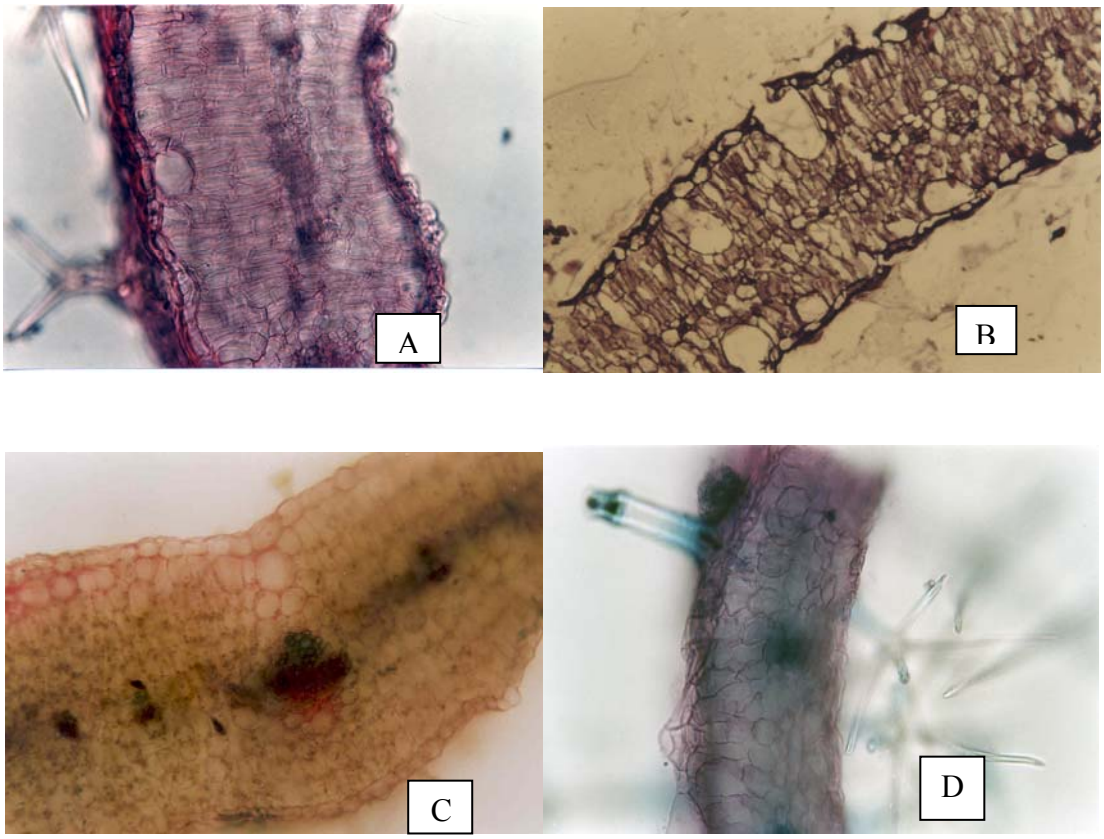

Fig. 1: A-C, Leaves of *Verbascum* species, mesophyll in transversal section; A, B, C: ×200 LM; D: ×100 LM; A, *V. oreophylum*; B, *V. agrimonifolium*; C. *V. mucronatum*; D. *V. agrimonifolium*.

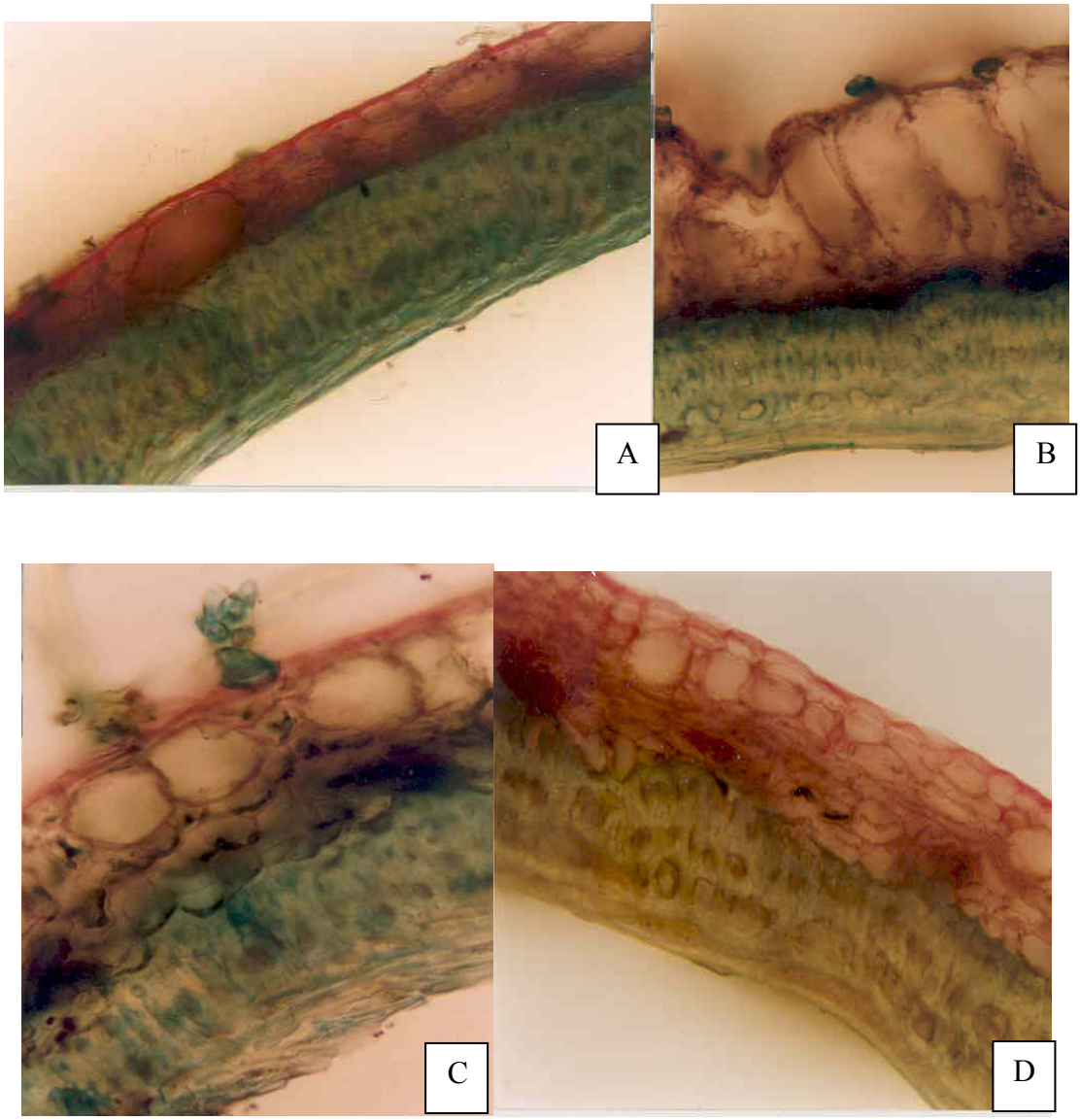

Fig. 2. A. LM micrographs of capsules, ×200. *Verbascum sinuatum*; B, *V. mucronatum*; C. *V. szovitsianum*; D. *V. macrocarpum*.

DISCUSSION

The anatomical study on leaves was carried out pursuing Lersten and Curtis (1997) who have reported mesophyllous idioblasts as a factor in identification within the genus *Verbascum*. According to the results obtained from the anatomy of leaves, the presence of idioblasts was distinct in four of our species, *V. agrimonifolium* (subsect. *Singuliflora*), *V. oreophilum* (subsect. *Fasiculata*), *V. szovitsianum* (subsect. *Fasiculata*) and *V. macrocarpum* (subsect. *Singuliflora*) (Fig. 1. A & B). The presence of idioblasts in *V. macrocarpum* and the lack of idioblasts in *V. mucronatum*, *V.sinuatum*, and *V. speciosum* are consistent with the data given by Lersten and Curtis (1997). Our study showed two types of mesophyll including dorsiventral (Fig. 1. C & D) and isobilateral (Fig. 1. A & B), which have not been reported before. Although the classification of the species based on anatomical features, due to low number of the species, was not possible however our results showed that the presence of idioblasts and the type of the mesophyll were useful in determining the species. We could not

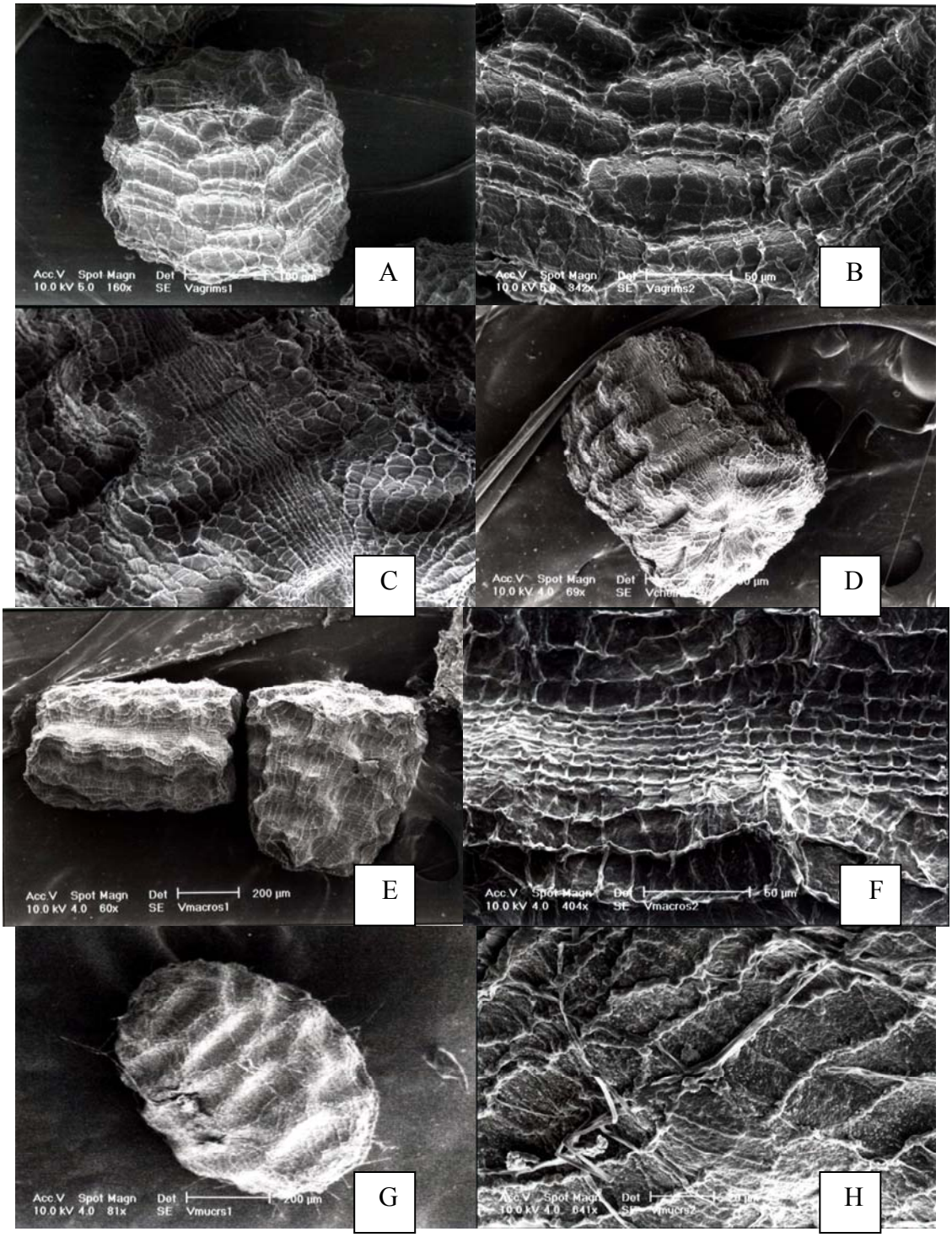

Fig. 3. A-H, SEM micrographs of seeds. A, B: *Verbascum agrimonifolium*; C, D: *V. cheirantifolium*; E, F: *V. macrocarpum*; G, H: *V. mucronatum*. Scale bar: 50 µm.

use the shape of idioblastic cells as it was a variable character.

There were some differences in the density of mesophyllous cells, for example palisadic parenchymateous cells in *V. sinuatum* had the least density and in *V. oreophilum* the most density in a well organized cells (Fig. 1. D, A). Having high rate of density among mesophyllous cells is a kind of compatibility for xerophytes to save the water in dry seasons (Fahn 1916). This ecological character can be seen in *V. oreophilum*, which grows on high hills of the mountains, while *V. sinuatum*, the species that has the loose mesophyll with much dentate cells grows in low altitudes and road sides (Fig. 1. D).

From the view point of capsular anatomy, our observations showed some distinctive differences in the density rate and shape of idioblastic cells in epicarp. For example in *V. sinuatum*, elliptic idioblasts elongated longitudinally with high density (Fig. 2. A); in *V. mucronatum*, elliptic idioblasts elongated perpendicularly, covering almost the whole epicarp (Fig. 2. B); and in *V. oreophilum*, rounded idioblasts with medium density (Fig. 2. C) and in *V. macrocarpum* round to elliptic idioblasts elongated longitudinally with low density (Fig. 2. D). Also two types of thickness ratio of endocarp to pericarp (2/3, 1/2) were distinguished (Table 2). Our findings support the data obtained by Juan et al. (1997), i.e.: the anatomical features of capsules was important in classification of the species within the genus *Verbascum*. However the study of anatomical features of capsules in more species allows separating morphologically similar species from each other.

Seed surface showed a reticulate pattern in all the studied species, which could be seen as alveolate or ridged in four features (reticulate-shallow pitted, reticulate-pitted, reticulate-wrinkled and reticulate-gemmate). The details of seed micromorphology are shown in Table 3. Presence of vesicles on the hexagonal or polygonal walls of the epidermis of seeds (Table 3) was in agreement with Attar et al. (2006) but the data on seed shape of some common species were in contrast with them. For example, the shape of seeds of *V. agrimonifolium* is distinguished as prismatic-oblong with obtuse beak in our investigation (Fig. 3. A, B). However, its shape was described as prismatic with apiculate beak by Attar et al. (2006). Also, some differences were found in the shape of some of our examined seeds with those described by Attar et al. (2006). For example *V. cheiranthifolium* showed deep and narrow alveolate ornamentation (Fig. 3. C, D), while it has been reported as deep and wide alveolate by Attar et al. (2006). A new finding could be seen in *V. speciosum*, in which the seed surface was densely covered by gemmas (Fig. 3. N). This characteristic has not been reported by others yet. It seems that the variability of seed shape and the width of alveoli or ridges were not useful in differentiating the species and it was in agreement with Attar et al. (2006). In contrast, the depth of depressions (alveoli or ridges) seems to be a constant character, the depressions were shallow in some species, while deep in others. The seed ornamentation of two species (*V. oreophilum and V. mucronatum*) has not been reported yet (Fig. 3. G and Fig. 4. J). The seed anatomy was in agreement with Elisens & Tomb (1983). No correlation was found among the examined characters (leaf anatomy, seed micromorphology and fruit anatomy) in our observations.

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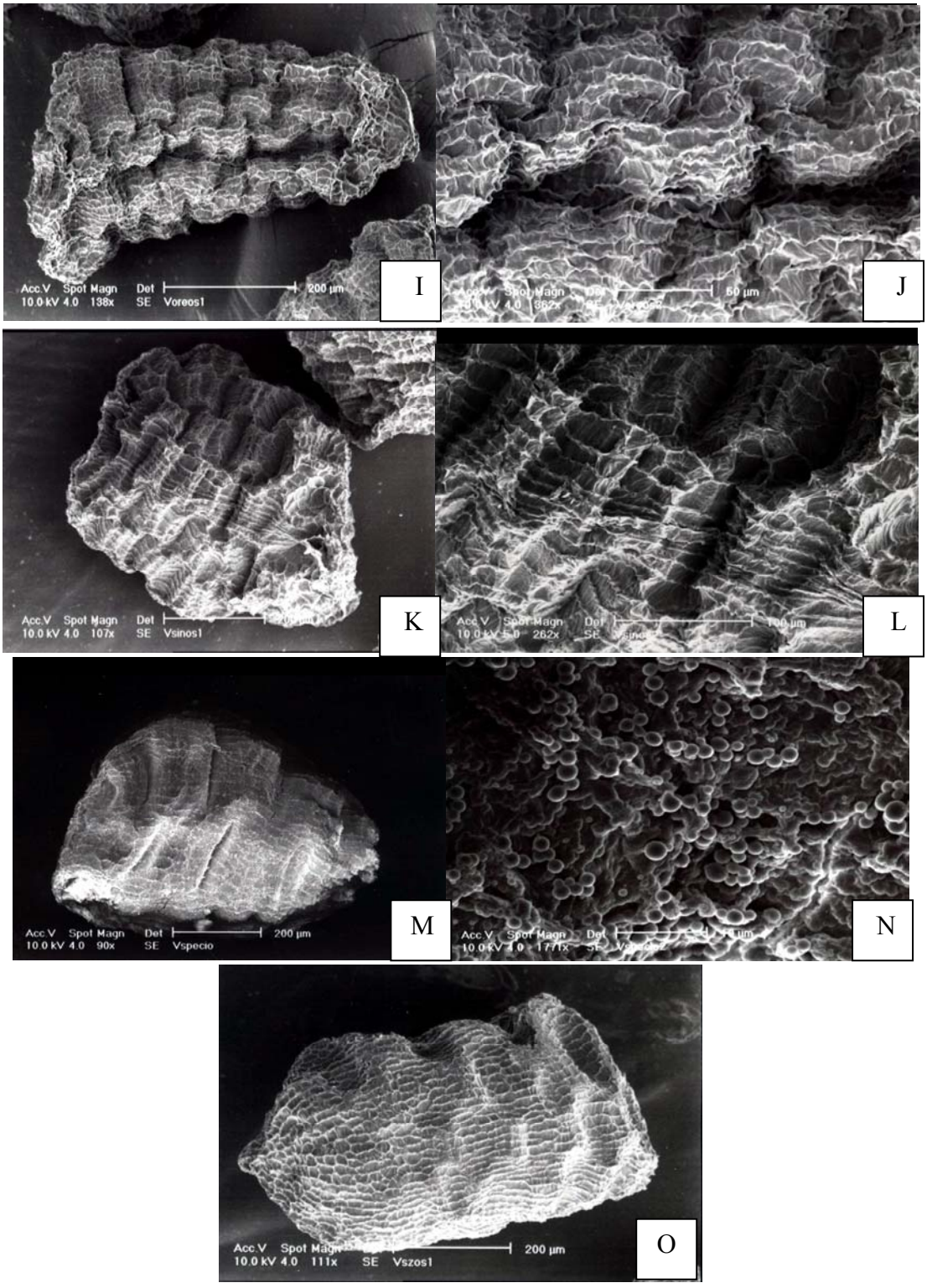

Fig. 4. I-O. SEM micrographs of seeds. I, J: *Verbascum oreophilum*; K, L: *V. sinuatum*; M, N: *V. speciosum*; O: *V. szovitianum*. Scale bar: 50 µm.

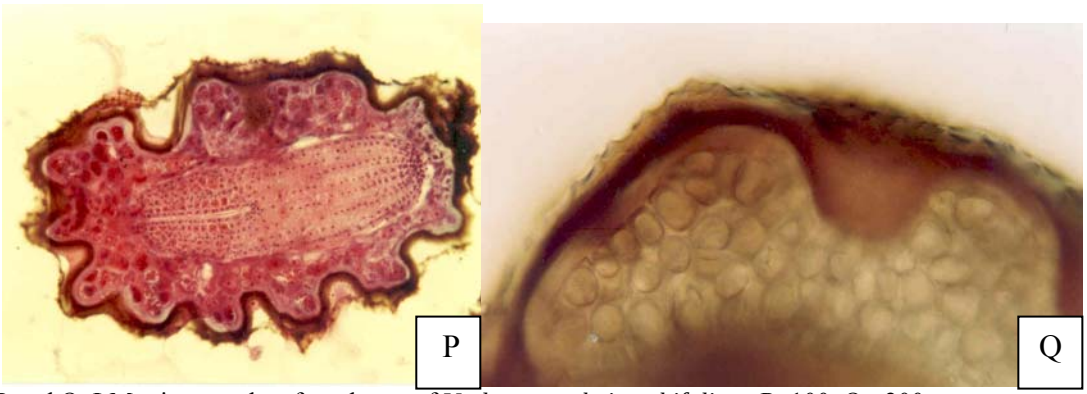

Fig. 5. P and Q. LM micrographs of seed coat of *Verbascum cheiranthifolium*. P×100, Q ×200.

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