

COMPARATIVE STEM ANATOMICAL AND PALYNOLOGICAL STUDIES ON *HYOSCYAMUS* L. SPECIES (SOLANACEAE) IN NORTHEAST OF IRAN

FOROUGH KHOSROMEHR, AZARNOOSH JAFARI* AND MOHAMMAD MAHDI HAMDI¹

*Department of Biology, Faculty of Sciences, Mashhad Branch,
Islamic Azad University, Mashhad, Iran*

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Abstract

Stem anatomical and palynological characteristics of some species of *Hyoscyamus* in NE Iran were examined. For anatomical study, manual cross sections of fresh and dried stems were prepared and stained by differential staining. In palynological study, the pollen were extracted from anther, acetolysed and observed by SEM. The results of internal structure of stem showed variation in the type cell of outer cortex, endodermis layer, secondary phloem diameter/cortex diameter and vessel arrangement. The palynological findings indicated trizoncolporate, reticulate, metareticulate, negative reticulum and striato-reticulate pollen in the species.

Introduction

The genus *Hyoscyamus* L. belongs to Solanaceae comprising 13 and seven species in Iran and Khorassan Razavi Province (NE Iran), respectively (Khatamsaz 1998, Chase and Reveal 2009). Despite reports about medicinal characteristics of this genus, internal structure and pollen studies have been made on a few of *Hyoscyamus* species e.g., studying of stem internal structure in Atlas stem anatomy of herbs, shrubs and trees (Schweingruber *et al.* 2013), pollen morphology of Solanaceae in Jordan (Al-Quran 2004), pollen morphology of Solanaceae from Pakistan (Perveen and Qaiser 2007) and pollen morphology of *Hyoscyamus* L. (Solanaceae) attracts *Hymenoptera* species as pollen visitors in Jordan (Al-Quran 2008). Comparative stem anatomical and palynological studies were carried on *Hyoscyamus* species in NE Iran to recognize variation in internal structure of stem and pollen micromorphological features among them.

Materials and Methods

For anatomical study, the base of stem from *H. arachnoides* Pojark, *H. kurdicus* Bornm., *H. pusillus* L., *H. reticulatus* L., *H. squarrosus* Griff. and *H. turcomanicus* Pojark were fixed in FAA solution, then cross sections were made and stained with green methyl and carmine. Finally observation of the cross sections were made under light microscope, Labomed model CMZ4 (Johnson 1940). The localities of the specimens from where they were collected are presented in Table 1. Anatomical characteristics of stem such as the number of cortex layer, endodermis layer, secondary phloem diameter, the type of secondary xylem and the arrangement of vessels were analyzed. For palynological study, the pollen of five species *Hyoscyamus* were extracted from the anther, dehydrated by glacial acetic acid, then acetolysed and studied by SEM and LM (Erdtman 1952, Moore *et al.* 1991). In this part, P/E ratio (polar axis length/equatorial axis length), the class and ornamentation of pollen were assessed at magnification 5000 and 20000. The pollen terminology is based on glossary of spores and pollen (Punt *et al.* 2007).

*Author for correspondence: <azarnoosh_djafari@mshdiau.ac.ir>. ¹Department of Biology, Faculty of Sciences, Central Tehran Branch, Islamic Azad University, Tehran, Iran.

Table 1. The locality of the studied species of *Hyoscyamus*.

Species	Locality
<i>H. turcomanicus</i>	10 km Shadmehr to Kashmar road, Khoumgi Darreh mount, 1250 m, Faghinihnia, 1996/4/5, 22331 (FUMH); Between Kashmar and Neyshabour, Chalpo, 1700 m, Hojat and Zangouei, 1997/4/28, 24014 (FUMH); South of Shirvan, Shirvan to Varak, 1850 m, Hosseinzadeh and Zangouei, 1997/4/25, 30274 (FUMH); West of Sabzevar, Mehrabad, 830 m, Khosromehr, 2010/5/3, 9153 (IAUM); Mashhad, Ladan Blvd, Ladan 33, Khosromehr, 2011/4/9, 9154 (IAUM).
<i>H. pusillus</i>	45 km Mashhad to Fariman, Khosromehr, 2011/4/26, 9156 (IAUM); 2 km Mashhad to Fariman, Khosromehr, 2011/4/26, 9157 (IAUM).
<i>H. arachnoides</i>	Sarakhs, Yartappeh, 300 m, Khosromehr, 2011/5/3, 9155 (IAUM); Kashmar, north east of Bardaskan, Sir village, Khosromehr, 1500 m, 2011/4/9, 9158 (IAUM).
<i>H. reticulatus</i>	75 km Mashhad to Torbat Heydarieh, before Asadabad, 1984 m, Rezaei and Zangouei, 1984/3/29, 8987 (FUMH); Binaloud mount, between Cheshmeh Sabz and Boujan, Khosromehr, 2011/5/5, 9152 (IAUM); Chenaran, between Abghad and Frizi, 1250 m, Khosromehr, 2011/5/7, 9151 (IAUM).
<i>H. kurdicus</i>	Mashhad, Zoshk, 1580 m, Khosromehr, 2011/3/24, 9159 (IAUM); Mashhad, Kardh dam, Faiz, 2011/4/9, 9160 (IAUM).
<i>H. squarrosus</i>	North east of Mashhad, Barzangiabad to Tabadkouh, 1300 m, Joharchi, 1995/4/5, 30277 (FUMH); Gonabad, 18 km Kakhk to Boskabad, 1900 m, Rafei and Zangouei, 1998/4/10, 26763 (FUMH); 20 km Bajestan to Noe, 1550 m, Rafei and Zangouei, 1998/4/10, 26883 (FUMH).

Results and Discussion

Anatomical results of stem revealed circular to elliptical and uni-seriate epidermal cell, outer and inner cortex including annular or angular collenchymatous and parenchymatous cells with idioblast. Compact and small collenchymatous cells in outer cortex was noticed in *H. turcomanicus*, *H. arachnoides*, *H. reticulatus* and *H. squarrosus* except *H. kurdicus* and *H. pusillus* which had large cells. *H. reticulatus* and *H. turcomanicus* had many idioblasts in inner cortex. Moreover, diffuse porous secondary xylem, solitary, radial chain pore and cluster vessels were noticed. Many xylary fiber with a few vessels were observed in *H. reticulatus*. The detailed information of internal structure of stem is presented in Table 2 and Figs 1A-F.

Table 2. The stem anatomical characteristics in the species of *Hyoscyamus*.

Species	Outer cortex layer	Inner cortex layer	S.ph/cortex	S.xy/s.ph	Vascular Ray	Arrangement of vessel
<i>Hyoscyamus arachnoides</i>	6	3	0.48	3.5	Uniseriate	R, Cl
<i>H. kurdicus</i>	4	2	3	2	Multiseriate	S, R
<i>H. pusillus</i>	4	4	0.33	4-7	Uni or biseriate	S, R
<i>H. reticulatus</i>	5	5	0.33	4	Uniseriate	S
<i>H. squarrosus</i>	3	7	0.25	5.5	Uni or biseriate	S, R
<i>H. turcomanicus</i>	4	3	0.9	5	Uni or multiseriate	S, R, Cl

S: Solitary vessel. R: Radial chain pore vessel. Cl: Cluster vessel. S.ph/cortex: Diameter of secondary phloem /cortex. S.xy/s.ph: Diameter of secondary xylem/secondary phloem.

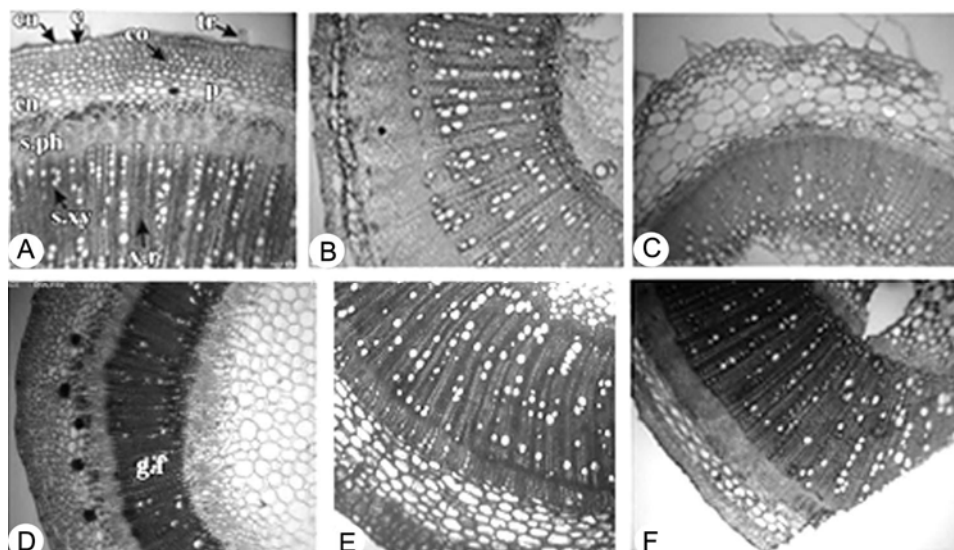


Fig. 1. Cross section of stem: A. *H. arachnoides*. B. *H. kurdicus*. C. *H. pusillus*. D. *H. reticulatus*. E. *H. squarrosus*. F. *H. turcomanicus*. Tr = Trichome, e = Epidermis layer, cu = Cuticle, co = Collenchymatous layer, p = Parenchymatous layer, en = Endoderm, s.ph = Secondary phloem, s.xy = Secondary xylem, v.r = Vascular ray, g.f = Xylary fiber.

Palynological results showed trizonocolporate pollen class. Elliptical and oblong triangular pollen was noticed in *H. squarrosus*, *H. turcomanicus* and *H. arachnoides*, *H. kurdicus* *H. pusillus* respectively. While in polar view the pollen shape was spherical or semi-spherical. Moreover, the ornamentation of pollen was observed reticulate, striato-reticulate, metareticulate and negative reticulum. In *H. kurdicus* and *H. squarrosus* reticulate ornamentation included a few spine with circular small lumina and many spin with large lumina, respectively (Table 3 and Figs. 2A-J).

Table 3. Pollen characters in the studied species of *Hyoscyamus*.

Species	Pollen class	Exine ornamentation	Lumina shape	P (μ)	E (μ)	P/E
<i>Hyoscyamus arachnoides</i>	Prolate Spheroidal	Negative reticulum	Rectangular	40.1	38.56	1.03
<i>H. kurdicus</i>	Prolate	Reticulate, echinate	Circular	36.28	19.73	1.83
<i>H. pusillus</i>	Subprolate	Striato-reticulate	Elliptical	37.14	31.42	1.18
<i>H. squarrosus</i>	Perprolate	Reticulate, echinate	Circular	47.71	22.85	2.08
<i>H. turcomanicus</i>	Subprolate	Metareticulate	Circular	38.56	32.85	1.17

In anatomical analysis of stem, the minimum and maximum number of outer cortex layers were noticed in *H. arachnoides* (6-layered) and *H. squarrosus* (3-layered). Only cortex in *H. kurdicus* was completely parenchymatous. The minimum and maximum number of inner cortex layers were observed in *H. kurdicus* (2-layered) and *H. squarrosus* (7-layered). In *H. reticulatus*, *H. pusillus*, *H. squarrosus*, *H. arachnoides*, and *H. turcomanicus* diameter of the secondary phloem

is less than cortex. Endodermis layer had variable casparian strip. Just *H. arachnoids* had no solitary vessels. Since solitary and cluster vessels are primitive and advanced characteristic, so *H. reticulatus* had primitive and *H. arachnoids*, *H. turcomanicus* had advanced vessel arrangement. Also, *H. reticulatus* had no radial chain pore vessel. While, Schweingruber showed one ring in an annual plant with mainly solitary vessels in *H. bohemicus*. Schweingruber believed differences in stem internal structure is related to growing period and age of plant so they are not significant features to identify studied species (Schweingruber *et al.* 2013).

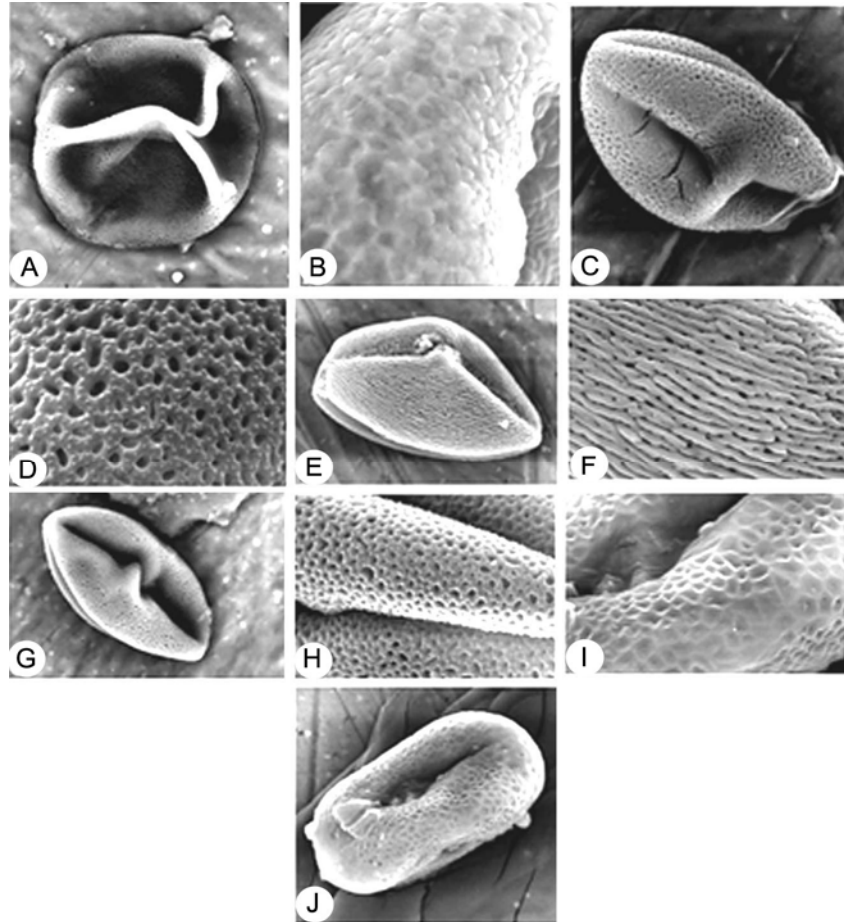


Fig. 2. The pollen shape in polar view $\times 5000$ and exine ornamentation $\times 20000$: A, B. *H. arachnoides* $\times 5000$ (in equatorial view), 20000. C, D. *H. kurdicus* $\times 5000$, 20000. E, F. *H. pusillus* $\times 5000$, 20000. G, H. *H. squarrosus* $\times 5000$, 20000. I, J. *H. turcomanicus* $\times 20000$, 5000.

The results of palynological study indicated the presence of prolate, subprolate, perperoplate and prolate spheroidal pollen class. Four types of ornamentation (reticulate, striato-reticulate, metareticulate and negative reticulum) were observed. The minimum and maximum P/E was perceived in *H. arachnoides* (1.03) and *H. squarrosus* (2.08), respectively. In the previous reports, Al-Quran reported pantoporate pollen for 11 species of *Hyoscyamus* collected from different parts

of Jordan with small differences i.e., the pollen class 3-zonocolporate in *H. reticulatus*, 3-loxocolporate in *H. albus* and 4-loxocolporate in *H. aureus* (Al-Quran 2004). Al-Quran found elliptic pollen in equatorial view and angular pollen in polar view of *H. aureus*, *H. albus*, *H. reticulatus*. He believed the pollen class, P/E ratio, pollen outline (polar view, equatorial view, P/E) are not diagnostic palynological criteria because of the high degree of similarities between the examined species (Al-Quran 2004). Moreover, Perveen and Qaiser (2007) mentioned suboblate with P/E (0.66) for *H. niger* pollen despite the results of the present study for another species.

In conclusion, there was no quite considerable variation in stem anatomy, pollen morphology and exine ornamentation so the pollen characteristics had no systematic value to recognize circumscription of the studied specimens.

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