

An investigation of the anatomy, palynology and trichome types of *Phlomis olivieri* (Lamiaceae)

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Abstract

The anatomy, palynology and trichome types of *Phlomis olivieri* were studied in order to understand the usefulness of these characteristics for systematic purposes. Results showed that vascular bundles were next to each other in the stem, the mesophyll was composed of 1-layered palisade and 3-4 layered spongy parenchyma. There were two collateral vascular bundles in the centre and two small subsidiary bundles in the corners of petiole and there were 4 small vascular bundles in the bracteole. Five main types of trichomes (peltate, capitate glandular, stellate, unicellular simple and dendroid trichomes) were observed. The capitate trichomes were subdivided into three groups: type 1 (short stalk), type 2 (medium or tri-cellular stalk) and type 3 (long or four-cellular stalk). Stellate trichomes were subdivided into two groups: group 1, sessile or short stalked trichomes and group 2, long stalked trichomes. The dendritic trichomes also were reported for the first time in this species. The pollen grains were tricolpate, relatively large, ovate and the exine ornamentation was reticulate and perforate.

Key words: Anatomy, Lamiaceae, Palynology, Trichome, *Phlomis*, Iran

Introduction

The genus *Phlomis* L. is one of the largest genera of subfamily Lamioideae (Lamiaceae) with more than 100 recognized species distributed in Asia, south Europe and north Africa that have been divided into two main sections: *Phlomis* and *Phlomoides* (Moench) Briq. (Rechinger, 1982). Section *Phlomis* was subdivided by Bentham (1834) into three subsections: *Dendrophlomis* Benth., *Gymnophlomis* Benth. and *Oxyphlomis* Benth. The diagnostic character for separating sections is corolla shape. Corolla in sect. *Phlomis* have a curved upper lip and a trifid lower lip with large median and smaller lateral lobes as opposed to the presence of a straight upper lip and a trifid lower lip with subequal lobes in sect. *Phlomoides* (Azizian and Moore, 1982). The genus represented by nearly 19 species in Iran including *Ph. olivieri* Benth. which grows wildly in the north, northwest, west and centre of Iran (Rechinger, 1982; Jamzad, 2012).

The taxonomic value of the indumentum as well as its implication in systematics and

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phylogenetics are well known in Lamiaceae and in the related families Acanthaceae, Bignoniaceae, Scrophulariaceae and Verbenaceae (Abu-Asab and Cantino, 1987; Ahmad, 1974, 1978; Cantino, 1990; El-Gazzar and Watson, 1968, 1970; Elias and Newcombe, 1979; Gairola *et al.*, 2009; Mathew and Shan, 1983; Metcalfe and Chalk, 1972; Rahn, 1992). Trichome micromorphology has been suggested to be useful in the phylogeny reconstruction (Abu-Asab and Cantino, 1987) and has been widely used in the systematics of Lamiaceae (Cantino, 1990; Kaya *et al.*, 2000; Erken, 2005; Satil *et al.*, 2007; Dinç and Öztürk, 2008; Güvenç and Duman, 2010; Celep *et al.*, 2011) as well as specific and subspecific levels (Bruni *et al.*, 1987; Giuliani *et al.*, 2008; Bini Maleci *et al.*, 1992; Sebebe and Harley, 1992; Servettaz *et al.*, 1992).

The importance of pollen morphology within the Lamiaceae has been proven as supplementary data to classification (Abu-Asab and Cantino, 1992, 1993, 1994; Dinç *et al.*, 2009). A comprehensive overview of the pollen survey within subfamily Lamioideae sensu Erdtman (1945) has been published by Abu-Asab and Cantino (1994). Moreover, some further examinations of pollen have been carried out, e.g. by Sebebe and Harley (1992: for *Stachys* L.), Dönmez *et al.* (1999: for *Teucrium* L.), Satil *et al.* (2005: for *Thymus* L.), Dinç and Öztürk (2008: for *Stachys* sect. *Ambleia* Benth.), Dinç *et al.* (2009: for *Lallemantia* L.) and Asadollahi *et al.* (2014: for *Salvia* L.). The pollen morphology generally supported the segregation of some genera of Lamiaceae such as *Marrubium* L., *Phlomis* and *Stachys*. However, its taxonomic value for infrageneric classification varies in the family Lamiaceae.

Up to now, some studies on *Phlomis* have been conducted (Bech, 1963; Azizian and Cutler, 1982; Azizian and Moore, 1982). But neither of them exactly investigated the anatomy, palynology and trichome types of *Ph. olivieri*. Therefore, the present study aims to investigate the trichome types, palynology and anatomy of *Ph. olivieri* as an important rangeland plant of Iran.

Materials and Methods

Specimens of *Phlomis olivieri* were prepared from fresh material collected from various wild populations from west of Iran and herbarium specimens. Voucher specimens (Table 1) were deposited in the herbarium of the University of Isfahan. Anatomical studies were performed using an average of 40 fresh specimens kept in 70% ethanol. All sections were made from the stems, leaves, petioles, calyces and bracteoles using commercial razor blades. Sections were stained with Methyl blue and carmine and mounted on the slides using Canada balsam. Samples were studied using an Olympus microscope model BX-41 light microscope with 40X to 400X magnifications. For identification of trichome types, trichomes were obtained from surface of stems, leaves, petioles, calyces and bracteoles were studied using an Olympus microscope model BX-41 light microscope with 40X to 400X magnifications. For palynological study, pollen grains were obtained from herbarium samples. The pollen slides were prepared according to Wodehouse (1935) technique. For LM investigation, measurements and observations were made using the Olympus microscope model BX-41 LM. For the scanning electron microscopy (SEM), the pollen grains were observed and photographed with a KYKY-3200 SEM to determine their exine ornamentation. Also pollen terminology has been used based on Punt *et al.*, 2007.

Table 1. Voucher specimens of *Phlomis olivieri* used in this study

No.	Collection site	Altitude (m)	Collector	Date
1	Hamedan: Asada Abad mountains	2180	Yousefi 19013 (HUI)	4 June 2011
2	Hamedan: Malayer, Lashkardar	2118	Yousefi 19011 (HUI)	7 June 2011
3	Hamedan: Nahavand, Giyan	1737	Yousefi 19005 (HUI)	5 June 2011
4	Hamedan: Razan, Boghaty Mountains	2148	Yousefi 19012 (HUI)	12 June 2011
5	Hamedan: Heidare village	2114	Yousefi <i>s.n.</i> (HUI)	4 June 2011
6	Hamedan: road of Touyserkan to Malayer	1891	Yousefi 19010 (HUI)	7 June 2011
7	Kermanshah: 95 km from Kermanshah to Kernerd Gharb	1425	Yousefi <i>s.n.</i> (HUI)	1 June 2011
8	Kermanshah: road of Biseton to Sonqor, Karkasar, Mooineh village, Dalakhani mountains	1800	Yousefi <i>s.n.</i> (HUI)	1 June 2011
9	Kurdestan: road of Hamedan to Sanandaj	1848	Yousefi <i>s.n.</i> (HUI)	2 June 2011
10	Kurdestan: Salavat Abad mountains	2016	Yousefi <i>s.n.</i> (HUI)	2 June 2011

Results

Phenology and habitat

Phlomis olivieri is a perennial herb species distributed in Iran and Iraq, and flowering in June to August. This plant grows on mountainous regions, adjacent to rocky slopes, steppe vegetation and the overgrazed rangeland soils of Irano-Turanian region and Hyrcanian district of Iran (Jamzad, 2012) and could be as one of important destroyed rangeland indicator together with *Stachys inflata* Benth. (Mozaffarian, 2005) (Figure 1).



Figure 1. General appearance of *Phlomis olivieri* (yellow flower) together with *Stachys inflata*

Anatomical characteristics

Stem

The stem was clearly quadrangular. The epidermis was covered by a thin cuticle. The epidermis consisted of a single layer of oval, squarish and rectangular cells. Underneath the epidermis, multi-layered collenchyma cells were located at the corners of the stem. The cortex was composed of 2-5 layers of irregular oval and rectangular parenchymatic cells with intercellular spaces. Vascular bundles were next to each other. Those located at the corners were slightly bigger in size than the others. Cambium was not visible. Primary and secondary xylem could be differentiated. Tracheae in the secondary xylem were denser and larger than in the primary xylem. The pith was large and comprised of hexagonal or orbicular parenchymatic cells with intercellular spaces in the centre of stem (Table 2, Figure 2).

Leaf

Transverse section of the lamina indicated that the upper and the lower epidermis were covered with a nearly thick cuticle layer. The thickness of both epidermises cuticles was nearly equal. Both epidermises consisted of 1 layered oval and rectangular cells. Upper epidermal cells were larger than lower epidermal cells or nearly equal to them. The mesophyll was composed of elongated rectangular palisade parenchyma and spongy parenchyma cells. The palisade parenchyma was 1 layered. The spongy parenchyma cells were 3-4 layers. The spongy parenchyma cells had intercellular spaces. Transverse section of the midrip showed that the adaxial surface was concave and the abaxial surface was convex. The epiderm was 1 layer. There was one large vascular bundle in the center that was surrounded by parenchymatic cells (Table 2, Figure 3). Stomata are diacytic.

Table 2. Anatomical measurement of various tissues of *Phlomis olivieri*

Material	Length Min-Max	Mean \pm S.D.	Width (μ m) Min-Max	Mean \pm S.D.
Stem				
Epidermis cell	0.5-1.1	0.78 \pm 0.25	0.4-0.7	0.54 \pm 0.11
Collenchyma cell	0.5-3	1.54 \pm 1.03	0.4-1.6	0.88 \pm 0.50
Parenchyma cell	1-2	1.42 \pm 0.37	0.5-1.1	0.8 \pm 0.25
Pith cell	1-3.5	2.48 \pm 0.97	1-3.3	2.16 \pm 0.90
Leaf				
Upper epidermis cell	0.7-1.6	1.26 \pm 0.35	0.3-1	0.68 \pm 0.25
Lower epidermis cell	0.4-1	0.72 \pm 0.21	0.4-0.7	0.52 \pm 0.13
Palisade parenchyma	2.5-2.8	2.70 \pm 0.12	0.4-0.5	0.44 \pm 0.05
Spongy parenchyma	-	-	0.5-1.2	0.88 \pm 0.25
Pith cell	1.1-2.1	1.64 \pm 0.37	0.8-2	1.52 \pm 0.44
Petiole				
Adaxial epidermis cell	0.6-1.4	0.86 \pm 0.31	0.4-0.7	0.54 \pm 0.11
Abaxial epidermis cell	0.4-1.4	0.84 \pm 0.37	0.5-0.9	0.66 \pm 0.15
Fiber cell	0.4-0.8	0.54 \pm 0.16	0.2-0.8	0.48 \pm 0.23
Parenchyma cell	1.5-3	2.14 \pm 0.65	1.3-2.1	1.72 \pm 0.37
Calyx				
Upper epidermis cell	0.2-0.6	0.48 \pm 0.16	0.2-0.5	0.34 \pm 0.11
Lower epidermis cell	0.7-1.5	1.26 \pm 0.39	0.6-0.7	0.66 \pm 0.05
Parenchyma upper fiber cell	0.5-0.8	0.64 \pm 0.13	0.3-0.7	0.53 \pm 0.17
Parenchyma lower fiber cell	2.1-3.9	3.12 \pm 0.79	1.5-1.9	1.64 \pm 0.19
Fiber cell	0.6-0.8	0.72 \pm 0.08	0.4-0.5	0.44 \pm 0.05
Bracteole				
Adaxial epidermis cell	0.7-2.5	1.26 \pm 0.72	0.3-0.8	0.64 \pm 0.20
Abaxial	0.5-0.8	0.60 \pm 0.12	0.4-0.5	0.44 \pm 0.05
Parenchyma cell	0.3-1.1	0.58 \pm 0.33	0.2-0.7	0.42 \pm 0.19

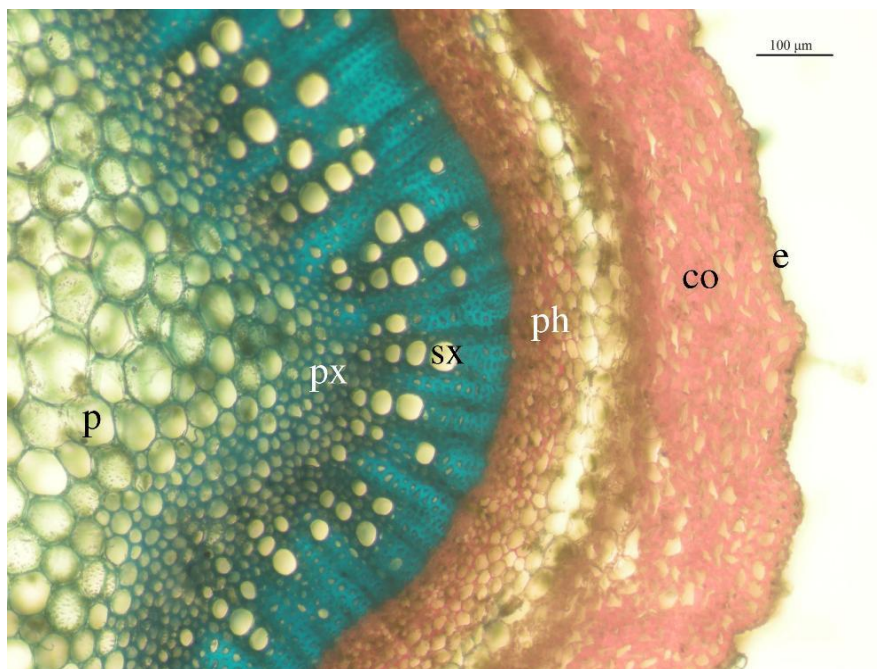


Figure 2. Cross-section of the stem of *Phlomis olivieri*. e. Epidermis; cp. Cortex parenchyma; ph. Phloem; sx. Secondary xylem; px. Primary xylem; p. Pith. Scale Bar= 100 μ m

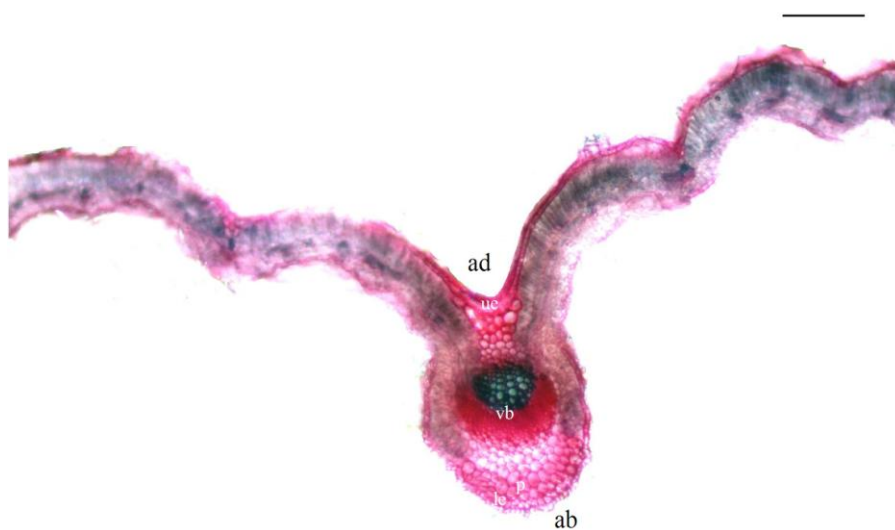


Figure 3. Cross-sections of the leaf blades of *Phlomis olivieri*. ab. Abaxial epidermis of midrib; ad. Adaxial epidermis of midrib; le. Lower epidermis; ue. Upper epidermis; vb. Vascular bundle. Scale Bar = 200 μ m

Petiole

The adaxial surface was concave and the abaxial surface was convex. Both epidermises were one layer and covered with a thick cuticle layer. The epidermal cells of both surfaces were more or less rectangular to oval. Under epidermis, 2-7 collenchyma layers had placed, as well as collenchyma density at the corner was more than other parts. Parenchyma cells were hexagonal or orbicular. There were two collateral vascular bundles in the centre and two small subsidiary bundles in the corners of petiole (Table 2, Figure 4).

Calyx

The both epidermises were one layer and covered with a thick cuticle layer. The upper epidermal cells were nearly longer than the lower epidermal cells. Parenchyma cells consisted

of 4-5-layer cells. A single layer of elongated continuous fiber cells was observed under parenchyma cells. A one small parenchyma bundle was visible in middle of fiber cells. The expanded parenchyma cells had located under fiber cells (see Table 2).

Bracteole

The upper and lower epidermises were one layer and covered with a thick cuticle layer. 3-4 parenchyma layers had placed after vascular bundles. The fiber cells were observed among of xylem cells (see Table 2).

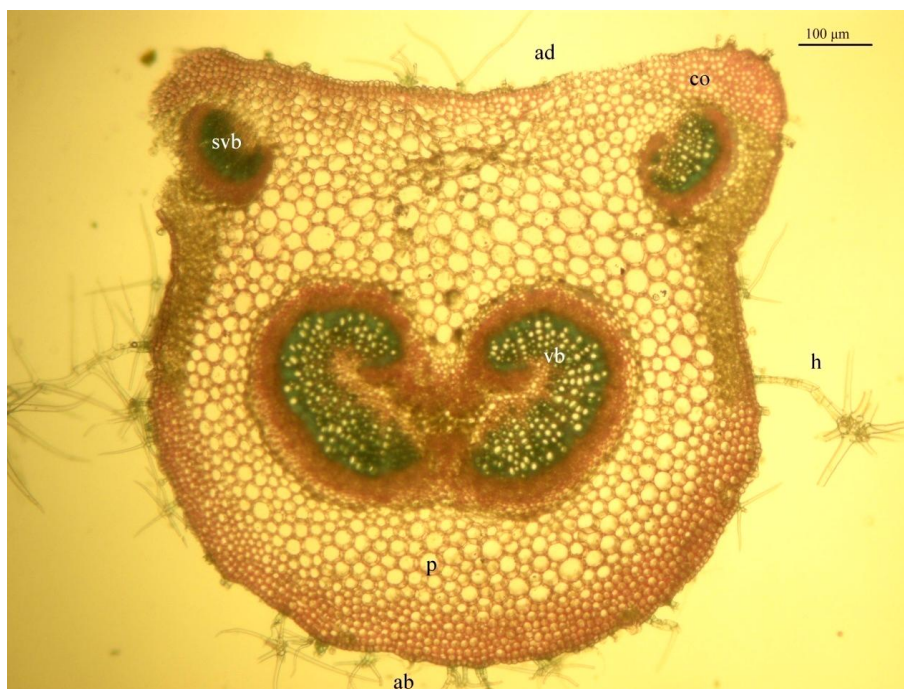


Figure 4. Cross-section of the petiole of *Phlomis olivieri*. ab. Abaxial epidermis; ad. Adaxial epidermis; co. Collenchyma; h. Hair; p. Parenchyma; svb. Subsidiary vascular bundles; vb. Vascular bundles. Scale Bar: 100 μ m

Trichome morphology

Five different trichome types on the stems, leaf blades, petioles, calyces and bracteoles of *Phlomis olivieri* were observed: peltate, capitate glandular, stellate, unicellular simple and dendroid trichomes (Figure 5). Capitate glandular and peltate trichomes could be distinguished by head size and stalk length (Ascensão and Pais, 1998). In the capitate trichome, the length of the stalk should be more than half the height of the head, whereas peltate trichomes were short with a uni- or bicellular stalk and a large secretory head with 4 to 18 cells arranged in one or two concentric circles (Werker *et al.*, 1985). Type 1 is the typical peltate glandular trichome and consisted of a basal epidermal cell, a very short unicellular stalk and a round multicellular secretory head consisting of four or eight cells. Type 2 was a capitate glandular trichome composed of a basal epidermal cell, unicellular to bicellular stalk of variable length and a large, unicellular or bicellular secretory head. Type 3 was a non-glandular (stellate) trichomes consisted of several basal epidermal cells, and branched at the tip of the stalk cell to form star-shaped that divided to two groups: group 1 (sessile or short stalked stellate trichomes) and group 2 (long stalked trichomes). The First group trichomes did not have stalk or have short stalk but the second group trichomes had long stalk to subdivided into three subgroups: stalks are uni-, bi- and multiseriate. Type 4 was dendroid trichomes that this kind of trichomes was branched along the stalk cell and the branching was

along the biserrate long stalk cells. Type 5, this type was composed of unicellular simple trichomes that were observed nearly in all of parts. The sessile or short stalk and multiseriate stalked stellate trichomes were on the adaxial surface of leaf but uni- and biseriate stellate trichomes mostly were observed on the abaxial leaf surface. The density of the stellate (especially, sessile and multiseriate) trichomes were in abundance on the abaxial surface of calyx. The glandular trichomes were present in abundance on the abaxial surface of leaf. The most density of glandular trichomes was observed on the adaxial surface of petiole and the most density of stellate trichomes was observed on the adaxial surface of bracteole (Table 3). The dendroid trichomes were only observed on abaxial surface of calyx. The dendritic trichomes were reported for the first time in this species.

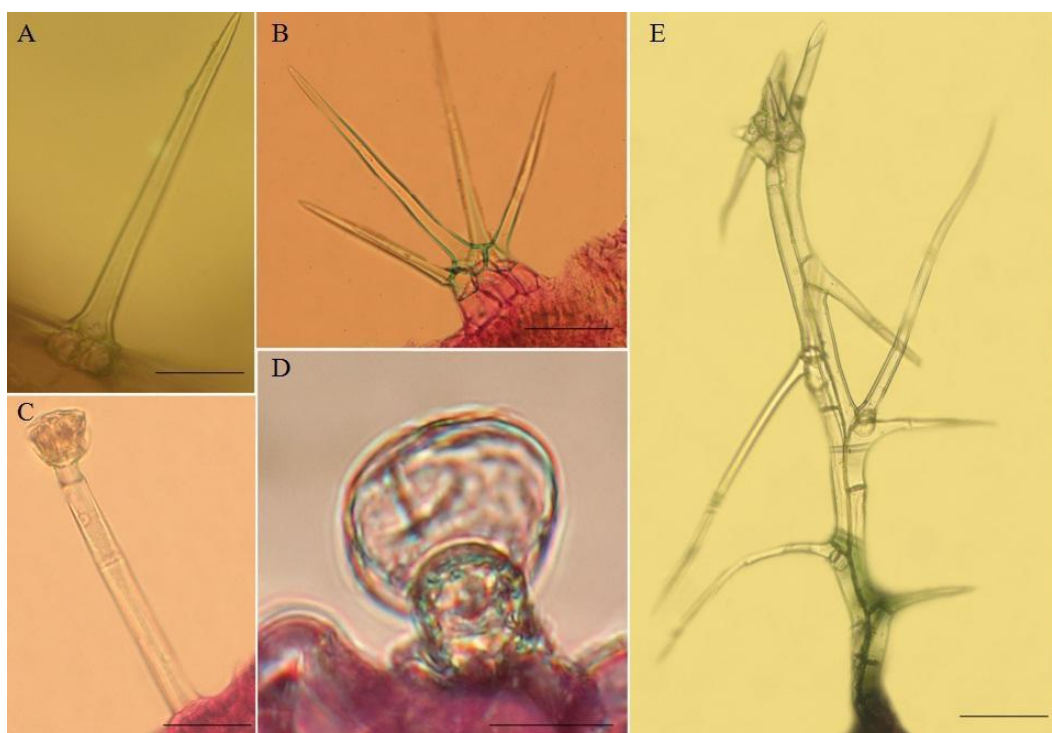


Figure 5. Types of trichome in *Phlomis olivieri*. A. Unicellular simple; B. Stellate; C. Capitulate glandular; D. Peltate glandular; E. Dendroid. Scale Bar = 10 µm

Table 3. Distribution and density of trichome types in *Phlomis olivieri*. - absent, ± scarce, + present, ++ abundant

Material	Peltate	Glandular type 1 capitate	Glandular type 2 capitate	Glandular type 3 capitate	Type 1 stellate	Type 2 stellate	Dendroid	Unicellular
Stem	±/+	+	+	-	-	+	-	+
Adaxial leaf side	++	++	±	±	±	++	-	+
Abaxial leaf side	++	++	±	++	++	±	-	+
Petiole	++	+	±	±	±	+	-	+
Abaxial calyx side	+	±	++	±	++	+	++	+
Adaxial bracteole side	+	±	-	-	++	++	-	+

Pollen characteristics

The pollen grains were tricolpate. Their shape was protate-spheroidal. The dimensions of polar axis and equatorial axis were 38.15-41.26 and 26.05-29.45 µm, respectively. The ratio P/E was 1.39-1.48. The exine thickness was 2.95-3.15µm. Exine ornamentation was reticulate and perforate (Figure 6, Table 4).

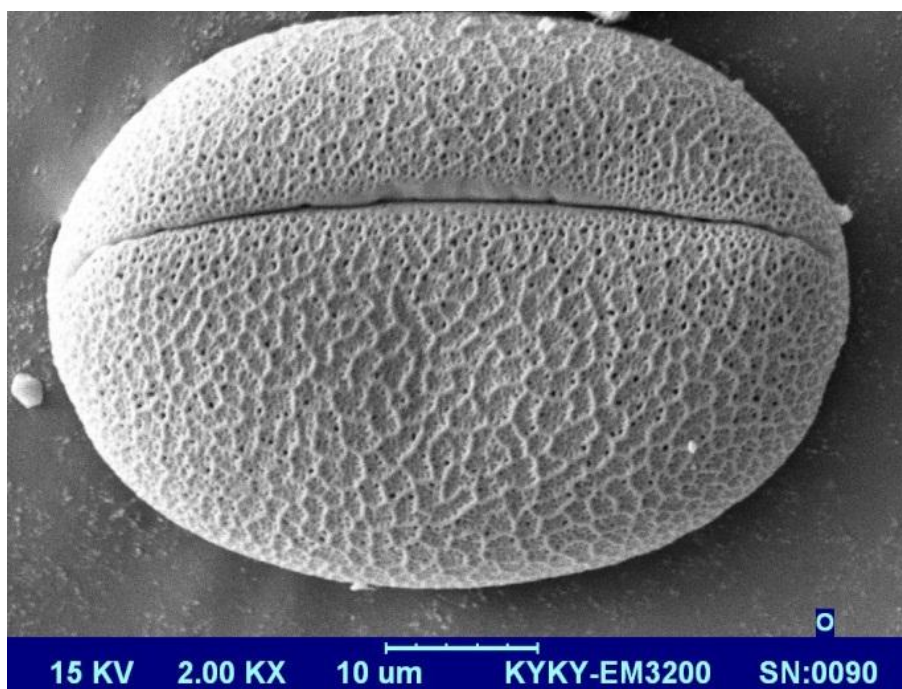


Figure 6. General appearances of the pollen grains in *Phlomis olivieri*

Table 4. Pollen morphological data of *Phlomis olivieri* (μm) showing minimum-maximum ranges and mean value \pm standard deviation. All measurements are in μm except for ratio of polar axis/equatorial axis

Characters	Minimum-maximum values	Mean \pm Standard deviation values
Polar axis	38.15-41.26	39.72 \pm 1.13
Equatorial axis	26.05-29.45	27.88 \pm 1.26
Ratio of polar axis/equatorial axis	1.39-1.48	1.43 \pm 0.03
Exine thickness	2.95-3.15	3.05 \pm 0.08

Discussion

The *Phomis olivieri* is an important rangeland plant in Iran also the anatomical characters, palynology and trichome types of this species were exactly examined. This is the first report from the anatomical characters of calyx and bracteole in this species. Metcalfe and Chalk (1972) pointed out that the structure of the vascular bundles in the petiole of the species of Lamiaceae could be used as a diagnostic character. In the petiole of this species, there is two-lobed vascular bundle in the centre and 1 small subsidiary bundle in petiolar wings. Therefore, the anatomical properties of the petiole may be useful characters for distinguishing and separation of this species from other species in the genus. Four vascular bundles were observed in bracteole. The number of vascular bundles in bracteole could be considered as a remarkable character. Five main types of trichomes were observed. Our findings about trichomes types are not consistent with Azizian and Cutler (1982). Our study about trichomes showed the presence of dendroid trichomes on abaxial surface of calyx. The dendroid trichomes are reported for the first time in *Ph. olivieri* from Iran whereas its importance remains unsound.

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بررسی آناتومی، گرده‌شناسی و انواع کرک گونه *Phlomis olivieri* از تیره Lamiaceae

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چکیده

در مطالعه حاضر، صفات آناتومی، گرده‌شناختی و انواع کرک گونه *Phlomis olivieri* به منظور شناخت آنها جهت به کارگیری در اهداف سیستماتیک بررسی شد. نتایج نشان داد که دستجات آوندی در ساقه در کنار هم قرار دارند. مزوفیل از یک لایه پارانشیم نرده‌ای و سه تا چهار لایه پارانشیم اسفنجی تشکیل شده است. دو دسته آوند همجوار در مرکز و دو دسته آوند فرعی کوچک در حاشیه دمبرگ و چهار دسته آوند کوچک در برگک وجود دارد. پنج نوع اصلی کرک (سپری، غده‌ای سرسان، ستاره‌ای، ساده تک سلولی و درختی) مشاهده شد. کرک‌های سرسان به سه گروه طبقه‌بندی شدند: نوع اول (پایه کوتاه)، نوع دوم (پایه متوسط یا سه سلولی) و نوع سوم (پایه بلند یا چهار سلولی). کرک‌های ستاره‌ای به دو گروه بدون پایک یا پایه کوتاه و پایه بلند طبقه‌بندی شدند. کرک‌های درختی نیز برای نخستین بار در این گونه گزارش می‌شود. دانه‌های گرده سه شیاری، نسبتاً بزرگ، تخم‌مرغی و تزئینات سطح لایه خارجی دانه گرده مشبک و روزن‌دار هستند.

واژه‌های کلیدی: آناتومی، تیره نعنائیان (Lamiaceae)، گرده‌شناسی، کرک، *Phlomis*، ایران