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POLLEN AND NUTLET MICROMORPHOLOGY IN *PHLOMIS ARMENIACA* WILLD. AND *P. SIEHEANA* RECH.F. (LAMIACEAE)

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Abstract

Phlomis sieheana and *P. armeniaca* species are known as two close taxa that are confused with each other when firstly observed in their natural habitats. In the current investigation, the morphological features of *Phlomis sieheana* and *P. armeniaca* belonging to the Lamiaceae family have been revised and their anatomical, palynological and micromorphological features have been presented. The pollen grains of the studied species are monad, isopolar, and radially symmetrical. The pollen shape of *P. sieheana* is subprolate, while *P. armeniaca* is prolate-spheroidal. Also, exine sculpture was observed as reticulate-perforate in both species. However, the types of pollen aperture of the species are determined differently. *P. sieheana* has pollen grains with tricolpate apertures, while *P. armeniaca* has both tricolpate and syncolpate ones. Nutlet micromorphology contains characters that support the differentiation of species. The nutlet coat is rugulate in *Phlomis sieheana* and reticulate in *P. armeniaca*. The characteristics of anatomical, palynological and micro morphological in *P. sieheana* and *P. armeniaca* are found valuable supporting the separation of these species.

Introduction

It is well known that plants are vital to human medicine, clothing, shelter, and sustenance. There are currently 300,000 recognized plant species in the world (Botaniska, 2025). While human beings nowadays benefit from around 70,000 plant species, the number of plant species cultivated is around 7000. Only 30 plants meet 90% of the world's nutritional needs (Arslan *et al*., 2014). Herbal pharmaceutical products and supplements derived from plants have been increasingly common in recent years due to their therapeutic features, low toxicity, simplicity of availability, and low cost (Zinicovscaia *et al*., 2020). As a result, plant researchers have recently concentrated their efforts on determining the properties of potential medicinal and aromatic plant sources with innovative and beneficial health effects (Chrysargyris *et al*., 2020; Yılmaz, 2020). The Lamiaceae family, which comprises a large number of medicinal and aromatic plants, is the fourth largest family in terms of species number; however the third largest one based on the taxon number in Türkiye. This huge family has 782 taxa through 48 genera, and of these, 346 taxa are endemic in Türkiye (Celep and Dirmenci, 2017). The consequence proves that Türkiye is one of the centres of biodiversity for Lamiaceae in the Old World. Therefore, Türkiye has about 10% of all Lamiaceae members worldwide. The genus *Phlomis* (Lamiaceae) comprises 53 taxa in Türkiye and the endemism ratio is 57%. In the List of Vascular Plants of Türkiye, the genus is listed as 46 species, 13 of which are hybrids, and 11 are varieties (Güner *et al*., 2012). *Phlomis* is the genus with the most hybrids in the Lamiaceae family because natural hybridization occurs often among its members (Davis *et al*., 1988). Members of the *Phlomis* are known for their tasty and stimulating leaves, which are used as teas, tonics, and carminatives in traditional medicine (Baytop, 1999; Gürbüz *et al*., 2003).

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Nowadays, palynotaxonomy, describing the pollen and spore morphology of plants, is popular, and as a branch of science with increased applicability. The family Lamiaceae has evaluated based on pollen morphology by many authors that given essential data to distinguish species (Abu-Asab and Cantino, 1994; Cantino *et al*., 1992; Celenk *et al*., 2008; Erdtman, 1945; Harley *et al*., 1992; Hassan *et al*., 2009; Moon *et al*., 2008a, b; Salmaki *et al*., 2008; Özler *et al*., 2011). However, a few studies have been conducted on the pollen morphology of some species of *Phlomis* genus (Oran, 2015; Özaltan and Koçyiğit, 2022). The pollen characteristics of *P. grandiflora* H. S. Thompson were determined by Özaltan and Koçyiğit (2022) in Türkiye.

Micromorphological studies on plant seeds are taxonomically significant in the mint family (Ryding 1994, 2010; Salmaki *et al.* 2008, Moon *et al*. 2009; Kahraman *et al*. 2011, Celep *et al.* 2014, 2022b) and nutlet coat morphological patterns have been evaluated as an important diagnostic character (Kamel, 2014; Firdous *et al*., 2015; Celep *et al*. 2022b). The genus *Phlomis* has examined with little example focusing on nutlet and pollen morphology (Oran, 2015) whereas the phytochemical and pharmacological studies are more (Sarkhail *et al*., 2006; Demirci *et al*., 2008; Demirci *et al*., 2009; Aghakhani *et al*., 2018; Göger *et al*., 2021; Okur *et al*., 2022; Nematian *et al*., 2024; Azhikhanova *et al*., 2025).

Phlomis armeniaca Willd. is an endemic widespread species however, the distributional area is limited generally in Inner Anatolia while *P. sieheana* Rech. is another endemic plant related to *P. armeniaca* adopted to dune and sandy habitats. Additionally, *P. armeniaca* is a species that can grow up to 60 cm in length, has subulate bracteoles and yellow flowers, and has a greater number of flowers on the verticillaster, whereas *P. sieheana* is a species with linear bracteoles, grows up to 30 cm in length, and has fewer flowers on the verticillaster. This study aims to determine the anatomical characters, pollen characters, and additionally nutlet micromorphological characters of *P. armeniaca* and *P. sieheana* species, and to determine the diagnostic value of these features in distinguishing the species.

Materials and Methods

Plant materials, location and collections

Plant specimens of *P. armeniaca* and *P. sieheana* were collected from natural populations in Konya and Antalya (Türkiye) the location coordinates (Fig. 1).

P. sieheana: C4 Konya: Karapınar, Akören Village, steppe, 1030 m., 05.07.2019, B. Çıtak 350-G.Yüksel.

P. armeniaca: C2 Antalya: Antalya, Akseki, Çimi Village, Ölümiyar province, 2100 m., 16.07.2019, B.Çıtak-359-G.Yüksel.

Some specimens of plants were separated for herbarium materials and voucher numbers were assigned them as B. Çıtak 350-G.Yüksel and B.Çıtak-359-G.Yüksel in KNYA herbarium. The remaining materials were fixed and stored in ethanol (70%).

Palynological and seed micromorphological investigations

Wodehouse's (1935) technique was applied to obtain pollen grains, and the herbarium samples were used. In this simple technique the anthers carefully were separated from flowers and transferred to clean slides. The anthers were treated with ethanol to move gum, mucilage or others. With a clean needle the anthers were opened and the pollen grains were poured to glass slides into glycerin-gelatin and covered with cover slip. Microscope slides were deposited in the pollen collection of the Plant Anatomy and Palynology Laboratory. Department of Biology, Selçuk University, Türkiye. The seeds of studied species were collected through the botanical trip surveys and treated with ethanol. Mature seeds were observed with a stereomicroscope and at least 20 grains were measured and analyzed.

Microscopical analysis

For LM investigation, measurements and observations were made using the Leica microscope model DM 1000 attached Kameram 21 programme. Pollen grains were measured both in polar and equatorial view. For morphometric assay, at least 30 numerical value randomly taken were used of equatorial and polar view, 15 measurements of length of colpus and width of colpus, 15 measurements of the apocolpium side, and 10 measurements of aperture length and with in equatorial view. Additionally, 15 measurements were saved of exine and intine thickness from both equatorial and polar view. For the scanning electron microscopy (SEM), the pollen grains were observed and photographed with a Zeiss Evo LS 10 SEM to determine their exine ornamentation. Also pollen terminology has been used based on Punt *et al.* (2007) and Halbritter *et al.* (2018).

Results and Discussion

Morphological characters

P. armeniaca Willd.*:* The plant is a perennial herbaceous plant, 16-20 cm tall. The stem is four-angled, compressed, densely stellate hairy, 10.5-19 cm. Basal leaves are ovate, oblong-lanceolate, 70-105×9-14 mm, stem leaves are linear-lanceolate, 43-53×7-15 mm. Petiole of basal leaves is 25-49 mm, stem leaves are 7-10 mm. The leaves, which are arranged in opposite directions, have glandular hairs in addition to stellate hairs, the lower surface of the leaves is whitish due to the hairs, the upper surface is grayish green. The leaf base is cuneate, the tips are obtuse, the edges are crenate-toothed. The raceme is 57-85 mm long, verticillates are 4-10-flowered, the flowers are concentrated in the upper part, the flowers are zygomorphic in symmetry. The calyx tube is 9-18×5-6 mm, five-toothed, the teeth are 7 mm acuminate. Corolla yellow, two-lipped; outer surface of lower lip stellate, inner surface simple, outer lip simple hairy. Bracteoles subulate and stellate-tomentose hairy 5-10 mm. Petals 22-30×9-18 mm yellow; stamens 21 mm, anthers 1.1 mm, cream colour. Pistil length 27-28 mm, ovary 2 mm. Fruit composed of 4 nutlets. Flowering June-July. Nutlet brown, 3.7-5.9×1.9-2.8 mm in size.

Phlomis sieheana Rech.f.*:* It is a perennial herbaceous plant. The plant is 19-26.2 cm tall, with a deep taproot supported by lateral roots. The stem is 5.5-16 cm long, variable from erect to ascending, with four-angled compressed stellate hairs. Basal leaves are oblong, oblong-lanceolate broadly shaped 50-90×13-23 mm, stem leaves are oblong-lanceolate, linear-lanceolate simple leaves with 31-53×6-12 mm. The base of the leaves is generally cuneate narrowing on both sides, the tips are obtuse, the edges are crenate-toothed. Both surfaces of the leaves are covered with stellate hairs as well as glandular hairs, the lower surface of the leaf is grayish green, the upper surface is green. Petiole of stem leaves is 6-10 mm. Inflorescence is verticillate with 4-8 flowers, the flowers are sessile, and the inflorescence is zygomorphic. Raceme length is 70-160 mm. Bracteoles linear 2-3 mm tomentose hairy. Corolla yellow outer surface stellate, inner surface simple sparsely hairy. Calyx tubular teeth 4-4.5 mm stellate hairy acuminate. Petals 23-25×7-13 mm yellow, upper lip two, lower lip three lobed. Stamens 22 mm, anthers 1 mm, cream-brown color. Pistil length 23-25 mm; ovary 2 mm. Fruit nutlet-shaped inside the calyx. Nutlet dark brown, 5.1-6.9×2.2-3.3 mm in size. Flowering June-July.

Pollen characters

The results obtained aid of LM and SEM microscopy studies showed that the pollen grains of *Phlomis armeniaca* and *P. sieheana* are monad, mainly tricolpate and radially symmetrical. However, the pollen grains of *P. armeniaca* has two types of aperture morphology both tricolpate (%96) and syncolpate (%4) that differ from *P. siehena* which has only tricolpate ones. The shapes

of pollen grains based on P/E ratio has determined prolate-spheroidal as in *P. armeniaca* and those of *P. sieheana* are subprolate. The structure of exine was detected subtectate among investigated species. Colpus with sunken formation traverses to poles and granulate colpus membrane is similar in both studied species. The intine layer is quite thin under exine. The outer border of pollen grains that exine sculpture is determined reticulate-perforate in two species (Figs 2-3).

Fig. 1. The morphological photographs of *P. armeniaca* (a-c) and *P. sieheana* (b-d)

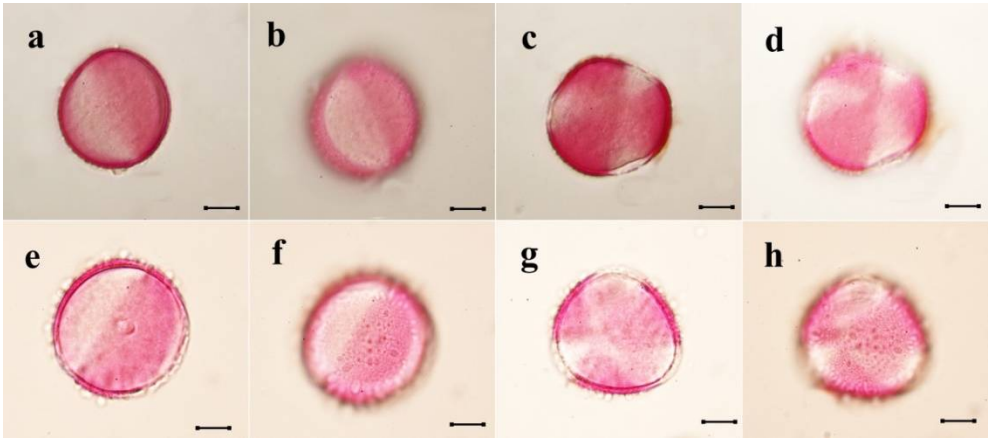

Fig. 2. The microphotographs of pollen grains (LM) *P. armeniaca* (a-d) and *P. sieheana* (e-f). a-b and e-f: equatorial view, c-d and g-h: polar view (scale bar:10 µm).

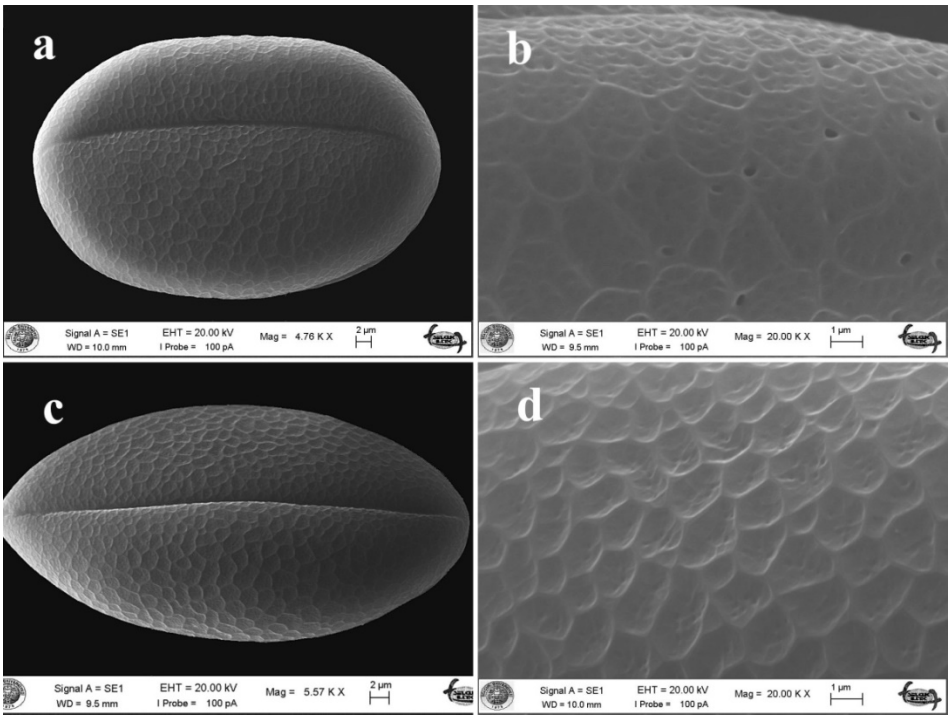

Fig. 3. The microphotographs of pollen grains (SEM) *P. armeniaca* (a-b) and *P. sieheana* (c-d). a-c: equatorial view, c-d: exine sculpture.

Nutlet characters

The studied taxa have globose shaped and brown coloured nutlet that *P. sieheana* are 5.1-6.9 mm long and 2.2-3.3 mm wide, 3.7-5.9 mm long and 1.9-2.8 mm wide in *P. armeniaca*. The nutlet shape is obovate in *P. armeniaca* and *P. sieheana.* Surface pattern of mature nutlets is reticulate with regular epidermis cells in *P. armeniaca* and not regular in *P. sieheana*. The sculpturing of surface of pattern of mature nutlets is reticulate in *P. armeniaca* and rugulate in *P. sieheana*. The nutlet surface of studied taxa is glabrous (Fig. 4).

It is well known that Erdtman (1945) is the foundation for research on identifying the pollen features of the family Lamiaceae. In order to categorize subfamilies by pollen type, he integrated his own study with that of other family experts. This method splits the family into two subfamilies: Nepetoideae and Lamioideae. Nepetoideae pollen has six colpi, whereas Lamioideae pollen has three (rarely four) colpi. Studies have also been conducted based on the identification of pollen characteristics in *Phlomis* (Oran, 2015). According to this study, species of the genus have pollen grains with subprolate, prolate-spheroidal, and reticulate ornamentation of the tricolpate aperture type. Pollen grains from *P. armeniaca* and *P. sieheana* species have both tricolpate and tetracolpate apertures, respectively, according to the current study. While reticulate sculpture is observed in *P. armeniaca*, reticulate-perforate ornamentation is remarkable in *P. sieheana*. On the other hand, the examined species were found to have comparable pollen diameters.

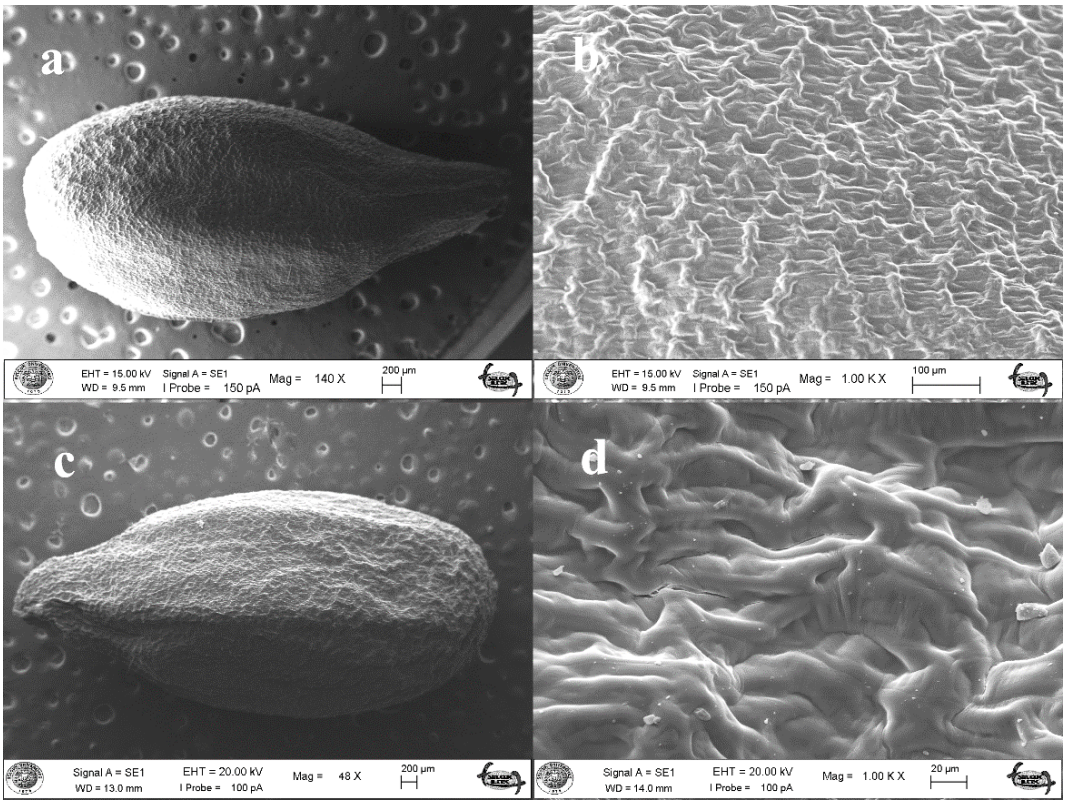

Fig. 4. The nutlet microphotographs of *P. armeniaca* (a-b) and *P. sieheana* (b-d). a-c: general view, c-d: nutlet surface in detail.

Fruits of the Lamiaceae family are called nutlets that exhibit various types of ornamentation on their outer surfaces. These structures are used in classification, as are the width and length of the nutlets, their color, and their shape (Mosquero *et al*., 2002; Kahraman *et al*., 2011; Ebadi *et al.*, 2021; Özker *et al*., 2023). Davis *et al.* (1988) reported that limited information on the fruit macromorphology and micromorphology of the *Phlomis* genus is available, and researchers have only provided information on the fruit and calyx. With this study, species can be distinguished from each other according to the characteristics of their capsule (fruit) and nut structures. There are differences in the appearance of the epidermal cells in the nutlet shell ornamentation of the species we studied. So, the nutlet shell of *P. sieheana* has rugulate ornamentation, the epidermal cells have indistinct borders, and an irregular appearance. The nutlet shell of *P. armeniaca* has reticulate ornamentation, the epidermal cells have well-defined borders, a regular appearance, and thick walls. The nutlet size of *P. armeniaca* is smaller than that of *P. sieheana*. Nutlet micromorphological characteristics reveal significant taxonomic differences. The two species are similar when examining the micropolar and chalazal poles. The seed coat ornamentation and epidermal cell appearance of the species we studied differ. The seed coat of *P. sieheana* has rugulate ornamentation, epidermal cell borders are indistinct, and irregular in appearance. The seed coat of *P. armeniaca* has reticulate ornamentation, epidermal cell borders are well-defined, regular in appearance, and thick-walled. Nutlet surface morphology can be used in addition to taxonomic characters to distinguish species.

As a conclusion, pollen and nutlet characteristics provide strong evidence for the taxonomic distinction between the species. *P. armeniaca* pollen characteristics differ significantly from *P. sieheana*. Aperture types have been identified in the pollen grains. In addition to the tricolpate aperture type, *P. armeniaca* pollen has also been observed with tetracolpate apertures. Therefore, we believe that aperture type can aid in species differentiation. It was concluded that nutlet micromorphology, along with pollen characters, supports species differentiation. Nutlet surface ornamentation, in particular, supports species differentiation. We believe that the results of this study will guide the determination of pollen and seed characteristics of other species of the genus *Phlomis*.

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