

THE ANATOMICAL AND MICROMORPHOLOGICAL CHARACTERISTICS OF THYMUS PERSICUS (LAMIACEAE)

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Thymus persicus is an endemic wild species of the genus *Thymus* in the Lamiaceae, grows in the northwest of Iran. In this research micromorphological feature of trichome, nutlet and pollen grain using light and scanning electron microscopes and anatomical characteristics was performed to evaluate taxonomic implication of these data. The leaf was dorsio-ventral in transverse section, including a one-layered epidermis, 2-3 layers of compact palisade parenchymatous cells, a large median vascular bundle with one medium-sized and one small vascular bundle in both side of the mesophyll. Vascular bundle cap was found to be dense sclerenchymatous fibre on the phloem side. Bundle sheath was composed of one-layered parenchymatous cells. Indumentum includes a one to five-celled short and long glandular trichomes, while glandular trichomes were of capitate and rarely peltate types. Stem had a single layer of epidermis covered with a cuticle as well as glandular and non-glandular trichomes and vascular bundles were separated by parenchymatous cells. Nutlets were 0.7×1.0 mm, brown, ovate in outline, glabrous and with star-shaped papillae ornamentation. The pollens were hexacolpate, isopolar and reticulate. The polar axis (P) and equatorial axis (E) of pollen were 37.4-40.4 and 24.8-28.1 μm , respectively. The ratio of P/E was found to be 1.3-1.5. The results showed that the leaf anatomical, nutlet and pollen micromorphological characteristics could be considered as taxonomic characters in species identification and circumscription of the genus *Thymus*.

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Keywords: *Thymus persicus*; anatomy; micromorphology; trichome; nutlet; Pollen

مطالعه صفات تشریحی و ریزریخت‌شناسی آویشن ایرانی (*Thymus persicus*)

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آویشن ایرانی با نام علمی *Thymus persicus* یکی از گونه‌های بومزاد سرده *Thymus* از تیره نعنائیان است. بررسی ویژگی‌های ریزریخت‌شناسی فندقچه و گرده با استفاده از میکروسکوپ‌های نوری و الکترونی نگاره و صفات تشریحی برای به‌دست‌آوردن صفات کمکی در شناسایی دقیق این گونه انجام شد. برگ در برش عرضی پستی-شکمی، شامل اپیدرم یک لایه، دو تا سه لایه پارانشیم نردبانی فشرده، یک دسته آوندی بزرگ در محل رگبرگ میانی به‌همراه دو دسته آوندی متوسط در طرفین و دو دسته کوچک در انتهای برگ که فیبرهای اسکلرانشیمی مترکم به‌صورت کلاهدک آوندی روی فلوئم وجود دارند. غلاف آوندی یک لایه و پارانشیمی و پوشش کرکی شامل کرک‌های غیرغده‌ای تک تا پنج سلولی و بندرت کرک‌های غده‌ای سبزی شکل است. سطح فندقچه قهوه‌ای، صاف به‌شکل تخم مرغی و ابعاد 0.7×1.0 میلی‌متر و دارای برآمدگی‌های ستاره‌ای شکل است. دانه گرده شش‌شیار، جورقطب، به‌صورت مشبک است. متوسط طول قطبی و محور استوایی دانه گرده به‌ترتیب $37.4-40.4$ و $24.8-28.1$ میکرون

و این نسبت ۱/۳-۱/۵ می‌باشد. نتایج نشان داد که صفات تشریحی برگ و ریزریخت‌شناسی بذر و گرده می‌تواند به‌عنوان صفات کمکی در آرایه‌شناسی این گونه کاربرد داشته باشند.

INTRODUCTION

Thymus L. (Lamiaceae) with common persian name of “*Avishan*”, is a well-known medicinal and aromatic perennial herb originated from Mediterranean region. Among 215 species of the genus grown in the world, 18 species are represented in flora of Iran, four of which are endemic (Jamzad, 2012). *Thymus persicus* (Ronniger ex. Rech. f.) Jalas is one of the endemic and endangered species of the genus, growing in northwest of Iran (West Azarbaijan and Zanzan Provinces) (Rechinger 1982; Morales 2002). Morphologically, *T. persicus* is a distinct species from the others. There are a number of anatomical studies of Lamiaceae family including *Lamium moschatum* (Baran & Özdemir 2011), *Teucrium* species (Ecevit-Genç & al. 2018) and micromorphological studies of Lamiaceae including nutlet micromorphology of *Salvia quezelii* (Celep & al. 2014) and some Lamiaceae taxa (Hassan & Al-Thobaiti 2015), Tabassi (2016), pollen micromorphology of *Marrubium* (Akgal & al. 2008) and *Salvia* species (Polat & al. 2017), trichome micromorphology of *Satureja laxiflora* (Sonboli & al. 2004). Analysis of nrDNA ITS sequences of *T. persicus* and its phylogenetic position and relationships with other *Thymus* species for authentication have been studied (Sonboli & al. 2013). Size, shape and ornamentation of pollen grains and nutlets are useful characters for taxonomic implications in the genus *Thymus*. As far as

our literature survey could as certain, the anatomical and micro-morphological characteristics of *T. persicus*, an endemic species in Iran, has not been reported yet. Therefore, the principal aim of this study were: (i) to examine stem and leaf anatomy and (ii) to investigate micromorphological characteristics of the trichome, pollen and nutlet of *T. persicus* by light microscopy (LM) and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Plant material was collected from Takab, Baderlu village (36°28' N, 47°13' E) at an altitude of 2500 m, in the northwest of Iran (fig. 1). The plant was identified and a voucher specimen deposited in the Herbarium of Medicinal Plants and Drugs Research Institute (MPH-1673) of Shahid Beheshti University, Tehran, Iran. Anatomical studies were carried out on stems and leaves of the specimens fixed in 70% ethanol. Transverse sections and surface preparations of stems and leaves were made manually. Pollen grains investigated were acetolyzed according to Reitsma's (1969) method. Several slides were made and photographed for each sample with light microscopes (LM, Olympus BX-51, Tokyo, Japan). For scanning electron microscopy (SEM), the samples mounted on stubs and coated with a thin layer of gold. The prepared samples were viewed with a KYKY-EM3200 SEM under a voltage of 26 kV.



Fig. 1. Flowering plant of *Thymus persicus* in its natural habitat.

RESULTS

Anatomy

Stem

Transverse section of the stem was quadrangular. Epidermis with a single layer of cells covered with a cuticle as well as glandular and non-glandular trichomes. The epidermal cells were either roundish or almost rectangular and square in shape. Upper and lower walls are thicker than lateral walls. The angular collenchyma located at the corners of the stem and composed of 2-3 layers of cells. The cortex formed of 6-7 layers of roundish cells and thin-walled parenchyma cells with intercellular spaces. Endodermis is single layered. Pericycle is indistinguishable. Phloem is 6-7 layered and consists of irregular cells. Pith consists of large orbicular or polyhedral parenchymatous cells (fig. 2A-C).

Leaf

In the transverse section of leaf, the epidermis was composed of single-layered cells, and almost square or roundish cells covered with a cuticle. Most of trichomes on both surfaces were 1-5 celled non-glandular. 2-3 layered spongy parenchyma consisted of smaller and variously shaped cells with large intercellular spaces. Vascular bundles are large, median and small size in both side of the mesophyll and they were surrounded by bundle sheath. Bundle sheath was composed of one-layered parenchymatous cells. Vascular bundle cap was found to be dense sclerenchymatous fibre on the phloem side. Vascular bundles are collateral type. There are 3-5 vascular bundles in leaf, 2-4 of which are on the sides, and a big in the middle. Vascular bundles at the corners are smaller and less-developed. Middle and small vascular bundles are surrounded by parenchyma cells. Multilayered sclerenchyma are seen on the phloem in median vascular bundles. The xylem faces towards the upper surface while phloem faces the lower epidermis. The phloem tissue is surrounded by thick sclerenchymatous tissue towards the lower epidermis (fig. 2D-V).

Trichome Micromorphology

The leaf and stem of *T. persicus* are covered by two major types of trichomes. Capitulate trichomes were composed of a round multicellular head consisting of two cells arranged in a single circle (20-60 μm) in diameter and a very short monocellular stalk a unicellular (20-90 μm long) or bicellular stalk (up to 150 μm). The peltate glandular trichomes were

common on the leaf. The eglandular ones are simple, needle-shaped, uniseriate, uni-multicellular (up to 5-celled), retrorse and have a cuticle with papillae. The non-glandular trichomes were including unicellular, bicellular and multicellular acicular/needle-shaped trichomes (20-300 μm) on their surfaces. The acicular trichomes were abundant on the stem and leaf (fig. 3A-I).

Nutlet and Pollen Micromorphology

Nutlets of *T. persicus* were brown, ovate in outline (fig. 3J, K). They were 1.0 mm long and 0.7 mm wide. In SEM micrographs, the nutlet surface was glabrous and with star-shaped papillae ornamentation (fig. 3L). The pattern of the nutlets is a useful characteristic for distinguishing the genera and species of the family Lamiaceae (Tarimcilar & al. 2013; Jamzad & al. 2009). Moon & al. (2009) pointed out that the nutlet shape, distribution of trichomes and surface sculpture can be used for species identification in Lamiaceae.

Thymus species show very similar palynological features by LM and SEM. Exine sculpturing was reticulate (fig. 3M-O). The pollens were hexacolpate and isopolar. The dimensions of polar axis (P) and equatorial axis (E) were 37.4-40.4 μm and 24.8-28.1 μm , respectively. The ratio of polar axis/equatorial axis (P/E) was 1.3-1.5 and their shape was oblate-spheroidal to prolate-spheroidal (fig. 4A, B). Pollen characteristics of the family Lamiaceae have been reported to be of considerable taxonomic importance (Erdtman 1945). Şakıyan (1998) reported that the shape of pollen grains are isopolar, hexacolpate, suboblate, oblate-spheroidal and prolate spheroidal, circular or hexagonal in the family Lamiaceae. Mártonfi (1997) showed that the pollen was suboblate, isopolar, oblate-spheroidal or prolate-spheroidal in the *Thymus* species investigated. Badamtsetseg & al. (2012) in the study on pollen morphology of the family Lamiaceae, reported that the exine ornamentation of pollen grains of Lamiaceae was classifiable to four types: tuberculate, microreticulate, bireticulate and polish. Our study with LM and SEM revealed that *T. persicus* pollen had reticulate ornamentation and bireticulate type. In conclusion, we aimed at providing a comprehensive micromorphological and anatomical description of *T. persicus*, an endemic plant in Iran.

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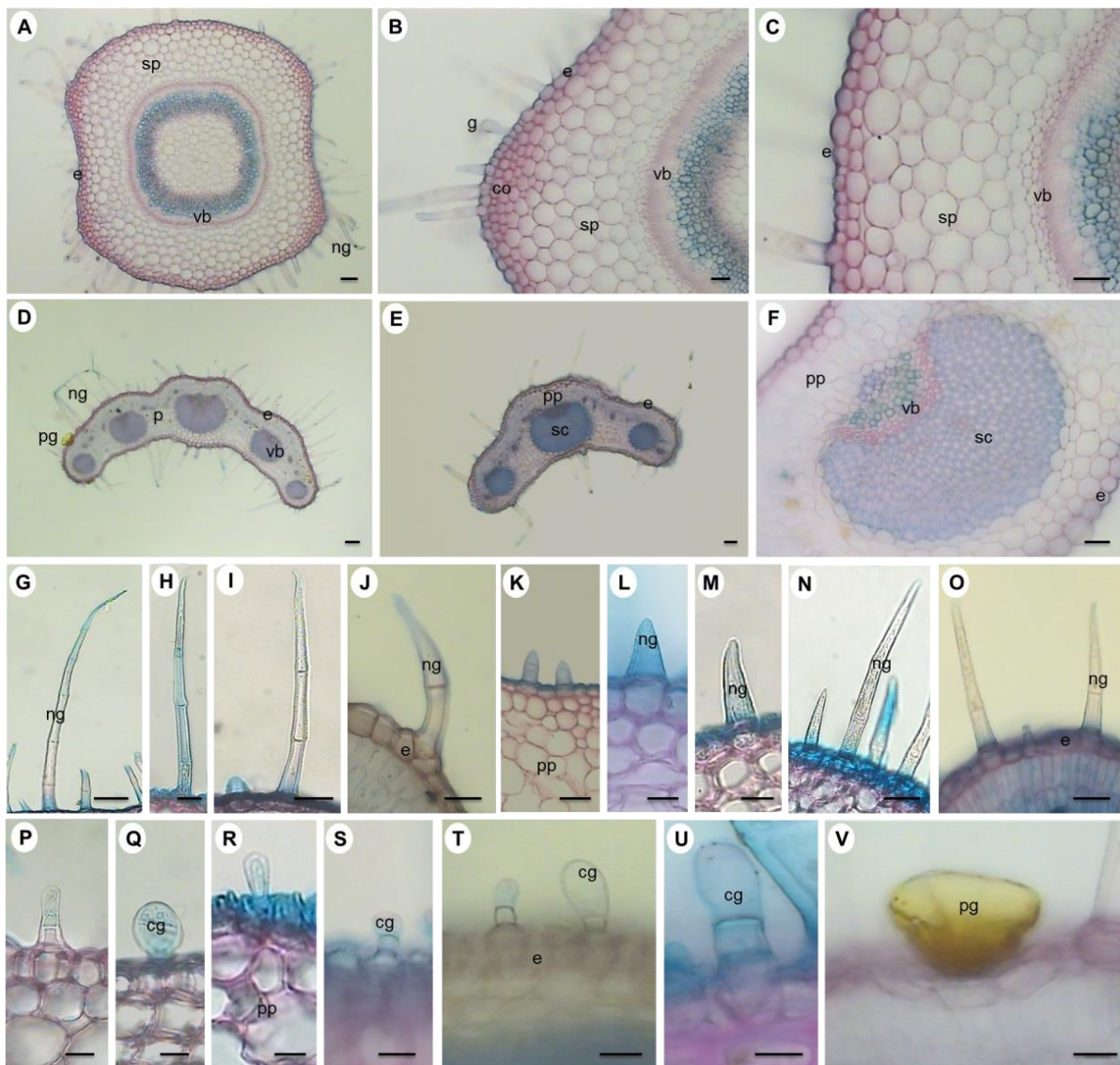


Fig. 2. LM micrographs of transverse sections of stem and leaf of *Thymus persicus*. A-C, Stem; D-V, Leaf. co, collenchyma; sc, sclerenchyma; e, epidermis; g, glandular trichome (cg, capitulate glandular and pg, peltate glandular); ng, non-glandular trichome; pp, palisade parenchyma; sp, spongy parenchyma; vb, vascular bundle. Bars: 100 μ m.

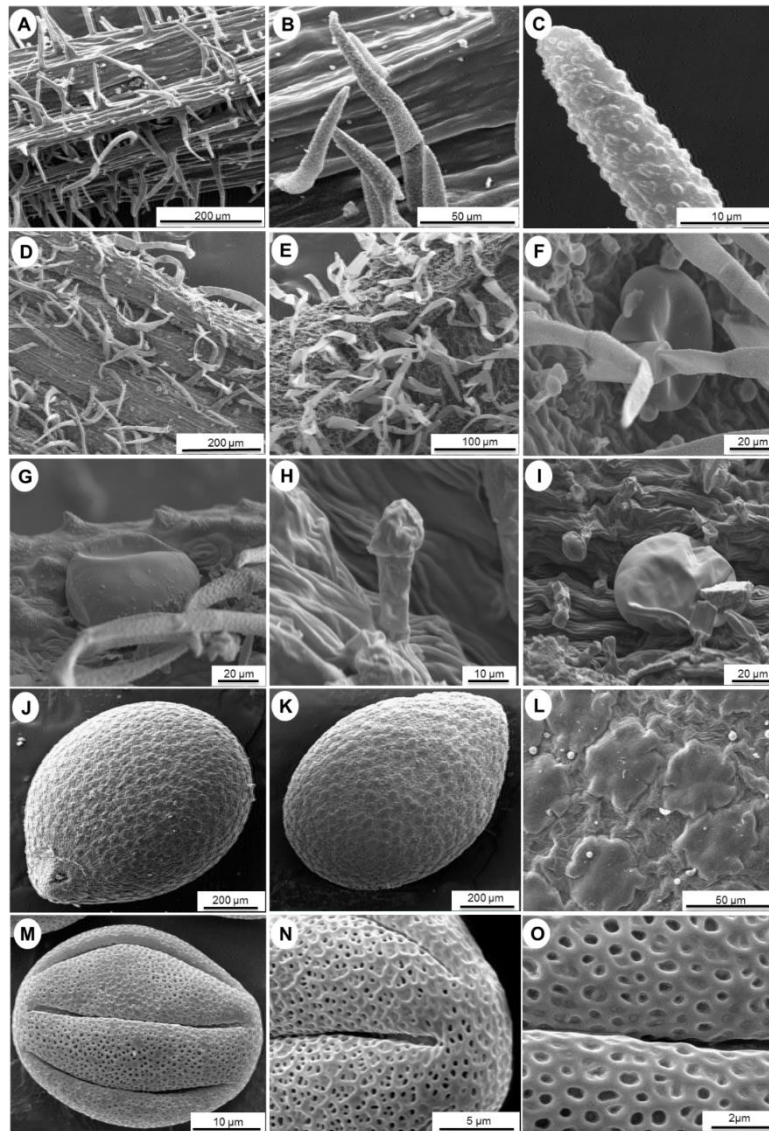


Fig. 3. SEM micrographs of *Thymus persicus*. A-C, non-glandular trichomes on the stem; D, leaf; E, corolla; F, peltate and capitate glandular trichomes on the corolla; G-I, leaf, abaxial epidermis; J-L, Nutlets and M-O, Pollen grains.

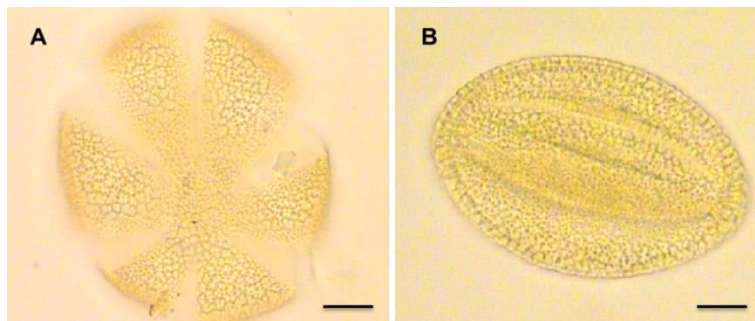


Fig. 4. LM micrographs of pollen grains of *Thymus persicus*. A, polar view of hexacolpate pollen; B, equatorial view in LM, bars = 10 μm.

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