

MORPHOLOGY AND ANATOMY OF ENDEMIC *THERMOPSIS TURCICA* KIT TAN, VURAL & KÜÇÜKÖDÜK

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Abstract

Morphology and anatomy of an endemic plant from Turkey, *Thermopsis turcica* Kit Tan, Vural & Küçüködük, belonging to Fabaceae have been investigated. The species had a perennial rhizome, an erect stem and compound leaves consisting of three leaflets. Morphological and anatomical research findings have been presented by photographs, drawings and tables.

Introduction

The family Leguminosae is divided into three subfamilies Papilionoideae (=Faboideae), Mimosoideae and Caesalpinoideae (Davis 1969). Most of the tropical Faboids are woody, while those out of tropics are usually herbaceous (Baytop 1991). Traditionally, the small tribe Thermopsidae of Faboideae includes 6 genera scattered through the Mediterranean basin and central to North-East Asia. The tribe as a whole is absent from the southern Hemisphere. The genus *Thermopsis* contains perennial herbs producing rhizomes (Wang *et al.* 2006). *Thermopsis* is a genus of about 25 species distributed in Asia and North-America, mostly in montane regions (Davis *et al.* 1988). Based on nuclear ribosomal DNA analysis, the tribe Thermopsidae is phylogenetically not a monophyletic group. The East Asian and North-American disjunct *Thermopsis* species are not monophyletic. According to the DNA analysis, there is a geographical division between the old world and the new world *Thermopsis* (Wang *et al.* 2006).

Thermopsis turcica is an endemic perennial herb with large rhizome in East Mediterranean region. *Thermopsis* is a genus confined to Central Asia and East United States, and it is represented by only one endemic species, *T. turcica* in Turkey. The unique character of *T. turcica* is the uniform occurrence of three free carpels. The polycarpellary condition of *T. turcica* is a clearly derived, rather than a primitive character since the whole Papilionoid pollination syndrome has obviously evolved around the presence of a single median carpel (Davis *et al.* 1988, Kit *et al.* 1983). Although presence of polycarpellary condition has been previously documented in normal members of the Mimosoideae and Caesalpinoideae, apart from teratological forms, this is the first record in the Faboideae (Kit *et al.* 1983).

There have been a number of morphological, anatomical and phylogenetic studies of the subfamily Faboideae (Aniszewski *et al.* 2006, Wang *et al.* 2006, Yiotis *et al.* 2006, Oliveira and Paiva 2005, Ainouche *et al.* 2003, Doyle *et al.* 2000, Ainouche and Bayar 1999, Pereira-Netto *et al.* 1999, Talavera and Gibbs 1997 and Crow *et al.* 1997).

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The literature searching of *Thermopsis* revealed that there has been no anatomical study. Investigations have been performed on the chemical constituents of *Thermopsis* species (Asilbekova 2004, Kotenko *et al.* 2001). Apart from the morphological description of *T. turcica* (Kit *et al.* 1983), there has been only one study on the alkaloids of the species (Rahman *et al.* 1991).

It is reported that some *Thermopsis* species contain alkaloids, flavanoids, vitamin C, macro- and microelements. The air-dried aerial part is used as a medicinal raw material for a cytisine preparation and as an expectorant (Asilbekova 2004, Kotenko *et al.* 2001). On the other hand, the genus *Thermopsis* consists of poisonous and harmful species with low feeding value (Li 1997, Zhang and Lui 1996). *Thermopsis* species have been suspected of causing livestock losses. In addition, series of human poisoning by *Thermopsis* has been reported. Twenty three suspected cases of *Thermopsis* exposures, some resulting in significant toxicity like vomiting, abdominal pain, drowsiness, oral irritation, tachycardia, tremors and other general symptoms are reported (Mcgrath-Hill and Vicas 1997). Some *Thermopsis* are also used as ornamentals in the United States (Lockhart 2005).

Since there is no report on the anatomical features of *Thermopsis*, morphological and anatomical characters of a Turkish endemic, *T. turcica* have been reported here in detail.

Material and Methods

Plant samples were collected from natural populations at the following location:

B3: Konya, Ak ehir, Gölçayır Village, Lake sides, 960m, 28.05.2004 (Küçüködük 1251).

Taxonomic description of the plants was done according to Kit *et al.* (1983) and Davis *et al.* (1988). Anatomical studies were carried out on the samples kept in 70% alcohol and using paraffin method (Algan 1981). The cross-sections were also taken by hand using razor blade. Results were presented by photographs, drawings and tables.

Results and Discussion

Morphological features

T. turcica is a perennial herb with long rhizome. Erect stem up to 35-80 cm in length and densely white-villous in the upper parts. Sterile branches leafy, striate, sheathing at the base. Sheaths scarious, passing into leaf-like stipules. Leaves alternate, digitately trifoliolate, white-sericeous or sometimes white-villous, becoming greyish-green. Stipules leaf-like, free, those on the main stem much larger than those at the leaf bases, acute. Petioles 07 - 2.5 cm in length. Leaflets elliptic-ovate, 11 - 40 × 4 - 16 mm in dimension, acute, densely white-villous on both surfaces. Inflorescence terminal, racemose, 12 - 35 cm. Flowers large, bracteate, zygomorphic, hermaphrodite. Bracts leaf-like, ovate, 7 - 15 × 3 - 11 mm, white-villous, the margins long-ciliate. Pedicels up to 24 mm. Calyx 9 - 13 mm in length, densely white-villous, unequally 5-toothed, bilabiate, the upper lip forming 2 teeth of ca. 6 × 8 mm, the obtuse lobe sometimes cleft to 1.4 mm; the lower lip forming 3 teeth, nearly equal, triangular-acute, 4.5 - 5.5 × 2.5 - 3.0 mm in dimension. Petals golden yellow in colour, imbricate, free except for partly connivent keels. Standard glabrous, 25 mm with a 5 mm claw and suborbicular, the retuse lamina ca. 20 × 25 mm, the wings glabrous, 2 × 9 mm (inc. 1.8 mm claw), the keel ca. 22 × 8 mm (inc. 7 mm claw) in dimension being dorsally coherent. Stamens 10 and free. Filaments filiform, not dilated, 18-20 mm in length, glabrous; anthers uniform, dorsifixed, 1.8 mm in length, yellow in colour. Disc

absent. Ovary 3-carpellate, carpels free, each 1-locular, nonseptate, white-villous, sessile; ovules 10 in number and on the adaxial suture; style ca. 12-13 mm, glabrous, recurved at apex, persistent in fruit; stigma terminal, small and capitate. Fruit a legume, 2-3 seeded, elliptic- or oblong-ovoid, 22-25 × 7-9 mm in dimension, slightly falcate, nearly straight at maturity, densely sericeous-villous, indehiscent. Seeds exarillate, subreniform in shape, 3.5-5 × 2.5-3 mm, smooth, not foveolate, pale purplish. Grows in marshy lakeside ca. 950-1050 m in altitude (Pl. 1).



Colour

Plate 1. General appearance of *Thermopsis turcica* Kit Tan, Vural & Küçüködük.
f. flower, s. stem, lg. legume, l. leaf and r. root.

Anatomical characteristics

Root: The outer surface of the root was covered by 3 - 5 layered periderm of which cells were dark coloured, crushed, broken up and sometimes fallen out. Cortex was 8 - 24 layered below the peridermis. Large or small sclerechyma groups were located on the well-developed phloem. Trachea cells were irregularly located in the xylem tissue that was below the phloem. Pith rays were 1 - 5-layered. A great number of pith rays, 1 - 2-layered were close together in the xylem tissue. The center of the root was filled up with parenchymatous pith cells (Pl. 2 A, B).

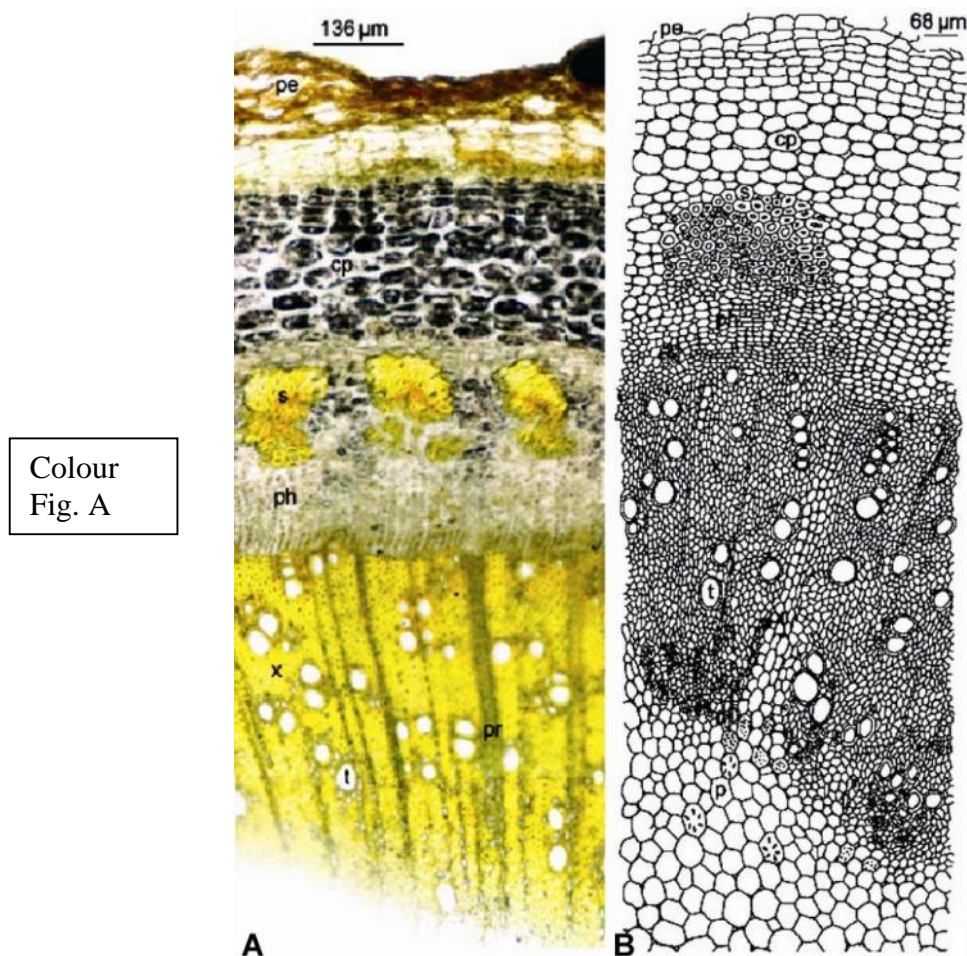


Plate 2. Cross-sections of root of *Thermopsis turcica*. A. Microphotograph.
 B. Camera Lucida drawing. pe: peridermis, ph: phloem, t: trachea, p: pith,
 cp: cortex parenchyma, pr: pith ray, s: sclerenchyma and x: xylem.

Stem: The cross-section of stem was circular or sometimes angular and nearly quadrangular. The outer surface of the cross-sections was slightly wavy exhibiting small, thin projections. A thick cuticle was present on the epidermal cells of which shape was quadrangular or rectangular. 1 - 5 layered collenchyma was located below the single layered epidermis. Parenchymatous cortex was present under epidermis which was 4 - 16 layered. Vascular bundles consisted of xylem, phloem and sclerenchyma patches. Sclerenchyma patches surrounding the phloem were 2 - 5 layered. The thick-walled cells forming sclerenchyma patches upon the phloem tissue were noticeably large, nearly as much as the size of the trachea. Phloem elements were clear. Cambium was 3 - 4 layered in woody stem, but indistinctive in young stem. Trachea and tracheids were arranged in regular rows. A large cavity was present at the middle of pith region. This cavity was formed by the broken parenchymatic cells that were thin-walled. Starch grains were frequently present in the parenchymatous pith cells (Pl. 3 A, B, C, D).

Petiole: Epidermis was 2-layered. A thick cuticle was present on the epidermal cells. 4-14 layered parenchyma was located between the epidermis and the vascular bundles. There was a very large, undivided vascular bundle at the center and one small bundle at both ends of the petiole. Phloem tissue was clear under the sclerenchyma patches. Trachea cells were regularly arranged in the xylem tissue. There was a 4-10 layered, thick-walled parenchyma below the xylem tissue, apart from other parenchymatous cells under the epidermis (Pl. 4 A,B).

Colour
Fig. A

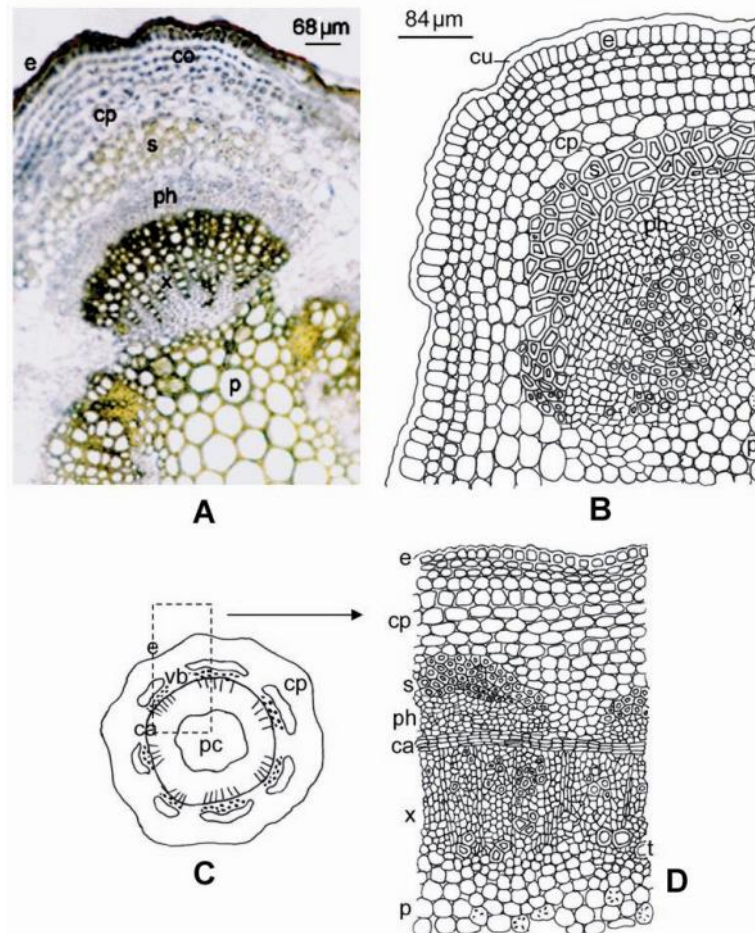


Plate 3. Cross-sections of young (A, B) and woody (C, D) stems of *Thermopsis turcica*.

A. Microphotograph. B, C and D Camera Lucida drawing. e: epidermis, cu: cuticle, vb: vascular bundle, ph: phloem, t: trachea, p: pith, pc: pith cavity, cp: cortex parenchyma, co: collenchyma, ca: cambium, s: sclerenchyma and x: xylem

Leaf: A thick cuticle was present on both abaxial and adaxial epidermis of leaf, being the thickest on the abaxial epidermal cells where the median vein was located. In contrast to the epidermal cells where the median vein was located, of which length size was longer than the width size, epidermis cells located at the other parts had usually longer width than the length size. Stoma cells were located at the adaxial and abaxial epidermis, being at the same line with epidermal cells. Capitulate glandular hairs with or without stalk cells were scarcely present near glandular hairs.

Although mesophyll was not clearly differentiated as palisade and spongy parenchyma, it was formed by 2 - 3-layered palisade parenchyma under both adaxial and abaxial epidermis and 1-2-layered spongy parenchyma hardly visible at the median part between them.

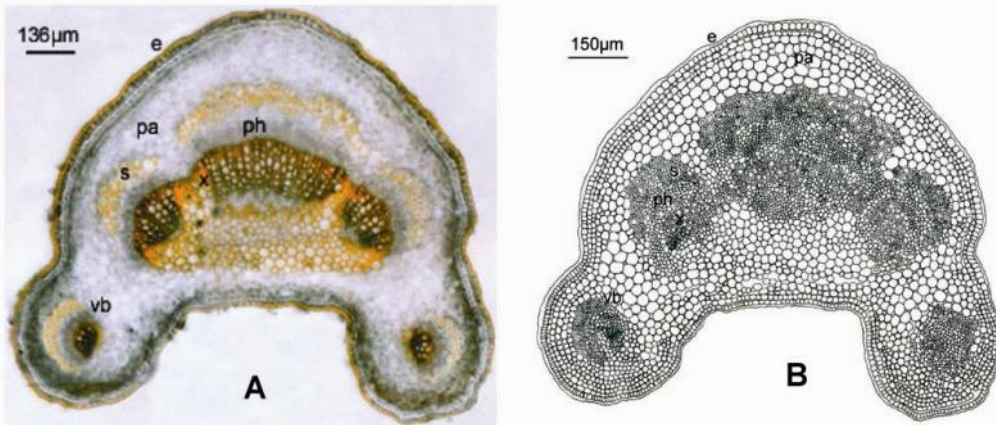


Plate 4. Cross-sections of petiole of *T. turcica*. A. Microphotograph. B. Camera Lucida drawing.
e: epidermis, ph: phloem, pa: parenchyma, vb: vascular bundle, s: sclerenchyma and x: xylem.

Mesophyll cells were with chloroplasts. Smaller vascular bundles in leaf were surrounded by 1-layered parenchyma of round shaped cells without chloroplasts. Vascular bundles forming the median vein were surrounded by round-shaped parenchyma cells mostly without chloroplasts (Pl. 5 A,B).

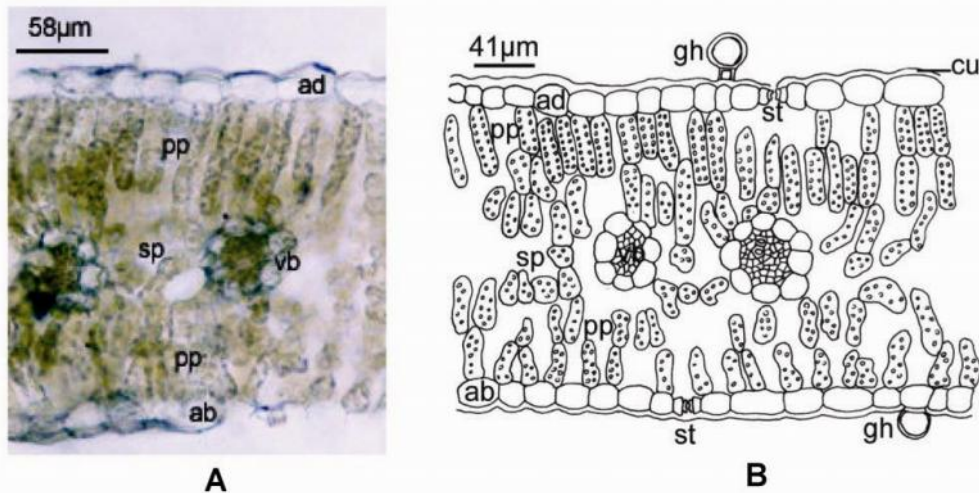


Plate 5. Cross-sections of leaf of *Thermopsis turcica*. A. Microphotograph. B. Camera Lucida drawing.
ad: adaxial epidermis, ab: abaxial epidermis, cu: cuticle, pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle, gh: glandular hair and st: stoma cell.

Seed: In the anatomical sections, seed was composed of exotesta, mesotesta and endosperm. There was a thick cuticular layer on the surface of exotesta. Thickness of cuticle was 5.4-16.6 µm.

Exotesta consisted of thick-walled macrosclereids which were longitudinally elongated. Macrosclereids were $96.4\text{-}128.6 \times 5.4\text{-}26.6 \mu\text{m}$ in dimension. This tissue was connected to mesotesta layer which consisted of thin-walled parenchymatous cells with intercellular spaces. Mesotesta was 2-layered. One layer was formed by large, semicircular or hourglass-shaped cells and the other was formed by much flattened cells. The compression of the mesotestal layers was clear. Endosperm had dense starch and fat cells and intercellular spaces (Pl. 6).

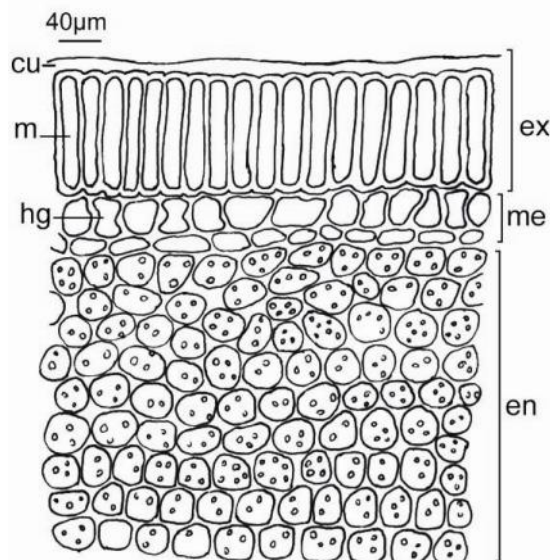


Plate 6. Cross-section of seed of *T. turcica*. cu: cuticle, m: macrosclereid cell, hg: hourglass cell, ex: exotesta layer, me: mesotesta layer and en: endosperm.

The anatomical characters of *T. turcica* that have been investigated, have some differences or similarities with some other members of the family. The stem of *Calicotome villosa* (Poiret) Link. (Leguminosae) is characterized by raised ridges and grooves (Yiotis *et al.* 2006). Vascular bundles of *Cytisus* species (Papilionoideae) form ribs on the stem surface (Talavera and Gibbs 1997). Although the stem ribs were absent in *T. turcica*, the epidermal cells which were accompanied by the cortex parenchyma cells formed a wavy stem surface. A thick cuticle was present on the wavy stem surface.

C. villosa leaves possess stomata on both sides, the abaxial and the adaxial. Leaf is furnished with palisade parenchyma on both sides (Yiotis *et al.* 2006). The leaflet of *Pueraria lobata* Willd. a member of Leguminosae, has two layers of palisade parenchyma and one layer of spongy parenchyma (Pereira-Netto *et al.* 1999). Leaves of *T. montana* have well-developed palisade and spongy parenchyma (Myers *et al.* 1997). Although there is a well-developed palisade parenchyma under the adaxial and the abaxial epidermis in *T. turcica*, the spongy parenchyma in the middle of the mesophyll has hardly been seen between the two palisade parenchyma. Stoma cells were present on both sides of the leaf of *T. turcica*. A thick cuticle was present on the epidermal cells. The vascular bundles of the leaf were surrounded by round-shaped mesophyll cells without chloroplasts.

There are some reports on the optical properties of *Thermopsis* leaves in the literature. Martin *et al.* (1991) investigated the lens properties of the leaf epidermal cells of *T. montana*. They found epidermis could concentrate light within the leaf amounting well in excess of ambient light

(Martin *et al.* 1991). It is reported that the spongy mesophyll is important in increasing internal reflectance of the leaves. The greater proportion of spongy parenchyma in leaves increases intercellular light scattering and thus absorbance (Delucia *et al.* 1996). It is also reported that the palisade tissue may help in distributing light more uniformly to chloroplasts within leaf (Vogelmann and Martin 1993). The leaves of *T. turcica* had a greater proportion of palisade parenchyma, being located under the two epidermal layers. This leaf character of *T. turcica* may be the result of an environmental effect like the habitat of the species.

Table 1. Anatomical measurement values of various tissues of *Thermopsis turcica*.

	Width (µm)		Length (µm)	
	Min. - Max.	Mean ± S.D.	Min. - Max.	Mean ± S.D.
Root				
Peridermis cell	13.2 - 105.9	55.6 ± 32.1	13.2 - 47.6	30.0 ± 13.8
Parenchyma cell	26.5 - 68.8	48.8 ± 12.9	21.2 - 50.3	31.5 ± 9.5
Trachea cell	7.9 - 79.4	41.9 ± 22.8		
Pith cell	31.8 - 84.7	59.1 ± 23.2		
Stem				
Epidermis cell	13.2 - 26.5	20.0 ± 4.8	15.9 - 42.4	28.8 ± 8.9
Parenchyma	10.9 - 65.5	35.1 ± 21.9	8.2 - 38.2	20.8 ± 10.9
Trachea cell	5.3 - 52.9	31.9 ± 19.9		
Pith cell	13.2 - 89.9	59.2 ± 32.6		
Petiole				
Epidermis	4.5 - 24.1	12.6 ± 7.1	17.0 - 40.7	26.9 ± 8.3
Parenchyma	10.2 - 57.8	32.8 ± 15.3		
Trachea cell	6.8 - 30.6	19.0 ± 9.2		
Leaf				
Cuticle	2.7 - 15.3	7.2 ± 3.1		
Adaxial epidermis cell	21.4 - 53.6	37.5 ± 11.6	10.7 - 32.1	23.9 ± 6.0
Abaxial epidermis cell	10.7 - 58.9	33.6 ± 15.6	7.9 - 47.6	25.4 ± 15.6
Palisade cell	10.7 - 21.4	14.9 ± 3.1	24.6 - 53.6	40.2 ± 9.3
Spongy cell	15.9 - 37.5	25.4 ± 6.1		

Pollinators typically visit more flowers on the plants with larger floral displays, which should present such attractive plants with significant pollen transport losses. Many-flowered plants with hermaphrodite flowers might reduce the costs of attractiveness by encouraging fewer sequential flower visits by pollinators (Biernaskie and Cartar 2004). *T. turcica* had a large floral display with their attractive and abundant flowers.

Leguminous plants (Fabaceae) have exotestal seeds. The term 'exotesta' is often used as an equivalent to the outer epidermis of the integument of the legume ovule and it differentiates as a layer of radially elongated macrosclereids, which are commonly called 'Malpighian cells' or palisade cells (Corner 1976, Werker 1997). Generally, two microscopic characters distinguish the *Fabaceae* testa: external palisade cells and hourglass cells (Corner 1951). The testa in legumes is usually composed of seven layers: cuticle 'light line' (absent in some legumes), epidermis (Malpighian layer), hypodermis (sclereid layer), parenchyma, remnant layers and endosperm as the deepest testa layer. However, the testa in legumes is highly differentiated and its anatomical structure is species-specific (Werker 1997). The exotesta of the mature seed of *Phaseolus lunatus*

is composed of a very thin cuticular layer, sclerenchyma containing a palisade cell layer and crushed cells. The palisade tissue is of exoepidermal sclerenchyma, constructed from large sclereids, 2-3-layered, which are palisade in form, relatively long. The palisade sclereids are connected to a very thin layer of crushed cells or an adhering layer between the exotesta and mesotesta. The mesotesta is composed of two different layers: thin-walled parenchyma cells with a combination of other cell types and a layer of crushed cells. Parenchyma cells of the mesotesta are large, semicircular, hourglass or stirrup-shaped, but elongated (Aniszewski *et al.* 2006). In contrast to the seed coat of *P. lunatus*, that of *T. turcica* had a thick cuticle and 1-layered palisade tissue consisting of macrosclereids. In the seed coat of *T. turcica*, the mesotesta with two different layers was composed of thin-walled parenchyma cells. The outer layer of mesotesta was formed by semicircular, hourglass or stirrup-shaped mesotestal cells as well as in *P. lunatus* and the inner layer was formed by flattened cells. No thin layer of crushed cells or an adhering layer between the mesotesta and exotesta of seed coat of *T. turcica* was observed in this investigation. The exotesta of the seed coat of *Pterodon emarginatus* presents radially elongated cells which form the palisade layer. In the mature seeds, the subepidermal layer of the testa differentiates into hourglass-cells; the mesotesta presents many intercellular spaces (Oliveira and Paiva 2005). In the seed coat of *T. turcica*, the mesotesta had intercellular spaces as seen in *P. emarginatus*.

The Rocky Mountains and intermountain regions are populated by the relatively variable and some widespread *Thermopsis* species in North America (Chen *et al.* 1994) while the habitat of endemic *T. turcica* is marshy lakeside of about 950 - 1050 m in altitude in Turkey.

The authors aimed to introduce endemic *T. turcica*, the first record of the genus *Thermopsis* for Turkey. *T. turcica* can be used as an ornamental plant, for their large, attractive and golden yellow flowers. Further studies are needed for cultural needs, physiology and possible conservation measures of this important endemic Turkish plant species which has medicinal values.

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