

## Morphology and anatomy of *Hedysarum pannosum* (Boiss.) Boiss. (Fabaceae)

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**Abstract** – The aim of this paper is to investigate morphological, anatomical, palynological, fruit and seed micromorphological properties of *Hedysarum pannosum* (Boiss.) Boiss. A detailed description of the species is reported for the first time in this study. The morphological features of the species have been compared with the results of previous investigations. Anatomical studies have been carried out on cross-sections of roots, stems, leaflets and petioles. The anatomical results show that the plants have secondary growth roots, protruding stems, amphistomatic and equifacial leaves with tannin, triangular shaped petioles. *Hedysarum pannosum* pollen are tricolpate, prolate and pollen exine ornamentation is reticulate. Fruits have trichomes on their setae and tomentose trichomes have papillae. Seeds are reniform and they have rugolo-reticulate ornamentation.

**Keywords:** anatomy, Fabaceae, fruit, *Hedysarum*, micromorphology, morphology, pollen, seed.

### Introduction

Fabaceae, represented by 650 genera and 18000 species in the world, are a well-known family and have an economic value (POLHILL 1981). The genus *Hedysarum*, was established by LINNAEUS (1753). It belongs to the tribe Hedysareae of the family Fabaceae. The genus *Hedysarum* is represented by 154 species and the main origin of distribution of the genus is Central Asia. This genus has also an important distribution in the Anatolian-Iranian-Caucasian triangle. In Turkey, the genus *Hedysarum* is represented by 22 species (DAVIS 1970). *Hedysarum pannosum* was a synonym of *Onobrychis pannosa* (BOISSIER 1849). Later, PONERT (1973) reported *H. pannosum* as *H. pogonocarpum* Boiss. subsp. *pannosum* in Feddes Repertorium. However AKTOKLU (2012) determined *Onobrychis pannosa* and *H. pogonocarpum* subsp. *pannosum* as a synonym of *H. pannosum*.

The genus *Hedysarum* is a legume plant which has photosynthetic metabolism and N<sub>2</sub>-fixing ability. Accordingly, investigations about the genus *Hedysarum* are concentrated on photosynthetic metabolism and N<sub>2</sub>-fixing but there are also studies based on various scien-

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tific fields: molecular biology and karyology (ARSLAN and ERTUGRUL 2010, ARSLAN et al. 2012), palynology (CIVELEK et. al. 1999, PAVLOVA and MANOVA 2000, YILDIZ et al. 2009, GHANAVATI and AMIRABADIZADEH 2012) and nuclear DNA content (AKPINAR and YILDIZ 1999). However, anatomical and micromorphological studies are very limited (CIVELEK et al. 1999). Anatomical characters are not always as useful as morphological characters for plant identifications. However, they are well-established criteria and can offer significant assistance in plant taxonomy (GUVENC and DUMAN 2010, RANJBAR et al. 2010, GUVENC et al. 2011). Furthermore, pollen exine ornamentation can also be used for plant taxonomy. The objectives of this investigation are to give a detailed account of the morphological, anatomical, pollen, fruit and seed micromorphological characteristics of *H. pannosum* and to evaluate their usefulness in the taxonomy of the genus.

## Material and methods

The specimens of *Hedysarum pannosum* were collected from Konya, Obruk, Kızıltepe, low mountain steppe, 1250 m, 38°01'N 32°56'E, on 5<sup>th</sup> July 2011, and were deposited in KNYA herbarium (Dural-3500). Taxonomical description of the species was followed according to DAVIS (1970) and our observations.

Anatomical investigations were performed using an average of 20 fresh specimens which were kept in FAA solution (5%, v/v formalin, 5%, v/v acetic acid, 50%, v/v ethanol). The paraffin method was used for cross-sections. Sections were taken by a Thermo Scientific Shandon Finesse 325 Rotary microtome, stained with safranin-fast green and mounted with entellan. Only root cross-sections were taken with a razor blade and were stained with fluoroglisin-HCl. Slides were observed by Leica DM 1000 light microscope. Measurements were made with Cameram 21 program and photos were taken with a Canon EOS 450D camera attached to the light microscope.

For palynological investigations, pollen samples were obtained from herbarium materials. For light microscope investigations the pollen slides were prepared according to the WODEHOUSE technique (1935). Measurements and observations were made using a Leica DM 1000 light microscope with a Canon 450D camera and Cameram 21 program. The polar length (P), the equatorial length (E), the colpus length (CLG), the colpus width (CLT), thicknesses of exine and intine were examined for 30 pollen grains and P/E ratios were calculated. For scanning electron microscope investigations, unacetolyzed pollen grains were first mounted on a double sided carbon tape affixed to aluminum stubs, were covered with gold by a Cressington Auto 108 sputter coater and were photographed with ZEISS EVO LS10 SEM. Pollen terminology was followed according to PUNT et al. (2007).

Size of fruits and seeds were screened with stereomicroscope and 30 mature fruits and seeds were measured. For SEM investigations, the mature fruits and seeds were placed on stubs directly and covered with gold and their diagnostic parts were photographed at several magnifications.

## Results

### Morphological characteristics

*Hedysarum pannosum* is an erect and perennial plant, up to 65 cm, stems branched at the base, stems surface densely covered with pannose trichomes. Leaves are mostly basal

with 4–10 pairs of ovate to elliptic or linear-lanceolate. Leaflets have a pannose indumentum on both surfaces. The length of leaflet is 0.4–2 cm. Upper surface of leaflets is greenish and more sparsely hairy than the lower surface, which is grayish-green. The apex of the leaflets is acute. Petioles are 3–11 cm. Stipules are 4–5 mm, broad triangular, brownish, density and type of trichomes are the same as those of the stem. Peduncles are sturdy, 4–7 cm. Inflorescence is 9–16 cm, dense raceme. Bracts are c. 2–3 mm and bracteoles 2–2.25 mm. Calyx is 5–6 mm with subequal teeth  $\pm$  same length and teeth are 2.5–3 mm. Corolla is yellow; standard size is 15–16 mm; wings are 8–9 mm; keel is 11–12 mm. Stamens are diadelphous, filaments are 11–15 mm, anthers are approximately 1 mm length. Ovary is pilose. Lomentum has 1–2(–3) elliptic-ovate segments, 20–25  $\times$  5–7 mm, fruit (juvenile) is pinkish-red setae, fruit (mature) is brownish-cream with densely 5–10 mm length setae.

## Anatomical characteristics

### Root anatomy

Root anatomy shows that plants have a disintegrated periderm on the outermost layer as a protective tissue, which has 8–9 layers and is composed of disintegrating or squashed cells. Width of periderm cells is 81.16–128.59  $\mu\text{m}$  (Tab. 1). Cortex, 7–8 layered, follows periderm towards to the center. There are sclerenchymatic cell groups in cortex and phloem (Fig. 1a). Phloem is well developed, phloem and xylem split up by 2–3 layered cambium. Vessels in xylem are irregular, according to METCALFE and CHALK (1957) classification vessel grouping. The centre of roots in transverse sections was covered with xylem (Fig. 1b).

### Stem anatomy

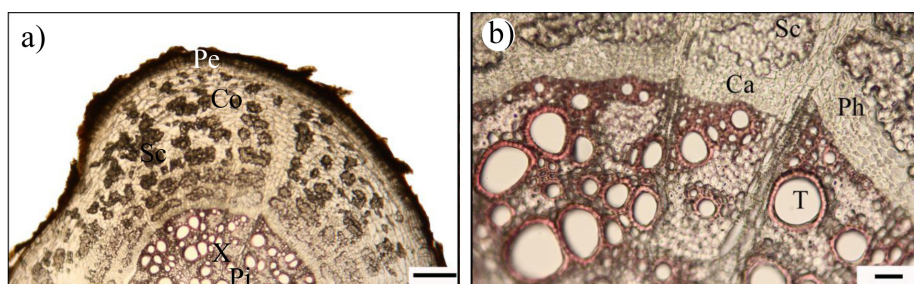
The shapes of the cross-sections of stem are oval but stem corners are protruding. It is evident on the transverse sections of stems that the one layered epidermis mainly consisted of rectangular, frequently arranged cells with a not thick cuticle (0.68–4.18  $\mu\text{m}$ ) (Tab. 1). The collenchyma is located below the epidermis; it has 6–7 layers at the corners of stem but it has 1–2 layers at the margins. The cortex which includes tannin is composed of 7–8 layered oval parenchymatic cells and their dimensions are 4.5–18.4  $\times$  7.6–30.3  $\mu\text{m}$  (Fig. 2a, Tab. 1). Phloem and xylem are well developed. Above the phloem, sclerenchymatic cells are present. Diameters of the tracheas are 11.2–33.3  $\times$  7.9–29.3  $\mu\text{m}$  (Tab. 1). The pith region of the stem consists of large parenchymatic cells and some of them include tannin (Fig. 2b).

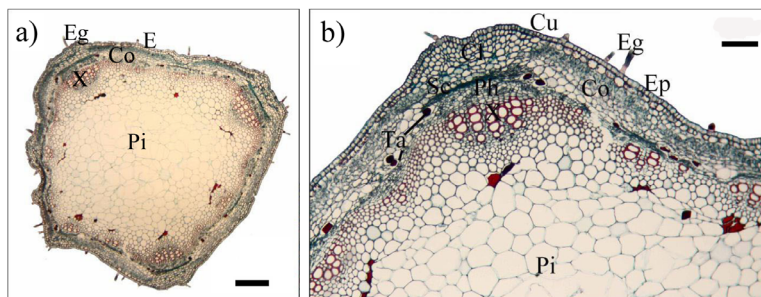
### Leaflet anatomy

The transverse sections of leaflets show that the upper and lower epidermis are made up of oval, rectangular or isodiametric cells with adaxial and abaxial cuticles (adaxial cuticle thickness is 0.69–4.18  $\mu\text{m}$ , abaxial cuticle thickness is 1.88–5.93  $\mu\text{m}$ ). Cells of the upper epidermis (8.63–50.45  $\mu\text{m}$  wide  $\times$  6.75–22.74  $\mu\text{m}$  long) are wider than those of the lower epidermis (5.70–42.68  $\mu\text{m}$  wide  $\times$  5.0–16.6  $\mu\text{m}$  long) (Tab. 1). The leaflet is amphistomatic and equifacial (Fig. 3a). The mesophyll thickness is 106.11–208.86  $\mu\text{m}$  (Tab. 1). The palisade parenchyma is 1–3-layered above and 1–2-layered below the mesophyll. The spongy parenchyma cells which are 1–3-layered are present among the palisade parenchymatic cells with large intercellular spaces. Leaflets of *H. pannosum* have hypodermis on the abaxial side. Vascular bundles are collateral types (Fig. 3b).

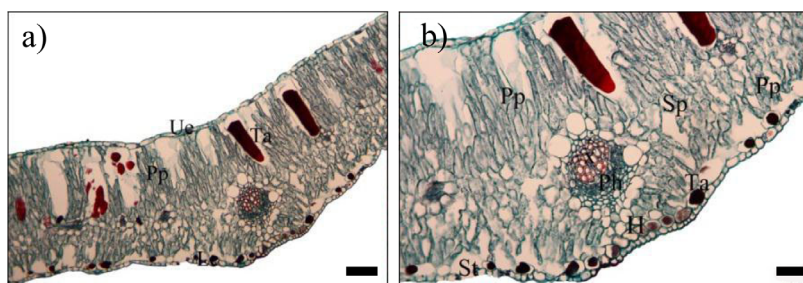
**Tab. 1.** The anatomical characteristics of *Hedysarum pannosum*. Mean value  $\pm$  standard deviation (SD), n = 20.

	Width ( $\mu\text{m}$ )		Length ( $\mu\text{m}$ )	
	Min–Max	Mean $\pm$ SD	Min–Max	Mean $\pm$ SD
<b>Root</b>				
Peridermis cell	81.16–128.59	105.0 $\pm$ 11.7		
Parenchymatic cell	214.6–348.8	275.3 $\pm$ 49.9		
Trachea cell	9.35–48.02	26.31 $\pm$ 8.74		
<b>Stem</b>				
Cuticle	0.68–4.18	2.35 $\pm$ 0.7		
Epidermis cell	5.9–25.9	12.7 $\pm$ 3.9	7.5–17.1	12.2 $\pm$ 1.9
Cortex parenchyma cell	7.6–30.3	15.0 $\pm$ 5.0	4.5–18.4	12.8 $\pm$ 2.9
Trachea cell	7.9–29.3	18.3 $\pm$ 4.1	11.2–33.3	23.0 $\pm$ 4.3
Pith cell	16.03–79.7	39.3 $\pm$ 15.9	16.8–74.1	37.1 $\pm$ 14.1
<b>Leaflet</b>				
Adaxial cuticle	0.69–4.18	2.34 $\pm$ 0.7		
Abaxial cuticle	1.88–5.93	3.48 $\pm$ 1.15		
Adaxial epidermis	8.63–50.45	22.3 $\pm$ 8.2	6.75–22.74	12.51 $\pm$ 2.97
Abaxial epidermis	5.70–42.68	16.87 $\pm$ 7.8	5.0–16.6	9.8 $\pm$ 2.41
Mesophyll	106.11–208.86	161.39 $\pm$ 25.5		
Palisade parenchyma	2.57–16.66	11.0 $\pm$ 2.8	11.66–77.31	44.3 $\pm$ 12.2
Spongy parenchyma	5.61–38.03	24.03 $\pm$ 6.85		
<b>Petiole</b>				
Cuticle	1.86–3.78	2.91 $\pm$ 0.52		
Adaxial epidermis	1.66–20.6	7.7 $\pm$ 3.2	3.6–16.6	8.76 $\pm$ 2.0
Abaxial epidermis	2.7–18.0	8.23 $\pm$ 3.0	5.15–14.78	8.83 $\pm$ 1.9
Parenchymatic cell	22.9–95.4	55.7 $\pm$ 13.9	18.4–102	58.1 $\pm$ 16.1
Trachea cell	4.8–29.88	19.71 $\pm$ 5.1		

**Fig. 1.** The transverse sections of the root of *H. pannosum*: a) Pe – periderm, Sc – sclerenchyma, Co – cortex, X – xylem, Pi – pith region (scale bar = 250  $\mu\text{m}$ ); b) Ph – phloem, T – trachea, Ca – cambium (scale bar = 25  $\mu\text{m}$ ).



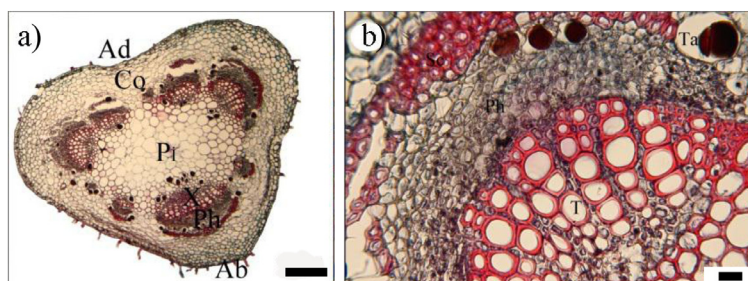
**Fig. 2.** The transverse sections of the stem of *H. pannosum*: a) Eg – eglandular hair, Ep – epidermis, Co – cortex, X – xylem, Pi – pith region (scale bar = 250  $\mu$ m); b) Ta – tannin, Cu – cuticle, Sc – sclerenchyma, Ph – phloem, Cl – collenchyma (scale bar = 100  $\mu$ m).



**Fig. 3.** The transverse section of the leaflet of *H. pannosum*: a) Ue – upper epidermis, Pp – palisade parenchyma, Ta – tannin, Le – lower epidermis (scale bar = 100  $\mu$ m); b) Sp – spongy parenchyma, H – hypodermis, X – xylem, Ph – phloem, St – stomata (scale bar = 50  $\mu$ m).

### Petiole anatomy

The general view of petiole cross section is triangular especially in sections which have been taken from the midrib of the petiole. In petiole transverse sections, the epidermis is composed of one layer and epidermal cells of both surfaces are rectangular to oval and have trichomes (Fig. 4a). Cortex parenchymatic cells which are located under the epidermis are orbicular shaped and are composed of 8–9 layers. The dimensions of cortex cells are 18.4–

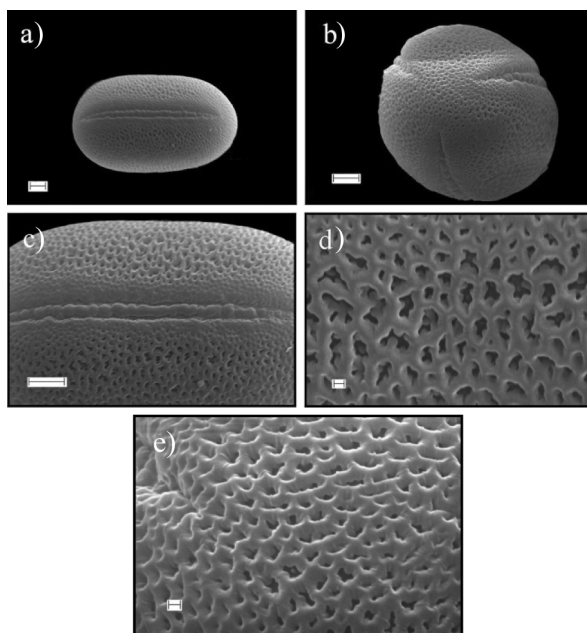


**Fig. 4.** The transverse sections of petiole of *H. pannosum*: a) Ad – adaxial epidermis, Ab – abaxial epidermis, Co – cortex, X – xylem, Ph – phloem, Pi – pith region (scale bar = 250  $\mu$ m); b) T – trachea, Ta – tannin, Sc – sclerenchyma (scale bar = 25  $\mu$ m).

$102 \times 22.9\text{--}95.4 \mu\text{m}$  (Tab. 1). There are three large primary collateral vascular bundles in the corners of the petioles, with small secondary bundles among them. On the vascular bundles there is sclerenchyma shaped like an arc (Fig. 4a). The pith is composed of parenchymatic cells which are orbicular shaped (Fig. 4b).

### Pollen characteristics

The pollen grains of *H. pannosum* are tricolpate, prolate and isopolar. The shapes of pollen grains are elliptical in equatorial view (Fig. 5a) and orbicular in polar view (Fig. 5b). The dimensions of the polar axis (P) and equatorial axis (E) are  $20.303\text{--}24.608 \mu\text{m}$  and  $13.493\text{--}17.737 \mu\text{m}$ , respectively. The ratio of P/E is  $1.350\text{--}1.792 \mu\text{m}$ . Colpus length is  $15.072\text{--}21.275 \mu\text{m}$  and colpus width is  $1.305\text{--}2.834 \mu\text{m}$ . Colpus membrane has large sculptural elements but some of them are fused especially in the middle of colpus (Fig. 5c). The exine thickness is  $0.604\text{--}1.058 \mu\text{m}$  and the intine thickness is  $0.447\text{--}0.84 \mu\text{m}$ . Exine ornamentation is reticulate in both of equatorial and polar view but pores in equatorial view are larger than those in polar view (Figs. 5d–e).

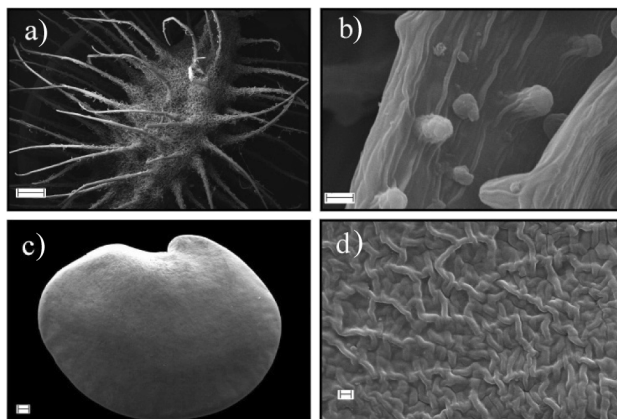


**Fig. 5.** SEM micrographs of the pollen of *H. pannosum*: a) equatorial view (scale bar =  $2 \mu\text{m}$ ); b) polar view (scale bar =  $2 \mu\text{m}$ ); c) colpus and colpus membrane view (scale bar =  $2 \mu\text{m}$ ); d) exine sculpturing in equatorial view (scale bar =  $200 \text{nm}$ ); e) exine sculpturing in polar view (scale bar =  $200 \text{nm}$ )

### Fruit and seed micromorphological characteristics

Stereomicroscope investigations show that mature fruits (lomentum) of *H. pannosum* have 1–2(–3) segments, ovate,  $20\text{--}25 \times 5\text{--}7 \text{mm}$ , and its lomentum has setae. Lomentum surface is densely covered with tomentose trichomes. Juvenile fruit setae are pinkish-red,

but mature fruits are brownish-cream and their length is 5–10 mm. The SEM investigations disclosed that both tomentose trichomes and setae are covered with papillae which are 1–2  $\mu\text{m}$  long (Figs. 6a–b). Mature seeds are reniform (Fig. 6c), brownish-yellow,  $6 \times 4$  mm, seed surface is rugolo-reticulate (Fig. 6d).



**Fig. 6.** SEM micrographs of the fruit and seed of *H. pannosum*: a) the general view of fruits (scale bar = 1 mm); b) the setae of fruits (scale bar = 20  $\mu\text{m}$ ); c) the general view of seeds (scale bar = 200  $\mu\text{m}$ ); d) seed surface ornamentation (scale bar = 2  $\mu\text{m}$ ).

## Discussion

The present study sought to provide useful information on the anatomy, pollen morphology, fruit and seed micromorphology of *H. pannosum*. This is the first report on the examined characteristics of the species. The morphological results (e.g. leaves, bracts, stipules dimensions and corolla color) are mainly congruent with the description reported in Flora of Turkey (DAVIS 1970). However the dimensions of some characters of the plant such as stems, petioles and lomentum segments are seem to be variable than previously and measurements of some characters of *H. pannosum* are such as filament and anther length are presented here for the first time (Tab. 2).

Little work appears to have been done on the anatomy of vegetative organs of *Hedysarum* species (WATARI 1934, CIVELEK et al. 1999). In the root of *H. pannosum* periderm cells have 8–9 layers, but the root of *H. aucheri* comprises 14–15 layers (CIVELEK et al. 1999). *H. pannosum* cortex parenchyma and pith ray cells do not contain starch. However, the pith rays and cortex cells of *H. aucheri* do have starch in their parenchymatic cells. Also cambium cells of *H. aucheri* are composed of 3–4 layers (CIVELEK et al. 1999). However *H. pannosum* are composed of 2–3-layered rectangular cells. The number of layers of cambium, periderm and the presence/absence of starch in roots can appear to be a taxonomically significant feature to distinguish the species.

The transverse sections of stem of *H. pannosum* are made up of four main tissues from outside to inside and their names are epidermis, cortex, vascular bundles and pith region, respectively. We found that at the corners of stem of *H. pannosum* there are 6–7 layered

**Tab. 2.** Comparison of morphological characters of *Hedysarum pannosum* based on the present study and 'Flora of Turkey' (DAVIS 1970).

	'Flora of Turkey'	Present study
Stem	Up to 30 cm	Up to 65 cm
Leaves	3–9 pairs ovate to elliptic on both surface pannose indumentum	Ovate to elliptic on both surface pannose indumentum
Leaflets	Not recorded	4–10 pairs of ovate to elliptic or ovate to linear-lanceolate. The biggest leaflet 0.4–2 cm. The both surfaces of leaflets pannose. Upper surface of leaflets is greenish and more sparsely hairy than lower surface, lower surface more hairy, grayish-green and leaflets apex acute.
Petiole	Not recorded	3–11 cm
Stipules	c. 3 mm	4–5 mm
Peduncles	Sturdy, much longer than leaves	4–7 cm
Inflorescence	Not recorded	9–16 cm, dense racem
Bracts and bracteoles	Not recorded	Bracts c. 2–3 mm; bracteoles c. 2–2.25 mm
Calyx	Calyx 4–5 mm with subequal teeth ± same length as tube	Calyx c. 5–6 mm, teeth approximately the same length and teeth c. 2.5–3 mm length
Corolla	Corolla yellow; standard 10–14 mm; wings 7–9 mm; keel 10–15 mm	Corolla yellow; standard 15–16 mm; wings 8–9 mm; keel 11–12 mm
Filaments	Not recorded	Stamens diadelphous, filaments 11–15 mm
Anthers	Not recorded	c. 1 mm
Ovary	Ovary glabrous or slightly pilose	Ovary pilose
Fruits (lomentum)	Lomentum with 1–2 ovate-orbicular segments, c. 8 × 8 mm, pubescent and densely covered with setae up to 5 mm long	Lomentum with 1–2(–3) segments, 20–25 × 5–7 mm, ovate, when fruit is juvenile pinkish-red and is mature brownish-cream with densely 5–10 mm length setae

collenchyma. Although *H. aucheri* has 4–5 layered (CIVELEK et al. 1999). Vascular bundles of *H. pannosum* are collateral type and over them there are sclerenchymatic groups like an arc. Furthermore the pith cells of *H. pannosum* have tannin but there is no tannin in *H. aucheri* (CIVELEK et al. 1999). The number of layers of collenchyma at the corners of stem, the presence/absence of tannin in stem anatomy can be useful characters for distinguishing the species.

Upper epidermis of *H. pannosum* is larger than lower epidermis in leaflet anatomy (Tab. 1). The leaflet of mesophyll of *H. pannosum* is equifacial that of *H. aucheri* is the same (CIVELEK et al. 1999). In leaflet anatomy of *H. pannosum* vascular bundles are of the col-



lateral type and among the upper palisade parenchymatic cells there are cylindrical tannin cells in lysigenous secretory cells. Also tannins are present between abaxially palisade parenchyma in *H. pannosum*. CIVELEK et al. (1999) observed tannin in *H. aucheri* leaflets.

WATARI (1934) emphasized that 'the petiolar base is one of the most important regions in the vascular course of foliar organs of Leguminosae.' He classified branching patterns of the vascular bundles at the petiolar bases into three types based on the number leaf traces and whether they are fused or free. In the present study leaf traces of *H. pannosum* are free. In petiole anatomy of *H. pannosum* there are three main median vascular bundles and also small secondary ones and they involve tannin. The shapes of petioles of *H. pannosum* show similarities with those of *H. micropterum* and *H. falconeri* (CHOI et al. 1999). METCALFE and CHALK (1957) pointed out that the stomata of Fabaceae is of the anizositic type and that those of *H. pannosum* and also *H. aucheri* (CIVELEK et al. 1999) are the same.

The results of our investigation show that pollen morphology of *H. pannosum* is comparatively homogenous and confirms the general description presented by OHASHI (1971), FERGUSON and SKVARLA (1981), FAEGRI and IVERSEN (1989), MOORE et al. (1991), CHOI and OHASHI (1996), CIVELEK et al. (1999), PAVLOVA and MANOVA (2000), and GHANAVATI and AMIRABADIZADEH (2012). *H. pannosum* pollen are tricolpate, prolate, circular in polar view and elliptical-elongated in equatorial view and ornamentation reticulate. While *H. tauricum*, *H. grandiflorum* and *H. aucheri* pollen grains are prolate, triangular-obtuse in polar view, elongated, rectangular-obtuse to elliptic in equatorial view and their ornamentation is finely reticulate (CIVELEK et al. 1999, PAVLOVA and MANOVA 2000). The pollen grains of *H. pannosum* colpus membranes have large sculptural elements but some of them are united especially in the middle of the colpus. PAVLOVA and MANOVA (2000) declared that *H. tauricum* and *H. grandiflorum* colpus membrane was covered by differently sized sculptural elements. Also GHANAVATI and AMIRABADIZADEH (2012) explained that *H. kopetdaghi* and *H. damghanicum* colpus membranes were covered by large and small sculptural elements.

There has been no investigation about the fruit micromorphology of *Hedysarum* genus. In this study, the fruit micromorphology of *H. pannosum* is presented for the first time. The fruits of the *H. pannosum* lomentum are oval shaped and have setae. The SEM investigations show that on tomentose trichomes of lomentum and setae there are papillae 1–2 µm in diameter. The investigation of the fruits of *Hedysarum* genus will be a guide for future discussions.

The seeds of *H. pannosum* are reniform, hilum small and the seed surface is rugolo-reticulate. The surface of seed coat sculpture is arranged irregularly (Fig. 6d). SA (2007) showed that the seeds of *H. jaxartucirdes* were reniform, hilum small at upper part of seed and seed coat sculpture mainly cerebelloid, highly irregular, cell wall is sinuate and without ornamentation. Also the seeds of *H. gmelinii*, *H. dahuricum* and *H. setigerum* were reniform, and their hilum region is grooved; the seed coat sculpture was irregular, the seed surface ornamentation was reticulate. Moreover *H. brachypterum* and *H. dasycarpum* seeds ornamentation were reticulate and seed coat cells were highly irregular. But *H. splendens* seeds coats were relatively smooth (SA et al. 2010). *H. aucheri* seeds were reniform and seed surface ornamentation was smooth (CIVELEK et al. 1999). Hence these features of seed surface micromorphology can suggest taxonomical diagnostic characters for distinguishing species.

Anatomical, palynological and micromorphological (pollen, fruit and seed) characteristics might be useful in the definition of the species investigated. Nevertheless, these characteristics will be more valuable if other species of *Hedysarum* are also examined.

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