Pak. J. Bot., 44(4): 1185-1192, 2012.

THE ANATOMY AND MORPHOLOGY OF THE MEDICINAL PLANT, *LILIUM CANDIDUM* L. (*LILIACEAE*), DISTRIBUTED IN MARMARA REGION OF TURKEY

FAZIL ÖZEN[1], HACI TEMELTAŞ[2] AND ÖZLEM AKSOY[1*]

[1]*Department of Biology, Faculty of Science and Literature, University of Kocaeli, Umuttepe Campus, Kocaeli, Turkiye*
[2]*Maltepe Askeri Lisesi Komutanlığı, 35314, Izmir, Turkiye.*
[*]*Corresponding author's e-mail: ozlem.aksoy@kocaeli.edu.tr; Tel: 90 262 3032902; Fax: 90 262 3032903*

Abstract

Lilium candidum L., was cultivated and has been used for different purposes like medical, cosmetics, commercial for thousands of years. It was naturally distributed around the city of Balıkesir (in Marmara region) and was investigated for its morphological, anatomical and phenological specifications during this study. Some of the morphological measurements were different from the measurements in Flora of Turkey. The measurements on bulb, stem leaves, basal leaves, bracte, pedicel, inner and outter tepals (separately), fruit, seed and pollen were given for the first time. Natural distribution of *L. candidum* in this region is the first record for the region and also the natural growth conditions for this species around Balıkesir city were determined for the first time.

Introduction

Historical records about lilies (*Lilium* L. spp.) indicate that there was cultivation of lilies in Knossos in the ancient Crete civilization thousands of years BC (Wilson *et al*., 1981), that the Egyptians used lilies in wreaths in funerals, and that Romans cultivated lilies together with daffodils and hyacinths in palace gardens for using in rituals. From the Sumerian tablets dating back to 5000 years before, it has been determined that the city Susa in Iran has been surrounded with lily fields and this city has been named after this plant (Uzun, 1984). Today, lilies are commercially used for medical and ornamental purposes and in perfumes (Baytop, 1984; Khawar *et al*., 2005; Saifullah *et al*., 2010). Several saponins have been isolated from the ethanolic extract of the bulbs and flowers of *L. candidum* (Haladova *et al.*, 2011). *L. candidum* extract is well known in folk medicine for the treatment of burns, ulcers, inflammations and for healing wounds (Kopaskova *et al*., 2012).

L. candidum L. is a herbaceous, bulbous perennial plant belonging to *Liliaceae* family. It is popularly known as "Madonna Lily" or "white lily". Having been gathered from nature and marketed for a long time on account of its fragrant and big flowers and its medically significant bulb, this plant has been successfully cultivated in the recent years in Europe, USA, Turkey and many other countries (Baytop, 1984; Khan *et al*., 2010).

L. candidum is an eastern Mediterranean element distributed in Turkey, Lebanon, Syria, Palestine, Greek Islands and the Balkans. It has been recorded from Aydın (C1), Muğla (C1,C2), Antalya (C3) İstanbul (A2) and Mardin (C8) in Turkey, and this study forms the first record in Balıkesir (B2) region. Although it grows naturally in Balkan Peninsula and Eastern Mediterranean, the origin of the plant is not exactly known. It is the only lily species having white flowers (Davis, 1988).

There was no study found in the literature about the anatomy and morphology of *L. candidum*. However, some studies about the species have been made on hybrid lilies grown as cut flowers in greenhouses (Uzun, 1981; Uzun, 1984). Besides, it has been determined that some biochemical and genetic studies have been made on *L. candidum* (Van-Tuyl *et al*., 1986). *L. candidum,* which has been known for thousands of years and been used for various purposes, was being grown extensively in gardens for ornamental purposes in Balıkesir area. But no scientific record were found about this species growing naturally in Balıkesir area.

With this study, the commercially significant genus *Lilium candidum* was recorded in Balıkesir area for the first time, and its anatomical, morphological and phenological characteristics were investigated. This study was made considering that these findings, together with ecological data, will contribute scientifically to facilitation of cultivation of this plant.

Material and Method

As research material, plant samples were collected from villages of Sarıçayır, Sarıfakılar (Sarfaklar), Keçidere, Mehmetler and Durak on rocky lands at altitudes of 150-460 meters in the vicinity of areas with dense deciduous shrubs and trees, and were identified using Flora of Turkey (Davis, 1988).

The changes in the phenology of the species (basal leaves, stem leaves, stem, bulb and flower) were observed in its habitat, required measurements were taken, and drawings were made. Then, the minimum and maximum values of these measurements obtained in the investigations were determined. We made use of Karasar's study in determining the mean and standard deviation (Karasar, 1994). Cross sections of the roots, bulbs, basal leaves, stems and stem leaves were taken, and stem and root cross sections were treated with flouroglycine in investigation of anatomical features (Yakar-Tan, 1982). The cross sections were covered with canada balsam for permanent preparation. The drawing of the cross sections were made using a Nicon Eclipse E600 binocular microscope, and measurements were taken using an ocular micrometer.

The features of the research area: This study has been made on *L.candidum* which has a natural distribution at altitudes of 150-460 meters in the villages Sarıçayır and Sarıfakılar (Sarfaklar) located at southeast of Kepsut town

which is located at 20km.'s east of Balıkesir city center, especially on south and southeast slopes. Great soil groups of this area are lime-free brown forest soil, brown forest soil and lime-free soil (Anonymus, 1985). The research area falls into Mediterranean floristic region in terms of vegetation formations (Akman, 1993), and is located in B2 square according to Davis's grid system (Davis, 1988) (Fig. 1).

Fig. 1. The distribution of *L. candidum* on the research area.

Results and Discussion

The morphological features of *L. Candidum*: Certain differences were determined between the data obtained in the research on external morphological features of the species and Flora of Turkey (Davis, 1988). However, in this study, separate inner and outer tepal measurements, together with biometric measurements of the bulb, stem and basic leaves, bract, pedicle, pollen, fruit and seeds were presented, and there was no study found about our findings.

L. candidum has fibrious roots, and can extend up to 45cm. The roots are yellowish white colored and hairy. Odorless, yellowish white bulbs are composed of succulent laminae. The measured dimensions are 1.2-7.6 x 0.8-2 cm. (Table 1). Its base is nearly smooth, wider in the middle, and conic at the tip (Uzun, 1981; Uzun, 1984). The succulent laminae look like roof tiles lined up on top of each other vertically in the ground (Fig. 2). It is stated that a long bulg can have up to 50 laminae, whereas in this study, 90 laminae have been determined (Uzun, 1981; Uzun, 1984). Although *L. candidum* is presented with a stem length of 50-150 cm. and purplish colored in the Flora of Turkey (Davis, 1988). The measurements have been 43-50 cm. in this study (Table 1) and it has been determined that the color has not been uniformly purple throughout the stem all the time (Fig. 3). The color of the stem turns purple bottom to up in parallel to the growing process, and this coloring increases during the generative phase. The stem is herbaceous and shows branching. The basic leaves appear starting from autumn and form rosettes that disappear after the winter during blooming season. They are usually lanceolate-oblanceolate shaped, high-colored, hairless, parallel-veined and entire. In this study, it has been determined that each plant can have 2-12 basic leaves with measured size 14-35 x 1.5 x 5cm. (Table 1). Basic leaves start dying in parallel to the development of stem and stem leaves and completely disappear during blooming season on most of the plants. Basic leaves can be found on some plants rarely during blooming season on relatively shady locations (Fig. 4a).

Fig. 2. The bulb of *L. Candidum*.

Stem leaves are ordered on the stem on alternating sides, are high-colored, hairless, usually shaped diversely from lanceolate to linear lanceolate, stalkless, parallel-veined and entire. The stem leaves get smaller as they move up the stem. Size measurements of stem leaves are 4-21.5 x 0.4-2.5 cm., and are presented in this study for the first time (Table 1). *L. candidum* blooms during leaved season. The stem leaves turn purple shortly before blooming season and then start dying. During blooming season, some of the leaves dry (Fig. 4b).

The blooming season is 5th month (May) according to the Flora of Turkey (Davis, 1988). In this study, it has been determined that blooming could occur between the end of May and the end of June depending on the climate conditions. The flowers of *L. candidum* are cone shaped, snow white colored, fragrant, actinomorphic and are arranged in a racemose inflorescence (Davis, 1988). In this study, 2-9 flowers have been observed per plant although the flower count reported in the Flora of Turkey (Davis, 1988) is 2-12. The pedicles arise from bract axils and have been measured to have size 1.5-4.8cm. The bracts have been lanceolate-ovate shaped with measured size 2.2-4.1 x 0.5-1.8 cm. (Table 1). The edges of the bracts turn purple during generative phase.

Flower cover perigon, there are 6 tepals which are petaloid and get together in 2 rings (Fig. 5). The size given in the Flora of Turkey (Davis, 1988) for all tepals is 55-65 (-80) x 6-13 (-20) mm. In this study, however, it has been determined that the sizes of the inner and outer tepals were different. The tepals were lanceolate-linear lanceolate shaped. The inner tepals' measured size was 47-70 x 12-22 mm., and outer tepals' 35-69 x 6-21 mm. (Table 1). But it has also been observed that the size of the inner and outer tepals could be same.

Table 1. Biometric measurements of *L.candium*

Number of measurements	Part of plant / parameter	Min.	Max.	Mean	Standard deviation
	Bulb				
55	Length (cm)	1.2	7.6	4.6	± 0.17
	Diameter (cm)	0.8	2	1.4	± 0.04
40	**Stem**				
	Length (cm)	43	150	87.82	± 4.25
	Basic Leaf				
35	Length (cm)	14	35	19.7	± 0.92
	Width (cm)	1.5	5	3.4	± 0.13
	Stem Leaf				
55	Length (cm)	4	21.5	14.75	± 4.11
	Width (cm)	0.4	2.5	1.5	± 1.98
36	**Pedicle**				
	Length (cm)	1.5	4.8	3.03	± 0.14
	Bract				
45	Length (cm)	2.2	4.1	2.99	± 0.08
	Width (cm)	0.5	1.8	1.2	± 0.04
	Outer Tepal				
36	Length (mm)	35	69	53	± 0.14
	Width (mm)	6	21	10.9	± 0.06
	Inner Tepal				
36	Length (mm)	47	70	59	± 0.10
	Width (mm)	12	22	18.6	± 0.05
55	**Anther**				
	Length (mm)	6	11	8	± 0.02
55	**Filament**				
	Length (mm)	30	59	48	± 0.08
30	**Ovarium**				
	Length (mm)	8	15	11	± 0.04
30	**Stilus**				
	Length (mm)	44	64	52	± 0.20
	Pollen				
30	Length (μ)	45	60	51.86	± 0.70
	Diameter (μ)	22	36	26.76	± 0.69
	Fruit				
30	Length (cm)	2	5	3.19	± 0.13
	Diameter(cm)	1.5	4	2.51	± 0.12
	Seed				
30	Length (mm)	10	15	12.9	± 0.22
	Diameter (mm)	6	11	8.8	± 0.24

Fig. 3. The appearance of *L.candidum* a.vegetative phase, b. generative phase.

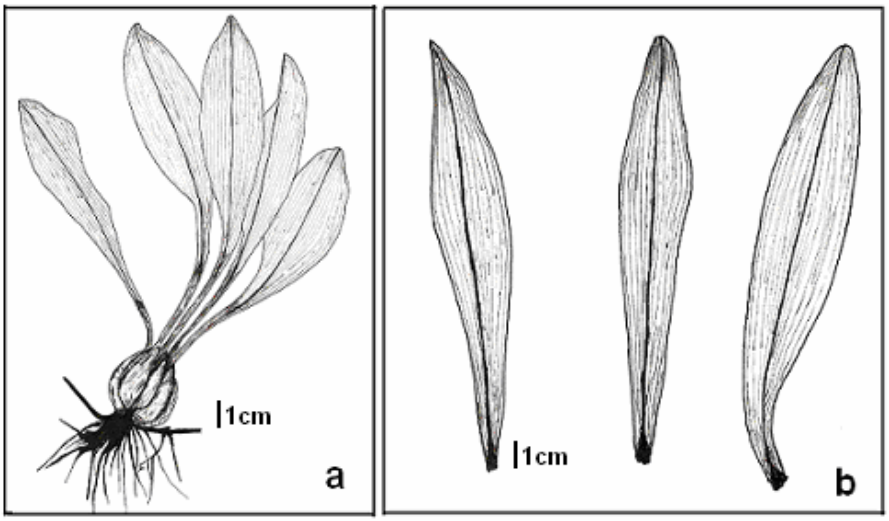

Fig. 4. Leaves of *L. candidum* a. basic leaves, b. stem leaves.

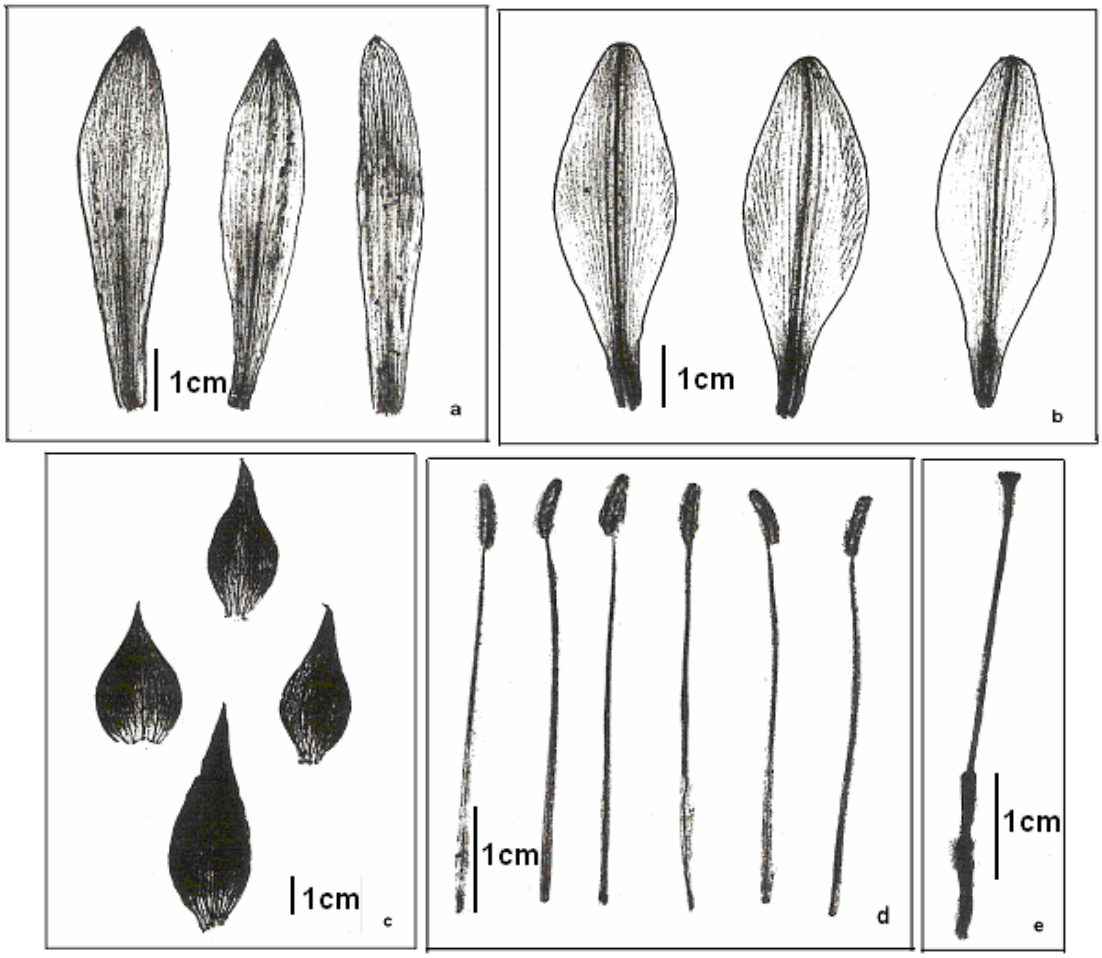

Fig. 5. The parts of *L. candidum* flower a. outer tepals, b. inner tepals, c. bracte, d. stamens, e. Pistil.

Stamen count is 6, are opposite the tepals and free. The size of the filaments given in the Flora of Turkey (Davis, 1988) is 45-50 (-57) mm., and the measured size in this study was 30-59mm. Anthers are versatile, tecas are parallel and open longitudinally. The basis of anther is obtus. The size of the anthers given in the Flora of Turkey (Davis, 1988) is 9-11 mm., and the measured size in this study was 6-11 mm. (Table 1). Pollens are golden yellow, elipsoid and have longitidunal lines. Polen dimensions are 45-60 x 22-36µ (Table 1) (Fig. 6). *L.*

candidum has one pistil which is shorter than tepals. Stilus is spherical and measured as 44-64 mm. It is given as 35-50 (60) mm in the Flora of Turkey (Davis, 1988). Stigma is three lobed. Ovarium is a sincarp, upper state with 3 carpels and 6 loculus. Dimensions are measured as 8-15 mm (Table 1). Plasentation is axial (Fig. 7a). The flower diagram is shown in Fig. 7b.

Fig. 6. The pollen grains of *L. candidum* (X1000) Trachae, Tr: Tracheid, P: Phloem, Cc: Companion cell, Sv: Sieve tube Le: Lower epidermis.

The fruit is loculucidal capsule in greenish colour and oblong shape (Fig. 8a). Dimensions are measured as 2-5 x 1.5-4 cm. Seeds are flat and numerous (Fig. 8b). There are approximately 120 seeds in a fruit. The dimensions of seed are measured as 10-15 x 6-11mm. (Table 1). The differences between the morphological chracteristics of L.candidum from the study area and the Flora of Turkey (Davis, 1988) may be due to the fact that they were collected from different areas and thus they may have effected by different ecological factors. However the descriptions of this species in the Flora of Turkey (Davis, 1988) were made from Syria and Paletsine.

The anatomical features of *L. candidum*: The results of the anatomical studies showed that the plant has a typical monocotiledonae root. Rectangular shaped and monolayered epidermis cells and root absorbent hairs were seen in the outer part of the root cross section. Cortex layer which consists of different dimensions and shapes of paranchymatic cells were placed under the epidermis. After the cortex endodermis lies which has monolayered, well shaped passage cells. Under the endodermis, phloem elements were seen which are alternately located with monolayered schleranchimatic pericycle cells. There was no paranchymatic core in the vascular system and the centre is full of metaxylem elements. Vascular beams in root are radial type (Fig. 9). The findings about the structure of root were similar with the features about the root anatomy of *Lilum* described by Yentür (1995) and Vardar (1982).

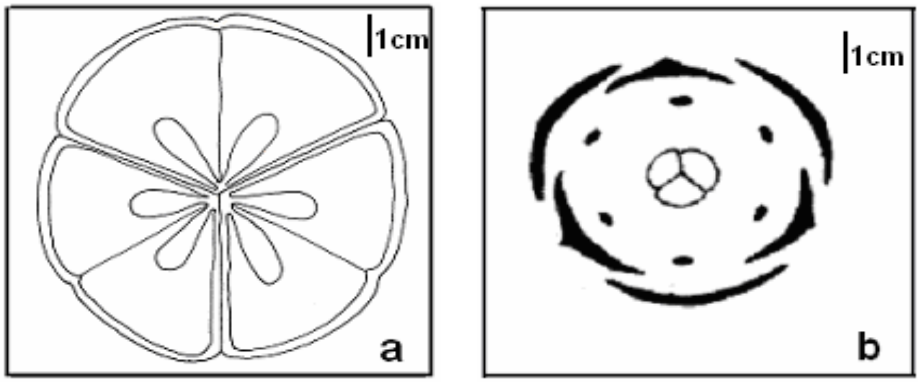

Fig. 7. a. The plasentation in cross section of ovarium, b. The flower diagram in *Lilium candidum* (The flower formula $T_{3+3}\ A_6\ G_{(3)}$).

Fig. 8. The fruit and seeds of *L. candidum* a. Fruit, b. Seeds (x1).

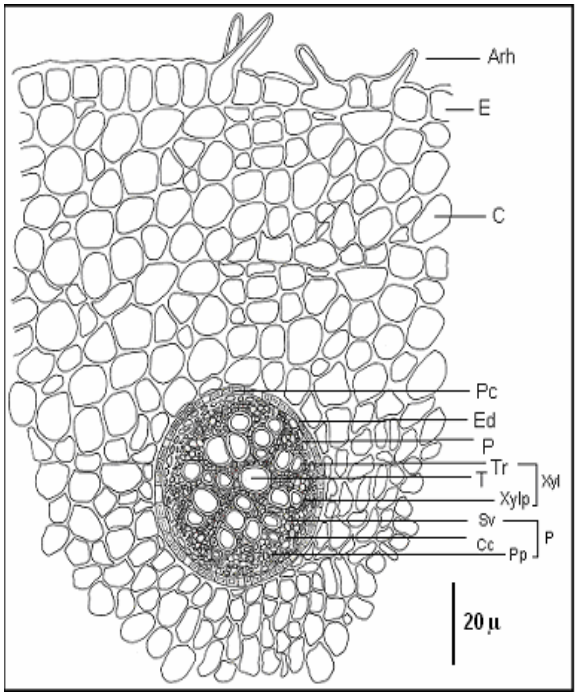

Fig. 9. Cross section of root; Ab: Absorbent root hairs, E: Epidermis, C: Cortex cell, Pc: Passage cell, Ed: Endodermis, P: Pericycle, Xyl: Xylem,T: Trachea, Tr: Tracheid, Xylp: Xylem parenchyma, P: Phloem, Sv: Sieve tube, Cc: Companion cells, Pp: Phloem parenchyma.

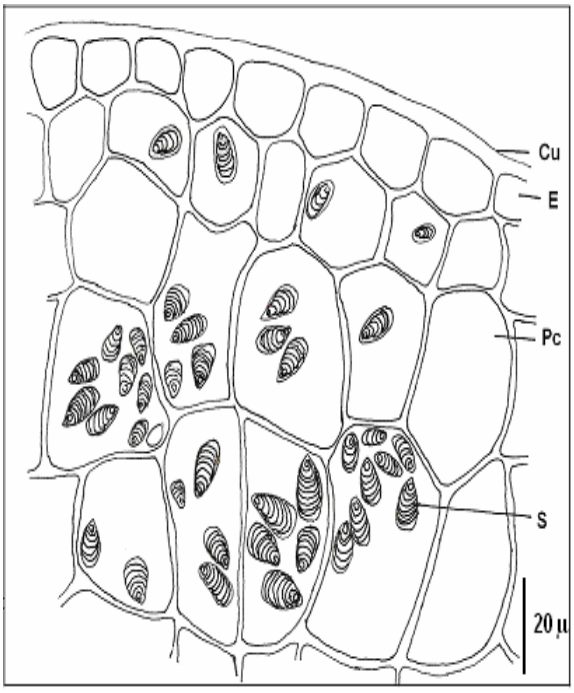

Fig. 10. Cross section of *L.candidum* bulb; Cu: Cuticle, E: Epidermis, Pc: Parenchymatic cell, S: Starch granules.

In the cross section of bulb epidermis layer was observed in the outer region which consists of large rectangular monolayered cells covered with cuticule. Large and square shaped paranchymatic cells which contain many starch granules were under the epidermis. Starch granules were conic shaped and densely located around hilum Vascular beam in bulb were scattered and closed colleteral type (Fig. 10).

In the cross section taken from the stem, outer region is cuticule, under the cuticle epidermis lies which has square-rectangular shaped monolayered cells. One celled simple covering hairs were rarely seen on the stem. Rectangular shaped four cell layered collenchima lies under the epidermis. Collenchyma is in the shape of plate collenchyma. The occurence of collenchyma cells gave a mechanical support to the plant which has a herbaceous stem in vegetative developmental period (Fahn, 1990; Vardar, 1982a; Vardar, 1982b; Yentür, 1995).

Under the collenchyma altough it is not significant in young plant stems, in mature plant stems it was observed that the cell walls of approximately 8 cell rows became thicker and they became schleranchymatic. The schleranchymatic cells formed by seconder wall development help the plant organs for bending, folding and give support for weight and pressure (Fahn, 1990; Vardar, 1982a; Vardar, 1982b; Yentür, 1995). Cortex paranchyma which is composed of big and spherical cells lies after the cortex cells that have been schleranchimatic. And then vascular beam lies without cambium. There are many closed colleteral type vascular beams scattered out. In vascular beam xylem elements are; in the outer part xylem schlerancyma, xylem parenchyma and trachae and tracheids inside. Phloem elements are; in the outer part phloem schleranchyma, phloem parenchyma and phloem companion cells (Fig. 11).

In the cross sections taken from stem leaf and basal leaves, monolayered upper and lower epidermis can be seen which is covered with cuticula in the outer part. Mesophyll tissue has many intercellular spaces and chloroplasts. Some of the cells of palisade parenchyma are lobed and can be distinguished from the sponge parenchyma cells (Figs. 12-13). Fahn (1990) reported that in *L. candidum* palisade parenchyma cells are lobed, Yentür (1995) and Vardar (1982a; 1982b) reported that palisade parenchyma cells are branched and lobed in *Lilium*. In this study we found the similar features in many palisade parenchyma cells.

Vascular beams in leaf were covered with monolayered paranchymatic cells and they were closed colleteral type. In superficial section of the leaf epidermis cells were long and had undulated cell walls. The undulated structure in cell walls known to be strengthen the relation between the cells (Yentür, 1995) (Fig. 14). Leaves are hypostomatic and amarrylis type. Guard cells are paralel to the neighbour cells and parasitic. In the investigations about the leaves, there was no anatomical difference between stem leaf and basal leaves, there was only morphological differences like dimensions, form and phenology. In the cross sections taken from tepals, rectangular shaped, monolayered upper and lower epidermis cells can be seen covered with cuticule. And also there were one celled hairy structures above the upper epidermis cells. There were paranchymatic cells with large intercellular spaces below the epidermis. Vascular beam in tepals were closed collateral type. There were nectarium channels in the basement of tepals (Fig. 15).

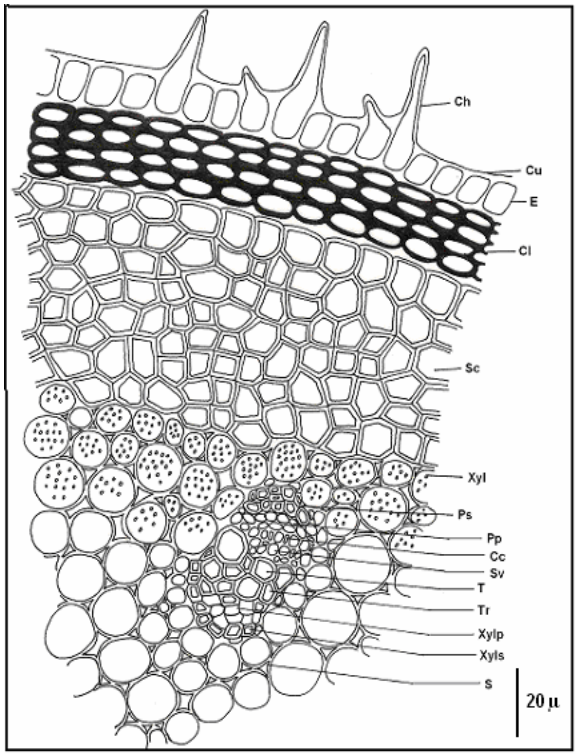

Fig. 11. Cross section of stem; Ch: Cover hair, Cu: Cuticle, E: Epidermis, Cl: Collenchyma, Sc: Schleranchymatic cortex cells, Cp: Cortex parenchyma, P: Phloem, Ps: Phloem schlerancyma, Pp: Phloem parenchyma, Cc: Companion cell, Sv: Sieve tube, Xyl: Xylem, T: Trachae, Tr: Tracheid, Xylp: Xylem paranchyma, Xyls: Xylem schlerancyma, S: Schizogen intercellular area.

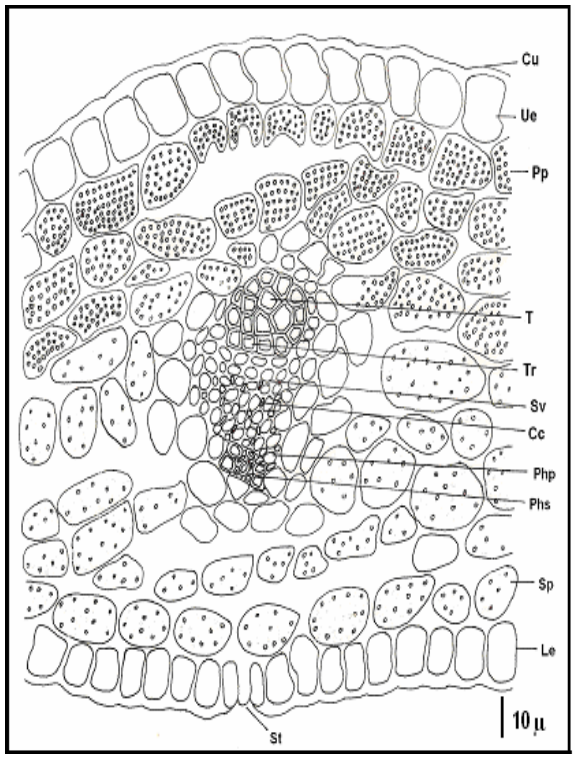

Fig. 12. The cross section of stem leaf; Cu: Cuticle, Ue: Upper epidermis, Pp: Palisade parenchyma, Xyl: Xylem, T: Trachae, Tr: Trachaeid, P: Phloem, Sv: Sieve tube, Cc: Companion cells, Php: Phloem parenchyma, Phs: Phloem schlerenchyma, Sp: Sponge parenchyma, Le: Lower epidermis, St: Stoma cell.

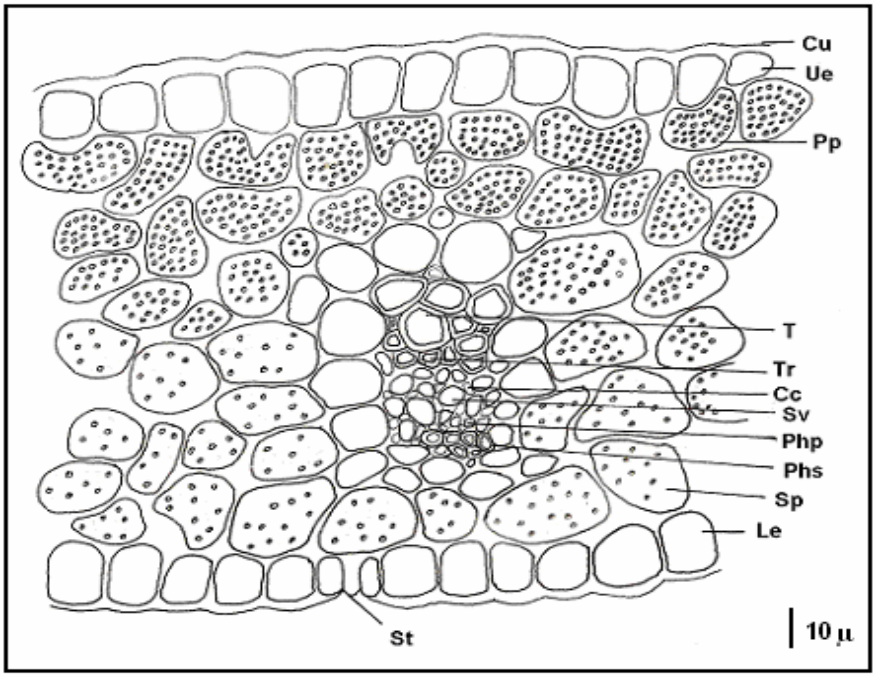

Fig. 13. The cross section of basal leaf; Cu: Cuticule, Ue: Upper epidermis, Pp: Palisade parenchyma, Xyl: Xylem, T: Trachae, Tr: Trachaeid, P: Phloem, Sv: Sieve tube, Cc: Companion cells, Php: Phloem parenchyma, Phs: Phloem schlerenchyma, Sp: Sponge parenchyma, Le: Lower epidermis, St: Stoma cell.

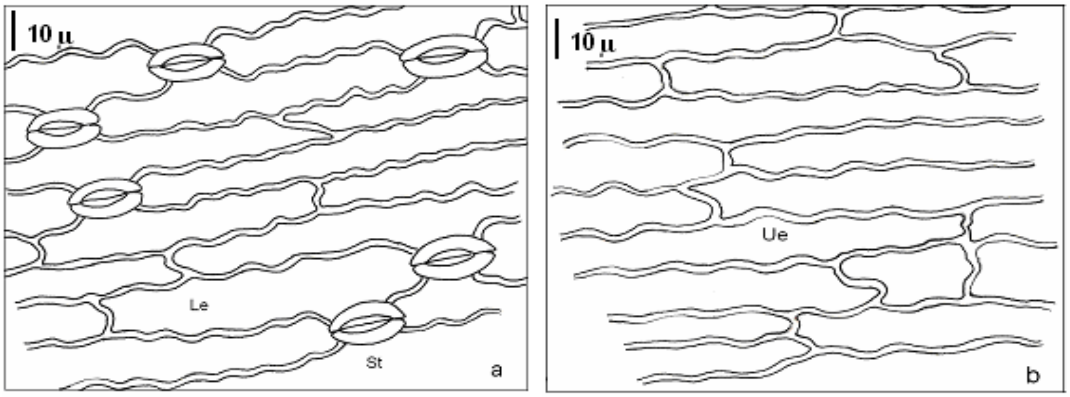

Fig. 14. The superficial section of the stem leaf; a. Lower surface, b. Upper surface, Le: Lower epidermis, Ue: Upper epidermis, St: Stoma cell.

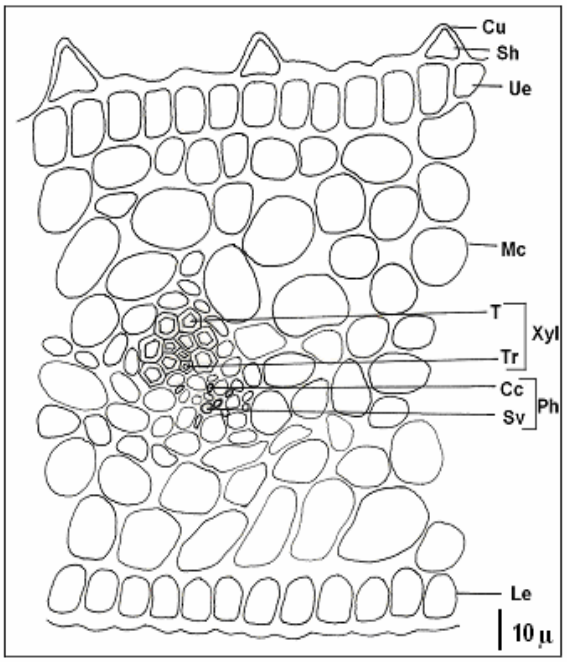

Fig. 15. The cross section of tepal; Cu: Cuticle, Sh: Simple hair, Ue: Upper epidermis, Mc: Mesophyll cells, Xyl: Xylem, T:

The phenological findings about *L. candidum*

The first development of basal leaves	:	September
The development of stem leaves and stem	:	March – end of May
Flowering	:	End of May – end of June
Drying of basal leaves	:	End of May – end of June
Formation of fruit	:	End of June - August
Maturation of seed	:	August - September
Dispersion of Seed	:	September – October
Drying of Aboveground parts	:	October-November

References

Akman, Y. 1993. *Biyocoğrafya*, Palme Yayınları, Ankara.

Anonymus. 1985. *Balıkesir İli Verimlilik Envanteri ve Gübre İhtiyaç Raporu*, T.C. Tarım, Orman ve Köy İşleri Bakanlığı Köy Hizm. Gn. Md. Yay., TOVEB Yay. No. 37, Gn. Yay. No. 779, Ankara.

Baytop, T. 1984. *Türkiye'de Bitkiler ile Tedavi*, İstanbul Üniversitesi, Yayın No: 3255, Eczacılık Fakültesi Yayın no: 40, İstanbul.

Davis, P.H. 1988. *Flora of Turkey and East Aegean Island*, 1-9, Edinburg University Press, Edinburg.

Fahn, A. 1990. *Plant Anatomy*, Linacra House, Jordan Hill, OX2 8 DP, A Division of Reed Educational and Professional Publishing Ltd. Oxford, England.

Haladova, M., P. Mucaji, M. Budesinsky, K. Vokac, J. Cvacka, D. Grancai and E. Eisenreichova. 2011. Spirostanol saponins from the bulbs of *Lilium candidum*, *Chem Nat Compd,* 46(6): 1004-1005.

Karasar, N. 1994. *Bilimsel Araştırma Yöntemi*, 3A Araştırma Eğitim ve Danışmanlık Ltd., Ankara.

Khawar, K.M., S. Çöçü, I. Parmaksiz, E.O. Sarihan, C. Sancak and S. Özcan. 2005. Mass proliferation of Madona Lilly (*Lilium candidum* L.) under *in vitro* conditions. *Pak. J. Bot.*, 37(2): 243-248.

Kopaskova, M., L. Hadjo, B. Yankulova, G. Jovtchev, E. Galova, A. Sevcovicova, P. Mucaji, E. Miadokova, P. Bryant and S. Chankova. 2012. Extract of *Lillium candidum* L. can modulate the genotoxicity of the antibiotic zeocin. *Molecules*, 17(1): 80-97.

Saifullah, K., N. Sheeba, R. Mariam, K. Naheed, N. Asma and S. Bushra. 2010. Cultivation of Lilies (*Lilium regale*) for commercialization in Pakistan, *Pak. J. Bot*., 42(2): 1103-1113.

Uzun, G. 1981. Çukurova Bölgesinde Mis Zambak (*Lilium candidum* L.) Yetiştiriciliği ve Sorunları Üzerinde Bir Araştırma, *Çukurova Üniversitesi Ziraat Fakültesi Yıllığı*, Yıl:12, Sayı: 1-4.

Uzun, G. 1984. *Zambak Yetiştiriciliği*, Tarımsal Araştırmaları Destekleme ve Geliştirme Vakfı, Yalova.

Van-Tuyl, J.M., A.J. Van-Dıjk and L.W.D. Van Raamsdok. 1986. Identification of Interspesific Hybrids and Determination of Relationships Between Species in the Genus *Lilium* by Isoelectric Focusing, *Acta Hortic*, 177(2): 601-605.

Vardar, Y. 1982a. *Bitki Anatomisi Dersleri I*, (Hücre ve Dokular) Ege Üniversitesi Fen Fak. Kitaplar Serisi No: 29, E.Ü.Basımevi, İzmir.

Vardar, Y. 1982b. *Bitki Anatomisi Dersleri II*, (Organlar) Ege Üniversitesi Fen Fak.Kitaplar Serisi No: 26, E.Ü.Basımevi, İzmir.

Wilson, H.F. and B. Mathew. 1981. *Bulbs* – The bulbous plants of Europe and Their Allies, William Collins Sons & Co.Ltd., 329-550.

Yakar-Tan, N. 1982. *Bitki Mikroskopisi Kılavuz Kitabı*, İstanbul Üniversitesi Yayınları Sayı: 2947, Fen Fakültesi No: 166, Fen Fakültesi Basımevi, İstanbul.

Yentür, S. 1995. *Bitki Anatomisi*, İstanbul Üniversitesi Yayınları, Sayı: 3308, Fen Fak.Yayın No: 227, İstanbul.

(Received for publication 19 November 2010)