Morpho-anatomical observations on *Colchicum boissieri* Orph. in Turkey

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Abstract

In this study, the morpho-anatomical features of *Colchicum boissieri* Orph. were demonstrated. The morphological characteristics of the corm, leaf, flower, fruit and seeds were presented and the habitus of the species were given by photographs. Corm lengths, cataphyll color and length, leaf color, outer-inner tepal dimension (length x width), perigonium tube dimension, outer-inner filament (length) and anther (length), styles color, seed number, shape, dimension and color were determined. The corm of *C. boissieri* is soboliferous, occasionally tooth-like projections, vertical, soboles horizontal, 2.3-4.5 cm in length and 0.6-1.4 cm in width. The neck (collum) of the corm is 3-5.5 cm in length. The cataphyll is yellowish-white in color, 3-7.5 cm in length. Perigonium tube is 4.4-13 cm in length. The seeds are that wrinkled surface subglobose to globose in shape, yellowish-brown in color, and (1.3-) 1.4-1.9 mm in diameter.
Anatomical characteristics of the corm, leaf, fruit and seeds were shown with photographs. Transverse sections from the middle portion of the corm, leaf, pericarp and seed testa were obtained. The upper and lower surface views of the leaf, pericarp and the seed testa were examined. Anatomical features of *C. boissieri* were discussed in this research for the first time. Anatomical features of *C. boissieri* are similar to some *Colchicum.* In the anatomical view of leaf, it has been determined that there are differences in the number of spongy parenchyma cell layers between the species. Testa of the seeds include epidermis, parenchyma and pigment layer cells.

Key words: *Colchicum*, morphology, anatomy, soboliferous
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Türkiye'deki *Colchicum boissieri* Orph. üzerinde morfo-anatomik gözlemler

Özet

Bu çalışmada *Colchicum boissieri* Orph. türünün morfo-anatomik özellikleri gösterilmiştir. Korm, yaprak, çiçek, meyva ve tohumun morfolojik özellikleri ile habitusu fotoğraflar eşliğinde ortaya konulmuştur. Korm boyu, katafil rengi ve boyu, yaprak rengi, dış- iç tepal boyutu (boy x en), perigonyum tüpünün boyu, dış- iç filament (boy) ve anter (boy), sitilus rengi, tohum sayısı, şekli, boyutu ve rengi tespit edilmiştir. *C. boissieri*' nin kormu sobelifer, genellikle diş benzeri dikey çıkıntılara sahip, yatay uzamış, 2.3-4.5 cm uzunluğunda, 0.6-1.4 cm genişliğindedir. Kormun kollumu 3-5.5 cm uzunluktadır. Katafil sarımsı-beyaz renkli 3-7.5 cm uzunluktadır.

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Pürüzlü yüzeye sahip olan tohumlar küresel ya da aşağı yukarı küresel, sarımsı-kahverengi ve (1.3-)1.4–1.9 mm çapındadır.
Gövde, yaprak, meyva ve tohumun anatomik özellikleri fotoğraflar eşliğinde sunulmuştur. Korm, yaprak, perikarp ve testanın orta bölgesinden enine kesitler alınmıştır. Yaprak, perikarp ve testanın üst ve alt yüzeysel kesitleri incelenmiştir. *Colchicum boissieri'* nin anatomik özellikleri bu araştırmada ilk kez ele alınmıştır. Türün anatomik özellikleri bazı *Colchicum* türleri ile benzerlik göstermektedir. Bu türlerden yaprak anatomisindeki sünger parenkiması hücre sırasının sayısı ile farklılık göstermektedir.

Anahtar Kelimeler: *Colchicum*, morpholoji, anatomi, sobolifer

Introduction

Colchicum belongs to the tribus *Colchiceae*, *Colchicaceae* family and the order *Liliales* (Dahlgren et al., 1985). Turkey has the highest contribution of *Colchicum* species which is about 90 species worldwide. According to the recent studies 39 *Colchicum* taxa are present in Turkey except the Eastern Aegean Islands (Brickell 1984; Persson 1988, 1998, 1999a, b, c, 2000, 2001, 2007; Akan and Eker 2005). Within such a high distribution, Turkey strikes out as the richest country with regard to *Colchicum* species. Systematical features of some *Colchicum* species in Turkey were reported and information about their distribution was given (Baytop 1976, 1977, 1987; Sütlüpınar 1983, 1988). In 1980's, 1990's and 2000's taxonomic revisions of the *Colchicum* species in Israel, Greece, Iran and Turkey were studied by a group of scientists (Baytop 1976, 1977, 1987; El-Hamidi and Fahmy 1961; Fell et al. 1965, 1966; Küçüker 1994; Persson 1991, 1992, 1993a, b, 1999c, 2007; Dinç and Sümbül 2007).

Morphological and anatomical investigations on the *Colchicum* species in Turkey have been started by Kasaplıgil (1961). Özyurt (1978 a,b) made his contribution to the literature of *Colchicum* species in Turkey by studies on the family of Liliaceae and Iridaceae.

During the last two decades, more attention has been paid on morphological and anatomical characteristics of the *Colchicum* species in Turkey. It has been initiated by morphological, anatomical and cytological studies on some *Colchicum* species (Küçüker 1985). Chromosome numbers of some *Colchicum* species have been reported for the first time (Küçüker 1984; Küçüker and Çelebioğlu 1986). For example, chromosome number of *C. boissieri* is given as 2n= 46 (EGE 16429) (Persson 2000; Şık and Küçüker 1998). Şık et al. (2009) continued studying on the same issue.

Up to now the morphological and anatomical features of organs such as leaf, fruit and seeds have been given in detail (Küçüker and Çelebioğlu 1988; Küçüker 1990b; Özcan 1995; Sungu 1993; Bölükler 2003; Akan and Satil 2005). Some morphological formations (laterally contracting pioneer root) that has been observed for the first time in Turkey have also been reported (Küçüker 1992). On the other hand, micromorphological features (SEM) of leaf, fruit, tunics and seed surface of some *Colchicum* species in Turkey have been released (Küçüker and Çelebioğlu 1988; Engel and Küçüker 1994, Küçüker and Engel 1994).

The description and the distribution of the species were presented within some flora studies. But the morphological and anatomical characteristics of the species were investigated inadequately. For this reason, detailed morphological and anatomical observations were established on *C. boissieri* .

Materials and Methods

Sample Collection

C. boissieri was collected from Manisa (Spil Mountain- Atalanı Location-1250 m) and İzmir (Bozdağ-Büyük Çavdar High Plateau-1330 m, from Bozdağ village to Ovacık- 1080 m). They were cultivated in the Botanical Garden of the Istanbul University and also were prepared as herbarium samples for the Herbarium of the

Faculty of Science, Istanbul University (ISTF 37439, 38490, 38491, 38492).

Systematic description of the species has been done according to Brickell (Brickell 1984) and this description has been checked out by comparing them with samples of the Herbarium of the Pharmacy Faculty, Istanbul University (ISTE).

Morphological Method

Changes during all growing stages of the plants were observed both in the Botanical Garden and simultaneously in their natural habitats as well. Thus annual growth stages were determined. The plants were observed during their leafy and flowery stages. Morphological appearances and the main features of organs such as corm, leaf, flower, fruit and seed were presented by photographs and these organs were also measured. Morphological measures were made according to Brickell and Persson (Brickell 1984; Persson 1988, 1991, 1999a, b, c, 2001).

Anatomical Method

Leaf samples were collected when the color of their tips turned to yellow. And the fruits were collected when their color turned to brown from purple-brown as an indicator of the maturation of the pericarp. We have selected dry and mature seeds for the anatomical studies. Fresh material fixed in FAA and the dry and mature seeds were soaked with 5% KOH at 25ºC for 4-6 hours to separate the layers forming the testa (Küçüker and Çelebioğlu 1988). For anatomical studies of the species transection and the surface images were taken by free-hand section. Transverse sections which were taken from corms, leaves, pericarp, seed testa and surface images which were taken from the upper parts of leaf (and the lower), pericarp and seed testa have been examined in "Sartur solution" (Çelebioğlu and Baytop 1949).

Anatomical investigations were conducted under the Reichert- Neopan light microscope. Photographs of the transections and the surface views were taken with Zeiss 47 30 28 and Olympus BH-2 photomicroscope.

Results and Discussion

Morphological features

The corm of *C. boissieri* is soboliferous, occasionally tooth-like projections, vertical, soboles horizontal, 2.3-4.5 cm in length and 0.6-1.4 cm in width. The tunics are pale reddish-brown in color, membranous. The neck (collum) of the corm 3-5.5 cm. The cataphyll is yellowish-white in color, 3-7.5 cm in length (Fig. 1.a). This species produce foliage between February and May. The leaves are 2-3 in number, hysteranthous, erect or at the base erect, linear in shape. The dimensions of leaves are (8-) 11-20 cm x 2-6 mm whereas the inner ones are shorter than the outer ones. The tips of leaves are obtuse to subacute, margins are glabrous, the color of the leaf is dark green (Fig. 1.b). The species yields flower from beginning to the middle of the fall (September-October). The flowers are 1 (-2) in number, campanulate to narrowly funnel-shaped. Perigonium segments bright rosy-lilac in color, very narrowly elliptic-ovate in shape. The measurements of the outer and the inner tepals are (2.2-) 3.2-4.9 cm x 7-13 mm and (2.2-) 3.1-4.7 cm x 6.5-11 mm respectively. The tips of the tepals are obtuse. Perigonium tube is 4.4-13 cm in length. The color of the filaments are white and yellowish- white at the base, glabrous. The length of the filaments are 8-20 mm (outer), and 13-24 mm (inner). The anther is yellow in color, 4-10 x 1 mm (outer) and 4.5-10 x 1mm (inner) in length, versatile and latrorse. The pollen is yellow in color. The stylus is white and 7-15 cm in length. The stigmas are punctiform (Fig. 1.a). The fruit appears on the ground (rarely underground). The light pink color of the fruit is closed at the beginning of the maturation and turns to purple brown in the course of maturation, thereafter the fruit dries and opens. The capsule is ellipsoid, the tips are acute, rostrate. The measurements of the capsules 1.5-2 x 0.8-1 cm, seed number 15-25 (-35) (Fig. 1.b). The seeds

are subglobose to globose in shape, yellowish-brown in color, and (1.3-) 1.4-1.9 mm in diameter. They become relatively elongated near caruncle and have wrinkled (rugosus) surfaces.

Five *Colchicum* species in Turkey have soboliferous corm (*C. boissieri*, *C. soboliferum* Stef., *C. minutum* K. Perss., *C. munzurense* K.

a **b**

Figure 1. *Colchicum boissieri*: (habitus). in flower (a); in leaves and fruit (b).

Perss. *C. leptanthum* K. Perss.).The morphological results we obtained are compared with studies in sobeliferous *Colchicum* species in Turkey. Morphological features of corm, tunics, neck, cataphyll, leaves, flowers, fruit in *C. boissieri* are almost the same with the others studies (Dinç and Sümbül, 2007). Besides, the place of the fruit and the seed amount which had been omitted by Dinç and Sümbül (2007) were given in this study. In this manner, we think that our investigation provides a recent data for the Flora of Turkey.

The leaf width of the sobeliferous species of genus *Colchicum*; *C. soboliferum*, *C. minutum*, *C. munzurense*, *C. lepthantum* are synanthous species. *C. boissieri* (hysteranthous) present in Turkey is considerably narrower than *C. soboliferum* (Persson 1992), *C. minutum*, *C. munzurense* (Persson 1999a) and wider than *C. lepthantum* (Persson 2001).

The size of periant part of *C. boissieri* are found longer and wider in terms of the filament and anther lengths than the other sobolifer species (Persson 1992, 1999a, 2001).

The perigonum tube of *C. boissieri* is remarkably longer than the other species. It was striking that the anther joint to filament in versatile form, and the stigma was shaped as punctiform where these characters were also shared with the other species (Persson 1992, 1999a, 2001).

It was found that the color of fruit stated by Brickell as light silver-brown turned to purple brown when it is ripe (Brickell 1984). We observed that the capsule is generally found at soil floor level which is rarely found immediately under the soil. This characteristic showed similarity with the species *C. minutum*, *C. munzurense* and differentiates from *C. soboliferum*, which capsule is generally found beneath the soil (Persson 1992, 1999a, 2001).

The seed size of *C. boissieri* is generally smaller and the number of seeds are higher than the other sobolifer species (Persson 1992, 1999a, 2001).

Anatomical features

Transection of the corm shows a single layered epidermis with widely square-like cells. Under the epidermis, parenchyma cell layers are usually polygonal in shape with numerous starch grains and thin cell walls, having narrow intercellular space. Vascular bundles are embedded within the parenchyma layers (Fig. 2.a).

Transection of the leaf shows an isolateral and amphistomatic leaf structure. Both the upper and the lower epidermises are formed with widely square-like cells. The lower epidermis cells are smaller. The upper and the lower palisade parenchyma have 2-3 layer of cells, long and oval in shape with a lot of chloroplasts. The spongy parenchyma contains 4-5 layers of cells commonly polygonal in shape having large intercellular space. Vascular bundles were embedded within the spongy parenchyma. The upper and the lower epidermal cells are covered with undulated cuticle (Fig. 2.b).

We have observed rather thick cells in the transection of the leaf margin cell walls and the cuticle layers of epidermal cells compared to those of the epidermal cells elsewhere in the leaf. The spongy parenchyma has 2-3 layers. The cuticle on the leaf margin is more undulated (Fig. 2.b).

In the upper and lower surface views, the epidermal cells seem to be long and rectangular in shape. The anomocytic stomata occur on the upper and the lower surface of the leaf. Lower epidermal cells are narrower than the upper ones. The cuticle is striated paralleled on the upper and the lower surface views (Figs 2.b.1, 2.b.2).

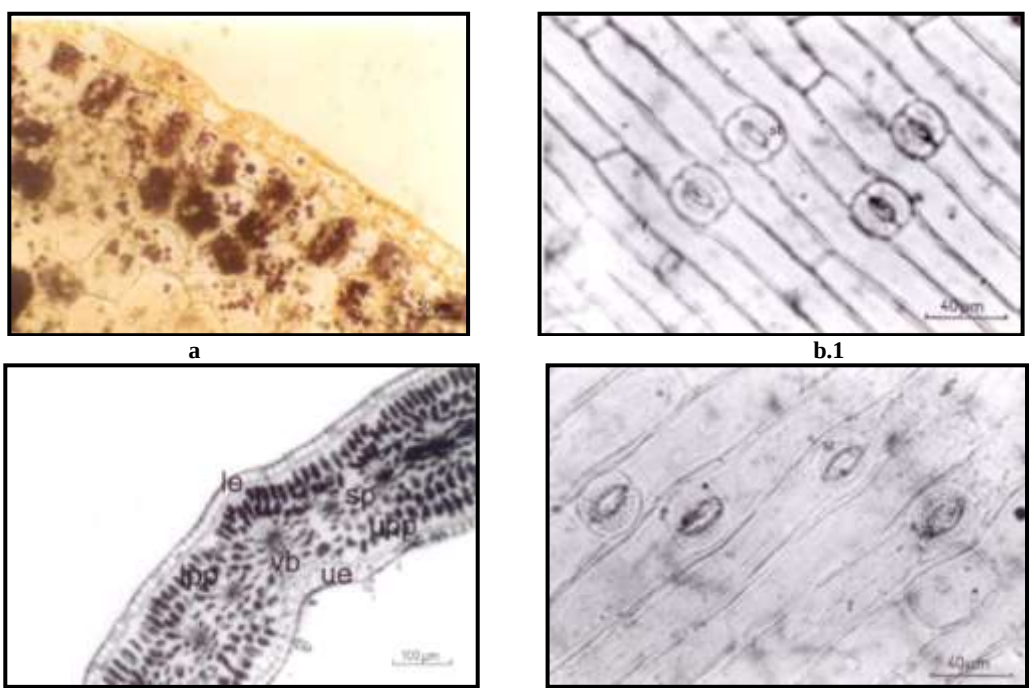

Figure 2. *Colchicum boissieri*: Transverse section of the corm (a) and the leaf (b); surface views: upper (b.1) and lower (b.2) parts of the leaf. e: epidermis, p: parenchyma, ue: upper epidermis, upp: upper palisade parenchyma, vb: vascular bundle, sp: spongy parenchyma, lpp: lower palisade parenchyma, le: lower epidermis, st: stomata.

In the transections, the exocarp contains single-layered more or less square-like epidermal cells. Exocarp is covered with a thin cuticle. Endocarp transections contain single layered, long-rectangular epidermal cells (Fig. 3.a).

Mesocarp comprises of 6-7 layers of parenchyma cells containing starch grains and chloroplasts which are commonly oval in shape (Fig. 3.a).

Under the exocarp 2-3 layers of mesocarp cells are wide and isodiametric in shape, become flattened narrow long rectangular and oval towards endocarp. Mesocarp cells contain a lot of chloroplast and starch grains. Vascular bundles are embedded within the mesocarp (Fig. 3.a).

In the surface views, exocarp consists of rectangular, penta or hexagonal cells ; but endocarp is formed commonly rectangular and elongated cells. Anomocytic stomata which occurs on the exocarp contains leucoplast (Figs 3.a.1, 3.a.2).

Testa of the seeds include epidermis, parenchyma and pigment layer cells. In transections epidermis comprises irregular, long and flattened cells. Under the epidermis parenchyma has 2-3 layer of cells more or less pentagonal with thick cell walls. Under the parenchyma the pigment layer consists of single layers of flattened cells having excessive flobafen pigments (Fig. 3.b).

The endosperm consists of wide and long rectangular parenchyma cells which have thickened cell walls and numerous simple pits. These cells contain at high amount of aleuron grains and lipid drops (Fig. 3.b).

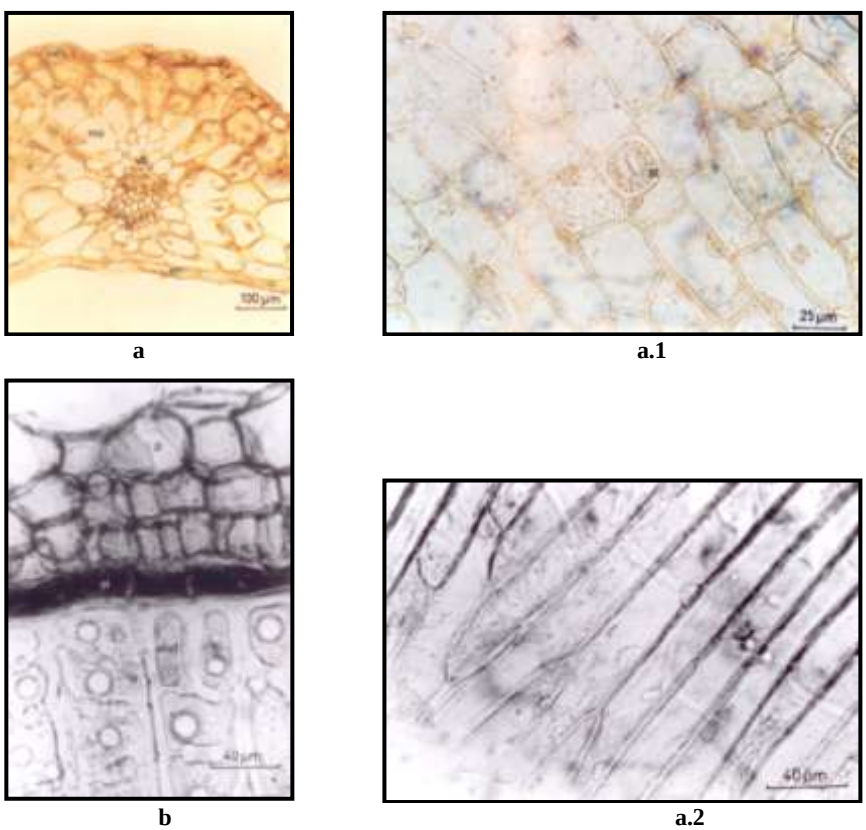

Figure 3. *Colchicum boissieri*: Transverse section of the pericarp (a) and the seed (testa) (b); surface views: the exocarp (a.1) and the endocarp (a.2). ex: exocarp, ms: mesocarp, vb: vascular bundle, en: endocarp, st: stomata, e: epidermis, p: parenchyma, end: endosperma.

In the literature no report has been found on the anatomical features of *C. boissieri* and other soboliferous *Colchicum* species. For this reason, an anatomical comparison has been made with the previously investigated *Colchicum* species (Çelebioğlu 1949; Fell et al. 1965; Özyurt 1978 a, b; Küçüker 1985, 1990 a, b; Küçüker and Çelebioğlu 1988; Özcan 1995).

Anatomical features of corm in *C. boissieri* are almost the same as *C. bivonae* Guss., *C. variegatum* L. (Özcan 1995), *C. stevenii* Kunth (Fell et al. 1965), *C. szovitsii* Fish & May (Özyurt 1978 a, b). Anatomical features of leaf in *C. boissieri* are similar to *C. bivonae, C. variegatum* (Özcan 1995), *C. chalcedonicum* Azn., *C. turcicum* Janka, *C. micranthum* Boiss., *C. baytopiorum* C.D.Brickell, *C. lingulatum* Boiss. et Spruner (Küçüker 1985, 1990 a, b), *C. szovitsii* (Özyurt 1978 a), *C. steveni* (Fell et al. 1965) species. In the anatomical view of leaf, it has been determined that there are differences in the number of spongy parenchyma cell layers between the species. We have not found a clear anatomical difference between the fruits of *C. chalcedonicum, C. turcicum, C. micranthum, C. baytopiorum, C. lingulatum* (Küçüker 1985, 1990 a, b). But there have been differences in the number of cell layers. In the anatomical views of seeds it has been observed that there are some similarities in the features of *C. lingulatum, C. chalcedonicum, C. turcicum, C. autumnale* L. and *C. boissieri* species (Çelebioğlu 1949; Küçüker and Çelebioğlu 1988). However differences have also been observed in the number of parenchyma cell layers (2-3) with the cell shapes being more or less pentagonal.

Up to now only systematical descriptions and distributions of the species have been given and the insufficiency of the detailed morphological appearances has been emphasized by Persson who had revised *Colchicum* genus (Persson 1988, 1991, 1992, 1993a, b, 1998, 1999a, b, c, 2000, 2001, 2007). In this survey, we have firstly shown the soboliferous corm characteristics of *C. boissieri* by the well-detailed photographs. On the other hand, we think that the anatomical features of the vegetative (leaf) and generative (fruit, seed) organs observed in this study would be helpful to the scientists in their future studies.

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