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Anatomical Investigation of Endemic *Crocus gilanicus*

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Abstract: The present investigation included the anatomical features of *Crocus gilanicus*, an endemic species in Iran. The specimens were collected from Guilan province. The determination of the anatomical characteristics presented in this investigation was the first data available in the literature. The anatomical investigation included transverse sections of root, stem, leaf, corm, anther and ovary which has been examined and supported by illustration. Most anatomical characters observed were similar to the usual anatomical features of Iridaceae anatomy. However, there were some particular characters could be used to distinguished this species.

Key words: *Crocus gilanicus* · Guilan · Anatomy

INTRODUCTION

Crocus gilanicus (Iridaceae) is natively growing in Guilan province in the north part of Iran. The genus *Crocus* L. includes about 85 species distributed mainly in the Mediterranean region [1]. At present about 8 taxa are recognized in Iran [2]. *Crocus* is a prennial plant containing the corm. Some *Crocus* species were used for making medicine [3-5]. No details of anatomical studies were recorded on *Crocus gilanicus* but some anatomical studies were investigated on other *Crocus* species [6-10]. The purpose of this study was to investigate the anatomical features of *Crocus gilanicus,* indigenous to Iran, in order to obtain more details descriptions for future works.

MATERIALS AND METHODS

Crocus gilanicus samples were collected from natural habitats (Guilan province in the north of Iran) in vegetative (April 2009) and generative (October 2009) stages. The living materials were fixed in F.A.A (10% Formalin+85% alcohol+5% Acetic acid) for anatomical studies. Cross-sections of the root, stem, leaf, corm, anther and ovary were manually taken for anatomical studies. Specimens were cleared by commercial sodium hypochlorite and acetic acid. The sections were stained with methyl green and kongorot and fixed with glycerin-gelatin [11]. All images were captured using Olympus DP71 camera attached to the light microscope. Identification of different cells and tissues were fulfilled on the images of each specimen.

RESULTS AND DISCUSSION

Root: Epidermis consists of two layered, which have thin-walled. The first layer of epidermal cells is larger than those of the second layer. Some of the cells in the first layer are prismatic shape but more cells are polyhedron and round shapes. Second layer of the epidermal cells are polyhedron and round shapes and nearly of the same size (Fig. 1). Studies on three subspecies of *Crocus speciosus* L. and *Crocus flavus* Weston subsp. flavus showed epidermal of single layer [6-7]. Most of the monocots have epidermal of single layer [12]. The cortex 7-8 layers, ovoid or round shapes of parenchymatic cells with intercellular spaces (Fig. 1), whereas the cortex composed of 4-6 layers in most of *Crocus* spp. [6-7]. In the same cases, the cortex composed of 15-20 layers in monocots [13]. Endodermis is single layer and containing Casparian strip. The walls of the endodermal cells are thick three sided and toward

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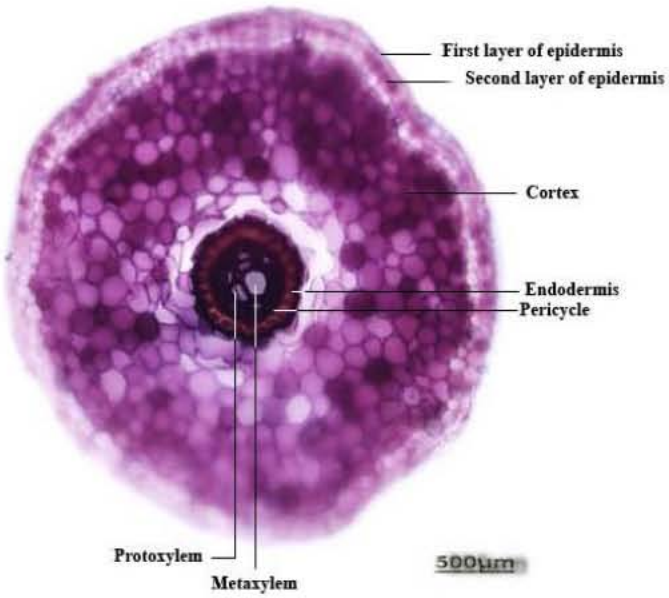

Fig. 1: Cross section of the root.

the pericycle (Fig. 1). Similar characterstics were also observed in the roots of *Crocus aerius* Herb., *Crocus speciosus* Bieb., *Crocus danfordiae* Maw., *Gladiolus atroviolaceus* Bioss. [6, 14, 15]. The wall thickness of the endodermal cells of *Crocus speciosus* subsp. *ilgazensis* were three-sided and toward the cortex. These types of the endodermal cells are common in the roots of monocots [12]. The thickness was clear on the walls of the endodermis cells. The thickness was not clear on the walls of the endodermal cells in *Crocus flavus* Weston subsp. flavus [7], while in *Crocus fleischeri* Gay and *Crocus danfordiae* Maw this thickness was clear [14]. Passage cells located between the endodermis cells. Pericycle wall is thin and located inside endodermis. Two metaxylems vessels were presented on the central part of vascular cylinder. Five or six protoxylems were presented on the periphery of the vascular cylinder and these strands reach to the pericycle (Fig. 1). The same feature has been reported on the root of *Crocus speciosus* subsp. speciosus [6]. In *Crocus flavus* Weston subsp. flavus, *Crocus speciosus* subsp. *ilgazensis*, *Crocus aerius* Herb., *Romulea columnae* Sebast. and Mauri subsp. columnae and *Crocus pulchellus* Herb., single metaxylem vessel was presented on the central part of vascular cylinder and four or six xylem strands was presented on the periphery of the vascular cylinder [6, 14-16]. The number of xylem strands and metaxylem in *Crocus speciosus* subsp. xantholaimos and *Lilium ledebourii* (Baker) Bioss. are four and seven or eight, respectively [6, 13]. The root avoided from pith, instead it has metaxylem vessels (Fig. 1).

Stem: Stem is surrounded by leaves (Fig. 2). In transverse section of the stem, epidermis was single layer. The ground tissue does not show distinct demarcation of hypodermis and cortex, ground tissue parenchymatous cells were polyhedron shape. Ten vascular bundles were found irregularly scattered in the ground tissue (cortex). The peripheral bundles were relatively smaller than those of central region. There is no differentiation of general cortex, endodermis, pericycle, pith and pith rays (Fig. 2). Many monocots have the same features in transverse section of their stem [12]. This feature has been observed in *Crocus pulchellus* [14], while vascular bundles in the stem of *Crocus flavus* Weston subsp. flavus, *Crocus fleischeri*, *Crocus danfordia* and *Romulea columnae* Seb. and Mauri subsp. *columnae* are located in the peripheral and central parts [7].

Leaf: Leaves have a central square or rectangular keel and two lateral arms with their margins slightly curved toward the keel (Fig. 3). Rudall and Mathew [8] examined the leaf anatomy of several *Crocus* species and they pointed out that the leaves of most species have a central square or rectangular keel with two lateral arms which may be considered as taxonomically significant. The same results obtained by other researchers [15, 17]. *Crocus* species that have the same leaf characters may be closely related to each other. In contrary, the leaf of *Crocus flavus* Weston subsp. flavus has a triangular keel [7]. There was a large air space in the center of the keel which surrounded by the parenchymatous cells.

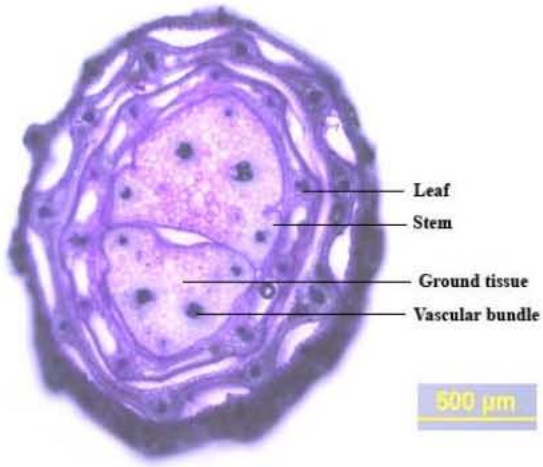

Fig. 2: Cross section of the aerial stem surrounded with three leaves.

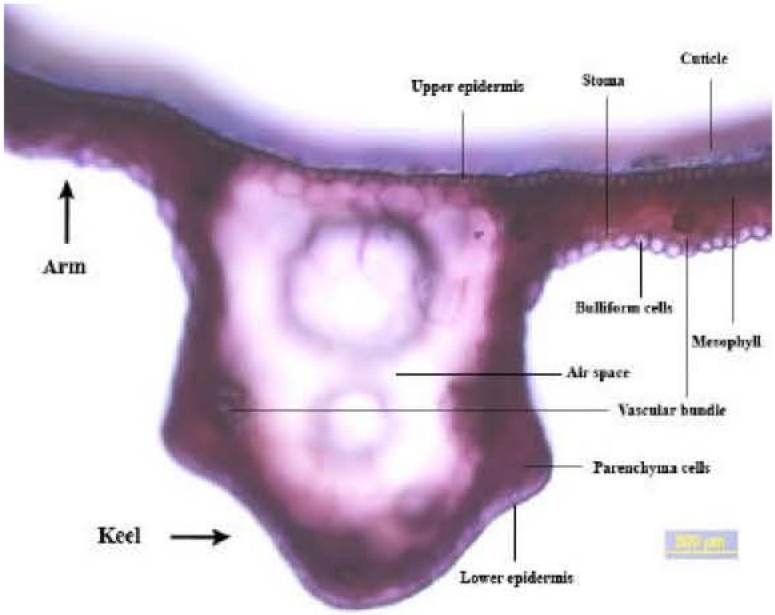

Fig. 3: Cross section of a part of the leaf.

The parenchymatous cells break down to form an air space (Fig. 3). This result is considered as a common feature in this genus [8]. Vascular bundles of the keel are located between the parenchymatous cells. Both adaxial and abaxial surfaces covered with a cuticle, but the thickness of the cuticle on the adaxial surface was thick than that of abaxial surface. The epidermis was single layer observed on both abaxial and adaxial surfaces. Some of the epidermis cells on abaxial surface were larger than other epidermal cells on abaxial and adaxial surfaces. These cells were the bulliform cells (Fig. 3). Some monocots such as *Zea mays* L. have bulliform cells. These cells play an important role in water storage [12]. Mesophyll cells are more or less uniform in shape and usually consist of 3-4 layers of spongy parenchymatic cells (Fig. 3). These features are similar to *Crocus leichtlinii* (D. Dewar) Bowles [9], spongy mesophyll cells were more or less uniform in the most monocots [12]. Vascular bundles were located in one row between the palisade and spongy layers. The bundle sheath consists of sclerenchymatic cells (Fig. 3). The same features were observed in many *Crocus* species and other monocots [8, 12, 13, 18]. Stomata presented only on the abaxial epidermal layer, while no stomata were observed on the adaxial epidermal layer (hypostomatic leaf). The same result has been reported in the leaves of *Crocus aerius, Crocus flavus* Weston subsp. flavus and *Lilium ledebourii* (Baker) Bioss. [7, 13, 15]. This is contrary to the most monocots which have stomata on the both surfaces of the leaves [12].

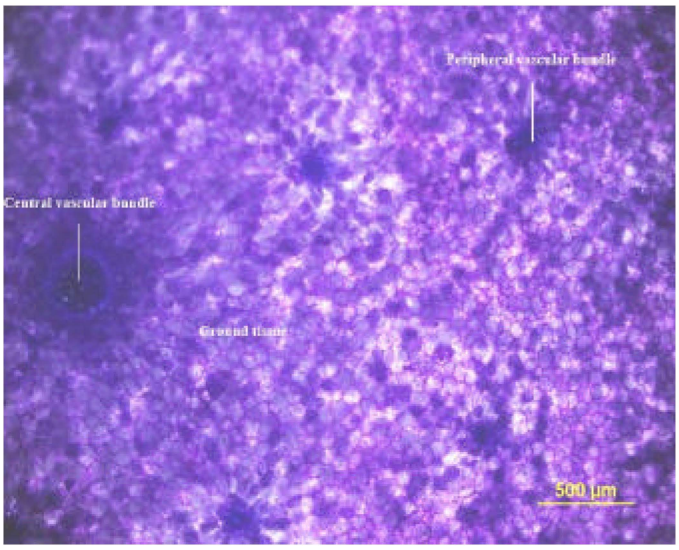

Fig. 4: Cross section of the corm, containing a vascular bundle in the central part and several vascular bundles in the peripheral part

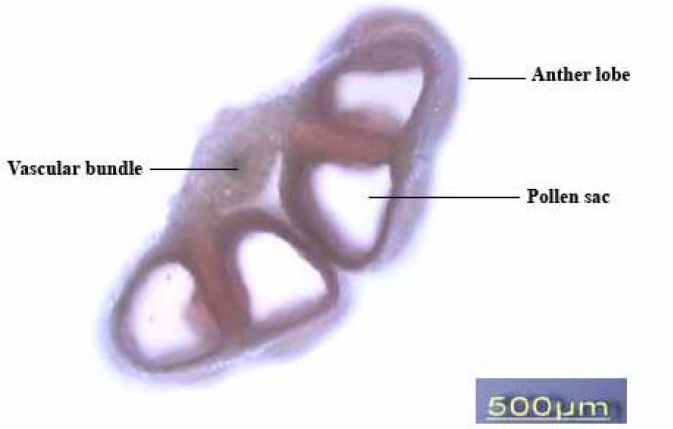

Fig. 5: Cross section of the anther, containing four pollen sacs.

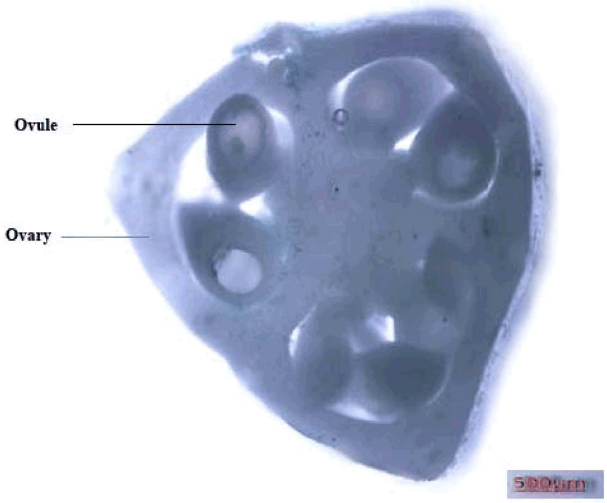

Fig. 6: Cross section of the ovaries with the axial placentation.

Corm: The corm was surrounded by scale leaves. The epidermis composed of one single layer and cubical shape. The cortex was multilayered with ovoid and polyhedron cells. Vascular bundles are arranged in three rings. The vascular bundles in the center were longer than those in the peripheral parts of the corm (Fig. 4). These findings are completely similar to those obtained by Özdemir *et al*. [7] studied on *Crocus flavus* Weston subsp. flavus.

Anther and Ovary: The cross-section of the anther revealed that, anther has two lobes and four pollen sacs (Fig. 5). The cross-section of the ovary showed that placentation was axial (Fig. 6). Many monocots and dicots have the same anther and placentation characters [12].

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