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ANATOMICAL STUDY OF THE GENERA *DACTYLORHIZA ELATA* AND *OPHRYS BOMBYLIFLORA* (ORCHIDACEAE) GROWING WILD IN IRAQ

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SUMMARY

The genus *Dactylorhiza elata* is a tuberous herbaceous perennial growing up to 50 cm, with magnificent spikes of purple flowers in spring. Meanwhile, the genus *Ophrys bombyliflora* is a pseudo tubers plant, 10–20 cm in height, with amazing bumblebee flowers. The stems of the genera *D. elata* and *O. bombyliflora* were circular within the cross-section. The outer first layer was the epidermis, consisting of one row of globular cells; however, the thickness varied in the taxa. In the stem, the sclerenchyma cells contrasted between both genera. The genus *D. elata* consists of many layers, and the thickness reached 133.6 µm, while two layers with a thickness of 43.5 µm in the genus *O. bombyliflora*. In both genera, the vascular bundles were in a collateral and closed type, with an arrangement as one row in the ground meristem, each consisting of xylem and phloem. The thickness of tissues varied between them, and the sclerenchyma cells covered the phloem only. The upper and lower epidermis of the leaves consists of one layer of cells covered with cuticle, and the cells' shapes were ovate. The leaf was hypostomata (the stomata located in only in the lower epidermis). The ordinary cells' shape was hexagonal in the upper epidermis and longitudinal in the lower one in the genus *D. elata*. However, it was triangular in the upper and tetragonal in the lower epidermis of the genus *O. bombyliflora*. Anomocytic types of stomata were evident in both taxa. The mesophyll differed, comprising 12–16 layers in the genus *D. elata* and 9–11 in the genus *O. bombyliflora*.

Keywords: Orchidaceae, *D. elata*, *O. bombyliflora,* sclerenchyma cells, meristematic tissues, epidermis

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Key findings: The collection of plant samples of the genera *D. elata* and *O. bombyliflora* through the field survey came from the AL-Sulaymaniyah District (MSU), especially from Penjwin, Iraq. The study comprised anatomical description and comparison of the genera's leaves and stems, even though both species belong to the same family Orchidaceae. However, the study observed anatomical features' differences between them.

INTRODUCTION

The Orchidaceae belongs to the monocotyledonous family and is the second-largest angiosperm family with five subfamilies, within the range of species, as well as, 736 genera. The Orchidaceae species dominate in all the ecosystems worldwide, particularly in South America (Chase *et al.,* 2015). However, all the genera of Orchidaceae are herbaceous (Koch *et al.,* 2014). It is a diverse and widespread group of flowering plants, with blooms often colorful and fragrant. Orchids are cosmopolitan plants, prevalent in almost every habitat on earth, except glaciers. The world's richest diversity of orchid genera and species are dominant in the tropics (Walsh and Michaels, 2017).

The recent orchids incorporating data from DNA analysis has fixed various sides of the confirmed classification; however, it has also provided some shocks for orchid taxonomists. First, the results have confirmed the monophyly about the evolutionary integrity that means the group includes all the taxa obtained from an ancestral genus of the orchid family, plus the apostasioids and cypripedioids. Second, they suggested the orchids are an old group, evolving in the great Southern continent of Gondwanaland before it separated (Jose and George, 2015).

Among the monocots, the orchids are the most varied group, cultured for their charming flowers (Hossain, 2009). The Chinese were the first to culture and provide details about orchids, also describing the orchids for medicinal use. Most orchids species are beneficial, utilized by ancient communities as ornamental plants, such as, species of the genus *Ophrys umbilicata* and *Arachnis* (Foge *et al.,* 2019), the genus *Vanilla planifolia* for food (Bhatnagar *et al.,* 2017), the genus *Rhynchostylis retusa* for medicines used by the peoples of India (Hinsley *et al.,* 2018), and *Vanda tessellata* and *Orchis latifolia* used by the Chinese (Chinsamy *et al.,* 2011).

Several medicinal species of the Orchidaceae family occurred to contain alkaloids, triterpenoids, flavonoids, and stilbenoids, with antimicrobial activities. Recently, the studies have focused on the isolation of anthocyanins, triterpenoids, and stilbenoids from the genus of the family Orchidaceae (Dobignard and Chatelain, 2010).

Dactylorhiza elata, also referred to as the robust marsh orchid, is a species of flowering plants in the family Orchidaceae, native to the Western Mediterranean region (Bateman and Rudall, 2018). *O. bombyliflora*, known as the bumblebee orchid (bee orchid), native to the Mediterranean region from Portugal and the Canary Islands to Turkey and Asian nations. The genus *Ophrys* is from the Greek language, meaning in signal to the furry lips of the flowers, the particular epithet *bombyliflora* is also from the Greek bombylios (bumblebee) relating to the looks of the flowers of this species (Aybeke *et al.,* 2010; Hassler *et al*., 2022).

This study aimed to make an anatomical study to describe the leaves and stems of the two genera *Dactylorhiza elata* and *Ophrys bombyliflora* and make a comparison to differentiate their anatomical features and determine which a monocotyledon is. This study, considered the first one in Iraq, is novel.

MATERIALS AND METHODS

The plant samples' collection of the two genera *D. elata* and *O. bombyliflora* through field survey came from AL-Sulaymaniyah District (MSU), particularly from Penjwin, Iraq (with longitude and latitude of 35.60522013358285 N and 45.973119411441786 E). The plant samples' preservation included the stem and leaves in the fixative solution of Formalin acetic

acid alcohol (FAA), prepared according to the methodology of Johanson (1940). After 24 hours, ethyl alcohol 70% replaced the previous solution.

The cross-sections of the plant parts comprised stems, leaf epidermis, and vertical sections of leaves, prepared by hand sectioning (Hutchinson, 1954), with some modifications according to Al-Hadeethi *et al.* (2021). At the beginning, cutting the stem specimens into small pieces had a length ranging from 5 to 7 cm. Then, placing these stem cuttings in sodium hypochlorite (5%) for 5 min removed the chlorophyll pigment. Later, the transfer of sections to a glass slide with a cover continued. The prepared slides bore examination under the usual KRÜSS microscope, then photographed with the camera installed on the microscope.

RESULTS AND DISCUSSION

Cross-section study of the stem

The genus *D. elata* is a tuberous herbaceous perennial growing to 50 cm in height, with exquisite spikes of purple flowers in spring (Figure 1). The genus *O. bombyliflora* is a pseudo tuber plant, 10–20 cm in height, with erect stems, glabrous, and the basal leaves in rosette, with amazing bumblebee flowers (Figure 1). The present investigations are consistent with past findings about flora of Turkey (Aybeke *et al.,* 2010).

The stems of the genera *D. elata* and *O. bombyliflora* were circular within the cross-section (Figure 2). The first layer of the stem was the epidermis, consisting of one row of globular cells, and the thickness reached to 25.3 µm and 37.5 µm in the genera *D. elata* and *O. bombyliflora,* respectively. The epidermis was cuticle-covered, and following the epidermis, the ground meristem consisted of two main types of cells. The first type was the sclerenchyma cells found below the epidermis in the genus *D. elata,* consisted of many layers, with the thickness reaching to 133.6 µm. The genus *O. bombyliflora* comprised two layers, and the thickness was 43.5 µm (Kaushik, 1983). The second type of ground meristem was the chlorenchyma cells; these in each genus contained several chloroplasts necessary within the process of photosynthesis and food preparations (Figure 2, Table 1).

In each genus, the vascular bundles were collateral and closed type, arranging in one row in the ground meristem, with each one consisting of xylem and phloem. The thickness of xylem in the *D. elata* is 112.8 µm and in *O. bombyliflora,* 99.1 µm. From the outside, the sclerenchyma layers surrounded the bundles, known as bundle sheath fibers, similar to the other monocot plants. However, in these two genera, the sclerenchyma cells have covered the phloem only. The presence or the absence of sclerenchyma layers covering the vascular bundles can be one of the important features of anatomical characteristics to differentiate them among the genera (Stern and Carlsward, 2009). After the ground meristem, a big cavity was evident in both genera (Figure 3, Table 1). These observations were also analogous to past findings by studying the structural adaptations of two sympatric epiphytic orchids of the family Orchidaceae (Moreira *et al.*, 2013).

Surface and cross-section study of the leaves

The leaf of each genus consists of an upper and lower epidermis, with each one coated by a cuticle. The cuticle plays an essential role in the interaction of plants with the environment, like reducing absorbed radiation and temperature by reflecting the daylight, and moderating transpiration (Koch and Barthlott, 2009; Domínguez *et al.,* 2011, Fan *et al.,* 2014).

In both genera, the stomata occurred in the lower epidermis only, meaning, these genera were hypostomatic type. In *D. elata*, the upper epidermis consisted of ordinary epidermal cells with hexagonal shapes, while the lower epidermis consists of ordinary epidermal cells with longitudinal shapes. However, in the genus *O. bombyliflora,* the ordinary epidermal cells were prevalent with trigonal and tetragonal shapes. The stomata type was anomocytic type (irregular celled) in

Figure 1. The shape of *D. elata* and *O. bombyliflora*.

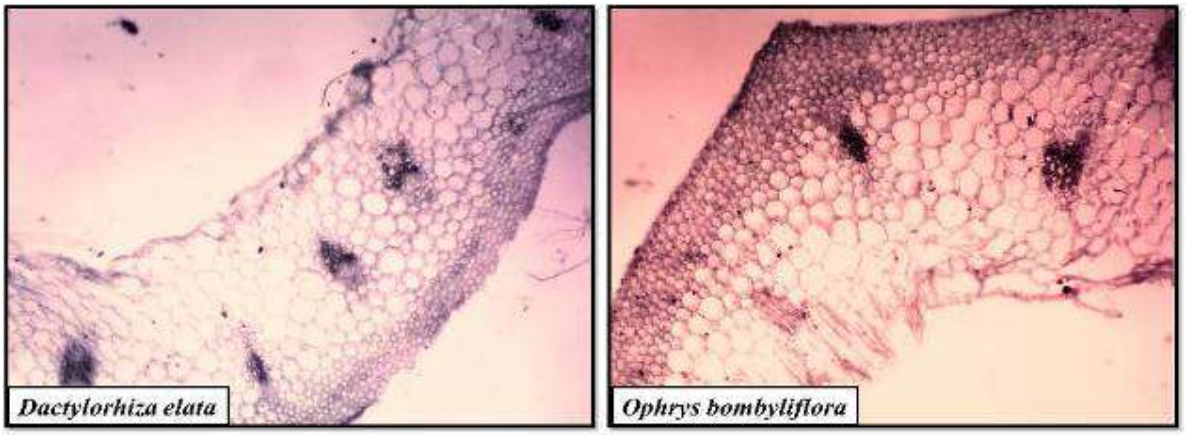

Figure 2. The cross-section of stems in *D. elata* and *O. bombyliflora*, magnified 10×.

Table 1. The quantitative features of the tissue in the stem and leaves of *D. elata* and *O. bombyliflora* genera in Micrometer.

Characters	*Dactylorhiza elata*	*Ophrys bombyliflora*
Stem epidermis thickness	22.5-27.4 (25.3)	30.2-39.4 (37.5)
Sclerenchyma layer in the stem thickness	130.1-136.2 (133.6)	40.7-45.2 (43.5)
Xylem in stem thickness	100.5-116.3 (112.8)	95.4-102.3 (99.1)
Phloem in stem thickness	118.5-123.4 (120.9)	70.2-77.6 (73.4)
Stomata long	29.5-36.1 (34.2)	16.6-25.3 (22.4)
Stomata width	30.1-36.4 (32.7)	12.6-17.4 (16.5)
Leaf upper epidermis thickness	20.3-26.1 (24.6)	31.2-46.7 (44.5)
Leaf lower epidermis thickness	26.9-33.1 (30.5)	25.5-38.4 (36.2)
Mesophyll thickness	300.1-318.2 (315.5)	225.1-230.9 (228.4)
Vascular bundle of leaf thickness	60.3-65.1 (62.7)	168.2-171.5 (169.3)

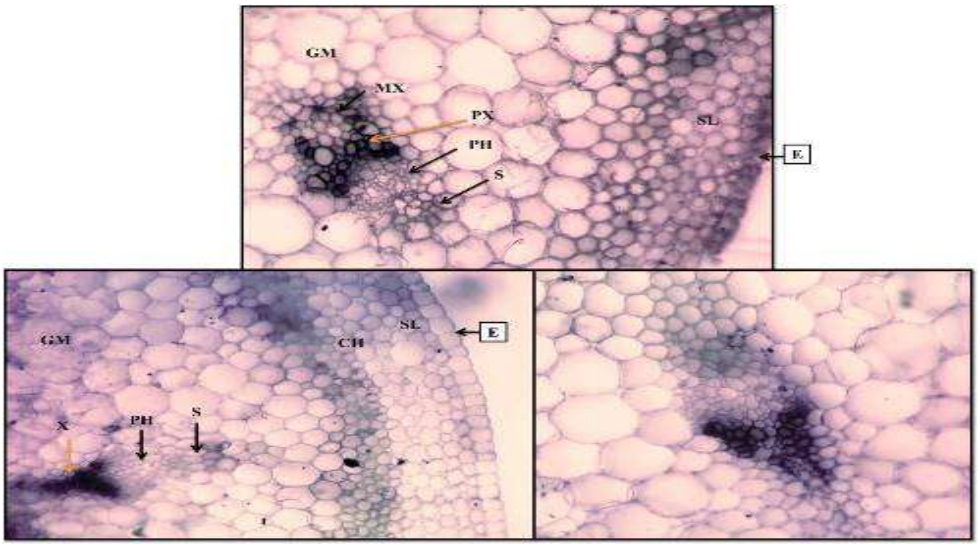

Figure 3. The cross-section of stem in *D. elata* and *O. bombyliflora* respectively, magnified 40×. E: epidermis, SL: sclerenchyma layers, CH: chlorenchyma layers, S: sclerenchyma tissue, PH: phloem, X: xylem, GM: ground meristem.

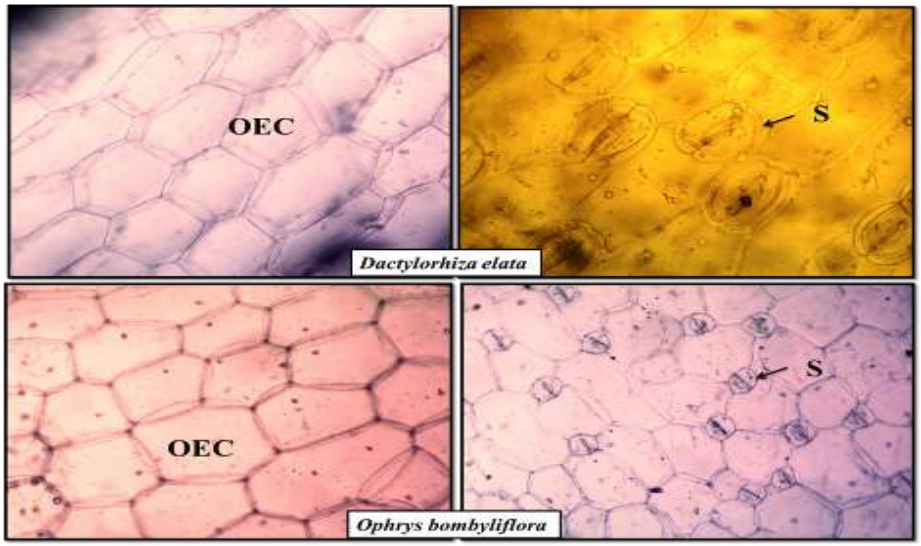

Figure 4. The epidermis in leaves of *D. elata* and *O. bombyliflora*, magnified 40×.

both genera (Figure 4). In the genus *D. elata*, the stomata length and width were 34.2 µm and 32.7 µm, respectively. In the genus *O. bombyliflora,* the length and width of stomata were 22.4 µm and 16.5 µm, respectively (Table 1). Stomata always have significant importance in plant physiology, evolution, and ecology, with the small stomata found more tolerant to drought conditions (Hetherington and Woodward, 2003).

The cross-sections of the leaves revealed two epidermises, the upper and lower epidermis. Each epidermis consisted of one layer of the cells, and the shape of the cells was ovate, also varying in thickness between the genera. In the genus *D. elata*, the upper

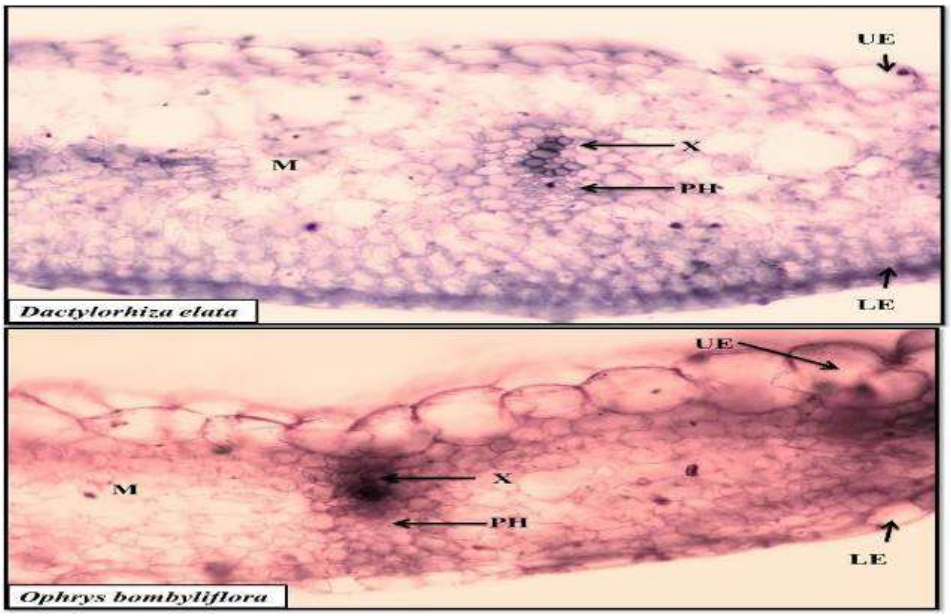

Figure 5. The leaf blade in *D. elata* and *O. bombyliflora*, magnified 10×. UE: upper epidermis, LE: lower epidermis, PH: phloem, X: xylem, M: mesophyll.

epidermis thickness was 24.6 µm and the lower epidermis thickness was 30.5 µm. In the genus *O. bombyliflora,* the upper epidermis thickness was 44.5, while the lower epidermis thickness was 36.2 µm (Figure 5, Table 1). The epidermises function as a barrier and protector to the leaves from the unfavorable environment, and are also vital for the response to external stimuli (Dietz and Hartung, 1996). Epidermis is crucial to save mesophyll from UV and reduce the heat capacity of leaves (Darling, 1989; Guan *et al.,* 2011).

The mesophyll tissue is an influential structure in the leaves, consisting of a vital component for photosynthesis, known as the chlorenchyma tissue, to assimilate the nutrients. The number of mesophyll layers also varied in both genera. In the genus *D. elata,* the mesophyll has 12–16 layers, while 9–11 layers in the genus *O. bombyliflora* (Figure 5, Table 1). The mesophyll thick layers support the leaves' succulence (Lack and Evans, 2001; Metusala *et al.,* 2017). They also preserve the capability for water storage to increase plants' tolerance to high irradiation (Carlsward *et al.,* 2006; Taib *et al*., 2023; Al-Shami *et al*., 2024).

The vascular bundle is a transport system containing the xylem and phloem. The thickness of vascular bundles was 62.7 µm in the genus *D. elata*, while in the genus *O. bombyliflora,* the said thickness was 169.3 µm, arranged in one row in the center of the mesophyll (Figure 6, Table 1). These results agree with past findings by studying the vegetative anatomy and systematics of the angraecoids of the family Orchidaceae (Carlsward *et al.*, 2006). The vascular bundles are responsible for transporting the water and nutrients (Stern and Carlsward, 2008; Moreira *et al.,* 2009).

CONCLUSIONS

This study centered on the anatomical features of two genera of the family Orchidaceae. In the presented investigations, carried out for the first time in Baghdad, Iraq, the genera *D. elata* and *O. bombyliflora* revealed considerably significant. The different anatomical characteristics scrutinized, in addition to ecological data, will contribute significantly to the facilitation of finding variations between these genera anatomically.

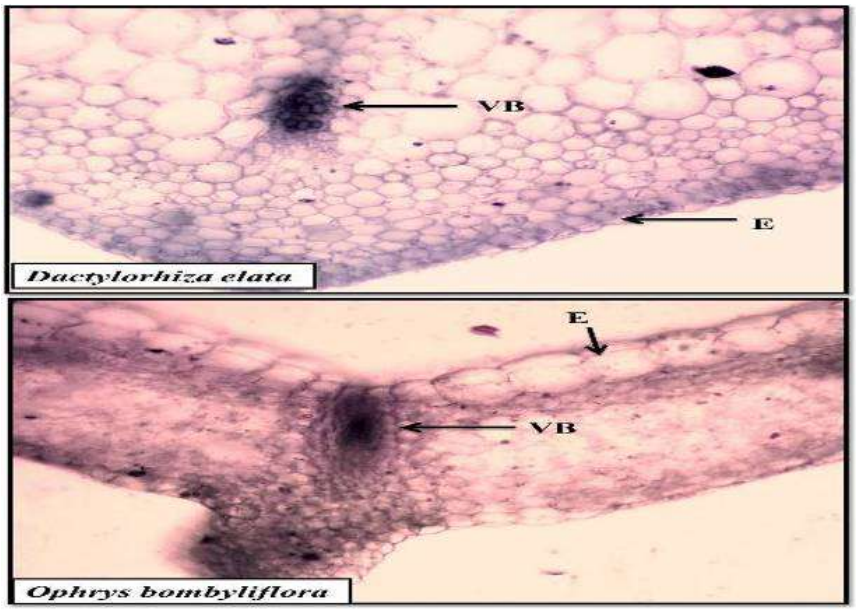

Figure 6. The midrib of leaf in *D. elata* and *O. bombyliflora*, magnified 10×. E: epidermis, VB: vascular bundle.

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