

Morphological, Anatomical and Ecological Study on Turkish Endemic *Viola yildirimlii* M. Dinç & Y. Bağcı

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Received: February 21, 2008
Accepted: April 04, 2008

Abstract

In this study, morphological, anatomical and ecological data about Turkish endemic *Viola yildirimlii* M. Dinç & Y. Bağcı, which was introduced to the scientific world from Adana Aladağ National Park in 2003, were given. Anatomical and morphological characteristics based on observation on the individuals belonging to the species, the cross sections of the root, stipule, peduncle, petiole and leaf parts, and the surface sections of the leaves and the literature data were presented. For ecological investigations, the chemical properties of the soil were analysed, and the accompanying species were given. Also, conservation status of the species is proposed according to our observation on its population size and the distribution area.

Key words: *Viola yildirimlii*, anatomy, morphology, ecology.

INTRODUCTION

The *Violaceae* is a medium-sized family of perennial or rarely annual herbs or shrubs, including the violets or pansies. The family is cosmopolitan, but more typical of the temperate regions and tends to be restricted to higher mountainous areas. It includes about 900 species belonging to 22 genera. The *Viola* L. is the largest genus of the family and has about 400 species in the world [1]. There are 33 natural *Viola* taxa belonging to 29 species and four subspecies in Turkey [2, 3, 4, 5, 6, 7, 8, 9, 10, 11]. The species without stolons of the section *Viola* L. subsection *Viola* L. are, traditionally considered as series *Eflagellatae* [12, 13]. Series *Eflagellatae*, including *V. yildirimlii*, consists of 11 species are all relictual with narrow endemic or disjunct distributions and native to the high mountainous and alpine regions of Central Europe, North Africa, and the Middle East [8, 10, 14, 15, 16, 17].

The genus *Viola* has a commercial significance. *Viola odorata*, included in section *Viola* like *V. yildirimlii*, is grown for essential oils used in manufacture of perfumes, flavorings, toiletries and the very sweet, violet-colored liqueur parfait amour. The flowers of many species are preserved in sugar as crystallized violets used largely for decoration. Also, many species of *Viola* are grown as ornamentals [1]. Morphological and anatomical aspects of some species of subsect. *Viola* L. and *Rostratae* Kuppfer were previously investigated [18, 19]. Here, we report on the anatomical and ecological features of local Turkish endemic *V. yildirimlii* for the first time.

MATERIALS and METHODS

Plant and soil samples of *Viola yildirimlii* were collected from its type locality, Aladağ National Park. The specimens were dried according to standard herbarium techniques and stored at the Selçuk University Herbarium (KNYA). The collecting locality of the species as follows;

Turkey. C5 Adana: Aladağ National Park, Aladağ, above Ulupınar village, rocky slopes, 1800 m, 22.04.2002, M. Dinç

1162 & Y. Bağcı; Ibid., 30.04.2001, M. Dinç 985 & Y. Bağcı; Ibid., 06.07.2004, M. Dinç 2386a & Y. Bağcı

For anatomical analysis, some samples were fixed in 70% alcohol. Anatomical investigations were performed on the cross-sections of the root, petiole, peduncle, stipule and leaves, and the surface sections of leaves. The cross and surface sections were painted with basic fuchsin and covered with glycerin-gelatin [20]. Photographs of them were taken with an Olympus BX-50 microscope. The stomatal index and stomatal index rate were calculated as described by Meidner & Mansfield [21]. Soil sample was taken from 0-20 cm. The analysis of soil sample was done according to organic matter Smith-Weldon method [22]; CaCO₃ Scheibler calcimeter method [23]; phosphorus Olsen method [24]; K, Ca, Mg, NH₄OAc method [22]; and Cu, Fe, Mn, Zn at DTPA extract ICP-AES with apparatus [25].

RESULTS

Morphological Properties (Figure 1)



Figure 1. *Viola yildirimlii* in the wild.

Acaulous perennial herb up to 8 cm high, lacking stolons. Rootstock 5 mm thick, woody and more or less branched, vertical, clothed with withered petioles. Leaves erect or suberect, ovate and very shallowly cordate at base when young; prostrate, narrowly triangular-ovate and truncate at base when mature; blade 6–55 x 10–35 mm, pubescent, thick, crenate-serrate. Petioles 15–70 mm, narrowly winged, pubescent. Stipules lanceolate to widely lanceolate, sometimes narrowly ovate, 4–12 x 1–3 mm, membranous, shortly glandular-fimbriate. Peduncle (of chasmogamous flowers) 25–35 mm long, pubescent; bracteoles 4–5 mm long, borne just above the middle of the peduncle, glandular-fimbriate below the middle; peduncle of the cleistogamous flowers much shorter. Flowers not fragrant, 8–10 mm long, violet with a white throat. Sepals narrowly oblong, 3–4 x 1–1.2 mm, pubescent; sepal appendages 0.8–1 mm long. Petals hairless, lower petal 8–10 mm, lateral and upper petals 8–9 mm; spur pale violet, 2–3 mm, curved upwards. Style 1.5–1.6 mm, geniculate at base, apex curved downwards, shortly beaked; stigmatic cavity brown at circumference. Capsules borne on peduncles which are procumbent at maturity, globose, compressed at apex, 8–10 mm in diameter, densely pubescent. Seeds 3.0 x 1.5 mm with distinctive elaisomes.

Periderm is multilayered on the outer surface of root. Phellem elements are brown. Cortex is present under the periderm. It is parenchymatous and consists of 30–35 layered flattened quadrangular secondary phloem and primary cortex cells. Cambium can be distinguished in some parts of the border of cortex and central cylinder. The xylem comprises trachea and tracheids. Pith rays are 1–2 layered. In the pith region, there is primary xylem tissue.

Anatomical Properties

Root (Figure 2)

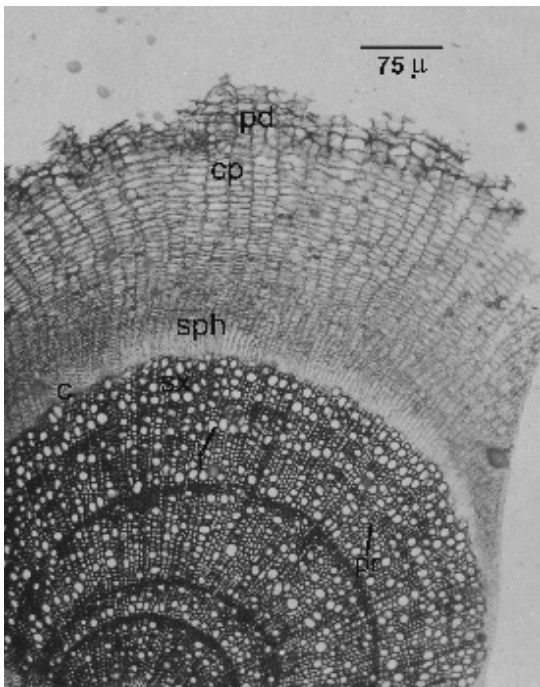


Figure 2. Cross-section of the root. pd) Peridermis, cp) Cortex parenchyma, sph) Secondary phloem, c) Cambium, sx) Secondary xylem, t) Trachea, pr) Pith ray.

Leaf (Figures 3-5)

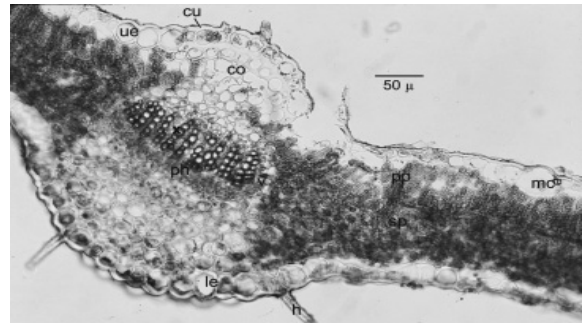


Figure 3. Cross-section of the leaf. h) Hair, cu) Cuticle, ue) Upper epidermis, mc) Mucilaginous cell, pp) Palisade parenchyma, sp) Spongy parenchyma, v) Vascular bundle, co) Collenchyma, le) Lower epidermis.

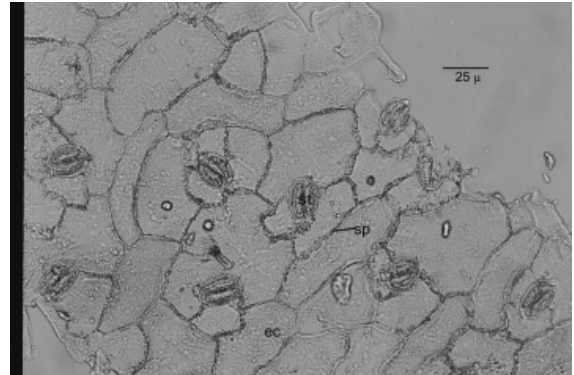


Figure 4. Surface section of the upper side of leaf. st) Stoma, ec) Epidermis cell.

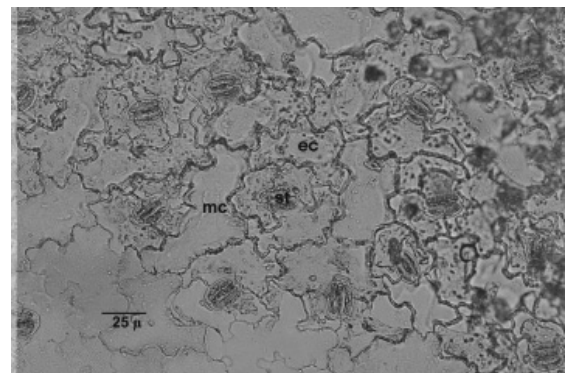


Figure 5. Surface section of the lower side of leaf. st) Stoma, ec) Epidermis cell, h) Hair.

Table 1. Some properties of leaf epidermis

	Upper surface	Lower surface
Number of stoma	96 ± 4	84 ± 4
Number of epidermis cells	162 ± 5	146 ± 5
Stoma length	21–25 μ	24–28 μ
Stoma width	18–20 μ	19–21 μ
Stoma index	37.2	36.5
Stoma index rate	1.02	-----

Leaves are covered by a thick cuticular layer on both surfaces. The upper and lower epidermis consists of a single row of ovoid, squarish and rectangular cells. There are some mucilaginous cells which are larger than the epidermis cells in both epidermis. Also, there are simple and one-celled hairs on both epidermis. Leaf is bifacial and amphistomatic. Stomata are anisocytic. The number of stomata is 96 ± 4 on the upper epidermis and 84 ± 4 on the lower epidermis of the leaf. The stomata index is 37.2 for the upper epidermis, and 36.5 for the lower epidermis. The stomata rate is 1.02 (Table 1).

There is a large vascular bundle on the median region of leaf. Parenchymatic bundle sheath surrounds the vascular bundle. The collenchyma is present in both epidermises. Lower collenchyma cells are 3-4 layered, and the upper 1-2-layered. Vascular bundles are collateral. Palisade parenchyma cells are 2-layered below the upper epidermis. Spongy parenchyma cells are 3-4 layered.

Stipules are covered by a thin cuticular layer on both surfaces. Epidermis consists of a single layered ovoid, squarish and rectangular cells on both surfaces. Stipule is unifacial. Mesophyll is not differentiated, and consists of 3-6 layered spherical parenchymatic cells. Parenchymatic cells have intercellular space, and lack chloroplast. Vascular bundle is near the upper epidermis. It is much reduced.

Stipule (Figure 6)

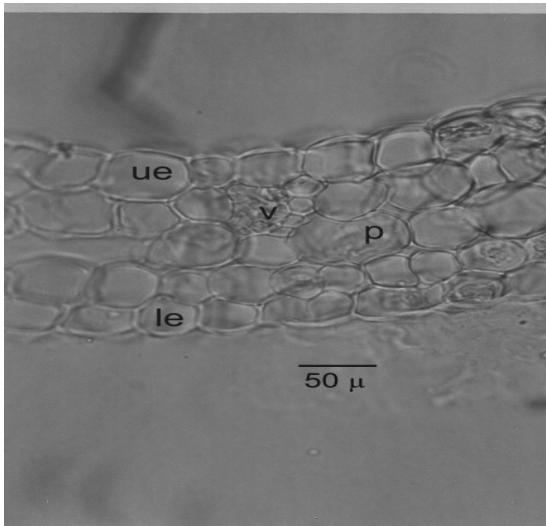


Figure 6. Cross-section of the stipule. ue) Upper epidermis, p) Parenchyma, v) Vascular bundle, le) Lower epidermis

Petiole (Figure 7)

Petiole is covered by ovoidal epidermal cells. There are simple and one-celled hairs on epidermis. The collenchyma is present under epidermis. Collenchyma consists of 3-5 layered cells at the corners of petiole, whereas it is 1-2 layered at the other parts. Parenchymatous cortex is present under collenchyma. Cortex cells are spherical, 8-20 layered and with intercellular space. There is a large crescent shaped vascular bundle on the median region of petiole. Also, the same shaped lateral small vascular bundle is present at each corner of petiole. All of three vascular bundles are collateral and covered by parenchymatic bundle sheath. The xylem turns towards upper side of petiole, and the phloem turns towards lower side of petiole. 2-4 layered sclerenchymatic cells occur between two points of the xylem of large vascular bundle.

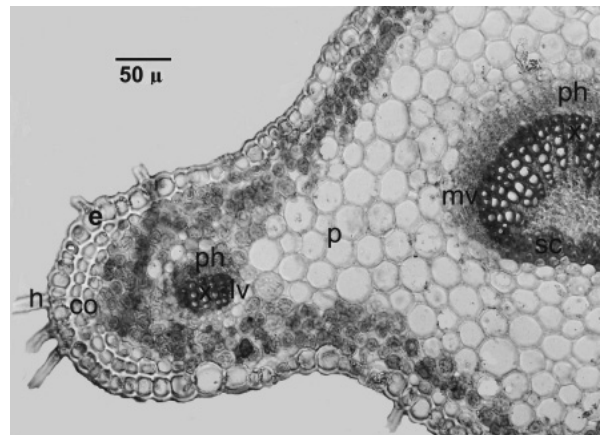


Figure 7. Cross-section of the petiole. h) Hair, e) Epidermis, co) Collenchyma, p) Parenchyma, mv) Median vascular bundle, lv) Lateral vascular bundle, ph) Phloem, x) Xylem, sc) Sclerenchyma

Peduncle (Figure 8)

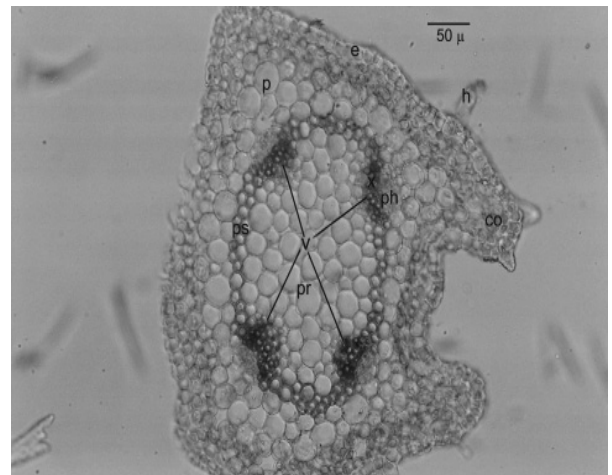


Figure 8. Cross-section of the peduncle. h) Hair, e) Epidermis, co) Collenchyma, p) Parenchyma, en) Endodermis, v) Vascular bundle, ph) Phloem, x) Xylem, pr) Pith region.

Peduncle is covered by ovoidal epidermal cells. There are simple and one-celled hairs on epidermis. The collenchyma consisting of 1-2 layered cells is present under epidermis. There is parenchymatous cortex below the collenchyma. Cortex cells are spherical, 4-10 layered and with intercellular space. There are 4 vascular bundles in the median region of the peduncle. Vascular bundles are collateral and situated on circular axle. Prosenchyma consisting of 1-2 layered cells is present between the vascular bundles. The xylem turns towards inside, and the phloem turns towards outside. Pith consists of 10-15 layered spherical parenchymatic cells.

Petal (Figure 9)

Petals are covered by cuticular layer on both surfaces. Epidermis consists of a single layered ovoidal cells on both surfaces, and lacks papilla. Mesophyll consists of 3-5 layered spherical parenchymatic cells. Parenchymatic cells have intercellular spaces. Vascular bundle is much reduced and occur near the middle region of petal in the cross-section.

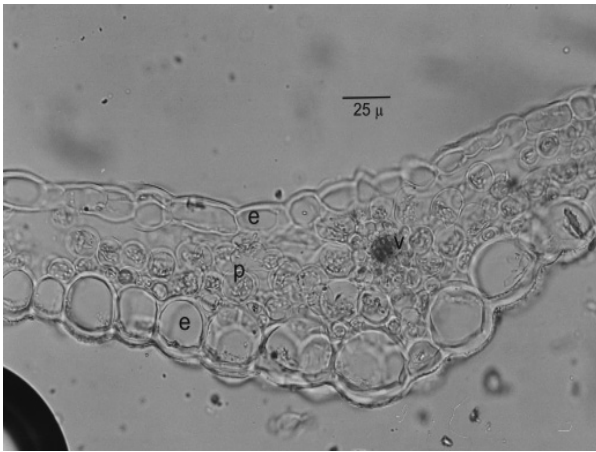


Figure 9. Cross-section of the petal. e) Epidermis, p) Parenchyma, v) Vascular bundle.

ECOLOGICAL PROPERTIES

Ecologically, chemical features of the soil on which the species naturally grow were investigated. The soil sample taking from 0-20 cm is found as rich by Fe, enough by organic matters, is medium by values pH (alchalic), sufficient with P_2O_5 , K, Mg, Na, Cu, Zn and Mn. Also, $CaCO_3$ are very poor and NaCl is medium quantity according to the the results of the analysis (Table 2).

Table 2. Soil chemical analysis of *Viola yildirimlii*

Analysis	Unit	Result
pH (1:2.5, soil:water)	---	7.8
$CaCO_3$	%	1.8
Organic matter	%	5.8
NaCl (1:5, soil:water)	mS/cm	2.7
Phosphorus (P_2O_5)	mg/kg	5.9
Potassium (K)	mg/kg	235
Calcium (Ca)	mg/kg	2985
Magnesium (Mg)	mg/kg	236
Sodium (Na)	mg/kg	65
Copper (Cu)	mg/kg	1.72
Iron (Fe)	mg/kg	160.6
Zinc (Zn)	mg/kg	1.18
Mangan (Mn)	mg/kg	69.17

V. yildirimlii grows on rocky slopes near melting snow at an elevation of 1800 m (Figure 10). It is found with *Corydalis rutifolia* (Sibth. & Sm.) DC. subsp. *erdalii* (Zucc.) Cullen & Davis, *Primula vulgaris* L. ssp. *vulgaris*, *Anemone blanda* Schott & Kotschy, *Ornithogalum lanceolatum* Labill., *Muscari azureum* Fenzl (endemic), *Gladiolus atroviolaceus* Boiss. at an altitude of 1800 m. The range of the new species is restricted to only one locality. The population include approximately 30–40 individuals. Its area is approximately 500 m². Therefore, we

suggest that *Viola yildirimlii* should be placed under the IUCN category *Critically Endangered* (CR) [26].

DISCUSSION

Morphological properties of *V. yildirimlii* was perviously given [10]. In this study, it was understood that the summer leaves reach to 55 x 10–35 mm in size, and the petioles to 70 mm long.

General anatomical characteristics of *Viola* species were pointed out that they have generally dorsiventral leaves with gland-tipped teeth, transition stoma form between *Cruciferous* and *Rubiaceous*, simple and one-celled trichomes, epidermis often with mucilaginous, aggregate calcium oxalate crystals [27]. Paracytic stoma is also known as *Rubiaceous* type, and anisocytic stoma also as *Cruciferous* type [28]. Our results basically agree with the characteristics pointed out by Metcalfe & Chalk [27]. But, the stomata of *V. yildirimlii* are all anisocytic.

The *Viola* species in Turkey divided into two sections as *Viola* L. and *Melanium* Gingins, and sect. *Viola* divided into two subsections as *Viola* and *Rostratae* according to Becker's sectional classification [29]. Akarsu stated that *Viola odorata*, included in sect. *Viola* subsect. *Viola*, has amphistomatic leaves with one layered palisade parenchyma and the petal epidermis without papilla [18]. Similarly, *V. yildirimlii*, one of the species of sect. *Viola* subsect. *Viola*, has amphistomatic leaves and the petal epidermis lacking papilla according to our results. But, the palisade parenchyma of its leaf consists of two layered cells in cross section differently. It was also reported that the petals have papillate epidermis in some species of sect. *Viola* subsect. *Rostratae*, and the petals lacks papilla on epidermis in some species of sect. *Viola* subsect. *Viola* [7, 19]. Consequently, it can be signified that papillate epidermis on the petals are important anatomical character of sectional classification of the genus *Viola*.

The stipule shape is one of the most important morphological character in classification of *Viola* species [2]. It is not similar to the leaf in sect. *Viola*. However, it is green in subsect. *Rostratae*, and not green and membranaceous in subsect. *Viola*. The results derived from the anatomical study on *V. yildirimlii* exhibited that the mesophyll is not differentiated as in leaf, and consists of 3-6 layered spherical parenchymatic cells without chloroplast in the stipule, conveniently morphological shape of the stipule in the sect. *Viola* subsect. *Viola*.

REFERENCES

- [1] Heywood, V.H. 1993. Flowering Plants of the World. London: Univ. Press.
- [2] Coode, M.J.E. & Cullen, J. 1965. *Viola* L. in Davis PH (ed.), *Flora of Turkey and the East Aegean Islands* 1: 524 - 533. Edinburgh: Edinb.Univ. Press.
- [3] Davis PH, Mill R.R, Tan K 1988. *Flora of Turkey and the East Aegean Islands*. vol.10, Edinburgh: Edinb.Univ. Press.
- [4] Yıldırım, Ş. 2000. *Viola* L. in: A Güner, N Özhatay, T Ekim and KHC Başer (eds.), *Flora of Turkey and the East*

- Aegean Islands*. 11: 43-44. Edinburgh: Edinburgh Univ. Press.
- [5] Dinç, M., Yıldırım, Ş., Ünal, A. 2000. *Viola alba* Besser subsp. *alba* (Violaceae), Türkiye Florası İçin Yeni Bir Alttür. *Ot Sistematiği Botanik Dergisi* 7 (2): 9 – 14.
- [6] Dinç, M., Yıldırım, Ş., Dural, H., Savran, A. 2001. A New violet record for The Flora of Turkey. *Viola jordanii* Hanry, *Ot Sistematiği Botanik Dergisi* 8 (1): 9 – 12.
- [7] Dinç, M. 2002. İç Anadolu Bölgesi'ndeki *Viola* L. Cinsinin Revizyonu, S. Ü. Fen Bilimleri Enstitüsü, Konya (in Turkey, unpubl.).
- [8] Dinç, M. & Yıldırım, Ş. 2002. A new species of *Viola* (Violaceae) from Turkey. *Botanical Journal of the Linnean Society* 138: 483-487.
- [9] Yıldırım, Ş., Dinç, M. 2002. A New Species of *Viola* L., *Viola ermenekensis* (Violaceae) from Ermenek-Konya-Turkey. *Ot Sistematiği Botanik Dergisi* 9 (1): 1–5.
- [10] Dinç, M., Bağcı, Y., Yıldırım, Ş. 2003. A New Species of *Viola* L. (Violaceae) from South Anatolia. *Botanical Journal of the Linnean Society* 141: 477 – 482.
- [11] Blaxland, K. 2004. A new species of *Viola* (Violaceae) from south-west Turkey. *Botanical Journal of the Linnean Society* 145: 505–509.
- [12] Okamoto, M., Okada, H., Ueda, K. 1993. Morphology and chromosome number of *Viola pilosa* and systematic position. *Taxon* 42:781-787.
- [13] Marcussen, T. & Borgen, L. 2000. Allozymic variation and relationships within subsection *Viola* (Violaceae). *Plant systematics and evolution* 223: 29-57.
- [14] Melchior, H. 1939. Ein neues Veilchen aus SW.-Anatolien und die Phylogenie der Sprossentwicklung innerhalb der Section *Nomimum*. *Feddes Repert.* 46: 39-42.
- [15] Contandriopoulos, J. & Quézel, P. 1976. Contribution à l'étude de la flore du Taurus et de l'Amanus. *Bull. Soc. Bot. France* 123: 419.
- [16] Yıldırım, Ş. 1994. *Viola bocquetiana*, a new species from Turkey. *Ot Sistematiği Botanik Dergisi* 1 (1): 1-4.
- [17] Marcussen, T. 1998. *Viola* L. subsection *Viola*: Variation, hybridization, and relationships among European taxa. *Biologisk Institutt, Oslo*.
- [18] Akarsu, F. 1989a. Batı Anadolu'da Doğal Yayılış Gösteren *Viola* L. (Syn: Subgen. *Nomimum* Ging.) Alt Cinsi Üzerinde Morfolojik ve Anatomik Araştırmalar I *Viola odorata* L. *Doğa Türk Botanik Dergisi* 13 (3): 522-529.
- [19] Akarsu, F. 1989b. Batı Anadolu'da Doğal Yayılış Gösteren *Viola* L. (Syn: Subgen. *Nomimum* Ging.) Alt Cinsi Üzerinde Morfolojik ve Anatomik Araştırmalar II. *Viola reichenbachiana* Jord. ex Bor. *Doğa Türk Botanik Dergisi* 13 (3): 530-537.
- [20] Vardar, Y. 1987. *Botanikte Preparasyon Tekniği*. İzmir: Ege Üniversitesi Fen Fakültesi Basımevi.
- [21] Meidner, H. & Mansfield, T.A. 1968. *Physiology of Stomata*. London: McGraw-Hill.
- [22] Bayraklı, F. 1987. *Toprak ve Bitki Analizleri*. O.M.Ü. Ziraat Fak. Yayınları, No: 17, Samsun.
- [23] Hızalan, E. & Ünal, H. 1966. *Toprakta Önemli Kimyasal Analizler*. Ankara Üniv. Ziraat Fak. Yay. no: 278, Ankara.
- [24] Olsen, S.R. & Sommers, L.E. 1982. Phosphorus in Methods of Soil Analysis, part 2, *Chemical and Microbiological Properties*. Agronomy Monograph, no 9, 2nd Ed., ASA-SSSA, Madison.
- [25] Soltanpour, P.N. & Workman, S.M. 1981. Use of Inductively-Coupled Plasma Spectroscopy for the Simultaneous Determination of Macro and Micro Nutrients in NH₄HCO₃-DTPA Extracts of Soils. in Barnes RM (ed). *Developments in Atomic Plasma Analysis*, 673-680, USA.
- [26] IUCN 2001. Red List Categories: Version 3.1. Prepared by the IUCN Species Survival Commission. Gland, (Switzerland) and Cambridge, (UK): IUCN.
- [27] Metcalfe, C.R. & Chalk, L. 1950. *Anatomy of the Dicotyledons I*. London: Oxford University Press.
- [28] Yentür, S. 1995. *Bitki Anatomisi*. İstanbul Üniversitesi. İstanbul: Fen Fakültesi. 118.
- [29] Becker, W. 1925. *Viola* L. in: Engler A. & Prantl K (eds), *Die natürlichen Pflanzenfamilien* 21: 363-376.