Araştırma Makalesi / Research Article

Morphological and Anatomical Properties of Endemic Iris nezahatiae Distributed in the North-East Anatolia Region (Turkey)

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Abstract
In this study, the morphological and anatomical properties of endemic Iris nezahatiae Güner & H. Duman were determined and discussed with Iris caucasica Hoffm. in terms of similar and different properties. I. nezahatiae is placed in juno irises. This species is a spring geophyte of Turkey and has flowers in varied colours. It distributes only in the vicinity of Yusufeli (Artvin) in Turkey and is under threat of exctinction. The species is a bulbous and perennial plant and has storage roots. The leaves and flowers of I. nezahatiae have different morphologic properties. In anatomical research, the differences were observed in the central cylinder of root, stem and mesophyll layer of leaf. Consequently, some morphologic and anatomic characters between two close taxa were determined that can be selecred as discriminative characters. Stomata index of the species was also calculated

Keywords: Iris nezahatiae, endemic, morphology, anatomy

1. Introduction

Turkey has a rich flora, because of its geographical location, climate, topographic features and situated at the cross section of three phytogeographic regions. This flora consists of approximately 10.000 vascular plants. In Turkey, 900 geophyte taxa grow naturally [1]. These plants create an important part of the floristic richness of Turkey. Mediterranean Basin is an important gene center for geophyte plants. Geophyte plants have tubers, bulbs and rhizomes. Generally, geophytes take place in Araceae, Liliaceae, Primulaceae, Iridaceae, Geraniaceae, Orchidaceae, Ranunculaceae, Amaryllidaceae families. In Turkey, some of the geophytes bloom their best colourful flowers in early spring and autumn. Furthemore, some of the geophytes have medical and economical properties [2, 3, 4]. The geophytes are widely used as ornamental plants in Turkey.The genus Iris belongs to the family Iridaceae and it includes over 300 species. In Turkey, this genus comprises 40 species and 49 taxa [6, 7, 8]. Iris taxa have an important place among the geophytes plants. Some of Iris taxa are used in dropsies, anti-spasmodic, emmengogue, stimulants as diuretic, aperient, gall bladder diseases, in fever and enlargement of the liver and for catarrhal ailment of children [5] and are grown in gardens, parks and balconies as ornamental plant. I. nezahatiae is placed in juno irises (subgenus Scorpiris Spach.). Seven species of this subgenus distribute in Turkey naturally and three of them are endemic to Turkey [6, 7, 9]. I. nezahatiae is a bulbous, perennial plant and has a length of 10-15 cm at flowering time. The number of flowers are 1-2 (3) and reddish brown colored. Because of its pretty colorful flowers, this species can be used as a ornamental plant in the gardens, balconies and parks in the future. However, it is distributed only in Yusufeli surroundings inTurkey. The construction of dams being built around Yusufeli will threaten the habitate of I. nezahatiae. Thus, threatened catecory I. nezahatiae was reported as critically endangered (CR) by Güner and Duman [9]. For this purpose, we wanted to put forward its morphological and anatomical properties in detail.

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2. Materials and Methods

Plant samples were collected from vicinity of Yusufeli (Artvin-Turkey) between 2012 and 2013 (Figure 1). The localities of plant samples collected are listed below;
1. A8 Artvin: Yusufeli, above Öğdem, road sides, 1700 m., 17 May 2013, Kandemir, 520.
2. A8 Artvin: Yusufeli, below Öğdem, around Hazara, metamorphic slopes, 1635 m., 17 May 2013, Kandemir, 521.
3. A8 Artvin: Yusufeli, below Çıralı Village, scrubby slopes, 770 m., 20 May 2013, Kandemir, 522.
Taxonomic description of I. nezahatiae was given according to Güner and Duman [9]. Anatomical studies were carried out on samples fixed and preserved in 70 % alcohol solution. The paraffin method was used for preparing cross-sections of the root, stem and leaf parts and surface-sections of the leaf [10]. The cross and surface-sections of these parts were photographed. The anatomical and morphological measurements were made with a micrometric ocular and ruler, respectively. The morphological studies were carried out 15 plant samples. Anatomical measurements of root, stem and leaf are given in Table 1. Stomata index was calculated according to the method described by Mesdner and Mansfield [11]. Mean of the number of epidermal and stoma cells in 1 mm2 were given in Table 2. Also, the measurement results of morphologic properties of I. nezahatiae and I. caucasica were given in Table 3.

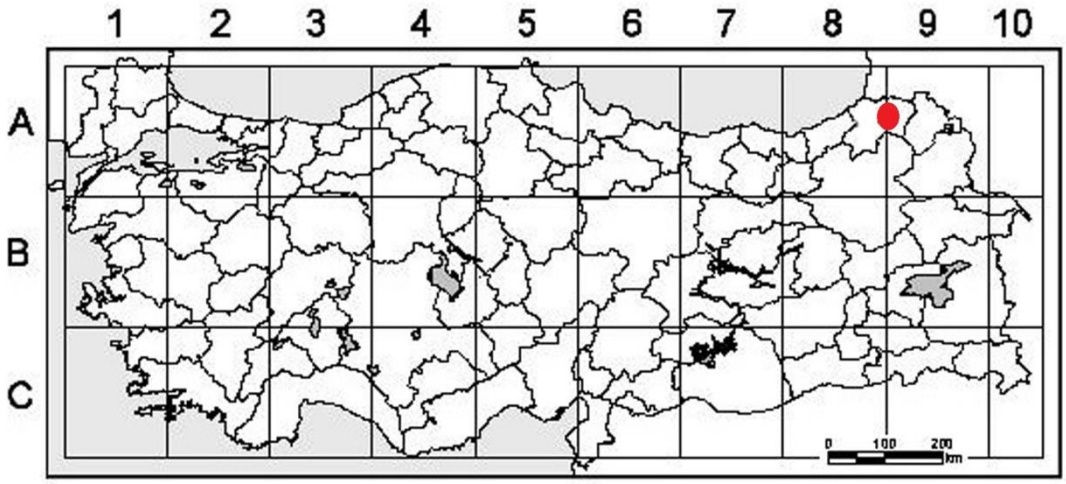

Figure 1. Map of the region where the plant is collected

● : *Iris nezahatiae*

3. Results

3. 1. Morphologic properties

Bulbous, perennial and herbaceous plants. Plant height is 10-15 cm; storage root fleshy, swollen, at depht of 5-10 cm and remains alive all year; bulb 3-3.5 x 1.7-2.0 cm, ovoid; tunic dark brown; stem 2.5-4.5 x 0.4-0.6 cm, unbranched, hidden by leaves and grows between leaves and bulb. Flowers on stem are 1-3 in number (Figure 2). Leaves 10-17 x 0.8-1.7 cm, falcate, canaliculate and linear-lanceolate, glossy green above, regulary veined below, margins white and wide at base, number of leaves 5-6, with paralel venation. Bract 4-6.3 x 1.3-1.7 cm, lanceolate, green, membranous at margins, acuminate at tip, back of bract slightly carinate and wraps the perianth tube. Bracteol 4.5 5.4 x 1.5-2.0 cm, ovate or lanceolate, green, above, membranous below, acute or acuminate at tip, not carinate. Flowers reddish brown; perigon arranged in two whorls, with three perigon segments in each whorl, three of them falls

and the other three standarts; perianth tube 2.5-3.6 cm length and sometimes hairy inside; falls (outer tepals) 3.5-4.1 x 1.4-1.8 cm, oblong; claw of falls 2.0-2.4 x 1.3-1.5 cm, winged with narrow yellow band in the centre; blade of falls 1.1-1.3 x 1.2-1.3 cm, oblong, slightly wide; crests 2.5-2.6 mm, twisted and yellow; standarts (inner tepals) 1.5-1.7 x 0.4-0.6 cm, lanceolate (Figure 2). Style
3 branches and petaloid, 3.1-3.6 x 1.1-1.4 cm, reddish brown and whitish at margins; stigma 0.2 x 0.4-

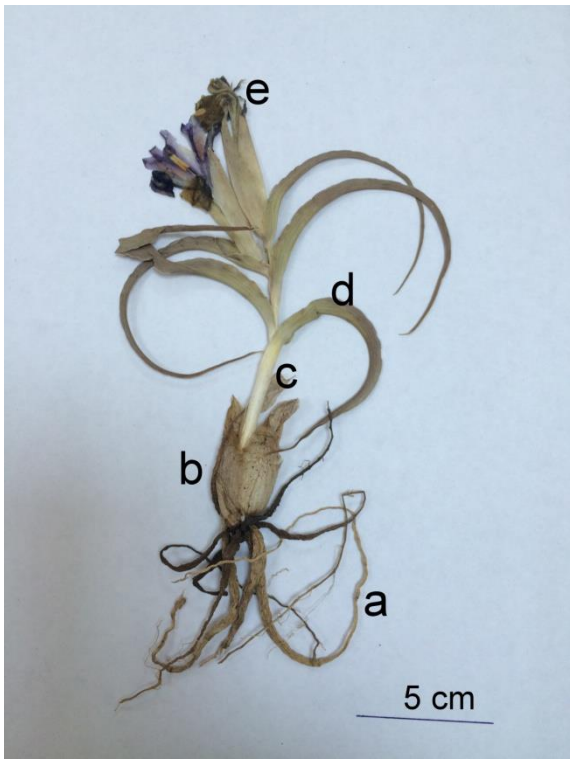

Figure 2. General appearance of herbarium sample (photo by Nezahat Kandemir) a) Root, (b) Bulb, (c) Stem, (d) Leaf, (e) Fower

0.5 cm, bilobed and retuse. Stamens 3; filaments 1.3-1.9 cm, creamy white; anthers 1.1-1.3 cm, creamy white. Capsule 2.0 x 1.0 cm, beaked; beak 0.9-1.0 cm. Seeds 4 mm, ovoid; testa rugose. The species distribute between 650 and 1700 m. Flowering time: May-June

3.2. Anatomic properties

3.2.1. Root: Epidermis is single layered and big celled. Shape of a cell is quadrange and 25-35 µ. Exodermis is 2-3 layered and 27-40 µ. Cortex is multilayered (7-8 layered) and parenchymatic. The parenchyma cells are 30-45 µ (Table 1). Cortex cells near the endoderma are small. Endoderma and pericycle are single layered and parenchymatic. The endodermis cells are 10-16 µ and the pericycle cells are 7-10 µ. The thickness in endodermis are towards pericyle. There is no thickness in the direction the cortex. Xylem have eight arks. There are three wide trachea in the pith (Figure 3).

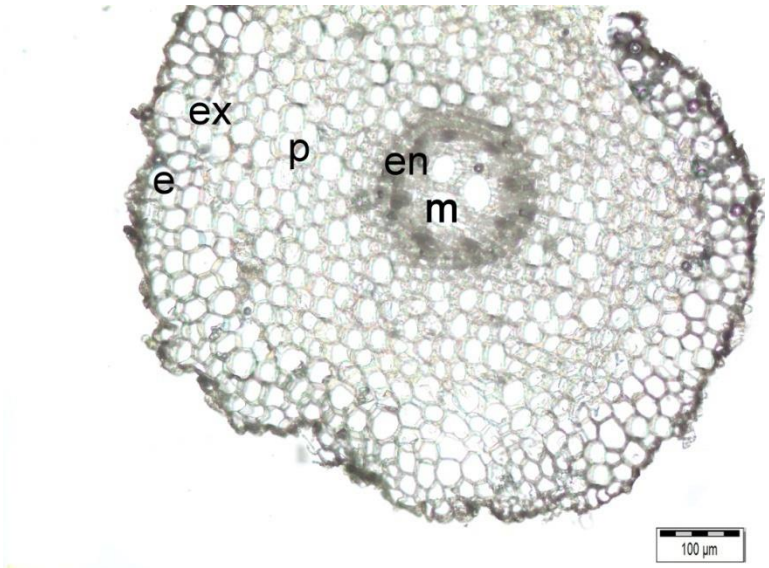

Figure 3. Cross section of root. (e) Epiderma, (ex) Exoderma, (p) Parenchyma, (en) Endoderma, (m) Metaxylem

3.2.2. Stem: Epiderma is single layered, 23-30 µ. Cells of epidermis are big, quadrange. Epiderma is covered by thick cuticular layer and 10-15 µ. Cortex consists of large or small oval parenchymatic cells. Parenchymatic cells are without chloroplast and 40-60 µ. Sclenchymatic sheath is located in the cortex and 3-4 layered. Vascular bundles are scartered (Figure 4). The vascular bundles are collateral type. There is a bundle sheath around the vascular bunsles. Xylem and phloem are obvious. Trachea of xylem is 20-25 µ. The phloem is 15-20 µ (Table 1). The pith consists of parenchmatic cells.

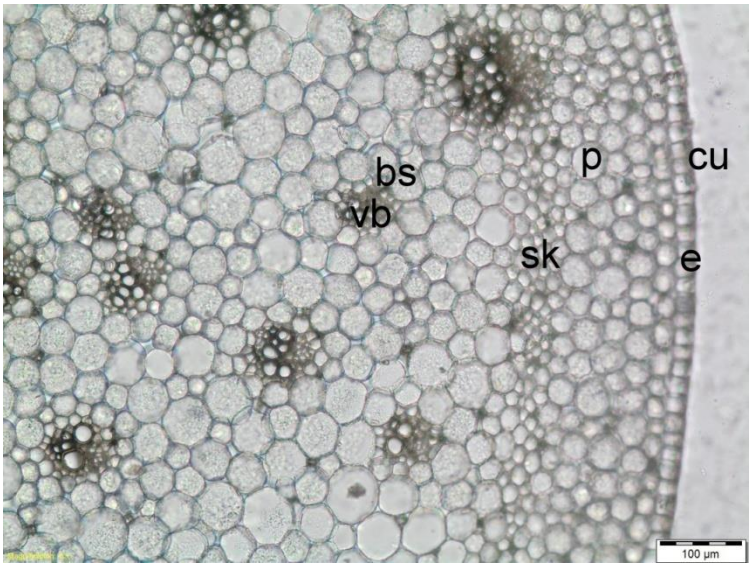

Figure 4. Cross section of stem. (cu) Cuticle, (e) Epiderma, (p) Parenchyma, (sk) Sclerenchyma, (vb) Vascular bundle, (bs) Bundle sheath

3.2.3. Leaf: Epiderma on upper and lower surface of leaf are single layered. Upper epidermis cells (50-90 µ) are larger than lower epidermis cells (22-30 µ). The cuticle layer is thick (10-14 µ) on both epiderma. There are rarely bulliform cells in upper epiderma. The micropapillae and papillae are dense in the cuticle and lower epiderma, respectively. But, the micropapillae and papillae are rare and less in the cuticle and upper epiderma, respectively. The side walls of the upper epidermis cells are wavy. While lower epidermis cells are quadrangle shaped, upper epidermis cells are in rectangular shape (Figure 5).

Stoma cells are more common in the lower epiderma. But, there are no stoma cells in the upper epiderma. Stoma cells are amaryllis type (Figure 6). Width of stoma is 29-31 µ and length 30-35 µ. Stomata are lower than the epidermis cells**.** The mean number of epidermal and stoma cells in mm^2 in lower and upper surfaces of the leaves were found to be respectively: 290 and 89; 250 and 0. Stoma index in the lower and upper epiderma are 23.48 % and 0.4 %, respectively (Table 2). The mesophyll is bifacial type (Figure 5). The mesophyll occurs large, oval shaped, with chloroplast and 3-4 layered palisade parenchyma. Spongy parenchyma is 1-2 layered, oval shape, small cells and with chloroplasts. Vascular bundles in mesophyll are single row. There is a bundle sheath around the vascular bundles. Xylem is towards adaxial surface and there are parenchyma cells on the xylem. These parenchyma cells do not have chloroplast. Phloem of vascular bundles is towards abaxial surface and there are dense sclerenchyma cells on the phloem. Also, sclerenchyma cells are at leaves margins The sclerenchyma cells are 8-11 µ in diameter. Xylem and phloem elements are apparent. Trachea of xylem is 16-22 µ in diameter and sieve tube of phloem is 9-12 µ in diameter (Table 1).

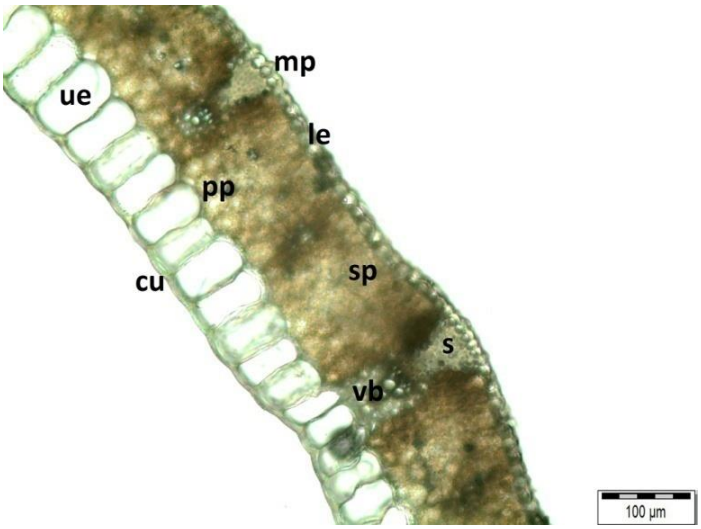

Figure 5. Cross section of leaf. (cu) Cuticle, (ue) Upper epiderma, (pp) Palisade parenchyma, (sp) Spongy parenchyma, (vb) Vascular bundle, (le) Lower epiderma (s) Sclerenchyma, (mp) Micropapillae

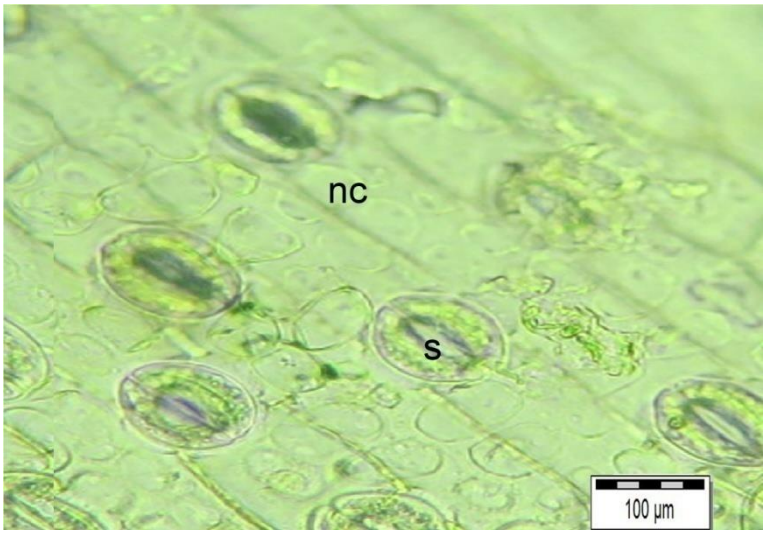

Figure 6. Surface section of leaf (s) Stoma cell, (nc) Neighbor cell

Table 1. Measurements of various tissues in cross-section.

		Width (µ)		Length (µ)	
		Minimum	**Maximum**	**Minimum**	**Maximum**
ROOT	Epidermis cell	25	35	25	34
	Exodermis cell	27	38	28	40
	Parenchyma cell	30	45	30	45
	Endodermis cell	10	14	12	16
	Pericyle cell	8	10	7	9
	Phloem cell	8	10	7	10
	Trachea cell	15	25	14	23
STEM	Epidermis cell	25	30	23	28
	Cuticle	10	15	-	-
	Diameter of parenchyma cell	40	55	40	60
	Trachea cell	20	25	22	25
	Phloem cell	18	20	15	18
	Pith parenchyma cell	55	70	40	60
LEAF	Cuticle	10	14	-	-
	Upper epidermis cell	50	65	70	90
	Palisade parenchyma cell	30	45	28	40
	Spongy parenchyma cell	25	30	24	32
	Trachea cell	16	22	16	20
	Phloem cell	10	12	9	12
	Stoma cell	29	31	30	35
	Lower epidermis cell	22	25	24	30
	Sclerenchymatous cell	9	11	8	11

Table 2. Stoma, epidermis cells numbers and stoma index in 1mm^2 of leaf surface section (S.H.S: Stoma cell number, E.H.S: Epidermis cell number, S.I: Stoma index)

Species name	**Lower surface**			**Upper surface**		
	S.H.S.	E.H.S.	S.I.	S.H.S.	E.H.S.	S.I.
Iris nezahatiae	89	290	23.48 %	0	250	0.4 %

4. Discussion

The juno irises are large and distinct group, with bulbs, fleshy persistent roots, reduced standards, well developed falls, seed and pollen morphology, petaloid style branches and bifacial leaf anatomy. They have a storage system and rootstock. Some taxa have a rugose seed surface while other taxa have prominent arils seed surface. Due to the above mentioned properties, they are unique among *Iris* taxa [12].

Although *Iris nezahatiae* is similar to *I. caucasica*, it is separated from *I. caucasica* with some morphologic and anatomic characters. Also, molecular similarities between these taxa were reported by

Ikinci et al.[13]. In Table 3, different morphologic characters between *I. nezahatiae* and *I. caucacica* were shown. It was determined that morphological characters such as bulb length, the shape of leaves and hair condition, perianth tube and style length, flower colour, the shape of standart and falls, stigma and testa characters, flowering time and altitude can be used as taxonomical characters to distinguish of these two taxa. On the other hand, our morphologic results generally showed similarities with the findings of Güner and Duman [9].

In the root anatomy of *I. nezahatiae,* the thickening in endoderma are towards pericyle, and there is no thickness in the direction of the cortex. Xylem have eight arks. Also, these properties were found in the root anatomy structure of *I. caucasica* by Özyurt [14]. There are three wide trachea in the pith of *I. nezahatiae*. However, *I. caucasica* has seven or eight trachea in the pith. In *I. nezahatiae,* 3-4 layered sclenchymatic sheath located in the stem cortex. But, in *I. caucasica* this property was no mentioned. Vascular bundles of stem are scartered both *I. nezahatiae* and *I. caucasica*. While bundles sheath around the vascular bundeles in the stem of *I. caucasica* are not seen, bundle sheath around the vascular bundles in the stem of *I. nezahatiae* are apparently seen. We though that these different anatomical properties in the root and stem can be used as taxonomical characters to distinguish two taxa. Bulliform cells in the upper epiderma, the micropapillae in the cuticle, papillae in the lower epiderma and wavy in the side walls of the upper epidermis cells in the laeves of *I. nezahatiae* were observed. But, these properties in the leaves of *I. caucasica* were not observed by Özyurt [14]. Rudall and Mathew [15], Kandemir [16] suggested that micropapillae on the cuticle layer and papillae in epiderma cells have some taxonomic significance. In *I. nezahatiae,* the mesophyll is bifacial type while the mesophyll of *I. caucasica* is isolateral type. Mesophyll include 3-4 layered palisade parenchyma with chloroplast, 1-2 layered spongy parenchyma in *I. nezahatiae*. On the other hand, mesophyll of *I. caucasica* include 2-3 layered palisade parenchyma and 1-2 layered spongy parenchyma. The leaves anatomy of the juno irises have been studied by Rudall and Mathew [17]. These researchers found that j uno irises have bifacial leaf anatomy. Our results about leaf structure are in accordance with the findings of Rudall and Mathew [17]. Actually, this subgenus are unique within Iridaceae having entirely bifacial leaves. We though that these different properties in the mesophyll can be used as taxonomical characters to distinguish of these two taxa.

The investigated species has xeoromorphic characters such as; thick cuticular layer on the epiderma of leaf and stem, bulliform cells on the upper epiderma of leaf, the edges of the leaves curved towards inside, stomata below than the epiderma cells, epiderma trichomes, lacking of stoma in the upper epiderma, palisade parenchyma more than spongy parenchyma, dense sclerenchyma cells in the vascular bundles and on the edge of the leaf, the the extending sclerenchyma as girders towards epiderma and the presence of bundle sheath. These chacacters were determined in Iridaceae and the other families [15, 16, 18, 19, 20, 21].

Consequently, morphologic and anatomic properties of I. nezahatiae which is under threat of exctinction were determined in detail. Since I. nezahatiae and I. caucasica are very close taxa, discriminative morphologic and anatomic characters which are important in terms of taxonomic of two taxa were also given.

Table 3. Comparison of morphological measurements of closely related two taxa

Morphological properties	***Iris nezahatiae***	***Iris caucasica***
Root	5-10 cm and fleshy	4-5 cm and fleshy
Bulb	1 number and 3-3.5 cm diameter	2 numbers and 2-2.5 cm diameter
Stem	2.5-4.5 x 0.4-0.6 cm	5-6 x 0.4-0.5 cm
Leaves	5-6 numbers, 10-17 x 0.8-1.7 cm, linear-lanceolate and margin white, sparsely hairy	4-6 number, 4.5-8x1-1.5 cm, lanceolate and margin white, densely hairy
Flower colour	reddish brown	yellow or greenish yellow
Perianth tube	2.4-3.6 cm length	3-4.7 cm length
Falls (outer tepals)	3.5-4.1cm x1.4-1.8 cm, oblong	3.8-4x1.3-1.5 cm, ovate
Standarts (inner tepals)	1.5-1.7 x 0.4-0.6 cm, lanceolate	2-2.1x0.5-0.6 cm, oblanceolate
Style	3.1-3.6 x 1.1-1.4 cm, reddish brown and whitish margins	3.7-3.8 x 1.5 cm, light yellow
Stigma	0.2 x 0.4-0.5 cm, bilobed and retuse	0.6 cm, crescent
Capsule	2.0 x 1.0 cm, beaked and oblong-cylindrical	3.5-4 x 0.8-1.3 cm, cylindrical
Seeds	4 mm, ovoid	4-5 x 2-2.5 mm, spherical
Testa of seeds	rugose	Rough
Flowering time in habitat	May-June	April-June
Altitude	650-1700 m	1200-3500 m

5. References

1. Koyuncu M., Alp Ş. 2014. New geophyte taxa described from Turkey at last decade, Yüzüncü Yıl University Journal of Agriculture Science, 24 (1): 101-110.
2. Baytop T. 1984. Therapy with plant in Turkey, İstanbul University Publication, 321pp. Istanbul.
3. Sargın A.S., Selvi S., Akçiçek E. 2013. Investigations of ethnobotanical aspect of some geophytes growing in Alaşehir (Manisa) and surrounding area, Erciyes University Journal of Institute ofScience and Technology, 29 (2): 170-177.
4. Atak A., Kaya E., Erken K. 2014. Determination of quantity and purity of some geophytes collected from the flora of Turkey, Asian Journal of Plant Sciences, 13 (3): 98-110.
5. Atta-ur R., Shama N., Irfan B., Saima J., İlkay O., Bilge S., Igbal M.C. 2003. Anti-inflammatory isoflavonoids from the rhizomes of Iris germanica, Journal Ethnopharmacology, 86: 177-180.
6. Mathew B. 1984. Iris L. in Flora of Turkey and the East Aegean Island, Edited by Davis PH, Vol.8, Edinburg: Edinburg Univ Press, 382-410.
7. Mathew B. 1988. Iris L. in Flora of Turkey and the East Aegean Islands, Edited by Davis PH, Mill RR, Tan K, Vol. 10, Edinburg: Edinburg Univ Press, 227-228.
8. Güner A. 2012. Iris L.in Turkey Plants List (Vascular Plants), Edited by Güner A, Aslan S, Ekim T, Vural M, Babaç MT, İstanbul: Nezahat Gökyiğit Botanical Garden and Flora Research Association Publication, 535-540.

9. Güner A., Duman H. 2007. A new Juno Iris from North-East Anatolia Turkey, Turkish Journal of Botany. 31: 311-315.
10. Algan G. 1981. The microtechnic for plant tissues, Fırat University Press, 93pp. Elazığ.
11. Mesdner H., Mansfield T.A. 1968. Physiolgy of stomata, Mc Graw-Hill, London.
12. Mathew B. 2001. Some aspects of the Juno group of irises, Annali di Botanica, 1: 113-122.
13. Ikinci N., Hall T., Lledo M.D., Clarkson J.J., Tillie N., Seisums A., Saito T., Harley M., Chase M.W. 2011. Molecular phylogenetics of the Juno irises, Iris subgenus Scorpiris (Iridaceae), based on six plastid markers, Botanical Journal of the Linnean Society, 167 (3): 281-300.
14. Özyurt S. 1978. Morphologic and ecologic investigations on some geophytes belonging Liliaceae and Iridaceae family of vicinity Palandöken Mountains, Atatürk University Publication, 93pp. Erzurum.
15. Rudall P., Mathew B. 1990. Leaf anatomy in Crocus (Iridaceae), Kew Bulletin, 45: 535-544.
16. Kandemir N. 2011. Comparative leaf anatomy of some endemic Crocus L. taxa fromTurkey, Bangladesh Journal of Botany, 40: 155-162.
17. Rudall P., Mathew B. 1993. Leaf anatomy of the bulbous Irises, Botanische Jahrbücher,115: 63-76.
18. Kandemir N. 2009. A morphology, anatomy and ecology of critically endangered endemic Crocus pestalozzae Boiss. (Iridaceae) in North-West Turkey, Bangladesh Journal of Botany, 38 (2): 127-132.
19. Kandemir N. 2010. A morphological and anatomical investigation about two rare and endemic Crocus taxa (Iridaceae) from Southern Anatolia, Eur-Asian Journal of BioSciences, 4: 54-62.
20. Kandemir N., Çelik A., Yayla F. 2012. Comparative anatomic and ecologicinvestigations on some endemic Crocus taxa (Iridaceae) in Turkey, Pakistan Journal of Botany, 44 (3): 1065-1074.
21. Rotondi A., Rossi F., Asunis C., Cesaraccio C. 2003. Leaf xeromorphic adaptations of some plants of acoastal Mediterranean macchia ecosystem, Journal of Mediterranean Ecology 4: 25-35.