Bangladesh J. Plant Taxon. **27**(2): 213-224, 2020 (December)

THE ANATOMICAL STRUCTURES OF THE GENUS *IBERIS* L. (BRASSICACEAE) IN TURKEY

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Keywords: Anatomy; Candytufts; Cruciferae; *Iberis*, Mustard family; UPGMA.

Abstract

The present study was designed to analyse the anatomy of the vegetative and reproductive parts of Turkish *Iberis* species from a systematic point of view. Samples of leaves, stems, roots, fruits, and seeds of each species were collected, fixed, and processed according to the paraffin method for light microscopy. The numerical analysis derived from 11 anatomical characteristics showed that the number and dimensions of vessels in the root, presence of aerenchyma in the leaf, number and dimensions of palisade parenchyma, and mesophyll type were useful for grouping the *Iberis* taxa. The testa was composed of four layers: the epidermis, subepidermis, compact tissue, and parenchyma. The testa thickness was a significant character to distinguish the investigated *Iberis* species. In this study, the traditional classification of Turkish *Iberis* species was mostly congruent with the dendrogram generated vegetative anatomical properties.

Introduction

The family Brassicaceae, also named as Cruciferae in reference to its four crossed petals, is commonly called the mustard family. This family contains 52 tribes, 341 genera, and 3997 species (Koch *et al.*, 2012; Kiefer *et al.*, 2014; BrassiBase: https://brassibase.cos.uni-heidelberg.de/, accessed 5 February 2018) distributed worldwide, primarily in temperate regions (Al-Shehbaz, 1984; Al-Shehbaz *et al.*, 2006). The family has economic importance (Franzke *et al.*, 2011; Al-Shehbaz, 2012; Huang *et al.*, 2016), as it includes the well-known model plant species *Arabidopsis thaliana* (Linn.) Heynhold, many crops (e.g., cabbage, cauliflower, turnip, rapeseed, canola, radish, and wasabi) and ornamentals (e.g., species of *Lobularia* Desv., *Iberis* L., *Hesperis* L., and *Matthiola* W.T. Aiton). Although the family is easily recognised by its morphological aspects, it is often difficult to assign an individual plant to a given genus, and there is tremendous controversy regarding its generic and tribal delimitations (Al-Shehbaz *et al.*, 2006; Al-Shehbaz, 2012). More specifically, obtaining plants with mature fruit is highly significant for separating genera in the family Brassicaceae. The genus *Iberis* is a small group in crucifers, with a total of 28 species in the world (Al-Shehbaz, 2012; Çilden and Zare, 2019). The total number of *Iberis* species in Turkey is nine (Mutlu, 2012; Oskay, 2017; Çıtak, 2019).

Systematic studies based on the anatomy of vegetative and reproductive parts can be useful to discriminate the species of Brassicaceae (Selvi and Paksoy, 2013; Atçeken *et al.*, 2016). Although quite a few palynological (Çıtak, 2019), embryological (Prabhakar and Vijayaraghavan, 1983), and floral (Busch and Zachgo, 2007) studies have been carried out on *Iberis*, the investigation of its comparative vegetative and reproductive anatomy remains insufficient for the genus. In order to gain knowledge of the anatomical relationship of Turkish *Iberis* species, the first comprehensive study, which included representatives of eight species, was conducted herein to evaluate their practicality in the taxonomy of the genus.

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Materials and Methods

Collection and storage of the plant materials

The plant specimens used in this study were collected during 2015 and 2019 from various localities in Turkey. The information about the voucher specimens is given in Table 1. The anatomical samples of the examined species were stored in the Plant Anatomy Laboratory of Biology Department of University of Selçuk, Konya, Turkey.

Table 1. Locations and collector information of the Turkish *Iberis* taxa.

Taxa	Location	Collector number
Iberis sempervirens L. (I1)	C4 Konya: Beyşehir, Dumanlı Mountain, 1800 m., 07.06.2018	B. Çıtak-340 (**)
	C3 Antalya: Akseki, Atlarkırı Mountain, 2100 m., 04.06.2019	B. Çıtak-348
I. carnosa Willd. (I2)	B5 Nevşehir: Ortahisar, 1300 m., 17.05.2015	B. Çıtak-167-a (**)
	Adana: Pozantı, Horozköy, 1000 m., 22.05.2018	B. Çıtak-332
I. odorata L.(I3)	C6 Kahramanmaraş: Pazarcık, 800 m. 23.04.2018	B. Çıtak-334 (**)
I. simplex DC. (I4)	B5 Nevşehir, Akdağ, 1300 m., 29.05.2016	B.Çıtak-180 (**)
	Adana: Pozantı, 1000 m.	B.Çıtak-333
I. carica Bornm. (I5)	C2 Muğla: Marmaris, 500 m., 25.05.2019	B. Çıtak-345 (**)
I. halophila Vural & H. Duman* (I6)	C4 Aksaray: Eskil, The Salt Lake, 920-950 m., 19.05.2018	B.Çıtak-335-a (**)
	C4 Konya: Tersakan Lake, 900 m., **10.06.2019**	B. Çıtak-351
I. saxatilis L. subsp. *saxatilis* (I7)	B1 Balıkesir: Edremit, Kaz Dağı, 1600 m., 25.05.2018	B.Çıtak-336 (**)
I. saxatilis subsp. *magnesiana* Oskay* (I8)	B1 Manisa: Soma, 1000 m., 26.05.2018	B.Çıtak-337 (**)

*Endemic taxa, (**) the photographing species.

Anatomical surveys

The paraffin method was used to prepare the permanent anatomical slides (Johansen, 1940). For each taxon, five plant samples were used and the experiments were done at least three times. The vegetative parts of the species were cut into small pieces. Next, they were treated through an increasing alcohol series to remove the water from the tissues. As the next step, a portion of paraffin was added to small glass flasks. The paraffin blocks were made and 12–16-µm-thick transverse sections were cut using a Thermo Scientific microtome with disposable blades. Under a light microscope (Leica DM 1000), the best sections were chosen and photographed at magnifications of 10x, 20x, and 40x. The measurements, which were made with the Kameram 21 software programme, were based on at least 30 or more cells per specimen. The mean values of the measurements of all of the investigated taxa were given. The root, stem, leaf, mature fruit and seed were used for anatomical studies.

Numerical analysis

For the numerical analysis, the qualitative and quantitative characters were scored. A total of 11 anatomical characters were used to evaluate the taxonomical similarities of the *Iberis* species (Table 2). A data matrix was set using the recorded qualitative and quantitative characters. Based on the anatomical characters, the coefficients of correlation among the eight species were determined and these species were grouped using the clustering analysis method (unweighted pair

group method with arithmetic mean, UPGMA, dissimilarity, standardised variables). The clustering analysis was based on Gower's (1971) general coefficient similarity (Sneath and Sokal, 1973), which was used directly with a mixture of character types (binary, qualitative, and quantitative. Untransformed, centred, and unstandardized data were used to create a covariance matrix. In addition, principal component analysis (PCA) was used to ordinate the variables and identify the valuable all the selected anatomical characters used in taxonomy (Table 2). MVSP 3.22 software was used for all of the computations.

Table 2. Anatomical characteristics of Turkish *Iberis* species used for the numerical analysis.

Vegetative organ	Acronyms	Definition of anatomical characteristics
Root	A1	Root structure type
	A2	Vessel diameter
	A3	Number of vessels
Stem	A4	Diameter of pith cells
	A5	Aerenchyma
Leaf	A6	Mesophyll thickness
	A7	Length of palisade cells
	A8	Width of palisade cells
	A9	Row of palisade tissue
	A10	Width of spongy parenchyma
	A11	Leaf cross-section shape (triangular: 0, linear: 1)

Results and Discussion

The general anatomical descriptions of the root, stem, leaves, fruits, and seeds of the eight taxa were prepared (Table 3). The selected anatomical images, which best represented the examined taxa, are shown in Figs. 1–8. All scores related to the anatomical characters are given in Table 3.

Table 3. Anatomical data of Turkish *Iberis* species used for the numerical analysis.

Species/Characters	I1	I2	I3	I4	I5	I6	I7	I8
A1 (µm)	652	407.32	459.58	625.66	304.43	1063.06	242.6	279.44
A2 (µm)	112.6	64.6	73.81	86.83	74.5	55.7	26.33	37.16
A3 (µm)	62.83	21.26	30.59	26.83	24.53	30.33	18.5	18.2
A4	0	0	0	1	0	1	0	0
A5 (µm)	42.66	30.84	44.28	41.73	23.96	29.26	21.66	38.24
A6 (µm)	361.9	821	996	34.03	53.96	623.3	335.6	33.1
A7	0	0	0	0	0	1	0	0
A8	0	0	0	0	0	1	1	1
A9 (µm)	77.3	50.12	36.72	45.8	33.13	48	31.86	32.24
A10	0	0	0	1	0	1	1	1
A11	0	0	0	1	0	1	1	1

Root anatomy

The cross-sections of the root had different layers as protective tissue. *I. saxatilis* subsp. *saxatilis*, *I. saxatilis* subsp. *magnesiana*, *I. sempervirens*, *I. halophila*, and *I. carnosa* had a peridermis, whereas *I. carnosa*, *I. odorata*, and *I. carica* had an epidermis (Figs 1-2). The cortex parenchyma was on a small area of the roots in the studied species. Only *I. halophila* had large cavities (aerenchymatic area) in the cortex cells. The phloem and xylem were well-developed. The pith region was fully filled with xylem elements. The vessels were quite reduced in *I. halophila*.

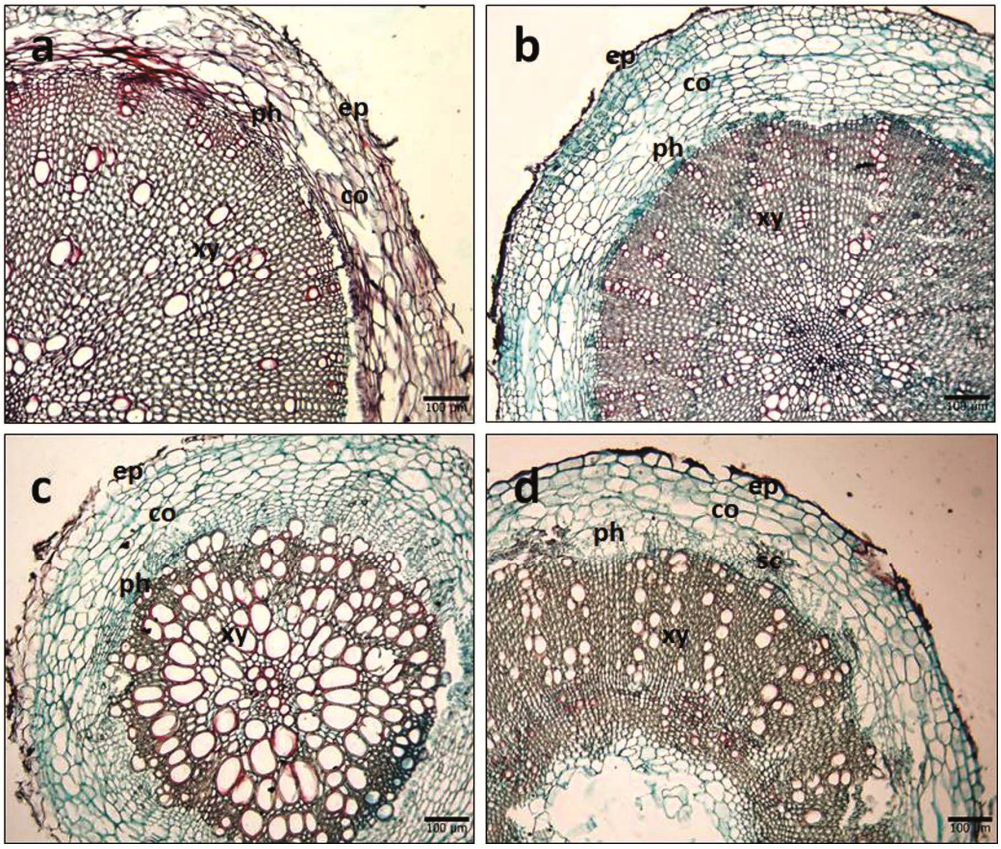

Fig. 1. Root anatomy of annual or biennial Turkish *Iberis* species: a. *I. carica*, b. *I. odorata*, c. *I. carnosa*, d. *I. simplex*. ep: epidermis, co: cortex parenchyma, ph: phloem, xy: xylem.

Stem anatomy

The transverse sections of the stem had an epidermis, cortex, and vascular bundles and a pith region towards the centre in all of the examined taxa. The epidermis was single-layered with oval–rectangular-shaped cells. The cortex parenchyma had chlorophyll pigments with oval-shaped cells. The ridges in the stem had collenchymatic cells. Phloem and xylem elements were found continuously in the stem. There were some sclerenchymatic cells over the phloem in the examined species, except in *I. carnosa*. The pith region was covered with oval-shaped parenchymatic cells (Figs 3-4).

Leaf anatomy

The general view of the leaf cross-section showed the presence of an epidermis, mesophyll, and vascular bundles. The epidermis was limited to the leaves from the abaxial and adaxial sides. The cylindrical-shaped palisade parenchyma cells contained abundant chlorophyll pigments and they were arranged in 2 or 3 layers on both sides of the leaves. Only *I. halophila* had a greater number of palisade parenchyma cells in its leaves. Vascular bundles were found in a single line and the midvein vascular bundle was larger than the others. The aerenchymal area was developed in *I. saxatilis* subsp. *saxatilis* (Figs 5-6).

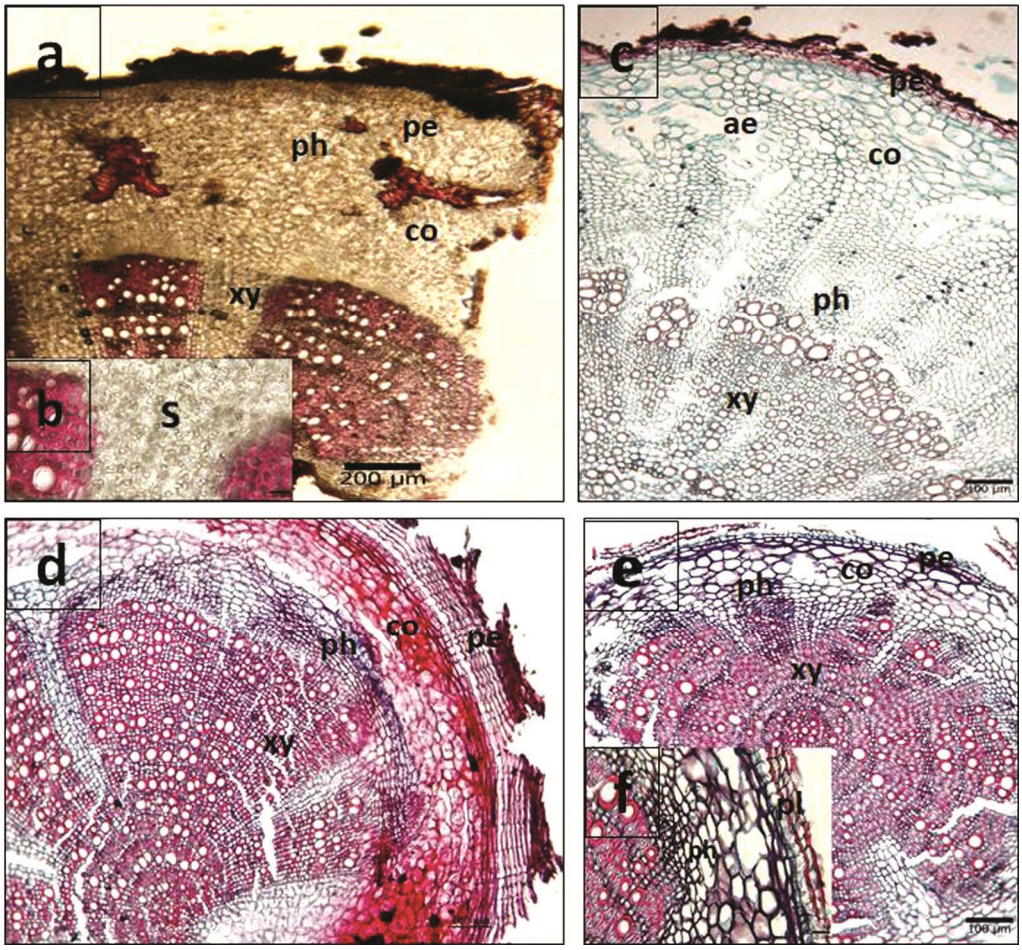

Fig. 2. Root anatomy of perennial Turkish *Iberis* species: a-b. *I. sempervirens*, c. *I. halophila*, d. *I. saxatilis* subsp. *saxatilis*, e-f. *I. saxatilis* subsp. *magnesiana*. ep: epidermis, co: cortex parenchyma, ph: phloem, xy: xylem, ae: aerenchyma, pl: phellem, s: starch.

Fruit anatomy

The findings of the anatomical studies of the fruit are shown in Table 3 and Fig. 7. The fruit wall was composed of 4 anatomical layers: the outer epidermis, parenchyma, sclerenchyma and endocarp. The wing contained abundant parenchyma cells. The mesocarp thickness varied in the studied species (Table 3).

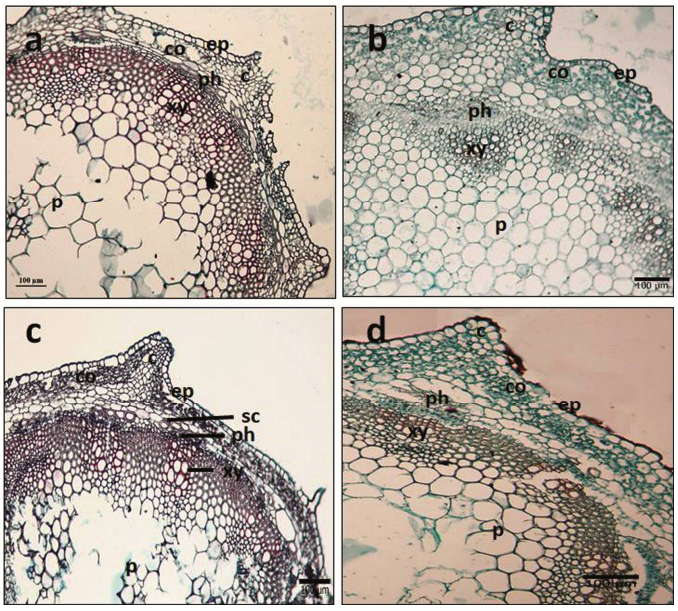

Fig. 3. Stem anatomy of annual and biennial Turkish *Iberis* species: a. *I. carica*, b. *I. carnosa* c. *I. odorata*, d. *I. simplex*. ep: epidermis, co: cortex parenchyma, ph: phloem, xy: xylem, c: collenchyma, p: parenchyma

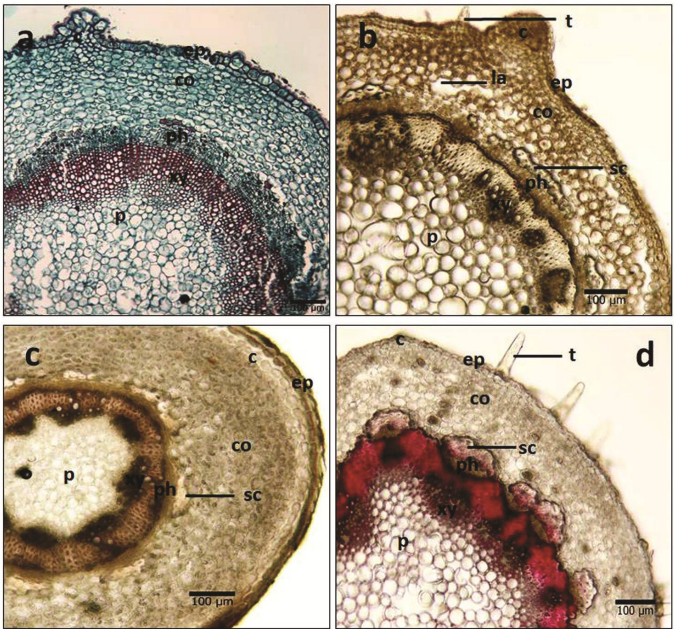

Fig. 4. Stem anatomy of perennial Turkish *Iberis* species: a. *I. sempervirens*, b. *I. halophila*, c. *I. saxatilis* subsp. *saxatilis*, d. *I. saxatilis* subsp. *magnesiana*. ep: epidermis, co: cortex parenchyma, ph: phloem, xy: xylem, c: collenchyma, p: parenchyma, t: trichome, sc: sclerenchyma, la: lacunae.

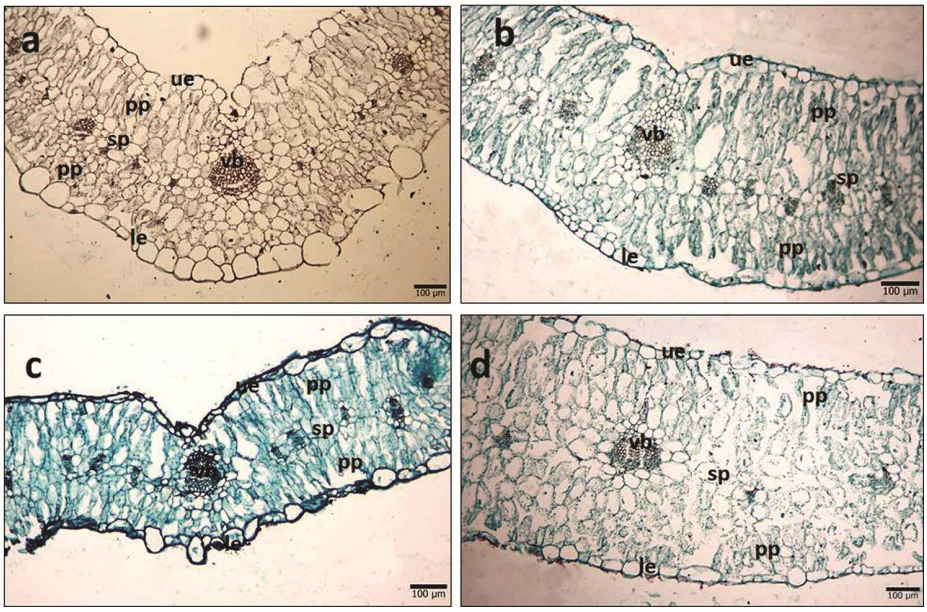

Fig. 5. The leaf anatomy of annual and biennial Turkish *Iberis* species: a. *I. carica*, b. *I. carnosa*, c. *I. odorata*, d. *I. simplex*. ue: epper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, le: lower epidermis, vb: vascular bundle.

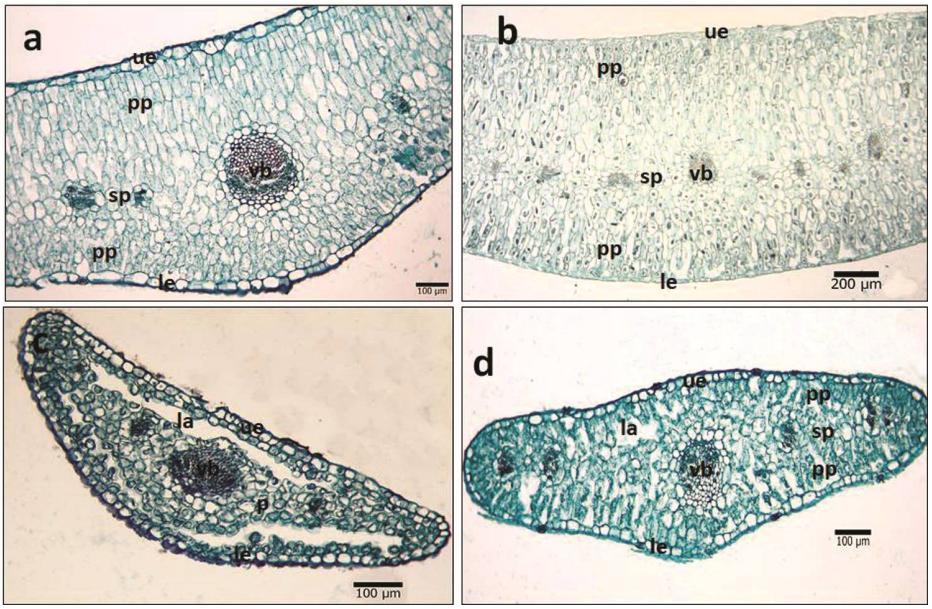

Fig. 6. The leaf anatomy of perennial Turkish *Iberis* species: a. *I. sempervirens*, b. *I. halophila*, c. *I. saxatilis* subsp. *saxatilis*, d. *I. saxatilis* subsp. *magnesiana*. ue: epper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, le: lower epidermis, vb: vascular bundle, la: lacunae, p: parenchyma.

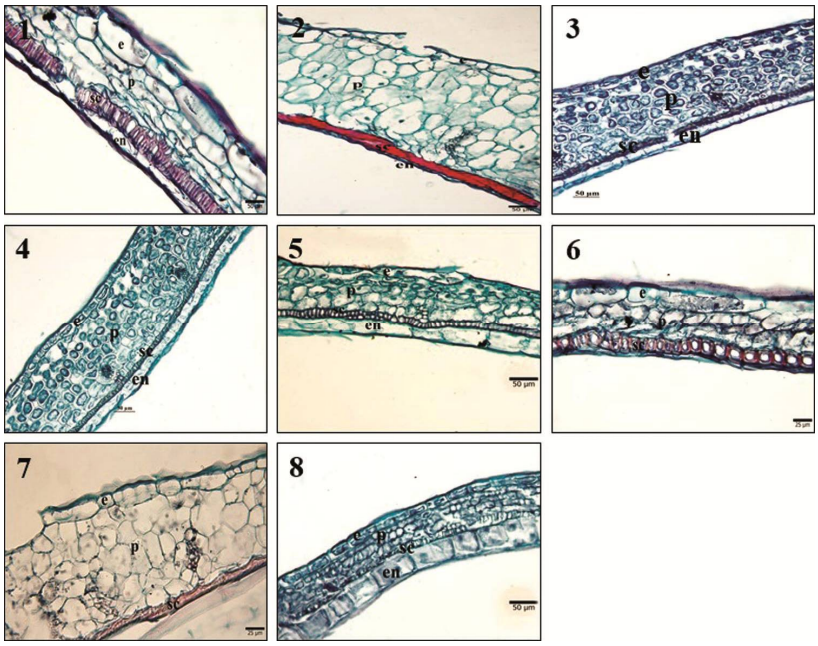

Fig. 7. The fruit wall anatomy of Turkish *Iberis* species: a. *I. sempervirens*, b. *I. halophila*, c. *I. saxatilis* subsp. *saxatilis*, d. *I. saxatilis* subsp. *magnesiana*. e: the outer epidermis, p: parenchyma; en: endocarp, sc: sclerenchyma.

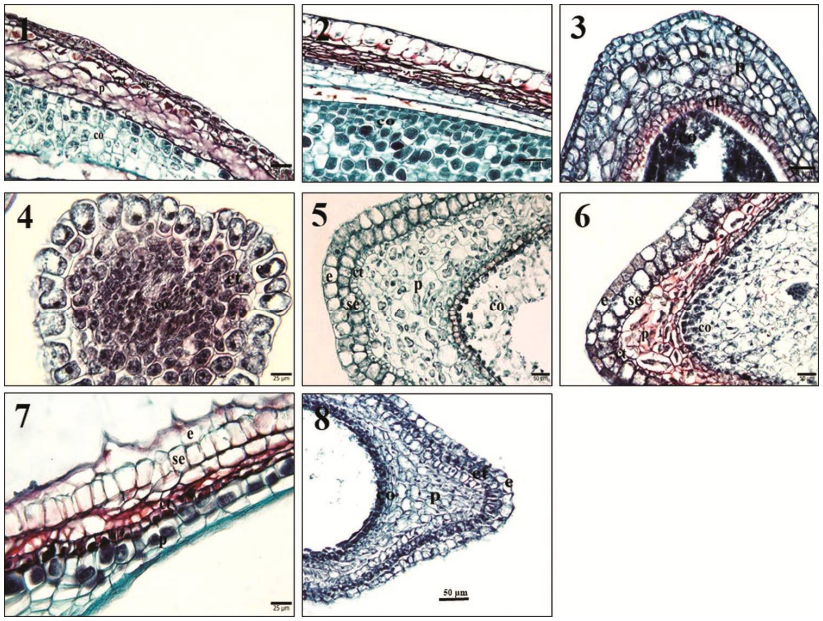

Fig. 8. The seed wall anatomy of Turkish *Iberis* species: a. *I. sempervirens*, b. *I. halophila*, c. *I. saxatilis* subsp. *saxatilis*, d. *I. saxatilis* subsp. *magnesiana*. e: the outer epidermis, se:subepidermis, p: parenchyma; ct: compressed tissue, co: cotyledon.

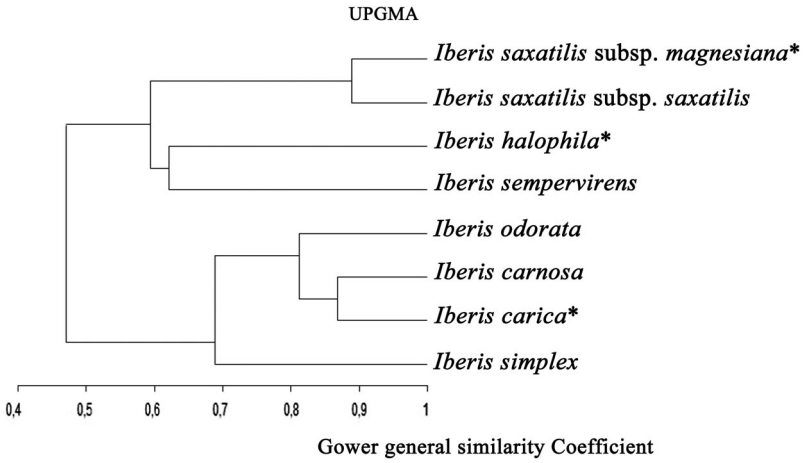

Fig. 9. Dendrogram based on anatomical traits showing the similarity and distance between *Iberis* species. Asterisks indicate endemic taxa.

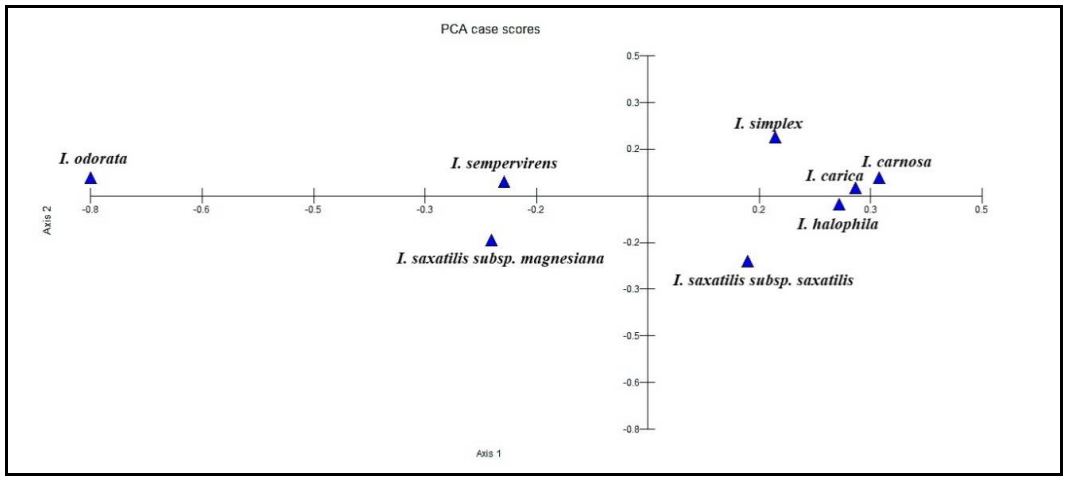

Fig. 10. Principal component analysis of examined *Iberis* species.

Seed anatomy

The findings of the anatomical investigations of the seeds are shown in Table 3 and Fig. 8. The seeds of the investigated taxa were composed of epidermis, subepidermis, compact tissue, parenchyma, and endosperm layers. The epidermis and subepidermis cells were oval or rectangular shaped in the examined species. The thickness of the testa was the thickest in *I. carnosa* and the thinnest in *I. sempervirens* (Table 3).

Numerical analysis of the anatomical character states

The dendrogram derived from the cluster analysis using the UPGMA based on the 11 anatomical variables of the eight *Iberis* species is presented in Fig. 9. This dendrogram reflected the similarities among the examined species. The dendrogram revealed two main groups: Group A (with 60% similarity) comprised four perennials of Turkish *Iberis*: *I. saxatilis* subsp. *saxatilis*, *I. saxatilis* subsp. *magnesiana*, *I. sempervirens*, and *I. halophila*. Group B (with 68% similarity)

comprised the remaining four taxa of the annuals and biennials of Turkish *Iberis*. Group A consisted of two main clusters, which were described further as Clusters A_1 and A_2. Cluster A_1 included subspecies of *I. saxatilis*, *I. saxatilis* subsp. *saxatilis*, and *I. saxatilis* subsp. *magnesiana* (with 88% similarity). Cluster A_2 included two species: *I. halophila* and *I. sempervirens* (with 62% similarity). Group B consisted of 2 main clusters: Clusters B_1 and B_2. Cluster B_1 included 2 subclusters: C_1 and C_2. C_1 contained *I. odorata* (with 81% similarity). C_2 included *I. carnosa* and *I. carica* (with 86% similarity). Cluster B_2 included only *I. simplex*.

The anatomical characteristics of the examination and designation of the family Brassicaceae has been based on the study by Metcalfe and Chalk (1950). In the current study, the diagnostic anatomical traits were determined as mesophyll, via the vessel diameter in the root and the presence of aerenchyma tissue. The species of *Iberis* were herbaceous annual and biennial, and perennial. The genus *Iberis* was not sufficiently identified by Hedge (1965). Çıtak (2019) wanted to trait the genus by its palynological data. Our results supported the discrimination of Turkish *Iberis* based on palynomorphological characteristics previously indicated by Çıtak (2019).

A generally accepted theory by Fahn (1990) was that the anatomy of the root was unchangeable, and the taxonomic value of the roots was very limited in many plant genera. The root anatomy of Turkish *Iberis* studied here was congruent with the life form of the species. The annual and biennial *Iberis* species had a primary root structure and the perennial ones had a secondary root structure. As can be seen from Table 3, the vessel diameter was the largest in *I. carnosa*, while the smallest was in *I. saxatilis* subsp. *saxatilis*. The number of vessels was the most crowded in *I. halophila* because of the characteristics of this halophytic species, not mentioned previously in Çilden and Zare (2019). Çilden and Zare (2019) indicated that *I. carica* had a perennial habit and isolateral leaf anatomy. However in this study, we confirmed that *I. carica* has a distinct annual herb with primary root anatomy. Additionally, *I. carica* has equifacial mesophyll type different from Çilden and Zare (2019)'s study.

The stem shape was rounded, semi-rounded, rectangle, circular, or irregular in the family Brassicaceae (Atçeken *et al*., 2016; Qader, 2018). The stem shape in the investigated *Iberis* taxa was circular, in addition to ridges with collenchymatic tissue. Cortex parenchymatic cells covered the small area in the cross-sections of stem. However, they contained starch molecules as stored materials. This arrangement has been declared by some researchers previously (Selvi and Paksoy, 2013; Atçeken *et al*., 2016). Sclerenchymatic tissue was found in the studied *Iberis* taxa, except in *I. carnosa*; however, their dimensions and amount were different among the species (Figs 4-5). Aerenchyma has generally been reported in the stems of marsh plants (Salisbury and Ross, 1985; Drew *et al*., 2000) or halophytic plants (Akcin *et al*., 2015). Accordingly, in the present study, aerenchymatic tissue was observed only in *I. halophila*, which grows in salty habitats. Yentür (2003) had declared that the arrangement of vascular bundles within the stem can be useful information for comparative anatomical studies. Our investigation of the stem cross-sections showed that in most of the taxa characterised by vascular bundles arranged in a ring, only *I. halophila* also had small cortical bundles among the main large vascular bundles (Figs 4e–4h, Table 3).

In the family Brassicaceae, the leaf anatomy has been used to characterise tribes and some genera (Selvi and Paksoy 2013). Interestingly, *I. saxatilis* subsp. *saxatilis*, and *I. saxatilis* subsp. *magnesiana* had aerenchymatic tissue in the leaf. Possibly, the moist habitat of these 2 subspecies could result in the aerenchymatic areas in leaf. In the present study, a unifacial mesophyll was only observed in *I. saxatilis* subsp. *saxatilis*, which grow in moist areas, while equifacial leaves were observed in the other investigated taxa, which mainly grow in the dry habitats of the Irano-Turanian phytogeographic region of Turkey. According to Yentür (2003), equifacial leaves were generally characteristic of xerophytic plants, which was in accordance with the observations made

herein. Accordingly, the number and volume of palisade parenchyma were larger in *I. halophila*, which grows in the salty habitats of Salt Lake.

The fruit and seed anatomical properties contained essential information about the taxonomy of the family Brassicaceae (Mummenhoff *et al*., 2008; Mühlhausen *et al*., 2010; Lenser *et al*., 2016). Karaismailoğlu (2019) declared that the testa thickness could be showed a great variation for *Aethionema* genus. In present study, the fruit and seed wall thicknesses of *Iberis* genus has been found as a great potential to separate its species.

The UPGMA dendrogram generated from the anatomical traits of the vegetative parts discriminated the species of *Iberis* according to their life forms. The positions of the *Iberis* species and their similarities reflected in the clusters were found to be agreeable with the previous large scale classification of the genus. *Iberis saxatilis* subsp. *saxatilis* and *I. saxatilis* subsp. *magnesiana*, which are local and/or very distinct endemic species, were in the same clade. The two subspecies could be easily separated from each other by the presence-absence of stem indumentum. In *I. saxatilis* subsp. *magnesiana*, the stem was retrorsely setulose, while the stem of *I. saxatilis* subsp. s*axatilis* was glabrous. *Iberis sempervirens* is a semi-shrub plant that has no close relative and *I. halopila*, which is a dwarf, perennial species, lives in salty habitats found in the same subclade, because of their similar anatomical characteristics. The other 4 annual or biennial Turkish *Iberis* species were positioned in the same clade. This position of the species was congruent with the Hedge (1965) classification system in Flora of Turkey.

PCA ordination and similarity matrix in accordance with anatomical traits of vegetative parts are shown in Fig. 10, in which *I. carica* and *I. halophila* are placed as the closest taxa, whereas *I. odorata* and *I. carnosa* as the most distant taxa. Additionally, the cumulative variance value of principal components achieved 81.8%.

In conclusion, vessels in the roots and stems, mesophyll type, aerenchymatic tissue, mesocarp and testa thickness are the most valuable variables for distinguishing *Iberis* species. In further investigations we propose that the systematic problems of *Iberis* taxa should maybe solved by providing morphological and more molecular studies.

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(Manuscript received on 12 June, 2020; revised on 5 November, 2020)