



Stem Anatomy of Some *Crepis* L. (Asteraceae) Taxa and Its Taxonomic Significance

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ABSTRACT

In this study, the stem anatomy of nine *Crepis* (Asteraceae) taxa was described for the first time from Türkiye. The anatomical data obtained were evaluated in terms of taxonomy with analysis of variance and principal components analysis. The stem mainly consists of a layer epidermis, parenchymatous cortex, collateral vascular bundles, and parenchymatous pith in all taxa examined. Glandular or non-glandular trichomes are found in the epidermal cells of the stem in some species examined. Even though all taxa examined have similar stem anatomy, quantitative traits such as the length or width of the anatomical characters significantly vary among the taxa. In particular, the xylem thickness considerably differs among the taxa studied, according to the results of analysis of variance. Besides, the xylem is thicker in caulescent species than in scapigerous taxa. According to the results of the principal components analysis, the xylem thickness and the epidermis cell length explain most of the total variation with about 82% value among the studied taxa. These results show that xylem thickness and epidermis cell length have a high taxonomic value.

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Bazı *Crepis* L. (Asteraceae) Taksonlarının Gövde Anatomisi ve Taksonomik Önemi

ÖZET

Bu çalışmada, dokuz *Crepis* (Asteraceae) taksonunun gövde anatomisi ilk kez Türkiye'den tanımlandı. Elde edilen anatomik veriler, varyans analizi ve temel bileşenler analizi ile taksonomik yönden değerlendirildi. İncelenen tüm taksonlarda gövde, başlıca tek tabakalı epidermis, parenkimatik korteks, kollateral iletim demetleri ve parenkimatik özden oluşmaktadır. İncelenen bazı türlerde, gövdenin epidermal hücrelerinde salgı veya salgısız tüyler bulunur. İncelenen tüm taksonlar benzer gövde anatomisine sahip olmakla birlikte, anatomik karakterlerin uzunluk veya genişlik gibi kantitatif özellikleri, taksonlar arasında önemli ölçüde değişir. Özellikle, ksilem kalınlığı taksonlar arasında oldukça farklıdır. Bununla birlikte, ksilem, gövdeli türlerde, skaplı taksonlara göre daha kalındır. Temel bileşenler analiz sonuçlarına göre, ksilem kalınlığı ve epidermis hücresi uzunluğu yaklaşık %82 değeri ile çalışılan taksonlar arasındaki varyasyonun çoğunu açıklar. Bu sonuçlar, ksilem kalınlığı ve epidermis hücresi uzunluğunun yüksek bir taksonomik değere sahip olduğunu gösterir.

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INTRODUCTION

Crepis L. with above 200 species belonging to the tribe Cichorieae is one of the taxonomically difficult genera of the family Asteraceae (Bremer, 1994; Enke, 2009). Its species are widely distributed in the Northern Hemisphere and Africa (Babcock, 1947a, b). Within *Crepis*, delimitation of the species is difficult due to the

lack of discriminating characters. High morphological plasticity in the *Crepis* species is also common, which has resulted in a profusion of published names (Iamonico & Iberite, 2023).

According to the classic works of Babcock (Babcock, 1947a, b), *Crepis* is a monophyletic genus. However, recent molecular studies indicate that the genus

Crepis is polyphyletic with split into three statistically well-supported clades (Enke & Gemeinholzer, 2008; Enke, 2009).

The taxonomic significance of stem anatomy has been confirmed in some groups of Asteraceae, such as *Inula* L. and *Tripleurospermum* Sch.Bip. (Karanović et al., 2022; Ozcan & Inceer, 2022). Anatomical characteristics of the stem have also taxonomic value for some members of Cichorieae (Carlquist, 1967; Metcalfe & Chalk, 1979).

The systematic significance of anatomical knowledge for *Crepis* was noted by Inceer et al. (2018) based on achene and leaf characters. However, anatomical data on the stem anatomy of *Crepis* are very scarce. According to current taxonomic literature (Yıldırım, 2021), 41 *Crepis* taxa are found in Türkiye, where its

endemism rate is about 22%. This study aims to contribute to the anatomical knowledge of *Crepis* with the evaluation of stem anatomical characters of nine *Crepis* taxa from Türkiye using univariate and multivariate analyses.

MATERIALS and METHODS

Plant Materials

The samples of the *Crepis* taxa were collected from natural populations in Türkiye (Table 1), and their stems were fixed in FAA (5 parts formalin: 5 parts acetic acid: 90 parts 70% ethyl alcohol) (Ozcan & Inceer, 2022).

Table 1. Collection data of the *Crepis* taxa examined

Çizelge İncelenen Crepis taksonlarının koleksiyon verileri

Taxon	Locality	Voucher**
<i>C. alpestris</i> (Jacq.) Tausch	Bursa: Uludağ, 2,230 m, 30.7.2013	Inceer 1046
<i>C. amanica</i> Babcock*	Adana: Misis Nur Mountain, 205 m, 18.5. 2013	Inceer 989
<i>C. armena</i> DC.*	Kahramanmaraş: Işık Mountain, 2,550 m, 2.7. 2013	Inceer 1031a
<i>C. aspera</i> L.	Hatay: Near Saint Pierre Church, 160 m, 20.5. 2013	Insert 994
<i>C. aurea</i> (L.) Cass. subsp. <i>olympica</i> (K. Koch.) Lamond*	Bursa: Uludağ, 2,035 m, 29.7. 2013	Insert 1043
<i>C. bithynica</i> Boiss.	Bursa: Uludağ, 2,210 m, 30.7. 2013	Inceer 1045
<i>C. dioritica</i> Schott & Kotschy ex Boiss.*	Niğde: Bolkar Mountains, between Kızıltepe and Karagöl, 2,600 m, 04.7.2013	Insert 1034
<i>C. dioscoridis</i> L.	Muğla: Datça, 80 m, 4.5. 2013	Inceer 984
<i>C. smyrnaea</i> DC. ex Froehlich	Ankara: Mahiye Tepe, 2,043 m, 30.6. 2013	Inceer 1012

*endemic to Türkiye, **vouchers are deposited in the KTUB herbarium

Stem Anatomy

For stem anatomy, transverse sections from the middle parts of the stems fixed in FAA were taken by hand using commercial razor blades (Inceer et al., 2016). The transverse sections were stained with safranin and then mounted in Entellan (Inceer et al., 2016). Five well-permanent slides belonging to five individuals for each taxa were performed (Inceer & Ozcan, 2021). The anatomical structures were examined under the Leica DM 4000B microscope.

Data Analysis

The data obtained from the anatomical characters (epidermis cell length, epidermis cell width, cortex thickness, row number of collenchyma, phloem thickness, xylem thickness, vascular bundle width) were evaluated with analysis of variance (one-way ANOVA, Duncan's multiple-range test), and principal components analysis (PCA). The PCA and ANOVA were performed with using Statistica version 12 and SPSS version 17, respectively.

RESULTS and DISCUSSION

Stem Anatomy

The stem anatomy of nine *Crepis* taxa is presented from Türkiye in detail for the first time. Systematic aspects for stem anatomy in the studied taxa were provided. The present results show that all taxa studied have similar anatomical structures (Figure 1), but there are significant differences in the dimensions of the anatomical characters among the taxa (Table 2). In particular, the results obtained from ANOVA show that there are considerable differences in the xylem thickness among the taxa studied. Besides, epidermis cell width has a less significant value than other anatomical characters among the taxa.

The stems in the transverse sections were generally more or less rounded in shape. The epidermis covered by a thin cuticle layer is single-layered. The length of the epidermal cells ranges from 12.61±0.93 µm in *C. bithynica* to 16.06±1.26 µm in *C. dioscoridis* (Table 2). The width of epidermal cells varies from 13.83±1.86 µm in *C. alpestris* to 20.54±5.46 µm in *C. aspera*. The cortex is composed of collenchyma, parenchymatous cells as well as endodermis. Its thickness ranges from 83.77±9.86 µm in *C. dioritica* to 147.82±7.76 µm in *C.*

aspera. Within the taxa examined, vascular bundles are collateral, phloem and xylem are clear. Cambium is not visible between the phloem and the xylem. The phloem thickness ranges from 44.94±7.95 µm in *C. dioscoridis* and 121.59±11.50 µm in *C. aspera*. The xylem thickness and vascular bundle wideness vary from 95.16±5.32 µm and 94.55±0.61 in *C. dioritica* to 400.57±4.03 µm and 214.11±1.44 in *C. aspera*, respectively (Table 2). The path is composed of large and round parenchymatic cells (Figure 1). Similar findings are reported from other species of *Crepis* (Metcalf & Chalk, 1979; Crivellaro & Schweingruber, 2015; Inceer et al., 2016).

Within the studied taxa, *C. alpestris*, *C. aurea* subsp. *olympica* and *C. bithynica* are found in the same ecological environment in the alpine region of Uludağ in Bursa. According to the results of ANOVA, there is no significant difference in all anatomical characters between *C. aurea* subsp. *olympica* and *C. bithynica*. On the other hand, significant differences are found in the

cortex thickness, the phloem thickness, and the vascular bundle width between *C. alpestris* and the other two taxa.

Some *Crepis* taxa are characterized by having scapiform, that is one-headed stems bearing few or no leaves (Babcock, 1947a, b; Lamond, 1975). The results obtained from stem anatomy indicate there is a significant difference in the xylem thickness between caulescent species and saliferous taxa. As seen in Table 2, except for *C. armena*, the xylem is significantly more thinner in scapigerous taxa, namely *C. alpestris*, and *C. aurea* subsp. *olympica*, *C. bithynica*, and *C. dioritica*, then caulescent species, namely *C. amanica*, *C. aspera*, *C. dioscoridis*, and *C. smyrnaea*.

The present results show that well-lignified sclerenchymatous cells are found between vascular bundles in *C. amanica* and *C. aspera* (Figure 1). A similar finding is reported from the endemic species *Crepis macropus* in Türkiye (Inceer et al., 2016).

Table 2. Differences in anatomical characters (mean value ±SD) of the stem in the *Crepis* taxa studied. Among the taxa in the same column, the mean values with different letters are significant at $p = 0.05$

Çizelge 2 Çalışılan *Crepis* taksonlarında gövdenin anatomik karakterlerindeki (ortalama değer±standart sapma) farklılıklar.

Taxa	Epidermis cell length (µm)	Epidermis cell width(µm)	Cortex thickness (µm)	Cr	Phloem thickness(µm)	Xylem thickness (µm)	Vascular bundle width(µm)
<i>C. alpestris</i>	12.81±1.06ab	13.83±1.86a	136.64±19.17ef	3-4	76.25±6.34cd	97.60±27.48a	131.76±26.42c
<i>C. amanica</i>	14.03±1.22abc	15.66±2.14a	111.02±14.80cd	3-6	63.64±10.07bc	219.40±6.69c	158.80±8.42d
<i>C. arena</i>	14.64±1.06bcd	16.06±0.93a	105.53±7.64bc	2-3	90.48±3.13e	144.16±10.32b	130.13±4.97c
<i>C. aspera</i>	15.66±0.29cd	20.54±5.46b	147.82±7.76f	2-5	121.59±11.50f	400.57±4.03d	214.11±1.44e
<i>C. aurea</i> subsp. <i>olympica</i>	13.62±1.54ab	14.03±1.61a	89.67±9.82ab	2-3	59.58±3.01b	95.57±7.07a	99.43±1.61ab
<i>C. bithynica</i>	12.61±0.93a	14.23±1.76a	89.06±6.01ab	2-3	54.70±7.35ab	103.50±14.49a	98.21±8.21ab
<i>C. dioritica</i>	13.83±1.27abc	14.84±0.93a	83.77±9.86a	1-2	51.04±4.97ab	95.16±5.32a	94.55±0.61a
<i>C. dioscoridis</i>	16.06±1.26d	20.13±2.36b	127.49±18.02de	2-4	44.94±7.95a	130.95±9.61b	117.73±9.60bc
<i>C. smyrnaea</i>	13.83±0.35abc	15.86±0.61a	111.43±14.64cd	2-3	77.88±12.11de	197.44±6.26c	195.40±13.16e
	$F_{8,18} = 3.782$ $p < 0.01$	$F_{8,18} = 3.963$ $p < 0.01$	$F_{8,18} = 11.843$ $p < 0.001$		$F_{8,18} = 27.843$ $p < 0.001$	$F_{8,18} = 148.384$ $p < 0.001$	$F_{8,18} = 45.935$ $p < 0.001$

Aynı sütundaki taksonlar arasındaki farklı harflere sahip ortalama değerler, $p = 0.05$ düzeyinde önemlidir
Cr: row number of collenchyma

Endemic species *Crepis dioritica* and *C. macropus* were grouped within clade V in the molecular phylogenetic context of the genus *Crepis* (Enke & Gemeinholzer, 2008; Enke, 2009). The present results show that *C. dioritica* has less lignified sclerenchymatous cells than *C. macropus* between vascular bundles. Besides, *C. dioritica* has more thin xylem thickness (95.16±5.32 µm) than *C. macropus* (194.18±15.29 µm, Inceer et al., 2016). On the other hand, the cambium is not visible in *C. dioritica*, while the cambium is visible in *C. macropus* (Inceer et al., 2016). These anatomical traits can be used as additional data to support the morphological separation of *C. dioritica* from its relative *C. macropus*. Glandular or non-glandular trichomes can be found in epidermal cells of the stem in some members of *Crepis* (Krak & Mraz, 2008). Within the studied taxa, the epidermis contains glandular (uniseriate with unicellular head) and non-glandular trichomes

(uniseriate filiform trichomes with elongated apical cells) (Figure 2), except for *C. aurea* subsp. *olympica* and *C. dioscoridis* have stem surfaces without hairs, which is in line with the results of Lamond (1975). Within the studied taxa, the epidermis of *C. armena*, *C. bithynica*, and *C. dioritica* have glandular trichomes. Likewise, glandular trichomes on the stems of *C. dioritica* were previously reported by Lamond (1975).

Within all *Crepis* taxa examined, the collenchyma is under the epidermis. In addition, the cells of this tissue cross vascular bundles. Similar results are reported for the species of *Scorzonera* L. (Makbul et al., 2011). On the other hand, the number of its rows differs among the taxa (Table 2). As seen in Table 2, the highest row number in the collenchyma is found in *C. amanica*, whereas the lowest row number in the collenchyma is present in *C. dioritica*. *Crepis amanica* is an annual species 38-60 cm long and its stem is erect as well as

rather stout (Babcock, 1947b; Lamond, 1975). Hence, the collenchyma as supporting tissue may play an

important role in tensile stress in this species.

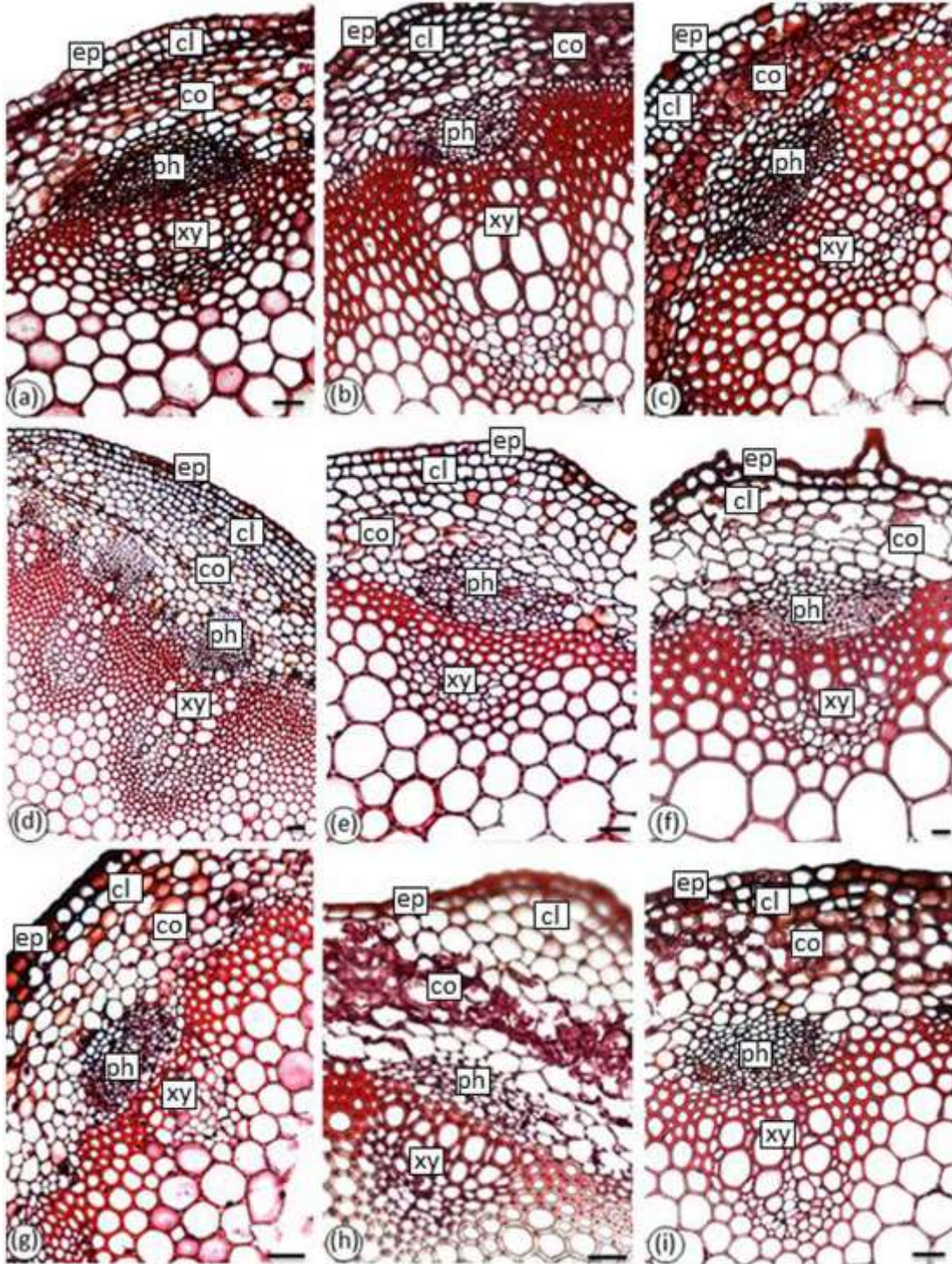


Figure 1. Transverse sections of the stems in the *Crepis* taxa examined; a: *C. alpestris*, b: *C. amanica*, c: *C. armena*, d: *C. aspera*, e: *C. aurea* subsp. *olympica*, f: *C. bithynica*, g: *C. dioritica*, h: *C. dioscoridis*, I: *C. smyrnaea*. ep: epidermis, cl: collenchyma, co: cortex, ph: phloem, xy: xylem. Scale bars: 30 μ m (a, b, c, e, g, h, i), 50 μ m (d, f)

Şekil 1. İncelenen *Crepis* taksonlarında gövdelerin enine kesitleri; a: *C. alpestris*, b: *C. amanica*, c: *C. armena*, d: *C. aspera*, e: *C. aurea* subsp. *olympica*, f: *C. bithynica*, g: *C. dioritica*, h: *C. dioscoridis*, I: *C. smyrnaea*. ep: epidermis, cl: kollenkima, co: korteks, ph: floem, xy: ksilem. Ölçekler: 30 μ m (a, b, c, e, g, h, i), 50 μ m (d, f)

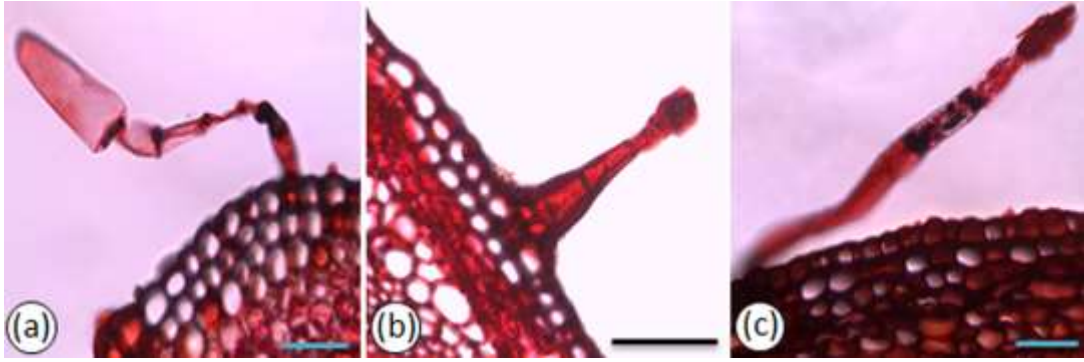


Figure 2. Trichomes in transverse section of the stem in *Crepis*: a: *C. armena*, b: *C. dioritica*, c: *C. smyrnaea*. Scale bars: 50 µm

Şekil 2. *Crepis*'de gövde enine kesitinde tüyler; a: *C. armena*, b: *C. dioritica*, c: *C. smyrnaea*. Ölçekler: 50 µm

Principal Components Analysis

The results obtained from PCA analysis indicate that PC1 and PC2 are composed of four groups based on anatomical characteristics of the stem (Figure 3). According to PCA data analysis, the first two PC factors accounted for about 82% of the total variance (Figures 3 and 4). As seen in Figure 4, the first factor accounts for about 66% of the total variance, with the mean thickness of the xylem having the highest negative correlation ($r = -0.92$). The second factor accounts for 16% of the total variance, with epidermis cell length showing a negative correlation ($r = -0.62$). These findings indicate that xylem thickness and epidermis cell length explain most of the total variation among the studied taxa.

xylem thickness and epidermis cell length have a high taxonomic value at an interspecific level to infer anatomical variations among the studied taxa. In particular, the xylem is thicker in caulescent species (*C. amanica*, *C. aspera*, *C. dioscoridis*, and *C. smyrnaea*) than scapigerous taxa (*C. alpestris*, *C. aurea* subsp. *olympica*, *C. bithynica* and *C. dioritica*). The caulescent species *C. amanica* and *C. aspera* have also well-lignified sclerenchymatous cells between vascular bundles in their stems. On the other hand, the collenchyma may play an important role in tensile stress in *C. amanica*.

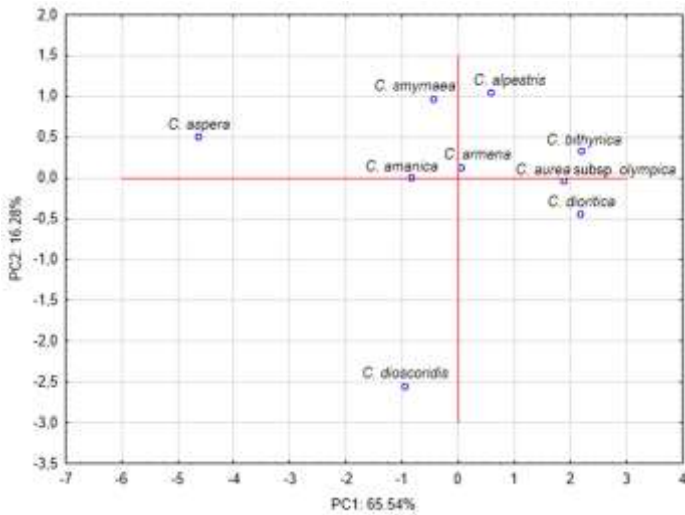


Figure 3. Results from the PCA of the *Crepis* taxa based on stem anatomy

Şekil 3. *Crepis* taksonlarının gövde anatomisine dayalı temel bileşenler analiz sonuçları

CONCLUSION

This is the preliminary study on the taxonomic evaluation of anatomical characters of the stem using analysis of variance and principal components analysis. According to the results of these analyses, the

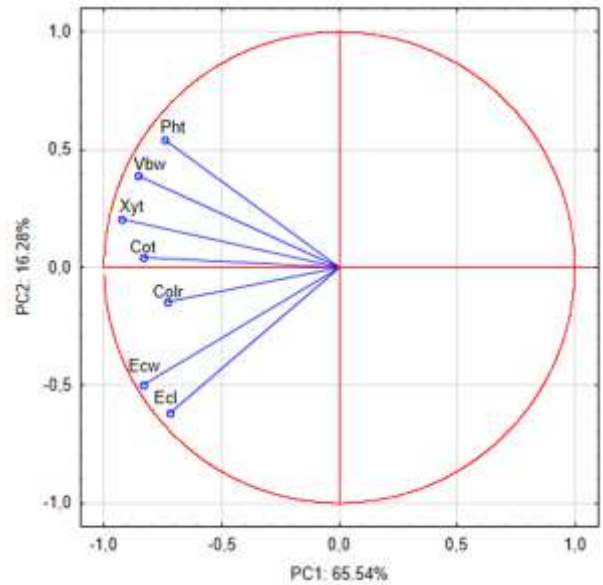


Figure 4. Results from the PCA of stem anatomy in *Crepis*: ct: cortex thickness, cr: row number of collenchyma, ecl: epidermis cell length, ecw: epidermis cell width, pht: phloem thickness, xyt: xylem thickness, vb: vascular bundle width

Şekil 4. *Crepis*'de gövde anatomisinin temel bileşenler analiz sonuçları; ct: korteks kalınlığı, cr: kollenkima sıra sayısı, ecl: epidermis hücre uzunluğu, ecw: epidermis hücre genişliği, pht: floem kalınlığı, xyt: ksilem kalınlığı, vbw: iletim demet genişliği

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Researchers Contribution Rate Declaration Summary

Huseyin Inceer: Conceptualization, Data curation, Visualization, Formal analysis, Investigation, Methodology, Software, Funding acquisition, Writing-original draft, Writing-review & editing. Ozge Ozgurluk: Methodology, Data curation.

Conflicts of Interest Statement

The article's authors declare that they do not have any conflict of interest.

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