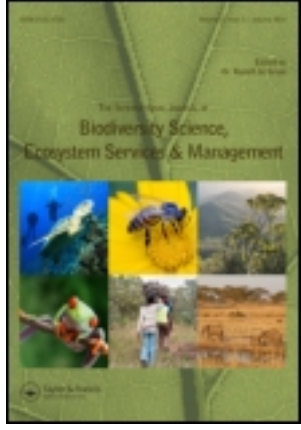


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# ***Centaurea* species in Turkey (B): Comparative studies of two closely related species, *C. kurdica* Reichardt and *C. sclerolepis* Boiss**

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Key words: *Centaurea*, *C. kurdica* Reichardt, *C. sclerolepis* Boiss., Achene, anatomy, ecology, morphology and palinology

## **SUMMARY**

Morphological, anatomical, palynological, achene structure, and ecological features of two endemic *Centaurea* L. species, *C. kurdica* Reichardt and *C. sclerolepis* Boiss., were studied in detail. Pollen studies showed that the pollen grains of the species could be described as prolate-spheroidal in *C. kurdica*, and prolate in *C. sclerolepis*. The SEM study of achene surfaces showed that the cell wall in *C. kurdica* is thicker than that of *C. sclerolepis*, especially on the margins. It was determined that the soil in which *C. sclerolepis* grows is richer in organic matter and Ca<sup>++</sup> and Mg<sup>++</sup> ions than that below *C. kurdica*. It was also found that the root anatomy shows significant variations between the species and can be used to differentiate them.

## **INTRODUCTION**

The genus *Centaurea* L. comprises approximately 500 to 600 species distributed all around the world, particularly in Asia, North Africa and America (Wagenitz 1975; Davis et al. 1988). The genus is the third largest genus after *Astragalus* and *Verbascum* in Turkey, represented by 187 taxa, of which 114 are endemic (endemism ratio: 60.9%) (Wagenitz 1975; Wagenitz et al. 1988; Davis et al. 1988; Güner et al. 2000; Duran and Duman 2002; Turkoğlu et al. 2003). The high endemism ratio shows that Turkey is one of the gene centres of the genus.

*C. kurdica* Reichardt and *C. sclerolepis* Boiss. are morphologically very similar species, separated using only the gross morphology of their leaves: leaves undivided and basal leaves oblong-ovate with more or less cordate base in *C. kurdica*, and basal and lower leaves lyrate with very large lanceolate to broadly ovate in *C. sclerolepis*.

The main objectives of this study are to carry out a multidisciplinary study to evaluate whether morphology, anatomy, palynology, achene structure and ecology of the species support their present classification.

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## MATERIAL AND METHODS

Plant specimens were collected from Elazığ, Mus, Batman and Diyarbakir (see Table 1 for voucher specimens). The plant specimens were identified according to Wagenitz (1975) and are kept in the Biology Department of Canakkale Onsekiz Mart University. The specimens were measured using a microscope and micrometer or ruler. Some features of the specimens were measured in the field. The pollen grains were obtained from dried herbarium specimens. Several unopened buds (to make sure alien pollen grains were not present) were placed in a watch glass and squashed, adding a few drops of wetting agent. The pollen grains were transferred to copper stubs, which were already prepared with double-sided adhesive tape, and the stubs were then coated with gold for 5–6 min for studying and taking pictures with a scanning electron microscope (SEM). A Jeol 100 x CXII SEM was used in this study. The terminology used is mainly that of Punt et al. (1994).

In the present study of the anatomy of root, stem and leaf, the wax embedding procedure was followed. Plant parts were first fixed by using formalin/acetic alcohol (FAA) (Cutler 1978; Menemen and Jury 2001). The samples were passed through an alcohol and histoclear series for dehydration. Paraplast was added to the histoclear and the samples placed in an oven. Cross sections of root, stem and leaf were taken with a rotary microtome to 7 µm thickness, and stained with safranin and crystal violet. Investigations were carried out under a light microscope and photographs taken with a microphotography apparatus (JENA).

Soil specimens (0–10, 10–20 and 20–30 cm deep) were collected from each site and brought into the laboratory for analysis (Table 1). The analyses were carried out according to Walkley and Black (1934), Jackson (1962), Chapman and Pratt (1961), Bouyoucos (1962) and Olsen and Sommers (1982).

## RESULTS

### Morphological characteristics

A total of 14 vegetative and floral characters were obtained from both *C. sclerolepis* and *C. kurdica* and compared (Table 2). No distinct differences are

**Table 1** Origins of the plant and soil specimens where they were collected

Taxa Population number	Locality	Date	Collector
<i>C. sclerolepis</i> Population I	Muş-Elazığ N38°.22' E 42°.46'	13.07.2002	S. Çelik
<i>C. sclerolepis</i> Population II	Diyarbakir-Batman N 38°.08' E 41°.12'	13.07.2002	S. Çelik
<i>C. kurdica</i> Population I	Bingöl-Elazığ N 38°.38' E 39°.43'	13.07.2002	S. Çelik
<i>C. kurdica</i> Population II	Urfa-Siverek N 37°.51' E 39°.35'	20.07.2002	S. Çelik

seen between *C. sclerolepis* and *C. kurdica* except for the leaves, which are undivided, oblong-ovate with more or a less cordate base in *C. kurdica* and divided, lyrate, lanceolate to ovate in *C. sclerolepis*.

### Pollen characteristics

The pollen grain in both species is tricolporate, with colpi tapering at both ends, and of spinulose ornamentation. The pollen in *C. sclerolepis* differs from *C. kurdica*, having a prolate shape and spinules 1.73 µm in length. Full pollen descriptions of the species *C. sclerolepis* and *C. kurdica* are provided below:

#### ***Centaurea sclerolepis* Boiss. (Sect: Cynaroides)**

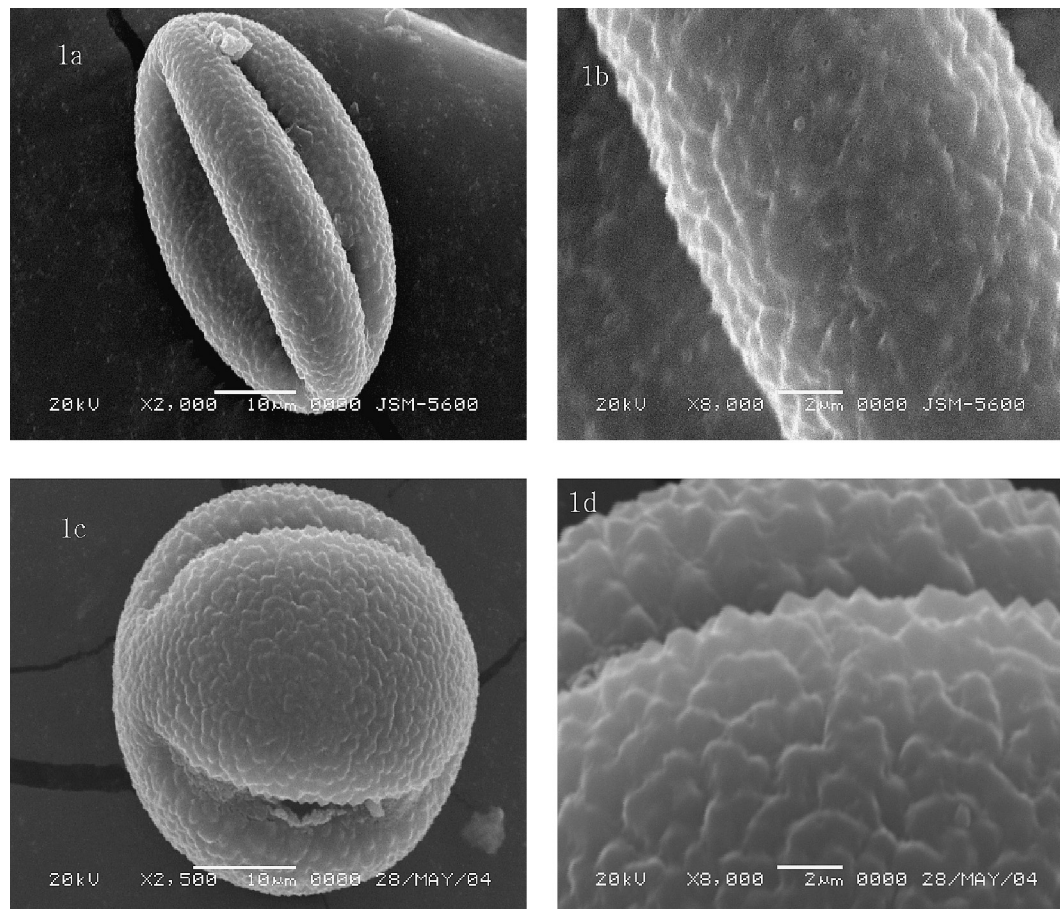
Tricolporate; polar axis (P) 46.15 µm, equatorial axis (E) 27.30 µm, P/E 1.69, prolate; colpi tapering at both ends; spinulose, spinules densely distributed, width of the spinules at base (W) 1.30 µm, height of the spinules (H) 0.50 µm, W/H 2.60, very large at base, apices sharp, with basal and subapical distinct perforations, pores irregularly distributed (Figure 1a, b).

#### ***Centaurea kurdica* Reichardt (Sect: Cynaroides)**

Tricolporate; polar axis (P) 33.82 µm, equatorial axis (E) 32.35 µm, P/E 1.05, prolate-spheroidal; colpi tapering at both ends; spinulose, spinules very densely distributed, width of the spinules at base

**Table 2** Comparison of morphological features of *C. sclerolepis* and *C. kurdica*

Morphological Characters	<i>Centaurea sclerolepis</i>	<i>Centaurea kurdica</i>
Duration ve height	biennial, 45–140 cm	biennial, 50–130 cm
Number of capitula per raceme	3–12	4–10
Leaf size (Basal)	45 × 100 mm	55 × 120 mm
Basal leaf shape	lyrat, lanceolate to ovate, divided	oblong-ovate, not divided
Terminal leaf shape	Lanceolate	wide lanceolate
Hairs	densely plumose	densely plumose
Capitulum size	40 × 47 mm	50–65 mm
Involucrum size	35 × 40–30–40 mm	30 × 40–35–45 mm
Spine length	8–14 mm	7–12 mm
Involucrum shape	orbicular to ovate	orbicular to wide ovate
Achene size	6.8–7.5 × 2.2 × 2.5 mm	6–7.3 × 1.8 × 2.1 mm
Achene shape	oblong to slightly obovate-rectangular	oblong to slightly obovate-rectangular
Pappus length	7.8–11.2 mm	7.8–11.2 mm
Flower colour	pink	pink

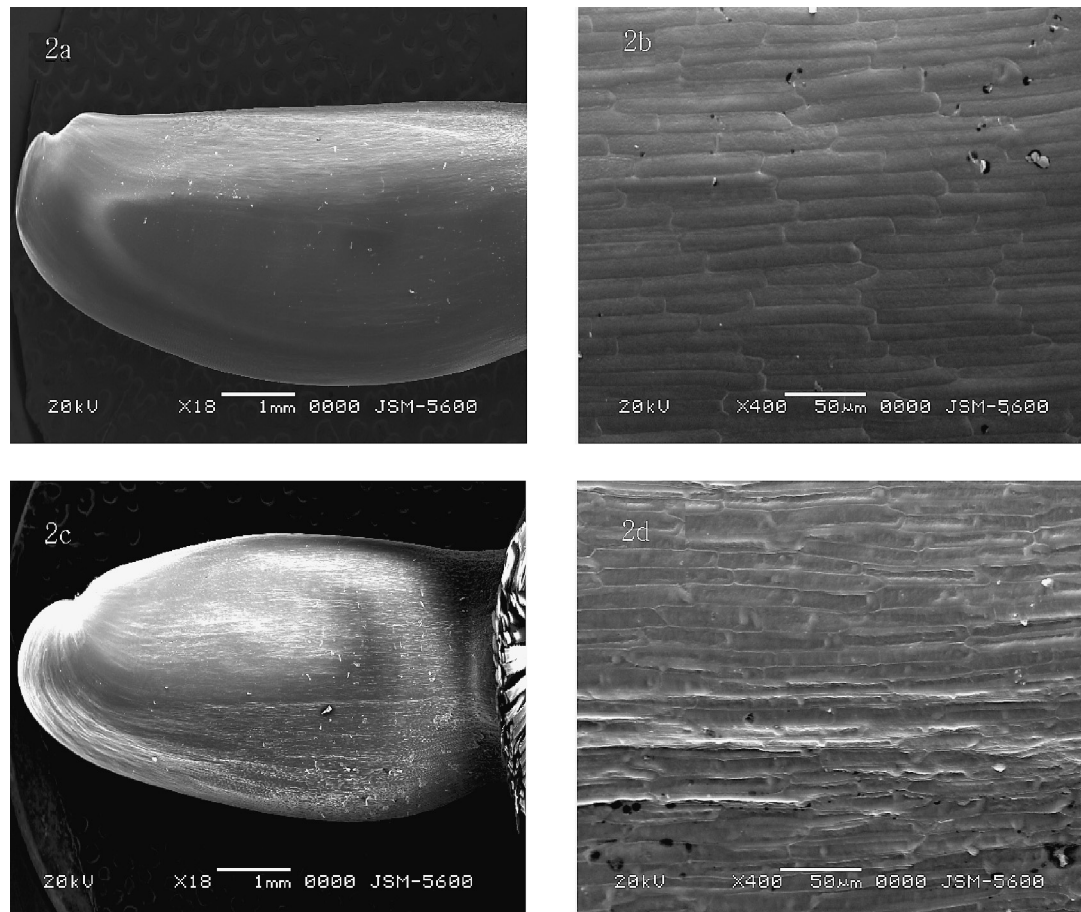
**Figure 1** SEM pictures of (a,b) *C. sclerolepis* and (c,d) *C. kurdica* pollen grains. a. and c. general view, b. and d. ornamentations

(W) 1.30  $\mu$ m, height of the spinules (H) 0.61  $\mu$ m, W/H 2.13, very large at base, apices sharp, with basal and subapical perforations, pores irregularly distributed (Figure 1c, d).

#### SEM study of achenes

The SEM study showed that all cells on the achene form a smooth surface in both species. The cell wall





**Figure 2** SEM pictures of (a,b) *C. sclerolepis* and (c,d) *C. kurdica* achenes. a. and c. general view, b. and d. achene surfaces

in *C. kurdica* is thicker than that of *C. sclerolepis*, especially on the margins. The indumentum in both species is glabrous. (Figure 2 a–d).

#### Anatomical characteristics

Cross sections of roots, stems and leaves from *C. sclerolepis* and *C. kurdica* were studied to see whether their anatomy supports the morphological delimitation.

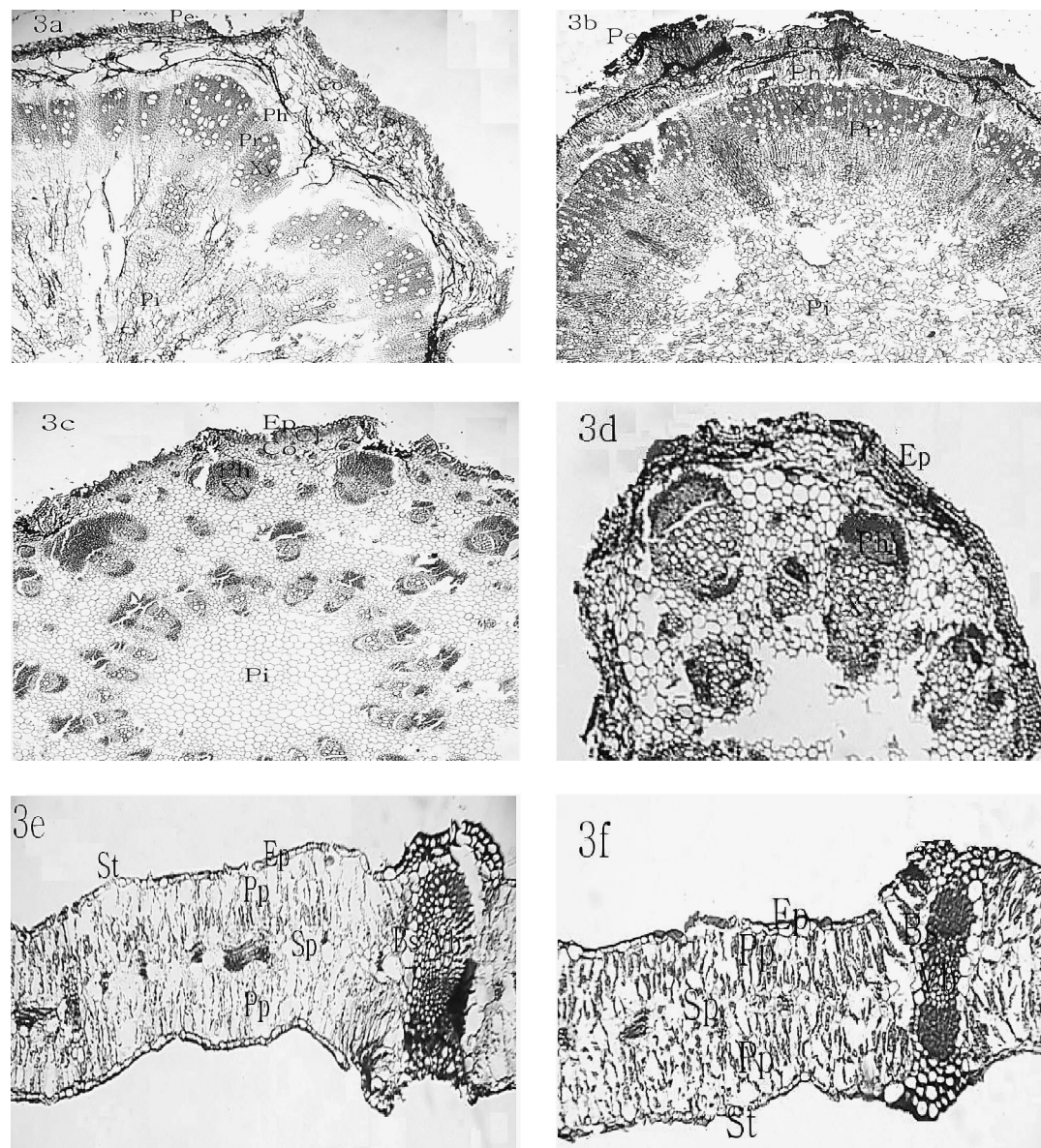
#### Root anatomy

*Centaurea sclerolepis* and *Centaurea kurdica* are perennial species. These plants have a disintegrated periderm on the outermost layer as a protective tissue. *C. sclerolepis* has cortex tissue with disordered, flattened and thin walled parenchymatic cells. The cortex tissue, with 2–3 layers of sclerenchyma cells, consists of 10–16 layers and occupies a wide arcade up to the endodermis. *C. kurdica* has cortex tissue

with small, oval-shaped and thin walled parenchymatic cells. The cortex in *C. kurdica* has 4–6 layers of sclerenchyma cells and is composed of 20 layers. In both species, the endodermis, vascular bundles and cambium are very similar. The endodermis is not easily distinguished. Phloem takes up a small part of the bundles, in contrast to the xylem which is located all around the parenchymatous pith. Pith rays lie in the cortex. Cambium is in a depressed form and has 2–3 layers (Figure 3a, b)

#### Stem anatomy

Stem anatomy is almost identical in both species, except for chlorenchymatic tissue, with 1–2 layers in *C. kurdica* and 2–3 in *C. sclerolepis*. In both species, the stem has a thick cuticle layer, followed by a single layered epidermis with sparsely single aglandular hairs and mesophytic amaryllis-type stomata. Vascular bundles are scattered in a circular manner below the parenchymatic tissue, with



**Figure 3** Cross sections of root, stem and leaf in *C. sclerolepis* and *C. kurdica*. Anatomy of root in (a) *C. sclerolepis* and (b) in *C. kurdica*; of stem (c) in *C. sclerolepis* and (d) in *C. kurdica*; of leaf (e) in *C. sclerolepis* and (f) in *C. kurdica* (Co: Cortex, Xy: Xylem, Ph: Phloem, Sc: Sclerenchyma, Ep: Epidermis, Cl: Chlorenchyma, Sc: Sclerenchyma, Pi: Pith, Pp: Palisade parenchyma, Sp: Spongy parenchyma, St: Stomata) (Magnification:  $10 \times 6.3$ )

phloem on the outer and xylem on the inner sides. The phloem, as in the roots, occupies a small area and xylem makes up most of the bundles, which are surrounded with sclerenchymatic tissue. The cambium is composed of two layers. The pith is of parenchymatous cells (Figure 3c, d)

#### Leaf anatomy

Anatomical features of the leaves of the two species are identical: an amphistomatic leaf with sparsely single aglandular hairs of equifacial type, in which

2–3 layers of palisade parenchyma are found on both sides of spongy parenchyma, occupying a small part in the middle, as in other xerophytes. A waxy cuticle lies on upper and lower sides of epidermis, with mesophytic stomata. The bundle is surrounded by a single parenchymatic cell layer; the largest bundle is in the midvein (Figure 3e, f).

#### Ecological characteristics

Although *C. kurdica* and *C. sclerolepis* are both endemic to Turkey, they are natural and common



species in the steppes of the southeast of the country. They are generally adapted to terrestrial climates and dominate over other species in the areas where they grow, especially in agricultural areas. Although the involucre are spiny, animals – especially cattle – prefer them for feeding because of their spineless basal leaves.

*C. sclerolepis* grows together with the following species: *Euphorbia virgata* Waldst. and Kit, *Anthyllis vulneraria* L. ssp. *boissieri* (Lang.) Bornm., *Coronilla varia* L. ssp. *varia*, *Lotus corniculatus* L. var. *corniculatus*, *Medicago sativa* L. ssp. *sativa*, *Melilotus officinalis* (L.) Pers., *Onobrychis altissima* Grossh., *Geranium collinum* Steph. ex Willd. ve *Hypericum armenum* Jaub. and Spach.

*Centaurea kurdica* grows with *Anchusa azurea* Mill. var. *azurea*, *Anthemis cretica* L. ssp. *albida* (Boiss.) Grierson, *Carduus adpressus* C.A. Mey., *Trifolium alpestre* L., *Trigonella fischeriana* Ser., *Vicia cracca* L. ssp. *cracca* and *Centaurea armena* Boiss.

In the course of studying ecological features of *C. sclerolepis* and *C. kurdica*, soil specimens were collected from two different areas where the populations grow naturally. Their chemical analyses were carried out in detail (Table 3). Both species grow on calcareous soils. Soil specimens under *C. sclerolepis* are rich in organic matter and nitrogen, slightly basic, normal in total  $\text{CaCO}_3\%$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{K}^+$  and poor in  $\text{P}_2\text{O}_5\%$ ,  $\text{K}^+$  and  $\text{Na}^+$ . Analyses of *C. kurdica* soil specimens showed that the soil is rich in organic matter and nitrogen, slightly basic, normal in total  $\text{CaCO}_3\%$ ,  $\text{K}^+$  and poor in  $\text{P}_2\text{O}_5\%$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and  $\text{Na}^+$ .

## DISCUSSION

*C. kurdica* and *C. sclerolepis* are morphologically very similar species, only separated using the gross morphology of their leaves. The present study showed that the leaf is divided and lyrate, and varies from large lanceolate to broadly ovate in *C. sclerolepis*. *C. kurdica* has a long pedicel, undivided and basal, oblong-ovate with more or less cordate base. Moreover, *C. sclerolepis* differs from *C. kurdica* in its longer and thinner involucre bracts.

It was found that the pollen grain features are useful in separating the species. The pollen grain in

**Table 3** Soil analyses of *C. sclerolepis* and *C. kurdica* Populations

	<i>C. sclerolepis</i>		<i>C. kurdica</i>	
	Pop. I	Pop. II	Pop. I	Pop. II
pH	7.70	7.60	6.80	6.90
$\text{CaCO}_3\%$ total	2.10	2.15	1.75	1.65
Organic Mat. %	4.90	4.10	2.85	2.75
$\text{P}_2\text{O}_5\%$	80	60	50	40
N%	1.85	1.55	1.10	1.05
$\text{Ca}^{++}$ ppm	1800	1450	1250	1300
$\text{Mg}^{++}$ ppm	1550	1200	950	1000
$\text{K}^+$ ppm	8000	6500	4000	4500
$\text{Na}^+$ ppm	35	30	15	18

both species is tricolporate, with colpi tapering at both ends and of spinulose ornamentation. The main differences between *C. sclerolepis* and *C. kurdica* are the pollen shapes and the lengths of spinules. The shape is prolate in *C. sclerolepis* and prolate-spheroidal in *C. kurdica*, the length of spinules is 1.73 and 0.61  $\mu\text{m}$  in *C. sclerolepis* and *C. kurdica*, respectively. The SEM study of achene surfaces showed that the cell wall in *C. kurdica* is thicker than that of *C. sclerolepis*, especially on the margins and could be a useful character in delimitation of the species.

The root anatomy of the species also shows variations which can be of taxonomic significance. Stem and leaf anatomies of the two species are almost identical, except for chlorenchymatic tissue in the leaf, with 1–2 layers in *C. kurdica* and 2–3 in *C. sclerolepis*.

Finally, both species are dominant in their distributional areas, but this is not a result of allelopathy. It was determined that the soil in which *C. sclerolepis* grows is rich in organic matter,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions, compared to the soil specimens from *C. kurdica*. Other soil and habitat features are very similar for both species.

In conclusion, it can be said that, in addition to morphology of the species, anatomical, palynological, achene structure, and ecological features support the present day classifications of *C. kurdica* and *C. sclerolepis*.

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