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Seed morphology of Cuscuta L. (Convolvulaceae) in Turkey and its systematic importance

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ABSTRACT
Seed morphologies of 14 Cuscuta taxa from Turkey were examined by light (LM) and scanning electron microscopy (SEM). Seed shapes, sizes, epidermal cell shapes, anticlinal/periclinal cell walls and surface patterns of macro and micro morphological features were determined. Seed epidermis cell shapes were generally either irregular or polygonal. The surface pattern was mostly reticulate. This research revealed that Cuscuta species in Turkey have different seed morphologies. The results obtained will aid in the taxonomic evaluation of morphologically closely related species.

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Cuscuta L.'nın (Convolvulaceae) Türkiye'deki tohum morfolojisi ve sistematik önemi

ÖZET
Türkiye'den 14 Cuscuta taksonunun tohum morfolojisi, ışık (LM) ve taramalı elektron mikroskobu (SEM) ile incelenmiştir. Makro ve mikro morfolojik özelliklere ait tohum şekilleri, boyutları, epidermal hücre şekilleri, antiklinal/periklinal hücre duvarları ve yüzey desenleri belirlendi. Tohum epidermisi hücre şekilleri genellikle ya düzensizdir ya da çokgendir. Yüzey deseni çoğunlukla retikulattır. Bu araştırma, Türkiye'deki Cuscuta türlerinin farklı tohum morfolojilerine sahip olduğunu ortaya koymuştur. Elde edilen sonuçlar, morfolojik olarak yakın ilişkili türlerin taksonomik değerlendirmesine yardımcı olacaktır.

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INTRODUCTION
Seed micromorphology presents various characteristics that demonstrate the relevance of seed shape in phylogenetic, evolutionary, and species identification. (Attar et al. 2007; Johnson et al. 2004; Rashid et al. 2021;). Most systematists believe that data on seed macrostructure and microstructure are critical for classifying angiosperm species (Khalik 2006). Taxa can be distinguished by differences in seed form, size, and testa ornamentation. (Aniszewski 2001; Karaismailoğlu 2022). Seed traits are less impacted by environmental factors, and so frequently represent genetic variations(2005 Hassan et al). The seed surface and cell shape are important differentiating characteristics at both the intraspecific and interspecific levels (Kubitzki et al. 2013). The relevance of scanning electron microscopy in investigating systemic difficulties, as well as providing highly significant information, has been demonstrated (Seggara and Mateu 2001; Bobrov et al. 2004; Hassan et al. 2005). Dodders (Cuscuta sp.) are keystone species in their natural ecosystems impacting the diversity, structure and dynamics of plant communities (Press and Phoenix 2005). There are about 200 species belonging to the genus Cuscuta L. worldwide (Stefanović and Olmstead 2004). Although 15-20 of these species cause problems in agricultural areas (Dawson et al. 1994), some narrowly distributed species need to be protected (Costea and Stefanovic 2009). Cuscuta parasitic

plants lack some vegetative organs (roots, leaves, etc.). This often creates problems in their identification (Demir et al. 2017). Therefore, seed micromorphological features can contribute to the solution of this problem.

The Cuscutaceae family has a variety of seed characters. These can play an important role in the evaluation of taxonomic decisions. (Khalik 2006; Costea et al. 2005). The studies on the seed morphology of this genus are few so far, and these studies are generally limited to a few species. Investigating the morphological and anatomical diversity of seeds will also be important for the systematics of Cuscuta due to the paucity of available morphological characters carrying a phylogenetic signal in this genus. Therefore, Cuscuta seeds with a larger taxonomic sampling are required to provide a comprehensive data source (Olszewski et al. 2020). Studies have been carried out for a taxonomic explanation of Cuscuta taxa in Turkey (Demir et al. 2020; Demir et al. 2017; Keskin et al 2017; Kaya et al. 2018; Taşar et al. 2022). This study was conducted to investigate the morphological and anatomical diversity of Cuscuta seeds, and to discuss and contribute to the importance of the genus in terms of taxonomy.

MATERIAL ve METHOD

Collection of seed samples

Seeds were collected from mature plants distributed in different localities of Turkey between 2013 and 2021 (except Cuscuta kotshyana). Plant samples taken from their natural environment were identified and only mature seeds were used for research (Table 1).

Table 1 Table 1 List of examined taxa
Çizelge 1. İncelenen taksonların listesi

Takson	Collecting site (Turkey)	Voucher No
C. hyalina Roth	Bitlis- Hizan	İ.D 2101
C.campestris	Mardin- Kızıltepe	İ.D 1755
C. babylonica var. babylonica	Malatya- Venk Hill	İ.D 1780
C. babylonica var. elegans	Van- Erek Mountain	İ.D 2103
C. approximata	Van-Hoşap	İ.D 1778
	Manisa-Spil Mountain	İ.D 1807
C. epithymum	Erzurum	İ.D 1795
C. brevistyla	Bitlis- Hizan	İ.D 1786
C. planiflora	İzmir-Bornova	İ.D 1796
C. kotshyana	Cumhuriyet University Herbarium (CUFH)	-
C. europae	Bitlis- Hizan	İ.D 1993
C. palaestina	Van- Gürpınar	İ.D 2095
C. kurdica	Hakkâri- Cilo Mountain	İ.D 1812
C. lupuliformis	Hakkâri- Zap Valley	İ.D 1767
C. monogyna	Muş- Mercimekkale	İ.D 2108

Light microscopy

The light microscope was used for the measurements of the seeds. For this purpose, 5-10 mature seeds for each taxon were measured in Leica EZ4D brand stereo microscope, and the minimum, average and maximum values of the measurements were calculated (Table 2).

Scanning electron microscopy

SEM was performed on dried seeds. The seed samples were then put directly on stapes using adhesive tape. The samples were gold-palladium coated before being studied by SEM (Model ZEISS Sigma 300). Each seed sample was assessed for diagnostic seed traits such as seed form, surface structure, and testa properties (epidermal cells, anticlinal and periclinal wall pattern) using terminology from prior studies (Bojnansky & Fargasova 2007).

RESULTS and DISCUSSION

Seed shape

Grammica and *Cuscuta* subgenus are elliptic or circiular, while *Monogynella* (*C. lupuliformis*, *C. monogyna*) is ovoid.

Seed size

Seed sizes in the examined taxa vary greatly. The species with the largest seed diameters are *C. lupuliformis* and *C. monogyna* (26-31 x 16-25 and 23-26 x 16-21 mm). Those with the smallest seed sizes; *C. planiflora* (4-6 x 4-5), *C. palaestina*, *C. approximata* (5-6 x 4-5) and *C. kurdica* (5-7 x 5-6). The rest have slightly larger seeds (6-12 x 5-12 mm).

Seed coat surface

The epidermal cell shapes and anticlinal wall boundaries possess a significant position in the systematics of taxa (Rashid et al. 2021). *C. campestris, C. kotschyana, C. kurdica, C. brevistyla, C. planiflora* and *C. approximata* (1807) have Isodiametric (4-5-6-polygonal) cell shapes, *C. monogyna, Cuscuta lupuliformis* and *C. approximata* (1178) Irregular to elongate in one direction, others have irregular polygonal epidermis cell shapes.

C. kotschyana, C. brevistyla and *C. monogyna* have channel shape, *C. approximata, C. babylonica* var. *elegans, C. europae, C. palaestina, C. lupuliformis* and *C. monogyna* species have raised-channeled others have raised anticlinal cell wall structure.

The periclinal cell walls of the majority of the species are convex or concav-shaped, *C. kotschyana* and *C. kurdica* flat, *C. babylonica* var. *elegans, C. palaestina* and *C. lupuliformis* concave to convex.

C. kotschyana, C. brevisyla and *C. monogyna* are rugose, *C. approximata* and *C. palaestina* are rugose-reticulate, *C. lupuliformis* is rugose-ruminate, others are reticulate.

This research revealed that the genus Cuscuta has a diverse seed morphology. The genus Cuscuta was divided into 3 subgenus by Yuncker (1932) and Engelmann (1859), especially according to their stylus and stigma shapes. These; *Grammica, Cuscuta* and *Monogynella*.

Although the *Cuscuta* and *Grammica* subgenus are quite different in macro morphology, they show similar seed morphology characteristics (with the exception of some species such as *C. kotschyana*). However, the seed characteristics of the *Monogynella* subgenus (*C. monogyna* and *C. lupuliformis*) are quite different from them. This difference has also been demonstrated in other previous morphological (Wright et al. 2011; Riviere et al. 2013) and DNA sequences (Costea et al. 2015) and karyological analysis studies (Taşar et al. 2022).

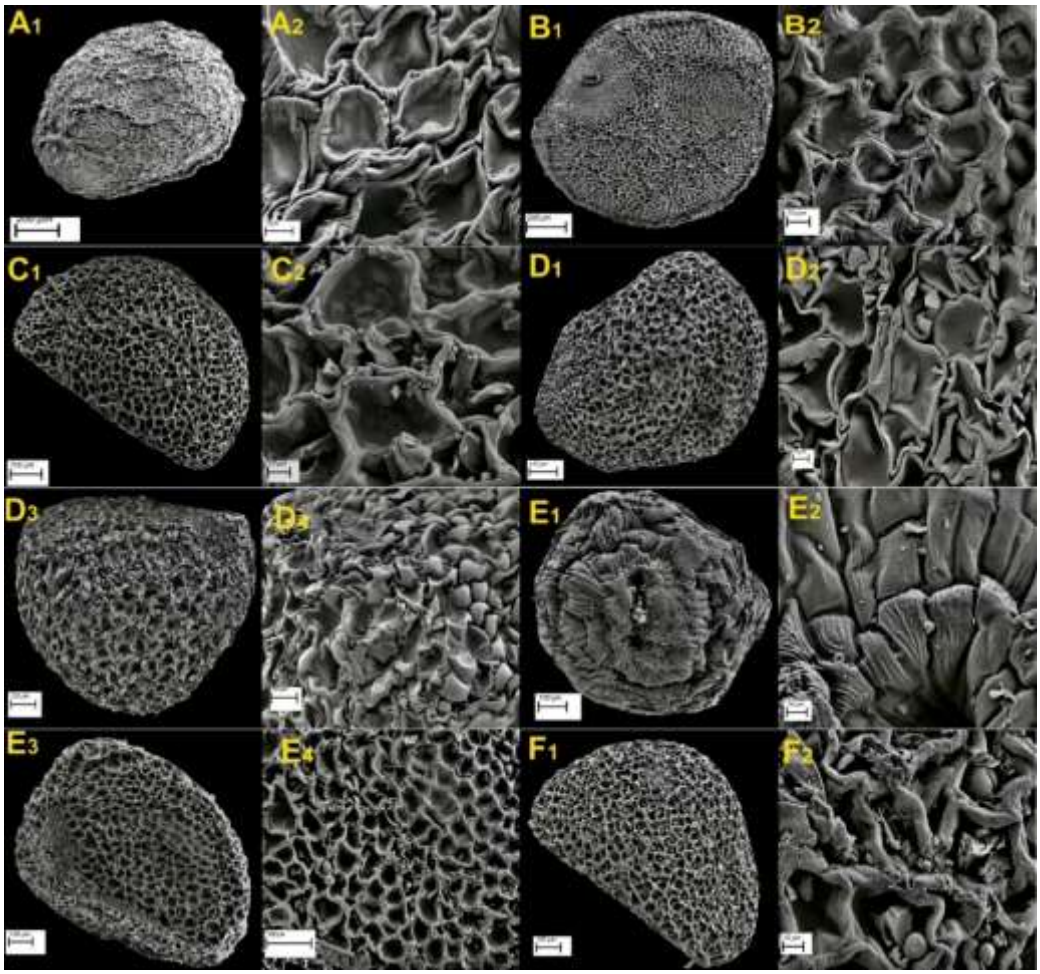

Figure1. Scanning electron microscope (SEM) micrographs of seed characters in Cuscuta. A= C. hyalina, B= C. campestris (1752); C= C. campestris (1755), D= C. babylonica (D1, D2= var. babylonica; D3, D4= var. *elegans), E= C. approximata (E1, E2= 1178; E3, E4= 1807), F= C. epithymum*

Şekil 1. Cuscuta'daki tohum karakterlerinin taramalı elektron mikroskobu (SEM) mikrografları. A= C. hyalina, B= C. campestris (1752); C= C. campestris (1755), D= C. babylonica (D1, D2= var. babylonica; D3, D4= var. elegans), E= C.yaklaşım (E1, E2= 1178; E3, E4= 1807) , F= C. epithymum

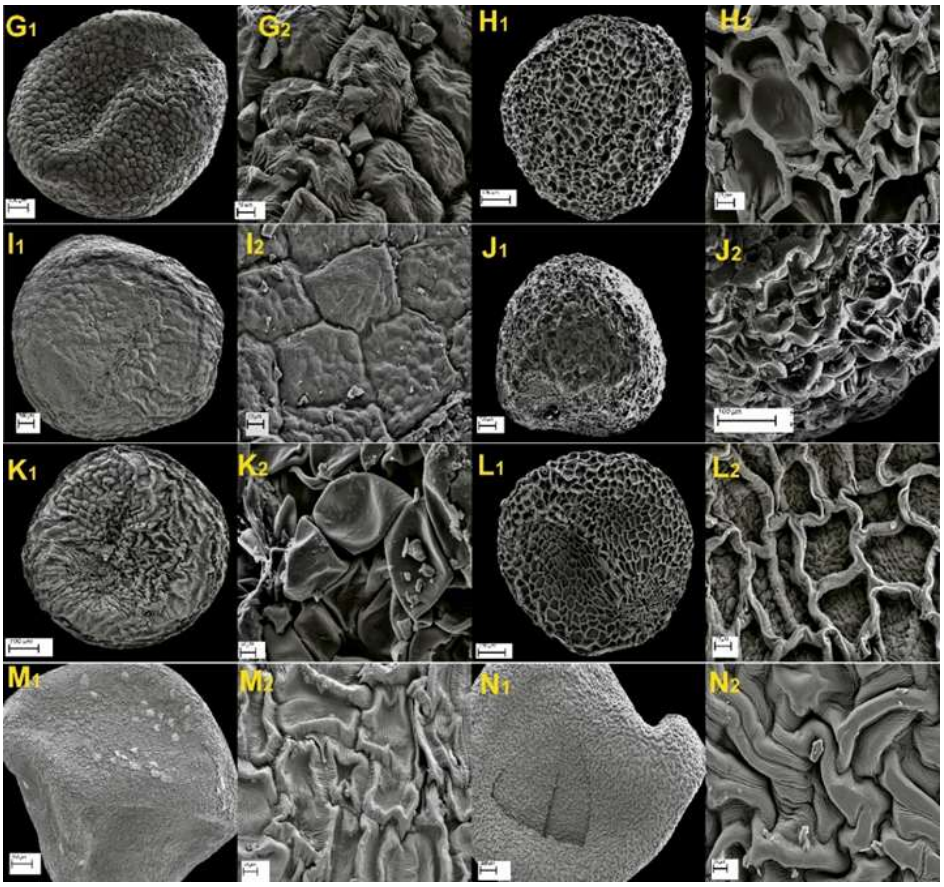

Figure1. Scanning electron microscope (SEM) micrographs of seed characters in Cuscuta. G= C. brevistyla, H= C. planiflora, I= C. kotschyana, J= C. europae, K= C. palaestina, L= C. kurdica, M= C. lupuliformis, N= C. Monogyna

Şekil 1. Cuscuta'daki tohum karakterlerinin taramalı elektron mikroskobu (SEM) mikrografları. G= C. brevistyla, H= C. planiflora, I= C. kotschyana, J= C. europae, K= C. palaestina, L= C. kurdica, M= C. lupuliformis, N= C. monogyna

C. campestris is a cosmopolitan species widely distributed throughout the world. Many species show close macro morphological similarity to this species (Yuncker 1932). Therefore, the seed morphology characteristics of this species may be an important taxonomic character that distinguishes it from other species.

Cuscuta subgenus is divided into *Epistigma, Babylonicae* and *Cuscuta* sections (Costea et al. 2015). The Sect. Epistigma is distinguished from other sections by the indistinct stylus. The seed characteristics of *C. kotshyana* found in this section also showed obvious differences compared to the others.

Sect. Babylonicae is easily distinguished from the others with its truncated calyx. The only species included in this section is *C. babylonica*. However, there are not enough macro morphological characters to distinguish the varieties of this species (var. babylonica and var. elegans) (Yuncker, 1972). In this study revealed that *C. babylonica* var. *babylonica* and *C. babylonica* var. *elegans* have different anticlinal and periclinal cell walls.

Sect. *Cuscuta* is the group with the most taxa and also the most taxonomically problematic group. Because most of the members of this section have very close flower characters to each other and it is often very difficult to distinguish them from each other.

C. kurdica, *C. palaestina* and *C. europaea* species are distinguished from the others by "flowers usually 4-merous" feature (Plitman 1978). These three species are macro morphologically very close to each other. However, in this study, it was revealed that the seed micro morphological characteristics were different (Fig 2).

Another group in the sect. Cuscuta, which are very close to each other, is *C. approximata, C. brevistyla, C. epithymum* and *C. planiflora*. *Cuscuta approximata* causes significant economic losses by causing parasitism in alfalfa. This species also has other wild plant hosts in nature. In this study, the sample taken from agricultural fields (sample no 1778) showed obvious seed morphological differences from that taken in the wild (sample no 1807) (Fig1,

Table 2). It was concluded that *Cuscuta approximata* should be re-evaluated taxonomically.

Species in the subgenus *Monogynella* have the largest seed sizes. Seeds of subg. *Monogynella*, exhibit the likely ancestral epidermis type consisting of elongated and interlocked cells, which are morphologically invariant and uninfluenced (Olszewski et al. 2020). The species in this group (*C. lupuliformis* and *C. monogyna*) have very similar flower structures, although their hosts are different. Their flowers differ from each other only by the size of the staminal bracts. However, the micromorphological features of these species vary. For example, pollen characteristics (Demir et al. 2017) differ markedly. Again in this study, there are differences between seed characteristics.

Table 2 SEM and LM results of seed morphological characteristics
Çizelge 2. Tohum morfolojik özelliklerinin SEM ve LM sonuçları

Taxon	Seed shape	Seed size (mm)	Seed epidermis cell shape	Anticlinal cell wall	Periclinal cell wall	Surface pattern
Cuscuta hyalina	E	8-10 x 5-7	Ir-pol:	R-S	Con	Rt
Cuscuta campestris	E	10-12 x 8-10	Iso,5-pol	R-S	Con	Rt
Cuscuta kotschyana	E	7-10 x 5-8	Iso,5-pol	R-S	F	Rg
Cuscuta babylonica	C	6-8 x 5-7	Ir-pol	R-S	Con	Rt
Cucuta babylonica var. *elegans*	O	7-9 x 5-7	Ir-pol	Rc-S	Con - Convx	Rt
Cuscuta europaea	C	7-9 x 6-7	Ir-pol	Rc-S	Con	Rt
Cuscuta kurdica	C	5-7 x 5-6	Iso,4-5-pol	R-S	F	Rt
Cuscuta palaestina	C	5-6 x 4-5	Ir-pol	Rc-S	Con - Convx	Rt-Rg
Cuscuta epithymum	E	7-8 x 5-6	Ir-pol	R-S	Con	Rt
Cuscuta brevistyla	C-E	8-12 x 6-8	Iso,4-5-pol	Ch-S	Convx	Rg
Cuscuta planiflora	C	4-6 x 4-5	Iso,5-6-pol	R-S	Con	Rt
Cuscuta approximata (1807)	E	5-6 x 4-5	Iso,4-5-pol	R-S	Con	Rt
Cuscuta approximata (1178)	C-O	8-9 x 5-7	Ir-el	Ch-S	Convx	Rg
Cuscuta lupuliformis	O	26-31x16-25	Ir-el	R-Ch-S	Con - Convx	Rt-Rg
Cuscuta monogyna	O	23-26x16-21	Ir-el	R-Ch-S	Convx	Rg

E: Elliptic, **C**: Circular, **O**: Ovoid,
Ir-pol: Irregular polygonal, **Iso**: Isodiametric, 5-pol, **Ir-el**: Irregular to elongate
R: Raised, **S**: straight to slightly sinuous, **R**: Raised, **Rc**: Raised-channeled, **Ch**: channeled
Con: Concave, **F**: Flat, **Conx**: Convex
Rt: Reticulate, **Rg**: Rugose

CONCLUSION

Recent studies in Turkey (pollen, molecular etc.) have contributed to the taxonomic evaluation of Cuscuta L. In this study, the seed morphologies of some taxa belonging to the genus Cuscuta collected from Turkey were revealed. It is also the first study from Turkey. There are very few studies on the seed morphology of this genus. Many species have not been evaluated in these studies. The seed morphologies of species such

as C. kurdica, C. palaestina were revealed for the first time. The results contributed to the distinguishing of species with similar characteristics in Turkey. Also, it is the most comprehensive study revealing the seed morphology of this genus.

Author's Contributions

The contribution of the authors is equal.

Statement Of Conflict Of Interest

Authors have declared no conflict of interest.

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