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Morpho-anatomical, palynological and karyological characterization of *Silene commelinifolia* var. *ruscifolia* (Caryophyllaceae)

Silene commelinifolia var. *ruscifolia* (Caryophyllaceae) türünün morfo-anatomik, palinolojik ve karyolojik karakterizasyonu

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Anahtar Kelimeler: Anatomi, Morfoloji, Polen, Karyoloji, *Silene*

ABSTRACT

A morphological, anatomical, palynological, and karyological of *Silene commelinifolia* var. *ruscifolia*, an endemic taxon for Türkiye, was examined in the present study. The anatomical approach described the root from outside to inside, by the periderm, cortex, endodermis, pericycle tissues, and vascular system. The stem is formed by cuticula layer, epidermis tissue, cortex tissue, sclerenchymatous rings and vascular system. The leaves, sepals and petals are formed by cuticula layer, epidermis tissue which is at the bottom, and the upper surface of the leaf and mesophyll tissue. The length of the root 30-130 mm and the diameter of the root 6-22 mm, type of root taproot, stem erect, and cylindric, the length of the stem is 46-156 mm and the diameter of stem 20-35 mm, intensive glandular puberulous, cauline leaves ovate-cordate, 6-34 x 1-25 mm, intensive glandular puberulous, basal leaves linear-oblongate, 6-69 x 1-9 mm, intensive glandular puberulous, the flower monocline, diclamideic, fruit 9-18 mm, denticid capsula, seeds reniform, brown, at the size of 1.62-2.17 x 1.25-1.67 x 0.75-1.125 mm. Pollen periporate, prolate-spherical (A/B=1.03), long axis of pollen (A) 31.18 µm, short axis of pollen (B) 30.14 µm, structure tektat, ornamentation microechinat-perforate, number of pores 24-34, the length of pore (Plg) 4.95 µm, the width of pore (Plt) 4.18 µm, distance between pores 5.34 µm, the width of ecsin 2.23 µm on the average. In our study, it was determined that the chromosome number of the species was 2n=24.

ÖZ

Bu çalışmada, Türkiye için endemik bir takson olan *Silene commelinifolia* var. *ruscifolia* türünün morfolojik, anatomik, palinolojik ve karyolojik özellikleri incelenmiştir. Anatomik yaklaşım kökü dıştan içe doğru, periderm, korteks, endodermis, perisikl dokular ve vasküler sistem ile tanımlamıştır. Gövde kütikula tabakası, epidermis dokusu, korteks dokusu, sklerenkimatik halkalar ve vasküler sistem tarafından oluşturulmuştur. Yapraklar, çanak ve taç yapraklar kütikula tabakası, altta bulunan epidermis dokusu ve yaprağın üst yüzeyi ile mezofil dokusundan oluşur. Kök uzunluğu 30-130 mm ve kök çapı 6-22 mm, kök tipi kazık kök, gövde dik ve silindirik, gövde uzunluğu 46-156 mm ve gövde çapı 20-35 mm, yoğun salgı tüylü, gövde yaprakları yumurtamsı-kordat, 6-34 x 1-25 mm, yoğun salgı tüylü, taban yaprakları linear-oblongat, 6-69 x 1-9 mm, yoğun salgı tüylü, çiçek monokline, diklamideik, meyve 9-18 mm, denticid kapsül, tohumlar reniform, kahverengi, 1.62-2.17 x 1.25-1.67 x 0.75-1.125 mm boyutlarında. Polenler periporat, prolate-küresel (A/B=1.03), polen uzun eksenini (A) 31.18 µm, polen kısa eksenini (B) 30.14 µm, yapı tektat, süsleme mikroekinat-perforat, por sayısı 24-34, por uzunluğu (Plg) 4.95 µm, por genişliği (Plt) 4.18 µm, porlar arası mesafe 5.34 µm, eksen genişliği ortalama 2.23 µm'dir. Çalışmamızda türün kromozom sayısının 2n=24 olduğu tespit edilmiştir.

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1. INTRODUCTION

The genus *Silene* L. is the largest genus in the Caryophyllaceae family (Yıldız, 1990). Two centers where the genus is densely found on Earth have been identified. These centers are the Balkan Peninsula and Southwest Asia. The genus is represented by 166 taxa in Europe. (Coode & Cullen, 1967; Greuter, 1995; Davis et al., 1988; Güner et al., 2012). The flora of Türkiye takes place approximately 180 *Silene* species belonging to 31 sections and the endemism rate is 48%. (Coode & Cullen, 1967; Davis et al., 1988; Greuter, 1995; Aytaç, 1998; Tan & Vural, 2000; Vural & Dönmez, 2002; Duran & Menemen, 2003; Aytaç & Duman, 2004; Deniz & Düşen, 2004; Genç et al., 2007; Yıldız & Dadandı, 2009; Kandemir et al., 2018; Yıldız & Erik, 2010; Hamzaoğlu et al., 2011; Budak & Koç, 2011; Güner et al., 2012; Hamzaoğlu, 2012; Yıldız, 2012; Aydın et al., 2014; Aytaç et al., 2015; Dadandı & Yıldız, 2015; Güner & Duman, 2016; Fırat & Yıldız, 2016a; Fırat & Yıldız, 2016b; Budak et al., 2018).

Many morphological, anatomical, palynological, and karyological studies on *Silene* species have been published over time. The more comprehensive study was by Chowdhuri (1957) revised *Silene* in world. Melzheimer (1977) systematically revised *Silene* taxa distributed in the Balkans. Wringley (1986) examined the sect. *Otites* Adanson from taxonomical, karyological, and palynological points of view. Yıldız (2006) investigated the morphology of *Silene* species from the flora of Türkiye. Further data on the genus *Silene* can be found in the studies on the whole family Caryophyllaceae, for examine; Yıldız & Minareci (2008) reported morphologic, anatomic, palynologic, and cytologic characters of *S. urvillei* Schott ex d'Urv.; Keshavarzi et al. (2014) examined anatomical characters of some *Silene* species belonging to the sect. *Lasiostemon* (Boiss.) Gürke; Bağcı & Biçer (2015) analyzed anatomical and morphological characters of the *S. cappadocica* Boissier & Heldreich and *S. spergulifolia* (Willd.) M. Bieb.; Punt and Hoen (1995) reviewed pollen morphology of Caryophyllaceae; Yıldız (2001, 2005, 2006) and Yıldız et al. (2010, 2011) studied pollen morphology of different *Silene* species in Türkiye. Also, Biosystematic Studies on genus *Silene* (Caryophyllaceae): Sectio *Brachypodeae* Boiss. and *Auriculatae* Boiss., Süleyman Demirel University, Doctoral Thesis, Isparta (Kılıç, 2007).

In this study, *Silene commelinifolia* var. *ruscifolia* Hub.-Mor. & Reese by examining its morphological, anatomical, palynological, and karyological features, to provide better recognition, contribute to the solution of taxonomic problems, to determine the population abundance of this taxon, update the current danger category and to contribute to the studies to be carried out in the field of botany.

2. MATERIALS AND METHODS

2.1. Plant materials

Plant specimens of *S. commelinifolia* var. *ruscifolia* were collected from the Kutlukaya-Boğazdere district of Sivas province during the flowering and seed-ripening months of May, June, July, and August 2012.

While collecting the samples, care was taken to ensure that each plant sample had roots, stems, leaves, flowers, and fruits. After the flowering period of the plants ended, the same areas were visited again to collect seeds. After the collected samples were measured, some of them were dried according to herbarium rules and turned into herbarium material. Herbarium specimens are kept in the Herbarium of Cumhuriyet University, Faculty of Science, Department of Biology (CUFH).

IUCN's Red List Categories were taken into consideration for conservation status (IUCN 2012). It is necessary to take live samples from threatened populations of this taxon and preserve them ex-situ in the botanical garden (Eminağaoğlu & Eminağaoğlu, 2018).

2.2. Anatomical studies

While collecting plant samples from the study area, some of the samples were placed in 70% ethyl alcohol for anatomical studies, then transverse sections and superficial sections were taken from the root, stem and leaf parts of these samples by hand and microtome in the laboratory.

The sections taken by hand were kept in a 1:1 mixture of Safranin-Alcian Blue dye for a few minutes, then placed on a slide, 1-2 drops of glycerin were added and the coverslip was closed. These preparations were examined and photographed under an Olympus BX 51 light microscope.

2.3. Pollen studies

Preparations prepared according to the Wodehouse (Wodehouse, 1935; Cabi, 2005) and Acetolysis method (Erdtman, 1960; Pınar, 1989) were used for the examination of pollen morphology and the terminology of Punt et al. were followed (Punt et al., 1994).

Before applying the Wodehouse method, glycerin gelatin with safranin, which will be used in this method, was prepared. For this purpose, gelatin plates were left in distilled water for 2-3 hours, then 1 part gelatin was mixed with 1.5 parts glycerin, safranin and 2-3% acid phenic was added to prevent mold. This mixture was heated to 80°C and poured into clean petri dishes at a certain consistency and cooled (Wodehouse, 1935).

To prepare the preparation by the Wodehouse method, pollen from the anthers of the plant samples was placed on a clean slide and 2-3 drops of 96% alcohol were added to dissolve the resin and oils. The preparation was placed on a heater to evaporate the alcohol and kept until the alcohol evaporated. A small amount of the previously prepared glycerin gelatin with safranin was placed on the pollen and heated slightly to melt it. The slide was covered with a coverslip, inverted and left to dry (Wodehouse, 1935). Since the intine and protoplasm are preserved in the preparations prepared by the Wodehouse method, it is difficult to distinguish some features of the pollen (Pınar, 1989). Therefore, in addition to this method, preparations were also prepared by the acetolysis method. In the preparations prepared by the acetolysis method, the intines and protoplasm of the pollen are removed and only the exine pollen is artificially fossilized and kept waiting. To 1 part of the softened gelatin, 1.5 parts of glycerin and 2-3% of the anti-mold acid phenic were added and heated to 80°C. The mixture was poured into a clean petri dish and allowed to solidify. The measurements of the pollen prepared by the acetolysis method were made in an Olympus CX 21 light microscope with ocular=10 x objective=100 magnification and photographed in an Olympus BX 51 light microscope. At this magnification, each range of the micrometer was calculated as 1.16 µm.

Pollen and seeds were photographed at Erciyes University Technology Research and Application Center. For this process, pollen and seeds were washed with an ether-alcohol mixture to remove contaminants on their surfaces, dried and placed on the sample holder stupa with double-sided adhesive tape. After gold plating with

a sputtering device, they were examined using a Leo 440 computer-controlled digital SEM (Scanning Electron Microscopy).

To determine the seed size, measurements were made on an Olympus CX 21 light microscope at a magnification of ocular=10x objective=4. At this magnification, each range of the micrometer was calculated as 25 µm.

The seeds in each capsule were counted, 1000 seeds with normal seed morphology were weighed on a precision balance and the value found was divided by 1000 to determine the average weight of the seeds.

2.4. Karyological studies

In order to determine the chromosome number of the plant, mature seeds were germinated under room conditions in petri dishes with filter paper. Germinated seeds were pre-treated with 0.5% colchicine for 1.5-5 hours and stored in a 3:1 ratio of ethyl alcohol - acetic acid mixture (Carnoy) in the refrigerator for at least 24 hours. The seeds were removed from Carnoy and kept in 1 N HCl for 7-10 minutes, to soften the roots. Root tips (0.5-1 cm) were taken and left to dye in 2% aceto-orcein for at least 20-40 minutes, and washed in 45% acetic acid for 2-3 minutes. A crushed preparation was made with acetic acid. The coverslip was separated with a 3:1 ratio of ethyl alcohol-glacial acetic acid mixture, and the edges were glued with entellan for a permanent preparation. They were examined with an immersion objective on an Olympus BX 51 light microscope, photographs were taken, and chromosome numbers were determined (Elçi, 1982; Yıldız & Mineraci, 2008).

3. RESULTS AND DISCUSSIONS

3.1. Morphological Features

We used *S. commelinifolia* var. *ruscifolia* Rept. Spec. Nov. Regni Veg. 52: 44 (1943)

Type: B6 Sivas; Sivas-Tecer Mountains, 27 km southeast of Sivas, steppe, 1450m, 17.05.1939, Reese & Skrivanek. (Holotype; Huber-Morath Herbarium, 13146)

A perennial bushy plant 8-15 cm high, densely grassy. A wood stem 5-10 mm, thick, up to 20 cm long, branched, covered with the remains of dead leaves, densely leafy at the apex. The stems of the flower are erect, robust, angled, thickened at the nodes, densely leaf, densely glandular hair, simple or sparsely branched, internodes

1-2 cm long. All the leaves are densely covered with sweet-hairy acorns; basal leaves cluster in a dense rosette. 1-3 nerved oblanceolate or linear-spathulate base gradually attached to the petiole, apex acute, 20-50 mm long 3-10 mm wide; cauline leaves soon larger broadly; lanceolate, ovate, or broadly ovate clustered, flowers usually numerous, arranged in sub-dichotomous, capituliform, cymes. Bracts large ovate long pointed; 10-20 mm long, 4-7 mm wide, very glandular. Bracteoles lanceolate ca. 10 mm long and 3 mm wide. pedicel 0.5-10 mm long. Calyx floriferous narrowly tubulose-clavate 18-23 mm long 3-4 mm wide, album branch aceous with 10 green veins, glandular. Calyx teeth 5-6 mm long, lanceolate-acuminate; fruiting calyx slightly inflated white petals 20-25 mm long bipartite, oblong-linear lobes glabrous auricles claws, oblong-linear crown. Capsule ovate accumulate 10-14 X 5-6 mm multi ovulate; carpophore 6-7 mm long. Seeds 1,5-2 mm serially obtusely tuberculate with flat back or subcannalulate with flat faces. (Figure 1).

Phenology: The flowering time is May and July, fruiting time is July and August.

Habitat: The habitat preference of the species is serpentine soil at 800–1800 m.

Conservation status: *S. commelinifolia* var. *ruscifolia* is an endemic species known in Türkiye, and is an Irano-Turanian element. According to the World Conservation Union (IUCN), categories are Least Concern (LC), widely distributed, and highly populated (IUCN 2012).



Figure 1. The general appearance of *S. commelinifolia* var. *ruscifolia* (Akpulat & Kutlukaya, 2012)

Distribution: *Silene commelinifolia* var. *ruscifolia* Hub.-Mor. & Reese. Besides Sivas, it is known from Ankara,

Konya, Van, Isparta and Kayseri. This species was identified from two different localities in Sivas. In the places where the species grows, the soil structure is dark green and is immediately noticeable. Populations were found between Ulaş, Kutlukaya-Boğazdere, and Divriği-Kangal. B4 Ankara: Beynam forest, Bilger 5164, Konya: Meram 950 m, Hub.-Mor. 14565, B6 Kayseri: Sapan Mounth, 15 km S of Pınarbasi, 1590-1620 m, Hub.-Mor. 10719, Sivas: Sivas to Ulaş, 1450 m, Hub.-Mor. 13146 (Coode & Cullen, 1967).

Konya, Dereköy 10 km West of Konya, 1140 m, Hub-Mor. 850. Ankara Beynam forest, Karamanoğlu 5164. Erzincan, Refahiye, serpentine rock, 1540-1560 m, Hub.-Mor. 12435. (Huber-Morath, 1967).

B4 Ankara: Beynam forest, openings of *Pinus nigra* forest, 1300- 1400m, 20.05.1989, GAZI, Duman 2606, B6 Sivas: Hafik, Günyamac locality, rocks, gypsiferous soils, 1470m, 06.07.1991, GAZI, E.H. 341, B7 Erzincan: Kolçekmez Mountain pass, steppe, 2400 m, 30.06.2003, ISTE 68721, B7 Erzincan: Kolçekmez Mountain pass, afforestation area, 1800 m, 27.05.1998, GAZI, A. Dönmez 6158, B9 Van: Van towards Çatak, 35-40 km before Çatak, steppe, 2150 m, 15.06.2002, Özçelik 9345, B9 Van: Van towards Çatak, steppe, 2000 m, 15.06.2002., Özçelik 9346b, C3 Isparta: Yalvaç, Bahtiyar village, eroded steppe slopes, 800 m, 14.05.1995, Özçelik 6985, C3 Isparta: Yalvaç, Bahtiyar village, eroded steppe slopes, 1000-1100 m, 10.07.2005, Kılıç & Özçelik 690 (Kılıç, 2007; Figure 2).

The life form is Chamaephytes

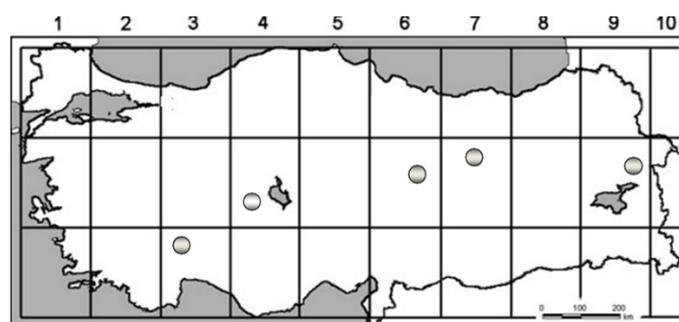


Figure 2. Distribution of *S. commelinifolia* var. *ruscifolia* in Türkiye

Other taxa coexisting with *Silene commelinifolia* var. *ruscifolia*: The main plant taxa common in the Kutlukaya-Boğazdere are: *Achillea sintenisii*, *A. sipikorensis*, *Cousinia sivasica*, *Helichrysum chionophilum*, *Scorzonera tomentosa*, *Arnebia densiflora*, *Aethionema*

caespitosum, *Erysimum repandum*, *Asyneuma rigidum* subsp. *rigidum*, *Dianthus crinitus* var. *crinitus*, *Silene commelinifolia* var. *ruscifolia*, *S. supina* subsp. *pruinosa*, *Minuartia corymbulosa* var. *corymbulosa*, *Convolvulus compactus*, *Scabiosa calocephala*, *Astragalus microcephalus*, *Ebenus laguroides* var. *laguroides*, *Hedysarum pestalozzae*, *Globularia trichosantha*, *Hypericum thymopsis*, *Phlomis oppositiflora*, *Salvia vermifolia*, *Ziziphora capitata*, *Atraphaxis grandiflora*, *Bellevalia gracilis* (Özhatay, 2006).

3.1.1. Root

In the studies, it has been determined that the root of *S. commelinifolia* var. *ruscifolia* Hub.-Mor. & Reese is

strong, woody, cylindrical, taprooted, 30-130 mm long and 6-22 mm in diameter, and the root length and diameter may vary according to the age and environmental conditions of the plant, and the measurement values related to the roots are shown in Table 1.

3.1.2. Stem

The plant has herbaceous stems, the stem is erect, cylindrical, with large distances between nodiums. It is densely glandular, puberulent. Body length is 46-156 mm, body diameter is 20-35 mm. The measurement values related to the body are given in Table 2.

Table 1. Root and stem measurement values of *S. commelinifolia* var. *ruscifolia*

Measured parameter	Number of samples	Minimum	Maximum	Average	Standard deviation
Root Length (mm)	25	30	130	82	24.9
Root Diameter (mm)	25	6	22	13	4.23
Stem Length (mm)	79	46	156	109	24.6
Stem Diameter (mm)	25	20	35	25	5.09

3.1.3. Leaf

On the plant base, there are two types of leaves: Basal leaves and cauline leaves. Basal leaves were linear-oblongate, 6-69x1-9 mm in size, simple, lamina complete, leaf margin integer, leaf tip acuminate, leaf base and connection decurrent, vascularity reticulated, dense glandular-puberulous. Stem leaves ovate-

chordate, 6-34x1-25 mm in size, simple, dense glandular-puberulous, reticulated vascular, lamina complete, leaf tip acuminate, leaf margin flat The base of the lamina surrounds the stem (amplexicaule), the leaves are opposite-arranged. The measurement values for the leaves are given in Table 2.

Table 2. Leaf Measurement values of *S. commelinifolia* var. *ruscifolia*

Measured parameter	Number of samples	Minimum	Maximum	Average	Standard deviation
Stem Leaf Width (mm)	235	1	25	8	5.54
Stem Leaf Length (mm)	207	6	34	19	6.71
Basal Leaf Width (mm)	132	1	9	3.48	1.74
Basal Leaf Length (mm)	132	6	69	32.69	13.46

3.1.4. Flower

Flowers in dichasium with 3-5 or more flowers, and sticky pedicels. The perianth is dichlamydiic: calyx and corolla are morphologically different.

Dense glandular-puberulent calyx synsepal (5), 7-31 mm long and 2-9 mm in diameter, actinomorphic

symmetrical, tubular shaped, with five pointed teeth at the tip (Figure 3d), the length of the calyx is approximately five times the length of the teeth, ten main veins are extending at the junction of the teeth, the calyx is permanent and persistent in the fructification, the middle part becomes swollen and surrounds the fruit without knuggling in the anthtophore. Calyx measurements are given in Table 3.

The corolla is also pentamerous and actinomorphic, coripetal, petals 20-30 mm long, auricula, white, with atrium, tongue and coronal scales, bifidus limbs, fragmented (Figure 3 b,c). Stamens emerge from the petals. Petal measurements are given in Table 3. The flower has an anthophore of 5-9 mm in length. The corolla, andrecheum, and guinea cheeum of the flower

emerge from the anthophore (Figure 3. f). It is wrapped in anthopher calyx.

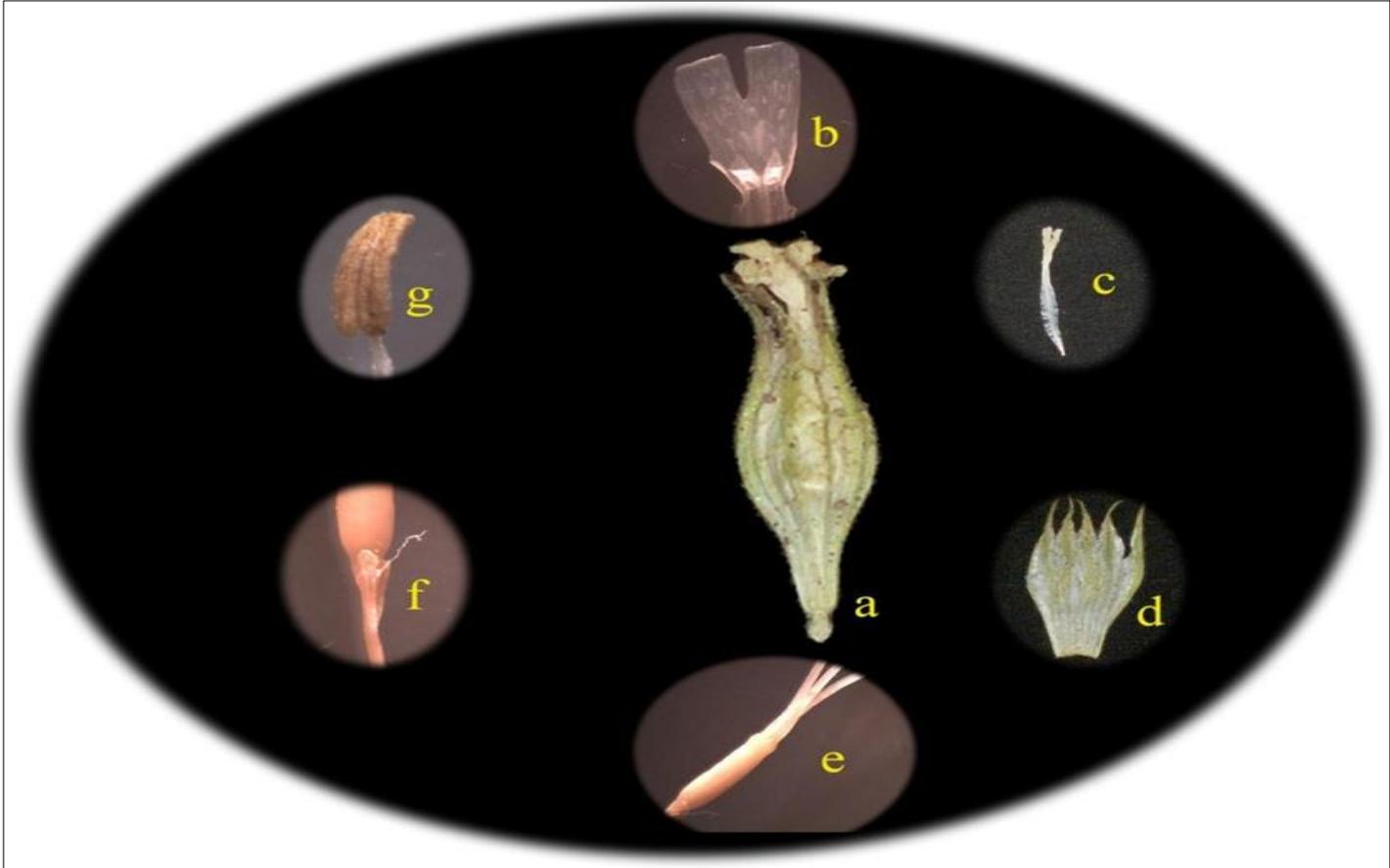


Figure 3. *S. commelinifolia* var. *ruscifolia* flower and parts a. Flower, b. Petal limb, c. Petal, d. Calyx, e. Ovarium and Style f. Anthophore, g. Stamen

In the anther, the thecae are parallel-linked, and the anther is obtus-based. The anther is opened with longitudinal slits (Figure 3. g). Stamen measurements are given in Table 3.

The flower bears 1 pistil. It is a monocline (hermaphrodite) because it carries the pistil and stamens together. The ovary is of the syncarp type with a single loculus and 3 carpels. A free central placenta is seen in

the ovary (Figure 14) Since the ovary is in the upper state, the flower is hypogynous. The stylus is in the form of a triple homostylus with a length of 1.5-4 mm. Stylus measurements are given in Table 3.

5 of the 10 stamens in the flower are free and 5 are attached to the petal. The length of the stamens of free is 2-20 mm, and the length of the attached stamens is 2-17 mm.

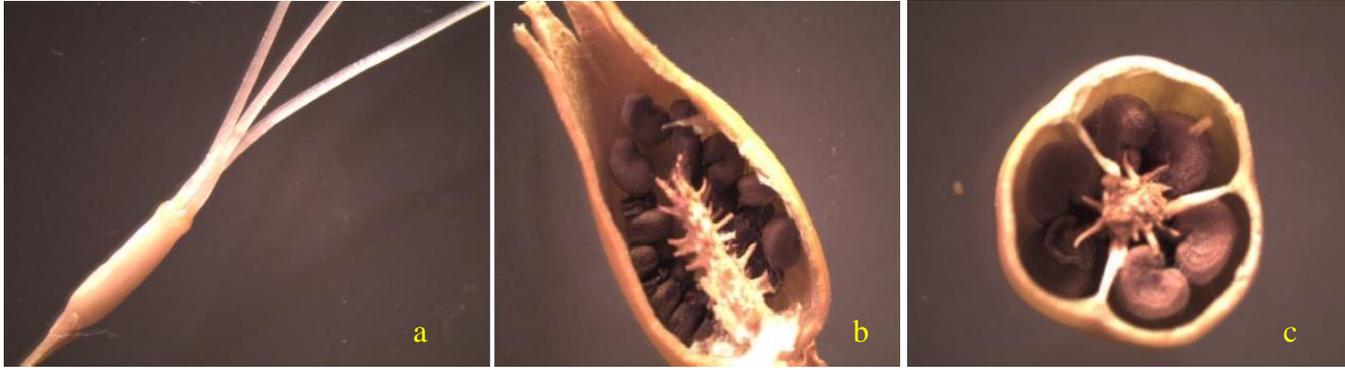


Figure 4. a. Ovary, b. style, c. placenta in *S. commelinifolia* var. *ruscifolia*

Table 3. Flower parts of *S. commelinifolia* var. *ruscifolia* measurement values

Measured parameter	Number of samples	Minimum	Maximum	Average	Standard deviation
Calyx length (mm)	123	7	31	20	4.3
Petal length (mm)	59	20	30	24	2
Anthophore length (mm)	60	5	9	7.08	1.16
Stamen length attached to petal (mm)	53	2	17	6.6	2.41
Free stamen length (mm)	58	2	20	11.3	3.83
Style length (mm)	50	1.5	4	2.6	0.67
Calyx diameter (mm)	123	2	9	4.92	1.94
Calyx tube (mm)	123	6	24	16.25	3.16
Calyx tooth (mm)	123	1	8	4.06	1.54

3.2. Anatomical features

3.2.1. Root

The anatomical structure that we examined in our study, showed secondary growth, it consists of periderm, secondary phloem, cambium, and secondary xylem sections from the outside to the inside (Figure 5).

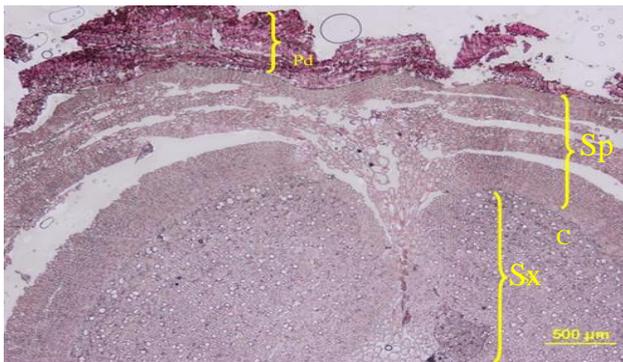


Figure 5. Anatomical structure in the cross-section of the root of *S. commelinifolia* var. *ruscifolia* (Pd: Periderm, Sp: Secondary phloem, C: Cambium, Sx: Secondary xylem)

3.2.1.1 Periderm

During secondary growth, the epidermis and hypodermis are replaced by the peridermic layer. With the development of the periderm at the woody root, the cortex undergoes abscission, and the fungal layer assumes the protective function, forming the periderm and secondary phloem shell (Figure 6).

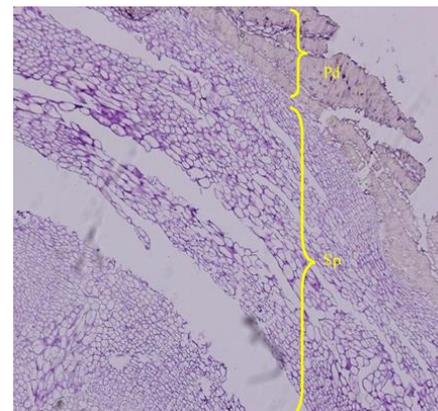


Figure 6. *S. commelinifolia* var. *ruscifolia* root cross-section, periderm (Pd: Periderm, Sp: Secondary phloem)

3.2.1.2. Secondary phloem

The vertical system consists of griddle tubes that provide longitudinal transmission of nutrients, friend cells, phloem parenchyma, fibers, storage in the horizontal system, and parenchyma cells involved in the displacement of nutrients. Rays are located in the secondary phloem layer, which contains abundant fibers (Figure 7).

3.2.1.3. Vascular cambium

It enables the formation of a large number of secondary xylems inwards and secondary phloem layers outwards, resulting in secondary growth of the root (Figure 7).

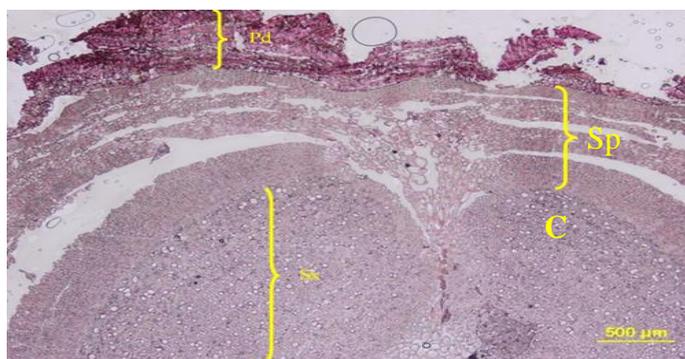


Figure 7. Subepiderm tissues in the cross-section of the root of *S. commelinifolia* var. *ruscifolia* (Pd: Periderm, Sp: Secondary phloem, C: Cambium, Sx: Secondary xylem)

3.2.1.4. Secondary xylem

It is formed by the inward division of the vascular cambium. It provides water and mineral transmission from the roots upwards with its large-diameter trache and narrow-diameter tracheid elements (Figure 8).

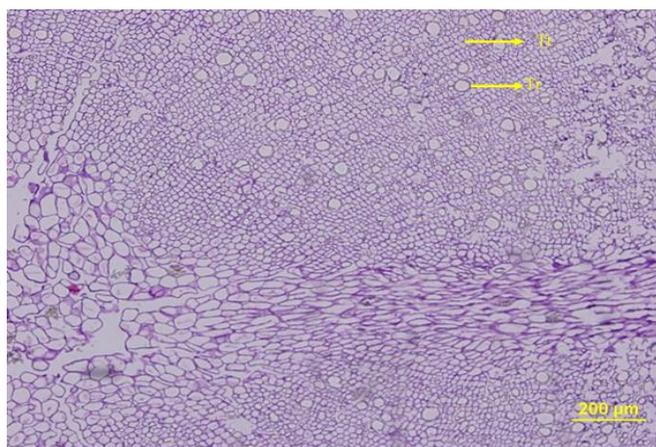


Figure 8. Secondary xylem in cross-section of the root of the *S. commelinifolia* var. *ruscifolia* (Tr: Trache, Tt: Tracheid)

3.2.2. Stem

The stem that develops from the plumula tissue of the embryo is usually the part of the plant that rises above the soil. It bears leaves and reproductive organs on it. It provides the exchange of substances between the root and the leaves (Yentür, 1995). Its anatomical structure consists of the epidermis, basic tissue, and vascular system (Figure 9).

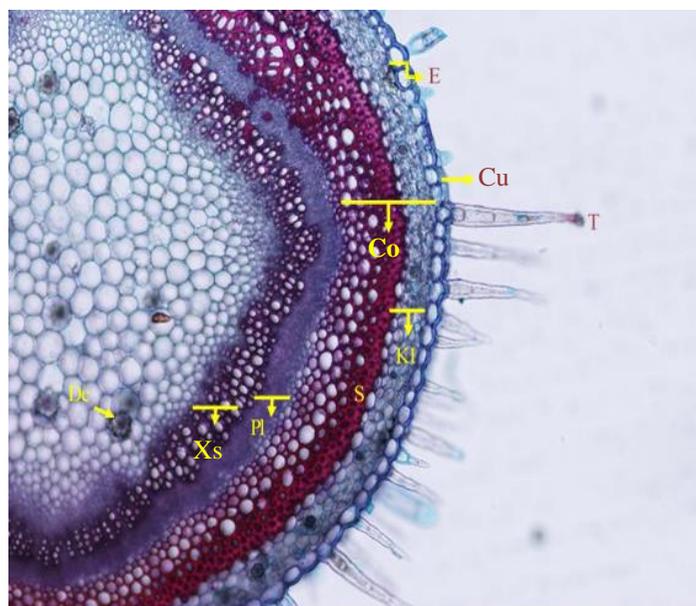


Figure 9. Anatomical structure in the cross-section of the stem of *S. commelinifolia* var. *ruscifolia* (Cu: Cuticle E: Epidermis, Kl: Klorenkima, S: Sclerenchyma, Co: Cortex, Pl: Phloem, Xs: Xylem, Dc: Druse crystal, T: Trichome, P: Pith)

3.2.2.1. Epidermis

It is the outermost tissue of the herbaceous stem. A thick layer of cuticle stands out on it and cuticle sampling is in this layer. It consists of a single layer of cells. There are no gaps between the rectangular-shaped cells. Densely populated hairs appear between its cells. The feathers show the characteristics of glandular hairs (Figure 10).

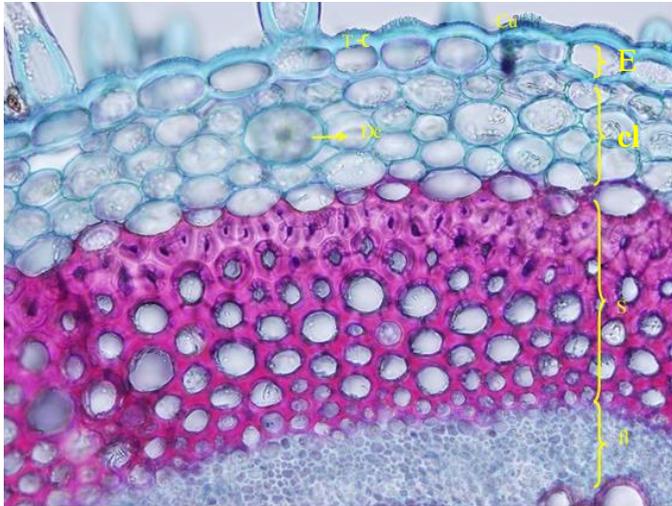


Figure 10. Epidermis and sub-epidermis in the cross-section of the body of *S. commelinifolia* var. *ruscifolia* textures (Cu: Cuticle, E: Epidermis, Kl: chlorenchyma, S: Sclerenchyma, Dc: Druse crystal, S: Sclerenchyma, fl: Phloem)

3.2.2.2 Basic tissues

It consists of the cortex, which is formed by 10-12 rows of cell layers just below the epidermis, and the core regions surrounded by the vascular system. The thin-walled parenchyma cells in the layer consisting of the first 4-5 cell rows of the cortex form the chlorenchyma layer because they have chloroplasts. Below this layer is the sclerenchyma layer, which consists of 2-3 rows of cells. The core region in the center of the trunk consists of thin-walled, loosely arranged parenchyma cells with gaps between them. Druse crystals stand out in the essence region. The stele type of the body is the ectofloic siphonostele (Figure 11).

3.2.2.3 Vascular system

The vascular system, which consists of open collateral conduction bundles, is in the form of a structure in which the xylem extends towards the essence where the cambium layer is located, and phloem tissue extends towards the cortex. In the xylem tissue, the core arms formed by several rows of parenchyma cells are seen (Figure 11).

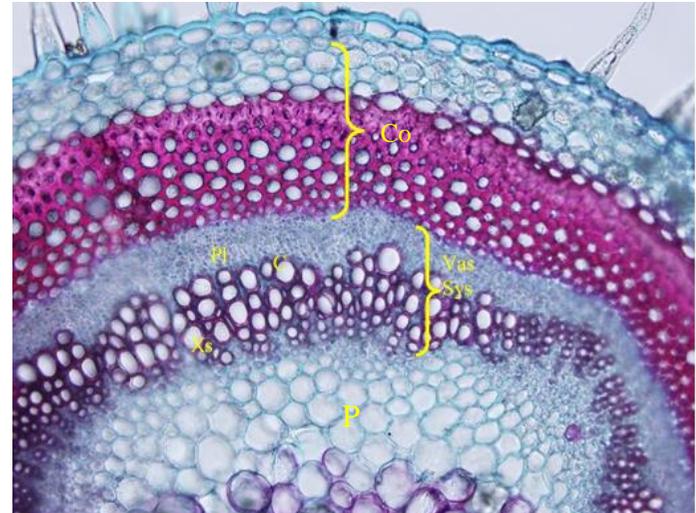


Figure 11. Basic tissue and vascular system in the cross-section of the body of *S. commelinifolia* var. *ruscifolia* (Co: Cortex, Vas sys: Vascular system, Xs: Xylem, Pl: Phloem, C: Cambium, P: Pith)

3.2.3. Leaf

The leaf cross-section clearly shows the 3 basic parts: epidermis, mesophyll tissue and vascular system. Epidermis cells appear over both sides of the leaf in a single layer. Stomata occur in the upper and lower epidermis. Hairs occur present on epiderm of both sides of the leaf. Mesophyll tissue is composed of two types of cells palisade and sponge parenchyma. Druse crystals are present in the leaf mesophyll.

3.2.3.1. Epidermis

It is located on the lower and upper surface of the leaf in the form of a single layer of cells. On it is the cuticle layer. The cells of the upper epidermis are more uniformly arranged, and the cuticle layer on them is also thicker. Epidermis cells include multicellular glandular hairs, covering hairs (Figure 12), and stomata (Figure 13). The leaf is amphistomatic and contains oval-shaped anemostic-type stomata between the epidermis cells on both the lower and upper surfaces (Figure 13).



Figure 12. Hairs in the superficial section of *S. commelinifolia* var. *ruscifolia*

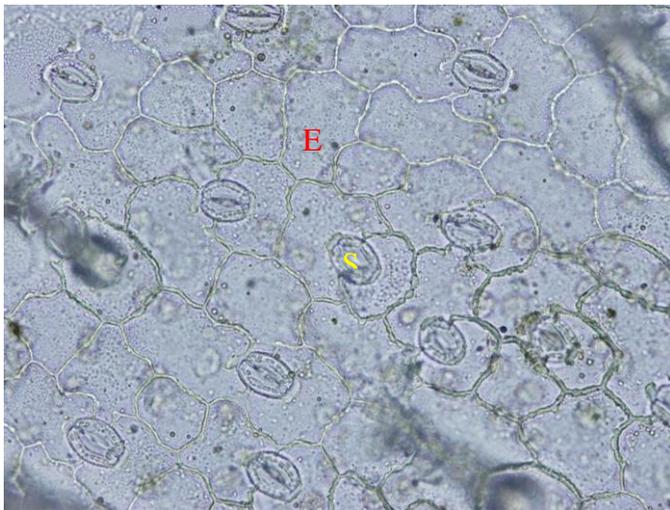


Figure 13. Stomata in the superficial section of *S. commelinifolia* var. *ruscifolia* (St: Stoma, E: Epidermis)

3.2.3.2. Basic tissue

It consists of the mesophyll, in which there are parenchyma cells with abundant chloroplasts, which remain between the two epidermis. Druse crystals are also found in the mesophyll (Figure 14).

3.2.3.3. Vascular system

It consists of collateral conduction bundles distributed in the mesophyll. Around the conduction bundles are the sheath of the bundle and the multi-row sclerenchyma (Figure 14).

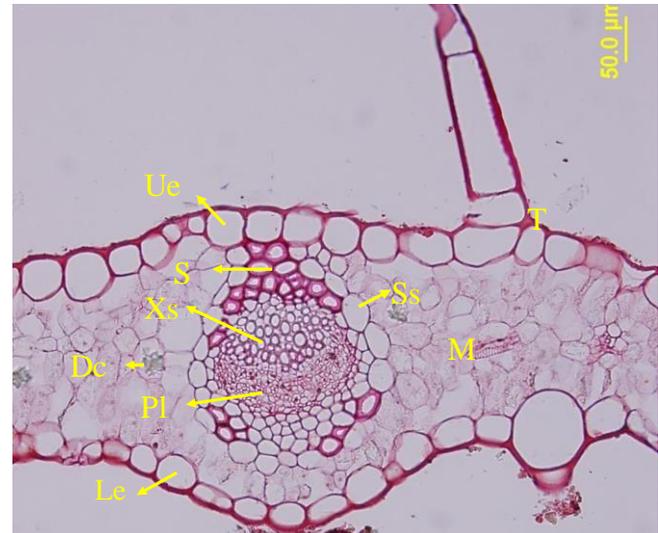


Figure 14. Cross-sectional anatomical structure in the leaf of *S. commelinifolia* var. *ruscifolia* (Cu: Cuticle, Ue: Upper epidermis, Le: Lower epidermis M: Mesophyll, Ss: Sheaf scabbard, Pl: Phloem, Xs: Xylem, S: Sclerenchyma, Dc: Druse crystal, T: Trikom)

3.2.4. Flower

In the anatomical examination of the flower, which is the reproductive organ of the plant, the green sepals that form the outer ring and the petals in the inner ring were taken. In the examinations, it is seen that the sepal and petal consist of the lower and upper epidermis bearing the cuticle on them and the mesophyll tissue between them, while the vascular system is located in the mesophyll (Figure 15) and petal (Figure 16).

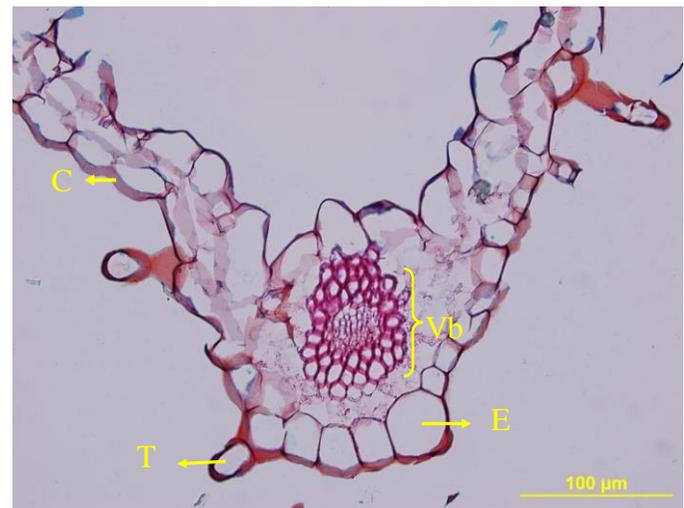


Figure 15. Anatomical structure in cross-section of *S. commelinifolia* var. *ruscifolia* (C: Cuticle, E: Epidermis, T: Trikom, M: Mesophyll, Vb: Vascular bundle)

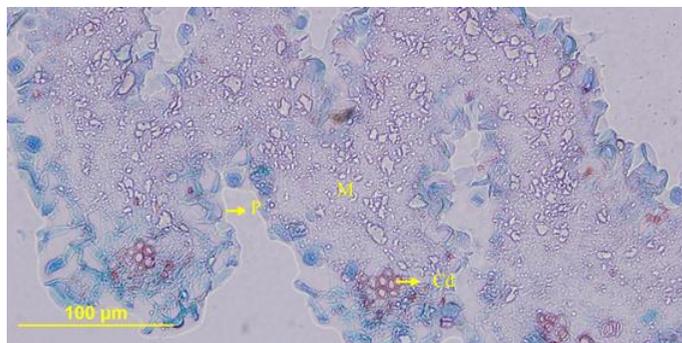


Figure 16. Anatomical structure in cross-section of the petal of *S. commelinifolia* var. *ruscifolia* a.(x10) b.(x40) (P: Papilla, Cd: Conduction bundle, M: Mesophyll)

3.2.5. Pollen

Studies have shown that pollen is of periporate type, prolate-spheroidal shape ($A/B=1.03$) (Figure 18). The long axis (A) of pollen is $31.18 \mu\text{m}$, and the short axis (B) is $30.14 \mu\text{m}$. The pollen structure is uniform, the sampling is microechinate-perforate, the echina is dense and pronounced, the perforation is irregular. The por count was 24-34, the pore length (Plg) was $4.95 \mu\text{m}$, the pore width (Plt) was $4.18 \mu\text{m}$, the interpore distance was $5.34 \mu\text{m}$, the annulus was prominent, the operculum structure was granular, the granules were large, prominent and dense (Figure 17). The mean thickness of the excise was $2.23 \mu\text{m}$, and the pollen measurement values are given in Table 4.

3.2.6. Fruit

The fruit is a dried fruit consisting of a single syncarp ovary, opened in denticid capsule type (Figure 18). The opening takes place at the junction of the teeth at the tip of the capsule. The capsule in the calyx is 9-18 mm long and oblong-ovoid in shape. The number of seeds in the capsules is highly variable and is 1-55 grains. Measurements related to the fruit are given in Table 5.

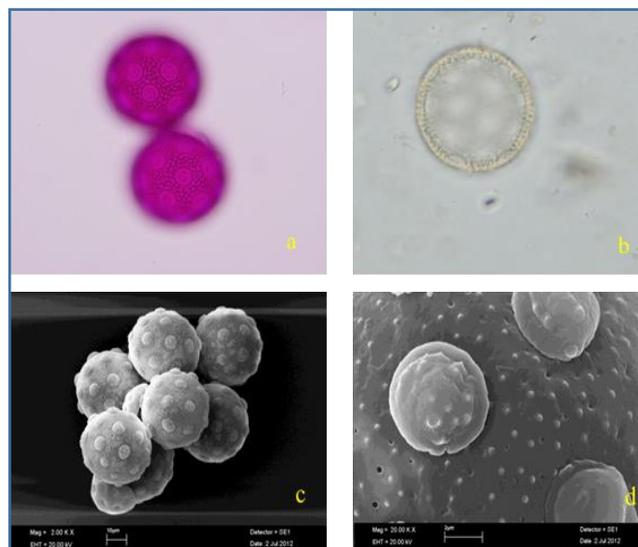


Figure 17. *S. commelinifolia* var. *ruscifolia* pollen a. General view in light microscope, b. optical section c. SEM general view d. SEM surface sampling and pores



Figure 18. *S. commelinifolia* var. *ruscifolia* fruit a. general view, b. cross section, c. longitudinal section

Table 4. Pollen measurement values of *S. commelinifolia* var. *ruscifolia*

Measured parameter	Number of samples	Minimum	Maximum	Average	Standard deviation
Pollen length axis (A) (μm)	51	26.3	36.5	31.18	2.12
Pollen width axis (B) (μm)	51	22.3	36.5	30.14	2.29
A/B		1	1.17	1.03	2.20
Por length (Plg) (μm)	101	3.55	8.12	4.95	0.76
Por width (Plt) (μm)	103	2.03	6.09	4.18	0.90
Number of por	54	24	34	29	2.40
Distance between pores (μm)	106	2.03	12.18	5.34	2.58
Exine (μm)	67	1.52	3.04	2.23	0.44

Table 5. Fruit measurement values of *S. commelinifolia* var. *ruscifolia*

Measured parameter	Number of samples	Minimum	Maximum	Average	Standard deviation
Capsule size (mm)	150	9	18	13.7	1.64
In each capsule number of seeds	109	1	55	24	13.16

3.2.7. Seed

The seeds are reniform in shape, brown in color (Figure 19.a). The surface shape is flat and concave, the dorsal part is flat or round, the surface is densely granular, the granules are large, the hilum indentation is prominent, and the walls of the shell cells are toothed (Figure 19).

Seed sizes are 1.62-2.17x1.25-1.67x0.75-1.125 mm, seed weight is 1.56 g. Seed measurement values are shown in Table 6. (Wodehouse, 1935).

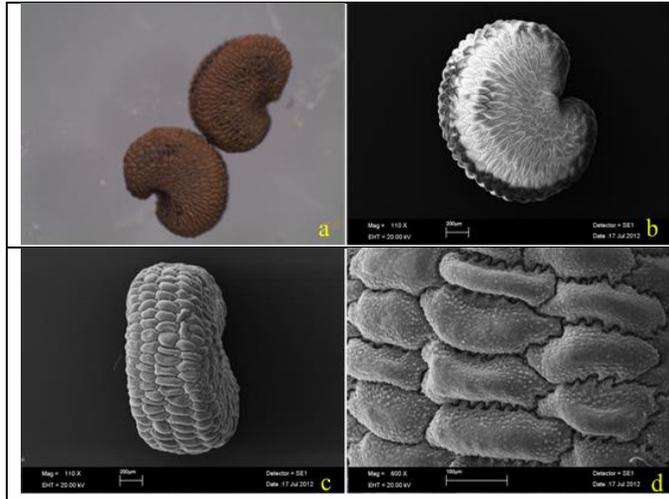


Figure 19. *S. commelinifolia* var. *ruscifolia* seed a. general view under stereoscopy, b. SEM general view c. dorsal view in SEM, d. detailed view of the surface

3.3. Karyological features

By examining the preparations prepared from the root tips of germinated seeds under a microscope, the chromosome number of *S. commelinifolia* var. *ruscifolia* was determined as $2n=24$. Photographs obtained from microscope images of chromosomes are shown in Figure 20.

4. DISCUSSION and CONCLUSIONS

In this study, *S. commelinifolia* var. *ruscifolia* which is located in the genus *Silene* with taxonomic problems and which has not been studied from this locality before. The morphological, anatomical, palynological, karyological and ecological characteristics of the taxon were to be revealed.

S. commelinifolia var. *ruscifolia* generally grows on serpentine bedrock in Sivas province, while it is found in different habitats (steppes) in other provinces. Therefore, we think that the reason for the differences seen in Kılıç's study may be due to the reflection of the change in environmental conditions on morphology. In morphological studies, it was determined that the root length was 30-130 mm, the root diameter was 6-22 mm, the calyx tube was 6-24 mm, the number of seeds in each capsule was 1-55 for the first time, and detailed photographs of the flower parts and fruit were presented by us for the first time (Table 7).

Table 6. Seed measurement values of *S. commelinifolia* var. *ruscifolia*

Measured parameter	Number of samples	Minimum	Maximum	Average	Standard deviation
length (mm)	50	1.62	2.17	1.91	0.13
width (mm)	50	1.25	1.67	1.44	0.10
Height (mm)	53	0.75	1.125	0.92	0.08

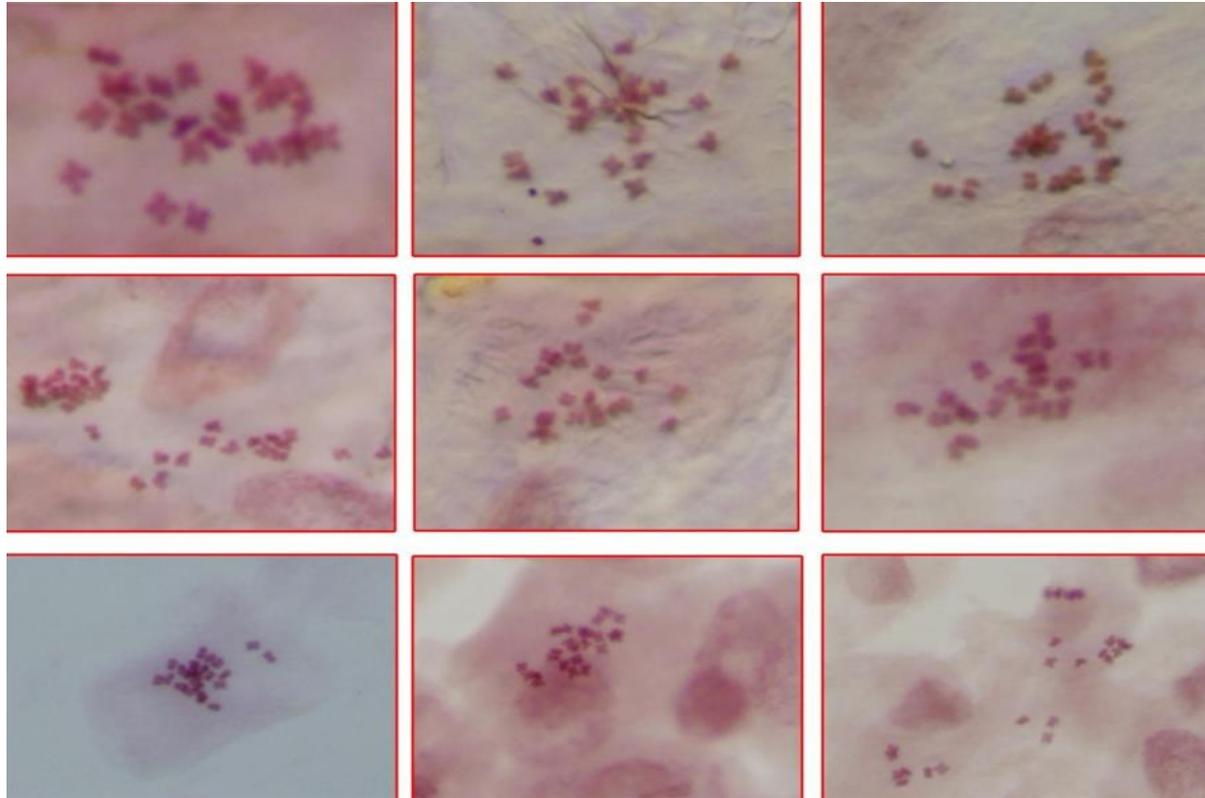


Figure 20. Mitotic metaphase chromosomes at the root tips of *S. commelinifolia* var. *ruscifolia* ($2n=24$)

Table 7. Comparison of findings with general morphological information of *S. commelinifolia* var. *ruscifolia* in the literature

Characters	Coode & Cullen (1957)	Kılıç (2007)	Results of this study
Body Length (cm) (min-max-avg)	10-15	8-17; 11.33	4.6-15.6; 10.9
Body feather condition	Intense puberty	Dense glandular puberulent	Dense glandular puberulent
Basal leaf shape	Oblanseolate	Linear-oblanseolat	Linear-oblanseolat
Basal leaf size (mm) length x width	30x3-4	30-85x3-10	6-69x1-9
Stem leaf shape	Ovate-cordate	Wide cordate	Ovate-cordate
Stem leaf size (mm) lengthxwidth (min-max)	From 2 times its width more	20-45 x 4-30	6-34x1-25
Leaf fluff condition	All puberulous	Dense glandular puberulous	Dense glandular puberulous
Leaf veining	3-5 veined	3-5 veined reticulate	3-5 veined reticulate
Flower condition	3 in a dichasiium and or more flowering	3 or 5-flowered compound dichaseium	3 or 5-flowered compound dichaseium
Calyx length (mm) (min-max)	18-20	18-26	7-31
Petal color	White	White	White
Limb fragmentation rate	3/4	3/4	3/4
Antophore length (mm) (min-max)	7	4-7.5	5-9
Antophore feather status	Hairless	Hairless	Hairless
Capsule shape	Ovoid	Oblong-ovoid	Oblong-ovoid
Petal Length (mm) (min-max)	Unspecified	18-24	20-30
Petal-bound stamens Length (mm) (min-max)	Unspecified	11-17	2-17
Free Stamen Length (mm) (min-max)	Unspecified	12-18	2-20
Stylus Length (mm) (min-max)	Unspecified	6,2-10,5	1.5-4
Calyx tooth length (mm) (min-max)	Unspecified	3.5-5.5	1-8
Capsule Length (mm) (min-max)	Unspecified	10-15	9-18

When the findings related to pollen and seed morphology were compared with the findings of Kılıç (2007), the length and width measurements of the seeds, which were determined to be reniform shaped and brown in color in both studies, were determined as 1.62-2.17x1.25-1.67 mm in our study, while in the study of Kılıç (2007) it was determined as 1.2-2x1-1.5 mm. In our studies on the pollen of the plant, it has been determined that the characteristics we have determined as the pollen shape prolate spheroidal, the type is periporate, the structure is monote, the structure of the

granules on the exine is dense and prominent, the operculum is large, covered with prominent and dense granules, and it is consistent with the findings of Kılıç. While the sampling type was determined as scabrate-punctate in Kılıç's study, it was determined as scabrate-microechinate in our study. Numerical values related to pollen measurements are presented in Table 8. While some findings such as average pollen and pore diameters are similar, some findings such as the average number of pores differ.

Table 8. Comparison of pollen measurement values of *S. commelinifolia* var. *ruscifolia* with literature information

Characters	Kılıç (2007)				Results of this study			
	Min	Max	Average	SD	Min	Max	Average	SD
Pollen Length Axis (A) (μm)	32	40	35.1	2.12	26.3	36.5	31.18	2.12
Pollen Width Axis (B) (μm)	30	38	33.75	2.09	22.3	36.5	30.14	2.29
A/B	1.05	1.06	1.06	2.10	1.00	1.17	1.03	2.20
Por Length (Plg) (μm)	4	6	4.9	0.71	3.55	8.12	4.95	0.76
Por Width (Plt) (μm)	4	6	4.55	0.6	2.03	6.09	4.18	0.9
Number of Pores	16	24	19.25	2.07	24	34	29	2.4
Distance Between Pores (μm)	3	8	5.25	1.42	2.03	12.18	5.34	2.58
Thickness of the Exine(μm)	2	3.5	2.42	0.46	1.52	3.04	2.23	0.44

*SD: Standard deviation

The anatomical structure of the body of *Silene* is characterized by the presence of multicellular hairs on the epidermis, calcium oxalate crystals in the phloem cortex parenchyma in the eigenregions of some taxa (Metcalf & Chalk, 1957), in which the structures were determined. It has been determined that both the lower and upper surfaces of the leaves are multicellular hairy, and the mesophyll, which is covered with abundant chloroplast-bearing parenchyma, contains dense calcium oxalate crystals (Metcalf & Chalk, 1957). In our findings, it was determined that the body of *S. commelinifolia* var. *ruscifolia* was densely hairy and there were druse crystals in the pedicel; In the anatomical structure of the leaf, it was determined that both surfaces of the leaf were multicellularly hairy and the presence of druse crystals in the mesophyll, as suggested by Metcalf and Chalk (1957).

It has been determined that the root, sepal and petal anatomical sections of *S. commelinifolia* var. *ruscifolia* have been examined by us for the first time. When the

anatomical findings were compared with the findings obtained from the topotype samples of Kılıç (2007), in our study, the cortex layer detected in the body in a sequence of 14-15 cells in the study of Kılıç was 10-12. The chlorenchyma layer, which was detected as cell sequential, 5-6 cell sequential, was detected as 4-5 cell sequential, and the sclerenchyma layer, which was detected as 1-2 cell sequential, was detected as 3-4 cell sequential, while it was stated that there was no druse crystal in chlorenchyma, it was determined that there was also druse crystal in chlorenchyma in our study. In our study area, it has been determined that the soil of the area is calcareous, low in organic matter and phosphorus, and loamy structure. We think that the druse crystals, which are abundant in both stem and leaf sections, are due to the gypsum nature of the land. According to our observations in our field studies, the soil where the plant was located was quite stony, sandy and covered with rock fragments. The fact that the sclerenchyma layer is more numerous in our study may be an adaptation of the plant to the negativity of the

environmental conditions in which it lives. Other anatomical findings are parallel to Kılıç's findings.

As a result of our karyological studies, the chromosome number of *S. commelinifolia* var. *ruscifolia* was determined as $2n = 24$. Of the 750 species described worldwide, 315 of the species in the genus *Silene* have had their chromosome numbers determined and their chromosome numbers were recorded as $2n = 20, 24, 30, 48, 72, 96$ (Yıldız et. al, 2009). Studies have shown that this genus has two different haploid chromosome numbers, $x=10$ and $x=12$ (Yıldız et. al, 2009; Darlington & Wylie, 1955; Moore, 1982; Yıldız & Gücel, 2006; Martin et. al, 2008; Löve & Löve, 1961; Federov, 1974). In karyological studies with *Silene* species, the rate of diploidy is 80% (Yıldız et al., 2009). In the karyological study of Yıldız et al. (2009) with species in the *Lasiostemon* section in the *Silene* taxon and in the biosystematic revision study of Melzheimer (1977) on the *Silene* genus in the Balkans (Yıldız et. al, 2009; Melzheimer (1977), the chromosome number of all species studied was determined as $2n=24$.

We determined that animal husbandry was common in the area because the land was not very suitable for agriculture, so the most important anthropological effect affecting the species was overgrazing. Important plant It is a necessity to give the area, which has the characteristics of an area (ÖBA), a conservation status where planned and alternating grazing is allowed. Otherwise, this special habitat will face the danger of extinction along with the biodiversity it hosts.

With our study, the flowering and fruiting specimens of the *S. commelinifolia* var. *ruscifolia* plant, which had not been studied from the specimens in this locality before and which were stated that the specimens in this locality could not be obtained from the national herbaria (Kılıç, 2007), were brought to the Cumhuriyet University Herbarium (CUFH) and therefore to the herbarium of the country. Some characteristics of the plant that have not been studied before were identified and the findings obtained from different localities were also studied from the samples in this locality and the deficiencies in this regard were eliminated.

Some of the findings that had been previously identified turned out to be erroneous. The number of chromosomes of the plant, which had not been determined before, was determined and data were

provided for both systematic and genetic studies to be carried out with the plant.

As a result of our observations in the field, we have determined that *S. commelinifolia* var. *ruscifolia*, which has a hazard category Least Concern (LC), is exposed to many threats. Some of these threats include the fact that the area is too close to the highway, overgrazing, and the Baku-Tbilisi-Ceyhan oil pipeline (BTC) passing through this area.

The Sivas plant protection unit has also been contacted for the protection of the endemic species that grows naturally in abundance in this area. We believe that all our findings will provide data for future revision studies on the *Silene* genus, which has taxonomic problems, including the *S. commelinifolia* var. *ruscifolia* species.

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