Morphological, Anatomical and Palynological Studies on Endemic *Matthiola anchoniifolia* Hub.-Mor. (*Brassicaceae*)

Mehmet TEKIN¹, Gülden YILMAZ²*, Esra MARTIN³

¹Çumhuriyet University, Faculty of Science, Department of Biology, 58140 Sivas, Turkey; mtekin2280@gmail.com

²Trakya University, Faculty of Science, Department of Biology, Balkan Campus, 22030, Edirne, Turkey; guldenyilmaz2009@yahoo.com (*corresponding author*)

³Neçmettin Erbakan University, Faculty of Ahmet Keleşoğlu Education, Department of Biology Education, 42090, Konya, Turkey; esramartin@yahoo.com

Abstract

In this paper, anatomical, palynological and seed micromorphological properties of an endemic plant *Matthiola anchoniifolia* Hub.-Mor. are recorded for the first time. A description and descriptive illustrations of the species are given based on the collected specimens for morphological study. Seed surface of *M. anchoniifolia* is examined by scanning electron microscope. The seed of *M. anchoniifolia* was compressed, brownish in colour and the cells of testa were nearly 60-80 μm in diameter and ranged from isodiametric, tetragonal or pentagonal. The anticlinal walls were straight or weakly curved while the outer periclinal walls were concave to flat with smooth surface. In anatomical study, cross sections of root, stem and stem leaf are examined. The root had secondary structure. Periderm consists of 5-8 layers of cells for phellem. Cortex consists of 9-12 layered parenchymatic tissue under the periderm. Secondary phloem ring-shaped, 6-9 layered and consists of companion cells and grouped sieve tubes. Stem had primary structure when analyzed. It is circular with a few irregular ribs in cross section. Cortex is 8-12 layered and parenchymatous. Stoma cells are present on both epidermis. Leaf is isobilateral. Palisade parenchyma cells are 1-2 layered and consists of companion cells and grouped sieve tubes. Stomata are present on both epidermis. Palisade parenchyma cells are 1-2 layered and spongy parenchyma cells are 5-12 layered. *M. anchoniifolia* has tricolpate pollen type, prolate pollen shape and reticulate exine ornamentation.

Keywords: anatomy, endemic, *Matthiola anchoniifolia*, morphology, palynology, seed, SEM

Introduction

*Brassicaceae* has been represented by 365 genus and 3250 species in the world and it is indicated as a huge family (Simpson, 2006). The major centres of distribution of the family are in the Mediterranean, Irano-Turanian and Saharo-Sindian regions (Hedge, 1976). In terms of the *Brassicaceae* family, Turkey is one of the richest country with 61 genera and 653 native species (Al-Shehbaz et al., 2007). The genus of *Matthiola* R.Br. (*Brassicaceae*) is distributing in Africa, Europe and Asia. This genus represented approximately 50 species in the world and with 10 species in Turkey (Cullen, 1965; Dirmenci et al., 2006; Heywood, 1993). Among the endemic taxa identified for Turkey is *Matthiola anchoniifolia* Hub.-Mor., *M. longipetala* (Vent.) DC. subsp. *pumilio* (Sibh. & Smith) P.W. Ball, *M. montana* Boiss. and *M. trojana* T. Dirmenci, F. Satı & G. Tümen (Dirmenci et al., 2006; Ekim et al., 2000). *M. anchoniifolia* has been distributed in Sivas province in Turkey (Cullen, 1965). According to Red Data Book of Turkish Plants, treat category of *M. anchoniifolia* Hub.-Mor. is lower risk/conservation dependent (LR / cd) (Ekim et al., 2000). There is no report on the anatomy, palynology and seed micromorphology of *M. anchoniifolia* which is an endemic species in Turkey. In this study, morphological, anatomical and palynological properties of *M. anchoniifolia* were given in detail for the first time.

Material and methods

The specimens which were examined in this study had collected in Sivas (Turkey) from Ulaş and Kangal town in Sivas. The localities were given as follows:

B6 Sivas: Ulaş town, Ziyarettepe, 1406 m, 39° 33' 08.9" N, 37° 01' 12,1" E, 12.05.2012, M. Tekin 1189; ibid. 12.06.2012, M. Tekin 1248.

B6 Sivas: Kangal-Gürün road 16. km, 1530 m, 39° 07' 52.2" N, 37° 14' 33,4" E, 21.06.2012, M. Tekin 1251.

Voucher specimens were deposited in the Cumhuriyet University Herbarium, Faculty of Science and Department of Biology (CUFH). Morphological features and taxonomic descriptions of the plant were carried out according to Cullen (1965). For anatomical studies, the root, stem and stem leaf of the some specimens were fixed and conserved in 70% ethyl alcohol. Hand sections from fixed material were taken with a razor blade and sections were stained with Alcian blue (Sigma) for pectic substances, Safranin (Sigma) for lignin in the rate of 3/2 (Yılmaz, 2009).
Sections were waited in blend waited in blend dye about 5 minutes for staining. The stained sections were mounted on herbarium specimens and were prepared by Wodehouse method (Wodehouse, 1965). For measurements of polar axis, equatorial diameter, exine thickness and lumina size of pollen grains were used 100× objective of Olympus light microscope CX21. Measurements were based on 50 pollen grains. For SEM studies, pollen grains coated with gold in a sputter coater. Morphological observations were made and micrographs were taken with a LEO 440 SEM at magnification 5000×; 8500×; 30000×. For morphological descriptions of pollen, followed the terminology of Punt et al. (1994). For seed micromorphology, seeds were mounted on aluminum stubs. Specimen stubs were fixed to the specimen holder of Scanning Electron Microscope (LEO Supra 35 VP FEG SEM) and maintained at accelerating potential voltage of 1.5 kV and photomicrographs were taken at 150× and 500× magnifications in Sabancı University.

Results and discussion

Morphological properties

*M. anchoniifolia* Hub.-Mor. in Bauhinia (1963) (Fig. 1, 2, 3)

Plant 15-45 cm and perennial. Stem herbaceous, woody at the base. Basal leaf 15-38 × 3-7 mm, sessile, linear-oblong to linear-oblanceolate, entire, canescent with dendraic unicellular eglandular hairs. Leaf apex subacute to oblong. Stem leaf 25-56 × 3-10 mm, sessile, linear to narrowly oblanceolate, canescent with unicellular branched hairs, entire and subacute at apex. Inflorescence raseome, 4-8 flowered. Flowers hermaphrodite, hypogynous with two planes of symmetry. Bracts absent. Pedicel 2-6 mm at anthesis. Calyx 8.8-10.1 mm. Calyx tube length 8-9 mm and diameter 2.5-3 mm. Calyx teeth 0.8-1.1 mm. Corolla cruciform; petals 4, 22-25 × 4-6 mm and brown colored. (Fig. 1, 2); (Tab. 1).

In the present study morphology, anatomy, palynology and seed micromorphology of *M. anchoniifolia*, a narrow deployed endemic species, was examined in detail. Some additions are required for morphological features (Cullen, 1965). Biometric measures belonging to the species is presented in Tab. 1. Cullen (1965) did not report any information about the length of plant, stem leaf, pedicel length at flowering time, inflorescence, calyx, corolla, stamen and pistil features.

In our study the length of plant was about 15-45 cm., stem leaf as 25-56 × 3-10 mm., inflorescence as raseome and 4-8 flowered. Pedicel length was 2-6 mm at flowering time. Bract absent, calyx tubular, sepal oblong and 8.8-10.1 mm, calyx tube 8-9 × 2.5-3 mm; calyx teeth 0.8-1.1 mm. Petal 22-25 × 4-6 mm, approximately oblanceolate and brown colored. Stamen tetradynamous, outer 2 short stamens 6.6-7 mm; inner 4 long stamens 9.2-10 mm. Pistil 3-3.2 × 0.7-0.8 mm, lanceolate, canescent and stigma green colored. Although Cullen (1965) reported in Flora of Turkey the basal leaf of *M. anchoniifolia* to be oblong-linear, we determined it to linear-oblong to narrowly linear-oblanceolate and the basal leaf 15-38 × 3-7 mm in size. Similarly, although Cullen (1965) reported that pedisel length was 7-10 mm at fruiting time, siliquae 8-12 × 0.3-0.45 cm and seed 5 × 3 mm, we measured pedisel length at fruiting time as 5-12 mm, siliquae 5-15 × 0.3-0.45 cm and seed 3-4.2 × 2-3 mm in size.

![Fig. 1. Matthiola anchoniifolia in natural habitat: a General view in flowering time; b General view in fruiting time](image1)

![Fig. 2. Drawings of Matthiola anchoniifolia: a General view (Scale bar: 2 cm); b Sepal (Scale bar: 1 mm); c Petal (Scale bar: 3 mm); d Stamens (Scale bar: 0.7 mm); e Pistil (Scale bar: 0.4 mm); f Fruit (Scale bar: 1 cm)](image2)
The shapes of the cells of testa ranged from isodiametric, tetragonal or pentagonal 60-80 μm in diameter in *M. anchoniifolia*. The anticlinal walls were observed to be straight or slightly curved while the outer periclinal walls were observed to be concave to flat with a smooth surface (secondary sculpture) and a slightly fibrous pattern in *M. anchoniifolia* (Fig. 3).

In previous studies, Tantawy *et al.* (2004) determined seed morphological characters of *M. longipetala* subsp. *bicornis* (Sibth) Ball., *M. longipetala* (Vent) DC. subsp. *hitra* (Conti) and *M. longipetala* subsp. *incana* R.Br. In *M. longipetala* subsp. *bicornis* the seed surface sculpture was reticulate with more or less broad and raised anticlinal walls. The periclinal walls were more or less raised with smooth surfaces. In *M. longipetala* subsp. *incana* the seed surface pattern was reticulate. The anticlinal walls were shallow raised with smooth irregularly arranged folds running in different directions. The periclinal walls were very narrow and deeply depressed. In addition, in the other examined taxa *M. longipetala* subsp. *incana* the seed surface pattern was reticulate. The anticlinal walls were more or less broad, raised with smooth surfaces. The periclinal walls were shallow depressed with smooth-papillate surface in certain areas and smooth-striated in others (Tantawy *et al.*, 2004).

Kasem *et al.* (2011) investigated seed exomorphic characters of 32 taxa of *Brassicaceae* by LM and SEM. In their study seed morphological characters of *M. longipetala* subsp. *incana* were determined again and they pointed out that seeds of this taxa were globose and yellowish in colour with glabrous texture. The seed size was 1.5 × 0.2 mm (Kasem *et al.*, 2011). In this study seed of *M. anchoniifolia* was compressed, brownish in colour and the cells of testa were nearly 60-80 μm in diameter. The anticlinal walls were straight or weakly curved while the outer periclinal walls were concave to flat with smooth surface...
Palynological properties

The shape of pollen grains is prolate. Exine surface ornamentation is coarsely reticulate. Exine thickness is 2.1 - 2.6 μm in range and mean 2.4 μm (Tab. 2, Fig. 7).

Brassicaceae is a stenopalynous family whose pollen grains are usually tricolpate and reticulate (Erdtman, 1972; Reile, 1992) but there are some differences between members of the same genus as in Hesperis L. in terms of some features, such as pollen shape (Pınar et al., 2009).

Properties of pollen morphology of M. anchoniifolia were determined with our present palynological measurements and examinations. Our results were compared with those in M. anchoniifolia. It was similar to M. longipetala subsp. incana with this feature.

Anatomical properties

The root had secondary structure when the plants collected. Periderm consists of three different tissues, phellem (5-8 layers of cells are brown due to suberised cell wall), phellogen (1 layer) and phelloderm (1 layer), from exterior to interior. Cortex consists of 9-12 layered parenchymatic tissue under the periderm (Fig. 4).

Parenchyma cells are 40-85 × 45-125 μm in size. Cambium is not distinguishable. Secondary phloem ring-shaped, 6-9 layered and consists of companion cells and grouped sieve tubes. Secondary xylem consist of trachea and tracheid. In the secondary xylem parenchyma cells could be observed between xylem vessels (Fig. 4).

Stem had primary structure when analyzed. Stem is circular with a few irregular ribs in cross section. Epidermis cells are 12-30 × 15-40 μm in size, ovoid to ovoid-oblong in shape. There are ramified unicellular eglandular hairs on epidermis. Cortex is 8-12 layered and parenchymatous. Cells of cortex are 20-70 × 25-100 μm in size and ovoidal. There are sclerenchyma fibers between xylem bundles. The pith is large and consists of parenchymatous cells. Pith cells are 35-115 × 45-140 μm in size and circular to circular-ovoid in shape (Fig. 5).

There is a single layered epidermis on the upper and lower surface of the leaf. Thickness of cuticle is 5-7 μm. The shape of epidermal cell is oblong-ovoid. There are unicellular and ramified hairs on both surface. Upper epidermis cells are 18-35 × 10-45 μm and lower epidermis cells are 15-30 × 8-40 μm. Stoma cells are present on both epidermis. Leaf is isobilateral. Palisade parenchyma cells are 1-2 layered, 12-27 × 35-70 μm and generally cylindrical shaped. Spongy parenchyma cells are 5-12 layered, 10-42 × 18-55 μm and circular, ovoid or rarely irregular shaped (Fig. 6).


**Tab. 2. The comparison of the pollen morphological characters obtained from *Matthiola anchoniifolia* with other species (P: polar axis; E: equatorial diameter; P/E: the ratio between the polar axis and equatorial diameter; Pt: Pollen type; Ps: pollen shape; Lumina ca.: lumina size)**

| Taxon                        | Pollen type (Pt) | Pollen shape (Ps) | Polar axis (P)(μm) | Equatorial diameter (E)(μm) | P/E ratio | Lumina ca. (μm) | Exine thickness (μm) | Ornamentation type |
|------------------------------|------------------|-------------------|--------------------|----------------------------|-----------|-----------------|---------------------|--------------------|
| Present study                | Tricolpate       | Prolate           | (20-29)            | (16-20)                    | 1.39      | 3.1             | 2.1-2.6             | Coarsely reticulate  |
| *M. arabica* (Abdel Khalik et al.) | Tricolpate       | Prolate           | (25-30)            | (17-20)                    | 1.53      | 3.4             | Not recorded        | Coarsely reticulate  |
| *M. fruticulosa* (Abdel Khalik et al.) | Tricolpate       | Prolate           | (27-32)            | (18-21)                    | 1.5       | 3.5             | Not recorded        | Coarsely reticulate  |
| *M. longipetale* subsp. longipetale (Abdel Khalik et al.) | Tricolpate       | Prolate           | (28-30)            | (20-22)                    | 1.38      | 2.4             | Not recorded        | Coarsely reticulate  |
| *M. parviflora* (Abdel Khalik et al.) | Tricolpate       | Subprolate        | (24-30)            | (20-30)                    | 1.27      | 3.9             | Not recorded        | Coarsely reticulate  |

from related species of the same genus studied by Abdel Khalik et al. (2002).

Our results confirm the existence of minor differences in pollen size, shape, apertures and exine ornamentation in pollen grains of the other members of *Matthiola* genus. In *Brassicaceae*, pollen shapes vary more or less among genera within tribes but very rarely among species within the same genus (Abdel Khalik et al., 2002). The pollen shape of *M. anchoniifolia* is prolate. The palynological study of Abdel Khalik et al. (2002) on *Matthiola* also included *M. arabica* Boiss, *M. fruticulosa* (L.) Maire, *M. longipetale* (Vent.) DC subsp. *longipetale*. According to this study, all these species had the same shaped prolate pollen grains with the exception of *M. parviflora* (Schousb.) R. Br. with subprolate grains. When polar axis and equatorial diameter of *M. anchoniifolia* was compared with other *Matthiola* species,

![Fig. 7. SEM photographs of pollen of *Matthiola anchoniifolia*: a Multiple view of pollen (Scale: 10 μm) ; b Equatorial view and aperture (Scale: 2 μm); c Polar view and aperture (Scale: 2 μm) ; d Ornamentation (Scale: 2 μm)](image-url)
it appeared that pollen grains of *M. anchonifolia* were the smallest (Tab. 2). Erdtman (1972), Rollins and Banerjee (1979), Lahham and Al-Eisawi (1987) reported slightly colpate or non aperturate pollen grains in a few *Matthiola* species. Abdel Khalik *et al.* (2002) reported especially in *M. arabica* that the colpi was less clear. However *M. fruticulosa*, *M. longipetala* subsp. *longipetala*, *M. parviflora* and in our study, the colpi of *M. anchonifolia* was clear. *M. anchonifolia* and all investigated *Matthiola* taxa in the study of Abdel Khalik *et al.* (2002) were tricolpate. Exine ornamentation type of *M. anchonifolia* is coarsely reticulate. Abdel Khalik *et al.* (2002) reported that exine ornamentation of *M. arabica*, *M. fruticulosa*, *M. longipetala* subsp. *longipetala*, *M. parviflora* was the same with *M. anchonifolia* and they were coarsely reticulate. Lumina size of *M. anchonifolia*, *M. arabica*, *M. fruticulosa*, *M. parviflora* is more or less similar value apart *M. longipetala* subsp. *longipetala*, which have smaller value of the others. There is no report about exine thickness of *Matthiola* species in the study of Abdel Khalik *et al.* (2002) but in this study mean thickness of exine of *M. anchonifolia* pollen grains was determined as 2.4 µm (Tab. 2).

### Conclusion

Morphological, anatomical, palynological and seed micromorphological properties of *M. anchonifolia* which was an endemic plant in Sivas province were reported in detail for the first time in this study.

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