Comparative study of the leaf surface micromorphology and mesophyll of *Platanus orientalis (Platanaceae)* plants originating from two localities

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Abstract. Leaf surface micromorphology and mesophyll structure of the *Platanus orientalis* plants were studied. The plants originated from two localities: Kresna (Bulgaria) and Sicily (Italy), and were grown together from seeds under controlled conditions. The mature leaves showed variations in cuticular ornamentation, shape and size of the epidermal cells, which could testify to resistant traits formed in the source plants as a result of the specific environment they inhabited. For all examined leaves, paracytic, laterocytic and anomocytic stomata on the abaxial epidermis were identified, as well as non-glandular candelabra and glandular capitate trichomes on both surfaces. Regarding histological organization of the leaf lamina, no differences were observed between the plants of the two origins.

Key words: bifacial leaf, environmental conditions, epidermis, glandular trichomes, stomata

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Introduction

Platanus L. is the single extant genus of the *Platanaceae* family. It comprises seven species spread in the northern hemisphere (Grimm & Denk 2008). The only native species of the genus in Europe is *Platanus orientalis* L. It is distributed in the eastern part of the Mediterranean region, southern part of the Balkan Peninsula and the Aegean Islands (Radoukova & al. 2015). Although *P. orientalis* is included in IUCN as a plant of Least Concern (IUCN 2016) and is also categorized as Near Threatened in the Red List of Bulgarian Vascular Plants (Petrova & Vladimirov 2009), its habitats are protected by the Biological Diversity Act of the Republic of Bulgaria and are included in the Habitats Directive (92/43/EEC) (Radoukova & al. 2015). *P. orientalis* is a deciduous tree and its communities are naturally distributed along river banks. In contrast to other riparian species, it grows on very limited soil layers thus depending mostly on soil hu-

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midity (Grueva & Zhelev 2011). Change of the stream courses for irrigation purposes, increase of arable land and enhanced anthropogenic pressure are regarded as the main threats for the survival of *P. orientalis* (Rosati & al. 2015, Radoukova & al. 2015, IUCN 2016). According Rosati & al. (2015), *P. orientalis* should be regarded as archaeophyte in the southern parts of Italy (Campania, Calabria, Apulia, and Sicily) and thus it should be considered as a plant of conservation concern, reported as Endangered in the recent National Red List. After analyzing genetic diversity of the central and western *Platanus* populations, Rinaldi & al. (2019) identified four groups belonging to two main phyletic groups that seem to have different biogeographic origins. In their study, Sicilian and Bulgarian populations belong to different lineages.

As an isoprene-emitting tree, *P. orientalis* has been subjected recently to extensive physiological and structural studies. Leaf anatomy and ultrastructure of the species has been intensively examined under high temperatures, elevated CO_2 (Velikova & al. 2009, Koleva & al. 2010), different light and watering regimes (Arena & al. 2016, Velikova & al. 2018). They proved to be determined not only by the genotype but also strongly affected by the environmental factors during leaf development. According to Gratani (2014), it is important to analyze the variations at phenotypic and genetic levels in particular species, especially in endemic and rare, because these variations could have drastic effects at an ecosystem level.

The present study of surface micromorphology and structure of the epidermis and mesophyll of *P. orientalis* leaves matured under the same light, temperature and humidity regimes aims to compare the morpho-anatomical leaf characteristics of plants originating from two genetically diverse populations, and thus contribute to the assessment of the adaptive potential of the species.

Material and methods

Plant material

Platanus orientalis L. plants were grown from seeds collected from two native localities in Kresna (Bulgaria) and in Francavilla di Sicilia, Sicily (Italy), respectively. The seeds were cultivated at the Institute of Plant Physiology and Genetics, BAS, Sofia, Bulgaria, in a climate chamber under controlled conditions: temperature 25°C ± 2 / 22°C ± 2 day/night, photoperiod 16/8 h, 350 mmol m^{-2} s^{-1} PPFD, and 60–65% air humidity (Velikova & al. 2018). Mature (fully expanded) leaves from ten plants, five of each origin (further in the text referred to as Kresna plants and Sicily plants) were used for morpho-anatomical analysis.

Scanning electron microscopy (SEM) and light microscopy (LM)

Surface micromorphology was studied on dried small segments of the middle part of the leaf lamina, attached to aluminum specimen stubs by double–sided carbon tape, sputtered with gold in vacuum-evaporator Jeol JFC-1200 fine coater, by scanning electron microscope Jeol JSM-5510. For light microscopy analysis of the epidermis, fragments from the central part of the leaves were rinsed with distilled water and bleached with commercial bleach. After rinsing with distilled water, the samples were placed in glycerol on microscopic slides and observed under Amplival 4 microscope (Carl Zeiss, Jena, Germany). Fifteen measurements of the length and width of the adaxial and abaxial epidermal cells and stomata were made by ocular micrometer. For each sample, the number of stomata was counted in fifteen microscopic fields. Microphotographs of the epidermis were taken in paradermal view by EcoBlue digital microscope (EC.1657) with integrated 5.0 MP USB-2 camera (Euromex, The Netherlands), at magnification of 100× and 400×.

For histological analysis, small segments (about 1–2 mm^2) from the middle part of the leaves were fixed in 3% (m/v) glutaraldehyde, in 0.1 M sodium phosphate buffer (pH 7.4). Handmade transverse cuttings were mounted on slides in glycerol. Leaf anatomy was observed and microphotographs were taken by light microscope and camera Nicon Eclipse 50i (Tokyo, Japan). The thicknesses of the leaf lamina, mesophyll, palisade parenchyma, spongy parenchyma, as well as adaxial and abaxial epidermis were measured by means of ImageJ (National Institutes of Health, USA). Thirty measurements for each parameter and each variant were made.

Results

SEM observations have shown dense cuticle striations covered by wax granules, well visible on the adaxial leaf surface of the Kresna plants; also, polygonal epidermal cells with curved anticlinal cell walls (Fig. 1A). The leaf cuticle of the Sicily plants had predominantly fine striations and deeply undulated outlines of the cell walls (Fig. 1B). Furthermore, the epidermal cells were significantly longer and higher than in the Kresna plants (Tables 1, 2). The cuticle of the abaxial epidermis was smooth and the cells were irregular, with undulated anticlinal cell walls in all studied leaves (Fig. 1C, D). The size of epidermal cells did not differ considerably but their average length and width were greater and the height was smaller in the Kresna as compared to the Sicily plants (Tables 1, 2).

The examined leaves were hypostomatous, with evenly distributed elliptic stomata (Fig. 1C, D). Arrangement of the subsidiary cells referred to the paracytic, laterocytic or anomocytic stomatal types, usually observed all together on the same leaf (Fig. 2B, D). Morphometric data did not show significant differences in the stomatal length, width or frequency between the Kresna and Sicily plants (Table 1).

For all examined leaves, non-glandular candelabra and glandular capitate trichomes were identified on both surfaces (Fig. 2A, B). Most candelabra hairs were shed off, but their trichome bases with thickened pore rims, about 20–24 μm in diameter, remained well outlined (Fig. 2A). Capitate hairs had unicellular globose heads, with yellowish content and 1-2 celled stalks

Fig. 1. SEM of *P. orientalis* leaf surface: A–adaxial epidermis, dense cuticle striations and epidermal cells with curved anticlinal cell walls (Kresna plants); B–adaxial epidermis, fine cuticle striations and epidermal cells with undulated anticlinal cell walls (Sicily plants); C–abaxial epidermis, trichome bases and stomata (Kresna plants); D–abaxial epidermis, stomata (Sicily plants).

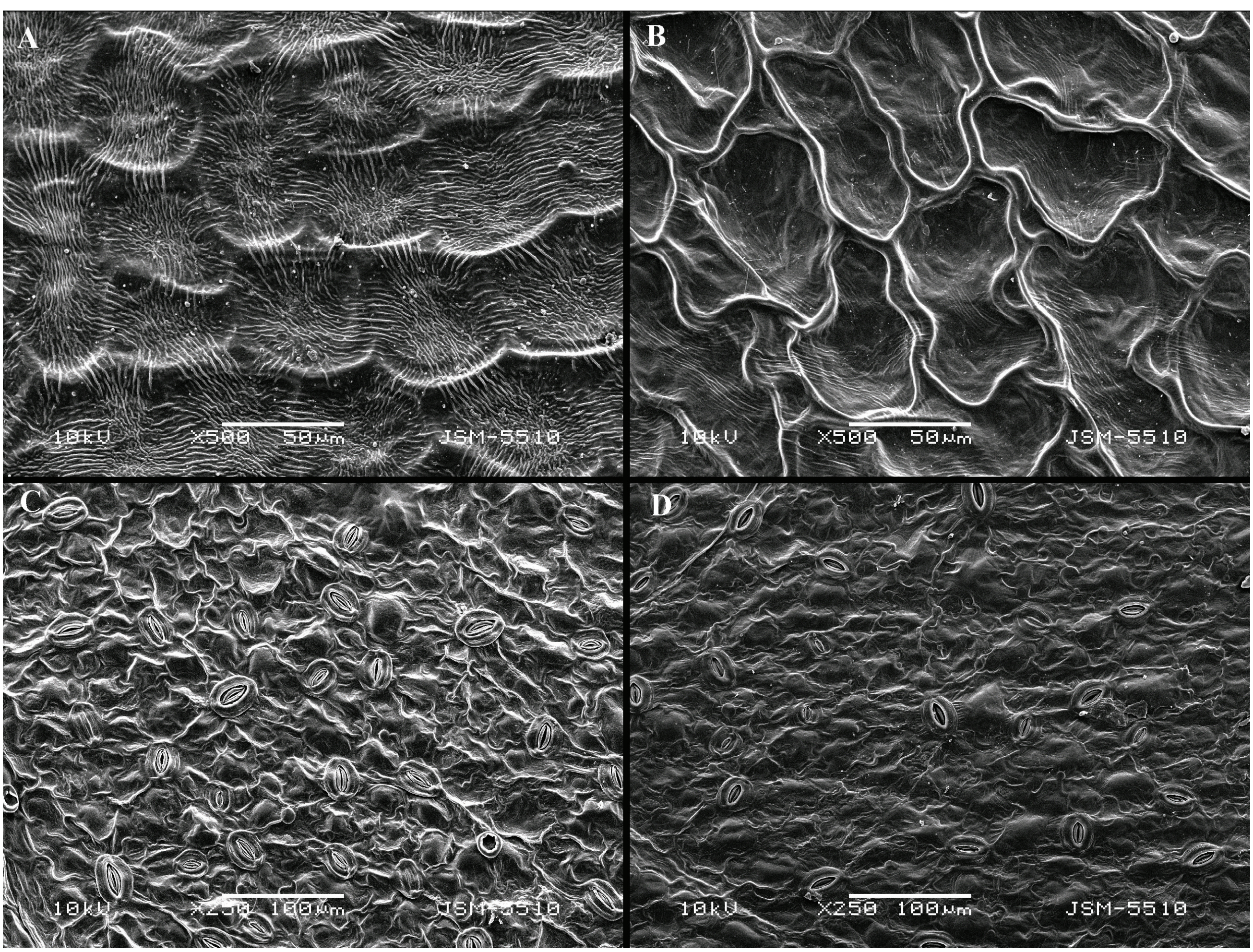


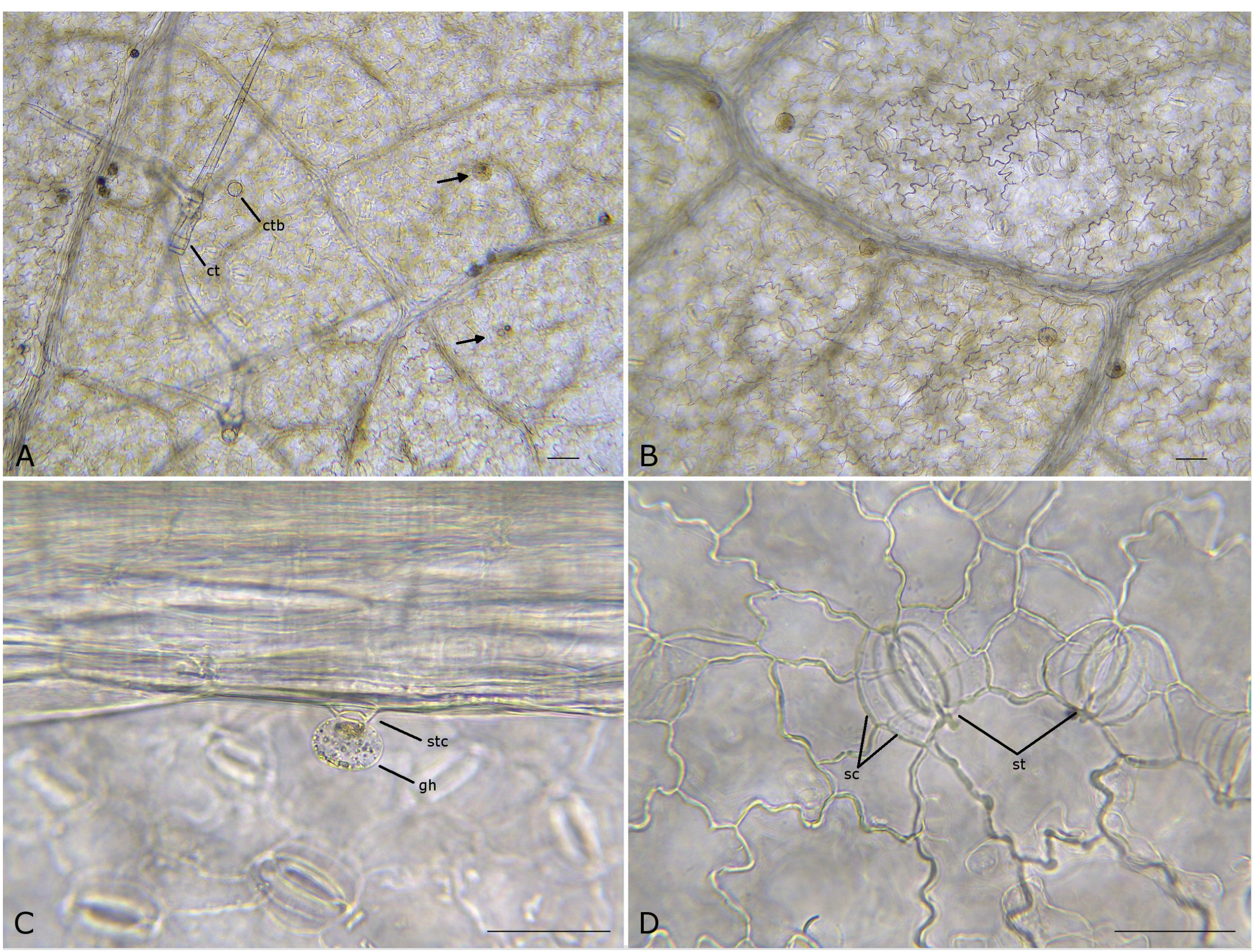
Table 1. Morphometric data of *P. orientalis* epidermis presented as the mean value ± STDEV

Platanus orientalis locality	Epidermal cells				Stomata		
	Adaxial		Abaxial				
	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Frequency Number per mm^2
Kresna (Bulgaria)	73.7±15.83	51.4±10.15	56.2±12.34	36.7±8.30	35.9±4.03	28.0±2.77	93
Sicily (Italy)	81.4±14.96	52.9±10.13	53.3±11.10	33.2±7.01	37.0±3.28	27.0±2.06	88

Table 2. Morphometric data of *P. orientalis* leaf tissues presented as the mean value ± STDEV

Platanus orientalis locality	Leaf lamina (µm)	Adaxial epidermis (µm)	Abaxial epidermis (µm)	Mesophyll (µm)	Palisade parenchyma (µm)	Spongy parenchyma (µm)
Kresna (Bulgaria)	174.8±10.43	30.9±9.14	14.5±3.50	132.5±7.75	70.4±7.13	61.2±9.64
Sicily (Italy)	182.6±13.70	34.2±10.97	16.0±3.11	134.6±13.26	80.9±13.18	57.6±5.58

Fig. 2. LM of *P. orientalis* epidermis: A-abaxial epidermis - candelabra trichomes (ct), candelabra trichome base (ctb), capitate glandular trichomes (arrows); B-capitate glandular trichomes on the abaxial epidermis; C-capitate glandular trichome – unicellular glandular head (gh), stalk cell (stc); D-abaxial epidermis – stomata (st), subsidiary cells (sc) (scale bar = 50µm).



(Fig. 2A, B, C). Diameter of the glandular trichome bases was about 12 µm, and diameter of the glandular head was about 32 µm. Both types of trichomes were distributed mainly above the veins, predominantly on the abaxial epidermis (Fig. 2A, B, C).

LM histological analysis showed that *P. orientalis* leaves were bifacial. The mesophyll consisted of one-layer palisade cells and three to four layers of spongy cells. The comparative study of Kresna and Sicily plants showed similar leaf histology and cell morphology (Fig. 3A, B). The palisade parenchyma was built of typically cylindrical cells. Their anticlinal walls were undulated, which increased the volume of intercellular spaces and reduced the symplast contact area between the cells. The spongy parenchyma consisted of irregular to almost spherical cells. They surrounded some distinct intercellular spaces, bigger beneath the stomatal complex. Light microscopy analysis of the leaf transverse sections did not reveal any variations in the anatomy of *P. orientalis* leaves. Morphometric data also confirmed the uniformity of the histological leaf structure in the examined plants (Table 2).

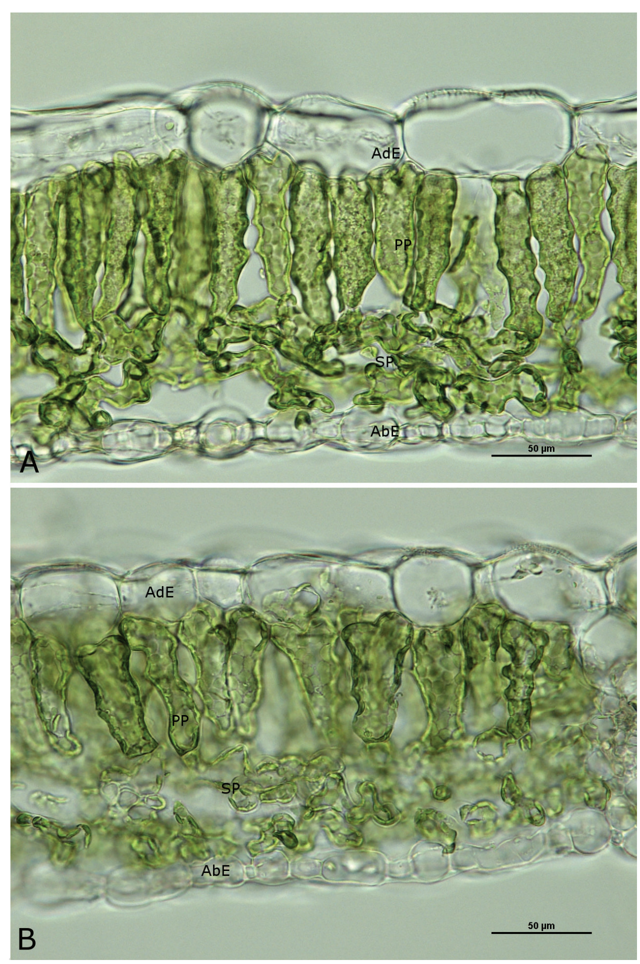
Discussion

Surface micromorphology of *P. orientalis* leaves showed typical characteristics of a mesophytic plant, such as cuticular ornamentation and undulation of the anticlinal cell walls of the epidermal cells. According to Stace (1965), in many mesophytes the cuticle forms striations and the epidermal cells have undulation of the anticlinal cell walls that increase the area of contact between adjacent cells. Differences in the cuticular ornamentation, shape and size of the epidermal cells in the leaves observed between Kresna and Sicily plants could be attributed to the specific environmental conditions during the formation of the initial plant genotypes. The examined *P. orientalis* plants originated from native populations which, according to the Koppen-Geiger classification, grow in a mild temperate climate with humid-hot summer in Kresna (https://en.climate-data.org/europe/bulgaria/kresna/kresna-194719), and with dry-hot summer in Francavilla di Sicilia, respectively (https://en.climate-data.org/europe/italy/sicily/francavilla-di-sicilia-114388/).

The present identification of different stomatal types for *P. orientalis* leaves matches the studies of Brett (1979) on the ontogeny of the stomatal complex of *Platanus* and the description of stomata made by Carpenter & al (2005). In the present study, in addition to the same types, the average stomatal size and frequency also did not show significant differences between the leaves from Kresna and Sicily plants. This could sustain the taxonomic value of stomata characteristics for identification of species.

The young leaves of *P. orientalis* are initially coated with hairs, which are subsequently shed off (Metcalfe & Chalk 1965, Baas 1969, Ntefidou & Manetas 1996).

Fig. 3. Leaf lamina transverse section of *P. orientalis*: Kresna plants (A); Sicily plants (B) – AdE-adaxial epidermis, AbE-abaxial epidermis, PP-palisade parenchyma, SP-spongy parenchyma.



Therefore, predominantly trichome bases instead of intact trichomes have been observed on both surfaces of the mature leaves. Almost in all plant species, the young leaves are covered with hairs, as the leaves are especially vulnerable to disruptive damage during the early stages of their ontogeny (Johnson 1975). It has been presumed that the non-glandular trichomes of *P. orientalis* protect the mesophyll against the photoinhibitory effect of excess light during the leaf development (Ntefidou & Manetas 1996). Non-glandular trichomes provide protection against UV-B radiation by behaving as optical filters, screening out the wavelengths that could damage the sensitive tissues (Karabourniotis & al. 2020). We presume that in *P. orientalis* after the loss of indumentum both the cuticle and waxes effectively protect the leaves. Moreover, in our previous study a combined application of elevated CO2 and high temperature caused an increase of wax formation on the leaf surface as an adaptive reaction in stress conditions (Koleva & al. 2010).

Although Metcalfe & Chalk (1965) reported for *Platanus* simple capitate glands and branched hairs with glandular endings on the leaves, other authors usually reported only non-glandular candelabrum (dendritic, stellate) type of trichomes (Baas 1969, Nixon & Poole 2003, Simpson 2019). Carpenter & al. (2005) described only large trichome bases for the leaf cuticles of *P. orientalis* as they did not find any glandular trichomes. In a leaf anatomy study of *P. orientalis* from Turkey (Madra Mts), Sargin (2021) did not observe glandular hairs on the lamina but registered a small number of capitate glandular hairs on the petiole. The latter resembled in type and structure the glandular trichomes described in this study on the lamina of leaves developed under controlled conditions. Thus, to the authors' best knowledge, these have been the first capitate glandular trichomes on *P. orientalis* mature leaf surface captured by light microscopy. Therefore, a further study could show whether the glandular trichomes would remain on the leaves of plants from their natural environment or, as Baas (1969) concluded, that type is deciduous at a very early stage of leaf development.

The present histological analysis showed that the leaves of *P. orientalis* were bifacial, with uniseriate palisade layer beneath the adaxial epidermis. Sargin (2021) also reported a dorsiventral structure of the mesophyll in *P. orientalis* plants naturally growing in Turkey, whereas Metcalfe & Chalk (1965) described isobilateral leaves in all investigated species of the *Platanaceae* family, with a single layer of palisade tissue towards both surfaces. This contradiction raises the question whether variations in the leaf architecture could be attributed to the growth conditions alone. A further study on the leaf anatomy of plants from the natural populations in Kresna and Sicily would supplement the present results and clarify the cause for the observed variation.

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