Available online at www.sciencedirect.com
SCIENCE DIRECT®

Flora 201 (2006) 102–107

FLORA
www.elsevier.de/flora

Leaf and green stem anatomy of the drought deciduous Mediterranean shrub *Calicotome villosa* (Poiret) Link. (Leguminosae)

Charilaos Yiotis, Yiannis Manetas, George K. Psaras*

Section of Plant Biology, Department of Biology, University of Patras, GR 265 00, Patras, Greece

Received 7 March 2005; accepted 28 April 2005

Abstract

Light and scanning electron microscopy were used to study leaf and stem fine structure of the drought deciduous green-stemmed Mediterranean shrub *Calicotome villosa* (Poiret) Link. (Leguminosae). Each leaf consists of three small obovate leaflets with abundant but small (16 μm length) anomocytic stomata on both surfaces. Adaxial surface exhibits more than double stomatal density ($440 \pm 8\,\mathrm{mm}^{-2}$) than the abaxial one ($185 \pm 4\,\mathrm{mm}^{-2}$). T-shaped trichomes ($36 \pm 3\,\mathrm{mm}^{-2}$) are present only on the abaxial leaf surface. Leaves are unifacial, furnished with palisade parenchyma on both sides. The stem is characterized by raised ridges and grooves. Beneath the one-cell-layered epidermis sclerenchyma is found on ridges, whereas stomata and palisade chlorenchyma are found in grooves. Hairs are abundant, especially in grooves. Stem and leaf palisade chlorenchymas are structurally similar. According to these data, photosynthesis could be efficiently supported by the stem.

Keywords: *Calicotome villosa*; Leaf anatomy; Mediterranean species; Stem anatomy; Stem photosynthesis

Introduction

Dominant perennial plant species native in the Mediterranean-climate areas belong to xerophytes (Fahn and Cutler, 1992) and may be classified as evergreen sclerophylls and drought deciduous shrubs (Margaris, 1981). Structural features of the drought-adapted leaf of xerophytes have been related to the whole plant physiology and adaptability (Shields, 1950). Small and thick leaves with multilayered mesophyll are considered to characterize evergreen sclerophylls (Fahn and Cutler, 1992) and sun species (Terashima et al., 2001, 2005). Drought deciduous species possess leaves that undergo senescence and desiccation during the period of dryness (Orshan, 1963). Amphistomaty and leaf compartmentation have been repeatedly evaluated concerning leaf xeromorphy. Amphistomaty, which is more common in xeric habitats (Fahn and Cutler, 1992; Parkhurst, 1978), shortens the distance of CO_2 diffusion to mesophyll cells (Parkhurst et al., 1988; Terashima et al., 2005). Small but abundant stomata are also believed to lower the CO_2 diffusion resistance toward the photosynthesizing tissue; thus, non-succulent species show increased stomatal density (Sundberg, 1986). Mesophyll compartmentalization is supposed to protect the leaf against water stress (Terashima, 1992), but, unavoidably, increases the CO_2 diffusion resistance of the tissue (Miyazawa and Terashima, 2001). Abundant palisade tissue is also believed to increase the CO_2 absorbing surface of the mesophyll (Rhizopoulou and

---

*Corresponding author. Tel.: +30 2610 996254;
fax: +30 2610 997411.
E-mail address: g.k.psaras@upatras.gr (G.K. Psaras).

0367-2530/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2005.04.007

Psaras, 2003; Terashima et al., 2005). Thus, leaf fine structure of a species, which provides the anatomical basis to explain physiological traits, always needs to be elucidated.

One of the xerophytic life forms distinguished by Oppenheimer (1960) is that of “leafless non-succulent switch shrubs” occurring in Mediterranean-climate areas as well. This group consists of perennial species possessing green stems which are the major or, periodically, the only source of carbon assimilation (Gibson, 1983). Information concerning stem anatomy of such species comes also from desert and semi-desert perennial species. According to Gibson (1983), such green-stemmed perennials show convergent stem anatomy, including a well-developed cuticle, sunken stomata, sclerenchyma, thick chlorenchyma and delayed periderm formation. He analyzed the stem structure of 30 non-succulent species of desert and semi-desert perennials of North America and classified them to the four basic designs distinguished previously (Böcher, 1972; Böcher and Lyshede, 1972; as cited by Gibson, 1983) on the basis of relative positions of sclerenchyma and chlorenchyma.

Though leaves are traditionally considered as the main photosynthetic plant organs, other plant organs like green stems, flowers, fruits and aerial roots photosynthesize as well. Photosynthesis of plant stems has been categorized into three types: (i) stem photosynthesis occurring in the green primary axis; (ii) crassulacean-acid metabolism (CAM) photosynthesis carried out by succulent species, and (iii) corticular photosynthesis in the bark after secondary thickening growth (Aschan and Pfanz, 2003; Nilsen, 1995). Although all three types of photosynthesis are carried out by stem tissues, the term *stem photosynthesis* is applied to the first type of photosynthesis, where the CO_2 is taken up through abundant stomata of the stem epidermis and carbon fixation occurs through the C_3 pathway (Nilsen, 1995). *Calicotome villosa* is a Mediterranean drought deciduous shrub possessing mature, green stems. The aim of the present work was to study the fine structure of both the leaf and the stem of the plant, providing, thus, the background knowledge for understanding further experimental work implicating physiological parameters.

Materials and methods

Plant material, i.e. mature leaves and stems, was collected from five shrubs of. *C. villosa* (Poiret) Link. (Leguminosae; common name: spiny broom) growing in an open field near the campus of Patras University (38°15′N, 21°44′E, 150 m above sea level) in southwestern Peloponnisos, Greece. The plant bears leaves from roughly November to May; therefore, plant material was collected in February (2003) and December (2004). Care was taken to collect branches with similar (south) position within the shrubs. Leaves and stems kept in air-tight bags were immediately transferred in the laboratory, carefully cut and fixed in 5% glutaraldehyde in phosphate buffer at pH 7 at room temperature for 2 h. Tissue was then post-fixed in 1% OsO_4 at 4 °C and dehydrated in acetone. For scanning electron microscope (SEM) the dehydrated tissue samples were critical point dried, mounted with a double-sided adhesive tape on stubs, sputter coated with gold and observed with a JEOL 6300 SEM (JEOL, Tokyo, Japan). SEM pictures were digitally recorded. For light microscopy, dehydrated tissue was embedded in Durcupan ACM (Fluka). Semi-thin sections (1–2 μm) of resin-embedded tissue made on a Reichert Om-U2 (Vienna, Austria) microtome using glass knives were stained with Toluidine Blue O. The sections were examined and digitally recorded with a Zeiss Axioplan microscope (Zeiss, Oberkochen, Germany) equipped with a video camera (Sony, SSC-DC58AP, Tokyo, Japan). Dimensions of stomata and trichomes number were determined from micrographs using the Image Tool 1.25 program (University of Texas Health Science Center, San Antonio, Texas, USA). Numerical values are means ± standard error.

Results and discussion

C. villosa has compound leaves composed of three small ($10.2 \pm 1.9\,mm^{-2}$) obovate leaflets each (Fig. 1A). Leaflets are ca. 170 μm thick (165 ± 6 μm) and they possess stomata on both sides, abaxial (Fig. 1B) and adaxial (Fig. 1C). However, stomatal density of adaxial surface is much higher ($440 \pm 8\,mm^{-2}$) than that of the abaxial one ($185 \pm 4\,mm^{-2}$, cf. also Figs. 1B and C). Stomatal length, on both sides, as determined from SEM micrographs is 16 ± 0.4 μm. As judged from their different pattern (Fig. 1D), it is concluded that stomata complexes are anomocytic, i.e., real subsidiary cells are absent. Abaxial epidermis possesses T-shaped trichomes ($36 \pm 3\,mm^{-2}$, Fig. 1B) which are totally absent from adaxial surface (Fig. 1C). As seen in cross sections, leaflets are equifacial, i.e., palisade parenchyma is found on both leaf sides (Fig. 1E). Examination of paradermal sections reveals the typical structure of palisade parenchyma with numerous cells forming small intercellular spaces and abundant chloroplasts arranged along exposed parts of the cell walls (Figs. 1F and G).

Reduced leaf size, increased thickness, thick external walls of epidermal cells, high stomatal density and palisade developed at the expense of spongy mesophyll are common features of plants grown in xeric