Limoniastrum monopetalum (L.) Boiss, a candidate plant for use in urban and suburban areas with adverse conditions. An anatomical and histochemical study.

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Abstract

Limoniastrum monopetalum L. is an interesting small shrub native to Greece and other Mediterranean countries with an attractive foliage, and blooming in the summer with pink flowers. In this work an anatomical and hystochemical study of the species were carried out in order to investigate the suitability of the *L. monopetalum* for use in urban and suburban areas as ornamental plant and its growth in hostile environments such as archeological sites, roof gardens, landscape restoration (e.g. quarries, roadsides, etc.). The results showed that the leaves of *L. monopetalum* are amphistomatic bifacial with leaf blade approx. 170 μm thick. The epidermal cells is relatively thick approximately 20 μm and covered by a 2 μm thick cuticle. Furthermore both surfaces of the leaves are entirely covered by salt crystals secreted by the salt glands, as this is revealed from fresh unprocessed specimens observed with the SEM.The histochemical tests showed the presence of terpenoids in the cell walls of the epidermal cells and in the leaf vascular tissues, phenolics, nitrites and alkaloids, flavonoids in the mesophyll cells, especially in the vacuoles, and lipids only in the cuticle. Both morphological anatomical and histochemical characteristics of the leaves indicate the xeromorphic character of the species which render *L. monopetalum* a suitable plant for ornamental use in a wide spectrum of areas with adverse conditions

Keywords: *halophytes, salt glands, phenolics, flavonoids, landscape restoration.*

Introduction

L. monopetalum (L.) Boiss, (Plumbaginaceae) is an evergreen shrub native to Greece and also found in other Mediterranean countries with dense foliage reaching a height of 0.3 – 1.0 m, with many branching shoots. Its foliage is green-gray and it blooms in the summer which are initially pink and turning violet at the end of the blooming season (Blamey & Grey-Wilson, 1993). *L. monopetalum* is a typical halophyte using the strategy of salt absorption and secretion through specialized structures on their leaves, the salt glands. *L. monopetalum* is also recommended for use for phytoremediation of soils polluted with Cd and Pb (Manousaki *et al*. 2014).

Aims and Objectives

Due to the ornamental characteristics and properties of *L. monopetalum* the anatomy and histochemistry of its leaves were investigated in order to assess the plant's suitability as ornamental for urban and suburban areas and its growth in problematic places such as archeological sites, roof gardens etc.

Materials and Methods

Light and scanning electron microscopy (SEM) were used for the study of the morphology, anatomy and histochemistry of *L. monopetalum* leaves which were collected from naturally

grown plants from the Island of Milos. For light microscopy and histochemistry (bright field and fluorescence), transverse sections of approximately 25 μm thickness were cut with a cryotome and stained with Nadi reagent for the detection of phenolics, Sudan IV (Jensen, 1962) for lipids, Dragendorff reagent for alkaloids, and $AlCl_3$ (Guerin *et al.*, 1971) for the enhancement of fluorescence of flavonoids. The samles were observed and photographed with an Olympus BX40 light microscope, equipped with an Olympus DP21 digital camera. For SEM observations small pieces of leaves (5X5 mm) were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2, washed in buffer, dehydrated in a series of acetone, critical point dried and sputter coated with gold. Samples were observed with a Jeol 6063 SEM. Some samples were examined fresh without any prior treatment. The thickness of the leaves, epidermis and cuticle were measured, as well as the number of stomata was counted from ten different photographs and mean values were calculated.

Results and Discussion

The leaves of *L. monopetalum* are amphistomatic and bifacial with a single layer of epidermal cells. The leaf blade is approx. 170 μm thick (Fig. 2A,B); on both sides the outer cell wall of epidermal cells is relatively thick, approx. 20 μm and covered by a 2 μm thick cuticle (Fig. 2C). Stomata are of the anamocytic type and their number is the same on both sides of the leaf which is 97±2 st/mm^2 (Fig. 1A,B). A number of irregularly shaped astrosclereids appear in the mesophyll with their size varying between 50 - 100 μm in length (Fig. 2A). Both surfaces of the leaves are entirely covered by salt crystals apparently secreted by the salt glands, as this is revealed from fresh unprocessed specimens observed with the SEM (Fig. 1B). The salt glands were approximately 6/mm^2, with a diameter of 100 – 300 μm. These anatomical features are characteristic of a xerophytic halophyte using the strategy of salt absorption and secretion through specialized structures on their leaves, the salt glands. The presence of large astrosclereids, the thick outer cell wall of the epidermal cells and the presence of salt on their surfaces protects them from the herbivores (Fahn, 1990).

The histochemical tests showed the presence of, terpenoids in the cell walls of the epidermal cells and in the leaf vascular tissues, phenolics, nitrites and alkaloids, flavonoids in the mesophyll cells, especially in the vacuoles, and lipids only in the cuticle (Fig. A-D). The presence of phenolics in this plant has also been verified by chemical analysis methods (Trabelsi *et al.,l* 2010). All these secondary metabolites contribute to the chemical defense against biotic and abiotic factors.

Conclusions

All these structural and chemical characteristics providing defense and protection seem to render *L. monopetalum* suitable for use in almost extreme environmental conditions such as drought, high salinity, high light intensity, and high temperatures as well as protection from

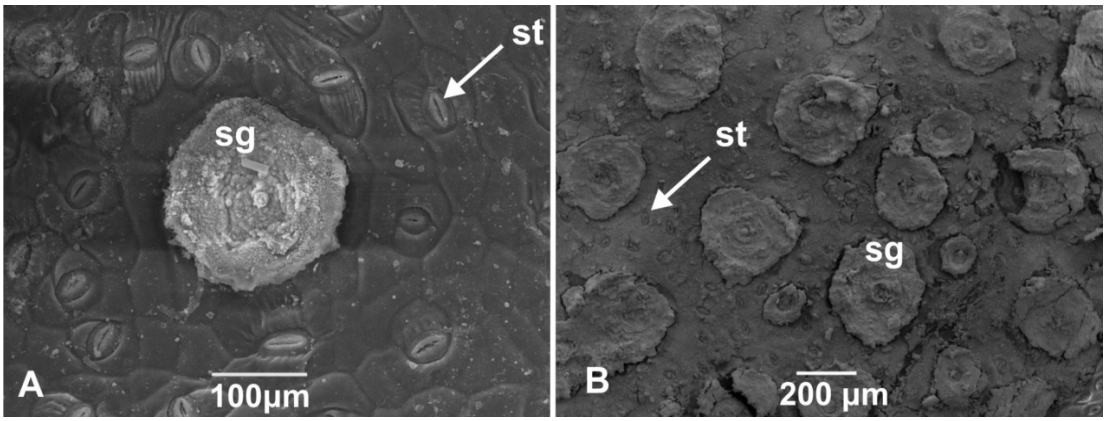

Figure 1. SEM from the leaf surface of *L. monopetalum*. **A**. processed sample. Due to the processing the salt deposits have been rinsed off. **B**. fresh, unprocessed sample where the leaf surface is covered with salt, secreted by the salt glands. **sg**: salt gland, **st**: stoma.

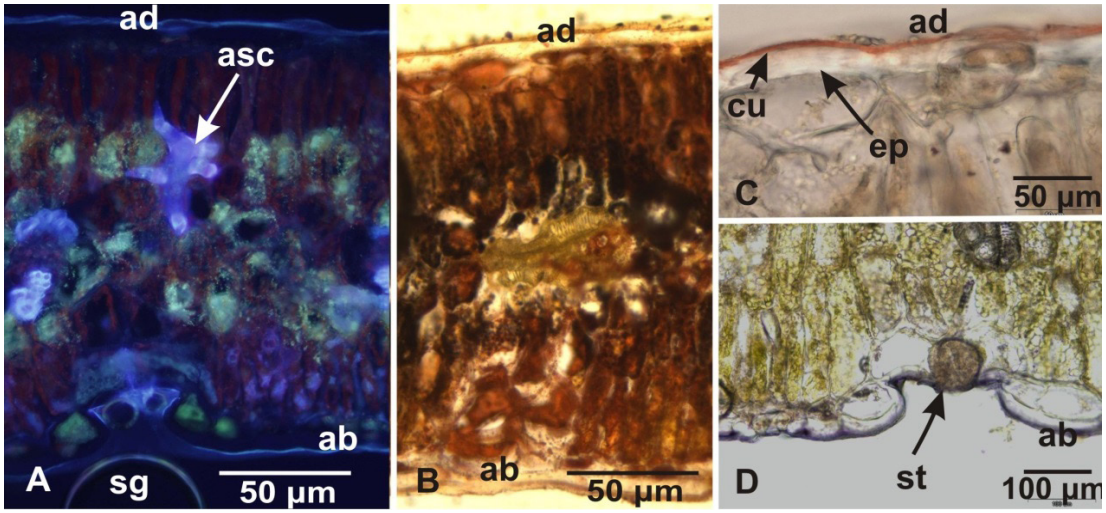

Figure 2. Transverse sections of *L. monopetalum* leaves as observed with fluorescence microscopy **(A**), yellow green and blue fluorescence is emitted by flavonoids; stained with Dragendorff reagent (**B**) reddish-orange colour indicates alkaloids; Sudan IV (**C**) stains red lipids, in this case the cuticle and Nadi reagent (**D**) stains purple terpenoid substances (epidermal cell walls and vascular tissue). **ad**: adaxial side of leaf, **ab**: abaxial side of leaf, **asc**: astrosclereid, **sg**: salt gland, **ep**: cell wall of epidermal cells, **cu**: cuticle.

herbivores and therefore suitable for ornamental use in modern urban and suburban areas such as archeological sites, restoration of quarries, roof gardens, and other disturbed areas.

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