

Paolo Colombo

Morpho-anatomical and taxonomical remarks on *Limonium* (*Plumbaginaceae*) in Sicily

Abstract

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The morpho-anatomical adaptations of *Limonium cosyrense* (Guss.) Kuntze, *L. tenuiculium* (Tineo) Pign., *L. bocconeii* (Lojac.) Litard., *L. flagellare* (Lojac.) Brullo, *L. ponzoi* (Fiori & Bég.) Brullo, *L. syracusanum* Brullo, *L. panormitanum* (Tod.) Pign., *L. albidum* (Guss.) Pign., *L. lopadusanum* Brullo, *L. hyblaeum* Brullo, *L. intermedium* (Guss.) Brullo, *L. mazararum* Pign., *L. virgatum* (Willd.) Fourr., *L. pignattii* Brullo & Di Martino, *L. catanzaroi* Brullo, *L. aegusae* Brullo, *L. secundirameum* (Lojac.) Brullo, *L. todaroanum* Raimondo & Pignatti, *L. minutiflorum* (Guss.) Kuntze, *L. lojacconi* Brullo, *L. melancolicum* Brullo, Marcenò & Romano, *L. optimae* Raimondo, *L. calcarum* (Tod.) Pign., *L. sinuatum* (L.) Miller, *L. serotinum* (Rchb.) Pign. are illustrated here. An explanatory table is also included showing ecological data pertaining to the above species and to their foliar organization.

Introduction

The morphological adaptations of plants to the environment confirm that the anatomical structures can intermix (Evert 1961; Dikison & al. 1978) and transform plants living under particular environmental conditions such as dry-windy climates (Stocker 1960), salty (Poljakoff 1975), rocky habitats etc. In the frame of researches on the endemics to Sicily and its archipelago, (Colombo & al. 1979, 1980, Marcenò & al. 1991a, 1991b, Trapani & Colombo 1992, Trapani & al. 1991), several studies specifically dealing with the anatomy of some species of *Limonium* Miller (Colombo & Trapani 1991, 1992a, 1992b, 1996, Trapani & Colombo 1997, Trapani & al. 1996, 1997) had been carried out. Here a comprehensive analysis of such genus is introduced. With this aim, *Limonium cosyrense* (Guss.) Kuntze, *L. tenuiculium* (Tineo) Pign., *L. bocconeii* (Lojac.) Litard., *L. flagellare* (Lojac.) Brullo, *L. ponzoi* (Fiori & Bég.) Brullo, *L. syracusanum* Brullo, *L. panormitanum* (Tod.) Pign., *L. albidum* (Guss.) Pign., *L. lopadusanum* Brullo, *L. hyblaeum* Brullo, *L. intermedium* (Guss.) Brullo, *L. mazararum* Pign., *L. virgatum* (Willd.) Fourr., *L. pignattii* Brullo & Di Martino, *L. catanzaroi* Brullo, *L. aegusae* Brullo, *L. secundirameum* (Lojac.) Brullo, *L. todaroanum* Raimondo & Pignatti, *L. minutiflorum* (Guss.) Kuntze, *L. lojacconi* Brullo, *L. melancolicum* Brullo, Marcenò & Romano, *L. optimae* Raimondo, *L. calcarum*

(Tod.) Pign., *L. sinuatum* (L.) Miller, *L. serotinum* (Rchb.) Pign. have been taken into consideration by analysing their morpho-anatomical characters.

As far as systematics and taxonomy of these species are concerned, particular reference to Brullo (1980) and Pignatti (1982) is made.

Pignatti (1982), taking into account that it is a hard polymorphous genus characterised by different levels of ploidy ($2n$, $3n$ and $4n$), divided *Limonium* in different "groups". This led Dolcher and Pignatti (1971) and later on Erben (1978) to hypothesize that triploids, being generated from the crossing of a diploid gamete with an aploid one, are to be considered apomictic. Consequently different subgroups have been defined, including agamospecies morphologically almost undifferentiated and endemic to very restricted areas. Some of these species with a very local occurrence in Sicily have recently been described (Raimondo 1993, Raimondo & Pignatti, 1986, Brullo & al. 1996). Aim of this work is to find additional anatomical characters suitable for a better discrimination of the groups and subgroups currently used in the above mentioned works.

Material and methods

A special attention has been devoted to the study of the leaf since it is particularly important in the ecophysiological evaluation and shows an excellent model of coordination between the network of veins and the conduction efficiency. The length of the xylematic elements allows us to speculate on the ecological adaptation of species (Carlquist, 1977). As a matter of fact both leaves and stems are nowadays considered as integrated elements of the "bud system". But considering that the anatomical structure of the stem and the panicle axes are almost undifferentiated and unsuitable from the taxonomical point of view, the present research concerns only the leaves, although some complementary details of the root and the panicle are also given.

The observations thus concern:

1. parameters of the epidermal cells;
 2. anatomy of the lamina and the related parts;
 3. foliar architecture;
 4. sclerenchimatous idioblasts.
1. By "parameters" of the epidermal cells we mean the length, the width and the thickness of the leaf epidermal cells, their number by mm^2 and the index of stomata. These parameters can be represented graphically in order to evaluate the overall differences among the different taxa. The resulting data have been obtained through epidermal replications, and have been confirmed by S.E.M. observations (Appendix 1).
 2. In order to illustrate and comment the anatomy of leaf and other related organ, the data have been obtained through staining-fresh sections, sections of the plant dipped in paraffin, discolorations through the classic histological methods (Safranin, Light green, Methyl green, Phloroglucin+ HCl, Zinc chloro-iodide, Sudan III etc.).
 3. The foliar architecture is proposed as a remarkable discriminating character for the species of *Limonium* here treated. The expression "foliar architecture" is used by Hickey (1971, 1973) with respect to the leaf and its shape, position of the elements mak-

ing up the structure of the leaf itself, the pattern of veins, the margins, the shape and size of the lamina, the position of the glands, etc. The foliar architecture hence represents the whole of the spatial organisation of the leaf and of the elements with regard to their histology, function, origin and homology. It is remarkable since the dicotyledonous leaves have special and well morphologically defined characteristics that are constant at every level, from the subclass to the species rank (Hickey & Wolfe, 1975).

The microscopic samples showing the foliar architecture were obtained by discoloration of the materials and by mounting the sections on cotton blue and on lactophenol in order to make the tridimensional vision of the sclereids more accessible. Subsequently the discoloured parts and the sections have been dehydrated through ethylic alcohol in progressing volume, then coloured with safranin, mounted on Canadian balsam and protected under sampling glass.

4. Generally speaking, almost all of the observed taxa show the presence of "idioblasts" in compound with the free branches of the areolas. The typology of the idioblasts has been studied by dipping some small pieces of the leaf in glycerine gel Johnsen (1940). The observed sclereids are typical idioblasts distributed along the different parts of the plant. Thanks to the thickness of their secondary walls, they give the plant a more solid and compact aspect. According to Tschirch (1985), the sclereids show a thick and tough texture. Francken (1890) argues that the brachisclereids very often have mechanical functions; Haberbladt (1914) asserts that the brachisclereids are structured so that they can strengthen the foliar architecture and thus help the plant to survive for longer periods of time. According to Stevens (1924), the sclereids "offer the plant a mechanical solution against the environmental impact by helping them to increase in size"

For the purposes of this study for each of the species taken into account specimens of comparatively uniform size were collected from the natural habitats in the period of their maximum vegetative development. The observations were made by taking comparable segments of the root and of the flowering stems and five leaves of different age from the middle part of the rosette. The materials were observed both as fresh sections and as paraffin preparations. The fresh sections were stained with safranin, zinc chloro-iodide, Lugol's solution, sudan III and ferric chloride; the ones prepared with paraffin were fixed in FAA, dehydrated and later stained with alcoholic safranin. The observations at the S.E.M. were made after pre-treatment at critical point. A group of leaves was fixed, cleared with 6% NaOH and stained with 1% alcoholic solution of safranin in order to study the xylem architecture. Some samples were covered with nail-polish to make prints that would show the morphological characteristics.

Measurements were taken with a micrometric ocular inserted on a Reichert Jung Microstar microscope. The anatomical terms are used according to Esaú (1965), while the leaf vascular terminology is that of Hickey (1973).

Results

Root

The root of the observed samples is made up of a multi-layered periderm with thick-walled cells containing tannin. The bark contains thin-walled reserve parenchymal cells.

The thickness varies according to the species and is interrupted by externally scattered schlerenchimatic islets that verging towards the centre form a unique and continuous ring. The number of the islets, their position and size vary depending on the species and on the age of single individuals but they do not have taxonomical value.

The phloem is made up on the external part of a continuous layer of thin proto and metaphloem elements and on the internal part of a great number of conducting elements with parenchymal cells and very few fibres. The cambium zone follows with some layers of meristematic cells and the secondary xylem at the furthest part which is made up of very small and yet well lignified vases running towards the outer part of the leaf and increasing in size towards the centre of the leaf. The parenchyma and the pith rays are very small or absent. The pith is generally small with parenchymal elements rich in tannin. The central part is generally made up of a combination of schlerentimatic cells with a thick wall.

Panicle

If the differences in the roots of the species are of little importance, the ones in the axe are even insignificant: a one-layered epidermis is always present with a thickly corrugated skin reaching the cellular walls (Fig. 2). The stomata and the salty glands are small and oblong due to the small size of the organ and its curvy shape. Beneath the epidermis is a chlorophyll parenchyma consisting of round cells with narrow intercellular spaces. Underneath that is a rich multi-layered schlerentimatic ring with narrow external vessels broad inner ones.

Cortical bundles are present in the chlorenchyma, on the external part of the sheath or nearby. Sometimes the sheath can reach the layers and forms broad bundles of cells with consistent walls similar to pith rays. The conduction apparatus is made up of closed pith bundles enclosed in a parenchymal sheath rich in tannoids. These bundles are generally more or less numerous depending on the axial diameter and are made up of proto and metaxylem elements forming a V-shape. Protoxylem is located in the inner part of this V-shaped formation. The phloematic elements are contiguous to the xylem and inside the V.

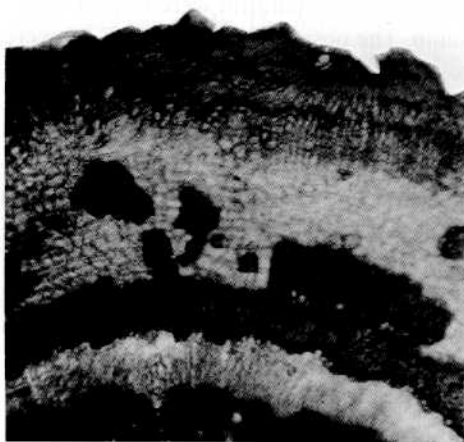


Fig. 1. *L. flagellare*: transverse section of the root.

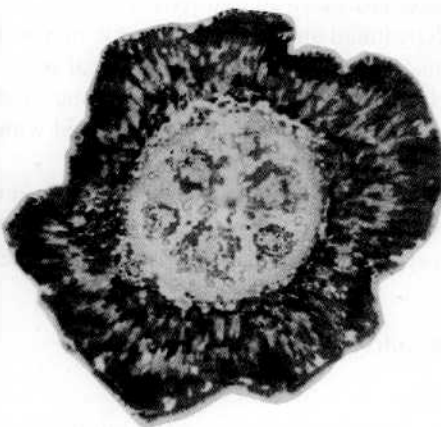


Fig. 2. *L. bocconeii*: transverse section panicle axis.

The pith can be observed, occupying the central part of the axis and is made up of thick parenchymal cells rich in tannin and some lignified ones.

Leaf

The most significant characteristic of the leaf is the presence of the Mettenius glands occupying the entire portion of the foliar lamina. These glands are slightly concave and surrounded by a crown of convex epidermal cells of evident and some tunnel-like ornamentations that are more evident on the upper blade and rather shallow on the lower one. They occur irregularly being small in number and large on the upper blade whereas they are small in size and numerous on the lower leaf. In table 1 the number, the position and the size of the glands are shown.

The leaf discoloured shows the cuticular structure of the glands. Its lower part is convex towards the mesophyll while the upper one is flat or slightly concave (Fig. 3).

Generally the structure of each gland resembles a cup, from its round brim four thin supporting columns branch off meeting in the basal part and forming a characteristic anchor shape.

The internal part consists of 12 to 16 cells (examples of 12-cell type species are *L. bocconeii*, *L. pignattii*, *L. lojacconi*; 16-cell types are *L. ponzoii*, *L. albidum*, *L. virgatum*, *L. aeu-gusae*) with different functions. For example, in the 12-cell types 4 of the cells are secretional, 4 internal and 4 external are in the shape of a calyx. Each internal cell bears a pore. The 4 pores, separated by a cross and enclosed in a ring (Fig. 4), are visible in the replicated leaf.

Outside the cutinized structure and at the base of the 12 and 16- cell groups are 4 storing cells with different shapes and functions in the different species (Fig. 5). These cells secrete the exceeding salts which are first accumulated in the storing cells and later pushed down the central part of the glands where they are elaborated and released through the above mentioned 4 pores. The salts build up on the foliar axis of the panicle so that the different species are white-greyish reflecting light and heat radiations thus helping the plant to withstand the xeroalophilous environment.

The analysis of foliar architecture shows a well defined pattern of characteristic main veins: basically the leaves are three-veined but the one-veined are also frequent whereas the pinnate rarely occur (Tab. 1). The three-veined leaves have a thicker central vein structure.

The secondary ones are commissural and smaller, running parallel to the midline towards the margins where they bisect forming thinner, second order veins. The secondary veins, that are acrodrome, imperfect-basal, form acute angles arising from the base to the apex and branching off towards the margins and thus forming heterogeneous intercostal panels. The tertiary

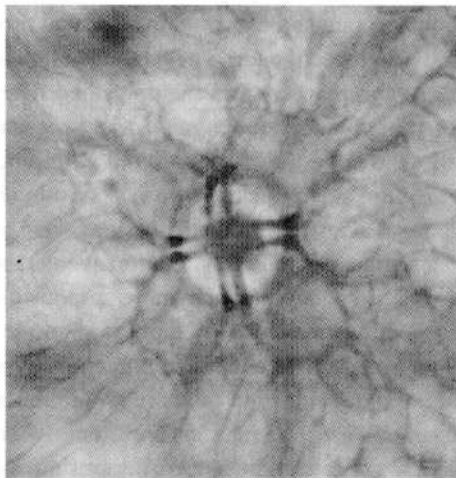


Fig. 3. Cutinized capsule in *L. panormitanum*.

