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SYSTEMATIC LEAF MORPHO-ANATOMY OF SIX *LIMONIUM (Plumbaginaceae)* SPECIES

SUMMARY

The anatomical study of the leaves of six species of *Limonium (Plumbaginaceae)* reveals features useful for the identification of species within *Limonium intermedium* group. A key for the identification of species of this group using these anatomical characters is given.

RIASSUNTO

Osservazioni sistematiche sulla morfo-anatomia fogliare di sei specie di Limonium (Plumbaginaceae). Vengono riportati i risultati di una indagine condotta sull'organizzazione morfo-anatomica delle foglie di sei specie di *Limonium (Plumbaginaceae)*, *L. albidum* (Guss.) Pignatti, *L. hyblaeum* Brullo, *L. intermedium* Brullo, *L. lopadusanum* Brullo, *L. mazarae* Pignatti e *L. panormitanum* (Tod.) Pignatti, endemiche della Sicilia.

I caratteri morfo-anatomici esaminati sono la distribuzione degli stomi, il numero degli strati del tessuto a palizzata, la forma e il tipo della guaina del fascio, la presenza/assenza e la forma delle sclereidi all'estremità dei rami liberi. Tali caratteri hanno consentito l'elaborazione di una chiave analitica per l'identificazione delle specie nell'ambito del gruppo *L. intermedium* che viene infine presentata.

Introduction

The cosmopolitan genus *Limonium* Miller is represented in the Mediterranean region by more than 250 species, mostly endemic (Greuter, Burdet & Long, 1989). The species recorded from the Italian peninsula, Sicily and

Sardinia show three levels of ploidy (2n, 3n, 4n). DOLCHER & PIGNATTI (1971) hypothesized an origin of the triploids through hybridization between diploids and tetraploids. Sucessively ERBEN (1978) suggested an apomictic origin of the triploids, due to the union of haploid gamete with a diploid one. Several agamospecies, distributed along coastal cliffs or inner salty areas, can be differentiated only by less marked characters.

A taxonomic revision of *Limonium* in Sicily has been furnished by BRULLO (1980) who recognized 39 intrageneric taxa in the island. A few more species were later described by RAIMONDO & PIGNATTI (1986) and RAIMONDO (1995).

The aim of the present paper is to evaluate the value of leaf anatomy in the systematics of six species within the *Limonium intermedium* group (i.e. *L. albidum* (Guss.) Pign., *L. lopadusanum* Brullo, *L. hyblaeum* Brullo, *L. intermedium* (Guss.) Brullo, *L. mazarae* Pign. and *L. panormitanum* (Tod.) Pign.). Following PIGNATTI (1982), *L. albidum* and *L. panormitanum* are to be regarded as the two extremes of the variability of this group in Sicily and the smaller islets of the Sicilian Channel.

DE FRAINE (1916) formerly underlined the taxonomic value of morpho-anatomical characters of the root, stem, inflorescence axis and leaf in two species of the genus *Limonium* (sub *Statice*). BOKHARI (1970) stressed the significance of shape, structure and size of leaf sclereids in the genus. Recently, COLOMBO & TRAPANI (1992), TRAPANI, COLOMBO & CHIFARI (1996) described the xeromorphic adaptations of some organs (root, inflorescence axis, leaf) of some species of *Limonium* from Sicily.

The leaves of *Limonium* are usually arranged in many-leaved to few-leaved rosettes. Some species show a reduced cushion-like habit, with leaves appressed or inserted on short branches. The leaves can be one-nerved, parallel-nerved or pinnately-nerved. Their reduced surface and xeromorphic features are to be regarded as adaptations to the coastal mediterranean climate.

MATERIALS AND METHODS

Specimens of the same size, belonging to the six investigated species, were collected in their natural habitat, in the period of their maximum vegetative development.

Observations were carried out on ten plants, using leaves of different age from the medium part of the rosette.

Both fresh material and paraffin-preserved material was studied. The fresh sections were coloured with specific stains (safranin, zinc chloroiodide, Lugol's solution, sudan III, ferric chloride) (JOHANSEN, 1940); those prepared with a paraffin technique were fixed first in FAA, then dehydrated and stained

with alcoholic safranin. A group of leaves was fixed, cleared with 6% NaOH and stained with 1% safranin alcoholic solution to study the xylematic architecture (FUCHS, 1963). Some samples were treated with transparent nail-polish to obtain leaf prints to show epidermal features. Measurements were made by a micrometric ocular inserted on a Reichert Jung Microstar microscope and the observations concerning the xylematic architecture were made under polarized light. Anatomical terms according to ESAU (1965) were adopted, while the leaf vascular terminology reported is that of HICHEY (1973).

RESULTS

All taxa have bifacial leaves, with differentiated mesophyll. The thickness of the lamina, measured halfway between the midrib and the margin, ranges in TS from 238 µm in *L. intermedium* to 428,8 µm in *L. panormitanum*.

However the shape of epidermal cells is polygonal; the abaxial epidermal cells are more irregular.

Table 1

Selected characters of leaf anatomy in the Limonium *species studied.*

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
L. albidum	3.36	1.00	341	1	1	B	245	109	56	19	2	+	268	+	+
L. hyblaeum	1.04	0.45	283	1	1	B	221	60	60	21	1	+	107	+	+
L. intermedium	3.15	0.60	238	1	2	B	167	83	56	20	2	+	116	+	0
L. lopadusanum	1.61	0.72	409	1	1	B	337	116	84	22	2	+	242	+	+
L. mazarae	2.16	0.46	308	1	1	B	235	76	76	20	1	+	104	+	+
L. panormitanum	2.40	0.70	483	1	2	B	384	154	81	35	2	+	177	+	+

Key: 1, leaf length, cm; 2, leaf width, cm; 3, lamina thickness, µm; 4, epidermis thickness (1 = adaxial thicker than abaxial); 5, stomatal distribution (1 abassial only; 2 predominantly abaxial); 6, lamina structure (b = bifaccial); 7, mesophyll thickness, µm 8, thickness of palisade chlorenchyma, µm; 9, length of palisade cells of the first row, µm; 10, width of palisade cells of the first row, µm; 11, number of palisade layers; 12, bundle sheath (− absent, + present); 13, midvein diameter, µm; 14, sclerenchyma caps (− absent; + present); 15, idioblasts in free veinelets (0 absent, + present).

As regards to the stomata, all taxa investigated show anamocitic guard cells. The stomata, placed within the epidermal cells, may occur on both surfaces in *L. intermedium* and *L. panormitanum*, while in other species are present only on the abaxial surface. The number of palisade adaxial layers ranges from one to two with cells of variable size length. One layer of palisade-like cells under the abaxial epidermis is present in *L. mazarae* (Fig. 3b); a spongy mesophyll constitued by roundish cells with reduced intercellular spaces is well

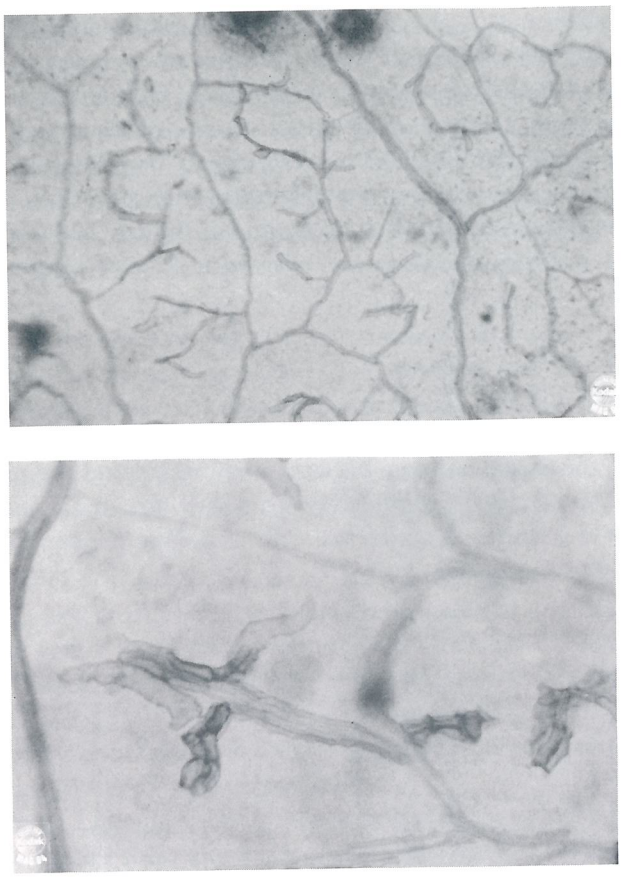

Fig. 1 — Areoles without sclereids in free veinlets in *L. intermedium* × 20 (a), with sclerotracheoids in *L. panormitanum* × 40 (b).

developed in *L. panormitanum*. The cells of the upper palisade row are oblong and their average length/width ratio ranges from 2.3 in *L. panormitanum* to 3.7 in *L. mazarae* and *L. lopadusanum*. The palisade/spongy thickness ratio ranges from 0,47 in *L. mazarae* to 1 in *L. intermedium*. The leaf margin is entire, recurved and apically pointed. The palisade cells at the margin are restricted to one of the sides. Vascular bundles are collateral, placed in one row subjacent to the palisade. The thickness of the midrib ranges from 104 to 268 μm. The profile of the adaxial and abaxial surfaces varies among the species due to protrusion of the midrib, measuring the vertical distance in μm from abaxial surface of the lamina to the extremite of midrib. More protruding midribs are found in *L. lopadusanum* and *L. albidum*, less

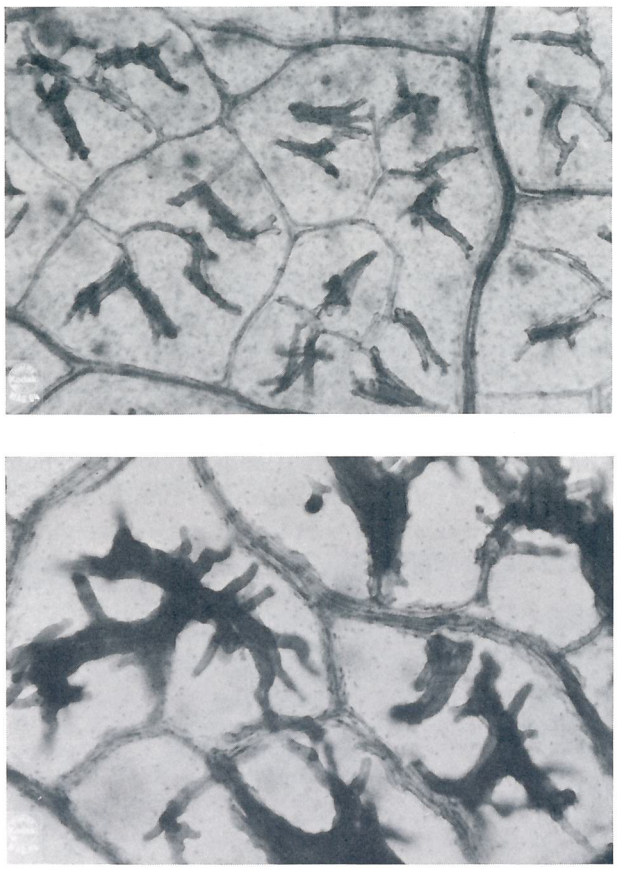

Fig. 2 — Areoles with terminal sclereids columnar ramiform in *L. albidum* × 20 (a), polymorphic in *L. lopadusanum* × 40 (b).

protrunding in *L. intermedium*. Sclerenchyma bundle caps were observed on the two extremites and a complete sheath surrounding the midvein is present in all species. In all taxa examined bundle-sheath extensions are absent. Schlerenchyma bundle caps are linked in some species by parenchymatous cells surrounding the bundle. Lateral ribs and superior veins are smaller than the midvein and have few schlerenchymatous cells.

Sclereids in free veinlets, absent in *L. intermedium* (Fig. 1a), are present in the other species with the following shapes: sclerotracheoids in *L. panormitanum* (Fig. 1b), ramiform in *L. hyblaeum* and in *L. mazarae* (Fig. 4a,b), columnar ramiform in *L. albidum* (Fig. 2a), polymorphic in *L. lopadusanum* (Fig. 2b), (Rao, 1991).

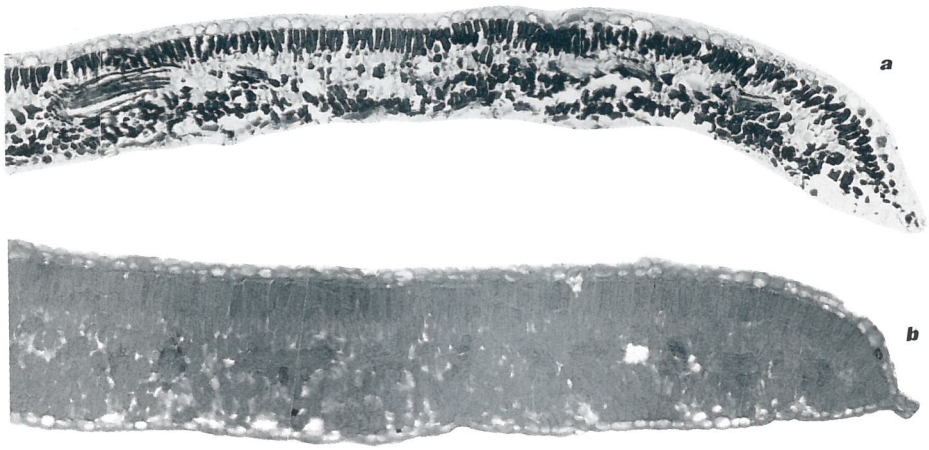

Fig. 3 — Leaf transverse sections × 10 : (a) *L. hyblaeum*, (b) *L. mazarae*.

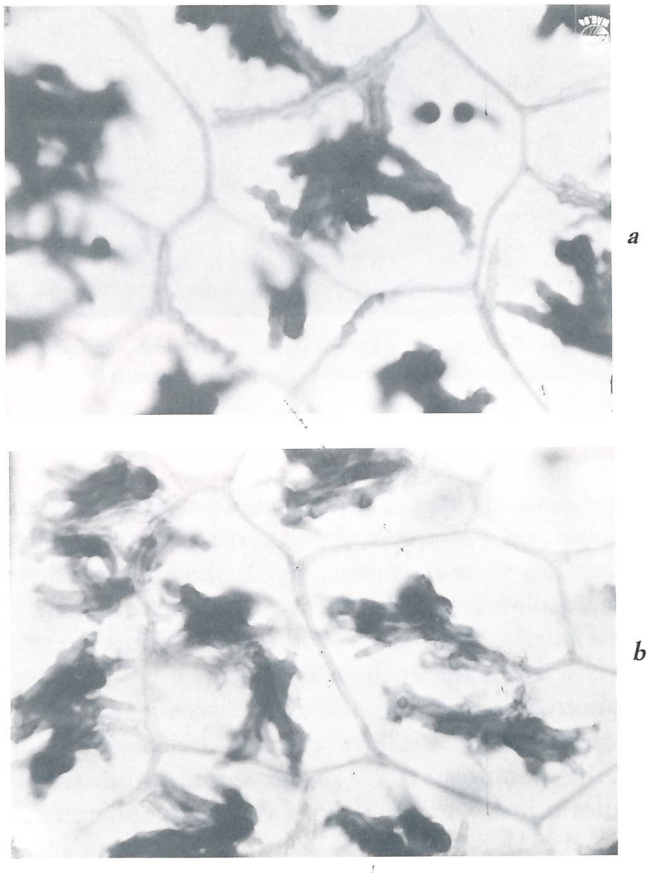

Fig. 4 — Areoles with sclereids ramiform × 40 in *L. hyblaeum* (a) and in *L. mazarae* (b).

Discussion and Conclusion

The leaves investigated provide a number of characters wich have proved to be taxonomically interesting. Epidermal cells in TS are rectangular or square, and are more numerous in adaxial epidermis. Stomata in all species are uniform and are present in both surfaces only in *L. intermedium* and *L. panormitanum*. The shape of the palisade cells under the adaxial epidermis is oblong in all species studied. The mesophyll is well differentiated in the palisade and spongy mesophyll, while in *L. mazarae* (Fig. 3b) the spongy mesophyll is reduced to the presence of one row of discontinous palisade cells under the abaxial epidermis. The upper palisade is formed by a row of cells in *L. hyblaeum* (Fig. 3a) and *L. mazarae* (Fig. 3b) and by two rows in some species. Sclerenchyma caps were observed on both sides of the midvein and the midvein is surrounded by a sclerenchymatous bundle-sheath in *L. hybaleum* and *L. lopadusanum* and by a parenchymatous bundle-sheath in the other species. The great variety of forms of terminal sclereids in the free veinlets of *Limonium* makes those characteristic valuable for taxonomy, but they must be used in conjunction with other characters (Bokhari, 1970).

A key for the identification of the species has been elaborated on the basis of the results discussed using leaf-anatomical characters.

Key for identification of species studied using leaf-anatomical characters.

1. Amphystomatic levaes	
1.1 Sclereids in free veinlets absent	*L. intermedium*
1.2 Sclereids in free veinlets present	*L. panormitanum*
2. Hypostomatic leaves	
2.1 Palisade cells 1 adaxial layer	
2.1.1 Abaxial palisade absent	*L. hyblaeum*
2.1.2 Abaxial palisade present	*L. mazarae*
2.2 Palisade cells 2 adaxial layers	
2.2.1 Terminal sclereids columnar ramiform	*L. albidum*
2.2.2 Terminal sclereids polymorphic	*L. lopadusanum*

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