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Anatomical studies on the Sicilian *Helianthemum* (Cistaceae)

Abstract

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The phytodermology on leaf and the stem anatomy were studied in *Helianthemum croceum* (Desf.) Pers., *H. canum* subsp. *nebrodense* (Heldr.) Arcang. and *H. cinereum* (Cav.) Pers.. In particular, the characters taken into account concern presence of trichomes, heterogeneity of chlorenchyma, presence of glandular hairs, epidermal foliar parameters by scanning electron and light microscopy, leaf architecture as well as stem and flower bud structure.

Introduction

In Sicily the genus *Helianthemum* Miller consists of *Helianthemum croceum* (Desf.) Pers., *H. nummularium* (L.) Miller, *H. apenninum* (L.) Miller, *H. sessiliflorum* (Desf.) Pers., *H. ledifolium* (L.) Miller, *H. salicifolium* (L.) Miller, *H. aegyptianum* (L.) Miller, *H. canum* subsp. *nebrodense* (Heldr.) Arcang., *H. cinereum* (Cav.) Pers. (Pignatti 1982); three of these species, i.e. *H. croceum*, *H. canum* subsp. *nebrodense* and *H. cinereum*, are all subshrubby chasmophytes occurring in the meadows and on the slopes of the northern mountainous ranges between Peloritani and the Madonie mountains, above 500 m a.s.l.

Helianthemum croceum has twisted and woody stems with white-tomentose, ascending branches; basal leaves are almost round, while the upper ones are elliptic-lanceolate, slightly revolute on the margin and white-tomentose on the lower blade.

Helianthemum canum subsp. *nebrodense* has woody, twisted basal stems, tightly entangled; the leaves are small and white-tomentose with hairs tightly interwoven.

Helianthemum cinereum has woody basal stems which are more or less grey-tomentose and often reddish in the upper part; petiolate leaves with ovate-elliptic blade rounded at the base, sub-glabrous on the upper surface and white-tomentose on the lower one.

In this paper the leaf morphology is analysed and compared in *Helianthemum croceum* (Desf.) Pers., *Helianthemum canum* subsp. *nebrodense* (Heldr.) Arcang. and *Helianthemum cinereum* (Cav.) Pers. with the aim to find anatomical features possibly suitable in the taxonomical delimitation of the genus *Helianthemum*, whose uncertain characters are well known. (Pignatti 1982).

Materials and methods

The observed materials were collected at the typical localities where the species occur. After some preliminary staining-fresh observations with relative discolouration, the materials were fixed in F.A. and later dehydrated, coloured with safranin or light green and dipped in paraffin. The 10-15 μm thin sections were obtained using a revolving type microtome and were later mounted on Canada balsam. The observations consist in determining both the number and the size of the epidermic cells, the number and the position of the stomata, the presence of hairs, the thickness and the number of strata forming the epiphyllous and the hypophyllus as well as the thickness of the leaf, of the mesophyll, of the palisade and of the spongy parenchyma. In order to highlight the fine pattern of veinlets in the leaves, the Fuchs method (1963) was applied. The terminology employed for the pattern of veins is that of Hickey (1973), whereas the anatomical terms are those employed by Easù (1965). The samples were later brought to critical point, metal sprayed and observed at the S.E.M.

Result

Leaf micromorphology of the three *Helianthemum* species was obtained by means of epidermal replications of the only upper surface, the lower one, in fact, being markedly tomentose, was studied at the S.E.M.

In *H. croceum*, (Fig. 1a, 1b) cells are present in large number (2922 by mm^2) while *H.*

Table 1. Epidermal foliar parameters (upper blade) of Sicilian *Helianthemum*: data and plots.

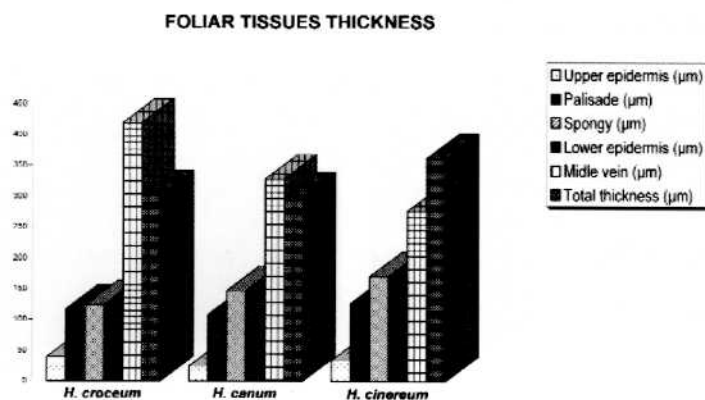
	<i>H. croceum</i>	<i>H. canum</i>	<i>H. cinereum</i>
N. cells $\times \text{mm}^2$	2922.4	2973	2888
N. stomata $\times \text{mm}^2$	274	570	468
Length cells (μm)	57.5	50.7	50
Width cells (μm)	30.5	30	37
Length stomata (μm)	32.5	37.5	35
Width stomata (μm)	22.5	26.5	25

EPIDERMAL FOLIAR PARAMETERS

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Table 2. Foliar tissues thickness (μm) of Sicilian *Helianthemum*: data and plot.

	<i>H. croceum</i>	<i>H. canum</i>	<i>H. cinereum</i>
Upper epidermis	39.5	24	33.5
Palisade	117	108	126
Spongy	123	147.5	170
Lower epidermis	22.5	19.9	33
Middle vein	419	328	276.5
Total thickness	301	299.5	362



canum and *H. cinereum* show slightly lower values (2888 by mm^2). The number of stomata is extremely variable: they are in a relatively low number in *H. croceum* (274 by mm^2) while higher values are present in *H. cinereum* (468 by mm^2) and *H. canum* (570 by mm^2); length and width of the epidermal cells are totally comparable in the three species, as well as length and width of the stomata (Table 1). Observed at S.E.M., *H. croceum* shows anomocytical stomata on both leaf surfaces (usually four epidermal cells surround each stoma), isodiametrical epidermal cells with more or less undulate walls, presence of sub-epidermal mucilage, hairs in small number (15 by mm^2) on the upper surface, with deeply-rooted foot into the epidermal thickness and the body made up of 2 to 4, smooth and on average 470 μm -long branching (Fig. 1c, 1d, 1e, 1f). The hair amount considerably increases towards the rounded leaf margin. The lower surface is instead markedly tomentose and whitish, with stellate hairs and a large number of branches (Fig. 1g-1h); such hairs are tightly interlaced; observed at the S.E.M., through the thick tomentum the stomata appear broader than in the lower surface.

H. canum subsp. *nebrodense*: amphistomatic leaf, with epidermal structure very similar to *H. croceum* (Fig. 2a, 2b, 2c); anomocytical stomata on both leaf surfaces (Table 1), hairs on the upper surface (16 by mm^2), slightly lignified at the base and cellulosic at the apex

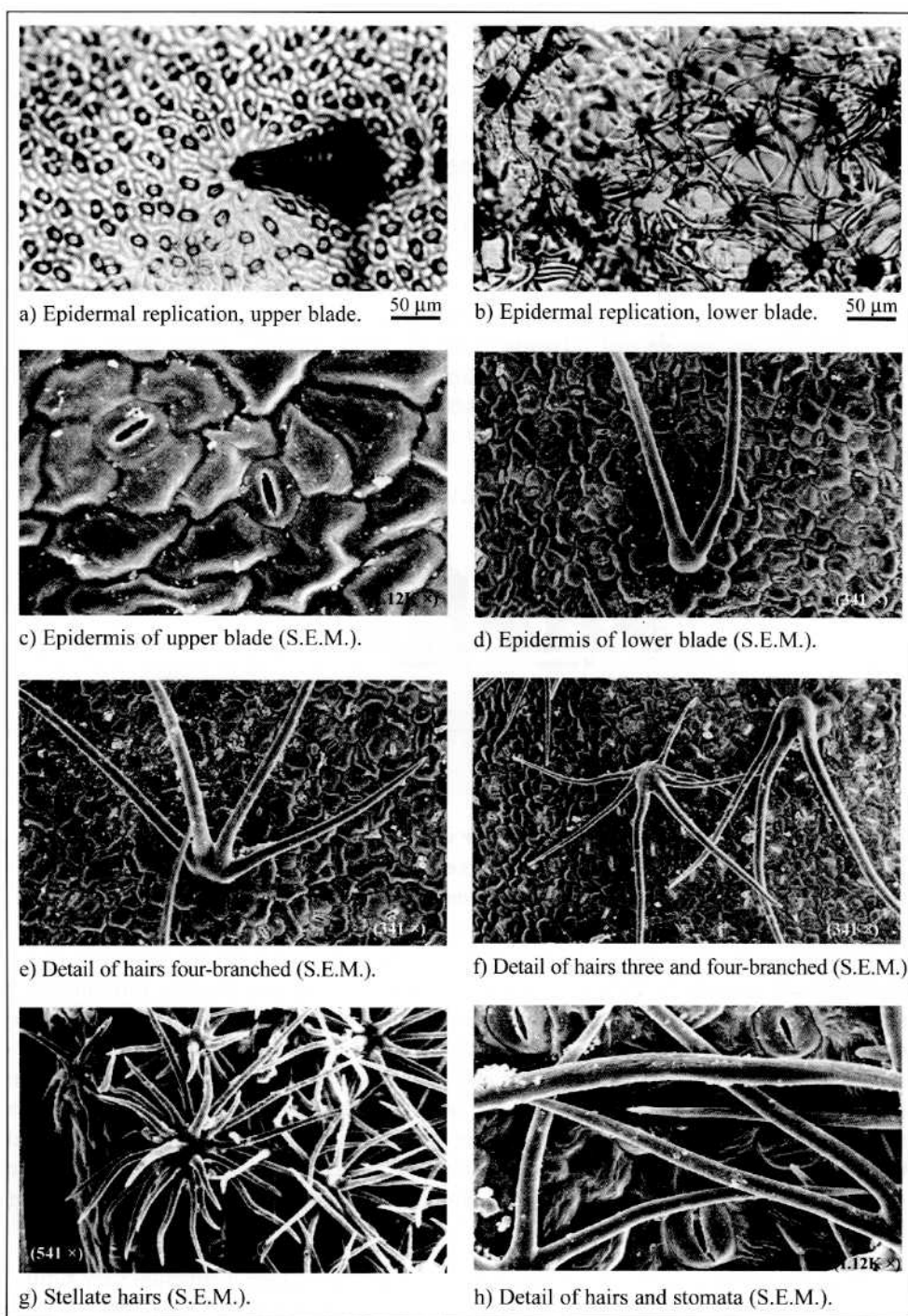


Fig. 1. *Helianthemum croceum*: leaf micromorphology.

as shown by the Schultze solution treatment. Hairs on the upper surface are either two- or four-branched as well as closer and longer than in *H. croceum* (Fig. 2d). The leaf lower surface is markedly tomentose with stellate hairs with a greater number of thinner and longer branches (Fig. 2e) than in *H. croceum*.

H. cinereum: subglabrous epidermis with scanty branched hairs (5 by mm^2), usually either two- or three-branched, shorter (294 μm) than the above examined ones (Fig. 3a,

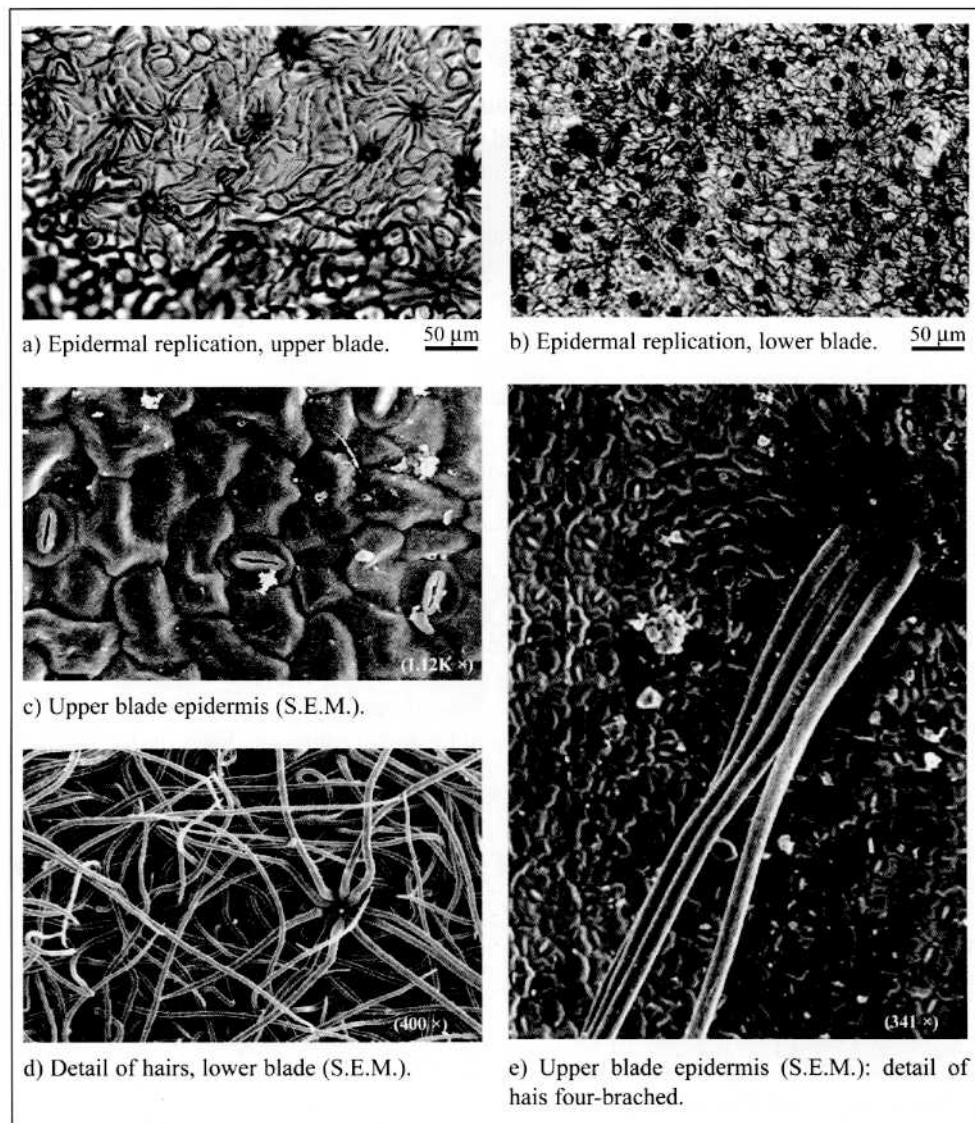


Fig. 2. *Helianthemum canum* subsp. *nebrodense*: leaf micromorphology.

3b). The scaly cuticle and the stomata are strongly cutinized and slightly protruding respect to the epidermal surface. On the lower surface, the hairs are both stellate and two- or three-branched, accordingly they can be defined as hair complex, following Watson and Dallwitz (1992). The leaf is amphistomatic with anomocytical stomata.

In the transverse section, *H. croceum*, *canum* e *cinereum* leaves are dorsiventral with cystoliths in the mesophyll, broad midrib, protruding towards the lower surface, minor leaf veins with phloem transfer cells. Values concerning the epidermal, palisade and spongy thickness are listed in Table 2. Margins are rounded and slightly revolute.

Leaf architecture

H. croceum: lower leaves ovate with obtuse basal part and pointed, somewhat obtuse apex (Fig. 7a, 7b). Strong midrib with lignified sheath where four pairs of secondary veinlets, forming an acute angle with slightly waved trend, depart. Camptodrome veining developing broad and almost regular inter-ribbing panels. Third-, fourth- and fifth-rank veins are present and scarcely characterized with a randomly netted arrangement. Small and irregular areoles with branches one- or two-branched with the ending tracheids scarcely lignified. The narrow and elongate upper leaves have the midrib scarcely lignified with parenchymatic sheath. Secondary veining (six pairs) is thinned and the third-rank one barely distinguishable.

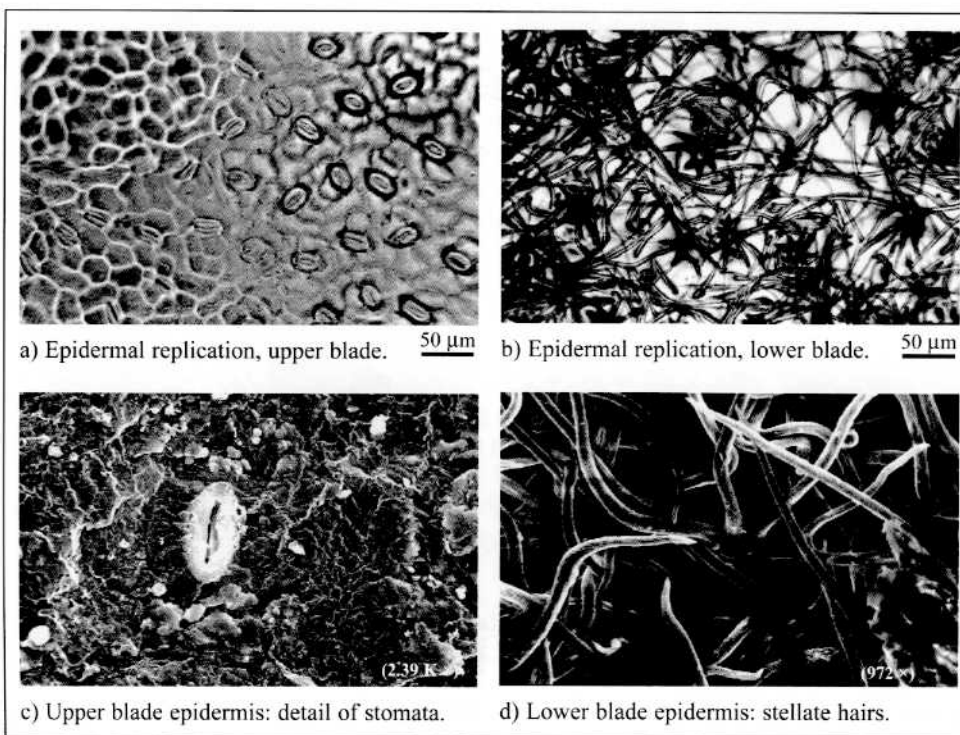


Fig. 3. *Helianthemum cinereum*: leaf micromorphology.

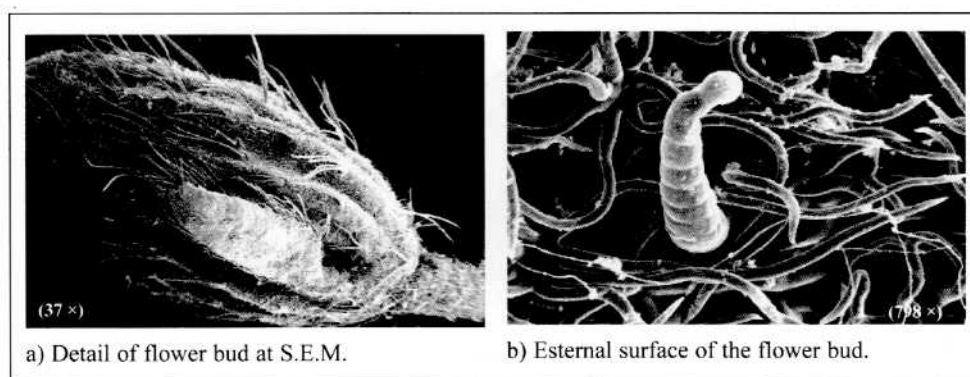


Fig. 4. *H. canum* subsp. *nebrodense*: flower bud micromorphology.

The areoles are very small and incomplete with one-branched free branches poorly lignified.

H. canum subsp. *nebrodense*: has ovate-subrounded leaves, with the midrib surrounded by a scarcely lignified sheath from which at least three pairs of second-rank, camptodrome veining branch off (Fig. 7c). The panels, originated from second-rank veining, are regular

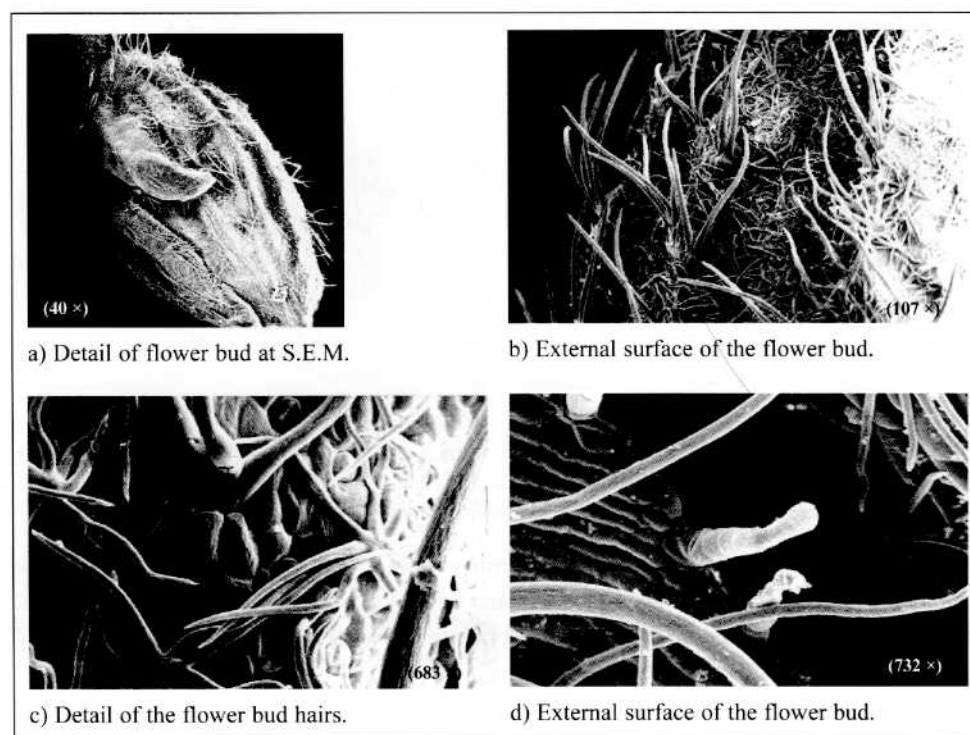


Fig. 5. *Helianthemum cinereum*: flower bud micromorphology.

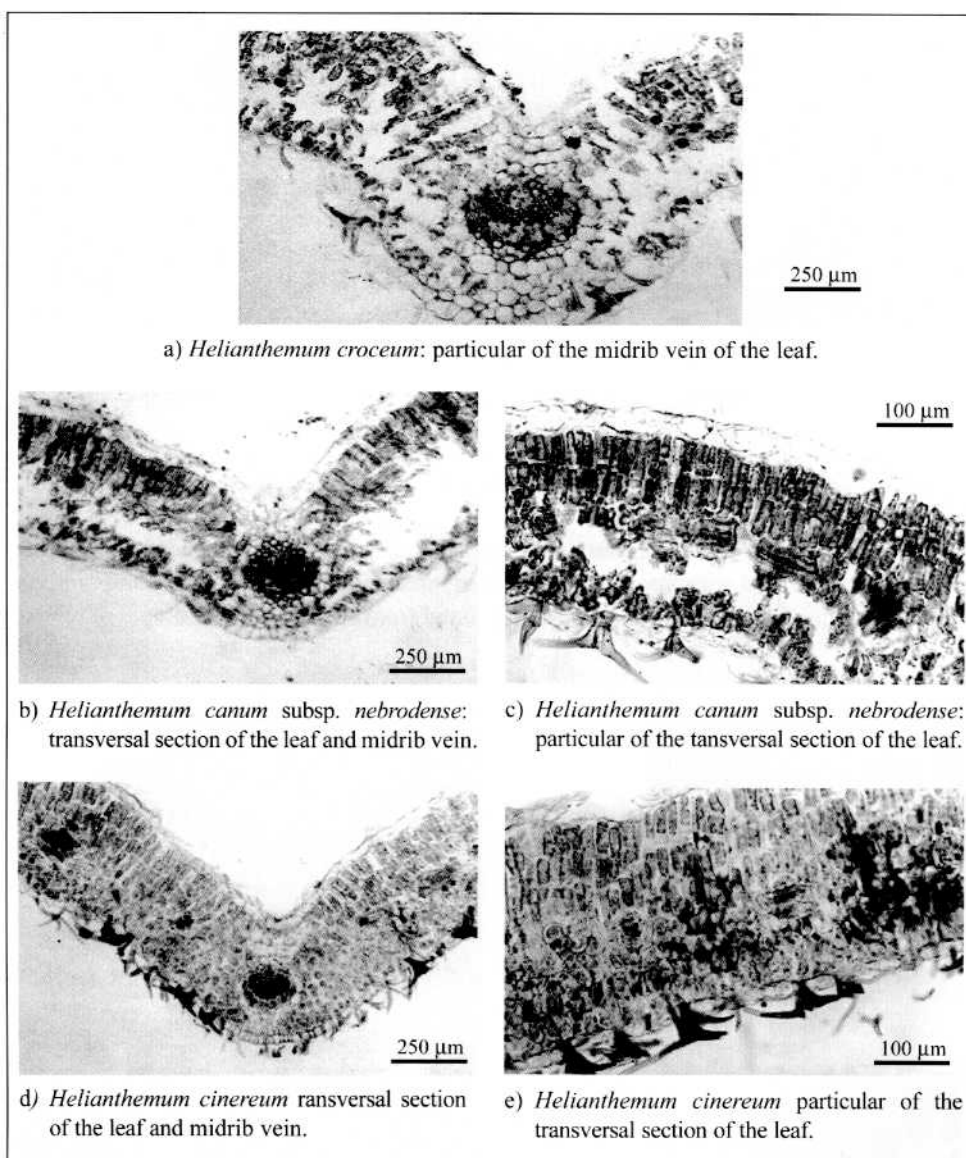


Fig. 6. Transverse section of the foliar lamina in some Sicilian *Helianthemum*.

and symmetrical. Third-, fourth-rank and minor veining are visible. On the whole, the xylematic pattern is poorly characterized and randomly netted; the areoles are small, polygonal, incomplete, with one- or two-branched free branches including short and scarcely lignified ending tracheids.

H. cinereum: the epidermal replications show 2888 cells by mm^2 . Stomata, present on both the leaf surfaces, are more evident on the subglabrous upper surface (468 by mm^2).

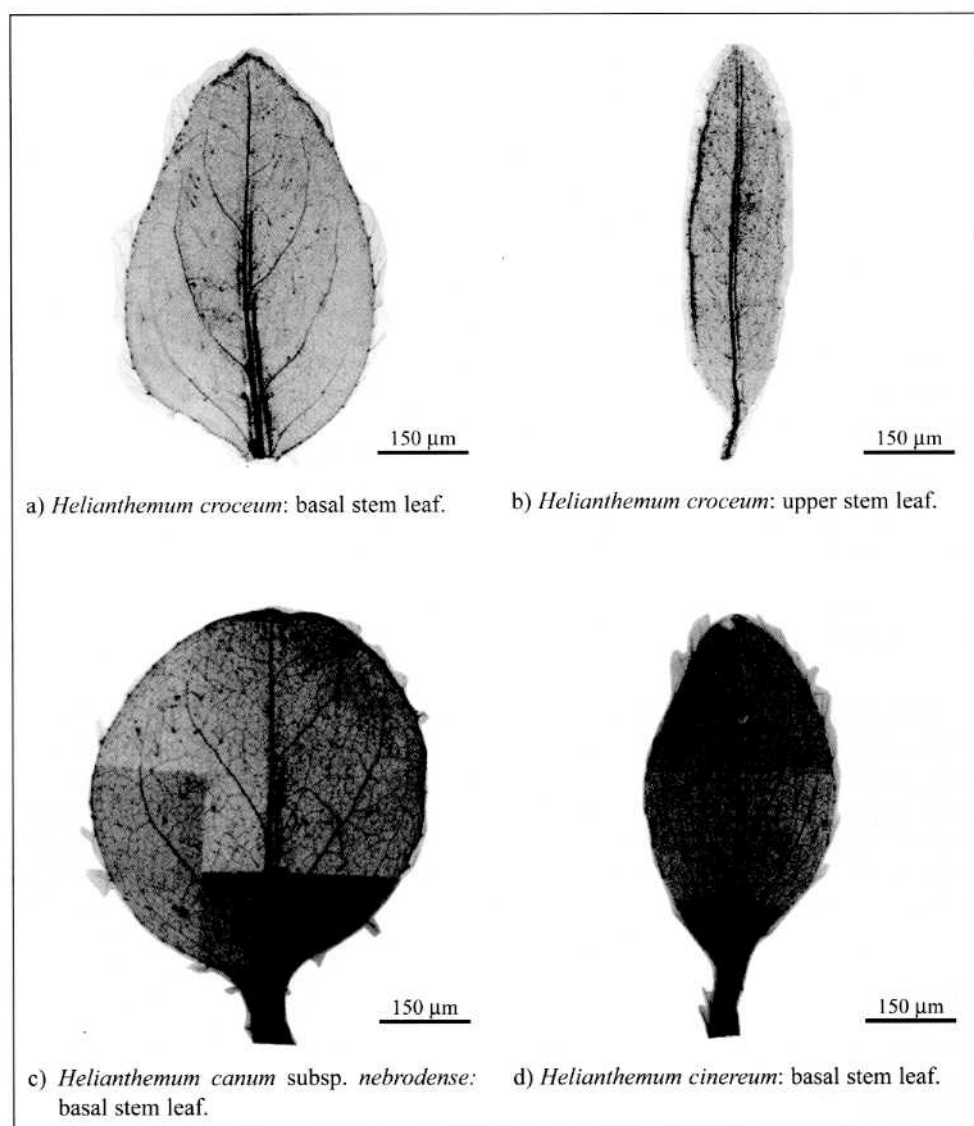


Fig. 7. Leaf architecture in some Sicilian *Helianthemum*.

On the white-tomentose lower surface, instead, they are not clearly visible and their presence has been confirmed by means S.E.M. observation through tomentum discontinuity. Further values concerning epidermal parameters are listed in Table 1. Leaves are amphistomatic, with anomocytical stomata and rare hairs, with a deeply-rooted foot into the epidermal thickness from which 2 or 3 very long branches originate. Hairs on the lower surface are either peltate or stellate. At S.E.M. the upper surface shows a scaled cuticle and slightly protruding stomata with strongly cutinized cleft (Fig. 3c). Hairs on the lower surface are

either stellate or peltate, white-tomentose and tightly interwoven (Fig. 3d). The leaf architecture shows ovate-elliptic blade rounded at the base. After clarification (Fig. 7d) the leaf reveals the first-rank midrib with lignified sheath at the base and a parenchymatic one in the 2/3 left. From this latter 3-4 pairs of lignified second-rank veins branch off delimiting regular panels where scarcely lignified third- and fourth-rank veining are visible. The areoles are of medium size and irregular with either one- or two-branched free branches with short and lignified ending tracheids. Minor veining are present.

Leaf anatomy

In the transverse section (Fig. 6a, 6b, 6c, 6d, 6e), the *Helianthemum croceum*, *H. canum* subsp. *nebrodense* and *H. cinereum* leaves are dorsiventral with monostratified epidermis, palisade two-stratified both in the *croceum* and in the *canum*, three-stratified in the *H. cinereum*, the spongy tissue thickness varies in the three species and is deeper in the *H. cinereum*, the lower palisade is generally monostratified in the three species. Cluster crystals abundant in the mesophyll. The midrib is present in examined species and its thickness ranges between 301 μm in the *H. croceum* and 362 μm in the *H. cinereum*.

Stem anatomy

Young stems cylindrical, cork cambium present formed by lower ringed corkcambium. The cortex is spongy but water-storage tissue frequently occurs in its inner part especially in the endodermal region. Pericycle including small strands of thick-walled fibres apparently more numerous. Xilem and phloem present in the form of a closed cylinder surrounding the pith. Vessel small, with simple perforation. Wood fibres with abundant bordered pits cluster crystals abundant. Solitary type less frequent. Pith is made up of broad thin-walled cells with cluster crystals.

Flower bud

Observed at the S.E.M., the *H. canum* (Fig. 4a, 4b) flower bud showed complexly tomentose bracts, on the external side, with thicker basal covering including multi-branched stellate hairs mixed with very long hairs in cluster with a varying number of branching (2 to 4). They are also numerous on the bracteoles' margin of the involucre and less abundant along the midrib of each bract; mixed with the whole hair cover, also short glandular hairs, articulated with a swollen head, can be observed.

The *H. cinereum* flower (Fig. 5a, 5b, 5c, 5d) bud shows bracteoles whose structure is very similar to the *H. canum* one, but with branched and stellate hairs in minor number; these are usually two-branched, always on the margin and along the midrib of the bracteoles. Glandular hairs, mixed with them, are in minor number, less articulated and with more elongated head. The transverse section of *H. croceum* flower bud at its different organisation stages is shown in Fig. 8. The flower bud apical section (Fig. 8a) displays the twisted and tightly interwoven flower involucres; Fig. 8b illustrates the calyx prefloration in

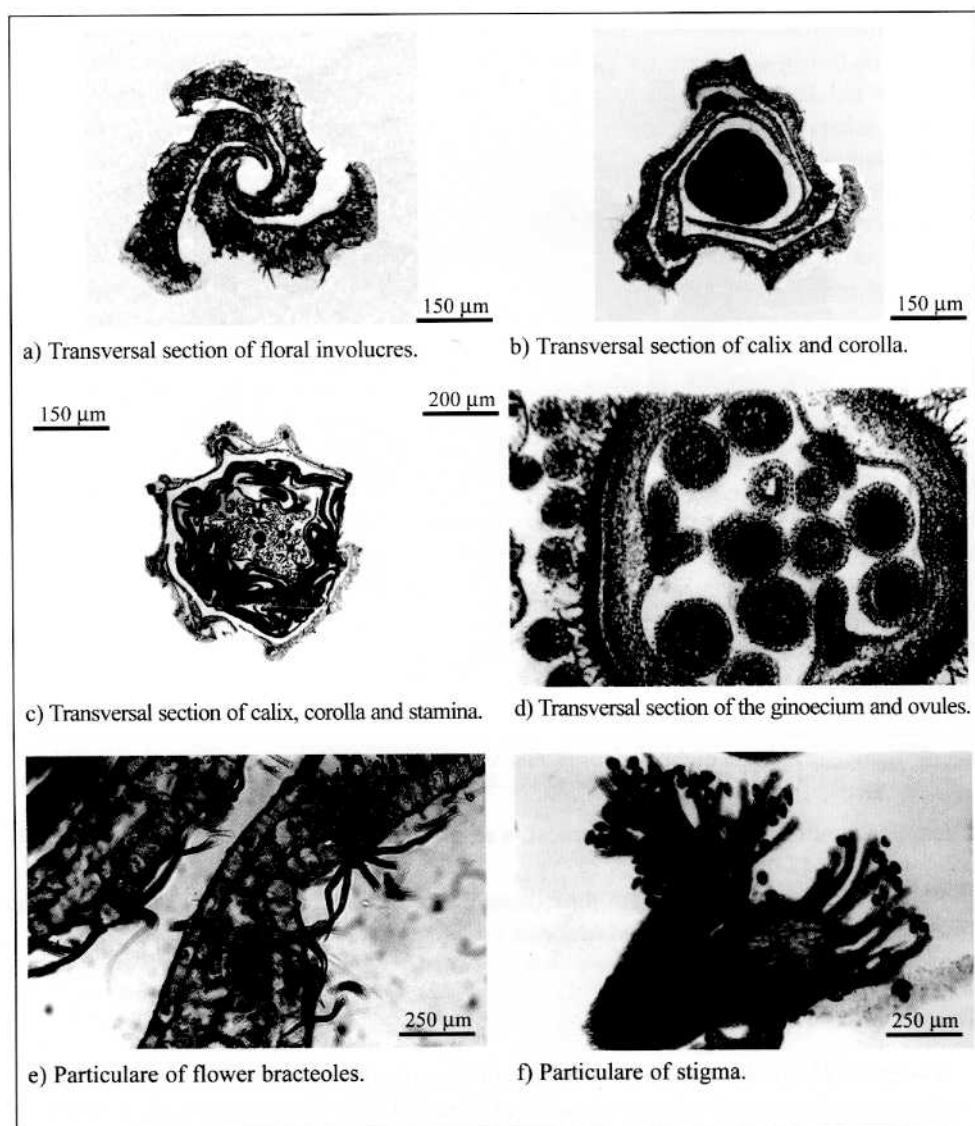


Fig. 8. Transversal section of the flower bud of *H. croceum*.

expansion with the imbricate involucre; while the corolla with the tightly closed petals is in centripetal position before the flower bud opening. Fig. 8c shows the basal part of the floral bud while calyx and corolla are clearly distending; in the middle, the androecium with four-sporanged fertile stamens are distinctly detectable.

Anthers are characterized by dehiscence longitudinal, epidermis persistent, microsporogenesis simultaneous, wall initially with one middle layer, tapetum glandular.

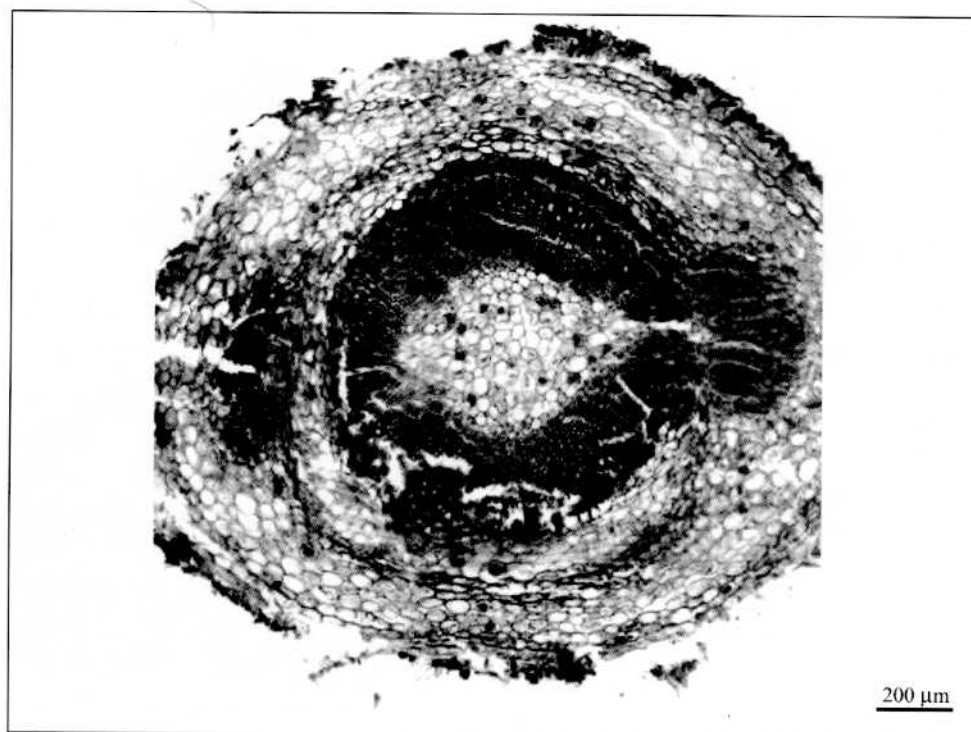


Fig. 9. Transversal section of the stem of *H. cinereum*.

The gynoecium is stilted (Fig. 8d) apical, a single stigma minute. Ovules are included in a single cavity 13-15.

The carpel involucres, as well as the external flower bracts, have glandular hairs different from the above described *H. croceum* ones.

Discussion and conclusions

The genus *Helianthemum* Miller is not a fully known group owing to uncertain characters, confusing synonymy and extreme variability (Pignatti 1982). The present study is therefore a contribution to the solution of some problems linked to the morpho-anatomy of the species here examined. The epidermal replications and the S.E.M. images provided data about shape and size of the epidermal cells as well as their number by per mm²; quantity of stomata by mm² and their size (length and width); quantity of hairs by mm². The observations at the S.E.M. evidenced the general appearance of the epidermis, the shape and the structure of the hairs both on the upper and lower surface, the shape and position of the glandular, stellate and non-stellate hairs both on the leaf and the bracts of the flower bud. The epidermal cells of *H. croceum* are isodiametrical, with moderately waved margin, almost smooth, slightly convex and in large number by mm². The hairs are two-, three, four-branched on the upper surface with branches markedly diverging and stellate with numerous

short branching on the lower surface; in *H. canum* subsp. *nebrodense* the epidermal cells are always isodiametrical, more numerous by mm² than *H. croceum*, less waved and slightly more convex. The hairs on the upper surface are three- and four-branched, but with very close branches. The ones on the lower surface are, instead, stellate, more regular and with longer branching. *H. cinereum* has shorter, less convex, isodiametrical epidermal cells, in minor number by mm², with a particularly scaled cuticle and an intermediate number of stomata between *H. croceum* e *H. canum* subsp. *nebrodense*, but with distinctly cutinized cleft; the hairs are two-, three-branched on the upper surface, while they are stellate but with few stout branches on the lower one. In the transverse section, the leaves are dorsiventral with very respectively thick midrib and a variable number of palisade tissue layers: two in *H. croceum* and *H. canum*, three in *H. cinereum*. All leaves have a thin palisade layer contiguous to the lower epidermis and oxalate crystals widespread in the mesophyll. Such organisation is typical of leaves under strong insulation degree. The species in question are clearly xerophytic showing a large number of both epidermal cells and stomata by mm², a reduced leaf surface, the mesophyll thickness, many layers of palisade and the widespread of the xylematic pattern. The hairs play an important role in protecting the leaves of all the examined species and, being unevenly spread on both the leaf surface confirm the shift in response to the different insulation degrees. Accordingly, early in the morning the leaves take the orthogonal sunbeams to the leaf surface, instead, during the insulation peak they are arranged in orthotropous position to take the parallel sunbeams to their surface. The lower surface points outwards, being more tomentose and whitish and, therefore, protecting the stomata against excessive transpiration. As a consequence, an "air space" develops between the leaf surface and the interlacement of stellate hairs, determining an appropriate humidity degree reducing further H₂O loss.

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