Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Elsa M. Paganelli Cappelletti, Giorgio Casadoro

Studies in plant morphology by scanning electron microscopy and applications to plant species of pharmaceutical interest.Leaves of Pilocarpus pennatifolius Lem.

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **58** (1975), n.3, p. 427–431.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1975_8_58_3_427_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1975.

SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Botanica. — *Studies in plant morphology by scanning electron microscopy and applications to plant species of pharmaceutical interest. Leaves of* Pilocarpus pennatifolius *Lem.* Nota di ELSA M. PAGANELLI CAPPELLETTI e GIORGIO CASADORO (*), presentata (**) dal Socio C. CAPPELLETTI.

RIASSUNTO. — Vengono riferiti i risultati di una ricerca, effettuata per mezzo del microscopio elettronico a scansione, sulla morfologia delle superfici fogliari di *Pilocarpus pennatifolius* Lem. Sono descritti i dettagli morfologici delle cellule epidermiche, dei peli ghiandolari e dei peli protettori, come pure la distribuzione e l'aspetto delle striature cuticolari. È infine discussa la presenza di alcune caratteristiche formazioni costituite da cellule epidermiche con parete esterna convessa e talora subpapillosa, formazioni che sono state messe in relazione con la presenza di sottostanti cavità secretrici.

The leaf surface morphology and cuticle ornamentation details being today generally regarded as valuable diagnostic characters [17, 4, 5, 6, 14, 15, 10, 1, 2, 7, 8], a research has been carried out on the leaflet surfaces of Paraguay Jaborandi (*Pilocarpus pennatifolius* Lem.) by means of scanning electron microscopy, which in a previous work on *Atropa belladonna* L. [13] proved to be a particularly useful tool.

The present paper represents a further contribution to a research on the details of leaf surfaces which we are carrying on with morphological and diagnostic purposes.

MATERIALS AND METHODS

Full-developed leaves from a 3 meters high specimen of *Pilocarpus pennatifolius* Lem. growing in a greenhouse in winter and in the open air in summer, were used.

Although less rich in alkaloids than *P. microphyllus* Stapf.—which is the species at present almost exclusively in commerce [17]—*P. pennatifolius* was studied, being the only *Pilocarpus* species available in the Botanical Garden of the University of Padua.

Preliminary observations having pointed out no significant difference in the morphological features of the epidermal cells among the leaflets of a single imparipinnate compound leaf, only the second leaflet pair from the leaf basis, was taken into account.

The samples were prepared for scanning electron microscope (SEM) observation following the method previously described [13] and namely: careful leaflet washing in buffer solution, fixation in 2% glutaraldehyde in cacodylate buffer pH 6.9 and dehydration through a series of ethyl alcohols at increasing concentrations. The samples, coated with carbon and gold in a vacuum evaporator type Jeol JEE 4 B, were observed with a Jeol Scanning Electron Microscope JSM-U3 at the «Centro Universitario Grandi Apparecchiature Scientifiche» (CUGAS) of the University of Padua at an accelerating voltage of 25 kv.

Although carefully washed with buffer solution before fixation, the leaflet surfaces showed some dirt, especially in proximity of glandular hairs and midribs.

(*) Istituto di Botanica e di Fisiologia Vegetale dell'Università di Padova.
(**) Nella seduta dell'8 marzo 1975.

Results

The adaxial surfaces are characterized by the absence of stomata and by epidermal cells showing polygonal outlines, flat outer walls and strongly striated cuticles (fig. 1). Well-defined cell outlines are apparent only when at the boundary between two contiguous epidermal cells a cuticle furrow lined by one ridge on one or on both sides, is present (fig. 2).

Within each epidermal cell, cuticle striations run nearly parallel; if the overall cuticle ornamentation pattern is however taken into account, one can realize that striations do not develop following a single direction, quite perpendicular striations sometimes occurring on contiguous epidermal cells (fig. 3). The cuticular striations on the adaxial leaflet surface of *Pilocarpus pennatifolius* are scarcely twisted and infrequently branched.

On the adaxial surfaces external glands—sometimes referred to as glandular hairs [12, 3]—situated in marked depressions of the epidermis [9], frequently occur (fig. 4). In proximity of these depressions, SEM micrographs (fig. 5) show radially arranged cuticle striations (sometimes seeming to end at the depression edge) assembled in groups by radially arranged furrows extending beyond the depression edge.

Light microscopy of transverse leaflet sections allows to state that the furrows define radially arranged rows of epidermal cells; the outlines of every single cell of a row are however hardly recognizable, the cuticle striations uninterruptedly extending over contiguous cells of the row (fig. 5).

The cells on the leaflet midrib are elongated, with convex outer walls and almost completely smooth cuticles, faint striations occurring only at the boundary between two contiguous cells of a row (fig. 6).

The abaxial epidermis is composed of polygonal cells with well-defined outlines, every cell being surrounded by a rised line. The cuticle is smooth and the outer walls are faintly convex (fig. 7) and sometimes with a lumpy surface (fig. 8).

On the abaxial surface stomata of different size are present: the larger ones are few and rather isolated, while the smaller ones are numerous and close together (fig. 8 and fig. 9). The guard cells are accompanied by small, tangentially elongated subsidiary cells which form a narrow surrounding ring (cyclocytic stomata after Stace [16]).

Glandular hairs in depressions of the epidermis, surrounded by radially arranged rows of cells devoid of cuticular striations, were observed on the abaxial surface (fig. 10 and fig. 11).

The cells on the leaflet midrib are flat, elongated and defined by a rised line (fig. 12).

On both the adaxial and the abaxial surfaces, unicellular covering trichomes with more or less warty walls according to the hair length, are present, most abundantly on the midribs (fig. 12, fig. 13 and fig. 14).

On both epidermises, groups of 2–6 cells with convex to papillose outer walls, situated in more or less marked depressions which are surrounded by radially arranged rows of cells, were observed. The presence of cuticular striations both on convex cells and on surrounding cells, allows to discriminate the groups found on the adaxial (fig. 15 and fig. 16) from those found on the abaxial epidermis (fig. 17).

An interpretation of these structures is provided by light microscopy of transverse leaflet sections, epidermal cells with convex outer walls interposed among normal epidermal cells, occurring where mesophyll secretory cavities are present just below the epidermis (fig. 18).

CONCLUSIONS

The study of the leaflet surface details by scanning electron microscopy points out that in *Pilocarpus pennatifolius* cuticular striations are present only on the adaxial epidermis, unlike *P. microphyllus*, which is reported to have strongly striated cells on the adaxial surface and less marked striations on the abaxial one [10]. SEM investigations on other *Pilocarpus* species appear therefore of great interest from a diagnostic point of view.

Unlike *Atropa belladonna* [13], no relation between cuticular striation arrangement and epidermal cell outline—which is often defined by a furrow—was observed in *Pilocarpus pennatifolius*. A similar pattern was reported for the adaxial surface of *Aesculus hippocastanum* by Martin and Juniper [11]. Moreover in *Pilocarpus pennatifolius* cuticle striations sometimes extend uninterruptedly over many contiguous cells (fig. 5).

Although histologically identical, in surface view the glandular hairs found on the adaxial surface show a quite different aspect from those found on the abaxial one, the radially arranged cuticle striations of epidermal cells representing an outstanding feature.

The occurrence of warty covering trichomes was reported by Jackson and Snowdon [10] for *Pilocarpus microphyllus*. By scanning electron microscopy the details of cuticle ornamentation of the warty covering trichomes of *P. pennatifolius* (fig. 13) were described and a certain relation between hair lenght and degree of cuticle wartness was pointed out.

The typical aspect (to the Authors' knowledge never previously put in evidence) of the epidermal cells which come into direct contact with underlying secretory cavities, was also pointed out.

Acknowledgments. We wish to express our gratitude to prof. Vittorio Gottardi for giving us the opportunity of making use of the Jeol Scanning Electron Microscope at the «Centro Universitario Grandi Apparecchiature Scientifiche» (CUGAS) and to mr. Gino Bacelle for technical help.

LITERATURE CITED

[1] AHMAD K. J. (1969) – *Pharmacognosy of the leaf and root of* Barringtonia acutangula *Gaertn.*, « Planta medica », *17*, 338–345.

[2] BALBAA S. I., HILAL S. H. and HAGGAG M. Y. (1970) – *A macro-and micro-morphological study of the leaves of* Digitalis mertonensis, « Planta medica », *18*, 84–89.

[3] DEZANI S. and GUIDETTI E. (1953) – *Trattato di Farmacognosia*, 2nd Ed., UTET, Torino, 250–253.

[4] FELL K. R. and HARKISS K. J. (1964) – *Structure of the aerial parts of* Linaria vulgaris *Mill.*, « Planta medica », *12*, 460–477.

[5] FELL K. R., RAMSDEN D. and TREASE G. E. (1965) – *The morphology and anatomy of the flowering plant of* Colchicum steveni, « Planta medica », *13*, 158–177.

[6] FELL K. R., RAMSDEN D. and TREASE G. E. (1966) – *Diagnostic anatomical features of certain species of* Colchicum, « Planta medica », *14*, 209–216.

[7] HARKISS K. J. (1972) – *Studies in the* Scrophulariaceae. *Part IV. Structure of the aerial parts of* Antirrhinum majus *L.*, « Planta medica », *20*, 302–313.

[8] HARKISS K. J. (1973) – *Studies in the* Scrophulariaceae. *Part VII. Structure of the aerial parts of* Antirrhinum orontium *L.* (Misopates orontium (*L.*) *Raf.*, « Planta medica », *23*, 182–189.

[9] HÉRAIL J. (1927) – *Traité de matière médicale. Pharmacographie*, Librairie J.–B. Baillière et Fils, Paris, 757–761.

[10] JACKSON B. P. and SNOWDON D. W. (1968) – *Powdered vegetable drugs. An atlas of Microscopy for use in the identification of some Plant Materials employed as Medicinal Agents*, Churchill Ltd. Ed., 66–67.

[11] MARTIN J. T. and JUNIPER B. E. (1970) – *The cuticles of plants*, Edward Arnold Ltd., 109.

[12] METCALFE C. R. and CHALK L. (1950) – *Anatomy of the Dicotyledons*. Vol. I., Clarendon Press, Oxford, 305–309.

[13] PAGANELLI CAPPELLETTI E. M. (1975) – *Studies in plant morphology by scanning electron microscopy and applications to plant species of pharmaceutical interest. Leaves of* Atropa belladonna *L.*, « Rend. Acc. Naz. Lincei, Cl. Sci. fis., mat. e nat. », (in press.).

[14] PATEL M. B. and ROWSON J. M. (1966) – *Investigations of certain Nigerian medicinal plants. Part VII. The anatomy of the leaf of* Hedranthera barteri (*Hook. f.*) *Pichon.*, « Planta medica », *14*, 111–119.

[15] RILEY G. S. (1966) – *The microscopical characters of the aerial parts of* Calotropis procera., « Planta medica », *14*, 443–452.

[16] STACE C. A. (1965) – *Cuticular studies as an aid to plant taxonomy*, « Bull. British Mus. Nat. Hist. », Bot., *4*, 3–78.

[17] WALLIS T. E. (1960) – *Text book of Pharmacognosy*. 4th Ed., J. and A. Churchill Ltd., London.

EXPLANATIONS OF PLATES I–III

PLATE I.

Fig. 1. – Adaxial surface, epidermal cells (×250).

Fig. 2. – Adaxial surface, epidermal cells (×400).

Fig. 3. – Adaxial surface, epidermal cells (×750).

Fig. 4. – Adaxial surface, light micrograph of a glandular hair in transverse section (×400).

Fig. 5. – Adaxial surface, glandular hair in surface view (×400).

Fig. 6. – Adaxial surface, epidermal cells on the midrib (×1200).

PLATE II.

Fig. 7. – Abaxial surface, epidermal cells and stomata (×600).

Fig. 8. – Abaxial surface, epidermal cells and stomata of different size (×200).

Fig. 9. – Abaxial surface, epidermal cells and stomata of different size (×350).

Fig. 10. – Abaxial surface, glandular hair in surface view (×300).

Fig. 11. – Abaxial surface, glandular hair in surface view (×600).

Fig. 12. – Abaxial surface, epidermal cells on the midrib (on the left), unicellular covering trichome with warty wall and epidermal cells and stomata (on the right) (×400).

PLATE III.

Fig. 13. – Abaxial surface, warty wall of a unicellular covering trichome (×4,500).

Fig. 14. – Adaxial surface, covering trichome with not warty wall (×1400).

Fig. 15. – Adaxial surface, group of 2 convex epidermal cells which are related with underlying secretory cavities (×300).

Fig. 16. – Adaxial surface, as in fig. 15, group of 3 convex epidermal cells (×550).

Fig. 17. – Abaxial surface, group of 4 papillose epidermal cells which are related with underlying secretory cavities (×300).

Fig. 18. – Abaxial surface, light micrograph of a transverse leaflet section showing convex epidermal cells with underlying secretory cavities (×400).

Fig. 1.

Fig. 2.

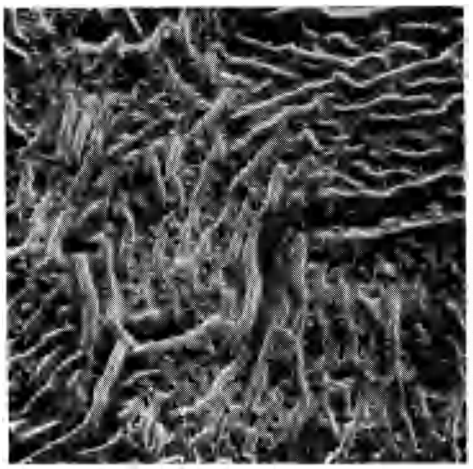

Fig. 3.

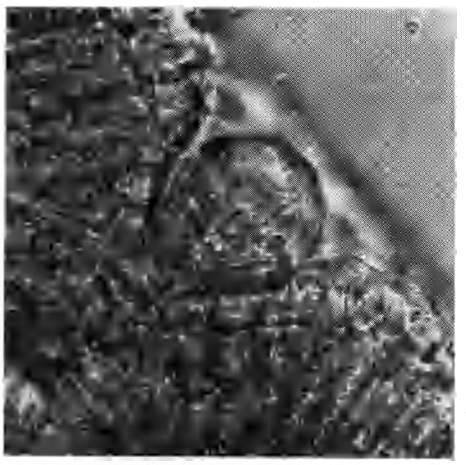

Fig. 4.

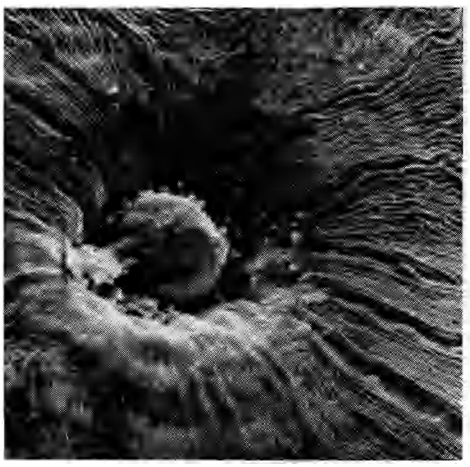

Fig. 5.

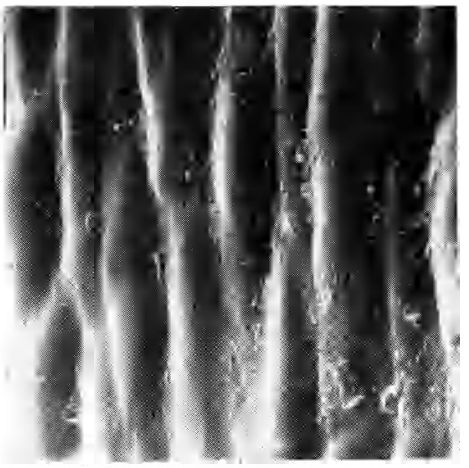

Fig. 6.

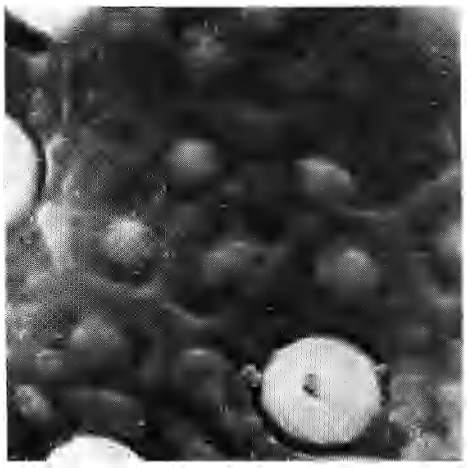

Fig. 7.

Fig. 8.

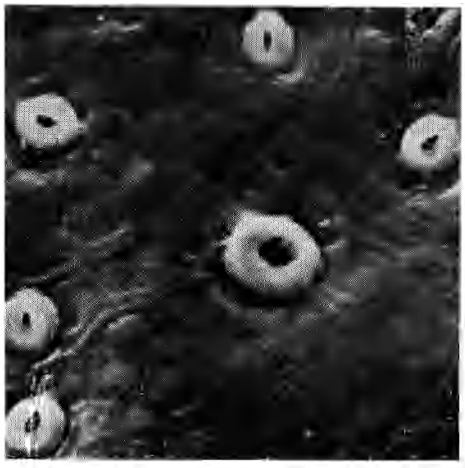

Fig. 9.

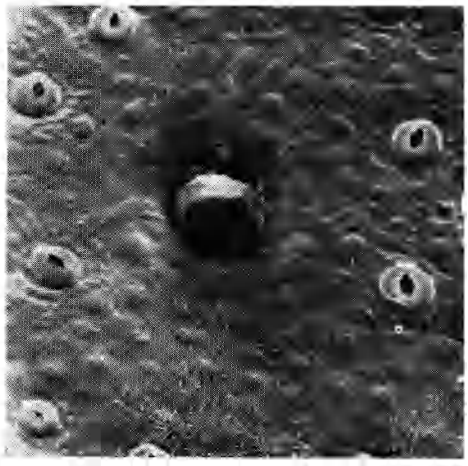

Fig. 10.

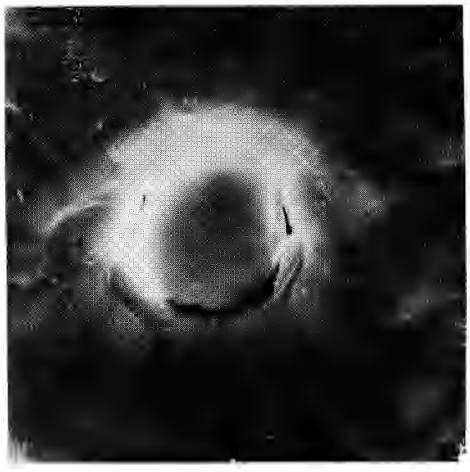

Fig. 11.

Fig. 12.

Fig. 13.

Fig. 14.

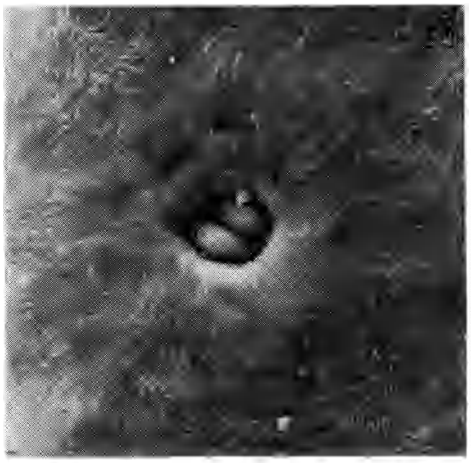

Fig. 15.

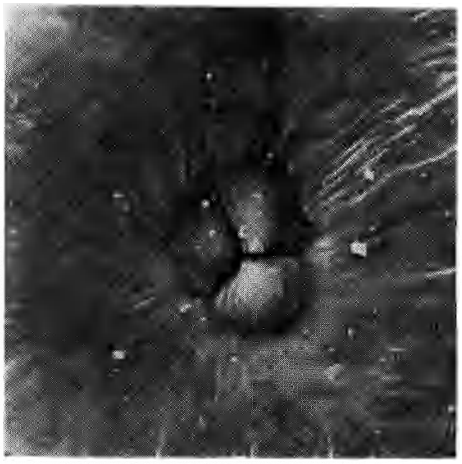

Fig. 16.

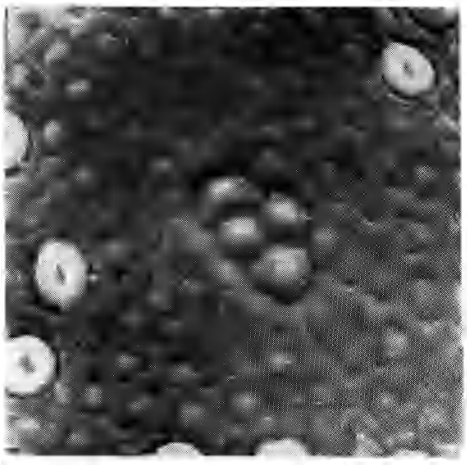

Fig. 17.

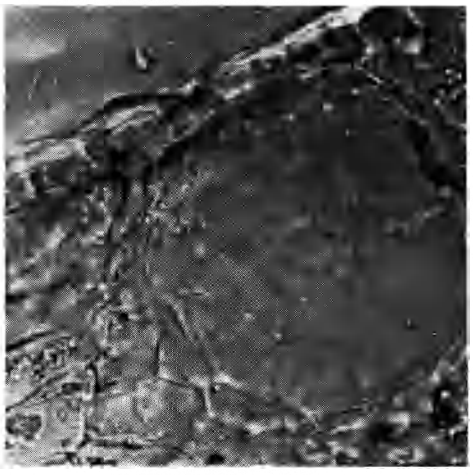

Fig. 18.