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Studies in plant morphology by scanning electron microscopy and applications to plant species of pharmaceutical interest. Leaves of Atropa belladonna L.

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Morfologia vegetale. — *Studies in plant morphology by scanning electron microscopy and applications to plant species of pharmaceutical interest. Leaves of* Atropa belladonna *L.* Nota di ELSA M. PAGANELLI CAPPELLETTI (*), presentata (**) dal Socio C. CAPPELLETTI.

RIASSUNTO. — Lo studio delle superfici fogliari di *Atropa belladonna* L. mediante il microscopio elettronico a scansione, ha permesso di definire i dettagli morfologici delle ornamentazioni cuticolari e di descriverne le modificazioni in funzione del tipo di pagina considerata e del livello di inserzione della foglia sul fusto.

INTRODUCTION

A careful and extensive study of leaf epidermis details of *Atropa belladonna* assumes valuable interest, being today generally accepted (Wallis, 1960; Jackson and Snowdon, 1968; Paris and Moyse, 1971) that cuticle striations of leaf cells represent a very valuable diagnostic character.

Denoël and co- workers (1968) showed by light microscope observations, that in *Atropa belladonna* cuticle striations vary with leaf position on the stem and moreover with culture conditions.

Therefore I intended to study in detail by scanning electron microscopy the morphology of leaf surfaces, with particular attention to cuticular ornamentation. The adaxial and abaxial epidermises of leaves at different levels on a single plant were comparatively examined.

MATERIALS AND METHODS

Spontaneous, full-blooming, 2 meters high plants of *Atropa belladonna* growing in a glade of the beech forest in the Cansiglio Plateau (Venetian Pre-Alps, about 1100 m a.s.l.) were used.

By the time of full-blooming, many of the lower alternate leaves below the first stem branching, had already fallen. The following leaves were therefore used: the lowest available leaf, at about 70 cm at ground level; a leaf in an intermediate position between the first available leaf and the first stem branching; a leaf at the first stem branching; a geminate leaf at the second stem branching and a geminate leaf at the first flower level on a terminal branch.

Preliminary observations having pointed out no significant difference in cuticular striation morphology between geminate leaves for both the adaxial and abaxial epidermises, only one of the geminate leaves for each level was examined, namely the smaller one which is situated on its own normal insertion level (Paris and Moyse, 1971).

Acrylic imprints following Cappelletti (1963) were used for light microscope surveys of the epidermises of a whole leaf.

For scanning electron microscope (SEM) observation, freshly excised leaves were dehydrated either by physical methods (freezing in liquid nitrogen followed by sublimation in a high vacuum evaporator) or by chemical methods (fixation in 2% glutaraldehyde in

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cacodylate buffer pH 6.9 with sucrose added for osmotic pressure adjustement and dehydration using a series of ethyl alcohols at increasing concentrations). The two methods providing quite comparable results, chemical dehydration was more extensively used.

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.

Results

Light microscopy of acrylic imprints shows the main morphological features of leaf surfaces: epidermal cells with more or less sinuous outline on both surfaces, in contrast with the straight outline of the cells situated on veins, rectangular shaped and with tapered ends. Only on the terminal vein branching, cell outline becomes sinuous. Anysocitic stomata, numerous on the abaxial epidermis, scarce and mainly located in proximity of veins on the adaxial one, are present.

Light microscopy of acrylic imprints allows to ascertain the absence or the presence of cuticular ornamentation and its main characters; in this way radial striations on the cells at hair basis and striations in the direction of the longitudinal axis of vein cells were observed.

For cuticle ornamentation details, SEM observation is particularly suitable. In some SEM micrographs, the leaf surfaces, although carefully washed with buffer solution before fixation, show some dirt and sometimes micro-organisms.

On the adaxial epidermises, cuticle striations are always apparent although their extension, topography and morphology may change depending upon leaf position on the stem. In fact in lower level leaves (Pl. I, fig. 1 and fig. 2) cuticle is not evenly striated over all the cell surface, and smooth areas are present together with striated ones (especially on infra-lobal portions). More evenly distributed and sometimes twisted (Pl. I, fig. 3 and fig. 4) cuticular striations were on the contrary observed on the adaxial epidermises of leaves at the first stem branching. The just described cuticle features, and even more marked, were found on the leaves at the second stem branching level (Pl. II, fig. 5 and fig. 6), while close and twisted cuticle ridges were observed on the last leaf sample examined (Pl. II, fig. 7).

The few stomata on adaxial epidermises, closely resembling those occurring on the abaxial ones, will be described later on.

The surface features of the epidermal cells on veins, always striated in the direction of the longitudinal axis (Pl. II, fig. 8), do not depend upon leaf position on the stem, no significant difference being evident between leaves of bottom and top levels.

Less marked cuticle striations in the abaxial surfaces than in the adaxial ones were observed.

The leaf position on the stem clearly affects cuticle appearance in the abaxial epidermises as well. Below the first stem branching smooth or faintly striated cuticles are present, only the cells surrounding the stomata having well marked cuticle ridges, extending perpendicularly to stomata aperture,

while the guard cells show faint concentric grooves (Pl. III, fig. 9 and fig. 10). At the first stem branching level, much more marked cuticular striations, which extend to almost the totality of cell surface, were observed (Pl. III, fig. 11). Similar cuticle features are present on leaves at the second stem branching level (Pl. III, fig. 12).

The last leaf taken into account shows cells of the abaxial surface with less marked cuticular ornamentation (Pl. IV, fig. 13) than the previous leaf considered.

The aspect of the cells on veins is quite similar (Pl. IV, fig. 14) to the corresponding cells on the adaxial surface and reveals no level effect in connection with leaf position on the stem.

DISCUSSION AND CONCLUSION

The study of leaf surface details by scanning electron microscopy points out in *Atropa belladonna* a relation between epidermal cell outline and cuticular striation arrangement: the ridges in fact follow the sinuous anticlinal walls of the cells; at the centre of the cells cuticle may be either smooth or provided with straight grooves or also with well marked and sometimes twisted ridges, depending on the degree of cuticular striation, which in turn is correlated with the leaf surface considered and the leaf position on the stem. In fact more marked cuticular striations on the adaxial than on the abaxial surfaces and in upper level than in lower level leaves were observed. The effect of level position on the stem on cuticular ridge extension and intensity was reported also by Denoël (1968).

Besides leaf position on the stem, leaf age must be taken into account. Cuticle ornamentations do not form until a relatively late stage of leaf development (Holloway, 1971) and therefore the faintly striated cuticle on the abaxial surface of the highest leaf examined (pointed out also by Denoël, 1968), could depend on its later origin compared with leaves inserted in lower positions on the stem.

An ontogenetic research with the aim to follow the forming of cuticular ornamentation appears therefore to be interesting, in order also to state at which time in leaf development environmental factors—known to affect deeply both cuticle thickness and morphology (Martin and Juniper, 1970)—may influence the cuticle morphological features.

An outstanding feature, particularly evident on faintly striated abaxial epidermises, is the difference occurring between the cells surrounding stomata and the remaining epidermal cells, the former having ridges at right angle to the stomatal apertures. A similar pattern was reported, for different plant species, by Patel and Rowson (1966) and by Ahmad (1969).

From a pharmacognostic point of view, cuticular striations have to be considered a valuable diagnostic character not only for *Atropa belladonna*, but for many other plant species of pharmaceutical interest (Wallis, 1960; Fell and Harkiss, 1964; Fell, Ramsden and Trease, 1965; Bradley and Fell,

1966; Fell, Ramsden and Trease, 1966; Patel and Rowson, 1966; Riley, 1966; Denoël, 1968; Jackson and Snowdon, 1968; Ahmad, 1969; Balbaa, Hilal and Haggag, 1970; Harkiss, 1972 and 1973). The scanning electron microscopy, allowing a careful morphological investigation on details of cuticle ornamentation, seems to be therefore the must useful tool for ecological and pharmacognostic researches.

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Literature cited

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

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.

Bradley J. M. and Fell K. R. (1966) – *The anatomy of the fruit of* Heracleum sphondylium, « Planta medica », *14*, 10–18.

Cappelletti E. M. (1963) – *Proposta di un nuovo metodo per ottenere impronte fogliari stabili*, « Atti Istituto Veneto Sci. Lett. ed Arti », *121*, 273–283.

Denoël A. (1968) – *La matière médicale, science interdisciplinaire*, Conférence faite au 2e colloque international consacré aux plantes médicinales les 6, 7 et 8 juillet 1968, « Plantes médic. et phytothérapie », *2* (3), 153–176.

Fell K. R. and Harkiss K. J. (1964) – *Structure of the aerial parts of* Linaria vulgaris *Mill.*, « Planta medica », *12*, 460–477.

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.

Fell K. R., Ramsden D. and Trease G. E. (1966) – *Diagnostic anatomical features of certain species of* Colchicum, « Planta medica », *14*, 209–216.

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

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.

Holloway P. J. (1971) – *The chemical and physical characteristics of leaf surfaces*, in: « Ecology of leaf surface micro-organisms », T. F. Preece and C. H. Dickinson Ed., Academic Press, Cap. 2, 39–53.

Jackson B. P. and Snowdon D. W. (1968) – *Powdered vegetable drugs. An atlas of Microscopy for use in the identification and authentication of some Plant Materials employed as Medicinal Agents*, Churchill Ltd. Ed., 1–203.

Martin J. T. and Juniper B. E. (1970) – *The cuticles of plants*, Edward Arnold Ltd., 1–347.

Paris R. R. and Moyse H. (1971) – *Matière Médicale. Collection de Precis de Pharmacie*, Masson et Cie Ed., Tome III, 149–150.

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.

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

Wallis T. E. (1960) – *Text book of Pharmacognosy*. 4th Ed., J. and A. Churchill Ltd., London, 1–640.

Fig. 1. – Lowest available leaf, adaxial surface (×1,300).

Fig. 2. – Leaf in intermediate position between the lowest available leaf and the first stem branching, adaxial surface (×650).

Fig. 3. – Leaf at the first stem branching, adaxial surface (×650).

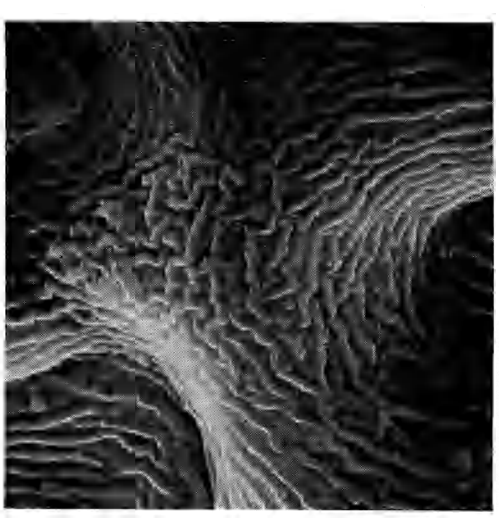

Fig. 4. – The same sample of fig. 3 at higher magnification (×1,950).

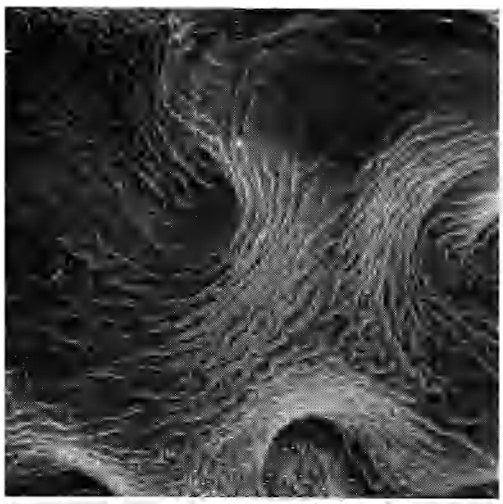

Fig. 5. – Geminate leaf at the second stem branching, adaxial surface (×1,300).

Fig. 6. – The same sample of fig. 5 at higher magnification (×3,250).

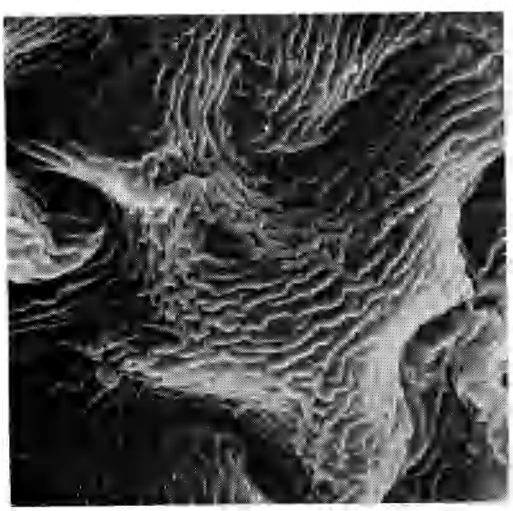

Fig. 7. – Geminate leaf at the first flower level on a terminal branch, adaxial surface (×1,950).

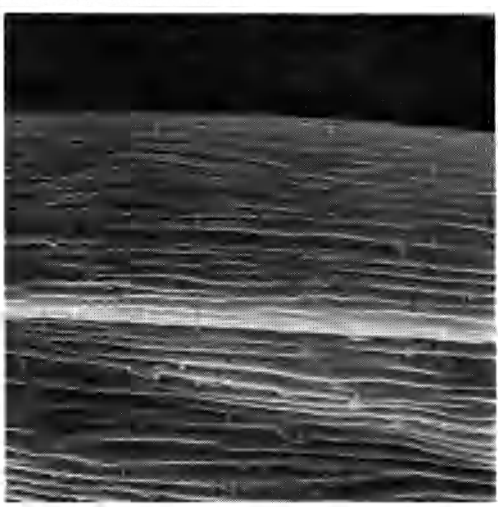

Fig. 8. – Aspect of epidermal cells on veins, leaf at the first stem branching, adaxial surface (×1,300).

Fig. 9. – Lowest available leaf, abaxial surface (×400).

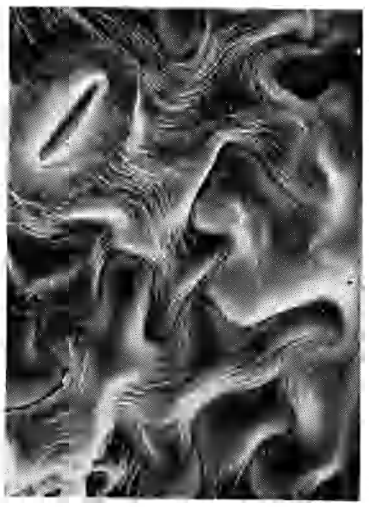

Fig. 10. – Leaf in intermediate position between the lowest available leaf and the first stem branching, abaxial surface (×650).

Fig. 11. – Leaf at the first stem branching, abaxial surface (×650).

Fig. 12. – Geminate leaf at the second stem branching, abaxial surface (×650).

Fig. 13. – Geminate leaf at the first flower level on a terminal branch, abaxial surface (×1,950).

Fig. 14. – Aspect of epidermal cells on veins; leaf at the first stem branching, abaxial surface (×1,300).