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COMPARISON OF DELPINO AMENTIFLORAE ON THE BASIS OF THE STRUCTURE OF LEAF EPIDERMIS

SZERÉN PATAKY

Institute for Plant Morphology and Systematic of the
Attila József University, Szeged

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Introduction

For delimiting taxonomic categories and evaluating ecologic effects, there are often employed epidermal marks, as well. On the basis of the ontogeny of epidermal cells and subsidiary cells, the development, structure and shape of cuticles, the relief of leaf epidermis, stoma type and number and other epidermal marks, there is a possibility for defining species and separating them from one another (Bandulska, 1924; Sveshnikova, 1966; Sitte-Renier, 1963; Hluchvsky-Srb, 1959; Priestley, 1943; Linskens, 1966; Ujhelyi, 1960; Maácz, 1956; Greguss, 1962; Maróti, 1965, 1966; Gulyás, 1961; etc.).

„The organizational characteristics developed in the course of the evolutionary history of plant species refer — according to Soó, too — to a relationship of plants; on the other hand, the peculiarities in accomodation are determined by the essential external conditions, throwing light upon the way of life, ecology of plants" (Soó, 1945; Shenikov, 1953; Simon—Wolcsánszky, E.—Molnáros, 1964; Maróti, 1965).

During our examinations we have compared *Amentiflorae* from different soils on the basis of some measurable and formal marks of their leaf epidermis.

Material and method

At our examinations living and herbarial material was used. For preparation we have got samples from the middle part of the leaf-sheet of fully developed leaves to obtain a real comparison (Zalensky, 1904; Simon-Wolcsászky, E.—Molnáros, 1964).

The investigation of the epidermal structure took place on preparations made immediately, resp. on epidermis prepared by maceration (Sárkány—Szalai, 1966; Ujhelyi, 1954). Staining: vesuvin, acid haematoxylin Ehrlich-f, *Sudan* III, by triple staining (Kisser, 1926; Sárkány—Szalai, 1966).

For comparison the following measurable and formal features have been used: length and width of guard cells, the distribution of adjacent or subsidiary cells expressed in percentage, stoma count, L/W ration of guard cells, (their shape), type of stomata.

The measurement data are the average of 50 fields of sight. For demonstrating the formal peculiarities microphotographs have been applied.

The measurable features were evaluated by variancy analysis, F-test and t-test (Yule-Kendall, 1964).

The following species have been examined:

Ordo. **Urticales**

1. Familia: *Moraceae:*
   Morus alba L.
   Maclura aurantiaca Nutt.
   (Ioxylon pomiferum Raf.)
   Ficus elastica Roxb.

4. Familia: *Ulmaceae:*
   Ulmus laevis Pall.
   Ulmus glabra Huds. (Mill).
   Celtis occidentalis L.

Ordo. **Fagales**

1. Familia: *Betulaceae:*
   Betula pendula Roth.
   Beula nana L.
   Corylus avellana L.
   Corylus colurna L.

TABLE 1

Family	*Moraceae*			*Ulmaceae*			*Betulaceae*			
Species	*Morus alba*	*Maclura aurantiaca*	*Ficus elastica*	*Ulmus laevis*	*Ulmus glabra*	*Celtis occidentalis*	*Betula pendula*	*Betula nana*	*Corylus colurna*	*Corylus avellana*
Number of stomata piece/sq. mm	738	195	157	229	282	456	162	91	48	129
Length of guard cells in μ	13,8	27,4	39,6	29,2	30,5	21,9	30,5	40,5	32,1	27,1
Width of guard cells in μ	12,9	20,2	34,8	16,9	21,4	12,3	25,9	32,3	25,6	21,5
Ratio l/W of guard cells	1,06	1,32	1,07	1,67	1,34	1,74	1,34	1,19	1,19	1,19

2. Familia: *Fagaceae:*

Fagus silvatica L.
Quercus robur L.
Quercus prinus L.
Quercus rubra L.
Quercus macranthera Fisch et Mey.
Castanea savita Mill.

Ordo. **Juglandales**

Familia: *Juglandeceae:*

Juglans regia L.
Carya alba K. Koch.
Pterocarya fraxinifolia Spach.

Ordo. **Salicales**

Familia: *Salicaceae:*

Salix alba L.
Salix fragilis L.
Salix arbuscula L.
Populus alba L.
Populus canadensis Mnch.

Populus tremula L.
Populus balsamifera Dur.
(Soó, 1963)

Discussion of results

In Table 1 mainly home plant species, belonging to six families are compared. The leaves of the examined species are hypostomatic, except some species of the family *Salicaceae (Salix alba, S. fragilis, Populus canadensis),* therefore the Table is containing but data of the lower epidermis.

Fagaceae			*Juglandaceae*			*Salicaceae*				SD		
Fagus silvatica	*Quercus robur*	*Castanea sativa*	*Juglans regia*	*Carya alba*	*Pterocarya fraxinifolia*	*Salix alba*	*Salix fragilis*	*Populus alba*	*Populus canadensis*	0,1%	1%	5%
209	357	338	161	424	232	309	175	402	92	143	98	68
36,6	38,4	32,8	30,5	29,6	28,7	28,7	33,2	24,2	31,1	4,7	3,1	2,2
29,3	29,4	23,2	21,4	24,7	21,4	19,7	26,0	18,4	21,0	4,4	3,0	2,0
1,19	1,24	1,37	1,37	1,14	1,34	1,39	1,22	1,24	1,44	0,31	0,21	0,15

Comparison on the basis of the measurable features of epidermis

The number of stomata (cf. Table 1) is partly of ecologic significance and partly it can be used to separate the species (Shenikov, 1953; Mrs. Simon—Molnáros, 1964; Maróti, 1965). In case of the species examined, its value for an area of 1 sq.mm has changed between 48 and 738.

Inside the family the differences between the single species are considerably smaller. There isn't any significant difference — except the family *Betulaceae* — inside the same family between species belonging to different genera, thus e.g., in the family *Moraceae* between the *Maclura aurantiaca* and *Ficus elastica*, in the family *Fagaceae* between *Quercus robur* and *Castanea sativa*. In the family *Betulaceae* the *Betula pendula* and. *B. nana*, the *Corylus avellana* and *C. colurna* are differring from each other on a five percent level, while some species of the two genera *(Betula nana—Corylus avellana* and *Betula pendula—Corylus colurna)* cannot be separated even on a five percent significance level. On the other hand, in the family *Salicaceae*, even inside the genera *Salix* and *Populus*, the species examined are differring essentially concerning their numbers of stomata (on a level of 1 and 0,1 percent).

Length of guard cells (cf. Table 1).

In case of species examined, it is changing between 13,8 μ and 40,5 μ. In the family *Juglandaceae* the length of guard cells of the species representing all the three examined genera, in the families *Ulmaceae* and *Fagaceae* those representing one genus *(Ulmus)* or more ones *(Fagus, Quercus)*, is not different from the others.

In contradistinction to them, between the examined species of *Moraceae, Betulaceae* and *Salicaceae*, in a great majority of cases, there are differences surpassing even the 0,1 percent SD values. At the same time, the lengths of guard cells of *Ulmus glabra, Betula pendula*, and *Juglans regia* are in a complete accordance with each other.

Width of the guard cells (cf. Table 1).

It changes between 12,3 μ and 34,8 μ (the joint width of two guard cells was measured with the pore of stoma between them). Inside the families *Betulaceae, Fagaceae* and *Juglandaceae*, the widths of the guard cells of some species are identical, however, on the basis of the available data, no general regularity can be drawn concerning the species examined.

L/W ratio of the guard cells (Table 1).

The shape of the guard cells may be well characterized by their L/W ratio. The shape of the guard cells of the species examined — except the family *Ulmaceae* — is elliptical. The extreme values of their L/W are: 1, 1 and 1,4.

The data are the most homogeneous inside the family *Betulaceae;* there is not any significant difference between the ratios L/W of guard cells of the single species.

In the family *Ulmaceae*, the guard cells of the species examined, in contrast to the above-mentioned ones, are longshaped (their L/W ratio being between 1,4 and 1,7).

Comparison on the basis of the formal features of epidermis

The upper and lower epidermis are divided into costal and intercostal fields. The radial wall of cells of the costal fields (above the vascular structure of leaves) is — apart from a few exceptions — generally straight, the cells are strongly lengthened in the direction of the course of the leaf vessels. The radial wall of epidermal cells of the intercostal fields is, viewed from above, straight (quadrangular, quinquangular, sexagonal), tortuous or undulatory. The stomata take place dispersed in the intercostal fields, being of acyclic or monocyclic type (the guard cells are surrounded only by epidermal cells, resp. subsidiary cells). The shape of guard cells is elliptic or lengthened (Plate I).

The number of the adjacent cells or subsidiary cells, connected with guard cells, is in the family *Salicaceae* generally four-five, in the other families, as a rule, five-six (Fig. 1).

Moraceae

The stomata of the *Morus alba* and *Maclura aurantiaca* are of acyclic type. The shape of guard cells is, in case of the species examined, elliptic, a difference occuring only concerning the size of cells (cf. Table 1). It is obvious in epidermal cells that the cuticle is striped, generally agreeing with the direction of the longitudinal axis of cells (Plate I. Fig. 1/4).

The stomata of *Ficus elastica* are characteristic of the plants of xerophyte type: the guard cells are to be found immersed on the bottom of small cavities, covered above by a cuticle film provided with orifices of a direction agreeing with the longitudinal axis of the pores of stomata (Plate I. Fig. 2).

Ulmaceae

The shape of guard cells is lengthened (Table 1). The stomata are of acyclic type, but the adjacent cells surrounding the guard cells of *U. glabra* are stained more strongly, thus separated from the other epidermal cells in respect of staining and, a little, of their shape.

The structure of stomata of the *Celtis occidentalis*, the shape of guard cells are corresponding to those of the two *Ulmuses*. It is anyhow very characteristic of the *Celtis occidentalis* that the number of the adjacent cells surrounding the stomata is, apart from a very few exceptions, four (Fig. 1), and it is obvious, too, how strongly wrinkled the thick cuticle is that is covering the lower epidermal cells. In the adjacent cells, the cuticle is triped generally at right angles to the guard cells (Plate II. Fig. 1).

Betulaceae

The stoma type of the plant species examined of the family is monocyclic, the wall of the subsidiary cells is thinner and stained less

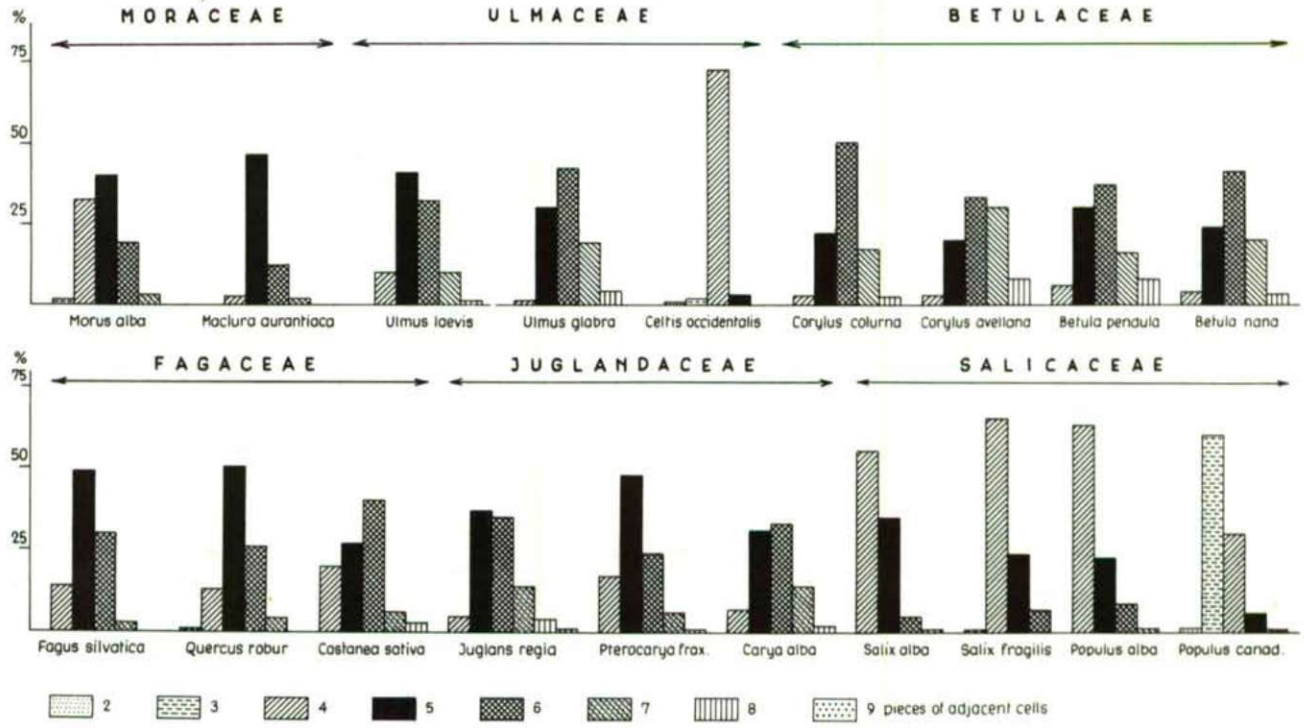

Fig. 1. Percentile distribution of the adjacent cells, resp. subsidiary cells connected with the guard cells in the lower epidermis.

than those of the proper epidermal cells (Plate I. Fig. 1./3). The shape of the guard cells is elliptic (Table 1).

Fagaceae

The morphologic marks of the species examined are highly various, save the shape of guard cells wich is in every case elliptic.

The stomata of *Fagus silvatica* and *Quercus rubra* are monocyclic. The subsidiary cells are stained more strongly; they are different, in shape too, from the other epidermal cells (Plate II. Fig. 2).

The stomata of *Quercs robur, Q. prinus, Q. macranthera* and *Castanea sativa* are of acyclic type.

Juglandaceae

The shape of the guard cells is elliptic (L/W ratio: 1,3; 1,1; 1,3), the stomata are definitely of acyclic type, in genera *Juglans, Carya, Pterocarya,* and *Engelhardtia.* A difference is to be found in the shape of epidermal cells and in the epidermal appendices (Plate I. Fig 1./2).

Salicaceae

The species examined are various in point of morphologic marks. The leaves of the *Salix alba* and *S. fragilis* are amphistomatic, the number of stomata is less in the upper epidermis, and much more in the lower one. The shape of guard cells is elliptic (cf. Table 1). The stoma structure is monocylic, beside the two lateral subsidiary cells there can often be found lateral coronal cells, as well (Plate III. Fig. 1).

The leaves of the *Salix arbuscula* are hypostomatic. The shape of the guard cells is elliptic, their stomata are of monocyclic structure.

The leaves of the *Populus alba* are hypostomatic. The shape of the guard cells is elliptic (cf. Table 1). Their stomata are monocyclic. In the lateral sybsidiary cells the cuticle is striped at right angles to the longitudinal axis of the guard cells.

The stomata of the *Populus tremula* are more differentiated, beside the lateral subsidiary cells also the lateral coronal cells can always be observed. The cuticle is triped much omre (Plate III. Fig. 2./1).

It is characteristic of the species of genus *Populus* that the cuticle covering the cells is strongly striped; on that basis they can well be separated from the species *Salix* and *Populus* inside the *Salicaceae* (Plate III. Fig. 1., 2).

The leaves of the *Populus canadensis* are amphistomatic, their stomata are of monocyclic type, beside the lateral subsidiary cells also a coronal cell can generally be found.

Evaluation of results

1. *On the basis of measurable features*

According to our examinations, a separation of the examined families cannot be solved on the basis of L/W ratio of the guard cells as the difference between the proportional numbers is surpassing the 1 percent level nearly in every family among the species examined. Inside the families, however, there is an opportunity in every case to determine

some species exactly on that basis. E.g., inside the family *Moraceae* the *Maclura aurantiaca*, inside the family *Ulmaceae* the *Ulmus glabra*, from the family *Betulaceae* the *Betula pendula*, from the family *Salicaceae* the *Populus canadensis* could be identified with due certainty. At some species, however, there isn't any difference concerning the L/W ratio of guard cells inside the family, and even between the families. E.g., the L/W ratio of the guard cells of the *Betula nana* and *Fagus sivatica* is completely corresponding (cf. Table 1).

The other features, because of the changes of considerable degree in the function of ecologic factors, can be used, in our opinion, for a sure identification but in extreme cases. E.g., it is probable that inside the family *Moraceae* the *Morus alba* can be separated because of a difference of considerable degree from the two other species even on the basis of the number of stomata (Table 1). Similarly, in the family *Ulmaceae*, the *Celtis occidentalis* can be separated because of the extremely high number of stomata. Concerning the number of stomata, we cannot see any possibility for a further determination among the species examined.

According to our earlier examinations, the influence of environmental factors on length and width of the guard cells is considerable, as well (Pataky, 1967). The families cannot be separated, even concerning these two features, on the basis of our investigations; on the other hand, in extreme cases, inside families, these features, too, can be employed for separating the species. E.g., inside the family *Moraceae*, the stomata of the *Ficus elastica* are nearly three times as large as those of *Morus alba*, and that of the *Maclura aurantiaca* is almost twice as large as that.

Larger taxonomic categories, e.g. families that are close to one another, cannot be separated on the basis of the measurable features examined: we think, however, so that this is generally a smaller problem than a reliable separation of the species inside a family or genus, being considerably helped by a statistic analyses of the measurable features. There are, namely, as a rule, so obvious differences between larger taxonomic categories concerning formal features that their separation is easier and more certain with the methods used in the taxonomy.

2. *On the basis of formal marks*

The results of our examinations are agreeing but partly with the literary statements. The formal marks, used by us, are namely more suitable to characterize greater taxonomic units, maybe families or genera (Plate I. Fig. 1), and are used but rarely for a reliable determination of species.

Thus, inside the family *Moraceae*, in the lower epidermis of the *Ficus elastica* the structure of stomata is the most characteristic, making possible a sure separation of it from the species examined. The stomata described at the *Ficus elastica* are characteristic of the genus *Ficus* (Bargagli, 1901).

The family *Ulmaceae* can be separated, on the basis of the shape of guard cells (L/W ratio: 1,4—1,7), from the other families examined. Inside the family, the *Celtis occidentalis* can be mentioned.

It can be separated inside the family as the thick cuticle layer covering its lower epidermis is characteristically wrinkled (Plate II. Fig. 1); but it cannot be separated from the species *Populus tremula* belonging to the family *Salicaceae* just because of that feature (Plate III. Fig. 2). On the other hand, it is a feature, characterizing exclusively the *Celtis occidentalis,* in the structure of stomata that the adjacent cells surrounding the guard cells are characterized in 73 percent by number four. At the other species, the percentile distribution of the number of adjacent cells is considerably more various.

Inside the family *Betulaceae,* in the (monocyclic) type of stomata, even the genera *(Betula* and *Corylus)* cannot be separated from each other. The shape of subsidiary cells, the thickness of radial walls related to the other epidermal cells, the weaker staining of these cells are characteristic of each of the species examined (Plate I. Fig. 1./3).

The most species and specimens have been examined inside the family *Fagaceae,* thus our results concerning this family are of more general validity. The type of stomata in the developed epidermis is acyclic at some *Quercus* and *Castanea* species, the stomata of *Fagus silvatica* and *Q. rubra* are, however, definitely of monocyclic type (Plate I. Fig. 1./1, Plate II. Fig. 2). The type of stomata is, therefore, in itself not suitable for separating the species from one another; anyhow, it may possibly be used to separate genera and families.

The species examined from the family *Juglandaceae* also cannot be separated from one another on the basis of the formal marks of stomata.

Inside the family *Salicaceae,* the two genera can be separated on the basis of the structure of stomata and the cuticles. The shape of the epidermal and subsidiary cells of species *Salix,* viewed from above, is concerned, beside the lateral subsidiary cells also lateral coronal cells are frequently to be found (Plate III. Fig. 1). On that basis they can be separated from every species examined.

In the lateral subsidiary cells of *Populus* species it is highly characteristic that the thick cuticle is strongly striped, generally at right angles to the longitudinal axis of pores (Plate III. Fig. 2). The striped cuticle, joint with other features of the epidermis, is possibly suitable for determining some species, in conformity with Hluchovsky-Srb's results (1959).

Summary

On the basis of our examinations it may be ascertained:

a) From the measurable features of the leaf epidermis mainly the L/W ratio of guard cells and the stoma count can be used for diagnostic purposes. These features change the least under ecologic influences (Pataky, 1967).

b) A feature alone is rarely enough for a reliable identification; it is generally necessary to have an analysis on the basis of more measurable features.

c) Several formal features together are suitable to define larger taxonomic categories (ordos, families, possibly genera).

d) For determing the species, a joint application of formal and measurable features seems to be the most suitable.

e) At comparison and separation of taxonomic categories it is practical to perform the examinations in the lower epidermis. In the upper epidermis namely, as experienced in each of our cases, there may occur greater differences in function of the environmental factors than in the lower epidermis (Pataky, 1967).

f) An analysis of the measurable features with a mathematic-statistical method is more exact, making possible to demonstrate smaller differences between the single species.

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Address of the author:

Dr. Szerén Pataky
Institute for Plant Morphology
and Systematic of the
A. J. Unversity, Szeged, Hungary

Plate I

Fig. 1. Lower epidermis with stomata dispersed in the intercostal fields *(Quercus rubra)*, M. x100; 2. epidermal cells of straight walls, stomata of acyclic type *(Engelhardtia Leschén)*, M. x800; 3. elliptic guard-cell shape surrounded by subsidiary cells, a) stoma of monoyclic type *(Betula)*, M. x800; costal and intercostal epidermal cells with the characteristic wrinkles of cuticles *(Maclura aurantiaca)*, M: x300.

Fig 2. *Ficus elastica.* Lower epidermis. M: x800.

Plate II

Fig. 1. *Celtis occidentalis:* lower epidermis, M: x1350.
Fig. 2. *Fagus silvatica:* lower epidermis. M: x800.
Fig. 3. *Juglans regia:* lower epidermis. M: x800.

Plate III

Fig. 1. 1. *Salix alba,* and 2. *Salix arbuscula:* lower epidermis. M: x300.

Fig. 2. 1. *Populus termula:* lower epidermis. M: x500. 2. *Populus balsamifera:* lower epidermis. M: x 800.

PLATE I

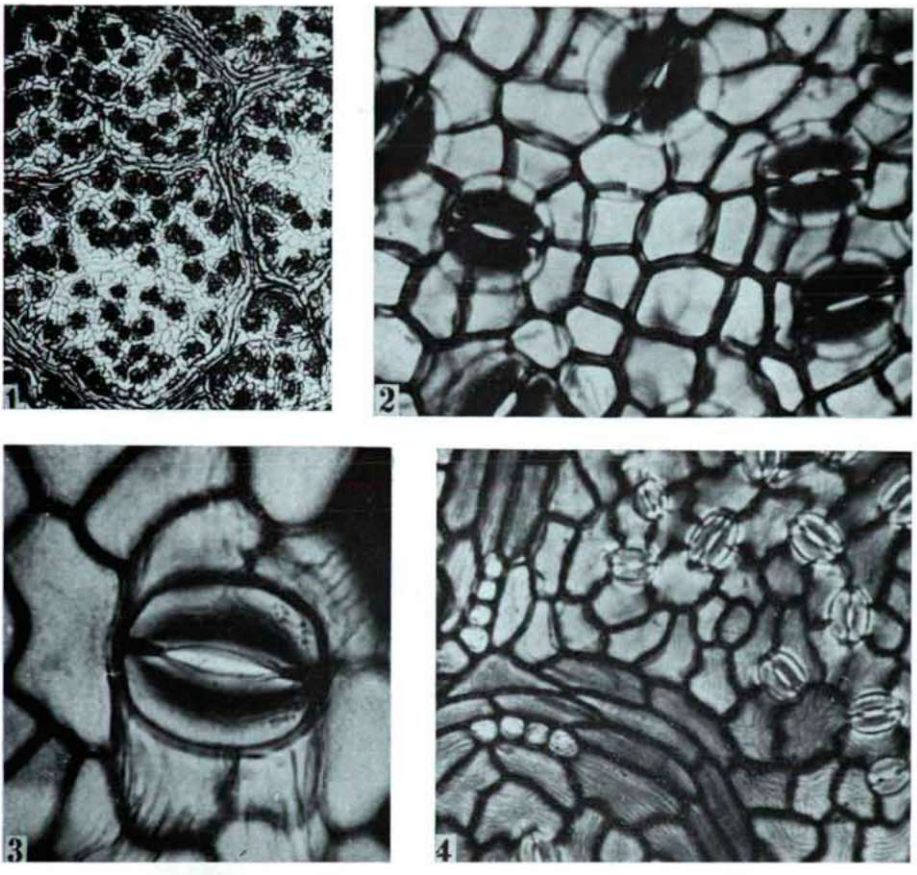


Fig. 1

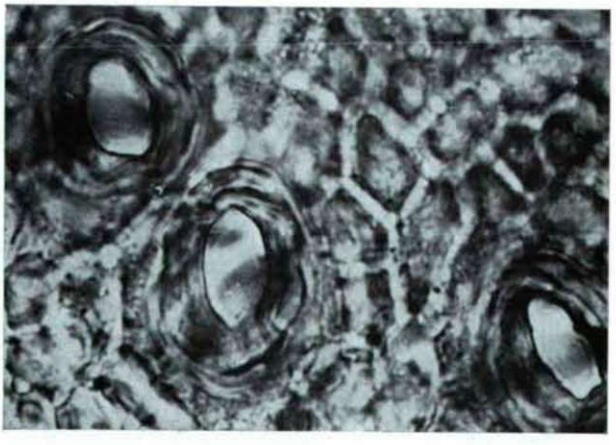


Fig. 2

PLATE II

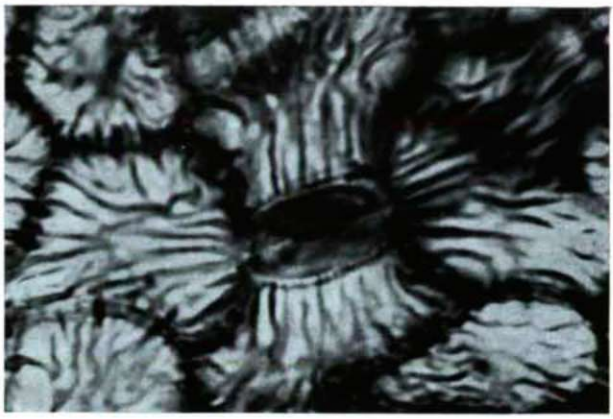


Fig. 1

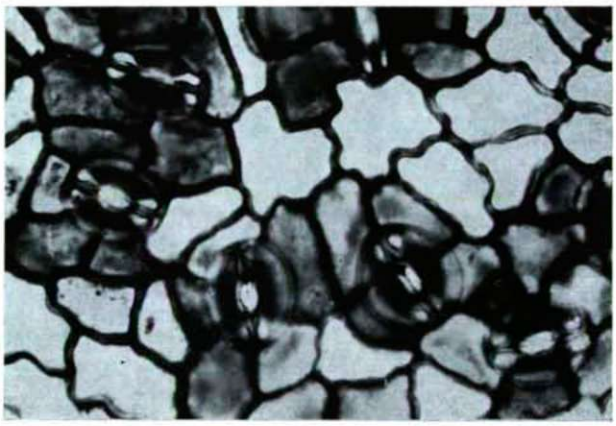
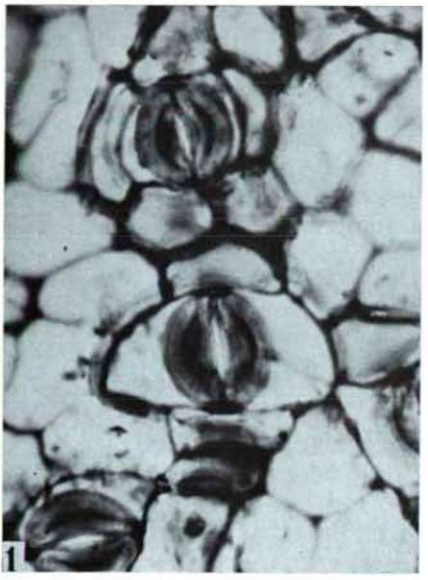


Fig. 2

Fig. 3

PLATE III



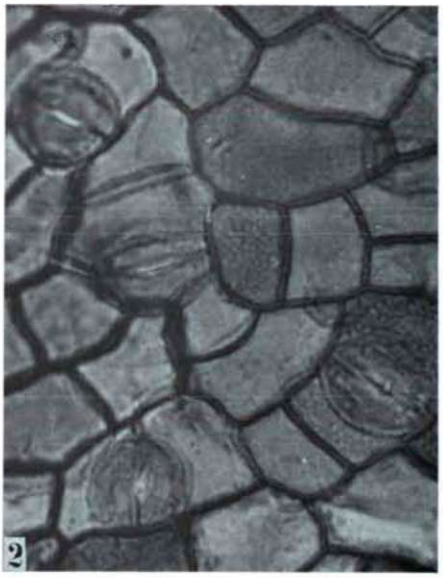


Fig. 1

Fig. 2