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ULTRASTRUCTURAL CHANGES IN *VITIS VINIFERA* L., CV. PINOT GRIS TISSUES INDUCED BY LEAFROLL DISEASE

AURELIA BREZEANU[1], ELENA BUCIUMEANU[2]

Ultrastructural studies of *V. vinifera* cv. Pinot gris with typical leafroll disease symptoms showed masses of filamentous particles which can be considered as virions belonging to a closterovirus. As the result of virus and cell interaction, modified organelles and destructive processes characteristic of phloem-limited viruses, were present in all kinds of tissues examined. Very often nuclei with partially broken nuclear envelope and microparticles (of about 70 nm in dia) were observed. Unusual modificated cells containing particular formations with regular shape and size were also present.

Key words: *Vitis vinifera*, leafroll disease, tissues electron microscopy.

Leafroll is the most widespread virus disease of grapevine, occurring in all viticultural areas. Seven phloem-limited closteroviruses, serologically unrelated, have been associated with the disease, but its aetiology is not certain (7). The presence of these viruses in grapevine either separately or in different combinations is sufficient and necessary for the symptoms of the disease to appear (1).

Cytopathology of leafroll-affected grapevine has been studied in grapevine plants belonging to different cultivars. The main cytopathic effects observed were the presence of bundles of flexuous viruses, vesiculating mitochondria (3, 4, 5) and altered chloroplasts (2). Various modifications of mitochondria may be considered as a diagnostic value (4).

The aim of this paper is to analyse the cytopathic effects observed in leafroll affected grapevine (*V. vinifera L.*, cv. Pinot gris).

MATERIAL AND METHODS

Leafroll virus affected grapevine with typical disease symptoms belonging to *V. vinifera L.*, cv. Pinot gris have been used in this study. Axillary buds, radicle meristems and leaf tissue specimens (0.5-1.0 cm) were taken from *in vivo* and *in vitro* grown plants (obtained by multiple axilary bud regeneration) and processed for transmission electron microscopy according to the standard procedure. Similar tissues were taken from symptomless vines as control. The samples of the tissue were fixed overnight at 4°C in 3% glutaraldehyde and postfixed in 2% OsO4 in

0.1 M phosphate buffer pH 7.2 at room temperature for 2 hrs. Dehydration was carried out in an ethanol series and embedding in Epon 812. Thin sections were cut on the ultramicrotome TESLA and stained with uranylacetate and lead citrate by Reynold's procedure. The sections were observed in the TESLA BS 500 and Phillips electron microscopes.

RESULTS AND DISCUSSIONS

The electronomicroscopical observations of *V. vinifera* cv. Pinot gris tissues with typical leafroll disease symptoms revealed numerous modifications of the cells and organelles structure as an effect of virus and plant cell interaction. Part of them resemble the modifications induced by stress factors but some of them are specific for virus disease.

The main cytopathic effect observed was the presence of masses of flexuous filaments of undetermined length that can be considered as virus particles belonging to a closterovirus (Plate I, Fig. 1).

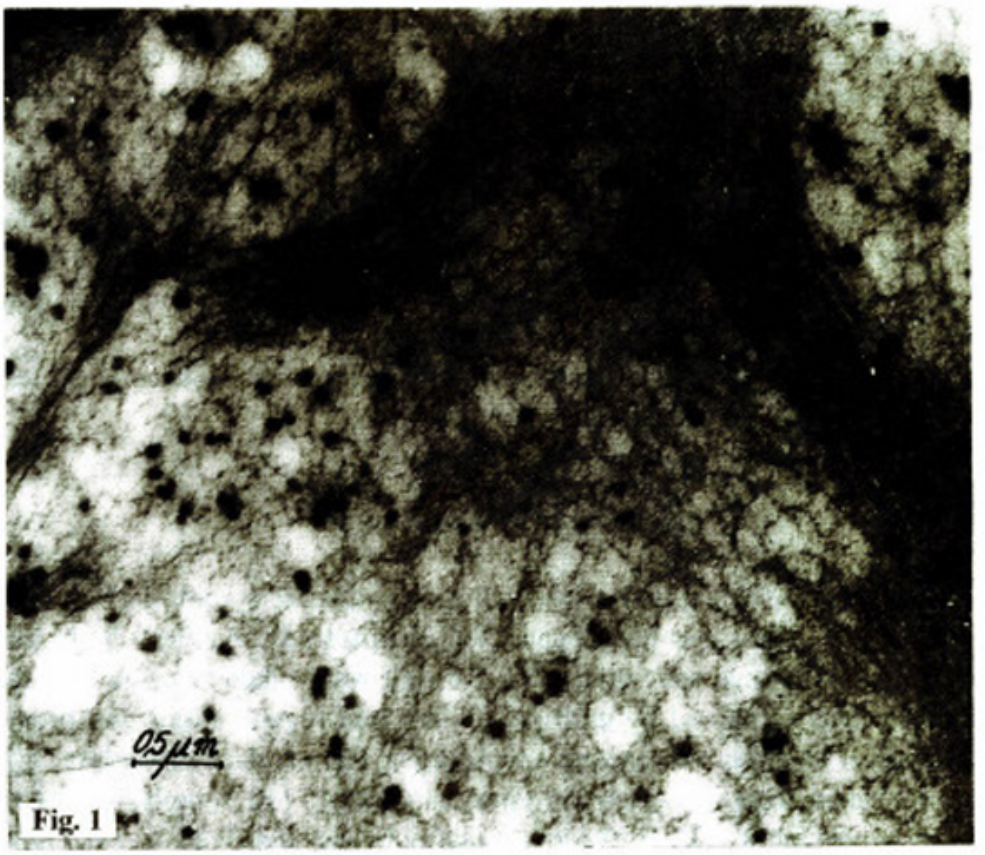

Plate I, Fig. 1. – A portion of the sieve elements that contains masses of filamentous particles in the cytoplasm.

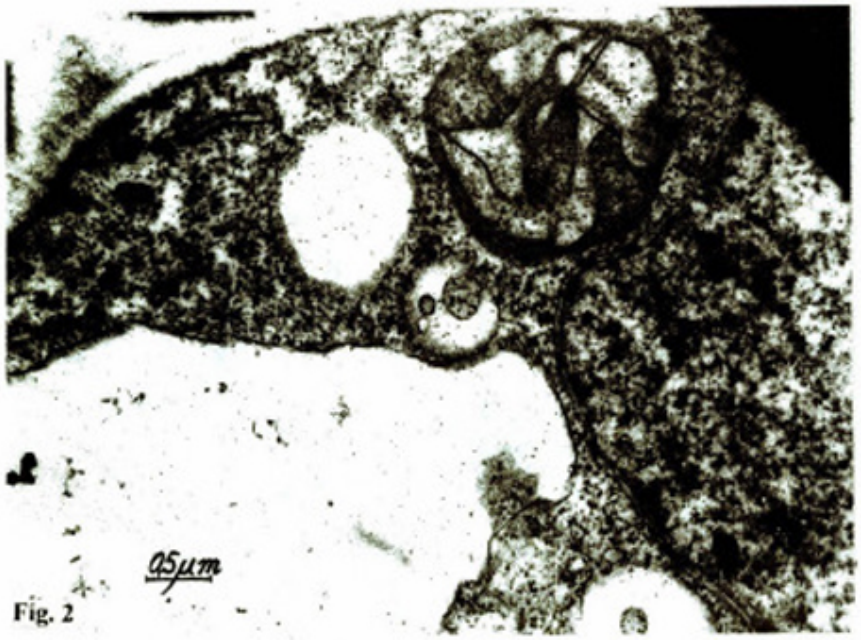

Plate I, Fig. 2. – Modified mitochondria with few cristae and vesiculated aspect. The cytoplasm of the cell is highly vacuolated.

The common cytopathic feature of phloem-limited viruses is the vesiculation of mitochondria; the mitochondria with a small number of cristae and vesiculated aspect were frequently observed in foliar tissue especially (Plate I, Fig. 2). Vesiculated mitochondria induced by grapevine leafroll-associated viruses type I and II (GLRaV-I and GLRaV-II) in the parenchyma phloem cells of naturally infected grapevine plants were also reported by FARAO et al. (6) who investigated their role in the infection process using cytochemical and immunocytochemical methods. They suggested that vesiculated mitochondria could be involved in the replication of the viral RNA but not in the assembly of virus particles. Moreover, as in the early stages of infection, vesicular mitochondria are easier to detect than virus particles, they could constitute a valuable aid for an early diagnosis of the grapevine leafroll disease.

Golgy bodies with intense secretory activity (with many vesicles to the ends) were also found in this tissue (Plate II, Fig. 3, see arrows). The plastids with starch inclusions were rarely observed. The cell wall tends to be regarded mainly as a physical supporting and barrier structure, but few abnormalities have been observed in or near the affected cell wall. Cell wall protrusions involving plasmodesmata with one or more canals filled with electron-dense material were found. Depositions of electron-dense material between the cell wall and the plasmalemma were observed on large areas (Plate II, Fig 1).

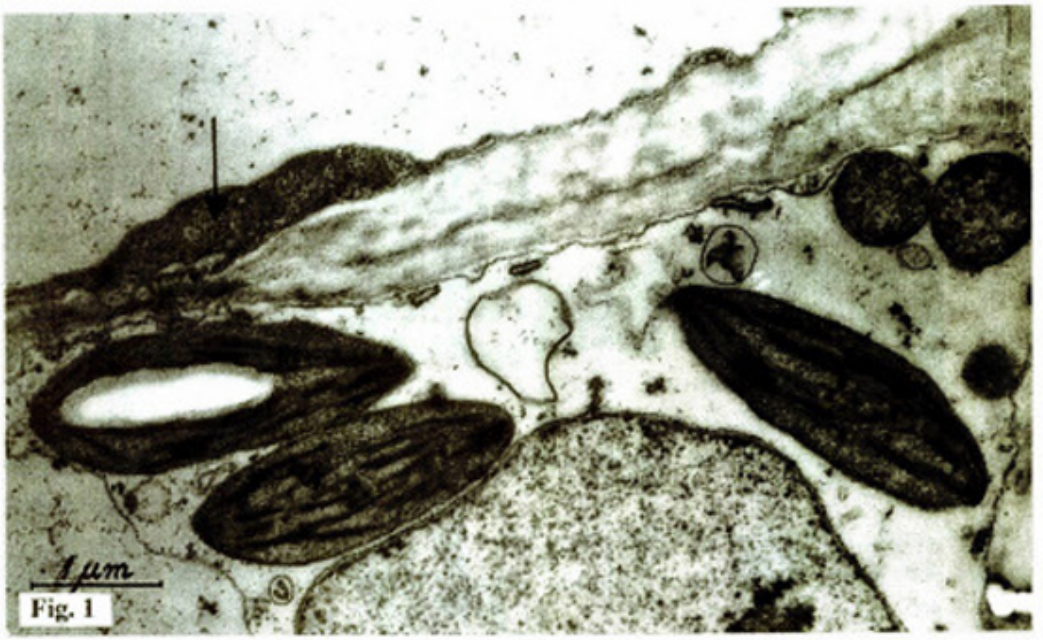

Plate II, Fig. 1. – Depositions of electron-dense material between the cell wall and the plasmalemma near the plasmodesmata (see arrow).

In the radicle meristem tissue the presence of myelin-like bodies was frequently observed either adjacent to the tonoplast or in the vacuoles together with phenol inclusions (Plate II, Figs. 3 and 4). It is possible that the penetration of these formations into the vacuoles and the accumulation of the phenols may take place in a later cellular stage of the infection and also the myelin-like bodies close by the tonoplast may involve other cell membranes in their formations. The presence of numerous ribosomes spread in the cytoplasm proved the disruption of the rough endoplasmic reticulum and the cytoplasm became very electron-dense. Neighbourhood double membrane vesicles with irregular shape were also observed.

In the axillary buds large nuclei with partially broken nuclear envelope and microparticles of about 70 nm in diameter, resembling NEPO viruses, were observed. However, the structure of nucleolus was preserved but with an irregular contour. Numerous vesicles were noticed nearby the plasmalemma either separately or connected to the membrane from which they appeared to originate (Plate III, Figs. 1, 2). In other cases, the chromatin was distributed to the nucleus periphery. Between the membranes of the nuclear envelope electron-dense roundish bodies of 80-300 nm were found (Plate IV, Fig 1). Very often disorganisation of cellular content was observed replaced by the appearance of unusual formations with regular shape and size (Plate IV, Figs. 2, 3). In these particular modifications of the cells the combined effect of a viral complex may be involved. In the samples of symptomless grapevine similar modifications were not found.

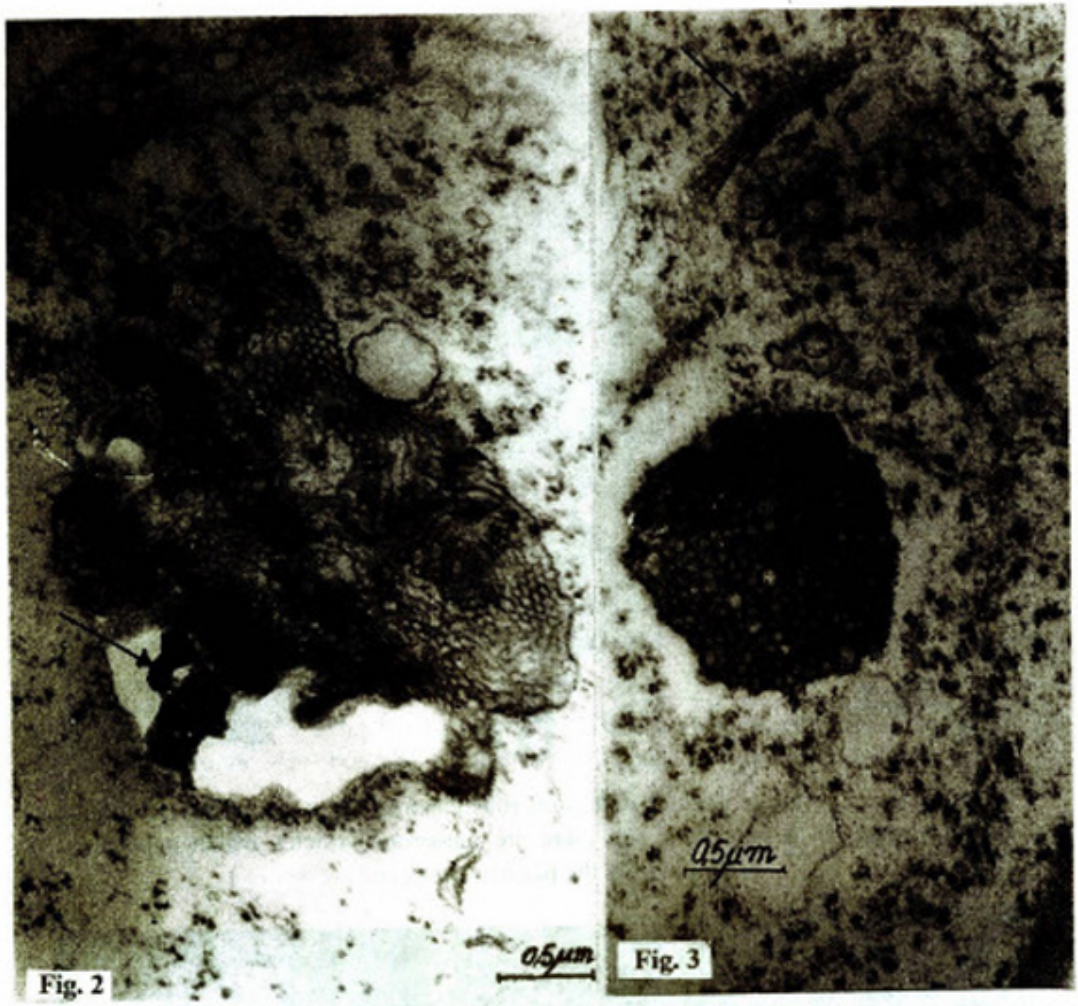

Fig. 2 Fig. 3

Plate II, Figs. 2-3. – Different myelin like bodies. In Fig. 2, a myelin like body with accumulation of phenols is observed (see arrow). In Fig. 5, a myelin body surrounded by cytoplasm optically less dense and the Golgi bodies with many vesicles to the end (see arrows) are evident.

CONCLUSIONS

1. In the axillary bud and leaf tissue the modifications produced by the virus infection were more numerous and more clear than in the radicle tissue.

2. The modifications of the organelles-like vesiculated mitochondria occurred in all infections with phloem-limited viruses. Due to the particular aspects of the nucleus, it can be concluded that the observed effects were produced by a viral complex.

3. Because of the difficulties in diagnosing the leafroll disease, a characteristic cytopathic feature may be a valuable aid in closterovirus diagnosis.

Acknowledgements. The authors gratefully thank Dr. P. Ploaie for helpful discussions and advice in material preparation.

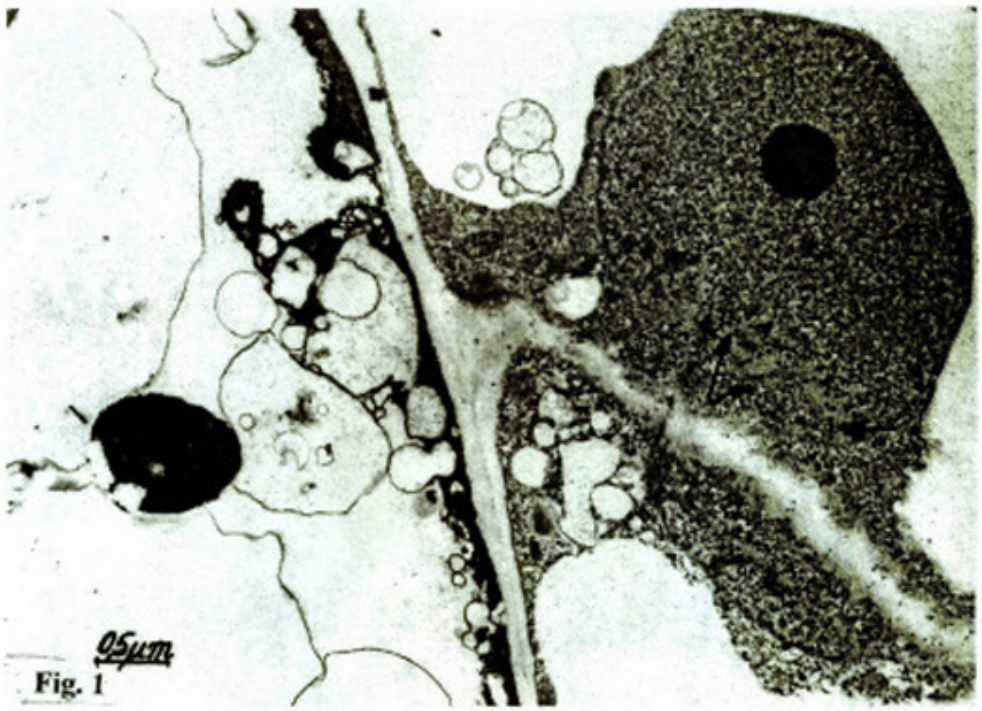

Plate III, Fig. 1. – Large nucleus with numerous electron-dense particles resembling NEPO viruses. In a neighbour cell there are numerous vesicles which appear to originate from the plasma membrane.

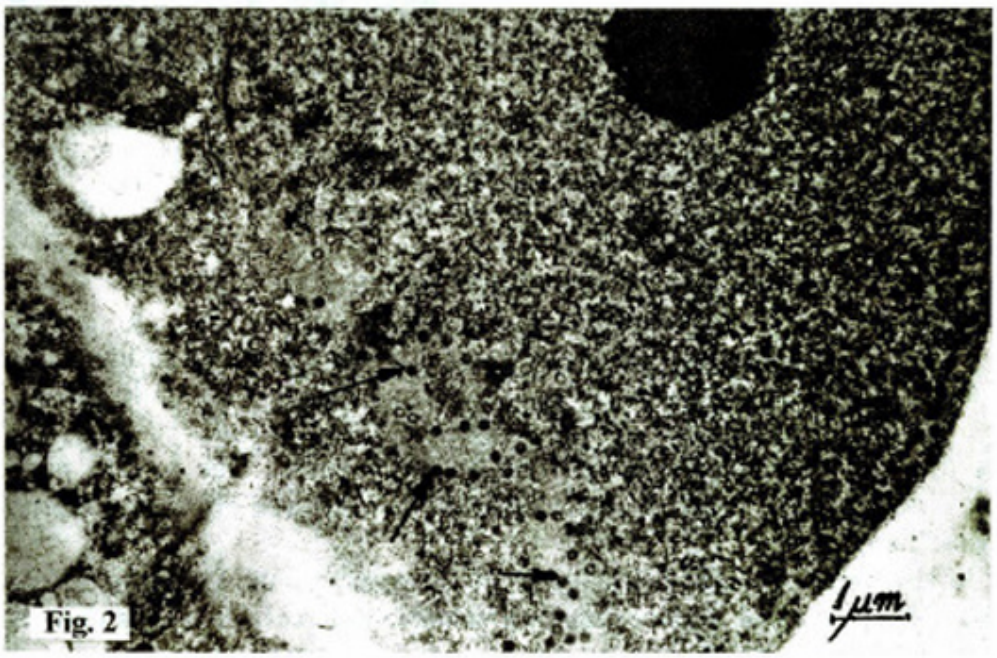

Plate III, Fig. 2. – A detail of the microvesicles from the nuclear envelope area (see arrows).

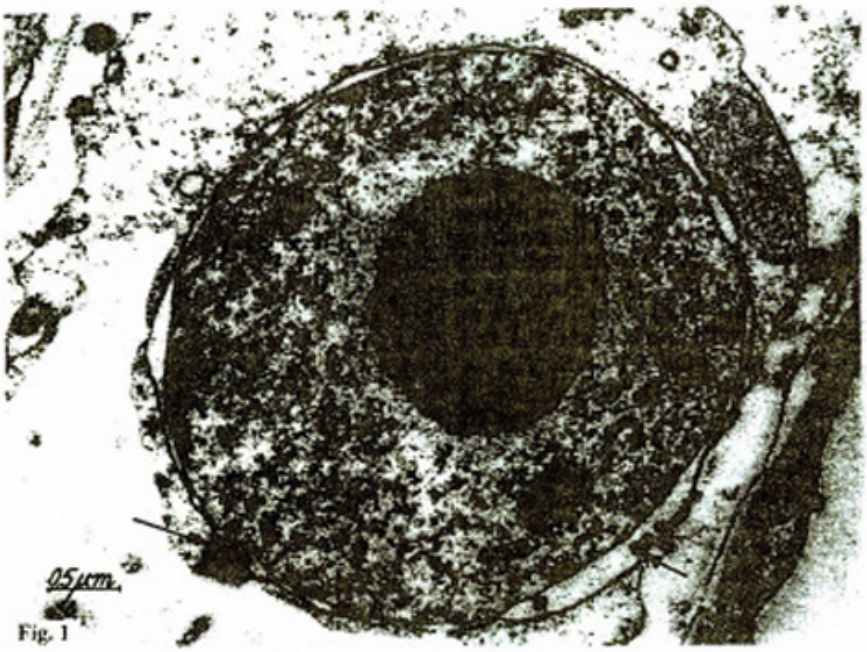

Fig. 1

Plate IV, Fig. 1. – A nucleus with large nucleolus and electron-dense bodies between the membranes of the nuclear envelope. The chromatin is directed to the nucleus periphery.

Fig. 2

Plate IV, Fig. 2. – Cell degradation and appearance of unusual formations with particular aspect (probably degenerative structures).

Plate IV, Fig. 3. – Detail of the cell degradations.

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SEX CHROMOSOMES IN SOME FLOWERING PLANTS OF THE ROMANIAN FLORA

VERONICA STOIAN, NICOLETA CONSTANTIN, ANDREEA CUNIȚĂ

Contrary to animal reproductive systems, which are mostly dioecious, the majority of the angiosperms are hermaphrodite. Nevertheless, the dioecious condition is peculiar to some angiosperm species or groups of species; sometimes, it is associated with sex heteromorphic chromosomes presence. We intend to study dioecious plants from the Romanian flora, either cultivated or spontaneous, in danger of extinction, and endemic in order to observe sex chromosome presence, structure and, ultimately, evolution. For the beginning, we chose *Silene alba*, *Cannabis sativa* and *Humulus lupulus;* their sex chromosome systems are presented and discussed.

Key words: sex chromosome, dioecious species, X/autosomes ratio.

Contrary to animal reproductive systems, which are mostly dioecious, the majority of the angiosperms are hermaphrodite. Nevertheless, the dioecious condition cannot be neglected; at least 1500 dioecious species, 1300 genera, and 160 families exist in the world flora; these species represent 4% of the dicots and 3% of the monocots.

The reproductive systems of angiosperms evolved from hermaphrodites and autoincompatibility toward bisexual reproduction and exogamy; two important "moments" of this process are autoincompatibility and the dioecious condition. The latter is usually peculiar to some angiosperm species or groups of species, and only occasionally features a whole subgenus (i.e. *Rumex*) or even a family (i.e. *Salicaceae*) (3).

As far as sex chromosomes are concerned, they are present only in some of these dioecious species, such as *Silene alba* (*Melandrium album*) and *Silene dioica* (*Melandrium rubrum*); the genetic formula of both of them is $2n = 24$, XX/XY. They belong to the large *Silene* genus, which contains 300 hermaphrodite and a few dioecious species (7). In order to study sex determination in this genus, polyploidy was induced by colchicine treatment, descendants were crossed and segregation analysis was performed; the results revealed that plants which have X/autosomes ratio 0.5-1.5 were female, if the Y chromosome was absent. This means that the reproductive system is "Y-active", as the presence of the Y chromosome confers masculinity; consequently, its presence is a prerequisite for the development of the male sex. Y chromosome value in sex determination approximately equals four Xs (3).

All of the species in fam. *Canabaceae* (*Cannabis sativa, Humulus lupulus*, and *Humulus japonicus*) have sex chromosomes. *Humulus* species are phenotypically similar, but they exhibit different chromosome number: the first one has $2n = 20$, is polytypic and the simplest sex chromosome system it carries is XX/XY, with Y smaller than X (both of them are euchromatic) (4). Other complex systems developed by translocation X/autosome or Y/autosome, e.g. *H.lupulus* var. *cordiofolius* female plants have the genetic formula for sex chromosomes $X_1X_1X_2X_2$, while the male - $X_1X_2Y_1Y_2$; fertility depends on regular disjunction of the four heterosomes in male meiosis (4, 7).

Sex chromosomes of *Cannabis sativa* usually are XX in female plants and XY in the male ones; Y is larger than X and the pair is the largest in the complement. In fact, *Cannabis* has a subdioecious condition: sex development is variable and sensitive to environmental factors (4)..

Angiosperm heteromorphic sex chromosomes were reported for the first time in *Rumex acetosa* and *Silene dioica*. Afterwards many other dioecious species chromosome complements were analyzed and pairs of heterosomes (or bivalents supposed to be heterosomes) were claimed to exist in 80-90 species (Table 1) (3, 4, 8). Most of the reports were not further supported by experimental data and correspondent species were eliminated; in other cases, results are still equivocal because of the different source of the material and/or technique. Few studies were carried out with modern molecular techniques on doubtful species; consequently, many of them were eliminated (4).

Table 1

Dioecious species which were investigated from the sex chromosome presence point of view (after Westergaard, 1958) (Grant V., 1975).

Species	Chromosomal constitution in	
	female plants	male plants
"Sure" species		
Cannabis sativa	XX	XY
Humulus lupulus	XX or $X_1X_1X_2X_2$	XY or $X_1X_2Y_1Y_2$
Humulus japonicus	XX	XYY
Rumes angiocarpus	XX	XY
Rumex tenuifolius (tetraploid)	(XX)XX	(XX)XY
Rumex acetosella (hexaploid)	(XXXX)XX	(XXXX)XY
Rumex graminifolius (octoploid)	(XXXXXX)XX	(XXXXXX)XY
Rumex hastatulus	XX	XYY
Rumex acetosa	XX	XYY
Rumex paucifolius (tetraploid)	XXXX	XXXY
Silene alba	XX	XY
Silene dioica	XX	XY
Coccinia indica	XX	XY

"Uncertain" species
Acer negundo (fam. Aceraceae)
Elodea canadensis (fam. Hydrocharitaceae)
Fragaria elatior (fam Rosaceae)
Ilex serrata (fam. Aquifoliaceae)
Morus bombycis (fam. Moraceae)
Populus sp. (fam. Salicaceae)
Salix sp. (fam. Salicaceae)
Silene otites, *S. densiflora* (fam. Caryophyllaceae)
Spinacia oleracea, *S. tetrandra* (fam. Chenopodiaceae)
Urtica dioica (fam. Urticaceae)
Valeriana dioica (fam. Valerianaceae)

With the same purpose, we intend to study dioecious plants from the Romanian flora, either cultivated or spontaneous, in danger of extinction, and endemic in order to observe sex chromosome presence, structure and, ultimately, evolution.

MATERIALS AND METHODS

- *Silene alba* (Mill.)E.H.L.Krause: a herbaceous, perennial, dioecious plant (1, 5). Flower buds and seeds were harvested from the spontaneous flora in the Botanical Gardens, Bucharest;
- *Cannabis sativa* L.: a herbaceous, perennial, dioecious plant (1, 5). Flower buds were harvested from the Frumuşani village area (Călăraşi);
- *Humulus lupulus* L.: a herbaceous, perennial, dioecious plant (1, 5). Seeds were harvested from the Muşeniţa village area (Suceava).

In order to observe meiotic chromosomes, we used immature anthers (approximately 1-2 mm), preserved in acetic ethanol 1:3; squashes were realized in 2% acetic carmine. Pollen mother cells in different meiotic stages were observed (6).

Mitotic sex chromosomes were analyzed in squashes of meristematic root tips from seeds germinated in semisterile conditions, 0.05% colchicine - treated (2 hours at room temperature) and fixed in acetic ethanol 1:3 for 24 hours at least. Hydrolysis was carried out at 60°C, for 15 min., in 1N hydrochloric acid. Chromosomes were colored with Schiff's reagent, for 1-2 hours (6).

We used an Amplival microscope to analyse slides and photos were taken with an Exacta-Varex device.

RESULTS

Silene alba (2n = 24, XX/XY) pollen mother cells in various meiotic stages were observed; in metaphase I, among the 12 bivalents, we observed X and Y

chromosomes, paired end-to-end (consequence of the presence of a homology region), and 11 were autosome pairs. X and Y heteromorphism allows us to differentiate them easily (Fig. 1); they are entirely euchromatic (4) (Figs.1, 2).

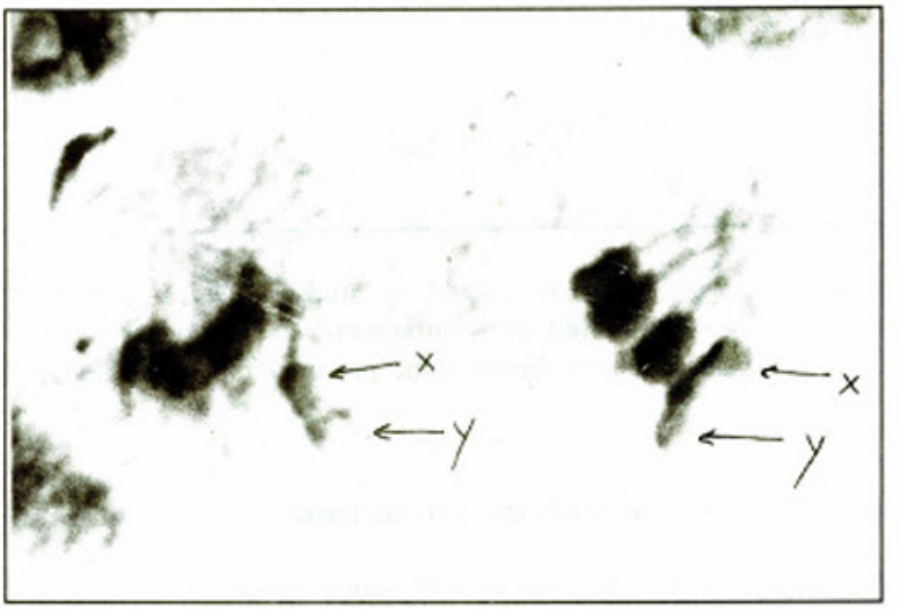


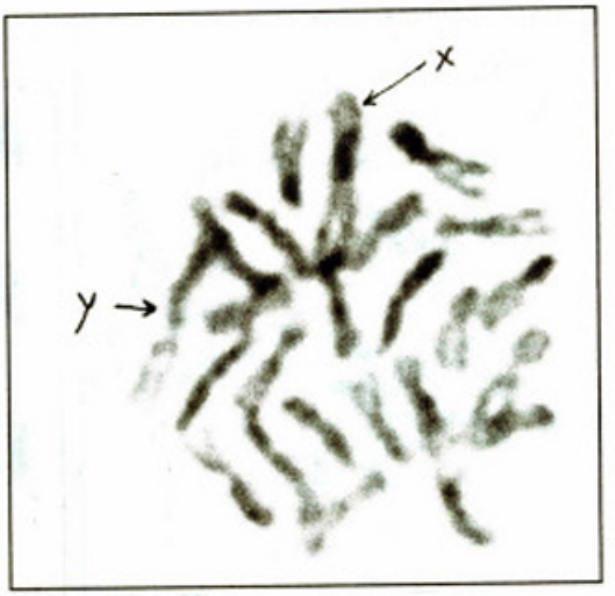
Fig. 1. – *Silene alba* meiotic metaphase I.

Fig. 2. – *Silene alba* meiotic meta-anaphase I.

In mitotic metaphase plates (Figs. 3, 5), one can observe that X and Y chromosomes are the biggest of the complement; Y is the largest metacentric, approximately twice larger than X, which is submetacentric; both of them are euchromatic.

Considering mitotic metaphasic chromosome analysis, we constructed the karyotypes in Figs. 4 and 6 (ordered in size decreasing order for each type): 11 homologous pairs of autosomes (4 of metacentrics, 7 of submetacentrics) and XY heteromorphic pair.

Fig. 3. – *Silene alba* mitotic metaphase.



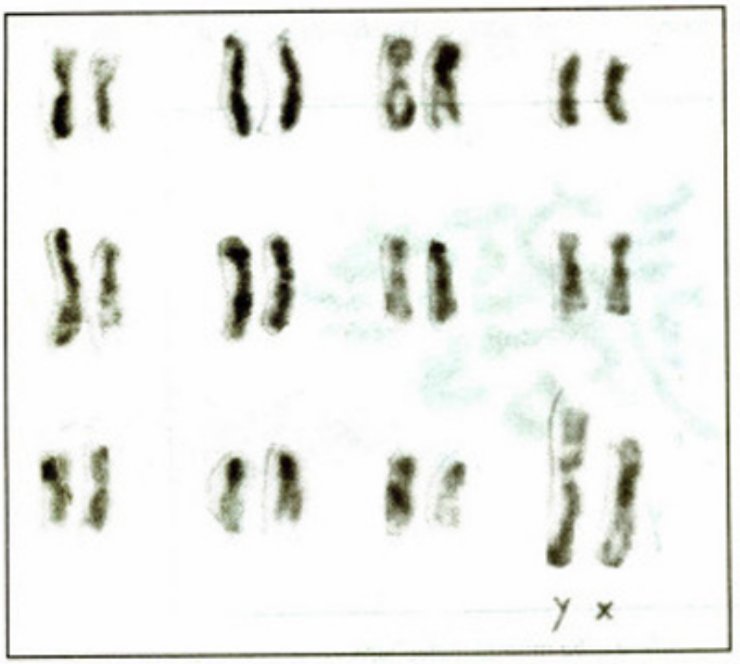


Fig. 4. – *Silene alba* karyotype.

In *Cannabis sativa* (2n = 20, XY) pollen mother cells in metaphase I (Figs. 8, 9), we observed end-to-end paired X and Y chromosomes. Y is larger than X and this pair is the largest of the complement (which also includes other 9 autosome pairs).

In *Humulus lupulus* (2n = 20, XX/XY), the karyotype in Fig.11 was realized based on mitotic metaphases; one of them is presented in Fig.10. Autosomes were grouped in 9 pairs, displayed in size decreasing order. Sex chromosomes are heteromorphic, euchromatic and X is larger than Y; heterosomes are the largest of the complement.

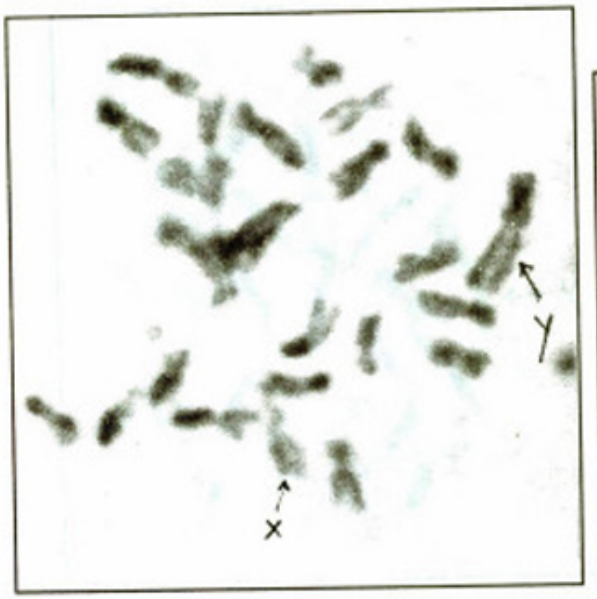


Fig. 5. – *Silene alba* mitotic metaphase.

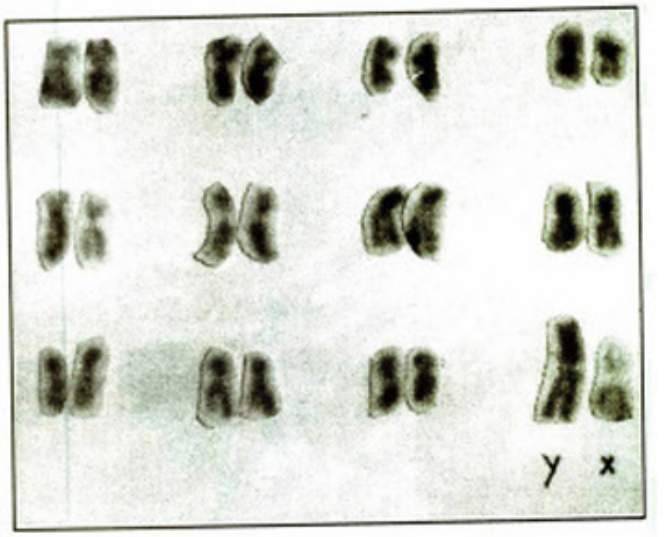


Fig. 6. – *Silene alba* karyotype.

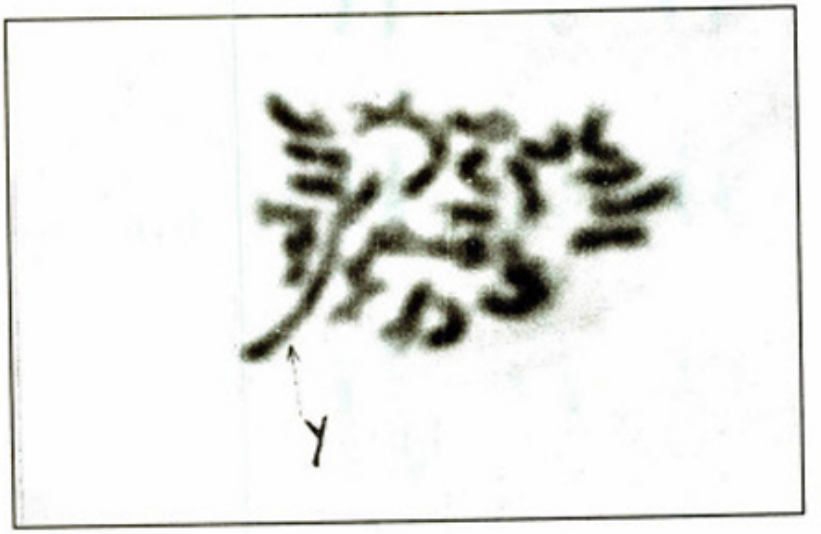


Fig. 7. – *Silene alba* mitotic metaphase.

CONCLUSIONS

1. The three spontaneous dioecious species studied exhibit well differentiated sex chromosomes, which are always the largest of the complement.

2. Considering our results and the literature data, we conclude as a rule that Y chromosome or their sum (if there are multiple Y chromosomes) is larger than X; nevertheless, we can observe one exception, *Humulus lupulus*.

Fig. 8 – *Cannabis sativa* meiotic metaphase I.

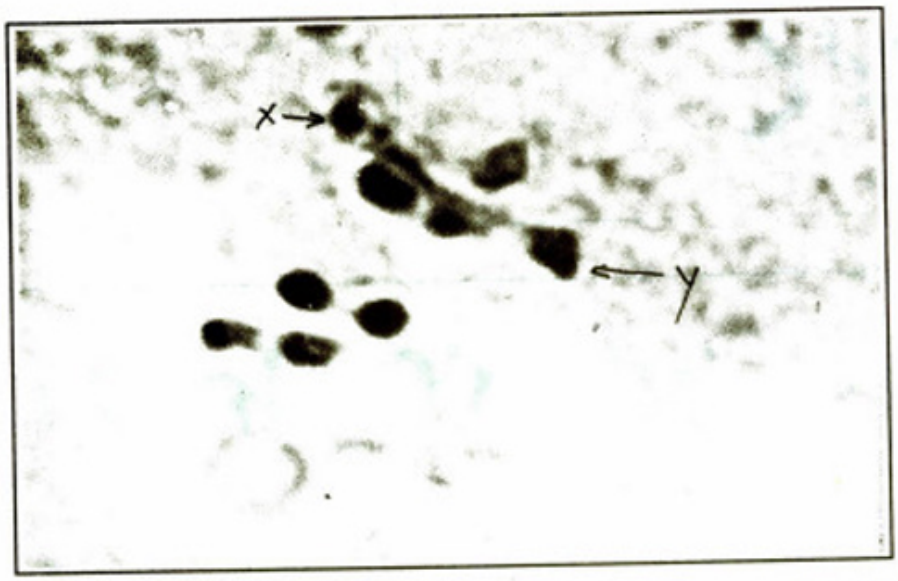


Fig. 9. – Part of a *Cannabis sativa* meiotic metaphase I. Arrow points toward sex bivalent.

3. Plant Y euchromatic chromosome differs from the standard animal model, where it is among the smallest chromosomes, mostly heterochromatic; considering X chromosome, as far as present data allow us to appreciate, there are no major differences.

4. Sex chromosomes evolution in plants seems to involve a rising in ADN content, possibly concurrent with dioecious condition origin (3).

Fig. 10. – *Humulus lupulus* mitotic metaphase.

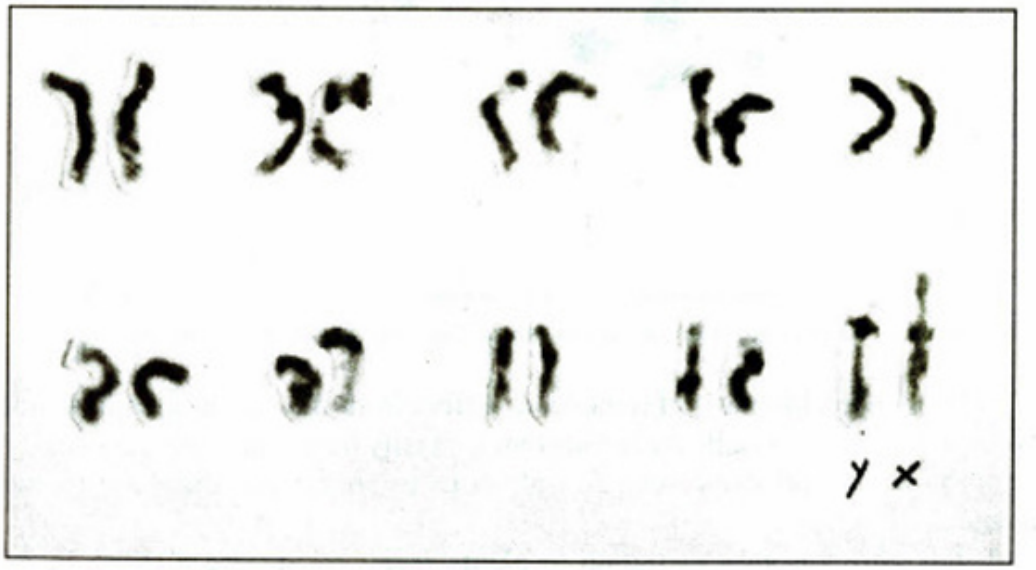


Fig. 11. – *Humulus lupulus* karyotype.

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HISTO-ANATOMICAL AND MICROMORPHOLOGICAL OBSERVATIONS CONCERNING THE LEAF OF SOME *MARANTACEAE* SPECIES

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Histo-anatomical characteristics of the leaf (petiole and lamina) in 10 *Marantaceae* species are described. Special references are made on the micromorphology of foliar surfaces, based on their investigations by scanning electron microscopy.

Key words. leaf, structure, micromorphology.

The *Marantaceae* family (*Zingiberales* Ord., *Liliatae* Cls.) is composed of over 30 genera and approximately 400 species distributed in tropical and subtropical areas (6); in our temperate climate, *Marantaceae* species are present just in greenhouse collections of botanical gardens or, rarely, in some private collections. They are cultivated for their special decorative qualities generated by their colours and the design of the foliar apparatus. From the 24 taxa which are cultivated in the greenhouse of the Botanical Garden of Iaşi (7), we investigated 10 species from 3 genera, for evidencing the constant histo-anatomical characteristics which could be used for taxonomical purposes and which complete the researches made until now on decorative plants.

HISTORICAL REFERENCES

The scientific treatises on the anatomy of *Monocotyledons* (2, 4, 5) and some general papers (1, 6) give us some general information referring to the structure of the representative plants of this family. We mention therefore data concerning cytological and histo-anatomical differences between the sectors with special foliar chromatic and some aspects referring to how aeriferous spaces of the petiole structure were formed (5); a special study was made on the functioning mode of articulare pulvinules (6).

The *Marantaceae* species have been little investigated from a histo-anatomical point of view; in Romanian papers these problems have not been investigated, until now.

As in some works the existence of some epicuticular wax was only mentioned (5), we intended, through investigations on foliar surfaces by scanning microscope, to bring some new observations relating to their micromorphology.

MATERIAL AND METHODS

The investigated material is represented by the leaves of 10 species of the *Marantaceae* family: *Maranta leuconeura E. Morr. "Fascinator", Maranta leuconeura E. Morr. "Kerchoveana", Calathea ornata (Lind.) Koern., Calathea bachemiana E. Morr., Calathea makoyana E. Morr., Calathea lancifolia Boom. (C. insignis), Calathea zebrina (Sims.) Lindl., Calathea lietzei E. Morr., Ctenanthe setosa (Rosc.) Eichler, Ctenanthe pilosa "Golden Mosaic", Stromanthe sanguinea.*

Sample fixation and obtention of *microscopial* preparations were realised through the known techniques used in vegetal histo-anatomical investigations.

For detailed investigation of foliar *micromorphology* the samples were dried through physical methods, metalised with Pt and examined on a Tesla BS-340 scanning microscope.

RESULTS AND DISCUSSIONS

Leaf structure of the 10 *Marantaceae* species was analysed concerning:

- **petiòle**: contour of transversal section in the middle third, epidermis organization, dimensions and disposition of the aeriferous cavities, presence or absence of oxaliferous cells in *fundamental* parenchyma, and the number, size, disposition and structure of vascular bundles;
- **lamina**: epidermis organization in superficial and transversal sections (at midvein level and between *secondary* veins), structure of the assimilating parenchyma and aquiferous parenchyma.

PETIOLE. The contour of the *transversal* section through petiole differs from approximately elliptical with the adaxial plane side, limited by 2 lower prominent ribs (*Calathea lancifolia*), incompletely elliptical with a superficial adaxial (*Ctenanthe setosa*) or large and deep (*Calathea ornata*) pit, semielliptical with a deep adaxial (*Ctenanthe pilosa*) or large (*Maranta leuconeura "Fascinator"*) pit, to elliptical (*Calathea zebrina*), ovoid (*Maranta leuconeura "Kerchoveana"*), almost circular (*Calathea makoyana*), or circular (*Calathea bachemiana*).

The epidermis is formed of isodiametrical little cells with all the walls, especially the external one, thickened in the majority of the investigated species; rarely, there are also slightly tangential (*Calathea lancifolia*) or radial (*Ctenanthe setosa*) elongated cells; among the usual epidermal cells there are stomata and unicellular trichomes (*Calathea bachemiana, Calathea makoyana*).

The fundamental parenchyma often contains cells with simple oxalate crystals (*Calathea lancifolia, Calathea zebrina, Ctenanthe pilosa, Calathea makoyana, Calathea lietzei*). Sometimes, in hypodermical position, some sclerenchyma cordons were observed which form a discontinuous ring, broken by

cellulose parenchymatic cells (*Calathea ornata*) or by isodiametrical vascular bundles (*Ctenanthe setosa*).

The continuity of the fundamental parenchyma is often interrupted by some relatively large aeriferous spaces (*Calathea lancifolia*), situated just abaxially (*Calathea zebrina*), of an irregular contour (*Ctenanthe setosa*) or filled with star-shaped cells (*Ctenanthe pilosa, Calathea lietzei*).

Vascular tissues (Plate I) form bundles of collateral closed type, their number, size and disposition being comparatively *different* in the studied species. The bundles are disposed on 3 (*Calathea lancifolia, Calathea lietzei*) or 2 concentric circles (*Ctenanthe setosa, Stromanthe sanguinea*), one of them is incompletely adaxial; in this case, there are also central little bundles formed just of sclerenchyma. In other investigated species, the disposition of the bundles is irregular, hardly distinguishing 1-2 external bundles arches and some other inner bundles. Usually, external bundles are small, with more or less sclerenchyma around them; the median bundles are radially elongated, with sclerenchyma just at the phloemic pole (*Maranta leuconeura "Fascinator"*) or at both poles, out more developed at the phloemic one (*Calathea ornata, Calathea lancifolia*); the inner bundles are thin, often isodiametrical, with few sclerenchyma on the outskirts of the phloem.

The sclerenchyma is composed of cells with very thick lignified walls (*Calathea zebrina, Ctenanthe setosa, Ctenanthe pilosa, Calathea makoyana*) or unlignified walls (*Calathea lancifolia*); in other species the sclerechyma is moderate or little thickened (*Maranta leuconeura "Fascinator"*).

LAMINA. The *epidermis* presents cells with an irregular contour and undulated lateral walls, the amplitude of the undulations is higher in *Maranta leuconeura "Kerchoveana"* or less higher in *Calathea bachemiana*; here and there in the lower epidermis stomata of biperigen type were observed.

Examined by the scanning electron microscope (Plate II), the foliar surfaces present some interesting *aspects* concerning micromorphology, the form and dimensions of the epidermal cells, the aspects and the thickness of the lateral walls, the form, dimensions and the density of the wax deposits.

In the upper epidermis, the cells present lateral walls which are relatively thickened and slightly undulated (*Calathea ornata*) or almost plane with polyhedral cells (*Ctenanthe setosa*); the undulations of the lateral walls can be rare and of low amplitude (*Maranta leuconeura "Fascinator"*); in some cases, the walls are very thickened, with undulations which are difficult to observe (*Calathea bachemiana*) or the limits of the walls are well marked by high and thin wax crests, more evident after reducing the cellular volume by drying (*Calathea zebrina*).

The epicuticular wax is situated on the surfaces of the upper epidermis as granules of different dimensions disposed relatively uniformly (*Calathea ornata*) or zonally (*Calathea lietzei*), with variable frequency; sometimes even missing (*Ctenanthe setosa*).

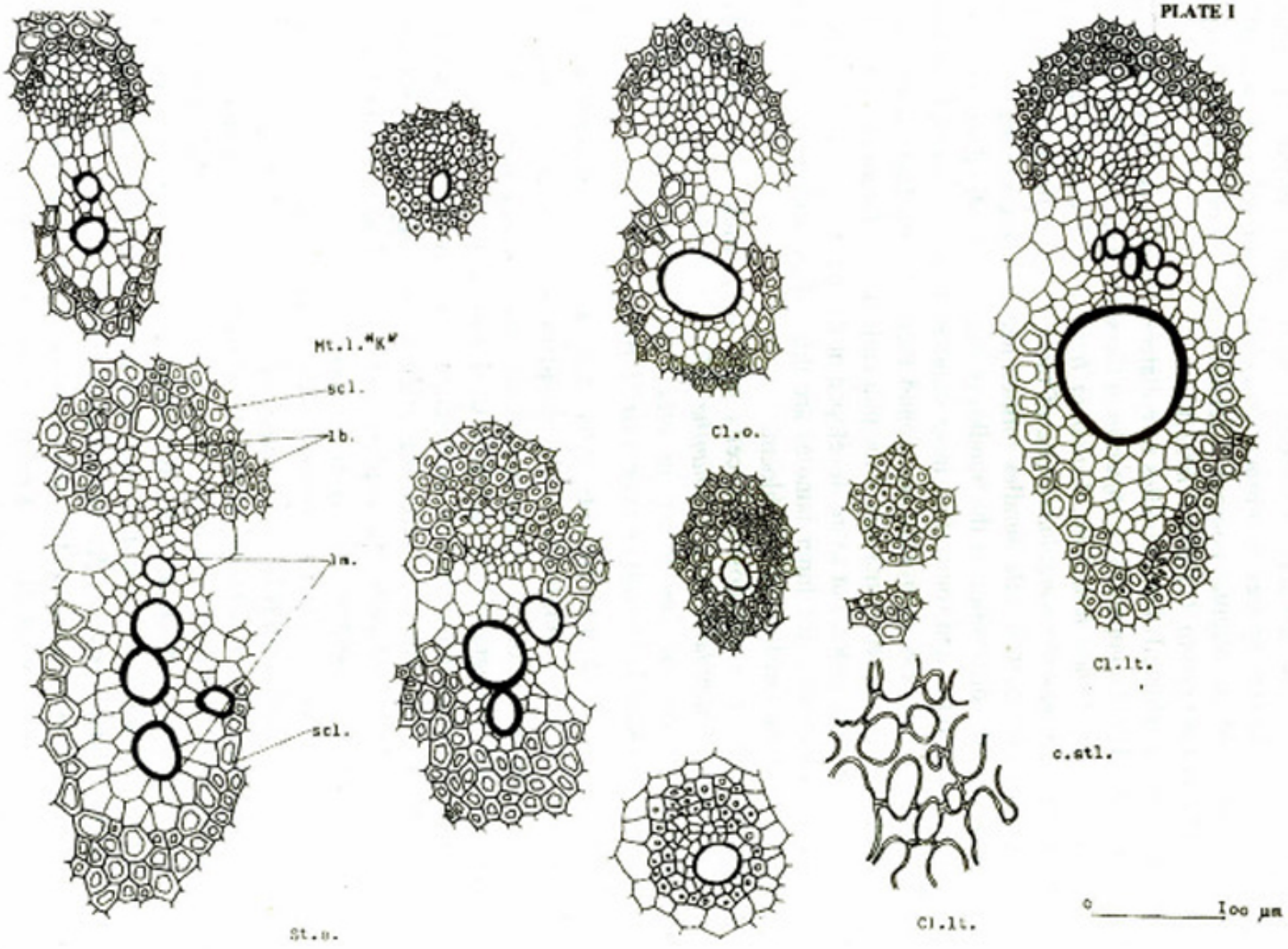

Plate I. The structure of the vascular bundles of the petiole in *Maranta leuconeura "Kerchoveana" (Mt.l "K")*, *Calathea ornata (Cl.o)*, *Calathea lietzei (Cl. lt.)* and *Stromanthe sanguinea (St.s.)*: scl. - sclerenchyma; lb. - liber (phloem); c.stl. - star-shaped cells.

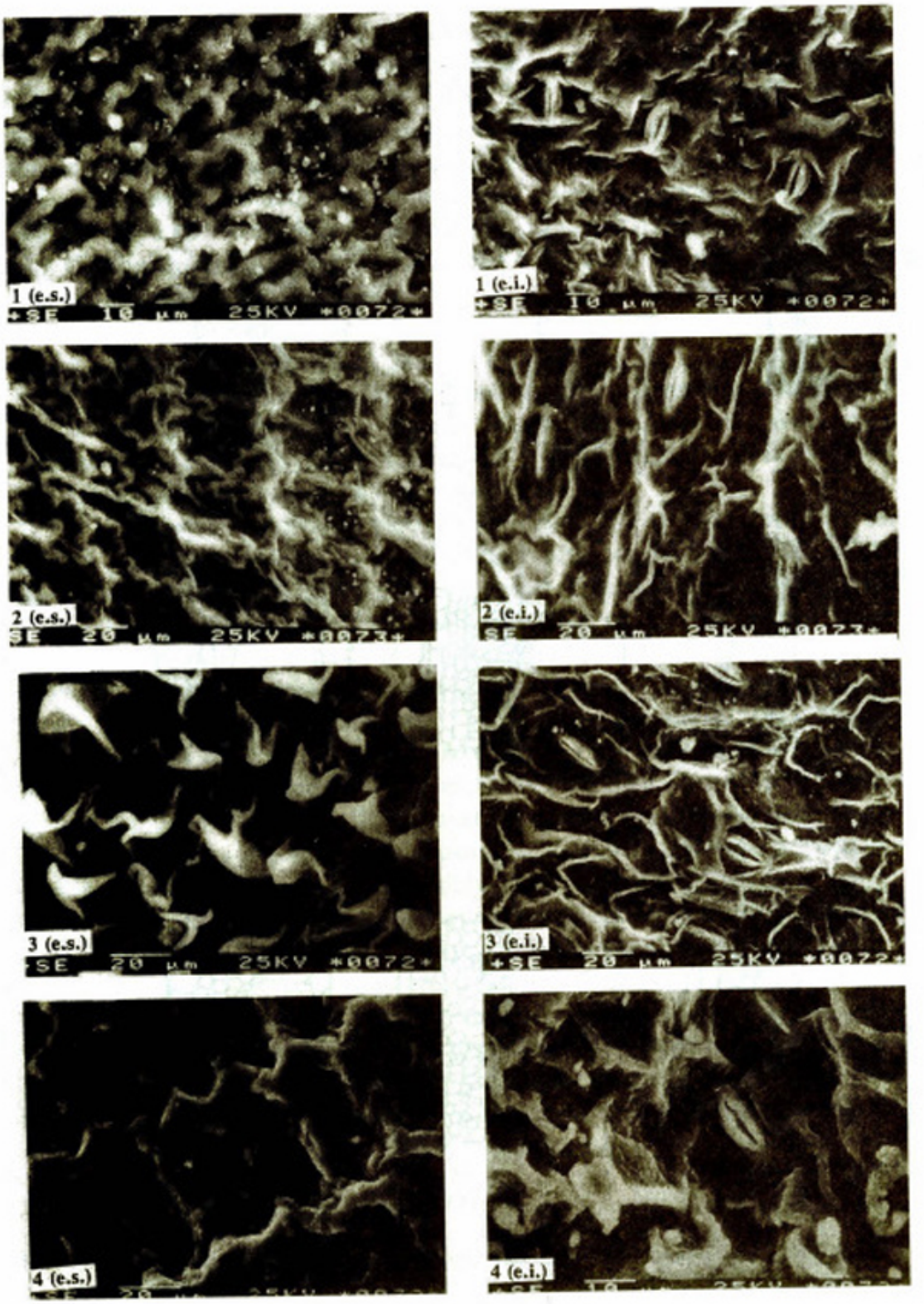

Plate II. Micromorphology of foliar surfaces in: *Calathea ornata (1), Calathea lietzei (2)* and *Calathea zebrina (3); Ctenanthe setosa (4)*: e.s.-upper epidermis; e.i.-lower epidermis.

PLATE III

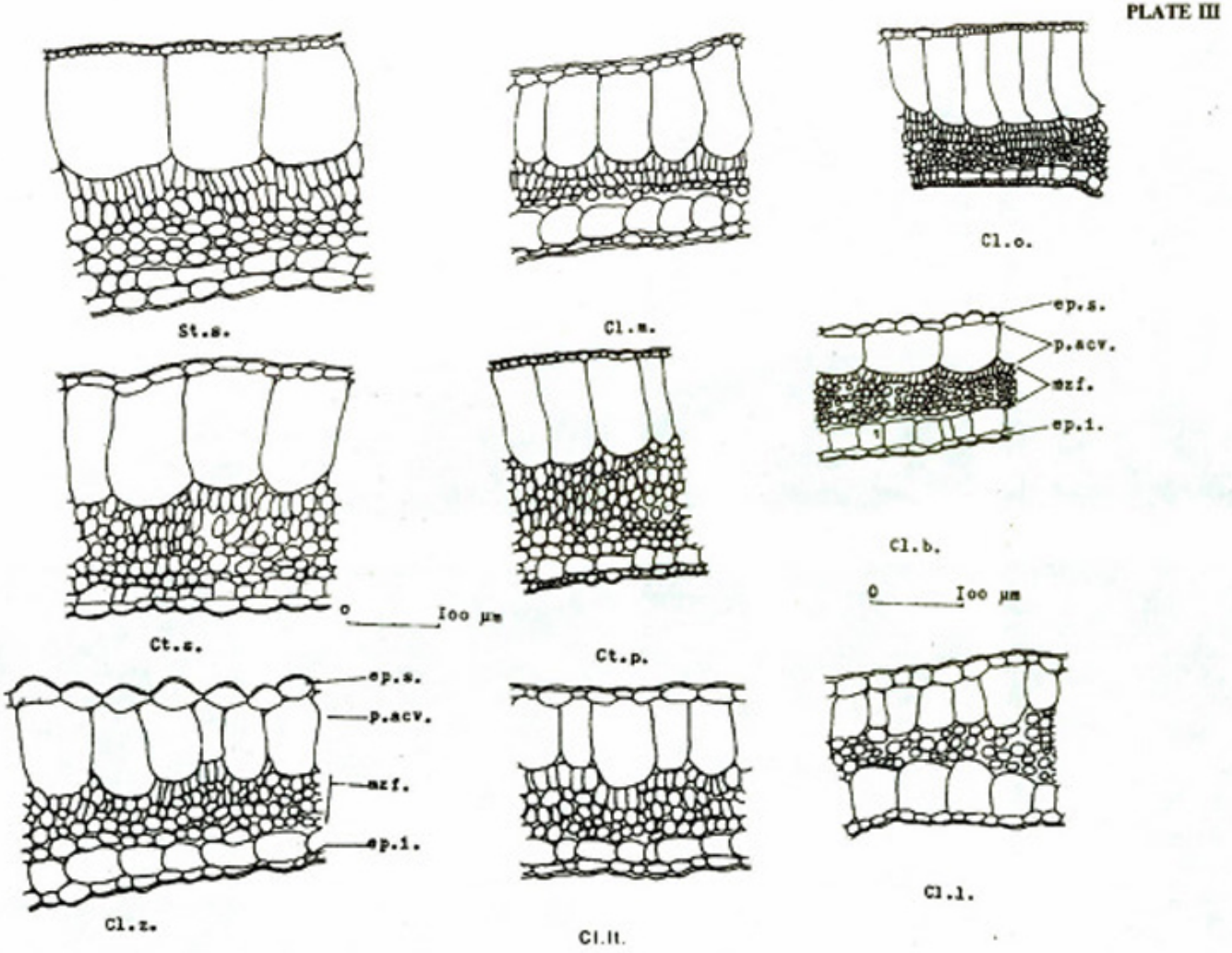

Plate III. The structure of the lamina in transversal section in: *Stromanthe sanguinea (St.s.); Calathea ornata (Cl.o.); Calathea makoyana (Cl. m.); Ctenanthe setosa (Ct.s.), Ctenanthe pilosa (Ct.p.); Calathea zebrina (Cl.z.); Calathea lietzei (Cl.lt.); Calathea lancifolia (Cl.l.)*: e.s. upper epidermis; e.i. lower epidermis; p.acv. aquiferous parenchyma; mzf. mesophyll.

The lower epidermis is formed of cells with a contour very difficult to identify, but with irregularities less evident; sometimes, the cells are of low elliptical form, with undulated walls and with some fragile and many granular wax deposits (*Stromanthe sanguinea*). The wax deposits can be both rare and strong (*Ctenanthe setosa*) or relatively little and frequent (*Maranta leuconeura "Fascinator"*).

In transversal section, the midvein is slightly prominent (*Calathea makoyana, Maranta leuconeura "Fascinator"*), moderately prominent (*Calathea ornata, Calathea lietzei*) or highly prominent (in the other species); at the lower side of the lamina, at the upper side of it, an adaxial little deep pit is formed (*Calathea ornata, Calathea lietzei*).

The epidermis presents very small isodiametrical or slightly tangential elongated cells (*Ctenanthe pilosa, Stromanthe sanguinea*); sometimes, the cells of upper and lower epidermis are slightly papiliferous (*Maranta leuconeura "Fascinator"*), or they have different sizes with a plane external wall in the lower epidermis (*Maranta leuconeura "Kerchoveana"*).

The hypodermal aquiferous parenchyma (Plate III) is unistratified at the lower face and uni- or bistratified, with bigger cells, at the upper face of the lamina; sometimes, the cells have a palisadic aspect (*Calathea zebrina*); rarely, the cells of the hypodermal tissue from the upper face of the lamina are small, rectangular, with rounded extremity (*Calathea makoyana*).

The mesophyll is thin, homogeneous, of 3-5 isodiametrical cell layers (*Maranta leuconeura "Kerchoveana", Calathea lancifolia*) or slightly heterogeneous, the cells of the adaxial layer have a palisadic aspect (*Calathea lancifolia*); sometimes, the mesophyll is relatively thickened, formed of 4-5 layers with small cells, the cells of the first 2 layers under the aqueriferous parenchyma having a palisadic aspect (*Calathea makoyana, Calathea zebrina, Stromanthe sanguinea*); rarely, the mesophyll is visibly more thickened (*Ctenanthe setosa, Calathea lietzei*). Thus, the structure of the lamina has a bifacial-isofacial structure (*Maranta leuconeura "Kerchoveana", Maranta leuconeura "Fascinator"*) with a slight tendency to a heterofacial structure in the other species.

With the analysis of the leaves of the 10 investigated species, this paper completes scientific data referring to the disposition of vascular bundles from the petiole and presents some conclusions about the epidermis structure in a surface view, the lamina anatomy in transversal section at midvein level and between lateral veins.

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ORIGINE, MORPHOLOGIE ET CLASSIFICATION DES ÉPINES CHEZ LES ESPÈCES DES *CACTACEAE*

RODICA RUGINĂ, DANIELA DORIN, C. TOMA

Les auteurs analysent la morphologie des aréoles et des épines des 95 espèces des Cactacées, cultivées dans les serres du Jardin Botanique de Iaşi, mettant en relief les caractères des épines selon lesquels les taxons peuvent être classifiés: forme, grosseur, fréquence, couleur, consistance, localisation sur les aréoles (marginale, centrale).

Mots clé: épines, morphologie, *Cactaceae*.

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Les Cactacées représentent une famille avec des espèces très nombreuses, répandues sur le continent américain entre 35^0 N et 54^0 S, contenant presque toutes les formes de relief. Backeberg C. [1], celui qui a réalisé la monographie de cette famille, cite 1900 espèces qui appartiennent à 189 genres, et Emberger L. (3) prend en considération 2000 espèces encadrées en 20-30 genres.

Ce sont des plantes adaptées aux valeurs extrêmes de la température (de 37-40 ^{0}C à 7-8^0 C) et des précipitations (400 mm au Mexique et 2000-6000 mm en Patagonie). Ces conditions ont conduit à l'apparition de formes plus variables, fait qui a rendu difficile l'intégration systématique et a mené à la création de nombreuses synonymies.

Pour l'analyse de la nomenclature des taxons étudiés dans cet article, l'ouvrage de référence a été celui de Backeberg C. [1], dans quelques situations seulement on a utilisé d'autres ouvrages [2, 3, 5, 11, 13, 14].

Pour caractériser les espèces de Cactacées, les traités de taxonomie font référence à la forme, à la consistance et à la position en espace de la tige, la présence ou l'absence des feuilles et des fleurs; les épines, les glochides et les poils, par leurs forme, grosseur, couleur et manière d'arrangement sur les aréoles et sur les mamelons complètent la diagnose.

Des commentaires sur l'origine des épines, des mamelons et des aréoles sont faits dans les ouvrages de: Emberger L. [3], Plantefol L. [11], Leinfellner W. [8] et même Metcalfe C. R. et Chalk L. [9], faisant appel à la littérature ou à des données personnelles.

Les études d'anatomie faites par Gravis A. (4) sur un nombre de 29 taxons de la famille *Cactaceae*, ont mis en valeur l'importance taxonomique et évolutive de la morphose des épines, en distinguant 7 groupes anatomo-éthologiques.

MATÉRIEL ET MÉTHODE DE TRAVAIL

Le matériel provenant des serres du Jardin Botanique de Iaşi, qui abrite 650 unités systématiques de la famille *Cactaceae*, appartient à 92 genres et 3 sous-familles.

Dans cette étude ont été analysés 97 taxons (90 espèces et 7 variétés) encadrés en 42 genres des sous-familles *Pereskioideae* (1 genre), *Opuntioideae* (4 genres) et *Cereioideae* (37 genres). Les observations, réalisées directement à l'oeil nu ou à la loupe, ont suivi: le nombre, le groupement en aréoles, la couleur, la forme et la consistance des épines ainsi que la forme et la couleur des aréoles. En forme synthétisée, les données ont été enregistrées dans le tableau 1, les taxons étant arrangés dans l'ordre alphabétique des genres et des espèces.

Dans la littérature consultée, roumaine et étrangère, des données pareilles par le mode d'approche manquent, fait qui nous a encouragés à entreprendre ce travail.

RÉSULTATS ET DISCUSSIONS

Les Cactacées sont considérées comme des plantes sans feuilles, bien que, en réalité, les feuilles soient représentées par des épines, qui sont groupées sur des surfaces petites, rondes ou ovales, dénommées aréoles. Sur ces surfaces, outre des épines, on peut observer aussi des glochides, semblables aux épines, mais avec la surface barbelée, ainsi que des poils courts et nombreux, qui forment un feutre épais à la base des glochides.

L'origine de ces surfaces a été interprétée de différentes manières, soit comme l'aiselle d'une feuille [4], soit comme un bourgeon [9], où l'épine est insérée à la base d'un axe secondaire, interprété donc comme une annexe de celle-ci. Plus vraisemblable est l'opinion du Nozeran R. et Neville P. [cf. 3], selon lesquels l'aréole est un axe avec des feuilles transfomées en épines, confirmant ainsi les affirmations de Leinfellner W. [8].

La majorité des aréoles sont rondes, mais elles peuvent êtres ovales (tab.: 8, 9, 36, 57, 64, 79, 92), triangulaires (tab.: 86) ou rhomboïdales (tab.: 42). Quelques-unes d'entre elles sont blanches (tab.: 36 taxons), blanc-jaunâtre (tab.: 16 taxons), blanc-argenté (tab.: 3, 4, 5, 6), blanc-gris (tab.: 14, 30, 38, 41, 80, 90), blanc-bordeaux (tab.: 21), blanc-marronâtre (tab.: 17, 26, 37, 85, 86) ou jaunes (tab.: 11 taxons), jaune-marronâtre (tab.: 59), orange (tab.: 68), marron (tab.: 11, 13, 40, 62, 66, 83, 87) et noires (tab.: 16). Parfois la couleur d'une aréole de la base de tige diffère de celle de sommet (tab.: 16) ou même les deux moitiés d'une même aréole, d'habitude, ovale.

Les épines, comme des éléments caractéristiques pour les Cactaceés, diffèrent par la forme, la couleur, la longueur, le nombre et le mode de groupement sur les aréoles (centrales ou marginales), définissant les genres et surtout les espèces de cette famille.

Tableau 1

Données numériques concernant la morphologie des épines

No.	ESPÈCE	ÉPINES CENTRALES (nombre/aréole, longueur)	ÉPINES MARGINALES (nombre/aréole, longueur)	COULEUR	FORME, CONSISTANCE	ARÉOLE
1	***Acanthocalycium klimpelianum*** (Wendl. et Werderm.) Bckbg.	1-2; 1,7 cm	6-8; 1,1 cm	blanc-jaunâtre, pointe roux-marron	minces, courbées	ovale, blanche, bords marron
2	***Acanthocalycium violaceum*** (Werderm.) Bckbg.	1; 2,4 cm	12; 1,7 cm	jaunâtre	minces, courbées	blanche
3	***Armatocereus matucanensis*** Bckbg.	-	8-9; 1-2: 1,5-10 cm 7-8: 0,7 cm	blanc-gris jusqu'à marron	droites, dures, pied élargi	blanche- argentée, marron sur les crêtes
4	***Astrophytum ornatum*** (DC) Web. ex Britt. et Rose	-	5-11; 0,8-2 cm	jaune-marron	grosses, poitues, droites	blanche, duveteuse
5	***Austrocilindropuntia subulata*** (Muhlpf.) Bckbg.	1 (2); 8 cm	quelques glochides	jaunâtre, glochides blanches	droites, dures	ovale, blanche, duveteuse
6	***Aylostera albipilosa*** (Ritt.) Bckbg.	1; 1-4 cm	nombreuses; 1-1,5 cm	blanc-marron	très minces, courbées	ovale, épaisse, blanchâtre
7	***Aylostera fiebrigii*** (Gurke) Bckbg.	1 (2); 1-4 cm	30-40; 0,7-2 cm	centrales: roux-noirâtres; marginales: blanchâtres	droites, très minces	petite, blanc-jaunâtre
8	***Aylostera pseudodeminuta*** (Bckbg.) Bckbg.	2-3; 1-1,5 cm	12; 1-5 cm	blanc	très minces	ovale, blanc-jaunâtre
9	***Aylostera spegazziniana*** (Bckbg.) Bckbg. var. ***atroviridis*** Bckbg.	2; jusqu'à 0,2 cm	jusqu'à 20; 0,4 cm	centrales: jaunâtres, pointe marron; marginales: blanches	très minces	ovale, jaunâtre, douveteuse
10	***Borzicactus morleyanus*** Britt. et Rose	1-4; 0,9-2,5 cm	18; 0,7-2 cm	jaunâtre jusqu'à marron	droites, minces, élastiques	ronde, blanc-jaunâtre, bordeaux aux environs du pied des épines
11	***Cephalocleistocactus ritteri*** (Bckbg.) Bckbg.	jusqu'à 5; jusqu'à 1 cm	nombreuses; 0,7 cm	centrales: jaunâtres; marginales: blanches	très minces	marron

No.	ESPÈCE	ÉPINES CENTRALES (nombre/aréole, longueur)	ÉPINES MARGINALES (nombre/aréole, longueur)	COULEUR	FORME, CONSISTANCE	ARÉOLE
12	***Cereus forbesii Otto***	1; 2,5-5 cm	2-4 (5); 1,5-2 cm	blanc-bleu foncé	droites, dures, pied élargi	petite, blanche
13	***Cereus peruvianus*** (L) Mill.	5-6; 3 cm	3-4; 1,5 cm	centrales: marron; marginales: blanc-bleu foncé	droites, dures	petite, marron
14	***Chamecereus silvestrii*** (Spegazz.) Britt. et Rose	-	10-12; 0,3-0,5 cm	blanc	minces, molles	ronde, blanc-gris, duveteuse
15	***Cleistocactus baumanni*** (Lem.) Lem.	1; 3 cm	~ 14; 0,8 cm	centrale: blanc-jaunâtre; marginales: jaunâtre-marron	dures	ronde, blanche
16	***Cleistocactus candelilla*** Card.	1-3 (4); 0,9-1,5 cm	7-13; 0,4-0,5 cm	au sommet de la plante: marron, à la base: blanche	droites, très minces	ovale, petite, au sommet de la plante: noirâtre, à la base:blanche
17	***Cleistocactus jujuyensis*** (Bckbg.) Bckbg.	1; 1,3-3 cm	nombreuses; 0,7-1,5 cm	centrale: marron, marginales: blanc-jaunâtre	minces	mi-blanche, mi-marron
18	***Cleistocactus smaragdiflorus*** (Web.) Britt. et Rose	1; 2 cm	~ 20; 0,5-1,5 cm	centrale: blanc-jaunâtre, pointe marron, marginales: base blanche, pointe marron	centrales: dures	elliptique, mi-jaunâtre-marron, mi-blanche
19	***Cleistocactus tupizensis*** (Vpl.) Bckbg.	1-2; 1,5-4 cm	nombreuses; 0,6 cm	centrale: marron, marginales: blanc-luisant	très minces	blanc-jaunâtre
20	***Cylindropuntia rosea*** (DC) Bckbg.	-	jusqu'à 20; 2,5-4 cm	blanc-jaunâtre jusqu'à marron, étui jaunâtre	droites ou courbées, dures	ovale-ronde, blanc sale
21	***Dolichothele sphaerica*** (A. Dietr.) Britt. et Rose	1; jusqu'à 0,4 cm	12-15; 0,7-0,9 cm	centrale: jaunâtre, marginales: blanc-jaunâtre	droites, minces	petite, laineuse, concentrique: blanc-bordeaux
22	***Echinocactus platyacanthus*** Link & Otto	1-3; jusqu'à 3 cm	4-5 (6); 2-4 cm	roux-bleu foncé ou noirâtre	légèrement courbées, dures, pied élargi	grande, blanc-jaunâtre
23	***Echinocereus cinerascens*** (DC) Rumpl.	1-4; 2 cm	8-10; 0,7-1,5 cm	blanchâtres, pointe rousse	droites	blanche
24	***Echinocereus polyacanthus*** Engelm.	2-4; jusqu'à 7 cm	8-12; 0,6-0,9 cm	blanc-bleu foncé ou gris-roux	droites, pointues, pied élargi	blanc-jaunâtre

No.	ESPÈCE	ÉPINES CENTRALES (nombre/aréole, longueur)	ÉPINES MARGINALES (nombre/aréole, longueur)	COULEUR	FORME, CONSISTANCE	ARÉOLE
25	***Echinofossulocactus gladiatus*** (Link & Otto) Lawr.	1; 2-3 (4) cm	4-7; 1-2,5 cm	rousses, pointe foncé	centrales: droites ou légèrement courbées, marginales: courtes, rondes, deux élargies	jaune
26	***Echinopsis bridgesii*** Salm.-Dyck.	-	2-6 (8); 0,5-1 cm	blanc-gris jusqu'à noir	dures, pointues	grande, blanc-marron, duveteuse
27	***Echinopsis leuchantha*** (Gill.) Walp.	1; 1-5 cm	3-5 (9); 0,8-3 cm	marron-jaunâtre	droites	blanc-jaunâtre, duveteuse
28	***Echinopsis mamillosa*** Gurke	1-4; 0,8-1 cm	8-10; 0,6-1 cm	blanc-jaunâtre, pointe marron	dures, base légèrement élargiée	blanche, duveteuse
29	***Echinopsis obrepanda*** (Salm.-Dyck.) K. Schum.	1-3; jusqu'à 5 cm	4-6 (9); 0,5-0,9 cm	blanches jusqu'à marron	très courbées, minces, molles	blanche, duveteuse
30	***Echinopsis oxigona*** (Link) Zucc.	1-5; 0,1-0,3 cm	8-10; 0,3-1,3 cm	ocre, pointe noire	-	grande, blanc-grise, duveteuse
31	***Epiphyllum darrahii*** (K. Schum.) Britt. et Rose	-	1-3; 0,4-0,8 cm	blanches	la grosseur d'un cheveu	petite, blanche
32	***Epiphyllum phylanthus*** (L.) Haw.	-	1-2; 0,4-0,6 cm	-	-	-
33	***Erdisia meyenii*** Britt. et Rose	1-2; jusqu'à 6 cm	9-11; jusqu'à 3 cm	bleu foncé, pointe noire	très grosses, dures, droites ou courbées	ronde, blanche
34	***Eriocereus bonplandii*** (Parm.) Ricc.	-	6-8; 0,3-3 cm	rouges jusqu'à gris	droites, dures	blanche
35	***Ferocactus emoryi*** (Engelm.) Bckbg.	1; 4 cm	5-8; 3,5-6 cm	blanches, bordeaux au sommet de la plante	dures, courbées	jaunâtre-marron, duveteuse
36	***Gymnocalycium ochoterenai*** Bckbg.	dans la moitié supérieure: noires, triangulaires; 0,2-0,4 cm dans la moitié inférieure: blanc-rose; 0,3-0,4 cm; base élargie, courbées				ovale, blanc-jaunâtre
37	***Gymnocalycium oenanthemum*** Bckbg.	-	5-7; 2,5 cm	grises	très courbées latéralement	blanc-marron

No.	ESPÈCE	ÉPINES CENTRALES (nombre/aréole, longueur)	ÉPINES MARGINALES (nombre/aréole, longueur)	COULEUR	FORME, CONSISTANCE	ARÉOLE
38	***Gymnocalycium mostii*** (Gurke) Britt. et Rose	1; 1-1,5 cm	5-6 (7); 2-2,5 cm	ocre, pointe marron	dures, pied courbe	blanc-grise
39	***Haagocereus aureispinus*** var. ***rigidispinus*** (Rauch & Bckbg.) Rauch & Bckbg.	1; jusqu'à 1 cm	30-40; 0,7-2,5 cm	centrale: jaune jusqu'au rouge, noires, marginales: jaunes	marginales élargies, courbes, une épine droite, ronde	ronde, blanc-jaunâtre
40	***Hylocereus undatus*** (Haw.) Britt. et Rose	-	1-3 (4); 0,2-0,4 cm	marron	dures, pied élargi	petite, marron
41	***Lobivia varians*** Bckbg.	1; 3,5-4,5 cm	7-9; 0,6-2 cm	centrale: blanc-bleu foncé, pointe rousse, marginales: rousses	droites ou courbées, relativement dures	forme irrégulière, blanc-grise
42	***Mamillaria amoena*** Hopf.	2; 0,7-1,5 cm	12-15; 2-5 cm	centrales: jaune-marron, marginales: blanches	minces, molles, légèrement courbées	rhomboïdale, laineuse, jaunâtre
43	***Mamillaria applanata*** Engelm.	1; jusqu'à 0,4 cm	15-20; 0,8-1,2 cm	blanches, pied et pointe roux	dures	blanche
44	***Mamillaria backebergiana*** Buch.	1-2; 0,6-1 cm	8-10; 0,6-0,8 cm	jaune-marron, pied marron	aciculaires, centrales droites	jaunâtre, glabre
45	***Mamillaria bocasana*** Poselg.	1; 0,5-0,8 cm	jusqu'× 25-30; 0,8-2 cm	centrale: jaune-marron, marginales: jaunâtres	centrale: courbée, marginales: duveteuses	très petite, blanc-jaunâtre
46	***Mamillaria bravae*** Craig	2; 0,6-0,8 cm	28-30; 0,3-0,7 cm	centrales: crème-rose, marron, marginales: blanches	centrales: minces, droites, aciculaires	très petite, blanc-jaunâtre
47	***Mamillaria centricirrha*** Lem.	1; 2,3 cm	4-6; 0,4-2 cm	centrale: jaune-verdâtre, marginales: marrons	centrale: dure, marginales: molles	blanc-jaunâtre
48	***Mamillaria centricirrha*** Lem. var. ***neumanniana*** (Lem.) Scheele	1; 1,5 cm	2-4; 0,4-0,8 cm	centrale: blanche, pointe noire	centrale: aciculaire, marginales: dures, légèrement courbées	blanche, duveteuse
49	***Mamillaria centricirrha*** Lem. var. ***recurva*** Lem.	1; 5,5 cm	4-5; 0,5-2 cm	blanches, jaunes, noires	centrale: dure, courbée, marginales: molles	blanche, très duveteuse
50	***Mamillaria coronata*** Scheidw.	4-8; 2 cm	~ 20; 0,7-0,8 cm	centrales: bordeaux, marginales: blanches	droites, dures	blanche, duveteuse

No.	ESPÈCE	ÉPINES CENTRALES (nombre/aréole, longueur)	ÉPINES MARGINALES (nombre/aréole, longueur)	COULEUR	FORME, CONSISTANCE	ARÉOLE
51	***Mamillaria elongata*** DC	1; 2,8 cm	~ 20; 0,7-1 cm	jaunâtre, pointe marron	dures, très minces	ronde, jaune
52	***Mamillaria elongata*** DC var. ***straminea*** Hort.	1; 2,8 cm	20; 0,7 cm	centrale: rousse, marginales: jaunâtres	très minces, les marginales sont caduques	ronde, jaunâtre
53	***Mamillaria formosa*** Galeotti	4-6; 0,7-0,9 cm	nombreuses; 0,3-0,7 cm	centrales: roses, pointe noire, marginales: blanches	centrales: droites, dures, marginales: minces, molles	très petite, blanche
54	***Mamillaria glassii*** Foster	nombreuses; 0,6 cm	nombreuses; 1-1,5 cm	blanches	minces, molles, les centrales inséparables	très petite, jaune
55	***Mamillaria gracilis*** Pfeiff. var. ***fragilis*** (Salm.-Dick.) Berger	2; 0,8-1,5 cm	14-16; 0,5-0,9 cm	centrales: blanches, pointe marron, marginales: blanchâtres	droites, très minces, molles	très petite, jaune
56	***Mamillaria hahniana*** Werderm.	1 (2); 0,4-0,8 cm	15-30; 0,5-1,5 cm	centrale: blanche, pointe marron, marginales: blanches	dures, centrale droite	blanc-jaunâtre
57	***Mamillaria hidalgensis*** J. A. Purp.	2 (4); 0,8-1 cm	-	roses, pointe rousse	dures, aciculaires, légèrement courbées	ovale, blanche
58	***Mamillaria longicoma*** (Britt. et Rose) Berger	4; 0,5-1,2 cm	nombreuses; 0,6-0,8 cm	centrales: orange, marron, marginales: blanches	centrales: aciculaires, courbées, minces, marginales: droites, dures	très petite
59	***Mamillaria marksiana*** Krainz	1; 1 cm	8-12; 0,7-0,8 cm	centrale: jaune doré, marginales: blanches	centrale: courbe, marginales: minces	jaune-marron
60	***Mamillaria microhelia*** Werderm.	4; 0,7-1 cm	20 (jusqu'à50); 0,4-0,6 cm	centrales: bordeaux jusqu'à marron, marginales: jaunes jusqu'à marron	centrales: courbées, dures, marginales: droites, molles	ronde, jaune
61	***Mamillaria moellendorffiana*** Shurly	2-6; 0,7-1,4 cm	20-28; 0,2-0,6 cm	centrales: blanches, pointe marron, marginales: blanches	centrales: courbées, marginales: droites, molles	très petite, blanche
62	***Mamillaria multiceps*** Salm.-Dyck.	6-8; 0,8-0,9 cm	30-50; 0,6-0,7 cm	centrales: jaunes jusqu'à marron, marginales: blanches	centrales: minces, droites	petite, marron

No.	ESPÈCE	ÉPINES CENTRALES (nombre/aréole, longueur)	ÉPINES MARGINALES (nombre/aréole, longueur)	COULEUR	FORME, CONSISTANCE	ARÉOLE
63	***Mamillaria nigra*** Ehrh.	4-7; 3,5-4 cm	16-18; 0,6-1 cm	blanches, base et pointe marron	droites ou légèrement courbées, dures	blanc-sale, laineuse
64	***Mamillaria obconella*** Scheidw.	4; jusqu'à 2,5 cm	-	violet jusqu'à noir ou jaunâtre jusqu'à orange	minces, courbées, molles	ovale, blanche
65	***Mamillaria pringlei*** (Coult.) Brand.	1-6; 2,5-4 cm	15-20; 0,7-1 cm	jaunâtres	centrales: courbées, marginales: aciculaires, en rosette	ovale-ronde, jaunâtre
66	***Mamillaria rhodantha*** Link & Otto	4-7; 0,7-2,5 cm	16-20; 0,5-1 cm	blanches ou jaunâtres	aciculaires, dures, centrales: 3 droites et 1 courbée	marron
67	***Mamillaria simplex*** Haw.	3 (5); 0,7-1 cm	10-16; 0,5-0,8 cm	centrales: marron-roux, pointe noire marginales: roux-gris	aciculaires	petite, blanche, laineuse
68	***Mamillaria tesopacensis*** Craig.	1; 1-1,2 cm	15-20; 0,5-0,7 cm	marron-roux, pointe noire	minces, droites, molles	petite, orange
69	***Mamillaria umbrina*** Ehrh.	1-2; 0,7-2,4 cm	22-24; 0,4-1,2 cm	centrales: rouge-marron, pointe noirâtre, marginales: blanchâtres	centrales: courbées, marginales: droites, dures	ovale, duveteuse, blanche
70	***Melocactus bahiensis*** (Britt. et Rose) Werderm.	4; 2-3,5 cm	7-10; 1-2,5 cm	centrales: marron-gris, cephalium marron	dures, ondulées, pied élargi	petite, blanc-sale
71	***Melocactus ernestii*** Vaup.	4; 1 (2): 0,7-2 cm 1: 5,5 cm	7; 0,8-1 cm	blanc-rose, cephalium court	3 centrales courbées, 6 marginales dures, pied élargi	ronde, blanche
72	***Neobuxbaumia polylopha*** (DC) Bckbg.	1; 0,8 cm	7-8; 0,8-2 (7) cm	jaunes, pointe marron	dures, pied élargi	petite, duveteuse
73	***Neochilenia esmeraldana*** (Ritt.) Bckbg.	1; 1 cm	4-12; 0,2-0,7 cm	marron-noir	minces, très dures, ondulées	ovale, grande, blanc-jaunâtre
74	***Neochilenia taltalensis*** (Hutch.) Bckbg.	6-12; jusqu'à 3 cm	8-12; 0,7-2 cm	centrales: marron-noir, marginales: blanches jusqu'à marron	très dures, ondulées	ovale, blanc-jaunâtre

No.	ESPÈCE	ÉPINES CENTRALES (nombre/aréole, longueur)	ÉPINES MARGINALES (nombre/aréole, longueur)	COULEUR	FORME, CONSISTANCE	ARÉOLE
75	***Neoporteria nidus*** (Sohr.) Britt. et Rose var. ***senilis*** (Phil.) Krainz.	1; 3,5 cm	nombreuses; 0,5-2 cm	blanc-grise, pointe noire	centrale: dure, marginales: emmêlées	blanche, duveteuse
76	***Notocactus apricus*** (Arech.) Berg.	3-4; 2 cm	12-20; 0,6-0,9 cm	centrales: rose, marginales: jaunes, pied rouge	centrales: ondulées, minces, relatif molles	ronde, jaune
77	***Notocactus mueller-melchersii*** Fric et Bckbg.	1(3); 2-3 cm	10-18; jusqu'à 0,8 cm	centrales: roux-ocre, marginales: blanc-jaunâtres	droites, pointe courbe	petite, blanc-jaunâtre
78	***Notocactus submammulosus*** (Lehm.) Bckbg.	2; 0,8 (1)-2 cm	7; 0,5-0,7 cm	blanc-jaunâtre, pointe de couleur foncée	centrales: droites, dures, grosses, marginales: minces, molles	ronde, blanche
79	***Opuntia microdasys*** (Lehm.) Pfeiff.	-	glochides nombreuses; 0,4 cm	jaune-marron	très minces, molles	très petite
80	***Opuntia phaeacantha*** Engelm.	1-2; 3,3-3,5 (4) cm	glochides nombreuses; 0,1-0,5 cm	centrales: blanc-marrons, glochides: jaune jusqu'à marron	centrales: droites, dures, glochides molles	ronde, blanc-grise
81	***Opuntia vulgaris*** Mill.	1-2; 1,5-2,2 cm	glochides nombreuses; 0,2-0,5 cm	centrales: jaune-marron, glochides: rousses	-	très petite
82	***Parodia aureispina*** Bckbg.	4; 1-1,5 cm	15-20; 1 cm	centrales: jaune-doré, en croix, marginales: blanches	relativement dures, minces, centrales: 3 droites et 1 courbée	ronde, blanche
83	***Peireskia aculeata*** Mill.	2 courtes; 0,7-0,8 cm; pied élargi; courbées 10 longues; 5 cm; pied légèrement élargi; droites; marron; dures				marron-grise, légère, duveteuse
84	***Pilosocereus leucocephalus*** (Poselg.) Britt. et Rose	1; 2-2,5 cm	18-19; 0,6-1 cm	jaune, pointe marron	droites, dures, pied légerement élargi	ronde, blanche
85	***Pseudolobivia aurea*** (Britt. et Rose) Bckbg.	1-4; jusqu'à 3 cm	10; 1 cm	centrales: blanches, pointe noir marginales: blanches	dures, pied élargi	blanc-marron, duveteuse

No.	ESPÈCE	ÉPINES CENTRALES (nombre/aréole, longueur)	ÉPINES MARGINALES (nombre/aréole, longueur)	COULEUR	FORME, CONSISTANCE	ARÉOLE
86	***Pseudolobivia polyancistra*** (Bckbg.) Bckbg.	1; 1-1,5 cm	nombreuses; 0,7-1,3 cm	blanche, pointe marron	minces, courtes, ondulées	triangulaire, blanc-marron
87	***Rebutia xantocarpa*** Bckbg.	-	15-20; 0,5-0,8 cm	blanc-jaunâtre	très minces, relativement dures	ovale, marron
88	***Ritterocereus eichlamii*** (Britt. et Rose) Bckbg.	1; 0,7-1 cm	7 (9); 0,7-0,8 cm	grises, pointe noire	pied élargi	ronde, blanc-grise
89	***Ritterocereus hystrix*** (Haw.) Bckbg.	3; 2-4 cm	11; 0,7-1,5 cm	blanc-gris ou blanc-rouge, pointe marron	droites, dures	ronde, proéminente, blanche
90	***Ritterocereus pruinosus*** (Otto) Bckbg.	1; 3 cm	5-7; 0,7-0,8 cm	gris, pointe marron	droites, dures, relatif grosses, pied élargi	petite, blanc-grise
91	***Roseocereus tephracanthus*** (Linl. & Otto) K. Schum.	1; 0,5 cm	4-7; 0,1-0,7 cm	blanchâtre, pointe marron	droites, dures	ronde, proéminente, blanche
92	***Stetsonia coryne*** (Salm.-Dick.) Britt. et Rose	1; 2,5-5 cm	7; 0,7-3 cm	jaunâtre-marron jusqu'à blanc	droites, dures, grosses	ovale, feutrée, blanche
93	***Tephrocactus articulatus*** (Pfeiff. & Otto) Bckbg.	-	glochides nombreuses, 0,4-0,7 cm	marron	-	ronde, proéminente, duveteuse
94	***Trichocereus thelegonus*** (Web.) Britt. et Rose	1; 2-4 cm	8-10; 0,5-0,8 cm	jaune jusqu'à marron	dures, courbées	ronde, blanc-sale
95	***Trichocereus werdermannianus*** Bckbg.	-	nombreuses; jusqu'à 0,7 cm	jaune-ocre jusqu'à marron	droites, dures, pied élargi	ovale, blanche
96	***Turbinicarpus pseudomacrochele*** (Bckbg.) Buxb. & Bckbg.	-	6-9; 1,5-2 cm	jaune-marron	très minces, ondulées	blanche, duveteuse
97	***Weingartia ambigua*** (Hildm.) Bckbg.	1; 1,5 cm	4-6 (8); 0,6-1,3 cm	centrale: marron, marginales: noires	droites, minces, relativement dures	irrégulière, blanc sale-jaunâtre

Pour la majorité des espèces, les épines sont groupées en deux catégories: centrales et marginales.

Les épines centrales, présentes chez 34 des taxons analysés (Tableau 1), sont plus longues que celles marginales; 21 taxons appartiennent au genre *Mamillaria* (moins *Mamillaria amoena, Mamillaria applanata* et *Mamillaria hahniana*, chez qui les deux catégories d'épines sont égales). Les 1-2 (4) épines centrales sont d'une même longueur (tab.: 76, 77; pl. V) ou ont des longueurs différentes (tab.: 50, 78, 80, 82, 93; pl. IV, V, VI); elles sont uniformes et légèrement courbées (tab.: 42, 50, 61, 93; pl. III, IV, V, VI), ondulées (tab.: 25, 70, 76, 86; pl. II, V), droites (tab.: 44, 46, 53, 56, 78; pl. IV, V) ou polymorphes, étant parfois même absentes (tab.: 3, 4, 14, 26, 31, 32, 34, 37, 40, 79, 87, 96; pl. I, II, III, V, VI).

La longueur de ces épines centrales varie de quelques millimètres (2 mm chez *Aylostera spegazziniana*) à quelques centimètres [5-7 (8) cm chez *Echinocereus cinerascens, Erdisia meyenii, Peireskia aculeata*]. Selon la longueur des épines, les espèces analysées par nous peuvent être encadrées en trois groupes:

1. avec les épines courtes, entre 0,2 cm et 1 cm; 12 des 21 taxons de ce groupe appartiennent au genre *Mamillaria*;
2. avec les épines de longueur moyenne, entre 1 et 3 cm (tab.: 35 taxons);
3. avec les épines longues, de 3 à 8 cm (tab.: 19 taxons).

Les épines marginales, au niveau d'une aréole, sont d'une même longueur (tab.: 23 taxons) ou de longueur variable (tab.: 49 taxons); seulement en deux situations elles peuvent manquer (tab.: *Mamillaria hidalgensis, Mamillaria obconella*). Chez la majorité des espèces les épines d'une aréole ont la même forme (droites, courbées ou ondulées), mais elles peuvent avoir aussi des formes différentes (tab.: 25, 33, 39, 71).

Pour les espèces les plus nombreuses (tab.: 49 taxons) les épines marginales ont entre 0,2 et 1,5 cm (24 taxons appartiennent au genre *Mamillaria*); seulement quelques espèces ont des épines de longueur comprise entre 1,5 et 3 (4) cm (tab.: *Cereus forbesii, Erdisia meyenii, Echinocereus platyacanthus*) ou entre 5 et 10 cm (tab.: *Armatocereus matucanensis, Neobuxbaumia polylopha, Peireskia aculeata*).

La couleur des épines d'une aréole est blanche (tab.: 8, 14, 23, 31, 32, 43, 48, 54, 56, 61, 75, 85, 86, 91) avec les nuances: blanc-jaunâtre (tab.: 1, 20, 26, 28, 42, 49, 66, 78, 87), blanc-gris (tab.: 3, 24, 37, 88, 89, 90), blanc-rose (tab.: 36, 57, 71), blanc-bordeaux (tab.: 12, 35), blanc-marron (tab.: 6, 29, 63), ou jaune (tab.: 2, 5, 39, 51, 65, 72), en différentes combinaisons: jaune-marron (tab.: 10, 27, 44, 84, 92, 94, 96), jaune-violet (tab.: 4, 64, 83) et jaune-orange (tab.: 30, 38, 95). Pour certaines espèces les épines ont la couleur: marron (tab.: 40, 68), marron-roux (tab.: 25, 34), marron-bordeaux (tab.: 13, 33, 73), marron-jaunâtre (tab.: 16, 79) ou noir (tab.: 22).

Pour 30% des taxons analysés les épines centrales et marginales sont différemment colorées. Les marginales peuvent êtres blanches et les centrales

marron-roux (tab.: 7, 41, 77), marron (tab.: 17, 19, 58), jaunes (tab.: 11, 18, 21, 59, 62, 82), jaune-marron (tab.: 9, 45, 46, 69, 70, 81), roses (tab.: 53, 76), bordeaux (tab.: 50) ou noires (tab.: 74, 55). Dans quelques situations les épines marginales sont jaunes et les centrales blanc-jaunâtres (tab.: 15, 47, 93), marron (tab.: 60, 80) ou marron-roux (tab.: 52).

Les épines marginales peuvent êtres toutefois rousses (tab.: 67) ou noires (tab.: 97), dans le centre de l'aréole prédominant la couleur marron.

En ce qui concerne la consistance des épines, on peut dire que les 97 taxons analysés ont des épines dures (tab.: 3, 4, 5, 12, 13, 15, 18, 26, 28, 33, 43, 83, 87, 89, 90, 91, 92, 93, 94, 95) ou molles et élastiques, les dernières étant aussi très minces (tab.: 1, 2, 6, 7, 8, 9, 11, 14, 16, 17, 29, 30, 79, 86, 96), caractère variable selon l'espèce.

Ainsi, pour *Mamillaria* (28 taxons) les épines sont:

1. molles et élastiques (tab.: 42, 45, 46, 51, 54, 58, 62, 64, 68);
2. dures (tab.: 43, 56, 57, 63, 65, 66);
3. dures – les épines centrales et molles – les épines marginales (tab.: 47, 48, 49, 53, 59, 60, 61, 69).

CONCLUSIONS

La famille *Cactaceae* a suscité l'intérêt par le haut degré de spécialisation morpho-structurale de ses nombreux représentants.

Particulièrement ont attiré l'attention les aréoles, les épines, les glochides et les poils dont l'origine a été beaucoup discutée.

Partant du fait que dans la littérature roumaine et étrangère consultée nous n'avons pas rencontré une étude comparative de morphologie des aréoles et des épines, dans cet ouvrage nous avons analysé les variations morphologiques des aréoles et des épines des 97 taxons qui appartiennent aux sous-familles *Pereskioideae, Opuntioideae* et *Cereiodeae*.

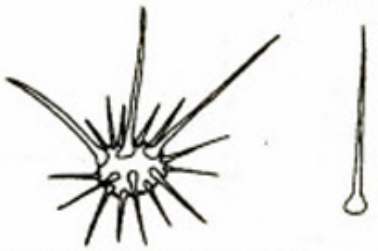
Ainsi, dans la majorité des espèces analysées, les aréoles sont rondes ou ovales, et très rarement elles sont triangulaires ou rhomboïdales. La couleur prédominante est le blanc ou le jaune, en différentes combinaisons avec marron, orange et noir. Les variations de couleur apparaissent, de même, en fonction de l'âge de la plante et de la position des aréoles sur la tige (base, sommet).

Les épines, unanimement reconnues comme des feuilles modifiées et situées dans ces aréoles, sont centrales ou marginales. Présentes ou absentes, elles donnent le caractère de l'espèce ou elles définissent une variété par le nombre, la longueur, la couleur, la forme et la consistance.

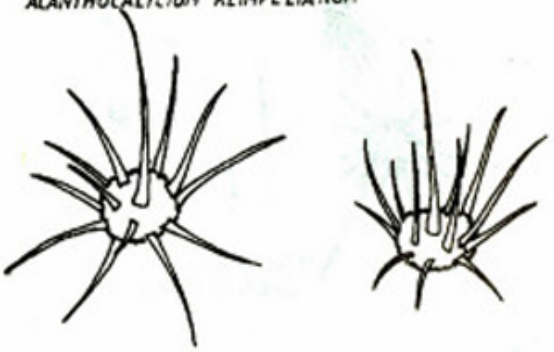
Les épines centrales [1-2 (4)] sont, d'habitude, plus longues (de 0,2 à 7 (8) cm) que les marginales (de 0,2 à 1,5 cm).

PLANCHE I

ACANTHOCALYCIUM KLIMPELIANUM



ARMATOCEREUS MATUCANENSIS



ACANTHOCALYCIUM VIOLACEUM

ASTROPHYTUM ORNATUM

AYLOSTERA FIEBRIGII

AYLOSTERA PSEUDODEMINUTA

AYLOSTERA SPEGAZZINIANA var. ATROVIRIDIS

AYLOSTERA ALBIPILOSA

CEREUS FORBESII

CEREUS PERUVIANUS

BORZICACTUS MORLEYANUS

PLANCHE II

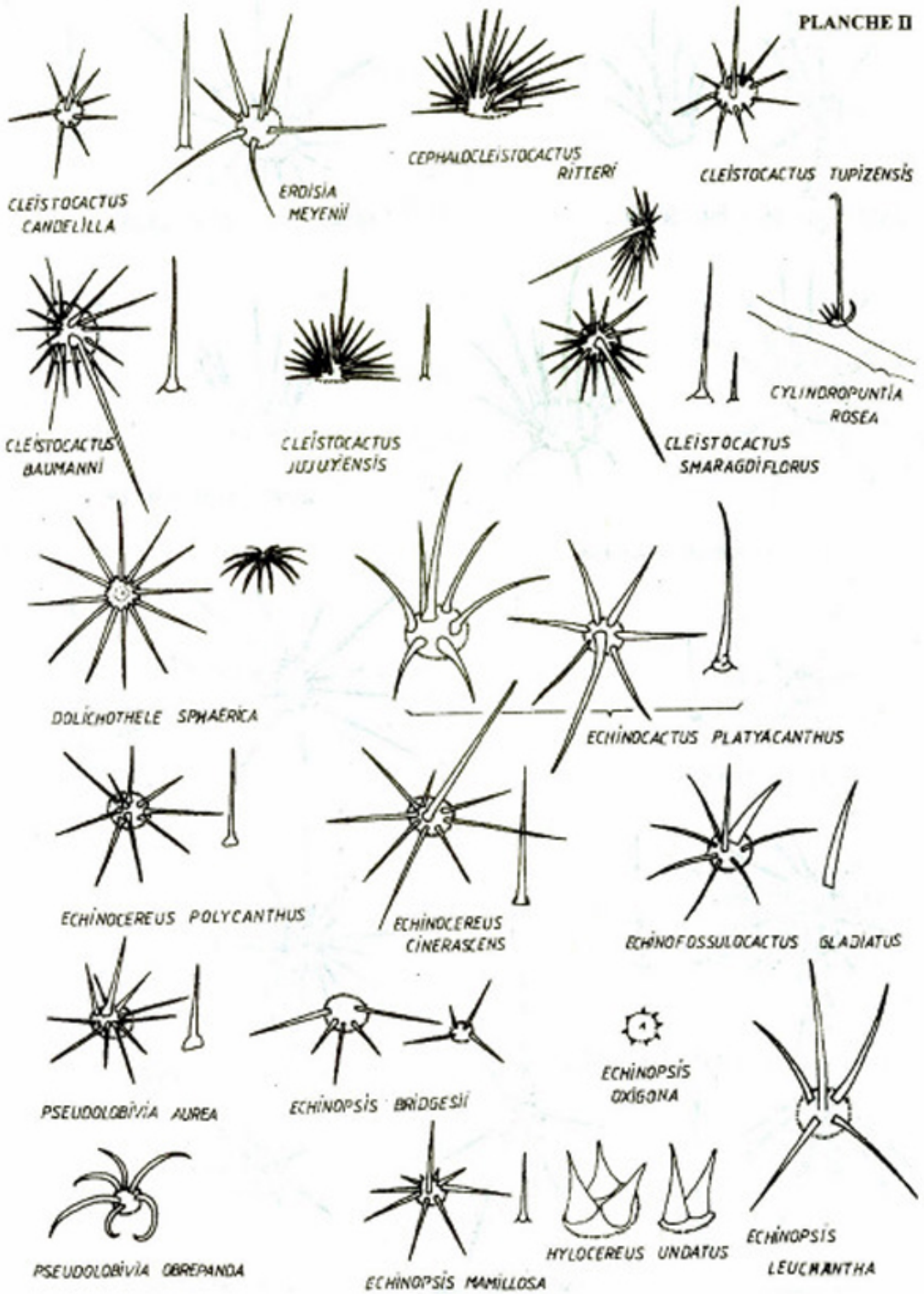

CLEISTOCACTUS CANDELILLA
ERDISIA MEYENII
CEPHALOCLEISTOCACTUS RITTERI
CLEISTOCACTUS TUPIZENSIS
CLEISTOCACTUS BAUMANNI
CLEISTOCACTUS JUJUYENSIS
CLEISTOCACTUS SMARAGDIFLORUS
CYLINDROPUNTIA ROSEA
DOLICHOTHELE SPHAERICA
ECHINOCACTUS PLATYACANTHUS
ECHINOCEREUS POLYCANTHUS
ECHINOCEREUS CINERASCENS
ECHINOFOSSULOCACTUS GLADIATUS
PSEUDOLOBIVIA AUREA
ECHINOPSIS BRIDGESII
ECHINOPSIS OXIGONA
PSEUDOLOBIVIA OBREPANDA
ECHINOPSIS MAMILLOSA
HYLOCEREUS UNDATUS
ECHINOPSIS LEUCHANTHA

PLANCHE III

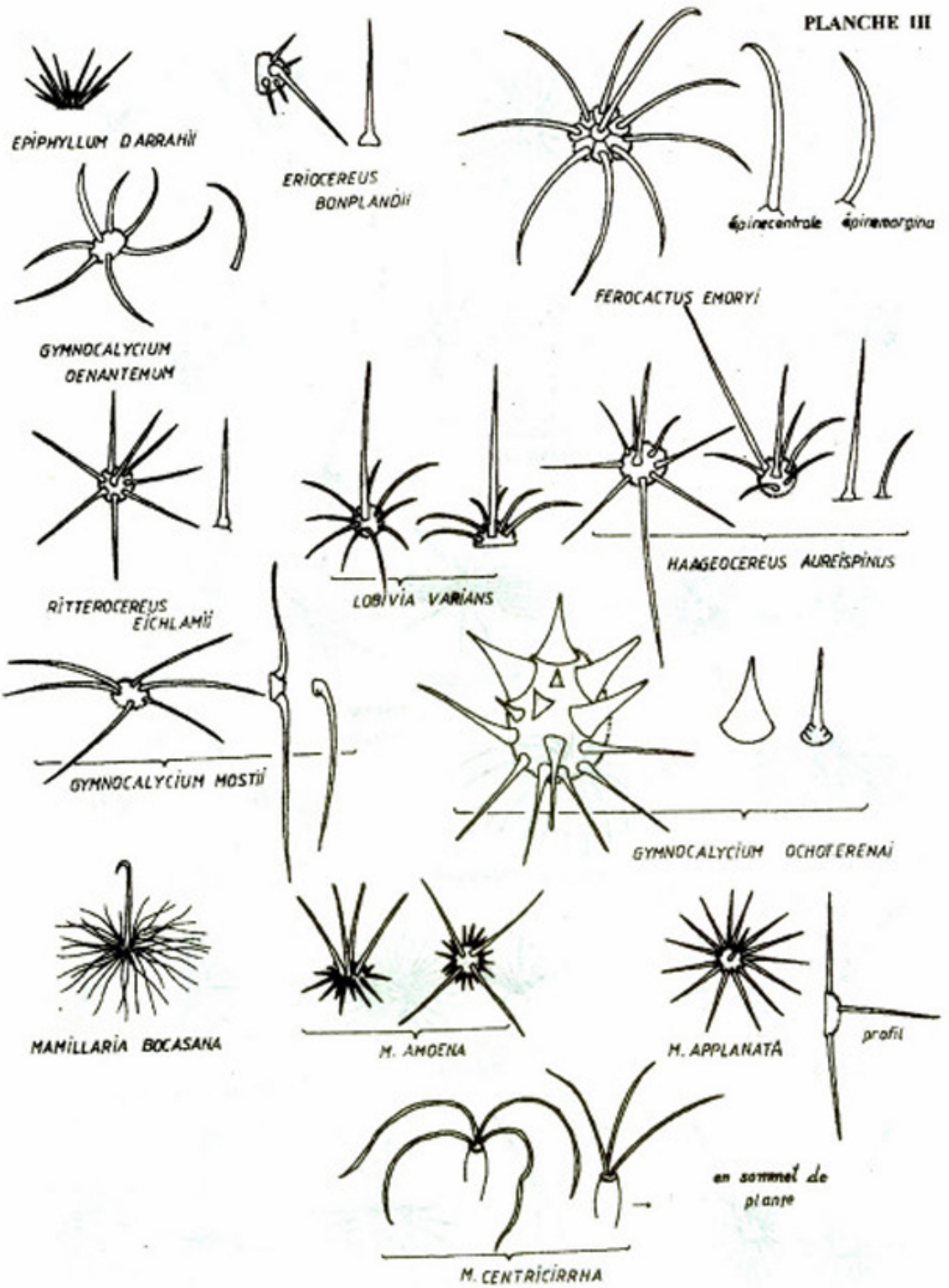

EPIPHYLLUM D'ARRAHII
ERIOCEREUS BONPLANDII
épine centrale
épine marginale
FEROCACTUS EMORYI
GYMNOCALYCIUM OENANTEMUM
RITTEROCEREUS EICHLAMII
LOBIVIA VARIANS
HAAGEOCEREUS AUREISPINUS
GYMNOCALYCIUM MOSTII
GYMNOCALYCIUM OCHOTERENAI
MAMILLARIA BOCASANA
M. AMOENA
M. APPLANATA
profil
M. CENTRICIRRHA
en sommet de plante

PLANCHE IV

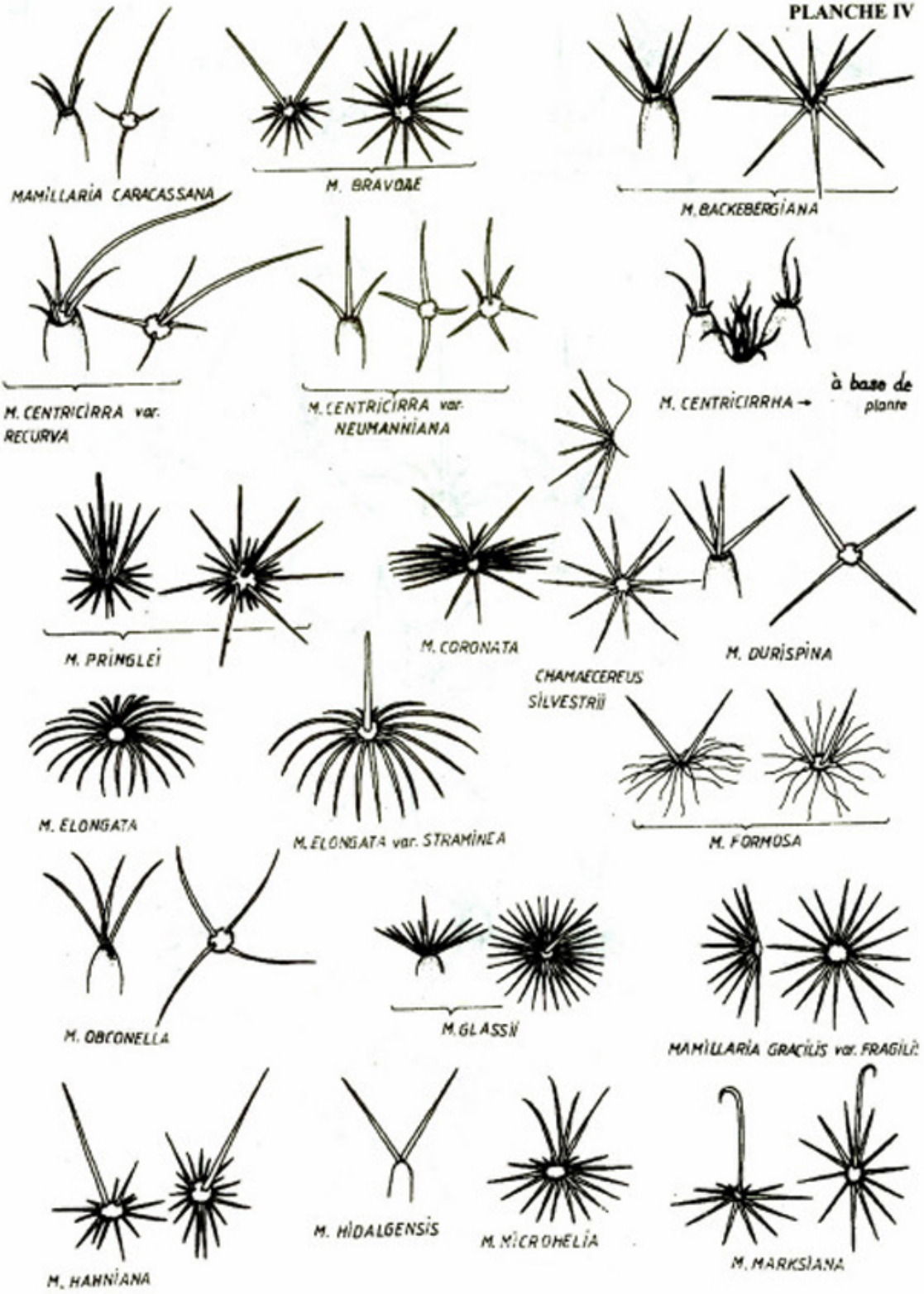

MAMILLARIA CARACASSANA
M. BRAVOAE
M. BACKEBERGIANA
M. CENTRICIRRA var. RECURVA
M. CENTRICIRRA var. NEUMANNIANA
M. CENTRICIRRHA →
à base de plante
M. PRINGLEI
M. CORONATA
CHAMAECEREUS SILVESTRII
M. DURISPINA
M. ELONGATA
M. ELONGATA var. STRAMINEA
M. FORMOSA
M. OBCONELLA
M. GLASSII
MAMILLARIA GRACILIS var. FRAGILIS
M. HAHNIANA
M. HIDALGENSIS
M. MICROHELIA
M. MARKSIANA

PLANCHE V

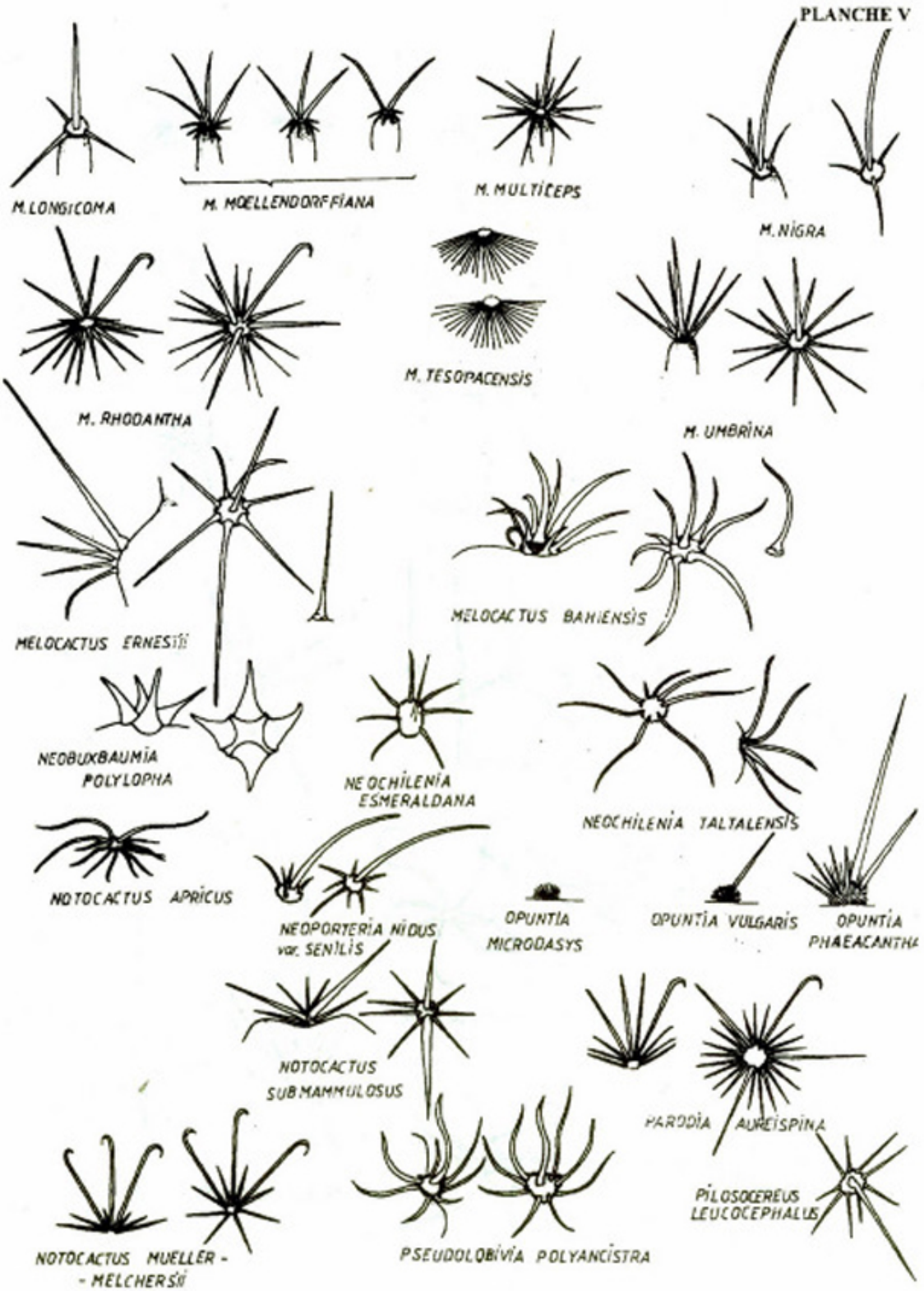
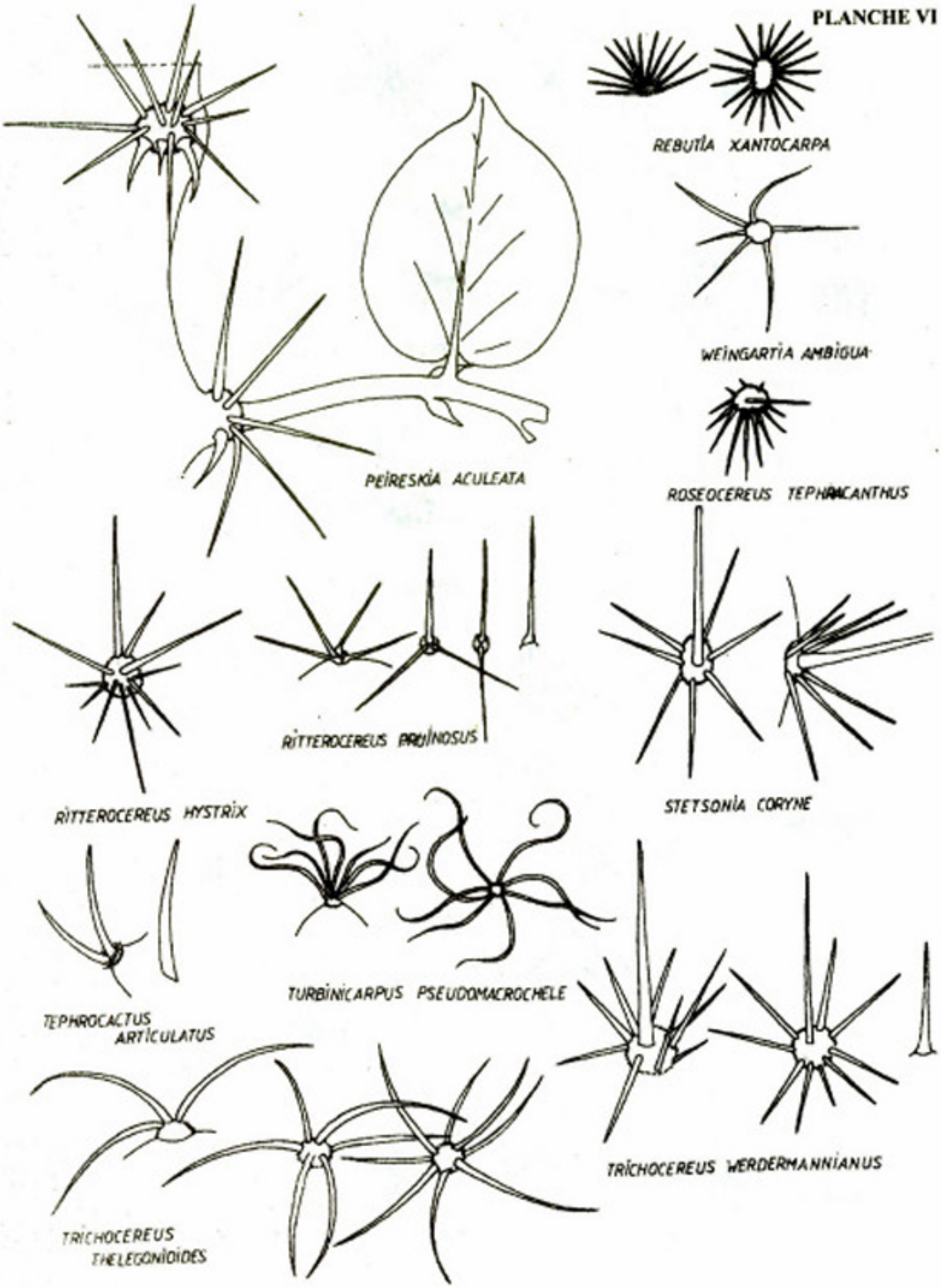

M. LONGICOMA
M. MOELLENDORFFIANA
M. MULTICEPS
M. NIGRA
M. RHODANTHA
M. TESOPACENSIS
M. UMBRINA
MELOCACTUS ERNESTII
MELOCACTUS BAHIENSIS
NEOBUXBAUMIA POLYLOPHA
NEOCHILENIA ESMERALDANA
NEOCHILENIA TALTALENSIS
NOTOCACTUS APRICUS
NEOPORTERIA NIDUS var. SENILIS
OPUNTIA MICRODASYS
OPUNTIA VULGARIS
OPUNTIA PHAEACANTHA
NOTOCACTUS SUBMAMMULOSUS
PARODIA AUREISPINA
PILOSOCEREUS LEUCOCEPHALUS
NOTOCACTUS MUELLER-MELCHERSII
PSEUDOLOBIVIA POLYANCISTRA

PLANCHE VI


REBUTIA XANTOCARPA
WEINGARTIA AMBIGUA
PEIRESKIA ACULEATA
ROSEOCEREUS TEPHRACANTHUS
RITTEROCEREUS PRUINOSUS
RITTEROCEREUS HYSTRIX
STETSONIA CORYNE
TURBINICARPUS PSEUDOMACROCHELE
TEPHROCACTUS ARTICULATUS
TRICHOCEREUS WERDERMANNIANUS
TRICHOCEREUS THELEGONIOIDES

La forme et la couleur des épines centrales diffèrent de celles des épines marginales, étant caractéristiques pour un taxon. Elles sont uniformément et légèrement courbées, droites ou ondulées, ou polymorphes. La couleur prédominante est blanc ou jaune en différentes combinaisons avec rose, bordeaux, violet, marron et noir. La majorité des épines sont grosses et dures, mais elles peuvent êtres aussi minces, molles et élastiques.

Même s'ils sont très variables, les caractères donnés par les aréoles et les épines définissent un taxon outre la forme de la tige, des mamelons et des côtes de celle-ci, ainsi que les caractères donnés de la fleur, du fruit et des semences.

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THE VARIABILITY OF SOME QUANTITATIVE TRAITS AT *CHELIDONIUM MAJUS* L. SPECIES

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The researches performed on *Chelidonium majus* L. individuals from micropopulations of Iaşi city area, revealed a significant variability for metrical traits (i.e. the length and the weight of some leaves equivalent samples, the petals and the stamina number, the capsula number and weight, the seed number and weight), statistically processed.

Key words: *Chelidonium majus* L., metrical traits, leaves, flowers, seed.

Chelidonium majus species has a big importance for the phytochemists, because it offers a wide "palette" of compounds (secondary metabolic products), with the most different use (from hepatic diseases treatment to the antitumoral one). That is why, in time, many investigations aimed at clearing up the chemical, physiological or biochemical aspects (alkaloids separation, the active principles of extraction for drugs), and less the aspects connected with the cytotaxonomy, cytogenetics or ecology of this species. Since the complete knowing of the species biology and, especially, of the ecogenetical and ecophysiological aspects offers increased chances for its melioration, we appreciated the importance of these investigations, which sometimes leads us to remarkable conclusions (5).

We have proposed to investigate (through statistical methods) the *Chelidonium majus* individuals quantitative traits variability, in order to understand "the strategy" of this species survival and perpetuation within its biocoenosis.

MATERIAL AND METHODS

Our investigation were aimed at establishing the correlations between the length and the width of leaves. For this purpose we carried out measurements on one hundred leaves of the same age, from the same individuals, as well as the weight/thickness ratio of 1 cm^2, from the basis, median zone and the tip of leaves.

At he same time, the fruits (siliquiform capsula) medium number per plant and the seed number and their medium weight per fruit were calculated.

Another problem was to establish whether there is a correlation between the number of stamens and that of petals in the same flower, and if these numbers have a tendency to increase or to decrease.

All the data have been statistically processed and interpreted.

RESULTS AND DISCUSSIONS

One aim of our investigations was to establish the variability of the amplitude of leaves shape. For this, we have measured both the maximum leaves length and width. After that, we calculated the length the length/width ratio. Simultaneously, we estimated the variability amplitude for each trait, establishing the $\bar{x}$, s, s %, sx and sx%. So, for the leaves length the mean ($\bar{x}$) was 11.36, the individual deviation (s) was 2.5 cm and the standard deviation of mean ($s\bar{x}$) was o.22 cm. Both values, correlated with that of the variation index (s%), pointed out a high variability of this trait (s% = 18.67). On the other hand, the percentage value of the deviation from the ideal mean ($s\bar{x}$ = 1.86) underlines a high degree of uncertainty, therefore a large enough amplitude of variability.

The correlation between the values dynamics of leaves length and the weight of a centimetre square excised from each leaf pointed out a small value (r = 0.06). However, the variability of the weight of one centimeter square excised from the leaf is large enough (for $\bar{x}$ = 8.86 mg, s = 2.09, $s\bar{x}$% = 2.36) suggesting much incertitude of conclusions. Consequently, when two traits display a large amplitude of variability it is normal to find a high correlation between them. At the same time, a high variability suggests the existence of a great flexibility and reactivity of species and, as a consequence, a good adaptability at the environment, a high fitness of individuals which will be able to survive in different habitats (Fig. 1, Table 1).

Table 1

Statistical indicators calculated on 100 individuals sample ($\bar{x}$ = arithmetic mean; s = standard deviation; $s\bar{x}$ = arithmetic mean standard deviation: s% = variations coefficient; $s\bar{x}$% = mean standard deviation percents expressed)

Analyzed trait	$\bar{x}$	s	$s\bar{x}$	s%	$s\bar{x}$%
Leaf length	11.56	0.22	2.15	18.67	1.86
Petals no./flower	4.09	0.43	0.04	10.43	1.04
Stamina no./flower	16.29	3.41	0.34	20.91	2.09
Fruits no./plant	83.31	53.31	5.33	63.99	6.40
Seeds no./fruit	34.23	0.97	9.70	28.32	2.83
Rondel weight average/leaf	8.86	2.09	0.21	23.58	2.36
Flower weight/plant	16.68	3.96	0.40	23.71	2.37
Seeds weight/fruit	37.28	15.24	1.52	40.88	4.09

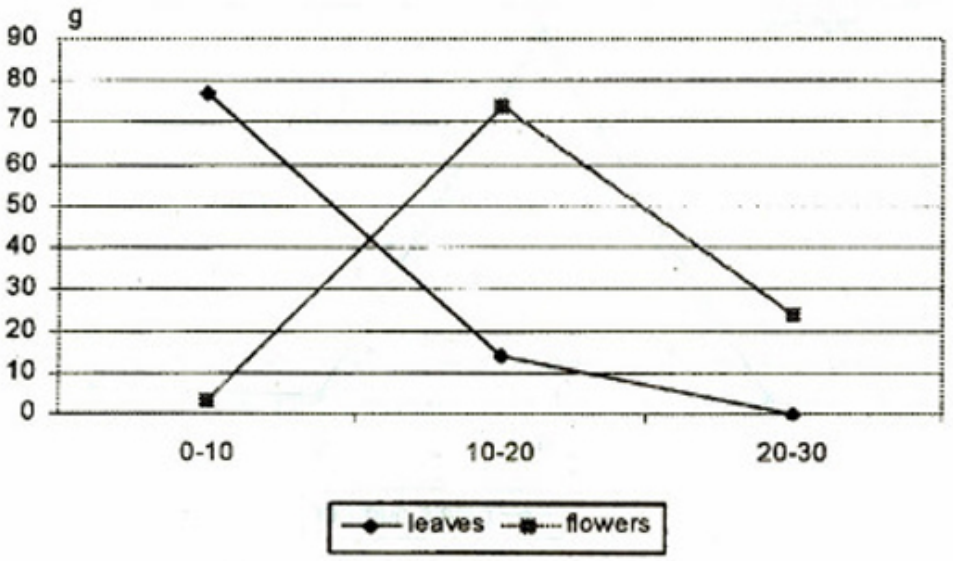

Fig. 1. – Leaves weight average and flowers weight average.

A low variability of the analyzed traits reflects either a high hereditary stability or a high uniformity of the environment, but, in our opinion, none of these suppositions has any chance to be realistic.

The number of petals is as variable as the leaves shape (the length/width ratio). It is indubitable that the petals (by number and colour) represent a very important element to assure the pollination by insects. On the other hand, in the case of pollination by wind, the petals have no significance and their presence in a large number can be a limiting factor (Fig. 2).

Consequently, it seems that the species has an adequate strategy for the maintenance of this trait variability amplitude, thus favouring the selection action. The curve for this trait variability has two peaks suggesting the existence of an incipient process of disruptive selection.

Futher analysis will establish if the directioned selection is possible, e.g. in case of an increased number of petals. Based on these observations we shall eventually be able to issue an assumption related to the "*floriplenum*" shape appearance.

Firmly bounded to the "number of petals" is the "number of stamina", as it is well known that these two "traits" are connected (the number of petals in *Papaveraceae* family may increase with the decrease in the number of stamina).

Analyzing the shown data, we may find that the number of stamina (which is much lower for *Chelidonium majus* as compared to *Papaver somniferum* for example) is variable enough, the mean of 16.29 recording individual deviations of 3.41 and a variability index of 20.91. The calculated average deviation from the ideal mean is also high.

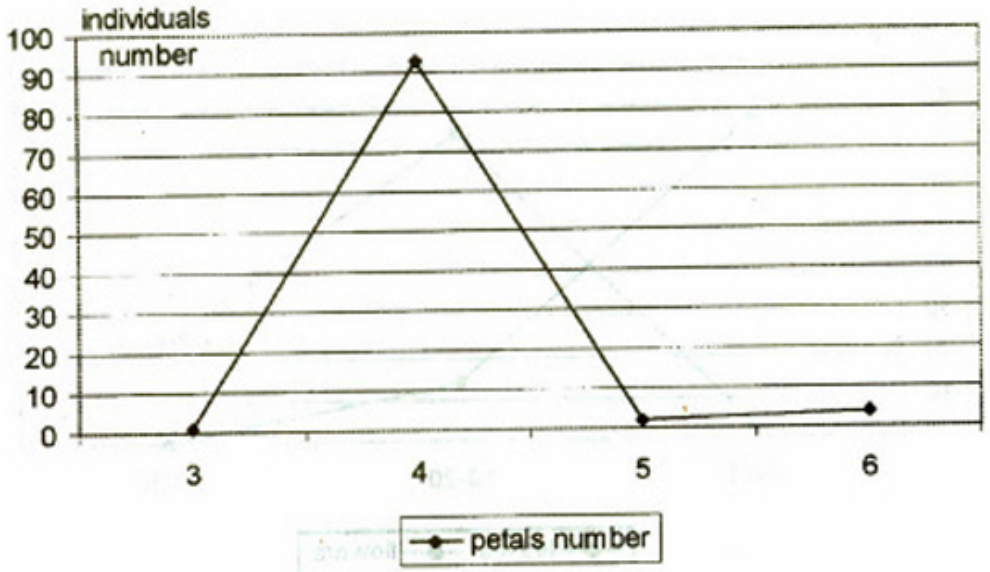

Fig. 2. – Average number of petals at 100 individuals.

Certainly, the number of staminas (Fig. 4) and therefore, the produced quantity of pollen have a greater importance for the species perpetuation than the number of petals. Therefore, a high variability amplitude of this trait is useful, because only a part of the pollen is able to perform its role. Considering that the flowers weight (Fig. 1) in complete state may offer some informations related to the species competitivity (this also including the pollen quantity and also the size of the ovary), we performed the trait quantification. We noticed that there was a great variability amplitude (s=3.9 and s=23.71), fact that can be explained by our previous assertions. The correlations between weight and the number of petals on the one hand, and between the flower weight and the number of staminas on the other hand, sustain the idea that in the second case the correlation is much stronger than in the first one. Analyzing this situation, the results seem relevant and important because as they increase in number, the staminas have a larger influence on the flower weight and, implicitly, the pollen quantity plays a greater role in the perpetuation of the population, as a whole.

Table 2

Correlation coefficients (r – regression coefficient) between measurements of different characters on the tagged plants

No	Analyzed trait	r	No	Analyzed trait	r
1	Leave weight mean and length	0.06	6	Seeds weight/fruit and seeds no./fruit	0.65
2	Flower weight and petal number /flower	0.06	7	Seeds weight/fruit and fruit no./plant	-0.06
3	Flower weight and stamina no/flower	0.41	8	Flower weight and leaf mean weight	0.22
4	Stamina no. and petals number/flower	0.24	9	Seeds weight/fruit and flower weight/plant	0.12
5	Seeds no./fruit and fruit no./plant	0.01	10	Seeds weight/fruit and leaf mean weight	-0.001

Normally, all these preliminary data should reflect on the number of fruits and on weight, which are major parameters for the density perpetuation of the population. The population, as a stong integrated system should be maintained through self adjusting, framed into enough stable parameters in some pretty variable environmental conditions. It seems that this "desideratum" is achieved by providing large enough individual variability amplitude, for the described traits.

So, the number of fruits (Fig. 3) shows such a high variability (s%=63.99) that practically, the ($s_{\bar{x}}$) mean deviation and the safety coefficient for the calculated mean ($s_{\bar{x}}$%) provide us indications according to which the veridicity of the results, obtained through investigations carried out on over 100 individuals, may be doubted. Analogous to that is the seeds number fruit behaviour (s%=28.32) only that here we have the calculation limit certitude ($s_{\bar{x}}$%=2.83).

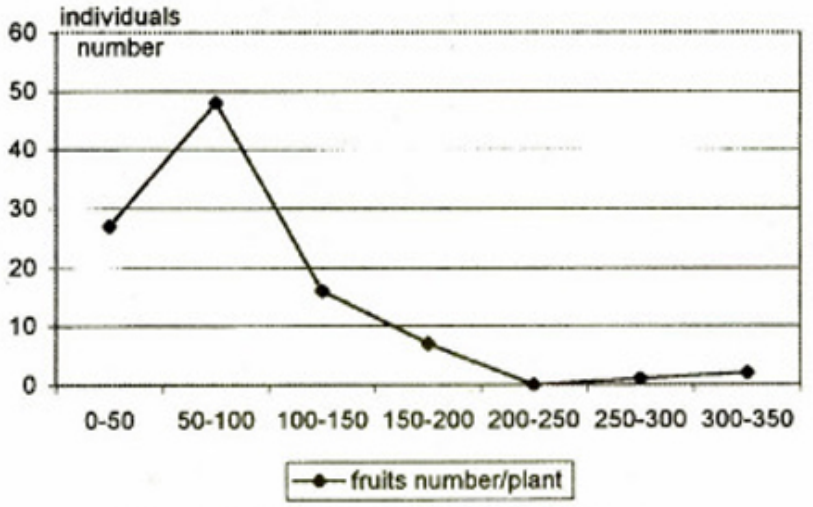

Fig. 3. – Fruits number/plant at 100 individuals.

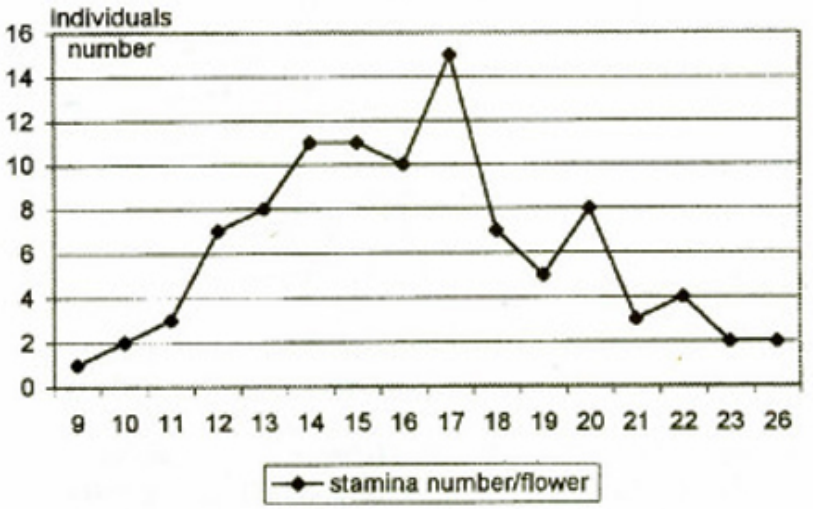

Fig. 4. – Average number of stamina's at 100 individuals.

Concerning the seeds weight/fruit (Fig. 6), we notice the existence of a high enough individual deviation (s=15.24 mg) and of a very variability coefficient (s%=40.88). In this case also, the percentage value of the calculated average deviation from the ideal mean is greater than 3, showing the uncertainty of the obtained data (s%=4.09).

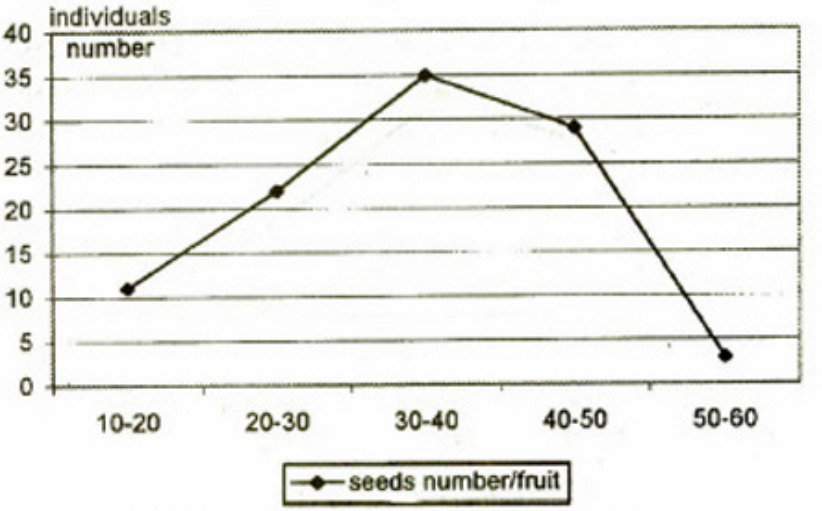

Fig. 5. – Seeds number/fruit at 100 individuals.

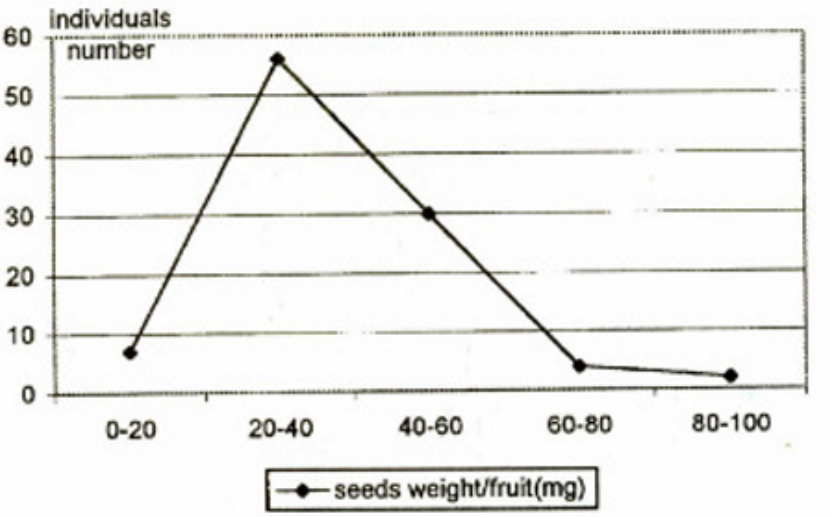

Fig. 6. – Seeds weight/fruit at 100 individuals.

All these results show, in our opinion, that the variability of all the quantitative traits examined is very high, providing therefore a very rich starting material for the natural selection. Because the environmental conditions for the area in which the species may be found are very variable, this being a highly anthropized environment, this accentuated variability, one is valuable for good future adaptability. On the other hand, we may see that even though extremely variable, the fruit number per

individual and the seeds number per fruit (Fig. 5) have a very small correlation, while the correlation between the seeds number per fruit and the seeds weight/fruit has an enough positive value (r=0.65). As we may expect, the correlation between the fruit number per plant and the seeds medium weight has a smaller value, so the conclusion is that: the more fruit, per individual, the more seeds – that is, a normal thing considering the adaptability process that we previously assessed.

CONCLUSIONS

The results of the statistical calculations performed over the "leaf length" and the sample weight of a centimetre square has shown a high enough variability, the correlation between these two parameters being relatively low.

The number of petals displays an important variability being observed the tendency to increase (in population) and this could be the basis for an explanation of the "*floriplenum*" shape appearance.

The number of petals is in correlation with the number of staminas (e.g. the flowers with 6 petals have, without any exception, between 20 and 24 staminas/flower).

Important correlations have been shown between the flowers weight and the number of staminas.

The number of fruits per individual, as well as the number of seeds per fruit are the traits with the highest variability.

The great variability of the examined traits offers to the *Chelidonium majus* species a better adaptability to environmental conditions as well as the possibility to conquer new ecological niches.

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FUNGI BIORHYTHMS: PRACTICAL IMPORTANCE

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Scientific researches regarding fungi biorhythms evidenced not only theoretical importance, but also possible applicabilities of these new discoveries. These data are useful to improve knowledge concerning the evolution of the biophenomena and are fundamental for the development of new domains in biological sciences, such as *Chronophytopathology, Biorhythmotherapy* or *Biorhythmotechnology*. Knowing that the living organisms are characterised by a certain temporal organization, the importance of the new concept "*starting time*" by laboratory experiments using *Penicillium chrysogenum* Thom as test-organism was proved.

Key words: biorhythms, fungi.

The investigations on plants biorhythms are now considered as having great theoretical and practical importance (1, 2, 12), but the literature concerning fungal biorhythms is still very scarce.

Bünning (cited by 12) investigated the biological cycles in *Pilobolus sphaerosporus* (Grove) Palla in continuous dark conditions and observed that the delivery of spores presented however clear circadian biorhythms, the acrophases being in the afternoon hours. The records made along several days evidenced the maximum amplitude values in the first 24 hours, followed by gradually reduced values, until 125 hours, when the delivery practically ceased.

Extensive investigations were accomplished in our country (3) in apple-trees orchards.The determinations regarding *Venturia inaequalis* Wint.(syn. *Endostigme inaequalis* (Cke.) Syd. evidenced the typical biorhythms for ascospores delivery, having the acrophases placed towards 6 p.m. for all the studied sorts of apple-trees. These acrophases are placed after the maximum values of the diurnal air temperature rhythms.

Seasonal biorhythms of fungi species can also be evidenced by analysing other literature data (10, 17), within periods of 25-30 days. The typical seasonal cycles, repeated at almost equal time intervals, are known as regards the cycle phases in the vegetative and reproductive life of many parasite species. The practical importance of these rhythmical biophenomena has not been considered till now.

Studies should include environmental factors rhythms concomitantly with those regarding the parasites and their host organisms, evidencing also the modifications produced by their interactions. They can agree or not with one another, and thus the effects are different. New methodologies of investigations on

the pathogeny, diseases cycles and cycles of epidemy depending on the biological time of organisms should be initiated.

The development of *Chronophytopathology* can be of great importance for crop protection.

One new domain in the biological sciences, with special practical importance, Biorhythmotherapy is also developing. This represents the rationalisation of pesticides applications to obtain optimum result, without supplementary treatments, having in view the fact that many of them were proved inefficient and polluting. As is known, for example, the ascospores number is appreciated as the main bioindicator for an open campaign of pesticide applications. Establishing their circadian and seasonal rhythms, the maximum value being placed at a certain time, this becomes thus predictable and this kind of data can be useful for administration time prognosis. The time of pesticide applications must be well established, because these agents can exert harmful influence on the cultivated organisms, modifying their normal physiological, biochemical, or even genetical biorhythms.

Among the fungi, there are many considered as very useful, in different domains. Certain *Penicillium, Aspergillus, Fusarium* and *Trichoderma* species have the biological capacity to degrade pesticides (20). A large field of scientific researches having direct practical importance in bioindustries is that of fungal bioproductivity, and of possibilities to improve this. As my view, the Biorhythmotechnology should develop for scientific management of biotechnologies. Experimental researches were performed in recent years having this aim, and thus evidencing the fungal circadian biorhythms, according to new biorhythmological concepts and laws.

MATERIALS AND METHODS

The laboratory experiments were accomplished according to the new methodology, biorhythmological, in a circadian biorhythm model, to prove the importance of the "starting time" concept. In this manner, the hour for beginning of experiments was the main variable factor. We present here the results recorded in three series of experiments, initiated on April 19, 1991, April 30, 1991, and respectively February 24, 1992.

The test-species utilised was *Penicillium chrysogenum* Thom, the strain 415, selected and utilised in the Antibiotics Factory of Iaşi (13, 14) to produce penicillin. The seeding was effectuated from homogeneous suspensions, by drops of 0.1 cm^3, each containing almost 15,000 conidia. To cultivate the mycelia, two different agarised media were used, these being the Special and Reistrich medium, in sterilized Petri boxes (5 boxes for each experimental time).

The air temperature degrees and luminosity as luces were recorded concomitantly, for the main experimental times. To determine the luminosity in the laboratory, a 150 U type Luxmeter was utilized.

After inoculation, the boxes were maintained in laboratory to incubate, having in view that majority of fungi species are mesophiles, developing in cultures between 5 and 37°C (16).

Periodically, after certain intervals, at the same hours, the cultural parameters were recorded and morphological differences compared.

RESULTS AND DISCUSSION

The results obtained in the first series of experiments evidenced differences between the cultures produced with different "starting times". After 72 hours the surfaces of the developed mycelia varied significantly, the best results were obtained when seeding was accomplished after 11 o'clock, on both culture media. The culture started at 8, 9 and 10 o'clock were less developed.

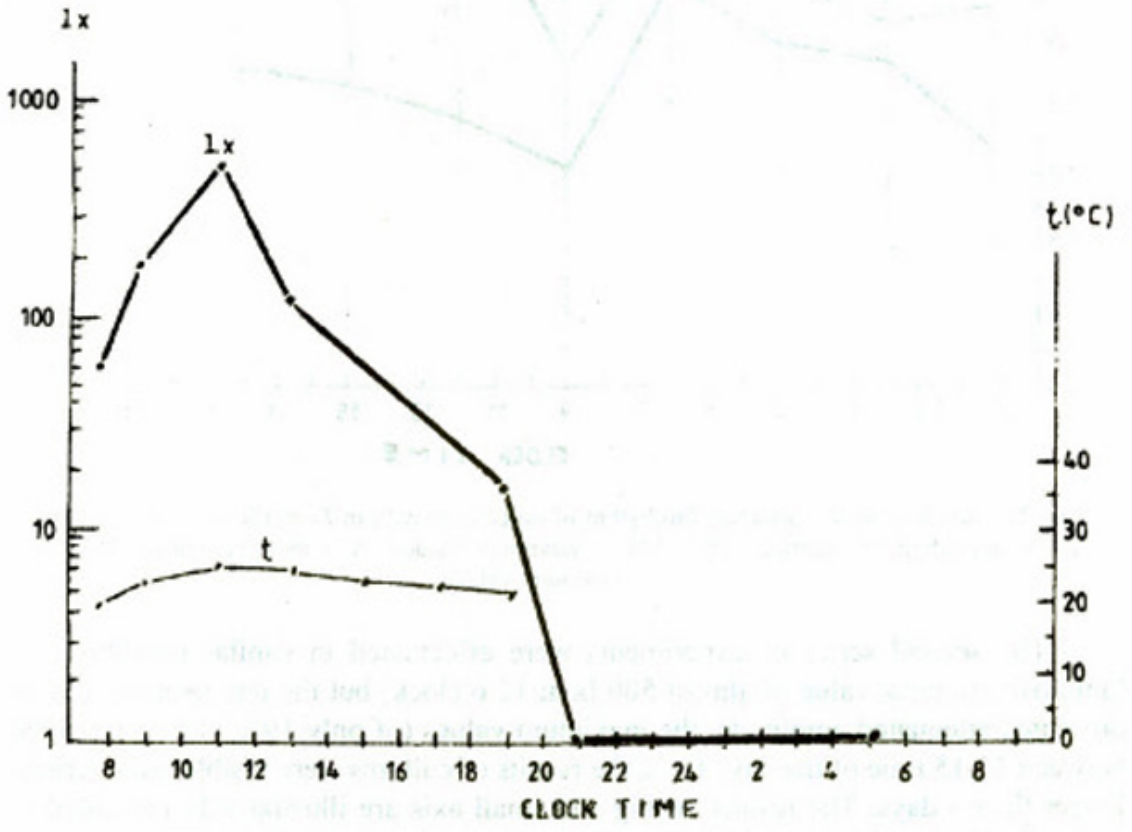

Fig. 1- Rhythms of air temperature (°C) and luminosity (lx) in laboratory.

The diurnal rhythms of luminosity and of air temperature are represented in figure 1. As one can see, the peak values of luminosity (almost 500 lx) were recorded at 11 o'clock and that of temperature (24 °C) between 11-13 local time of day.

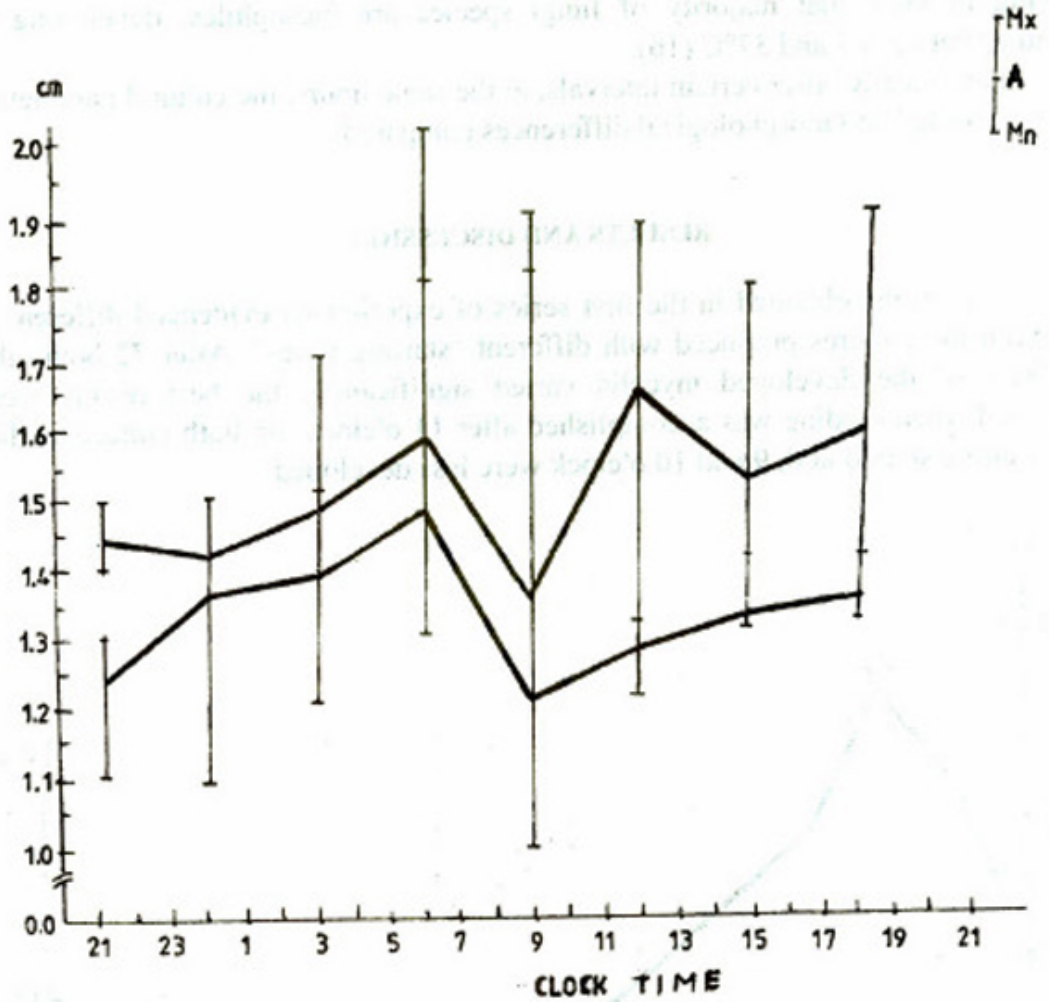

Fig. 2- Variations in the circadian biorhythm of mycelia growth in *Penicillium chrysogenum* Thom according to "starting time". Mx = maximum values; A = average values; Mn = = minimum values.

The second series of experiments were effectuated in similar conditions of luminosity (a peak value of almost 500 lx at 12 o'clock) but the temperature rhythm presented attenuated amplitude, the maximum values (of only 19°C) being recorded between 12-15 time of the day. Thus, the results of cultures were visible after periods longer than 3 days. The results, as big and small axis are illustratively presented in figure 2. The maximum and minimum values recorded for each "starting time" are also marked. The circadian biorhythms were again evidenced, less culture surfaces

being developed if the "starting time" was around 9 o'clock and the best developed cultures when the "starting time" was at 6 a.m., 12 a.m. or 6 p.m.

Figure 3 represents the results recorded in the third series of experiments, for both variants (light and dark). The maximum values of luminosity were over 10,000 lx (at 12 a.m.) and air temperature values varied between 20-23 °C. Again, the best "starting time" were 6 a.m. and mainly 6 p.m. The light presented stimulating action, but only at certain hours, at others actioning even as an inhibiting factor.

It is clear that the fungi present circadian biorhythms of growth, and that the "starting time" is important, the differences between different experimental times being significant. For the strain of *Penicillium chrysogenum* Thom tested, the interval between 8 a.m. – 9 a.m. seems to be "the resting time" in the growth activity of cells. Interesting is the fact that growing is the greatest in the afternoon hours, especially at 6 p.m., as for other biological activities evidenced by different fungi species.

Along the time (Fig. 3) the pattern of circadian biorhythms can change similarly with other organisms, thus the biological age is also an important factor and must be known in experiments to compare the results obtained.

Regarding the action of irradiating external factor, it is known that these, especially UV have inhibitory action. Certain authors (18) mentioned the inhibition of fungi by culture media previously exposed to light. But also this action is specifical, negative upon the growth of *Armillariella mellea* (Wahl.:Fr.) Karst, *Rhizoctonia solani* Kühn, *Phytophthora capsici* Leonian and not for other species as *Fusarium oxysporum* (Schlecht.) Sn et Hansen, *Verticillium albo-atrum* Reinke et Berth, and *Alternaria alternata* (Fr.) Keissler. Also they stated that the inhibition is produced only in the beginning of growth and not in the multiplication speed.

Lungu (12) discussed the data presented by Hastings and Sweeney in 1957 revealing the endogenous periodicity of spores delivery at many species of fungi, influenced by the blue light.

Because the fungi are vegetal organisms lacking photoassimilating pigments, it can be supposed that light is not a directly synchronising factor but, as it was proved, the visible irradiation has stimulating action for the biological time with peak activity.

The importance of certain phases in the development of microorganisms cultures is known (11) and used in industrial practice, for example the exponential phase of growth. The new discoveries regarding the biorhythms of microorganisms proved that these "classical growth phases" are different at different biological times, and there are possibilities to predict them.

The new concept "starting time" (4) was proved as having not only theoretical, but also practical importance. The differences between cultures are significantly varied and these can be great at industrial scale.

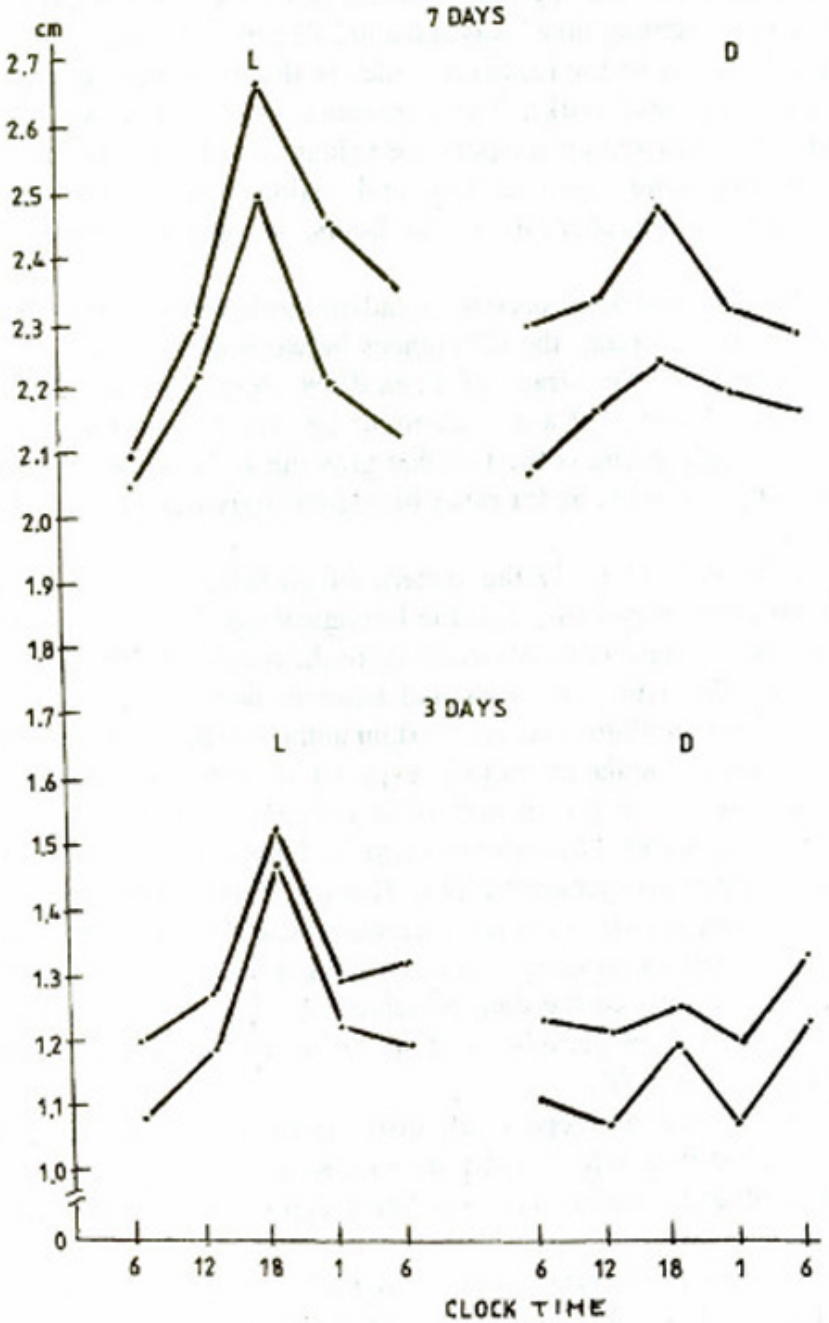

Fig. 3 – Variations according to "starting time" in the circadian biorhythms of mycelia growth in light (L) and dark (D) *in Penicillium chrysogenum* Thom.

The explanation of these biophenomena will be established by Molecular Biochemistry, the molecular structure is very important, and the configurational information, the assemblage, rearrangement change (8). In the living organisms,

these represent periodical changes in their cells dynamics. Importance must be granted also to the possibility of finite deformation through rotation of molecules (9). Also, recent studies (19) have evidenced interesting thermodynamic aspects regarding protein folding.

The "starting time" concept can receive new applications, regarding the cell cycles as periodical production of biochemical new products, the variations between certain biological times being quantitatively and also qualitatively significant. Knowing the optimum "starting time" for each cultivated species, the best results can be obtained for the principal and secondary bioproducts. As for other species of fungi (7) the mycelium can be attempted to be utilized as an enzyme source. Having in view the great volume of investigations for the selection programme (13), rationalisation of the researches to state the best activity according to the studies on biorhythms of selected strains may be useful. The biological time can be utilized as key-factor of variability even for clones, and thus to increase the productivity.

The results evidenced new interesting aspects, which may contribute to the development of one new applied domain in the biological sciences, as Biorhythmotechnology.

CONCLUSIONS

1. The circadian biorhythm of growth at *Penicillium chrysogenum* Thom evidenced periods of 12 hours, the peak values being obtained at 6 p.m.

2. External factors, such as temperature and luminosity, determine different directions of action at different biological times.

3. The "starting time" concept has had not only theoretical importance, but also many practical valuable applications.

The new taxonomic denominations and their authors according to Prof. dr. M. Mititiuc (Faculty of Biology, Iaşi) and dr. Al. Manoliu, Dr. Vera Bontea, 1997 (15).

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THE ANALYSES OF THE DEPENDENCE OF SOME PHYSIOLOGICAL PROCESSES AND A VEGETAL PRODUCTION AS OPPOSED TO THE NUTRIENTS IN SOIL

RADU STANCU

This paper presents the analysis of the addiction of some physiological processes (the intensity of the photosynthesis, the accumulation of total chlorophyll, the accumulation of biomass) and of the production of autumn grain on a acid soil as opposed to the mineral fertilizers having N, P, and K and the manure. The experimental results one can find here are the result of five years of work in the open and in the lab and they can be statistically analysed through the method of multiple correlation. This analysis was done with the aid of the correlation index *r*.

The numbers thus obtained are important and in several cases more than important.

The interpretation of the results with the aid of the Fisher test (F) developed some conclusions except for a few exceptions caused by the small number of freedom degrees especially when it comes to two independent variables (Table 1).

Key words: photosynthesis, total chlorophyll, index *r*.

The studying of the mineral nutrition of plants on acid soils is very important as these soils are one percent of the arable area. In Romania, these acid soils cover almost two million ha (20.4% of the country's arable soil) out of which almost 400,000 ha are in the area designated by the Olt and the Dîmbovița rivers.

In these acid soils some nourishing elements, essential for plants (N, P, Ca, K, Mg, Mo, s.a.) are harder to find, but there is a higher incidence of most microelements and heavy metals, which are toxic for plants. Beside these effects, the raised acidity of the soil modifies the populations and the activity of the microorganisms that mineralize the organic compound with N, P, and S and immobilizes the active substance in the mineral fertilizers added to the soil.

Many experiments with nourishing substances have shown that the absorption, the translocation and the use of the nourishing substances by the plants are influenced by the relative quantity of some substances in the environment.

Although very important, the results of the experiments with nourishing substances cannot always be replaced by the plants grown in different types of soils, where the relations between themselves are more complex and the results can be utterly different. When it comes to nourishing soils, the food regimen is

more monotonous than in regular soil, where it is difficult to say which factor influences the physiological process in question.

In this paper we present the results of some experiments carried out at the Research Centre for Cereals and Technical Plants Albota, Argeş, experiments that revealed the effect of the small and average dosage of mineral fertilizers with N, P and K and of the manure on some physiological processes and on the production of the autumn grain.

MATERIALS AND METHODS

In order to study the influence of the nourishing substances existing in the environment on some physiological processes on the autumn grain on acid soil, we organized a field experience with mineral and organic fertilizers in twelve variants with five repetitions, following the method of the randomized blocks.

There have been used the following dosages and combinations of mineral and organic fertilizers*: V_1 = unfertilized (the control variant), $V_2 = N_{50}$, $V_3 = N_{100}$, $V_4 = P_{35}$, $V_5 = P_{70}$, $V_6 = N_{50} + P_{35}$, $V_7 = N_{100} + P_{70}$, $V_8 = N_{100} + P_{70} + K_{80}$, V_9 = 10 t manure/ha/year, V_{10} = 10 t M + N_{50}, V_{11} = 10 t M + P_{35}, V_{12} = 10 t M + N_{50} + P_{35}.

The determinations were made in five phases of vegetation: phase I - union; phase II - the appearance of straws; phase III - the appearance of ear; phase IV - blooming, fecundation; phase V - baking.

The methods for studying the physiological processes were those usually utilized in the labs of plant physiology.

The intensity of photosynthesis was discovered through the Warburg manometric method for plants that grow on land modified by Sălăgeanu (1962).

The total chlorophyll was determined through a spectrophotometrical method, and the foliar area was a little modified by us (according to the gravimetrical and parametrical methods - Niciporovici, 1961), that is, we divided the grain leaf in the area where the margins are parallel and a triangle in the ending area.

The dry biomass (at 104^0 C, supraterrane areas only) and the agricultural production were determined by weighing.

The reported data are the average of experiments in the open and in the lab, the yearly fluctuations, being omitted, partially some of them being unimportant and some of them accidental.

RESULTS AND DISCUSSION

In two previous works we have shown the influence of the mineral nutrition system on the intensity of photosynthesis and breathing (10) and on the dynamics

* N_{50}, N_{100}, P_{35}, P_{70}, K_{80} stands for the quantity of active nitrogen, phosphorus and potassium in kg/ha, incorporated in the salt used as a fertilizer, and 10 t M means 10 t of manure spread each year on a ha.

of the assimilating pigments and of the foliar surface (11). In order to determine the variation of the researched phenomenon and to establish the degree of significance of this variation, the interpretation of the results was made by analysis of the variation, which offered the possibility of statistical interpretation of a single variable on several samples. When studying the analysis of the dependency between one or another of the studied physiological processes and the conditions of mineral nutrition experimentally provided, we used the method of simple correlation.

Now we are presenting the results of the analysis of the dependence of the intensity of the photosynthesis, of the accumulation of total chlorophyll, of the index of the foliated surface, of the dry biomass and of the production (variables depending on y), as opposite to the mineral and organic fertilizers experimentally provided (independent variables x_1, x_2, x_3), an analysis made through the method of multiple correlation.

The dependence between the variables was determined through the multiple regression equation having the form: $y = a + bx_1 + bx_2 + cx_3 + ... + zx_n$. As in this case the graphics would have become difficult to understand, the results are presented in the correlation Tables 1, 2 and 3.

Table 1 expresses the correlation of the productions, of the total surface index, of the photosynthesis of the biomass and the total chlorophyll (as variables depending on y), as opposed to the chemical fertilizers with N and P (as variables independent of x_1 and x_2).

In Table 2, the dependence of the same variables on y is discussed, as opposed to three independent variables (N, P, K), and in Table 3, as opposed to the four independent variables (N, P, K and M).

The analysis was made with the help of the correlation index r. The ultimate values of the correlation index r for degrees of freedom 6, 7 and 11 and the probabilities of transgression α were counted through interpolation in connection to the data in literature (see Ceapoiu 1968 p. 523, Table 11), getting the values mentioned at the foot of any table as one can see, all the results are important - most of them very significant, and only a few distinctly significant, the correlation being even more obvious when we have a higher number of independent variables (Tables 2 and 3), an element explained by the interaction between the fertilizers and the higher number of freedom degrees .

The interpretation of the results through the Fisher test (F) led to the same conclusions with a few exceptions due to the low number of freedom degrees, especially in the case of two independent variables (Table 1) which is entirely obvious, as between the correlation index r and the value F there is a close connection which, in the case of two groups, can be expressed as:

$$F = \frac{(n-2)r}{I - r^2},$$

where n is the number of the freedom degrees.

Table 1

The multiple correlation between some physiological processes and the chemical fertilizers with N and P at the autumn grain growing in an acid soil (the average of the values over 5 years)

$y = a + bx_1 + cx_2$

Nr. crt.	y (variable)	Index of the regression of independent variable			n	r	s	F	GL
		a	b(N)	c(P)					
1.	Production (the average in 5 years)	8.9841	0.1311	0.1078	7	0.911^{xx}	3.85	9.75^{x}	2 + 4
2.	The index of the foliar area phase I	0.2382	0.0002	0.0009	7	0.921^{xx}	0.02	11.22^{x}	2 + 4
3.	" " " " " " " II	0.4190	0.0038	0.0056	7	0.950^{xxx}	0.10	18.63^{xx}	2 + 4
4.	" " " " " " " III	1.3423	0.0101	0.0101	7	0.872^{xx}	0.41	6.36	2 + 4
5.	" " " " " " " IV	1.0448	0.0079	0.0079	7	0.920^{xx}	0.34	11.04^{x}	2 + 4
6.	" " " " " " " V	0.6223	0.0009	0.0008	7	0.946^{xxx}	0.18	16.98^{x}	2 + 4
7.	The photosynthesis phase I	5.5266	0.0064	0.0064	7	0.848^{xx}	0.57	5.13	2 + 4
8.	" " " II	6.4441	0.0181	0.0181	7	0.900^{xx}	0.80	8.50^{x}	2 + 4
9.	" " " III	7.2433	0.0359	0.0359	7	0.882^{xx}	1.39	6.99^{x}	2 + 4
10.	" " " IV	5.9703	0.0193	0.0193	7	0.876^{xx}	1.52	6.57	2 + 4
11.	" " " V	4.0072	0.0063	0.0063	7	0.931^{xxx}	0.83	13.06^{x}	2 + 4
12.	The dry biomass phase I	3.5379	0.0242	0.0242	7	0.749^{x}	1.50	2.56	2 + 4
13.	" " " " II	5.6241	0.0617	0.0617	7	0.939^{xxx}	1.62	14.95^{x}	2 + 4
14.	" " " " III	25.0420	0.2236	0.2236	7	0.877^{xx}	9.22	6.64	2 + 4
15.	" " " " IV	31.4040	0.2454	0.2454	7	0.869^{xx}	10.74	6.18	2 + 4
16.	" " " " V	38.6962	0.3377	0.3377	7	0.891^{xx}	14.27	7.72^{x}	2 + 4
17.	" " " " VI	43.4646	0.3407	0.3407	7	0.905^{xx}	15.12	9.07^{x}	2 + 4
18.	The total chlorophyll phase I	1.1469	0.0022	0.0022	7	0.897^{xx}	0.26	8.25^{x}	2 + 4
19.	" " " " II	1.5769	0.0013	0.0013	7	0.907^{xx}	0.25	9.28^{x}	2 + 4
20.	" " " " III	1.8277	0.0019	0.0019	7	0.932^{xxx}	0.24	13.20^{x}	2 + 4
21.	" " " " IV	1.5958	0.0013	0.0013	7	0.934^{xxx}	0.25	13.71^{x}	2 + 4
22.	" " " " V	0.7358	0.0132	0.0006	7	0.926^{xxx}	0.30	12.04^{x}	2 + 4

α 5% $r = 0.707^{x}$, α 1% $r = 0.834^{xx}$, α 0.1% $r = 0.924^{xxx}$; α 5% $F = 6.94^{x}$, α 1% $F = 18.00^{xx}$, α 0.1% $F = 26.28^{xxx}$

α = the level of significance; r = the index of correlation; s = the standard deviation of the estimation; F = the Fisher test; GL = the freedom degrees; n = the number of variants.

Table 2

The multiple correlation between some physiological processes and the chemical fertilizers with N, P and K at the autumn grain growing in an acid soil (the average of the values in 5 years)

$y = a + bx_1 + cx_2 + dx_3$

Nr. crt.	y (variable)	Index of the regression of independent variable				n	r	s	F	GL
		a	b(N)	c(P)	d(K)					
1.	Production (the average in 5 years)	0.9841	0.13107	0.10782	0.07229	8	0.952^{xxx}	3.86	12.99^{x}	3 + 4
2.	The index of the foliar area phase I	0.2382	0.00023	0.00093	0.00005	8	0.943^{xxx}	0.02	10.72^{x}	3 + 4
3.	" " " " " " " II	0.4190	0.00327	0.00564	0.00078	8	0.968^{xxx}	0.10	20.05^{xx}	3 + 4
4.	" " " " " " " III	1.3423	0.01073	0.01006	0.00388	8	0.922^{xxx}	0.41	7.60^{x}	3 + 4
5.	" " " " " " " IV	1.0446	0.01316	0.00790	0.00359	8	0.950^{xx}	0.34	12.36^{x}	3 + 4
6.	" " " " " " " V	0.6223	0.00938	0.00938	0.00017	8	0.957^{xx}	0.18	14.52^{x}	3 + 4
7.	The photosynthesis phase I	5.5266	0.01592	0.00640	0.00038	8	0.882^{xx}	0.57	4.66	3 + 4
8.	" " " II	6.4441	0.02685	0.01283	0.01283	8	0.944^{xxx}	0.80	10.84^{x}	3 + 4
9.	" " " III	7.2433	0.03952	0.03390	0.01616	8	0.930^{xxx}	1.39	8.58^{x}	3 + 4
10.	" " " IV	5.9703	0.04824	0.01929	0.01453	8	0.919^{xxx}	1.53	7.27^{x}	3 + 4
11.	" " " V	4.0072	0.03781	0.00626	0.01889	8	0.960^{xxx}	0.82	15.52^{x}	3 + 4
12.	The dry biomass phase I	3.5379	0.02455	0.02424	0.00435	8	0.821^{xx}	1.50	2.77	3 + 4
13.	" " " " II	5.6241	0.06501	0.06176	0.02418	8	0.965^{xxx}	1.62	17.93^{xx}	3 + 4
14.	" " " " III	25.0420	0.25208	0.22557	0.13205	8	0.931^{xxx}	9.21	8.68^{x}	3 + 4
15.	" " " " IV	31.4040	0.28678	0.24545	0.17852	8	0.930^{xxx}	10.74	8.56^{x}	3 + 4
16.	" " " " V	38.6962	0.43979	0.33769	0.11020	8	0.931^{xxx}	14.27	8.64^{x}	3 + 4
17.	" " " " VI	43.4646	0.52378	0.34067	0.16130	8	0.942^{xxx}	15.12	10.42^{x}	3 + 4
18.	The total chlorophyll phase I	1.1469	0.00929	0.00226	0.00079	8	0.923^{xxx}	0.26	7.68^{x}	3 + 4
19.	" " " " II	1.5769	0.00961	0.00136	0.00016	8	0.926^{xxx}	0.25	8.06	3 + 4
20.	" " " " III	1.8277	0.01088	0.00169	0.00053	8	0.945^{xxx}	0.24	11.17^{x}	3 + 4
21.	" " " " IV	1.5958	0.01205	0.00131	0.00106	8	0.950^{xxx}	0.25	12.19^{x}	3 + 4
22.	" " " " V	0.7358	0.01321	0.00061	0.00131	8	0.937^{xxx}	0.30	9.56^{x}	3 + 4

α 5% $r = 0.666^{x}$, α 1% $r = 0.798^{xx}$, α 0.1% $r = 0.898^{xxx}$, α 5% $F = 6.59^{x}$, α 1% $F = 16.69^{xx}$, α 0.1% $F = 24.26^{xxx}$

α = the level of significance; r = the index of correlation; s = the standard deviation of the estimation; F = the Fisher test; GL = the freedom degrees; n = the number of variants

Table 3

The multiple correlation between some physiological processes and the chemical fertilizers with N and P at the autumn grain growing in an acid soil (the average of the values in 5 years)

$y = a + bx_1 + cx_2 + dx_3 + ex_4$

Nr. crt.	y (variable)	Index of the regression of independent variable					n	r	s	F	GL
		a	b(N)	c(P)	d(K)	e(G)					
1.	Production (the average in 5 years)	8.198	0.1607	0.0937	0.0592	0.0121	12	0.928^{xxx}	4.37	10.78^{xxx}	4 + 7
2.	The index of the foliar area phase I	0.235	0.0004	0.0009	0.0000	0.0034	12	0.915^{xxx}	0.02	9.93^{xx}	4 + 7
3.	" " " " " " " II	0.470	0.0040	0.0051	0.0006	0.0446	12	0.951^{xxx}	0.11	16.45^{xxx}	4 + 7
4.	" " " " " " " III	1.315	0.0125	0.0034	0.0034	0.0825	12	0.907^{xxx}	0.38	8.14^{xx}	4 + 7
5.	" " " " " " " IV	0.969	0.0155	0.0023	0.0023	0.0775	12	0.930^{xxx}	0.36	11.26^{xxx}	4 + 7
6.	" " " " " " " V	0.579	0.0107	0.0006	0.0006	0.0187	12	0.940^{xxx}	0.19	13.16^{xxx}	4 + 7
7.	The photosynthesis phase I	5.415	0.0177	0.0023	0.0023	0.0894	12	0.898^{xxx}	0.47	7.70^{x}	4 + 7
8.	" " " II	6.334	0.0309	0.0200	0.0200	0.2095	12	0.936^{xxx}	0.76	12.75^{xxx}	4 + 7
9.	" " " III	6.873	0.0513	0.0010	0.0010	0.3370	12	0.894^{xxx}	1.63	6.94^{x}	4 + 7
10.	" " " IV	5.577	0.0591	0.0080	0.0080	0.2952	12	0.899^{xxx}	1.61	7.32^{x}	4 + 7
11.	" " " V	3.488	0.0481	0.0102	0.0102	0.2239	12	0.908^{xxx}	1.29	8.26^{xx}	4 + 7
12.	The dry biomass phase I	3.240	0.0334	0.0006	0.0006	0.2105	12	0.813^{xxx}	1.47	3.41	4 + 7
13.	" " " " II	5.372	0.0787	0.0200	0.0200	0.7121	12	0.939^{xxx}	2.05	12.89^{xxx}	4 + 7
14.	" " " " III	23.682	0.3055	0.1964	0.1094	0.0225	12	0.915^{xxx}	9.15	9.05^{xx}	4 + 7
15.	" " " " IV	29.925	0.3503	0.1539	0.1539	0.0259	12	0.912^{xxx}	10.84	8.60^{xx}	4 + 7
16.	" " " " V	38.078	0.5149	0.0998	0.0998	0.0331	12	0.908^{xxx}	14.71	9.19^{xx}	4 + 7
17.	" " " " VI	41.858	0.6238	0.1346	0.1346	0.0392	12	0.916^{xxx}	16.53	9.17^{xx}	4 + 7
18.	The total chlorophyl phase I	1.035	0.0113	0.0011	0.0011	0.0440	12	0.898^{xxx}	0.28	7.30^{x}	4 + 7
19.	" " " " II	1.491	0.0112	0.0013	0.0013	0.0257	12	0.914^{xxx}	0.25	8.91^{xx}	4 + 7
20.	" " " " III	1.725	0.0129	0.0023	0.0023	0.0345	12	0.920^{xxx}	0.27	9.58^{xx}	4 + 7
21.	" " " " IV	1.487	0.0145	0.0008	0.0006	0.0367	12	0.917^{xxx}	0.31	9.26^{xx}	4 + 7
22.	" " " " V	0.639	0.0154	0.0029	0.0029	0.0225	12	0.921^{xxx}	0.31	9.78^{xx}	4 + 7

α 5% $r = 0.553^{x}$, α 1% $r = 0.684^{xx}$, α 0.1% $r = 0.8004^{xxx}$, α 5% $F = 4.12^{x}$, α 1% $F = 7.85^{xx}$, α 0.1% $F = 10.05^{xxx}$

α= the level of significance; r = the index of correlation; s = the standard deviation of the estimation; F = the Fisher test; GL = the freedom degrees; n = the number of variants.

In the literature there are less references to the statistical analysis through the method of multiple correlation of the addition of the physiological processes to the mineral and organic fertilizers at the autumn grain grown on acid soils.

Yet some mentioned data correspond to the results presented by us, even if in most cases other species of plants were experimented on (Gregori et al.; Richards; Richards and Templeman quoted by Steward 1963; Sălăgeanu and Pristavu, 1966; Pocinak 1967; Marinescu et al., 1984; Sebanek, 1985, 1992; Salisbury and Ross, 1991; quoted by Burzo et al., 1999).

CONCLUSIONS

1. The mineral and organic fertilizers influence the physiological processes discussed in this study in different ways, according to the dosage and the combination used.

2. The best combination of fertilizers proved to be the moderate dosages of mixed N, P,and K or small dosages of N and P, mixed with moderate dosages of manure.

3. The statistical analysis done through the method of multiple correlations shows the addition of some essential physiological processes: photosynthesis, the foliar area, the accumulation of chlorophyll, the accumulation of biomass and the agricultural production as opposed to the mineral nourishing conditions in the soil.

4. In the clay gathered by water having an acid reaction it is better to administrate chemical fertilizers with N, K and P, in moderate dosages, or organic fertilizers mixed with small or moderate dosages of N and P.

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POLLINATION ECOLOGY, FERTILIZATION AND EMBRYOGENESIS AT *ORCHIS PROVINCIALIS* BALB. ON THE SOUTH COAST OF CRIMEA

A.A. CHEBOTARU*, O.I. LAGUTOVA**

Biology of flowering, pollination ecology, fertilization and embryogenesis of a Crimean orchid species (*Orchis provincialis* Balb.) has been investigated. Specific evolutionary coadaptive relationships between the attractant plant, insect pollinator and orchid have been investigated. Formation of generative organs, microsporogenesis and megasporogenesis occur without any visible anomalies. Embryogenesis is completed with formation of a spherical embryo not differentiated into organs. Instances of polyspermy against a background of syngamy are described. Endosperm formation is extremely reduced and results in the formation of a primary triploid nucleus which is resorbed soon.

Key words: pollination, fertilization, embryogenesis, *Orchis*.

The process of propagation by seeds in *Orchidaceae* is characterized by extremely high specialization degree of certain constituent stages. Complicated evolutionary-ontogenetical correlations between the flower and its pollination agent (3, 10), embryostructure and way of seed germination (13), protocorm and mycorrhizaforming fungus (6), fertilization and seed structure (12, 13) are well known. European orchid species are studied best from a perspective of reproductive biology (4, 9, 5). As regards the Crimean orchids, only some main stages of seed reproduction of a single species have been investigated (8). Presently, there are 46 orchid species in the Crimean Peninsula. The study of the reproduction by seeds of orchids is especially important because most representatives of *Orchidaceae* are ascribed to the category of rare and threatened plants and they all are included in the "Red Book of Ukraine" (Vakhrameyeva and Denisova, 1980). In this paper results of investigating sexual reproduction in a coenopopulation of *Orchis provincialis* Balb. are given.

MATERIAL AND METHODS

The coenopopulation of *Orchis provincialis* in Crimea was studied during 1989-1991. The site of observations was situated on the southern slope of the Crimean Mountains' Main Ridge near village Oreanda, at an altitude of 420 m

above sea level. It is located in an oak-hornbeam thickened forest, with an admixture of *Fraxinus excelsior* and *Arbutus andrachne* Shrub. layer is represented by *Laburnum anagyroides* Medik. and *Ruscus ponticus* Woronow ex Grossh. Embryological studies were carried out according to the generally used procedure (11). The investigation was effected both on permanent and temporary preparations using temporal material fixation according to the M.S. Navashin method (7).

Procedures used by foreign scientists (1) have been assumed as a basis of studying dynamics of flowering and pollination.

RESULTS OF THE DISCUSSION

Generative shoots of *Orchis provincialis* appear usually above soil surface in the middle second decade of March, being a typical monopodium type orchid with 4-10 flower buds, which are at the stage of initiating stamen primordia. A single primordium increases its dimensions and assumes outlines of tetrasporangial anther to early April. As a result of cell division within the multicellular archesporium, a large amount of microsporocytes are formed, which are combined in separate units cells. Entering meiosis, the cells of one unit divide synchronously while cells of other units may be at different stages of meiosis (Fig. 1 A, B). Tetrads of microspores form according to a simultaneous pattern. In one microsporangium T-shaped, isobilateral, tetrahedric, and tetrads were observed (Fig. 1C).

The wall of the young anther at the microspore mother cell stage consists of 4 layers: epidermis, endothecium, middle layer, and tapetum. Uniserial and uninucleate tapetum already begins to degenerate at the stage of meiosis. Two-celled male gametophyte forms in the microspore mother cells as a result of two successive rounds of mitosis.

Gynoecial development remains behind that of the androecium significantly. At the time of anthesis, ovule primordia can be found on the placenta of a three carpelar paracarpious ovary. These primordia consist of an axial series of 6-8 cells of nucellus surrounded by epidermis (Fig. 2 A). In transverse section, the placenta of each carpel is branched dichotomically. Each branch, when viewed in the plane of the section through the middle of the ovary, consists of 8-12 primordia. Further development of the primordia takes place only after the pollination.

Blossoming period of *Orchis provincialis* in the south coast of Crimea begins usually in the middle of April, continuing to later May. The stage of most profuse flowering occurs between 26 April and 6 May when the maximum air temperature is 20-21°C. Female bumble-bees (*Bombus hortorum*) have been caught. *Orobus aureus* Stev. (Fabaceae) is blossoming at the same time with

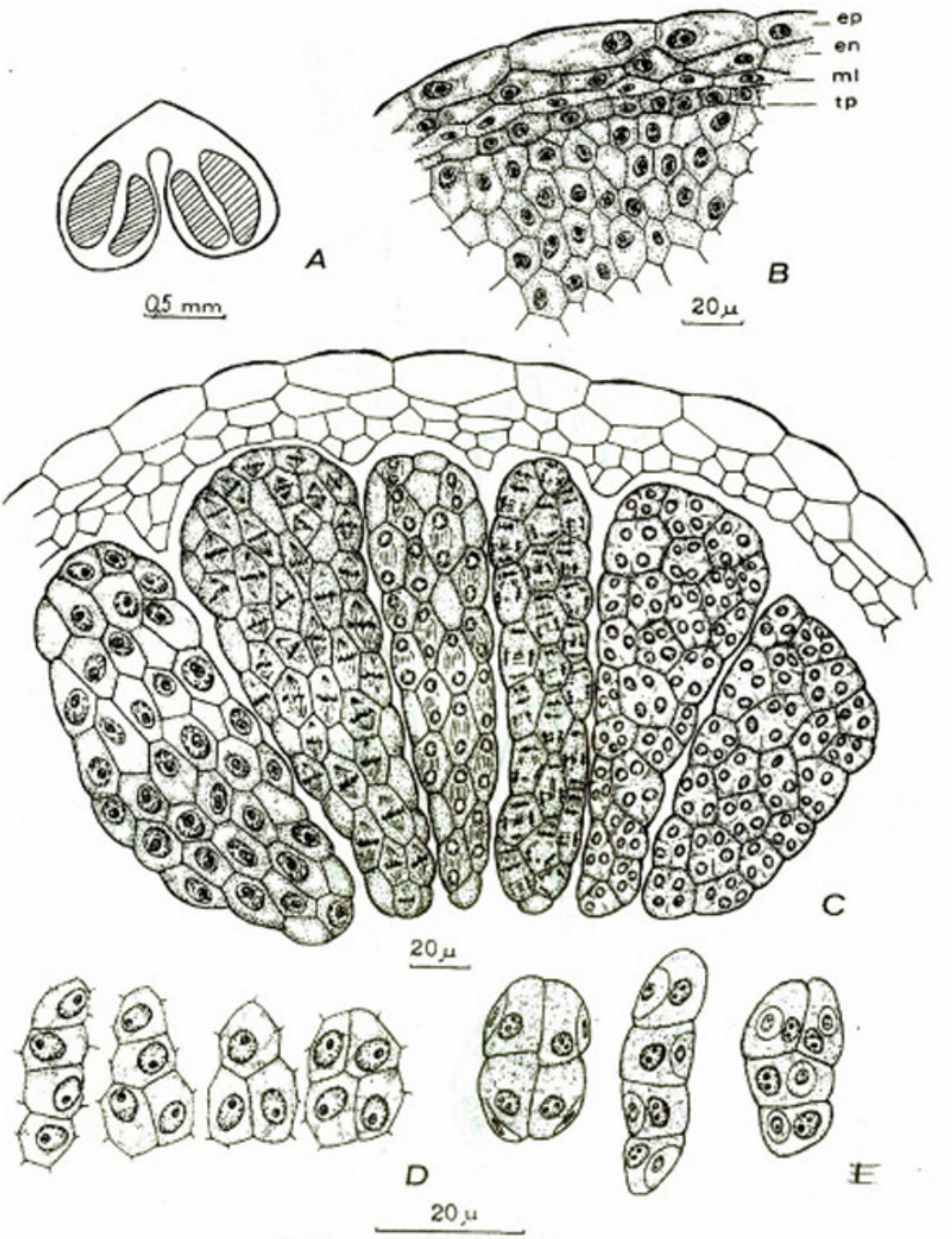

Fig. 1. – Microsporogenesis and pollen grain development at *Orchis provincialis*: **A** - Section of anther at the stage of microspore mother cell; **ep** - epidermis, **en** -endothecium, **ml** - middle layer, **tp** - tapetum; **B** - Asynchronous nature of meiosis in different sets of microspore mother cells; **C** – Tetrads of microspores; **D** - Different development periods of male gametophyte.

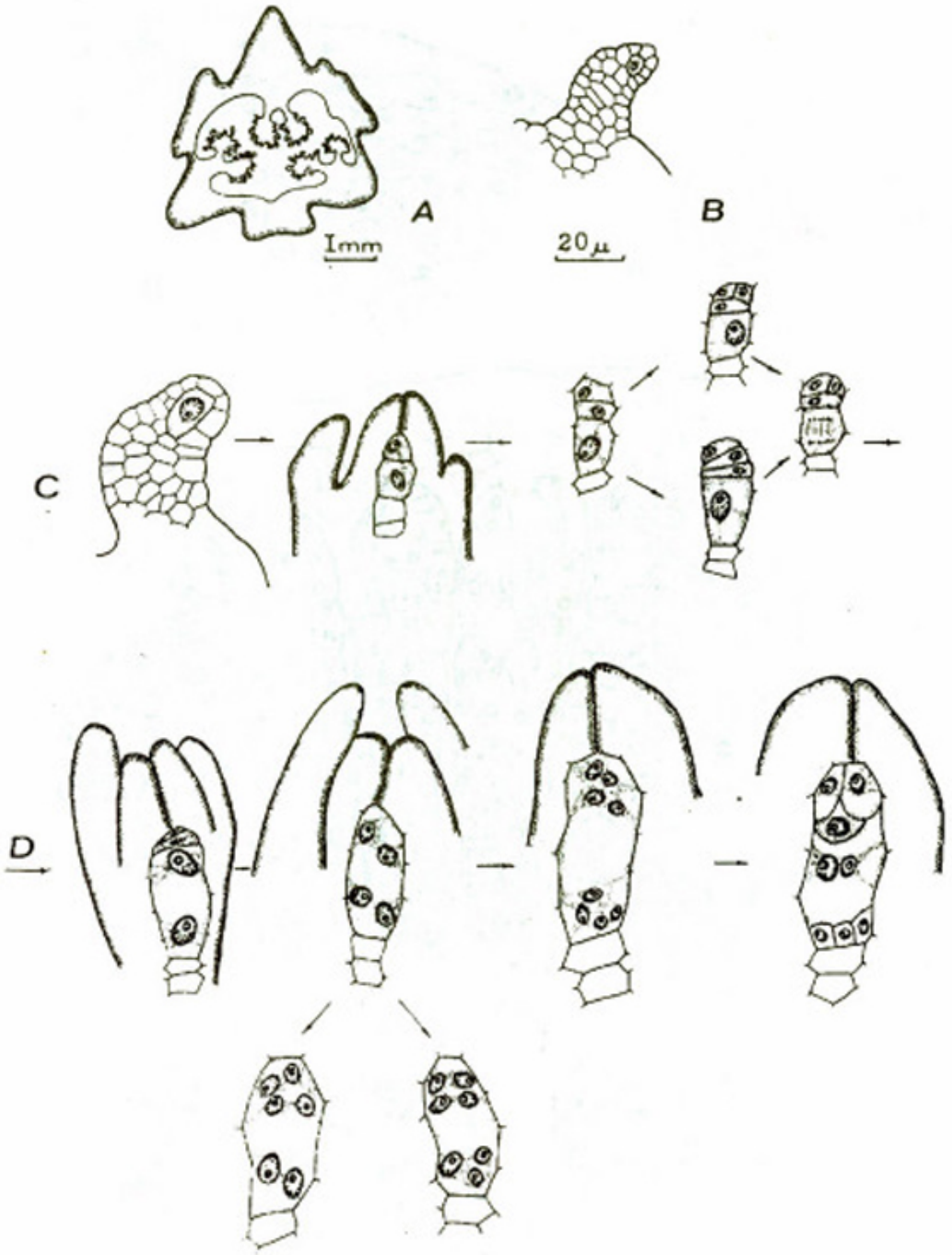

Fig. 2. – Megasporogenesis and female gametophyte development of *Orchis provincialis*: **A** - Transverse section of ovary; **B** - Ovule primordium at the archesporial cell stage; **C** - Megasporogenesis; **D** - Embryo sac development.

orchid prevails in the grass cover of orchid habitat. Flowers of *Orobus aureus* are given marked preference by the pollinators being rewarding in contrast to *Orchis*.

At the beginning of flowering of *Orchis provincialis* its flowers were visited readily by the insects owing to the vivid search aspect of the orchid. Visits of flowers took place mainly when bumble-bee females appeared after hibernation and the feed constancy was not yet formed. Certain likeness between flowers of *O. provincialis* and *O. aureus* and their arranging ways in inflorescences also promoted active visits. The mimicry likeness cannot be called complete as there is difference in coloration of the corolla.

Fruiting level in *O. provincialis* is 29% which is considerably higher than in orchids using only "inexperience" of the insects (5%), and lower in those which use full mimicry likeness (32-62%) (2). Observations show that *O. provincialis* can attract insect-pollinators by two ways: by bright appearance and superiority in flower display, as compared to the other plants blossoming at the same time, and by partial mimicry likeness with fodder plants of the pollinators. Pollen germination and growth of pollen tubes activate subsequent development of unicellular primary archesporium of ovule primordia which later turns into megaspore mother cell (MCM). As a result of meiotic division of MCM, on 20-22 days after pollination, a dyad consisting of two unequal cells is formed (Fig. 2 C). A second division of the dyad's micropylar cell can take place both in longitudinal and transversal directions forming linear or T-shaped tetrads of megaspores.

The chalazal dyad cell divides always transversally into two unequal megaspores, the lower one of which forms embryo sac of *Polygonum* type (6-, 7- or 8- nucleate) (Fig. 2 D) following three mitotic divisions. Its egg-apparatus consists of two pear-shaped synergids and an egg-cell. The polar nuclei most often fuse into the central nucleus before fertilization occurs. Antipodal apparatus is represented by small cells or nuclei which begin to degenerate at the very moment of fertilization. The interval between pollination and fertilization is 27-29 days. Fertilization is porogamous. Pollen tube having passed microphyle pour out its content into one of the synergids. Before fertilization, the sperm nuclei are poorly condensed: chromosomes are stained faintly. Sperm nucleus enlarges in volume before gamete fusion, nucleolus becoming larger (Fig. 3).

Fusion of the second sperm can take place both with the fused nuclei pair and central nucleus with one of polar nuclei. The resulting primary triploid nucleus of the endosperm does not divide further. The observed cases of polyspermy can be attributed to deviations from the course of normal fertilization. Sometimes, the contents of a few pollen tubes are discharged into the embryo sac. Zygote contains dense cytoplasm, a large nucleus, a nucleolus and some micronucleoli. It is pershaped and most often is located at an angle 25-30°C to the long axis of embryo sac. The zygote is directed towards the micropylar end to that synergid into which the pollen tube contents were discharged. The dimensions of the zygote increase and, after the rest period, it divides by means of a transversal wall into two cells: basal and terminal ones. As a result of transversal division of the basal cell, a three-celled proembryo is formed comprised of terminal, middle and

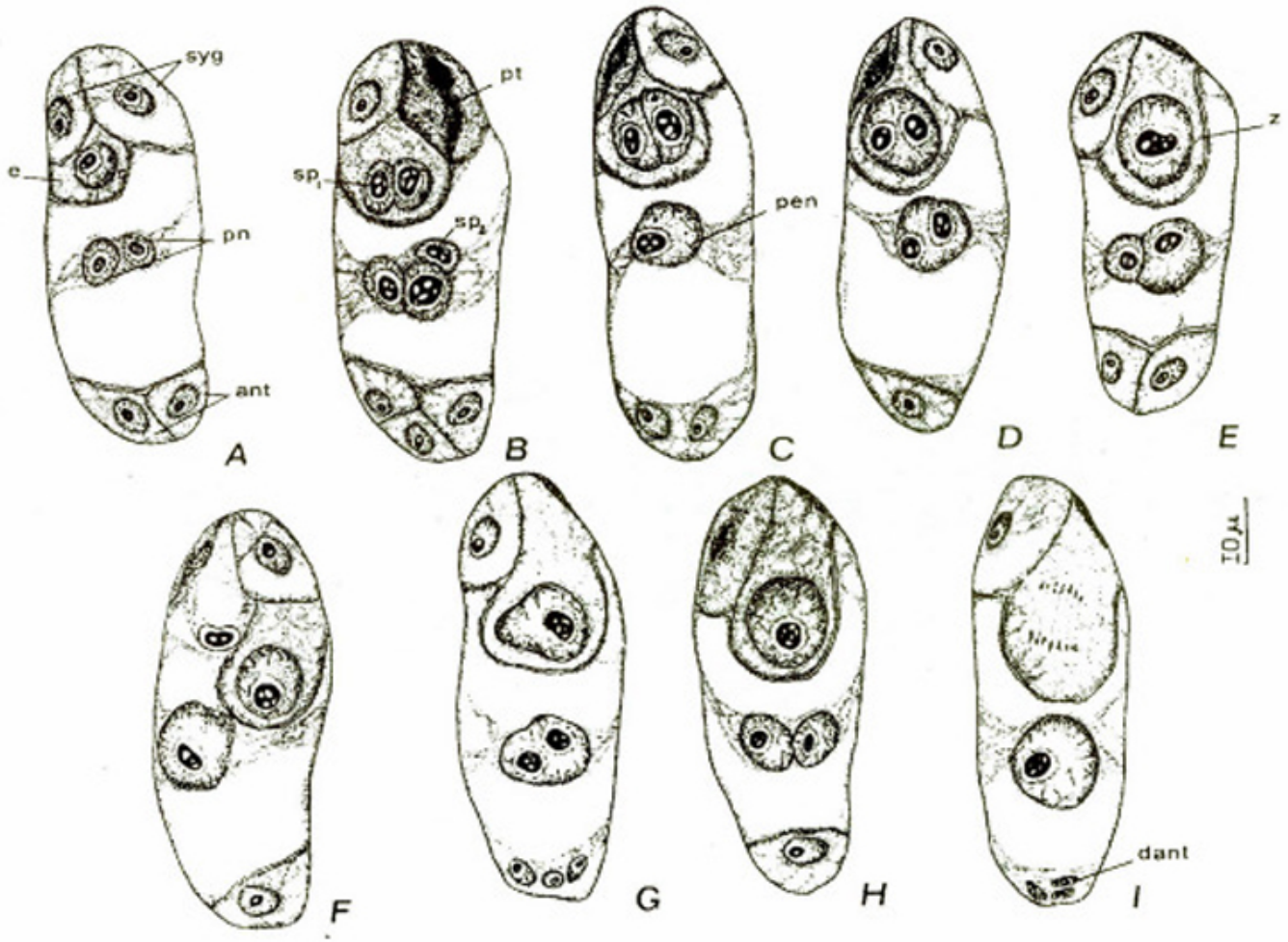

Fig. 3. – Different forms of double fertilization in *Orchis provincialis*: **A** - Mature embryo sac; **ant** - antipodal cells; **e** - egg-cell; **pn** - polar nuclei; **syg** - synergid; **B** - Sperm in contact with the egg-cell nucleus and a polar nucleus; **pt** - pollen tube; **sp** - sperm; **C, D** - Fusion of egg-cell nucleus with sperm; **pen** - primary endosperm nucleus; **E, F, G, H** - Zygote in rest period; **z** - zygote; **I** – First zygote division; **dant** - degenerating antipodes.

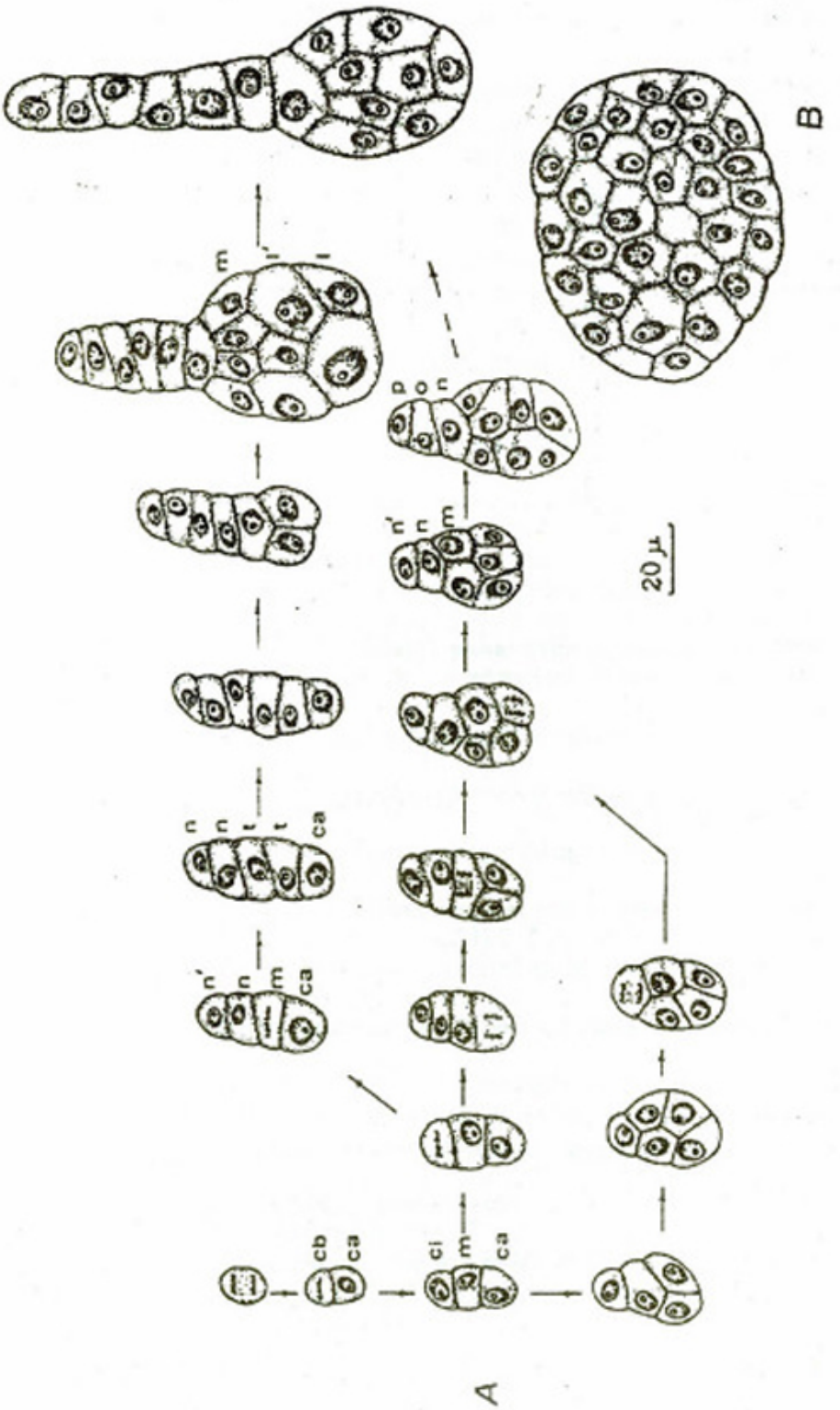

Fig. 4. – Embryogenesis stages at *Orchis provincialis*: **A** - Embryogenesis ways: **cb** - basal cell; **ca** - apical cell; **m** - middle cell; **ci** – initial suspensor cell; **n**, **n'**, **p**, **o** - basal cell derivatives, **t**, **t'** - middle cell derivatives; **B** - Mature embryo.

suspensor initial cells. Further embryo development takes place according to the *Onagrad* type, that is, the terminal and middle cells take part in the formation of the mature embryo (Fig. 4).

The developing embryo at early developmental stages is fed by endosperm and at later stages by suspensor. Cases of polyembryony have been found. Apparently, additional apomictic embryos are formed from the synergids. In this report we have retracted the changes occurring in the orchid flower from the bud stage to mature fruit. Development of male and female generative organs in *O. provincialis* proceeds without visible anomalies and ensures normal sexual reproduction. Since differentiation of female sexual structures ensues mainly after pollen is deposited by the insect pollination onto the stigma, the pollination process is the most important link of the reproductive cycle of the orchid under study.

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PROFESSOR DR. KATHERINE ESAU
(April 3, 1898 - June 4, 1997)

The *grande dame* of the American botany as she is considered, a brilliant representative of the plant anatomy worldwide, Katherine Esau would have turned one hundered years on April 3, 1998, being one of the most longevous naturalists of all times. But it was not to be so; her life broke off ten months earlier: the famous plant anatomist died on June 4, 1997, in Santa Barbara, California. With her passing away the biological community of all continents lost one of those distinguished scientists, an excellent researcher and schoolmaster, a model of scrupulosity, hard work, abnegation and honesty.

Katherine Esau was born on April 3, 1898, in the town of Yekatherinoslav, now Dnepropetrovsk, in Ukraine, living there till she was 20 years old, when she and her family fled to Germany during the Bolshevik Revolution.

Katherine's great-grandfather Aron Esau had immigrated to Ukraine in 1804 from Prussia. Her grandfather Jacob Esau lived in the so-called colony of Gnadenfeld, where her father John Esau was born. Both her father (a mechanical engineer) and her uncle (an eye doctor) left the colony to study in Russian schools. Finally, Katherine's father became the mayor of Yekaterinoslav, where he built the city water works, a streetcar line, and large city buildings, including several schools. Katherine's mother was born in the same town, in a family also emigrated from Prussia. Both families were descendants of the German Mennonites (advocates of an anabaptist sect founded by the Dutch reformer Menno Simons - 1496-1561 -, especially spread today in Holland, the U.S.A. and Germany) invited to Russia by Katherine the Great to promote agriculture on the steppes of Ukraine.

Katherine Esau attended a Mennonite primary school in the native town of Yekaterinoslav, learning very well both Russian and German. When she was 11 years old, she entered the Gymnasium which she graduated in 1916; that fall she entered the Golitsin Women's Agricultural College in Moscow, starting with studies in natural sciences, physics, chemistry and geology. The Revolution of October 1917 interrupted her schooling after the first year, at the end of two semesters; wainting for the departure from Russia, Katherine studied English, took piano lessons, attended a gardening school, and collected plants that she was supposed to present at school in the second year.

When the war ended in 1918, at the same time with the retreat of the German army (which had occupied Ukraine in 1917), Katherine Esau's family fled to Berlin. Here, she continued the studies begun at the Agricultural College and she enrolled in various agricultural courses, finishing them with good results and passing an exam in plant breeding given by the then famous geneticist Erwin Baur; subsequently, she worked in a model seed breeding station for wheat.

After graduation in October 1922, the Esau family immigrated to the United States and settled in Reedly, California, the location of a Mennonite community. Her father talked about bying a farm to apply Katherine's agricultural training, but she persuaded him that it would be wiser for her to find a job in a seed company. That is why she left for Fresco, where she was engaged to do housework, and then for Oxnard where she took a job in a seed selection and production ranch, with sugarbeet seed of prime interest. The beginning was rough and Katherine Esau had a great many responsibilities. Among them she had to hire Mexicans to plant selected seed by hand; this required knowledge of Spanish so that she could communicate with the workers.

Shortly after the Oxnard episode which lasted only one year, Katherine Esau was hired by the Spreckels Sugar Company at Spreckes near Salinas. Her main task was to develop a sugarbeet resistant to curly-top disease. The name curly-top referred to the curling of leaves on diseased plants, which were also severely stunted. The disease was then already recognized as a virus transmitted by the beet leafhopper. But she was not content only with that. So she began to consider continuing her education, thinking about doing graduate work at Davis. Dr. W.W. Robbins, the chairman of the Botany Division, offered her assistantship in his division, where she could continue her research on sugarbeet.

In the spring of 1928, nearly 30 years old, Katherine Esau registered as a graduate student with the Botany Division and her field of study was botany. Professor T.H. Goodspeed, the nicotine cytologist at the Botany Department of Berkeley, who was her adviser, prepared a list of courses to strenghten her botanical background; she also took courses in organic and physical chemistry and in French language. Her scholastic standing was evaluated by the Graduate Dean, Charles B. Lipman, as being equivalent to a master degree at the University of California.

In September 1930, when she was 32 years old, Katherine Esau took her qualifying examination at the University of Berkeley, and after that she was advanced to Ph. D. candidacy. In that time there were no anatomists at either Davis or Berkeley; for this reason her research area was plant anatomy, or, more specifically, pathological anatomy, replacing her original project with a study on the effect of the curly-top virus upon the anatomy of the beet. Her final examination was held at Berkeley and her Ph.D. degree was awarded in December 1931. Upon graduation, Dr. Esau was assigned to teach plant anatomy, systematic botany, morphology of crop plants, and microtechnique; at the same time she occupied the position of instructor in botany and junior botanist at the Experimental Station of the Berkeley College of Agriculture.

During a period of 17 years, Dr. Esau ascended all the degrees of the didactic hierarchy until the full professorship in 1949, when she was 51.

During all this time she continued the research regarding the effect of curly-top virus upon the anatomy of beet and the phloemic tissue whose structure degenerated by the virus action.

In the early 1960s, Dr. Esau turned to electron microscopy, which greatly enhanced the understanding of virus-plant host relations. As to the phloem itself, the electron microscopy began to reveal the role of the unique features of the sieve element in the function of the cell as a food conduit. Together, these two aspects of phloem research came to dominate her interest in plant science.

While studying both noninfected and infected sugarbeets, Dr. Esau discovered that what had previously been indentified solely as pericycle (considered to play an entirely supportive function in early development) included the first functional sieve tubes and companion cells, i.e. the protophloem sieve tubes and companion cells; as bundle development progressed, these sieve tubes and companion cells became obliterated and the remaining phloem parenchyma cells became difficult to distinguish from the cells of the pericycle. This discovery was of great significance for the understanding of the relation of the curly-top virus with the tissues of the plant: the localization of the first degenerative effect of the disease upon the tissue indicated that the virus was invading the phloem. Therefore, in producing a systemic infection in the plant, the curly-top was transported in the phloem in the same direction and at similar velocities as the sugar solution, or the assimilate stream.

Further studies by Dr. Esau on the effect of the curly-top virus in the tobacco plant strengthened the concept of the dependence of this virus on the phloem tissue for initiating the infection and spreading it through the plant. The virus was transported from leaf to leaf through the phloem of the leaf traces.

The research on diseased plants was interspersed with developmental studies on healthy plants, including celery, tobacco, carrot, pear, grapevine, a.s.o. The tobacco article is especially notable because in it Dr. Esau tackled head-on several general problems of developmental anatomy, including the characters used to

distinguish between procambium and cambium and between primary and secondary vascular tissues, the origin of internal phloem, leaf-trace differentiation, and the concept of pericycle. Almost all of Dr. Esau's studies involved crop plants.

Dr. Esau was introduced to electron microscopy at Davis and her move to the University of California, Santa Barbara, led to the development of electron microscopy in the Biology Department of electron there. As before, the main topics of Dr. Esau's research at the University of California, Santa Barbara, were the structure and development of the phloem, especially of the sieve element, and the appearance and fate of the virus in plant cells. One of these studies contributed to the characterization and the development of the P-protein, a common component in the sieve elements of dicotyledons (tobacco, squash, mimosa, cotton, bean, a.s.o.); other aspects of development were also examined, including development of sieve-plate pores, of the nucleus and endoplasmic reticulum, and of nuclear crystalloids, especially in the Boraginaceae.

Beet yellow disease virus was the first virus studied by Dr. Esau with the electron microscope. Filamentous virus particles, also called virions, were found in mature sieve elements, where they often filled the sieve-plate pores in the place of P-protein, or combined with it. Virions were also found in the plasmodesmata connecting the sieve elements with parenchymatous elements.

The book *Plant Anatomy* was begun in the late 1940s and was published in 1953. It immediately became a classic and the bible for structural botanists worldwide. In this book, Dr.Esau introduced a new and exciting developmental approach to the structure of plant tissues and organs. In 1961, *Anatomy of Seed Plants* was published as a synthesis of the former text. In her book, Dr. Esau standardized and unified the terminology and usage of the entire vocabulary of plant anatomy.

As a crowning of her remarkable research upon the phloem tissue, in 1969 the famous synthesis *The phloem* is published in the prestigious collection "Handbuch der Pflanzenanatomie".

For her entire remarkable scientific activity in the field of plant anatomy, Dr. Esau was elected member to many societies and academies: the National Academy of Sciences, the American Academy of Arts and Sciences, the American Philosophical Society, the Swedish Royal Academy of Science; she received much recognition and many awards, including the President's National Medal of Science in 1989. At the same time, Dr. Esau served as President of the Botanical Society of America in 1951, and in 1956 was one of the original recipients of the Merit Award of the Society at its Fiftieth Anniversary Meeting (which was held at the University of Connecticut), for her numerous contributions on tissue development of vascular plants and in particular for her outstanding studies on the structure, development and evolution of phloem.

In 1963, two years before she was to retire, Dr. Esau moved to the University of California, Santa Barbara, to continue her collaborative research on the phloem

with Vernon I. Cheadle, the Chancellor of the Santa Barbara campus. She remained actively engaged in research for another 24 years and she considered her period at Santa Barbara to be her most productive and rewarding.

One of the authors of this article became acquainted with Dr. Esau's books 40 years ago. As an assistant, he got into the possession of the crystal-clear *Plant Anatomy*. When he was advanced to the candidacy for the Ph.D. degree (in 1962), he asked Dr. Esau about the literature for his research; very kindly, she sent him excerpts of her main papers and adviced him to insist on the development of mechanical tissue in crop plants under mineral fertilizers influence. Subsequently, he also read the second important book, *Anatomy of Seed Plants*, written with the same precision and clarity. Both of these unequalled books republished and translated in several languages, considered to be the most important works of structural botany in the second part of this century, lie at the basis of our plant anatomy textbooks.

Those who knew Dr. Katherine Esau and wrote about her emphasized that she was a superb professor because she genuinely liked students and showed that. With her friendly, relaxed attitude, keen sense of humor, talent and enthusiasm to spread the good news, gift of an excellent story-teller, she was respected and loved by both students and co-workers.

Dr. Katherine Esau was the personification of excellence and integrity in education and research, providing a special model for women in science.

PUBLICATIONS by KATHERINE ESAU

Journal Articles:

1930 - *Studies of the breeding of sugar beets for resistance to curly-top*. Hilgardia, **4** : 417-441.

1933 - *Pathologic changes in the anatomy of the leaves of the sugar beet Beta vulgaris L., affected by curly-top*. Phytopathology, **23** : 679-712.

1938 - *Ontogeny and structure of the phloem of tobacco*. Hilgardia, **11** : 343-424.

1941 - *Phloem anatomy of tobacco affected with curly-top and mosaic*. Hilgardia, **13** : 437-490.

1943 a - *Origin and development of primary vascular tissues in seed plants*. Bot. Rev., **9** : 125-206.

1943 b - *Vascular differentiation in the pear root*. Hilgardia, **15** : 229-324.

1946 - *Morphology of reproduction in guayule and certain other species of Parthenium*. Hilgardia, **17** : 61-120.

1948 - *Phloem structure in the grapevine and its seasonal changes*. Hilgardia, **18** : 217-296.

1950 - *Development and structure of the phloem tissue*. Bot. Rev., **16** : 67-114.

1954 - *Primary vascular differentiation in plants*. Biol. Rev., **29** : 46-86.

1956 - *An antomist's view of virus diseases*. Am. J. Bot., **43** : 739-748.

1957 - *Phloem degeneration in Gramineae affected by the barley yellow-dwarf virus*. Am.J. Bot., **44** : 245-251.

1967 - Cranshaw J., Esau K., *Tubular and fibrillar components of mature and differentiating sieve elements*. J. Cell. Biol., **34** : 801-815.

1972 - Esau K., Gill R.H., *Nucleus and endoplasmic reticulum in differentiating protophloem of Nicotiana tabacum L.* J. Ultrastruct. Res., **41** : 169-175.

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1982 - Esau K., Thorsch J., *Nuclear crystalloids in sieve elements of species of Echium*. J. Cell. Sci., **54** : 149-160.

1985 - Esau K., Thorsch J., *Šieve plate pores and plasmodesmata, the communication channels of the symplast: ultrastructural aspects and developmental relation*. Special Paper. Am. J. Bot., **72** : 1641-1653.

1991 - Esau K., Gill R.H., *Distribution of vacuoles and some other organelles in dividing cells*. Bot. Gaz., **152** : 397-407.

Books:

1953 - *Plant anatomy*. Wiley, New York, 735 p.

1960 - *Anatomy of seed plants*. Wiley, New York, 376 p.

1961 - *Plants, viruses and insects*. Harvard University Press, Cambridge, Mass., 110 p.

1965 - *Plant anatomy*. 2-nd ed., Wiley, New York, 767 p.

1965 - *Vascular differentiation in plants*. Holt, Reinehart and Winston, New York, 160 p.

1968 - *Viruses in plant hosts: form, distribution and pathologic effects*. University of Winsconsin Press, Madison, 225 p.

1969 - *The phloem*. In Handbuch der Pflanzenanatomie, V. **2**, *Histologie*. Gebrüder Borntraeger, Berlin-Stuttgart, 505 p.

1977 - *Anatomy of seed plants*, 2-nd ed. Wiley, New York, 550 p.

C. TOMA, MIHAELA NIŢĂ – *"Al.I.Cuza" University, Iaşi*

CONSTANTIN TOMA, RODICA RUGINĂ, ***Anatomia plantelor medicinale* (*Atlas*), (Anatomy of medicinal plants. An atlas), Editura Academiei Române, Bucureşti, 1998, 320 p., 155 pl., ISBN 973-27-0601-5.**

The publishing of this book represents a very important editorial event to all those interested in plant anatomy aspects.

This atlas gathers the efforts made by its authors for a long period of time, in the field of plant anatomy, with special reference to those plants possessing therapeutical value.

A very important reason supporting the necessity of such a book refers to the fact that microscopic diagnosis of plant organs is required in various domains of human activity such as: vegetal taxonomy, silviculture, paleontology, stratigraphy, archaeology, criminology, food industry, pharmaceutics, cellulose and paper industry, a.s.o.

The volume is structured in two parts: a general and a special one; the theoretical notions offered in the general part of the atlas are to be further applied, in the chapters belonging to its special part, where the plants are analyzed in the alphabetical order of their families, genera and species, starting with *Pteridophytes* and going on with *Gymnosperms* and *Angiosperms*.

The general part contains a few notions of vegetal histology and anatomy, helping a better understanding of the structures presented in the special part.

The chapter devoted to histology discusses first the meristems, followed by a presentation of the definitive (primary and secondary) tissues they generated: protective, absorbant, assimilatory, aeriferous, storage, mechanical, vascular and secretory; the last ones grouped together only by their common secretion function, are more thoroughly dealt with, as having a special significance in the study of medicinal plants.

In the chapter devoted to the anatomy of the vegetative organs, the main problems presented are: primary and secondary structures of the root and stem, transition from one organ to another; structural variations at different groups of plants (ferns, gymnosperms, angiosperms); structure of the leaf over its ontogenetic and phylogenetic development (mosses, ferns, gymnosperms, angiosperms); types of leaves as function of the lamina's structure: bifacial (heterofacial, isofacial) and monofacial; for angiosperms, some notions of ecological anatomy are also provided, in parallel with evidencing the influence of certain environmental factors (especially light) upon these organs, characterized by a special plasticity.

Some general notions on the structure of the seeds and fruits have been also considered of interests, as well as information on the chemical composition of the vegetal cell.

The special part of the atlas presents the structure of the vegetative organs (root, rhizome, stem, leaf) and, in some cases, of the reproductive organs (flower, seed, fruit) of 128 species (111 native and 17 exotic ones; 97 herbaceous and 31 ligneous ones; 2 ferns, 4 gymnosperms and 122 angiosperms: 115 dicotyledons and 7 monocotyledons) belonging to 108 genera and 50 families. The descriptive data are accompanied by drawings (1154 original and 285 taken over from the literature), grouped in 155 plates. This part includes, too, two keys for the determination of organs, organ fragments and powders.

For each organ, both descriptions and interpretations are provided on: rhizodermis and epidermis; the types of hairs (trichomes and secretory) and stomata; cortex and the central cylinder from axial organs (the type and number of vascular bundles, forming of the phloem and of the primary and secondary xylems, width of the medular rays, presence or absence of the central aerial cavity); the structure of the leaf sheath, petiole and limb (the epidermis with hairs and stomata, the mesophyll, the mechanical and

vascular tissues). Special mention is made of cyto-histological characters with taxonomic diagnosis value, such as: shape and arrangement of the suber cells, type structure of the trichomes and secretory hairs, type of mechanical elements (collenchyma and sclerenchyma), of oxaliferous, tanniniferous and mucilagenous cells, localization of the secreting cavities and canals, form of the assimilating cells, localization of the mechanical sheaths and their relation with the vascular bundles or rings, etc.

When the analysis performed involves several species from the same genus, or several genera from the same family the structures put into evidence are compared, and the peculiarities by which they differ from a taxonomic point of view, especially when only organs, fragments of organs or vegetal powder are offered, are outlined.

The study ends with a rich bibliographical list (40 Romanian and 38 foreign references), an index providing explanations on the scientific and popular denominations of plants, as well as an index explaining the scientific terms met in the book.

Compilation of such a study was necessary indeed, the more so that literature of the field- published both in Romania and abroad - is quite scarce, or even absent. Against such a background, it was the authors' intention to offer, to all those interested, useful information for the determination of organs fragments and powders, starting from cyto-histo-anatomic characteristics.

The histo-anatomical analysis including all the plant organs recommends the book to specialists from various branches of biology, pharmaceutics, agronomy, silviculture, as well as to students, teachers and researchers interested in the fascinating world of medicinal plants.

Considering these observations, the book may be viewed as a real treatise, filling the vacant place in the present Romanian literature of the field and thus creating a necessary bridge between the theoretical approaches and the treatises of systematic anatomy.

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** *Angela Toniuc - Botanical Garden, Iași*

AVIS AUX COLLABORATEURS

La «Revue roumaine de biologie – Série végétale» publie des articles originaux des domaines suivants de la biologie végétale: biologie moléculaire, cytologie, cytogénétique, morphologie, physiologie, génétique, microbiologie, systématique, chorologie, géobotanique, écologie végétale et phytopathologie. La sommaire est complété par les rubriques: 1. La vie scientifique, qui traite des manifestations scientifiques dans le domaine de la biologie (symposiums, conférences, etc); 2. Comptes rendus des plus récentes parutions dans la literature.

Les auteurs sont priés de présenter leurs articles en double exemplaire et espacés à double interligne. Le contenu des articles sera introduit sur des disquettes dans un langage connu, préférablement Word 6.0. La composition et la mise en vedette seront faites selon l'usage de la revue: caractères de 11/13 points pour le texte, de 12/14 points pour le titre de l'article et de 9/11 pour les annexes (tableaux, bibliographie, explication des figures, notes, etc.) et le résumé en anglais de 10 lignes au maximum, qui sera placé au début de l'article. Il est obligatoire que sur les disquettes il soit spécifié le nom des fichiers ainsi que le programme utilisé.

Le matériel graphique sera envoyé sur disquette, scanné, avec les mêmes spécifications. En l'absence d'un scanner, le matériel graphique sera exécuté en encre de Chine sur papier calque.

Les tableaux et les illustrations seront numérotés en chiffres arabes dans l'ordre de l'apparition. Les titres des revues seront abrégés conformément aux usages internationaux.

Les textes ne doivent pas dépasser 10 pages (y compris les tableaux, la bibliographie et l'éxplication des figures).

La responsabilité pour le contenu des articles revient exclusivement aux auteurs.

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