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SOMMAIRE

- ANDREI, Ein Beitrag zur Kenntnis des Leitgewebes bei den
Moosen 3
- SIMA BHATTACHARYA, Chromosome study of some members
of the tribe *Ajugoideae* (*Labiatae*) with reference to their
taxonomic elucidation 7
- I. ȚUCRA and MARCELA NEACȘU, Criteria of elaborating
permanent meadow types in Romania 13
- M. KEUL, GEORGETA LAZĂR-KEUL und AL. POLIZU,
Feulgen-cytophotometrische und kernvolumetrische Un-
tersuchungen über die Wirkung der Thirahexalin-Kompo-
nenten auf Weizenwürzelmeristeme 17
- GH. POPOVICI and O. BOLDOR, Lab. pilot culture of cyano-
bacteria *Spirulina platensis* under continuous flow 23
- AURELIA BREZEANU, Ultrastructure of *Helianthus annuus* L.
callus during the growth, differentiation and aging processes 29
- G. G. PLOAIE and TR. I. ȘTEFUREAC, A transmission elec-
tron microscope study of the ultramorphology of the spores
of some *Bryophyta*. 37
- ECATERINA T. TÓTH, G. I. GHIORGHITĂ and ELVIRA V.
GILLE, Some biochemical peculiarities in plants of *Digi-
talis lanata* Ehrh. modified after successive treatments with
gamma rays and ethylmethanesulfonate 41
- V. STOIAN, P. RAICU, D. DUMA, L. GAVRILĂ, D. MU-
REȘAN, S. SILJAK-YAKOVLEV, R. GORENFLOT,
D. CARTIER et S. MEDJEDOVIĆ, L'organisation supra-
chromosomique chez quelques espèces animales et
végétales 49
- ELENA BADEA and PETRE RAICU, Experimental androgene-
sis in *Datura innoxia* plants with different levels of ploidy
(2n, 4n, 6n) 59

SCIENTISTS AND PEACE

REV. ROUM. BIOL.-BIOL. VÉGÉT., TOME 28, N° 1, P. 1-66, BUCAREST, 1983



EIN BEITRAG ZUR KENNTNIS
DES LEITGEWEBES BEI DEN MOOSEN

VON
M. ANDREI

Using the maceration technique, by means of 20% HNO_3 , the author reveals in the leptoidic and parenchymatous cells of *Plagiomnium undulatum* a system of microcapillaries, not previously mentioned in the literature. The microcapillaries traverse the cell walls through their characteristic thickenings, establishing a transverse circulation of plastic matter.

Das Fachschrifttum enthält zahlreiche Hinweise auf den Bau der Gewebe bei den Moosen [1], [7].

In neuerer Zeit wurde dem Leitgewebe der Moose besondere Aufmerksamkeit geschenkt u.zw. in den tiefeschürfenden Arbeiten von Ch. Hebant [2—4]. Eine systematische und substanzielle Studie über das Leitgewebe des Gametophyten der Moose stammt von I. Kawai [5], [6].

Die Ergebnisse der anatomischen Untersuchung wurden zu physiologischen Studien in Beziehung gebracht. Es konnte so der Leitungsvorgang im Leitgewebe der Moose nachgewiesen werden, sowie die wichtige Rolle der äusseren Leitung vermittelt Kapillarität.

Umfangreiche anatomisch-physiologische Untersuchungen über Taxa der *Polytrichaceae* wurden durchgeführt, insbesondere über die Gattungen *Polytrichum* und *Dawsonia*, die sich durch ihren Riesenwuchs auszeichnen.

Die bearbeiteten Arten (*Polytrichum commune*, *P. alpinum*, *P. juniperinum*, *P. formosum*, *Atrichum undulatum*, *A. lingulatum* u.a.) wurden sowohl mit dem Lichtmikroskop, als auch mit dem elektronischen Mikroskop studiert. Es wurden auf diese Weise Hydroide und Leptoide nachgewiesen, welche Wasser und plastische Stoffe leitende Strukturen darstellen, die dem Hadrom bzw. Phloëm ähnlich sind.

Je nach Vorhandensein oder Fehlen der Hydroide und Leptoide wurden 5 verschiedene Strukturtypen unterschieden:

1. Moose, bei denen beide Generationen Hydroide und Leptoide enthalten (*Polytrichum* ssp., *Atrichum* ssp. u.a.).
2. Moose, bei denen der Gametophyt nur Hydroide, und der Kapselstiel Hydroide und Leptoide enthält (*Funaria* ssp., *Splachnum* ssp. u.a.).
3. Moose, bei denen der beblätterte Stengel und auch die Seta nur Hydroide enthalten (*Dicranum* ssp., *Brachythecium* ssp. u.a.).
4. Moose, bei denen der Gametophyt kein Leitgewebe aufweist, die Seta jedoch ein solches besitzt (*Neckera* ssp., *Buxbaumia* ssp. u.a.).
5. Moose ohne Leitgewebe im Gametophyten und der Seta (*Orthotrichum* ssp. u.a.).

MATERIAL UND ARBEITSWEISE

Von uns wurde Material der Art *Plagiomnium undulatum* (Hedw.) Kop. untersucht, welches aus dem Bucegi-Gebirge stammt (leg. R. Wallfisch) und von Gh. Dihoru bestimmt worden ist.

Aus unter- und oberirdischen Stengeln wurden mittels der üblichen Mazeration in 20%-iger HNO_3 Präparate hergestellt.

Die Präparate wurden an einem Zeiss-Mikroskop untersucht (Ausrüstung: Ok. 10x, Obj. 90x).

ERGEBNISSE, DISKUSSION

Bei der Untersuchung einer grossen Anzahl von Präparaten wurden in den Zellen des Leptoid-Parenchyms Mikrostrukturen festgestellt, die in dem verfügbaren Schrifttum keine Erwähnung finden. Es handelt sich dabei um Mikrokapillaren, welche gewöhnlich Parenchym- und Leptoidzellen durchqueren und derart zwischen diesen eine enge Verbindung herstellen.

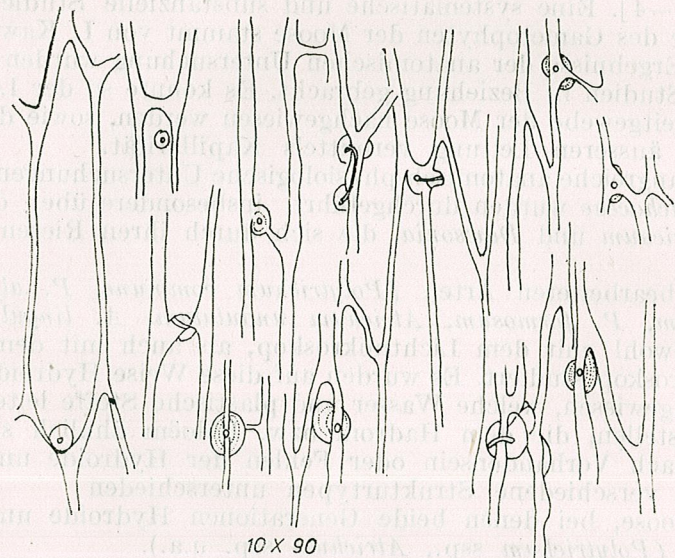


Abb. 1. — Parenchym- und Leptoidzellen bzw. Zellteile mit charakteristischen Verdickungen.

Die Mikrokapillaren verlaufen von einer Zelle in die andere und durch die gemeinsame Zellwand (Abb. 1, 2). An der Durchdringungsstelle weisen die Zellwände mehr oder minder mächtige, asymmetrische Verdickungen auf. Innerhalb der Verdickung erscheinen die Kapillaren als gerade oder gekrümmte Röhren, deren Enden bei Verwendung eines Immersionsobjektivs als zwei Poren sichtbar werden (Abb. 3).

Es wurde ausserdem noch beobachtet, dass die Wandverdickungen geschichtet sind, woraus hervorgeht, dass sie durch aufeinander folgende Ablagerungen von Zellulose-Material entstanden sind. (Phlorogluzin + HCl gab keine Lignin-Reaktion).

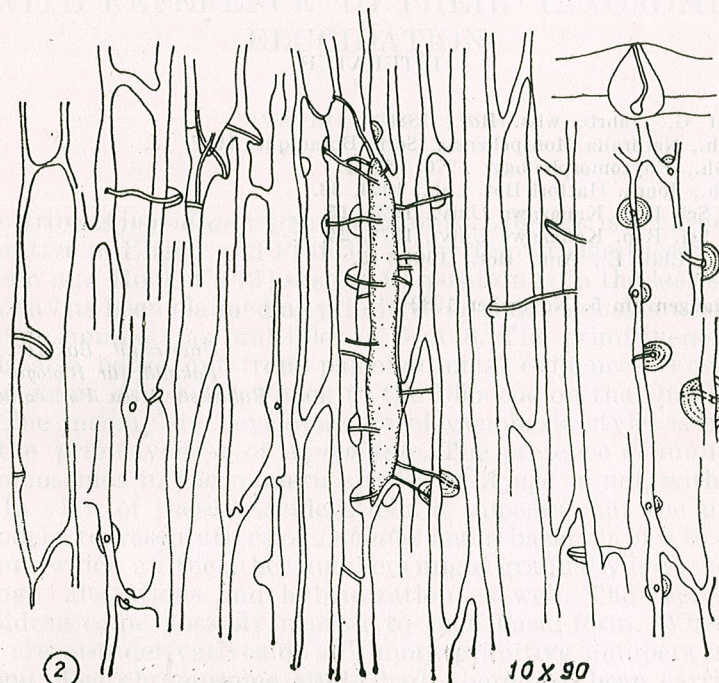
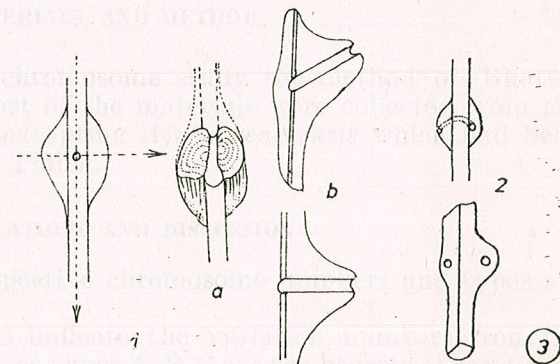


Abb. 2. — Parenchym- und Leptoidzellen bzw. Zellteile mit charakteristischen Verdickungen und Mikrokapillaren.

Abb. 3 — Modellhafte Darstellung der Zellwandverdickungen: 1 a: symmetrische Verdickung, im Querschnitt; 1 b: dieselbe, im Längsschnitt; 2: asymmetrische Verdickung mit gekrümmtem Röhren.



Die Zahl und Gestalt der Verdickungen längs der Zellwände ist schwankend; in gewissen Fällen dringen sie in das Zellumen vor, indem sie 75% desselben ausfüllen (Abb. 1, 2).

Wir nehmen an, dass die beschriebenen Mikrokapillaren ganz besonders in den Transport der plastischen Stoffe in Querrichtung eingreifen. Die grosse Zahl von Mikrokapillaren in bestimmten Abschnitten der Stengel entspricht einer hohen Transportaktivität in diesen. Es bleibt weiteren Untersuchungen vorbehalten, die Rolle dieser Gebilde genau zu bestimmen.

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CHROMOSOME STUDY OF SOME MEMBERS OF THE TRIBE *AJUGOIDEAE* (*LABIATAE*) WITH REFERENCE TO THEIR TAXONOMIC ELUCIDATION

BY

SIMA BHATTACHARYA

The tribe *Ajugoideae* under the family *Labiatae* is regarded as the most primitive in Engler and Prantl's [1] system of classification whereas in Bentham and Hooker's [2] system its position is in the last series. The tribe, which has been claimed as primitive on many considerations, shows chromosome numbers as multiples of 6 or 8. The primitiveness of *Ajugoideae* is also borne out from paleobotanical evidences. Fossil record [3] of the genus *Ajuga* dates back to the Pliocene on the Dutch-Prussian Border. The incomplete developments of gynobasic style is also in favour of the primitiveness of *Ajugoideae*. The presence of multiples of 6 or 8 chromosomes in the modern species of *Ajuga* is not without significance. In view of these considerations it appears that the progenitors of the modern representatives of *Labiatae* had a basic set of 6 to 8 chromosomes from which all the other numbers might gradually have been derived through alterations and hybridization as well. The representatives of *Ajugoideae* come possibly nearest to such basic form. Whether these numbers are also derivatives of still more primitive numbers is yet to be ascertained. The chromosome study had, therefore, been carried out in a few taxa belonging to this tribe with a view to getting an idea about the members at the chromosome level.

MATERIALS AND METHOD

For a somatic and meiotic chromosome study the method of Bhattacharya [5] was followed. Most of the materials were collected from the Eastern Himalayan regions excepting *Ajuga genevensis* which had been procured in Soviet Georgia, Tbilisi.

OBSERVATIONS AND DISCUSSION

The materials with their respective chromosome numbers and types are tabulated in Table 1.

The numbers in parentheses indicate the variation numbers from the modal number. The chromosome types A, B, C are the bearers of secondary constrictions or satellite. The type D represents the submedian and type E represents the metacentric chromosomes (Fig. 6). Accessory or B-

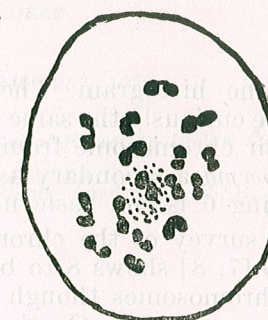
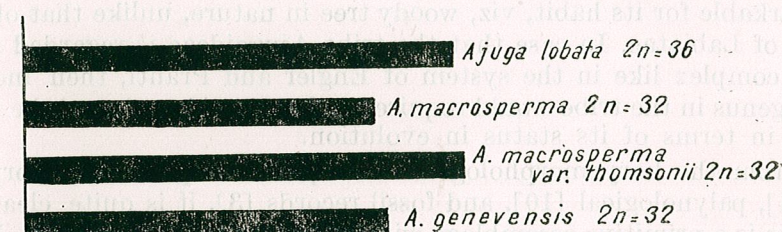
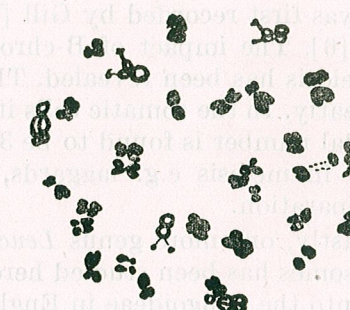
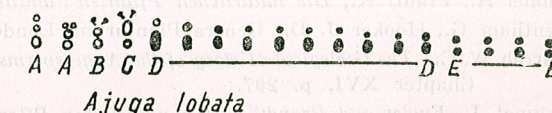
chromosomes are found in *A. genevensis*. Pollen sterility in this taxon counted was 16%.

Table-1

Name of the species	Chromosome number and type	Range of chromosome length (μ).
1. <i>Teucrium stoloniferum</i> Roxb.	$n = 16$ (13, 15)	
2. <i>Ajuga genevensis</i> L.	$n=16$, (14, 19); $2n=32$ (28, 36) Chromosome formula — ($D_{12} + E_{20}$) + B-chromosomes (0–5)	1–2.9 μ and 0.3–0.7 μ (B-chrom.)
3. <i>A. lobata</i> D. Don	$2n = 36$ (32, 34) ($A_4 + B_2 + C_2 + D_{20} + E_3$)	0.8–2.1 μ
4. <i>A. macrosperma</i> Wall var. <i>thomsonii</i> Hook. f.	$n = 16$, $2n = 32$ ($A_2 + B_4 + D_{24} + E_2$)	1.05–2.1 μ
5. <i>A. macrosperma</i> Wall.	$n = 16$, $2n = 32$, ($A_2 + B_4 + D_{20}E_2$)	0.7–2.1 μ
6. <i>Leucosceptrum canum</i> Sm.	$n = 22$ (16, 28)	

Previous literatures [7, 8] have revealed several chromosome numbers in the genus *Teucrium*. Most of them are multiples of 13 or 16. Intermediate numbers, too, such as 30, 81 etc. are also on record. The present investigation in *T. stoloniferum* also shows $n = 16$ chromosomes in this species. Polyploidy has also been recorded in several species of this genus. Even though, apparently the genus appears to be polybasic in nature because of the presence of different chromosome numbers, a critical study of the counts shows that it is not so. This is because of the fact that even in the same species such as *T. montanum* 13, 15, 16 and 30 chromosomes have been reported by several workers [7, 8]. Such intraspecific variations indicate that all these numbers are distinctly related. It appears, on the basis of evidences so far available, that the genus might have had a low base number, between 6 and 8, from which other numbers might have been derived either through aneuploidy or hybridization or through both.

Similar to *Teucrium*, three species and one variety of *Ajuga* have been studied. The two varieties of *Ajuga macrosperma* have shown $n = 16$ and $2n = 32$ chromosomes whereas in *A. lobata* ($2n = 36$) and *A. genevensis* ($2n = 32$) they have been found in the body cells. The karyotype of the genus *Ajuga* appears as very symmetrical, chromosomes are mostly median to submedian constricted and very short in length. In gross morphology there is no difference, i.e. identical but minute differences (vide figures) characterize its species indicating thereby the importance of structural alterations of chromosomes in evolution. Figure 4

Fig. 1.—Diakinesis ($n=16$) in *Teucrium stoloniferum*. Ca. 1800 $\times$.Fig. 2.—Karyotype ($2n = 36$) in *Ajuga lobata*. The secondary constriction bearing chromosomes are outlined only. Ca. 2000 $\times$.Fig. 3.—Karyotype ($2n = 32$) in *A. macrosperma*. Ca. 2000 $\times$.Fig. 4.—Histogram showing total chromatin length in haploid complements of *A. lobata*, *A. macrosperma*, *A. macrosperma* var. *thomsonii* and *A. genevensis* respectively. Ca. 2850 $\times$.Fig. 5.—Diakinesis showing bivalents in *Leucosceptrum canum*. Ca. 1800 $\times$.Fig. 6.—Idiogram of *Ajuga lobata* showing different types of chromosomes.

shows the histogram. The modal chromosome number and the histogram are curiously the same in *A. macrosperma* and *A. genevensis* ($2n = 32$) but their chromosome formulae are quite different (vide Table 1). In *A. macrosperma* a secondary association of bivalents has also been recorded suggesting 6 is the basic number for the genus.

A survey of the chromosome number so far reported in the genus (*Ajuga*) [7, 8] shows 8 to be the deep-seated number. So multiples of 8 or 16 chromosomes though occasionally $2n = 28, 30, 36$ chromosomes have been reported. On the basis of these data it appears that 8 is the deep-seated number which might have been responsible for further diversification through aneuploidy and polyploidy. Whether this number 8 is also secondarily derived from a still primitive number 6 as indicated from secondary association requires confirmation from different aspects of study.

The occurrence of B-chromosomes in few taxa of the family Labiatae was first recorded by Gill [9] and in *A. genevensis* by the present author [6]. The impact of B-chromosomes on the somatic cells as well as in meiosis has been revealed. The chromosome number and behaviour vary greatly. In the somatic cells it varies between $2n = 28$ and 36 though the modal number is found to be 32. Pollen sterility (16%) indicates irregularity in meiosis e.g. laggards, unequal separation of chromosomes, early separation.

Lastly, one more genus *Leucosceptrum canum* (Fig. 5) with $n = 22$ chromosomes has been studied here for the first time. This genus, though it falls into the Ajugoideae in Engler and Prantl's system of classification is remarkable for its habit, viz. woody tree in nature, unlike that of other genera of Labiatae. In case that the tribe Ajugoideae is regarded as primitive complex like in the system of Engler and Prantl, then inclusion of this genus in the tribe would require further consideration of the woody nature in terms of its status in evolution.

From the karyomorphological standpoint coupled with morphological [4], palynological [10], and fossil records [3], it is quite clear that the tribe is a primitive assemblage within the family, as treated by Engler and Prantl.

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CRITERIA OF ELABORATING PERMANENT MEADOW TYPES IN ROMANIA

BY

I. ȚUCRA* and MARCELA NEACȘU**

This work contains the categories and the criteria of typological meadow classification in Romania, which can be used in agricultural practice.

The typological meadow units are characterized by site conditions, vegetation and technology elements. The typological units of the higher level (the formation of meadow types), the intermediate level (the meadow type) and the lower level (the meadow subtype and variant) are differentiated.

The development of animal breeding in Romania requires experts to obtain ever greater quantities of fodder, first of all by the better evaluation and increased productivity of the natural meadows.

In order to attain this aim it is necessary to have a detailed knowledge of the characters of meadow land, their present production capacity, on one hand, and the quality and quantity of meadow vegetation, on the other, in order to elaborate the correct improvement technologies. So the meadow research was ecosystemic, meadows being classified by typological units, which are characterized also by the elements of vegetation, site conditions and technology, this kind of classification being useful in agricultural practice.

MATERIAL AND METHODS

It is well known that the plant association is the basic unit in phytosociological research (named thus, for the first time, at the Congress of Botany—Brussels, 1910). But the plant association has not enough elements to be used for establishing meadow productivity and improvement technologies.

In Europe, there is an increasing preoccupation to find new solutions for the study of meadow vegetation with application to practice [4], [5], [6].

The "type" concept was introduced in science for the first time by Lorenz, 1958, while Stebler and Schröter used the concept of "meadow type" in 1893, for the characterization of hay-fields in Switzerland.

In Romania, the "meadow type" concept was introduced by Safta I., 1939, and it is more and more used and characterized [1], [2]. In his works, Anghel established the basic principles for the elaboration of the meadow typology, thinking that in the study of vegetation the floristic

composition represents an important element of research, necessary but insufficient for establishing the types, therefore what is needed is information concerning the quality and quantity of vegetation, site conditions and improvement technology.

Starting from these basic theoretical principles the Romanian typological meadow classification is based on the ecosystem conception regarding the interdependence relations established between the meadow vegetation and its site conditions.

The object of research and classification is the meadow phytocenosis in the frame of the meadow elementary site.

The meadow elementary sites and phytocenosis, with features varying almost within the same limits, are grouped in variants and subtypes. At this level type, a field technological characterization is made in order to apply the melioration technologies.

The meadow type is pointed out and characterized on a minimum surface of 1 ha, which is at the same time the reference unit regarding yield and nutritive capacity (number of cows/ha).

The classification of typological meadow units is made after "The soil classification system for lower level categories", 1976.

The number of animals on a meadow can be established by evaluating the vegetation.

It is possible to estimate the basic natural type of meadow and the derived type, knowing the site conditions of vegetation.

RESULTS

Our researches were aimed at establishing the typological meadow categories and their criteria of differentiation.

The typological classification uses the categories of higher, intermediate and lower level.

The higher level is the meadow type formation; this unit falls within the Roumanian vegetation levels and expresses the bioclimatic conditions in this country. It is a very important unit in establishing the fundamental natural types, which can be mapped on charts, scales 1:1.000.000 and 1:500.000.

The "formation" concept in typology must not be confused for the concept of "formation" or "biom" as used in the American ecological literature, indicating the dominant forms of vegetation growth (herbaceous, woody, etc.), nor for the "formation" concept indicating the physiological structure in the Scandinavian literature.

The intermediate level is the meadow type that corresponds to the orotopographic units according to the French ecological school of Montpellier [6] and to the ecological conditions of soil and forest types and forest ecological stations in Romania. These units can be mapped on charts, scales 1:200.000 and 1:50.000.

The lower levels are the meadow subtype and the meadow variant.

The meadow subtype corresponds to the edaphic units, to the phytocological groups of the French school and to the elementary mea-

dow sites in Romania [3], [8] and in the German Democratic Republic [7]. These categories can be mapped on charts, scales 1:25.000—1:5.000. The melioration technologies are established on the subtype level.

The meadow typological variant refers to the local vegetation and site characters. It is mapped on charts, scales 1:50.000—1:2.000.

Criteria of differentiating meadow type formations:

- herbaceous vegetation, given by the dominant edifying species;
- bioclimatic level;
- soil class or subclass;
- specific ecological determinants;

Criteria of differentiating meadow types:

- dominant edifying species;
- relief;
- soil type or soil ecological regime;
- productivity;
- minimum melioration requirement

Criteria of differentiating meadow subtypes:

- co-dominant species;
- relief elements and forms, the slope;
- nature of the substrate;
- soil features or ecological regime;
- productivity;
- specific technologies.

Criteria of differentiating meadow typological variants:

- ecologically indicating species;
- local ecological factors and determinants;
- productivity;
- technological variants.

The conspectus of meadow typological units in Romania comprises 16 formations, 35 types, 54 subtypes, and 58 variants, while the soil classification comprises 10 classes, 22 subclasses, 39 types, 241 subtypes and over 5.000 variants.

The list is considered closed for the meadow formations and meadow types, but another 9 subtype and variant units can be added to each actual typological unit, because the system is decimal.

Here is an example of meadow typological units in Romania:

7. The *Festuca rubra* formation from spruce fir on oligotrophic cambisols;

7.1. The *Festuca rubra* type on very mildly sloping land, with acid brown soils, oligotrophic-mesotrophic, moist, average productivity;

7.1.1. Subtype *Agrostis tenuis*, on very gently sloping land, with acid brown soils, mesotrophic, moist, average productivity that requires fertilization;

7.1.1.1. Variant with *Festuca pratensis*, *Lolium perenne* obtained by fertilization.

7.1.2. Subtype *Agrostis tenuis* on sharply sloping lands with acid brown soils, mesotrophic, dry-moist, low productivity.

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FEULGEN-CYTOPHOTOMETRISCHE UND KERNVOLUMETRISCHE UNTERSUCHUNGEN ÜBER DIE WIRKUNG DER THIRAHEXALIN-KOMPONENTEN AUF WEIZENWURZELMERISTEME

VON

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The paper describes the specific effects of thirahexalin compounds on the mean DNA nuclear content and on the nuclear volume in wheat root meristem. The treatment with thirahexa (thiuram + hexachlorobenzene) and hexachlorobenzene induced no significant effects on the DNA content, while the nuclear volume is slightly increased. Under the influence of lindane, both DNA content and nuclear volumes increased many folds as a consequence of its known polyploidization action. The results demonstrate that lindane is the compound responsible for the phytotoxic action of thirahexalin on wheat plants.

In einer früheren Untersuchung wurde gezeigt, daß die phytotoxischen Wirkungen des kombinierten Samen-Beizmittels Thirahexalin (50% Thiuram + 10% Hexachlorbenzol + 20% Lindan) auf Weizenkeimlinge die charakteristischen Merkmale der Lindanwirkung auf Pflanzen aufweisen [19]. Die durch Thirahexalin induzierten Symptome äußern sich ähnlich der Lindanwirkung [2], [10], [11], [15], [16], [18] durch die Blockierung des Wurzelwachstums, die Entwicklung stark verkürzter und verdickter Wurzeln mit subapikalen Anschwellungen und verzweigten Wurzelhaaren, die Entstehung von Riesenzellen und überdimensionierten Zellkernen mit hohem DNS-Gehalt, durch Störungen des Zellzyklus und eine hohe Polyploidisierungsrate der Meristemzellen. Vergleichende Untersuchungen über die Wirkung von reinem Lindan und Thirahexalin gleichen Lindangehaltes ergaben identische Ergebnisse hinsichtlich der Hemmung des Wurzelwachstums [18] und gleichartige cytologische und strukturelle Veränderungen im Bereich der verschiedenen Wurzelzonen [16]. Hingegen zeigten die Versuche mit Hexachlorbenzol [17] und Thirahexa (Thiuram + Hexachlorbenzol) [5] keine bzw. nur geringe Hemmungen des Wurzelwachstums. Diese Ergebnisse führten zur Schlußfolgerung, daß die Symptome der Thirahexalin-Wirkung durch die Lindan-Komponente verursacht werden.

Zur weiteren Aufklärung des Wirkungsmechanismus von Thirahexalin auf Weizenpflanzen soll in vorliegender Arbeit die spezifische Wirkung der Thirahexalin-Komponenten auf den DNS-Gehalt, das Kernvolumen und den Zellzyklus im Weizenwurzelmeristem untersucht werden.

MATERIAL UND ARBEITSMETHODE

Winterweizenkaryopsen (*Triticum aestivum* L. ssp. *vulgare* (Vill., Host.) Mac Key, cv. „Bezostaia“) wurden in Linhardt-Schalen auf Filterpapierunterlagen mit destilliertem Wasser bei $21 \pm 1^\circ\text{C}$ im Brutschrank und im Dunkeln zur Keimung angesetzt.

Bei der Berechnung der Dosen wurde der prozentuale Anteil der einzelnen Pestizide in der Thirahexalin-Zusammensetzung berücksichtigt. Die Behandlung mit Thirahexa (Thiuram + Hexachlorbenzol), Hexachlorbenzol und Lindan erfolgte nach dem bisher angewandten Verfahren [11], [16], indem die für 20 Karyopsen/Linhardt-Schale umgerechneten Dosen entsprechend einer Aufwandsmenge von 125, 250 und 500 g Thirahexalin/100 kg Karyopsen in je 3 ml Aceton gelöst und auf die Filterpapierunterlagen aufgetragen wurden. Die Filterpapierunterlagen der Kontrolle wurden mit 3 ml Aceton durchtränkt.

Die Wirkung der Pestizide wurde nach andauernder Behandlung in drei aufeinanderfolgenden Wachstumsphasen der Keimpflanzen untersucht: I = am 2. Tag nach dem Ansetzen; II = nach dem Erscheinen der Koeoptile am 4.–5. Tag nach dem Ansetzen; III = nach der Ausbildung der zwei ersten Blätter am 8. Tag nach dem Ansetzen.

Der relative DNS-Gehalt der Interphasenkerne wurde Feulgen-cytophotometrisch nach der Zwei-Wellenlängen-Methode (vgl. [7], [9], [14]) in Arbeitseinheiten (AE) mit dem Ortholux-Mikroskop mit Photometeraufsatz MPE und Geradsicht-Spiegelmonochromator (Leitz) gemessen. Die beiden Wellenlängen (533 und 495 nm) wurden aufgrund des mikrospektrophotometrisch aufgenommenen Absorptionsspektrums von 10 Feulgen-gefärbten Interphasekernen ermittelt. Fixiert wurde 2 Stunden in einem Äthylalkohol-Eisessig-Gemisch (3:1), anschließend in 5 N HCl bei Zimmertemperatur 1 Stunde lang hydrolysiert und im Schiff-Reagens 1 Stunde gefärbt. Die Auswertung erfolgte in Quetschpräparaten an mindestens 100 Interphasenkernen pro Variante. Die Interpretation der Ergebnisse erfolgte anhand der in der Telophase gemessenen 2C-Referenzwerten (vgl. [9]). Die Referenzwerte der höheren Ploidiestufen wurden rechnerisch ermittelt.

Das Volumen der Zellkerne wurde nach der Ausmessung der beiden Durchmesser der Kernfläche mittels eines Mikrometer-Schraubenokulars (Zeiss, Jena) berechnet [14].

ERGEBNISSE UND DISKUSSION

Wie aus den in Abb. 1 und 2 dargestellten Ergebnissen hervorgeht, hat die andauernde Behandlung der Weizenkeimlinge mit Thirahexa und Hexachlorbenzol im Bereich der untersuchten Dosen keinen Einfluß auf den mittleren DNS-Gehalt der Zellkerne im Wurzelmeristem. Die (graphisch nicht dargestellten) DNS-Häufigkeitsverteilungen für die cytophotometrierten Zellpopulationen zeigen keine wesentlichen Veränderungen im Verhältnis der einzelnen Zellzyklusphasen gegenüber der Kontrolle. Es wurden keine DNS-Werte höheren Ploidiegrades gemessen. Diese Feststellungen belegen, daß Thirahexa und Hexachlorbenzol im

Gegensatz zu Thirahexalin [19] nur einen geringen Einfluß auf den DNS-Stoffwechsel und die Mitoseaktivität im Weizenwurzelmeristem ausüben.

Die Ergebnisse der kernvolumetrischen Messungen (Abb. 1 und 2) lassen im Vergleich zur 5 bis 10fachen Vergrößerung des Zellkernvolumens

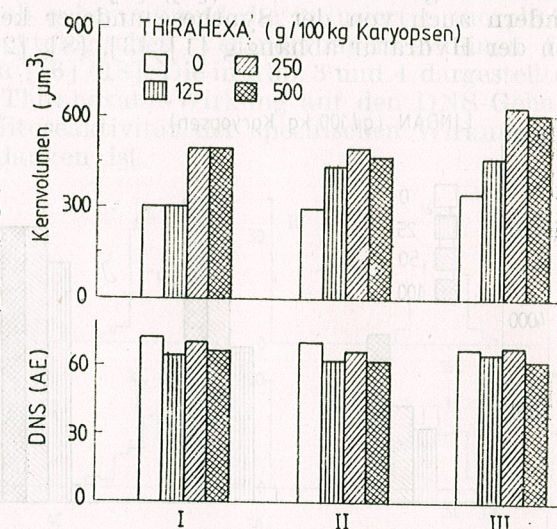


Abb. 1. — Die Wirkung von Thirahexa (Thiuram + Hexachlorbenzol) auf den mittleren DNS-Gehalt in Arbeitseinheiten (AE) und das mittlere Volumen der Zellkerne im Weizenwurzelmeristem. I, II und III = Wachstumsphasen der Keimpflanzen (vgl. Text).

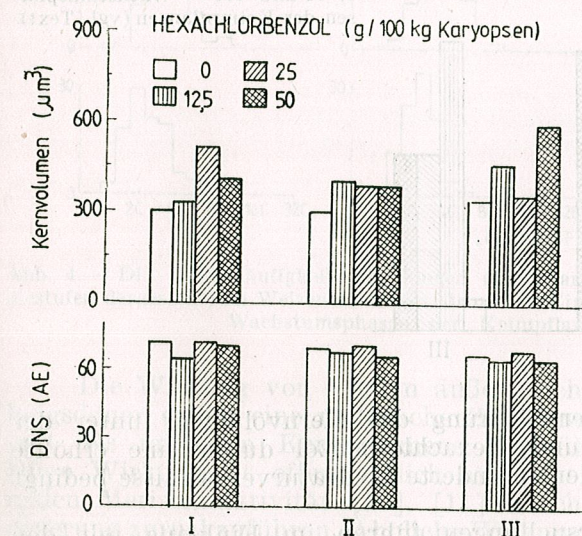


Abb. 2. — Die Wirkung von Hexachlorbenzol auf den mittleren DNS-Gehalt in Arbeitseinheiten (AE) und das mittlere Volumen der Zellkerne im Weizenwurzelmeristem. I, II und III = Wachstumsphasen der Keimpflanzen (vgl. Text).

durch Thirahexalin [19] nur eine relativ geringe Beeinflussung der Kerngröße durch Thirahexa und Hexachlorbenzol erkennen. Das mittlere Zellkernvolumen wird durch Thirahexa und Hexachlorbenzol je nach Dosis und Behandlungsdauer bis höchstens auf den doppelten Wert gegenüber der Kontrolle erhöht. Während die Vergrößerung des Kernvolumens

durch Thirahexalin eindeutig auf einer endopolyploidisierenden Wirkung beruht, ist die leichte Zunahme der Kerngröße durch Thirahexa und Hexachlorbenzol nicht mit höheren DNS-Werten korreliert.

In proliferierenden Pflanzengeweben ist die Kerngröße erheblichen Schwankungen unterworfen [1], [9] und nicht allein vom DNS-Gehalt, sondern auch von der Synthese anderer kerneigener Substanzen, sowie von der Hydratur abhängig [1], [3], [8], [20]. Aufgrund dieser Befunde

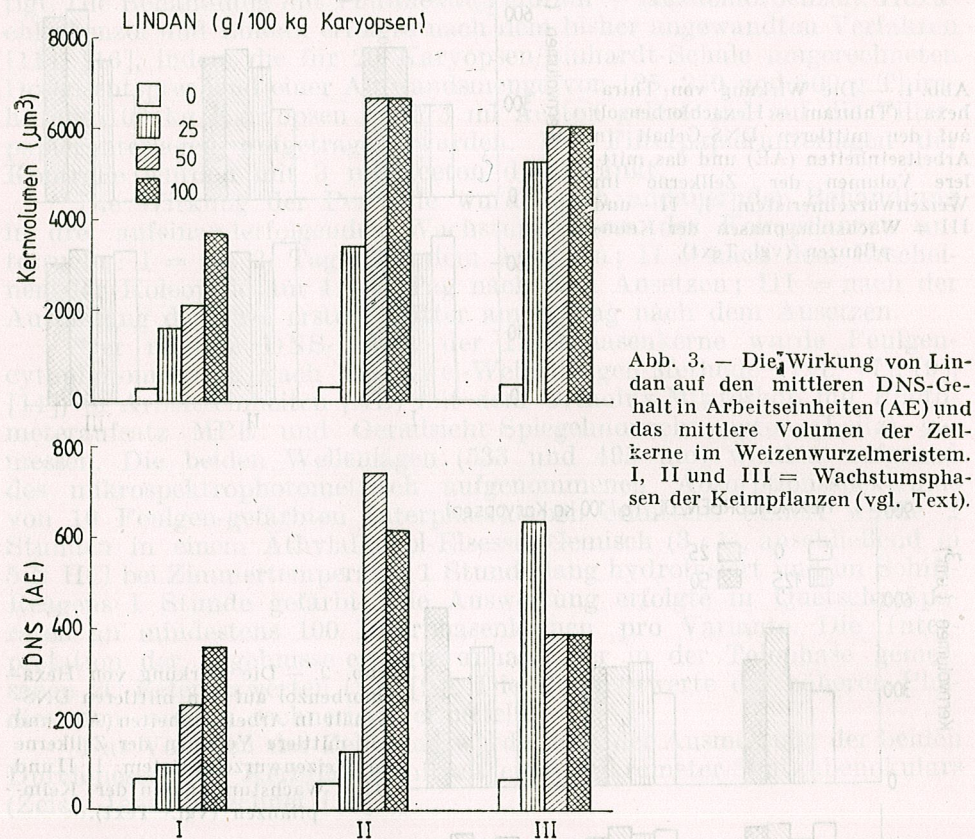


Abb. 3. — Die Wirkung von Lindan auf den mittleren DNS-Gehalt in Arbeitseinheiten (AE) und das mittlere Volumen der Zellkerne im Weizenwurzelmeristem. I, II und III = Wachstumsphasen der Keimpflanzen (vgl. Text).

ist anzunehmen, daß die Vergrößerung des Kernvolumens unter der Einwirkung von Thirahexa und Hexachlorbenzol durch eine erhöhte Nukleoproteinsynthese und/oder veränderte Hydraturverhältnisse bedingt wird.

Die vorliegenden Untersuchungen führen im Einklang mit den Befunden über den geringen Einfluß von Thirahexa [5] und Hexachlorbenzol [17] auf das Wurzelwachstum zur Schlußfolgerung, daß die phytotoxischen Symptome der Thirahexalin-Wirkung nicht durch die Komponenten Thiuram und Hexachlorbenzol verursacht werden. Nach den Angaben anderer Autoren ist die phytotoxische Wirkung dieser Fungizide auf höhere Pflanzen im allgemeinen gering [4], [12], [13].

Die bisher durchgeführten Untersuchungen führen übereinstimmend zur Erkenntnis, daß die phytotoxischen Nebenwirkungen von Thirahexalin in entscheidendem Maße durch die Lindan-Komponente verursacht werden. Nach Behandlung von Weizenkeimlingen mit reinem Lindan und Thirahexalin gleichen Lindan-Gehaltes wird das Wurzelwachstum in gleichem Ausmaß gehemmt und die induzierten morphologischen, strukturellen und cytologischen Veränderungen stimmen für beide Präparate völlig überein [16] [18]. Die in Abb. 3 und 4 dargestellten Ergebnisse belegen, daß die Thirahexalin-Wirkung auf den DNS-Gehalt, das Kernvolumen und die Mitoseaktivität der spezifischen Wirkung der Lindan-Komponente zu verdanken ist.

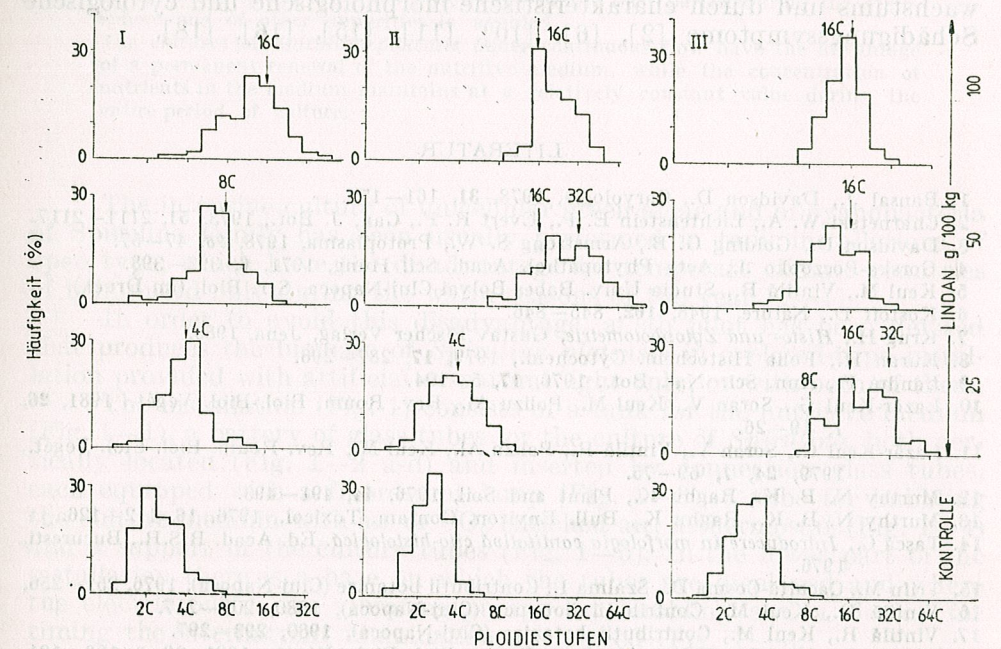


Abb. 4. — Die DNS-Häufigkeitsverteilungen (in logarithmischer Klassenbreite nach Ploidiestufen dargestellt) im Weizenwurzelmeristem nach Einwirkung von Lindan. I, II und III = Wachstumsphasen der Keimpflanzen (vgl. Text).

Die Wirkung von Lindan äußert sich je nach Dosis und Behandlungsdauer durch eine mehrfache Zunahme des mittleren DNS-Gehaltes und des mittleren Kernvolumens gegenüber der Kontrolle (Abb. 3). Diese Wirkung ist offensichtlich auf eine drastische Störung der normalen Meristemaktivität [10], [11] durch zunehmende Endopolyploidisierung zurückzuführen. Ähnliche Wirkungen werden durch Thirahexalin induziert [19]. Bei kürzeren Behandlungszeiten sind die Effekte proportional mit der Dosis. Bei höheren Dosen und längeren Behandlungsdauern nimmt sowohl das Kernvolumen als auch der DNS-Gehalt ab und die Zellkerne weisen eine fortschreitende Desorganisation auf.

Der Mechanismus der endopolyploidisierenden Wirkung von Lindan beruht auf einer Hemmung des Spindelapparates [6] bei ungehindert

weiterlaufender DNS-Reduplikation. Die DNS-Häufigkeitsverteilungen in Abb. 4 veranschaulichen die Zunahme des Ploidiegrades der Zellkerne mit der Dosis und der Behandlungsdauer, wobei Gipfel bis zu 32 C erscheinen. Die erneute Reduzierung des Kernvolumens und des DNS-Gehaltes unter der Einwirkung hoher Dosen nach längeren Behandlungszeiten ist zweifellos eine Folge der allgemeinen Desorganisation der Zellstruktur.

Über polyploidisierende Wirkungen verschiedener Lindan-haltiger Beizmittel auf Getreidearten wurde von anderer Seite berichtet [21]. Die polyploidisierende Wirkung von Lindan und Lindan-haltigen Kombi-Präparaten äußert sich phänotypisch durch die Hemmung des Wurzelwachstums und durch charakteristische morphologische und cytologische Schädigungssymptome [2], [6], [10], [11], [15], [16], [18].

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LAB. PILOT CULTURE OF CYANOBACTERIA *SPIRULINA PLATENSIS* UNDER CONTINUOUS FLOW

BY
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The continuous production of *Spirulina* was achieved in a thermostated installation, with an adjustable flow rate input of the nutritive medium; the system being of the closed type, the penetration of the dust particles from the atmosphere and of other impurities is avoided.

The cultures of *Spirulina platensis* under continuous flow have the advantage of a permanent renewal of the nutritive medium, while the concentration of nutrients in the medium maintains at a relatively constant value during the entire period of culture.

The intensive culture of unicellular green algae and of cyanobacteria of *Spirulina* family has been usually performed in tank installations of open type, which have the disadvantage for temperate climate countries of being used only during the warm seasons of the year [1—4].

In order to avoid this disadvantage, a lab pilot has been devised that produces the biomass of *Spirulina platensis* in a closed-type installation provided with artificial or natural light only, or with both of them.

The installation (Fig. 1) consists of a tank for the nutritive medium (Fig. 1—1), a battery of glass tubes for the culture of *Spirulina*, both vertically located (Fig. 1—2 a-d) and inserted by connection glass tubes, each equipped with a fluorescent lamp (Fig. 1—3), a tube for bubbling the culture medium with air or CO₂ enriched air, a thermostat (Fig. 1—4) and a support of the culture tubes (Fig. 1—5). In the lower part of the installation there is a base on which the tubes are displayed and where the electric outfit, the vibrating pumps for bubbling and a device for timing the intermittent illumination are placed (Fig. 1—6).

In order to obtain a suspension of *Spirulina*, with an optimum harvesting density, the culture tubes filled 1/3 of the volume with nutritive medium are inoculated with a suspension in the exponential stage of growth, that is ensured with increasing optical densities from the tube *a* on the side of the tank containing the nutritive medium to the collecting tube *d*.

After inoculation, the tubes are filled with culture medium up to the overflow level (Fig. 1—7 a-d) and the bubbling, the inside lighting with fluorescent lamps and the thermostat are set running by the switches (Fig. 1—8) of each culture tube.

In order to get a *Spirulina* suspension with an optimum harvesting density, the rate of the culture medium inlet is adjusted by a special device (Fig. 1—9), as to correspond to the growth rate of the culture which

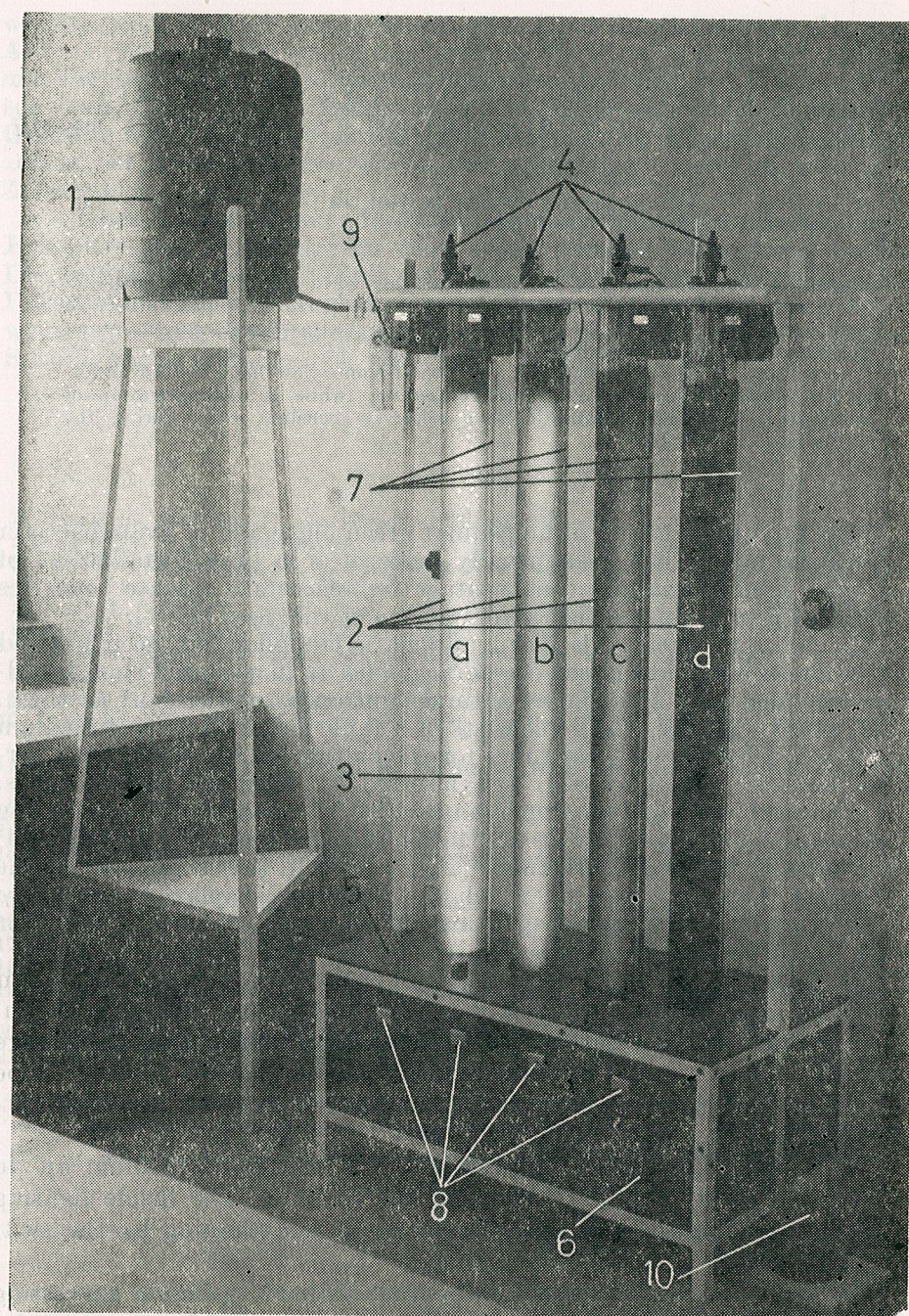


Fig. 1. — The general aspect of the installation for the culture of *Spirulina platensis* under continuous flow (the detailed description in the text).

makes possible to get a suspension of the best constant density in the harvesting vessel (Fig. 1—10). The installation was patented under No. 77.127/1981.

RESULTS AND DISCUSSION

In comparison with the classical cultures, achieved in tanks, *Spirulina* cultures under continuous flow have the advantage of the permanent renewal of the nutritive medium, which is enjoyable for the cyanobacteria.

Consequently, the pH values of the nutritive medium maintain at a practically constant level, close to the optimum value (pH = 9.5), while the concentration of nutrients in medium remains at a relatively constant value along the whole culture cycle.

In case we are aiming at obtaining a high field of biomass per suspension volume there occurs a new, essential parameter, namely the circulation rate of the suspension after the culture medium was admitted into the installation.

The experiments performed in laboratory conditions, by using additional illumination for the cultures, show that the optimum admission value of the nutritive medium and hence the circulation rate of the suspension is 200 ml/h. This rate makes it possible for obtaining a dry biomass of 3.02 g/l (table 1).

Table 1

The effect of the nutritive medium circulation rate on the biomass yield in *Spirulina* culture under continuous flow

The circulation rate of the medium (ml/h)	Dry biomass (g/l)
1	2
40	1.78
80	1.95
150	2.24
175	2.75
200	3.02
250	2.85
300	2.72
350	2.54

At lower circulation rates of the culture medium the accumulation of biomass decreases while at higher ones it also shows a decreasing tendency.

In the case the cultures are performed in installations under natural conditions, the accumulation of biomass depends on the result of the

simultaneous action of all vegetation factors. The continuous flow culture under natural conditions brings about an accumulation of biomass of 1.48–2.20 g/l per day (table 2).

The results concerning the accumulation of biomass by the method described in the present paper represent a premise in the efforts of obtaining an intensive culture of *Spirulina platensis*.

Table 2

The effect of the continuous flow culture on the accumulation of biomass under natural conditions

The moment of collection (days after the inoculation of the medium)	Dry biomass (g/l per day)	Temperature °C		Illumination (lux)
		Max.	Min.	
1	2	3	4	5
the 6th	1.48	28	15	5 700
the 7th	1.58	28	15	19 900
the 8th	1.60	28	15	26 600
the 9th	1.69	26	15	44 600
the 10th	1.99	26	15	6 600
the 11th	1.75	24	14	8 600
the 12th	1.75	25	15	6 400
the 13th	1.75	17	16	6 200
the 14th	2.20	21	6	2 100
the 15th	2.20	16	11	6 200
the 16th	1.54	16	11	8 500

The installation for the culture of spirulina presents the following advantages:

- achieves a continuous culture, with possibilities of adjusting the admission rate of the culture medium;
- makes use of natural or artificial light, or of both of them;
- offers the possibility of thermostating the nutritive medium, which allows to obtain cultures throughout the year, even in the unfavourable seasons;
- occupies a limited space due to the vertical location of the culture vessels;
- being of a closed type it reduces the contamination with the impurities and the dust particles from the atmosphere that could affect the culture;

The installation as well as the method used could represent a basis in the launching of an industrial technological process for obtaining the biomass of *Spirulina*.

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ULTRASTRUCTURE OF *HELIANTHUS ANNUUS* L. CALLUS, DURING THE GROWTH, DIFFERENTIATION AND AGING PROCESSES

BY

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The cells growth, differentiation and aging in the callus of *Helianthus annuus* L. are connected with ultrastructural modifications of cell organelles which are similar from many points of view with those found in intact plants. The onset of senescence was marked by the reduction of the cytoplasmic content, the differentiation of the vacuolar system (lysosomes) and the presence of myelin figures, lytic intracytoplasmic areas, plastids with abnormal structure and microbodies with large crystalline inclusions.

The assumption that aging represents part of the cell differentiation process and its genetic determination are also discussed.

Morphological modifications of cells 'in vitro' cultures were analysed mainly as regards the process of cell growth and differentiation [2], [3], [8], [10], [13], [14], [16], [17], [24], [26], [27]. There are few studies on the ultrastructural modifications accompanying the ageing process [4], [9]. The present paper intends to make a cytological characterization of *Helianthus annuus* L., during the ageing process, and to discuss some aspects of cell differentiation and ageing 'in vitro'.

MATERIAL AND METHOD

Seeds of *Helianthus annuus* L., variety Record, were superficially sterilized in a solution of 2% bromine water, washed three times in sterile distilled water, submersed for 5 minutes in ethyl alcohol 70%, washed four times in sterile distilled water and germinated in aseptic conditions on a solidified White medium. The germination took place in the darkness at 25–27°C. Seedlings after 5 days of germination were used for the beginning of callus formation; explants of 5 mm × 2 mm were collected from their cotyledons and inoculated on a basal medium B₅ [13] containing 30 g/l sucrose, 0.2 g/l hydrolyzated caseine, 0.5 g/l malt extract and 0.5 mg/l 2,4-D; the pH was adjusted to 5.5 before autoclaving. The medium was solidified by addition of agar 0.8% g. The incubation was performed at 27±1°C and a photoperiod of 16:8 hours with a light intensity of 4,500 lux, using a fluorescent white light source.

For electronmicroscopic studies, the cells were fixed at 3, 10, 15, 20 days since inoculation in glutaraldehyde 3% in phosphate buffer 0.05 M (pH = 6.8) for 3 hours, washed and included in agar buffer 2.5%,

postfixed in a solution of OsO_4 2% for 2 hours at room temperature, then included in araldite. The sections were contrasted according to the Reynolds method and examined under a JEM 7 electronmicroscope, at an acceleration voltage of 80 kV.

RESULTS AND DISCUSSIONS

The growth of *Helianthus annuus* L. cells in callus culture is produced according to a Gaussian curve, with two peaks (at 10 and 20 days since inoculation), with a gradual reduction of the dry matter between 25 and 30 days [5]. According to the culture age the morphological aspect of cells is sensibly modified.

Therefore, after 3 days since inoculation the culture has a relatively uniform aspect. Most cells are characterized by a rich cytoplasmic content, a nucleus with central localisation and an irregular, sinuous, lobated shape. Inside the nucleoplasm, chromatine is relatively diffuse and uniformly spread. The cytoplasm contains the whole complement of organelles, characteristic of the plant cell (endoplasmic reticulum, mitochondria, Golgi apparatus, ribosomes). Plastids in an incipient stage of differentiation are most frequent (Pl. 1 A). Part of them contain starch granules and membranary inclusions (tubular or vesicular) originating from the internal membranae invaginations of the plastidial cover and interpreted as precursors of the thylacoidal system. The Golgi apparatus is characterized by a reduced number of vesicles. The ribosomes are generally freely spread in the cytoplasm, sometimes in association with the profiles of the endoplasmic reticulum. The mitochondria are characterized by a typical well-differentiated internal structure. The numerically reduced vacuoles are small in size.

10 days since inoculation the callus shows a heterogeneous aspect, characterized by 3 distinct types of cells. Most of them are of a parenchymatic nondifferentiated type, characterized by a thin primary cell wall, little cytoplasm with parietal disposition and a large central vacuole. Plastids in various stages of differentiation, mitochondria, Golgi bodies and profiles of the endoplasmic reticulum (Pl. 1 B) with a characteristic structure are present inside the cytoplasm.

The second category of cells is represented by vascular elements (tracheids and phloem cells) in various stages of differentiation (Pl. 1,

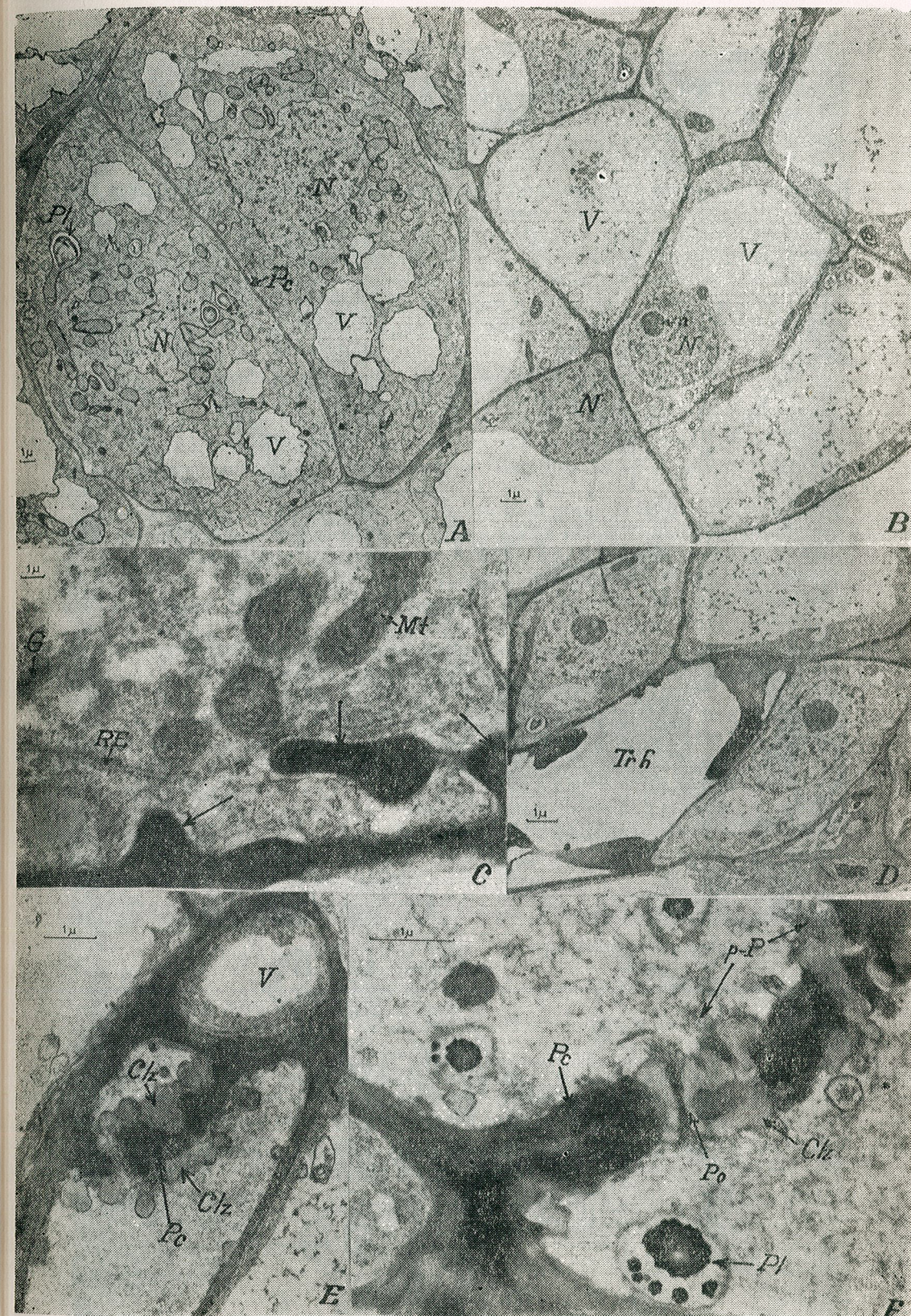


PLATE 1 A. — Ultrastructural characteristics of young cells in a juvenile callus of *Helianthus annuus* L. (3 days after inoculation) ($\times 3,100$);

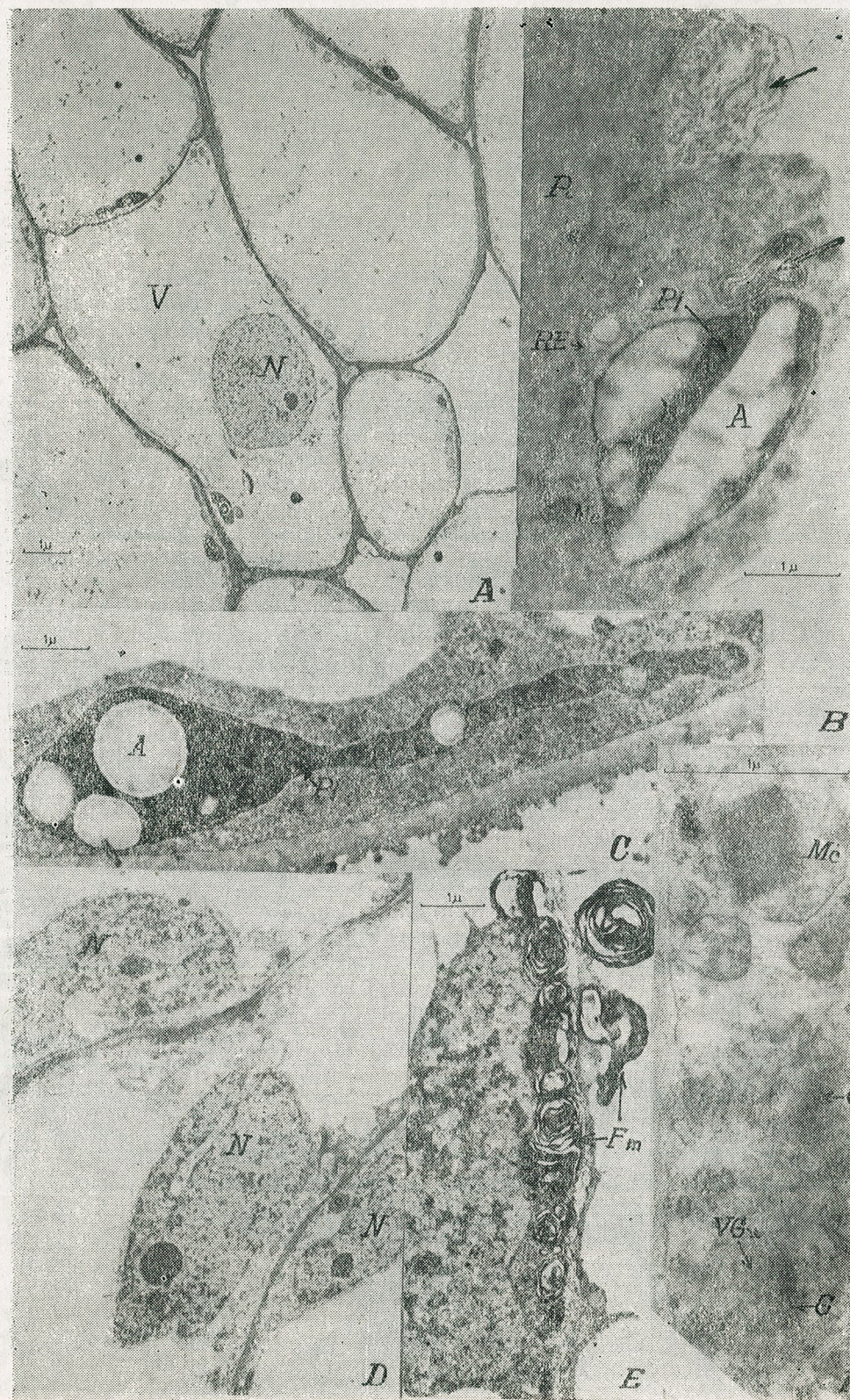
B. Nondifferentiated parenchyma cells in the callus of *H. annuus* L. (10 days after inoculation) ($\times 5,300$);

C. Cell fragment during tracheidal differentiation. Secondary thickenings of the primary cell wall (arrow) ($\times 3,500$);

D. Completely differentiated tracheidal cell. Secondary modifications of the primary cell wall and the complete lysis of the cell content ($\times 6,500$);

E. A deposit of callose on both sides of the cell wall during the sieve plate differentiation ($\times 11,800$);

F. Completely differentiated sieve plate. Abundance of P-protein fibrils in the vicinity of the cell wall and inside the pore of the sieve plate and the particular aspect of plastids ($\times 8,200$).



C, D, E, F). In the case of future tracheids, for example, a secondary thickening of the primary cell wall occurs (Pl. 1 C, arrow). At the same time, the cytoplasm undergoes a progressive process of lysis, up to a complete disappearance of the cell content (Pl. 1 D). The differentiation of sieve plates begins with a deposit of callose on both sides of the cell wall. Gradually, the process of lysis advances on both sides, up to the level of medium lamella, between the two adjacent cells, with a differentiation of a communication channel (Pl. 1 E, F). Among the modifications of organelles of phloematic cells under differentiation those at the plas-tids level are significant, because of their particular, typical aspect (Pl. 1 F). The presence of P-protein is also noticed, close to the sieve plate inside its pore (Pl. 1 F).

The third category contains the meristematic cells grouped in 'meristemoids'; they are centers of callus proliferation.

15 days after inoculation, cells with modifications characteristic of the ageing process appear besides the cell types previously described. 20 days after inoculation they are very frequent, and after 25–30 days, they are prevailing.

One of the essential characteristics of ageing cells is represented by a quantitative reduction of cytoplasm and a proportional development of the vacuolar system (lysosomal) in comparison with cell sizes (Pl. 2 A). The vacuoles originate from Golgi vesicles and profiles of the endoplasmic reticulum. They are hypertrophied and have an intense hypersecretory activity. The autophagic vacuoles are predominant. They are formed by tonoplast invagination and surrounding of some cytoplasm portions with the component organelles, followed by the gradual digestion of cytoplasm by enzymes of the vacuolar sap.

The phenomenon is similar to that described by Matile [18] and Marty [20] for some species of higher plants 'in vivo'. Oliveira [22], [23] noticed in the brown algae *Ectocarpus* that during the ageing process, a strong lysis of the cytoplasm content takes place, similar to that described before; they appreciate — according to morphological and cytochemical observations — that the inclusions resulted from this process of lysis are related to lipofuscin 'ageing pigment' described in protozoa and various types of animal cells.

PLATE 2. — Modifications of the structure of cell organelles during the natural ageing process (20–25 days after inoculation).

- A. Group of old cells. The reduction of the cell content and the development of the vacuolar system ($\times 9,000$);
 - B. Multivesicle bodies (arrow) and areas of intracytoplasmic lysis in one old cell (double arrow) ($\times 19,000$);
 - C. Plastids with an abnormal aspect, a strongly electrondense stroma and a slightly differentiated lamellar system ($\times 13,450$);
 - D. Irregularly shaped nucleus from an old cell ($\times 13,000$);
 - E. Abundance of myelin figures derived from the plasmalemma and the tonoplast in an advanced ageing cell ($\times 12,150$);
 - F. Microbody with a crystalline, well differentiated inclusion and Golgi bodies with numerous Golgi vesicles (arrow) ($\times 37,000$).
- A — amyloiferous inclusion; Clz — calloses; Fm — myelin figures; G — Golgi apparatus; Mc — microbodies; Mt — mitochondrias; N — nucleus; n — nucleolus; Pc — cell wall; Pl — plastid; Po — pore; p — P-proteins; RE — endoplasmic reticulum; Trh — tracheidal cell; V — vacuole.

Wilhelm and co-workers [30] remarked that the accumulation of some pigments of lipofuscine type in the chloroplasts of old leaves of *Phaseolus vulgaris* present fluorescence properties similar to those of animal tissues. The phenomena may occur similarly in cells of both regna. The accumulation of some substances of lipofuscine type in the intracellular membranes (Golgi bodies, endoplasmic reticulum, plastids) from animal cells may induce degradation of their functionality leading to senescence and finally to the cell death.

Another characteristic of old cells is represented by the appearance of myelin figures and multivesicle bodies as a result of the degradation of lipoproteic components. As in the case of old cells of *Triticum monococcum* and *Oryza sativa* [4], [9] the proplastids and the structure of amyloplasts underwent several modifications. Therefore, the inhibition of the lamellar system was noticed as well as the hydrolysis of their protein structure, enlargement in size and number of amyloplasts. It is possible that the inhibition of the lamellar system of plastids from old cells should be the result of some modifications in the transcription of plastidial ADN. The increased number and size of starch granules are indicative of metabolic alterations which probably affect the conversion of carbohydrates. A reduction in the efficiency of chlorophyll for light could also be possible. The microbodies, seldom noticed in young cells of *H. annuus* L., were frequent in old cells. In all cases they contain crystalline inclusions identical with those described by Frederick and Newcomb [11] in many species of plants grown 'in vivo'. Cronshaw [7], Davey and Street [8], Sutton-Jones and Street [26], Matsushima [19] described in cells grown 'in vitro' conditions such 'bodies with crystalline inclusions', corresponding to microbodies described by Frederick [11].

The high frequency of these organelles in plant cells with a reduced metabolic activity (for example, in lipid storage cells from cotyledons) would suggest that they are indicative of functional inactivity. Gomez et al. [13] noticed a higher number of microbodies and an increase of the cytochemical reaction with DAB in old tissues a phenomenon similar to that noticed by Vasil [28] in the beans endosperm old cells. The microbodies content in enzymes implied in the degradation of nucleic acids and glycoproteins, in the oxidation of fat acids and NAD and in the photorespiration could justify their role in the phenomena of cells senescence. It was noticed by Fowke and Setterfield [10] in the callus of *Helianthus tuberosus* cells and by Brezeanu and Davis [4], [9] in cell suspensions of *O. sativa* and *T. monococcum* that the microbodies were not identified after the addition of 2,4-D to the nutritive medium, the latter exerting an inhibitory effect on the differentiation of microbodies or on the accumulation of catalase. The presence of such formations in the old cells, although the 2,4-D was used in the culture medium, makes us believe that the microbodies are implied in the senescence processes and the concentration of 2,4-D is not an inhibitory factor for their differentiation or crystalloid degradation.

Special attention should be paid to establish the interrelations between senescence and differentiation. One of the characteristics of callus culture is the rapid onset of cellular differentiation. This would make difficult to delimit the two processes, all the more so since some subcelul-

lar morphological modifications are identical (e.g. the structural modifications of plastids and the formation of the lysosomal system during the normal differentiation of the vascular tissue). The difference may consist in the fact that while the structural and functional modifications during the differentiation induce a specialization of cells to perform certain functions, those during senescence lead to the cell death. Senescence would represent in fact a continuation of the cell differentiation or at least part of a differentiation process in which protein degradation exceeds protein synthesis or produces errors in the protein syntheses.

We conclude that ageing is a complex phenomenon involving several metabolic processes. Although the peroxidative reactions of lipids may have the most important role in the onset of ageing, the real causes are more complex, implying also genetic control [15], [19].

The existence of a system of gene repression is supported to exist in the final stage of cell ageing. The hypersecretory activity of dictyosomes correlated with the development of lysosomes during the ageing process should imply such a genetic control. Part of the genome is responsible for senescence manifestation.

Factors of the environment such as reduction in nutrients, an increased quantity of CO₂, pH modifications and an accumulation of some products of metabolic degradation in the environment represent physiological stress factors implied in the phenomena of cell autolysis or decompensation of the injured parts of the cell.

The stress factors only cannot produce the ageing of the cells, but can facilitate the onset of senescence in a critical moment of the cell.

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A TRANSMISSION ELECTRON MICROSCOPE STUDY OF THE ULTRAMORPHOLOGY OF THE SPORES OF SOME *BRYOPHYTA*

BY

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Images of six species of *Bryophyta* spores, obtained with TEM, are given in this paper. The spore surface is covered with characteristic ornamentations of conic, mushroom or spherical shape. The taxonomic significance of these ornamentations is discussed.

Transmission electron microscopy has provided a better understanding of the complex morphology of the spore wall of the families *Polytrichaceae* [8], [10], *Splachnaceae* [6], *Funariaceae* [9] and *Buxbaumiaceae* [11]. These studies are based on a method of direct observation of spores*, without any treatment, developed and used by us since 1967 to elucidate the fine morphological and structural nature of the spore wall and its role for diagnostic separation within the *Bryophyta* [6].

The purpose of this paper is to describe the fine morphology of a new series of *Bryophyta* spores.

MATERIALS AND METHODS

The moss spores examined belong, according to Gams [2], to the following species: *Buxbaumia aphylla* Hedw. (Fam. *Buxbaumiaceae*), *Philonotis calcarea* (B.S.G.) Schimp. (Fam. *Bartramiaceae*), *Pleurozium schreberi* (Brid.) Mitt. (Fam. *Entodontaceae*), *Amblystegium juratzkanum* Schimp. (Fam. *Amblystegiaceae*), *Hypnum cupressiforme* Hedw. and *Ptilium crista-castrensis* (Hedw.) De Not. (Fam. *Hypnaceae*).

The spores were collected from herbarium material (Collection of Prof. dr. doc. Tr. I. Ștefureac), prepared by the previously described method* and subsequently examined on a TESLA BS 500 electron microscope TEM.

RESULTS AND DISCUSSIONS

Although we have utilised a transmission electron microscope, the images obtained show the presence of a characteristic ornamentation on the exine surface.

* Ploaie G.G., Ultrastructure researches in the ornamentations of the sporoderm of *Bryophyta*. Diploma paper, University of Bucharest, 1971.

The features of the exine of spores are presented and illustrated in the following electron micrographs.

Buxbaumia aphylla. The shape of spores is sphaerical or ovoid, having 8–12 μm in diameter, and the exine is transparent for the electron beam. The exine ornamentations are prominent, 170–200 nm high and conic in shape (plate I, A). An ornamentation is composed from 6–7 dense superposed discs, separated by less electron dense discs. The diameter of the basal disc is about 70–90 nm and decreases to the peak of ornamentation, which is conic and elongated, sometimes curved (Plate I, B).

The disposition of ornamentation on the exine surface is irregular and the space between ornamentations is bigger than the basal disc diameter.

Philonotis calcarea. The spores are spherical, 15–17 μm in diameter. The exine is very transparent to electron beam and is covered by dense ornamentations. They are irregularly disposed, associated in clusters and separated by an empty space (Plate I, C). The shape of the ornamentations resembles a young stage of a mushroom having a cylindrical part, covered by an ovoid, and a dense part with a 100–120 nm diameter. The terminal part surface is smooth, the secondary ornamentation is absent (Plate I, D).

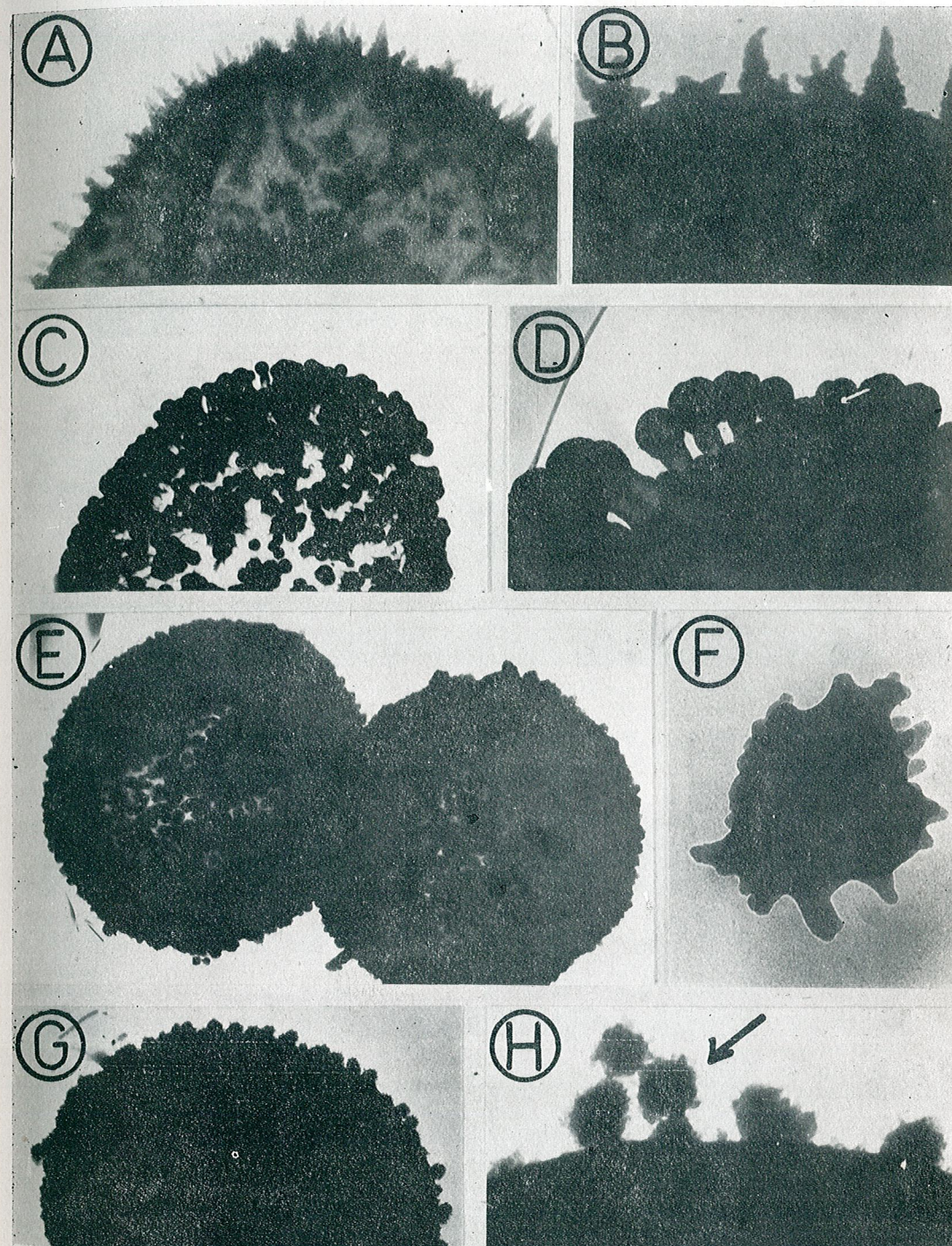
Amblystegium juratzkanum. The spores are spherical in shape, 12–14 μm diameter, and the exine is very opaque to the electron beam but the ornamentations are visible at the spore bordure (Plate I, G). These ornamentations are spherical, 140–160 nm in diameter, and can be easily separated from the exine (Plate I, H arrow). Small conic papillae or secondary structures, 15–20 nm high and 8–10 nm basal diameter are visible on spherical ornamentations. The disposition of these papillae is irregular.

Pleurozium schreberi. The spores are spherical, 18–20 μm in diameter, and covered with very dense spherical ornamentations, with a 130–160 nm diameter. Sometimes a regular disposition of the ornamentations on the exine, in a hexagonal pattern, was visible (Plate I, E).

Under electron beam the ornamentations can be separated from the exine and can be examined at high magnification (Plate I, F). These ornamentations are not smooth their surface been covered by small conic papillae with a diameter of 8–10 nm high, irregularly disposed. These are secondary ornamentations.

Hypnum cupressiforme. The spores, 12–14 μm in diameter, are spheric or triangular-elliptic in shape. The exine is transparent to the electron beam, and the ornamentations are regularly disposed in a hexagonal pattern, visible in some regions. Sometimes they present pentagonal patterns (Plate II, B). The ornamentations are spherical, 150–160 nm in diameter, and very electron dense. On their surface, there are present small conic papillae, 20–22 nm high, visible on the distal faces, which constitute secondary ornamentations (Plate II, C).

Ptilium crista-castrensis. The spherical spores, 13–17 μm , are always preserved. The exine is traversed by an electron beam and the ornamen-



Plate, I, A — A sector from a spore of *Buxbaumia aphylla* with conic ornamentations, $\times 14,500$
 B — Some discs composed ornamentations of *Buxbaumia aphylla* spores, $\times 57,000$
 C — A sector from a spore of *Philonotis calcarea* with clusters of ornamentations, $\times 9200$
 D — The ornamentations of *Philonotis calcarea*, mushroom-like shape, young stage, $\times 26,5000$
 E — Two spores of *Pleurozium schreberi* with spherical ornamentations, $\times 14,700$
 F — An ornamentation of *Pleurozium schreberi* with conic papillae irregularly disposed, $\times 103,000$
 G — The *Amblystegium juratzkanum* sector of a spore, covered by spherical ornamentation, $\times 8200$
 H — Some ornamentations of *Amblystegium juratzkanum*, bordered by conic secondary ornamentations, $\times 42,000$

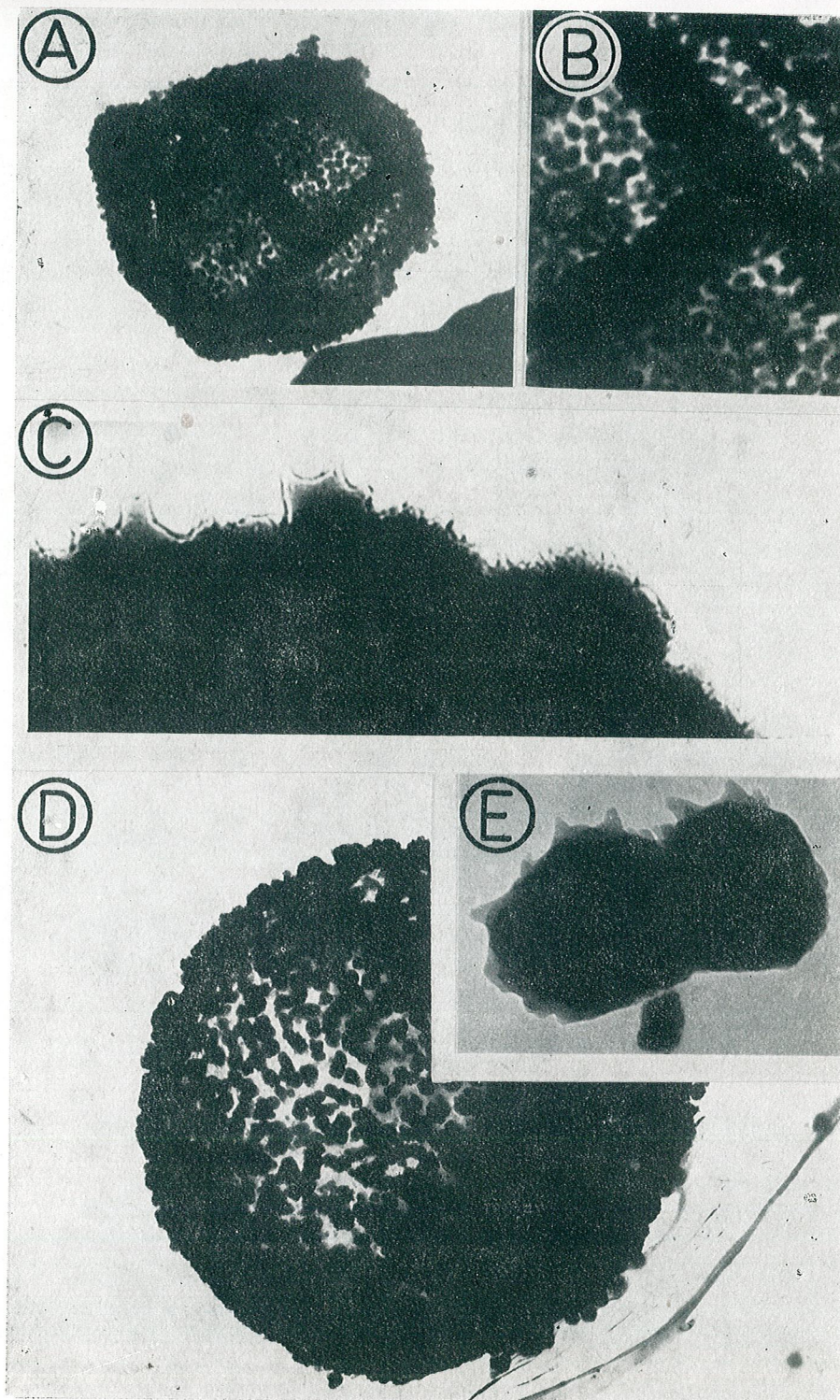


Plate II, A — The image of a *Hypnum cupressiforme* spore, $\times 12\,500$
 B — The disposition of ornamentations on the spore surface in *Hypnum cupressiforme*, $\times 18\,800$
 C — The ornamentations of *Hypnum cupressiforme* covered by conic papillae, $\times 48\,300$
 D — A sector from a spore of *Ptilium crista-castrensis* with dense ornamentations irregularly disposed, $\times 23\,200$
 E — A double ornamentation with conic papillae, $\times 94\,000$

tations are clearly visible on the spore surface both in the central region and the border of the spore profile (Plate II, D). The ornamentations are nearly spherical, 130–140 nm, sometimes associated two by two (Plate II, E) and can take off from the exine. On their surface there are visible secondary ornamentations, with a conic shape, 15–17 nm high and transparent.

DISCUSSION

All five species of moss have spherical, sometimes ovoid spores, transparent to the electron beam. Spore surface is richly ornamentated with prominent ornamentations. The *Buxbaumia aphylla* spores are covered by conic ornamentations, composed discs that suggest a "Christmas tree". These ornamentations resembling those of *Polytrichum strictum*, *P. piliferum* and *P. juniperinum* described by Ștefureac, Ploaie and Ploaie [8], [10] in TEM and SEM and *Polytrichum juniperinum* described by Schiavone [7] in SEM. It is necessary to point out that this ornamentation is not similar to those of *Buxbaumia viridis* [11].

The ornamentations of *Philonotis calcarea* spores are characteristic of this species and are not common among *Bryophyta* at least up to now.

The spherical shape of ornamentations of *Amblystegium juratzkanum*, *Pleurozium schreberi*, *Hypnum cupressiforme* and *Ptilium crista-castrensis* spores is common for these species, and also frequent for *Bryophyta*.

Vitt and Hamilton [12] have described this ornamentation shape, in *Encalypta* species from North America and Járαι-Komlódy and Orban [3] from European *Encalypta* species.

Boros and Járαι-Komlódy [1] present images of this shape (verrucae, bubble-like elements) on the spores of *Conocephalum conicum*, *Acaulon triquetrum* and *Pottia heimi*. Koponen [4] reports this type of ornamentation in *Tayloria kilimandscharica* and *T. lingulata*. We have also observed spherical ornamentation in *Polytrichum alpinum*, *P. formosum* and *P. gracile* spores [8], [10]. It appears that this ornamentation shape is very common in *Bryophyta* and this way new phylogenetic and biosystematic considerations are possible. Recently, Lloyd [5] described with SEM this type of ornamentation in fern spores, and suggested a similarity in spore ornamentation. The images obtained by us can be used to establish the new affinities in uncertain taxa.

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SOME BIOCHEMICAL PECULIARITIES IN PLANTS OF *DIGITALIS LANATA* EHRH. MODIFIED AFTER SUCCESSIVE TREATMENTS WITH GAMMA RAYS AND ETHYLMETHANESULFONATE*

BY

ECATERINA T. TÓTH, G. I. GHIORGHITĂ and ELVIRA V. GILLE

The alternative treatments with gamma rays and ethylmethanesulfonate (EMS) of *Digitalis lanata* seeds (along two generations) induced a great morphological and biochemical variability of the plants. The plants changed after the applied treatments generally presented higher values of the cardenolide content and a modified isoenzyme pattern.

The study of the behaviour of medicinal and aromatic plants exposed to chemical and physical mutagens could make important theoretical contributions to the field of radiobiology and chemical mutagenesis. This, of course, taking into account their biological and biochemical peculiarities. But, the mutagen agents may also successfully be used to obtain new plant forms, having improved characteristics needed for pharmaceuticals [5–10]. The results of our recent researches have shown that this insufficiently explored route is promising enough. By means of repeated gamma ray irradiation, we obtained (in the second generation) plants of *Vinca minor* and *Atropa belladonna* morphologically changed, which differed from the control plants by their content of active principles, peroxidase and esterase isoenzyme [3].

We, however, make such investigations in other species of medicinal plants too such as: *Datura innoxia*, *Cynara scolymus* and *Digitalis lanata*. They revealed the fact that the gamma ray treatment generally determined the increase in the content of active principles (the main index considered) in the leaves of the first generation plants [2], [11]. In the case of cultivated *Digitalis* (treated again in the second generation) we rendered evident an extremely great morphological variability of the plants. We made a series of individual analyses on some plants with modified phenotypic aspect. The results of these analyses are presented in this paper.

MATERIAL AND METHODS

Digitalis lanata Ehrh. seeds harvested from plants irradiated in the first generation (1979–1980) with doses of 3, 6, 10, 15 and 20 kR were either irradiated again or treated with solutions of 0.075, 0.15 and

* The paper was presented at the XIIIth annual meeting of ESNA, Aberdeen-Scotland.

0.30% ethylmethanesulfonate (EMS) in the second generation. The applied treatments, especially with EMS (which lasted for 12 hours) induced important changes in the morphology of the plants. Plants appeared with green or yellowish stems (instead of dark brown), their leaves ranging from long and narrow with parallel borders, to short and broad ones, leaves with protuberances on the nervation undulations, with modified thickness and colour (from light green to yellow-green), flowers with 2, 3, 4 and 5 lips a.o. In the first half of July 1981, we harvested samples (leaves) from some of these plants in their second year of vegetation, in order to analyse their total content of cardenolides, soluble sugars, free-amine nitrogen and proteins with peroxidase and esterase activity (by gel-electrofocussing). The methods used were presented in previous papers [1], [3].

We also fixed in ethanol the leaves of isolated plants in order to determine the histo-anatomical changes induced by the treatment with the mutagens used.

The results of our investigations are shown in Tables 1 and 2, as well as in figures 1–3. The tables also comprise the average values of the analysed biochemical parameters regarding the experimental variants that contained morphologically changed individuals.

RESULTS

We first noticed that the average cardenolide content of the re-irradiated variants recorded, in the second year of vegetation, values similar to those of the first year (when the harvesting of leaves for industry is recommended). A second remark is that the individuals with changed morphological aspect (analysed by us) present values of the total cardenolides markedly higher than the average scored by the variants which comprised them and the control plants (Table 1).

Table 1

The values of some biochemical parameters in *Digitalis lanata* plants modified by gamma irradiation in two successive generations

No.	The treatment		Free amine nitrogen % d.s.	Reducing sugars % d.s.	Total sugars % d.s.	Total cardenolides % d.s.
	first generation	second generation				
1	Control		0.83	1.90	7.69	1.12
2	10 kR	10 kR	0.82	1.94	8.44	1.17
3	11–28	10 kR	—	—	—	1.24
4	19–38	10 kR	0.68	1.89	12.69	1.19
5	15 kR	15 kR	0.66	1.72	8.38	1.16
6	12–30	15 kR	0.91	2.30	12.42	1.79
7	12–32	15 kR	0.91	2.15	12.40	1.36
8	20 kR	20 kR	1.29	2.38	12.30	0.98
9	13–33	20 kR	0.88	2.12	12.47	1.70

The most valuable individual was no. 12–30, resulted from the variant irradiated with 15 kR in both generations, its glycoside content being about 54% higher (1.79%) than the average of the plants in the

variant (1.17%). But the highest growth per variant compared to the average was recorded by individual no. 13–33 (1.70%) i.e. by 73% higher than the average of the variant (0.98%).

Along several years, the behaviour of the metabolism of sugars and of some amine compounds in different conditions and in variously irradiated plants has been examined. Considering the fact that the active principles of *Digitalis* are represented by glycosides, we had one more reason to study the effect of mutagen treatments on the soluble sugars, to follow an eventual relation between these two parameters. Examining the data in Table 1 we may conclude that the analysed individuals normally present values of the total sugars content greater than the average of the variant and control plants. But we cannot assert that there is a direct correlation between the total sugars and total cardenolides values.

Analysing the zymograms representing the forms of the identified esterases in the case of the individuals tested (Fig. 1), we notice the existence of significant differences between the control plants and those with a changed morphological aspect. The isoenzymatic spectrum of the latter is richer, a fact that, under certain conditions, could confer them some advantages. The isolated individuals of the variant treated with 10 kR (11–28; 19–38) differ from the control plants only by the appearance of an isoesterase that acts at a pH of about 5.8. This isoenzyme is also present in the case of the modified plants of the variant treated along 2 generations with 15 kR (12–30; 12–32). The latter presents

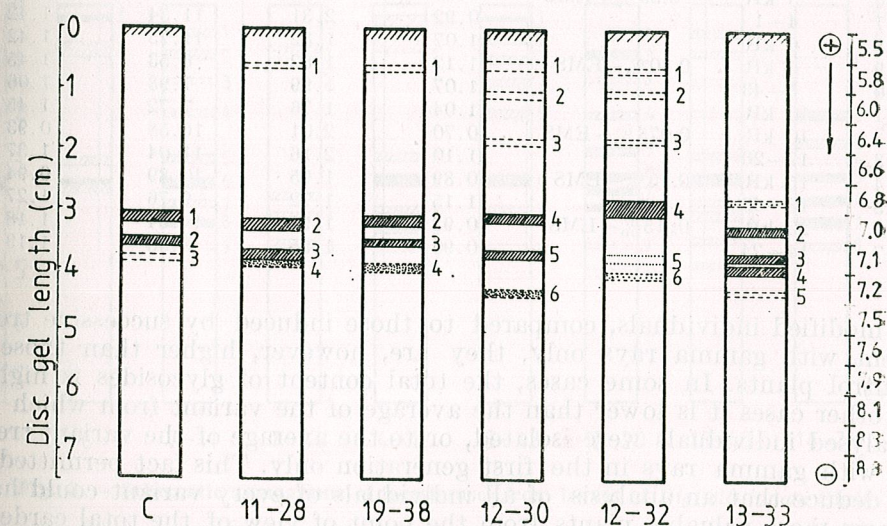


Fig. 1. — Schematic zymograms of isoesterases from leaves of *Digitalis lanata* Ehrh.

6 isoenzymes with esterase activity among which only 4 are common, namely those that act at pH 5.8–6.0, 6.4 and 7.1 — the first three being present in the control plants as well. Individual no. 12–30 has an iso-

esterase corresponding to $\text{pH} = 7.2$ and plant no. 12-32 presents a band very active at $\text{pH} = 6.8$.

It is interesting to notice that plant no. 13-33, isolated in the variant irradiated for two successive generations with 20 kR, presents, besides the isoesterase characteristic of the control plants, the two iso-enzymes that differ in plants no. 12-30 and 12-32; it must also be noticed that those in the domain of $\text{pH} = 5.6-6.8$ disappear.

From the multitude of plants changed by means of alternative treatment with gamma rays and EMS we selected some and made the same set of analyses. The data presented in Table 2 show the fact that although the values of the cardenolide content are reduced, in the case

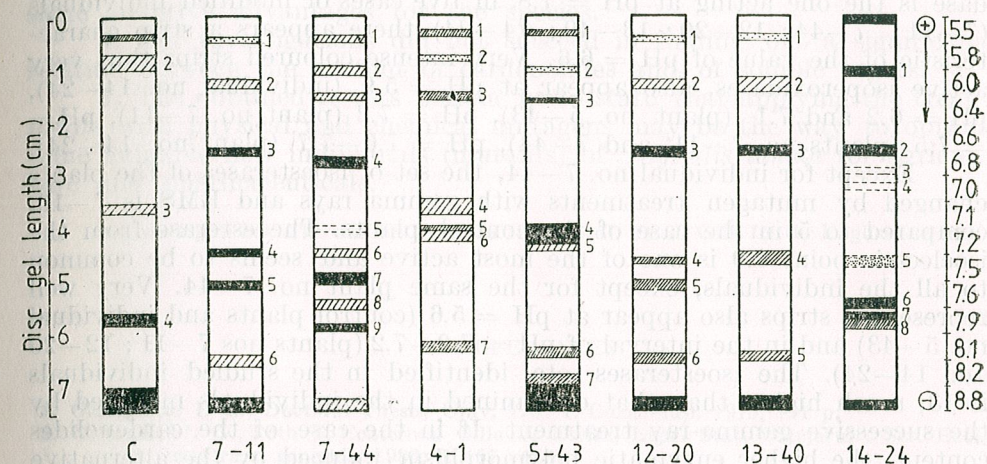
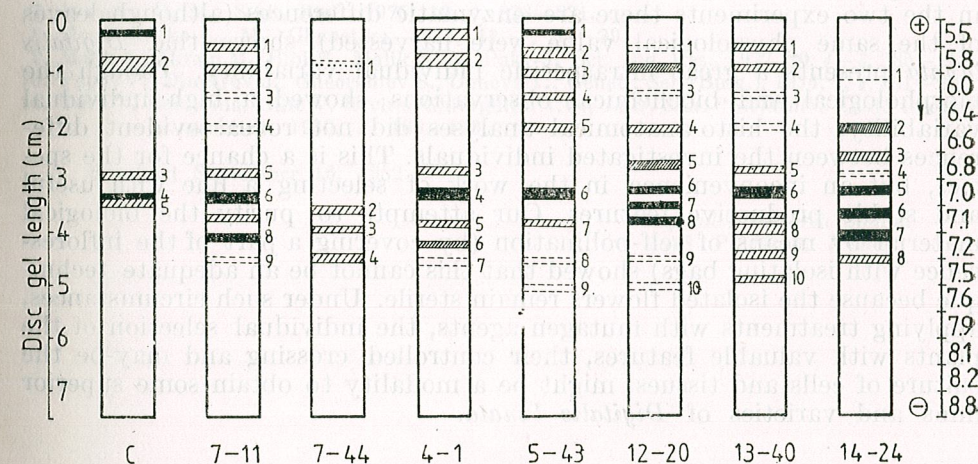
Table 2

The values of some biochemical indices in *Digitalis lanata* plants modified by alternative treatment with gamma rays and ethyl-methanesulfonate

No.	The treatment		Free amine nitrogen % d.s.	Reducing sugars % d.s.	Total sugars % d.s.	Total cardenolides % d.s.
	first generation	second generation				
1	Control		0.83	1.90	7.69	1.12
2	3 kR		0.94	1.93	12.70	0.98
3	3 kR	0,15 %—EMS	1.10	1.93	11.67	0.93
4	7-11		0.76	1.93	11.33	1.12
5	7-44		—	—	—	1.10
6	3 kR	0,30 %—EMS	0.88	1.72	9.88	1.33
7	4-1		0.92	2.31	11.34	1.42
8	6 kR		1.07	1.82	11.73	1.42
9	6 kR	0,30 %—EMS	1.12	1.62	6.53	1.45
10	5-43		1.07	1.99	7.98	1.06
11	10 kR		1.04	1.76	7.72	1.45
12	10 kR	0,075 %—EMS	0.70	2.01	16.58	0.93
13	12-20		1.10	2.16	11.04	1.37
14	15 kR	0,15 %—EMS	0.89	1.95	10.89	0.94
15	13-40		1.15	1.72	9.76	1.27
16	20 kR	0,15 %—EMS	0.93	1.94	14.84	1.18
17	14-24		0.90	1.55	8.38	1.19

of modified individuals, compared to those induced by successive treatment with gamma rays only, they are, however, higher than those in control plants. In some cases, the total content of glycosides is higher, in other cases it is lower than the average of the variant from which the analysed individuals were isolated, or to the average of the variant treated with gamma rays in the first generation only. This fact permitted us to deduce that an analysis of all individuals of every variant could have shown more valuable plants from the point of view of the total cardenolides compared to that of the individuals investigated by us. The highest level of cardenolides was recorded by plant no. 4-1 (1.42%) originated from the variant treated with 3 kR in the first generation and with 0.3% EMS in the second one. We did not establish a correlation between the values of cardenolides and the other investigated parameters in this experiment.

The analysis of the zymograms, representing the isoperoxidases and the isoesterases identified in the phenotypically modified plants (Figs 2, 3), revealed again a broader spectrum of isoenzymes compared to the control plants. The applied treatments induced an evident enzymatic polymorphism, which, in our opinion, represents an advantage —

Fig. 2. — Schematic zymograms of isoperoxidases from leaves of *Digitalis lanata* Ehrh.Fig. 3. — Schematic zymograms of isoesterases from leaves of *Digitalis lanata* Ehrh.

from the point of view of adaptability — for these individuals and for the species of course. In the pH interval, ranging between 5.6 and 8.2, we identified a number of approximately 17 isoenzymes with peroxidase activity at the level of the analysed individuals (13 new isoperoxidases compared to the control plants).

The richest sets of isoperoxidases were shown by individuals no. 7-44 and 14-24 (with 9 and 8 isoenzymes, respectively), individuals that did not as yet present high values of the total cardenolides (1.10 and 1.19% respectively). As the isoenzymatic differences among the investigated individuals are very high, we limit ourselves in asserting that while in the case of the control plants the most representative peroxidase is the one acting at pH = 7.8, in five cases of modified individuals (7-11; 7-44; 12-20; 13-40; 14-24) there appears a strip characteristic of the value of pH = 6.6. Very intense coloured strips thus very active isoperoxidases, also appear at pH = 5.9 (individual no. 14-24), pH = 6.2 and 7.1 (plant no. 5-43), pH = 7.2 (plant no. 7-11), pH = 7.5 (plants no. 7-11 and 7-44), pH = 7.6-7.7 (plant no. 14-24).

Except for individual no. 7-44, the set of isoesterases of the plants changed by mutagen treatments with gamma rays and EMS is 7-10, compared to 5 in the case of the control plants. The esterase from the isoelectric point 6.9 is one of the most active and seems to be common to all the individuals, except for the same plant no. 7-44. Very well represented strips also appear at pH = 5.6 (control plants and individual no. 5-43) and in the interval of pH = 7.0-7.2 (plants nos 7-11; 12-20 and 14-24). The isoesterases total identified in the studied individuals is 13, much higher than that determined in the individuals modified by the successive gamma-ray treatment. If in the case of the cardenolides content, the higher enzymatic polymorphism induced by the alternative treatment with gamma rays and EMS had no positive effects, this biochemical peculiarity contributed to a more vigorous growth and a better development of the plants. The fact that even among the control plants in the two experiments there are enzymatic differences (although leaves of the same physiological value were harvested) shows that *Digitalis lanata* presents a great intraspecific individual variability. Though the morphological and biochemical observations showed a high individual variability, the histo-anatomical analyses did not reveal evident differences between the investigated individuals. This is a chance for the species, but an inconvenience in the work of selecting a line with useful and stable productive features. Our attempts to purify the biological material by means of self-pollination (by covering a part of the inflorescence with isolating bags) showed that this cannot be an adequate technique because the isolated flowers remain sterile. Under such circumstances, applying treatments with mutagen agents, the individual selection of the plants with valuable features, their controlled crossing and may-be the culture of cells and tissues, might be a modality to obtain some superior lines and varieties of *Digitalis lanata*.

CONCLUSIONS

1. The alternative treatments with gamma rays and EMS of *Digitalis lanata* Ehrh. seeds (along 2 generations) induced a greater morphological and biochemical variability of the plants than in the case of treating them successively with gamma rays alone.
2. The plants changed by the applied treatments presented generally higher values of the cardenolide content compared to control plants. The

successive treatments with radiations are more adequate for the proposed aim. They determined higher values of the total cardenolides than alternative treatments with radiations and EMS.]

3. The mutagen treatments caused the activation of some isoperoxidases and isoesterases that were absent in the control plants. The treatments determined a pronounced enzyme polymorphism that could confer some adaptive advantages for the species.

4. Our investigations did not succeed in finding out a significant relation between the content of cardenolides and of soluble sugars.

5. The obtained results enable us to state that applying the treatments with physical and chemical mutagens may be the way to obtain some valuable new individuals (mutants) of *Digitalis lanata* for agriculture and pharmaceuticals.

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L'ORGANISATION SUPRACHROMOSOMIQUE CHEZ QUELQUES ESPÈCES ANIMALES ET VÉGÉTALES

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Data obtained by different techniques especially about interchromosomal fibres between telomeres and centromeres may be interpreted as an evidence of super-chromosomal organization of genetic material in the cell.

The interconnecting fibres of homologous or nonhomologous chromosomes explain the non-random distribution of chromosomes in mitosis and meiosis, genome segregation, non-equilibrated anaphase and the variation of chromosome number in meristematic and in the pollen mother cells.

A partir du modèle original proposé par Du Praw [14] selon lequel une chromatide est formée d'une seule macromolécule géante d'ADN pliée longitudinalement et transversalement, il semblait que le problème de l'organisation de la chromatine en chromosomes chez les Eucaryotes était résolu. Les chromosomes pouvaient être considérés en tant qu'unités complètement indépendantes, ayant une position aléatoire dans le noyau et donc une disjonction également aléatoire lors de l'anaphase, leur nombre correspondant au nombre de groupes de linkage.

Mais les recherches ultérieures sur la structure des chromosomes faites en microscopies optique et électronique, les expériences de microchirurgie sur des chromosomes métaphasiques isolés à partir de cellules vivantes, ainsi que l'analyse statistique de la position accidentelle de certains chromosomes dans la plaque métaphasique, ont démontré que les chromosomes sont, en réalité, reliés entre eux par des fibres télomériques, centromériques et intercalaires et que leur position n'est pas le fait du hasard.

En microscopie électronique [33] il a été observé qu'au niveau des télomères, les fibres de chromatine n'ont pas d'extrémités libres, mais tout au contraire qu'elles se plient et se retournent à l'intérieur de la chromatide. De plus, ces fibres lient les chromosomes les uns aux autres, bout à bout ou latéralement au niveau des centromères [48]. Les expériences de microchirurgie [26] pratiquées sur des cellules humaines, ont démontré que les chromosomes ne sont pas indépendants dans le noyau,

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du moins physiquement. Enfin, la réunion des chromosomes D et G chez l'Homme et le Chimpanzé, démontrée en interphase et en métaphase par plusieurs auteurs [16], [53], [12], [13], [1], le voisinage des chromosomes homologues au cours de la mitose chez les Rongeurs [22], ainsi que la distribution particulière des chromosomes dans la plaque métaphasique rencontrée, tant chez les animaux [51], [25], [6], que chez les végétaux [17], [50], révèlent un certain ordre dans lequel sont maintenus les chromosomes, ordre qui ne peut être expliqué que par l'existence de liens les unissant et par leur fixation à la membrane nucléaire.

A la suite de ces preuves obtenues à l'aide de techniques de grande résolution, il est donc facile de comprendre que l'on est passé du modèle de la fibre pliée de la chromatide, au modèle d'une organisation suprachromosomique, modèle selon lequel la fibre géante d'ADN-protéines pourrait dépasser le cadre d'un chromosome. Ainsi, chez les Eucaryotes, la même molécule géante d'ADN passerait d'un chromosome à l'autre pouvant, par exemple, former un set haploïde complet.

Cette nouvelle hypothèse de Du Praw [15] se base sur de nombreuses observations récentes effectuées en microscopie électronique, mais aussi sur d'autres fournies par la microscopie optique, certaines très anciennes, reprises à la faveur de cette hypothèse.

Les fibres de chromatine qui réunissent les chromosomes ne peuvent être observées par les méthodes classiques, du fait de l'action de la colchicine et d'autres substances utilisées dans les prétraitements, des chocs hypotoniques, ainsi que de la fixation par l'alcool-acétique. En effet, tous ces traitements provoquent plus ou moins la rupture des liens interchromosomiques.

MATÉRIEL ET MÉTHODES

Afin de mettre en évidence l'existence d'une organisation suprachromosomique chez les Eucaryotes, nous avons cherché à employer des matériels biologiques différents et à varier les techniques en fonction du matériel. Ainsi, nous avons étudié les chromosomes mitotiques et méiotiques chez quelques espèces de Rongeurs (*Mesocricetus auratus*, *Mus musculus*, *Cricetus cricetus*, *Rattus rattus*, *Citellus citellus*), les chromosomes interphasiques, prophasiques et métaphasiques chez *Allium cepa*, *Centaurea cuspidata*, *Lilium bosniacum*, *Solanum melongena*, *Crepis pantocekii* et *Crepis conyzaeifolia*, ainsi que les chromosomes géants des glandes salivaires de *Chironomus plumosus*.

Les techniques employées sont les suivantes :

1) *Recherche des micronoyaux à l'aide d'une coloration May-Grunwald Giemsa* [3] [23].

Les animaux sont sacrifiés sans être soumis à un prétraitement ; la moelle osseuse fémorale est prélevée et appliquée sur lame sous forme d'empreintes légères. On réalise également des frottis de testicules. Les lames, préalablement séchées à l'air, sont plongées dans une solution May-Grunwald (3 min.), puis lavées à l'eau bidistillée (1 min.) et colorées (30 min.) dans une solution de Giemsa (10%).

2) *Technique des bandes C modifiée pour mettre en évidence les fibres interchromosomiques* [31].

Cette technique a été utilisée chez le Hamster doré (*Mesocricetus auratus*), à partir d'empreintes ou de frottis. La moelle osseuse ou le testicule sont mis sous forme d'empreintes sur lames. Après séchage, ces lames sont traitées par l'alcool absolu (10 min.), séchées et plongées (5 min.) dans l'eau distillée. Ensuite, les préparations sont placées à la température de la pièce dans une solution saturée d'hydroxyde de baryum (5 min.), lavées à l'eau du robinet (30 min.) et traitées par $2 \times \text{SSC}$ à 65°C (2 h). La coloration est effectuée en solution de Giemsa (10%) pendant 15–20 minutes ; les lames, lavées à l'eau du robinet, sont séchées à l'air et montées en Eukitt.

Après une fixation légère (10 min.) de moelle osseuse ou de testicule par l'alcool acétique (3 : 1), le matériel est centrifugé (10 min.), puis étalé sur lame sous forme de frottis. Après séchage, les lames sont traitées conformément à la méthode des bandes C décrite ci-dessus.

3) *Technique des bandes T employée pour les végétaux*

Nous avons utilisé la technique de Stack et Clark [47] :

les racines non prétraitées sont fixées pendant une heure à la température de la pièce par l'alcool acétique (3 : 1). Le squash est fait dans l'acide acétique à 45% et les lamelles sont ôtées après avoir été gelées à -20°C [41]. Les lames sont alors séchées à l'air, puis traitées par une solution saturée de $\text{Ba}(\text{OH})_2$ à la température de la pièce (10 min.), lavées à l'eau distillée (5 min.) et placées à 60°C pendant une heure dans le tampon KH_2PO_4 0,12 M, pH 6,8. Nous avons réalisé ensuite la coloration avec une solution de Giemsa à 10% dans le même tampon. Le montage est fait en Eukitt.

4) Pour mettre en évidence, dans de meilleures conditions, les liaisons interchromosomiques chez *Centaurea cuspidata*, nous avons appliqué la technique de la coloration différentielle au Giemsa [34], en prolongeant la coloration jusqu'à 24 h [45].

5) *La coloration des chromosomes polytènes de Chironomus plumosus* a été faite par la technique habituelle d'étalement des glandes salivaires dans une goutte de carmin acétique.

RÉSULTATS ET DISCUSSIONS

Les résultats des observations faites sur le matériel étudié nous conduisent à discuter quelques aspects considérés comme des preuves d'une organisation suprachromosomique. Nous avons groupé ces résultats selon les techniques employées.

1) *L'arrangement des chromosomes et des fibres interchromosomiques en mitose et en méiose chez quelques Rongeurs* (*Mesocricetus auratus*, *Mus musculus*, *Rattus rattus*, *Citellus citellus*, *Cricetus cricetus*) observé par la technique des micronoyaux.

D'après le modèle classique, les chromosomes des Eucaryotes seraient constitués d'un réseau de fibres de plus en plus contractées, de

l'interphase jusqu'à la métaphase. Il a été décrit, par plusieurs auteurs et nous-mêmes, des configurations métaphasiques ordonnées dans lesquelles tous les chromosomes sont situés sur une circonférence. Celles-ci, dénommées configurations radiales, ont été observées chez diverses espèces, par exemple chez *Vicia faba* [29] [38], dans les tumeurs de Souris [4], etc. D'abord considérées comme des aberrations induites par différents agents mutagènes physiques ou chimiques, ces configurations peuvent également être interprétées d'une manière différente. Malgré l'application de la préfixation, du choc hypotonique et de la fixation, ou même de quelques traitements mutagéniques sur les chromosomes, quelques cellules à configurations radiales auraient pu échapper à l'effet de ces traitements, ou bien ces traitements auraient maintenu de telles configurations. Ce genre de configurations radiales, maintenues ou induites par des agents mutagéniques, peut être observé sur des préparations réalisées selon la technique conventionnelle [18] chez la Souris (fig. 1 et 2).

En outre, grâce à la technique des micronoyaux, nous avons remarqué que toutes les configurations métaphasiques radiales observées (fig. 3) sont des configurations normales des chromosomes surprises à un moment donné de la division, quand ils sont attachés sur les fibres du fuseau nucléaire. Cette affirmation est possible car, dans cette technique, il n'a été utilisé ni traitements à base de colchicine, ni fixateur, ou solutions hypotoniques.

Dans les configurations radiales, bien que les chromosomes soient très proches les uns des autres, on observe des fibres interchromosomiques et des boucles qui se replient à l'intérieur de la chromatide, ce qui fait penser à une continuité de la fibre de chromatine. Dans la métaphase mitotique de *Cricetus cricetus* de la fig. 4, dans la diacinèse de *Mesocricetus auratus* de la fig. 5, on remarque des liaisons interchromosomiques indiquées par des flèches. De telles liaisons ont été signalées par d'autres auteurs [7], [49], [30], [9].

Ces fibres contiennent probablement de l'ADN répétitif sans gènes détectables [24]. Observées, tant en microscopie optique qu'en micro-

Fig. 1 et 2. — Configurations mitotiques radiales chez *Mus musculus* après traitements par des pesticides.

Fig. 3. — Configuration mitotique radiale chez *Mesocricetus auratus* mise en évidence par la technique des micronoyaux.

Fig. 4. — Métaphase mitotique chez *Citellus citellus*: nombreuses liaisons entre les chromosomes (technique des micronoyaux).

Fig. 5. — Diacinèse chez *Mesocricetus auratus*: nombreux liens entre les bivalents (techniques des micronoyaux).

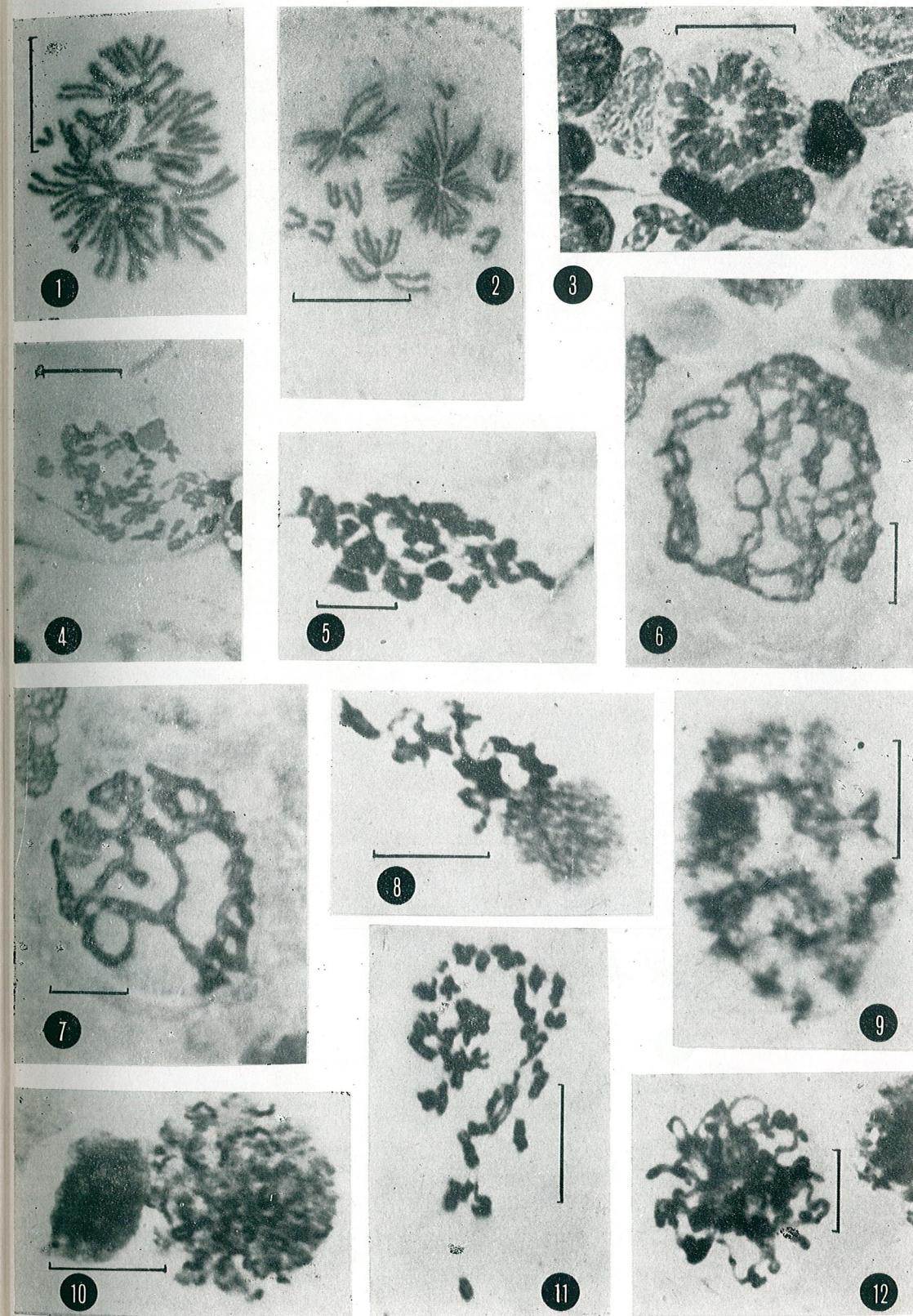
Figs. 6 et 7. — Diplotène (6) et diacinèse (7) chez *Rattus rattus*: disposition circulaire continue des chromosomes (technique des micronoyaux).

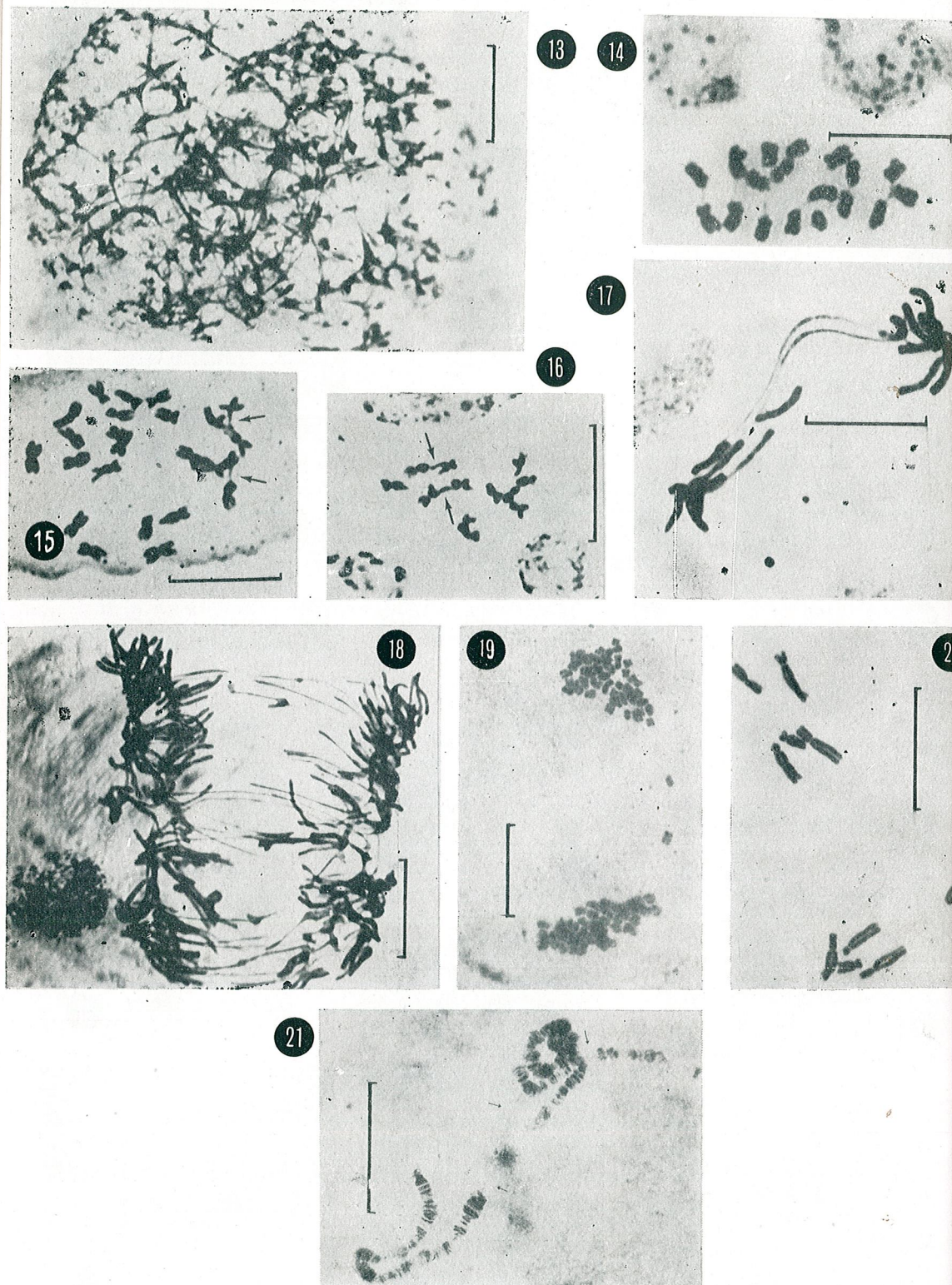
Fig. 8. — Diacinèse chez *Mesocricetus auratus*: liaisons entre les chromosomes (technique de bandes C, sans hypotonie et sans fixation).

Fig. 9 et 10. — Le stade diffus de la méiose chez *Mesocricetus auratus*: liaisons entre les chromosomes et chromatine disposée en réseau.

Fig. 11. — Fibres interchromosomiques dans la métaphase mitotique de *Mesocricetus auratus* (technique de bandes C modifiée).

Fig. 12. — Télomères condensés disposés à la périphérie du noyau chez *Allium cepa*. On peut remarquer l'aspect diffus du matériel génétique à l'intérieur du noyau et la continuité de la fibre de chromatine (technique de bandes T).





scopie électronique, elles constituent une réalité physique qui n'est pas un artefact, mais leur signification précise n'étant pas connue, on peut émettre à leur propos différentes hypothèses.

Particulièrement significatives sont les figures 6 et 7 qui peuvent appuyer l'idée de base de Chiarelli [8] [10] pour qui les deux génomes d'une cellule diploïde sont organisés en deux unités, la fibre de chromatine étant continue et circulaire pour chacune d'elles. Chez le Rat, cette fibre, en diplotène (fig. 6) et en diacinese (fig. 7), n'est pas du tout interrompue, les chromosomes étant liés l'un à l'autre et constituant une double chaîne fermée, poussée vers la périphérie du noyau.

La position non accidentelle des chromosomes dans le noyau est presque la même pendant toute la durée de la mitose. Elle suppose l'attache des chromosomes à la membrane nucléaire en interphase et partiellement en prophase d'une part et l'existence de fibres de liaison d'autre part [11] [39]. Il est probable que, en anaphase, ces liens sont rompus parce qu'ils sont très minces et n'ont pas de fonctions de transcription [2]. Ainsi, les chromosomes peuvent migrer vers les pôles en positions bien déterminées, celles-ci étant les mêmes qu'en interphase. En télophase, dès le commencement de la réorganisation de la membrane nucléaire, les chromosomes apparaissent de nouveau liés à celle-ci en des lieux spécifiques.

2) *Fibres interchromosomiques en mitose et en méiose chez le Mesocricetus auratus observées sur des préparations effectuées par la technique des bandes C modifiée*

Nous devons tout d'abord souligner le fait que cette technique a été appliquée sur du matériel mitotique et méiotique n'ayant pas subi de choc hypotonique. Klasterska et Ramel [32] ont, en effet, démontré que le choc hypotonique pratiqué dans la technique de préparation des chromosomes de Mammifères, entraîne la rupture des liens entre les chromosomes. Au contraire, l'absence de choc hypotonique permet de conserver les fibres de chromatine de la surface des chromosomes formant un réseau interchromosomique. En éliminant l'hypotonie et en employant la technique des bandes C, il est possible de mettre en évidence de nombreux détails de la structure chromosomique, principalement au stade diffus de la méiose, ce que ne permettent pas les techniques habituelles.

Fig. 13. — Blocs d'hétérochromatine en interphase méiotique chez *Lilium bosniacum*.

Fig. 14. — Fibres interchromosomiques dans une métaphase mitotique de *Centaurea cuspidata*.

Fig. 15 et 16. — Fibres interchromosomiques dans une métaphase mitotique de *Solanum melongena* à l'état diploïde (fig. 15), à l'état haploïde (fig. 16).

Fig. 17. — Ponts chromosomiques et micronoyaux (*Crepis conyzaeifolia*).

Fig. 18. — Anaphase II (*Lilium bosniacum*) avec des ponts.

Fig. 19. — Métaphase mitotique en culture de tissus, avec ségrégation des génomes (*Nicotiana tabacum*).

Fig. 20. — Métaphase mitotique en méristèmes racinaires (*Crepis pantocephala*).

Fig. 21. — Liaisons interchromosomiques entre les chromosomes géants (*Chironomus plumosus*).

L'échelle représente 10 μ m.

Nous avons ainsi remarqué, en diacinèse, les liaisons interchromosomiques (fig. 8) au stade diffus de la méiose (pachytène retardé), l'aspect en réseau de la fibre de chromatine en surface des chromosomes, ainsi que les liens entre chromosomes (fig. 9). Le même aspect en réseau a été observé également dans des préparations faites selon la méthode conventionnelle pour les chromosomes méiotiques, mais sans prétraitement à la colchicine (fig. 10).

A l'aide de la même technique des bandes C, sur des préparations obtenues sans choc hypotonique et avec seulement une fixation légère (10 min.), il a été possible d'observer des fibres interchromosomiques en mitose (fig. 11). On peut également mettre en évidence des chromosomes en chaîne ayant tendance à occuper la périphérie du noyau en division.

3) *La présence de blocs d'hétérochromatine à la périphérie du noyau interphasique et prophasique chez les végétaux*

La présence de masses d'hétérochromatine à la périphérie du noyau interphasique et prophasique est un phénomène bien connu. Elle peut expliquer l'attachement des chromosomes à la membrane nucléaire interne par l'intermédiaire de télomères et de centromères d'origine hétérochromatique. Ce fait a été mis en évidence, en particulier par des études de microscopie électronique [52], [27], à la suite desquelles ce dernier auteur a proposé l'hypothèse du « body guard chromatin ». L'euchromatine fibrillaire du noyau a comme origine les masses hétérochromatiques périphériques. L'intérieur de celui-ci prend ainsi un aspect diffus. En prophase mitotique, chez *Allium cepa*, nous avons observé distinctement des blocs d'hétérochromatine à la périphérie du noyau, tandis qu'à l'intérieur de celui-ci, l'euchromatine est diffuse (fig. 12). Le même phénomène a été décelé chez *Lilium bosniacum* en interphase méiotique (fig. 13).

4) *Les liaisons interchromosomiques chez les végétaux*

Comme nous l'avons déjà signalé plusieurs fois chez différentes espèces végétales [37], [43], des liaisons interchromosomiques existent, aussi bien en mitose qu'en méiose, chez les diploïdes et les haploïdes.

Ainsi, grâce à une coloration différentielle (bandes C) prolongée chez *Centaurea cuspidata*, des fibres interchromosomiques, probablement d'origine hétérochromatique, sont visibles (fig. 14). Elles se révèlent très solides puisqu'elles résistent à différents traitements cyostatiques, fixation et « squash ». Chez cette espèce, les liaisons entre les chromosomes homologues satellifères s'effectuent au niveau des satellites qui sont de nature hétérochromatique. Le même phénomène a été remarqué chez *Reichardia picroides* [42]. L'hétérochromatine pourrait donc avoir un rôle important dans l'organisation suprachromosomique.

Chez *Solanum melongena* [44], il existe des liaisons interchromosomiques à l'état diploïde (fig. 15), comme à l'état haploïde (fig. 16). Ces liaisons sont surtout présentes entre les chromosomes qui montrent un retard dans le cycle de spiralisation.

5) Un autre problème concernant l'organisation du matériel génétique est l'origine de deux sortes de ponts chromosomiques en anaphase :

— les uns produits par la rupture/réunion de chromosomes, phénomène ayant pour résultat un chromosome dicentrique et un fragment

acentrique, et qui peuvent être considérés comme des aberrations chromosomiques (induites ou spontanées) donnant naissance en interphase aux micronoyaux (fig. 17);

— les autres constitués par des fibres reliant les chromosomes entre eux, de l'interphase à la métaphase. Ces fibres peuvent s'étirer et se rompre, les chromosomes arrivant intacts aux pôles de la cellule (fig. 18). On ne peut pas considérer ce genre de ponts comme des aberrations chromosomiques, parce qu'ils n'impliquent ni la rupture des chromosomes, ni des échanges. De tels ponts ont été signalés [20] chez *Secale cereale*.

6) *La tendance à la séparation des génomes dans les cellules somatiques végétales*

Chez les espèces amphiploïdes, la ségrégation des génomes, dans le cas des cellules somatiques en culture « in vitro », suggère une organisation suprachromosomique du matériel génétique. Ainsi, nous avons observé une tendance à la séparation des génomes dans les cellules des pieds de Tabac obtenus par androgenèse et diploïdisés par la colchicine [36] (fig. 19).

Il est probable que des liaisons existeraient entre les chromosomes de chaque génome, celles-ci ayant pour conséquence un comportement unitaire de chacun d'eux. Chez *Crepis pantocekii*, espèce diploïde à $2n = 2x = 10$, la ségrégation des génomes a été mise en évidence. En effet, grâce à la morphologie différente de chaque paire chromosomique, il est facile de reconnaître les homologues dans chacun des deux lots (fig. 20).

7) *Fibres interchromosomiques entre les chromosomes polytènes chez le Chironomus plumosus*

Dans ce cas, des connexions concernent des bandes non homologues du même chromosome ou de chromosomes différents. Ce type de liaison a été nommé [28] « associations non homologues de chromosomes », alors que [46] on a proposé pour ce phénomène le terme de « connexion ectopique ». De telles liaisons ont été décrites pour la première fois chez *Drosophila melanogaster* [5]. Le phénomène a été observé à nouveau chez *Anopheles stephensi* [35] et les *Aphidoletes sp.* [21]. Pour *Chironomus plumosus*, nous avons retrouvé des associations comparables entre chromosomes non homologues (fig. 21). Elles se font bout à bout (end-to-end) et peuvent être interprétées conformément au modèle de l'organisation suprachromosomique. Ainsi, quelques chromosomes d'un génome ou tous les chromosomes d'un set chromosomique sont liés entre eux.

CONCLUSION

L'organisation suprachromosomique du matériel génétique de la cellule des Eucaryotes, dont nous avons présenté diverses manifestations, a conduit Du Praw (*loc. cit.*) à penser que tous les chromosomes d'un génome ne seraient peut-être constitués que d'une seule molécule géante d'A.D.N. Certes, toutes les espèces ne révèlent pas, par exemple, de liaisons interchromosomiques, du moins à l'aide des techniques habituelles, mais il n'est pas interdit de penser que, dans ce cas, ces techniques pour-

raient être en cause. Si la notion d'organisation suprachromosomique devait se généraliser, elle nous ramènerait, en grande partie, à celle du spirème qui a prévalu pendant longtemps. Selon cette conception, l'individualisation des chromosomes serait précédée par la formation d'un long filament chromatique unique, le spirème, qui secondairement se scinderait transversalement en autant de tronçons que de chromosomes. Mais cette interprétation contestée a été abandonnée, les auteurs pensant que le spirème ne correspondrait seulement qu'à une impression de continuité entre des filaments chromatiques en réalité discontinus.

On doit se demander si l'existence de liaisons interchromosomiques pourrait avoir des conséquences sur le déroulement normal de la mitose et de la méiose. En fait, il est possible d'envisager certaines perturbations. En effet, si les fibres de liaison ne se brisent pas toutes avant ou au cours de l'anaphase, la répartition des chromosomes mitotiques ne sera pas équationnelle, ce qu'illustrent les cas d'hétéropléidie au sein des méristèmes, à la suite d'anaphases asymétriques. Même au niveau des grains de pollen le nombre chromosomique n'est pas toujours constant. La disjonction non équilibrée pourrait être, au moins dans certains cas, en rapport avec une organisation suprachromosomique. Sans multiplier les exemples, rappelons que le genre *Crepis* montre, à la fois, une variabilité chromosomique des méristèmes racinaires [19], du pollen [40] et une ségrégation des génomes.

Certes, l'organisation suprachromosomique n'est pas démontrée pour toutes les espèces et de nombreux travaux restent à faire mais, sans nul doute, cette notion doit être prise en compte, en particulier chez les végétaux où la variabilité chromosomique des cellules végétatives et même des cellules reproductrices est souvent si déconcertante.

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EXPERIMENTAL ANDROGENESIS IN *DATURA INNOXIA* PLANTS WITH DIFFERENT LEVELS OF PLOIDY (2n, 4n, 6n)

BY
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The androgenetic ability of 4n and 6n *Datura innoxia* plants and the cytogenetic variability of the plants obtained by the anther culture were studied. The variation of the ploidy level of androgenetic plants is correlated with the ploidy level of the donor plants.

By the culture of anthers coming from diploid plants, haploids, diploids, triploids, tetraploids in a variable number were obtained, and by the culture of anthers coming from 4n and 6n plants, almost exclusively diploid and respectively triploid plants, were obtained, which proves that the nuclear fusions that frequently occur in haploid spores are more scarce in the diploid and triploid spores.

In 1964, Guha and Maheshwari proved, for the first time with *Datura innoxia*, the pollen ability of developing "in vitro" anther cultures and of generating haploid plants.

Subsequently, a series of researches made in this species with high androgenetic ability have been aimed at establishing the action of some physical and chemical factors on the development of embryoids [5], [11]–[13], [16], [17], the role of nuclear fusions on pollen embryogenesis [16], the ultrastructure of pollen in anther cultures [1]–[3], the correlation between flower button size and the level of ploidy in androgenetic plants [4].

While the first plants obtained were haploid ones [7], subsequent researches have shown that by the culture of anthers with *Datura innoxia* (2n = 24), 2n (10), 3n, 4n (4) and even 6n plants were obtained as a result of some nuclear events taking place in the culture and which involve vegetative and generative nuclei [14], [15].

Our researches were aimed at establishing the androgenetic ability of *Datura innoxia* plants with an even number of chromosomes (2n, 4n, 6n). We also followed if the mechanisms which act in the case of haploid pollen are similarly efficient in the case of diploid and triploid pollen and if the cytogenetic variability of the plants obtained by experimental androgenesis with *Datura innoxia* 4n and 6n is as high as that of 2n plants.

MATERIALS AND METHODS

The materials used had three levels of ploidy: diploid (2n = 24), tetraploid (4n = 48) and hexaploid (6n = 72) plants obtained by anther culture or by regeneration from callus culture.

The medium used for the anther culture was the Nitsch and Nitsch [9] basic medium supplemented with 0.1 mg/l indole-acetic acid. All the anther cultures were kept for 16 hours under light and for 8 hours in darkness.

Plantlets produced by the anther culture were transplanted into the Murashige-Skoog medium [8] without any growth regulators, for rooting.

Chromosomes were counted in the root tip squashes after Feulgen staining.

RESULTS AND DISCUSSIONS

By the culture of anthers which come from *Datura innoxia* plants with an even number of chromosomes (2n, 4n, 6n), we obtained n, 2n, 3n, 4n, 6n plants and aneuploids with varied frequency (Table 1).

Table 1

Chromosome number of androgenetic plants produced by anther culture of plants with an even ploidy chromosome number

Chromosome number of donor plant	No. of plants examined	No. of plants with a varied level of ploidy and aneuploids					
		n	2n	3n	4n	6n	aneuploid
2n = 24	72	15	45	11	1		
2n = 48	50	—	47	—	2		1
2n = 72	66	—	2	56	—	3	5

The yield capacity of anthers (number of plants per anther) was higher in cultures coming from 2n and 4n plants than in those from 6n plants.

The time of plant development from pollen was not influenced by the level of ploidy of the donor plant. In all cultures, after 12–14 days, there appeared, through the side openings of anthers, numerous embryoids, in different development stages, which were immediately parted to avoid their concrescence.

From the data presented in Table 1 it can be seen that the *Datura innoxia* plants with an even number of chromosomes have androgenetic ability and the variation of the ploidy level of the plants obtained by anther culture is correlated with the level of ploidy of donor plants.

The cytogenetic study of androgenetic plants has also shown that as the genome number increases, the nuclear fusions which generate this variability do not take place with the same frequency [4].

So, by the culture of anthers coming from diploid plants, we obtained n, 2n, 3n, 4n plants, in a variable number, and by the culture of anthers coming from 4n and 6n plants, we obtained only diploid plants, respectively triploid ones, which proves that the nuclear fusions which frequently occur in the haploid spores are more scarce in the diploid and triploid spores. In the androgenetic descendents coming from

tetraploids and hexaploids, a small number of aneuploids was obtained with 34, 35 and 38 chromosomes having normal growth and development. In the culture of *Datura innoxia* anthers coming from 6n plants, abnormal plantlets were observed which did not develop, or got a teratologic aspect in time. These abnormal plantlets came from genetically nonequilibrated gametes which appear more frequently at the hexaploid level than at the tetraploid or diploid one and which do not permit a normal cytodifferentiation.

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THE WORKING SESSION OF THE REPRESENTATIVES
OF THE NATIONAL COMMITTEES FROM SOCIALIST
COUNTRIES FOR THE INTERNATIONAL UNION
OF BIOLOGICAL SCIENCES (IUBS)
BUCHAREST, 22-24 JUNE 1982

After the meeting held in June 1981 in Budapest, this 6th working session was organized on 22-24 June in Bucharest, under the sponsorship of the Academy of the Socialist Republic of Romania. The members of the Romanian National Committee were pleased to welcome the following guests: acad. prof. V. Landa from Czechoslovakia, prof. J. Salanki and dr. P. Biro from Hungary, prof. A. Urbanek from Poland, acad. prof. M. S. Ghilarov and dr. S. G. Vassetzky from USSR. The agenda of this session included such topics as the participation of delegations from the socialist countries to the XXI IUBS General Assembly in Ottawa, Canada, August 1982, the election of the officers of the new Executive Committee of the IUBS and the problems of the future structure of the IUBS divisions. There were also discussed proposals for different research projects of general or regional interest to be carried out within the framework of IUBS, i.e. the establishment of a group of systematic and evolutionary biology, at the request of the Polish National Committee and completion of the necessary research for drawing up the vegetation map of Europe. Plans were submitted for the organization in 1984, in the USSR, of a Symposium on the early stage of ontogeny and of a European conference on cell biology in Hungary, in 1986. Wishes were expressed for a closer cooperation between the scientific institutions of the socialist countries, especially in the following fields: publication of scientific journals by an international editorial board, production of scientific equipment and chemicals, rearing of laboratory animals, genetically controlled. Visits were paid to the Institute of Cellular Biology and Pathology, the Institute of Biological Sciences, the "Fundulea" Research Institute for Cereals and Technical Plants and the "Gr. Antipa" Natural History Museum, or others, optionally.

During all these contacts the stringent need appeared for a permanent climate of peace absolutely necessary to assure human welfare and the progress of scientific work through the cooperation of world biologists within IUBS in relation with UNESCO and ICSU to firmly act against the threats of armed conflagrations and especially of a nuclear war.

The following opening address by acad. prof. Radu Codreanu, on behalf of the Romanian National Committee for IUBS, summarizes the scientific trends and the pacific aims of biologists from socialist countries who gathered in Bucharest.

Dear Colleagues,

It is a great honour and pleasure for the Academy of the Socialist Republic of Romania to offer its hospitality to the present working session of the prominent representatives of the National Committees for the International Union of Biological Sciences, IUBS, from socialist countries. We are happy to express the warmest welcome to Bucharest to our guests from Czechoslovakia, Hungary, Poland, the Soviet Union, and we regret the absence of our colleagues, biologists from Bulgaria, Yugoslavia and the German Democratic Republic.

The goal of the International Union of Biological Sciences is the better coordination and cooperation between the activities of biological organizations from different countries. Since geographic proximity raises many common problems for biological research, periodic exchanges of our results proved to be very useful.

Modern biology is highly diversified, including topics on molecular processes in the living cells as well as questions of the dynamics of the ecosystems of the biosphere. This multiplicity of branches of biological knowledge should be reflected more and more adequately so as to be employed by the IUBS divisions in bringing to the fore the vital importance of advances in fundamental and applied biology for the development of human society.

The use of physical and chemical technologies was certainly decisive for the revolutionary progress of biology in this century. But, it must not be forgotten that all scientific activities are due only to the creative powers of the human brain in our capacity of rationally acting beings. Therefore, the field of biological implications is much more extensive and acquires a basic significance during our endeavours to clear up the major factors of interest in human evolution. Indeed, biology is able to supply us the theoretical elements for the understanding of man's place within the evolutionary history of life on earth and also to solve, by means of its applied disciplines, many important practical problems, such as man's health, the preservation of the natural equilibrium, the increase of the biological productivity confronted with the demographic overflow.

The present complexity of the difficult relationship between mankind and its environment runs the risk of heavy deterioration as a result of the energy crisis and the nuclear arms race. Let us hope that the strong movements for peace recently started throughout the world and which have been long and firmly promoted by the President of Romania, Nicolae Ceaușescu, shall prove successful and efficient.

The setting up, in the last year, after the 16th International Congress of the History of Science held in Bucharest, of an official body called "Scientists and Peace", presided over by Academician Elena Ceaușescu, D. Chem. Eng., first-vice-prime minister of the Government of the Socialist Republic of Romania, President of the National Council for Science and Technology, gives an optimistic suggestion that the International Union of Biological Sciences may also constitute an important background for the defence of peace since biologists are best qualified to appreciate the wonderful unicity of life and the human species on our planet.

The evolution of the biosphere was a multi-millenary way that led to the emergence of human consciousness which has the unique privilege of reflecting universal reality. Without mankind, there will be nothing left to attest the eternal organizing movement of matter, or our splendid cultural values. It is therefore our most justified common duty to preserve humanity and the biosphere against the radical destructions of a nuclear war.

The trends for scientific cooperation of the International Union of Biological Sciences are both pacific and transdisciplinary.

With all this in mind, we wish our participants to broach with fruitful achievements the topics listed on the agenda of our three-day meeting and believe that the following report of acad. M. S. Ghilarov on the last session of the IUBS Executive Committee, Paris, April 1982, will introduce us into the most topical problems before the next General Assembly to be held on August 22–27, in Ottawa, Canada.

The evolution of the biosphere was a multi-millenary way that led to the emergence of human consciousness which has the unique privilege of reflecting its own development. It is therefore our duty to preserve the conditions that have made possible the development of the biosphere and the human mind. The evolution of the biosphere was a multi-millenary way that led to the emergence of human consciousness which has the unique privilege of reflecting its own development. It is therefore our duty to preserve the conditions that have made possible the development of the biosphere and the human mind.

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AVIS AUX AUTEURS

La « Revue roumaine de biologie - Série de biologie végétale » publie des articles originaux d'un haut niveau scientifique, de tous les domaines de la biologie végétale : morphologie, systématique, géobotanique, physiologie, écologie, génétique, microbiologie, phytopathologie. Les sommaires des revues sont complétés par d'autres rubriques, comme : 1. La vie scientifique, qui traite des manifestations scientifiques du domaine de la biologie : symposiums, conférences, etc.; 2. Comptes rendus des livres de spécialité parus en Roumanie. Les auteurs sont priés d'envoyer leurs articles, notes et comptes rendus dactylographiés en deux exemplaires. Les tableaux et l'explication des figures seront dactylographiés sur pages séparées et les diagrammes seront exécutés à l'encre de Chine noire, sur papier calque.

Les tableaux et les illustrations seront numérotés avec des chiffres arabes. La répétition des mêmes données dans le texte, dans les tableaux ou dans les graphiques sera évitée.

Les références bibliographiques, citées par ordre alphabétique, comporteront le nom de l'auteur, l'initiale du prénom, le titre de la revue, abrégé conformément aux usances internationales, l'année, le tome, le numéro, la page. Les travaux seront accompagnés d'un court résumé, de maximum 10 lignes, en anglais. Les textes des travaux ne doivent pas dépasser 7 pages dactylographiées (y compris les tableaux, la bibliographie et l'explication des figures). La responsabilité concernant le contenu des articles revient exclusivement aux auteurs.