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SOMMAIRE

CONSIDÉRATIONS CRITIQUES SUR QUELQUES TAXONS DU GENRE *OROBANCHE* L.

PAR

TRAIAN I. ȘTEFUREAC et LIVIA UNGUREAN

L'étude morpho-anatomique et taxonomique effectuée sur tous les organes des plantes de *Orobanche ramosa, O. brassicae, O. mutelii* a permis d'établir le rapport qui existe entre *O. ramosa* et *O. brassicae*. Les taxons *O. ramosa* et *O. brassicae* peuvent être considérés comme des espèces indépendantes, présentant de nombreux caractères différenciels très significatifs.
En ce qui concerne l'espèce *Orobanche brassicae* dont la morphologie est identique à celle de *O. mutelii*, les auteurs croient qu'on devrait plutôt la considérer comme ssp. *brassicae* Novopokr. de *O. mutelii* Schultz.

Outre les caractères morphologiques, la spécificité du parasite pour la plante-hôte a un rôle important dans l'identification des taxons parasites du genre *Orobanche* L.

Selon I. V. Novopokrovski [4], ce caractère suffit pour délimiter certaines espèces. Ainsi l'espèce *Orobanche brassicae* Novopokr. est connue comme un parasite monophage de *Brassica oleracea* L. Novopokrovski l'a fait dériver de l'espèce parasitaire polyphage *O. mutelii* Schultz, tout d'abord (1928) [1] comme sous-espèce *(O. mutelii* Schultz ssp. *brassicae* Novopokr.), puis (1929) [4] comme espèce *(O. brassicae* Novopokr.).

Orobanche brassicae Novopokr. est connue dans la flore de Roumanie depuis 1955 quand C. Zahariadi et Gh. Anghel l'ont signalée dans la culture de choux de Băneasa, près de Bucarest [6]. Avec le même binôme il est considéré aussi par Al. Buia (Flora R. S. România, VIII, 1961, p. 40) [1].

Le taxon avait été cité antérieurement mais sous d'autres noms : *Phelipaea ramosa* L. var. *mutelii* Boiss. (I. Grințescu, 1914) [3]; *O ramosa* L. (I. Prodan, 1938) [5].

Vu que *Orobanche mutelii* est absente de la flore de Roumanie, nous n'éprouvons pas de difficultés provenant de l'absence des caractères différentiels entre *O. mutelii* et *O. brassicae*. En échange, la présence des deux taxons dans la flore d'un pays est la cause de certains inconvénients que A. O. Chater et D. A. Weeb, qui ont analysé et commenté les espèces de la fam. *Orobanchaceae* dans la Flora Europaea (t. 3, 1972) [2], ont évité en éliminant les espèces *O. mutelii* Schultz et *O. brassicae* Novopokr. et en réactualisant le taxon *O. ramosa* L. ssp. *mutelii* (Schultz) Couthino, décrit par Couthino en 1913.

Sans minimaliser la valeur des taxons acceptés par Chater et Weeb, mais en même temps sans surestimer la valeur des autres taxons *(O. mutelii*,

O. brassicae) nous croyons qu'il faut garder le nom des taxons dont les limites taxonomiques peuvent être déterminées à l'aide des caractères morphologiques et anatomiques évidents, surtout les particularités de la structure de la tige (fig. 1) et de la testa des graines (fig. 2).

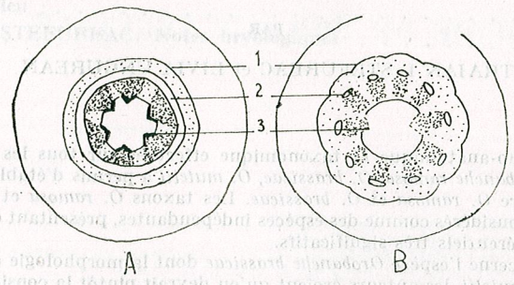

Fig. 1. — Schéma de la structure de la tige chez *Orobanche ramosa* L. (A) en comparaison de *O. mutelii* Schultz et *O. brassicae* Novopokr. (B) : 1) sclérenchyme extrafasciculaire annulaire (A), calotté (B) ; 2) libre annulaire (A) et insulaire (B) ; 3) gaine de sclérenchyme (orig.).

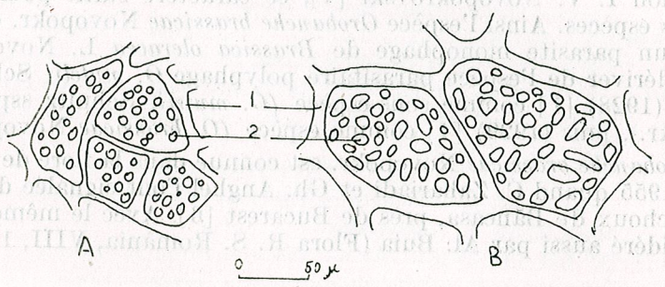

Fig. 2. — Fragment de la testa chez *Orobanche ramosa* L. (A) en comparaison de *O. mutelii* Schultz et *O. brassicae* Novopokr. (B) : 1) réticule principal ; 2) réticule secondaire (orig.).

Dans ce but on a comparé, du point de vue morphologique et anatomique, les taxons : *Orobanche ramosa*, *O. brassicae*, *O. mutelli* et les résultats sont présentés dans le tableau 1.

L'étude morpho-anatomique des trois espèces met en évidence l'existence de plusieurs caractères différentiels significatifs entre les taxons *Orobanche ramosa* et *O. mutelii* d'une part et entre *O. ramosa* et *O. brassicae* d'autre part et leur absence entre les espèces *O. brassicae* et *O. mutelii*. Ces résultats ne nous permettent pas de considérer l'espèce *O mutelii* comme sous-espèce de *O. ramosa*. En échange, comme l'espèce *O. brassicae* Novopokr. ne présente pas d'autres caractères différentiels par rapport à *O. mutelii* excepté sa spécificité plus accentuée pour la plante-hôte *Brassica ole-*

Tableau 1

Caractères différentiels entre les espèces analysées

No.	*O. ramosa* L.	*O. brassicae* Novopokr.	*O. mutelli* Schultz
	A. Caractères morphologiques et leur signification		
1.	Tiges ramifiées, jaunes	Tiges ramifiées, bleues-violacées ***	Comme chez *O. brassicae*
2.	Fleurs de 15—17 mm	Fleurs de 20—25 mm ***	
3.	Corolle jaune avec des lèvres lilas	Corolle bleue ***	
4.	Anthères avec des touffes de poils laineux sur le connectif	Anthères glabres ou avec des poils courts***	—„—
5.	Style glanduleux à poils apicaux et basaux	Style glabre ***	—„—
6.	Capsule noire avec des valves rostrées, de dimensions pareilles au calice	Capsule brune avec des valves émarginées plus grandes que le calice	—„— —„—
7.	Graines de 318 μ	Graines de 460 μ	—„—
	B. Caractères anatomiques et leur signification (fig. 1, 2)		
8.	Tige avec sclérenchyme extrafasciculaire annulaire, libre annulaire, gaine de sclérenchyme dans la partie périmédullaire des fascicules	Sclérenchyme extrafasciculaire, calotté ***, gaine de sclérenchyme seulement chez quelques fascicules **	—„— —„—
9.	Réticule principal (cellules de la testa) de 79 μ	Réticule principal de 107 μ	—„—
10.	Réticule secondaire (les pores des cellules de la testa) de 6 μ	Réticule secondaire de 12 μ	—„—
	C. Caractères biologiques et leur signification		
11.	Parasite polyphage	Parasite plutôt monophage	Parasite polyphage **

** caractère distinct significatif
*** caractère très significatif

racea L., nous croyons qu'il est plus juste de la maintenir comme sous-espèce de l'espèce *O. mutelii* Schultz, comme elle a été décrite par I. V. Novopokrovski en 1928 *(O. mutelli* Schultz ssp. *brassicae* Novopokr.).

CONCLUSIONS

1. Le nombre important de caractères morpho-anatomiques et leur signification justifient le maintien des taxons *Orobanche ramosa* L. et *O. mutelii* Schultz comme espèces indépendantes.

2. L'absence des caractères morpho-anatomiques différentiels entre *Orobanche mutelii* Schultz et *O. brassicae* Novopokr. impose le retour au taxon décrit initialement par I. V. Novopokrovski (1928) [1] — *Orobanche mutelii* Schultz ssp. *brassicae* Novopokr.

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CYTOTAXONOMICAL AND CHOROLOGICAL INVESTIGATIONS ON THE ENDEMIC SPECIES *POLYSCHEMONE NIVALIS* SCHOTT, NYM. & KOTSCHY

BY

TRAIAN I. ȘTEFUREAC and AURICA TĂCINĂ

The paper presents a new contribution to the study of endemites in Romanian flora. The investigations refer to two endemic populations of *Polyschemone nivalis* Schott, Nym. & Kotschy, a monotype genus, belonging to subfam. *Silenoideae* A. Br., fam. *Caryophyllaceae* Juss., a plant strictly limited to the Rodna Mountains. The results refer to the species with respect to its taxonomy, morpho-anatomy, palinology, the ecology, cenology, chorology and caryology, establishing also, for the 1st time, the caryotype ($2n = 24$). Since this stenochory endemite in the Rodna Mountains, the Eastern Carpathians of Romania, enjoyed a much disputed taxonomic position, the results presented in the paper further contribute to consider this endemic taxon as an independent, relict species *Polyschemone nivalis* Schott, Nym. & Kotschy and not included in genus *Lychnis* L.

INTRODUCTION

Among our investigations on endemic plants in Romanian flora, a special place is occupied by the only stenochory endemite of a superior rank, *Polyschemone nivalis* Schott, Nym. & Kotschy, subfam. *Silenoideae* A. Br., fam. *Caryophyllaceae* Juss. The species is confined to a small area in the Rodna Mountains, the Eastern Romanian Carpathians.

In the Romanian flora (II, 1953, 141), [19], this endemite is present as an independent monotype genus, with taxonomic justification by M. Guşuleac [7]. In Flora Europaea (I, 1964, 156, 157) [20], this plant included in genus *Lychnis* L., as *Lychnis nivalis* Kit., further commented on by A. D. Chater [3], described with his diagnosis in 1814 (in Schultes Oesterr. Fl. I., 1814, 698). The taxonomic value of its name *Polyschemone* results also from the fact that within genus *Silene* L. Sect. *Pleiogyne*, it is delimited as Subsect. III *Polyschemone* Pax & Hoffm. (Engler & Prantl, Nat. Pflanzenfam. III, 2 (1934) 346 (6), and within genus *Melandrium* Röhl. Sect. a it is called *Polyschemone* Nym. (Consp., 1879, 86, (9).

The wealth of synonyms : Lychnis nivalis Kit., Silene siegeri Baumg., Agrostemma nivalis G. Don, Lychnis siegeriana Schur, Viscaria siegeri Gris. & Schenk, Melandrium nivale Nym., Silene nivalis (Kit.) Rohrb., Viscaria nivalis Simk. [2], [7], [19], [20], denotes evidently the fact that this taxon was, taxonomically and nomenclatorically, much disputed for more than 70 years (1814—1884), belonging to five different genera; not even after 170 years (1814—1984), has its position been fully established.

The analysis of generic characteristics, in comparison with the other genera, allowed prof. M. Gușuleac [7] to consider it an independent genus in our flora on the ground of the floral elements polymorphism and polymery.

MORPHO-ANATOMICAL AND PALINOLOGICAL CONSIDERATIONS

The uncertainty of the unclear taxonomic position of the plant subfamily *Silenoideae* A. Br. incited much interest due to the value, among genera, of some morpho-anatomical characteristics especially the reproducing apparatus. Therefore Schott considered for a while that this plant was an intermediary one, between genus *Viscaria* and genus *Melandrium*, due to the 5-locular capsule and often 10-indented. Other authors such as Rohrbach and Pax supported its ascribtion to genus *Silene* because of the bidented valves of capsule. The investigations performed by Gușuleac [7] based on a rich herbarium material lead to the remark that in *Viscaria vulgaris* the capsule opens its valvae after maturity and therefore the plant can not be ascribed to this genus.

Since in our botanical literature, as well as in general, an inconsistency is noticed as regards the correct name of this endemite and its authors, it made us better investigate, in terms of certain aspects, the value of some differential, morpho-anatomical characteristics of the main floral organisational elements including the caryological-cytotaxonomical elements as well as the pollen peculiarities, all., between *Polyschemone* and *Lychnis*. These qualitative characteristics considered comparatively, namely: calicium, petals, stamens, gynoeceum, capsule, pollen, justify, once more, the inclusion of the plant in genus *Polyschemone*. The differentiations are clearly distinct in comparison with the three species of genus *Lychnis*, in our flora *(L. coronaria* (L.) Desr., *L. flos-cuculi* L., and *L. chalcedonica* L.).

Table 1

Differentiation of genus *Polyschemone* and *Lychnis* in terms of the morphotaxonomic aspect (according to bibliography and data by authors)

Polyschemone	*Lychnis*
— membranous calicium, swollen, smooth, 5—9 (10) rounded and ciliated teeth	— tubulous campanulate 10 — crossed
— ligulated petals (in number just as sepals), pink or white colour, without coronule	— whole emarginate with 2—4 red or white fidatea with coronule
— 10—18 (20) stamens	— 10 stamens
— polymeric gynaeceum, 5—7 — mer with basal loculus	— unilocular ovary, pediculated, 5 style, rarely 3 or 6
— capsule on a short stipelated carpofore, opens with bidented valvules	— capsular fruit with 5 teeth
— pollen with pores margin with annulus thickened, optic section of the exine pilate simpilate	— pores margin with annulus somehow less thickened, pilated optic section of exine

Al. Borza (Consp. I., 1947, 81) [2] considers this endemite as *Silene nivalis* (Kit.) Rohrb. (2) : I. Morariu & Al. Beldie (Flora XIII, 1976, 103), [8], Al. Beldie (Flora I, 1977, 135) [1], C. Toma [17] all. considered it as *Lychnis nivalis* Kit., as well as in Flora Europaea (I, 1964, 156, 157), (3), (20); E. Țopa — 1960 [18] (with Romanian name multicoloured rushlight), Tr. I. Ștefureac — 1971 [13], Tr. I. Ștefureac & A. Tăcină — 1978, 89 [14], [16], Protection of Maramureș nature, 1977, 25 [21] and in Romanian flora (II, 1953, 141) [19] is of *Polyschemone nivalis* Schott, Nym. & Kotschy (Fig. 1).

Fig. 1 — Popul ation of *Polyschemone nivalis* Schott, Nym. & Kotschy on the northern slope of the Pietrosu Mare (Rodna Mountains), 1950 m s.m. high (foto St. Hornung).

Histoanatomical investigations on the vegetation organs and phenomenon of anatomic longitudinal symmetry were performed in this endemite by C. Toma [17] ascribing the plant according to Chater [3] to genus *Lychnis* and not *Polyschemone* according to Romanian flora (II, 1964), [19].

Although it is considered an old relictary type [7] species *Polyschemone nivalis* presents however some nonsignificant variability, 5 forms (according to Schur and Zapalowitcz) being delimited : *quadrifolia* Zap., *diminuta* Zap., *plena* (Zap.) A. & G., *versicolor* (Schur) Gușul., *laciniata* (Schur) Gușul., *multiplicata* (Schur) Gușul. [19] based on a series of unimportant, labile characteristics such as : size type of inflorescence, biannual plant, uniflora stem, variable colour, large number of petals, sometimes laciniated, etc, relevant for the name itself of this taxon — *Polyschemone*, that is a plant with a high and variable number of floral elements which does not allow the classification of the species into another genus.

Al. Borza in Conspect (I, 1947, 81) [2] remarks within the variability limits of the species *Polyschemone nivalis* (Silene nivalis (Kit.) Rohrb.) the forms : *rosea*, *liliacea*, *albiflora*, *purpurea* (by Schur) and *quadriflora* and *deminuta* (by Zapalowictz) (in Flora II, 1953, it is called *quadrifolia* and *diminuta*) mentioning however "sine ulla valore taxon" (2, p. 81).

From a p a l i n o l o g i c point of view, the pollen analysis [12], [15] further adds to the differential features between genera *Polyschemone* and *Lychnis*, based on the following two characteristics : pore margin has a thick annulus in *Polyschemone*, while in *Lychnis* the annulus is less thick around the pore ; the exine optic section is pilated, in *Lychnis* and pilated-simpilated in *Polyschemone* (Table 1 ; Pl. II, Fig. 2 a, b) [1].

CARYOLOGIC-CYTOTAXONOMIC ANALYSIS

The caryologic investigation was performed on two endemic populations of *Polyschemone nivalis* (Fig. 1), from two stations (margin Iezer, 1800 m s. m., and superior slope, 2000 m s.m.) in the Pietrosu Rodnei (VIII — IX, 1982, 1983). The working material consisted of repeated fixation of root apexes and foliary primordia. The data were processed according to Feulgen with some suitable modifications required by the samples under analysis. The micrographs were obtained at a size of 400 ×.

Our caryological analysis on the taxon *Polyschemone nivalis* pointed out the diploid set of chromosomes 2n = 24 (x = 11) grouped in 12 pairs of homologous chromosomes, pairs 1, 2, 7 being formed of metacentrical chromosomes and pairs 3, 4, 5, 6, 8, 9, 10, 11, 12 of submetacentrical ones (Pl. I, Fig. 1—2 ; Pl. II, Fig. 1).

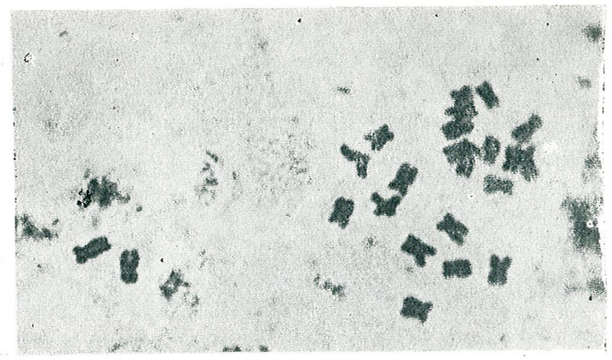
After the analysis of numerous metaphase plates and after establishing the caryotype we noticed the stability of the basic chromosome number (x = 12) as well as the absence of numeric variations and chromosomial anomalies. The diploid character and the symmetric caryotype are in agreement with the morpho-anatomic data and support the idea that species *Polyschemone nivalis* is an independent taxon within fam. *Caryophyllaceae*.

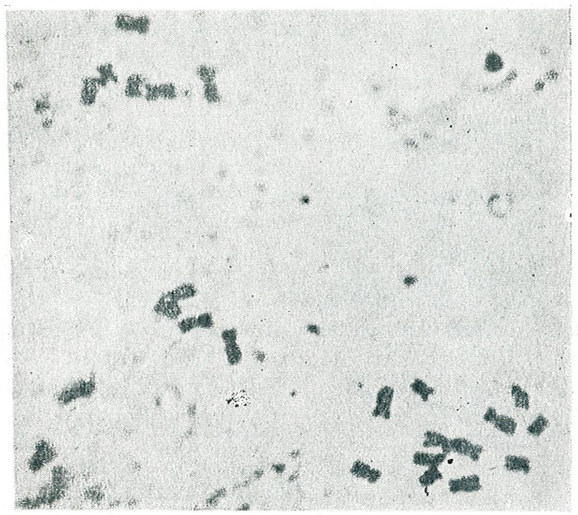
The geographic isolation of this stenochory endemic taxon attests to its age, conditioned by the stational ecologic factors. The presence of the two morphologic types of chromosomes with species *Polyschemone nivalis* defines the symmetric caryotype specific to the paleoendemites where speciation stopped.

ECOLOGY

Polyschemone nivalis grows in rocky, wet herbous places on slopes and plateaus, sometimes in places where snow remains for a longer time. The geological substratum is gneiss, granite, lime, with a certain edafic

[1] We are grateful to prof. G. Şerbănescu-Jitariu who offered us the original drawings for copying, as well as to him and D. Rădulescu, D. Biol., for useful talks on the morphological peculiarities of pollen.





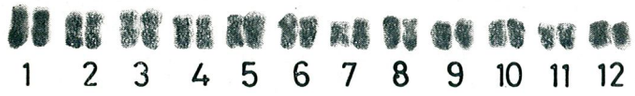


Plate I :

Fig. 1—2 — Metaphases plates and caryotype in endemite *Polyschemone nivalis* Schott, Nym. & Kotschy (2n = 24), 400 × (orig.)

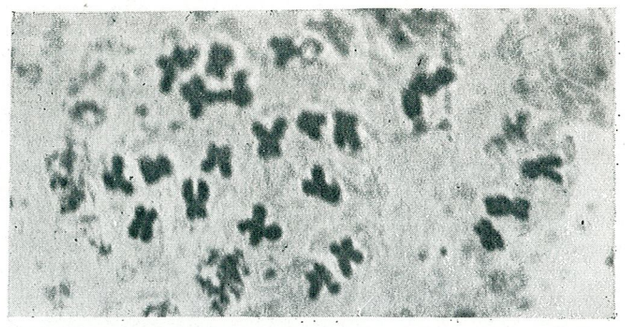


Fig. 1

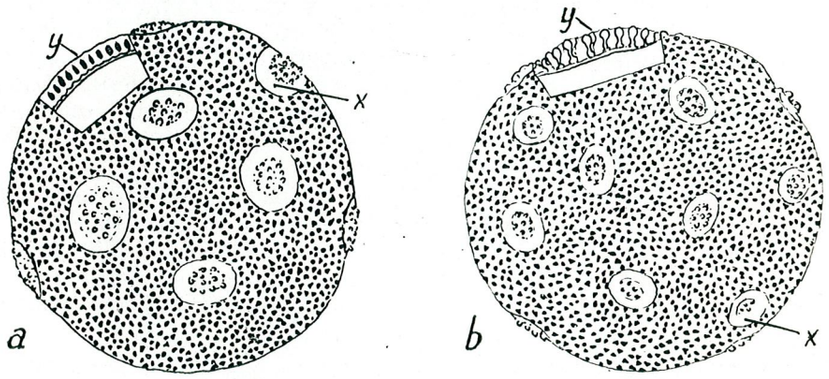


Fig. 2

Plate II:

Fig. 1 — Metaphase plate and caryotype in endemite *Polyschemone nivalis* Schott, Nym. & Kotschy, 400 × (orig.)

Fig. 2 a — *Polyschemone nivalis* Schott, Nym. & Kotschy: pollen ornamentation, presenting pores with thickened annulus (x) and exine in optic pilate-simpilate section (y) (× 1280, G. Şerbănescu-Jitariu original drawing, copy)

Fig. 2 b — *Lychnis coronaria* (L.) Desr.: pollen ornamentation presenting pores with annulus somehow less thickened (x), pilated optic section of exine (y) (× 1280, G. Şerbănescu-Jitariu original drawing, copy)

ecological amplitude, soil-podsol, skeleton-rich; it occurs in the Rodna Mountains with abundant rains and fog.

The variability on the vertical line of stations populated with this endemite is generally assumed to range between 1820—2200 m s.m.; A. Coman [5] delimits it in the Rodna Mountains between 1602—2280 m s.m.

The soil test (10—15 cm depth), podsol, with rock fragments (crystalline) from the Iezer area (1800 m s.m.) below the Pietrosu Rodnei (collected in 1983, IX) highlights the following characteristics: very acid reaction (pH = 4.31), rich content in gross humus (9.70%), less active with low humified organic matter, low degree of saturation in bases (VAh% — 26,77), changeable bases sum (SB) of 5.54, and hydrolitic acidity (Ah) of 15.15 miliequivalents for 10 g soil, total capacity cationic (T) of 20.69 and carbonates because of intense levigation under conditions of low rain regime [2].

Ecologically, *Polyschemone nivalis* is a Carpathian hemicryptophit endemic of Romania, with humidity index of (U) — 2.5 (xeromesophil, hygrophyl), temperature index (T) — 1 (hechistoterm, cryophyl) and soil reaction (R) — 3,5 (acid-neutrophyl, neutro-basiphyl) [11].

CENOLOGICAL DATA

The endemite *Polyschemone nivalis* is present on meadows, fixed rocky soils, slopes rich in gross humus, rocky places (crystalline), in the subalpine and alpine level in the *Pinus mugo* communities area or above it.

The phytocenoses with populations of *Polyschemone nivalis* belong to al. *Rhododendro-Vaccinion* Br.—Bl. 1926, Cl. *Caricetalia curvulae* Br.—Bl. in Jenny 1926 em. Krajina 1933 [11], peculiar cenosis being delimited, characterised by this valuable Carpathian stenochory endemite of the Eastern Carpathians (the Rodna massive), a fully justified classification.

CHOROLOGY

The analysis of herbarium materials in the collections of various institutes in this country permitted to better know the minimum areal of the plant *Polyschemone nivalis* distribution in the Rodna massive and also to complete the stations' inventory with this endemite in the Romanian flora (Flora R. P. Română, II, 1953, 141). Out of the 80 herbarium plates, most of them belong to the Inău mountain, this being the main place of occurrence, followed by the Iezer margin, under the Pietrosu Rodnei, Cişia, Galaţului peak, Obîrşia Rebrei, the Iezer mountain, Laptelui peak, Gemenea, Gărgălău peak, Repedea peak, Omu peak, Stol, Lala Valley and Puzdrea viseconească, mt. Faţa Meselor (A. Coman) etc. (2), (4), (5),

[2] We are endebted for these data to Prof. N. Barbu, D. Biol., from the University "Al. I. Cuza", Jassy, N. Geambaşu, D. Biol., from the Experimental Station of Spruce Fir Culture, Cîmpulung Moldovenesc and Gh. Pânzaru (Borşa).

(7), (8), **(10)**, (16). All stations are confined to the area between Pietrosu Rodnei and mt. Inău, geographically meaning N. latitude 47°42′ — 47°37′ and E. longitude 24°22′ — 24°54′ (see chorologic diagram Fig. 2).

Polyschemone nivalis was much herbarised by various botanists in the country and abroad : Fl. Porcius (?) [3], A. P. Alexi (?) I. Prodan (?),

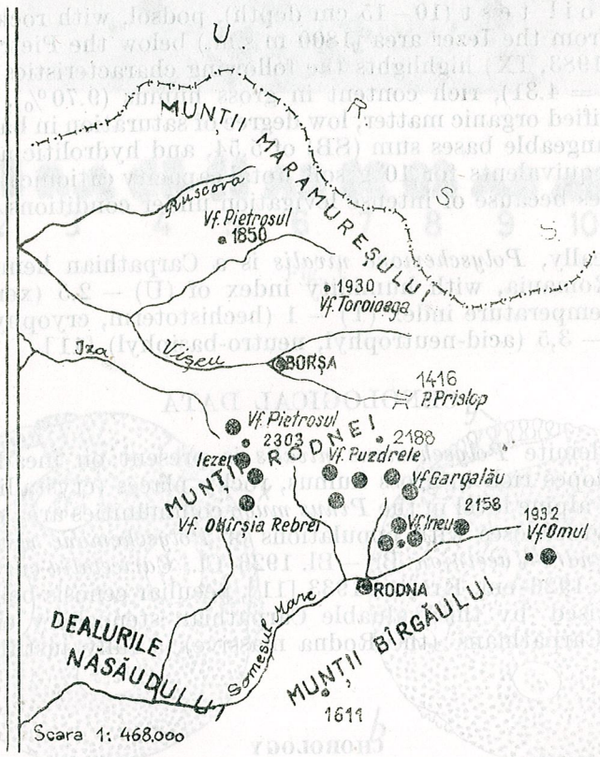


Fig. 2 — Chorological diagram representing (●) the main stations populated by the endemite *Polyschemone nivalis* Schott, Nym. & Kotschy in the Rodna Mountains (orig.).

A. Procopianu-Procopovici (1899), E. I. Nyárády (1907, 1918, 1919, 1942), Al. Borza (1923, 1925), A. Coman (1924, 1936, 1938, 1942, 1950, 1962 etc.), J. Ch. Baumgarten (1828, 1829), Reckert (1850—1860), M. Fuss (1842, 1852), A. Czetz (1843, 1858, 1862), L. Haynald (1860), V. Janka (1876), J. Römer (1883), E. R. Missbach (1896), A. Degen (1902), Kümmerle (1904), K. Ungar (1907), Gh. Grințescu (1935), Á. Nyárády (1932, 1937, 1949, 1950, 1955, some of them in collaboration with E. I. Nyárády), Kladni(?) etc. and frequently by contemporany botanists. Some checkings and remarks on the herbarium material with this taxon were carried

[3] Stations without the date of collection.

out by M. Gușuleac, others by Al. Beldie. It is significant and important that in 1850 (July 21), *Polyschemone nivalis* was collected from the main place by one of the three authors who defined (1854) the genus and species *Polyschemone nivalis* in the Rodna Mt., included in herbarium Schott (nr. 538119, Herb. Univ. Cluj-Napoca).

Polyschemone nivalis is a dizonal phytogeographic element, subalpine and alpine with confined area, limited only to the Rodna Mountains of the Romanian Eastern Carpathians.

The chorologic list of stations populated by the endemite *Polyschemone nivalis* in the Rodna Mountains is obtained by means of herbaristic data in the country belonging to the following herbaria with their abbreviations :

Herbarium institutions : BUC-H. University Bucharest ; BUCA-H. Institute of Biological Sciences in Bucharest ; BUCF-H. Institute of Forest Research and Experiments (forest amelioration) in Bucharest ; HIAB-H. Agricultural Institute, Bucharest ; CL- H. University Cluj-Napoca ; HIACL — H. Agricultural Institute Cluj-Napoca ; I — H. University "Al. I. Cuza" Jassy ; IAGB — H. Botanical Gardens Jassy ; IASI — H. Agricultural Institute, Jassy : SIB — H. Department of Natural Sciences, Brukenthal Museum, Sibiu ; HFSB — H. Faculty of Silviculture, Brașov ; HMNSB — H. Department of Natural Sciences, Bacău Museum ; HAC — H. A. Coman, Sighetu Marmației Museum.

Chorological enumerations : 2316 (BUCF) Maramureș, Borșa, Pietrosu over Iezer, 15. VII, 19 ., leg. A. Coman ; 15279 (BUCF), Maramureș, Pietrosu-Iezer, alt. 1860 m s.m., 22. VII, 1936, leg. A. Coman ; 38946, FRE (BUCF), Maramureș, Mt. Pietrosu Mare, in graminosis et saxosis ad "Iezer" alt. 1850—1885 m s.m. exp. N, VII, 1942, leg. et det. A. Coman ; 38589 (BUCF) Mt. Rodnei, Galați peak, 10. VIII, 1946 leg. I. M. Sor ? ; 45795 (BUCA), Mt. Rodnei, Obîrșia Rebrei, leg. et det. A. P. Alexi ; 20865 (BUCA), Mt. Rodnei, Inău peak, 11. VIII, 1935, leg. Gh. Grințescu, det. Al. Beldie, 1962 ; 22728 (BUCA), Mt Rodnei, Pietrosu-Iezer, alt. 1865 m s.m., 19. VII, 1950, leg et det. A. Coman ; 2751 (UBCA), Maramureș, Pietrosu, in graminosis et saxosis ad "Iezer" alt. 1850—1889 m s.m., exp. N., 21. VII, 1962, leg et det. A. Coman ; 20864 (BUCA), Mt. Iezer, alt. 2000 m s.m., 3. VII, 1915, rev. M. Gușuleac ; 45241 (BUCA), Mt. Rodnei, leg. et det I. Prodan ; FRE(IAGB), Iezer, 1850—1885 m s.m., 21. VII, leg. et det. A. Coman ; 135, 536, 537 (IAGB), Borșa, alt. 1820 m s.m. ; 1828 (HIACL), Mt. Rodnei, Galați peak, 1900 m s.m., 9. VIII, 1955, leg. et det. Á. Nyárády ; 1825 (HIACL), Montes Rodnensis, Laptelui peak, alt. cca 2000 m s.m., 16. VIII, 1950, leg. Á. Nyárády ; 1830 (HIACL), Gărgălău, N. slope, 16. VIII, 1950, leg. et det. Á. Nyárády ; 1831 (HIACL), Mt. Rodnei, rocky slopes, top Inău-Căldarea, 9. IX, 1949, leg. et det. Á. Nyárády-K. Sandor ; 1832 (FRE, nr. 2751), Maramureș, Montibus Pietrosu Mare, in graminosis et saxosis ad "Iezer", alt. 1850.—1889 m s.m., exp. N, 21.VII, 1942, leg. et det. A. Coman ; 05042 (HFSB), 11. VIII, leg. J. Römer ; 05018 (HFSB), Rodna, Matten an Inău, 3. VIII, 1896, leg. E. R. Missbach-Dresda ; 050180 (HFSB), Rodna, auf den Alpen : Pietrosu (Maramureș), Gemenea, Inău, 2000—2200 m s.m., leg. A. P. Alexi ; 013136 (HFSB), Borșa : Pietrosu-Iezer, exp. N. alt. 1865 m s.m., 19—20, VII,

1950, leg. A. Coman ; 034242 (HFSB), Mt. Rodna-Pietrosu Mare, 22. VII, 1969, leg. I. Morariu et M. Danciu ; 034244 (HFSB), 2751, FRE, Montibus Pietrosu Mare, exp. N., 21. VII, 1942, leg. A. Coman ; FHE, Cent. IX, 837, II, 425124 (CL), distr. Bistrița-Năsăud, in jugo Mt. Cișia, in herbidis calcareis, alt. 1500—2000 m s.m., solo gneissico, 16. I, 1918, leg. E. I. Nyárády ; 212380 (CL), Mt. Rodnensis Transsilvaniae, in decl. septentr., Repede peak, alt. 1850—1900 m s.m., 8. VII, 1942, leg. Á et E. I. Nyárády, det. Á. Nyárády (CL), Maramureș, Pietrosu peak, N. slope, Borșa, 5. VIII, 1925, leg. Al. Borza ; 50494 (CL), Transilvania, distr. Bistrița-Năsăud, in monte "Inău" supra balneas valley Vinului, alt. cca 1900—2280 m s.m., 21. VIII, 1923, leg. Al. Borza ; FRE, 209418, 617897 (CL), Maramureș, distr. Maramureș, Mt. Pietrosu Mare, in graminosis et saxosis ad "Iezer", alt. 1850—1889 m s.m., exp. N., 21. VII, 1942, leg. et det. A. Coman ; 448842 (CL), România, Maramureș, Pietrosu Mare, "Iezer", alt. cca 1840—1860 m s.m., 15. VII, 1938, leg. A. Coman ; 47072, 15429 (CL), Transilvania septentr.-orientalis, in alpibus Rodnensibus, ad nives perennes mt. Inău, alt. 2200 m s.m., leg. Fl. Porcius ; 425123 (CL), distr. Bistrița-Năsăud, in herbidis alpis Inău, super pag. Rodna (Borberek), 20. VII, 1860, locus classicus, leg. Á. Nyárády ; 99268 (CL), ad nives perennes mt. Inău (Kuhhorn), 12. VIII, 1889, leg. Procopianu-Procopovici ; 21556, 34067 (CL), in alpib. granit. Rodnensibus in Transsilvania, leg. J. v. Kováts ; 28261, FHE (CL), Transilvania septentr.- orient., in alpibus Rodnensibus ad nives perennes mt. Inău, alt. 2200 m s.m., leg. Porcius ; 37127 (CL), Transilvania, alp. Inău prope Rodna, 6500—7200 p . VII, leg. Czetz, ex Herb. J. C. Equitis Tittoni a Dannenfeldt ; 34064 (CL), in alp. calc. Rodnensibus, Transilvania, VIII, 1862, alt. 7200 p., leg. Czetz ; 34060 (CL), alp. Rodnensis Inău, VIII, leg. Czetz ; 34065 (CL), Mt. Rodna, western slopes of Inău peak, leg. Czetz ; 34061 (CL), 1843, without place, leg. Czetz ; 99277 (CL) in pascuis declivibus septentr. in Inău, prope Rodna, Transilvaniae ad comitatus Maramureș confines siti, 13. VIII, 1876, leg. Ianka ; 134063 (I), in alpibus Rodnensibus Transs., 6500—7200 p., leg. Czetz ; 34066 (I), Inău, 17. VIII, 1858, leg. Czetz ; R. Soó FHE, 538121 (Cent. IX, I, 832), distr. Bistrița-Năsăud, in herbidis alpis Inău, supra pag. Rodna, 26. VII, 1860, locus classicus leg. L. Haynald ; FHE, Cent. IX, 837, II, 538120, distr. Bistrița-Năsăud, in jugo mt. Cișia, in herbidis calcareis, alt. 1800—2000 m s.m., solo gneissico, 16, VIII, 1918, leg. E. I. Nyárády ; 538118 (CL), Bistrița-Năsăud, Alpis Rodnensis in mt. Inău, cca 2100 m s.m., 15. VIII, 1907, leg. Nyárády ; H. Schott, 538119 (CL), in herbidis mt. Inău, prope Bistrița copiosa, alt. 6800 p., 21. VII, 1850, leg. Th. Kotschy ; 538117 (CL), Bistrița-Năsăud. in alpes Cișia prope balneas Rodna, 22. VIII 1904, leg. Kümmerle ; H. Porcius, 47073 (CL), Transilvania borealiorientalis, in summis alpibus Rodnensibus (Inău, Cloca, Galați, Gemenea, Pietrosu) leg. Porcius ; H. M. Fuss, 11387 (SIB), Rodnaer Alpen, Inău, leg. M. Fuss 22.VIII (?) ; 11388 (SIB), Inău, VIII, 1842, 3.VIII 1852, leg. M. Fuss ; 11389 (SIB), Inău, leg. Reckert (without data, but certain between 1850—1860) ; H. K. Ungar, 37755 (SIB), Inău, 3. VIII, 1852, leg. M. Fuss ; 37756 (SIB), Inău, 25. VIII, leg. M. Fuss ; 37752 (SIB), Inău, 3. VIII, 1907, leg. Ungar ; H. Soc. de Șt. nat. Sibiu (mape 521) ; 44002 (SIB), Pietrosu, mt. Rodna, alt. 2000 m s.m. (without data) leg Alexi ; 44004 (SIB), Rodnaer Alpen, alt. 1850 m s.m., leg Kladni ; 44005 (SIB), Inău,

3. VIII, leg. M. Fuss ; H. Barth (SIB), 49161 (SIB), Inău, 17. VIII, 1902, leg. A. Degen ; H. Lic. Gh. Lazăr, 75727 (SIB), Rodnaer Alpen, VIII, 1828, leg. Baumgarten ; H. E. Kisch, 78336 (SIB), Inău, Rodnaer Gebirge, VIII, 1907, leg. Ungar ; H. Fl. Porcius, 81525 (SIB), Inău, Galați, Obîrșia Rebrei, Stol, Gemenea, Pietros, leg. Porcius ; 4228 (SIB), Inău, Mt. Rodna, alt. 2280 m s.m., 15. VIII, 1907, leg. E. I. Nyárády, rev. M. Gușuleac ; 4229 (SIB), mt. Rodnense, in rupestribus ad cacumine mt. Inău, alt. cca 2000—2280 m s.m., solo gneisacio, 13. VIII, 1919, leg. E. I. Nyárády ; 4230 (SIB), Transilvania, Mt. Rodna, in graminosis alpinis saxosisque mt. Cișia versus meridionalem spectantibus alt. 2000 m s.m., 1937, VII, 1922, leg. Á. Nyárády ; 4231 (SIB), FHE, nr. 521, Transilvania septentr.-orient., in alpibus Rodnensibus, ad nives perennes mt. Inău, alt. 2000 m s.m., leg. Porcius ; 4232 (SIB), Mt. Rodnensis, in cacumine Omu peak supra balneas Rodnaborserek, alt. cca 2100 m s.m., solo gneisaceo, 16. VIII, 1918, leg. E. I. Nyárády ; 4233 (SIB) distr. Bistrița-Năsăud, Mt. Rodnenses, in rupestribus ad cacumine Inău, alt. cca 2100—2200 m s.m., solo gneisaceo, 13. VIII, 1918, leg. E. I. Nyárády ; 4234 (SIB), Mt. Rodnenses in Transilvania, in declivibus graminosis mt. Cișia. alt. cca 2000 m s.m., solo granit., 13, VIII, 1918, leg. E. I. Nyárády ; 4235 (SIB), Inău peak, alt. 2050—2280 m s.m., 20. VII, 1932, leg. Á. Nyárády et E.I. Nyárády ; 4236 (SIB), valley Lala, alt. 1750—2150 m s.m., 21. VII, 1932, leg. Á. Nyárády et E. I. Nyárády ; 4237, FRE-2751 (SIB), distr. Maramureș, mt. Pietrosu Mare, in graminosis et saxosis ad Iezer, alt. 1850—1889 m s.m., exp. N., 21. VII, 1942, leg. et det. A. Coman ; 4238, FEAH, nr. 2510 (SIB), Transilvania septentr.-orient., in alpibus Rodnensibus, ad nives perenne mt. Inău, alt. 2200 m s.m., leg. Porcius ; 4239, FHE, I, 837 (SIB), distr. Bistrița-Năsăud, in herbidis alpis Inău supra pag. Rodna, 26. VII, 1860, locus classicus, leg. L. Haynald ; 837, II (SIB), in jugo mt. Cișia, in herbidis calcareis. alt. 1900—2000 m s.m., solo gneissico, 16. VIII, 1918, leg. E. I. Nyárády ; 4240 (SIB), Mt. Rodnense, in declivibus septentr. ad locis saxonis et graminosis mt. Cișia, alt. 1900—2060 m s.m., solo calc., 16. VIII, 1918, leg. E. I. Nyárády ; H. A. Coman (Mus. Maramureșan-Sighet), 4432 (HAC), Borșa, Pietros-Iezer, exp. N., alt. 1865 m s.m. ,20, VII, 1950 ; 4641 (HAC), f. *quadrifolia*, Borșa, Pietros, Iezer, exp. N., alt. 1865 m s.m., 20. VII, 1950 ; 4424, Borșa, Laptelui peak, exp. N., alt. 1600—2000 m s.m., 12. VII, 1963 ; 4425, Borșa, Pietros, Iezer, exp. N., alt. 1896 m s.m., 20. VII, 1945 ; 4640, Borșa, Maramureș, Pietros, Iezer, exp. N., alt. 1895 m s.m., 22. VII, 1936 ; 4417, Borșa, Pietros, Iezer, exp. N., alt. 1895 m s.m., 22. VII, 1936 [4].

Exiccata : FRE (nr. 235 a, b ; 2751 under *Silene*) ; FEAN (nr. 521, 2510 under Lychnide) ; HN (nr. 3025) ; FHE (nr. 837, I, II under *Silene*).

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[4] The authors are grateful to : Prof. O. Rațiu, D. Biol., and V. Fărcașiu (Botanical Gardens Cluj-Napoca), D. Pázmany, D. Biol. (Agricultural Institute Cluj-Napoca), Gh. Mihai D. Biol. (University "Al. I. Cuza", Jassy), E. Schneider-Binder, D. Biol. (Department of Natural Sciences, Brukenthal Museum Sibiu), M. Danciu, D. Biol. (Faculty of Silviculture, Brașov), M. Bereș (Natural Sciences Museum, Sighetu-Marmației) who offered us the herbarium data. A large part of the herbarium data were processed by the present authors.

CONCLUSIONS

The distinct morpho-anatomical characteristics (Table 1) cleary differentiate the genus *Polyschemone* from the genus *Lychnis* while the geographical isolation, the caryological and cytotaxonomic aspect, with the presentation for the first time of the caryotype in this stenochory endemite, are new evidence to support this generic monotype independent taxon, well-defined from the related genera, delimiting species *Polyschemone nivalis* Schoot, Nym. & Kotschy and not belonging to genus *Lychnis* L. as *Lychnis nivalis* Kit.

The results of our investigations, regarded as a whole, come to support a consistent nomenclature, in the study of flora in this country and also in general. This endemite is included on the list of species (I) forming the ecological study of the endemites relicts, and rare species in Romanian flora in order to protect them (21, p. 25).

It is remarkable that *Polyschemone nivalis* Schott, Nym & Kotschy and *Saussurea porcii* Deg. and *Pulmonaria rubra* Schott ssp. *filarskyana* (Jáv.) Domin (the last two also known in the Forest Carpathians in the Soviet Union) are the three valuable Carpathian species in the north of our country. They enrich the genofond of the Rodna Mountains [13], [22] and the rezervation Pietrosu Mare ; besides other species they justify its existence as a monument of biosphere as UNESCO (1979) called it.

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CONTRIBUTION À L'ÉTUDE PALYNOLOGIQUE DE LA FAMILLE *LORANTHACEAE*

PAR

GABRIELA ȘERBĂNESCU-JITARIU

The morphological characteristics of the analyzed pollen grains (shape, size, structure of the sporoderm, a.s.o.) provide an accurate outline of the genera and even of the species of the *Loranthaceae* family extant in the Romanian flora.

La famille *Loranthaceae* est incluse dans l'ordre des *Santales* tant dans l'ouvrage de synthèse *Syllabus der Pflanzenfamilien* [1], interprété par Dr. W. Schultze-Motel, que dans la *Flora Europaea* [3] et la *Flora R.P.R.* [4] qui ont constitué la base de la présentation du matériel dans nos ouvrages de palynologie.

Dans la flore spontanée du globe la famille mentionnée comprend environ 40 genres avec 1 400 espèces, surtout dans les zones tropicales. Dans la flore de Roumanie la famille *Loranthaceae* est représentée seulement par deux genres avec deux espèces.

Les observations morpho-polliniques des taxons compris dans la famille citée ont été effectuées sur du matériel d'herbier. Les grains de pollen ont été analysés dans l'eau et dans le chloralhydrate pour mettre en évidence la couleur, la grandeur, la forme, l'épistructure et l'épaisseur du sporoderme.

Les grains des deux genres regardés au microscope dans l'eau ont une couleur orange, et dans le chloralhydrate ils prennent une nuance jaune clair. En ce qui concerne l'épaisseur, les grains sont de taille moyenne, rarement grande ; la forme est prolé-sphéroïdale jusqu'à prolé. L'exine des deux genres est crassisexinée à surface verruqueuse, et chez *Viscum* elle est pourvue de bacules et d'épines. Le sporoderme en coupe optique est simplement tégilé-baculé.

Nous présentons dans ce qui suit les résultats de nos observations, avec des détails pour chaque genre et des caractères généraux pour les espèces.

LORANTHUS L.

Pollen sincolpé, sous-prolé-prolé, de taille petite jusqu'à grande, fréquemment moyenne. Le sporoderme verruqueux, aux verrues disposées en rangées parallèles ; en coupe optique, tégilé-baculé.

REV. ROUM. BIOL.—BIOL. VÉGÉT., TOME 30, N° 1, P. 19—21, BUCAREST, 1985

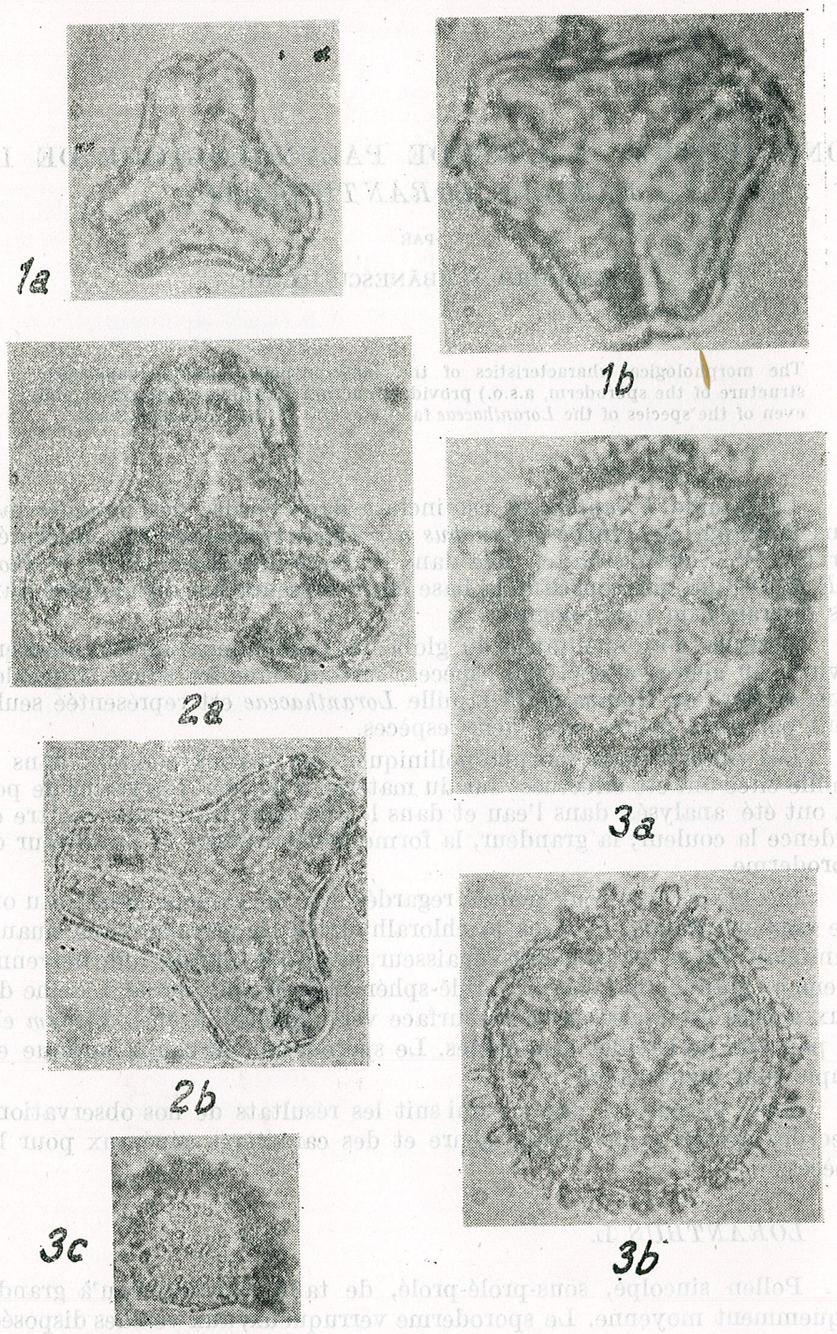

Fig. 1. — *Loranthus europaeus* Jacq. : a, vue polaire, ornementation et coupe optique ; b, idem, colpes et coupe optique (a, 400 × ; b, 1008 ×, orig.).
Fig. 2. — *Loranthus flavidus* Hook. f. : a, vue polaire, ornementation ; b, idem, coupe optique (a, b 1008 × , orig.).
Fig. 3. — *Viscum album* L. : a, vue polaire, ornementation ; b, idem, coupe optique ; c, idem, en partie ornementation (400 ×, orig.).

Loranthus europaeus Jacq. (Herb. Fl. Rom. Exs. nº 948)

Les grains de pollen en vue apicale ont 26— 28,6 μ de diam., en vue de profil la hauteur est de 23,4 — 33,8 μ et la largeur de 15,6 — 20,8 μ. Le sporoderme a 2,6 μ d'épaisseur (Pl. I fig. 1 a, b).

Loranthus flavidus Hook. f. (Herb. gen. Grăd. Bot. Buc. nº 279106).

Les grains de pollen en vue apicale ont 36,4 — 52 μ de diam., en vue de profil la hauteur est de 26 — 62,4 μ et la largeur de 18,2 — 52 μ. Le sporoderme a 2,6 μ d'épaisseur (Pl. I fig. 2 a, b).

VISCUM L.

Pollen tricolporé, certains grains tricolporoïdés, prolés-sphéroïdaux, de taille moyenne jusqu'à grande, fréquemment moyenne. Le sporoderme verruqueux et pourvu de bacules jusqu'à 5,2 μ de longueur et d'épines. En coupe optique, tégilé-baculé.

Viscum album L. (Herb. Grăd. Bot. Buc. nº 279253).

Les grains de pollen en vue apicale ont 36,4 — 52 μ de diam., en vue de profil la hauteur est de 41,6 — 52 μ et la largeur de 36,4 — 46,4 μ. Le sporoderme a 3,9 μ d'épaisseur (Pl. I fig. 3 a — c).

CONCLUSIONS

En général, le pollen des unités systématiques analysées de la famille *Loranthaceae* a un caractère hétérogène en ce qui concerne la morphologie des grains, fait souligné également par Erdtman [2] pour d'autres espèces de la même famille analysées du point de vue morpho-pollinique.

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INTERSPECIFIC ASSOCIATIONS WITHIN THE HERBACEOUS AND SHRUB LAYERS IN THE UPPER BELT OF SPRUCE FOREST (THE RETEZAT MOUNTAINS)

BY

LUCIA STOICOVICI

With a view to studying the species relationships, a xerophilous sub-alpine meadow situated between 1890 and 1900 m altitude on Fața Retezatului (Retezat Mountains) was chosen. For an objective representation of interrelationships, numerous quadrats were randomly placed in the field and using the correlation method it has been resorted to the hierarchichal arrangement of the species in the dendrogram. The 29 herbaceous species and dwarf shrubs with high frequency included in the dendrogram form distinct ecological groupings.

The purpose of this article was to investigate the structure of species groups and the species sociological behavior expressed by the degree of association between herbaceous plants and dwarf shrubs in the upper range of spruce forest. The data may contribute to a better knowledge of the phytosociological conditions in which the forest vegetation establishes itself in stands at hight altitude.

SITE DESCRIPTION

An area on Fața Retezatului (the Retezat Mountains), a surface comprised between 1890 m and 1900 m altitude where *Picea abies* * occurs at its upper range not exceedig 256 cm in height, was chosen as a study site. The woody vegetation is made up of isolated individuals of *Pinus cembra* with a low cover degree. Patches of *Pinus mugo* occurring at its lower growing zone are relative numerous. The understorey is also formed of *Juniperus communis* ssp. *nana* with high frequency and isolated individuals of *Salix silesiaca*. The ericaceous shrubs and the herbaceous plants are irregularly spread between scree and igneous blocks, making up the sub-alpine xerophilous meadows [4]. The humo silicate soil prevails. The surface has a south-western exposition, is open, windy and exposed to strong insolation in summer time.

MATERIAL AND METHODS

On the herbaceous vegetation a number of 150 quadrats, each of 50 × 50 cm, were randomly laid out. Following the recording of the spe-

* The species nomenclature follows Flora Europaea [7].

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cies presence in quadrats, for each species the percentage frequency has been calculated. These values were used for the computation of the correlation coefficients between species in pair. With regard to the statistical data-processing there were retained only those species which occur more than 6 times in the total quadrats. It has been resorted to an objective classification type of vegetation units — the hierarchical arrangement — which sets up a number of species in descending order of their importance and separates groups of plants with phytosociological and ecological possible significance [2], [3], [6].

RESULTS AND DISCUSSION

All the possible positive and negative interractions between 29 herbaceous plants and ericaceous shrubs with high frequency in the study site are synthetized in the dendrogram (Fig. 1). Other species with low frequency or with nonsignificant cover abundance values (from the viewpoint of the Braun-Blanquet values scale) have been recorded in quadrats. We still mention their presence in the context of the researches into plant relationships. In the structural network of groupings, the species position should not be considered as invariable. The changes of positive and negative relationships (their number, quality and significance) characterize the phytocoenose dynamics. It is also important to mention these species when the same statistical analysis is repeated over several years. The following enumeration does not include the species in Fig. 1 which were subjected to statistical processing. *Phyteuma vagneri, Ph. confusum, Knautia longifolia, Crepis viscidula, C. conyzifolia, Ranunculus nemorosus, Botrychium lunaria, Viola dacica, Silene nutans* ssp. *dubia, Carex pallescens, Cerastium fontanum, Thesium alpinum, Poa pratensis, Ligusticum mutellina, Agrostis rupestris, Thlaspi dacicum, Trollius europaeus, Coeloglossum viride, Orobanche flava, Omalotheca norvegica, Luzula sudetica, Gentiana acaulis, Hieracium aurantiacum, H. pseudocaesium, H. kotschyanum, H. atratum, Veronica bellidioides, Nardus stricta.*

The hierarchical arrangement of the relationships between the 29 species relies on 812 correlation coefficients (Fig. 1). The greatest number of these coefficients, either positive or negative, is nonsignificant ($P \geqslant 5$). The coefficients become significant above $r = 0.36$ or $r = -0.36$ (which are still nonsignificant). As it was supposed, the number of the positive coefficients exceeds that of the negative ones (Fig. 1). On the vertical axis the positive and negative correlation coefficients (r) are represented and on the horizontal axis the species which enter into combination (from species 1 to 29).

Generally, in the dendrogram are recognized 5—6 principal groups and several subgroups of species positive associated and three large groups negative associated. According to other studies [2] in the analysis of species groups there are identified group characterizing species. Thus the first nucleus in which also the highest positive correlation coefficient, $r = 0.98$; $P < 0.1$ is registered, is formed of the species in pair *Carlina acaulis* and *Geum montanum*. These are followed in descending order by other group

or subgroup characterizing pairs associated at positive significant levels (sometimes of the same rank). These species are : *Hieracium hoppeanum* + + *Anthoxanthum odoratum*, $r = 0.69$; $P < 0.1$; *Euphrasia minima* +

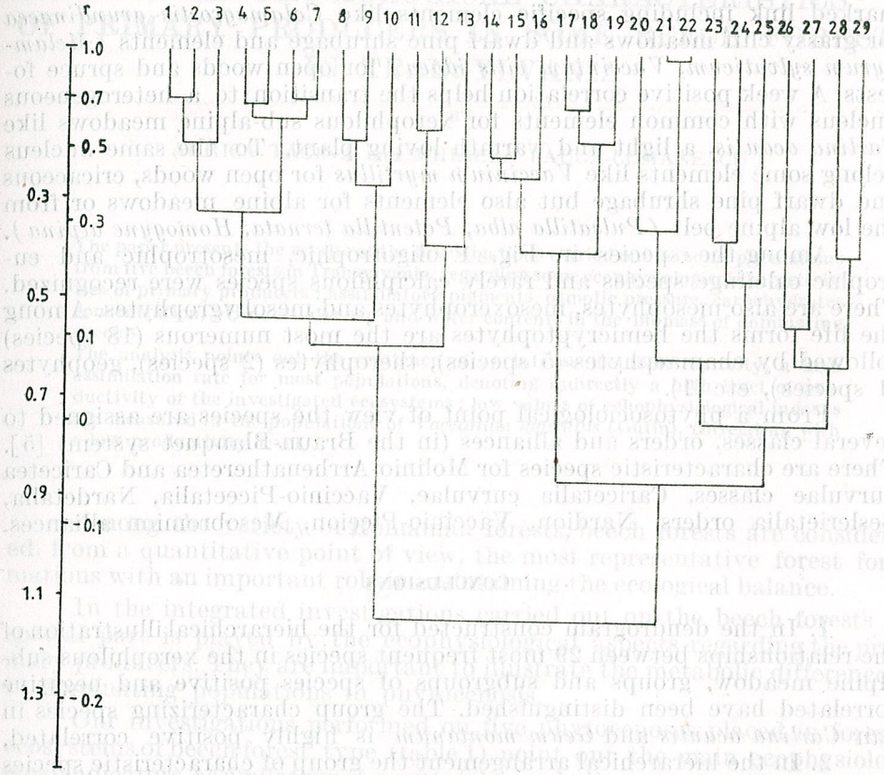

Figure 1. — Hierarchical arrangement of the species. Numbers on the horizontal axis identify species (as listed below), while numbers on the vertical axis represent L^2 values corresponding to r values (correlation coefficients). 1. *Hieracium hoppeanum*, 2. *Anthoxanthum odoratum*, 3. *Thymus praecox* ssp. *polytrichus*, 4. *Centaurea stenolepis* ssp. *nervosa*, 5. *Achillea stricta*, 6. *Euphrasia minima*, 7. *Campanula patula* ssp. *abientina*, 8. *Festuca violacea*, 9. *Bruckenthalia spiculifolia*, 10. *Rhinanthus alpinus*, 11. *Laserpitium krapfii* ssp. *krapfii*, 12. *Avenula versicolor*, 13. *Hypochoeris uniflora*, 14. *Vaccinium vitis idaea*, 15. *Antennaria dioica*, 16. *Scorzonera purpurea* ssp. *rosea*, 17. *Calamagrostis arundinacea*, 18. *Melampyrum sylvaticum*, 19. *Deschampsia flexuosa*, 20. *Vaccinium myrtillus*, 21. *Carlina acaulis*, 22. *Geum montanum*, 23. *Potentilla ternata*, 24. *Homogyne alpina*, 25. *Campanula serrata*?, 26. *Luzula luzuloides*, 27. *Hieracium sparsum*? 28. *Gymnadenia conopsea*, 29. *Pulsatilla alba*.

+ *Campanula patula* ssp. *abietina*, $r = 0.69$; $P < 0.1$; *Centaurea stenolepis* ssp. *nervosa* + *Achillea stricta*, $r = 0.67$; $P < 0.1$; *Calamagrostis arundinacea* + *Melampyrum sylvaticum*, $r = 0.66$; $P < 0.1$, etc.

As regards their site ecological requirements, most of these species range from the sub-zone of mixed beech-spruce forests to the low alpine region. Fig. 1 highlights the separation by a negative link of species group

characteristic of xerophilous sub-alpine meadows (from species 1 to 13), saxicolous, chionophylous and subtermophilous species (*Thymus praecox* ssp. *polytrichus*, *Festuca violacea* and *Bruckenthalia spiculifolia*) from a larger group (from species 14 to 29) also divided by a negative still not so marked link including specific elements like *Calamagrostis arundinacea* for grassy cliff meadows and dwarf pine shrubage and elements (*Melampyrum sylvaticum*, *Vaccinium vitis idaea*) for open woods and spruce forests. A week positive correlation helps the transition to a heterogeneous nucleus with common elements for xerophilous sub-alpine meadows like *Carlina acaulis*, a light and warmth loving plant. To the same nucleus belong some elements like *Vaccinium myrtillus* for open woods, ericaceous and dwarf pine shrubage but also elements for alpine meadows or from the low alpine belt (*Pulsatilla alba*, *Potentilla ternata*, *Homogyne alpina*).

Among the species in Fig. 1 oligotrophic, mesotrophic and eutrophic calcifuge species and rarely calciphilous species were recognized. There are also mesophytes, mesoxerophytes and mesohygrophytes. Among the life forms the hemicryptophytes are the most numerous (18 species) followed by chamaephytes (5 species), therophytes (2 species), geophytes (1 species), etc (1).

From a phytosociological point of view the species are assigned to several classes, orders and alliances (in the Braun-Blanquet system) [5]. There are characteristic species for Molinio-Arrhenatheretea and Caricetea curvulae classes, Caricetalia curvulae, Vaccinio-Piceetalia, Nardetalia, Seslerietalia orders, Nardion, Vaccinio-Piceion, Mesobromion alliances.

CONCLUSIONS

1. In the dendrogram constructed for the hierarchical illustration of the relationships between 29 most frequent species in the xerophilous sub-alpine meadow, groups and subgroups of species positive and negative correlated have been distinguished. The group characterizing species in pair *Carlina acaulis* and *Geum montanum* is highly positive correlated.

2. In the hierarchical arrangement the group of characteristic species for xerophilous meadow is separated by a negative link from a group of characteristic species for grassy cliffs, dwarf pine shrubage and open spruce forest. There is also recognized a heterogeneous nucleus including specific elements for the former groups mentioned before, to which are added some specific elements for alpine and lower alpine meadows.

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SOME ECOPHYSIOLOGICAL CHARACTERISTICS OF PRIMARY PRODUCERS IN SOME BEECH FORESTS IN TRANSYLVANIA

BY

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The paper presents the results of the investigations carried out on some populations from five beech forests in Transylvania, regarding some ecophysiological characteristics of primary producers: assimilatory pigments, osmotic pressure, carbohydrates concentration, pH of cellular sap and water contents in the biomass of dominating species.

The analysis points out the existence of an intense metabolic activity, a high assimilation rate for most populations, denoting indirectly a high level of productivity of the investigated ecosystems; low values of echophysiological indexes are measured in the populations of *Vaccinium myrtillus* (Gutin), correlating with a low productivity level.

Among the variety of Romanian forests, beech forests are considered, from a quantitative point of view, the most representative forest formations with an important role in maintaining the ecological balance.

In the integrated investigations carried out on the beech forests a special part is played by the ecophysiological aspects regarding the primary producers. They are important to illustrate the metabolic differences in dominating populations in phytocenosis.

Our investigations performed on five phytocenoses placed in forest ecosystems of beech forest type (table 1) point out the main ecophysiological indicators, assimilatory pigments (chlorophylian, carotenoid pigments), osmotic pressure, carbohydrates content, pH of cellular sap, water content in the dominating species, having an important role in characterising the production capacity of some stations.

MATERIAL AND METHOD

For each species, samples were collected in the stage of highest biomass quantity, and the determination of the content of assimilatory pigments was performed according to the Comar and Zscheille method; the water content was determined by the gravimetric method, the carbohydrates concentration by the refractometric method, the pH by the potentiometric one with mixed electrode and the osmotic pressure of the cellular sap by the cryoscopic one.

Table 1

Relationship between types of forest and plant associations against a geographical and ecological background

Type of forest	Association of plants	Geographic location	Ecological subregion	Class of production	Height
Carex pilosa beech forest	*Carici pilosae Fagetum* Oberd. 57	Sighișoara Podișul Tîrnavelor	I_2	II	535
Festuca drymeia mountain beech forest	*Festuco (drymeae) Fagetum carpathicum* Morariu et all. 68	Lemnia M. Nemirei	C_1	III	940
Festuca drymeia beech forest	*Festuco (drymeae) Fagetum carpaticum* Morariu et all. 68	V. Drăganului M. Vlădeasa	G_3	III	900
Mull flora beech forest	*Dentario-Fagetum sylvaticae* Hartm. 48 em. Pass. 68.	Butin M. Gutii	A_1	II-I	670
Vaccinium myrtillus beech forest	*Luzulo-Fagetum myrtilletosum* Beldie 51. Soó 62.	Guti M. Gutii	A_1	V	800

RESULTS AND DISCUSSIONS

The assimilatory pigments are synthesised in large quantities in all the analysed populations; the quantity of total chlorophyll is lower in vernal (spring) species in comparison with the estival (summer) ones, while the carotenoid pigments reach the highest values in vernal species (tables 2—6).

The highest values of assimilatory pigments were measured in *Fagus sylvatica* in *Carex pilosa* beech forest (table 2) and the lowest ones of this ecophysiological index were recorded in the *Vaccinium myrtillus* beech forest (table 6).

The average quantity of total chlorophyll (165×10^{-4} g/g. dry matter) synthesised in leaves of *Fagus sylvatica* is comparable with the values of this index recorded in the beech forests of Banat and superior to the beech forests in Muntenia [4].

We noticed high values in the content of total chlorophyll (a + b) in species of *Gramineae* and *Cyperaceae* families in all the analysed beech forests.

The relationship chlorophyll a/b as well as chlorophyll/carotin generally varies round the theoretical value [3].

The high quantity of synthesised assimilating pigments reflects the evolution of a normal process of assimilation, inducing therefore a highly specific level of productivity for most of the analysed beech forests, excepting the beech forest with *Vaccinium myrtillus* of low productivity.

Osmotic pressure indicative of the capacity of absorbtion presents high values in vernal species in comparison with estival ones (tables 2—6). The values of osmotic pressure in trees are low to moderate, in comparison with data given by Walter (1960) [1]. Although the values of osmotic pressure vary with the species, this being a determining factor, in the ana-

Table 2

Ecophysiological indicators characterising the primary producers in the *Carex pilosa* beech forest — Sighișoara

Species	Osmotic pressure (bari)	Carbohydrates (%)	pH	Total content of chlorophyll ($\times 10^{-4}$ g/g dry matter)	Ratio chlorophyll a/b	Carotenoid pigments ($\times 10^{-4}$g/g dry matter)	Ratio chlorophyll/carotin	Water contents (%)
Vernal phenaspect								
Ranunculus ficaria	9.76	5.5	5.4	60	3.55	20	3.05	89
Anemone nemorosa	17.08	9.0	5.2	73	2.97	33	3.71	85
Cardamine bulbifera	17.44	8.0	6.5	93	3.03	31	3.02	86
Estival phenaspect								
Fagus sylvatica	23.68	8.0	6.2	193	2.88	45	4.28	69
Lamium galeobdolon	17.20	5.0	5.8	117	2.64	30	3.85	85
Asperula odorata	17.08	5.0	5.6	101	3.22	24	4.20	84
Oxalis acetosella	14.68	5.0	1.7	65	2.76	15	4.37	88
Viola reichenbachiana				123	2.84	30	4.11	82
Carex sylvatica				121	3.66	33	3.67	75
Asarum europaeum	17.32	5.0	5.9	102	3.47	28	3.71	85
Carex pilosa	17.80	5.0	5.9	147	3.62	36	4.10	75

Table 3

Ecophysiological indicators characterising the primary producers in the *Festuca drymeia* beech forest — Lemnia

Species	Osmotic pressure (bari)	Carbohydrates (%)	pH	Total contents of chlorophyll ($\times 10^{-4}$g/g dry matter)	Ratio chlorophyll a/b	Carotenoid pigments ($\times 10^{-4}$g/g dry matter)	Ratio chlorophyll/ carotin	Water content (%)
Vernal phenaspect								
Cardamine bulbifera	16.84	9.0	5.0	63	3.68	29	2.15	87
Cardamine glanduligera	10.46	6.0	5.5	56	3.76	28	2.03	87
Anemone nemorosa	19.24	10.0	5.3	53	3.73	24	2.14	84
Estival phenaspect								
Fagus sylvatica	23.20	7.0	6.1	167	3.10	43	3.83	62
Festuca drymeia	16.12	5.5	5.7	124	3.01	31	3.94	74
Carex pilosa	10.96	6.5	6.0	124	3.01	31	3.94	71
Asperula odorata	15.88	6.0	5.6	82	2.72	20	3.48	84
Luzula luzuloides	17.92	5.0	5.8	81	2.72	20	3.96	77
Oxalis acetosella	17.44	7.0	1.7	84	2.14	19	4.80	87
Fragaria vesca	19.24	7.5	5.0	80	2.60	21	3.76	74

lysed communities, the stational character is still evident especially in *Vaccinium myrtilus* beech forest (table 6).

The lower values of osmotic pressure recorded in most herbaceous species and trees in comparison with those belonging to beech forests in

Table 4

Ecophysiological indicators characterising the primary producers in the Festuca drymeia beech forest — V. Drăganului

Species	Osmotic pressure (bari)	Carbohydrates (%)	pH	Total content of clorophyll ($\times 10^{-4}$g/g dry matter)	Ratio chlorophyll a/b	Carotenoid pigments ($\times 10^{-4}$g/g dry matter)	Ratio chlorophyll/carotid	Water content (%)
Vernal phenaspect								
Fagus sylvatica	13.35	6.5	4.1	102	2.83	19	5.13	74
Cardamine bulbifera	16.35	6.0	6.0	69	2.81	17	4.01	85
Pulmonaria rubra				66	3.06	14	4.61	89
Estival phenaspect								
Fagus sylvatica	21.28	8.0	5.5	156	2.96	39	3.96	63
Festuca drymeia	16.12	6.0	6.0	60	3.54	14	2.36	74
Luzula luzuloides	15.04	4.0	5.9	82	2.96	20	4.02	75
Asarum europaeum	16.84	4.5	5.9	124	3.00	30	4.89	84
Calamagrostis villosa	15.04	5.0	5.9	126	2.46	25	4.98	75

Table 5

Ecophysiological indicators characterising the primary producers in the mull flora beech forest — Butin

Species	Osmotic pressure (bari)	Carbohydrates (%)	pH	Total content of chlorophyll ($\times 10^{-4}$ g/g dry matter)	Ratio chlorophyll a/b	Carotenoid pigments ($\times 10^{-4}$ g/g dry matter)	Ratio chlorophyll/carotin	Water content (%)
Vernal phenaspect								
Cardamine bulbifera	16.50	6.0	5.7	41	2.83	11	3.53	84
Cardamine glanduligera	14.20	6.0	5.5	71	2.36	27	2.61	86
Anemone nemorosa	16.36	6.0	6.0	67	1.95	21	3.23	79
Estival phenaspect								
Fagus sylvatica	18.64	11.0	5.4	146	3.13	36	4.07	60
Oxalis acetosella	12.28	5.0	2.0	62	3.00	17	3.73	90
Carex sylvatica	16.36	6.0	6.7	101	2.74	26	3.88	78
Asperula odorata	13.96	7.0	5.7	85	2.22	18	4.72	83
Mycelis muralis	11.80	7.0	6.0	57	2.77	12	4.64	91
Salvia glutinosa	12.02	3.0	6.0	124	2.77	20	6.17	
Dryopteris phaegopteris	14.20	4.5	6.0	103	2.52	24	4.25	83

Muntenia and Banat [4], point out the well-balanced water regime, destermined by the climatic conditions all along the year under study and the specific level of beech forests with high productivity; the cellular sap concentration in carbohydrates, expresses, in general, the level of osmotic

pressure, higher values being recorded in vernal species and the dominating wooden species *(Fagus sylvatica)* (tables 2—6). The values of this ecophysiological index expressing the level of osmotic pressure are lower in comparison with the ones measured in populations of Banat and Muntenia [4], [2].

Table 6

Ecophysiological indicators characterising the primary producers in the Vaccinium myrtillus beech forest — Gutin

Species	Osmotic pressure (bari)	Carbohydrates (%)	pH	Total content of chlorophyll ($\times 10^{-4}$ g/g dry matter)	Ratio chlorophyll a/b	Carotenoid pigments ($\times 10^{-4}$ g/g dry matter)	Ratio chlorophyll/carotin	Water content (%)
Vernal phenaspect								
Fagus sylvatica	16.20	8.0	3.8	50	4.74	16	3.04	78
Vaccinium myrtillus	19.60	13.0	4.8	77	3.64	19	4.00	64
Luzula luzuloides	13.96	7.0	5.9	55	3.50	15	3.59	77
Estival phenaspect								
Fagus sylvatica	19.84	11.0	5.4	140	3.33	35	3.95	59
Vaccinium myrtillus	20.20	12.0	4.0	76	2.98	19	3.87	62
Luzula luzuloides	13.96	7.0	5.9	73	2.86	19	3.97	72
Luzula sylvatica	16.48	4.5	6.0	50	3.23	13	3.80	73
Calamagrostis villosa	14.68	5.0	5.8	101	3.23	25	4.08	79

Acidity of cellular sap (pH) is high both in herbaceous species and in *Fagus sylvatica* and expresses an intense metabolic activity (tables 2—6). The high metabolical activity compensates the effect of environment conditions, being confirmed by the high productive level both of the herbaceous layer and of the tree one.

The water content in the assimilatory tissue is found at a high level in most analysed phytocenoses, the average being 82%—87%, according to season, an average lower value (84—83%) being noticed in the forest with *Vaccinium myrtillus* (table 6), determining in this case not a higher productivity but the beech survival at the limit of its existence. The water reserve in the herbaceous biomass reflects on the one hand a permanent and good water supply and on the other hand a floristic composition favourable to the maintenance of water in the living structure of biomass.

CONCLUSIONS

1) In all the types of analysed beech forest the total chlorophyll content is high, showing a better assimilation process and therefore a high productive level.

2) The species of *Gramineae* and *Cyperaceae* families show higher values of chlorophyll content in all the analysed communities, lower values of this index being recorded in the *Festuca drymeia* beech forest, as

an effect of the stationary conditions affecting the assimilation process in the population of *Festuca drymeia*.

3) The values of the osmotic pressure and carbohydrates content in the cellular sap are different according to the species, station and the stage of momentary developement of the population under study.

4) The high acidity of the cellular sap in all the analysed beech forests reveals the high level of metabolic acidity, indicative of the high productive capacity of the analysed forests.

5) The rich water content, related to the low average osmotic pressure, supports the idea of a good water supply in the stations with a moderate water regime.

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EFFECT OF NITROGEN DEFICIENCY ON THE CELLULAR ULTRASTRUCTURE IN *SPIRULINA PLATENSIS*

BY

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Nitrogen chlorosis in *Spirulina platensis* was obtained by incubating a culture for 48 hours in a medium with a suboptimal dose of sodium nitrate and consists of rapid modification in colour and ultrastructure of cyanobacteria. In most organisms the phycobilisome disintegration occurs together with the polyglucoside and cyanophycin accumulation. In very few cells degenerative modifications take place consisting in dilatations and fragmentations of thylakoids, disintegration of polyhedral bodies; still cyanophycin granules remain intact.
Inside some cells in the nitrate poor culture large structures were also found, delimited by a thick wall and rich in storage products which can be considered resistance forms (microcytes). All the above-mentioned changes are reversible after the addition of nitrate.

Boresh [2] demonstrated, for the first time, that the nitrogen deficiency in the culture medium induces a change in colour in *Oscillatoria* sp., and after addition of nitrate to the substrate, the alga regained the blue-green colour. This loss of the normal colour was called by him nitrogen chlorosis. Similar changes of colour induced by nitrogen deficiency were described in other blue green algae as well [4], [6]. Allen and Smith [1] showed that under the conditions of nitrogen deficiency *Anacystis nidulans* contains normal amounts of chlorophyll *a* and carotenoids but they also noticed the loss of phycocyanin. Ultrastructural and biochemical changes, induced by the nitrogen deficiency, were described by Vasconcelos and Fay [19] in *Anabaena cylindrica*; they showed that under the same condition, a diminution in phycobiliprotein content takes place. The relationship between the content in phycobiliproteins and the phycobilisome frequency was demonstrated by Gantt and his co-workers [7—8] in the red alga *Porphyridium purpureum* and by Yamanaka and Glazer [21] in *Synechococcus sp.*

Few papers were published on the influence of various doses of nitrogen on the cellular ultrastructure of *Spirulina platensis*. Van Eykelenburg described the cellular ultrastructure in *Spirulina platensis* (Lake Nakuru) in relation with temperature, light intensity and nitrogen concentration [17—18]. He noticed that for doses lower than 3 mmol/l sodium nitrate chlorosis occurs after a few days, but the paper presents ultrastructural data only in cultures with 3,30 and 120 mmol/l nitrate.

The present paper describes the results of an electron microscopic study of the nitrogen chlorosis induced by a suboptimal dose of nitrogen in *Spirulina platensis*.

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MATERIAL AND METHOD

Spirulina platensis (ISBB-80) was grown on a standard mineral [22] at a light intensity of 5000 lux at 25 ± 2°C. The medium deficient in nitrogen, contained 0.05 g/l $NaNO_3$ (1/40 of the nitrate amount from the standard medium).

The preparation of specimens for electron microscopic analysis was carried out according to Wildman and Bowen [20]. 5—10 ml suspension samples were centrifugated for 10 min. at 5000—7000. g. The removal of the supernatant was followed by the fixation with 4% glutaraldehyde in phosphate buffer 0.1 M, pH 7.4 for 3—5 hours at 4°C. The postfixation was performed with 1% osmium tetroxyde in the same buffer for 2 hours at 4°C. The specimens were carefully washed in buffer. Another series of specimens were pelleted and embedded in 3% agar. On coiling, the solidified agar was cut into 1-mm cubes and stained in block with 10% uranyl acetate. Further, all samples were dehydrated in the series of alcohols and embedded in Epon 812 [12]. The ultrathin sections were obtained with a Tesla BS 491 A ultramicrotone, stained for 10—20 min. in uranyl acetate 4% and lead citrate (16) and examined with an JEM-7 electron microscope.

RESULTS

General morphology shows that *Spirulina platensis* is a helix (Fig.1) whose length and pitch varies according to the culture conditions [11] [17]. In our case, at 25°C and 5000 lux, in the exponential phase of growth, the helix has a length of 150—400 μm and consists of 4—7 spires with the diameter of 30—35 μm. The pitch is 40—45 μm and reduces to the extremities of the helix.

48 hours after inoculation the nitrate deficiency induces changes in colour from blue-green to yellow-brown with a stop in growth. These changes are reversible 24 hours after nitrate was added.

The electron microscopic analysis in the control samples is presented in Figs 2—6; the almost equal distribution of membranous and granular organelles is evident.

The photosynthetic apparatus is well-developed and consists of unstacking thylakoidal membranes with a radial display from the nucleoplasmic area to the cytoplasmic membrane. The phycobilisomes have a granular aspect, are in rows and are attached to the thylakoid membrane.

In face-view the phycobilisome are almost semicircular, generally flattened in the association region with the thylakoidal membranes. The diameter of phycobilisomes is of 24—28 nm. Rows of phycobilisomes are generally parallel to each other with a variable center-to-center spacing (60—120 nm) in face-view.

In some electron micrographs the thylakoids are closely spaced and the phycobilisomes are nearly abutting (Fig. 4).

The polyglucoside granules either elipsoidal or circular in profile, are located between photosynthetic thylakoids.These inclusions are regularly placed in one layer only between the closely spaced thylakoids (Fig. 4) or in a rosette shape when distance between adjacent thylakoids is larger than 100 nm (fig. 3, G). In Figs 5 and 6, the polyglucosidic inclusions are

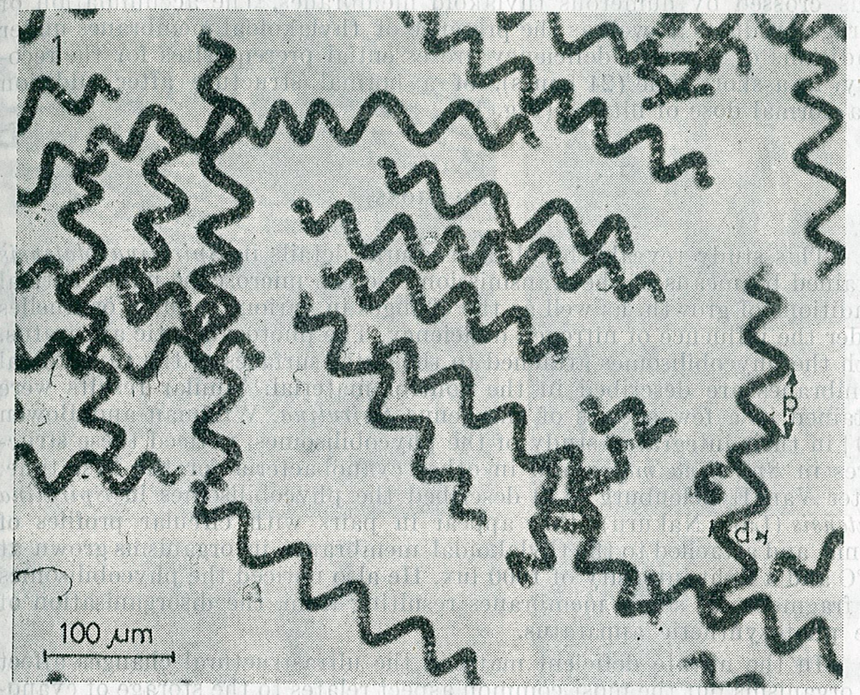


Fig. 1. — Helical filaments of *Spirulina platensis*; *p* is the pitch and *d* the outer diameter of the helix.

absent as a consequence of their disintegration under the action of uranyl acetate in case the specimens were stained in block.

Polyhedral bodies are noticed close to the nucleoplasma or are even sometimes enclosed in it (Fig. 6, PB).

The polyglucosidic inclusions and structured granules are predominantly in the nitrogen deficient material. The first are filamentous or elipsoidal in shape (Fig. 7) and generally follow the thylakoidal membranes (Fig. 8). In the nucleoplasmic region polyhedral and cylindrical bodies and lipid droplets are noticed (Figs. 9, 10).

The diminution in frequency or the absence of polyglucisidic inclusions were noticed in few cells and in such cases intact thylakoidal membranes are visible, but fragmented or swelling thylakoids devoid of phycobilisomes also occur. Important alterations are noticed at the level of the cytoplasmic membrane, but structured granules and nucleoplasma remain intact.

Some cells contain ovoid structures with a thick electron-dense wall. The wall of the "mother cell" is generally interrupted (Fig. 14) or disintegrated (Fig. 15). It is possible that these structures should repre-

sent resistance forms ready to be ejected from the cells in which they are formed and to generate a new organism under favourable conditions of growth. The content of these structures consists of polyglucosidic inclusions crossed by numerous thylakoid membranes. The accumulation of storage products as well as the presence of thylakoidal membranes under conditions of nitrogen deficiency are essential prerequisites for the recovery, in a short time (24 hours), of a normal structure after addition of a normal dose of nitrate (Fig. 13).

DISCUSSIONS

This study reveals the ultrastructural details in *Spirulina platensis* obtained by means of the transmission electron microscope under normal conditions of growth, as well as the changes in various cellular organelles under the influence of nitrogen deficiency. The photosynthetic apparatus, with the phycobilisomes attached to the outer surfaces of the thylakoidal membranes are described in the control material. Similar results were obtained in a few species of the genus *Spirulina*. Wildman and Bowen [20] in their integrated study of the phycobilisomes, noticed these structures in *Spirulina major* and in other cyanobacteria for the first time. Later Van Eykelenburg [17] described the phycobilisomes in *Spirulina platensis* (Lake Nakuru); they appear in pairs with circular profiles of 22 nm and attached to the thylakoidal membranes in organisms grown at 38°C and a light intensity of 1000 lux. He also noticed the phycobilisomes on fragments of single membranes resulting from the disorganisation of the photosynthetic apparatus.

In the nitrate deficient material the ultrastructural changes affect all organisms but the most common aspect relates to the storage of cyanophycin and polyglucosidic granules. The photosynthetic apparatus is present under the form of thylakoidal membranes, but because of the high frequency of the glycogen granules the phycobilisomes are not visible. Van Eykelenburg [18] showed that at lower doses of nitrate *Spirulina platensis* contains numerous polyglucosidic and cyanophycin granules. In *Anabaena cylindrica* deficient in ammonia Vasconcelos and Fay [19] noticed the storage of polyglucosidic and lipidic products as well as the thylakoid veziculation.

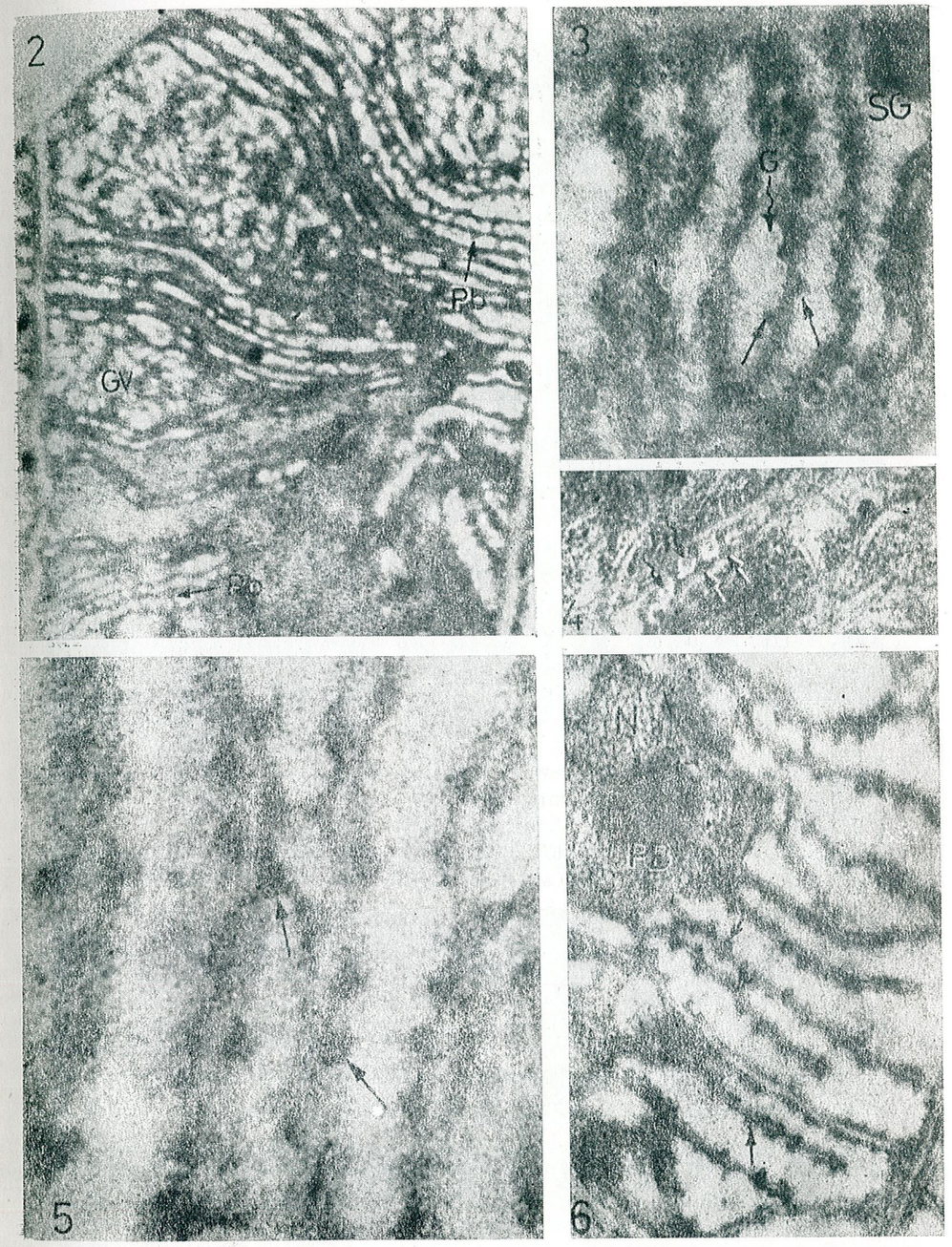
Fig. 2. — Cell ultrastructure at low magnification with thylakoids disposed in parallel. Phycobilisomes on adjacent thylakoids appear head to head, sometimes near abutting; 27,000 ×.

Fig. 3. — Interthylakoidal glycogen granules (G) are distinguished from darker staining phycobilisomes attached to thylakoids; 86,000 ×.

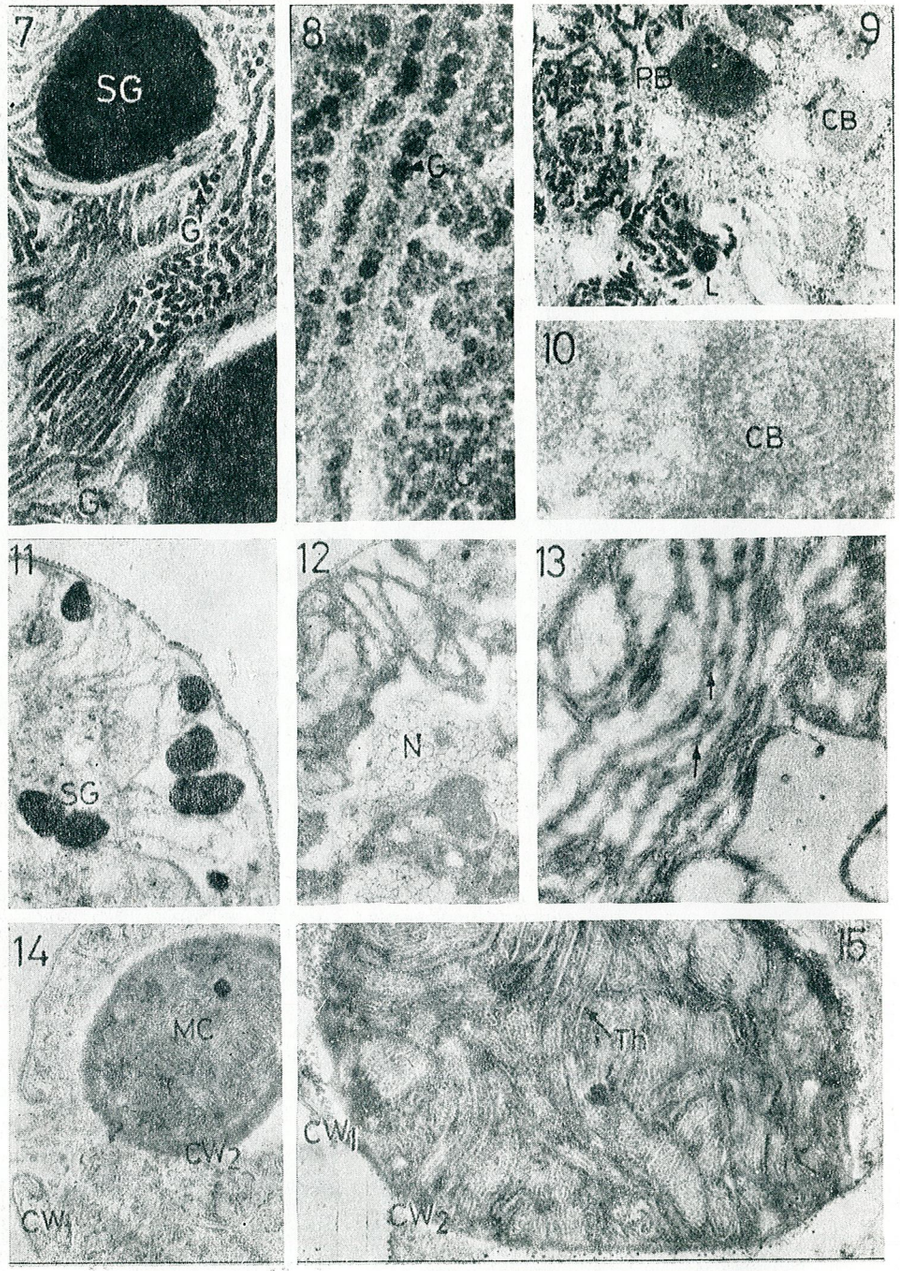
Fig. 4. — Phycobilisomes in face-view (right arrows) appear on both outer surfaces of adjacent thylakoids and alternate with glycogen granules (wavy arrows); 63,000 ×.

Fig. 5. — Face-view phycobilisomes at high magnification appear to have a subunit structure (arrow); 306,000 ×.

Fig. 6. — A less dense cytoplasmic background permits easy visualization of phycobilisomes. PB, polyhedral body are inclosed in the nucleoplasm; 63,000 ×.



Figs 2—6 — *Spirulina platensis*. Phycobilisomes and other cellular organelles in the control material. Pb, phycobilisomes; SG, structured or cyanophycin granules; G, polyglucoside (glycogen) inclusions; PB, polyhedral body; GV, gas vacuoles.



Figs 7—15 — *Spirulina platensis* from nitrate deficient culture L, lipid droplets; CB, cylindrical bodies; MC, microcyte-like structure.

A few number of organisms from our material present degenerative modifications consisting in the phycobilisome disintegration, the thylakoids swelling and fragmentation; still the structured granules, nucleoplasma and ribosomes remain intact. These data can be correlated with the physiological and biochemical data previously published by Allen and Smith [1] on the nitrogen chlorosis in *Anacystis nidulans*; in this case after 18 hours of nitrate deficiency the phycocyanin was lost in parallel with an increase of glucids and nucleic acids. The electron microscopic investigations performed by Gantt [8] reveal the disappearance of phycobilisomes in nitrate deficient cultures of *Porphyridium purpureum* and the reappearance of these structures after adding an optimal dose of nitrogen. Other authors [15] demonstrated that the nitrogen deficiency induces the degradation of apoproteic subunits of phycocyanin in *Synechococcus sp.*

Another aspect mentioned by us in *Spirulina platensis* refers to the occurrence in some organisms of small cells with thick walls, rich in storage products and thylakoidal membranes. In its work on the developmental stages in blue-green algae Poljansky [13] mentions the presence of microcytes in *Gloeotrichia natans*. Guerin-Dumartrait and Moyse [11] mention that resistance forms may occur in *Spirulina* organisms under unfavourable conditions, namely trichomes with a thick sheath as well as cells rich in storage products. Although we did not investigate the evolution of the microcytes that we found in *Spirulina platensis* we may suppose that the abundance of storage material and membranous elements contained inside microcytes is the main prerequisite for the development of new organisms if a normal nitrate dose is added to the culture medium. This hypothesis will be confirmed by the microcytes' future isolation and growth in standard medium.

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Fig. 7. — Filamentous and globular polyglucoside inclusions SG, structured granules; 62,500 ×.
Fig. 8. — Polyglucoside granules in rows are parallel to unstained thylakoids; 130,000 ×.
Fig. 9—10 — Polyglucoside inclusions, lipid droplets and cylindrical and polyhedral bodies are more frequent in nucleoplasmic regions 33,000; 100,000 ×.
Fig. 11—12 — Degradative changes of cells; still the structured granules and nucleoplasma remain intact; 20,000 ×.
Fig. 13 — Recovery of phycobilisomes following addition of nitrate; 59,000 ×.
Fig. 14 — Microcyte-like structure with thick wall (CW_2). The wall of the "mother-cell" (CW_1) is interrupted; 20,000 ×.
Fig. 15 — Microcyte-like structure very rich in storage products and with numerous thylakoids; 38,000 ×.

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ULTRASTRUCTURAL CHARACTERISTICS OF THE FOLIAR PARENCHYMA CELLS IN DIFFERENT GENOTYPES OF *LYCOPERSICON ESCULENTUM* MILL.

BY

CONSTANTIN CRĂCIUN *, GABRIEL C. CORNEANU ** and VERONICA CRĂCIUN*

The ultrastructure of foliar palisadic parenchyma cells of four tomato genotypes is presented. The differences regarding the amount of cytoplasm and cytoplasmatic organites as well as the arrangement of the grana groups in chloroplast were evidentiated. The normal and adulterated ultrastructure of the nucleus is presented. The structure of the loose nuclear bodies is described as well as their relation with the nucleolus and their possible functions.

Morphological, physiological, biochemical differences, as well as ultrastructural differences between different genotypes in the framework of a species were described. On the ultrastructure of parenchyma foliar cells in tomatoes there are relatively few studies [1], [2], [3], [10]. Biogenesis and chloroplast ultrastructure are both under *gh* and *nv* nuclear gene control [1], [2], and of *Pl–alb–1* plastid gene [10]. Ultrastructural changes of the chloroplast and mitochondria between different genotypes of tomatoes [2], [10] as well as between different chlorophyllous mutants [1], [3] were found.

The nuclear structure in tomatoes was described both in different genotypes [2], [10] and in some chlorophyllous mutants [1], [3]; in the nucleus of some chlorophyllous mutants we noticed the existence of a corpuscle of the "loose nuclear body" types [3], similar with that described by some authors in other species [4], [6], [7], [8], [9], [12], [13], [14] was noticed.

In the present paper the chloroplast, mitochondria and nucleus ultrastructure of four different tomato genotypes were described. The existing relation between nucleolus, loose nuclear body and nucleolar organizer are discussed.

MATERIAL AND METHOD

The Red Cherry and Severianin sorts as well as the Angela and H-14 hybrids of tomatoes *(Lycopersicon esculentum* Mill.) were utilized. The Red Cherry sort is an androsterile sort (ms^{32} gene) with determined growth. The Severinian sort is a partenocarpic sort with determined growth. The Angela hybrid produced by the Enza Zaden firm (Holland) and resistant to TMV (presents the *Tm* group genes), displayed an undetermined

growth. H-14 hybrid produced at SCL-Iṣalniţa (Craiova) has no total resistance to TMV having an undetermined growth.

For the study of the ultrastructural characteristics of the foliar palisadic parenchyma cells, fragments from the middle part of the median foliole of the leaf which is above the second inflorescence of the blooming plants were gathered. Leaf fragments of about 1 mm^3 were prefixed in 3% glutaraldehyde (3 h), postfixed in the 1% Millonig solution $\left(1\frac{1}{2}\text{ h}\right)$, and then included in vestopal W. Seriated sections of about 800—900 Å in thickness were performed with the LKB Ultratome III ultramicrotome, contrasting of the sections being performed with uranyl acetate and lead citrate. Analysis of the ultrastructural characteristics of the cells was performed with the TESLA BS-613 electronic microscope of the biology department at the Cluj-Napoca University.

RESULTS AND DISCUSSIONS

Cell aspect. The foliar palisadic parenchyma cells usually present a central vacuole, the cytoplasm with cellular organites being disposed marginally along the cell walls. Between the different genotypes analysed, differences regarding the amount of cytoplasm and cytoplasmatic organites and their disposition in cells were found. Thus the Angela hybrid has a rich peripheral cytoplasm including cytoplasmatic organites disposed along the cellular walls. The Severianin sort cells have a similary ultrastructure. But the H-14 hybrid has a thin layer of cytoplasm on the margins of the cell walls; the cell organites surrounded by a thin layer of cytoplasm are not disposed on all the cell walls. The Red Cherry sort cells presented a similar aspect.

The chloroplast at tomatoes is of the granum type, usually having a lenticular-elongated shape. Granum groups oriented along the length of the chloroplast are made up of a variable number of thylakoids, bigger in the Angela hybrid (5—52) and smaller in the H-14 hybrid (3—30). With mature chloroplasts the strome thylakoids are slightly dilated, a few plastoglobules and numerous granules having synthesis substances (Fig. 1). In the aged chloroplasts the dilatations of stroma thylakoids increase thus breaking up the groups of grana thylakoides.

Between the analysed tomato genotypes there are differences in the sense that if the Angela hybrid has, as a rule, the typical structure of the chloroplast, the H-14 hybrid generally presents a desorganised chloroplast structure, a character found—to a lesser extent — in the Severianin sort, too. In addition in the Severianin sort with desorganized grana, the stroma thylakoides are U-shape or circle disposed (Fig. 2).

The mitochondria in tomatoes have an ovoid-elongated shape, of the crista type, displaying electron-dense dilatations of the cristas, less numerous in the Red Cherry and the Severianin sorts. In Fig. 3 the transversal division of a mitochondrium is presented.

The nucleus, of an elongated shape, is located at the periphery of mature cells. Inside it has a finely dispersed nuclear euchromatin and electron-dense heterochromatin zones along the internal membrane of the nuclear envelope. The outlying electron-dense heterochromatin makes

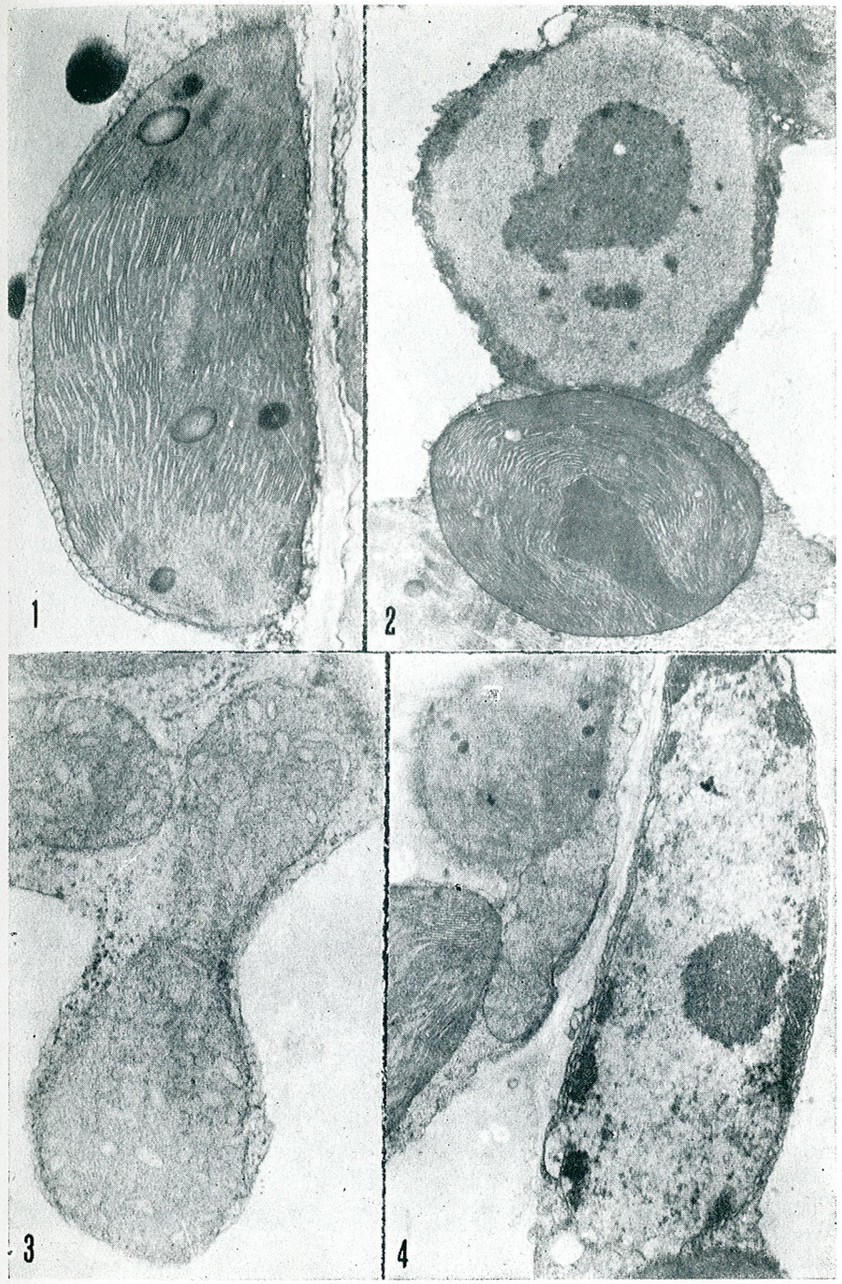

Plate I.

Fig. 1. — H—14 hybrid. Mature normal chloroplast with stroma thylakoids slightly dilated. × 20,250.
Fig. 2. — Severianin sort. Disorganized grana chloroplast and U-shaped stroma thylakoids. The heterochromatin in the nucleus lies both at the periphery (bodyguard) and in the central zone. × 15,000.
Fig. 3. — Angela hybrid. Mitochondrium in division. × 36,200.
Fig. 4. — Red Cherry sort. Nucleus with heterochromatin blocks disposed along the internal margin of the nuclear envelope and fine inner cords. × 17,900.

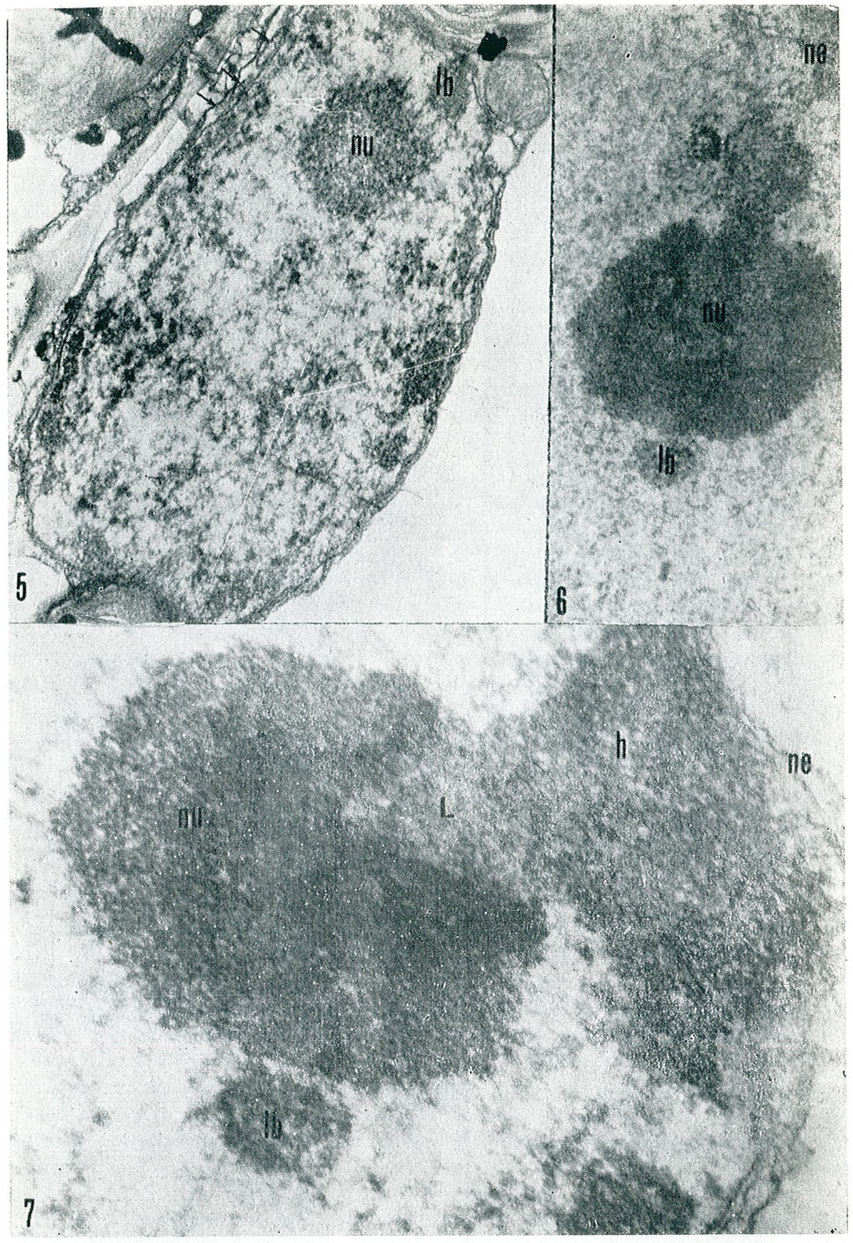

Plate II.

Fig. 5. — H—14 hybrid. Nucleus with nucleolus (nu) and loose nuclear body (lb) free in the nucleus. The dilated space between the two membranes of the nuclear envelope includes structured corpuscles (arrows). × 18,100.
Fig. 6. — H—14 hybrid. Nucleolus in association with loose nuclear body. × 32,000.
Fig. 7. — Severianin sort. Nucleolus with loose nuclear body. The nucleolar organizer (L) is in close relation both with the granular zone of the nucleolus and with the heterochromatin (h) disposed along the nuclear envelope (ne). × 67,400.

up the "bodyguard" (Figs 2, 4) in the conception of T.C. Hsu [5]. It is allegedly formed from repetitive DNA playing the role of protecting the euchromatic genes especially against chemicals and viruses. In case of an increased synthetic activity, the nucleus has fine cords of heterochromatin inside (Red Cherry sort, Fig. 4).

The abnormal nucleus in Fig. 2 (Severianin sort) probably presents a depolimerization process, the heterochromatin being found both at the internal margin of the nuclear envelope, and in the central zone of the nucleus which displays vacuolarization zones.

In the H-14 hybrid some nuclei present dilatations of the space separating the two membranes of the nuclear envelope where there are elongated structures of about 0.3 μm with an electron-dense central part and an external membrane, electron-dense parallel striations being present in their matrice (Fig. 5, arrows). On the internal part of the nucleus envelope there are small electron-clear cisterns of spherical-to-triangular shape involved probably in the nucleus-cytoplasme substance exchanges. The heterochromatin, which has no longer any direct contact with the internal membrane of the nuclear envelope but usually lies a little farther away, forms a net inside nucleus (Fig. 5). This suggests a stimulation of the synthetic activity. The general aspect of the nucleus is similar to that recorded in the case of the viral infection.

The nucleolus has a fibrilar-granular structure, the fibrilar zone lying in its center (Figs 6, 7). The nuclear organizer (*L* zone, the chromatin part containing the genes implied in r-RNA synthesis) is in connection both with the granular zone of the nucleolus, and of the heterochromatin zone of the nucleus periphery (Fig. 7).

In the Red Cherry and Severianin sorts as well as in the H-14 hybrid we noticed the presence in the nucleus, near nucleolus, of "loose nuclear body" type corpuscles (Figs 5, 6, 7). They were remarked either free in the nucleus near the nucleolus (Fig. 5), or in association with it (Figs 6, 7). The loose nuclear body has a spheric-to-elongated-spheric shape, being made up of fibres disposed relatively in parallel in an electron-clear matrice (Fig. 7). Every fibre has a diameter of 24—30 nm and is made up of several fibrils of about 16 Å in diameter (E. C. Jordan [8], C. Crăciun et al. [4]). Its structure is similar to that of a nucleolus because the nucleolonema fibrils have 15 Å in diameter (L. Recher et all. [11]).

The loose nuclear bodies were noticed in several species of plants of different origin (ferns, gymnosperms, angiosperms), identified in the normal cells (especially in the meristematic tissues) or experimentally stimulated, as well as in those derived from ill organisms. Similar formations were evinced in the hormonally stimulated or virus infested animals.

J. G. Lafontaine [9] has pointed out their presence in the meristematic cells of *Allium cepa, Vicia faba, Raphanus sativus*. E. G. Jordan [8] has met them in the phloem parenchyma cells of the *Daucus carota* root. Similar formations were described by other authors in *Pisum sativum, Spirogyra* sp., *Plantago ovata, Zea mays, Crepis capillaris, Beta vulgaris*. C. Crăciun and G. C. Corneanu [3] noticed them for the first time in *Lycopersicon esculentum* after analyses of the chlorophyllous mutants. C. Cră-

ciun et all. [4] revealed their presence in the callus cells and in the leaflets of *Dianthus* sp. grown "in vitro"; their number is bigger in the plants grown in culture media with addition of procaine and β-naphtoxiacetic acid (growth stimulators).

Some authors associated their presence to a viral infection [8], [12], [14]. E. G. Jordan and J. M. Chapman [6], [7] and A. M. Vagner-Capodano et al. [13] demonstrated an increasing r-RNA synthesis in their presence.

It is sure that both in case of a viral infection and synthetic stimulation (a characteristic state of the meristematic cells that undergo active mitotic division), the substance synthesis increases in the cell, the loose nuclear bodies being able to get involved in the substance synthesis or in their transport.

CONCLUSIONS

1. The chloroplast ultrastructure in tomatoes, determined by nuclear and plastid genes, is dependent on their genotype.
2. The presence in the nucleus of the loose nuclear body points out an increased synthetic activity.
3. The loose nuclear body is formed of fibrils similar to the nucleolonema fibrils.

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IN VITRO MORPHOGENESIS IN DIPLOID, TRIPLOID AND TETRAPLOID GENOTYPES OF WATERMELON – *CITRULLUS LANATUS* (THUMB.) MANSF

BY

ION ANGHEL and ANA ROŞU

A study was carried out on "in vitro" morphogenesis of watermelon, in order to assess the potentialities of such techniques for obtaining an increased number of plants belonging to valuable genotypes.
Adventitious bud induction from cotyledonary explants was achieved on MS basal medium with various combinations of growth substances and vitamins. Long-term cultures from diploid and tetraploid genotypes produced abundant adventitious shoots from which functional plants were regenerated. Meristem cultures were initiated also on the MS basal medium with two optimal combinations of growth regulators : BAP (2.25 mg/l) plus IAA (0.18 mg/l) or BAP (2.0 mg/l) plus NAA (0.1 mg/l). Shoot apices from diploid, triploid and tetraploid lines of watermelon were successfully reared "in vitro" and yielded shoots usable for rooting. The importance of producing a high percentage of triploid plants with seedless fruits in a more economical way is discussed.

The demonstration of the hormonal control of the cytodifferentiation and morphogenesis "in vitro" opened new research avenues for rapid clonal propagation and breeding of a wide range of economically important plant species. For some genotypes whose vegetative multiplication by conventional methodology is too laborious or even impossible, the establishing of the new methods for clonal propagation by using tissue culture techniques proved to be essential for achieving a high economic efficiency [3], [4], [8].

To our knowledge little research has been done on "in vitro" morphogenesis in watermelon [2] as well as on other cucurbits [7].

The main aim of our experiments was the use of tissue culture techniques as a means to overcome difficulties and consequently obtain a sufficient number of triploid watermelon plants. Their obtainment by conventional methods is laborious and the yield is very low. The interest for increasing the number of triploid plants is justified by the superior qualities of their fruit : higher levels of sugars, vitamin C, proteins and reduced quantities of malic acid and cellulose, which may affect the taste ; the triploid plants have a longer period of vegetation than the diploid ones and a better resistance to pests and diseases. Beside these advantages that are the result of a combination of traits determined by poliploidy and the heterosis phenomenon, recognized in all morphologic, physiologic and biochemical characteristics, the triploid fruits enjoy also a special commercial priority, because of their lack of seeds [1].

MATERIAL AND METHOD

For meristem cultures, explants of 0.5—1.0 mm (the meristematic dome plus 2—3 leaf primordia) were harvested either from plantlets obtained from germinating seeds in the laboratory or from field-grown plants. Surface sterilization was performed by using 0.05% mercuric chloride for 25—30 min., followed by 3—5 rinses in sterilized distilled water. The meristems were detached and transferred to Petri plates containing 10 ml of Murashige and Skoog basal medium (MS) with two optimal combinations of growth regulators : 2.25 mg/l BAP and 0.18 mg/l IAA or 2.0 mg/l BAP and 0.1 mg/l NAA.

For adventitious bud induction, watermelon seeds were surface sterilized in 1.0% mercuric chloride for 20—30 min., washed three times with sterile distilled water and germinated under aseptic conditions on wet cotton. When the plantlets had the epycotyls just emerging, the shoot apices were carefully removed from the cotyledonary node and the cotyledons were cross-sectioned in explants about 0.2—0.5 cm wide. The explants were placed in Petri plates containing 10 ml agar media (table 2) with the

Table 1

Adventitious shoots from cotyledonary explants

Cultivar	Nutrient media (Basal medium MS)						
	Vitamins	BAP (mg/l)	Kin (mg/l)	IBA (mg/l)	IAA (mg/l)	2,4-D (mg/l)	Sucrose (g/l)
Arad XIV-2 (2n)	Staba	1.0	—	—	—	0.1	20
	MS	2.25	—	—	—	—	30
Arad VII-1001 (4n)	Staba	1.0	—	—	—	0.1	20
	MS	2.25	—	—	—	—	30
	Staba	1.0	—	0.2	—	—	20
Arad XIV (4n)	Staba	1.0	—	—	—	0.1	20
Timpuriu de Canada (2n)	Staba	1.0	—	—	—	0.1	20
	MS	2.25	—	—	—	—	30
	MS	—	0.044	—	0.018	—	30
Baby Sugar (2n)	MS	2.25	—	—	—	0.0221	30
Sweet (2n)	MS	2.25	—	—	—	—	30
Graybelle (2n)	MS	2.25	—	—	—	—	30

MS = the basal medium Murashige and Skoog (1962)
BAP = 6-benzyl-aminopurine
IAA = 3-indoleacetic acid
IBA = 4-(3-indolyl)-butyric acid
2,4D = (2,4-dichlorophenoxy)acetic acid
Kin = kinetin

pH adjusted to 5.6—5.7 before autoclaving at 121°C for 20 minutes. The cultures were grown at 25°C (±2°C) with a daylength of 16 hours at an illuminating intensity of 7.000 lx.

For light microscopic examination the material was fixed in the Navashin fixative, embedded in paraffin, sectioned at 13 μm and double stained with hemalaun Mayer and eosin.

RESULTS AND DISCUSSION

I. Meristem culture

Looking for a new method to improve the obtaining of the triploid forms of watermelon, the meristem culture was firstly attempted. In order to assess the potentialities of this method for clonal propagation of watermelon, some diploid and tetraploid forms were also experimented. The effects of a range of nutrient media were studied and the final medium

Table 2

Cultivars propagated by meristem cultures

Explant sources	
Field-grown plants	Plantlets from germinating seeds
Varianta 1 4n × Graybelle 2 n (3n = 33)	Timpuriu de Canada(2n)
Varianta 1 4n × Princeton Sweet 2n (3n = 33)	Arad XIV — 2 (2n)
Varianta 1 4n × Baby Sugar 2n (3n=33)	Baby Sugar (2n)
Varianta 1 4n	Arad VII (4n)
Graybelle 2n	Varianta 1 — 102 (4n)
	Varianta 1 — 101 (4n)
	Arad XIV (4n)
	Brăila XI — 1022 (4n)
	Brăila VIII — 1061 (4n)
	Princeton Sweet (2n)

chosen was that of Murashige and Skoog (1962) (5) with BAP (2.0—2.25 mg/l) in combination with NAA or IAA (0.10—0.18 mg/l) which allowed maximum proliferation. All genotypes of the genus *Citrullus* cultivated "in vitro" (table 2) were able to develop multiple shoots by meristem cultures.

Buds and shoots appeared after 4 weeks (Plate I, 1) and the morphogenetic cultures were maintained "in vitro" for prolonged periods of time by periodic subcultures on fresh media (Plate I, 2 ; Plate II, 1,2). Good results were obtained also by using the whole shoot apex (1.0 cm long) as explant, on the same nutrient media, which stimulated multiple shoot development (Plate III, 1, 2, 3).

Shoots of 1.0—2.0 cm were detached from the culture and transferred to the rooting media (Plate IV, 1) that consisted either of the basal nutrient medium of MS without growth substances or of the same medium supplemented by 1.8 mg/l IAA and 0.022 mg/l Kinetin. The rooted plantlets (Plate IV, 2, 3) were then transferred to pots with sterilized soil and after gradual conditioning they were able to grow in the open environment (Plate IV, 4).

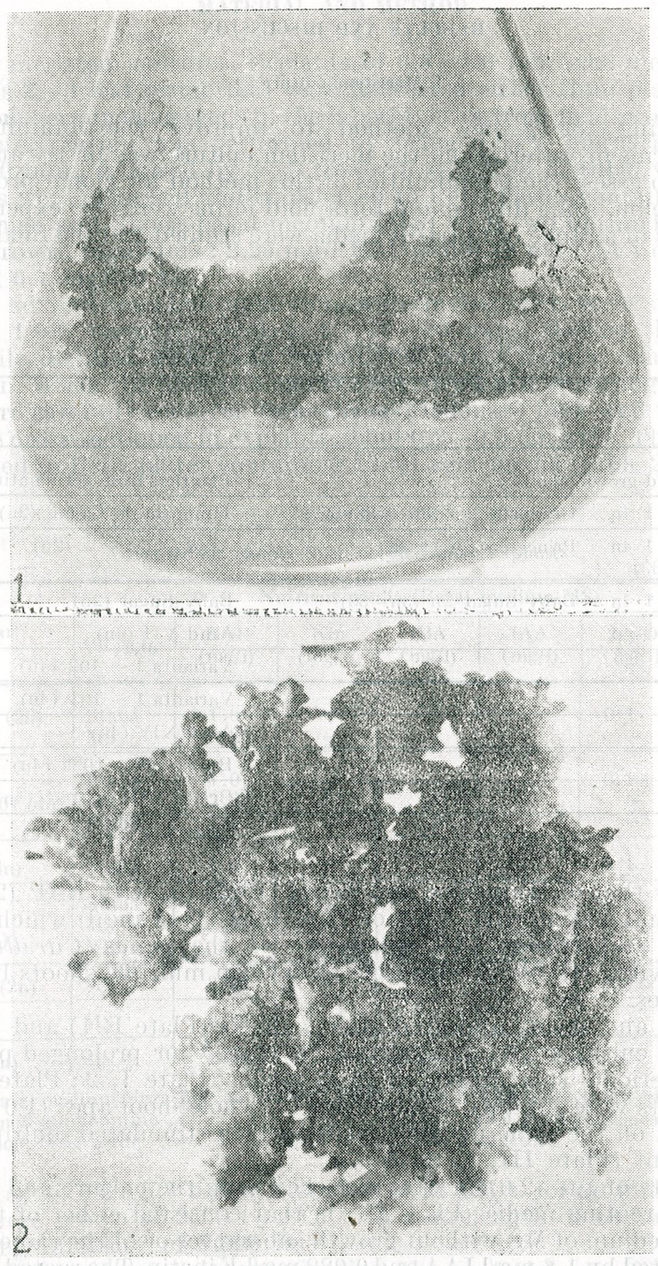
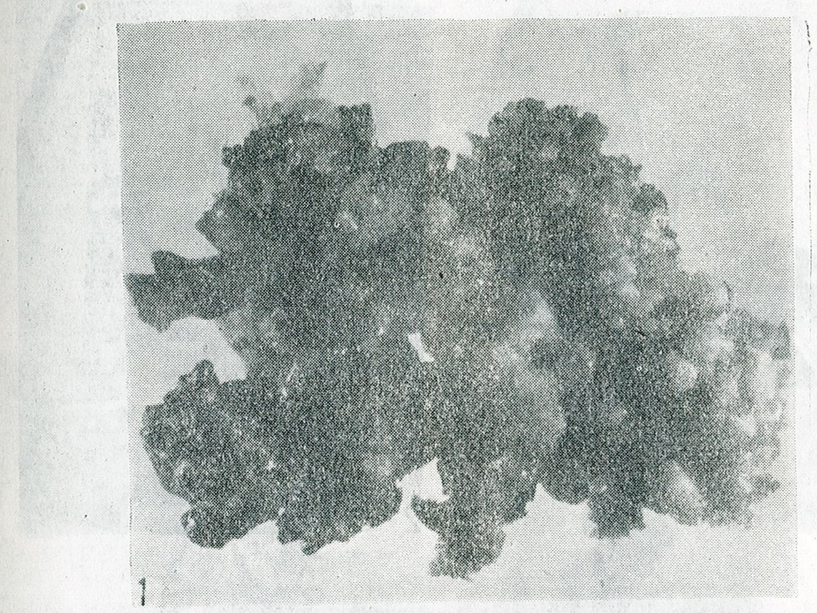


Plate I — Bud and shoot differentiation by meristem culture from the diploid cv. "Princeton Sweet".

Fig. 1 — Morphogenetic culture with the development of the first shoot primordia.
Fig. 2 — Advanced stage of culture with numerous buds and shoots.



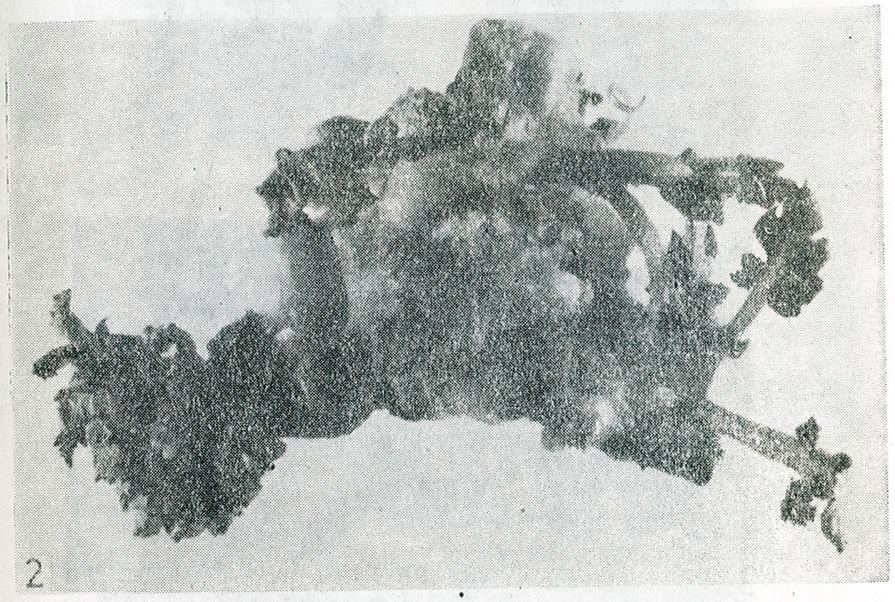


Plate II — Differentiation of buds and shoots in meristem cultures from the triploid hybrid "Varianta 1 (4n) × Baby Sugar (2n)" (Fig. 1) and from the tetraploid cv. "Arad XIV" (Fig. 2).

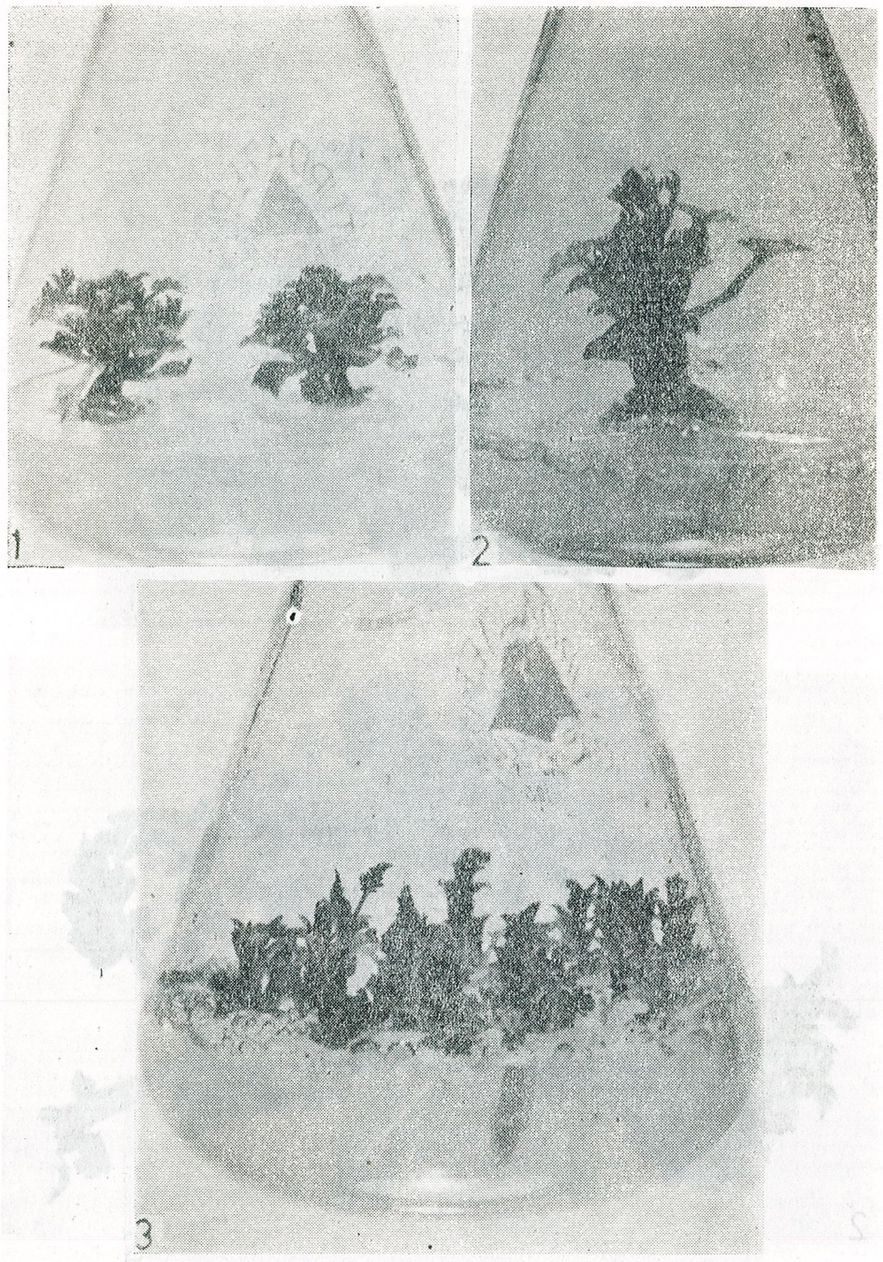


Plate III — Multiple shoot development from shoot apices.

Figs 1, 2 — Shoots developed from the cotyledonary node in the cv. "Princeton Sweet" (2n) (Fig. 1) and in the cv. "Varianta 1" (4n) (Fig. 2). Fig. 3 — Shoots separated from the culture and transferred to the rooting medium.

Plate IV — Successive stages of plant regeneration by meristem culture.

Fig. 1 — Detached shoots placed on the rooting medium. Figs 2, 3 — Rooted plants ready to be transplanted into the soil. Fig. 4 — Plant adapted to the open environment.

Though the genetic studies are still under way, by observing the morphologic features of the regenerated plants, we expect that they retain the genetic characteristics of the parent plants.

II. Adventitious shoot induction

Due to the fact that the adventitious shoots can be used with good results not only in plant propagation but also in mutation breeding, another series of our experiments was meant to achieve plant regeneration from cotyledonary explants cultured "in vitro", by adventitious bud induction. The attempts to obtain the same phenomenon by using hypocotyl explants were not successful [7], the only morphogenetic response of these explants being the callus and roots (Plate V, 1, 2, 3, 4, 5). Although on some nutrient media the callus was successfully induced both from hypocotyl and from cotyledonary explants, we encountered some difficulties in obtaining long-term callus cultures, because of a senescence which became evident after a few subcultures. More than that, the "in vitro" cultures often remain infected with latent bacterial contaminants that manifest during the subsequent subcultures.

Some preliminary experiments with cotyledonary explants showed the morphogenetic potential of this tissue [6], [7]. The media used with positive results in this respect as well as the diploid and tetraploid genotypes used with varying degrees of success in adventitious shoot induction are presented in table 1. The phenomenon occurred regardless of the ploidy degree of the donor plant. The BAP seems to be an obligatory prerequisite for adventitious bud induction. Buds developed in the presence of the cytokinin, alone or in combination with auxins. They protruded to the surface after four weeks of culture (Plate VI, 1, 2, 3) and some of them elongated into shoots from which complete plants were regenerated (Plate VI, 4). The effect of the genotype and of the position in the organ from which the explants was harvested on the morphogenetic processes were described in a previous paper [7]. The first striking event noticed after one week of culture "in vitro" was the excessive enlargement of the cotyledonary explants under the effect of the BAP. The histological studies showed significant morphological changes in the tissues structure, expressed by the individualization of large zones of cells with meristematic characteristics, from which buds emerged (Plate VII, 1, 2, 3, 4). Some explants displayed high morphogenetic capacities and we were able to establish long-term cultures that generate buds and shoots continuously (Plate VIII, 1). After the rooting of the adventitious shoots, the reconstituted functional plants (Plate VIII, 2, 3) were transferred into the soil in the same way as described for plants obtained from meristem cultures.

Our experimental data show the efficiency of meristem culture and adventitious shoot induction for clonal propagation of various watermelon forms with different levels of ploidy.

We may conclude that by combining the "in vitro" techniques with the conventional methods the number of watermelon plants belonging to valuable genotypes could be considerably increased.

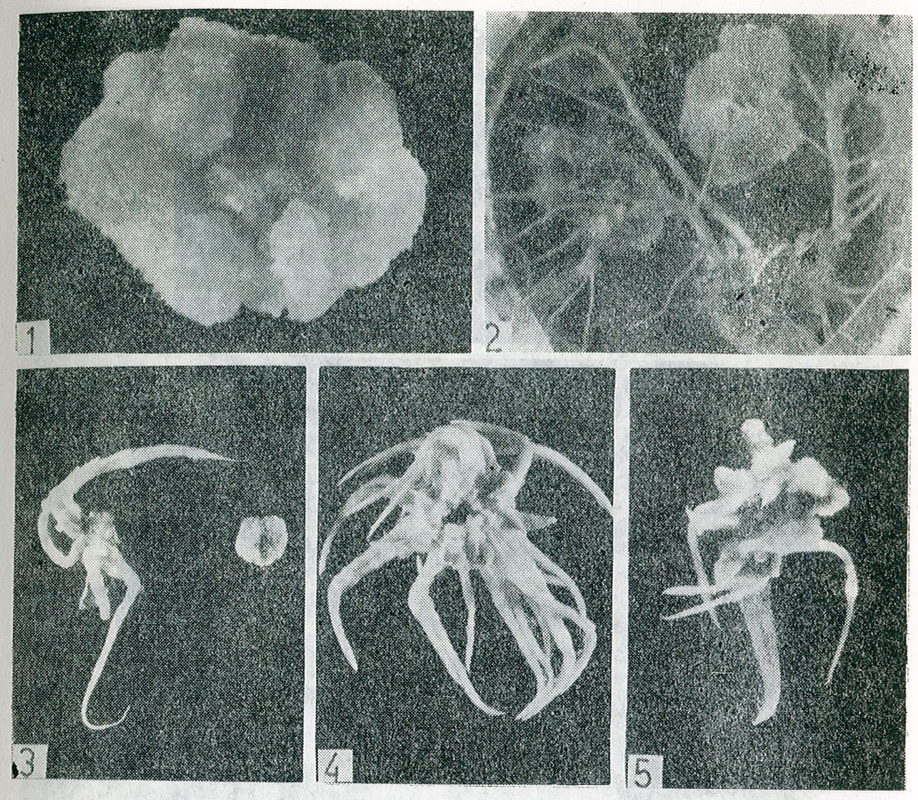


Plate V — Aspects of morphogenesis in hypocotyl explant cultures.

Fig. 1 — Proliferation of callus. Fig. 2 — Rhysogenesis from callus. Figs 3, 4, 5 — Rhysogenesis directly from hypocotyl explants.

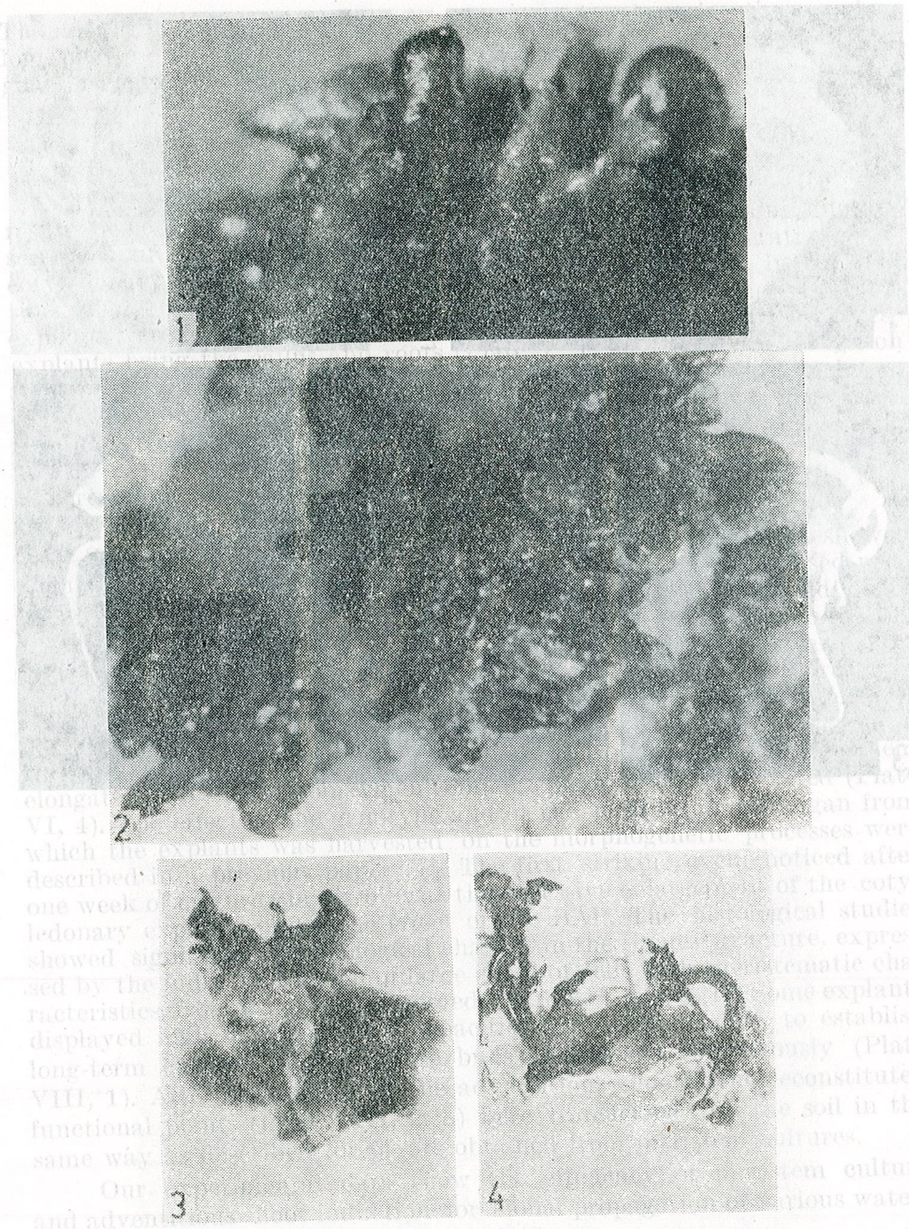


Plate VI — Successive stages in the differentiation of adventitious buds from cotyledonary explants.

Figs 1, 2, 3 — Incipient phases in bud development (Fig. 1, 2, × 5) cv. "Graybelle" (2n)
Fig. 4 — Elongated shoot apt for rooting, cv. "Princeton Sweet" (2n).

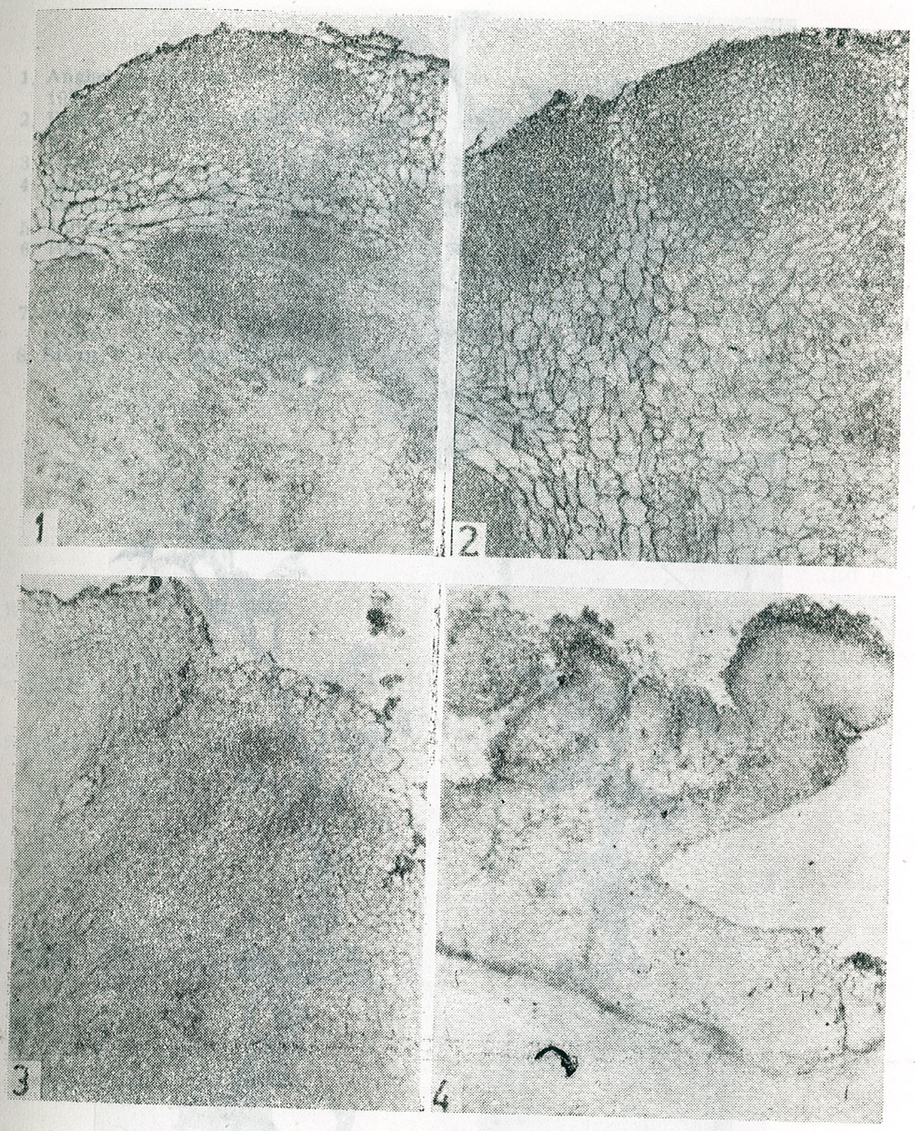


Plate VII — Histological aspects of the morphogenetic cotyledonary explants.

Figs 1, 2 — Individualization of cell zones with meristematic characteristics (meristemoids). (Fig. 1 — × 80; Fig. 2 — × 100)
Fig. 3 — The beginning of organogenesis (× 100).
Fig. 4 — Developing adventitious shoot from the cotyledonary explant (× 80).

Plate VIII — Functional plant regeneration from adventitious shoots.
Fig. 1 — Long-term culture with a high morphogenetic capacity — cv. "Arad VII" (4n).
Fig. 2 — Adventitious shoot rooted in the test-tube — cv. "Varianta 1" (4n).
Fig. 3 — Autonomous plant adapted to the open environment — cv. "Timpuriu de Canada" (2n).

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PLEIOTROPIC ACTION OF *nv* GENE IN TOMATO (*LYCOPERSICON ESCULENTUM* Mill.)

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The ultrastructural features of palisade parenchyma cells of the mature leaves, the amount of carotenoid and chlorophyll pigments as well as the size and chemical composition of the fruits in two tomato lines with different degrees of zygosis for *nv* gene were studied. The research results indicate that the chloroplast ultrastructure is a polygenic character, being determined both by plastid genes (*Pl — alb 1*) and pleiotropic genes placed on nuclear DNA (*gh* and *nv*). The *nv* gene has a pleiotropic action, affecting also the nucleus and mitochondria ultrastructure, and implicitly some morpho-physiological and biochemical characters (the fruits size and their chemical composition, the amount of carotenoid and chlorophyll pigments a.o.).

Out of more than 600 known genes in tomato [12], the *Tm-2* and *nv* genes lie on the chromosome 9, they are linked and have 22 "map units" [10]. The *Tm-2* gene renders the plants resistant to tobacco mosaic [8] and the *nv* gene (netted virescent) affects mainly the leaf colour and plant vitality.

For the purpose of knowing the *nv* gene action, this study of two tomato lines of different degrees of zygosis for *nv* gene was performed.

MATERIAL AND METHOD

Biological material

The *Tm-2 nv/Tm-2 nv* and *Tm-2 nv/++* tomato lines were cultivated under identical condition at the Vegetable Growing Research Station Işalnița (Craiova). Due to presence of *nv* gene in a homozygote state, the *Tm-2 nv/Tm-2 nv* line has a greenish colour of the leaves, total lack of vigour and fertility [9]. The presence of *nv* gene in a heterozygote state (*nv/+*) within the *Tm-2 nv/++* line determines a normal development of the plants.

Working method

The ultrastructural characteristics of the palisade parenchyma cells, the amount of chlorophyll and carotenoid pigments from the leaves, as well as the size and chemical composition of the fruits in the two tomato lines were studied.

The mean values of the fruits were calculated on the basis of 50 measurements. The chemical analyses of the fruits were carried out according to the classical methods.

The chlorophyll and carotenoid pigments amount in the leaf was determined by the spectrophotometrical method.

The parenchyma cell ultrastructure of the mature leaves from the two tomato lines was studied by means of a TESLA BS-613 electronic microscope (Biology Dept, Cluj-Napoca University). The about 1 mm large pieces of fresh leaves were prefixed in 3% glutaraldehyde (2 h), postfixed in the 1% Millonig fixing solution (1 1/2 h) and then included in vestopal W. The seriated sections, about 800—900 Å thick, were performed at the ultramicrotome LKB Ultratome III, the sections being contrasted with uranyl acetate and lead citrate.

RESULTS AND DISCUSSIONS

The previous researches [2] established that between the two tomato lines the differences recorded regarded the epidermic cell dimensions of the leaves, of the stomata and trichomes on both faces of the leaf, generally presenting lower values in the homozygote line for the *nv* gene. The stomata are much less dense on the homozygote line *(nv/nv)*, distinct and very significant differences existing between the two studied lines in both faces of the leaf [2].

The ultrastructure of the palisade parenchyma cells is different in the two tomato lines.

Tm-2 nv/++ line (Plate 1). The cells have a high quantity of cytoplasm with numerous chloroplasts, mitochondria, ribosomes, dictyosomes, elements of the endoplasmic reticulum, a.o. (Fig. 1), along all the walls of the cell. The nucleus (Fig. 2) has an elongated shape being situated near plasmalemma in mature cells. It has an electrondense feature because of the small particles of heterochromatin that are disposed among its own euchromatin. Around the inner nuclear envelope its heterochromatin is concentrated in very electrondense blocks (Fig. 2). The mature chloroplasts (Figs 1, 3) display grana groups formed of 5—30 thylakoids, orderly oriented along their length. Inside there are also pyrenoid corpuscles and accumulated substances.

Tm-2 nv/Tm-2 nv line (Plate 2). The palisade parenchyma cells of the homozygote line for the *nv* gene contain a lower amount of cytoplasmic constituents which are not disposed on all the walls of the cells (Fig. 4). The chromatin of the nucleus does not contain (in the homozygote line) heterochromatic particles dispersed in it, the number of heterochromatic blocks near the nuclear envelope being also lower (Fig. 5). In the caryoplasm, near the nucleolus, there is a corpuscle of the "loose body" type (Fig. 5), similar with those described by E. G. Jordan [5] in *Daucus carota*, C. Crăciun et al. [3], [4] in *Dianthus caryophyllus* and *Lycopersicon esculentum*, sector normal-viridis mutant and other authors. The presence of the corpuscles of the „loose body" type in the nucleus can be the result of one cellular hyperactivity determined by the action of the stimulating hormone (C. Crăciun et al., 1981 a.o.) or by a viral infection (E. G. Jordan,

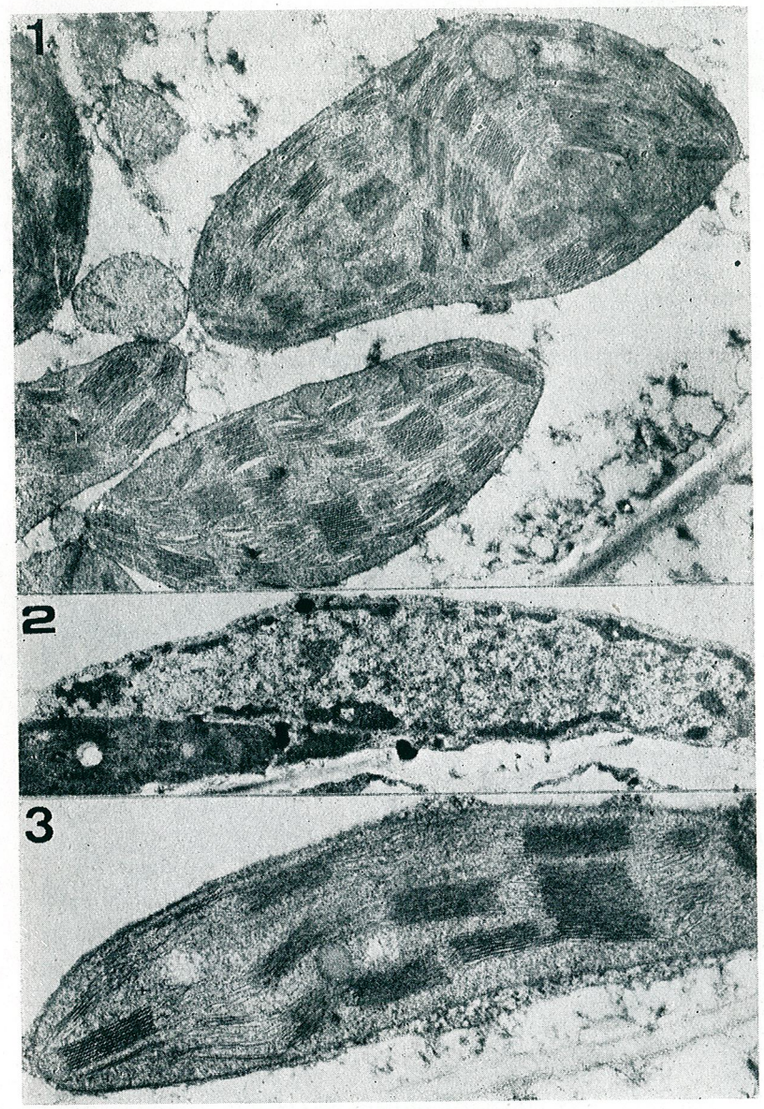

Plate I. The *Tm-2 nv/++* tomato line.

Fig. 1. — The palisade parenchyma cell with normal chloroplasts and mitochondria. × 15,500.
Fig. 2. — The interphasic nucleus. × 9,500. Fig. 3. — The mature chloroplast. × 28,500.

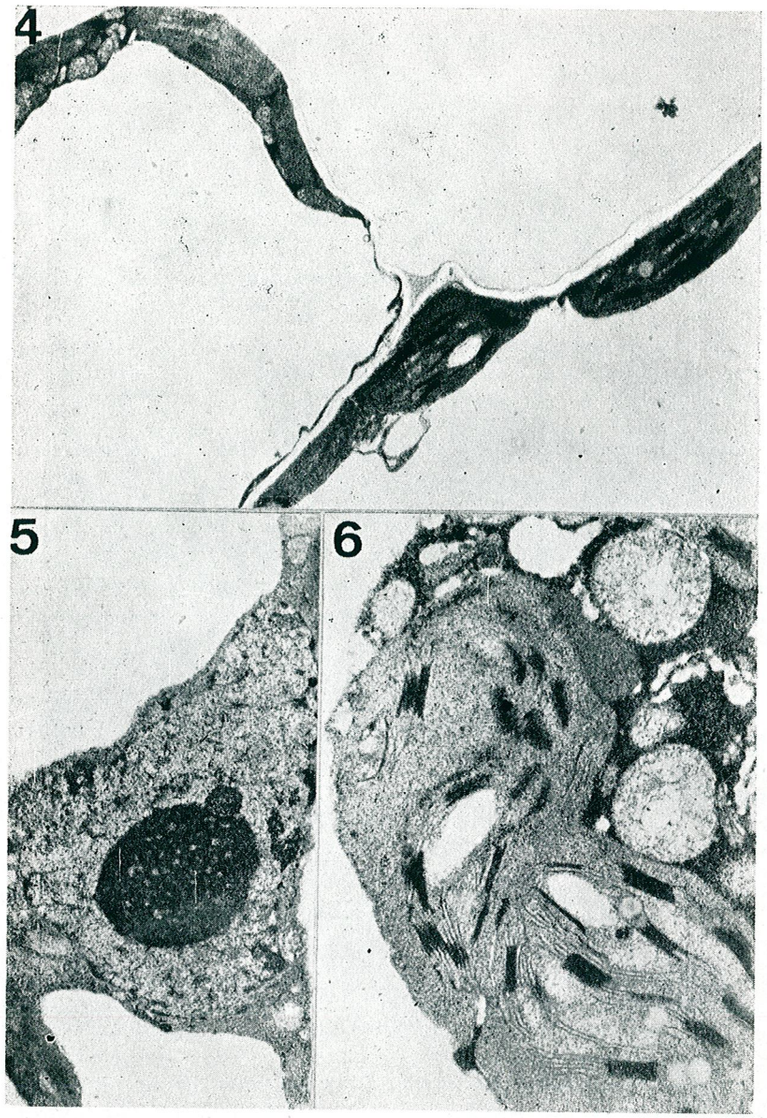

Plate II. The *Tm-2 nv*/*Tm-2 nv* tomato line.

Fig. 4. — Ensemble palisade parenchyma cells with a little quantity of all citoplasmic constituents. × 7,600. Fig. 5. — The nucleus with the corpuscle of "loose body" type. × 8000. Fig. 6. — The chloroplasts and mitochondria with disorganized structure. × 18,000.

1976; C. Crăciun, G. C. Corneanu, 1980 a.o.). In this case their presence is determined by a viral infection. The chloroplast ultrastructure, and that of mitochondria is strongly modified in the homozygote line for the *nv* gena (Fig. 6). Most of the chloroplasts have an altered ultrastructure, the grana groups being oriented disorderly, and the number of thylakoids in the grana is lower (Fig. 6). The mitochondria generally have fewer cristas as well as some dilatations and clarifications in their matrix (Fig. 6).

As a result of the alteration of the chloroplast ultrastructure, the content of chlorophyll and carotenoid pigments is lower in the homozygote line, especially the amount of B-chlorophyll and A-chlorophyll (Table 1).

Due to processes of low synthesis, the fruits' dimensions and weight are smaller in the homozygote line (Table 1). The chemical composition of the mature fruits in the two tomato lines shows different aspects, depend-

Table 1

Some features of the leaves and fruits in two tomato lines with different degrees of zygosis

Feature	*Tm-2nv*/++line	*Tm-2 nv*/*Tm-2 nv* line
The amount of chlorophyll and carotenoid pigments in leaves (mg/l g dry substance)		
A-Chlorophyll	0.69	0.33
B-Chlorophyll	0.13	0.016
Carotenoid pigments	0.36	0.29
The size fruits ($\bar{x} \pm s_{\bar{x}}$)		
Fruit's diameter (mm)	51.24±2.34	42.66±1.15
Fruit's height (mm)	47.73±1.16	38.31±0.76
Fruit's weight (g)	68.33±7.67	38.20±2.47
Chemical composition of the fruits		
Total dry substance (g/100 g fresh fruits)	7.86	6.07
Soluble dry substance (%)	4.80	5.10
C-Vitamin (mg/100 g fresh substance)	15.00	18.50
Acidity (g/100 g fresh substance)	0.44	0.50
Sugar (g/100 g fresh substance)	3.98	2.43
Tannin (g/100 g fresh substance)	0.02	0.03

ing on the analysed substance (Table 1). Thus the total dry substance amount is higher in the heterozygote line, while the soluble dry substance has higher values in the homozygote line. The acidity and C-vitamin amount have higher values in the homozygote line, while the sugar amount in the fruits has higher values in the heterozygote line (Table 1).

The alteration of the chloroplast ultrastructure, the recorded changes in the structure of the nucleus and mitochondria, the presence of a smaller amount of the cytoplasmic organelles in the palisadic parenchyma cells of the leaves, reveale the pleiotropic action of the *nv* gene in homo-

zygote state. As a result of these ultrastructural changes, the homozygote line for the *nv* gene has a lower content of chlorophyll and carotenoid pigments, as well as smaller fruits, at the same time the chemical composition of the fruits being modified as a result of the alteration of the processes of biosynthesis.

The biogenesis and the maintenance of the chloroplast function are both under the control of the nuclear genes and of the chloroplast genome [7]. Previous researches have pointed out that the ultrastructure and the function of the chloroplast, in tomato, are both under the control of the nuclear gene (the study of *gost*-mutant by W. R. Andersen et al., 1974), and the plastid genes (the study of plastom mutation *P1-alb 1* by M. S. Odintsova et al., 1978 and M. S. Turischeva et al., 1980).

Combining these findings with our results, it may be safely concluded that, in tomato, the ultrastructure and the function of the chloroplast are pleiotropic characters, determined both by the plastid genes (the gene *Pl-alb 1*), and the pleiotropic genes placed on the nuclear DNA (the genes *gh* and *nv*).

CONCLUSIONS

1. The study of the ultrastructural characteristics of the palisade parenchyma cells in tomato reveals that the *nv* gene contributes, together with other nuclear genes *(gh)* or plastid genes *(Pl-alb 1)*, to the maintenance of the ultrastructure and of the function of the chloroplast.

2. The *nv* gene has a pleiotropic action affecting also other ultrastructural characteristics of the palisade parenchyma cells (the ultrastructure of the nucleus and of the mitochondria, the quantity of cell organelles from the cell a.s.o.).

3. As a result of the ultrastructural changes induced by the *nv* gene, the processes of cell synthesis decrease and modify in the homozygote line, affecting mainly the mature leaves' content of chlorophyll and carotenoid pigments, as well as the size and chemical composition of the fruits.

4. The *Tm-2 nv/ Tm-2 nv* line does not put up a total resistance to virus infection. In the respective caryoplasm, near nucleolus, there is a corpuscle of the "loose body" type, its presence being determined (in this case) by a viral infection.

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INDUCTION OF ANDROGENESIS IN ANTHER CULTURE OF *DIGITALIS LANATA*

BY

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Using Murashige and Skoog medium supplemented with 1.0 mg/l BAP and 0.01 mg/l NAA pollen development in anther culture of *Digitalis lanata* was induced. Cold treatment at 5°C, for 5 days, enhanced frequency of responsive anthers. The anthers responded in culture quite differently by forming green, compact calli from which only one bud and shoot developed, yellowish, friable calli, in which embryoids in various stages of development were identified, as well as abnormal embryoids, their number ranging from 1 to 14 per anther. Excepting one, all types of calli and embryoids failed to develop.

Successful application of cell and tissue culture technology to crop improvement hinges on the ability of each species to grow and to regenerate plants *in vitro*.

In *Digitalis lanata* — a medicinal plant that represents an important source of cardiac glycosides — the differentiation was achieved as somatic embryogenesis in cell suspensions derived from calli originating in anther filaments [6], as indirect somatic organogenesis in calli developed from protoplasts [8], and as direct organogenesis when shoot apices were used as explants [2].

Digitalis lanata is an allogamous plant in which the attainment of pure lines represents one of the main aims of the breeding programs. Our researches aimed to assess the potentialities of the anther culture technique for obtaining haploid plants — the shortest way for the rapid production of homozygous lines.

MATERIAL AND METHOD

Flower buds were picked up from one or two year old plants, in full state of flowering. The donor plant from which the anthers were collected were grown in the field of S.C.P.M.A. — Fundulea.

After harvesting, the inflorescences were kept for 11 days at 5°C. Beginning with the forth day of storage, tens of flower buds were detached for culture every day. The surface sterilization was performed in 1‰ mercuric chloride or in a solution of 7% sodium hypochlorite for 15 and 10 minutes, respectively. In both cases a wetting agent was added (Tween 80). After the application of the sterilizing solution, the flower buds were repeatedly washed in sterile distilled water. For achieving our aim — recovering plants from pollen — a great number of cultures was

necessary, this rendering difficult the cytological examination of pollen grains. In this situation, anthers of various sizes, containing microspores in all stages of development, were put into culture. By dissecting the flower buds, 25 aseptically detached anthers were placed in each culture vessel. The cultures were grown on solid agarized medium, in Petri plates of 6 cm. in diameter. For anther culture, the basal media of Murashige and Skoog [4] and of Nitsch [5] were used with four hormonal variants (Table 1). The cultures were maintained at 25—30°C, with a daylength of 16 hours, at an illuminating intensity of 1000 lx.

For a histochemical study, the material was fixed for 24 hours in Navashin-Bruun solution, embedded in paraffin, sectioned at 13 μm and stained with 1% toluidine blue.

RESULTS AND DISCUSSION

The results regarding the influence of various hormonal combinations on the reponse of the anthers cultivated on two basal media are presented in table 1.

Table 1

The influence of the culture medium on the anther response

The basal media	Hormonal combination mg/l	No. of inoculated anthers	No. of responsive anthers	% responsive anthers
Two year old donor plant				
Nitsch	1.0 BAP—0.01 NAA	395	1	0.25
Nitsch	0.2 BAP—0.1 NAA	729	2	0.26
MS	1.0 BAP—0.01 NAA	1128	10	0.81
MS	1.0 NAA—0.1 BAP	269	—	—
One year old donor plant				
Nitsch	1.0 BAP—0.01 NAA	370	—	—
Nitsch	0.2 BAP—0.1 NAA	400	—	—
MS	1.0 K—0.01 NAA	620	—	—
MS	1.0 BAP—0.01 NAA	672	1	0.15

BAP = 6 benzyl aminopurine; NAA = 2 naphthylacetic acid;
K = Kinetin; MS = the basal medium Murashige and Skoog (1962).

The data presented show that for inducing pollen androgenic development the presence of both types of hormones in the culture medium is required, in a ratio favoring the cytokinin. In addition, the anthers responded in a higher percentage (0.81) on the basal medium MS than on the medium Nitsch.

The diagrammatic presentation of the results in fig. 1 illustrates that a 5—6 days pre-treatment at 5°C stimulates the response of the anthers to *in vitro* culture.

Most of the anthers retained the normal colour during the period of incubation regardless of the variant of the culture medium and the incidence of anther wall proliferation with the formation of callus was not noticed.

At the site of the anther filament incission, providing it was in contact with the nutrient medium, sometimes a callus developed that differentiated shoots on the variants with a high cytokinins content and roots in the presence of a high auxins content. Calli or embryo-like structures appeared after an incubation period that lasted 11—21 days. Especially interesting is the fact that the "products" of this process were completely detached from the anther, being sometimes placed even at some distance on the culture medium (Fig. 2). The productivity of the anthers was generally low, ranging from 1 to 14 embryo-like structures per anther. With one exception, all the structures that appeared in anther cultures of *Digitalis* failed to develop further.

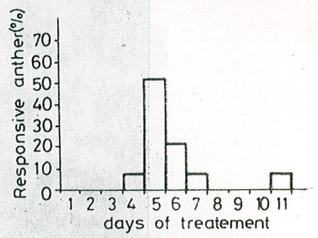


Fig. 1 — Influence of the length of the pretreatment applied to inflorescences upon the anther response.

"The responses" of the anthers in culture belong to three distinct categories: green, compact calli that stopped their evolution very soon or underwent an organogenic process, developing a single shoot (Fig. 3); embroids with atypical features, 1—14 per anther in number, that did not continue to develop on any of the tested media (Fig. 4); friable, achlorophyllous calli, associated with embryo-like structures (Fig. 5). In the latter case, the dissociation of a small piece of callus containing small structures, visible with the naked eye, in a drop of water, allowed the revealing of an intensive process of embryogenesis, numerous embryoids in early stages of development being observed. The very young embryoids were attached by means of the suspensor (Fig. 6) to a globular structure (Fig. 7) differentiated from the embryogenic callus (Fig. 8). It is perhaps the case of an intensively adventive embryogenic process. Histological investigation confirmed both budding of pro-embryoids from periphery of embriogenic callus (Fig. 9) and their abnormal anatomy (Fig. 10—11). The abnormal shape of the embryoids could be the consequence of their origin from pollen, whose chromosomal constitution does not permit the normal process of differentiation, a frequent phenomenon noticed in anther cultures derived from *Datura innoxia* plants with an uneven number of genomes [1]. It is also possible that in our experimental conditions the normal and complete development of the embryos did not take place.

The results of the researches regarding androgenesis in various species revealed the fact that is it possible to obtain plants from pollen either directly (pollen-embryoid-plant), or indirectly, after passing through an intermediary callus stage (pollen-callus-plant) [7]. In the calli derived from pollen, the differentiation may occur either by organogenesis or by embryogenesis, depending on the species. In *Datura meteloides*, Geier and Kochlenbach [3] described a particular way of pollen development in anther cultures: pollen-embryoids-callus-plants; the differentiation in callus took place through the embryogenic pathway.

The obtainment of the two types of pollen calli in which, in connection with their totally different aspect, either the organogenetic process

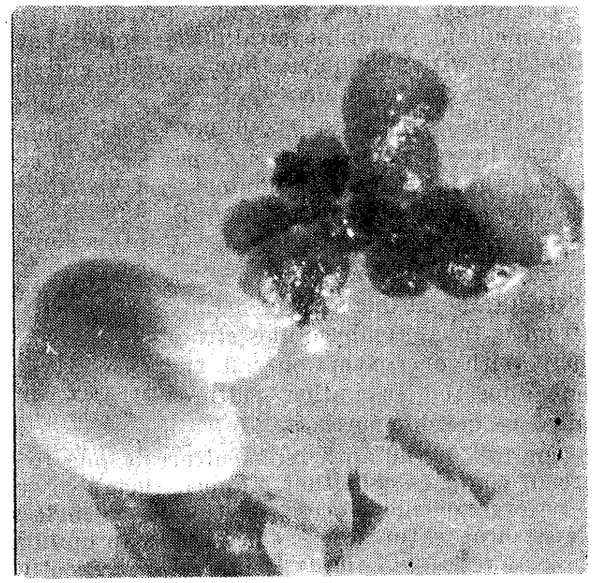


Fig. 2 — Embryoids completely detached from the anther wall, "thrown" on the surface of the culture medium.

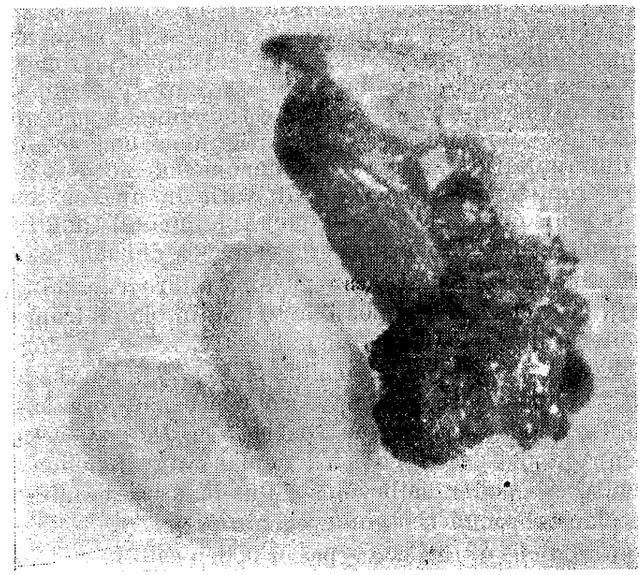


Fig. 3 — Shoot differentiated from callus derived from pollen.

or the embryogenic one occurs, represents a particular case that we noticed for the first time in *Digitalis lanata*. The results of further researches under way, regarding the influence of other biological, chemical and physical factors on the behaviour of anthers in culture, will show, if possible, the induction in this species not only of the androgenic development, but also the obtainment of plants from pollen.

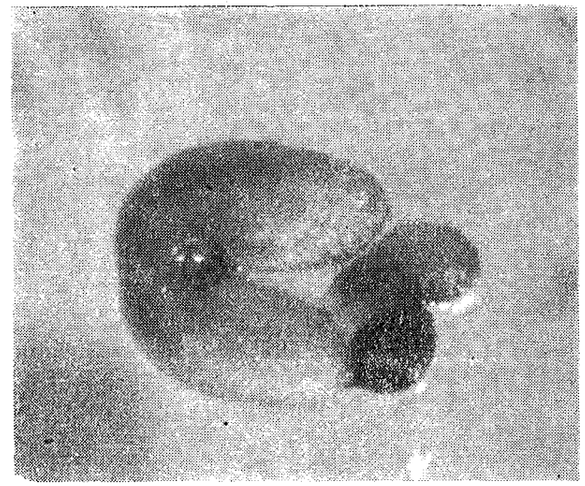


Fig. 4 — Embryoid with abnormal aspect originating from pollen.

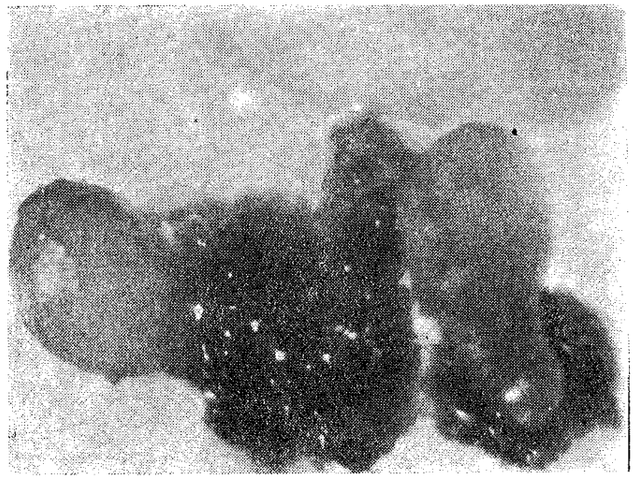


Fig. 5 — Direct and indirect androgenesis in the same anther.

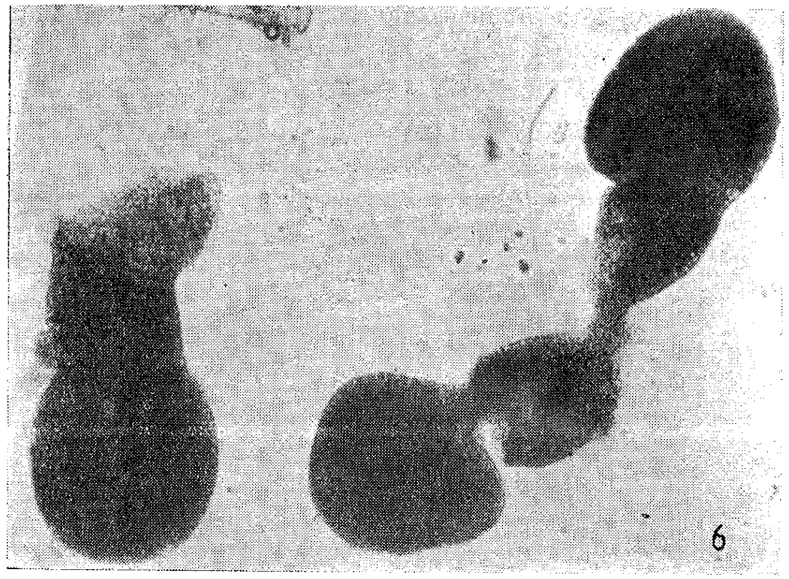


Fig. 6 — 7 — Embryoids differentiated from callus originating from pollen.

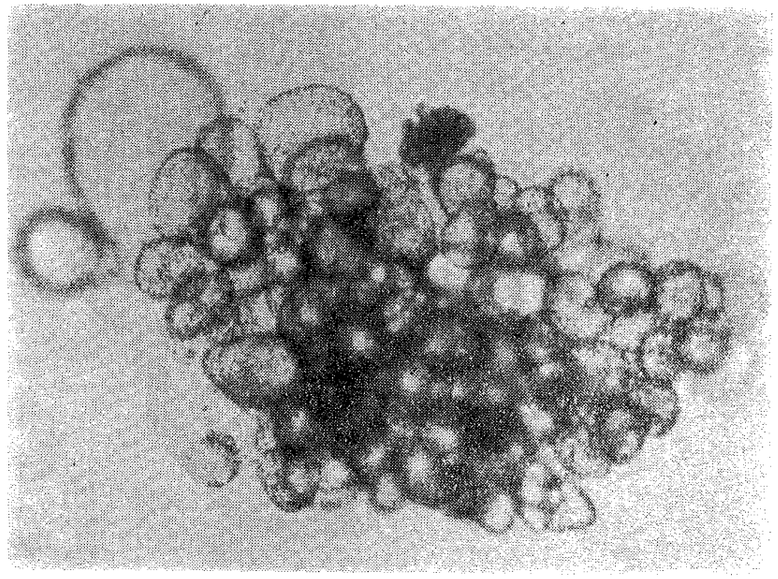


Fig. 8— Embryogenic callus.

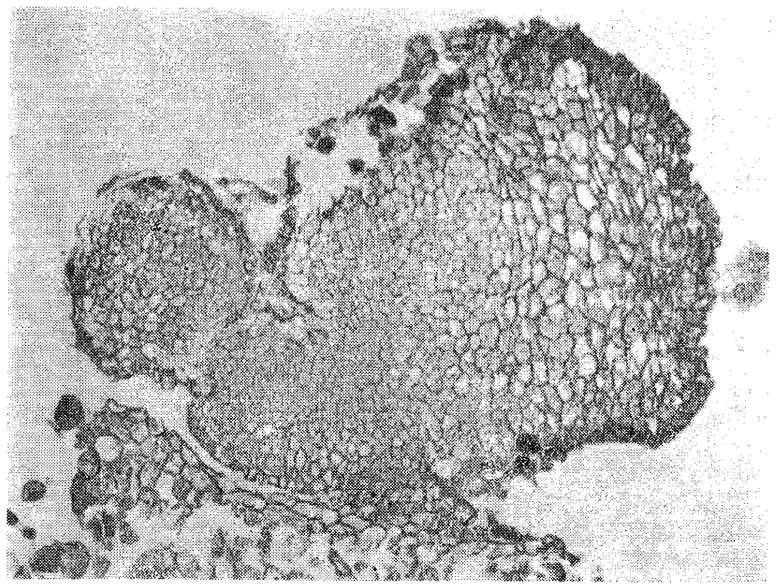
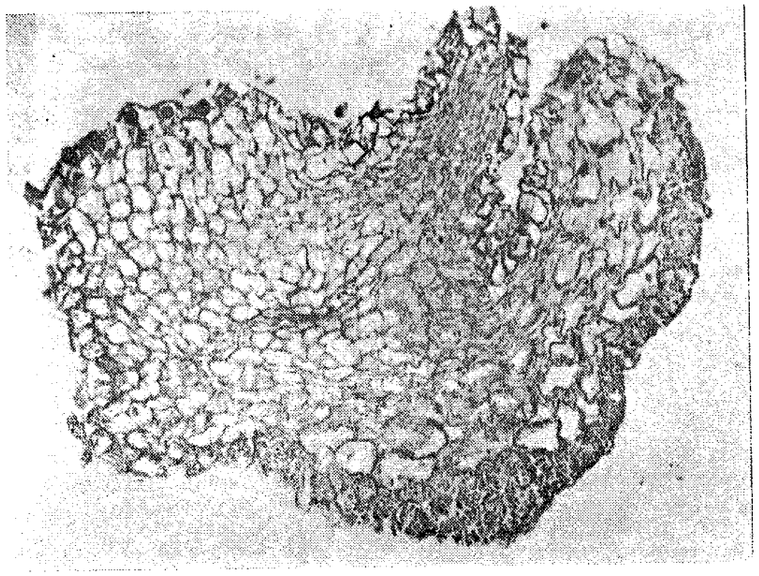


Fig. 9 — Budding of a pro-embryoid from the periphery of an embryogenic callus.



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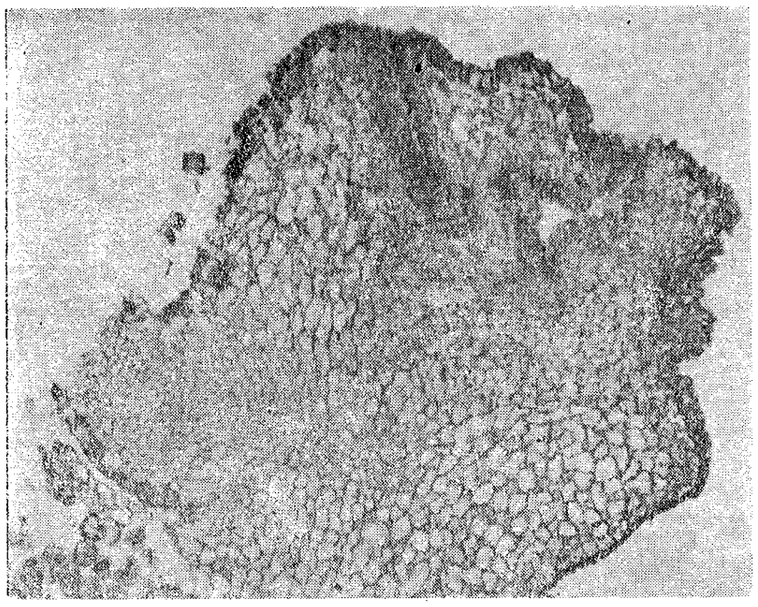

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Fig. 10 — 11 — The anatomy of normal and abnormal embryoids differentiated from pollen callus which stopped their development.

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THE EFFECT OF THE TREATMENTS FOR THREE SUCCESSIVE GENERATIONS WITH GAMMA RAYS AND ALKYLATING AGENTS ON THE MORPHINE CONTENT OF *PAPAVER SOMNIFERUM* L.

BY

ELVIRA V. GILLE*, G. I. GHIORGHIȚĂ* and GEORGETA PÎNZARU**

The mutagen treatments repeted for 3 successive generations induced a high variability of the morphine content in poppy capsules. The values of this index varied between 0,14 and 0.84% d.m. A number of 20 capsules (individuals) produced a content of over 0,50% morphine, a valuable material for the melioration activity of poppy under this aspect.
In the pedoclimatic conditions of Secuieni (Neamț) the plants produced a higher morphine content in the capsule than the plants of Pingărați (Neamț).

Several investigations on *Papaver somniferum* L. [4]—[8] showed that the treatments with ionizing radiations and chemical mutagens induce changes in the morphine content of plants and may contribute to obtain new forms of plants more productive from this point of view. These results were also attested by our experiments in which we observed the behaviour of poppy plants in the second and third generations, after a successive treatment with gamma rays ethylmethanesulfonate (EMS) and diethylsulfate (dES) in the first two generations [1], [2]. The applied treatments induced a great amplitude of plant variability, concerning the dimensions of the capsule, the morphine content, the soluble sugars, the free amine nitrogen and total nitrogen in the capsule. We also proved the importance of the pedo-climatic conditions under which the plants are cultivated in order to produce a certain phenotype by the genotype treated with mutagens. We isolated individuals with a valuable morphine content of their capsules.

In this paper we present the behaviour of poppy plants (their morphine content especially) that were treated for three successive generations with gamma rays and alkylating mutagens.

MATERIAL AND METHODS

Seeds of *Papaver somniferum,* the cultivar *Extaz,* harvested from the plants freely pollinated in 1981 and that had been treated with gamma rays, EMS and dES in the first generation (1980) and with gamma rays in the second (1981), were treated with gamma rays in the third generation (1982). We made two experiments. In the first experiment, the poppy

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Table

Capsule dimensions and its morphine content with *Papaver somniferum* plants treated for three

No.	Treatments			Capsule dimensions			
	1st generation	2nd generation	3rd generation	The height (mm)		The diameter (mm)	
				$\bar{x} \pm s_{\bar{x}}$	s%	$\bar{x} \pm s_{\bar{x}}$	s%
1	Control			50.35 0.91	15.71	25.52 0.61	20.84
2	5 kR			45.84 0.89	13.04	27.76 0.78	23.49
3	10 kR	5 kR		48.83 1.13	14.85	27.25 0.78	18.42
4	0.1% EMS	10 kR	5 kR	49.92 0.71	11.14	27.87 0.73	20.70
5	10 kR	10 kR	5 kR	50.13 0.60	9.23	29.04 0.79	20.97
6	15 kR	10 kR	5 kR	49.26 0.80	11.61	26.75 0.72	19.29
7	10 kR+0.1% EMS	10 kR	5 kR	51.38 1.00	13.88	28.62 0.88	21.98
8	15 kR+0.1% EMS	10 kR	5 kR	48.45 0.77	12.24	25.92 0.69	20.45
9	10 kR+0.1% dES	10 kR	5 kR	46.94 0.87	12.48	26.19 0.81	20.73
10	15 kR+0.1% dES	10 kR	5 kR	49.55 0.74	11.42	27.26 0.76	21.24

Table

Capsule dimensions and its morphine content with *Papaver somniferum* plants treated for

No.	Treatment			Capsule dimensions			
	1st generation	2nd generation	3rd generation	The height(mm)		The diameter (mm)	
				$\bar{x} \pm s_{\bar{x}}$	s%	$\bar{x} \pm s_{\bar{x}}$	s%
1	Control			49.61 0.79	11.21	31.35 0.89	20.03
2	8 kR			48.98 0.77	9.98	29.23 0.90	19.53
3	12 kR			48.35 0.76	11.68	27.53 0.81	21.94
4	10 kR	8 kR		45.77 2.01	17.61	29.07 1.41	19.43
5	10 kR	12 kR		49.27 0.78	11.52	29.69 0.78	19.23
6	10 kR	10 kR	8 kR	48.51 0.77	12.90	28.52 0.65	18.65
7	10 kR	10 kR	12 kR	50.38 1.00	13.71	32.62 0.78	16.03
8	15 kR	10 kR	8 kR	47.50 1.05	14.34	27.93 0.92	21.34
9	15 kR	10 kR	12 kR	50.27 0.66	9.27	31.90 0.97	21.32
10	0.1% EMS	10 kR	8 kR	50.29 0.94	11.87	29.41 0.76	16.46
11	0.1% EMS	10 kR	12 kR	50.54 0.98	9.20	29.01 0.77	15.97
12	10 kR+0.1% EMS	10 kR	8 kR	49.24 1.22	10.48	28.83 1.09	16.09
13	10 kR+0.1% EMS	10 kR	12 kR	48.87 1.31	15.39	28.00 1.11	22.68
14	15 kR+0.1% EMS	10 kR	8 kR	48.91 1.00	13.47	26.98 0.68	16.53
15	15 kR+0.1% EMS	10 kR	12 kR	47.52 0.79	13.76	27.35 0.54	16.34
16	10 kR+0.1% dES	10 kR	8 kR	50.20 1.03	12.31	27.28 0.79	17.37
17	10 kR+0.1% dES	10 kR	12 kR	47.13 0.97	14.13	24.74 0.76	21.10
18	15 kR+0.1% dES	10 kR	8 kR	47.23 0.88	11.14	27.33 0.88	19.24
19	15 kR+0.1% dES	10 kR	12 kR	46.73 0.99	12.20	27.42 0.72	14.99

1

generations successively with gamma-rays and alkylant agents (Pingărați, Neamț, 1982)

r	Weight of the capsule separately analyzed (gr.)		Morphine content in the capsule (% d.s.)			
			Free pollinated plants		Autopollinated plants	
	Free pollination	Autopollination	Average on variant	The biggest capsule	Average on variant	The biggest capsule
0.46	2.05	—	0.41	0.51	0.41	—
0.43	2.72	2.10	0.33	0.72	0.30	0.59
0.52	1.23	2.81	0.45	0.60	0.19	0.26
0.58	2.46	2.85	0.30	0.45	0.45	0.44
0.45	1.82	3.50	0.24	0.50	0.14	0.36
0.33	2.32	2.82	0.28	0.69	0.30	0.30
0.73	2.51	2.60	0.20	0.55	0.29	0.33
0.56	3.67	1.60	0.26	0.64	0.32	0.29
0.35	1.72	1.80	0.31	0.75	0.29	0.34
0.66	2.20	1.81	0.40	0.37	0.43	0.29

2

3 generations successively with gamma-rays and alkylant agents (Secuieni, Neamț, 1982)

r	Weight of the capsule separately analyzed (gr.)		The morphine content in the capsule (% d.s.)			
			Free pollinated plants		Autopollinated plants	
	Free pollination	Autopollination	Average on variant	The biggest capsule	Average on variant	The biggest capsule
0.44	2.20	2.00	0.34	0.52	0.48	0.60
0.32	1.96	1.63	0.49	0.41	0.45	0.29
0.48	1.41	2.20	0.41	0.37	0.37	0.36
0.23	2.10	—	0.34	0.57	0.31	—
0.41	2.40	2.25	0.61	0.39	0.32	0.41
0.56	1.90	2.25	0.41	0.34	0.23	0.45
0.42	2.75	2.10	0.37	0.48	0.45	0.31
0.55	1.82	2.00	0.32	0.52	0.41	0.31
0.31	2.31	2.06	0.45	0.82	0.31	0.52
0.43	1.60	1.70	0.60	0.50	0.52	0.49
0.46	2.09	1.96	0.45	0.41	0.34	0.31
0.17	1.71	1.44	0.45	0.52	0.34	0.84
0.73	2.20	—	0.31	0.37	—	—
0.35	1.73	2.03	0.41	0.42	0.31	0.55
0.50	1.70	1.85	0.49	0.42	0.26	0.62
0.52	2.10	1.65	0.37	0.45	0.31	0.27
0.58	2.30	1.70	0.55	0.45	0.45	0.45
0.58	2.00	0.92	0.37	0.44	—	0.18
0.43	1.40	—	0.32	0.31	0.34	—

seeds were irradiated in the third generation with 5 kR and were cultivated in the experimental field from Pîngărați (Neamț). In the other experiment the seeds were irradiated with 8 and 12 kR and cultivated in the experimental field from Secuieni (Neamț). In the experiment performed at Pîngărați we made 9 experimental variants (Table 1), in that of Secuieni (Table 2) — 19 variants. The plants of variants 2 in Table 1 and of variants 2, 3 in Table 2 were in 1982 after the first year of treatment, and those of variants 3 (Table 1) and 4, 5 (Table 2) had received a gamma rays treatment for two generations.

About their flowering time we isolated 10—12 individuals of each experimental variant with paper bags in order to prevent free pollination. When the plants reached their maturity, we harvested their capsules, measured the dimensions (diameter and height) and determined their morphine content. The morphine was dosed into four categories of samples from each variant i.e. : average samples obtained from the self-pollinated capsules, average samples from the free-pollinated capsules and from the greatest capsule selected out of the two categories of capsules. The analysis of the morphine content was made after the method of Gyéresi and Rácz [3]. The values of capsule dimensions were statistically processed.

The data of our investigations are presented in Tables 1 and 2.

RESULTS

Although the poppy seeds were treated with mutagens for 3 successive generations, the growth of the plants was not significantly perturbed, the dimensions of the mature capsules of the treated variants being comparable to those of the control plants. The dimensions of the capsules of the plants of the treated variants are even higher than the controls at Pîngărați (Table 1). The first two mutagen treatments induced a certain resistance of the biological material to a new treatment of this kind. This assertion is also proved by the values, generally lower, of the variability index (s%) of the capsule dimensions in plants treated with mutagens for three successive generations as compared to those treated for two generations alone [2].

In the pedo-climatic conditions of Pîngărați, the highest capsule ($\bar{x}$ = 51.38 mm) was found among the plants treated with 10 kR + 0.1% EMS in the first generation, with 10 kR in the second and with 5 kR in the third generation (Var. 7, Table 1), and the greatest diameter ($\bar{x}$ = 29.40 mm) was achieved by the plants of the variant irradiated with 10 kR in the first two generations and with 5 kR in the third (Var. 5, Table 1).

If the growth of the poppy plants from Pîngărați is not too much disturbed as a result of the three successive mutagen treatments, the morphine content of the plants there was influenced. With a few exceptions, the average morphine content of the variants treated with mutagens, irrespective of their way of pollination, is lower than that of the controls (Table 1). The individual analyses of the greatest capsule from each variant render evident a different situation, at least in the case of free pollinated plants. Except for the capsules selected from variants 4 and 10, all the others have the morphine value over 0.50% d.m. The highest level

of morphine (0.75% d.m.) was attained by the individual selected from the variant treated with 10 kR + 0.1% dES. in the first generation with 10 kR in the second and with 5 kR in the third. The individual analyses on the capsules of the self-pollinated plants revealed much more reduced values of morphine content as compared to the free-pollinated plants. If we make an average of the morphine content of the free pollinated individuals separately analysed and an average of the self-pollinated ones, we obtain two figures which express the markedly high value of the former (0.58% d.m. as to 0.35% d.m. respectively). This result refutes our previous affirmation [1] according to which the self-pollinated plants score higher levels of the morphine content in the capsules. The data in Table 1 as well reveal that although the capsules analysed individually had the greatest dimensions in the respective variants, we cannot state the existence of any relation between the size of the capsule and its morphine content. Thus, for example, in the case of the free-pollinated plants, two capsules whose weight and dimensions (1.23 gr — Var. 3 and 3.67 gr. — Var. 8) are markedly different, have a fairly equal morphine content (0.60 and 0.64% d.m. respectively).

The appreciations concerning the effects of the third successive treatment with mutagens on the growth of poppy plants, on the dimensions of their capsules and of their variability remain the same under the pedo-climatic conditions at Secuieni (Neamț) even if the irradiation doses used in the third generation has greater values — 8 and 12 kR (Table 2). The greatest dimensions of the capsules were registered by the plants irradiated with 10 kR in the first two successive generations and with 12 kR in the third ($\bar{x}$ = 50.38 mm for their height and $\bar{x}$ = 32.62 mm for their diameter). The most pronounced individual variability of the analysed morphological parameters was induced by the treatments with 10 kR + 0.1% EMS in the first generation, with 10 kR in the second and with 12 kR in the third generation (s% = 15.39 for their height and 22.68 for the diameter of the capsules). It must be noticed that this combination of treatments determined the best correlation of those two parameters.

The pedo-climatic conditions at Secuieni favoured the accumulation of greater quantities of morphine in the capsules as compared to those at Pîngărați. When calculating the average of the average morphine content per variant, the situation presents as follows : 0.44% for free-pollinated plants and 0.36% for self-pollinated ones in the conditions at Secuieni, as to 0.32 and 0.31% d.m. respectively at Pîngărați. If we take into account the fact that the variants that entered the average are twice as many at Secuieni than at Pîngărați, our assertion comes to be even more relevant. The highest average values of the morphine content per variant, both in the conditions of free-pollination and self-pollination, were attained by the capsules harvested from the plants treated with 0.1% EMS in the first generation, with 10 kR in the second and with 8 kR in the third generation (0.60% and 0.52% d.m. respectively, Var. 10, Table 2). The individual analyses we undertook furnished a valuable biological material concerning the morphine content. Among the free-pollinated plants, the one selected out of the variant irradiated with 15 kR in the first generation, 10 kR in the second and with 12 kR in the third generation (Var. 9) has a morphine content of 0.82% d.m. in the capsule.

The capsule analysed separately from the variant treated with 10 kR + 0.1% EMS in the first generation, with 10 kR in the second and with 12 kR in the third generation (Var. 12) has a morphine content of 0.84% d.m. (Table 2). Without taking into consideration the way of pollination, a total of other 9 capsules (individuals), analysed separately revealed values over 0.50% d.m. of the morphine content. If this character keeps stables in the following generations, one may expect to obtain poppy lines with a higher bio-productivity than those cultivated in our country at present. We could not render evident any relation between the weight of the capsule and its morphine content in the experiment performed at Secuieni either.

CONCLUSIONS

— The treatments for three successive generations with solutions of EMS and dES (in a concentration of 0.1% for 10 hours) and with gamma rays (doses between 5 and 15 kR) did not cause significant perturbations in the growing processes of the plants of *Papaver somniferum*, Extaz cultivar. The repeated mutagen treatments for successive generations led to the diminishing and not to the growing of the variability amplitude of the dimensions of poppy capsules.

— The mutagen treatments applied to the seeds induced a high variability of the morphine content in poppy capsules. The value of this index, in the experiments made at Pîngărați and Secuieni (Neamț) varied between 0.14 and 0.84% d.m. As a consequence of the individual analyses there were isolated 20 capsules (individuals) having a morphine content of over 0.50% d.m., a valuable biological material for poppy melioration under this aspect.

— In the pedo-climatic conditions at Secuieni (Neamț) the plants produced higher values of morphine in the capsule than the plants at Pîngărați (Neamț).

— The investigations undertaken did not show any sure relationship between the morphine content of the poppy capsules on the one hand, and the applied mutagen treatments, the plants' pollination, the weight and dimensions of their capsules, on the other hand.

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EINE NEUE VEGETATIONSKARTE VON RUMÄNIEN

VON

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The new vegetation map of Romania on a scale of 1 : 2 500 000 shows the potential vegetation comprised in 24 zonal units and 14 intrazonal units. These units and their limits have been established by reconstruction on the basis of the existing natural vegetation and of the ecological characteristics of the territories lacking such vegetation. These units are regional variants (Carpathian, Danubian, Balkanic, etc.) of the corresponding general European units.
The 38 units are included in 9 great vegetation units at a European level : B and C Alpine meadows, mountain pine elfin wood and subalpine light forests (1 unit) ; D Cold-temperate and temperate climate resinous forests (1 unit) ; E Hemiboreal and mountain-continental resinous-deciduous forests (1 unit) ; F Mesophilous deciduous and resinous-deciduous forests (9 units) ; G Xerothermophilous deciduous and resinous-deciduous forests (8 units) ; K Steppes (6 units) ; M Coast and halophilous vegetation (5 units) ; N Flood plain and marsh vegetation (7 units).
Forest vegetation units (24 exclusively forest units and 7 mixed forest and meadow or brush units) are predominant in Romania's potential vegetation.

Im Rahmen einer umfassenden Zusammenarbeit der Vegetationskündler aus mehreren europäischen Ländern wurde, in den letzten Jahren, die allgemeine Legende für eine Vegetationskarte von Europa imkleinen Maßstab (1 : 2 500 000 — 1 : 3 000 000) erarbeitet [2].

Diese Legende enstand aus nationalen Vorschlägen die auf regionaler Ebene abgestimmt und in 17 europäischen Großeinheiten eingereiht wurden.

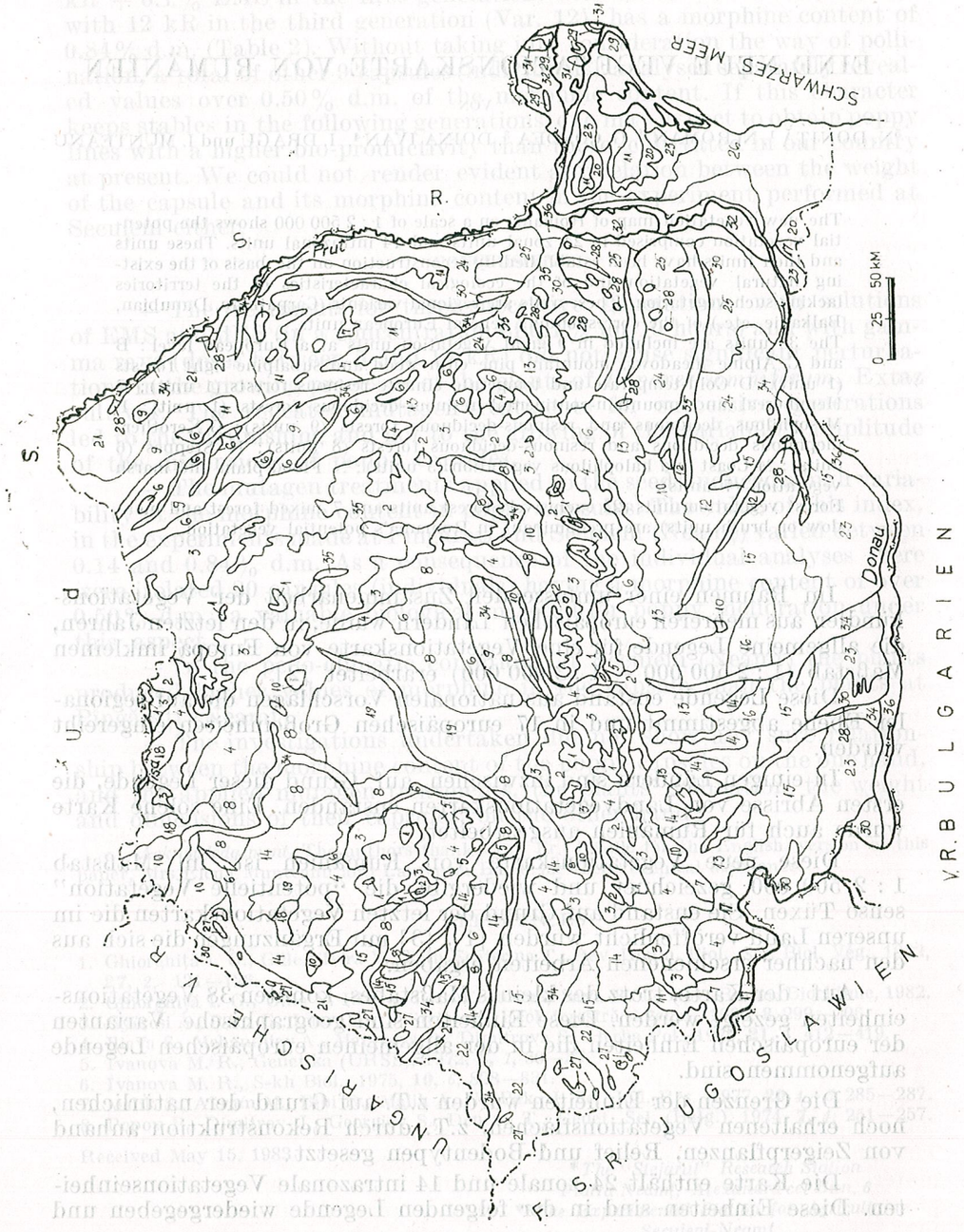
In einigen Ländern sind inzwischen, auf Grund dieser Legende, die ersten Abrisse von Landvegetationskarten enstanden. Eine solche Karte wurde auch für Rumänien ausgearbeitet.

Diese neue Vegetationskarte von Rumänien ist im Maßstab 1 : 2 500 000 gezeichnet und wiedergibt die "potentielle Vegetation" senso Tüxen. Sie enstand auf Grund der letzten Vegetationskarten die im unseren Land veröffenlicht wurden [1], [3] mit Ergänzungen die sich aus den nachher erschienenen Arbeiten ergaben.

Auf der Karte, trotz des kleines Maßstabes, konnten 38 Vegetationseinheiten gezeigt werden. Diese Einheiten sind geographische Varianten der europäischen Einheiten die in der allgemeinen europäischen Legende aufgenommen sind.

Die Grenzen der Einheiten wurden z.T. auf Grund der natürlichen, noch erhaltenen Vegetationsflächen, z.T. durch Rekonstruktion anhand von Zeigerpflanzen, Relief und Bodentypen gesetzt.

Die Karte enthält 24 zonale und 14 intrazonale Vegetationseinheiten. Diese Einheiten sind in der folgenden Legende wiedergegeben und



LEGENDE DER VEGETATIONSKARTE VON RUMÄNIEN (Maßstab 1 : 2 500 000).

B.C. * ALPENMATTEN, SUBALPINE LEGFÖHRENGEBÜSCHE UND LICHTWÄLDER

1. Karpatisches Hochgebirgskomplex mit Alpenmatten, subalpinen Legföhrengebüschen und Lichtwäldern *(Caricetalia curvulae + Junipero — Pinetalia mughi)*.

D. KALTTEMPERIERTE UND TEMPERIERTE NADELWÄLDER

2. Ostkarpatische Fichtenwälder *(Hieracio (transilvanico) — Piceetum)*.

E. HEMIBOREALE UND KONTINENTAL — MONTANE LAUBNADELWÄLDER.

3. Ostkarpatische neutrophile Buchen — Tannen — Fichtenwälder *(Pulmonario (rubrae) — — Abieto — Fagetum + Chrysanthemo — Piceeto — Fagetum)*.

F. MESOPHILE LAUB- UND LAUBNADELWÄLDER

4. Ostkarpatische neutrophile Buchenwälder *(Symphyto (cordatae) — Fagetum,* im Süd-Westen *Aremonio — Fagetum)*.
5. Karpatische acidophile Buchenwälder *(Luzulo — Fagetum)*.
6. Dazische neutrophile Hainbuchen — Buchenwälder *(Carpino — Fagetum)*.
7. Moldauische Silberlinden — Hainbuchen — Buchenwälder *(Tilio (tomentosae) — Fagetum)*.
8. Dazische Hainbuchen — Traubeneichenwälder *(Lathyro (hallersteinii — Carpinetum)*.
9. Moldauisch — podolische Linden — Hainbuchen — Stieleichen — Mischwälder *(Tilio (cordatae) — Carpinetum)*.
10. Karpatische Terassen — Stieleichenwälder *(Melamphyro — Carpinetum)*.
11. Moldauische Silberlinden — Hainbuchen — Traubeneichen — Mischwälder *(Tilio (tomentosae) — Carpinetum)* in Dobrudscha z.T. mit Orienthainbuchen. *(Nectaroscordo — Tilietum)*.
12. Danubische Silberlinden — Hainbuchen — Stieleichen — Mischwälder *(Tilio robori — Carpinetum)*.

G. XEROTHERMOPHILE LAUB- UND LAUBNADELWÄLDER

13. Balkanisch-danubische Traubeneichenwälder *(Quercetum dalechampii-polycarpicae)*.
14. Vorkarpatische Zerreichen-Traubeneichenwälder (z.T. mit *Tilia tomentosa*) *(Quercetum petraeae-cerris)*.
15. Balkanisch-danubische Zerreichen-Balkaneichenwälder *(Aceri tatarico — Quercetum confertae-cerris)*.
16. Balkanisch-danubische Balkaneichenwälder *(Quercetum confertae)*.
17. Balkanische Blumeneschen-Traubeneichenwälder *(Orno-Quercetum)*.
18. Vorkarpatische Tatarenahorn-Traubeneichenwälder *(Aceri tatarico — Quercetum petraeae)*.
19. Dazische Tatarenahorn — Traubeneichen — Stieleichenwälder *(Aceri tatarico — Quercetum robori-petraeae)*.
20. Pontische Orienthainbuchen-Flaumeichenwälder *(Paeonio peregrinae — Carpinetum orientalis)*.

K. STEPPEN

21. Moldauisch-podolisches Waldsteppenkomplex mit Tatarenahorn — Stieleichenwäldern und Steppenschwingel-Trockenrasen *(Aceri tatarico — Quercetum roboris + Medicagini — Festucetum valesiacae)*.
22. Pannonisches Waldsteppenkomplex mit Tatrarenahorn — Flaumeichen — Stieleichwäldern und gefurchten Schwingeltrockenrasen *(Aceri tatarico — Quercetum robori — pubescentis + Festucetum sulcatae-valesiacae)*.
23. Danubisches Waldsteppenkomplex mit Tatarenahorn — Graueichenwäldern und Steppenschwingel — Bartgras-Trockenrasen *(Aceri tatarico — Quercetum pedunculiflorae + Medicagini — Festucetum valesiacae + Chrysopogonetum grylli)*.

* Großeinheiten der Vegetationskarte von Europa.

24. Danubisches Waldsteppenkomplex mit Graueichen-z.T. Flaumeichenwäldern und ukrainischen Federgras-Trockenrasen *(Aceri tatarico — Quercetum pedunculiflorae + Galio dasypodi — Quercetum pubescentis + Stipo ucrainicae — Festucetum valesiacae).*
25. Danubische Steppentrockenrasen mit ukrainischem Federgras *(Stipo ucrainicae — Festucetum valesiacae).*
26. Dobrudscha-Steppenkomplex mit Löß- und Steinsteppen *(Stipo ucrainicae — Festucetum valesiacae + Pimpinello thymion zygoidi).*

M. KÜSTEN- UND HALOPHYTENVEGETATION

27. Pannonisches Halophytenkomplex *(Achilleo — Festucetum pseudovinae).*
28. Danubisches Halophytenkomplex *(Salicornietum + Suaedo-Kochietum).*
29. Pontisches Küstenkomplex mit Sanddünen- und Halophytenvegetation *(Scabioso ucrainicae — Festucetum cinereae + Aeluropetum + Salicornietum).*
30. Pannonisches Binnendünenkomplex mit Sanddünenvegetation und Stieleichenwäldern *Festucem vaginatae + Convallario-Quercetum).*
31. Danubisches Binnendünenkomplex mit Sanddünenvegetation und Graueichen-Stieleichenwäldern *(Scabioso ucrainicae — Festucetum cinereae + Quercetum robori-pedunculiflorae).*

N. AUEN- UND MOORVEGETATION

32. Danubisches Wasservegetationskomplex (in den Donauauen und -Delta) *(Phragmitetea, Potametea).*
33. Karpatisches Schwarzerlen-Bruchwälderkomplex.
34. Zentral-europäisches Auenkomplex mit Schmallblatteschen-, Stieleichen-, Weißpappel- und Silberweidenwäldern *(Fraxino pannonicae — Ulmetum + Populetalia).*
35. Danubisch-balkanisches Auenkomplex mit Flaumeschen-Graueichen-Stieleichenwäldern, sowie Weißpappel- und Silberweidenwäldern *(Fraxinetum pallisiae + Populetalia).*
36. Auenkomplex mit Weißpappel- und Silberweidenwäldern *(Populetalia).*
37. Karpatische Schwarzerlen-Auenwälder *(Aegopodio — Alnetum).*
38. Pontische Tamariskengebüsche *(Calamagrosti — Tamaricetum).*

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auf der anliegenden Vegetationskarte aufgezeichnet. Die Nummern der Vegetationseinheiten auf der Karte entsprechen denen in der Legende.

Die Eingliederung der Vegetationseinheiten, die in Rumänien vorkommen, in die Grundeinheiten der Vegetation von Europa erlaubt einige allgemeine Betrachtungen über den Charakter der Vegetation unseres Landes.

Vor allem soll hervorgehoben werden, daß in der Vegetation von Rumänien einige spezifische, nur diesem Raum eigene Einheiten vorhanden sind. Das betrifft die sogenannten dazischen Waldeinheiten und zwar die Buchen-Nadel- und Buchenwälder mit *Dentaria glandulosa, Pulmonaria rubra, Symphytum cordatum,* die die mittleren Höhen der Karpatengebirge einnehmen, so wie die Traubeneichenwälder mit *Lathyrus hallersteini* und die Stieleichenwälder mit *Melampyrum bihariense,* die in den innerkarpatische Hochebenen vorkommen. Auch die Fichtenwälder mit *Hieracium transsilvanicum* können z.T. als spezifisch für die rumänischen Karpaten betrachtet werden. In den perikarpatischen Hochebenen und Ebenen sind auch einige charakteristische Einheiten zu verzeichnen und zwar die Eichenmischwälder mit *Tilia tomentosa* und *Carpinus betulus* (z.T. mit *Quercus petraea* ssp. *petraea* und ssp. *dalechampii,* z.T. mit *Q. robur)* so wie die Graueichenwälder aus *Quercus pedunculiflora* mit *Acer tataricum.* Es gibt aber auch viele übergreifende Einheiten die auch in den

Nachbarländern auf größeren Flächen vorkommen. Vorwiegend sind es balkanische Einheiten die in den niederen Lagen verbreitet sind (mit *Quercus cerris, Q. frainetto, Q. petraea* ssp. *polycarpa, Q. pubescens* u.a. Arten). Einige kommen aber auch in höheren Lagen vor. (Verbindungsglieder *Rhododendron kotschyi, Bruckenthalia spiculifolia* u.a.). Bezeichnend sind auch die danubisch-pontischen Einheiten in der Steppe und Waldsteppe in denen kontinentale aber auch balkanische Elemente vorkommen.

Die neue Vegetationskarte, obwohl im kleinen Maßstab, wiederspiegelt sehr gut die große Verschiedenheit der Vegetation von Rumänien, die zum größten Teil mit der Anwesenheit der Karpatenkette im unseren Land verbunden ist. Diese Kette bildet eine wichtige Klimascheide, die Grenzen zwischen verschiedenen Klimaeinflüsse setzt, aber zugleich auch höhen- und reliefbedingte Klimaänderungen verursacht. Das alles wirkt sich stark in der Vegetationsdecke aus.

Die neue Vegetationskarte von Rumänien ist mit denen der Nachbarländern abgestimmt, sodaß die Grenzen der Vegetationseinheiten sich gut überdecken.

Die Karte gibt einen guten Überblick der natürlichen potentiellen Vegetation des Landes und kann für verschiedene praktische Zwecke verwendet werden (zonale und regionale Planung in der Land- und Forstwirtschaft, Ausbau des Naturreservennetzes das zur Erhaltung der Gen- und Ökopools nötig ist u.a.)

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RELICTES DANS LA FLORE DE ROUMANIE

I

Sphagnum wulfianum Girg. in Arch. f. d. Naturk. Liv. — , Ehst. —u. Kurl., 2 (1860) 173, Fam. *Sphagnaceae*.

(S. cuspidatum Ehrh. var. patens Aongstr. ap. Lindberg, 1862 ; S. pycnocladum Aongstr., 1864).

Espèce de la Section monotypique *Polyclada* Russ., sous-genre *Lithophloea* Russ.

Fig. 1. — *Sphagnum wulfianum* Girg., habitus.

La tige haute de 20—30 cm, fréquemment ramifiée, 5-angulaire, noirâtre, friable, avec des branches (7—13) en bouquets (faisceaux), polyclade, terminée par un grand glomérule sphérique, compact, formée de branches courtes (fig. 1). Les feuilles de la tige, petites-triangulaires-lingulées, celles des branches lancéolo-allongée (±arquées), coupées court, cellules hyalines sur le côté interne, presque sans pores, sur le côté extérieur avec des pores petits, annelés, cellules chlorophyliennes en section (coupe) elliptiques, centrées par les cellules hyalines. La couleur d'habitude verdâtre, brun-verdâtre, rarement rougeâtre ; n = 19(Sorsa, 1955).

Relicte sous-arctique caractéristique de l'aréal boréal circumpolaire, 48—69° latitude Nord et 25—55° longitude Est, connu (entre les années 1890—1977) dans la zone nord-carpatique de Roumanie (915—1100 m s.m.) dans six stations, dont 5 en Bucovine : Coşna (J. Dörfler, 1890, det. J. Breidler) ; Poiana Stampei (A. Mühldorf, 1925 ; M. Guşuleac et A. Mühldorf, 1925 ; Tr. I. Ştefureac avec l'identification de nouvelles bryocénoses, ¡1977) ; Drăgoiasa-Păltiniş (1959, 1960) ;Valea Stînii — Cîrlibaba (1969) ; Grădiniţa (1973) (Tr. I. Ştefureac, 1959—1973), toutes dans le département de Suceava, et une en Transylvanie (Apa Roşie, département de Covasna) (Tr. I. Ştefureac et V.Barabaş, 1975 ; Tr. I. Ştefureac, 1976). Elément relicte, conservé surtout dans les réserves naturelles de marais tourbeux eutrophes du système périglaciaire nord-carpatique (monts Călimani — Rodnei) (fig. 2).

Pas encore signalé dans les Carpates méridionales de la Roumanie ni dans le mont Balkan (Bulgarie).

Elément photo-sciaphile, tourbicole, acidophile, formant des bryocénoses ± monospécifiques, isolées (rarement associé à *Helodium blandowii* et autres, Drăgoiasa-Păltiniș), caractéristique des formations de

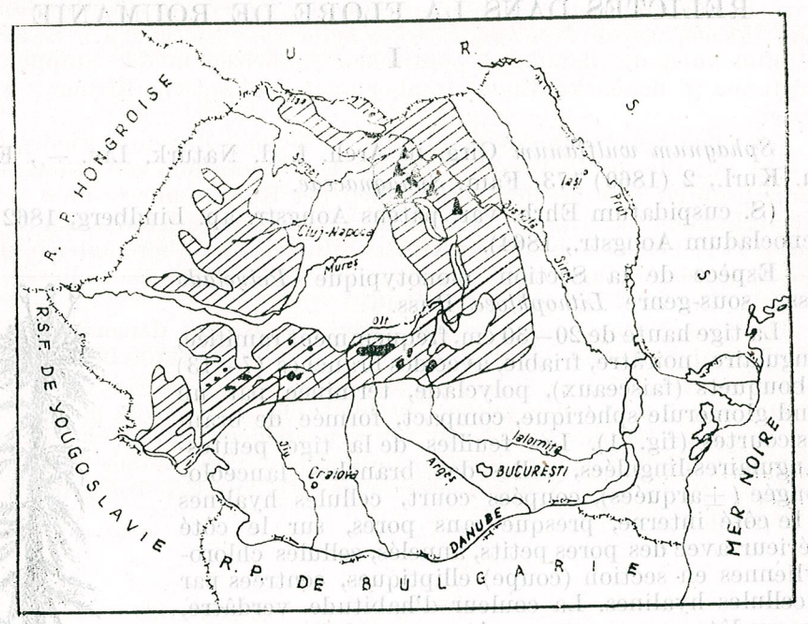

Fig. 2. — Esquisse corologique : ▲ — *Sphagnum wulfianum* Girg. (orig.) ; ● — relief glaciaire carpatique (Al. Roșu, 1980).

forêts d'épicéa sphagnétisées et des enclaves à pin *(Pinus sylvestris)* des marais eutrophes, humides ; l'association *Sphagneto (wulfiani) — Piceetum turfosum* Ștefureac, 1977.

En Roumanie il détient la limite sud de l'aréal.

L'aréal général de cette espèce comprend l'Europe septentrionale, la Sibérie et l'Amérique du Nord.

Traian I. Ștefureac

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.

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