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19410



CHOROLOGIC, KARYOLOGIC AND ANATOMICAL  
STUDIES ON *NIGELLA* L. GENUS  
(FAM. *RANUNCULACEAE*) IN ROMANIAGABRIEL C. CORNEANU\*, VASILE D. SIMEANU\*\*,  
GHEORGHE G. POPESCU\*\* and FELICIAN MICLE\*\*\*

In this paper the chorology as well as karyologic and anatomical aspects of the three *Nigella* L. species present in the Romanian flora are presented. *N. arvensis* L. ssp. *arvensis* is an archaeophyte species spread in the fields and hilly zones of Romania, in segetal, ruderal and xerophyllous associations. *N. damascena* L. "single flower" and "double flower" as well as *N. sativa* L. are cultivated in parks and gardens and are used as ornamental, aromatic and medicinal plants. The chromosome features as well as the main anatomical features of taxonomic importance (stem and leaf structure, epidermic cells features, stomata type and their density a.o.) were, noticing the differences between the four analyzed genotypes of the *Nigella* L. genus.

*Nigella* L. genus (Helleboreae tribe, *Ranunculaceae* family) comprises almost 20 annual species, found in the Mediterranean region (11), (12). In recent decades, anatomical studies (5), (6) and chromosome studies (4), (7), (10), (11) on some species of *Nigella* genus were performed.

In Romania's flora, the *Nigella* genus is represented by three species (8), which were studied partially (1), (2), (3), without using the anatomic criteria in the taxonomy of the species.

In the present paper, anatomic and chromosome features in the taxonomy of three *Nigella* species (four genotypes) were used, also establishing their chorology.

## MATERIAL AND METHOD

The species *Nigella arvensis* L. ssp. *arvensis*, *N. damascena* L. "single flower" and "double flower" and *N. sativa* L. taken from the same zone of the country (Craiova) were studied. To establish the taxonomy of these species, mature plants were collected (after flowering) on which anatomical studies were performed (the structure of the stem and the leaf; the shape and size of the epidermal cells; the type and density of stomata) as well as chromosome studies.

The stem structure was studied on cross sections in its median zone and the internode II under the flower. The determination of the structural characteristics of the leaves was performed in the cauline leaves of the median zone of the stem. The epidermis on both faces of the foliar limb was detached mechanically (by peeling) and analyzed under the



microscope. The foliar limb structure was analyzed on cross sections in the median zone of the terminal ramifications of the cauline leaf. The obtained data (100 observations/character) were interpreted statistically. In order that the obtained results should be comparable, the portions of vegetative organs on which the analyses were performed were collected from the same zones of the plants.

The study of chromosome characteristics was performed on preparations of squash type, Feulgen stain, obtained from the tops of radicles. For each species 12 metaphases were analyzed, noticing the length of the arms of the chromosomes and the satellites, the chromosome pairs which bear the satellites, the centromere index and the ratio of the arms.

The chorology of the species was established on the basis of the field observations, on existing materials in the herbariums in Romania and the data from the specialized literature.

## RESULTS AND DISCUSSIONS

### SPECIES CHOROLOGY

*Nigella arvensis* L. is a Pontic-Mediterranean species, met spontaneously in Romania. Of the four sub-species described by A. Strid (11), only *Nigella arvensis* L. ssp. *arvensis* was met in Romania. It is widespread in fields and hilly regions (up to 300 m altitude) on either side of the Carpathian arc. It vegetates on cultivated grounds, being met also in ruderal places and dry meadows near cultures, on sandy and clayey-sandy soils. In Romania's vegetation, *N. arvensis* L. ssp. *arvensis* was rendered obvious in the following vegetal formations:

Cl. SECALIETEA Br. — Bl. 1951 (segetal associations): ass. *Tribulo-Tagetum* Soó et Timar 1945; *Aristolochie-Convolvuletum arvensis* Ubrizsy 1965; *Consolido (orientali)-Vicetum striatae* (Slavnič 1944) Soó 1947 *biforetum radiantis*;

Cl. CHENOPODIETEA Br. — Bl. 1951 em. Soó (ruderal associations): ass. *Carduetum acanthoidis* Morariu 1939;

Cl. FESTUCO-BROMETEA Br. — Bl. et Tx. 1943 (xerophilous meadows): ass. *Adropogonetum ischaemi* Krist 1937; *Plantaginetum indicae* Păun 1964; *Trifolietum echinati* Morariu 1969; *Artemisiae (austriacae)*. — *Poëtum bulbosae* I. Pop 1971; *Convolvuletum lineatus* Morariu 1967.

*Nigella damascena* L. is cultivated in gardens and parks as an ornamental plant under different horticultural forms. The "double flower" form presents a recessive gene as compared with the "single flower" natural form (1), (9).

*Nigella sativa* L. is cultivated as an aromatic and medicinal plant, especially in the south-east of Romania (Constanța and Brăila districts). When growing in wilderness, it appears like a weed in the crops or on the sunny hills, especially in southern Romania.

### STEM ANATOMY

In cross sections performed in the median zone and in the internode II under the flower the stem of the analyzed species reveals a primary structures.

The stem epidermis is unistrate, having a cuticula outside. The bark is little developed and is present under the form of assimilatory chlorenchyma. This is continuous in *Nigella arvensis arvensis* (Figs 1,5), while in the other species it is interrupted in front of the stem edges by the angular chlorenchyma (Figs 2—4, 6—8). The stele is well developed, displaying leading libero-ligneous fascicles of open collateral type, the big ones alternating with the small ones. Outwards these fascicles are protected by sclerenchyma cords (Figs 1—8). In the median zone of the stem, the highest number of sclerenchyma cords are to be found in *Nigella arvensis arvensis*, and the lowest number in *Nigella sativa*, while in the internode II zone, the highest number is to be found in *Nigella arvensis arvensis* and in *N. damascena* "double flower", and the lowest number in *N. damascena* "single flower" (Table 1). In the median zone of the

Table 1

Number and size of sclerenchyma cords in Romania's *Nigella* L. genus species

| Species                             | Sclerenchyma cords |                                 |                                     |
|-------------------------------------|--------------------|---------------------------------|-------------------------------------|
|                                     | Number             | Width (μm)<br>$\bar{x} \pm s_x$ | Thickness (μm)<br>$\bar{x} \pm s_x$ |
| Stem median zone                    |                    |                                 |                                     |
| <i>N. arvensis arvensis</i>         | 27                 | 155.87 ± 18.00                  | 139.35 ± 14.00                      |
| <i>N. damascena</i> "single flower" | 25                 | 148.32 ± 11.75                  | 118.92 ± 1.55                       |
| <i>N. damascena</i> "double flower" | 25                 | 139.56 ± 14.30                  | 119.18 ± 11.80                      |
| <i>N. sativa</i>                    | 21                 | 187.55 ± 17.70                  | 85.65 ± 10.10                       |
| Internode II zone                   |                    |                                 |                                     |
| <i>N. arvensis arvensis</i>         | 20                 | 117.03 ± 15.48                  | 79.38 ± 11.42                       |
| <i>N. damascena</i> "single flower" | 14                 | 106.95 ± 13.38                  | 96.64 ± 14.43                       |
| <i>N. damascena</i> "double flower" | 20                 | 91.15 ± 8.69                    | 98.54 ± 10.54                       |
| <i>N. sativa</i>                    | 16                 | 117.82 ± 13.57                  | 83.72 ± 11.75                       |

stem, the dimensions of sclerenchyma cords (Table 1) are bigger in *N. sativa* (the width) and *N. arvensis arvensis* (the thickness), while in the internode II zone the utmost values occur in *N. arvensis arvensis* and *N. sativa* (the width) and in the two forms of *N. damascena* (the thickness). In *N. sativa* the sclerenchyma cords reveal bigger width/thickness ratio than in the other studied species, this being 1.4 in the internode II zone and 2.2 in the median zone of the stem.

In the central zone of the sclerenchyma cords of the stem of the two forms of *N. damascena* are to be found cells with thin walls (Figs 2, 4, 6, 7) whose function is difficult to specify.



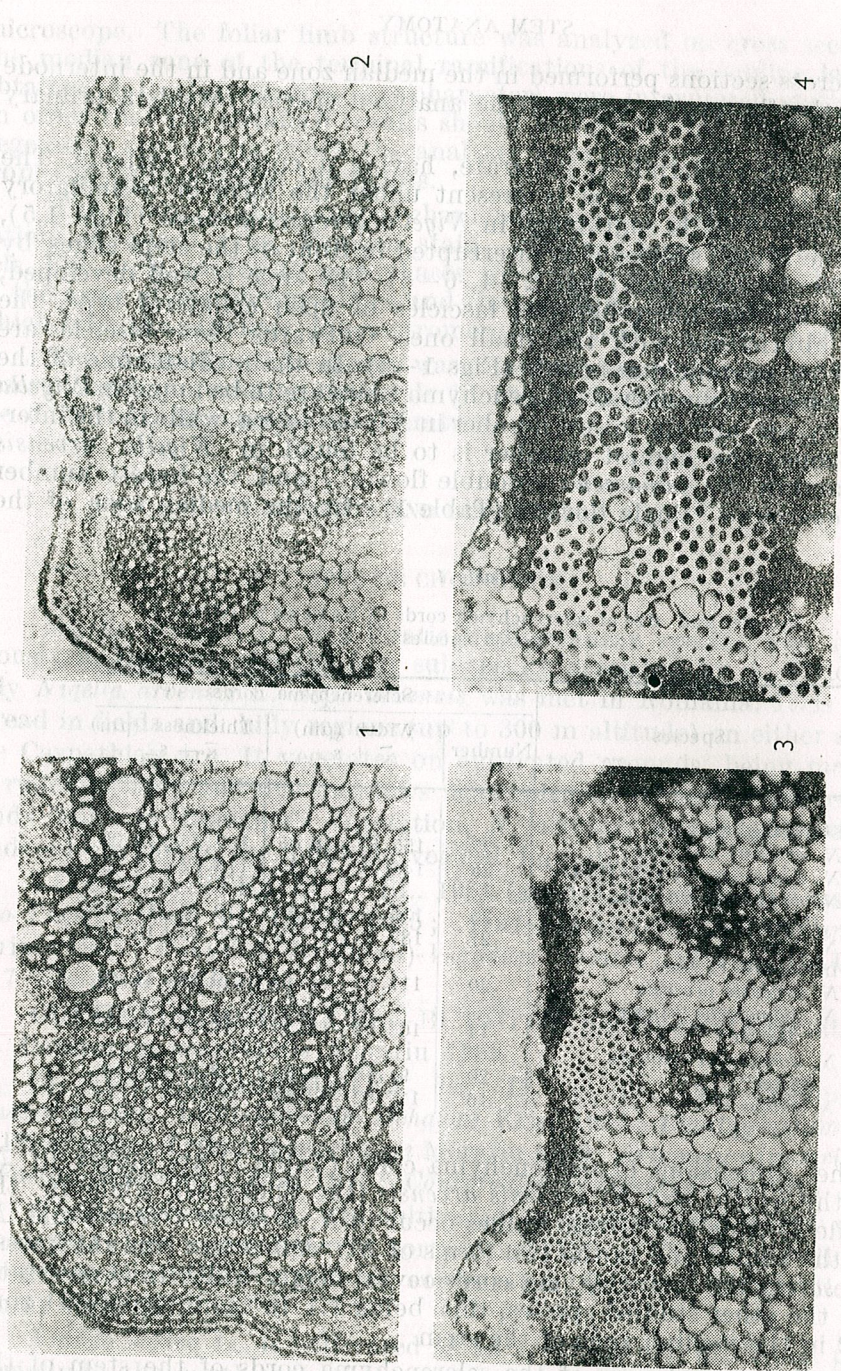
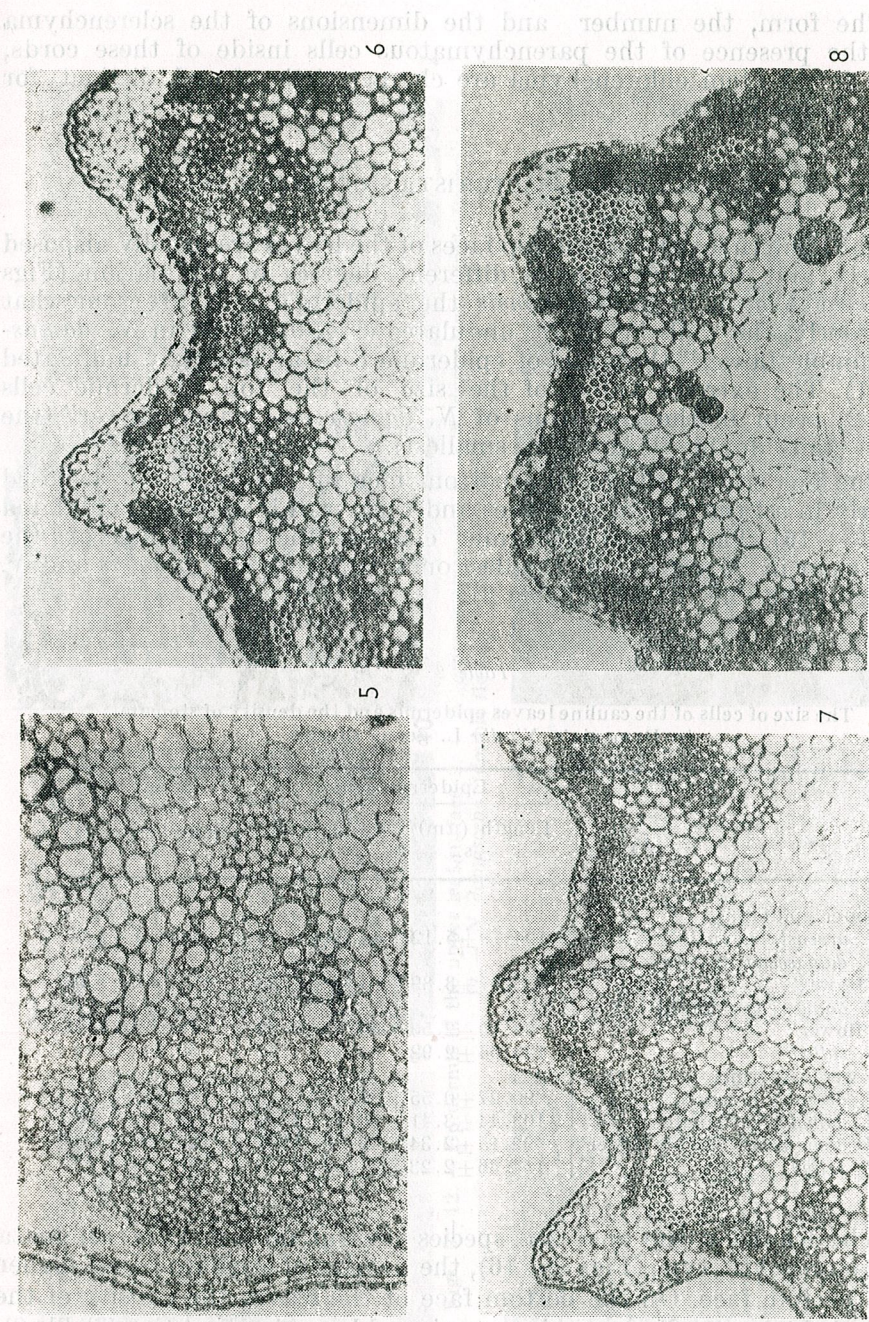


Fig. 1-4 — Portions of stem cross-section (median zone) 1. — *N. arvensis arvensis*; 2. — *N. damascena* "single flower"; 3. — *N. damascena* "double flower"; 4. — *N. salvia*.



Figs 5-8. — Portions of stem cross-section (internode II zone) 5. — *N. arvensis arvensis*; 6. — *N. damascena* "single flower"; 7. — *N. damascena* "double flower"; 8. — *N. salvia*.



The form, the number and the dimensions of the sclerenchyma cords, the presence of the parenchymatous cells inside of these cords, as well as the stem chlorenchyma are characteristic of and distinct for each analyzed species.

#### STRUCTURAL CHARACTERISTICS OF THE LEAF

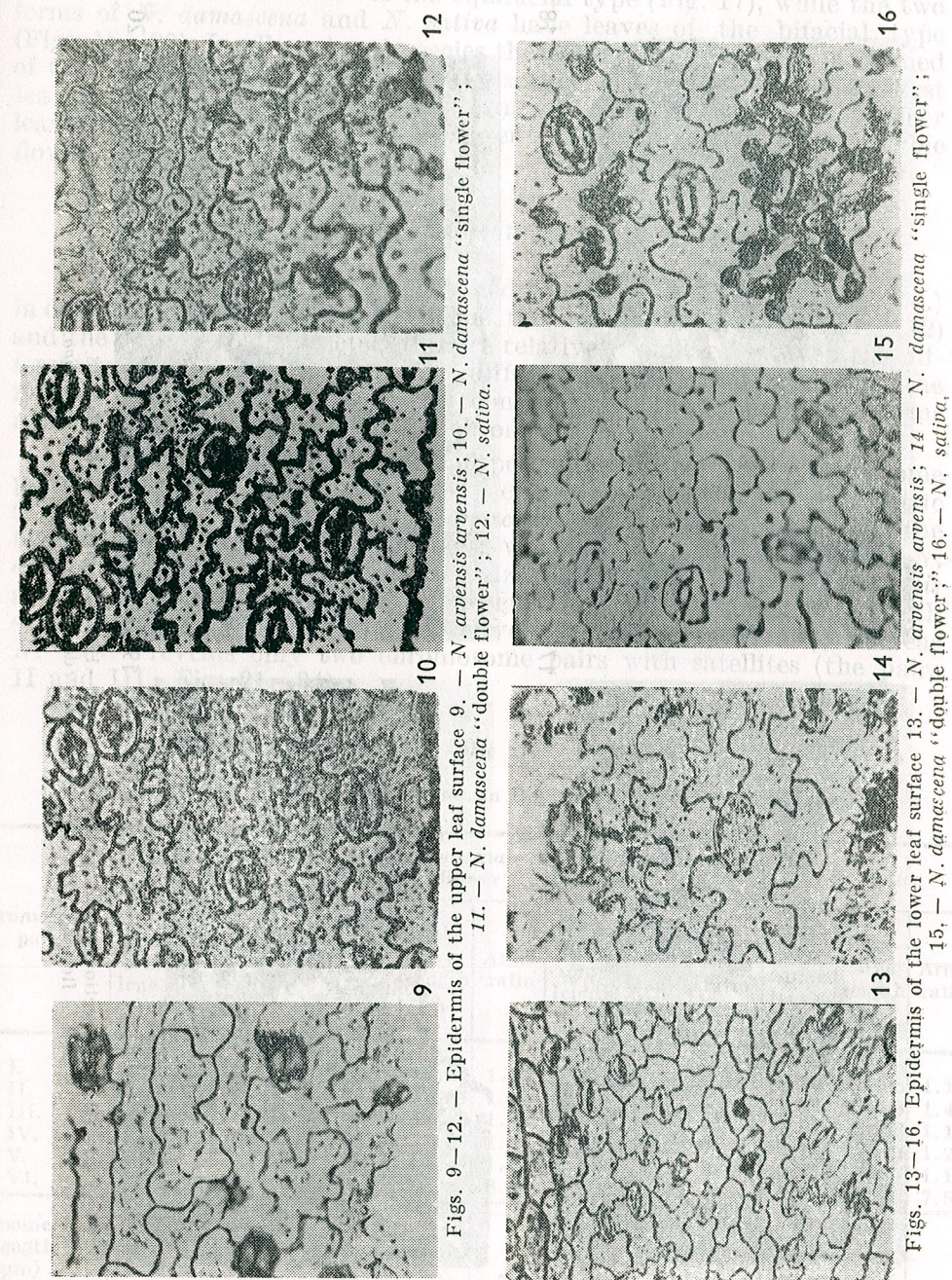
The epidermic cells on the top faces of the leaves are usually disposed unorderedly and their walls have different degrees of undulation (Figs 9–12). In *Nigella arvensis arvensis* the epidermic cells are somewhat more orderly, the walls being less undulated (Fig. 9), while in *N. damascena* "double flower" the walls of epidermic cells are strongly undulated (Fig. 11). The extreme values of the size of the top epidermic cells (Table 2) occur in the two forms of *N. damascena*: "single flower" (the biggest) and "double flower" (the smallest).

The epidermic cells on the bottom face of the leaf are disposed unorderedly in all the analyzed species and their wall is strongly undulated (Figs 13–16). The biggest epidermic cells on the bottom face of the leaves occur in *N. sativa*, the smallest ones in *N. arvensis arvensis* and *N. damascena* "double flower" (Table 2).

Table 2  
The size of cells of the cauline leaves epidermis and the density of stomata in Romania's *Nigella* L. genus species

| Species                             | Epiderma cells                                        |                                                      | Stomata density<br>(number/mm <sup>2</sup> )<br>$\bar{x} \pm s_{\bar{x}}$ |
|-------------------------------------|-------------------------------------------------------|------------------------------------------------------|---------------------------------------------------------------------------|
|                                     | Length ( $\mu\text{m}$ )<br>$\bar{x} \pm s_{\bar{x}}$ | Width ( $\mu\text{m}$ )<br>$\bar{x} \pm s_{\bar{x}}$ |                                                                           |
| Upper epidermis                     |                                                       |                                                      |                                                                           |
| <i>N. arvensis arvensis</i>         | 104.79 $\pm$ 5.12                                     | 44.83 $\pm$ 0.99                                     | 105.31 $\pm$ 4.67                                                         |
| <i>N. damascena</i> "single flower" | 138.87 $\pm$ 3.89                                     | 64.69 $\pm$ 1.23                                     | 27.16 $\pm$ 1.08                                                          |
| <i>N. damascena</i> "double flower" | 99.10 $\pm$ 2.50                                      | 50.27 $\pm$ 0.82                                     | 112.13 $\pm$ 4.71                                                         |
| <i>N. sativa</i>                    | 126.64 $\pm$ 2.92                                     | 64.89 $\pm$ 1.57                                     | 50.94 $\pm$ 2.34                                                          |
| Lower epidermis                     |                                                       |                                                      |                                                                           |
| <i>N. arvensis arvensis</i>         | 90.27 $\pm$ 0.55                                      | 39.64 $\pm$ 0.76                                     | 148.57 $\pm$ 5.89                                                         |
| <i>N. damascena</i> "single flower" | 103.44 $\pm$ 3.41                                     | 59.87 $\pm$ 1.10                                     | 119.36 $\pm$ 4.88                                                         |
| <i>N. damascena</i> "double flower" | 92.15 $\pm$ 2.34                                      | 45.98 $\pm$ 0.71                                     | 127.33 $\pm$ 5.23                                                         |
| <i>N. sativa</i>                    | 114.36 $\pm$ 2.23                                     | 71.34 $\pm$ 1.73                                     | 89.30 $\pm$ 4.08                                                          |

The leaves of the analyzed species present on either face stomata of the anomocytic type (Figs 9–16), the density of stomata being higher on the bottom face. On the bottom face of the leaves the density of the stomata is higher in *N. arvensis arvensis* and lower in *N. sativa* (Table 2). On the top face of the leaves the highest density of stomata is found in *N. damascena* "double flower", and the lowest density in *N. damascena* "single flower" (Table 2).

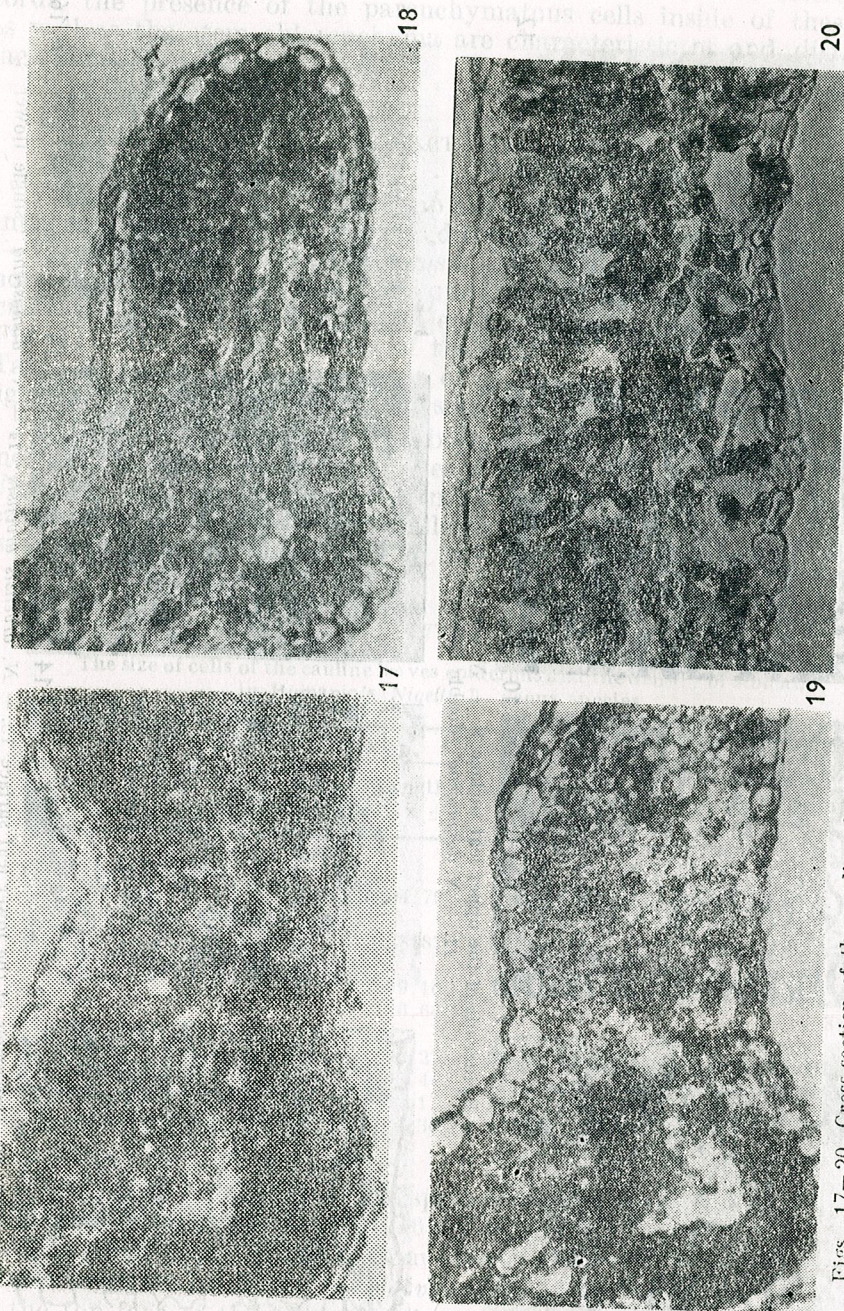


Figs. 9–12. — Epidermis of the upper leaf surface 9. — *N. arvensis arvensis*; 10. — *N. damascena* "double flower"; 11. — *N. damascena* "single flower"; 12. — *N. sativa*.

Figs. 13–16. Epidermis of the lower leaf surface 13. — *N. arvensis arvensis*; 14. — *N. damascena* "double flower"; 15. — *N. damascena* "single flower"; 16. — *N. sativa*.

\* Chromosomes with satellites





Figs. 17-20. Cross-section of the cauline leaf blade 17. — *N. arvensis arvensis*; 18. — *N. damascena* "single flower"; 19. — *N. damascena* "double flower"; 20. — *N. sativa*.

On the top face of the leaves the highest density of stomata is found in *N. damascena* "double flower", and the lowest density in *N. damascena* "single flower" (Table 2).

The study of the cross sections through the leaf points out that *N. arvensis arvensis* has leaves of the equifacial type (Fig. 17), while the two forms of *N. damascena* and *N. sativa* have leaves of the bifacial type (Figs. 18-20). In all analyzed species the palisade parenchyma is formed of two cell layers. *N. arvensis arvensis* and *N. sativa* present the thickest leaves (201.67  $\mu\text{m}$ ), while the two forms of *N. damascena* have thinner leaves (165.00  $\mu\text{m}$  in the "single flower" and 174.17  $\mu\text{m}$  in the "double flower").

#### CHROMOSOME CHARACTERISTICS

As it was shown in the specialized literature (4), (7), (10), (11) a.o.) in different species of the *Nigella* genus, the chromosome number ( $2n = 12$ ) and the karyotype characteristics are relatively similar. Between the different species of the *Nigella* genus, differences were found regarding the number and position of secondary constrictions, the value of the arms ratio, the relative length of the chromosomes.

The analyzed *Nigella* genotypes present the first chromosome pairs with median centromere, the last chromosome pair being acrocentric (Figs 21-24; Table 3). *Nigella damascena* "single flower" presents the biggest length of the chromosomes, whereas the chromosomes in *N. arvensis arvensis* have the smallest size (Table 3). *N. arvensis arvensis* and *N. damascena* (both forms) present three chromosome pairs with satellites (the pairs I, II, III; respectively the pairs II, III, IV), whereas *N. sativa* reveals only two chromosome pairs with satellites (the pairs II and III; Figs 21-24).

Table 3

The characteristics of the chromosomes in Romania's *Nigella* L. genus species.

| Chromosome pair                 | <i>N. arvensis arvensis</i>         |                     |            | <i>N. damascena</i> "single flower" |                     |            | <i>N. damascena</i> "double flower" |                     |            | <i>N. sativa</i>                    |                     |            |
|---------------------------------|-------------------------------------|---------------------|------------|-------------------------------------|---------------------|------------|-------------------------------------|---------------------|------------|-------------------------------------|---------------------|------------|
|                                 | Chromosome length ( $\mu\text{m}$ ) | Relative length (%) | Arms ratio | Chromosome length ( $\mu\text{m}$ ) | Relative length (%) | Arms ratio | Chromosome length ( $\mu\text{m}$ ) | Relative length (%) | Arms ratio | Chromosome length ( $\mu\text{m}$ ) | Relative length (%) | Arms ratio |
| I.                              | 9.70*                               | 19.72               | 1.24       | 15.75                               | 21.20               | 1.06       | 13.68                               | 20.42               | 1.04       | 12.90                               | 20.48               | 1.19       |
| II.                             | 90.08*                              | 18.46               | 1.27       | 13.85*                              | 18.64               | 1.27       | 12.40*                              | 18.51               | 1.18       | 12.52*                              | 19.88               | 1.46       |
| III.                            | 8.60*                               | 17.48               | 1.30       | 13.10*                              | 17.63               | 1.25       | 12.09*                              | 18.05               | 1.16       | 11.95*                              | 18.98               | 1.19       |
| IV.                             | 8.33                                | 16.94               | 1.09       | 12.85*                              | 17.29               | 1.06       | 11.56*                              | 17.26               | 1.24       | 10.15                               | 16.12               | 1.26       |
| V.                              | 7.88                                | 16.02               | 1.06       | 12.20                               | 16.42               | 1.03       | 10.90                               | 16.27               | 1.06       | 9.55                                | 15.17               | 1.12       |
| VI.                             | 5.60                                | 11.38               | 9.18       | 6.55                                | 8.82                | 8.36       | 6.36                                | 9.49                | 8.94       | 5.90                                | 9.37                | 7.53       |
| Genome length ( $\mu\text{m}$ ) | 49.19                               | —                   | —          | 74.30                               | —                   | —          | 66.99                               | —                   | —          | 62.97                               | —                   | —          |

\* Chromosomes with satellites



## CONCLUSIONS

1. In Romania's spontaneous flora there occurs only *Nigella arvensis* L. ssp. *arvensis*, whereas the *N. damascena* L. and *N. sativa* L. species are cultivated as ornamental, medicinal and aromatic plants.

2. All the analyzed species have a primary structure in the stem. *N. arvensis arvensis* displays a continuous stem chlorenchyma, whereas the other species have it interrupted on a level with the stem edges by the angular chlorenchyma. The number and the dimensions of the sclerenchyma cords are characteristic of each analyzed species.

3. The analyzed species present on both faces of the cauline leaves stomata of the anomocytic type. The form, disposal and the dimensions of the epidermic cells on both faces of the leaves are characteristic of each analyzed species.

4. The analyzed *Nigella* genotypes, although they have the same chromosome number ( $2n = 12$ ), differ from one another by the length of the chromosomes, the arms ratio and the chromosome pairs which bear the satellites.

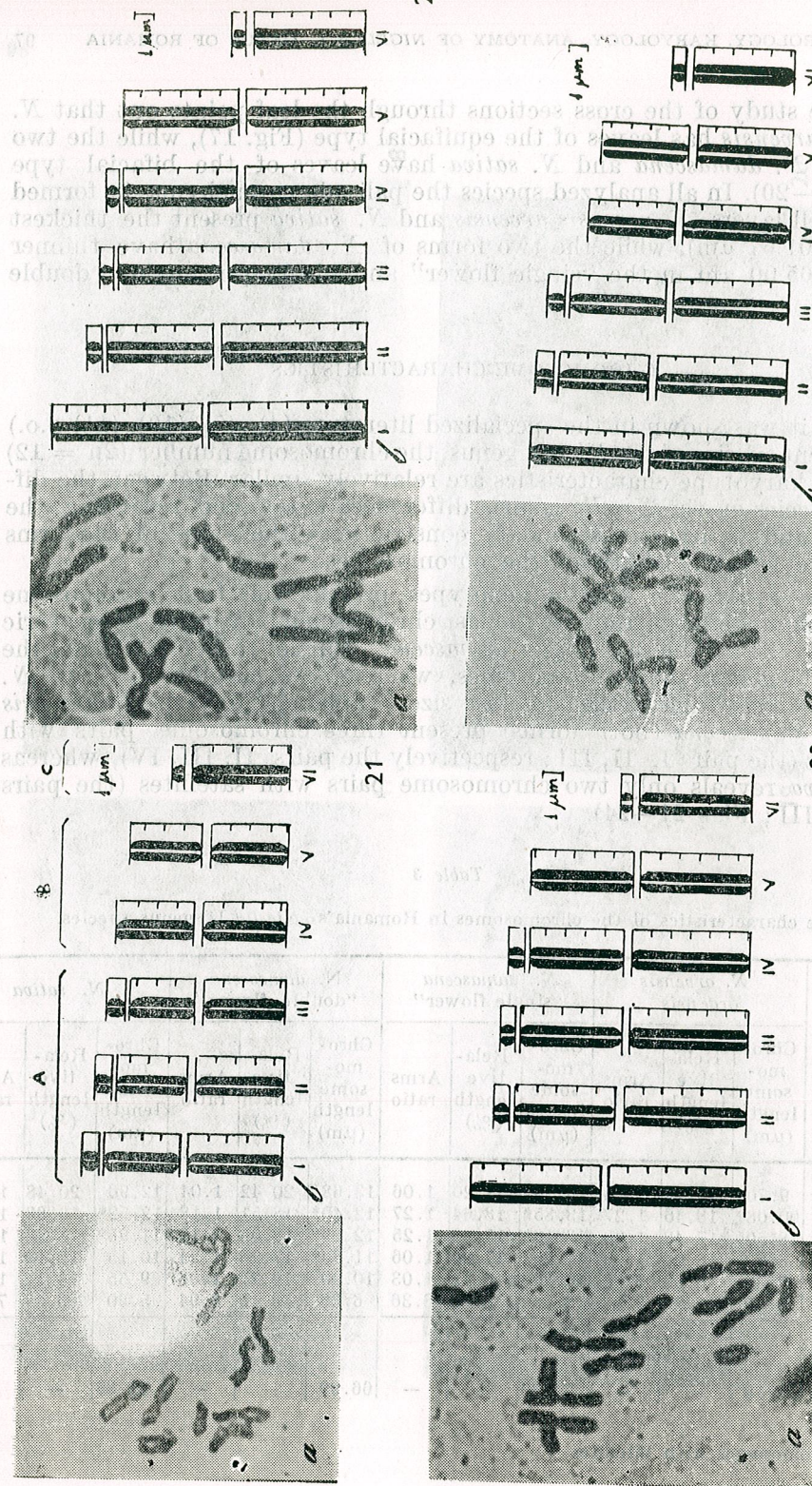
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Figs. 21-14. - Metaphasic chromosomes (a) and idiogram (b) 21. - *N. arvensis arvensis*; 22. - *N. damascena* "single flower"; 23. - *N. damascena* "double flower"; 24. - *N. sativa*.





# NEUE SCHNEEBODENGESELLSCHAFTEN IM RODNA-GEBIRGE

GH. COLDEA

The paper presents, from a floristical and ecological point of view, three new syntaxa of the class *Salicetea herbaceae*, which are characteristics for the Rodnei Mountains (the Eastern Carpathians), The syntaxa *Soldanello hungaricae-Ranunculetum crenati* ass. nova and *Poo-Cerastietum cerastioidis chrysosplenietosum* alpinae subass. nova are specific to the stations with are acid substratum, while *Soldanello hungaricae-Salicetum kitaibelianae* ass. nova is specific to the stations with a calcareous substratum. Based on the floristical differences and resemblances to the composition of the described associations from the neighbouring regions, the syntaxonomic position of these associations in the Eastern Carpathians is defined.

Die von uns in den letzten Jahren in Schneetälchen der Rodna-Gebirge durchgeführten geobotanischen Forschungen ermöglichen die Identifizierung einiger Phytozönosen, die sich hinsichtlich ihrer floristischen Zusammensetzung größtenteils sowohl von ähnlichen Phytozönosen in Zentral- [1], [4], [5], [10], [11], [13], [16], [22]\* und Südeuropa [6], [8] [9], [21], als auch von den jenen der Karpaten unterscheiden. Die besonderen Eigentümlichkeiten dieser Phytozönosen des Rodna-Gebirges werden durch die Gegenwart der chiono-hygrophilen Arten *Soldanella hungarica* ssp. *hungarica*, *Silene nivalis* und *Poa granitica* ssp. *disparilis* (= *Poa deyllii*) verliehen, deren Verbreitungsareal sich nur auf einige Gebirgsmassive der Südostkarpaten begrenzt. Neuere von Pawlowska [15], Chrték und Jirásek [7] und Nyárády [12] durchgeführten taxonomischen und chorologischen Forschungen heben den endemischen Charakter von *Silene nivalis* und *Soldanella hungarica* ssp. *hungarica* für das Rodna-Gebirge, bzw von *Poa granitica* ssp. *disparilis* für die orientalischen und südlichen Karpaten hervor [2]. Höchstwahrscheinlich fiel die morphologische und genetische Differenzierung dieser oligothermen Arten mit den harten arktischen Klimabedingungen der Pleistozäneiszeit zusammen und setzte sich bis Ende der Eiszeit, als dessen Genom vollkommen entwickelt war, fort. Somit ist die aktuelle zönotische Affinität dieser endemischen Arten für die chionophilen Phytozönosen der Ordnung *Salicetalia herbaceae* erklärlich.

In vorliegender Arbeit beschreiben wir drei neue Syntaxa der Schneebodengesellschaften des Rodna-Gebirges, die floristisch im Zusammenhang mit denen der geographischen Nachbarregionen analysiert werden.

1. *Soldanello hungaricae-Ranunculetum crenati* Ass. nova (Nomenklatorischer Typus : Tabelle 1, Aufnahme 1).

Syn. : *Agrosteto (alpinae) - Ranunculetum crenati* Resm. 1975; *Agrosteto (rupestris) - Ranunculetum crenati* Resm. 1975 em. 1978;

\* Das bibliographische Studium dieser Arbeit wurde anlässlich einer vom DAAD erhaltenen Einladung im Jahre 1983 vorgenommen, wofür wir unseren Dank aussprechen.



*Soldanello (pusillae)-Plantaginetum gentianoidis* Resm. 1976 non Boşcaiu 1971; *Soldanello (pusillae)-Ranunculetum crenati* Resm. 1983 (n.n.)

Die Phytozönosen dieser Assoziationen werden häufig im Rodna-Gebirge auf den nördlichen Schattenabhängen hoher Gipfel (1800–2150m) wie Pietrosul, Rebra, Anieş, Gărgălău und Inău angetroffen, wo sie als

Tabelle 1

*Soldanello hungaricae* – *Ranunculetum crenati* Ass. nova

| Nr. der Aufnahme                                              | 1    | 2    | 3    | 4    | 5    | 6    | 7   | 8    | 9    | 10   |     |
|---------------------------------------------------------------|------|------|------|------|------|------|-----|------|------|------|-----|
| Höhe (× 10 m ü.d.M.)                                          | 200  | 215  | 208  | 208  | 180  | 212  | 185 | 210  | 210  | 188  |     |
| Exposition                                                    | NV   | N    | N    | NV   | N    | N    | N   | N    | N    | NE   |     |
| Neigung                                                       | 30   | 5    | 15   | 15   | 10   | 10   | 20  | 35   | 15   | 15   |     |
| Deckungsgrad (%)                                              | 100  | 100  | 90   | 90   | 100  | 100  | 75  | 100  | 90   | 100  |     |
| Aufnahmeffläche (m <sup>2</sup> )                             | 100  | 10   | 10   | 100  | 10   | 100  | 100 | 25   | 25   | 100  | K   |
| <b>C. – Ass.</b>                                              |      |      |      |      |      |      |     |      |      |      |     |
| <i>Ranunculus crenatus</i>                                    | 3.5  | 3.5  | 3.5  | 2.5  | 4.5  | 4.5  | 4.5 | 2.5  | 4.5  | 3.5  | V   |
| <i>Soldanella * hungarica</i>                                 | 2.5  | 2.5  | + .5 | + .5 | + .2 | 1.4  | 1.5 | + .5 | 1.5  | + .5 | V   |
| <b>VC – Salicion herbaceae und OC – Salicetalia herbaceae</b> |      |      |      |      |      |      |     |      |      |      |     |
| <i>Luzula alpino-pilosa</i>                                   | +    | + .5 | 1.5  | 1.5  | +    | + .2 | 1.5 | + .3 | 1.5  | 1.5  | V   |
| <i>Gnaphalium supinum</i>                                     | + .5 | 1.3  | 1.5  | + .5 | 1.3  | + .5 | +   | +    | +    | 1.5  | V   |
| <i>Salix herbacea</i>                                         | 1.2  | .    | .    | .    | .    | 1.3  | .   | .    | .    | .    | I   |
| <i>Kiaeria falcata</i>                                        | 2.5  | 3.5  | .    | .    | .    | .    | 3.5 | .    | 2.5  | 2.5  | III |
| <i>Kiaeria starkei</i>                                        | .    | 2.5  | .    | 3.5  | 2.5  | 2.5  | .   | +    | .    | .    | III |
| <b>KC – Salicetalia herbaceae</b>                             |      |      |      |      |      |      |     |      |      |      |     |
| <i>Sedum alpestre</i>                                         | +    | .    | +    | .    | .    | .    | +   | .    | .    | + .5 | II  |
| <i>Poa * disparilis</i>                                       | 1.5  | +    | .    | 1.5  | .    | .    | +   | 1.5  | .    | 2.5  | III |
| <i>Oligotrichum hercinicum</i>                                | 2.5  | .    | .    | .    | .    | .    | .   | 3.5  | .    | 2.5  | II  |
| <i>Anthelia jurazkana</i>                                     | .    | 1.3  | +    | 2.5  | .    | .    | .   | 1.3  | +    | .    | III |
| <i>Polytrichum sexangulare</i>                                | 1.5  | .    | 2.5  | 2.5  | 1.3  | 1.3  | .   | +    | .    | +    | IV  |
| <b>Begleiter</b>                                              |      |      |      |      |      |      |     |      |      |      |     |
| <i>Ligusticum mutellina</i>                                   | +    | 1.5  | + .3 | .    | .    | + .5 | 1.5 | + .2 | 2.5  | .    | IV  |
| <i>Primula minima</i>                                         | + .3 | + .3 | 1.5  | 1.5  | + .3 | + .4 | +   | .    | +    | .    | IV  |
| <i>Geum montanum</i>                                          | .    | + .3 | +    | .    | +    | .    | +   | .    | + .3 | +    | III |
| <i>Carex curvula</i>                                          | +    | .    | +    | 1.3  | .    | .    | .   | .    | .    | .    | II  |
| <i>Deschampsia caespitosa</i>                                 | .    | .    | .    | .    | +    | .    | 1.5 | .    | +    | 1.5  | II  |
| <i>Nardus stricta</i>                                         | .    | .    | .    | .    | .    | .    | +   | .    | +    | .    | I   |
| <i>Diplophyllum taxifolium</i>                                | .    | +    | 1.3  | .    | +    | .    | .   | .    | .    | +    | II  |
| <i>Dicranella heteromalla</i>                                 | .    | .    | .    | .    | .    | +    | 1.3 | +    | .    | .    | II  |

Arten, die nur in einer Aufnahme vorkommen: *Sesleria disticha* 1: +, *Veratrum album* 2: +, *Gentiana punctata* 2: +, *Agrostis rupestris* 5: +, *Chrysosplenium alpinum* 7: +, *Alchemilla alpestris* 7: +, *Saxifraga stellaris* 8: +, *Ranunculus montanus* 9: +, *Arabis alpina* 9: +, *Saxifraga androsacea* 9: +, *Drepanocladus uncinatus* 9: +; X

Ort der Aufnahmen: 1 – Gărgălău 19.07.1982; 2 – Pietrosul Mare 19.07.1977; 3 – Buhăiescu Mare 24.07.1967; 5 – Puzdra – Anieşu Mare 27.07.1976; 4 – Gărgălău 19.07.1982; 6 – Buhăiescu 8.08.1979; 7 – Anieşu Mic 17.07.1982; 8 – Inău 17.08.1982; 9 – Anieşu Mare 15.08.1982; 10 – Galaţ 18.07.1982.

\* In der „Flora Europea“ als Unterarten behandelt.

Einschlüsse die kleinen Mulden auf Säuresubstrat besiedeln in denen sich der Schnee bis Ende Juli hält. Die Böden, worauf solche Zönosen vegetieren, sind oberflächliche, saure (pH = 5,3–5,5), an feinem Gestein reichhaltige und an organischen Stoffen arme Böden. Charakteristische Kennarten der Assoziation sind *Ranunculus crenatus* und *Soldanella hungarica* ssp. *hungarica*. Sie verzeichnen eine Geländebedeckung von 35–60%. Das Fehlen von *Soldanella pusilla* in der Rodna-Gebirgsflora und das häufige Auftreten, stellenweise sogar mit hoher Bedeckung, von *Soldanella hungarica* in diesen Phytozönosen, veranlaßten uns, sie als eine neue Assoziation zu beschreiben, die wir als eine geographisch ostkarpatische Variante der in mehrfachen Gebirgsmassiven der Südkarpaten [3], [17], [24] angeführten Assoziation *Soldanello pusillae-Ranunculetum crenati* (Borza 1931) Boşcaiu 1971 betrachten. Die Assoziation *Agrosteto alpinae-Ranunculetum crenati* Resm. 1975, die als aus der Eiszeit stammend beschrieben und nachträglich von ihrem Verfasser unter derselben Benennung [19] oder als *Agrosteto rupestris-Ranunculetum crenati* Resm. 1975 em. 1978 veröffentlicht wurde [18], [20], kann nicht in Betracht gezogen werden, da in ihrer Zusammensetzung manche Arten wie *Soldanella pusilla*, *Agrostis alpina* und *Plantago gentianoides* vorkommen, die in Rodna-Gebirge nicht vegetieren. Das häufige Auftreten von charakteristischen Arten des Verbandes *Salicion herbaceae* und der Ordnung *Salicetalia herbaceae* in der von uns beschriebenen Assoziation bestätigen deren Einordnung in diese syntaxonomischen Einheiten.

2. *Poa supinae-Cerastietum cerastioidis* (Söyr. 1954) Oberd. 1957 – *chrysosplenietosum alpinae* Subass. nova (Nomenklatorischer Typus: Tabelle 2, Aufnahme 7). Auf Mikrovertiefungen, auf manchen Schattenabhängen (Anieş, Coasta Netedă-Inău, Nedeia Corongiş) und in einigen Gletschertälern (Bila, Galaţ) haben wir Phytozönosen dieser Assoziation auf beschränkten Flächen identifiziert. Ähnlich den aus Zentraleuropa [13] beschriebenen Phytozönosen, kommen auch in denjenigen des Rodna-Gebirges *Cerastium cerastioides* und *Poa supina* als die Charakterarten vor. Sie erzielen auf den analysierten Geländen eine Durchschnittsdeckung von 30%. Im floristischen Aufbau der Assoziation sind die charakteristischen Arten für *Salicion* und *Salicetalia herbaceae* gut vertreten, was deren Einreihung in diese syntaxonomischen Einheiten [13] bedingt, nicht aber in den Verband *Arabidion caeruleae* bzw. in die Ordnung *Arabidetalia caeruleae*, so wie Passarge [14] es empfahl. Im untersuchten Gelände werden in solchen Phytozönosen an Stellen mit Feuchtigkeitsüberschuß in der Nähe von subalpinen Quellen manche fontinale, hygrophile Arten wie *Chrysosplenium alpinum*, *Saxifraga stellaris* und *Epilobium anagallidifolium* angetroffen. Auf Grund solcher ökologischer Differentialarten sondern wir eine neue Subassoziation für die Karpaten ab – *chrysosplenietosum alpinae* (Tabelle 2, Aufnahme 5–10). Derartige Phytozönosen wurden in der Karpatenzone nur aus den Gebirgen Ţarcu, Godeanu und Cerna beschrieben [3].

3. *Soldanello hungaricae-Salicetum kitaibelianae* Ass. nova (Nomenklatorischer Typus: Tabelle 3, Aufnahme 5). Chionophile durch *Salix kitaibeliana* aufgebaute Phytozönosen werden häufig im Rodna-Gebirge in den Zonen mit kalkhaltigem Substrat mancher Gebirgsspitzen wie: Pietrosul, Rebra, Cormaia, Anieş, Puzdra, Gărgălău und Inău angetroffen.



Tabelle 2

*Poa supinae* — *Cerastietum cerastioidis* (Söyr. 1954) Oberd. 1957  
— *chrysosplenietosum alpinae* Subass. nova

| Nr. der Aufnahme                                              | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |     |
|---------------------------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Höhe (× 10 m ü.d.M.)                                          | 202 | 201 | 183 | 184 | 184 | 188 | 189 | 177 | 173 | 180 |     |
| Exposition                                                    | N   | N   | V   | NE  | NE  | NV  | NV  | N   | N   | N   |     |
| Neigung                                                       | 5   | 5   | 5   | 10  | 5   | 45  | 40  | 5   | 25  | 20  |     |
| Deckungsgrad (%)                                              | 70  | 80  | 75  | 75  | 65  | 80  | 80  | 100 | 65  | 60  |     |
| Aufnahmeffläche (m²)                                          | 25  | 25  | 25  | 10  | 10  | 4   | 4   | 100 | 100 | 25  | K   |
| <b>C. — Ass.</b>                                              |     |     |     |     |     |     |     |     |     |     |     |
| <i>Cerastium cerastioides</i>                                 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 4.5 | 3.5 | 3.5 | V   |
| <i>Poa supina</i>                                             | .   | .   | 1.3 | 1.5 | +   | .   | +   | 1.3 | +   | +.2 | IV  |
| <b>D. — Subass.</b>                                           |     |     |     |     |     |     |     |     |     |     |     |
| <i>Chrysosplenium alpinum</i>                                 | .   | .   | .   | .   | .   | 1.2 | 2.5 | 1.3 | +   | +.2 | III |
| <i>Saxifraga stellaris</i>                                    | .   | .   | .   | .   | .   | 1.4 | +   | +   | +   | +   | II  |
| <i>Epilobium anagallidifolium</i>                             | .   | .   | .   | .   | .   | +   | +   | +   | +   | +   | II  |
| <b>VC — Salicion herbaceae und OC — Salicetalia herbaceae</b> |     |     |     |     |     |     |     |     |     |     |     |
| <i>Ranunculus crenatus</i>                                    | 1.5 | 2.5 | +.5 | .   | .   | +   | .   | +.5 | +.3 | +   | IV  |
| <i>Luzula alpino-pilosa</i>                                   | +.3 | +.5 | .   | .   | .   | +   | +   | +.5 | +.5 | +   | III |
| <i>Gnaphalium supinum</i>                                     | .   | +   | 1.5 | +.3 | +.4 | +   | +   | 1.5 | 2.5 | +   | IV  |
| <i>Soldanella * hungarica</i>                                 | +.2 | +.3 | +   | +.2 | .   | .   | .   | +.4 | +   | +   | IV  |
| <i>Salix herbacea</i>                                         | +   | 1.2 | .   | .   | .   | +   | .   | .   | +   | +   | I   |
| <i>Kiaeria starkei</i>                                        | +   | .   | .   | .   | .   | .   | .   | .   | .   | .   | II  |
| <b>KC — Salicetea herbaceae</b>                               |     |     |     |     |     |     |     |     |     |     |     |
| <i>Veronica alpina</i>                                        | +   | +.3 | .   | +   | .   | +   | .   | .   | .   | .   | III |
| <i>Sedum alpestre</i>                                         | .   | +   | +   | 1.5 | 1.5 | .   | .   | +   | 1.5 | 1.5 | IV  |
| <i>Silene nivalis</i>                                         | .   | +   | .   | .   | .   | .   | .   | .   | .   | .   | IV  |
| <i>Polytrichum sexangulare</i>                                | 1.5 | 1.5 | .   | .   | .   | .   | .   | .   | +   | +   | I   |
| <i>Oligotrichum hercinicum</i>                                | .   | .   | 1.5 | 1.5 | .   | 2.5 | 2.5 | 1.5 | .   | +   | II  |
| <b>Begleiter</b>                                              |     |     |     |     |     |     |     |     |     |     |     |
| <i>Poa alpina</i>                                             | +   | +.4 | +   | +.3 | .   | +.3 | +   | 1.5 | +   | +   | V   |
| <i>Geum montanum</i>                                          | +.3 | +.5 | +   | +.5 | 1.5 | +   | +   | 1.5 | +.3 | +.5 | V   |
| <i>Lingusticum mutellina</i>                                  | +.5 | 1.5 | +.5 | 2.5 | 1.5 | +   | .   | 1.5 | .   | .   | III |
| <i>Alchemilla apestis</i>                                     | .   | .   | .   | .   | .   | +   | +   | 1.3 | 2.5 | .   | III |
| <i>Deschampsia caespitosa</i>                                 | 1.5 | 1.5 | 2.5 | +   | +.3 | .   | .   | +.5 | .   | .   | III |
| <i>Potentilla * lernata</i>                                   | .   | .   | .   | .   | .   | .   | .   | .   | .   | .   | III |
| <i>Phleum alpinum</i>                                         | +   | .   | +   | +   | .   | .   | .   | +.5 | .   | .   | III |
| <i>Polygonum viviparum</i>                                    | .   | +   | .   | .   | .   | .   | .   | .   | .   | .   | I   |
| <i>Agrostis rupestris</i>                                     | .   | .   | .   | .   | .   | .   | .   | .   | .   | .   | I   |
| <i>Anthoxanthum odoratum</i>                                  | .   | .   | .   | .   | .   | .   | .   | .   | .   | .   | I   |
| <i>Campanula polymorpha</i>                                   | .   | .   | .   | +   | +   | .   | .   | +   | +   | +   | II  |
| <i>Arabis alpina</i>                                          | .   | .   | .   | +   | +   | .   | .   | .   | .   | .   | I   |
| <i>Festuca picta</i>                                          | .   | .   | .   | .   | .   | .   | .   | +.3 | +   | +   | I   |
| <i>Saxifraga androsacea</i>                                   | .   | .   | .   | .   | .   | .   | .   | +   | +.3 | +   | I   |
| <i>Primula minima</i>                                         | .   | .   | .   | .   | .   | .   | .   | +   | +   | +   | I   |
| <i>Pohlia ludwigi</i>                                         | .   | .   | .   | .   | .   | .   | .   | +   | +   | +   | I   |
| <i>Cratoneuron commutatum</i>                                 | .   | .   | .   | .   | 2.5 | 1.5 | .   | .   | .   | .   | I   |
| <i>Drepanocladus uncinatus</i>                                | .   | .   | .   | .   | .   | 1.5 | .   | .   | .   | .   | I   |

Arten, die nur in einer Aufnahme vorkommen: *Saxifraga aizoides* 6: +, *Cardamine pratensis* 7: +, *Viola biflora* 7: +, *Campanula abietina* 7: +, *Philonotis fontana* 7: 1; *Soldanella* × *major* 9: +, *Salix reticulata* 9: +, *Saxifraga hieracifolia* 9: +, *Doronicum carpatum* 10: +, *Rhododendron kotschy* 10: +;

Ort der Aufnahmen: 1,2 — Coasta Netedă — Inău, 8.08.1981; 3 — Căldarea Gălaț 18.07.1981; 4,5 — Nedeia Corongiș 28.08.1982; 6,7 — Buhăiescu Mare 7.09.1979; 8 — Săua Anieș 23.09.1982; 9,10 — Căldarea Bila 12.08.1983;

Tabelle 3

*Soldanella hungaricae* — *Salicetum kitaibelianae* Ass. nova

| Nr. der Aufnahme                                     | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |     |
|------------------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Höhe (× 10 m ü.d.M.)                                 | 210 | 190 | 212 | 205 | 208 | 206 | 211 | 205 | 227 | 210 |     |
| Exposition                                           | NV  | N   | N   | N   | N   | N   | NV  | N   | NE  | N   |     |
| Neigung                                              | 10  | 5   | 5   | 15  | 35  | 25  | 35  | 15  | 15  | 10  |     |
| Deckungsgrad (%)                                     | 80  | 80  | 100 | 100 | 90  | 85  | 100 | 85  | 100 | 100 |     |
| Aufnahmeffläche (m²)                                 | 6   | 25  | 100 | 25  | 100 | 10  | 100 | 100 | 10  | 25  | K   |
| <b>C. — Ass.</b>                                     |     |     |     |     |     |     |     |     |     |     |     |
| <i>Salix kitaibeliana</i>                            | 4.5 | 3.5 | 4.5 | 4.5 | 4.5 | 3.5 | 4.5 | 3.5 | 5.4 | 4.5 | V   |
| <i>Soldanella * hungarica</i>                        | +.4 | +   | +.3 | +   | 1.5 | +.5 | 1.5 | 1.5 | +.3 | 1.5 | V   |
| <b>VC — Arabidion caeruleae</b>                      |     |     |     |     |     |     |     |     |     |     |     |
| <i>Saxifraga androsacea</i>                          | .   | .   | .   | .   | +   | +   | +   | .   | .   | .   | II  |
| <b>OC — Salicetalia und KC — Salicetea herbaceae</b> |     |     |     |     |     |     |     |     |     |     |     |
| <i>Luzula alpino-pilosa</i>                          | 1.3 | 1.5 | .   | +   | 1.5 | +   | +.4 | 1.5 | +   | 1.5 | V   |
| <i>Salix herbacea</i>                                | .   | .   | .   | .   | 1.5 | +   | .   | .   | +   | +   | II  |
| <i>Poa * disparilis</i>                              | .   | .   | .   | .   | +   | +   | .   | .   | +   | .   | II  |
| <i>Silene nivalis</i>                                | .   | .   | .   | .   | +   | .   | .   | +   | .   | .   | I   |
| <b>Häufigste Begleiter:</b>                          |     |     |     |     |     |     |     |     |     |     |     |
| <b>Androsacetalia alpinae</b>                        |     |     |     |     |     |     |     |     |     |     |     |
| <i>Doronicum elusii</i>                              | +   | .   | +   | +   | +   | +.3 | 1.5 | +.4 | .   | +.4 | V   |
| <i>Veronica baumgartenii</i>                         | .   | .   | .   | +.4 | +   | .   | .   | +   | +.5 | +   | III |
| <i>Saxifraga bryoides</i>                            | .   | .   | .   | .   | +   | +   | .   | .   | .   | +   | II  |
| <i>Saxifraga carpathica</i>                          | .   | .   | .   | .   | .   | .   | .   | .   | .   | +   | I   |
| <b>Seslerietalia</b>                                 |     |     |     |     |     |     |     |     |     |     |     |
| <i>Sesleria bialzii</i>                              | .   | .   | .   | +   | 1.5 | .   | .   | 1.5 | .   | .   | II  |
| <i>Pedicularis verticillata</i>                      | .   | +   | +   | +   | +   | .   | .   | .   | +   | +.3 | III |
| <i>Polygonum viviparum</i>                           | +.3 | +   | +.5 | +.3 | +.5 | +.5 | +   | +.5 | +.5 | +   | V   |
| <i>Saxifraga moschata</i>                            | .   | .   | .   | .   | +   | .   | .   | .   | +   | .   | I   |
| <i>Dryas octopetala</i>                              | .   | 2.3 | .   | .   | .   | .   | .   | +   | .   | .   | I   |
| <i>Carex atrata</i>                                  | .   | +   | .   | +   | +   | +.3 | .   | +   | .   | .   | II  |
| <i>Carex sempervirens</i>                            | .   | 1.5 | +.4 | .   | .   | .   | .   | .   | .   | .   | I   |
| <i>Ranunculus oreophilus</i>                         | .   | .   | .   | .   | +   | .   | .   | .   | .   | .   | I   |
| <b>Sonstige Arten</b>                                |     |     |     |     |     |     |     |     |     |     |     |
| <i>Sesleria disticha</i>                             | 1.5 | .   | +   | 1.5 | +.5 | 1.5 | 1.5 | 1.5 | +   | +   | V   |
| <i>Sedum roseum</i>                                  | .   | .   | +   | +   | +   | .   | .   | +   | 1.3 | 1.3 | III |
| <i>Rhododendron kotschy</i>                          | +   | +   | +   | +   | +   | 2.5 | +   | 1.3 | +   | .   | IV  |
| <i>Saxifraga paniculata</i>                          | .   | 1.3 | .   | .   | .   | .   | .   | +   | 1.3 | .   | II  |
| <i>Festuca airoides</i>                              | .   | +   | +   | .   | .   | .   | .   | +   | 1.3 | 1.5 | III |
| <i>Campanula alpina</i>                              | .   | .   | .   | +   | .   | +   | +   | +.3 | .   | +   | III |
| <i>Helictotrichon versicolor</i>                     | +   | +   | .   | .   | +   | .   | +   | +   | .   | +   | III |
| <i>Geum montanum</i>                                 | +   | .   | .   | .   | +   | .   | .   | .   | .   | .   | I   |
| <i>Homogyne alpina</i>                               | 1   | .   | .   | +   | +   | .   | .   | .   | .   | .   | II  |
| <i>Ligusticum mutellina</i>                          | +.4 | .   | .   | .   | +   | .   | .   | .   | .   | .   | I   |
| <i>Primula minima</i>                                | +   | .   | 1.5 | +   | .   | +   | 1.5 | 1.5 | .   | .   | III |
| <i>Anthoxanthum odoratum</i>                         | .   | .   | .   | .   | +   | .   | .   | .   | .   | +   | I   |
| <i>Huperzia selago</i>                               | .   | .   | +.3 | +   | +   | +   | +   | .   | .   | .   | III |
| <i>Viola biflora</i>                                 | .   | .   | 1.5 | +   | 1.5 | +   | .   | 1.5 | +.4 | +   | IV  |
| <i>Pulsatilla alba</i>                               | .   | .   | .   | +   | +   | 1.2 | +   | 1.5 | .   | +   | III |
| <i>Hieracium alpinum</i>                             | .   | +.3 | +   | .   | .   | .   | .   | +   | .   | .   | II  |
| <i>Salix hastata</i>                                 | .   | 1.3 | .   | .   | .   | .   | .   | +.4 | .   | .   | I   |
| <i>Saxifraga aizoides</i>                            | .   | +   | .   | .   | +   | .   | .   | .   | .   | .   | I   |
| <i>Polytrichum alpinum</i>                           | 1.4 | .   | 2.5 | 2.5 | 1.5 | 2.5 | 2.5 | +   | 2.5 | +   | V   |
| <i>Hylocomium splendens</i>                          | +   | 1.5 | .   | .   | .   | +   | .   | 1.3 | .   | .   | II  |



(Fortsetzung Tabelle 3)

|                                 | 1 | 2   | 3   | 4   | 5 | 6 | 7   | 8   | 9 | 10  |     |
|---------------------------------|---|-----|-----|-----|---|---|-----|-----|---|-----|-----|
| <i>Rhitiadelphus triquetrus</i> | + | 1.3 | .   | .   | + | + | .   | 1.5 | . | +   | III |
| <i>Pleurozium schreberi</i>     | + | .   | .   | .   | . | . | .   | .   | . | 2.5 | I   |
| <i>Dicranum scoparium</i>       | . | +   | 1.5 | .   | . | . | .   | .   | . | +   | II  |
| <i>Dicranella heteromalla</i>   | . | .   | +   | .   | + | . | .   | .   | . | .   | I   |
| <i>Cetraria islandica</i>       | + | .   | .   | 2.5 | . | . | 2.5 | .   | . | +   | III |

Arten, die nur in einer Aufnahme vorkommen: *Anthemis carpatica* 2: +, *Trisetum fuscum* 2: +, *Festuca rupicola* ssp. *saxatilis* 2: +, *Doronicum carpaticum* 2: +, *Arabis alpina* 2: +, *Dianthus superbus* 2: +, *Festuca versicolor* 3: +, *Cardaminiosus arenosa* 5: +, *Cerastium lanatum* 9: +, *Myosotis alpestris* 8: +, *Hedysarum hedysaroides* 8: +, *Cerastium arvense* 8: +, *Saxifraga cymosa* 10: +; *Drepanocladus uncinatus* 3: +;  
 Ort der Aufnahmen: 1 — Rebra 17.07.1977; 2 — Cormaia 8.09.1979; 3 — Puzdra 15.08.1982; 4, 5 — Anieșu Mare 17.07.1982; 6, 7 — Gărgău 19.07.1982; 8 — Vf. Clăilor 20.07.1982; 9 — Pictrosul Nare 3.08.1982; 10 — Inău 17.08.1982;

Hier besiedeln sie die Felsengürtel oder leicht geneigtes Gelände von nördlichen Schattenabhänge, wo der Schnee bis Sommeranfang verbleibt. Die Böden worauf diese vegetieren sind, im allgemeinen, oberflächliche Lithosole, mit schwach saurer oder neutraler Reaktion (pH = 6,2 — 6,7) und einem geringen Gehalt an organischen Stoffen. Die Charakterart der Assoziation ist *Salix kitaibeliana* mit einer Durchschnittsbedeckung von 65%. Gegenüber anderen Phytozönosen der Südkarpaten mit *Salix retusa*, die auf die Assoziation *Anemono-Salicetum retusae* Horv. 1953 [3], [23] bezogen wurden, nehmen diejenigen des Rodna-Gebirges eine Sonderstellung ein, da in ihrem floristischen Aufbau karpatisch-endemische Arten wie: *Silene nivalis*, *Poa granitica* ssp. *disparilis* und *Soldanella hungarica* ssp. *hungarica* vorkommen. Das reichliche Vorhandensein von *Soldanella hungarica* in diesen Phytozönosen einerseits, und das Fehlen von *Anemone narcissiflora* andererseits, veranlaßten uns, sie als eine neue Assoziation zu beschreiben. Übrigens bleibt die Assoziation *Anemono-Salicetum retusae* Horv. 1953 ap. Boșc. 1971, bzw. *Anemono-Salicetum retusae* Horv. 1949 ap. Schneider-Binder und Voik 1979, zweifelhaft, da in einer kürzlich erschienenen Synthese selbst Horvat und Mitarbeiter [9] nichts von dieser Assoziation erwähnen. Die von uns beschriebenen Assoziation unterscheidet sich floristisch durch diese Differentialarten sowohl von den aus Zentraleuropa unter den Benennungen *Salicetum retusae-reticulatae* [4], [5], [10], [11], [13], [16] und *Saxifraga-Salicetum retusae* [25] beschriebenen Assoziationen, als auch von den aus Südeuropa bekannten Assoziationen *Soldanello alpinae-Salicetum retusae* [9] und *Carici nigrae-Salicetum retusae* [21]. Unter syngenetischem Aspekt bewerten wir die von uns beschriebene Assoziation als eine geographisch-ostkarpatische Variante dieser aus verschiedenen Regionen Europas bekannten Assoziationen. Das Vorhandensein charakteristischer Arten für *Androsacetalia alpinae* und *Seslerietalia* in der floristischen Zusammensetzung dieser Variante wird besonders auf den Kontakt mit einigen diesen Ordnungen einverleibten Assoziationen zurückgeführt.

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# RECHERCHES BRYOSPÉOLOGIQUES SUR CERTAINES GROTTES DE ROUMANIE

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The paper brings new data on the Bryophyta in the vestibular and prevestibular area of some caves in Romanian Carpathians. Bryospéologically were identified 62 taxa (12 sp. — *Hepaticae*; 50 sp. — *Musci*). Taxonomically, some adaptive morpho-anatomical modifications of some taxa to ecological and cavern conditions (substratum, light, humidity etc.) are insisted upon. The bioforms, as well as the ecological and geographical categories of analysed Bryophyta are indicated. From a bryocenological point of view two muscinal associations in the cave "Cetatea Rădesei", Bihor Mountains (Occidental Carpathians): *Mnio (thomsonii)* — *Orthothecium intricati* ass. nova and *Cratoneuro (filicini)* — *Chiloscyphum pallescentis* ass. nova are described. We ascribe these associations from a cenotaxonomic point of view in calcinal epilithic muscinal associations, established by Al. v. Hübschmann (1984).

Bien que sur le territoire de la Roumanie soit connu un large réseau de grottes de divers types signalées et étudiée par des spéologues consacrés et amateurs, pourtant les connaissances sur les Cryptogames (algues, champignons, lichènes et mousses), comme sur certains groupes de Cormophytes, sont encore très restreintes (Tr. I. Ștefureac, 1970) [14]. L'intensification de ces recherches aussi basées méthodiquement sur les conceptions introduites dans la spéologie universelle par le savant roumain E. G. Racoviță [11] vont compléter la gamme des études spéologiques en Roumanie.

Des études bryospéologiques sur certaines grottes d'Europe sont mieux connues et même dans quelques pays voisins, à savoir : la Tchécoslovaquie : Á. Boros — 1964 [4]; J. Šmarda — 1967 [12]; la Pologne : A. Żmuda — 1954 [18]; la Hongrie : Á. Boros — 1964 [4], L. Vajda — 1966 [16]; la France : G. de Lamerdière et J. Maheu — 1901 — 1948 [8]; l'Autriche : L. Lammermayr — 1911 — 1915 [9]; l'Italie : U. Tosco — 1957 — 1958 [15] et autres. En Roumanie, les premières données bryospéologiques sont mentionnées dans les grottes des Monts Bihor (M. Péterfi — 1908 [10], du bassin des vallées Sebeș-Criș (Á. Boros — 1942) [3] et Bihor) [2], du massif Piatra Craiului (Tr. I. Ștefureac — 1951) [13] de même qu'avec des données plus nombreuses de certaines grottes de Dobroudja, d'Olténie et de Transilvanie (Tr. I. Ștefureac — 1970) [14] avec l'indication en même temps de la bibliographie sur encore d'autres groupes de Cryptogames (algues, bactéries).

Dans la présente étude nous enrichissons de nouvelles données la connaissance de la flore bryologique de quelques autres grottes de Roumanie, à savoir : la grotte Cetatea Rădesei du bassin supérieur du Someș Cald (entrée, gorges, sortie) des Monts Bihor (leg. Tr. I. Ștefureac —



1971); la grotte de Vadu Crișului des Monts Pădurea Craiului (versant gauche du Défilé Crișul Repede) (leg. M. Neacșu — 1968, Tr. I. Ștefureac — 1985) des roches, du sol et des pierres sous l'eau; la grotte Gaura de Capu-Baciului (Sudol III) des Monts du Banat, le bassin Riul Mare, la Vallée Baciului, affluent de Birzava, versant droit du Cleanțu au sud de Reșița, et les grottes Lizlonea et Văleaga de la Vallée du Caras, localité Carașova (leg. A. et Șt. Negrea — 1970). Tout le matériel a été identifié par nous (1972).

A mentionner, les Bryophytes de ces grottes ont été, en général, recoltées dans la zone liminaire et sous-liminaire des grottes étudiées de leur côté prévestibulaire et certaines de celui vestibulaire.

L'analyse du matériel bryologique, de la collection d'environ 250 échantillons, a réussi à identifier 62 taxons (56 espèces et 6 infrataxons), à savoir 12 espèces *Hepaticae* (3-euthalliques; 9-cormoïdes) et 44 espèces de *Musci* (à 6 infrataxons) avec des formes acrocarpes et pleurocarpes. La totalité des espèces de Bryophytes, arrangées systématiquement, est notée avec la nomenclature actualisée, dans le tableau no. 1, représentant l'énumération de Bryophytes des grottes étudiées.

Dans le tableau est marquée (avec +) la présence des espèces de divers endroits des grottes et des variés substratum (roches-parois, sol et pierres sous l'eau) sur lesquels elles se développent. On remarque en même temps une différenciation des espèces dans les grottes étudiées, par rapport aux conditions écologiques, au degré de luminosité, à l'exposition des parois, à la nature géologique, à la position territoriale-géographique, y compris les formations de végétation de leur proximité.

Ainsi, les espèces signalées dans les trois grottes (principales) sont peu nombreuses: *Pedinophyllum interruptum*, *Thamnobryum alopecurum*; mais les espèces qui proviennent des deux premières grottes (Cetatea Rădesei et Vadu Crișului) sont nombreuses: *Pellia endiviifolia*, *Chiloscyphus pallescens*, *Pedinophyllum interruptum*, *Plagiomnium elatum*, *P. affine*, *Thamnobryum alopecurum*, *Amblystegium subtile*, *Eurhynchium hians*, *Rhynchostegium confertum*, *Ctenidium molluscum* et autres, mais leur écrasante majorité appartient, séparément, chacune d'elles, à une seule grotte (tableau 1).

Sous l'aspect taxonomique, un fait significatif est à remarquer: chez certains taxons des modifications morpho-anatomiques adaptatives à la vie ± cavernicole déterminées écologiquement et physiologiquement, à la différence de leurs espèces typiques. Bien souvent on rencontre des difficultés dans la délimitation des taxons à cause de leur polymorphisme accentué, de la variabilité qui intéresse leur analyse et révèle les conditions de vie où elles se développent. Ces modifications ainsi résultées ne constituent pas des infrataxons. Une partie du matériel bryologique comme celui appartenant aux genres *Cephaloziella*, *Barbula*, *Bryum* etc. profondément modifiés et à une faible présence (— c), n'ont pu être identifiés et situés parmi certains taxons spécifiques.

Ces formations nouvelles adaptatives de l'appareil végétatif (gametophyte) chez les Bryophytes des grottes peuvent être considérées comme résultées tant chez les formes aquatiques, que chez celles aérophyles,

Tableau 1

Bryophytes des grottes des Carpates de Roumanie

| BRYOPHYTA                                                                        | I |   |   | II |   |   | III |
|----------------------------------------------------------------------------------|---|---|---|----|---|---|-----|
|                                                                                  | a | b | c | d  | e | f | g   |
| 1                                                                                | 2 | 3 | 4 | 5  | 6 | 7 | 8   |
| <i>Hepaticopsida</i> (Hepaticae)                                                 |   |   |   |    |   |   |     |
| <i>Conocephalum conicum</i> (L.) Lindb.                                          | + | — | — | +  | + | — | —   |
| <i>Pellia endiviifolia</i> (Dick.) Dum.                                          | + | — | — | +  | + | — | —   |
| <i>Pellia neesiana</i> (Gott.) Impr.                                             | — | + | — | —  | + | — | —   |
| <i>Chiloscyphus pallescens</i> (Schimp. ex Hoffm.) Dum.                          | — | — | + | —  | — | — | —   |
| <i>Lophozia ventricosa</i> (Dick.) Dum.                                          | + | — | — | —  | — | — | —   |
| <i>Lophozia collaris</i> (Nees in Mart.) Dum.                                    | — | + | + | —  | — | — | —   |
| <i>Lophozia turbinata</i> (Raddi) Steph.                                         | — | + | + | —  | — | — | —   |
| <i>Lophozia bantriensis</i> (Hook.) Steph.                                       | + | + | + | —  | — | — | —   |
| <i>Jungermannia atrovirens</i> Dum.                                              | + | — | — | +  | + | — | +   |
| <i>Pedinophyllum interruptum</i> (Nees) Kaal.                                    | + | — | — | —  | — | — | —   |
| <i>Cephalozia lunulifolia</i> (Dum.) Dum.                                        | + | — | — | —  | — | — | —   |
| <i>Cololejeunea rossettiana</i> (Mass.) Schiffn.                                 | — | — | — | +  | — | — | —   |
| <i>Bryopsida</i> (Musci)                                                         |   |   |   |    |   |   |     |
| <i>Pogonatum urnigerum</i> (Hedw.) P. Beauv.                                     | + | — | — | —  | — | — | —   |
| <i>Dichodontium flavescens</i> (With.) Lindb.                                    | — | — | — | +  | — | — | —   |
| <i>Seligeria trifaria</i> (Brid.) Lindb.                                         | — | + | — | —  | — | — | —   |
| <i>Distichium capillaceum</i> (Hedw.) B., S. & G.                                | + | — | — | —  | — | — | +   |
| <i>Fissidens cristatus</i> Wils. ex. Mitt.                                       | — | — | — | —  | + | — | —   |
| <i>Fissidens pusillus</i> (Wils.) Milde                                          | — | — | — | —  | — | — | —   |
| <i>Euccladum verticillatum</i> (Brid.) B., S. & G.                               | — | — | — | +  | — | — | —   |
| <i>Gymnostomum aeruginosum</i> Sm.                                               | + | + | — | —  | — | — | —   |
| <i>Gymnostomum calcareum</i> Nees & Hornsch.                                     | + | + | + | —  | — | — | —   |
| <i>Tortula muratis</i> (Hedw.) Gaertn.                                           | — | — | — | +  | — | — | —   |
| <i>Mniobryum wahlenbergii</i> (Web. & Mohr) Andr.                                | + | + | + | —  | — | — | —   |
| <i>Mnium stellare</i> Hedw.                                                      | — | — | — | +  | + | — | —   |
| <i>Mnium thomsonii</i> Schimp.                                                   | + | + | — | —  | — | — | —   |
| <i>Plagiomnium elatum</i> (B. & S.) T. Kop.                                      | — | — | + | +  | — | — | —   |
| <i>Plagiomnium affine</i> (Bland.) T. Kop.                                       | — | — | — | +  | — | — | —   |
| <i>Plagiomnium cuspidatum</i> (Hedw.) T. Kop.                                    | — | — | — | +  | — | — | —   |
| <i>Rhizomnium punctatum</i> (Hedw.) T. Kop.                                      | + | — | — | —  | — | — | —   |
| <i>Timmia bavarica</i> Hessel.                                                   | + | — | — | —  | — | — | —   |
| <i>Neckera pennata</i> Hedw.                                                     | — | — | — | +  | — | — | —   |
| <i>Neckera webbiana</i> (Mont.) Düll                                             | + | — | — | —  | — | — | —   |
| <i>Neckera pennata</i> Hedw. (cavernicola)                                       | + | — | — | —  | — | — | —   |
| <i>Thamnobryum alopecurum</i> (Hedw.) Nieuwl.                                    | — | — | — | —  | — | — | —   |
| <i>Thamnobryum alopecurum</i> (Hedw.) Nieuwl. f. <i>protensum</i> (Turn.) Moenk. | — | — | — | —  | — | + | +   |
| <i>Leskea nervosa</i> (Brid.) Nyh.                                               | — | — | — | +  | — | — | —   |
| <i>Anomodon rugelii</i> (C. Müll.) Kreissler                                     | — | — | — | +  | — | — | —   |
| <i>Anomodon attenuatus</i> (Hedw.) Hüb.                                          | — | — | — | +  | — | — | —   |
| <i>Anomodon viticulosus</i> (Hedw.) Hook. & Tayl.                                | — | — | — | +  | — | — | —   |
| <i>Campylium stellatum</i> (Hedw.) J. Lange & C. Jens.                           | — | + | + | —  | — | — | —   |
| <i>Campylium halleri</i> (Hedw.) Lindb.                                          | — | — | — | +  | — | — | —   |
| <i>Cratoneuron commutatum</i> (Hedw.) G. Roth v. <i>irrigatum</i> (Zett.)        | — | + | — | —  | — | — | —   |
| <i>Brothia fluctuans</i> (B., S. & G.) Moenk.                                    | + | + | + | —  | — | — | —   |
| <i>Cratoneuron filicinum</i> (Hedw.) Spruce v. <i>gracilescens</i> Schimp.       | + | + | + | —  | — | — | —   |
| <i>Amblystegium subtile</i> (Hedw.) B., S. & G.                                  | — | — | + | —  | — | — | —   |
| <i>Calliergon richardsonii</i> (Mitt.) Kindb. ( <i>acuminatum</i> )              | — | — | — | —  | — | — | —   |
| <i>Brachythecium rutabulum</i> (Hedw.) B., S. & G.                               | — | + | — | —  | — | — | —   |



(Suite Tableau 1)

| 1                                                                                          | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--------------------------------------------------------------------------------------------|---|---|---|---|---|---|---|
| <i>Eurhynchium hians</i> (Hedw.) San de Lac.<br>incl. v. <i>atrovirens</i> (Swartz) Moenk. |   |   |   |   |   |   |   |
| <i>Eurhynchium pumilum</i> (Wils.) Schimp.                                                 | + | + | - | - | + | - | - |
| <i>Rhynchostegium confertum</i> (Dicks.) B.S. & G.                                         | + | - | - | - | - | - | + |
| <i>Rhynchostegium riparioides</i> (Hedw.) Card.                                            | + | - | - | - | + | - | - |
| <i>Rhynchostegiella tenella</i> (Dicks.) Limpr.                                            | - | - | - | + | - | + | - |
| <i>Rhynchostegiella curviseta</i> (Brid.) Limpr.                                           | - | - | - | - | - | - | * |
| <i>Cirriphyllum crassinervium</i> (Tayl.) Loeske & Fleisch.                                | - | - | - | + | - | - | - |
| <i>Orthothecium intricatum</i> (Hartm.) B.S. & G.                                          | + | - | - | - | + | - | - |
| <i>Orthothecium rufescens</i> (Brid.) B.S. & G.                                            | - | - | + | - | - | - | - |
| <i>Ctenidium molluscum</i> (Hedw.) Mitt.                                                   | - | - | + | + | - | - | - |

I = Cetatea Rădesei : a — entrée, b — gorges, c — sortie

II = Vadu Crișului : d — roches, e — sol, f — pierres sous l'eau

III = Gaura-Capu Baciului : sol et roches

\* = *Lizlonea* et *Vălcaga*

dans des conditions de  $\pm$  aérobentoniques. A l'entrée des grottes, on ne distingue pas d'espèces de Bryophytes troglobie, non plus trogrophiles proprement dites, mais seulement subtroglaphiles<sup>1</sup>. Nous interprétons toutes ces modifications dans le sens de J. Amann [1] comme des biomorphoses variées, surtout chez les Mousses subtroglaphiles, comme photomorphoses, sciomorphoses etc. par rapport aux facteurs écologiques et spécifiques déterminants.

Parmi ces modifications, nous remarquons pourtant dans le matériel analysé de la grotte Cetatea Rădesei les suivantes : *Neckera-pennata* Hedw. (*cavarnicola*), ayant les feuilles disposées imbriquées sur les tiges et celles des rameaux sont souvent arquées ; *Calliergon richardsonii* (Mitt.) Kindb. (*acuminatum*), correspond, selon la forme des feuilles, la nervure et les oreillettes distincte de l'espèce type, mais se distinguant par les feuilles terminal-acuminées et non arrondies, caractère non mentionné.

En dehors de ces deux cas on remarque chez d'autres espèces de la même grotte les modifications adaptatives suivantes : *Mnium thomsonii* Schimp. avec la disposition très rare des feuilles, celles-ci de taille très réduite, semblable à la variété *lycopodioides* (Hook.) Husnot (M. lycopodioides (Schwaegr.) Podp.); *Mniobryum wahlenbergii* (Web. & Mohr) Andr., ayant souvent la couleur des feuilles bronzé à la base, comme chez *Mnium thomsonii* Schimp. (M. orthorrhynchum auct.), avec lesquelles elle croît dans les mêmes bryocénoses, difficile à différencier macroscopiquement ; *Lophozia turbinata* (Raddi) Steph. avec des tiges filamenteuses, allongées, rarement foliées à 2 (rarement 3) lobes et des cellules de la lamine grandes, croissant en bryocénoses avec *Lophozia ventricosa* (Dicks.) Dum.

<sup>1</sup> Pour certaines informations et discussions de spécialité, nous exprimons nos vifs remerciements aux spéologues Dr. A. et Dr. Șt. Negrea, Dr. C. Tăbăcaru et au pédologue Dr. M. Neacșu.

Dans la grotte de Vadu Crișului (Bihor), on remarque, parmi des modifications qui proviennent fréquemment dans le matériel analysé, ce qui suit : *Thamnobryum alopecurum* (Hedw.) Nieuwl., avec les feuilles variées comme taille, longueur et largeur, le degré varié de denticulé du sommet des feuilles, par rapport à l'espèce type, diffère aussi selon le système de ramification des tiges, les cellules de la lamine plus courtes ou plus longues, en général pauvres en chloroplastes ; compte tenu de la majorité des caractères différentiels, ce matériel marque certaines transitions vers *Thamnobryum angustifolium* (Holt) Crundw., ainsi que certaines différences, par rapport à *Thamnobryum alopecurum* (Hedw.) Nieuwl. f. *protensum* Moenk. Chez *Pedinophyllum interruptum* (Nees) Kaal., on n'a pas remarqué des amphigastres et l'euthalle chez *Conocephalum conicum* (L.) Lindb. est beaucoup plus mince et incrusté de calcaire, caractère remarqué aussi chez quelques Mousses (*Cratoneuron*, *Gymnostomum* etc), de même que chez certaines algues.

Dans la grotte Gaura-Capu Baciului on remarque, d'après l'analyse du matériel chez *Pedinophyllum interruptum* (Nees) Kaal. de grandes ressemblances avec *P. stableri* (Pears.). K. M. (I<sup>e</sup> édit., p. 780) et des ressemblances (K. M., III<sup>e</sup> édit., p. 899) comme synonyme au *Plagiochila tridenticulata* (Hook.) Dum. (= *P. corniculata* (Dum.) Dum.). Dans cette grotte est remarquée une modification aussi chez *Thamnobryum alopecurum* (Hedw.) Nieuwl. cf. f. *protensum* Moenk., avec les feuilles apparemment pennées, disposées pareillement à *Neckera* (*neckerodeum*).

Comme bioformes, les espèces de Bryophytes appartenant à la Cl. *Musci*, de ces grottes, s'intègrent notamment, comme types de croissance dans la catégorie Brr — *Bryochamaephyta reptantia* (21 espèces), suivies de Brchc- *Bryochamaephyta caespitosa* (14 espèces), Brch- *Bryochamaephyta* (5 espèces) et Brchp- *Bryochamaephyta pulvinata* (2 espèces) ; toutes les espèces de la Cl. *Hepaticae* (12 espèces) appartiennent seulement à la catégorie Brr, *Bryochamaephyta*.

Du point de vue écologique l'écrasante majorité des espèces des Hépatiques, aussi que certaines des Mousses sont adaptées à des conditions de luminosité réduite, température basse, humidité grande (souvent excessive), avec une relative constance annuelle, courants d'air, émanations radioactives, substances chimiques et autres, du substratum organique et minéral et autre représentent des formes avec une prépondérance mésophylles, sciaphylles saxi-calciophiles, certaines terri-humicoles ; dans un pourcentage notable il y a des formes hygro- et hydrophylles, rarement et surtout dans les endroits prévestibulaires il y a des méso-xérophiles, qui proviennent souvent des formations de végétation environnantes.

Il est à remarquer le fait que, parmi les espèces de Bryophytes seulement très peu d'entre elles sont en partie  $\pm$  cavernicoles ; leur majorité est constituée d'espèces qui se développent dans la végétation forestière et autres, à proximité des entrées des grottes et pénètrent souvent quantitativement, dans les endroits prévestibulaires et vestibulaires, par rapport à l'orientation des ouvertures des grottes, à leur exposition et à leur degré d'éclairage.

Sous l'aspect corologique, la répartition des espèces de Bryophytes des grottes est assurée par les courants aériens, par l'eau qui



ruisselle sur les parois des roches, comme par celle des cours souterrains et autres, y compris par certains non vertébrés et quelques vertébrés cavernicoles. Cette répartition se réalise plus rarement par la formation de spores, mais surtout par des propagules. La formation des organes de reproduction sexuée (gamétocystes) est rarement rencontrée, de même que la formation de sporogones (capsules à spores). Ainsi, *Thamnobryum alopecurum* (Vadu Crișului), avait seulement de nombreux pédoncules sans capsules (ils se forment parfois en hiver), et chez *Seligeria tristicha* (Cetatea Rădesei), comme chez *Rhynchostegiella tenella* (Lizlonea), on a identifié des pédoncules et capsules mûres. On a pu observer des propagules chez *Cololejeunea rossettiana* (en forme de disques pluricellulaires) et chez *Dichodontium flavescens* (propagules filiformes), les deux de la grotte Vadu Crișului; la deuxième espèce, avec une grande variabilité, est considérée par certains auteurs, seulement comme une variété (var. *flavescens* (Dicks) Moenk.) de *Dichodontium pellucidum* (Hedw.) Schimp.

Du point de vue bryogéographique, la majorité des espèces de Bryophytes (Hépatiques et Mousses), analysées de ces grottes appartiennent à la catégorie des éléments phytogéographiques circumpolaires, fréquemment montagneux, parfois à caractère disjoint, tels : *Gymnostomum aeruginosum*, *Tortula muralis*, *Cratoneuron filicinum*, *Rhynchostegium riparioides*, *Calliergon richardsonii*, *Brachythecium rutabulum*; rarement circumpolaire alpin boréal (*Orthothecium intricatum*) ou circumpolaire à caractère nordique (*Pellia neesiana*).

Mais il est à remarquer qu'outre les éléments eurasiatiques et centr-européen-, on constate de manière pregnante l'infiltration des éléments à caractère sud-européen et occidental (méditerranéen, sous-méditerranéen, atlantique, sous-atlantique) souvent bien représentés, auxquels appartiennent 4 espèces parmi les Hépatiques : *Cololejeunea rossettiana*, *Pedinophyllum interruptum*, *Jungermannia atrovirens*, *Lophozia turbinata* et entre les Mousses 12 espèces : *Rhynchostegiella tenella*, *R. curviseta*, *Eurhynchium pumilum*, *Fissidens cristatus*, *F. pusillus*, *Neckera pennata*, *N. webbiana*, *Gymnostomum calcareum*, *Pogonatum urnigerum*, *Thamnobryum alopecurum*, *Eucladium verticillatum*, *Rhynchostegium confertum* etc.; à relever notamment les espèces du genre *Rhynchostegiella* : *R. tenella* (Lozlonea et Văleaga) et *R. curviseta* (Vadu Crișului).

Du point de vue bryocénologique deux associations muscinales ont été analysées, notées et décrites dans la grotte Cetatea Rădesei, à savoir :

Ass. *Mnio(thomsonii)-Orthothecietum intricati* ass. nova, notée dans deux relevés à l'entrée dans la grotte (l'ouverture), sur des parois rocheuses, 0.5 m<sup>2</sup>.

|                                | I     | II   |                                | I    | II   |
|--------------------------------|-------|------|--------------------------------|------|------|
| <i>Mnium thomsonii</i>         | 3-4.3 | 3.3  | <i>Gymnostomum aeruginosum</i> | + .2 | + .1 |
| <i>Orthothecium intricatum</i> | 2-3.4 | 2.2  | <i>Gymnostomum calcareum</i>   | + .2 | + .1 |
| <i>Distichium capillareum</i>  | 1-2.4 | + .2 | <i>Mniobryum wahlenbergii</i>  | + .2 | +    |
| <i>Amblystegium subtile</i>    | 1-2.3 | +    | <i>Conocephalum conicum</i>    | +    | +    |
|                                |       |      | <i>Timma bavarica</i>          | +    | +    |

|                                      |       |      |                               |   |   |
|--------------------------------------|-------|------|-------------------------------|---|---|
| <i>Pedinophyllum interruptum</i>     | 1-2.2 | 1.2  | <i>Eurhynchium hians</i>      | + | + |
| <i>Cratoneuron filicinum</i>         | 1-2.2 | +    | var. <i>atrovirens</i>        | + | + |
| <i>Jungermannia atrovirens</i>       | 1.2   | + .1 | <i>Lophozia collaris</i>      | + | + |
| <i>Neckera pennata</i> (cavernicola) | 1.2   | +    | <i>Cephalozia lunulifolia</i> | + | + |
| <i>Rhynchostegium confertum</i>      | + .2  | +    | <i>Pogonatum urnigerum</i>    | + | + |

L'association a, en partie, certaines ressemblances avec l'ass. *Pedinophyllum interrupti* Herzog & Höfler 1944 [6], mais s'en distinguant nettement par la présence des deux espèces caractéristiques dominantes (*Mnium thomsonii* et *Orthothecium intricatum*), comme par une série d'espèces qui les accompagnent (Hépatiques et Mousses), sur la base desquelles on peut l'insérer dans l'Al. *Seligerio-Fissidentio pusilli* v. Hübschmann 1984 [7].

Ass. *Cratoneuro (filicini)-Chiloscyphetum pallescentis* ass. nova, sur la même paroi, à l'entrée de la grotte, endroit plus humide, faible luminosité, notée dans deux relevés, à 0.5 m<sup>2</sup> :

|                                                     | I     | II   |                                            | I    | II |
|-----------------------------------------------------|-------|------|--------------------------------------------|------|----|
| <i>Chiloscyphus pallescens</i>                      | 2.3   | 3.4  | <i>Eurhynchium hians</i>                   | + .1 | +  |
| <i>Cratoneuron filicinum</i>                        | 1-2.2 | 2.3  | var. <i>atrovirens</i>                     | + .1 | +  |
| <i>Lophozia ventricosa</i>                          | 1.2   | +    | <i>Lophozia bantriensis</i>                | +    | +  |
| <i>Lophozia turbinata</i>                           | 1.2   | +    | <i>Seligeria trifaria</i>                  | +    | +  |
| <i>Mnium thomsonii</i>                              | 1.2   | +    | <i>Brachythecium rutabulum</i>             | +    | +  |
| <i>Jungermannia atrovirens</i>                      | 1.2   | +    | <i>Ctenidium molluscum</i>                 | +    | +  |
| <i>Mniobryum wahlenbergii</i>                       | 1.2   | + .1 | <i>Calliergon richardsonii</i> (acuminata) | +    | +  |
| <i>Gymnostomum calcareum</i>                        | 1.2   | +    | <i>Rhizomnium punctatum</i>                | +    | +  |
| <i>Cratoneuron commutatum</i> var. <i>irrigatum</i> | + .2  | +    | <i>Plagiomnium affine</i>                  | +    | +  |
| <i>Orthothecium rufescens</i>                       | + 1   | +    | <i>Plagiomnium elatum</i>                  | +    | +  |
| <i>Campyllum stellatum</i>                          | +     | +    | <i>Anomodon viticulosus</i>                | +    | +  |

Cette association est caractérisée, outre la présence d'un grand nombre de Hépatiques, par quelques espèces des genres *Mnium*, *Plagiomnium*, ainsi que l'association (±) avec *Seligeria trifaria* (= *S. tristicha*), élément boréal montagneux ayant des ressemblances avec l'association pionnière *Seligeretum tristichae* Herzog & Höfler 1944(6) sans être associée directement à *Seligeria pusilla*, *Fissidens pusillus*. *Pedinophyllum interruptum* et autres; mais ce qui est significatif, c'est la présence de l'espèce *Jungermannia atrovirens* (= *J. tristis*) appartenant aussi à l'Al. *Seligerio-Fissidentio pusilli* v. Hübschmann 1984 [7].

Mais les deux associations muscinales ont un caractère différentiel adaptatif aux conditions ± cavernicoles se situant dans l'Ord. *Cnidetalia mollusci* Šmarda et Hadač 1944 de la Cl. *Tortulo-Homalothecieta sericei* Hertel 1974, du système bryocénotaxonomique des associations muscinales épilitiques calcicoles récemment établi (1984) pour l'Europe Centrale, par Al. v. Hübschmann [7].



En dehors de ces deux associations muscinales nous pouvons encore noter, dans la grotte Cetatea Rădesei, la bryocénose avec *Jungermannia atrovirens*, en association avec *Pedinophyllum interruptum* et *Amblystegium subtile*, et dans la grotte de Vadu Crișului, à l'entrée, sur la roche, lieu à luminosité réduite, la bryocénose caractéristique de *Plagiomnium affine* en association avec *P. elatum*, *Neckera webbiana*, *Anomodon attenuatus*, *Cololejeunea rossettiana*, *Amblystegium subtile*, *Dichodontium flavescens*; sur le sol humide, à la base de la roche, s'est installée la bryocénose avec *Pedinophyllum interruptum*, *Mnium stellare*, *Eucladium verticillatum*, *Fissidens pusillus*, *Conocephalum conicum*; sur les roches ruisselantes d'eau, exposition nordique, se développe la bryocénose formée de : *Plagiomnium affine*, *P. elatum*, *Tortula muralis*, *Neckera webbiana*, *Cirriophyllum crassinervium*, *Rhynchostegium riparioides*; à l'entrée dans la grotte a été notée la bryocénose avec *Anomodon rugelii*, *Ctenidium moluscum*, *Plagiomnium cuspidatum*, *Leskea nervosa*, *Compylium halleri*; on remarque fréquemment la bryocénose avec *Pedinophyllum interruptum* et *Mnium stellare*.

Dans la grotte Gaura-Capu Baciului prend contour la bryocénose formée de : *Thamnobryum alopecurum* f. *protensum*, *Eurhynchium pumilum*, *Rhynchostegium confertum*, *Pedinophyllum interruptum* et autres; tant dans la grotte Lizlonea, que dans celle de Văleaga a été identifiée l'espèce d'intérêt bryogéographique *Rhynchostegiella tenella* (*R. algeriana*).

À l'identification des Bryophytes de diverses grottes on a fréquemment signalé, dans différentes bryocénoses, la présence de certains genres parmi les Cyanophycées : *Oscillatoria*, *Chroococcus* et autres, ainsi que parmi les Chlorophycées : *Ulothrix*, *Vaucheria* et autres.

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## ASPECTS OF VEGETATION ON THE BANKS OF THE DANUBE SFÎNTU GHEORGHE BRANCH (THE DANUBE DELTA)

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Among the 23 associations analysed on the banks of Sfintu Gheorghe branch, a number of 3 new associations for the Danube Delta : *Lepidietum latifolii*, *Rumicetum limosae* and *Salsolietum sodae* are mentioned.

The biomass determination of the main phytocenoses in the Danube Delta pointed out the fact that it varies a lot according to : number of component species, their density and the biotop on which it develops.

Therefore the highest quantity of dry biomass was recorded in *Scirpo-Phragmitetum* (4950.6 g/m<sup>2</sup>) and in the psamophyte associations of *Apera maritima* and *Secale silvestre* only of 48 g/m<sup>2</sup>, and 103.2 g/m<sup>2</sup> respectively.

The Danube Delta, unique in Europe for its beauty, is from an ecological point of view a biome, an ensemble of ecosystems in interdependence and functional interaction. Among the ecosystems of this young and complex territory the aquatic and paluster ones are prevailing and account for the characteristic features of the Danube Delta. The various origins and ages of these aquatic basins, the differentiated salinity as well as the degree of colmatation, supported the development and differentiation of a specific vegetation for each analysed biotop.

The terrestrial ecosystems on the delta banks, different in origin, age and structure, enjoy, consequently, specific biocenoses.

In order to point out the variety of paluster, psamophyte and halophyte cenotaxa, as well as the dynamics and evolution of vegetation according to the multitude of some factors (rainfalls, soil humidity, evapotranspiration, wind speed) the banks round Sfintu Gheorghe branch were investigated as follows : the village Sfintu Gheorghe, bank Ciotic, island Sacalin, bank Ivancea and canal Ivancea.

On the sands round village Sfintu Gheorghe close to the harbour, a representative phytocenosis of *Lepidietum latifolii* was identified. Florița Diaconescu (1978) (2) pointed out, for the first time, this association in Bahlui bassin, on bare fields with debris and rubbish, in the SE of town Jassy, near the barrier Tomești and at Tg. Frumos, where they live on heavy soils, with excess of humidity and not very salted.

The phytocenosis at Sfintu Gheorghe was noticed on a surface of 10 m<sup>2</sup> covered as much as 80% and 130 cm high. *Lepidietum latifolium* (AD = 4) vegetates together with : *Rumex crispus* (+), *Atriplex hastata* (+), *Poa annua* (+), *Chamomilla recutita* (+), *Chenopodium urbicum* (+), *Polygonum aviculare* (+), *Convolvulus arvensis* (+) and *Poa pra-*

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*tensis* (+). The association above has not been mentioned for the Danube Delta so far.

Both at Sfintu Gheorghe and on the Ciotic bank, the cenotaxon *Argusio-Petasitetum spuriae* Dihoru et Negrean 1976 (3) described at Sulina is in continuous expansion. At Sfintu Gheorghe, *Petasites spurius* forms compact phytocenosis on noncultivated fields, close to houses, vegetating together with a series of nitrophyle species such as *Linaria vulgaris*, *Tanacetum vulgare*, *Artemisia vulgaris*, *Rumex dentatus* ssp. *halácsyi*, etc. At Ciotic (Fig. 1) it interposes between phytocenoses of



Fig. 1. — Phytocenoses dominated by *Petasites spurius* on the sands at the Ciotic.

*Carici (distantis) — Festucetum arundinaceae* occupying the lower spaces, the microdepressions with higher humidity and psamophyle vegetation represented by *Aperetum maritimae* and *Secaletum silvestre*.

At the end of summer *Petasites spurius* becomes more evident on the sands surrounding the fishery, as the meadows populated with *Apera maritima* are grazed and those populated with *Secale silvestre* are destroyed being mostly trampled by animals. The absence of the second diagnosis species of association *Argusia sibirica* from the investigated phytocenoses in Ciotic and Sfintu Gheorghe allows us to identify a new sub-association — *petasitetosum spuriae* — with its characteristic species: *Petasites spurius*, *Gypsophila perfoliata*, *Apera maritima*, *Plantago arenaria*, *Secale silvestre*. The phytocenoses of *Petasites spurius* at Ciotic represent a psamophyle variant of this association.

On the marine banks of the Sacalin island, the compact meadows of *Acorellus pannonicus* noticed by us in 1975 (5) which vegetated on the deposits of the Sfintu Gheorghe branch waters are at present much diminished. Instead, the phytocenoses made of *Rumex limosus* developed very well. *Rumicetum limosae* was described for the first time by St. Grigore (4) in Timiș flood plain at Uliuc, on hydromorphic or slightly hydromorphic soils with increased humidity over the whole years.

The phytocenoses on Sacalin island (Fig. 2) have few species identical to those described in Banat; so, excepting the significant species *Rumex limosus*, the following can be mentioned: *Ranunculus sceleratus* and *Bidens tripartita*. The structure of phytocenoses on Sacalin, based on five surveys, identified on a surface of 200 m<sup>2</sup>, is the following: average vegetation layer 60–70%, height of vegetation 75–80 cm. *Rumex limosus* (AD = 4), *Acorellus pannonicus* (+–1), *Rumex maritimus* (+–1), *Chenopodium glaucum* (+), *Aster tripolium* (+), *Salicornia europaea* (+), *Chenopodium botrys* (+), *Puccinellia limosa* (+), *Bassia hirsuta* (+), *Bidens tripartita* (+), *Spergularia media* (+), *Tamarix ramosissima* (+), *Polygonum maritimum* (+), *Eleocharis parvula* (+), *Ranunculus sceleratus* (+), *Oenanthe aquatica* (+), *Polypogon monspeliense* (+). The abundance of halophyle species in these phytocenoses is to be noticed; that can be accounted for by the high content in mineral salts of the sands due to marine waters. The association has never been mentioned so far in the Danube Delta.

The other halophyle associations noticed in 1975 *Bassietum sedoidis*, *Juncetum maritimi*, *Suaedetum maritimae* and *Salicornietum europaeae* were also replaced in most cases with phytocenoses of the association *Salsoletum sodae* covering the sandy slopes in close neighbourhood of the Black Sea only for 40–45%. *Salsoletum sodae* (Fig. 3) is as high as 30–35 cm and dominates most exclusively (AD = 3–4), only accidentally meeting rare individuals such as *Tamarix ramosissima* and *Elymus racemosus* ssp. *sabulosus*.

The halophyle meadows on bank Ivancea dominated in the past by *Puccinellia limosa* are at present represented mostly by *Cynodonetum dactyloni* and *Carici (distantis) — Festucetum arundinaceae*. Between them is inserted in lower places the association *Aeluropetum littoralis* (where *Aeluropus littoralis* presents AD = 4), accompanied by species such as *Salicornia europaea* (+), *Bolboschoenus maritimus* (+), *Suaeda maritima* (+), *Atriplex littoralis* (+) and *Spergularia media* (+).

The stamping down of the field and intense grazing favored the association *Atriplicetum tataricae* which has never been mentioned so far but at Chilia Veche (8). Within these phytocenoses prevalently dominated by *Atriplex tatarica* (AD = 5) one can also find: *Hordeum murinum*, (+–+1), *Lepidium latifolium* (+), *Cynodon dactylon* (+) and *Lotus tenuis* (+). The center of the recently drained microdepressions is occupied by *Salicornietum europaeae*, and the banks by *Spergularia media*. The banks of ponds delimiting the halophyle meadows, mostly covered by water over the year, accommodate phytocenoses of *Sparganietum ramosi* and *Glycerietum maximae* (Fig. 4). The dry fields are partly populated with *Agrostidetum stoloniferae* and *Aeluropetum littoralis*.

The central part of this pools is dominated by phytocenoses of *Oenanthe aquatica* and *Rorippa amphibia* (*Oenanthe-Rorippetum*), gradually replaced by *Scirpo-Phragmitetum medioeuropaeum* occupying wide areas. The associations *Typhetum angustifoliae* and *Schoenoplectetum lacustris* are inserted within *Oenanthe-Rorippetum* and *Scirpo-Phragmitetum medioeuropaeum*.



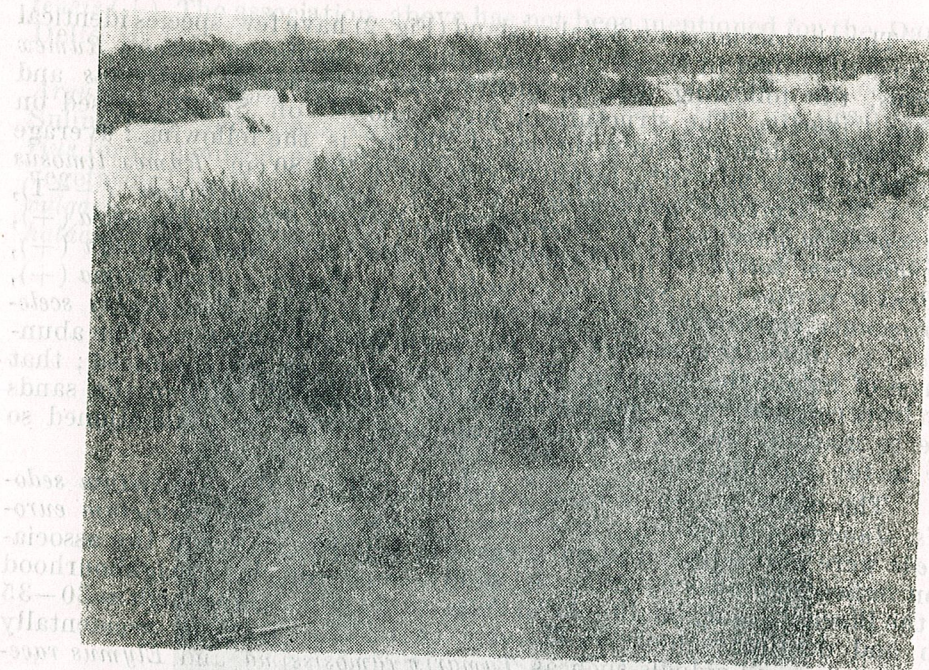


Fig. 2. — Aspect of hygrophyle vegetation dominated by *Rumex limosus* (Sacalin Island).

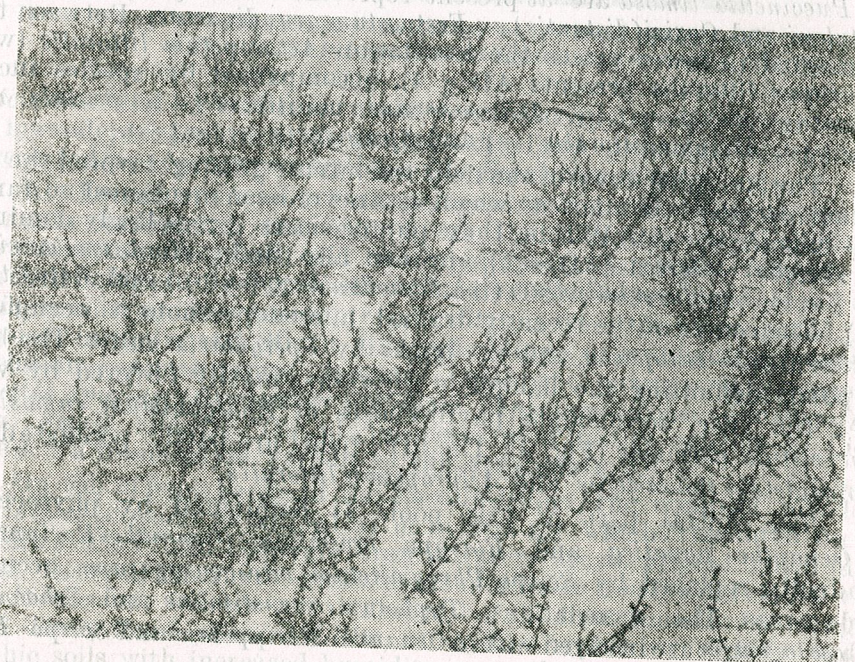


Fig. 3. — *Salsolietum sodae* monodominant on Sacalin island.

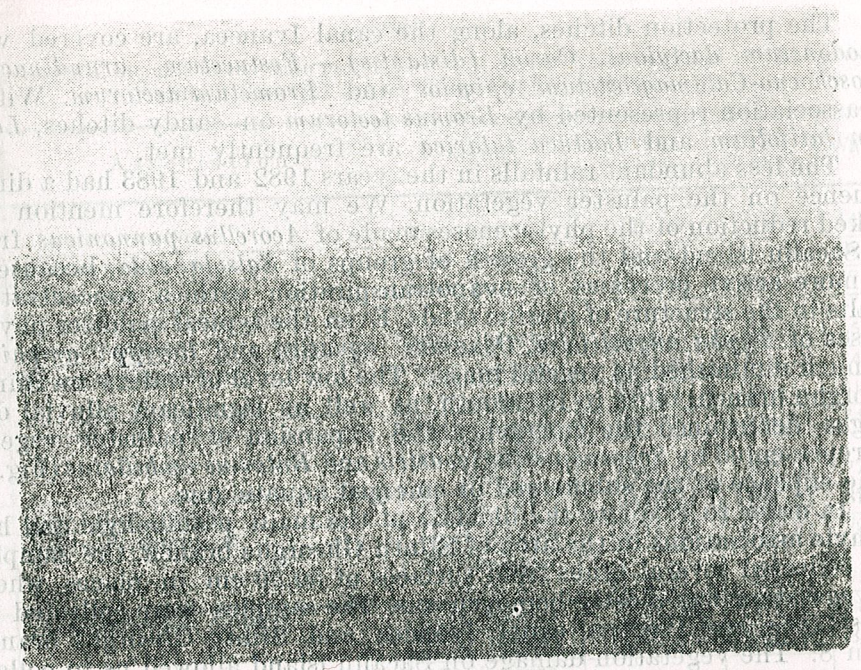


Fig. 4. — *Glyceria maxima* well developed on bank Ivancea.



Fig. 5. — Aspect of aquatic and paluster vegetation on Sacalin island, dominated by *Butomus umbellatus*.



The protection ditches, along the canal Ivancea, are covered with *Cynodonetum dactyloni*, *Carici (distantis) - Festucetum arundinaceae*, *Holoschoeno-Calamagrostetum epigeios* and *Brometum tectorum*. Within the association represented by *Bromus tectorum* on sandy ditches, *Lepidium latifolium* and *Lactuca tatarica* are frequently met.

The less abundant rainfalls in the years 1982 and 1983 had a direct influence on the paluster vegetation. We may therefore mention the marked reduction of the phytocenoses made of *Acorellus pannonicus* from the Sacalin island and the growth of groups of *Salsola soda*, because of the more active processes of evapotranspiration with a concentration of salts in the structure of marine sands. Even the typical paluster phytocenoses of *Typha angustifolia*, *Oenanthe aquatica* and *Rorippa amphibia* are much diminished as vegetal mass. The low level of waters on Sfintu Gheorghe branch, close to its mouth, as well as significant alluvial discharges determined the emergence and expansion of paluster vegetation represented by *Schoenoplectus lacustris* and *Butomus umbellatus* (Fig. 5) at the expence of the submerged or emerged aquatic one.

In order to evaluate the biomass of the main psamophyle and hygrophyle associations in the area of Sfintu Gheorghe branch, the samples were collected several times from surfaces of 50/50 cm in places where the vegetation was mostly homogeneous. The samples were collected on banks Ciotic and Ivancea, canal Ivancea and Sfintu Gheorghe branch at km 8. The vegetation damage on Sacalin island allowed us to collect biomass only in the case of *Rumicetum limosae*, the only association which resisted the repeated floodings in the last period.

The green and dry (80°C) biomass determination was also performed as well as the determination of the water content of the species in the structure of the analysed associations. Of the 11 associations which were the object of our study, two of them are psamophyle (*Aperetum maritimae* and *Secaletum silvestre*), three are halophyle or almost halophyle (*Aeluropetum littoralis*, *Carici(distantis) - Festucetum arundinaceae* and *Cynodonetum dactyloni*). From the group of the mesophyle associations *Agrostidetum stoloniferae* and *Rumicetum limosae* were studied, and from the paluster ones *Scirpo-Phragmitetum*, *Schoenoplectetum lacustris*, *Typhetum angustifoliae* and *Sparganietum ramosi*.

The lowest biomass values, both green and dry, were found in the psamophyle associations, made of graminea species with low density. Remarkable is also the very low biomass quantity in the association *Aperetum maritimae* of only 115.47 g/m<sup>2</sup> green mass and 48.1 g/m<sup>2</sup> dry mass. The water content of the species in this association is low, of about 44.15 %, the lowest water content in all the analysed associations. Almost equal to the values above are the phytocenoses of *Secale silvestre* developing on poor sands along the sea coast.

Higher biomass values were found in the halophyle associations. Therefore in *Aeluropetum littoralis* there was found 1218.29 g/m<sup>2</sup> green biomass and 490.79 g/m<sup>2</sup> dry biomass. Within associations growing on wet lands the water content in plants reaches as much as 77.29 %.

The small depressions with excessive water mainly in spring are covered almost 95 % by *Agrostidetum stoloniferae*, with a quantity of green biomass of 1396.78 g/m<sup>2</sup> and dry biomass 379.68 g/m<sup>2</sup>.

Table 1

Average values of biomass in the main psamophyle and hygrophyle association in the area of Sfintu Gheorghe branch of the Danube (g/m<sup>2</sup>).

| Number | Association                                        | Station           | Green biomass | Dry biomass | Water |
|--------|----------------------------------------------------|-------------------|---------------|-------------|-------|
| 1.     | <i>Aperetum maritimae</i>                          | bank Ciotic       | 115.47        | 48.10       | 44.15 |
| 2.     | <i>Secaletum silvestre</i>                         | bank Ciotic       | 271.98        | 103.21      | 55.86 |
| 3.     | <i>Aeluropetum littoralis</i>                      | bank Ivancea      | 1218.29       | 490.79      | 77.29 |
| 4.     | <i>Carici(distantis) - Festucetum arundinaceae</i> | bank Ivancea      | 1282.77       | 425.28      | 65.26 |
| 5.     | <i>Cynodonetum dactyloni</i>                       | bank Ivancea      | 1686.82       | 671.43      | 64.61 |
|        |                                                    | canal Ivancea     | 638.44        | 251.5       | 61.70 |
|        |                                                    | bank Ivancea      | 593.41        | 214.16      | 74.36 |
| 6.     | <i>Agrostidetum stoloniferae</i>                   | Sf. Gheorghe Km 8 | 1396.78       | 379.68      | 77.68 |
|        |                                                    | Sacalin           | 3851.24       | 1027.24     | 81.02 |
| 7.     | <i>Rumicetum limosae</i>                           | canal Ivancea     | 15574.56      | 4950.64     | 79.96 |
| 8.     | <i>Scirpo-Phragmitetum</i>                         | Sf. Gheorghe Km 8 | 8172.68       | 2107.51     | 75.92 |
|        |                                                    | bank Ivancea      | 5386.41       | 1518.32     | 80.07 |
| 9.     | <i>Schoenoplectetum lacustris</i>                  | canal Ivancea     | 11137.03      | 2743.68     | 83.83 |
|        |                                                    | Sf. Gheorghe Km 8 | 10262.88      | 1324.12     | 89.04 |
|        |                                                    | bank Ivancea      | 17540.28      | 3894.43     | 84.15 |
| 10.    | <i>Typhetum angustifoliae</i>                      | Sf. Gheorghe Km 8 | 16693.81      | 3142.62     | 81.56 |
|        |                                                    | bank Ivancea      | 6504.89       | 1404.32     | 84.78 |
| 11.    | <i>Sparganietum ramosi</i>                         | bank Ivancea      |               |             |       |

The highest values are found in paluster plants and especially in *Phragmites australis* up to 15574.56 g/m<sup>2</sup> green mass and 4950.64 g/m<sup>2</sup> dry mass. Significant biomass quantities are found in phytocenoses of *Typha angustifolia*, *Schoenoplectus lacustris* and *Sparganium ramosum* (Table 1).

The hygrophyle species growing in biotops with excessive humidity along the whole vegetation period, have a high water content of about 89.04 %.

The biomass accumulation within the same association indicates a stationary behaviour, well correlated with species density. Therefore, within the association *Cynodonetum dactyloni*, both the green biomass and the dry one of the phytocenoses on bank Ivancea are higher than the one on the ditch along canal Ivancea (Table 1). Differences of biomass are found within the association *Schoenoplectetum lacustris* collected in various biotops. Therefore the phytocenoses at Km 8 growing in water as deep as 30 cm, are vigorously developed, the green biomass being of 10262.88 g/m<sup>2</sup>, and the dry one of 1324.12 g/m<sup>2</sup>. The phytocenoses on bank Ivancea, growing on dry fields, have a green biomass of only 5386.41 g/m<sup>2</sup> and a dry one of 1518.32 g/m<sup>2</sup>.

The biomass values of the vegetal associations vary according to the phytocenoses structure and the specific stationary conditions in which they develop.



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Institute of Biological Sciences  
Bucharest, Splaiul Independenței 296STUDY OF MACROSPOROGENESIS  
IN *CITRULLUS LANATUS* (Thumb.) Mansf. 3nION ANGHEL, DOINA CÂRCIUMĂRESCU, TATIANA VASSU  
and ANCA SÂRBU

The comparative study of various diploid, triploid and tetraploid lines of *Citrullus lanatus* revealed the existence of a high correlation between the ploidy level and the morphoanatomical (macro- and microscopical), biochemical and physiological features.

Modification of the nucleus/cytoplasm ratio, by increasing the chromosome number, produced strong modification during cell division, fecundation and seed formation.

The cytogenetic studies showed that the low fertility of tetraploid lines and the complete sterility of the triploid ones are due to the chromosomal abnormalities occurring during the reductional cell division (micro- and meiosis) and morphophysiological, biochemical and genetic disturbances following fecundation and embryo formation.

The cytogenetic studies carried out in various tetraploid and triploid lines, belonging to various species (*Oryza sativa*, *Beta vulgaris*, *Hordeum vulgare*, *Secale cereale*, *Citrullus lanatus*, etc.) showed that the low fertility of tetraploid lines and the complete sterility of the triploid ones are due to the chromosomal abnormalities occurring during the reductional cell division (homologous chromosomes association under the form of multivalents — tetra- and trivalents — cells with univalents lagging chromosomes, asynchronic divisions, chromatic bridges, micronuclei, multipolar cells — triads, pentads —). Because of these abnormalities the chromosomal disjunction in anaphase and telophase develops irregularly; therefore after division and the resulted morphophysiological modifications, non-functional aneuploid gametes occur.

Without denying the possible correlation between the meiosis process and plants fertility, the investigations carried out between 1965 — 1985 showed that the low fertility of tetraploid plants (no more than 50% in comparison with the diploid ones) and total or almost total sterility of triploid lines are due in great part to abnormalities occurring during meiosis and morphophysiological, biochemical and genetic disturbances following fecundation and embryo formation; these are the reasons that we studied meiosis in various triploid hybrids of *Citrullus lanatus*.

## MATERIAL AND METHOD

The female flowers and fruit of triploid plants, collected in various stages of development, at various intervals after pollination, were fixed in Navasın fixator for 48 hours and preserved in 70% alcohol. The material

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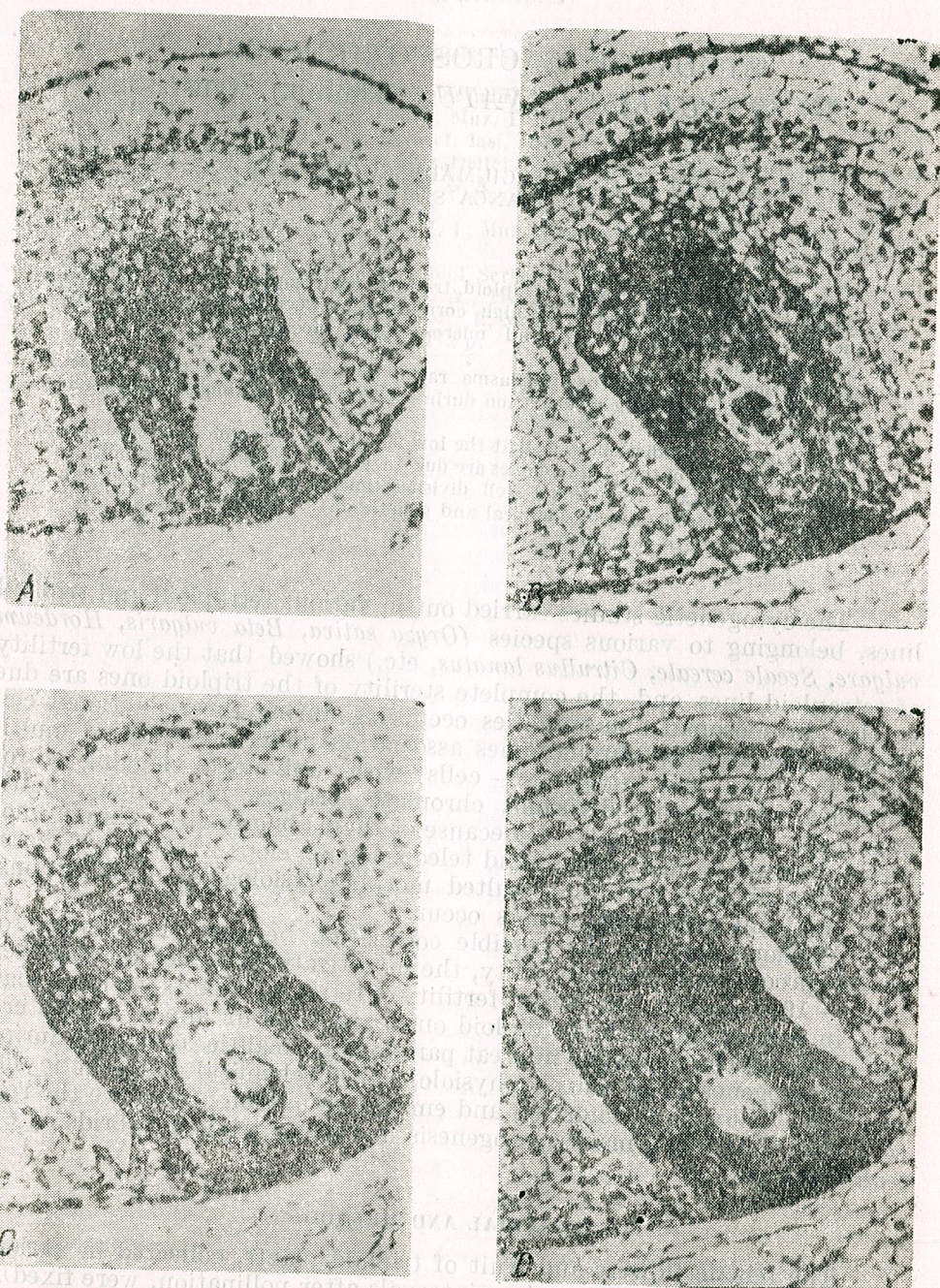


Plate 1 A, B. — Stages of the macrosporogenesis in *Citrullus lanatus* (Thumb) Mansf 3n; C, D. The beginning of the fecundation of the secondary nucleus of the embryo sack.

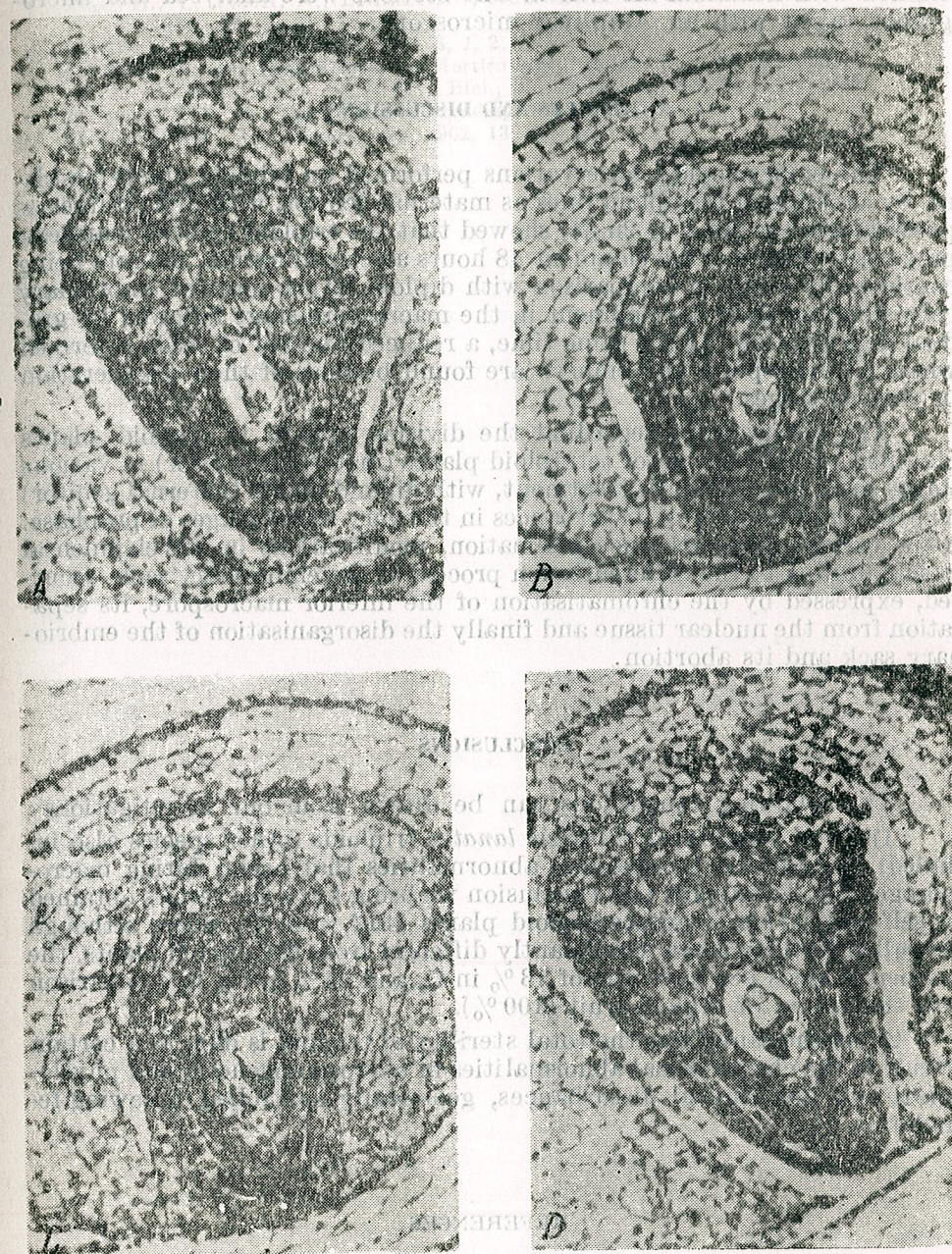


Plate 2. — Stages following the formation of the embryo sack.



was processed by means of paraffin fixation, sectioned with a microtom and stained with hematoxiline Erlich. The sections were analysed and micrographs taken with an Amplival microscope.

### RESULTS AND DISCUSSIONS

The embryological observations performed on triploid plants (resulted by crossing of tetraploid lines as maternal genitor, with diploid lines as paternal genitor ( $4n \text{♀} \times 2n \text{♂}$ )) showed that the triploid embryo manifests progressive stages of development 48 hours after pollination, the rate being considerably lower in comparison with diploid forms, most of embryonic cells stopping their development in the macrospore stage followed by gradual degeneration. At the same time, a reduced number of seeds is formed where no endosperm and embryo are found because of their degeneration in various stages.

The observations regarding the division process in triploid plants obtained by hybridation of tetraploid plants (maternal genitor) in various stages since the colchicine treatment, with diploid plants (paternal genitor) pointed out a number of disturbances in the very initial stages of prophase. After various intervals since pollination, modifications in developmental events leading to the differentiation process of the embryonic sack occurred, expressed by the chromatisation of the inferior macrospore, its separation from the nuclear tissue and finally the disorganisation of the embryonic sack and its abortion.

### CONCLUSIONS

The following conclusions can be drawn from our investigations:

1) Total sterility of *Citrullus lanatus* triploids (other species also included) are due to chromosomal abnormalities that occur during macrosporogenesis. To support this conclusion we bring forth the results obtained by self-pollination of autotetraploid plants ( $4n \text{♂} \times 4n \text{♀}$ ), where although the pollen fertility is not significantly different from the diploid plants, the number of seeds per fruit was of 13% in  $C_0$  and 34% in  $C_{20}$  in comparison with the values of diploid fruit (100%).

2) At the same time the total sterility of triploid is due, to a certain extent, to the chromosomal abnormalities in microsporogenesis and physiological and biochemical disturbances, genetically controlled, following fecundation.

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The paper presents the influence of the thermal factor on the protoplasmic streaming and the cellular viability in the root hairs and staminal filaments of wheat (*Triticum aestivum* L.).

It was found that protoplasmic streaming is not evident below 0°C. It starts in the root hairs at +6°C and in the staminal filaments at +8°C. By the gradual raising of the temperature the protoplasmic streaming stops in the root hairs at +37°C and in the staminal filaments at +34°C. The thermal factor influences the cellular viability depending on the dimensions of the root hairs and the duration of the treatment.

This research is concerned with the effect of higher and lower temperature on protoplasmic streaming within root and staminal filament cells of winter wheat (*Triticum aestivum* L., variety Bezostala 1). The experiments performed have proved that the reaction of protoplasmic movements to temperature, especially to higher temperature, depends on the age of the cells. The highest velocity of protoplasmic streaming was recorded in the mature root hairs, 300—600 μm long. These root hairs show a rotational type of streaming; about half of the highest value was recorded in the case of the smaller and younger root hairs, 50—150 μm long. These root hairs show a circulation type of streaming. The oldest root hairs, 2000—2500 μm long, showed a higher rate of rotational streaming at the very beginning but this suddenly ceased after the temperature had risen above +26°C. The lower temperature arrested the rotational streaming at +13°C and the shifting movements at +6°C. Within the hairs of staminal filaments there could not be seen or noticed any kind of protoplasmic movements or streaming at 0°C.

There are known several data about the effect of higher and lower temperature on protoplasmic streaming (for exhaustive information see (3)). These show that against the variation of the surrounding temperature, the average rate or velocity of protoplasmic movements or streaming increases and decreases according to the optimal principle (4).

The protoplasmic movements cannot be recorded at 0°C. The rise of the surrounding temperature causes the increase in the velocity of protoplasmic streaming which reaches the highest value at certain temperature known as the optimal temperature. Afterwards the velocity of streaming decreases again or else the protoplasmic movements may suddenly

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The following conclusions can be drawn from our investigations:  
 1) Total sterility of *Citrullus lanatus* triploid (other species also included) are due to chromosomal abnormalities that occur during macrosporangogenesis. To support this conclusion we bring forth the results obtained by self-pollination of autotetraploid plants (4n3 x 4n3) where although the pollen fertility is not significantly different from the diploid plants, the number of seeds per fruit was of 13% in C<sub>3</sub> and 34% in C<sub>2</sub>, in comparison with the average of seed per fruit of diploid fruit (100%).  
 2) At the same time the total sterility of triploid is due to a certain extent, to the chromosomal abnormalities in microsporangogenesis and physiological and biochemical disturbances, genetically controlled, following fecundation.

### CONCLUSIONS

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## THE EFFECT OF HIGHER AND LOWER TEMPERATURE ON PROTOPLASMIC STREAMING OF WHEAT (*TRITICUM AESTIVUM* L.)

ALISA DONOSE-PISICA and V. SORAN

The paper presents the influence of the thermic factor on the protoplasmic streaming and the cellular viability in the root hairs and staminal filaments of wheat (*Bezostaia* 1).  
 It was found that protoplasmic streaming is not evident below 0°C, it starts in the root hairs at + 6°C and in the staminal filaments at 8°C.  
 By the gradual raising of the temperature the protoplasmic streaming stops in the root hairs at 37°C and in the staminal filaments at 34°C.  
 The thermic factor influences the cellular viability depending on the dimensions of the root hairs and the duration of the treatment.

This research is concerned with the effect of higher and lower temperature on protoplasmic streaming within root and staminal filament cells of winter wheat (*Triticum aestivum* L., variety *Bezostaia* 1). The experiments performed have proved that the reaction of protoplasmic movements to temperature, especially to higher temperature, depends on the age of the cells. The highest velocity of protoplasmic streaming was recorded in the mature root hairs, 300—600 μm long. These root hairs show a rotational type of streaming; about half of the highest value was recorded in the case of the smaller and younger root hairs, 50—150 μm long. These root hairs show a circulation type of streaming. The oldest root hairs, 2000—2500 μm long, showed a higher rate of rotational streaming at the very beginning but this suddenly ceased after the temperature had risen above + 26°C. The lower temperature arrested the rotational streaming at + 12°C and the shifting movements at + 6°C. Within the hairs of staminal filaments there could not be seen or noticed any kind of protoplasmic movements or streaming at 0°C.

There are known several data about the effect of higher and lower temperature on protoplasmic streaming (for exhaustive information see: (2), (3)). These show that against the variation of the surrounding temperature, the average rate or velocity of protoplasmic movements or streaming increases and decreases according to the optimal principle (4).

The protoplasmic movements cannot be recorded at 0°C. The rise of the surrounding temperature causes the increase in the velocity of protoplasmic streaming which reaches the highest value at a certain temperature known as the optimal temperature. Afterwards the velocity of streaming decreases again or else the protoplasmic movements may suddenly



be arrested. The optimal temperature for the highest rate of protoplasmic streaming mainly depends on species and secondly on other factors. From among them, the effect of ageing is still very little known. One of the aims of our paper has been to reveal some aspects of this interesting phenomenon.

#### MATERIAL AND METHODS

In our experiments we have used the variety of winter wheat (*Triticum aestivum* L.) "Bezostoa 1".

There were gathered the hairs of staminal filaments from flowering plants grown at Lespezi and Trifesti (distr. Jassy, Roumania). The authors studied the root cap cells and root hairs of 3-days old wheat seedlings grown in our laboratory.

The organ scraps including the cells under investigation were put into a glass bucket on a microscopic slide and covered by a glass lid. The glass bucket was surrounded by a close circuit of water at a given temperature. The temperature of the water could be adjusted to the degree desired between  $-6^{\circ}\text{C}$  and  $+60^{\circ}\text{C}$ . By means of a copper-constant thermocouple there was measured the water temperature near the glass bucket and inside the glass bucket.

The plant material under investigation could be gradually chilled or warmed to any desired temperature between  $-6^{\circ}\text{C}$  and  $+60^{\circ}\text{C}$ . The cells studied were maintained at a given temperature about 10 minutes.

In keeping with S. Strugger's (5) method, there has been measured the velocity or rate of protoplasmic streamings over these 10 minutes. It has been rendered in  $\mu\text{m.s}^{-1}$ .

By using the method of vital staining, the vitality of the cells, has been proved. There were counted the stained cells with neutral red against unstained cells for a given temperature.

The results have been expressed in per cents of the living cells.

#### RESULTS AND DISCUSSIONS

The microscopic observations reveal that at  $20^{\circ}\text{C}$  temperature of control, the protoplasmic movements are present within various kinds of wheat cells. These movements can be properly measured within root hairs if the latter are longer than  $50\ \mu\text{m}$ . There could be noticed some oscillatory or shifting movements within the root cap cells and the youngest or very small root hairs,  $5-20\ \mu\text{m}$  long. With such small cells the velocity of protoplasmic movements has not been measured. We have only noticed the presence of a kind of protoplasmic movements.

The effect of the temperature rise on the rate of protoplasmic streaming has been studied only in case of the hairs between  $50$  and  $2500\ \mu\text{m}$  in length. The results are shown in figure 1.

Our results have proved that the wheat root hairs can be classified in three groups according to their resistance in protoplasmic streaming or behaviour against the temperature rise.

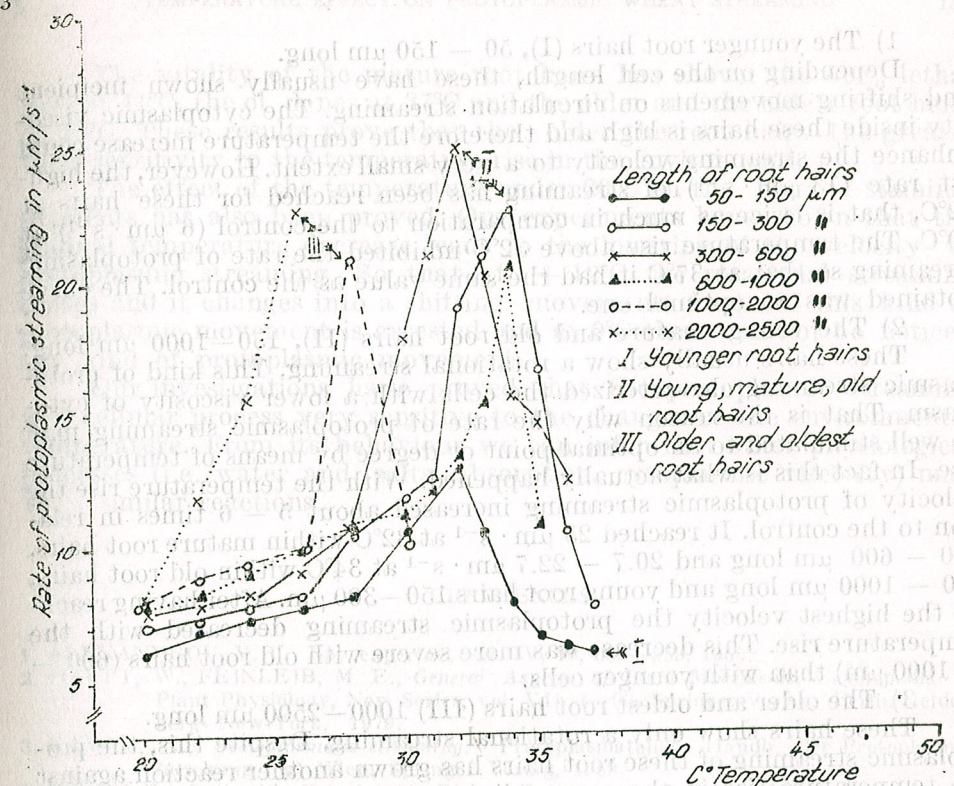


Fig. 1. — The effect of higher temperature on the rate of protoplasmic streaming within wheat root hairs

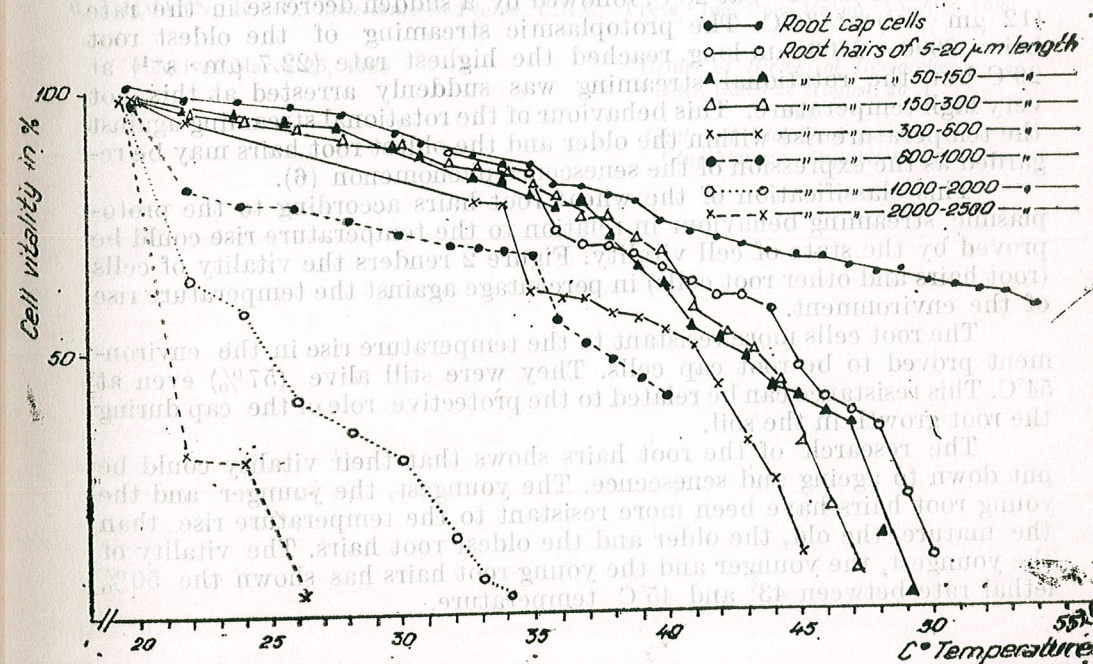


Fig. 2 — The vitality of wheat cells, root cap cells and root hairs (stained with neutral red) under the action of higher temperature



1) The younger root hairs (I), 50 — 150  $\mu\text{m}$  long. Depending on the cell length, these have usually shown incipient and shifting movements on circulation streaming. The cytoplasmic viscosity inside these hairs is high and therefore the temperature increase could enhance the streaming velocity to a very small extent. However, the highest rate ( $13 \mu\text{m} \cdot \text{s}^{-1}$ ) of streaming has been reached for these hairs at  $32^\circ\text{C}$ , that is twice as much in comparison to the control ( $6 \mu\text{m} \cdot \text{s}^{-1}$ ) at  $20^\circ\text{C}$ . The temperature rise above  $32^\circ\text{C}$  inhibited the rate of protoplasmic streaming so that at  $37^\circ\text{C}$  it had the same value as the control. The curve obtained was an optimal one.

2) The young, mature and old root hairs (II), 150—1000  $\mu\text{m}$  long. These hairs usually show a rotational streaming. This kind of protoplasmic streaming characterized the cells with a lower viscosity of cytoplasm. That is the reason why the rate of protoplasmic streaming may be well stimulated to an optimal point or degree by means of temperature rise. In fact this is what actually happened. With the temperature rise the velocity of protoplasmic streaming increased about 5 — 6 times in relation to the control. It reached  $25 \mu\text{m} \cdot \text{s}^{-1}$  at  $32^\circ\text{C}$  within mature root hairs, 300 — 600  $\mu\text{m}$  long and  $20.7 - 22.7 \mu\text{m} \cdot \text{s}^{-1}$  at  $34^\circ\text{C}$  within old root hairs, 600 — 1000  $\mu\text{m}$  long and young root hairs 150—300  $\mu\text{m}$ . After having reached the highest velocity the protoplasmic streaming decreased with the temperature rise. This decrease was more severe with old root hairs (600 — 1000  $\mu\text{m}$ ) than with younger cells.

3) The older and oldest root hairs (III) 1000—2500  $\mu\text{m}$  long. These hairs show only a rotational streaming. Despite this, the protoplasmic streaming of these root hairs has grown another reaction against the temperature rise in the surrounding environment. The protoplasmic streaming of the older root hairs 1000—2000  $\mu\text{m}$  long reached the highest velocity ( $20.8 \mu\text{m} \cdot \text{s}^{-1}$ ) at  $28^\circ\text{C}$ , followed by a sudden decrease in the rate ( $12 \mu\text{m} \cdot \text{s}^{-1}$ ) at  $30^\circ\text{C}$ . The protoplasmic streaming of the oldest root hairs 2000—2500  $\mu\text{m}$  long reached the highest rate ( $22.7 \mu\text{m} \cdot \text{s}^{-1}$ ) at  $26^\circ\text{C}$  but the rotational streaming was suddenly arrested at this not very high temperature. This behaviour of the rotational streaming against the temperature rise within the older and the oldest root hairs may be regarded as the expression of the senescence phenomenon (6).

This classification of the wheat root hairs according to the protoplasmic streaming behaviour in relation to the temperature rise could be proved by the state of cell vitality. Figure 2 renders the vitality of cells (root hairs and other root cells) in percentage against the temperature rise of the environment.

The root cells more resistant to the temperature rise in the environment proved to be root cap cells. They were still alive (57%) even at  $54^\circ\text{C}$ . This resistance can be related to the protective role of the cap during the root growth in the soil.

The research of the root hairs shows that their vitality could be put down to ageing and senescence. The youngest, the younger and the young root hairs have been more resistant to the temperature rise than the mature, the old, the older and the oldest root hairs. The vitality of the youngest, the younger and the young root hairs has shown the 50% lethal rate between  $43^\circ$  and  $45^\circ\text{C}$  temperature.

The vitality of the mature root hairs has shown the 50% lethal rate at  $41^\circ\text{C}$ , the older at  $37^\circ\text{C}$  and the older and the oldest root hairs at  $25^\circ\text{C}$ . These results prove that the older the root hairs the greater their sensitivity to the temperature rise in the environment.

The effect of the temperature under  $20^\circ\text{C}$  on the hairs of staminal filaments has also been proved. Our experiments have shown that the gradual temperature decrease leads to the lowering of the velocity of protoplasmic streaming. So that at  $+12^\circ\text{C}$  the rotational streaming ceases and it changes into a shifting movement. At  $+6^\circ\text{C}$  this kind of protoplasmic movement is arrested and at  $0^\circ\text{C}$  there can not be noticed any kind of protoplasmic movement.

Our investigations have proved that the protoplasmic streaming is a cellular process very sensitive to the changes of the environmental temperature. From its behaviour we can infer that other physiological processes (i.e. water and salts absorption, cytoplasmic viscosity) may show similar reactions.

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## SELECTION OF SOME YEAST STRAINS FOR ABILITY TO USE INULIN

VICTORIA HERLEA

The ability to use inulin as a sole source of carbon in 29 various yeast strains belonging to several genera and species, in order to select the most efficient ones, was investigated.

The results showed that 7 strains can be further used for direct enzyme assays of inulinase activity and, therefore, for the bioconversion of phytomass rich in inulin into products useful from an economic point of view.

To establish the growth potential of some yeasts in the presence of inulin, as the sole source of carbon, is of real interest if there is a series of agricultural sources rich in carbohydrate which can be used as a fermentation substrate. Therefore, among compositae containing inulin as sugar reserve (chicory, dandelion, dahlia and others), Yersusalem artichoke (*Helianthus tuberosus*) is remarkable as a robust plant producing a fine harvest of tubers rich in inulin, that can serve in ethanol production (7). This aspect is of great interest in the framework of the research of new sources of energy. In a first stage, it is necessary to select some strains capable to grow on a carbohydrate source-inulin — a plant fructosan formed by a linear chain of about 25—30 molecules of fructose linked by  $\beta(1 \rightarrow 2)$  bonds and terminated by a glucose residue (again  $\beta(1 \rightarrow 2)$ )).

### MATERIALS AND METHODS

**Yeast strains** — The 29 strains investigated for various abilities to use inulin as the sole C source (Table 2) belong to the collection of the Microbiological Laboratory of the Faculty of Biology — University of Bucharest. The yeast strains were kept on agar Y.P.G. medium (11) under a paraffin layer, at 4°C.

**Assessing the ability to use inulin** was carried out by mainly one of the techniques recommended by Barnett et al. (3, 4) in order to estimate the capacity to utilize certain organic compounds, each as the sole major source of carbon for aerobic growth. Among these techniques, often called "assimilation" tests, the procedure in liquid medium was used.

The tests are done in test tubes (180 mm by 16 mm) each containing a standard amount of liquid medium represented by Yeast Nitrogen Base (Y.N.B.) modified by Abadie (1, 2), with a content of 0.5% inulin Merck (5; 6).

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Table 1

Composition of chemically defined media \* in testing the ability of yeasts to use inulin

| Formula ingredients per liter        | Yeast nitrogen base (Y.N.B.) for inulin utilisation test | Y.N.B. without carbon source (negative control) | Y.N.B. with glucose (positive control) |
|--------------------------------------|----------------------------------------------------------|-------------------------------------------------|----------------------------------------|
| Nitrogen source :                    |                                                          |                                                 |                                        |
| Ammonium sulphate                    | 2 g                                                      | 2 g                                             | 2 g                                    |
| Carbon sources :                     |                                                          |                                                 |                                        |
| Bacto-dextrose                       | none                                                     | none                                            | 5 g                                    |
| Inulin                               | 5 g                                                      | none                                            | none                                   |
| Vitamins :                           |                                                          |                                                 |                                        |
| p-aminobenzoic acid                  | 200 µg                                                   | 200 µg                                          | 200 µg                                 |
| Biotin                               | 20 µg                                                    | 20 µg                                           | 20 µg                                  |
| Folic acid                           | 2 µg                                                     | 2 µg                                            | 2 µg                                   |
| Inositol                             | 10 mg                                                    | 10 mg                                           | 10 mg                                  |
| Niacin                               | 400 µg                                                   | 400 µg                                          | 400 µg                                 |
| Calcium pantothenat                  | 2 mg                                                     | 2 mg                                            | 2 mg                                   |
| Pyridoxine hydrochloride             | 400 µg                                                   | 400 µg                                          | 400 µg                                 |
| Riboflavin                           | 200 µg                                                   | 200 µg                                          | 200 µg                                 |
| Thiamine hydrochloride               | 400 µg                                                   | 400 µg                                          | 400 µg                                 |
| Compounds supplying trace elements : |                                                          |                                                 |                                        |
| Boric acid                           | 500 µg                                                   | 500 µg                                          | 500 µg                                 |
| Copper sulphate                      | 40 µg                                                    | 40 µg                                           | 40 µg                                  |
| Potassium iodide                     | 100 µg                                                   | 100 µg                                          | 100 µg                                 |
| Ferric chloride                      | 200 µg                                                   | 200 µg                                          | 200 µg                                 |
| Manganese sulphate                   | 400 µg                                                   | 400 µg                                          | 400 µg                                 |
| Sodium molybdate                     | 200 µg                                                   | 200 µg                                          | 200 µg                                 |
| Zinc sulphate                        | 400 µg                                                   | 400 µg                                          | 400 µg                                 |
| Salts :                              |                                                          |                                                 |                                        |
| Potassium phosphate monobasic        | 850 mg                                                   | 850 mg                                          | 850 mg                                 |
| Potassium phosphate dibasic          | 150 mg                                                   | 150 mg                                          | 150 mg                                 |
| Magnesium sulphate                   | 500 mg                                                   | 500 mg                                          | 500 mg                                 |
| Sodium chloride                      | 100 mg                                                   | 100 mg                                          | 100 mg                                 |
| Calcium chloride                     | 100 mg                                                   | 100 mg                                          | 100 mg                                 |

\* The media are based on the Wickerham formulae (11).

The pH of the media has been raised from 5.2 to 5.6 by the phosphates listed in the above table.

For each strain the sample with base nutritive medium (Y.N.B.) containing inulin is accompanied by the negative control (Y.N.B. free of any C source) and the positive control (Y.N.B. with D glucose as C source) (Table 1). The media were sterilised by filtration (bacteriological glass filters LABOR-THERM 17G5) to prevent the hydrolysis of C sources at high temperatures. According to some previous results (8, 9, 10), which are also in agreement with Abadie's results, we reduced from the medium formula histidine, methionine and tryptofane using also 2 g ammonia sulphate and 5 g D glucose/l; these quantities were sufficient to assure a good growth of yeasts.

Table 2

List of tested strains for ability to use inulin

| Strain Coll. No. | Genus and Species * Source**                             | O.D. (A <sub>640 nm</sub> ) |               |                         |
|------------------|----------------------------------------------------------|-----------------------------|---------------|-------------------------|
|                  |                                                          | Negative control sample     | Inulin sample | Positive control sample |
| 115              | <i>Candida guilliermondii</i> B.K.M.                     | 0.06                        | 0.11          | 0.86                    |
| 132              | <i>C. guilliermondii</i> I.P.                            | 0.08                        | 0.18          | 0.32                    |
| 113              | <i>C. macedoniensis</i> B.K.M.                           | 0.03                        | 0.36          | 0.32                    |
| 62               | <i>C. membranaefaciens</i> C.B.S. 1952                   | 0.05                        | 0.09          | 0.29                    |
| 63               | <i>C. parapsilosis</i> 213 Ly                            | 0.03                        | 0.05          | 0.65                    |
| 4                | <i>C. utilis</i> 13 I.C.A.                               | 0.08                        | 0.10          | 0.39                    |
| 24               | <i>C. utilis</i> C 14 I.P.                               | 0.09                        | 0.08          | 0.43                    |
| 25               | <i>C. utilis</i> 321 — N.Y.C.                            | 0.15                        | 0.16          | 0.35                    |
| 26               | <i>C. utilis</i> var. <i>major</i> . I.C.A.              | 0.09                        | 0.09          | 0.48                    |
| 27               | <i>C. utilis</i> U.V. — INCEF                            | 0.10                        | 0.10          | 0.62                    |
| 111              | <i>Debaryomyces hansenii</i> 64165 I.P.                  | 0.11                        | 0.11          | 0.48                    |
| 87               | Isolate from wood hydrolisate INCEF                      | 0.05                        | 0.06          | 0.95                    |
| 92               | Isolate from mixt inoculum INCEF                         | 0.01                        | 0.02          | 0.38                    |
| 84               | Isolate from wood hydrolisate INCEF                      | 0.04                        | 0.17          | 0.36                    |
| 91               | Isolate from wood hydrolisate INCEF                      | 0.01                        | 0.02          | 0.67                    |
| 102              | <i>Saccharomyces fragilis</i> S.S.I.                     | 0.08                        | 0.27          | 0.36                    |
| 104              | <i>S. lactis</i> A—94 I.P.                               | 0.08                        | 0.06          | 0.46                    |
| 106              | <i>S. marxianus</i> 88 I.P.                              | 0.06                        | 0.29          | 0.34                    |
| 116              | <i>S. pseudotropicalis</i> (var. <i>lactosa</i> ) B.K.M. | 0.05                        | 0.18          | 0.20                    |
| 109              | <i>Schizosaccharomyces pombe</i> C.E.R.I.A.              | 0.01                        | 0.01          | 0.2                     |
| 6                | <i>Torulopsis famata</i> C 120 I.P.                      | 0.15                        | 0.13          | 0.37                    |
| 28               | <i>Torula utilis</i> 2 — I.C.A.                          | 0.06                        | 0.10          | 0.43                    |
| 29               | <i>Torula utilis</i> 4 — I.C.A.                          | 0.08                        | 0.06          | 0.60                    |
| 12               | <i>Torulopsis utilis</i> B. — I.C.A.                     | 0.09                        | 0.11          | 0.41                    |
| 13               | <i>T. utilis</i> stamm Reindl I — I.C.A.                 | 0.13                        | 0.12          | 0.39                    |
| 15               | <i>T. utilis</i> Brenner-Rippel — I.C.A.                 | 0.06                        | 0.20          | 0.38                    |
| 14               | <i>T. utilis</i> 1 a Hefe Virg — I.C.A.                  | 0.10                        | 0.10          | 0.42                    |
| 19               | <i>T. utilis</i> T 10 — I.C.A.                           | 0.09                        | 0.16          | 0.80                    |
| 21               | <i>T. utilis</i> von Sulf Ablauge — I.C.A.               | 0.10                        | 0.15          | 0.30                    |

\* The nomenclature complies with the original one on the acquired collection tube.

\*\* B.K.M. = Institute of Microbiology, Moscow, U.S.S.R.

I.P. = Institute Pasteur, Paris, FRANCE

C.B.S. = Centraalbureau voor Schimmelcultures, Delft, HOLLAND

LY = Laboratoire de Biologie — University of Lyon, FRANCE

I.C.A. = Collection of the Institute for Food Research, Bucharest, ROMANIA

N.Y.C. = National Collection of Yeast Culture, GREAT BRITAIN

INCEF = Institute for Forest Research, Bucharest, ROMANIA

S.S.I. = Statens Serum Institut, Copenhagen, DENMARK

C.E.R.I.A. = Institute de recherches, BELGIUM.

The aminoacids deletion from Y.N.B. is also recommended by Barnett et al. (4) in order to reduce the growth of some yeasts in the negative controls.

For inoculation, the yeast strains under study are brought to an active state by two passages at an interval of 48 h on slope Y. P. G. medium



and incubation at 28°C. Suspensions in Y.N.B. devoid of any C source (negative control) obtained from these cultures, so standardised as to have the same concentrations cells/ml, estimated with a photoelectric nephelometer FEK 56M in optical density (O.D.) —  $A_{640} \approx 0.2$  — were used. Each tube (of the set of 3 for each of the 29 strains) is inoculated with 100  $\mu$ l standardised suspension. The tubes are incubated at 28°C and shaken by hand every day for a week.

The yeast growth and therefore the ability to use inulin as a sole C source is nephelometrically appreciated relative to the controls for each strain separately, 7 days after incubation.

## RESULTS AND DISCUSSION

The inulin utilization is quantitatively appreciated by the degree of development expressed in O.D. of each of the 29 tested strains. The estimation of the results, presented in Table 2, was made taking into account on the one side the O.D. of the negative control and on the other side the O. D. of the positive control. Table 2 shows that the O.D. of the negative control, inoculated under the same conditions, varies within certain limits demonstrating therefore that the inoculum, although extremely poor (when seen with the naked eye it is absolutely transparent), the composition of the base medium as well as the intracellular reserve in the inoculum support in a way the development in certain yeasts. As the specialists pointed out, it does not influence the validity of that results as long as for each strain the results take into consideration the control under experimental conditions, identical for all tested strains. The O. D. values, in case of positive control (Table 2) attest the accuracy of the experiment, being known that glucose is the best C source for most known yeasts.

After selection, out of the 29 investigated strains, the most efficient ones regarding the inulin utilization in comparison with glucose utilization were the following strains: *Saccharomyces fragilis*, *S. marxianus*, *S. pseudotropicalis* (var. *lactosa*) and *Candida macedoniensis*; in the inulin sample they have an almost equal O.D. or higher than O.D. in the sample with glucose. Out of the other tested strains, if we take into account the growth in inulin sample relative to negative control, the following can be pointed out: *Torulopsis utilis* Brenner-Rippel, *Candida guilliermondii* and strain 84 isolated from wood hydrolysate; in the inulin sample O.D. is clearly superior to the negative control.

Table 2 also shows that at the level of the same species the ability to use inulin is a variable character for different strains, a fact accepted by taxonomists asserting therefore the introduction in the experiment of a various number of strains belonging to the same species.

## CONCLUSIONS

— Out of the 29 strains tested to use inulin as a sole C source, the following can be selected:

- 4 strains (*Saccharomyces fragilis*—102, *S. marxianus*—106

*S. pseudotropicalis* (var. *lactosa*)—116; *Candida macedoniensis*—113) which according to the present nomenclature (4) are synonymous to *Kluyveromyces marxianus*;

- 3 strains: *Torulopsis utilis* Brenner-Rippel—15, *Candida guilliermondii*—132 (under the present nomenclature as *Hansenula jadinii* and respectively *Pichia guilliermondii*) and the strain 84 isolated wood hydrolysate.

— The selected strains may be sources of direct enzyme assays for inulinase activity and implicitly for bioconversion of phytomass rich in inulin.

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# DIE BEDEUTUNG DER PHENOLKÖRPER DER TANNENSAMEN FÜR DIE HERKUNFTSIDENTIFIZIERUNG UND ZUR SCHÄTZUNG MIT IHNEN KORRELIERTER MERKMALE

D. SIMON und MONIKA KONNERT

Studies on n-butanol soluble phenolics in fir seeds showed that between four populations, located in distinct parts of the Romanian fir range, there are clear quantitative and qualitative differences.

The combination of different phenolic fractions is characteristic of each studied population thus opening possibilities for the provenance identification of the fir seed-lots.

The correlations found between different phenolics and the biological and biometrical characteristics of trees, cones, seeds and seedlings indicate possibilities of indirect evaluations.

Eines der wichtigsten Probleme des Waldbaues besteht in der Auswahl geeigneter Provenienzen bei der Bestandesbegründung und der Reglementierung des Samentransfers. Für einzelne Fälle scheint aber eine Zertifizierung über biochemische, physiologische oder morphologische Methoden angebracht, die eine direkte Erkennung des Saatgutes ermöglicht.

Als Folge dieser Erkenntnis wurde untersucht, ob sich Tannenherkünfte anhand der Phenolkörper, die in den Tannensamen enthalten sind, erkennen lassen. Die Untersuchung der Phenolkörper bot sich an, weil diese in den Tannensamen analysierbare Konzentrationen erreichen und weil frühere Forschungen [6], [7], [11] für Fichte eine hinreichend hohe Heritabilität, beziehungsweise starke genetische Kontrolle der Phenolkörper festgestellt haben.

## MATERIAL UND METHODE

Um eine Übersicht der Variation, sowohl zwischen als auch innerhalb der Populationen zu bekommen, wurde in vier Forstämtern (Tabelle 1) von je 30 Bäumen getrennt Samen geerntet, dem in Kältekammern bei 0°C aufbewahrt wurde. Die Bäume wurden zufallsmäßig aus dem Bestand herausgegriffen, sodaß sie ungefähr gleichmäßig über den ganzen Bestand verteilt waren.

Die Phenolkörper wurden chromatographisch wie folgt dargestellt: 1.0 g frische, geschälte, im Mörser zerkleinerte Samen wurden in 50 ml



destilliertem Wasser am Rückfluß 3 Stunden lang extrahiert [7], [10], [12]. Das Extrakt wurde filtriert und die Phenolkörper dreimal mit je 25 ml n-Butanol ausgeschüttelt (die Extraktion mit n-Butanol wurde wiederholt um einen totalen Übergang in die organische Phase zu gewährleisten). Die organische Phase wurde auf 1 ml eingedampft [7]. Je 10 µl Konzentrat wurden auf Zellosoeschichten (Merck) strichförmig aufgetragen und eindimensional mit dem Fließmittel n-Butanol-Essigsäure-Wasser (4:1:5) getrennt [8], [12].

Tabelle 1

Geografische Beschreibung der untersuchten Tannenherkünfte

| Forstamt | Lage          | Geografische Koordinaten | Seehöhe (m) |
|----------|---------------|--------------------------|-------------|
| Väliug   | Banater Berge | 45°15'—22°00'            | 755         |
| Cimpeni  | Westkarpaten  | 46°20'—23°05'            | 880         |
| Vidraru  | Südkarpaten   | 45°20'—24°35'            | 885         |
| Rodna    | Ostkarpaten   | 47°25'—24°50'            | 885         |

Die getrennten Phenolkörper wurden durch Besprühen mit tetraazotiertem Benzidin sichtbar gemacht [4] und erschienen als rot oder gelb gefärbte Bänder auf weißem Grund. Die Chromatogramme wurden densitometrisch ausgewertet und der Anteil der n-butanollöslichen Fraktionen durch Planimetrieren der entstandenen Kurven in Prozenten der Gesamtfläche bestimmt. Die Ergebnisse wurden einerseits verwendet um die Möglichkeiten der Herkunftsidentifizierung zu klären und andererseits um mögliche Korrelationen zu anderen Merkmalen der Bäume, Zapfen, Samen und Sämlinge zu untersuchen. Es wurden zu diesem Zweck folgende gemessene und beobachtete Daten von 29 Bäumen der Herkunft Väliug verwendet: Höhe des Baumes (m), Höhe bis zum ersten nadeltragenden Ast (m), astfreier Teil des Stammes (m), Brusthöhen-durchmesser (cm), mittlerer Durchmesser der Kronenprojektion (m), mittlere Länge und Breite des Zapfens (cm), Tausendkorngewicht (g), Anteil der trockenen Samen (%), Anteil der nicht lebensfähigen Embryonen (%), Anteil der lebensfähigen Embryonen (%), Gehalt an reduzierendem Zucker in den Samen (% des Trockengewichtes), Fettgehalt der Samen (% des Trockengewichtes), Proteingehalt der Samen, Farbe der Samenflügel (kodifiziert) und Zahl der Phenolkörperfraktionen.

Der Gehalt an reduzierendem Zucker wurde durch die volumetrische Methode nach Bertrand bestimmt, der Fettgehalt durch Extraktion im Soxhlet-Apparat nach der indirekten Methode Ionescu-Ferando und der Proteingehalt nach der Kjeldahlmethode, wobei der proteische Stickstoff nach Gräser [3] mit 6,25 multipliziert wurde.

#### ERGEBNISSE

Die chromatographische Trennung der n-butanollöslichen Phenolkörper führte zur Identifizierung von 5 klar unterscheidbaren Peak-Zonen (A—E) und innerhalb dieser zur sicheren Ansprache von noch

je 1—3 peaks deren Trennung nicht immer vollständig war. Insgesamt wurden in den vier Herkünften 13 erkennbare Fraktionen nachgewiesen, deren Anteil sehr unterschiedlich war. In der Population Rodna wurden 12 Fraktionen, in der Population Vidraru 10, in der Population Väliug 12 und in der Population Cimpeni 8 Fraktionen festgestellt.

Die offenkundige Variation sowohl zwischen den Bäumen als auch zwischen den Populationen legte eine Untersuchung der Beziehung zwischen der Zahl der festgestellten Fraktionen pro Herkunft und der Herkunft nahe. Da wegen Verlusten bei der Samenernte und während der chromatographischen Trennung nicht von jeder Herkunft 30 Bäume erhalten blieben, wurde die folgende Untersuchung auf je 26 Bäume pro Population bezogen.

Mit Hilfe des G-Testes nach Weber [9] (Tabelle 2) wurde gezeigt, daß zwischen der Zahl der Fraktionen pro Herkunft und der Herkunft eine statistisch abgesicherte Verbindung besteht.

Tabelle 2

G-Test auf Unabhängigkeit zwischen den untersuchten Tannenherkünften

| Herkunft | Phenolkörperfraktion |    |     |    |    |     |    |     |     |    |     |    |     | Summe |
|----------|----------------------|----|-----|----|----|-----|----|-----|-----|----|-----|----|-----|-------|
|          | A                    | A' | A'' | B  | B' | C   | C' | C'' | D   | D' | E   | E' | E'' |       |
| Väliug   | 24                   | 6  | 2   | 24 | 1  | 26  | 23 | 1   | 26  | 0  | 26  | 19 | 1   | 179   |
| Cimpeni  | 17                   | 0  | 0   | 20 | 0  | 26  | 19 | 26  | 26  | 0  | 23  | 11 | 0   | 168   |
| Vidraru  | 23                   | 12 | 4   | 22 | 0  | 26  | 11 | 0   | 26  | 0  | 26  | 23 | 10  | 183   |
| Rodna    | 23                   | 9  | 2   | 25 | 8  | 26  | 9  | 0   | 26  | 1  | 26  | 20 | 19  | 194   |
| Summe    | 87                   | 27 | 8   | 91 | 9  | 104 | 62 | 27  | 104 | 1  | 101 | 73 | 30  | 724   |

$$G = 2 \left[ \sum_{i=1}^4 \sum_{j=1}^{13} z_{ij} \ln z_{ij} - \sum_{i=1}^4 \left( \sum_{j=1}^{13} z_{ij} \right) \ln \left( \sum_{j=1}^{13} z_{ij} \right) - \sum_{j=1}^{13} \left( \sum_{i=1}^4 z_{ij} \right) \ln \left( \sum_{i=1}^4 z_{ij} \right) - n \ln n \right] =$$

$$= 161,632 > \chi_{ab}^2$$

FG = 36

Diese globale Feststellung gestattet noch keine Identifizierung der Herkunft, ist aber ein Hinweis darauf, daß Identifizierungsmöglichkeiten bestehen. Zu diesem Zweck wurden zu den qualitativen Aussagen betreffend die Zahl der nachgewiesenen Fraktionen auch die quantitativen Daten über die Beteiligung jeder Fraktion hinzugezogen und eine Diskriminanzanalyse unternommen. Es fand das Programm 7M der Sammlung BMD der Universität Kaliforniens, das eine stufenweise Diskriminanzanalyse ermöglicht, Verwendung.

Wie aus Tabelle 3 zu ersehen ist, ergab die Diskriminanzanalyse 4 gut unterscheidbare Typen von Phenolkörpergruppierungen, die mit A—D bezeichnet wurden. Jeder dieser Typen kommt mehrheitlich in einer einzigen Herkunft vor und ist somit charakteristisch für diese.

Es wurde für jeden untersuchten Baum aufgrund der jeweils größten Wahrscheinlichkeit die Zugehörigkeit zu einem der vier Typen bestimmt und in Tabelle 3 die Variabilität innerhalb und zwischen den Herkünften dargestellt. Die homogenste Population ist jene aus Cimpeni, wo alle Bäume zum Typus B gehören, gefolgt von Väliug, wo 90 % zum



Typus C gehören; Vidraru, wo 82% zum Typus A gehören und Rodna, wo 65% zum Typus D gehören.

Der am weitesten verbreitete Typus ist C, charakteristisch für Văliug und der seltenste D, charakteristisch für Rodna.

Die Ergebnisse der Diskriminanzanalyse werden in Abbildung 1 veranschaulicht. Es ist offensichtlich, daß die Herkunft Cimpeni eindeutig erkennbar ist, während die anderen Herkunft zwar noch gut erkennbar sind, aber eine Überlappung aufweisen. In Tabelle 4 wurden mit Hilfe des

Tabelle 3

Verteilung der charakteristischen Phenolkörpermuster über die untersuchten Herkunft

| Herkunft | Zahl der untersuchten Bäume | Zahl der den verschiedenen Mustern zugeordneten Bäume |          |         |         |
|----------|-----------------------------|-------------------------------------------------------|----------|---------|---------|
|          |                             | A                                                     | B        | C       | D       |
| Văliug   | 29                          | 0                                                     | 0        | 26(90%) | 3       |
| Cimpeni  | 26                          | 0                                                     | 26(100%) | 0       | 0       |
| Vidraru  | 28                          | 23(82%)                                               | 0        | 3       | 2       |
| Rodna    | 26                          | 3                                                     | 1        | 5       | 17(65%) |

Tabelle 4

Paarweise Untersuchung der Unterschiede zwischen den Mittelwerten der 4 untersuchten Herkunft mit Hilfe des F-Testes

|   | Phenolkörpermuster |          |         |
|---|--------------------|----------|---------|
|   | A                  | B        | C       |
| B | 19.20***           |          |         |
| C | 6.92***            | 16.71*** |         |
| D | 6.07***            | 15.99*** | 4.85*** |

FG = 13 und 93

Tabelle 5

Statistisch signifikante Korrelationen zwischen Phenolkörperfraktionen und gemessenen und beobachteten Daten

| Fraktion | Merkmal                          | Korrelationskoeffizient |
|----------|----------------------------------|-------------------------|
| B        | Durchmesser der Kronenprojektion | -0.374*                 |
| A        | mittl. Zahl der Kotyledonblätter | 0.510**                 |
| C        | mittl. Zahl der Kotyledonblätter | 0.387*                  |
| C'       | Anteil der leeren Samen          | 0.554**                 |
| D        | Anteil der leeren Samen          | -0.362*                 |
| E'       | Gehalt an reduzierendem Zucker   | -0.416*                 |
| A''      | Gehalt an Proteinen              | -0.379*                 |
| B'       | Gehalt an Proteinen              | 0.423*                  |
| B        | Farbe der Samenflügel            | 0.382*                  |
| E        | Zahl der Phenolkörperfraktionen  | -0.400*                 |
| E'       | Zahl der Phenolkörperfraktionen  | 0.377*                  |
| E''      | Zahl der Phenolkörperfraktionen  | 0.461*                  |

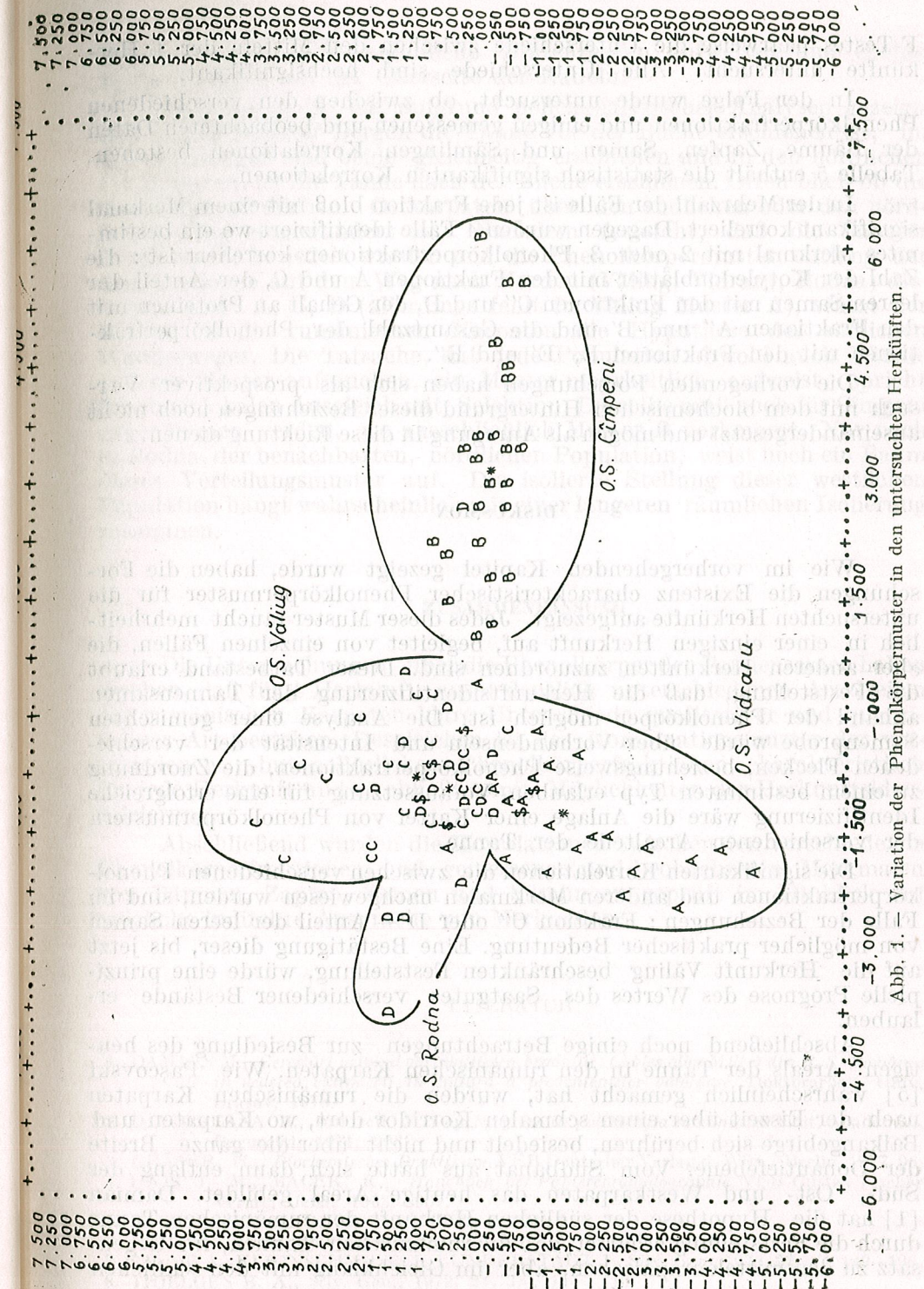


Abb. 1. — Variation der Phenolkörpermuster in den untersuchten Herkunft.



F-Testes paarweise die Unterschiede zwischen den Mitteln der 4 Herkünfte untersucht. Alle Unterschiede sind hochsignifikant.

In der Folge wurde untersucht, ob zwischen den verschiedenen Phenolkörperfraktionen und einigen gemessenen und beobachteten Daten der Bäume, Zapfen, Samen und Sämlingen Korrelationen bestehen. Tabelle 5 enthält die statistisch signifikanten Korrelationen.

In der Mehrzahl der Fälle ist jede Fraktion bloß mit einem Merkmal signifikant korreliert. Dagegen wurden 4 Fälle identifiziert wo ein bestimmtes Merkmal mit 2 oder 3 Phenolkörperfraktionen korreliert ist: die Zahl der Kotyledonblätter mit den Fraktionen A und C, der Anteil der leeren Samen mit den Fraktionen C' und D, der Gehalt an Proteinen mit den Fraktionen A' und B' und die Gesamtzahl der Phenolkörperfraktionen mit den Fraktionen E, E' und E''.

Die vorliegenden Forschungen haben sich als prospektiver Versuch mit dem biochemischen Hintergrund dieser Beziehungen noch nicht auseinandergesetzt und mögen als Anregung in diese Richtung dienen.

#### DISKUSSION

Wie im vorhergehenden Kapitel gezeigt wurde, haben die Forschungen die Existenz charakteristischer Phenolkörpermuster für die untersuchten Herkünfte aufgezeigt. Jedes dieser Muster taucht mehrheitlich in einer einzigen Herkunft auf, begleitet von einzelnen Fällen, die eher anderen Herkünften zuzuordnen sind. Dieser Tatbestand erlaubt die Feststellung, daß die Herkunftsidentifizierung der Tannensamen anhand der Phenolkörper möglich ist. Die Analyse einer gemischten Samenprobe würde über Vorhandensein und Intensität der verschiedenen Flecken, beziehungsweise Phenolkörperfraktionen, die Zuordnung zu einem bestimmten Typ erlauben. Voraussetzung für eine erfolgreiche Identifizierung wäre die Anlage einer Kartei von Phenolkörpermustern der verschiedenen Arealteile der Tanne.

Die signifikanten Korrelationen die zwischen verschiedenen Phenolkörperfraktionen und anderen Merkmalen nachgewiesen wurden, sind im Falle der Beziehungen: Fraktion C' oder D – Anteil der leeren Samen von möglicher praktischer Bedeutung. Eine Bestätigung dieser, bis jetzt auf die Herkunft Väliug beschränkten Feststellung, würde eine prinzipielle Prognose des Wertes des Saatgutes verschiedener Bestände erlauben.

Abschließend noch einige Betrachtungen zur Besiedlung des heutigen Areals der Tanne in den rumänischen Karpaten. Wie Paşcovski [5] wahrscheinlich gemacht hat, wurden die rumänischen Karpaten nach der Eiszeit über einen schmalen Korridor dort, wo Karpaten und Balkangebirge sich berühren, besiedelt und nicht über die ganze Breite der Donautiefebene. Vom Südbanat aus hätte sich dann entlang der Süd-, Ost- und Westkarpaten das heutige Areal gebildet. Damian [1] hat die Hypothese der südlichen Herkunft der rumänischen Tanne durch die Feststellung erhärtet, daß die rumänischen Tannen, im Gegensatz zu den mitteleuropäischen, aber im Gleichklang mit den südlichen

Formen häufig rote männliche Blütenzapfen aufweisen und ebenso Spaltöffnungen auf der Oberseite der Nadeln.

Diaconeasa [2] hat aufgrund pollenanalytischer Analysen gezeigt, daß in den Banater Bergen die Tanne vor der Buche erschienen ist, im Karpatenbogen sind beide gleichzeitig erschienen und in den nördlichen Ostkarpaten ist die Tanne nach der Buche erschienen. Offen blieb ob die Tanne der nördlichen Ostkarpaten über den südlichen oder den nördlichen Weg eingewandert ist. Nachdem feststeht, daß die Banater Berge zuerst besiedelt wurden und die Phenolkörpermuster entlang der drei Stationen auf dem Weg nach Norden (Väliug, Vidraru, Rodna) dieselben sind: A, C, D (allerdings fehlt in Väliug Muster A), stützt der Befund für die rumänischen Karpaten die Hypothese des südlichen Wanderweges. Die Tatsache, daß jede Population obwohl auch Bäume anderer Muster auftauchen, ein Muster mehrheitlich aufweist, spricht für einen lokalen Ausgleich mit Selektion. Dasselbe muß auch für Cimpeni angenommen werden, wo ausschließlich Muster B vorkommt. Nur noch in Rodna, der benachbarten, nördlichen Population, weist noch ein Baum dieses Verteilungsmuster auf. Die isolierte Stellung dieser westlichen Population hängt wahrscheinlich mit einer längeren räumlichen Isolierung zusammen.

#### ZUSAMMENFASSUNG

Die Untersuchungen über die Phenolkörper der Tannensamen haben ergeben, daß für 4 Herkünfte aus 4 deutlich unterschiedenen Arealteilen der rumänischen Karpaten klare Unterschiede qualitativer und quantitativer Art bestehen. Desgleichen ist das Kombinationsmuster der verschiedenen n-butanollöslichen Phenolkörperfraktionen charakteristisch für jede Herkunft und erschließt somit Möglichkeiten der Herkunftsidentifizierung.

Abschließend wurden die Korrelationen zwischen den verschiedenen Phenolkörperfraktionen und gemessenen und beobachteten Merkmalen von Bäumen, Zapfen, Samen und Sämlingen geprüft im Hinblick auf mögliche indirekte Ansprache von Merkmalen.

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## THE INFLUENCE OF THE PHYSIOLOGICAL STATE OF THE SEEDS AT THE IRRADIATION MOMENT ON THE EFFECTS INDUCED BY SMALL DOSES OF GAMMA RAYS ( $\text{Co}^{60}$ ) IN *TRITICUM AESTIVUM* L.

G. I. GHIORGHITĂ, ECATERINA T. TÓTH and T. T. POPESCU

The irradiation of the wheat seeds (*Fundulea-29* cultivar) with small doses of gamma rays (100-1 000 R) revealed that the cytogenetic, physiological and biochemical effects induced, essentially depended on the physiological state of the seeds at the irradiation moment. The most profound injuries in plants were induced when the seeds were irradiated after 24 hours of water moistening. The irradiation determined important changes of the peroxidase and catalase activity, of the isosterases and especially isoperoxidases spectrum in the 2 and 5 day-old seedlings.

In a series of previous investigations (15), (18), (19), (33), (34), we showed some of the cytogenetic, physiological and biochemical effects induced by single and combined treatments with gamma rays and alkylating agents in two-row barley and wheat, mainly observing the changes in the spectrum and the activity of some enzymes. The isoenzyme studies have become important in the investigation of phylogeny, polyploidy and cross-breeding, in discovering the genes which cause the synthesis of some enzymes and regulation of the cell metabolism, in the appreciation of the tissue differentiation stage etc. (1), (13), (28), (30). There are some data (21), (25), (28), (30) revealing that the spectrum and the enzyme activity differ from an organ of the plant to another, and they modify during the ontogenesis and with the health state of the organism.

Regarding the behaviour of the multiple molecular forms of enzymes to the action of mutagen agents we have few data, and these are contradictory. While some authors noticed that the irradiations may modify the spectrum and the activity of some enzymes (20), (31), others, on the contrary, proved that the zymograms of the enzymes in the irradiated plants do not differ from that of the controls (12), (14). In the investigations of two-row barley (unpublished data) and wheat (19), (33), (34) we noticed that both the gamma rays and some alkylating agents (EMS and dES) produce changes in the spectrum and the activity of the isoesterases and of the isoperoxidases especially. In the surviving plants, these change are generally eliminated by the 5th day of life.

In a previous paper (19) we mentioned that the irradiation of wheat seeds (after their moisture in water for 6-24 hours) with doses ranging between 1,000 and 10,000 R caused minor disturbances in the spectrum and the activity of the isoesterases. The isoperoxidases registered impor-

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tant changes of their spectrum and activity in the first 5 days of ontogenesis. These changes were influenced by the irradiation dose and the water content of the seeds at the irradiation moment. As the values of the gamma rays doses were relatively high, we intended to show that the same results are obtained when applying low irradiation doses (not over the value of 1,000 R).

#### MATERIAL AND METHODS

Seeds of wheat, *Fundulea-29* cultivar, were firstly moistened in water for 6, 12 and 24 hours (water content = 24.2%, 29.9% and respectively 34.4%, as to 9.2% in the control) and then irradiated with 100, 300, 500, 750 and 1000 R — gamma rays ( $\text{Co}^{60}$  — flow capacity = 1480 R/h). We produced 16 experimental variants (presented in Tables 1 and 2) which were cultivated at intervals of two days, for the study of isoesterases and isoperoxidases (the first interval: variants 1–6; the 2nd: var. 7–11; the 3rd: 12–16). After the treatments, the seeds (300 per variant) were cultivated on filter paper in Engelhardt germinators and put into a climatized box (temperature about 22°C; relative air humidity about 65–70%; luminosity of 4000 lux — continuous light). During the first two days, the germinators were kept in darkness. For the analysis of the aberrant chromosomal anaphases and telophases in the root mitoses, the roots of 50 individuals from each variant were harvested when the plants were 2 days old. The experiment lasted for 124 hours. By the isoelectric focussing method on polyacrylamide gel there were studied the

Table 1

The values of some physiological and cytogenetic indices in wheat plants (*Fundulea-29* cultivar) obtained from gamma irradiation of moistened seeds

| No. | The treatment   | The root length (mm)   |                        | The stem length (mm)   |                        | Dry subst. % fr.s. | Total A + T aberrants       |                             |
|-----|-----------------|------------------------|------------------------|------------------------|------------------------|--------------------|-----------------------------|-----------------------------|
|     |                 | $\bar{x} \pm s\bar{x}$ | $\bar{x} \pm s\bar{x}$ | $\bar{x} \pm s\bar{x}$ | $\bar{x} \pm s\bar{x}$ |                    | $\bar{x} \% \pm \bar{x} \%$ | $\bar{x} \% \pm \bar{x} \%$ |
| 1   | Control         | 43.50                  | 1.10                   | 56.97                  | 1.18                   | 11.00              | 5.75                        | 0.03                        |
| 2   | TA—6 h+ 100 R   | 44.74                  | 0.94                   | 58.11                  | 1.02                   | 11.68              | 8.76                        | 0.02                        |
| 3   | TA—6 h+ 300 R   | 43.78                  | 0.97                   | 56.36                  | 0.97                   | 10.19              | 9.98                        | 0.03                        |
| 4   | TA—6 h+ 500 R   | 45.56                  | 1.02                   | 57.62                  | 0.99                   | 11.97              | —                           | —                           |
| 5   | TA—6 h+ 750 R   | 48.68                  | 0.91                   | 58.01                  | 0.94                   | 10.55              | 10.59                       | 0.02                        |
| 6   | TA—6 h+ 1000 R  | 41.57                  | 0.98                   | 53.56                  | 1.08                   | 10.63              | 10.23                       | 0.04                        |
| 7   | TA—12 h+ 100 R  | 40.19                  | 0.83                   | 47.06                  | 0.92                   | 10.43              | 10.34                       | 0.04                        |
| 8   | TA—12 h+ 300 R  | 40.41                  | 0.95                   | 47.74                  | 0.83                   | 10.53              | 10.03                       | 0.06                        |
| 9   | TA—12 h+ 500 R  | 35.60                  | 0.77                   | 47.32                  | 0.91                   | 10.47              | 11.74                       | 0.03                        |
| 10  | TA—12 h+ 750 R  | 40.70                  | 0.81                   | 49.71                  | 0.90                   | 11.52              | 10.75                       | 0.04                        |
| 11  | TA—12 h+ 1000 R | 39.15                  | 0.88                   | 49.02                  | 0.86                   | 11.15              | 16.82                       | 0.05                        |
| 12  | TA—24 h+ 100 R  | 40.66                  | 1.17                   | 40.71                  | 0.85                   | 10.71              | 18.32                       | 0.04                        |
| 13  | TA—24 h+ 300 R  | 40.55                  | 1.08                   | 43.37                  | 0.76                   | 11.48              | 25.36                       | 0.06                        |
| 14  | TA—24 h+ 500 R  | 42.04                  | 1.17                   | 43.05                  | 0.77                   | —                  | 22.77                       | 0.04                        |
| 15  | TA—24 h+ 750 R  | 34.77                  | 0.94                   | 42.77                  | 0.78                   | 11.98              | 31.55                       | 0.06                        |
| 16  | TA—24 h+ 1000 R | 37.08                  | 0.98                   | 40.84                  | 0.84                   | 12.95              | 37.53                       | 0.05                        |

TA = tap water

A + T = anaphases + telophases

Table 2

The catalase and peroxidase activities in wheat germs and seedlings (*Fundulea-29* cultivar) originating from gamma irradiation of water moistened (6, 12, 24 hours) seeds

| No. | The treatment   | 48 hours                                   |            |                                            |            | 124 hours                                  |            |                                            |            |
|-----|-----------------|--------------------------------------------|------------|--------------------------------------------|------------|--------------------------------------------|------------|--------------------------------------------|------------|
|     |                 | Catalase activity                          |            | Peroxidase activity                        |            | Catalase activity                          |            | Peroxidase activity                        |            |
|     |                 | H <sub>2</sub> O <sub>2</sub> mg/gr. fr.s. | Sp. activ. | H <sub>2</sub> O <sub>2</sub> mg/gr. fr.s. | Sp. activ. | H <sub>2</sub> O <sub>2</sub> mg/gr. fr.s. | Sp. activ. | H <sub>2</sub> O <sub>2</sub> mg/gr. fr.s. | Sp. activ. |
| 1   | Control         | 21.58                                      | 2.855      | 41.250                                     | 5.457      | 27.39                                      | 4.015      | 27.416                                     | 4.019      |
| 2   | TA—6 h+ 100 R   | 43.16                                      | 5.740      | 40.416                                     | 5.375      | 29.54                                      | 4.856      | 23.333                                     | 3.440      |
| 3   | TA—6 h+ 300 R   | 44.82                                      | 5.960      | 38.500                                     | 5.913      | 29.88                                      | 5.006      | 25.833                                     | 4.328      |
| 4   | TA—6 h+ 500 R   | 48.14                                      | 6.750      | 40.000                                     | 5.609      | 37.35                                      | 5.702      | 30.666                                     | 4.681      |
| 5   | TA—6 h+ 750 R   | 56.44                                      | 7.506      | 40.833                                     | 5.430      | 39.84                                      | 5.907      | 31.666                                     | 4.695      |
| 6   | TA—6 h+ 1000 R  | 58.10                                      | 9.369      | 39.583                                     | 6.383      | 39.01                                      | 6.941      | 33.333                                     | 5.913      |
| 7   | TA—12 h+ 100 R  | 43.99                                      | 6.236      | 42.500                                     | 6.024      | 42.33                                      | 7.281      | 28.500                                     | 4.902      |
| 8   | TA—12 h+ 300 R  | 41.50                                      | 6.298      | 39.160                                     | 5.944      | 48.82                                      | 8.030      | 27.833                                     | 4.987      |
| 9   | TA—12 h+ 500 R  | 45.65                                      | 6.731      | 39.416                                     | 5.813      | 41.50                                      | 9.080      | 22.500                                     | 5.048      |
| 10  | TA—12 h+ 750 R  | 58.93                                      | 8.447      | 40.000                                     | 5.891      | 43.99                                      | 7.584      | 26.166                                     | 4.501      |
| 11  | TA—12 h+ 1000 R | 59.76                                      | 8.662      | 39.583                                     | 5.745      | 45.65                                      | 7.870      | 33.333                                     | 5.657      |
| 12  | TA—24 h+ 100 R  | 44.16                                      | 7.679      | 32.916                                     | 5.857      | 52.29                                      | 7.844      | 30.416                                     | 4.567      |
| 13  | TA—24 h+ 300 R  | 61.42                                      | 8.902      | 40.166                                     | 5.822      | 53.95                                      | 7.648      | 32.333                                     | 4.583      |
| 14  | TA—24 h+ 500 R  | 64.74                                      | 8.980      | 41.666                                     | 5.787      | 47.31                                      | 7.735      | 30.833                                     | 6.029      |
| 15  | TA—24 h+ 750 R  | 65.57                                      | 8.684      | 39.583                                     | 5.242      | 56.95                                      | 8.761      | 25.416                                     | 3.904      |
| 16  | TA—24 h+ 1000 R | 68.00                                      | 8.773      | 41.250                                     | 5.321      | 58.99                                      | 9.006      | 25.833                                     | 3.944      |

TA = tap water

isoesterase and the isoperoxidase spectrum and activity in the 2 day-old germs and in the 5 day-old plantlets. The catalase and peroxidase activity was quantitatively determined. The methods of analysis were presented in other papers (16), (17), (32). We also determined the content of dry substance, and the dimensions of the roots and stems of 124 hour-old plants, in order to appreciate the effect of gamma rays on plant growth. The results obtained are presented in tables 1–2 and figures 1–4.

#### RESULTS AND DISCUSSION

Our investigations clearly pointed out the impact of the water content of the seeds at the irradiation moment on the effects induced in the plants by the gamma rays. The longer the moistening period before irradiation, the higher the frequency of the induced cytogenetic disturbances, and plant growth was more strongly inhibited (Table 1), although the irradiation doses were rather reduced. Our results confirm the data obtained by many authors (2)–(7), (9)–(11), (23), (24), (27), (31) who studied the relation: radiosensitivity — water content of the seeds for different species, under different experimental conditions.

Between some limits of the water content, characteristic of the repose state, the seeds show a lower radiosensitivity. Excess of these limits, either in the sense of reducing the water content, or increasing, is usually associated with an obvious increase of the seed radiosensitivity.



The low doses of gamma rays, as these used by us, and even greater ones, have, as many authors proved (8), (22), (26), (29) a stimulating effect on the physiological and biochemical processes in plants. In our experiment, the doses used induced a slight stimulation of the plant growing processes only in the case of 6 hours moistened seeds (water con-

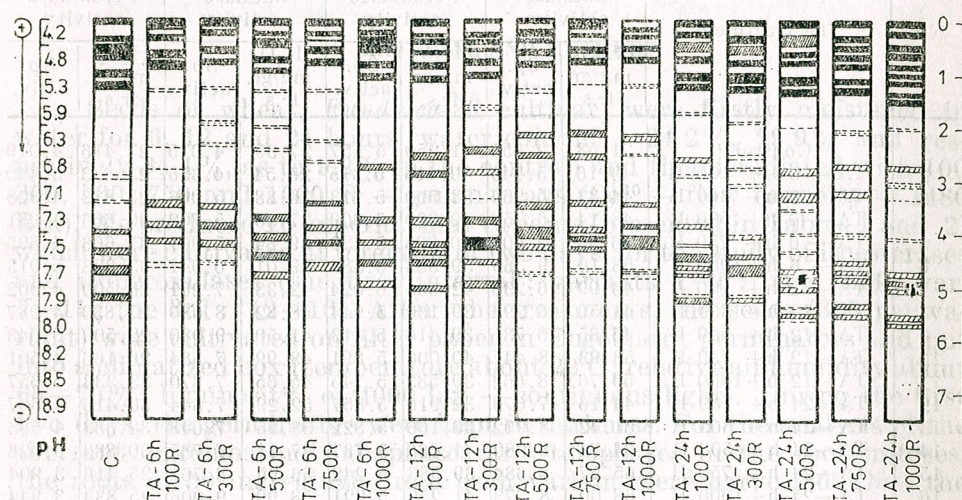


Fig. 1 — The zymograms of isoenzymes in 2 day-old wheat germs (*Fundulea 29* — cultivar) originating from gamma irradiation of water moistened (6, 12, 24 hours) seeds.

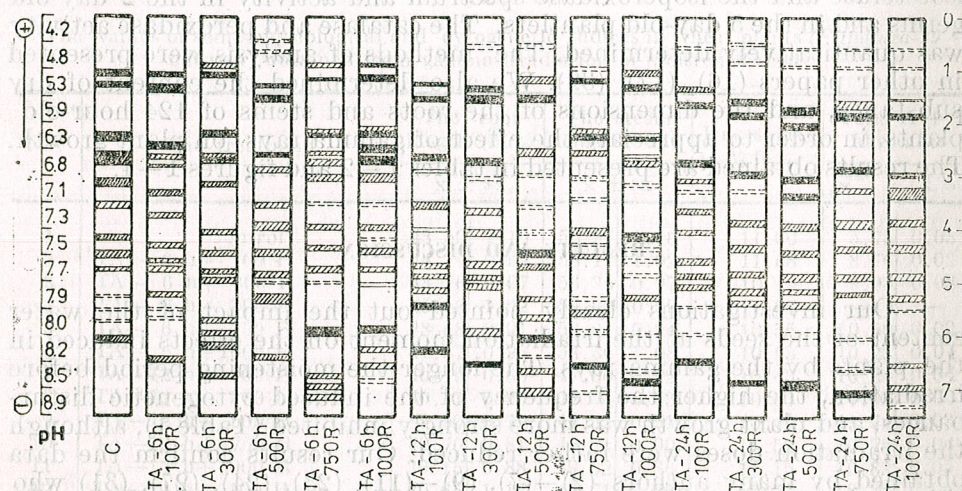


Fig. 2 — The zymograms of isoperoxidases in 2 day-old wheat germs (*Fundulea 29* — cultivar) originating from gamma irradiation of water moistened (6, 12, 24 hours) seeds.

tent of 24.2%), and only in the dose interval of 100 — 750 R. It is interesting to notice that this stimulation takes place in the circumstances in which the frequency of chromosomal aberrant cells in these plants is

higher than in the control ones. But, paradoxically, although the frequency of chromosomal aberrations in the plants irradiated after 12 hours of water moistening (water content of 29.9%) is approximately the same with that of the plants originated from seeds irradiated after 6 hours of moistening, their growth is obviously inhibited. Probably the

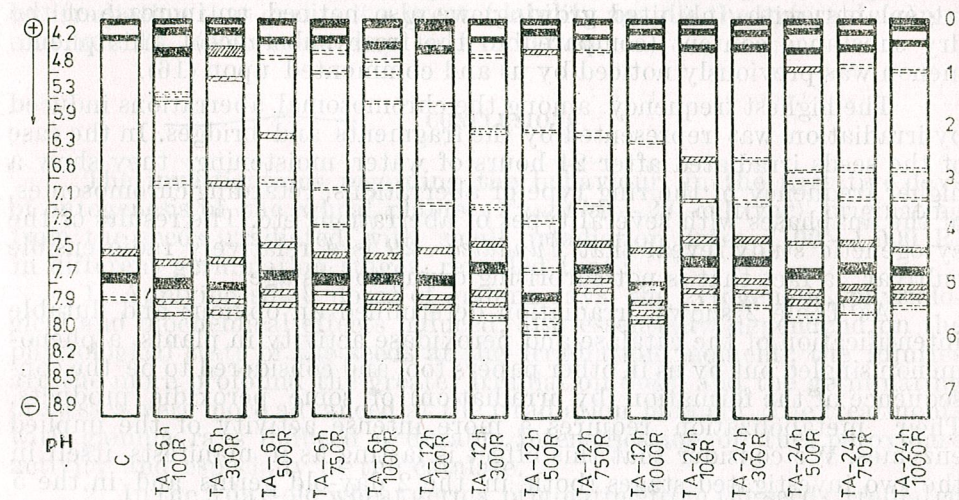


Fig. 3 — The zymograms of isoenzymes in 5 day-old wheat seedlings originating from gamma irradiation of water moistened seeds.

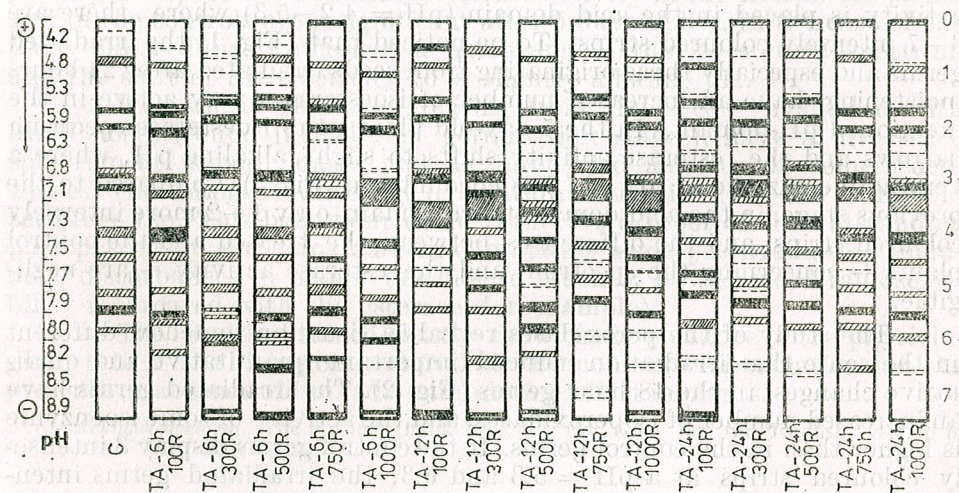


Fig. 4 — The zymograms of isoperoxidase of 5 day-old wheat seedlings originating from gamma irradiation of water moistened seeds.

postirradiation recovering processes were more profoundly disturbed. Irradiation of the seeds after 24 hours of water moistening (when the metabolic processes that provide germination are very active) induced a



very high frequency of aberrant cells, affecting, for examples when using 1000 R, 37.53 % of the analyzed anaphases and telophases. The cytogenetic injuries caused by the gamma radiations, probably associated with diminishing recovering processes, led to difficulties in the physiological and biochemical processes, in the plants, which finally had a negative effect on the growth (Table 1). In the plants irradiated after 24 hours of moistening (plants with inhibited growing) we also noticed an increase of the dry substance content (compared to the fresh substance). This phenomenon was previously noticed by us and commented upon (16).

The highest frequency among the chromosomal aberrations induced by irradiation was represented by the fragments and bridges. In the case of the seeds irradiated after 24 hours of water moistening, they show a higher frequency of the ring type of aberrations, retarding chromosomes, of the anaphases with several types of aberrations etc. The results of the cytogenetic study reveal that *Fundulea-29* is a relatively radiosensible cultivar, a fact that is not surprising owing to its age.

As Table 2 shows, irradiation determined an obvious and durable intensification of the catalase and peroxidase activity in plants, a phenomenon singled out by us in other papers too, and considered to be the consequence of the formation (by irradiation) of some peroxidic products. Their metabolization requires a more intense activity of the implied enzymes. We consider that this effect is lasting as it manifests itself in the two investigated stages (both in the 2 day-old germs and in the 5 day-old seedlings).

The isoesterase spectrum of the irradiated and non-irradiated germs does not differ very much. In this ontogenetic stage, the esterase activity is placed in the acid domain ( $\text{pH} = 4.2-5.3$ ) where there are 3-7 intensely coloured strips. To be noticed that (Fig. 1) the irradiated germs and especially those originating from seeds irradiated after 24 hours moistening, have an increased number of isoesterases very active in the mentioned pH-domain. In the 5 day-old plants, the isoesterase spectrum narrows and the esterase activity shifts to slight alkaline pH, where a very active enzyme at  $\text{pH} = 7.7$  is encountered (Fig. 3). Compared to the previous stage, in the acid domain there remain only 1-2 more intensely coloured strips, and the differences between the treated and the control plants — concerning the spectrum and the esterase activity — are negligible.

The study of the peroxidases reveals a situation somehow different in the sense that irradiation induced important quantitative and qualitative changes in the 48 hour-germs (Fig. 2). The irradiated germs have an increased number of isoperoxidases, and the activity of some isoenzyme is higher than in the control germs. If the control germs display 3 intensely coloured strips at a  $\text{pH} = 5.3$  and 6.3, the irradiated germs intensify some isoenzymes acting in the alkaline pH (the isoenzymes with  $\text{pH} = 7.3; 8.0; 8.2$ ), (Fig. 2). The differences regarding the spectrum and the isoperoxidase activity are also notable in the 5 day-old wheat seedlings (Fig. 4). The irradiated seedlings have a broader isoenzymatic spectrum as compared with the control, and the number of active isoperoxidases is also higher. If the controls have only 4 intensely coloured strips

( $\text{pH} = 5.9; 6.8; 7.9$ ), the irradiated seedlings have a series of active isoperoxidases in the pH interval of 7.3-7.7 and 8.0-8.2 (Fig. 4).

The present results confirm our previous data according to which the peroxidase of wheat is an enzyme more sensitive to irradiation as compared to the esterase. Probably it is the more important part played by the peroxidase in eliminating some toxic metabolic products induced by irradiation that grants it a greater lability against the external agents, and not its lower resistancy to irradiation.

## CONCLUSIONS

The investigations regarding the behaviour, in the first five days of ontogenesis of the wheat plants (*Fundulea-29* cultivar) originating from the seeds irradiated with small irradiation doses (100-1.000 R) in different germination stages, revealed that:

1. Applying small doses of gamma rays, the cytogenetic, physiological and biochemical effects induced, also essentially dependent on the physiological state of the seeds at the irradiation moment; the injuries are the more profound the greater irradiation dose, and the germinating processes were more advanced at the irradiation moment. The treatments with gamma rays induced a durable intensification of the peroxidase activity and especially of the catalase.

2. In the 2 day-old wheat germs, originating from the seeds irradiated after 24 hours of water moistening, the number and the activity of the isoesterases from the pH interval of 4.2-5.3 increase. The differences regarding the spectrum and the isoesterase activity in the irradiated plants and in the controls are insignificant after 5 days of life. In the case of the 5 day-old seedlings we noticed a narrowing of the isoesterase spectrum and the shift of their activity to a slightly alkaline pH.

3. The wheat plants originating from the seeds irradiated with small doses (found in different germination stages), have a diversified spectrum of isoperoxidases and an intensified activity of some isoenzymes. The changes induced by irradiation maintained in the 5 day-old seedlings too. This behaviour could rather be a proof of the outstanding part played by the peroxidase in eliminating some postirradiatory metabolic disturbances in plants and not so much of its increased radiosensitivity (compared with the esterase, for example).

4. The results obtained clearly show the importance of the physiological state of the biological material at the moment of irradiation for the presence or absence of the radio-stimulating effect in plants.

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## IS THE NUCLEOLUS AN INTRANUCLEAR ORGANELLE OR A FUNCTIONAL TRANSIENT ELEMENT OF CHROMOSOMAL PHENOTYPE?

LUCIAN GAVRILĂ and GRIGORE MIHĂESCU

The investigations performed with different types of cells in microorganisms, plants and animals, especially in insects, using light- and electron-microscopy, allowed us to reach the conclusion that the nucleolus is rather a functional transient element of chromosomal phenotype, than an intranuclear organelle, because its appearance, shape and size are largely dependent on the type and level of cell activity.

### INTRODUCTION

The existence of nucleolus was signalled by Fontana in 1774 and its description was done by Bowman in 1840, but its functional significance remained unclear for many years. In 1934, Barbara McClintock [8] established that the nucleolus is organized at the level of secondary constrictions in *Zea mays* chromosomes, any alterations in such constrictions leading to the appearance of corn mutants [9]. Such secondary constrictions involved in the organization of nucleolus have been designated as nucleolar-organizing regions (NORs), while the chromosomes which bear such regions have been called nucleolar-organizing chromosomes (NO-chromosomes). Now it is very well documented that in the NORs there are localized the cistrons for 18 S, 28 S and 5,8 S rRNAs [1-3], [10]. Every diploid cell contains, as a rule, two NO-chromosomes, one for every haploid chromosomal complement [2]. Consequently, there are two nucleoli per diploid nucleus. On the other hand, instances are found in which as much as five NO-chromosomes are involved in the organization of the same nucleolus, as in the human being where the chromosomes from the 13th, 14th, 15th, 21st, 22nd chromosomal pairs are NO-chromosomes. There is also a direct correlation between the number of nucleoli per nucleus and its degree of ploidy. As a common rule, at the end of prophase and the beginning of metaphase the nucleolus is breaking down, its reorganization taking place at the end of mitotic division. It has been considered that the nucleolus "disappears" in the period when chromosomes are in the distributive period of the cell cycle, when they reach an extreme condensed state and consequently are non-functioning structures. At the end of division, between telophase and  $G_1$  period of cell cycle, the nucleolus is "organized". This period corresponds to that part of cell cycle when the chromosomes are in their uncondensed



state and consequently are functional structures. Only in some unicellular organisms as in *Euglena*, the nucleolus, called here endosome, plays an important role in orientation and segregation of daughter-chromosomes in the particular type of nuclear division which is called englenoid division. In this process the endosome (i.e. nucleolus) plays an important role in orientation and segregation of duplicated chromosomes assuming a dumb-bell shape and in this way the dividing cell is polarized.

Our investigations in different types of organisms and cells have imposed the conclusion that the nucleolus is rather a functional transient element than an intra nuclear organelle in its "classical" meaning.

#### MATERIAL AND METHODS

As biological material we have used the following organisms: *Prorocentrum micans* (mesokaryote alga), *Euglena terricola* and *E. clara* (lower eukaryotes, unicellular photosynthetic flagellate green-algae), root-type nodules cells of *Trifolium pratense* (leguminose spermatophytic plant), polytenic nuclei from salivary gland cells of *Rhagoletis cerasi*, *Mycetophila agarici*, *Corethra* sp. and *Chironomus tentans* (Dipteran insects) and endoploid cells of silk glands from silkworm *Philosamia ricini* (Lepidopteran insects). The biological material has been worked out according to standard methods for investigation of nuclei, chromatin and chromosomes by light- and electron-microscopy (TEM).

#### RESULTS AND DISCUSSIONS

The nucleolus in mesokaryotes (dinoflagellate algae) has a typical structure similar to that encountered in typical eukaryotes (Fig. 1A). Our investigations in mesokaryote alga *Prorocentrum micans* have revealed for the first time the existence of more than one nucleolus-organizing chromosome which are involved in the organization of the same large nucleolus (Fig. 1 B). On the phylogenetic scale, the dinoflagellates represent the first group with cellular organization in which the organization and individualization of a nucleolus take place. This is the most primitive group of cellular organisms in which appears also a nucleus that encloses the genetic material in a specific space delimited by a nuclear envelope. Like eukaryotic nucleolus, the mesokaryote nucleolus has a fibrillar component and a granular one (Fig. 1A). At the level of nucleolar-organizing region of nucleolar-organizing chromosome there is a decondensed fibrillar structure similar to that which is met in the puffs or Balbiani rings from polytenic dipteran chromosomes. In the remaining parts of mesokaryote nucleolar-organizing chromosome there is a typical banded structure characteristic of mesokaryote chromosomes, representing series of nested arcs of DNA fibrils in an anorthospiral arrangement of eight figures. Considering the nucleolar-organizing regions of nucleolar-organizing

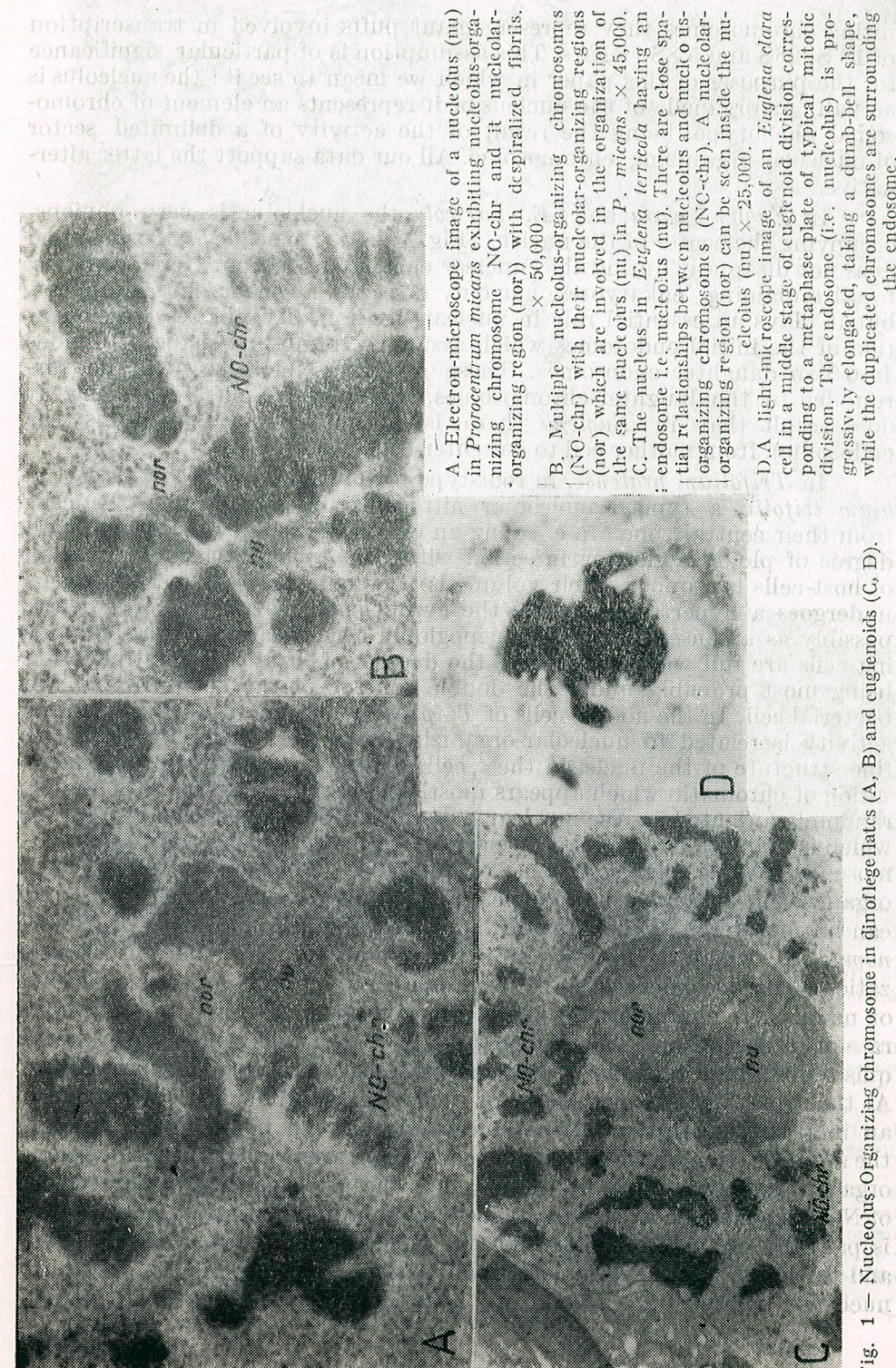


Fig. 1 — Nucleolus-Organizing chromosome in dinoflagellates (A, B) and euglenoids (C, D).



nizing chromosomes they represent giant puffs involved in transcription of 18 S, 28S and 5.8S rRNAs. This assumption is of particular significance for the purposes of this paper in which we mean to see it: the nucleolus is actually an organelle of the nucleus or it represents an element of chromosomal phenotype, being the result of the activity of a delimited sector of nucleolar-organizing chromosome. All our data support the latter alternative.

In *Euglena clara* and *E. terricola* the nucleolus is very obvious, occupying the center of the nucleus (Fig. 1.C.D). It is called endosome and does not disappear during the nuclear euglenoid division. Here, contrary to all remaining eukaryotes, not only does the nucleolus not disappear, but it plays an essential role in nuclear division. By progressive elongation of the initial endosomes which assume a dumb-bell shape, it divides into two daughter endosomes. These daughter-endosomes will be surrounded by the daughter-chromosomes, two daughter-nuclei resulting. It does result that in *Euglena* there is a continuity of nucleolus (the endosome), from mother cell to daughter-cells.

In *Trifolium pratense*, in root-type nodules in symbiosis with *Rhizobium trifolii*, a typical molecular nitrogen-fixing bacterium, the cells from their central zone are entering an endocycle so they achieve a high degree of ploidy. Following infection with *R. trifolii*, the multiplication of host-cells is stopped, their volume is very much increased, the nucleus undergoes a hypertrophy, while the cytoplasmic matrix becomes dense, possibly as a consequence of leghemoglobin accumulation. Such nondividing cells are full with bacteroids, the degree of bacterial multiplication being most probably under the double control of the host-cell and the bacterial cell. In the nodule cells of *T. pratense*, the most obvious nuclear activity is related to nucleolar-organizing function (Figs. 2 A-D). The fine structure of the nuclei in these cells exhibits an extensive decondensation of chromatin which appears mostly as fine fibrils of 100 Å diameter, randomly oriented in the nuclear space. The only chromatic structure which is maintained in a condensed state is the nucleolar-organizing chromosome. Its nucleolar-organizing region (NOR) involved in the nucleolus organization exhibits a more or less relaxed distribution of its fibrils and can be considered as a giant puff, as in polytenic chromosomes of *Chironomus* sp., where the largest puff is that connected with nucleolus organization. The decondensed state of chromatin in the cells in the central zone of nodules, is characteristic of an intensive gene activity, i.e. of a high rate of transcription. The endopolyploidy of host cells appears as a prerequisite for a high capacity of protein synthesis, especially of leghemoglobin. At the same time it is necessary for such high rates of synthesis to achieve an increased quantity of ribosomes and this can be obtained through the amplification of the ribosomal genes which are located in the nucleolar-organizing region of NO-chromosome. The nucleolar-organizing region of NO-chromosome is located submedianly. In fine sections it looks as if it is partially or totally penetrating the nucleolus, depending on the plane and thickness of the section. In some instances one can see the release of nucleolar bodies — a common cytologic sign of ribosomal gene ampli-

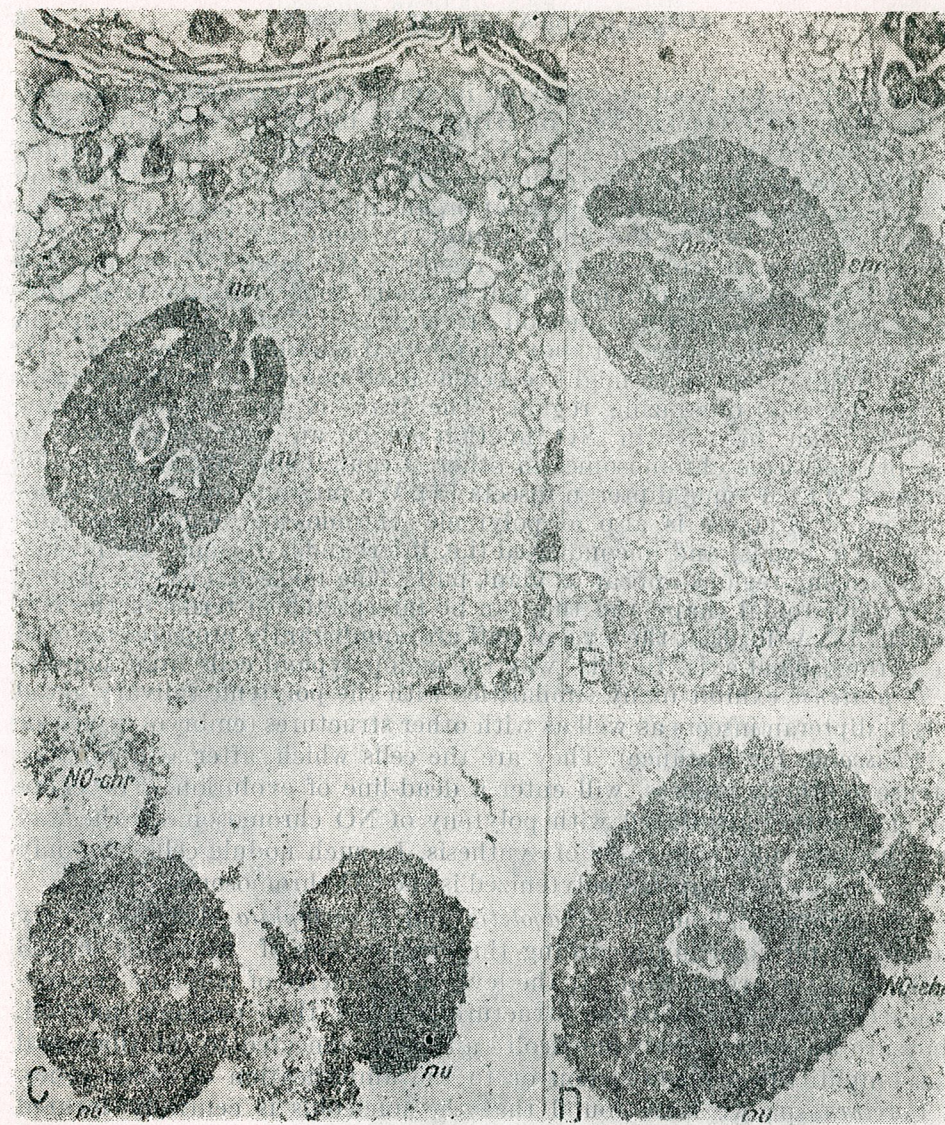


Fig. 2. — The nucleolus and nucleolar-organizing chromosome in the central cells of root-type nodules of *Trifolium pratense* in symbiosis with nitrogen-fixing bacteria *Rhizobium trifolii*.

- A. An overall image of a nucleus (N) with nucleolus (nu) in which several nucleolar-organizing regions (nor) can be seen. The nucleolar-organizing region appears as a giant chromosomal puff. R-bacteroids of *R. trifolii*.  $\times 22,000$ .
- B. A sector of a cell from a nodule having in its nucleus a nucleolus which is penetrated by the nucleolar-organizing region (nor) of the nucleolar-organizing chromosome (Chr). This chromosome is attached to the inner membrane of the nuclear envelope. In cytoplasm a multitude of bacteroid prophiles of *R. trifolii* (R).  $\times 25,000$ .
- C. Nucleolar-organizing chromosome (NO-Chr) from a nodule cell exhibiting a banded structure (i.e. polyteny). This chromosome has two separated sites in which two independent nucleolar-organizing regions (nucleolar-organizing region duplication) are involved in the organization of two distinct nucleoli (nu). The active nucleolar-organizing sites, representing giant nucleolar puffs, are largely separated by a region in which the banding pattern is irregular.  $\times 50,000$ .
- D. The nucleolus (nu) and a sector from the nucleolar-organizing chromosome (NO-Chr) displaying two profiles of its nucleolar-organizing region.  $\times 55,000$ .



fication which has been encountered in amphibian oogenesis [4], as well as in experimental conditions in HEp2 cells infected with viruses, or treated with thyoacetamide [6], [7]. It is also possible that, paralleling the endopolyploidization (i.e. endocycles) of the cells, an extrareplication of ribosomal cistrons from nucleolar-organizing region takes place. This assumption is based on the identification in some instances of a multiplication of the nucleolar-organizing region of the same NO-chromosome (fig. 2C). Here, in two different, well spatially separated nucleolar-organizing regions, two distinct nucleoli are organized. The same assumption can be supported by the fact that the released nucleolar-bodies (Fig. 2D) bear a fibrillar core which might represent itself one of the multiple copies of the nucleolar-organizing region. The parts of the NO-chromosome which are not involved in NO function exhibit an aspect very close to that of polytenic chromosomes in other Leguminosae like in *Phaseolus coccineus* [11] or some dipteran insects [5]. We presume this NO chromosome in *T. pratense* is also a polytenic chromosome, exhibiting condensed (i.e. bands) and uncondensed (i.e. interbands) regions. Its nucleolar-organizing regions appear as giant puffs. The nucleolus has an almost perfect spheroidal shape and this can be interpreted in terms of the fact that the DNA fibrils at the level of puff are equidistantly projected around the chromosomal axis. In this way the endopolyploid cells from nodules of *T. pratense* exhibit many similarities with the polytenic salivary gland cells in dipteran insects as well as with other structures (embryo suspensor in *Phaseolus* for instance). They are the cells which, after a short, very intense synthesis process, will enter a dead-line of evolution. The endopolyploidization associated with polyteny of NO chromosome is the way for achieving such high rates of synthesis. In such nodule cells, the only chromosome which appears polytenized is the NO chromosome.

In salivary glands of *Rhagoletis* and *Mycetophila* there is a very interesting phenomenon regarding the organization of nucleolus (Fig. 3 C, D). It is the organization at the level of telomeres of polytene chromosomes of some nucleolus-like structures, larger than common nucleoli. They are known as "Nebennucleoli" and are connected to the ribosomal gene amplification phenomenon or to the amplification of other genes. In *Corethra* sp; we have found that the nucleolus is centrally located inside the nucleus (Fig. 3 E), and instances with multiple nucleoli and nucleolar puffs are sometimes encountered (unpublished results).

In *Chironomus tentans*, the chromosome IV is the nucleolar-organizing chromosome (Fig. 3 A, B). The nucleolus is organized at the level of giant symmetrical puffs. At the level of the nucleolar-organizing region, the chromatin fibers are looping from the chromosomal axis and return to it in precise sites. The nucleolus organization takes place at the end of the third larval stage and by the end of the fourth larval stage it is completed. In these insects, like in *Bombyx mori* and *Triturus vulgaris* oogenesis, typical aspects of ribosomal gene amplification have been encountered, which result in the appearance of multiple nucleoli. Similar aspects have also been encountered in silk glands of silk worms. [4].

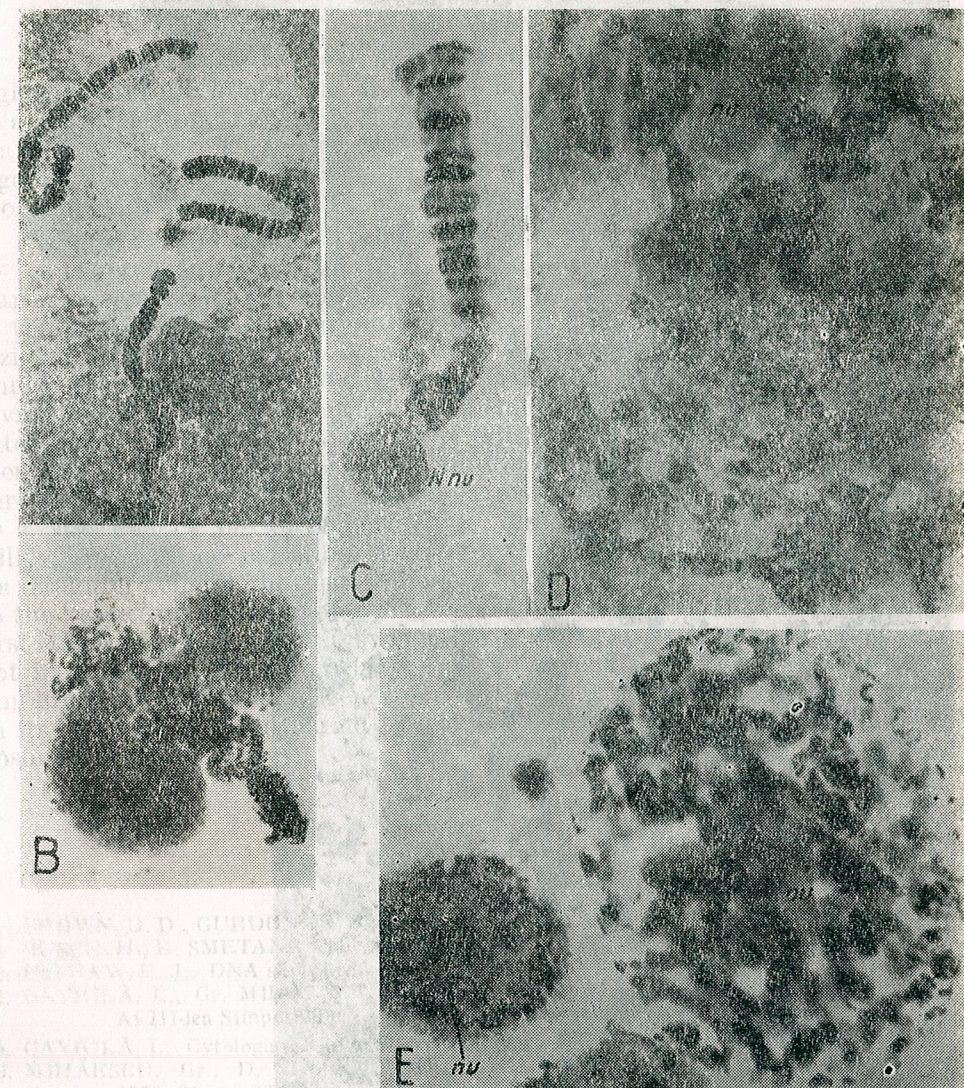


Fig. 3. — Nucleolus-organizing chromosome in polytene nuclei of salivary-gland cells of *Chironomus Tentans* (A, B), *Mycetophila garici* (C), *Rhagoletis Cerasi*, (D) and *Corethra* Sp. (E) larvae.

nu-nucleolus; Nnu — "Nebennucleoli". See the text for more details.

It is very important to note that in silk gland cells of *Phylosamia ricini* (fig. 4) the endopolyploidization is associated with polyteny of chromosomes as well as with clear aspects of ribosomal gene amplification revealed by the presence of a rich population of extrachromosomal multiple nucleoli.



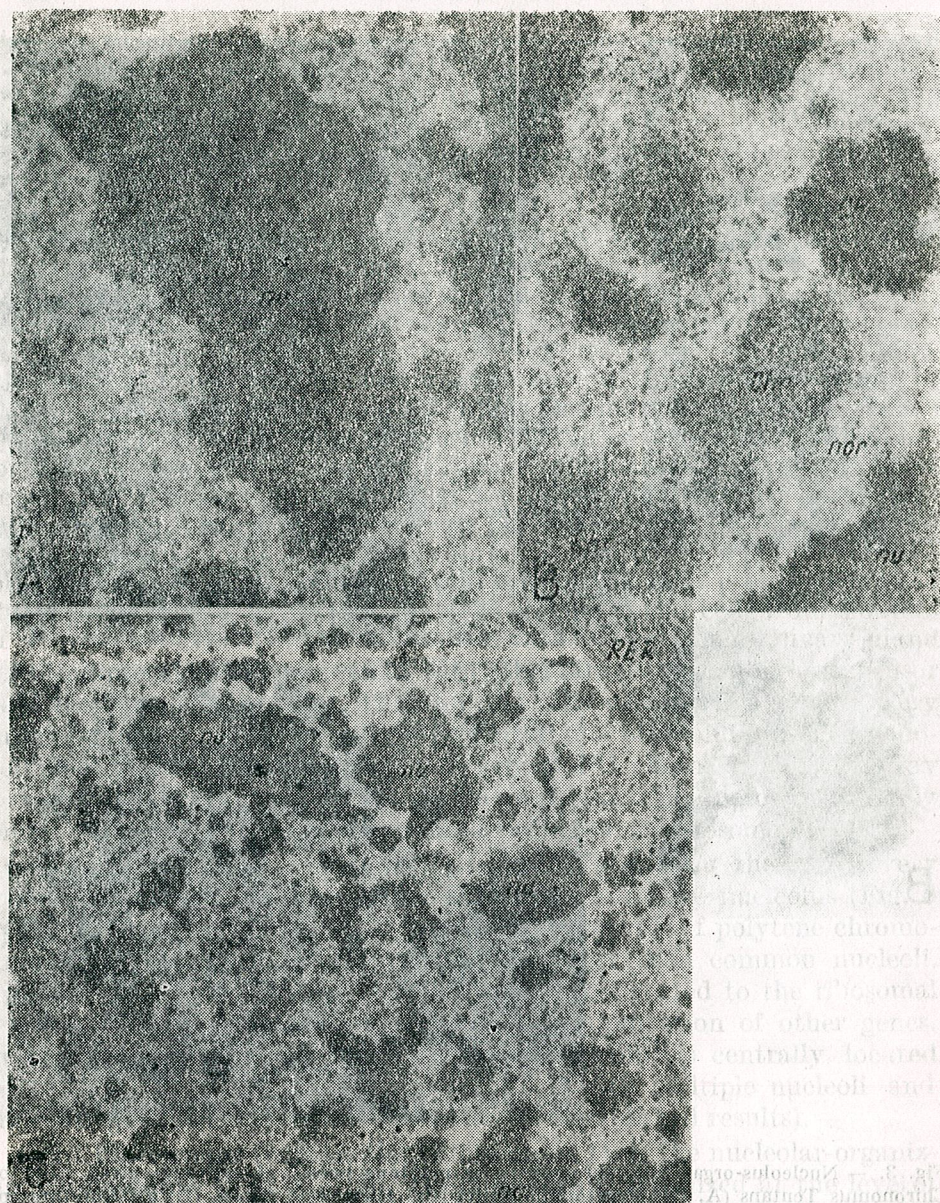


Fig. 4. — Ribosomal-gene amplification expressed by the presence of multiple extra-chromosomal nucleoli in silkglands of silkworm *Phyllosamia ricini*.  
 A. A small portion from a polymorphic nucleus of a silkgland cell with a large extra-chromosomal nucleolus (nu), heterochromatin (H) in intimate contact with nuclear envelope and diffuse euchromatin (E) randomly distributed inside the nucleus. In the cytoplasm there is a richness of polysomes (P). The cell is highly synthesizing.  $\times 50,000$ .  
 B. Nucleolus-organizing chromosome (Chr) from the nucleus of silkgland cell with its nucleolar-organizing region (nor) in intimate structural relationships with a part of a giant nucleolus (nu). This chromosome is a polytene one revealing a specific banded pattern with an alternation of blocks (i.e. bands) which differ in their electron-density (i.e. differing in the condensation of chromatin fibres).  $\times 65,000$ .  
 C. A part from a polymorphic nucleus of a silkgland cell in which multiple extra-chromosomal nucleoli (nu) are randomly distributed. In the cytoplasm there is a richness of rough endoplasmic reticulum (RER).  $\times 40,000$ .

## CONCLUSIONS

In all the biological systems we have studied, from Mesokaryota to higher eukaryotic organisms, the nucleolar-organizing function proved to be an essential one and is performed in identical ways. It does suggest that this function is an ancestral one, appearing as a consequence of the organization of nucleate organisms, remaining unchanged throughout evolution, any alteration of it being lethal.

All our results are converging to the conclusion that the nucleolus must be considered not as an intranuclear organelle, but rather as a transient element of chromosomal phenotype. The nucleolus is the result of the transcription of ribosomal genes, situated in the nucleolar-organizing region of NO-chromosomes. This conclusion is based also on the "nucleolus disappearance" in the Prophase-Metaphase of nuclear mitotic division and its "reappearance" at the end of the division, especially in interphase. There is not actually a "disappearance" of nucleolus, but merely a more or less uniform distribution of nucleolar ribonucleoproteins throughout the mixoplasm (i.e. the mixture of nucleoplasm and cytoplasm as a consequence of nuclear envelope disorganization) and this process will allow an equal partition of ribosomal reserves of the mother cell to its daughter-cells in the plasmodieresis process. It is very much similar to the "care" of oocyte to assure a reserve of ribosomal components in the first stages of zygote evolution when this is engaged in cleavage divisions, not in syntheses. This "mother care" at molecular level will lead to the amplification of ribosomal genes in oocyte due to the fact that the zygote in its cleavage division period is engaged in rapid divisions, — not in ribosomal synthesis.

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# FUNCTIONAL SIGNIFICANCE OF CELL STRUCTURES FROM ROOT NODULES IN *TRIFOLIUM PRATENSE*

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Root nodule cells of *T. pratense* exhibit peculiar features of fine nuclear and nucleolar structure. They lack heterochromatin and possess a compact nucleolar organizing chromosome. These are highly functionally specialized cells representing the link between two partners of association: the bacteria of the *Rhizobium* genus and the host plant. The ribosomal gene amplification from the nucleolar organizing chromosome signifies a high rate of rRNA transcription necessary for cell syntheses. The absence of heterochromatin can be interpreted in terms of a selective endopolyploidization of functional euchromatin. The heterochromatin elimination during successive cell divisions might be considered as an alternative way of selective endopolyploidization. Both phenomena are mechanisms which lead to an extreme functional specialization of root nodule cells.

The root nodules of legume plants are structures which represent a result of an abnormal, but harmonious, differentiated and limited tissue growth, qualitatively different from the other plant organs. The nodule is a result of the interaction between root cells of the host plant and infecting, nitrogen fixing bacteria of the *Rhizobium* genus, leading to the most complex and perfect symbiotic association, in which the plant offers its photosynthetic carbohydrates, while the bacteria, becoming bacteroids, use the energy which results from sugar degradation, to reduce  $N_2$  to  $NH_3$ .

## MATERIAL AND METHODS

Efficient nodules were prefixed in 2% glutaraldehyde buffer and postfixed in 1% osmium tetroxide. After dehydration, the pieces were embedded in Epon. The thin sections were stained with uranyl acetate and lead citrate and examined under a JEM 7 EM.

## RESULTS

The central zone of the nodule constitutes its main part. Immediately after infection the root meristematic cells undergo endopolyploidization (3), reaching a very large volume (Fig. 1). The bacteroids occupy the whole cytoplasmic space. The nucleus and vacuole are located in the central area of the cell. In *T. pratense* the bacteroids do not form abundant populations in the nodule cells. Their number is estimated around



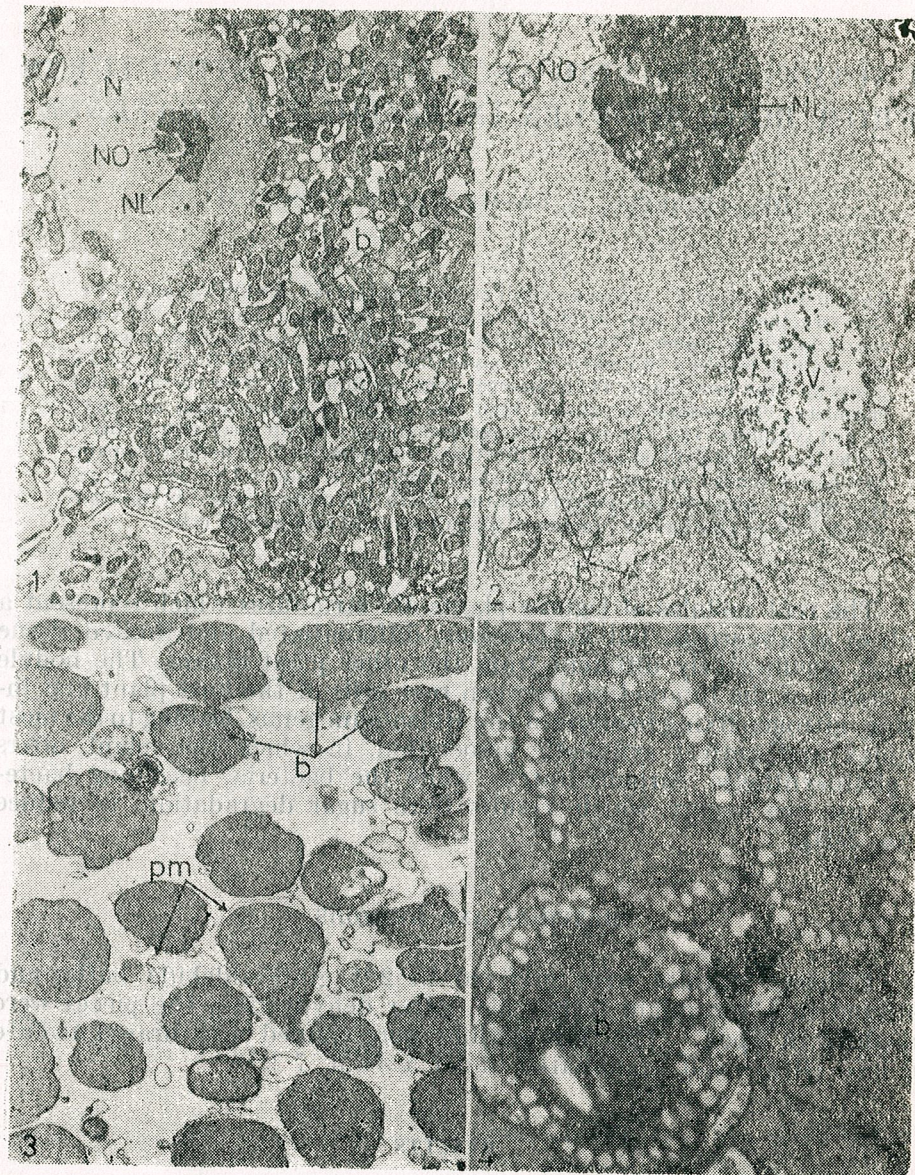


Fig. 1. — The overall view of nodule cell filled with bacteroids (b). In the nucleus (N), the sole electron-dense structures are the nucleolus (NL) and the nucleolus organizing chromosome (NO),  $\times 5400$ .

Fig. 2. — The nucleus has close spatial relationships with the vacuole (V). In the center bottom, a bacteroid of Y shape,  $\times 8500$ .

Fig. 3. — Every bacteroid is surrounded by a fine peribacteroidian membrane (pm),  $\times 18600$ .

Fig. 4. — At the periphery of large bacteroids, the invaginations of the plasmatic membrane form numerous clear vacuoles,  $\times 32000$ .

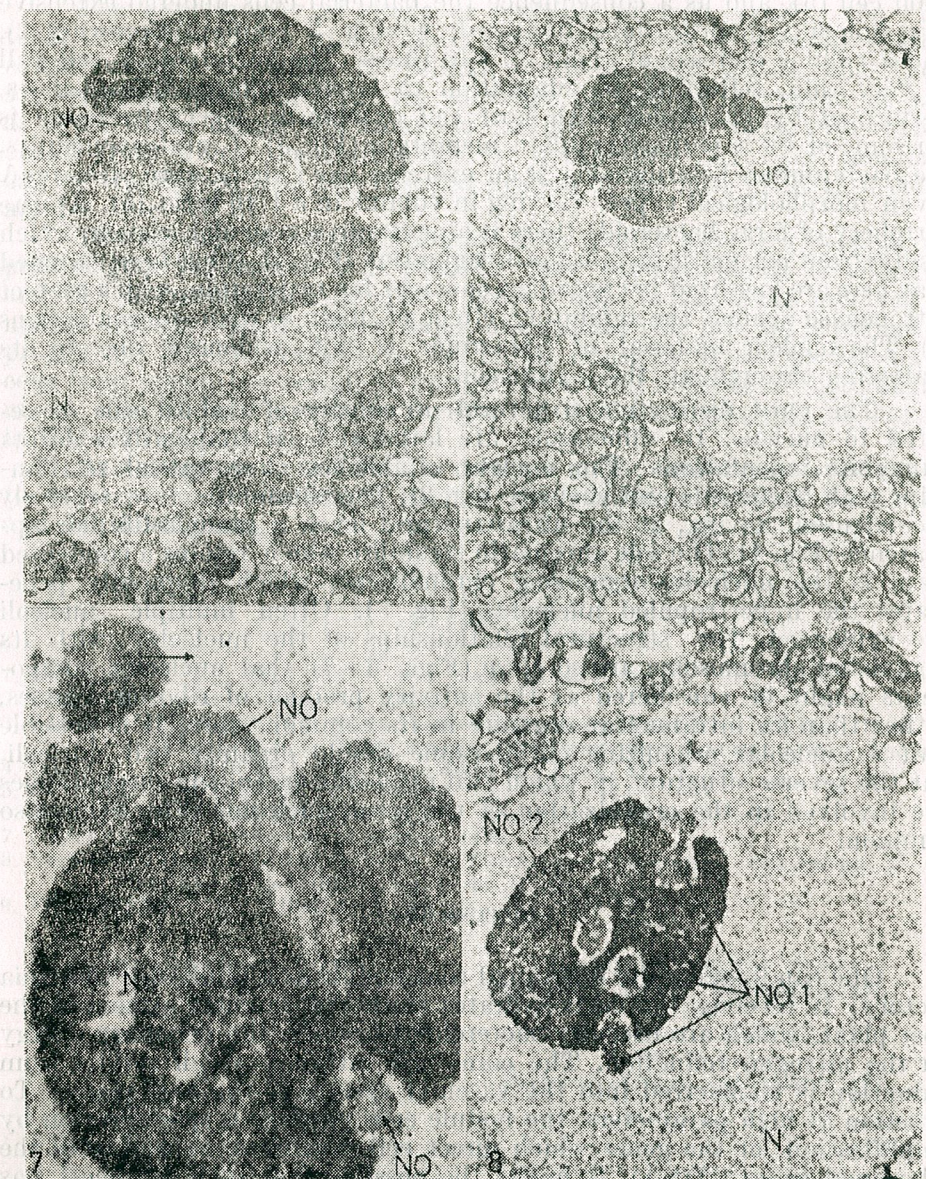


Fig. 5. — The nucleolus organizing chromosome penetrates almost completely the nucleolus,  $\times 18500$ .

Fig. 6. — Nucleolar body release from nucleolus is taking place (arrow). The nucleolus organizing chromosome is well evidenced,  $(\times) 12500$ .

Fig. 7. — A detail of a serial section of Fig. 6. The nucleolar organizer is evidenced along almost its whole length,  $(\times) 40000$ .

Fig. 8. — The nucleolus organizing region is doubled (NO 1 and NO 2),  $\times 8500$ .



1000 cell (5), and as a consequence the bacterial cells undergo extensive morphological modifications, frequently having Y configurations (Fig. 2), and a volume increase of about 30–40 times. The bacteroidian cell-wall is very thin, reflecting an adaptation to intense molecular exchanges, performed by bacteroids with host cell cytoplasm. Every bacteroid is surrounded by a peribacteroidian membrane (Fig. 3). *T. pratense* nodules, the bacteroidian heterogeneity is an extreme one: there were described seven morphological types, varying in volume in a 1/40 ratio (6). At the periphery of large bacteroids there is an extensive vesicular system, which results from plasmatic membrane invagination (Fig. 4). It has been assumed that here are localized bacteroidian enzymes for respiration, which protect nitrogenase against the inhibitory effect of high oxygen concentrations (4). The nuclear material of bacteroids is not evidenced due to its scattering throughout the cytoplasm.

The most distinctive structural peculiarity of nodule cells is related to nucleus. Its dimensions are large and it frequently exhibits numerous indentations. The nucleus establishes close spatial relationships with bacteroids and vacuole (Fig. 2). The chromatin is extensively decondensed, having, at the ultrastructural level, a granulo-fibrillar appearance (Fig. 1). The sole chromatin structure which retains a condensed state is the nucleolus-organizing chromosome, which frequently penetrates the hypertrophied nucleolus (Fig. 5). Often multiple nucleoli are evidenced. The structural relationships of the nucleolus with its organizing chromosome are so close (Figs. 5–7) that nucleolar components might be considered as the activity product of ribosomal genes, rather than an intranuclear organite in its common sense. It is possible that a nucleolar organizing chromosome should organize two nucleoli, but the reverse situation, in which two nucleolus organizing chromosomes are involved in the organization of the same nucleolus (Fig. 8), is also common.

#### DISCUSSIONS

One can imagine the functional root nodule as a miniature ammonia factory. It receives the carbohydrates from the vascular system of the host plant, which are used by bacteroids to obtain the necessary energy for the nitrogen reduction. The combined nitrogen which results from reduction is transferred into the vascular system of the host plant. To function at high parameters, the nodule cells undergo a specialization by amplification of the genes which direct cell syntheses, as well as of the ribosomal genes (rDNA). It might be possible that such a process has been accompanied by an elimination of unfunctional genes, located at the level of heterochromatin. The ribosomal genes, i.e. rDNA, by amplification, constitute a compact structure having close relationships with the nucleolus. Their amplification is firstly the result of the endopolyploidization process of root cortex cells, at the beginning of infection. On the other hand, the extrareplication of ribosomal cistrons localized in nucleolar organizing region cannot be ruled out. This assumption relies on the fact that sometimes the nucleolus organizing region is multiplied (Fig. 8).

The hypertrophy of single or multiple nucleoli signifies the existence of intense rRNA transcription processes as well as assembly of ribosomal subunits, necessary for protein syntheses from nodule cells. There are enzymes which perform the cleavage of sucrose in glucose as well as those which couple the ammonia excreted by bacteroids, with carbon chains from which the aminoacids are synthesised (1,2). The ammonia can be transferred into the vascular system of the host plant as ureides, amides, etc. (7, 8).

The absence of heterochromatin from nodule cells is a peculiar aspect of gene regulation. It is possible that endopolyploidy is selective, and the extrareplication process is limited only to the genes involved in nodule activity. On the other hand, one cannot rule out an elimination process of heterochromatin in the initial divisions of the meristematic cells from which the nodule is formed. The total absence of heterochromatin can be interpreted as facilitating nucleo-cytoplasmic exchanges in the cells of functional nodule.

As a conclusion, the nodule cells are highly functionally specialized, to serve as an intermediary step in the symbiosis between bacteroids and the host plant.

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## RELICTES DANS LA FLORE DE ROUMANIE

## II

*HELODIUM BLANDOWII* (Web. & Mohr) Warnst., Krypt. Fl. Brandenburg, 1905.

Les synonymes appartiennent ( $\pm$  chronologiquement) aux genres *Hypnum*, *Thuidium*, *Helodium*, dont nous mentionnons : *Hypnum lanatum* Stroem. (1791), *H. affine* Crome (1806), *H. blandowii* Web. & Mohr (1807), Sturm (1809), *H. lanatum* Stroem. ex Brid. (1819), *H. filicinum* var. *lanatum* Brid. (1827), *H. laricinum* Wils. in Hook. & Arn. (1831), *H. abietinum* var. *paludosum* Wahlenb. (1831), Meyl. (1921); *Thuidium blandowii* (Web. & Mohr) B., S. & G. (1852), *T. lanatum* Hagen (1897, 1907), *T. lanatum* (Brid.) Hagen (1897), *T. lanatum* (Stroem.) Hagen (1897), *T. pseudo-abietinum* Kindb. (1902), *T. lanatum* (Stroem.) Moenk. (1927); *Helodium lanatum* Stroem. (1791), *H. lanatum* (Sull.) Warnst. (1905), *H. lanatum* (Stroem.) Broth. (1908, 1925), *H. lanatum* (Brid.) Broth. (1908) et autres, nom. illeg., parmi lesquelles les dénominations suggestives qui rendent l'aspect morphologique, avec l'épithète spécifique de *lanatum* et écologique sous celui de var. *paludosum*.

Dans la flore de l'Amérique du Nord, par A. J. Grout (III, 4, 1934, p. 180) est connue l'espèce *Helodium paludosum* (Sull.) Aust. (1870), départ. Ohio (Gray Herb. Harvard University), étant décrite aussi la var. *heloldioides* (R. & C.) Grout (1929).

Au sujet des synonymes de l'espèce *Helodium blandowii*, voyez les notes infrapaginales 3 — 4 (p. 238 — 239) (Tr. I. Ștefureac, 1956), ouvrage où l'on apporte en même temps de nouvelles précisions et certains compléments morpho-anatomiques à sa diagnose. Les données se réfèrent surtout à nos observations de terrain et d'analyse, quantitative et qualitative, de l'espèce *Helodium blandowii* de toutes les stations connues jusqu'à présent en Roumanie.

Le genre *Helodium* Warnst. nom. cons., comprend 4 espèces frig.-temp. \* dont une seule est représentée en Europe, comme chez nous : *H. blandowii*, fam. *Thuidiaceae* Kindb., sous-fam. *Helodioideae* d'après Brotherus — 1925 (sect. *Elodium* Sull., ou sous-genre *Thuidiella* Schimp.).

L'habitus de l'espèce a, du point de vue macroscopique, des ressemblances avec *Abietinella abietina* (Hedw.) B., S. & G., se distinguant par des tiges et des feuilles molles (non pas raides ou épineuses). Mais elle diffère, morphologiquement et écologiquement, car elles croissent, hygrophiles, en touffes  $\pm$  compactes ou isolée parmi d'autres mousses, 5 — 10 (20) cm en hauteur, faiblement luisantes, d'un vert foncé, dans des lieux ombragés et vert-jaunâtre, jaunâtre ou bien souvent jaunes-brunes, dans des marais  $\pm$  ouverts; mousse pléurocarpe, à croissance en général érecte (touffes denses), tiges simplement pennées, ramifiées régulièrement ou es-



pacées (comme chez *Ctenidium*, *Ptilidium* etc.); les feuilles caulinaires grandes,  $\pm$  cordiformes, larges à la base, légèrement pliées, révolutes aux bords et la nervure arrivant au delà du milieu de la lamine; elle présente, comme caractère spécifique à la base des feuilles, ainsi que sur les tiges et sur les rameaux, de nombreux paraphylles filiformes (blancs-bruns) longs et abondamment ramifiés, couvrant la tige d'un feutre dense (*lanatum*), caracté-

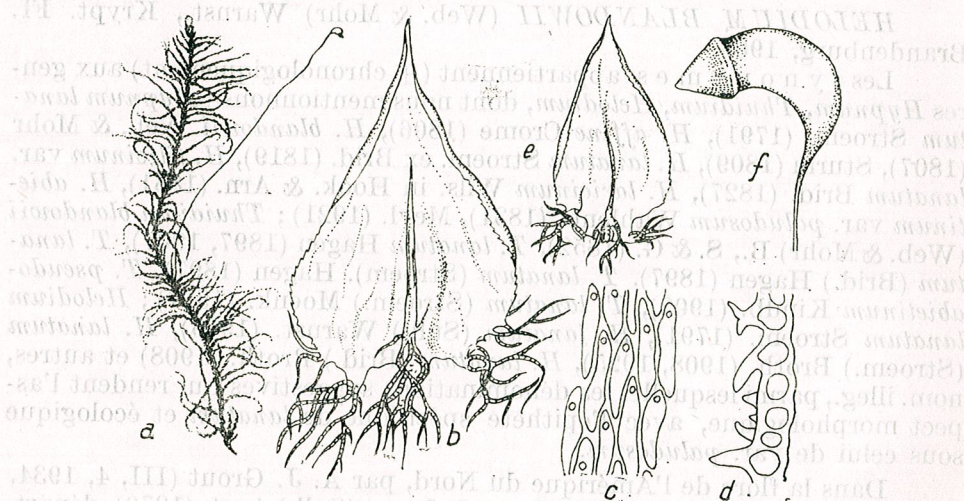


Fig. 1. — *Helodium blandowii* (Web. & Mohr) Warnst. a — habitus (gaméophyte et sporophyte); b — feuille caulinare avec des paraphylles; c — cellules du milieu de la lamine pourvues de papilles sur la partie dorsale (abaxiale), sous-terminal; d — coupe (p.p.) par la feuille caulinare avec des papilles; e — feuille raméale; f — capsule avec l'opercule (Tr. I. Ștefureac, 1956).

tere adaptatif hydrique; les cellules du milieu de la lamine, des feuilles des tiges, sont 7–9 fois plus longues que larges ( $\pm$  transparentes), sur la partie inférieure (adiaxiale), presque lisses, mais prévues sur la partie dorsale (abaxiale), sous-terminalement, d'une papille conique allongée, oblique ou droite, simple ou ramifiée; les feuilles raméales ovales, beaucoup plus petites, brièvement acuminées, faiblement denticulées et légèrement pliées avec la nervure jusqu'au milieu de la lamine (fig. 1).

Espèce monoïque; la capsule, relativement fréquente, grande (avec le pedicelle à 3–5 cm), sous-horizontale, oblique-cylindrique-allongée, arquée, étranglée sous l'orifice, brune; l'opercule conique, petit, aigu ou bref rostré, anneau large; les dents externe du péristome longues, jaune-oranges, celles internes jaunâtres, complètes ou étroitement perforées, les cils bien développés; les spores 10–16  $\mu$  avec le sporoderme net ou finement papilleux, jaunâtre, jusqu'au vert-olive faible; la maturation en juin-juillet (fig. 1). n = 12 me (Holmen 1958) — Danemark (R. Fritsch — 1982).

Écologiquement, *Helodium blandowii* est un élément hygrophile, photophile et  $\pm$  sciaphile, terricole, turfophile, acidophile, le pH de la tourbe entre 5,0 et 6,0, rarement sous ou au-dessus de ces valeurs, avec

une relativement légère amplitude; les altitudes entre 700 et 1300 m s.m. avec des variations d'environ 5–600 m, constituant, en général, un élément monozonal (némoral) de la zone des forêts de *Picea abies*, *Pinus sylvestris*, *Alnus incana*, plus rarement *Fagus sylvatica*, avec des marais tourbeux eutrophes, de transition et avec quelquefois des sources minérales, comme dans les aunaies  $\pm$  acides jusqu'à faiblement alcalines, sur un sol tourbeux marécageux; mentionné sporadiquement (Alpes) dans des marais calcaires (H. Gams).

Bryocénologiquement, dans les stations de Roumanie *Helodium lanatum* est associée à de nombreuses espèces de *Sphagnum* (*S. warnstorffii*, *S. rubellum* etc.) ainsi qu'à d'autres mousses ayant  $\pm$  la même écologie (semblable p. p. dans les pays de l'Europe du Nord), telles que: *Homalothecium nitens* (*Tomenthypnum nitens*), *Aulacomnium palustre*, *Drepanocladus vernicosus*, étant décrite (Lucina) l'association avec *Helodium blandowii* Ștefureac 1956, est l'ass. *Helodietum blandowii* ass. nova corr. Ștefureac 1985, de la phytocénose relictairre avec *Ligularia sibirica*, *Calamagrostis neglecta* et autres. Mais particulièrement caractéristiques et uniques dans notre pays sont les bryocénoses relictaires (Drăgoiasa-Pălteniș) où *Helodium lanatum* est associée avec *Sphagnum wulfianum*, *Pseudobryum cinclidioides*, *Homalothecium nitens* (et, à proximité, *Sphagnum balticum*), qui constitue l'ass. *Sphagneto (wulfiani) — Helodietum turfosum* ass. nova. À remarquer en même temps le fait que dans cette station (1020–1030 m s.m.), *Helodium blandowii* connaît, par endroits, dans les bryocénoses avec *Carex appropinquata*, *Angelica sylvestris*, *Ligularia sibirica* et autres, un développement optimal avec une croissance de 20–25 cm et avec la zonation caractéristique, évidente, du processus de tourbification (comme chez *Sphagnum*); toujours dans cette station ce relict a pu être identifié aussi comme sous-fossile dans les dépôts de tourbe (à une profondeur de 50 cm) et cela, sur la base des caractères morpho-anatomiques de la tige, l'absence du faisceau central, et autres.

Du point de vue phytocénologique, les bryocénoses avec *Helodium blandowii* de notre pays appartiennent fréquemment à l'ass. *Alnetum incanae* Aich. & Siegr. 1930, Al. *Alno-Padion* Knapp 1942 em. Medwecka Kornaš 1959, par endroits et à l'ass. *Scirpo-Phragmitetum* W. Koch 1926 (s. l.) *sphagnetosum* Soó 1926. Fréquemment *Helodium blandowii* se trouve dans les cénoses à *Salix cinerea*, *S. aurita*, *Betula pubescens*, *Alnus incana*, *Picea abies*, *Pinus sylvestris*, *Fagus sylvatica*, plus rarement *Rhamnus frangula* et quelquefois dans le voisinage de *Alnus viridis* (Maramureș) à une basse altitude, d'environ 700 m s.m., espèces qui indiquent de même le caractère relictairre boréal.

Corologiquement, *Helodium blandowii* a été découverte jusqu'à présent dans la bryoflore de Roumanie (1903–1980) et surtout, pendant les dernières décennies dans 10 stations (fig. 2), la majorité dans la partie nord-carpatique du pays, représentant un élément  $\pm$  monozonal dans les formations sylvestres montagneuses (némorales) de trois provinces floristiques: 6 stations en Bucovine (départ. de Suceava); Coșna, Lucina, Drăgoiasa-Pălteniș, Răchitișu Mare, Valea Stînii, Criștior; 2 stations en Maramureș (départ. de Maramureș): Tăul Morărenilor et le marais «La Tăcinoasa»; et 2 stations en Transylvanie: Săcărîmb-Monts Apuseni (départ. de Hunedoara) et les Monts Bodoc (départ. de Covasna). Considé-



rée d'abord très rare, cette espèce relicte a été, ultérieurement, découverte plus fréquemment dans certaines régions de notre pays, peu étudiée par ses bryologistes.

Du nombre total des stations à *Helodium blandowii* de Roumanie, ce taxon a été trouvé dans une station par M. Péterfi et dans une station par

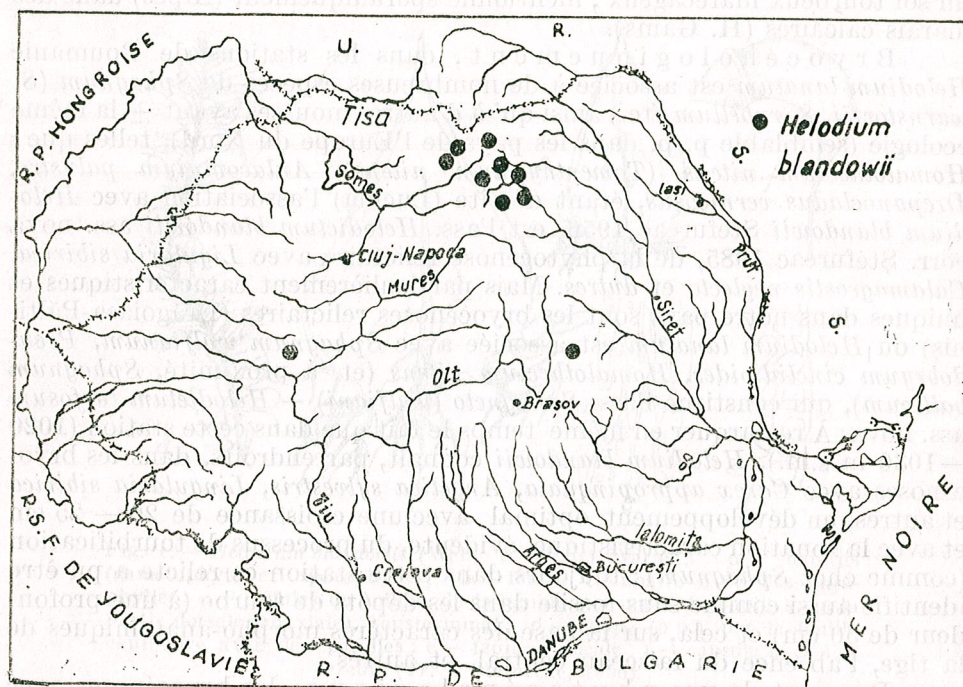


Fig. 2. — Esquisse corologique avec l'indication des stations avec *Helodium blandowii* (Web. & Mohr) Warnst. dans les Carpates de Roumanie, connues jusqu'à 1980 (orig.).

Á. Boros, et plus tard dans 6 stations par Tr. I. Ștefureac, et dans une station par L. Lungu. Quelques-unes des premières stations (Săcărîmb, Coșna) et surtout celles de Lucina sont notées aussi par E. Pop dans son études de synthèse sur les marais de tourbe de Roumanie (1960).

Énumération corologique (chronologique) des stations ayant *Helodium blandowii* de Roumanie :

1. Săcărîmb (sous *Thuidium blandowii*), dans un petit marais sous la colline Haitău, commune Certeju de Sus, à la limite sud-ouest des Monts Apuseni (départ. de Hunedoara), 700 m s.m., (— c), leg. M. Péterfi 1899 (publié en 1903, 1903—1904), vu dans « Herb. Péterfianum » de l'Herb. du Musée Nat. des Transylvanie (nr. 13025, leg M. Péterfi, 21. VI, 1899) ; disparue, n'ayant plus été retrouvée dans cette station (Tr. I. Ștefureac, 1935), mais certaines espèces de sa  $\pm$  bryocénose étant conservées, par fragments, sporadiquement : *Homalothecium nitens*, *Aulacomnium palustre* et même des traces de *Sphagnum* sp.

2. Coșna, ruisseau Teșna (sous *Thuidium lanatum*), marais tourbeux du bassin de Dorna (départ. de Suceava), 889 m s.m. (sporadiquement + c), rarement dans des touffes pures d'habitude parmi d'autres mousses : *Homalothecium nitens*, *Meesea triquetra*, *Drepanocladus vernicosus* ; lieu insuffisamment précisé, (près de Coșna), leg. Á. Boros, 1942 ; signalée aussi par nous dans le marais de Podul Coșnaei (Tr. I. Ștefureac, 1960).

3. Lucina, complexe de marais tourbeux (europhees et oligotrophes) des plateaux « Obcina Mestecăniș » (sous *Helodium lanatum*), commune Moldova Sulița (départ. de Suceava), dans plusieurs stations : vallée de Camni-onca (sous le mont Chicera), la vallée de Hostineț et dans les marais du pied de la colline Știrbu et autres ; pH 4,5 — 5,0, altitude 1026 — 1300 m s.m., (fréquemment + c), à la plus grande altitude du pays, leg. Tr. I. Ștefureac (1951, 1953 etc., publié en 1956, 1958), en association avec *Sphagnum warnstorffii*, *S. rubellum*, *Homalothecium nitens*, *Drepanocladus vernicosus* et autres, analysée phytocénologiquement (voyez sa bryocénologie).

4. Drăgoiasa-Păltiniș (sous *Helodium lanatum*), du bassin de Dorna (départ. de Suceava), dans le complexe de marais tourbeux intracarpatiques Drăgoiasa—Bilbor—Borsec (« Triconfinium » entre la Bucovine, la Moldavie et la Valachie), dans plusieurs stations, altitude entre 1020 et 1030 m s.m., (+ c), leg. Tr. I. Ștefureac (1962, 1963, 1967, etc.), en association avec *Sphagnum wulfianum*, *Pseudobryum cincildroides* etc. (voyez sa bryocénologie), formateur de tourbe, identifié et sous-fossile.

5. Răchitișu Mare (Dealul Glodu) (sous *Helodium lanatum*), village Benia, commune Moldova Sulița (départ. de Suceava), à l'altitude d'environ 1000 m s.m., (+ c), dans une petite aulnaie montagnaise avec *Alnus incana*, *Salix cinerea* etc., sous la réserve naturelle, avec *Arctostaphylos ura-ursi* des phytocénoses avec *Pinus sylvestris* sur le serpentín, leg. Tr. I. Ștefureac (1967, 1968, 1970 etc.).

6. Valea Stînii (sous *Helodium lanatum*), dans la ravine entre Fundu Moldovei et Cîrlibaba (départ. de Suceava), marais tourbeux avec *Picea abies*, *Betula pubescens* et sporadiquement *Pinus sylvestris*, altitude 1100 m s.m., isolée et rare, (— c), parmi d'autres mousses, station où l'on a identifié aussi *Sphagnum wulfianum*, leg. Tr. I. Ștefureac (1960, 1968, 1969, 1970).

7. Cristișor-Neagra Broștenilor, les Monts Bistrița (départ. de Suceava) (sous *Thuidium lanatum*), très rare, marais tourbeux avec *Alnus incana*, peuplé ou non d'épicea, altitude 815 m s.m., leg. L. Lungu (1971).

8. Tăul Morărenilor (départ. de Maramureș) (sous *Helodium lanatum*), en aulnaie avec *Alnus incana* et *Rhamnus frangula*, marais eutrophe sous la montagne Gutii, à 800 m s.m., (+ c), ayant tout près *Alnus viridis* (à une petite altitude, environ 700 m s.m.), leg. Tr. I. Ștefureac (1971, 1974 etc.).

9. Marais « La Tăcinoasa » (départ. de Maramureș) (sous *Helodium lanatum*), dans plusieurs stations, (+ c), marais tourbeux à l'altitude de 800 m s.m. en association avec *Alnus incana*, *Salix cinerea*, *Ligularia sibirica*, *Molinia caerulea*, *Sphagnum contortum*, *Aulacomnium palustre* etc. leg. Tr. I. Ștefureac (1971, 1974).

10. Les Monts Bodoc de la Courbure des Carpates (sous *Helodium lanatum*), marais tourbeux (« Bufogó »), au nord du mont Puturosu (« Büdös ») (départ. de Covasna), altitude 925 m s.m., (+ c), dans la zone avec



*Fagus sylvatica*; dans une station, leg. Al. Kovács, det. Tr. I. Ștefureac (1975) et dans deux stations (1967, publié en 1977), leg. Tr. I. Ștefureac et Al. Kovács.

Sous l'aspect aréalo graphique, *Helodium blandowii* représente un élément phytogéographique, circumpolaire boréal, relict sous-arctique glaciaire (Th. Herzog), prépondérant dans les zones arctiques jusqu'en Groenlande, répandu aussi en Europe surtout au nord (Bretagne du Nord, Belgique, Danemark, Scandinavie, surtout la Finlande), Asie du Nord, — Sibérie du Nord jusqu'en Kamtschatka et Sakhaline avec sa limite boréale de 68° 20' (en Norvège à 70° 30'). Sporadique dans l'Europe centrale (Allemagne, Suisse et autres) et de l'Est (Roumanie, Hongrie, Tchécoslovaquie, Pologne). La limite sud de l'aréal général est dans les Alpes suisses (très rarement, —c) dans le marais Saas-Fée (canton Wallis, I. Amann, 1944) à 70° 30' latitude nord, comme en Allemagne (Vogelsberg de Rhön, après G. Roth).

En Roumanie, *Helodium blandowii* se trouve dans les stations de Lucina (Bucovine), à 47° 39' lat. n. et 25° 12' long. est; toujours à la limite sud elle se trouve à Săcărimb (Monts Apuseni), lat. n. 45° 50' et long. est 23° 5' et dans les Monts Bodoc en Transylvanie, lat. n. 45° 55' et long. est 25° 54' comme les plus méridionales de notre pays, étant signalée, dernièrement aussi en Bulgarie (Sl. Petrov, 1955, sous *Thuidium lanatum*). Tout cela représente des stations de la frontière du sud, carpato-balcanique, de l'aréal général de ce relict sous-arctique glaciaire.

La documentation paléontologique l'atteste plus fréquente dans la végétation quaternaire (H. Gams) dans les régions exposées aux glaciations. En Roumanie, elle est conservée surtout dans certaines réserves naturelles du côté nord-carpatique (Bucovin, Maramureș) telles que : Lucina, Drăgoiasa-Păltiniș et Răchitișu Mare (départ. de Suceava) et Tăul Morărenilor (départ. de Maramureș).

Tr. I. Ștefureac

## AVIS AUX AUTEURS

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