La « Revue Roumaine de Biologie — Série de biologie végétale » paraît deux fois par an. Toute commande de l'étranger (fascicules ou abonnements) sera adressée à RODIPET S.A., Piața Presei Libere nr. 1, P.O. Box 33—57, București, România, ORION SRL., Splaiul Independenței 202 A, București 6, România, PO BOX 74—19 București, Tx 11939 CBTxR, Fax (40) 13122425, AMCO PRESS SRL., Bd. Nicolae Grigorescu nr. 29 A., ap. 66, Sect. 3, București, C.P. 57—88, Fax 13124569.

Les manuscrits ainsi que toute correspondance seront envoyés à la rédaction et les livres et publications proposés en échange à INSTITUTUL DE ȘTIINȚE BIOLOGICE, 79651, București, Splaiul Independenței 296.

REVUE ROUMAINE DE BIOLOGIE
SÉRIE DE BIOLOGIE VÉGÉTALE
Calea Victoriei 125
R—79717, București, România
téléphone 650. 76. 80

EDITURA ACADEMIEI
ROMÂNE
Calea 13 Septembrie nr. 13
R—79717, București
România, téléphone 631.74.00

REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE VÉGÉTALE

TOME 39 — N° 2, 1994

juillet — décembre

SOMMAIRE

REV. ROUM. BIOL.—BIOL. VÉGÉT., TOMÉ 39, N° 2, P. 77—144, BUCAREST, 1994

POPULUS ALBA L. VITROPLANTS PRODUCTION AND THEIR BEHAVIOUR IN FIELD TRIAL

MARGARETA IORDAN COSTACHE*, V.I. BENEA**, I. COMAN*

The paper deals with the research results obtained (1986—1993) regarding the micropropagation of four clones of *Populus alba* L. (Ro—D18, Ro—345, Ro—361 and Ro—366) with very poor vegetative macropropagation, the biotechnologies applied, the behaviour of the vitroplants in laboratory, and greenhouse as well as the biometrical data achieved in the field trial. The main results are : the occurrence of a differentiated morphogenetic potential depending on the clones and culture conditions, expressed by various propagation and growing rates, a specific aclimatizing rate strongly correlated with leaves structure during this process rejuvenation of shoots and genetic stability of vitroplants : related to their adaptability under environmental conditions and biometrical data, the clones are ranked : Ro—345, Ro—361, Ro—D18, and Ro—366.

INTRODUCTION

The White poplar *(Populus alba* L.) and its natural hybrid, the Corey poplar *(Populus × canescens Sm.)*, have a quite considerable importance in the applied silviculture and national economy. They cover more than 60.000 ha as natural stands in the plain and hill river valleys, in the Danube valley and Delta, as pure or mixed forest with other native tree species (Black poplar, willows). Owing to their large ecological area, they grow in a great diversity of climatical conditions, on the heavy compact salty soils with a low water regime, but also, on the short-flooded river banks (2), (3), (5).

Their woods is light, soft, generally of white colour, excellent for cellulose, matches, drawing boards, particle and fibre boards, domestic things. They are reproduced mainly by root sprout and very poorly by macropropagation in the field, which is a great inconvenience both for conservation and promotion of valuable genotypes.

The first results obtained by cloning the White poplar using the micropropagation "in vitro" and their growing in the field conditions in the period of time 1986—1993 are presented in the paper.

MATERIALS AND METHODS

Four white poplar *(Populus alba* L.) Romanian clones, namely : Ro—361, Ro—345, Ro—D18, Ro—366, have been tested. As explant source stem nodal segments of 1.0—1.5 cm in length with axillary buds taken from actively growing shoots have been used ; These shoots are produced from dormant cuttings which are brought into growth in the greenhouse or growthroom. Sterilisation of the explants was performed using mercuric chloride ($HgCl_2$) solution 0.1% w/v plus 2—3 drops of Twin 20, for 15 minutes or calcium hypochlorite (CaClOH) 4% w/v solution for 30—40 minutes. The sterilisation agent was removed 3—4 times in sterile distilled water.

REV. ROUM. BIOL. — BIOL. VÉGÉT., TOME 39, Nº 2, P. 79—84, BUCAREST, 1994

The explants were inoculated in 6 cm Petri dishes on the surface of ml aliquotes of agar solidified (0.8% w/v) MS based medium (8) or ACM medium (1) supplemented with Gambord (B_5) vitamins and plant growth regulators (Table 1).

Table 1

Nutrient media used by "in vitro" micropropagation of *Populus alba* clones

Type of medium	Standard media	Plant growth regulators	Vitamin (mg/1)	Sucrose	Results
Induction and proliferation	MS (1962)	1·0—2·0 BAP 0·2 IAA	B5	30	Axillary budding and branching
Elongation	MS (1962) (1/2) macroelements)	0·5 BAP 0·2 NAA	B5	20	Elongation of shoots Sporogenous rooting
Elongation	ACM*.	0·5 BAP 0·2 NAA	B5	20	Elongation of shoots Sporogenous rooting
Rooting	MS (1962) (1/2—1/4 macroelements)	0·5—1·0 IBA	B5	20	Elongation and rooting

The shoots elongation and their rooting were performed using MS medium with 1/4—1/2 concentration of macronutrients and the low concentrations of hormones (0.5—1.0 mg/1 IBA) or even free-hormones media. Cultures were kept under 16 hours daylight regime at 23 +2°C and transferred at 3 to 4 week intervals. For acclimatization to septical conditions, the vitroplants were transplanted into the mixture of soil, sand and perlite (1 : 1 : 1 v/v/v) and grown under high humidity (95—98%) for 10—12 days.

For histological analysis the segments of leaves detached from "in vitro" plantlets, then from platlets 7—8 days after transfer to septical conditions under high humidity and, finally, tree weeks later, after the plants acclimatization to environmental conditions, were used. The leaf samples were fixed in Navahin's solution, according to the standard method, embedded in paraffin, sectioned at 10—12 μm and stained with toluidine blue. The cytogenetic studies were accomplished using squash samples of root tips of the regenerated plants after Feulgen staining. In spring 1988 vitroplants of 15—20 cm in length were pointed out.

In the field trial the plants were distributed in a randomised system with tree repetitions at 4×3 m space, 12 individuals from each clone. The culture technique was similar to those used in silviculture practice. The histological and biometrical characteristics were collected using the methods and procedures applied normally in the forest researches, expressed by quantitative (mm, cm, m), qualitative (1, 2 symbols) and proportional (%) figures. The field trial was realised at the experimental

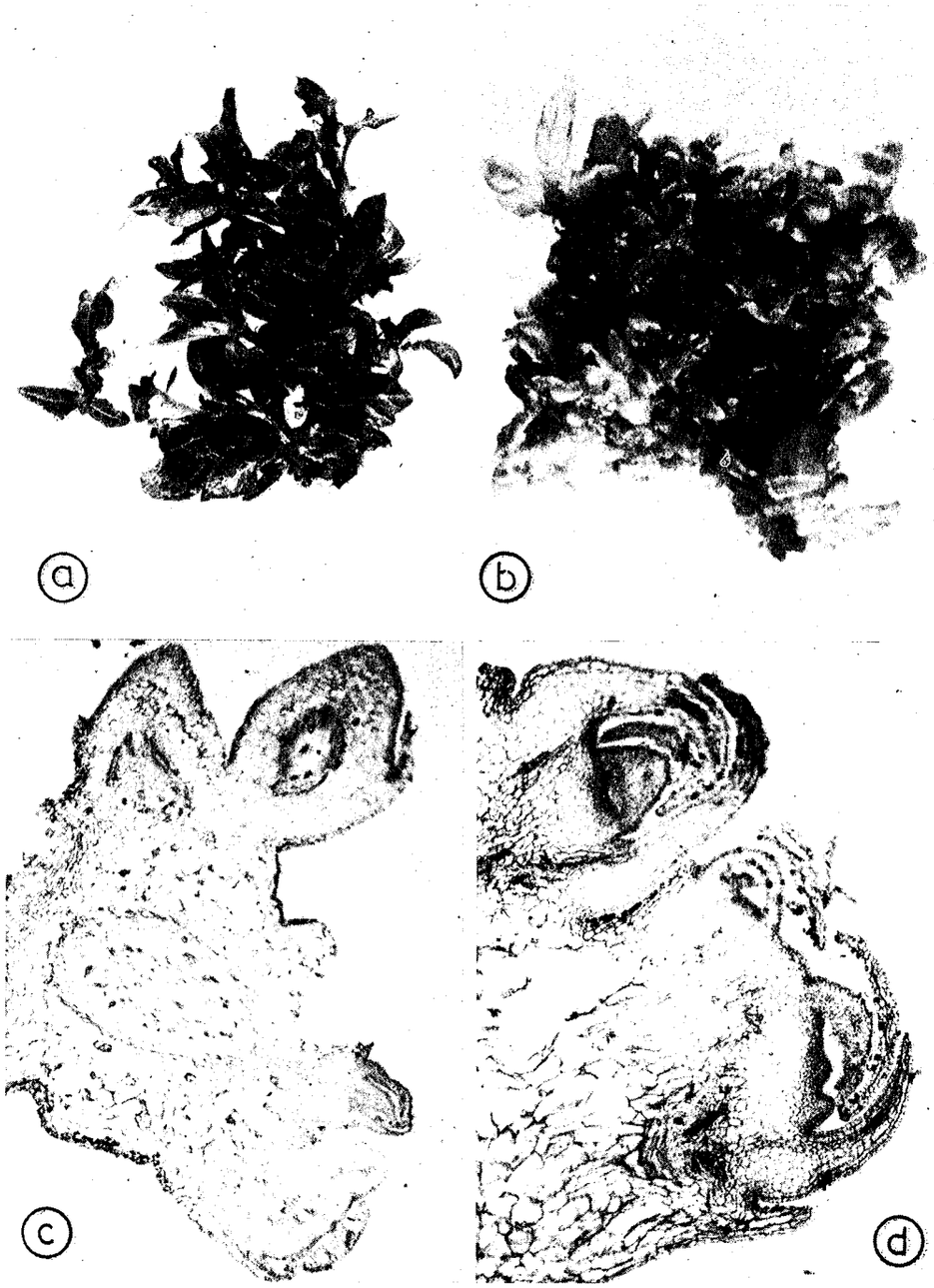


Plate I. – Aspects of *Populus alba* L. plant regeneration by direct organogenesis. a. axillary budding and branching of initial explant (nodal segment) after being 3 times subcultured on the proliferative culture medium. b. Macroscopical aspect of initial explant transformed into bud and shoot proliferative tissue. c, d. histological aspects of incipient stages of axillary budding.

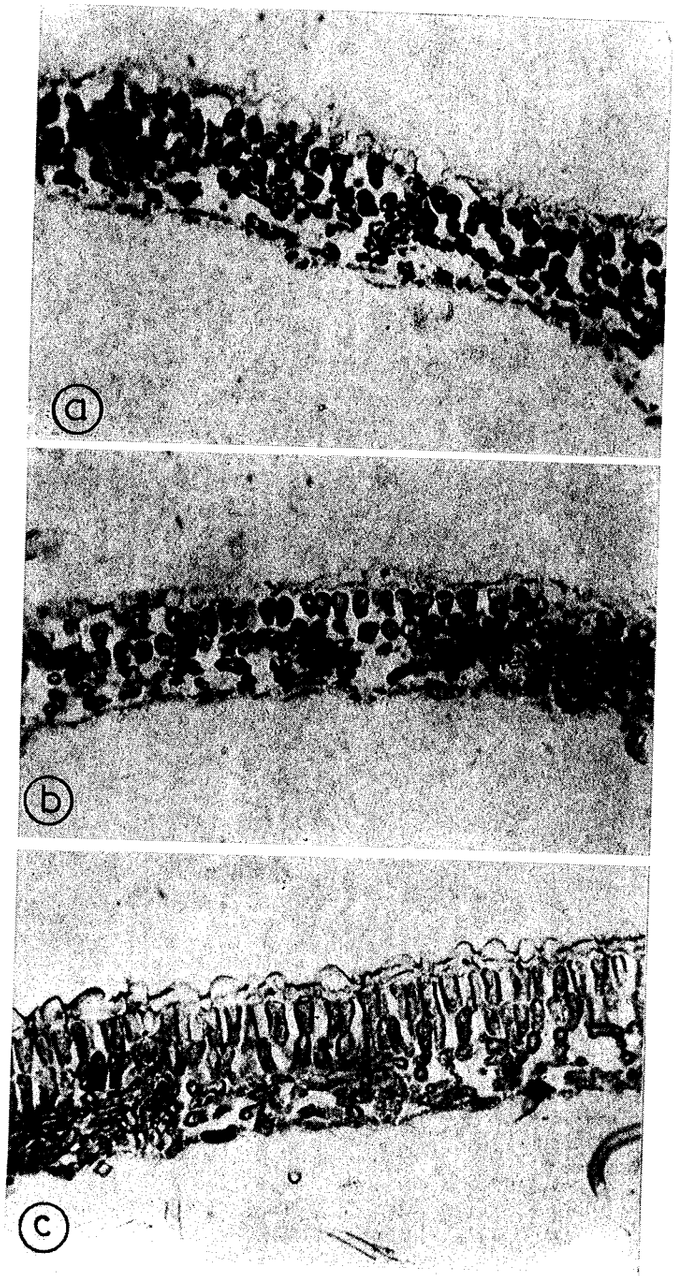


Plate II. – Histological aspects of *Populus alba* L. leaf structure (clone 345). a,b. "In vitro" leaf structure prior the transfer of plantlet to the septic conditions. c. Normal leaf structure, three weeks after acclimatization of vitroplants to the greenhouse conditions.

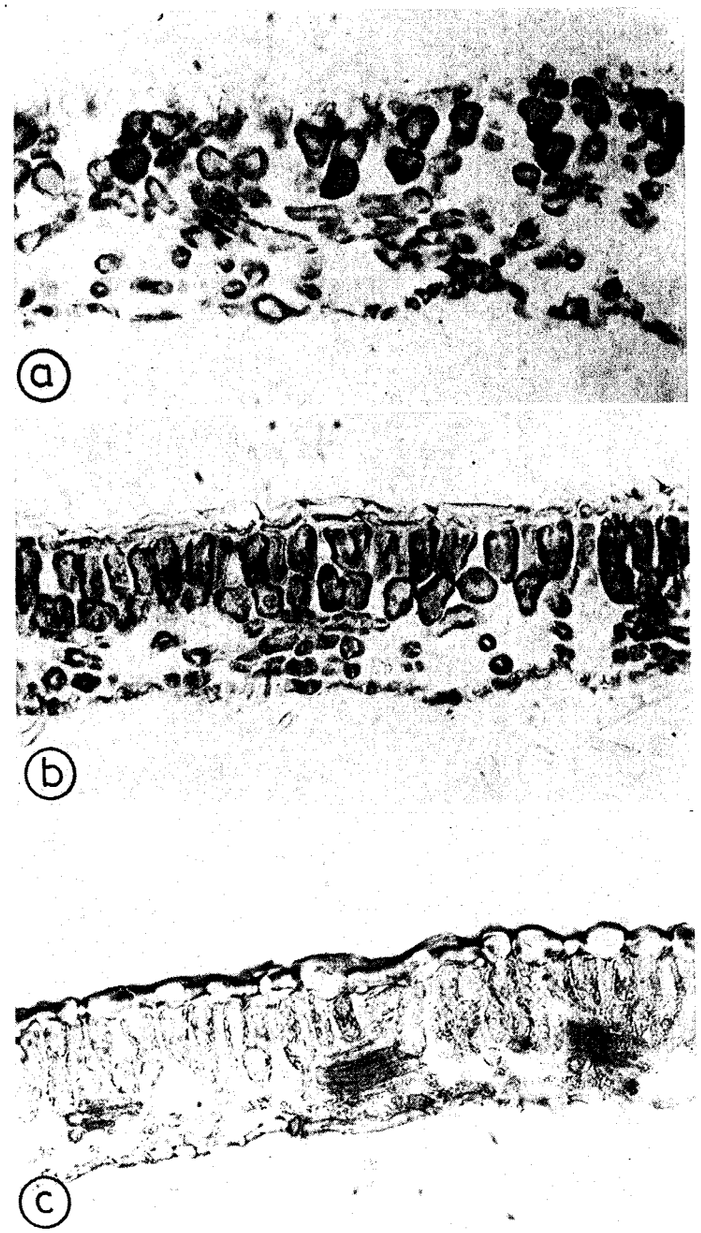


Plate III. – Histological aspects of *Populus alba* L. leaf structure (clone 361). a-c. successive stages of leaf structure differentiation during acclimatization to the septical conditions. d. normal leaf structure in greenhouse conditions.

Plate IV. – Vitroplants of white poplar *(Populus alba* L.) in aseptic conditions (a) versus septic (b, c) conditions during micropropagation by the bud cultures.

field of the Firest Research Station-Cornetu, located about 20 km South-West from Bucharest, at 100 m height above the Back Sea. The climate is continental with an annual mean temperature of +10.9°C (max. abs + + 41.1°C; min. abs. — 30°C) and 550 m rainfalls (380 m in a growing season).

The site is yellowish-brown soil, medium to rich bonity, along the bank of the Argeş river, with a water table under 6 — 7m in summer time.

RESULTS AND DISCUSSION

Stem nodal segments of experimenting *Populus alba* clones developed shoots from axillary buds within 3—4 weeks of "in vitro" culture. In the primary culture each inoculum performed one shoot which was detached and used both for rooting or minicuttings made up for a new inoculation. The initial nodal explant recultured on the same nutritive medium (with citokinines) produced new shoots owing to the new meristematic cells and bud formation zones. After 4—6 successive subcultures, the whole explant transformed itself into a compact proliferative tissue with many buds of various sizes from which 4—10 (25) shoots were detached every 20—25 days (Plate 1, fig. 1). A histological analysis of the proliferative areas reveals the origin of these buds and shoots in the cambial tissue as well as in the primary meristems of the axillary buds situated at the bottom of the detaching shoots from the explant (Plate 1, fig. 1,2,3). Because of the intense proliferation of the explant its fragmentation into 2—4 parts was necessary by every subculture that was an additional way of micromultiplication. The shoots of 3—5 cm in length were subcultured on the rooting medium (Plate 1, fig. 4) or cut each of 4—6 nodal segments, and inoculated on the elongation-rooting medium. The multiplication rate of *Populus alba* investigated clones was very highly being in agreement with the prior results obtained by Whitehead et al. (9) and Lubrano (7) to other poplars, which offers the possibility to obtain more than 1 million plants/explant/year.

The plantlets so obtained have been transplanted in greenhouse throughout the year using a sand soil and perlite based layer (1 : 1 : 1 v/v/v) and mist conditions. Some differences between *Populus* clones regarding their velocity of acclimatization to environmental conditions have been noticed, clone Ro—345 being very sensitive in this respect. These aspects are very interesting correlated with the cytoanatomical leaves structure (Plate 3, figs. 1—3) with the ability of genotype to develop its typical leaves having a normal structure for mesophyllum cuticle, stomata distribution, etc.

During "in vitro" culture of white poplar clones it was also remarked the rejuvenation of the shoots expressed by: a higher capacity of minicuttings proliferation (frequently 100% after 3—4 subcultures compare with cca. 50% in the primary culture); a superior number of shoots/explant after 3—4 successive subcultures and 95—100% rhysogenetic ability. In the growing plot the vitroplants showed a high vigour and growing rhythm (Plate 3) (e.g. 86—128 cm in length after two months

Table 2

Survival (S), diameter at 1,30 m (D), height (H), stem form (SF) and sanitary conditions (SC) of the cultivars/clones of *Populus alba* L. vitroplants, 1–6 years old – tested in the field trial

Cultivar clone	1988			1992			1993			1991			SF/1993					Ran king order
	S %	D cm	H m	D cm	H m	S %	D cm	H m	S %	D cm	H m	S %	1	2	3	4		
Cornetu Ro–361	100	2.52	4.11	7.40	6.60	66.7	7.82	7.79	73.9	6.00	5.94	100	95.7	4.3	—	—		2
Cornetu Ro–D18	100	2.55	4.03	7.50	6.50	61.1	8.61	7.46	81.0	6.09	6.30	100	85.6	4.8	4.8	4.8		3/4
Cornetu Ro–345	69	1.67	3.14	7.10	6.30	63.9	8.28	7.27	85.2	5.37	5.85	69	96.2	7.4	—	3.8		1
Cornetu Ro–366	73	1.94	3.32	7.90	6.60	52.8	8.53	7.34	72.2	5.81	6.33	73	94.7	—	—	5.3		3/4
Average	85.5	2.17	3.63	7.48	6.60	61.1	8.31	7.46	78.1	5.82	6.11	85.5	93.1	2.3	1.2	3.4		—

Legenda

Stem form (SF): 1 = straight
2 = slightly sinuous
3 = forked
Sanitary conditions (SC): 1 = healthy
2 = insect attack
3 = pathogen attack
4 = mechanical wind wounds

and 135–240 cm in height after 6 months, it depending on clones) an orthotropic form and a good health. The main characteristics of the plants investigated in the field conditions such as the survival ability, the diameter at 130 cm, the height of plants as well as the tree-form and their health are presented in Table 2.

It can be concluded, mainly, the followings :

— Regarding the survival we constated that even in the first year after planting (1988) the vitroplants have been divided into two groups : the first, with 100% (Ro–361 and Ro–D18) and the second, with 69–73% (Ro–345 and Ro–366) of survival, which is maintained until the sixth growing season (1993). Then a heavy and long drought has been lost 33.3–38.9% from the first group whereas at the second group the loss was of only 7.4–27.2%;

— According to diameters, in the last growing season (1993) the average diameters are between 7.82 cm (Ro–361) and 8.61 cm (Ro–D18), with a test average of 8.31 cm, exceeded by the clones Ro–D18 (8.61 cm) and Ro–366 (8.53 cm). The highest annual average increases (1.66 cm) were obtained in the 6th growing season (1993);

— The increase in height shows that in the last investigated growing season (1993) the average values are situated between 7.27m (Ro–345) and 7.79 m (Ro–361), with an average of 7.46 m, suppressed only by one clone – Ro–361 (7.79 m). The biggest annual average increases (1.71 m) were realised, such as the diameter, in the 6th growing season (1993).

The tree stem form is represented by straightness, sinuosity and forking.

The straightness is generally good, being ranked between 72.2% (Ro–366) and 85.2% (Ro–345) with a test average of 78.1%, exceeded by Ro–345 (+9.1%) and Ro–D18 (+3.7%).

The sinuosity occurs in average at 17.9% of all stems, the fewest being at the clone Ro–345 (7.4%) the rest of the clones equalizing/exceeding the average with 6.1%–55.3%.

The forking of the stems appears only at the clones Ro–361 and Ro–345, with 8.7% and 7.4% respectively, from all the trees.

Taking into consideration the above mentioned about stem forms, the clones Ro–D18 and Ro–366 are situated on the first places.

As regards the health of trees, the clones Ro–345 and Ro–366 have not signs of insect or pathogen attacks, only some mechanical wounds. But, the clone Ro–D18 shows some susceptibility to insects (4.8%) and pathogens (4.8%) and Ro–361 to insects (4.3%).

CONCLUSIONS

The results confirm :

— The possibility of micropropagation on large-scale of *Populus alba* L. clones (Ro–361, Ro–354, Ro-D18);

— Genetic stability of plants based on the cytogenetical and phenological analysis;

— The occurrence of a differentiated morphogenetical potential depending on the clones and culture conditions, expressed by various

propagation and growing rates as well as by the adaptability under environmental coditions;

— Related to their behaviour in field trial and biometrical data, the clones are ranked: Ro—345, Ro—361, Ro—D18, and Ro—366.

SELECTED LITERATURE

1. Ahuja R., 1983, Somatic cell differentiation and rapid clonal propagation of aspen, Silvae genetica, **32**, 3—4.
2. Benea V., Nicolae C., 1988, Selecția și tehnologia de cultură a plopilor și sălciilor pentru producerea de lemn industrial, pe zone ecologice (Selection and culture techniques of the poplars and willows for industrial wood production, by ecological zones), ICAS, Ref st. final, 15, tab. 1.
3. Benea V. et al., 1991, Selecția plopilor, sălciilor și răchitelor cu calități tehnologice superioare și rezistență sporită la boli și dăunători (Selection of poplars, willows and asiers with superior technological qualities and higher resistance to harmal insects and diseases) ICAS, Ref. st. final, 19, tab. 2
4. Chalupa V., 1979, In vitro propagation of some broadleoved forest tree, Comm. Inst. For. Czech.
5. Dumitriu-Tataranu I., Benea V., 1978., *Dendrologie și ameliorarea arborilor* (Dendrology and forest tree breeding), Ed. Did. și Pedagogică, Bucharest, 60—62.
6. Iordan-Costache M.,Erezeanu A., Benea V.,1991, Direct and indirect organogenesis in *Populus alba* L. cultivated "in vitro". Rev. Roum. Biol. — Biol. Végét., **36**, 1—2, 39—43.
7. Lubrano L., 1981. Micropropagation du peuplier coll Intern. sur la culture "in vitro" des essencis foresters, 81—86.
8. Murashige T., Skoog I., 1962., A revised medium for rapid growth and bioassay with tobacco tissue culture, Physiol. Plant., **15**, 473—497.
9. Whitehead C. M., Giles K.L., 1977, Rapid propagation of poplars by tissue culture methods, N.Z. I. Sci., **7** (1). 40—43.

Received April 12, 1994

*Institute of Biological Sciences, Bucharest, Splaiul Independenței 296 Romania

** Institute of Agricultural and Forestry Research, Bucharest, Romania

LE RÔLE DES LYSOSOMES DANS L'AMPLIFICATION DES EFFETS RADIOBIOLOGIQUES DÉTERMINÉS PAR LES RADIATIONS IONISANTES GAMMA DANS DES CELLULES MÉRISTÉMATIQUES DE SOJA [*Glycine hispida* (MNEH) MAXIM]

I.R. CIOBANU, Liliana GREGORIAN, Atena SCRIPCARU

Les radiations gamma provoquent dans les systèmes biologiques des effets directs pendant l'irradiation au niveau moléculaire et des effets secondaires causés par les radicaux libres, issues par l'impact des radiations avec la matière vivante. Ce type d'effets mènent à la formation des « radio-toxines » qui sont capables de modifier les structures cellulaires, aussi bien que l'intégrité et la pérmeabilité des membranes des organites. La destabilisation des membranes lysosomales favorise l'activation des enzymes hydrolithiques ce qui conduit à l'amplification des effets radiobiologiques. Il est convenu que les effets produits par les radiations gamma sont toujours proportionnels aux doses utilisées.
Mots clés : Glycine hyspida, les radiations gamma, les lysosomes.

Les recherches des effets radiobiologiques des radiations ionisantes ont connu un grand développement à cause de l'utilisation en biologie, en médicine et agriculture, aussi bien que l'augmentation du fonds radioactif naturel comme résultat direct des explosions atomiques expérimentales.

Les modifications radiobiologiques au niveau cellulaire sont strictement dépendantes du type des radiations utilisées, par leurs doses et aussi par une série de facteurs mésologiques qui interfèrent avec les particularités biologiques de chaque genre ou espèce et même de chaque individu. D'autre part chaque type de tissu, chaque type de cellule après son état physiologique réagit particulièrement à l'impact des radiations, spécialement à celles ionisantes.

Les radiations ionisantes provoquent dans les systèmes biologiques des effets directs en ce qui concerne la transformation, la dénaturation et l'inactivation de certaines biomolécules et des effets secondaires qui se manifestent par les altérations morphologiques et fonctionnelles des organites cellulaires.

L'essentiel dans l'impact des radiations avec la matière vivante c'est la production des radicaux libres qui sont de molécules extrêmement actives et qui provoquent des modifications importantes parfois irréparables au niveau des structures cellulaires.

L'altération de la perméabilité membranaire et, comme suite, la libre circulation des enzymes hydrolithiques nous a suggéré d'attribuer auxs lysosomes un rôle essentiel dans l'amplification des effets radiobiologiques, observation qui a déterminé l'étude ci-présente.

MATÉRIEL ET TECHNIQUE

Comme matériel biologique on a utilisé les méristèmes radiculaires de soja. Ceux-ci proviennent des graines irradiées par les radiations gamma ^{60}Co aux doses : 5000, 7500, 10000 et 15000 r ainsi que des méristèmes des graines non irradiées (témoin). Ces méristèmes ont été fixés dans une solution de glutar-aldéhyde 2%, tamponnée à pH=7,4 par tampon cacodylate 0,1 M pou 6 heures à la temprérature de 4°C. Après la fixation, les échantillons ont été lavés 3 fois par tampon cacodylate.

Une partie des échantillons a été lavé à la suite par 3 bains d'eau distillée et puis on l'a passé dans un milieu d'incubation (GoMoRI, 1952). Ensuite tous les méristèmes ont subi la post-fixation, dans une solution d'acide osmique 2% en tampon cacodylate 0,1 M pour 6 heures, à la tempéiature de 4°C. Après plusieurs lavages à l'eau distillée, le matériel a été déshydraté par des séries d'alcools, traité avec de l'oxyde de propylène et inclus dans l'araldite. L'échantillon témoin a subi le même traitement.

Les sections ont été effectuées à l'ultra-microtome Tesla. On a realisé la coloration des sectionnements à l'acétate d'uranyle et sels de plomb.

Les observations et les microphotographies ont été réalisées à l'aide d'un microscope électronique Tesla.

RÉSULTATS

Les recherches de microscopie électronique éffectuées sur des cellules méristématiques radiculaires des plantes témoin (pl. I, fig. 1), aussi bien que des plantes irradiées par 5000 r ont montré qu'il n'y a pas de différences ultra-structurelles notables.

Le cytoplasme fondamental (C) est riche en ribozomes et d'autres organites ; les mitochondries (M) sont en particulier ovales, prévues avec des crêtes différentiées. Les proplastides (PR) sont allongés et leur stroma est plus ou moins électrono-dense. Les lysosomes primaires (LP) se présentent sous la forme de vésicules avec du matériel électrono-dense à l'intérieur. Le noyau présente la forme ovale avec l'enveloppe du noyau intégrée ; la chromatine est constituée d'un réseau de fibrilles enchaînées avec des prochromosomes (PC) en cours de naissance Les fibrilles (FC) sont directement liées avec le nucléole. Le nucléole a une structure granulaire fibrillaire.

Un aspect ultrastructural au niveau cytoplasmatique des cellules méristématiques des plantes irradiées avec 7500 r est presenté dans la pl. I, fig. 2. On constate que de vastes territoires de la cytoplasme sont englobés par des lysosomes secondaires (LS) dans lesquels on observe les dépôts de matériel électrono-dense et des débris de matériel cellulaire lysé.

Dans la pl. II, fig 1 est montré aussi un aspect ultra-structural des cellules méristématiques des plantes irradiées avec 7500 r ; on constate que le noyau de la cellule centrale a la forme lobée, ce qui indique l'existence des perturbations du métabolisme cellulaire, conjugué avec

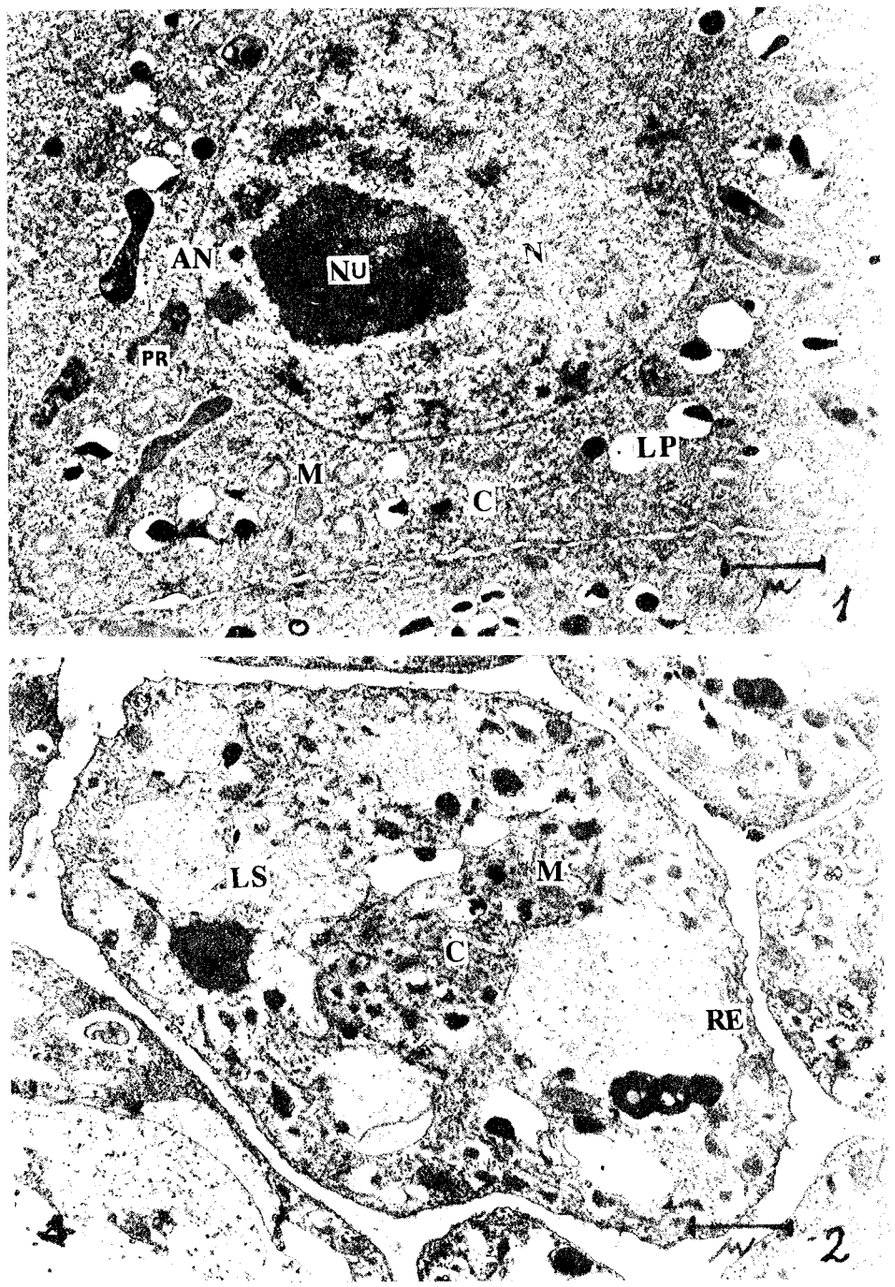


Planche I

Fig. 1. – L'aspect ultrastructural d'une cellule du méristème radiculaire; N = noyau; Nu = nucléole; AN = enveloppe du noyau; C = cytoplasme; M = mitochondrie; LP = lysosome primaire; PR = proplastide; FC = fibrilles de chromatine.

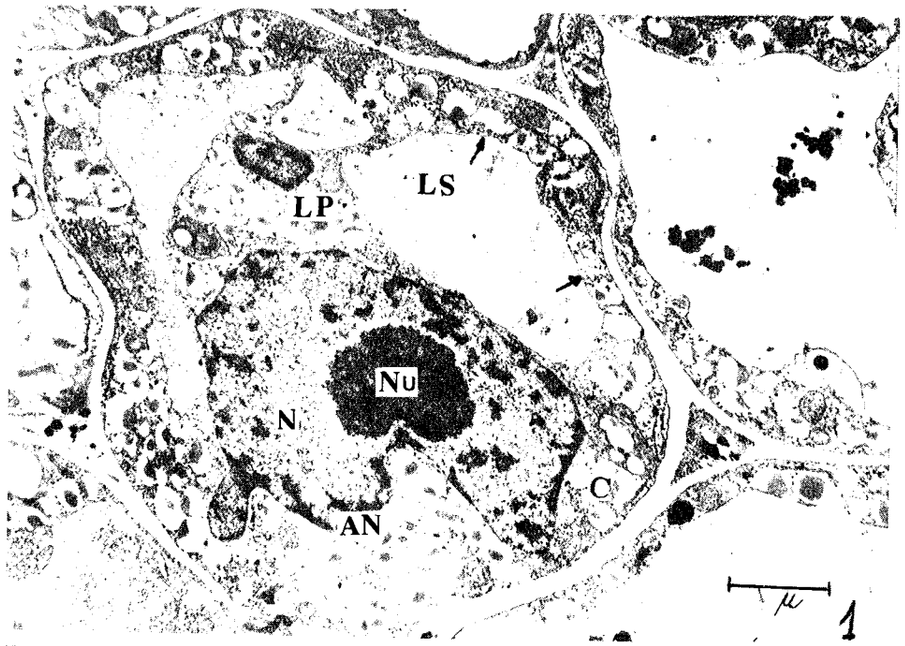


Planche II

Fig. 1. – L'aspect ultrastructural d'une cellule méristématique des plantes irradiées par 7500 r; N = noyau; Nu = nucléole; M = mitochondrie; LP = lysosome primaire; LS = lysosomes secondaires; M = mitochondrie.

Fig. 2. – Pour la même dose: RE = réticule endoplasmatique qui sillonne la formation d'un lysosome secondaire (LS);

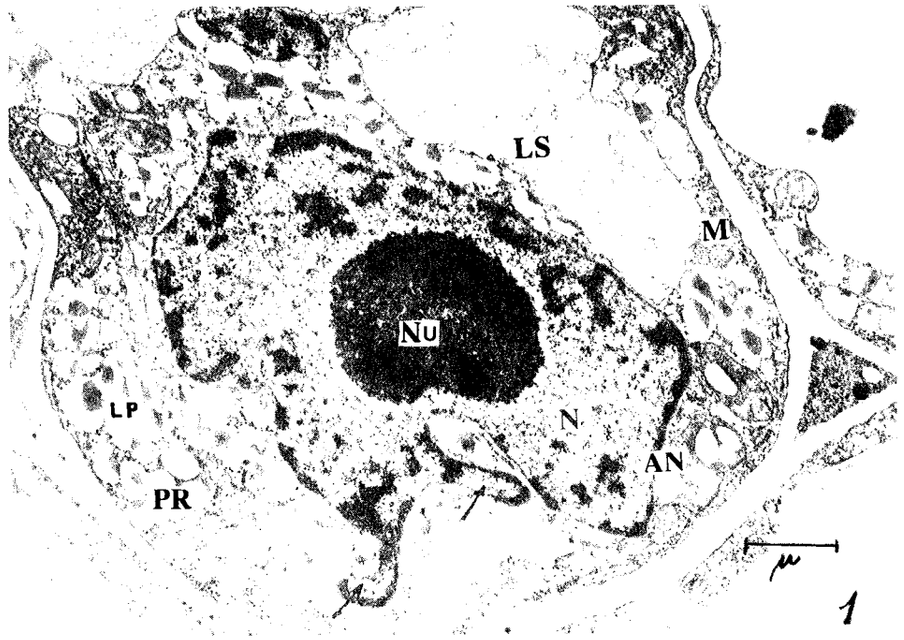
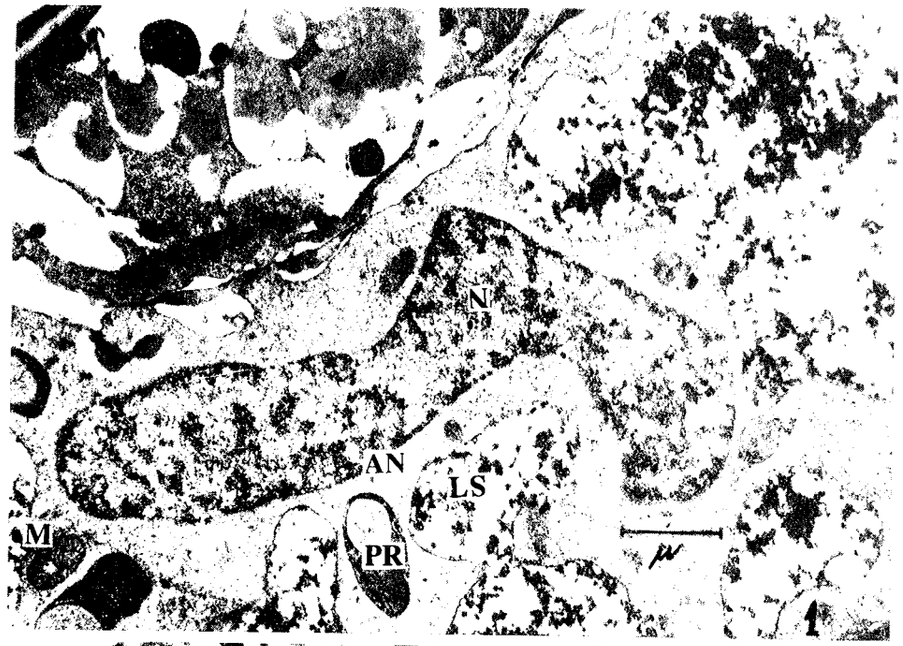


Planche III

Fig. 1. – L'aspect ultrastructural d'une cellule méristématique provenant des plantes iradiées à 10000 r: PR = proplastide; M = mitochondrie; C = cytoplasme.

Fig. 2. – Pour la même dose: PR = proplastide; LS = lysosomes secondaire; M = mitochondrie; C = cytoplasme.



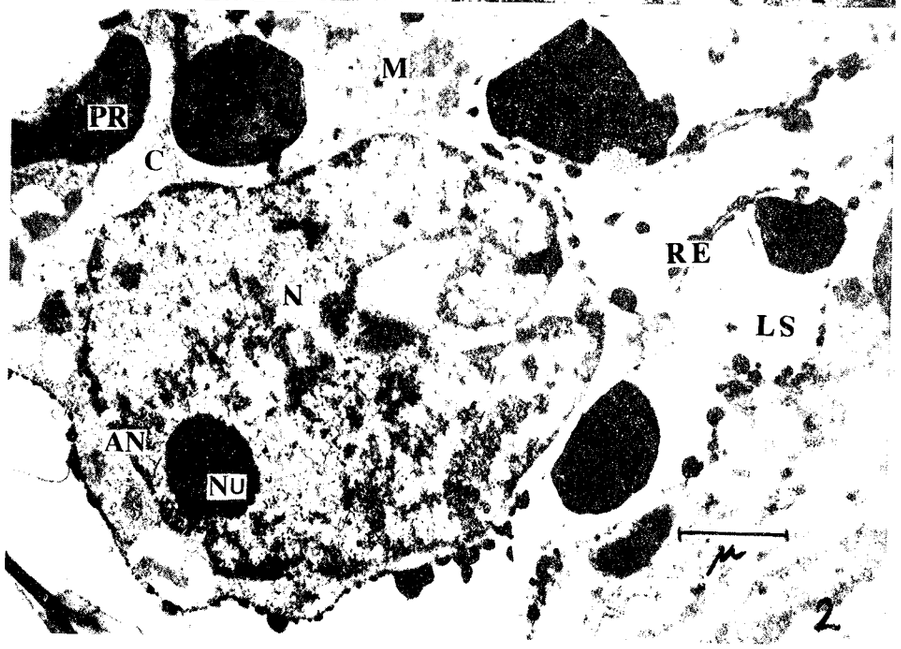


Planche IV

Fig. 1. – La cellule mérismatique des plantes irradiées par 15000 r: N = noyau; AN = enveloppe du noyau; M = mitochondrie; PR = proplastide; LS = lysosomes secondaire.

Fig. 2. – Autre aspect ultrastructural pour la même dose: N = noyau; Nu = nucléole; AN = enveloppe du noyau; RE = réticule endoplasmatique; M = mitochondrie; PR = proplastide; LS = lysosomes secondaire.

l'augmentation des lysosomes secondaires (LS) qui entourent le noyau. A l'intérieur des lysosomes secondaires il y a du matériel lysé ainsi que des organites cellulaires (mitochondries) en train de lyse. Les lysosomes primaires (LP) ont la membrane tangente à l'enveloppe du noyau; par leur fusion se développent les lysosomes secondaires qui vont détruir l'enveloppe du noyau par leurs enzymes hydrolithiques.

Dans la pl. II. fig. 2 on observe le modèle par lequel les profils de réticule endoplasmatique (RE) sillonnent (flèche) les zones lysées à cause des hydrolases lysosomales.

Les effets radiobiologiques produits par 10000 r sont présentés dans la pl. III. fig 1 ; le noyau est plus lobé et sa partie déformée est incluse à l'intérieur d'un lysosome secondaire (la flèche). Autour de l'enveloppe du noyau on trouve des lysosomes primaires (LP) très sérés les uns contre les autres. La formation des lysosomes secondaires autour du noyau provoquera sa lyse et donc la destruction cellulaire.

Les effets radiobiologiques à la dose 10000 r sont plus amples (pl. III, fig. 2). Les lysosomes secondaires (LS) envahissent la plupart des cellules et les lysosomes primaires restent dans la région pariétale du cytoplasme. Parmi certaines cellules on observe, à l'intérieur des lysosomes secondaires, des mitochondries aux crêtes dilatées.

Dans la pl. IV, fig 1, 2 sont présentés des aspects ultra-structuraux des cellules des plantes irradiées avec 15000 r. On remarque partout des lysosomes secondaires (LS) qui occupent des aires étendues du cytoplasme. Dans les lysosomes secondaires il y a des précipités de plomb, soit à l'intérieur (fig. 1), soit adhérents à la membrane lysosomale (fig. 2).

Les organites cellulaires sont plus ou moins rares et leur structure est affectée, les crêtes mitochondriales sont dilatées tandis que les ribosomes sont presque inexistants. Le noyau prend la forme allongée-lobée (fig. 1) ou ovale-lobée (fig. 2) avec des fibrilles chromatiniques denses et le nucléole compact.

De toute évidence, les lysosomes volumineux provoquent la perturbation du métabolisme cellulaire et, par conséquent donc, peu à peu, la modification structurale de toutes les organites cellulaires. En outre, la désorganisation moléculaire des membranes lysosomales ainsi que leur déchirement conduisent à la libération des enzymes lysosomales qui, par la dégradation cytoplasmatique, détermineront la mort des cellules.

DISCUSSIONS

L'amplitude et la complexité des effets des radiations gamma sur l'ultrastructure cellulaire se trouvent en corrélation avec l'amplification de la dose (BACQ et ALEXANDER, 1963 ; CIOBANU, 1972 ; CIOBANU et col., 1984, 1993), l'état de latence des grains (CIOBANU, 1973 ; ALSHITS et col., 1981) et enfin le degré de leur humidité (CIOBANU, 1972 ; CIOBANU et col., 1984, 1993 ; SILVY, 1984 ; SHEPPARD et EVENDEN, 1986 ; ARABI et col., 1991).

Tenant compte que les graines au contenu normal d'humidité ont les germes à l'état de latence, dans nos recherches l'irradiation a été effectuée après 24 heures d'hydratation des graines.

Ainsi par l'humectation on a déterminé le renforcement du métabolisme général, l'activation des systèmes enzymatiques et les divisions cellulaires ; par suite, la radiosensibilité augmente à cause des modifications métaboliques. En plus dans les cellules hydratées, les radiations gamma provoquent l'apparition des radicaux mis en liberté (OH^- et H^-) comme résultat de la radiolyse de l'eau, ainsi que des ruptures des liaisons entre C—N et C—H, qui, dans la présence de l'oxigène, produisent des péroxydes avec une activité accrue sur la perméabilité des membranes plasmatiques

Dans les travaux concernant cet aspect, certains auteurs (MERGEN et col., 1964 ; NILAN, 1976 ; MAHAMA et SILVY, 1982) montrent que la radiosensibilité des graines se dévellope proportionnellement au degré d'humectation. Les autres soutiennent que l'eau joue un rôle protecteur par son action dans la mobilité des radicaux libres qui, par leur recombinaison, se neutralisent réciproquement.

Nos recherches ont prouvé que l'hydratation des graines détermine l'accroissement de la radiosensibilité cellulaire. Les doses modérées (5000, 7500 r) provoquent la perturbation de la mitose et alors on remarque des ponts chromatiques, des fragments chromosomaux et des chromosomes retardataires, qui deviennent des micro-noyaux pendant l'interphase.

Au niveau du cytoplasme on remarque : les mitochondries aux crêtes dilatées, le réticulum endoplasmatique et des saccules dictyosomales qui sont vésicularisés (HAUSWIRTH et ROUX, 1970 ; TOKIN, 1970 ; BESPALOVA et MITUSHIN, 1970 ; GULAEV, 1970 ; JACOBS, 1969, 1970 ; CIOBANU, 1972 ; CIOBANU et col., 1984, 1993) ; la vésicularisation même de l'enveloppe du noyau qui, ainsi désorganisée, favorise le contact du cytoplasme avec le nucléoplasme (JACOBS, 1970 ; CIOBANU, 1972 ; CIOBANU et col., 1993).

Pour les grandes doses (10000, 15000 r), les radiations ionisantes provoquent de graves perturbations pour certaines cellules. A notre avis, dans ces situations, les radiations gamma par l'action sur les macromolécules d'ADN mènent à des lésions irréparables. Ainsi, les radiations gamma sont capables de produire la destruction des liaisons d'hydrogène, la dépolymérisation de l'ADN, des erreurs de duplication et donc de la translation et des conséquences négatives sur le métabolisme, sur la structure et sur la division cellulaire.

Tout de même, le concept de radiosensibilité dépasse largement les altérations génétiques. La cellule renferme de nombreux organites structurés comme des membranes pluristratifiées ; celles-ci prennent environ 80% de l'énergie des radiations gamma et, ensuite, la stabilité et la perméabilité membraneuses se réduisent substantiellement.

BACQ et ALEXANDER (1963) ont expliqué l'action des radiations par la libération des enzymes de diveres structures cellulaires. D'après KUZIN et VAGABOVA (1981) (12) la plupart des transformations sont d'une manière secondaire déterminées par les radiotoxines qui se trouvent en corrélation avec l'altération des structures cellulaires responsables de quelques activités enzymatiques.

A notre avis, la modification de la perméabilité des membranes des lysosomes permet la fonction des enzymes lysosomales qui, par leur activité lithique vacuolisent les structures biologiques affectées. Les images présentées dans ce travail prouvent que les phénomènes de lyse sont le résultat de l'activité de cette catégorie d'enzymes.

Pour conclure, l'activité lithique lysosomales semble le dernier effort cellulaire d'arrêter et de localiser les perturbations secondaires provoquées par les radiations en vue de rétablir le métabolisme normal s'il est encore possible.

BIBLIOGRAPHIE

1. Alshits L.K., Kulikova N.V., Sherchenko V.A., Yushkov P.I., Radiobiologhia, 1984, **3**, 459—464.
2. Arabi M. J., Barrault G., Sarrafi A., Albertini L., Canad. J. Bot., 1991, **2**, 311—315.
3. Bacq Z. M., Alexander P., *Fundamentals of Radiobiology*, Pergamon Press, Oxford-London — New York — Paris, 1963.
4. Bespalova L., Mitushin V., Congr. Intern. Microscop. Electro., Grenoble, 1970, 1, 661—662.
5. Ciobanu R. I., Rev Roum. Biol. Ser. Bot., 1972, **3**, 151—156.
6. Ciobanu R.I., Scripcariu A., Dumitrescu R., Gregorian L., Contract M.E.I., 1984.
7. Ciobanu R.I., Scripcariu A., Gregorian L., Gheorghe E., Adrian Paul, Stud și Cercet. Biol., 1973.
8. Gulaev V. A., 7e Congr. Intern. Microscop. Electro. Grenoble, 1970, 1, 663—664.
9. Hauswirth N., Roux J.C., — 7e Congr. Intern. Microscop. Electro., Grenoble, 1970, **1**, 667—668.
10. Jacobs M., Rad. Bot., 1969, **9**, 251—268.
11. Jacobs M., 7e Congr. Intern. Microscop. Electro. Grenoble, 1970, **1**, 669—670.
12. Kuzin A.M., Vagabova M.E., Radiobiologhia., 1981, **1**, 117—122.
13. Mahama A., Silvy A., Environ. Exp. Bot., 1982, **22**., 233—242.
14. Mergen F., Johasen T.S., Rad. Bot., 1964, **4**, 417—427.
15. Nilan R.A., Conf. Rad. Isotopes in agric., TIB 7512, U.S. Atomic Energy Commission, 1976.
16. Nilan R.A., Konzak C.F., Harle J.R., Heiner R. E., Strahlentherapie (suppl)., 1962, **51**, 171—182.
17. Sheppard S. C., Evenden W. G., Canad. J. Plant Sci., 1986., **66**, 431—442.
18. Silvy A., Environ. Exp. Bot., 1984, **24**, 75—90.
19. Tokin I.B., 7e Congr. Intern. Microscop. Electr., Grenoble, 1970, **1**, 653—654.

Reçu le 11 avril 1994

Institut de Sciences Biologiques
Bucarest, Splaiul Independenței 296
Roumanie

BRYOPHYTA — MUSCI IN THE ROMANIAN FLORA

G. DIHORU

From a of total 1230 papers, we have formed the preliminary *Conspectus of Musci* from bryoflora of Romania. There is a total number of 729 species grouped in 211 genera and 58 families. All the important synonyms, 1360, are also in our manuscript.

The Romanian bryological literature contains several partial syntheses of the various historical regions : Baumgarten 1846, Schur 1866, Fuss 1878, Papp 1943, Ştefureac et al. 1973, Ştefureac et al. 1981, Mohan 1988 and even for the whole country (Papp 1970, Mohan 1984), but all these books were poory distributed. European bryologists have little or no information on the bryophytes of the Romanian flora, except for Cl. *Hepaticopsida* which was published not long ago (Mihai 1983) in a journal with a narrow circulation. The author mentions 200 Hepaticae species to which we add other 3—4 new species.

This paper results from reading 1230 papers on Romanian bryoflora and several hundred foreign papers of recent years. All the information were processed on computer (2199 items with species and synonyms) except for the infrataxa cited from Romania.

The information was processed observing the following principles : (1) acceptance of microspecies and microgenera, (2) utilization of the most recent nomenclature, (3) utilization of new families indicated in the literature, (4) inclusion of only those infrataxa which gave epithets to some species (ex. *Lescurea stricta* var. *saxicola* = *L. saxicola*) or are simply synonyms of other species (ex. *Mnium affine* var. *rugicum* = *Plagiomnium ellipticum*), (5) estimation of the corollogy.

Our paper covers 729 species grouped in 211 genera and 58 families, indicated to genera in brackets.

As concerns the recent European bryology, two trends can be distinguished, (1) merging species into large genera (especially the English bryologists) and, (2) splitting some classical genera (especially the Scandinavian bryologists). We have adopted the latter because we noticed that some English scientists prefer this trend too (Corley et al. 1991). Thus, the small genera resulting from dividing large genera such as : *Mnium*, *Drepanocladus*, *Calliergon*, *Barbula*, *Tortula*, *Amblystegium* etc., were recognized.

We must also underline the fact that there are many species of which we are not certain they exist in the Romanian bryoflora : those mentioned only once, those from very old papers, those discovered recently and described without an arealogic giving their distribution, but also some species described for the first time here in Romania.

Conventional marks

CM — common species	SP — sporadic species
FR — very rare species	6 — species to be excluded
FV — very frequent species	? — doubtful species
RR — rare species	

REV. ROUM. BIOL. — BIOL. VÉGÉT., TOME 39, Nº 2, P. 91—107, BUCAREST, 1994

THE FAMILIES OF MUSCI IN ROMANIA

1. *AMBLYSTEGIACEAE*
2. *ANDREAEACEAE*
3. *ANOMODONTACEAE*
4. *ARCHIDIACEAE*
5. *AULACOMNIACEAE*
6. *BARTRAMIACEAE*
7. *BRACHYTHECIACEAE*
8. *BRUCHIACEAE*
9. *BRYACEAE*
10. *BUXBAUMIACEAE*
11. *CATOSCOPIACEAE*
12. *CINCLIDOTACEAE*
13. *CLIMACIACEAE*
14. *CRATONEURACEAE*
15. *CRYPHAEACEAE*
16. *DICRANACEAE*
17. *DIPHYSCIACEAE*
18. *DISCELIACEAE*
19. *DITRICHACEAE*
20. *ENCALYPTACEAE*
21. *ENTODONTACEAE*
22. *EPHEMERACEAE*
23. *FABRONIACEAE*
24. *FISSIDENTACEAE*
25. *FONTINALACEAE*
26. *FUNARIACEAE*
27. *GRIMMIACEAE*
28. *HEDWIGIACEAE*
29. *HELODIACEAE*
30. *HOOKERIACEAE*
31. *HYLOCOMIACEAEA*
32. *HYPNACEAE*
33. *LEMBOPHYLLACEAE*
34. *LESKEACEAE*
35. *LEUCOBRYACEAE*
36. *LEUCODONTACEAE*
37. *MEESIACEAE*
38. *MNIACEAE*
39. *MYRINIACEAE*
40. *NECKERACEAE*
41. *ORTHOTRICHACEAE*
42. *PLAGIOTHECIACEAE*
43. *POLYTRICHACEAE*
44. *POTTIACEAE*
45. *PTYCHOMITRACEAE*
46. *RHABDOWEISIACEAE*
47. *RHYTIDIACEAE*
48. *SCHISTOSTEGACEAE*
49. *SELIGERIACEAE*
50. *SEMATOPHYLLACEAE*
51. *SPHAGNACEAE*
52. *SPLACHNACEAE*
53. *TETRAPHIDACEAE*
54. *THAMNOBRYACEAE*
55. *THELIACEAE*
56. *THUIDIACEAE*
57. *TIMMIACEAE*
58. *TRICHOSTOMACEAE*

ENUMERATION OF MUSCI SPECIES

Abietinella (56)[1]
— *abietina* (Hedw.) Fleisch. CM
— *histricosa* (Mitt.) Broth. FR
Acaulon (44)
— *muticum* (Spruce) C.Müll. RR
— *triquetrum* (Spruce) C. Müll. RR
Aloina (44)
— *ambigua* (B. & S.) Limpr. FR
— *rigida* (Hedw.) Limpr. RR
Amblyodon (37)
— *dealbatus* (Hedw.) B. & S. FR
Amblystegium (1) [2]
— *humile* (Beauv.) Crundw. FV
— *juratzkanum* Schimp. FV
— *serpens* (Hedw.) B.S.G. CM
Amphidium (46)
— *lapponicum* (Hedw.) Schimp. FR
— *mougeotii* (B. & S.) Schimp. FV
Anacamptodon (23)
— *splachnoides* (Fröl. ex Brid.) Brid. RR
Andreaea (2)
— *alpestris* (Thed.) Schimp. FR
— *frigida* Hüb. FR
— *nivalis* Hook. FR
— *rothii* Web. & Mohr FR
— *rupestris* Hedw. SP

Anisothecium (16)[3]
— *grevilleanum* (Brid.) Broth. RR
— *rigidulum* (Hedw.) C. Jens. RR
— *rufescens* (Dicks.) Lindb. RR
— *schreberianum* (Hedw.) Dix. SP
— *vaginale* (Dicks.) Loeske FR
— *varium* (Hedw.) Mitt. FV
Anoectangium (58)
— *aestivum* (Hedw.) Mitt. SP
Anomobryum (9)
— *julaceum* (Gaertn., Meyer & Schreb.) Schimp. FR
Anomodon (3)[4]
— *attenuatus* (Hedw.) Hüb. CM
— *longifolius* (Brid.) Hartm. SP
— *rostratus* (Hedw.) Schimp. SP
— *rugelii* (C. Müll.) Keissl. FV
— *viticulosus* (Hedw.) Hook. & Tayl. CM
Antitrichia (36)
— *curtipendula* (Hedw.)Brid. SP
Archidium (4)
— *alternifolium* (Hedw.) Schimp. FR
Arctoa (16)
— *fulvella* (Dicks.) B.S.G. FR
— *hyperborea* (Dicks.) B.S.G. FR
Astomum (58)
— *crispum* (Hedw.) Hampe RR
— *intermedium* Péterfi FR
Atrichum (43)
— *angustatum* (Brid.) B. & S. SP
— *haussknechtii* Jur. & Milde FV
— *tenellum* (Rohl.) B. & S. SP
— *undulatum* (Hedw.) Beauv. CM
Aulacomnium (5)
— *androgynum* (Hedw.) Schwaegr. FR
— *palustre* (Hedw.) Schwaegr. FV
— *turgidum* (Wahlenb.) Schwaegr. FR
Barbula (58)
— *acuta* (Brid.) Brid. FR
— *asperifolia* Mitt. FR
— *cylindrica* (Tayl.)Schimp. FV
— *fallax* Hedw. CM
— *glauca* (Ryan) Mol. FR
— *icmadophila* C. Müll. FR
— *reflexa* (Brid.) Brid. FV
— *sinuosa* (Mitt.) Grav. FR
— *unguiculata* Hedw. CM
— *vinealis* Brid. CM
Bartramia (6)
— *halleriana* Hedw. FV
— *ithyphylla* Brid. FV
— *pomiformis* Hedw. FV
Blindia (49)
— *acuta* (Hedw.) B.S.G. SP
Brachydontium (49)
— *trichodes* (Web.) Milde FR
Brachythecium (7)
— *albicans* (Hedw.) B.S.G. SP
— *campestre* (C. Müll.) B.S.G. SP
— *capillaceum* (Web. & Mohr) Giac. FR
— *erythrorhizon* B.S.G. FR
— *geheebii* Milde RR
— *glaciale* B.S.G. FR
— *glareosum* (Spruce) B.S.G. FV
— *mildeanum* (Schimp.) Schimp. ex Milde[5] FV
— *nelsonii* Grout FR
— *oedipodium* (Mitt.) Jaeg. & Sauerb. FR
— *olympicum* Jur.[6] FR
— *oxycladum* (Brid.) Jaeg.[6] SP
— *plumosum* (Hedw.) B.S.G. SP
— *populeum* (Hedw.) B.S.G. CM
— *reflexum* (Starke) B.S.G. FV
— *rivulare* B.S.G. CM
— *rutabulum* (Hedw.)B.S.G. CM
— *salebrosum* (Web.& Mohr) B.S.G. CM
— *starkei* (Brid.) B.S.G. RR
— *trachypodium* (Brid.) B.S.G. FR
— *velutinum* (Hedw.) B.S.G. CM
Breidleria (32)
— *pratensis* (Rabenh.) Loeske SP
Brotherella (50)
— *lorentziana* (Mol. ex Lor.)

Loeske ex Fleisch. FR
— *nemorosa* (Brid.) Loeske FR

Bryoerythrophyllum (58)
— *alpigenum* (Vent.) Chen FR
— *recurvirostrum* (Hedw.) Chen FV
— *rubrum* (Jur.) Chen FR

Bryum (9)
— *algovicum* Sendtn. ex C. Müll. FV
— *alpinum* Huds. ex With. FV
— *arcticum* (R. Br.) B.S.G. FR
— *argenteum* Hedw. CM
— *bicolor* Dicks. SP
— *bimum* (Schreb.) Turn.[7] FV
— *caespiticium* Hedw. CM
— *calophyllum* R. Br. FR
— *capillare* Hedw. CM
— *creberrimum* Tayl. SP
— *elegans* Nees ex Brid. FV
— *erythrocarpum* × *B. bimum* FR
— *erythrocarpum* × *B. mixtum* Podp. FR
— *funckii* Schwaegr. RR
— *hazslinszkyanum* Péterfi ? FR
— *imbricatum* (Schwaegr.) B. & S. RR
— *intermedium* (Brid.) Bland. SP
— *klinggraeffii* Schimp. FR
— *knowltonii* Barnes FR
— *mildeanum* Jur. FR
— *muehlenbeckii* B.S.G. FR
— *neodamense* Itzigs. ex C. Müll. FR
— *pallens* Sw. FV
— *pallescens* Schleich. ex Schwaegr. CM
— *pallescens* × *B. warnstorfii* Ruthe FR
— *pseudotriquetrum* (Hedw.) Gaertn., Meyer & Schreb. CM
— *purpurascens* (R. Br.) B.S.G. FR
— *radiculosum* Brid. FR
— *rubens* Mitt.[8] FR
— *ruderale* Crundw. & Nyh. FR
— *schleicheri* Lam. & DC. SP
— *subapiculatum* Hampe FR
— *subelegans* Kindb. CM
— *torquescens* Bruch ex De Not. SP
— *turbinatum* (Hedw.) Turn. SP
— *uliginosum* (Brid.) B. & S. SP
— *versicolor* A. Br. ex B. & S. FR
— *weigelii* Spreng. FR
— × *mixtum* Podp. FR

Buxbaumia (10)
— *aphylla* Hedw. RR
— *viridis* (Meng. ex Lam. & DC.) Brid. ex Moug. & Nestl. RR

Callicladium (50)
— *haldanianum* (Grev.) Crum[9] SP

Calliergon (1)[10]
— *cordifolium* (Hedw.) Kindb. FV
— *giganteum* (Schimp.) Kindb. FV
— *richardsonii* (Mitt.) Kindb. ex Warnst. FR
— *stramineum* (Brid.) Kindb. FV
— *trifarium* (Web. & Mohr) Kindb. FR

Calliergonella (32)
— *cuspidata* (Hedw.) Loeske CM
— *lindbergii* (Mitt.) Heden. CM

Camptothecium (7)[11]
— lutescens (Hedw.) B.S.G. CM

Campyliadelphus (1)
— *chrysophyllus* (Brid.) Kanda FV
— *elodes* (Lindb.) Kanda FR
— *polygamus* (B.S.G.) Kanda SP
— *protensus* (Brid.) Kanda[12] FV
— *radicale* (Beauv.) Dihoru SP
— *stellatus* (Hedw.) Kanda CM

Campylium (1)
— *calcareum* Crundw. & Nyh.[13] FV
— *sommerfeltii* (Myr.) J. Lange[13] SP

* *Campyliadelphus radicale* (Beauv.) Dihoru, comb. nova. Basionym: Hypnum radicale Beauv. 1805, Prodr.: 68.

Campylophyllum (1)
— *halleri* (Hedw.) Fleisch. SP

Campylopus (16)
— *flexuosus* (Hedw.) Brid. SP
— *fragilis* (Brid.) B.S.G. RR
— *pyriformis* (Schultz) Brid. SP
— *schwarzii* Schimp. FR
— *subulatus* Schimp. FR

Campylostellum (45)
— *saxicola* (Web. & Mohr) B.S.G FR

Catoscopium (11)
— *nigritum* (Hedw.) Brid. FR

Ceratodon (19)
— *conicus* (Hampe ex C. Müll.) Lindb. FR
— *purpureus* (Hedw.) Brid. CM

Cinclidium (38)
— *stygium* Sw. FR

Cinclidotus (12)
— *aquaticus* (Hedw.) B. & S. SP
— *fontinaloides* (Hedw.) Beauv. FV
— *nigricans* (Brid.) Wijk & Marg. SP

Cirriphyllum (7)
— *cirrosum* (Schwaegr. ex Schultes) Grout FR
— *piliferum* (Hedw.) Grout FV
— *tommasinii* (Sendtn. ex Boul.) Grout FV

Climacium (13)
— *dendroides* (Hedw.) Web. & Mohr CM

Cnestrum (16)
— *schisti* (Web. & Mohr) Hag. FR

Conardia (1)
— *compacta* (C. Müll.) Robins. FR

Conostomum (6)
— *tetragonum* (Hedw.) Lindb. FR

Coscinodon (27)
— *cribrosus* (Hedw.) Spruce RR

Cratoneuron (14)
— *curvicaule* (Jur.) Roth FR
— *filicinum* (Hedw.) Spruce CM

Crossidium (44)
— *squamigerum* (Viv.) Jur. RR

Cryphaea (15)
— *heteromalla* (Hedw.) Mohr FR

Ctenidium (32)
— *molluscum* (Hedw.) Mitt. CM

Cynodontium (16)
— *bruntonii* (Sm.) B.S.G. FR
— *fallax* Limpr. FR
— *gracilescens* (Web. & Mohr) Schimp. FR
— *polycarpon* (Hedw.) Schimp. SP
— *strumiferum* (Hedw.) Lindb. FR
— *tenellum* (B.S.G.) Limpr. FR

Cyrtomnium (38)
— *hymenophylloides* (Hüb.) Nyh. ex Kop. FR
— *hymenophyllum* (B.S.G.) Homen FR

Desmatodon (44)
— *cernuus* (Hüb.) B. & G.[6] FR
— *heimii* (Hedw.) Mitt. RR
— *latifolius* (Hedw.) Brid. SP

Dichelyma (25)
— *falcatum* (Hedw.) Myr. FR

Dichodontium (16)
— *flavescens* (With.) Lindb. FR
— *pellucidum* (Hedw.) Schimp. FV

Dicranella (16)
— *banatica* Hampe ? FR
— *cerviculata* (Hedw.) Schimp. FV
— *heteromalla* (Hedw.) Schimp. CM
— *subulata* (Hedw.) Schimp. FV

Dicranodontium (16)
— *asperulum* (Mitt.) Broth. FR
— *denudatum* (Brid.) Britt. ex Williams FV

Dicranoweisia (16)
— *cirrata* (Hedw.) Lindb. ex Milde FR
— *compacta* (Schwaegr.) Schimp. FR
— *crispula* (Hedw.) Milde SP

Dicranum (16)
— *acutifolium* (Lindb. & Arn.) C. Jens. ex Weimn. FR

— *bergeri* Bland. ex Hoppe SP
— *bonjeanii* De Not. ex Lisa FV
— *brevifolium* (Lindb.) Lindb. FR
— *congestum* Brid. RR
— *drummondii* C. Müll. FR
— *elongatum* Schleich. ex Schwaegr. SP
— *fragilifolium* Lindb. FR
— *fulvum* Hook. FR
— *fuscescens* Sm. FV
— *groenlandicum* Brid. RR
— *majus* Sm. SP
— *muehlenbeckii* B.S.G. SP
— *scoparium* Hedw. CM
— *spurium* Hedw. RR
— *viride* (Sull. & Lesq.) Lindb. SP

Didymodon (58)
— *cordatus* Jur. FR
— *luridus* Hornsch. ex Spreng. SP
— *rigidulus* Hedw. CM
— *spadiceus* (Mitt.) Limpr. SP
— *tophaceus* (Brid.) Lisa FV

Diobelon (16)
— *squarrosum* (Schrad.) Hampe SP

Diphyscium (17)
— *foliosum* (Hedw.) Mohr FV

Discelium (18)
— *nudum* (Dicks.) Brid.[6] FR

Distichium (19)
— *capillaceum* (Hedw.) B.S.G. FV
— *inclinatum* (Hedw.) B.S.G. SP

Ditrichum (19)
— *cylindricum* (Hedw.) Grout FV
— *flexicaule* (Schwaegr.) Hampe CM
— *heteromallum* (Hedw.) Britt. FV
— *lineare* (Sw.) Lindb. FR
— *pallidum* (Hedw.) Hampe SP
— *pusillum* (Hedw.) Hampe FV
— *subulatum* (B.S.G.) Hampe FR

Drepanocladus (1)[14]
— *aduncus* (Hedw.) Warnst. CM
— *capillifolius* (Warnst.) Warnst. FR
— *cossonii* (Schimp.) Loeske[15] FR
— *revolvens* (Sw.) Warnst. FV
— *sendtneri* (Schimp. ex H. Müll.) Warnst. SP
— *tundrae* (H. Arn.) Loeske FR

Dryptodon (27)
— *patens* (Hedw.) Brid. RR

Encalypta (20)
— *affinis* Hedw. fil. FR
— *alpina* Sm. FR
— *ciliata* Hedw. SP
— *rhaptocarpa* Schwaegr. SP
— *spathulata* C. Müll. FR
— *streptocarpa* Hedw. FV
— *vulgaris* Hedw. FV

Entodon (21)
— *cladorrhizans* (Hedw.) C. Müll. FR
— *concinnus* (De Not.) Par. SP
— *schleicheri* (Schimp.) Demet. FR
— *transsilvanicus* Demet.? FR

Entosthodon (26)
— *fascicularis* (Hedw.) C. Müll. RR
— *hungaricus* (Boros) Loeske SP
— *obtusus* (Hedw.) Lindb. FR

Ephemerum (22)
— *cohaerens* (Hedw.) Hampe FR
— *recurvifolium* (Dicks.) Boul. FR
— *serratum* (Hedw.) Hampe RR

Eucladium (58)
— *verticillatum* (Brid.) B.S.G. SP

Eurhynchium (7)
— *angustirete* (Broth.) Kop. SP
— *crassinervium* (Wils.) Schimp. FV
— *flotowianum* (Sendtn.) Kartt.[16] FV
— *hians* (Hedw.) Sande Lac. CM
— *meridionale* (Schimp.) De Not. FR

— *pulchellum* (Hedw.) Jenn. SP
— *pumilum* (Wils.) Schimp. FR
— *schleicheri* (Hedw. fil.) Jur. FV
— *speciosum* (Brid.) Jur. FV
— *striatum* (Hedw.) Schimp. CM

Fabronia (23)
— *ciliaris* (Brid.) Brid. FR
— *pusilla* Raddi FR

Fissidens (24)
— *adianthoides* Hedw. FV
— *bryoides* Hedw. RR
— *crassipes* Wils. ex B.S.G. FR
— *dubius* Beauv. FV
— *exilis* Hedw. RR
— *gracilifolius* Brugg. Nann. & Nyh. FR
— *incurvus* Starke ex Rohl.[17] FR
— *obtusifolius* Wils. FR
— *osmundoides* Hedw. FR
— *pusillus* (Wils.) Milde[18] FR
— *rivularis* (Spruce) B.S.G. FR
— *rufulus* B.S.G. FR
— *taxifolius* Hedw. CM
— *viridulus* (Sw.) Wahlenb. FR

Fontinalis (25)
— *antipyretica* Hedw. CM
— *hypnoides* Hartm. FR
— *squamosa* Hedw. FR

Funaria (26)
— *hygrometrica* Hedw. CM
— *hygrometrica* × *Physcomitrium pyriforme* FR
— *muehlenbergii* Hedw. fil. ex Turn. FR
— *transsilvanica* Péterfi? FR

Geheebia (58).
— *gigantea* (Funck) Boul. FR

Grimmia (27)
— *anodon* B. & S. FR
— *apiculata* Hornsch. FR
— *atrata* Miel. ex Hoppe & Hornsch. FR
— *crinita* Brid. FR
— *decipiens* (Schultz) Lindb. FR
— *donniana* Sm. RR
— *elatior* Bruch ex Bals. & De Not. FR
— *elongata* Kaulf. ex Sturm. FR
— *funalis* (Schwaegr.) B. & S. SP
— *hartmanii* Schimp. SP
— *incurva* Schwaegr. SP
— *laevigata* (Brid.) Brid. FR
— *montana* B. & S. SP
— *orbicularis* Bruch ex Wils.[19] FV
— *ovalis* (Hedw.) Lindb. FV
— *plagiopodia* Hedw. FR
— *pulvinata* (Hedw.) Sm. CM
— *sessitana* De Not. FR
tenerrima Ren. & Card. RR
— *teretinervis* Limpr. FR
— *tergestina* Tomm. ex B.S.G. SP
— *torquata* Grev. FR
— *trichophylla* Grev. RR
— *unicolor* Hook. ex Grev. FR

Gymnostomum (58)
— *aeruginosum* Sm. SP
— *calcareum* Nees & Hornsch. SP

Gyroweisia (58)
— *tenuis* (Hedw.) Schimp. RR

Habrodon (23)
— *perpusillus* (De Not.) Lindb. FR

Hamatocaulis (1)
— *vernicosus* (Mitt.) Heden. FV

Haplocladium (56)
— *angustifolium* (Hampe & C. Müll.) Broth. FR

Haplohymenium (34)
— *triste* (Ces. ex De Not.) Kindb. FR

Hedwigia (28)
— *ciliata* (Hedw.) Beauv. CM

Helodium (29)
— *blandowii* (Web. & Mohr) Warnst.[20] SP

Herzogiella (42)
— *seligeri* (Brid.) Iwats. CM
— *striatella* (Brid.) Iwats. FR

Heterocladium (56)
— *dimorphum* (Brid.) B.S.G. RR
— *heteropterum* B.S.G. RR

Homalia (40)
— *besseri* Lob. FV
— *trichomanoides* (Hedw.) B.S.G. CM

Homalothecium (7)
— *philippeanum* (Spruce) B.S.G. FV
— *sericeum* (Hedw.) B.S.G. CM

Homomallium (32)
— *incurvatum* (Brid.) Loeske [21] FV

Hookeria (30)
— *lucens* (Hedw.) Sm. RR

Hydrogrimmia (27)
— *mollis* (B.S.G.) Loeske FR

Hygroamblystegium (1)
— *fluviatile* (Hedw.) Loeske SP
— *tenax* (Hedw.) Jenn. SP
— *varium* (Hedw.) Mönk. CM

Hygrohypnum (1)
— *alpestre* (Hedw.) Loeske FR
— *alpinum* (Lindb.) Loeske FR
— *duriusculum* (De Not.) Jameson RR
— *eugyrium* (Schimp.) Broth. RR
— *luridum* (Hedw.) Jenn. CM
— *molle* (Hedw.) Loeske RR
— *ochraceum* (Turn. ex Wills.) Loeske RR
— *styriacum* (Limpr.) Broth. FR

Hylocomium (31)
— *brevirostre* (Brid.) B.S.G. SP
— *pyrenaicum* (Spruce) Lindb. RR
— *splendens* (Hedw.) Schimp. in B.S.G. CM
— *umbratum* (Hedw.) B.S.G. SP

Hymenostomum (58)
— *microstomum* (Hedw.) R. Br. FV
— *squarrosum* Nees & Hornsch. FR
— *tortile* (Schwaegr.) B.S.G. FV

Hymenostylium (58)
— *recurvirostrum* (Hedw.) Dix. FR

Hyocomium (32)
— *armoricum* (Brid.) Wijk & Marg. FR

Hypnum (32)
— *andoi* A. Sm. CM
— *bambergeri* Schimp. SP
— *callichroum* Funck ex Brid. SP
— *cupressiforme* Hedw. CM
— *fertile* Sendtn. SP
— *hamulosum* B.S.G. FR
— *imponens* Hedw. RR
— *jutlandicum* Holmen & Warncke SP
— *pallescens* (Hedw.) Beauv. FV
— *procerrimum* Mol. FR
— *recurvatum* (Lindb. & Arn.) Kindb. [22] RR
— *reptile* Michx. [23] SP
— *revolutum* (Mitt.) Lindb. RR
— *vaucheri* Lesq. FV

Isopterygiopsis (42)
— *muelleriana* (Schimp.) Iwats. SP
— *pulchella* (Hedw.) Iwats. SP

Isothecium (33)
— *alopecuroides* (Dubois) Isov. CM
— *myosuroides* Brid. SP

Kiaeria (16)
— *blyttii* (B.S.G.) Broth. FR
— *falcata* (Hedw.) Hag. SP
— *starkei* (Web. & Mohr) Hag. FV

Kindbergia (7)
— *praelonga* (Hedw.) Ochyra SP

Leptobryum (9)
— *pyriforme* (Hedw.) Wils. CM

Leptodictyum (1)
— *riparium* (Hedw.) Warnst. CM

Leptodon (40)
— *smithii* (Hedw.) Web & Mohr FR

Leptodontium (58)
— *flexifolium* (Dicks.) Hampe FR
— *styriacum* (Jur.) Limpr. FR

Lescurea (34)
— *mutabilis* (Brid.) Lindb. ex Hag. FR
— *saxicola* (B.S.G.) Mol. ex Lor. FR

Leskea (34)
— *polycarpa* Hedw. CM

Leskeella (34)
— *nervosa* (Brid.) Loeske CM

Leucobryum (35)
— *glaucum* (Hedw.) Ångstr. CM
— *juniperoideum* (Brid.) C. Müll. RR

Leucodon (36)
— *sciuroides* (Hedw.) Schwaegr. CM

Meesia (37)
— *hexasticha* (Funck) Bruch FR
— *longiseta* Hedw. SP
— *triquetra* (Richter) Ångstr. SP
— *uliginosa* Hedw. SP

Mielichhoferia (9)
— *mielichhoferiana* (Funck) [6] Loeske FR

Mniobryum (9)
— *atropurpureum* (Wahlenb.) Hag. FR
— *delicatulum* (Hedw.) Dix. FV
— *ludwigii* (Schwaegr.) Loeske FR
— *pulchellum* (Hedw.) Loeske ? FR
— *wahlenbergii* (Web. & Mohr) Jenn. SP

Mnium (38)
— *ambiguum* H. Müll. SP
— *blyttii* B.S.G. FR
— *hornum* Hedw. FV
— *marginatum* (Dicks.) Beauv. FV
— *spinosum* (Voit) Schwaegr. FV
— *spinulosum* B.S.G. RR
— *thomsonii* Schimp. RR

Molendoa (58)
— *sendtneriana* (B.S.G.) Limpr. [24] RR
— *tenuinervis* Limpr. FR

Myrinia (39)
— *pulvinata* (Wahlenb.) Schimp. FR

Myurella (55)
— *julacea* (Schwaegr.) B.S.G. SP

Neckera (40)
— *complanata* (Hedw.) Hüb. CM
— *crispa* Hedw. CM
— *pennata* Hedw. FV
— *pumila* Hedw. RR

Nyholmiella (41)
— *gymnostoma* (Brid.) Holmen & Warncke FR
— *obtusifolia* (Brid.) Holmen & Warncke FV

Octodiceras (24)
— *fontanum* (B. Pyl.) Lindb. FR

Oligotrichum (16)
— *hercynicum* (Hedw.) Lam. & DC. SP

Oncophorus (16)
— *virens* (Hedw.) Brid. SP
— *wahlenbergii* Brid. SP

Orthodicranum (16)
— *flagellare* (Hedw.) Loeske SP
— *montanum* (Hedw.) Loeske FV
— *tauricum* (Sap.) Smirn. FR

Orthothecium (21)
— *intricatum* (Hartm.) B.S.G. SP
— *rufescens* (Brid.) B.S.G. SP
— *strictum* Lor. FV

Orthotrichum (41)
— *affine* Brid. CM
— *alpestre* Hornsch. ex B.S.G. RR
— *anomalum* Hedw. CM
— *cupulatum* Brid. SP
— *diaphanum* Brid. FV
— *elegans* Schwaegr. ex Richards. FV
— *fastigiatum* Bruch ex Brid. [25] SP
— *lyellii* Hook. & Tayl. SP
— *pallens* Bruch ex Brid. SP
— *patens* Bruch ex Brid. FR
— *philibertii* Vent. FV
— *pulchellum* Brunt. FR
— *pumilum* Sw. FV
— *rivulare* Turn. FR

— *rogeri* Brid. FR
— *rupestre* Schleich. ex Schwaegr. RR
— *scanicum* Grönv. FR
— *speciosum* Nees CM
— *speciosum* × *O. elegans* FR
— *stellatum* Brid. FR
— *stramineum* Hornsch. ex Brid. SP
— *striatum* Hedw. SP
— *tenellum* Bruch ex Brid. RR
— *urnigerum* Myr. FR

Oxystegus (58)
— *tenuirostris* (Hook. & Tayl.) A. Sm. RR

Paludella (37)
— *squarrosa* (Hedw.) Brid. RR

Palustriella (29)
— *commutata* (Hedw.) Ochyra CM
— *decipiens* (De Not.) Ochyra SP

Paraleucobryum (16)
— *enerve* (Thed.) Loeske SP
— *longifolium* (Hedw.) Loeske FV

Phascum (44)
— *curvicolle* Hedw. FR
— *cuspidatum* Hedw. FV
— *floerkeanum* Web. & Mohr FR

Philonotis (6)
— *arnellii* Husn. FR
— *calcarea* (B. & S.) Schimp. FV
— *caespitosa* Jur. FV
— *fontana* (Hedw.) Brid. FV
— *fontana* × P. *tomentella* FR
— *marchica* (Willd.) Brid. RR
— *rigida* Brid. FR
— *seriata* Mitt. FV
— *tomentella* Mol. RR

Physcomitrella (26)
— *patens* (Hedw.) B.S.G. FR

Physcomitrium (26)
— *eurystomum* Sendtn. RR
— *pyriforme* (Hedw.) Brid. CM
— *sphaericum* (Ludw.) Brid FR

Plagiobryum (9)
— *demissum* (Hook.) Lindb. FR
— *zieri* (Hedw.) Lindb. RR

Plagiomnium (38)
— *affine* (Bland. ex Funck) Kop. CM
— *cuspidatum* (Hedw.) Kop. CM
— *elatum* (B. & S.) Kop. FV
— *ellipticum* (Brid.) Kop. SP
— *medium* (B. & S.) Kop. SP
— *rostratum* (Schrad.) Kop. CM
— *undulatum* (Hedw.) Kop. CM

Plagiopus (6)
— *oederiana* (Sw.) Crum & Anders. FV

Plagiothecium (42)
— *cavifolium* (Brid.) Iwats. CM
— *curvifolium* Schlieph. ex Limpr. RR
— *denticulatum* (Hedw.) B.S.G. CM
— *donianum* (Sm.) Jaeg. FR
— *laetum* B.S.G. FV
— *latebricola* B.S.G. FR
— *neckeroideum* B.S.G. FR
— *nemorale* (Mitt.) Jaeg. FV
— *piliferum* (Sw. ex Hartm.) B.S.G. FR
— *platyphyllum* Mönk. SP
— *ruthei* Limpr. FR
— *succulentum* (Wils.) Lindb. SP
— *undulatum* (Hedw.) B.S.G. SP

Plasteurhynchium (33)
— *striatulum* (Spruce) Fleisch. SP

Platydictya (1)
— *jungermannioides* (Brid.) Crum RR
— *subtilis* (Hedw.) Crum CM

Platygyrium (32)
— *repens* (Brid.) B.S.G. FV

Pleuridium (19)
— *acuminatum* Lindb. RR
— *subulatum* (Hedw.) Rabenh. SP

Pleurochaete (59)
— *squarrosa* (Brid.) Lindb. RR

Pleurozium (21) [26]
— *schreberi* (Brid.) Mitt. CM

Pogonatum (43)
— *aloides* (Hedw.) Beauv. FV
— *dentatum* (Brid.) Brid. FR
— *nanum* (Hedw.) Beauv. RR
— *urnigerum* (Hedw.) Beauv. CM

Pohlia (9)
— *ambigua* (Limpr.) Broth. FR
— *andalusica* (Hohn.) Broth. FR
— *annotina* (Hedw.) Lindb. RR
— *bulbifera* (Warnst.) Warnst. FR
— *cruda* (Hedw.) Lindb. FV
— *drummondii* (C. Müll.) Andr. RR
— *elongata* Hedw. FV
— *erecta* Roth ex Corr. ? FR
— *longicollis* (Hedw.) Lindb. SP
— *ludwigii* (Spreng. ex Schwaegr.) Broth. RR
— *minor* Schwaegr. FR
— *muyldermansii* Wilcz. & Dem. [6] FR
— *nutans* (Hedw.) Lindb. CM
— *obtusifolia* Osterw. FR
— *proligera* (Lindb. ex Breidl.) Lindb. ex H. Arn. FR
— *sphagnicola* (B.S.G.) Broth. RR

Polytrichastrum (43)
— *alpinum* (Hedw.) G. Sm. CM
— *formosum* (Hedw.) G. Sm. CM
— *longisetum* (Brid.) G. Sm. CM
— *pallidisetum* (Funck) G. Sm. FV
— *sexangulare* (Brid.) G. Sm. SP

Polytrichum (43)
— *commune* Hedw. CM
— *commune* × *P. commune* var. *perigoniale* FR
— *formosum* × *P. longisetum* FR
— *hyperboreum* R. Br. FR
— *juniperinum* Hedw. CM
— *juniperinum* × *P. commune* FR
— *piliferum* Hedw. CM
— *strictum* Menz ex Brid. CM
— *strictum* × *P. juniperinum* var. *delicatum* FR
— *stefureacii* Plăm. (*P. commune* × *P. strictum*) = *P. commune* var. *perigoniale* ? FR

Pottia (44)
— *bryoides* (Dicks.) Mitt. FR
— *caespitosa* (Bruch ex Brid.) C. Müll. FR
— *crinita* Wils. ex B.S.G. FR
— *davalliana* (Sm.) C. Jens. FR
— *intermedia* (Turn.) Furnr. FV
— *lanceolata* (Hedw.) C. Müll. FV
— *truncata* (Hedw.) B. & S. FV

Pseudephemerum (16)
— *nitidum* (Hedw.) Reim. RR

Pseudobryum (38)
— *cinclidioides* (Hüb.) Kop. FR

Pseudocalliergon (1)
— *lycopodioides* (Brid.) Heden. RR

Pseudocrossidium (58)
— *hornschuchianum* (Schultz) Zand. RR
— *revolutum* (Brid.) Zand. RR

Pseudoleskea (34)
— *atrovirens* B.S.G. [27] FR
— *incurvata* (Hedw.) Loeske RR
— *patens* (Lindb.) Kindb. FR
— *radicosa* (Mitt.) Mac. & Kindb. FR
— *saviana* (De Not.) Latz. FR

Pseudoleskeella (34)
— *catenulata* (Schrad.) Kindb. SP
— *tectorum* (Brid.) Kindb. ex Broth. FR

Pseudoscleropodium (7)
— *purum* (Hedw.) Fleisch. FV

Pseudotaxiphyllum (42)
— *elegans* (Brid.) Iwats. SP

Pterigynandrum (34)
— *filiforme* Hedw. CM

Pterogonium (36)
— *gracile* (Hedw.) Sm. FV

Pterygoneurum (44)
— *lamellatum* (Lindb.) Jur. FR
— *ovatum* (Hedw.) Dix. SP
— *subsessile* (Brid.) Jur. FR
Ptilium (32)
— *crista-castrensis* (Hedw.) De Not. CM
Ptychodium (34)
— *plicatum* (Web. & Mohr) Schimp. RR
Pylaisiella (32)
— *polyantha* (Hedw.) Grout CM
Pyramidula (26)
— *tetragona* (Brid.) Brid. FR
Racomitrium (27)
— *aciculare* (Hedw.) Brid. RR
— *affine* (Schleich. ex Web. & Mohr) Lindb. FR
— *aquaticum* (Schrad.) Brid. RR
— *canescens* (Hedw.) Brid. CM
— *ericoides* (Web. ex Brid.) Brid. FR
— *fasciculare* (Hedw.) Brid. RR
— *heterostichum* (Hedw.) Brid. SP
— *lanuginosum* (Hedw.) Brid. FV
— *microcarpon* (Hedw.) Brid. FR
— *sudeticum* (Funck) B. & S. SP
Rhabdoweisia (46)
— *crispata* (Dicks.) Lindb. RR
— *fugax* (Hedw.) B.S.G. SP
Rhizomnium (38)
— *magnifolium* (Horik.) Kop. SP
— *pseudopunctatum* (B.&S.) Kop. SP
— *punctatum* (Hedw.) Kop. CM
Rhodobryum (38)
— *ontariense* (Kindb.) Kindb. FR
— *roseum* (Hedw.) Limpr. SP
Rhynchostegiella (7)
— *curviseta* (Brid.) Limpr. FR
— *jacquinii* (Garov.) Limpr. FR
— *tenella* (Dicks.) Limpr. RR
— *tenuicaulis* (Spruce) Kartt. RR
Rhynchostegium (7)
— *confertum* (Dicks.) B.S.G. FV
— *megapolitanum* (Web. & Mohr) B.S.G. SP
— *murale* (Hedw.) B.S.G. CM
— *riparioides* (Hedw.) Card. FV
— *rotundifolium* (Brid.) B.S.G. RR
Rhytidiadelphus (47)
— *loreus* (Hedw.) Warnst. SP
— *squarrosus* (Hedw.) Warnst. CM
— *subpinnatus* (Lindb.)Kop. FR
— *triquetrus* (Hedw.) Warnst. CM
Rhytidium (47)
— *rugosum* (Hedw.) Kindb. CM
Saelania (19)
— *glaucescens* (Hedw.) Broth. SP
Sanionia (1)
— *uncinata* (Hedw.) Loeske in Nitardy CM
Sarmentypnum (1)
— *sarmentosum* (Wahlenb.) Tuom. & Kop. SP
Schistidium (27)
— *apocarpum* (Hedw.) B.&S. CM
— *atrofuscum* (Schimp.) Limpr. FR
— *brunnescens* Limpr. RR
— *confertum* (Funck) B.S.G. [28] SP
— *papillosum* Culm. FR
— *pulvinatum* (Hedw.) Brid. FR
— *rivulare* (Brid.) Podp. RR
— *strictum* (Turn.) Loeske ex Mart. [28] FR
Schistostega (48)
— *pennata* (Hedw.) Web. & Mohr SP
Scorpidium (1)
— *scorpioides* (Hedw.) Limpr. RR
Scorpiurium (7)
— *circinatum* (Fleisch. & Loeske) B.S.G. FR
Seligeria (49)
— *acutifolia* Lindb. ex Hartm. FR

— *calcarea* (Hedw.) B.S.G. FR
— *donniana* (Sm.) C. Müll. FR
— *pusilla* (Hedw.) B.S.G. SP
— *recurvata* (Hedw.) B.S.G. RR
— *trifaria* (Brid.) Lindb. RR
Serpoleskea (1)
— *confervoides* (Brid.) Kartt. RR
Sphagnum (51)
— *affine* Ren. & Card. FR
— *angustifolium* (C. Jens. ex Russ.) C. Jens. SP
— *annulatum* Lindb. fil. ex Warnst. FR
— *balticum* (Russ.) Russ. ex C. Jens. RR
— *capillifolium* (Ehrh.) Hedw. CM
— *centrale* C. Jens. FV
— *compactum* Lam. & DC. FV
— *contortum* Schultz SP
— *cuspidatum* Ehrh. ex Hoffm. FV
— *denticulatum* Brid. RR
— *fallax* (Klinggr.) Klinggr. FV
— *fimbriatum* Wills. SP
— *flexuosum* Dozy & Molk. CM
— *fuscum* (Schimp.) Klinggr. CM
— *girgensohnii* Russ. CM
— *jensenii* Lindb. fil. FR
— *magellanicum* Brid. CM
— *majus* (Russ.) C. Jens. RR
— *molle* Sull. FR
— *obtusum* Warnst. FR
— *palustre* L. FV
— *papillosum* Lindb. FR
— *platyphyllum* (Lindb. ex Braithw.) Sull. ex Warnst. RR
— *pulchrum* (Lindb. ex Braithw.) Warnst. FR
— *quinquefarium* (Lindb. ex Braithw.) Warnst. FV
— *riparium* Ångstr. FR
— *rubellum* Wills. FV
— *russowii* Warnst. FV
— *squarrosum* Crome CM
— *subnitens* Russ. & Warnst. SP
— *subsecundum* Nees FV
— *tenellum* (Brid.) Bory FR
— *teres* (Schimp.) Ångstr. FV
— *warnstorfii* Russ. CM
— *wulfianum* Girg. RR
Splachnum (52)
— *ampullaceum* Hedw. SP
— *luteum* Hedw.[6] FR
— *rubrum* Hedw. FR
— *sphaericum* Hedw. SP
Sporledera (19)
— *palustris* (B. & S.) Hampe[6] FR
Stegonia (44)
— *latifolia* (Schwaegr.) Vent. SP
Stellariomnium (38)
— *stellare* (Hedw.) Bow. FV
Streblotrichum (58)
— *convolutum* (Hedw.) Beauv. FV
— *croceum* (Brid.) Loeske FR
Stylostegium (49)
— *caespiticium* (Web. & Mohr) B.S.G. FR
Syntrichia (44)
— *alpina* (B.S.G.) Jur. FR
— *inermis* (Brid.) Bruch ex Hüb. FR
— *laevipila* (Brid.) Schultz FR
— *latifolia* (Bruch ex Hartm.) Hüb., non Lindb. FR
— *montana* Nees SP
— *mucronifolia* (Schwaegr.) Brid. FR
— *norvegica* Web. RR
— *papillosa* (Wills.) Jur. SP
— *pulvinata* (Jur.) Jur. RR
— *ruraliformis* (Besch.) Card. RR
— *ruralis* (Hedw.) Brid. CM
— *subulata* (Hedw.) Web. & Mohr CM
Taxiphyllum (42)
— *densifolium* (Lindb.) Reim. FR
— *wissgrillii* (Garov.) Wijk & Marg. RR
Tayloria (52)
— *acuminata* Hornsch. RR
— *froelichiana* (Hedw.) Mitt. ex Broth. RR

— *hornschuchii* (Grev. & Arnott) Broth. FR
— *lingulata* (Dicks.) Lindb. RR
— *serrata* (Hedw.) B. & S. RR
— *splachnoides* (Schleich. ex Schwaegr.) Hook. FR
— *tenuis* (Dicks.) Schimp. RR

Tetraphis (53)
— *pellucida* Hedw. CM

Tetraplodon (52)
— *angustatus* (Hedw.)B.& S. RR
— *mnioides* (Hedw.) B. & S. FR
— *urceolatus* B. & S. ex Schimp. FR

Tetrodontium (53)
— *brownianum* (Dicks.) Schwaegr. FR

Thamnobryum (54)
— *alopecurum* (Hedw.) Gang. SP

Thuidium (56)
— *erectum* Duby CM
— *minutulum* (Hedw.) B.S.G. FR
— *philibertii* Limpr. FV
— *recognitum* (Hedw.)Lindb. FV
— *recognitum T.* × *erectum* FR
— *tamariscinum* (Hedw.) B.S.G FV

Timmia (57)
— *austriaca* Hedw. SP
— *bavarica* Hessl. RR
— *comata* Lindb. & Arn. FR
— *megapolitana* Hedw.? FR
— *norvegica* Zett. FR

Tomentypnum (7)
— *nitens* (Hedw.) Loeske FV

Tortella (58)
— *bambergeri* (Schimp.) Broth. FR
— *densa* (Lor. & Mol.) Crundw. & Nyh. FR
— *flavovirens* (Bruch) Broth. FR
— *fragilis* (Drumm.) Limpr. RR
— *humilis* (Hedw.) Jenn. FR
— *inclinata* (Hedw. fil.) Limpr. SP
— *nitida* (Lindb.) Broth. FR
— *tortuosa* (Hedw.) Limpr. CM

Tortula (44)
— *aestiva* (Hedw.) Beauv. RR
— *atrovirens* (Sm.) Lindb. FR
— *canescens* Mont. FR
— *muralis* Hedw. CM
— *obtusifolia* (Schwaegr.) Math. FR

Trematodon (8)
— *ambiguus* (Hedw.) Hornsch. FR

Trichostomum (58)
— *brachydontium* Bruch RR
— *crispulum* Bruch ex F. Müll. SP

Trochobryum (49)
— *carniolicum* Breidl. & Beck [6] FR

Ulota (41)
— *bruchii* Hornsch. ex Brid. RR
— *coarctata* (Beauv.) Hamm. RR
— *crispa* (Hedw.) Brid. CM
— *hutchinsiae* (Sm.) Hamm. RR

Warnstorfia (1)
— *exannulata* (B.S.G.) Loeske CM
— *fluitans* (Hedw.) Loeske ex Nitardy [14] SP
— *pseudostraminea* (C. Müll.) Tuom. & Kop. [14] FR

Weisia (58)
— *controversa* Hedw. FV
— *crispata* (Nees & Hornsch.) C. Müll. FV
— *rutilans* (Hedw.) Lindb. FR

Zygodon (41)
— *dentatus* (Breidl. ex Limpr.) Kartt. FR
— *gracilis* Wills. FR
— *viridissimus* (Dicks.) Brid. RR

BRIEF ANNOTATIONS

1. Although branching is an unimportant and unstable character, the large number of simple-pinnate species convinced us as other authors, to keep the genus *Abietinella* (Vohra 1983).
2. *Amblystegium enerve* B. & S. similar to the smaller forms of *A. serpens* (Hedw.) B.S.G., but the leaves have no nerves; we do not know to which species it is synonymous, but we think it may be *Platydictia* spp.
3. According to the nerve pattern and to the thickness of the lamina, however, we kept genera *Anisothecium* and *Dicranella* separate, like other authors did.
4. The genus *Anomodon*, previously placed in various families, is now separated in *Anomodontaceae* family (Przywara et al. 1991).
5. *Brachythecium mildeanum* (Schimp.) Schimp. ex Milde is a species rather difficult to separate from *B. salebrosum* (Web. & Mohr) B.S.G. when the latter grows in mesic areas it has slightly plicated leaves.
6. The species indicated after the binomial in figure 6 are proposed to be excluded from Romanian bryoflora until they are reconfirmed (also see "Conventional marks").
7. Recently, some authors (Berg et al. 1991) considered this taxon to be synonymous to *Bryum pseudotriquetrum* (Hedw.) Gartn., Meyer & Schreb., but, in our opinion, sexuality has great taxonomic importance for the representatives of this large *Bryum* genus, so we have decided to keep it at the species level.
8. *Bryum erythrocarpum* agg. is considered to be an aggregate species, in Romania this species has been cited as : *B. erythrocarpum* Schwaegr. s. str. (syn. : *B. atrovirens* Brid.), as it is by the Ukrainian bryologists which consider *B. rubens* Mitt. to be its synonym.
9. *Callicladium haldanianum* (Grev.) Crum — was mistaken in Romania for *Sematophyllum substrumulosum* (Hampe) Britt. (Mihai 1986).
10. Inside *Calliergon* genus several genera (Toumikoski, Koponen 1979; Hedenäs 1990) were recognized.
11. Both the gametophyte and the sporophyte have diagnostic characters which plead for keeping *Camptothecium* genus, the same opinion also has other supporters (Vohra 1983).
12. The opinions on the individuality of this taxon are divided, same researches considering it to be just a variety of *Campyliadelphus stellatus* (Hedw.) Kanda and others keep it as species, *C. protensum* (Brid.) Kanda, as we did.
13. What the European bryologists have named *Campylium sommerfeltii* (Myr.) J. Lange is now named *C. calcareum* Crundw. & Nyh., and what they have named *C. hispidulum* (Brid.) Mitt. in Europe is *C. sommerfeltii* (Myr.) J. Lange.
14. *Drepanocladus* genus is divided into several genera, most of them accepted. Some authors (Kanda 1976) consider it to be synonymous to *Drepanocladus pseudostramineus* (C. Müll.) Roth considers it to be synonymous to *D. fluitans* (Hedw.) Warnst., but other authors (Irealand et al. 1987) keep them as species.

15. Although there are authors which consider *D. intermedius* (Lindb. ex Hartm.) Warnst. to be synonymons to *D. revolvens* (Sw.) Warnst., other consider it to be synonymous to *D. cossonii* (Schimp.) Loeske.
16. More recently, *Eurhynchium flotowianum* (Sendtn.) Kartt. was brought back to the old genus, as *Cirriphyllum flotowianum* (Sendtn.) Ochyra but we have kept it as *Eurhynchium*.
17. According to some authors (Berg et al. 1991) *Fissidens incurvus* Starke ex Rohl. is a legitimate binomial, but according to others (Ireland et al. 1987) it would be a synonym of *F. viridulus* (Sw.) Wahlenb.
18. *Fissidens pusillus* (Wils.) Milde and *F. gracilifolius* Brugg.-Nann. &Nyh. (= *F. minutulus* auct.) are kept as species (Berg et al. 1991) or considered to be synonymous to *F. bryoides* Hedw. (Ireland et al. 1987) leaving only *F. bryoides* and *F. viridulus*. The truth is that these small species, with marginated leaves, have only small differences which most times are difficult to render evident.
19. If *Grimmia orbicularis* Bruch in Wils. is synonymous, as it seems to be, to *G. pulvinata* var. *africana* (Hedw.) Hook. fil., then it loses its priority, being replaced by *G. africana* (Hedw.) Arnott.
20. *Helodium blandowii* (Web. & Mohr) Warnst. is included together with *Palustriella* in *Helodiaceae* family (Przywara et al. 1991).
21. *Homomallium incurvatum* (Brid.) Loeske was sometimes mistaken at us for *Sematophyllum substrumulosum* (Hampe) Britt. (Mihai 1983).
22. *Hypnum recurvatum* (Lindb. & Arn.) Kindb. is excluded from Romanian bryoflora and only *H. fastigiatum* Brid. species were kept (Mihai 1986), but they are synonymous.
23. Many authors consider *Hypnum reptile* Michx. to be synonymous to *H. pallescens* (Hedw.) Beauv., or consider it to be a variety of this species.
24. G. Mohan indicates *Molendoa sendtneriana* (B.S.G.) Limpr. with the diagnosta character (in the text and iconography) of *M. hornschuchiana* (Hook.) Lindb. (Mohan 1984).
25. Many authors consider *Orthotrichum fastigiatum* Bruch ex Brid. to be synonymous to *O. affine* Brid., but as many authors consider it to be a species (Hinneri 1976).
26. The systematic position of the common species *Pleurozium schreberi* (Brid.) Mitt. is not yet stable; the species can be found in the families *Entodontaceae*, *Hypnaceae*, *Hylocomiaceae*, *Brachytheciaceae* and *Amblystegiaceae* (Kanda 1976). We have kept it in *Entodontaceae*.
27. Most bryologists consider *Pseudoleskea atrovirens* B.S.G. to be synonymous to *P. incurvata* (Hedw.) Loeske.
28. Recently, some authors (Baciurina, Melniciuk 1988—1989) keep the individuality of the microspecies *Schistidium confertum* (Funck) B.S.G. and *S. strictum* (Turn.) Mårt. (= *S. gracile* (Schleich.) Limpr.), which is what we have doue.

SELECTED REFERENCES

1. Abramova A., Abramov I., *Conspect flori mhov Mongolskoi Naradnoi Respubliki*, Izd. "Nauka", Leningrad, 1983.
2. Arts T., Mém. Soc. Roy. Bot. Belg., 1990, **12**, 20—36.

3. Augier J., *Flore des Bryophytes*, Editions Paul Lechevalier, Paris, 1966.
4. Baciurina G., Melniciuk V., *Flora mohiv Ukrainskoi R.S.R.*, **2**, **3**, Kiiv Naukova Dumka, Kiev, 1988—1989.
5. Baumgarten J., *Enumeratio Stirpium Magno Transsilvaniae Principatui. 4*, (curante M. Fuss), Cibinii, 1846 (1860).
6. Berg C., Meinunger L., Gleditschia, 1991, **19**, *2*, 315—343.
7. Boros A., Vajda L., Rév. Bryol. et Lichenol., 1967, **35**, *1—4*, 216—253.
8. Corley M. et al., J. Bryol., 1981, **11**, 609—689.
9. Corley M., Crundwell A., J. Bryol., 1991, **16**, *3*, 337—356.
10. Cortini Pedrotti Carmela, Aleffi M., Lista rossa delle Briofite d'Italia, in: F. Conti et al. (edit.), *Libro rosso delle piante d'Italia*, 1992, 559—637.
11. Frahm J.-P., Frey W., *Mossflora*. Verlag Eugen Ulmer Stuttgart, 1983.
12. Fuss M., Archiv. des Vereins für Siebenbürggische Landeskunde. Neue Folge, 1878, 14, 3, 627—708, Hermannstadt.
13. Gams H., *Die Moos- und Farnpflanzen*, Fünfte, erweiterte Auflage, in Kleine Kryptogamenflora, Bd. IV, Gustav Fischer Verlag, Stuttgart, 1973.
14. Hedenäs L., Lindbergia, 1990, **16**, *3*, 80—99.
15. Hinneri S., Ann. Univ. Turku., Ser. A., II. Biol.-Geogr.-Geol., 1976, **58**, 1—37.
16. Husnot T., *Muscologia gallica, descriptions et figures des Mousses de France et des contrées voisines*. Paris, 1884—1890.
17. Ireland R. et al., Lindbergia, 1987, **13**, *1—2*, 1—62.
18. Kanda H., Journal of Science of the Hiroshima University, Series B, Div. 2, 1975, **15**, *2*, 201—276; 1976, **16**, *1*, 47—119.
19. Karttunen K., Ann. Bot. Fennici, 1992, **29**, *2*, 121.
20. Koponen T., Isoviita P., Lammes T., *The Bryophytes of Finland: an annotated checklist*, in *Flora Fennica*, 6, 1977. Helsinki, 1977.
21. Mihai G., Anal. St. Univ. "Al. I. Cuza" Iași, Secț. II, a. Biol., 1983, **29**, 31—34.
22. Mihai G., Anal. St. Univ. "Al. I. Cuza" Iași, Secț. II, a. Biol., 1986, **33**, 36—37.
23. Mohan G., *Briofilé (Determinator ilustrat al brioflorei României)*, (Litogr.). Univ. București, București, 1984.
24. Mönkemeyer W., *Die Laubmoose Europas*, Akad. Verlagsgesellschaft m. b. H., Leipzig, 1927.
25. Ochyra R., Fragm. Flor. Geobot., 1992, **37**, *1*, 211—214.
26. Ochyra R., Pócs T., Fragm. Flor. Geobot., 1992, **37**, *2*, 379—388.
27. Ochyra R., Szmajda P., Fragm. Flor. Geobot., 1978, **24**, 1, 93—145.
28. Papp C., *Briofitele din Republica Socialistă România (Determinator)*, in Anal. St. Univ. "Al. I. Cuza" Iași, Secț. II, a. Biol. Monografii, 3, 1976. Iași 1970.
29. Petrov S., *Opredelitel na mâhovete v Bălgaria*. Izd. Bălg. Akad Nauk. Sofia, 1975.
30. Pilous Z., *Bryophyta — Sphagnidae*, Academia Naklad. Ceskoslov. Acad. Ved., Praha, 1971.
31. Przywara L., Kuta Elzbieta, Ochyra R., Fragm. Flor. Geobot., 1991, **36**, *1*, 89—104.
32. Savici-Liubitskaia L., Smirnova Z., *Opredelitel listostebelnâh mhov SSSR — Verhoplodnâe mhi*, Izd. "Nauka", Leningrad, 1970.
33. Schimper W., *Synopsis Muscorum Europaeorum. I, II*. Sumtibus Librariae E. Schweizerbart (E. Koch), Stuttartiae, 1876.
34. Schur F., *Enumeratio Plantarum Transsilvaniae*, Apud Guilielmum Braumüller, Vindobonae, 1866.
35. Smith A., *The Moss Flora of Britain and Ireland*., Cambridge University Press, Cambridge, 1980.
36. Szafran B., *Mchy (Musci). I, II*. Państwowe Wydawnictwo Naukowe, Warszawa, 1957, 1961.
37. Tuomikoski R., Koponen T., Ann. Bot. Fennici, 1979, **16**, 213—227.
38. Vohra J., *Hypnobryales suborder Leskeineae (Musci) of the Himalayas*, Botanical Survey of India, Calcutta, 1983.

Received April 30, 1994

Institute of Biology, Bucharest,
296 Spl. Independenței, Romania

THE ANNUAL DYNAMICS OF THE HERBACEOUS BIOMASS FROM SOME FORESTRY ECOSYSTEMS OF THE BUCEGI MOUNTAINS

V. SANDA, A. POPESCU, GABRIELA FIŞTEAG, GH. ŞERBĂNESCU

The work analyses the herbaceous biomass from the *Leucanthemo waldsteinii-Piceo-Fagetum* Soó 1964 and *Hieracio rotundati-Piceetum* Pawl. et Br.—Bl. 1939 associations situated on the Prahova slope of the Bucegi mountains, emphasizing on phenoaspects, the dynamics of the bioaccumulations at the herbaceous layer level, process which characterizes each investigated station.

Within the analysis of the forestry ecosystems the herbaceous synusy, by the specificity of flowering elements, determines not only the classification and the defining of the structural unities, but participates at the same time, in an active manner, at the acceleration of the pedogenesis processes by annual bioaccumulations. Also, an important role is played by the herbaceous layer in the consolidation and fixation of the soil on the sloped surfaces, protecting it from its rapid washing and draining.

MATERIALS AND METHODS

The collection of the vegetal material has been effected seasonally, in order to watch the biomass accumulations, on 0.25 m^2 surfaces, in one hundred repetitions.

In view of determining the biomass quantity on surface unit, a number of 25—50 individuals have been collected, depending on the gravimetric dimensions of plants, which were weighed with the analytic balance, in fresh or dried (85°C) state.

Knowing the individual medium weight for every species, as well as their frequency and density on square meter the quantity of biomass (fresh and dried) on surface unit was determined.

The phytocenoses have been classified according to the speciality literature (*1*), (*2*) and the biomass determinations have been effected according to the present methodology (*3*), (*4*).

RESULTS AND DISCUSSIONS

The phytocenoses of beech and spruce mixture provenience have been found and analysed by us on the left side of Valea Morarului at an approx. 1200—1250 m altitude, where they form a zonal vegetation. These woods vegetate on deep, acidic, brown soils, rich in humus and in total nitrogen. The two arborescent, edifying species *Fagus sylvatica* and *Picea abies* are in codominance relations.

REV. ROUM. BIOL.—BIOL. VÉGÉT., TOME 39, Nº 2, P. 109—117, BUCAREST, 1994

In the vernal phenophase, the herbaceous layer biomass is realized mainly by the following species: *Dentaria glandulosa*, *Athyrium filix-femina*, *Valeriana montana*, *Chaeropyhyllum hirsutum*, *Symphytum cordatum*, characteristic both for the *Symphyto-Fagion* alliance and also for the edifying cenotaxons which form the high weeds on the mountainous valleys penetrating these biotops (Table 1).

The biggest quantity of herbaceous biomass from the *Leucanthemo waldsteinii-Piceo-Fagetum* Soó 1964 association has been obtained in the aestival phenophase, namely 39.760 gr dried matter/m² (Table 2). The species which dominate and participate the most in the realization of this biomass are: *Senecio fuchsii*, *Valeriana montana*, *Oxalis acetosella*, *Cirsium erisithales*, *Athyrium filix-femina*, *Dryopteris filix-mas*.

In the autumnal season, the herbaceous biomass starts decreasing, owing to the beginning of the drying process of plants, reading 25.961 g dried matter/m², but the values are superior to the spring ones, the produced bioaccumulations being evident (Table 3).

The species which significantly participate in the realization of this autumnal season biomass are: *Calamagrostis arundinacea*, *Senecio fuchsii*, *Athyrium filix-femina*, *Valeriana montana* and *Oxalis acetosella*.

The species with great frequency and density in the Valea Morar station are weeds characteristic of the moisty, mountainous valleys as: *Senecio fuchsii*, *Chaerophyllum hirsutum*, *Valeriana montana*, *Leucanthemum waldsteinii*, *Calamagrostis arundinacea*, species which also have an increased individual biomass, thus explaining the production of a greater quantity on surface unit, than in the typical phytocenoses.

The second analyzed station is represented by the spruce woods on the Valea Brădet, situated at approx. 1350 m altitude. These vegetate on strong acidic, moist, podzolic, brown soils. The slopes on which the phytocenoses are cantonated are strongly inclined (26–35°) with various, generally western or north-western expositions. The herbaceous species are characteristic of the *Vaccinio-Piceion* alliance and the *Vaccinio-Piceetalia* order, but also the infiltration of many elements of *Symphyto-Fagion* and *Adenostyletalia* has been found.

The vernal synusy of the *Hieracio rotundati-Piceetum* Pawl. et Br.–Bl 1939 association is dominated by *Symphytum tuberosum*, *Ranunculus carpaticus*, *Dentaria glandulosa* and *Mercurialis perennis* which participate in the realization of the highest percentage of this season's biomass, the values reaching 15.108 g dried matter/m² (Table 4).

In summer, the quantity of biomass significantly increases, being the biggest of the whole year, namely 28.285 g dried matter per m² (Table 5). The species which participate with high values in the realization of this percentage are the same as in the vernal season, to which: *Senecio fuchsii*, *Mycelis muralis*, *Veronica urticifolia*, *Helleborus purpurascens*, *Astrantia major* and *Fragaria vesca* must be added.

In autumn, the quantity of biomass, with the value of 20.795 g dried matter/m² is realized mainly by: *Athyrium filix-femina*, *Rubus idaeus*, *Calamagrostis arundinacea*, *Geranium robertianum*, *Urtica dioica* and *Mercurialis* perennis (Table 6).

In the Brădet station, the species: *Oxalis acetosella*, *Galium odoratum*, *Mycelis muralis*, *Adoxa moschatellina*, *Hieracium rotundatum* and

Table 1

The frequency, density and vernal herbaceous biomass (May 1993) from *Leucanthemo waldsteinii-Piceo-Fagetum* Soó 1964 (Valea Morarului)

Species	Frequency %	Density ind./m²	Biomass (g/m²)	
			green	dried
Oxalis acetosella	89	114.05	11.861	0.912
Dentaria glandulosa	77	13.05	27.274	2.818
Chaerophyllum hirsutum	56	7.95	18.165	1.574
Stellaria nemorum	54	6.75	7.492	0.627
Valeriana montana	56	9.40	23.227	2.304
Leucanthemum waldsteinii	55	6.15	3.880	0.461
Soldanella hungarica	45	9.95	4.497	0.626
Ranunculus carpaticus	34	9.10	18.300	1.965
Athyrium filix-femina	26	5.40	23.036	2.629
Aconitum toxicum	19	1.75	10.036	0.955
Aegopodium podagraria	20	1.05	2.478	0.955
Adoxa moschatellina	37	6.50	1.144	0.007
Chrysosplenium alternifolium	19	2.95	0.705	0.007
Symphytum cordatum	17	3.10	14.508	1.174
Pulmonaria rubra	14	1.25	3.031	0.034
Galeobdolon luteum	12	1.26	0.732	0.142
Senecio fuchsii	29	2.10	4.592	0.386
Veronica urticifolia	14	0.75	0.202	0.024
Myosotis sylvatica	11	1.70	3.294	0.324
Tussilago farfara	16	2.00	7.966	0.598
Clemalis alpina	10	0.75	0.530	0.065
Primula officinalis	11	0.60	2.466	0.249
Gymnocarpium dryopteris	7	2.25	0.949	0.123
Saxifraga cuneifolia	10	1.40	1.145	0.270
Milium effusum	16	4.40	1.892	0.268
Doronicum austriacum	7	0.55	1.419	0.118
Cirsium erisithales	9	0.35	1.121	0.088
Calamagrostis arundinacea	11	7.55	1.419	0.302
Viola biflora	5	0.60	0.135	0.015
Ajuga reptans	4	0.30	0.282	0.039
Mycelis muralis	4	0.35	0.064	0.005
Dentaria bulbifera	1	0.10	0.053	0.006
Isopyrum thalictroides	2	0.30	0.118	0.018
Huperzia selago	4	0.50	0.464	0.147
Anemome nemorosa	2	0.20	0.079	0.012
Fragaria vesca	4	0.35	0.090	0.020
Hepatica transsilvanica	1	0.10	0.056	0.007
Pyrola secunda	1	0.10	0.026	0.011
Cortusa matthioli	5	0.55	0.503	0.050
Hieracium rotundatum	5	0.43	0.231	0.037
Rubus idaeus	9	0.45	2.947	0.822
Heracleum palmatum	6	0.40	6.728	0.640
Galium odoratum	1	1.50	0.534	0.051
Paris quadrifolia	2	0.45	0.207	0.625
Total biomass/m²			209.218	20.870

Table 2

The frequency, density and aestival herbaceous biomass (July 1993) from *Leucanthemo-waldsteinii-Piceo-Fagetum* Soo 1964 (Valea Morarului)

Species	Frequency %	Density ind./m²	Biomass (g/m²)	
			green	dried
Senecio fuchsii	41	3·75	48·293	6·109
Valeriana montana	45	6·20	22·221	2·883
Aegopodium podagraria	25	3·35	5·129	0·700
Leucanthemum waldsteinii	44	6·10	3·750	0·400
Gentiana phlogifolia	1	0·05	0·225	0·037
Oxalis acetosella	85	121·35	13·227	1·577
Myosotis sylvatica	14	2·70	4·012	0·707
Galeobdolon luteum	2	4·05	1·300	0·206
Soldaaella hungarica	21	8·65	1·894	0·311
Stellaria nemorum	50	8·40	5·048	0·546
Tussilago farfara	16	2·30	17·353	1·861
Cirsium erisithales	9	1·25	40·494	4·749
Heracleum palmatum	7	1·10	32·451	3·129
Rubus idaeus	14	0·85	3·699	1·029
Geum urbanum	4	0·25	0·357	0·068
Delphinium elatum	14	1·15	13·249	1·881
Symphytum cordatum	4	0·25	0·110	0·008
Primula officinalis	7	0·35	3·931	0·500
Thalictrum aquilegiifolium	5	0·55	1·570	0·312
Chrysosplenium alternifolium	5	0·55	1·323	0·180
Athyrium filix-femina	19	3·00	37·020	6·219
Adoxa moschatellina	1	0·85	0·179	0·014
Calamagrostis arundinacea	6	5·10	3·182	0·790
Viola biflora	10	1·05	0·429	0·044
Ranunculus carpaticus	26	3·40	8·701	0·870
Veronica urticifolia	11	0·95	1·966	0·334
Valeriana tripteris	4	0·45	1·118	0·169
Dryopteris filix-mas	7	1·05	10·820	1·994
Pulmonaria rubra	17	2·10	7·279	0·876
Cortusa matthioli	6	0·45	1·065	0·115
Saxifraga cuneifolia	7	4·35	2·332	0·361
Actaea spicata	2	0·10	0·070	0·005
Paris quadrifolia	1	0·15	0·084	0·008
Pyrola secunda	1	0·10	0·091	0·026
Hepatica transsilvanica	2	1·00	0·581	0·103
Melandrium nemorale	1	0·05	0·895	0·091
Urtica dioica	2	0·10	0·454	0·065
Epilobium montanum	1	0·10	0·167	0·032
Fragaria vesca	2	0·10	0·049	0·015
Gymnocarpium dryopteris	1	0·25	2·576	0·475
Total biomass/m²			298·702	39·760

Table 3

The frequency, density and autumnal herbaceous biomass (September 1993) from *Leucanthemo waldsteinii-Piceo-Fagetum* Soo 1964 (Valea Morarului)

Species	Frequency %	Density ind./m²	Biomass (g/m²)	
			green	dried
Pulmonaria rubra	13	2·05	6·753	0·863
Calamagrostis arundinacea	24	17·30	13·909	3·062
Senecio fuchsii	44	3·55	22·919	2·999
Dryopteris filix-mas	9	0·65	2·862	0·610
Tussilago farfara	29	3·30	12·240	1·594
Athyrium filix-femina	31	6·10	40·034	6·740
Gymnocarium dryopteris	14	2·05	0·492	0·110
Aegopodium podagraria	11	0·90	0·732	0·010
Valeriana montana	51	8·55	45·067	5·891
Oxalis acetosella	70	92·70	11·680	1·390
Leucanthemum waldsteinii	17	0·85	1·863	0·362
Galeobdolon luteum	17	2·50	0·770	0·107
Soldanella hungarica	16	4·20	0·861	0·139
Myosotis sylvatica	12	2·80	4·603	0·459
Rubus idaeus	14	0·85	2·598	0·707
Veronica urticifolia	14	1·00	0·909	0·186
Saxifraga cuneifolia	10	3·40	2·890	0·456
Fragaria vesca	2	0·15	0·074	0·022
Ranunculus carpaticus	6	0·80	1·023	0·126
Cortusa matthioli	4	0·50	1·183	0·127
Total biomass/m²			173·462	25·961

Table 4

The frequency, density and vernal herbaceous biomass (May 1993) from *Hieracio rotundati-Piceetum* Pawl. et Br.-Bl. 1930 (Brădet)

Species	Frequency %	Density ind./m²	Biomass (gr/m²)	
			green	dried
1	2	3	4	5
Oxalis acetosella	70	49·24	2·117	0·197
Mycelis muralis	44	3·68	0·680	0·058
Galium odoratum	37	7·92	1·987	0·435
Fragaria vesca	31	4·54	1·375	0·402
Symphytum tuberosum	32	7·84	13·053	1·426
Galeobdolon luteum	31	3·28	1·906	0·371
Ranunculus carpaticus	28	15·48	15·572	1·656
Euphorbia amygdaloides	21	1·72	4·642	0·964
Senecio fuchsii	20	2·04	8·735	0·724
Dentaria glandulosa	25	5·92	7·080	0·858
Dentaria bulbifera	22	4·16	2·229	0·262
Hieracium rotundatum	16	1·52	0·557	0·074
Luzula luzuloides	31	7·80	3·408	0·751
Veronica montana	15	0·96	0·264	0·029
Rubus idaeus	14	0·64	4·179	1·436
Poa nemoralis	12	7·60	0·661	0·129
Epilobium montanum	14	1·24	0·229	0·019
Adoxa moschatellina	53	20·12	3·681	0·321

Table 4 (continued)

1	2	3	4	5
Isopyrum thalictroides	12	2·72	1·283	0·274
Anthriscus nitida	11	0·92	2·171	0·220
Valeriana montana	10	1·40	0·413	0·049
Mercurialis perennis	16	9·20	14·858	1·738
Urtica dioica	15	2·24	3·608	0·454
Myosotis sylvatica	8	0·60	1·162	0·114
Soldanella hungarica	5	0·48	0·275	0·044
Bromus benekenii	5	1·36	0·350	0·073
Hepatica transsilvanica	7	1·36	0·767	0·096
Anemone ranunculoides	7	1·88	0·753	0·112
Helleborus purpurascens	6	0·72	3·852	0·433
Lilium martagon	7	0·36	2·048	0·153
Pyrola secunda	5	1·00	0·265	0·096
Geranium robertianum	7	0·48	0·912	0·139
Leucanthemum waldsteinii	4	0·40	0·252	0·030
Stachys sylvatica	3	0·20	0·435	0·036
Symphytum cordatum	2	0·52	2·433	0·197
Athyrium filix-femina	2	0·88	3·754	0·428
Veronica urticifolia	2	0·28	0·077	0·009
Aegopodium podagraria	2	0·12	0·283	0·028
Campanula abietina	2	0·48	0·133	0·015
Dryopteris filix-mas	1	0·04	0·170	0·019
Tussilago farfara	1	0·12	0·478	0·035
Calamagrostis arundinacea	1	0·68	0·125	0·025
Carex sylvatica	1	0·08	0·027	0·004
Homogyne alpina	2	0·08	0·036	0·005
Ribes petraeum	1	0·08	0·327	0·146
Total biomass/m²			113·622	15·108

Table 5

The frequency, density and aestival herbaceous biomass (July 1993) from *Hieracio rotundati-Piceetum* Pawl. et Br.-Bl. 1939 (Brădet)

Species	Frequency %	Density ind./m²	Biomass (g/m²)	
			green	dried
1	2	3	4	5
Mycelis muralis	69	8·65	13·347	1·854
Veronica urticifolia	35	5·20	10·551	1·445
Isopyrum thalictroides	5	0·85	0·386	0·059
Bromus benekenii	2	0·85	2·598	0·589
Oxalis acetosellla	85	81·95	3·934	0·410
Myosotis sylvatica	10	1·05	1·620	0·223
Senecio fuchsii	29	1·85	32·188	3·522
Valeriana montana	5	0·20	0·291	0·028
Hieracium rotundatum	31	5·30	3·097	0·559
Lilium martagon	4	0·15	1·860	0·181
Euphorbia amygdaloides	12	1·65	4·207	0·836
Galeopsis tetrahit	4	0·20	0·141	0·014
Soldanella hungarica	7	3·70	0·721	0·118
Ranunculus carpaticus	17	5·55	7·204	0·910
Dryopteris filix-mas	5	0·65	11·746	2·445
Rubus idaeus	12	1·05	4·998	1·441
Helleborus purpurascens	5	0·55	6·730	1·087

Table 5 (continued)

1	2	3	4	5
Geranium robertianum	4	3·30	6·369	0·832
Poa nemoralis	17	18·15	1·398	0·381
Epilobium montanum	15	0·60	0·429	0·088
Galeobdolon luteum	27	5·00	3·945	0·650
Astrantia major	10	2·70	10·473	1·761
Fragaria vesca	1	6·20	6·553	1·897
Urtica dioica	9	0·75	5·538	0·807
Symphytum tuberosum	21	6·20	21·836	2·598
Adoxa moschatellina	25	2·65	0·776	0·066
Moehringia trinervia	22	15·70	0·832	0·785
Mercurialis perennis	19	7·35	13·818	2·191
Aegopodium podagraria	4	0·25	0·819	0·110
Leucanthemum waldsteinii	2	0·15	0·171	0·024
Geranium phaeum	4	0·55	1·594	0·234
Saxifraga cuneifolia	1	0·55	0·244	0·083
Pyrola secunda	4	0·75	0·515	0·073
Total biomass/m²			180·929	28·285

Table 6

The frequency, density and autumnal herbaceous biomass (September 1993) from *Hieracio rotundati-Piceetum* Pawl. et Br.-Bl. 1939 (Brădet)

Species	Frequency %	Density ind./m²	Biomass (g/m²)	
			green	dried
Euphorbia amygdaloides	9	1·40	2·974	0·623
Fragaria vesca	21	4·45	2·194	0·659
Soldanella hungarica	6	0·75	0·140	0·023
Hieracium rotundatum	21	2·40	0·802	0·130
Oxalis acetosella	54	53·90	4·581	0·647
Poa nemoralis	6	3·90	0·292	0·105
Rubus idaeus	22	2·15	3·601	1·049
Veronica urticifolia	15	1·80	1·636	0·335
Calamagrostis arundinacea	9	8·00	4·552	1·096
Senecio fuchsii	26	1·50	5·707	0·738
Luzula luzuloides	7	2·10	1·357	0·323
Saxifraga cuneifolia	2	0·85	0·149	0·021
Athyrium filix-femina	9	2·50	16·407	2·762
Mycelis muralis	40	5·40	5·373	0·853
Galeobdolon luteum	15	1·85	1·036	0·198
Bromus benekenii	4	0·50	0·251	0·059
Geranium roberlianum	42	8·21	19·277	2·389
Urtica dioica	12	1·85	20·111	3·001
Mercurialis perennis	19	7·15	15·658	2·595
Epilobium montanum	11	1·50	1·732	0·325
Myosotis sylvatica	7	1·55	0·980	0·122
Galeopsis tetrahit	6	1·35	3·515	0·655
Helleborus purpurascens	10	0·75	3·670	0·614
Leucanthemum waldsteinii	6	0·75	1·644	0·319
Astrantia major	5	0·85	3·630	0·730
Ranunculus carpaticus	4	0·60	0·271	0·034
Symphytum tuberosum	6	0·65	1·009	0·118
Symphytum cordatum	1	0·40	1·029	0·084
Dryopteris filix-mas	1	0·20	0·880	0·188
Total biomass/m²			124·458	20·79

Ranunculus carpaticus are characterized by an increased individual frequency and density, thus contributing to a great extent to the formation of the herbaceous biomass in this phytocenosis.

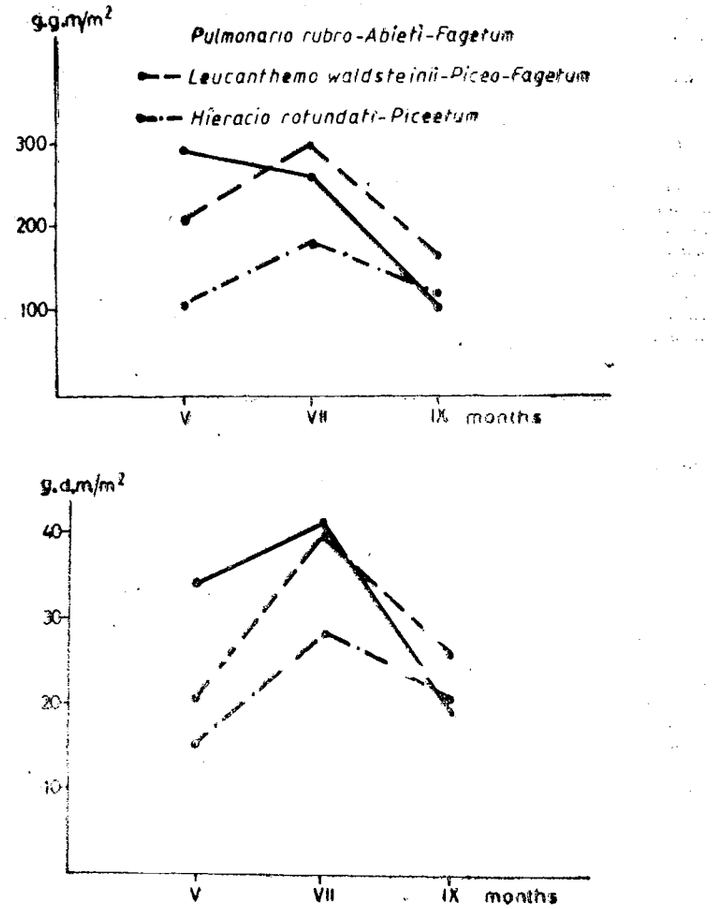


Fig. 1. — The herbaceous biomass from some forestry ecosystems of the Bucegi mountain chain (g/m²) g.m = green matter; d.m = dried matter

Analysing the common species of the herbaceous layer of the two investigated associations, a differently depending on the station biomass is identified, namely much higher values are found in the beech-spruce mixtures, as compared to the pure spruce woods. This is explained first of all by the existence of much more favorable microclimatic conditions on the Valea Morariului: the luminosity and humidity are much more accentuated, creating favorable conditions for the good development of the herbaceous synusy, as well as for the installation of some mountainous valley characteristic species as: *Heracleum palmatum*, *Calamagrostis*

arundinacea, etc., which abundantly participate in the formation of the herbaceous layer.

Investigating the repartition of the biomass quantity of the herbaceous layer in three forestry association types, it has been found that the biggest quantity of accumulated substance per surface unity is to be found in the beech-spruce mixtures (*Leucanthemo waldsteinii-Piceo-Fagetum*) amounting to 681.38 g dried matter/m² and in the beech-fir woods (*Pulmonario rubro-Abieti-Fagetum*) of 663.10 g dried matter/m² greatly differing of the pure spruce woods (*Hieracio rotundati-Piceetum*), where the biomass quantity decreases very much, reaching 419.10 g dried matter/m² (Fig. 1).

REFERENCES

1. Beldie Al., *Flora și vegetația munților Bucegi*, Editura Academiei, București, 1967.
2. Coldea Gh., *Prodrome des associations végétales des Carpates du sud-est (Carpates Roumaines)*, Camerino, 1991.
3. Pauca-Comănescu Mihaela, Tăcină Aurica, St. și Cerc. de Biol. Seria Biol. veget., **44** (2): 179—184, 1992.
4. Popescu A., Sanda V., Fișteag Gabriela, Tăcină Aurica, Șerbănescu Gh., Revue roum. de Biol., Sér. de Bot., **38** (2): 101—112, 1993.

Received March 15, 1994

Institute of Biology
Splaiul Independenței, 296
Bucharest

RECOVERY OF PHOTOSYNTHETIC ACTIVITY IN REHYDRATED LEAVES OF A RESURRECTION PLANT

FODORPATAKI LASZLO

Xerophyta scabrida is one of the very few poikilochlorophyllous monocotyledonous angiosperms, which exhibit the very specific behaviour of a resurrection plant regarding the recovery of its photosynthetic activity with the onset of the wet season. Upon rehydration, leaves which survived in anabiosis during the long dry season, unfold quickly and start to rebuild the photosynthetic apparatus of chloroplasts. In the presence of light they synthesize intensely a new set of light harvesting pigment-protein complexes. This is fellowed by recovery of the photosynthetic electron transport activity in thylakoids and finally by the efficient CO_2 fixation, which reaches its maximal level 3 days after the beginning of rehydration.

During their evolution some plants adapted to extremely arid environments by different mechanisms. Some of them, such as succulents, retain strongly and store high quantities of water for a long time, others, like xerophytes, minimize water loss by decreasing transpiration. Besides succulents and xerophytes there is a very limited number of vascular plant species, which are capable of tolerating drastic water loss approaching desiccation. These species are termed poikilohydric or resurrection plants, and during the long periods of drought suffered in their natural habitats they are almost completely dehydrated. They endure in a state of anabiosis with their metabolism arrested, and when water becomes available again, they rehydrate and physiological functions are quickly reactivated.

Desiccation tolerance requires biomembranes to withstand the chemical and mechanical stresses which accompany shrinkage of cells during water loss, and cellular expansion on reabsorption of water. Usually, high concentrations of salts, which create a chemical stress situation, dissociate extrinsic proteins from biomembranes. Because of the dissociation of the CF_1 of ATP-synthase, thylakoids lose the capability for light-dependent ATP synthesis (photophosphorylation) and for the formation of transthylakoidal proton gradient. During the slow desiccation of poikilohydric leaves polysaccharides are hydrolyzed and soluble sugars accumulate, which protect biomembranes against chemical inactivation (Schwab and Heber 1984). The fact that resurrection plants survive after desiccation and rehydration indicates that in these species either membrane rupture does not occur or can be repaired.

Even among the few poikilohydric species only a limited number is represented by poikilochlorophyllous resurrection plants. One of these rare species is *Xerophyta scabrida* (Pax) Th. Dur. et Schinz (Liliatae, Velloziaceae), which originates in Madagascar and lives in tropical and semi-desert areas of Southern and Eastern Africa. During the months of the dry season photosynthetic processes are completely ceased, but they recover quickly and function efficiently after rehydration of leaves. In the present work we investigated the dynamics of recovery of photosynthetic activity in this very special type of plants highly adapted to survive in long-term water stress conditions.

REV. ROUM. BIOL.—BIOL. VÉGÉT., TOME 39, Nº 2, P. 119—128, BUCAREST, 1994

MATERIAL AND METHODS

Experiments were carried out with leaves of *Xerophyta scabrida* collected by the end of the dry season in a rocky semidesert area in Tanzania, 650 m above sea level. Leaves were stored in dry state in air-tight polythene bags. For rehydration 20 leaves with the approximately same dimensions were put on a filter paper which covered the surface of the water (27 ± 1°C) of a 2 dm^3 vessel. During rehydration and regreening of leaves the water was changed every 12 hours. The leaves were illuminated continuously from above with light bulbs from a distance which avoided excessive heating and ensured an illumination of the upper surface of leaves with 600 μmole photons/m^2s photosynthetically active radiation. For all measurements disks with a diameter of 7 mm (0.385 cm^2 area) were detached from the middle third of the leaf blades' length, avoiding the removal of the middle vein.

Variations of water content during rehydration were detected by measuring the weight of 20 disks (with 7 mm diameter) before and after drying them for 4 hours at 105°C, in closed glass vessels with precisely known mass.

Photosynthetic pigment contents were determined spectrophotometrically as described by Arnon (1949), using an UV—Vis spectrophotometer made by Shimadzu. Chlorophylls were extracted from leaf disks (7 mm diameter) with 4 ml 80% (v/v) acetone (in dim light) and carotenoids in petroleum ether. Xanthophyll and β-carotene quantities were calculated according to Hager and Meyer-Bertenrath (1966). All extractions were performed at 5°C Extracts were centrifuged also at 5°C for 5 minutes at 2000 rpm and only the supernatants were used for measurements carried out in 10 mm wide cuvettes.

Fluorescence induction transients were measured with a chlorophyll fluorometer according to the method described by Hsu et al. (1989), after a dark incubation of leaf disks for 5 minutes.

Rates of CO_2 fixation were determined during illumination with saturating light of leaf disks for 1 hour, after an incubation for 15 minutes in darkness, in a closed chamber with constant supply of radioactively marked $^{14}CO_2$, followed by a postincubation for 15 minutes in darkness, in the same chamber. Radioactivity of completely dried leaf disks was measured with a scintillation counter, 0.5 min. for each sample in toluene cocktail. The method was applied according to Stitt and ap Rees (1978).

All the experimental set was repeated 3 times, each time with 20 leaves with the same size and general aspect.

RESULTS AND DISCUSSION

Desiccated leaves of *Xerophyta scabrida* are able to live in anabiosis even for more years. They are folded along the main vein, with the two halves of the blade's upper (adaxial) surface covering each other. The adventive roots which cover the thin stem are dead but they are able to fix the plant in the soil. The perennial leaves are 25—30 cm long and 6—8 mm wide in completely dried state, their abaxial face (which comes

in direct contact with the sunrays) is brown or dark grey, while the adaxial surface has a golden colour.

When the plant comes in contact with water, the leaves absorb it intensely through their entire surface, they unfold so that the adaxial face of the blade becomes visible, they grow rapidly in width (reaching 12—19 mm) and their consistence becomes slightly coriaceous. During rehydration the darker colour of the abaxial surface disappears and the leaves become uniformly bright straw-yellow before their regreening

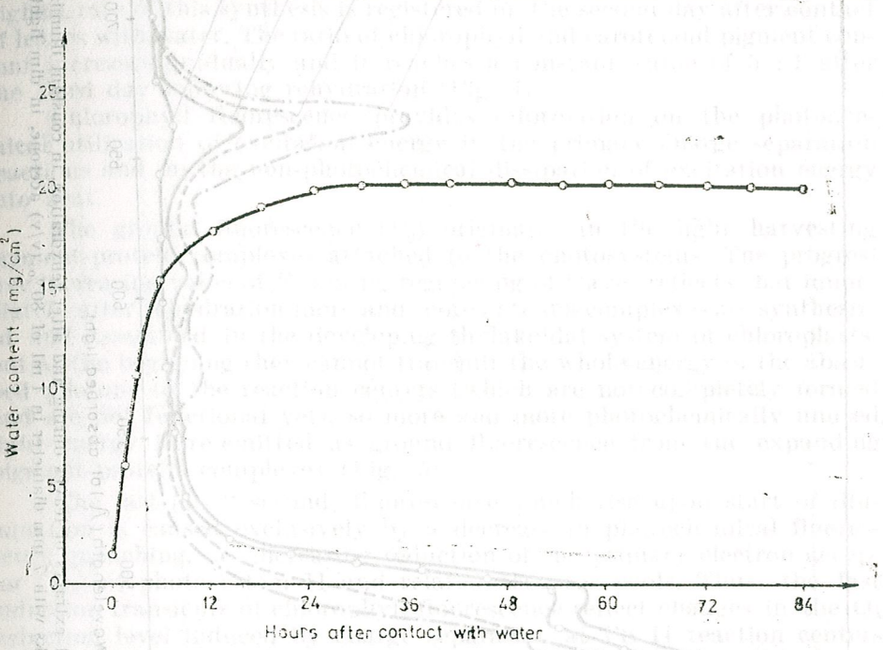


Fig. 1. — Variation of water content of *Xerophyta scabrida* leaves during rehydration.

starts. The pre-existent adventive roots cannot absorb water, and a new series of living, normally functioning roots will be formed during the wet season.

Rehydration of leaves is completed in approximately 24 hours and in the next few hours their light green colour appears obviously if they are illuminated, especially along both sizes of the main vein. Dynamics of water content of leaves during rehydration is shown in Fig. 1.

In the beginning of the dry season the total amount of chlorophylls is degraded before leaves enter in anabiosis, but a part of the carotenoid content is maintained. Carotenoid pigments probably protect the desiccated leaves against photodamage in conditions of high light intensities during the months of the dry season.

Synthesis of a new amount of photosynthetic pigments starts immediately after rehydration of leaves if they are illuminated. The progressive accumulation of *a* and *b* chlorophylls is reflected by the absorption spectra of chlorophyll extracts of leaf disks after different periods from the

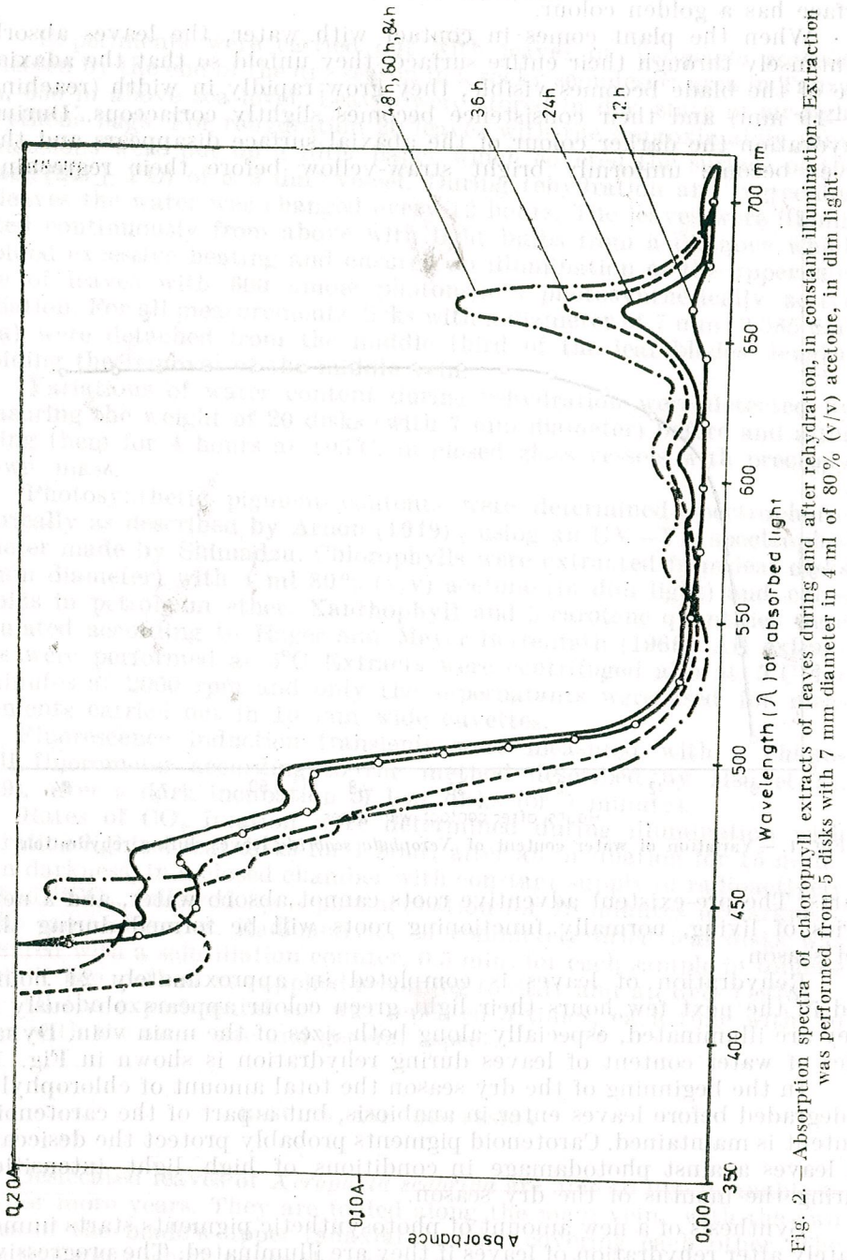


Fig. 2. — Absorption spectra of chloropuyll extracts of leaves during and after rehydration, in constant illumination. Extraction was performed from 5 disks with 7 mm diameter in 4 ml of 80% (v/v) acetone, in dim light.

beginning of contact with water, in conditions of continuous illumination with the same incident photon flux density and under the same 27 ± 1°C temperature value (Fig. 2).

Variation of xanthophyll + β-carotene content and of the chlorophyll *a* + *b* content of leaf disks during and after rehydration of these poikilochlorophyllous resurrection plants can be compared with the aid of Fig. 3. It is also noticeable that intense chlorophyll synthesis starts when leaves already reached their completely hydrated state, and the highest rate of this synthesis is registered on the second day after contact of leaves with water. The ratio of chlorophyll and carotenoid pigment content increases gradually and it reaches a constant value of 5 : 1 after the third day following rehydration (Fig. 4).

Chlorophyll fluorescence provides information on the photochemical utilization of excitation energy in the primary charge separation reactions and on the non-photochemical dissipation of excitation energy into heat.

The ground fluorescence (F_0) originates in the light harvesting pigment-protein complexes attached to the photosystems. The progressive increasing value of F_0 during regreening of leaves reflects that immediately after rehydration more and more antenna complexes are synthesized and assembled in the developing thylakoidal system of chloroplasts, but at the beginning they cannot transmit the whole energy of the absorbed photons to the reaction centers (which are not completely formed and are not functional yet), so more and more photochemically unused light energy is re-emitted as ground fluorescence from the expanding pigment-protein complexes (Fig. 5).

The fast (1—2 second) fluorescence which rise upon start of illumination is caused exclusively by a decrease in photochemical fluorescence quenching, i.e. increasing reduction of the primary electron acceptor (Q_A) of photosystem II and related acceptor pools. Thus, the fast induction transients of chlorophyll fluorescence reflect changes in the Q_A reduction level induced by charge separation at PS II reaction centers and the interplay of electron donation from the water splitting complex and electron acceptance by the plastoquinone pool in the thylakoids. In general, upon onset of illumination there is a biphasic rise of fluorescence from F_0, with the first phase representing the primary reduction of Q_A and the second phase reflecting reduction of the plastoquinone pool. As the latter is possible only with an intense electron transport from the donor side, its loss accompanied by a smaller maximal fluorescence (F_{max}) is an expression of lowered water-splitting activity.

Results shown in Fig. 5 reflect that in dehydrated leaves photosynthetic charge separation was completely suppressed and rehydration accompanied by illumination causes a delayed recovery of primary PS II reactions and of overall electron transport in the thylakoids of this resurrection plant. This fact is also reflected by the decreasing difference between consecutive variable fluorescence (F_{var}) values during recovery of photosynthetic activity. Considering that F_{var} is sensitive to the rate of electron transport through the reaction center of PS II and to the changes in thylakoid membrane structure, it can be concluded that the increase of F_{var} is due to the lowered rate of reoxidation of Q_A, electron

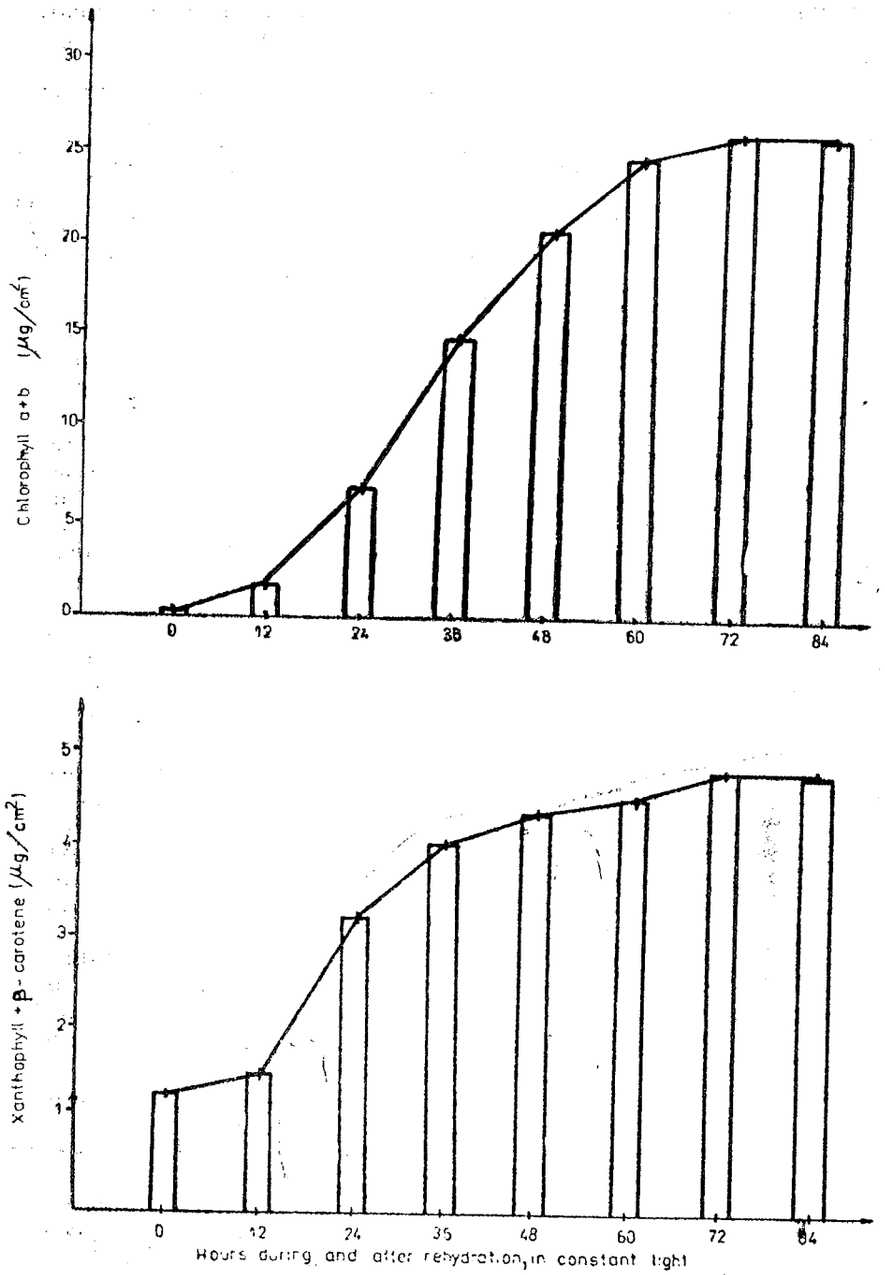


Fig. 3. — Comparison between the main carotenoid pigment and chlorophyll pigment content variation during rehydration and recovery of photosynthetic activity of *Xerophyta scabrida* leaves.

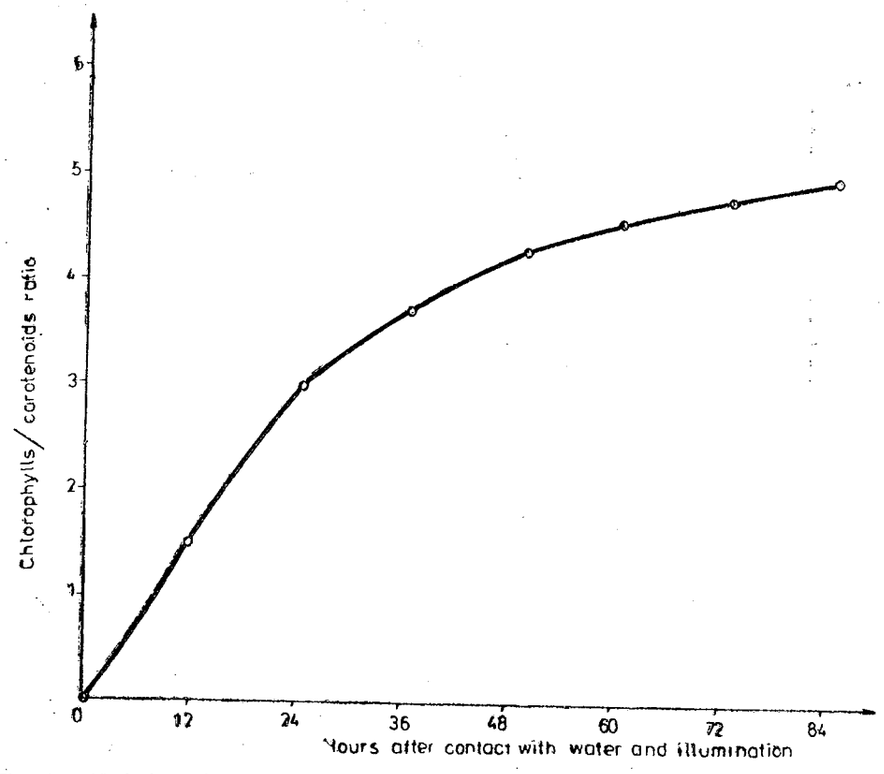


Fig. 4. — Variation of chlorophyll/carotenoid pigment ratio upon rehydration of leaves in constant illumination.

transport between Q_A and $NADP^+$ through the PS I being impaired by the degraded thylakoid components which have to be resynthesized and reassambled before being able to carry out the normal photosynthetic function.

The F_{var}/F_{max} ratio, considered as a measure of PS II effectiveness in the primary photochemical reactions, increases very obviously during the first 2 days after contact of desiccated leaves with water (Fig. 6).

Fig. 7 shows the net $^{14}CO_2$ incorporation into leaf structures, without taking into account respiratory CO_2 release and a possible photorespiratory loss of CO_2 during illumination. Dynamics of CO_2 fixation after rehydration reveals that the carbon reduction cycle starts its function only after the stroma of chloroplasts regains its normal hydration status (compare Fig. 1 with Fig. 7). One can also notice that biochemical reactions of the dark phase of photosynthesis recover when the light harvesting pigment complex is already built up in the thylakoids and photochemical reactions of the light phase of photosynthesis are restored (compare Fig. 3, 6 and 7), being able to supply the chemical energy and the reducing power requested for the synthesis of organic compounds.

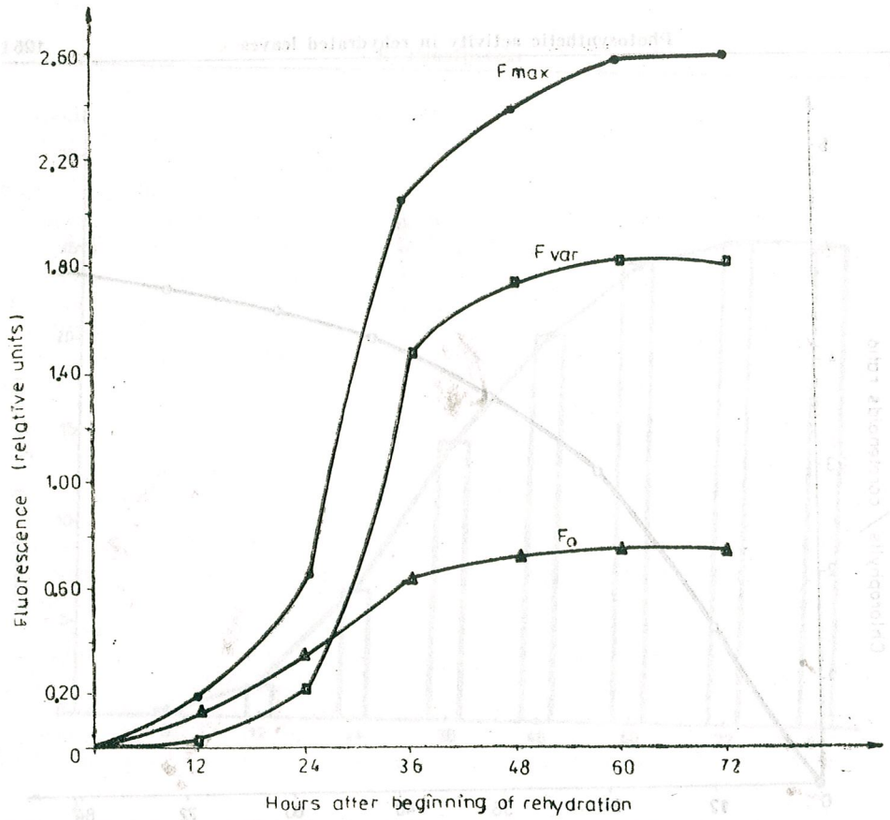


Fig. 5. — Fast (1.5 sec.) fluorescence induction parameters of leaf disks in subsequent phases of recovery of photosynthesis components in thylakoids (F_0 — ground fluorescence ; F_{max} —maximal fluorescence ; F_{var} — variable fluorescence).

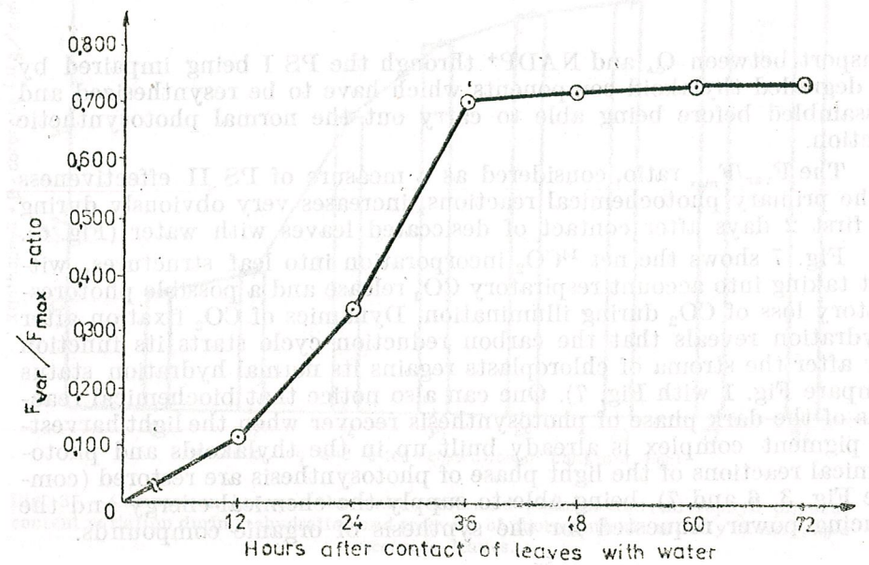


Fig. 6. — Modification of F_{var}/F_{max} ratio in fast fluorescence induction during recovery of photosynthesis in constantly illuminated *Xerophyta scabrida* leaves upon rehydration.

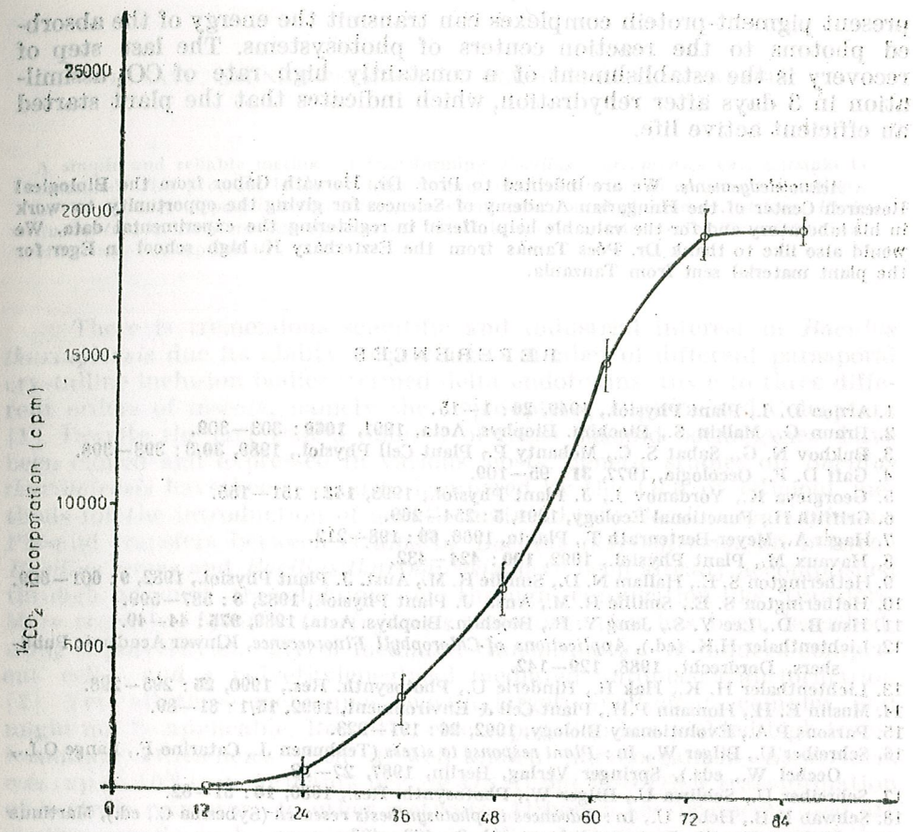


Fig. 7. — Dynamics of $^{14}CO_2$ fixation of *Xerophyta scabrida* leaves after rehydration in continuous light.

CONCLUSIONS

Xerophyta scabrida, a poikilohydric and poikilochlorophyllous vascular plant, has the capacity to re-enter very quickly in the active physiological state upon rehydration, even after a very long anabiotic life during the months of the dry season. Leaves of this plant show a specific dynamics of rebuilding the photosynthetic apparatus, a process which consists of a series of distinct phases.

When rehydration begins, leaves unfold, increase their width and exhibit a straw-yellow colour due to lack of chlorophylls and the presence of a certain amount of carotenoid pigments. When rehydrated leaves are illuminated, the synthesis of a new set of photosynthetic pigments is activated. Within 1—2 days the new components of the thylakoidal photosynthetic electron transport chain are assembled and the already

present pigment-protein complexes can transmit the energy of the absorbed photons to the reaction centers of photosystems. The last step of recovery is the establishment of a constantly high rate of CO_2 assimilation in 3 days after rehydration, which indicates that the plant started an efficient active life.

Acknowledgements. We are indebted to Prof. Dr. Horváth Gábor from the Biological Research Center of the Hungarian Academy of Sciences for giving the opportunity to work in his laboratory and for the valuable help offered in registering the experimental data. We would also like to thank Dr. Pócs Tamás from the Eszterházy K. high school in Eger for the plant material sent from Tanzania.

REFERENCES

1. Arnon D. I., Plant Physiol., 1949, **20**: 1—15.
2. Braun G., Malkin S., Biochim. Biophys. Acta, 1991, **1060**: 303—309.
3. Bukhov N. G., Sabat S. C., Mohanty P., Plant Cell Physiol., 1989, **30/3**: 393—398.
4. Gaff D. F., Oecologia, 1977, **31**: 95—109.
5. Georgieva K., Yordanov I., J. Plant Physiol., 1993, **142**: 151—155.
6. Griffith H., Functional Ecology, 1991, **5**: 254—269.
7. Hager A., Meyer-Bertenrath T., Planta, 1966, **69**: 198—212.
8. Havaux M., Plant Physiol., 1992, **100**: 424—432.
9. Hetherington S. E., Hallam N. D., Smillie R. M., Aust. J. Plant Physiol., 1982, **9**: 601—609.
10. Hetherington S. E., Smillie R. M., Aust. J. Plant Physiol., 1982, **9**: 587—599.
11. Hsu B. D., Lee Y. S., Jang Y. R., Biochim. Biophys. Acta, 1989, **975**: 44—49.
12. Lichtenthaler H.K. (ed.), *Applications of Chlorophyll Fluorescence*, Kluwer Academic Publishers, Dordrecht, 1988, 129—142.
13. Lichtenthaler H. K., Hak R., Rinderle U., Photosynth. Res., 1990, **25**: 295—298.
14. Muslin E. H., Homann P.H., Plant Cell & Environment, 1992, **15/1**: 81—89.
15. Parsons P.A., Evolutionary Biology, 1992, **26**: 191—223.
16. Schreiber U., Bilger W., In: *Plant response to stress* (Tenhunen J., Catarino F., Lange O.L., Oechel W., eds.), Springer Verlag, Berlin, 1987, 27—53.
17. Schreiber U., Schliwa U., Bilger W., Photosynth. Res., 1986, **10**: 51—62.
18. Schwab K.B., Heber U., In: *Advances in photosynthesis research* (Sybesma C., ed.), Martinus Nijhoff, Dr. W. Junk Publishers, vol. 3: 403—406.
19. Schwab K. B., Schreiber U., Heber. U., Planta, 1989, **177**: 217—227.
20. Stitt M., ap Rees T., Phytochemistry, 1978, **17**: 1251—1256.
21. Tuba Z., J. Plant Physiol., 1984, **115**: 331—338.

Received April 15, 1994

"Babeş-Bolyai" University
Department of Plant Biology
Str. M. Kogălniceanu nr. 1
3400 Cluj-Napoca

ELECTROPORATION OF *BACILLUS THURINGIENSIS* VAR. KURSTAKI Cry⁻ WITH pC194 PLASMID

L. SAVU, CORNEA PETRUȚA CĂLINA, F. MARCU, I. VATAFU

A simple and reliable method of transforming *Bacillus thuringiensis* var. kurstaki is described. This protocol based on high voltage electrotransformation (electroporation), in the presence of poly-ethyleneglycol, allows introduction of plasmid pC194 from *Bacillus subtilis* in *Bacillus thuringiensis* var. kurstaki cry⁻ vegetative cells. Transformation efficiency was up to 10^3.

There is tremendous scientific and industrial interest in *Bacillus thuringiensis* due its ability to produce a number of different parasporal crystalline inclusion bodies (termed delta-endotoxins) toxic to three different orders of insects, namely the Lepidoptera, Diptera and Coleoptera [1]. Despite the fact that a number of these plasmid borne genes have been cloned and expressed in various hosts, genetic studies on *Bacillus thuringiensis* have been seriously hampered by the lack of efficient methods for the introduction of genetic material into *Bacillus thuringiensis*. Plasmid transfers between strains of *Bacillus thuringiensis*, or between *Bacillus cereus* and *Bacillus thuringiensis* have been already accomplished through plasmid transductions or through conjugation-like transfers. More recently, *Bacillus thuringiensis* transformation has been described, using a polyethylene glycol mediated transformation of induced competent cells, and a polyethyleneglycol mediated autoplast transformation [2]. Yet, all these techniques remain largely inefficient, complex and might not be applicable. Recently, electroporation has yielded high transformation efficiencies with a well-known microorganism *Escherichia coli* (up to 10^{10} transformants per μg DNA) but also DNA transformation of bacteria refractory to other methods. Although the protocols used are similar, usually each organism need specific adaptations of one or another parameter. This paper describes the use of electroporation for transforming *Bacillus thuringiensis*.

MATERIALS AND METHODS

Organisms. Bacillus thuringiensis var. kurstaki was grown on GYS medium as described by Crickmore et al (1990) [3]. *Bacillus subtilis* PVS 035 harbouring the plasmid pC 194 was cultivated in LB broth supplemented with chloramphenicol (10 μg/ml).

Plasmid preparation. Plasmid DNA was isolated according to Sambrook et al. (1989) [4] and subjected to agarose gel electrophoresis (1%) at 5 V/cm for 3 h.

Plasmid curring of Bacillus thuringiensis var. *kurstaki*

Plasmid curring was performed according to Aronson et al (1986) [5]. Briefly the *Bacillus thuringiensis* culture was subjected to thermal shocks

at 40°C for several times and plated on nutrient agar until the bacteria lack crystals.

Transformation. The transformation procedure is similar to that outlined by Masson et al (1989). Briefly, 100 µl of cells were thawed at room temperature then placed on ice, the DNA was added and immediately transferred to a 0.2 cm gap electroporation cuvette prechiled on ice. After pulsing, 1 ml of GYS medium was added to the cuvette, and the cells subsequently transferred to a fresh tube. The cells were allowed to recover for 2 —10 hours with shaking. The cells were then placed on nutrient agar

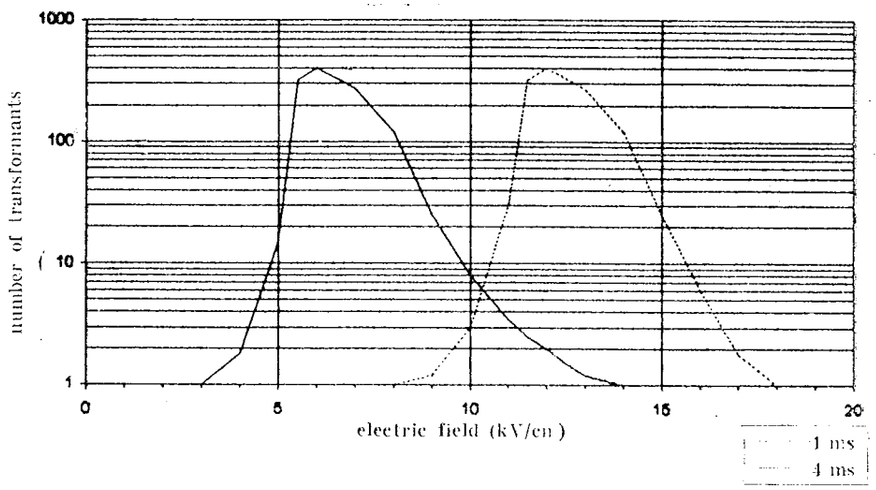


Fig. 1. — Electrotransformation efficiency of *Bacillus thuringiensis* cells with pC 194.

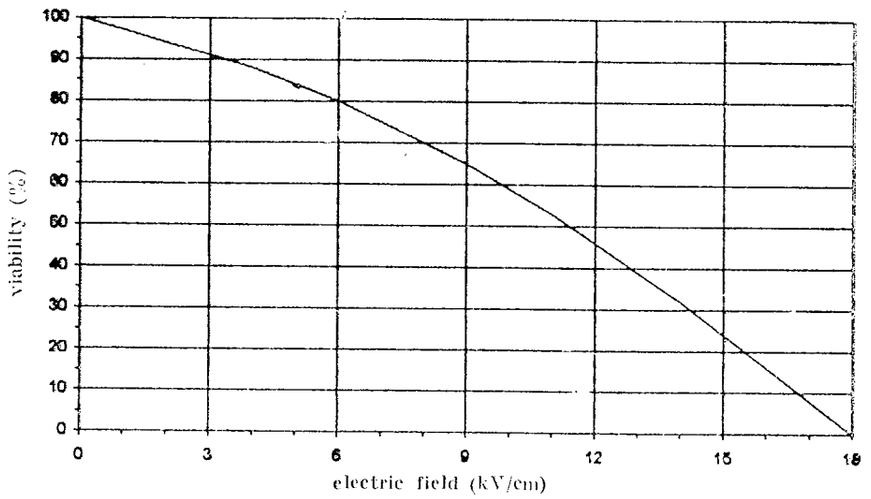


Fig. 2. — Viability curve of *Bacillus thuringiensis* cells exposed to the electric field.

plates containing 10 µg/ml chloramphenicol. The plates were incubated at 30°C overnight and the transformation efficiencies determined. Electroporation mixture was glycerol 10% prepared in distilled water.

RESULTS AND DISCUSSION

In the present paper we discuss the introduction of pC194 plasmid in *Bacillus thuringiensis* var kurstaki by electroporation. The efficiency of transformation by electroporation is known to be dependent on a number of parameters : namely, the bacterial strain examined, field intensity and the pulse length. To make much easier the experiments for the transformation we used a plasmid curred strain of *Bacillus thuringiensis*. This strain was subjected to a large range of field intensity (0 —20 kV/cm) at two pulse lengths to determine the optimized conditions for electroporation. The electroporation apparatus was capable to deliver pulses of 10 ns to 100 ms from 0 to 20 kV/cm in a 0.2 Biorad cuvette. *In Fig. 1* is shown the transformation efficiency at 1 ms and 5 ms. The highest efficiency was obtained at 12 kV/cm for 1 ms pulse and at 6 kV/cm for 5 ms. In Fig. 2 is shown the viability curve for the 1 ms pulse length. Chloramphenicol-resistant clones of *Bacillus thuringiensis* were examined for their plasmid content. The etidium bromide stained agarose gel showed the presence of pC194 in all the colonies tested that confirmed that they were transformed with plasmid DNA. The 2.8 kb DNA band corresponding to pC194 was absent in the untransformed *Bacillus thuringiensis* control. No differences between the original plasmid isolated from *B. subtilis* and that from *Bacillus thuringiensis* were observed.

Our results demonstrated that this method is simple and the transformation efficiencies make it an attractive technique to complement genetic studies on *Bacillus thuringiensis*.

REFERENCES

1. Masson, L., Prefontaine G., Brousseau, R., FEMS Microbiol. Letts, 1989, **60**, 273
2. Heierson, A., Landen, R., Lovgren, A., Dalhammar, G., Boman, H. G., J. Bacteriol., 1987, **169**, 1147.
3. Crickmore, N., Nichols, C., Earp, D. J., Hodgman, C., Ellac, J., Biochem. J., 1990, **270**, 133.
4. Sambrook, J., Fritsch, E. F., Maniatis, T., *Molecular cloning. A laboratory manual*, 1989, **1**, Cold Spring Harbor Laboratory Press.
5. Aronson, A. I., Beckman, W., Dunn, P., Microbiol. Rev., **50**, 1.

Received May 16, 1994

Institute of Biology, **296**
Spl. Independenței Bucharest
Romania

ISOLATION AND CHARATERIZATION OF SOME CONDITIONALLY-DOMINANT LETHAL MUTANT ALLELES OF *SACCHAROMYCES CEREVISIAE* GENE CIN8

LUMINIȚA PARAOAN

Conditionally-dominant lethal mutants of CIN8, a *Saccharomyces cerevisiae* gene that encodes a polypeptide related to the heavy chain of the microtubule-based force-generating enzyme kinesin, were induced and isolated.
At non-permissive temperatures cin8 alleles had a dominant lethal effect on both CIN8 and KIP1 — another kinesin-related gene. No such effect was observed when more copies of CIN8 were present. The respective phenotypes indicate possible ways of interaction of these molecules in performing their role(s) in mitosis at yeast.

About a decade ago it was discovered the fact that kinesin and cytoplasmic dynein are motor proteins able to generate force for motility along the microtubules. During the following years extensive families of kinesines and dyneins have been discovered and the importance of microtubule-based motility has been more and more revealed (9).

Microtubule-based motor proteins perform different types of intracellular movements and, consequently, they are obvious candidates as force-generating molecules responsible for mitotic movements. These molecules have the ability to convert the stored chemical energy into direct movement along the microtubules, in the presence and via a microtubule-dependent nucleosid-triphosphatase activity (usually ATPase). The polar structure of the microtubules suggests that two different types of microtubule-dependent molecular motors exist — plus-end directed and minus-end directed motors.

The classical microtubule-dependent plus-end directed motor is kinesin which has first been identified by its ability to translocate membrane attached organelles along the microtubules (10). The heavy chain of kinesin contains a globular motor domain with the elements needed for microtubule binding and ATP hydrolysis. The motor domain is sufficient for motility in vitro (11).

The kinesin superfamily is composed of proteins that show homology in the motor domain (300—400 amino acids) and that have been discovered a few years after kinesin sequence has been published, by showing that the mutations causing defects in mating at yeasts, in mitosis at fungi, in meiosis, at Drosophila and in axonal transport at worms are localized in genes which codify proteins similar to the kinesin heavy chain. Based on the homology with kinesin motor domain, one supposes that kinesin-like proteins act as microtubule motors.

With a view to understand the molecular basis of the chromosome segregation process and mitosis, the yeast *Saccharomyces cerevisiae* offers an excellent experimental system, being particularly suited to genetic and cell biological techniques and analysis. At *Saccharomyces cerevisiae* there have been identified two genes, CIN8 and KIP1, whose products

REV. ROUM. BIOL. — BIOL. VEGÉT., TOME 39, Nº 2, P. 133—138, BUCAREST, 1994

are related to the microtubule-based force generating protein kinesin and are required for mitotic spindle assembly and normal chromosome segregation (2). The deletion of both genes is lethal. One wild copy of either one is sufficient for viability under normal growth conditions. The two functions are redundant, but may be not identical. They are required at the stage when the duplicated spindle poles separate and, after spindle assembly they maintain its structural integrity (7). The removal of this activity causes the cells to arrest having the spindle collapsed, with the previously duplicated and separated spindle poles now back together. Cin8p was localized on the microtubules between the poles and appears as a component of the mitotic spindle. The sequences of CIN8 and KIP1 were reported (2) and (5).

The purpose of the experiments described in this paper was to generate and to isolate cin8ts dominant lethal alleles and to characterize their effect in strains with different genotypes (regarding the presence or absence of either CIN8 or KIP1). This approach is part of the effort to further the understanding of the way of function and interaction with one another and to describe the mitotic roles of the molecules encoded by these genes.

MATERIAL AND METHOD

In order to perform a random mutagenesis analysis of the CIN8 gene, the following strategy and methods have been used. A plasmid containing CIN8 gene was mutagenized with hydroxylamine (the plasmid designated pMA1208, as well as the yeast strains used were constructed in Dr. M.A. Hoyt laboratory); after assessing the degree of mutagenized DNA by transformation into an appropriate bacterial strain, the mutagenized DNA was amplified in *E. coli* and then used for spheroplast transformation of a yeast strain CIN8 kipl-Δ. The mutants causing a conditionally dominant lethal effect were screened directly by replica plating mutagenized-plasmid transformants to different temperatures.

The ts dominant lethal mutants were then analyzed in different backgrounds by transforming the respective plasmids in strains with relevant genotypes — CIN8 KIP1; cin8::URA3 KIP1; cin8::URA3 kipl::HIS3 kept alive by a CIN8 on a CEN plasmid (see Table 1). In all cases when a ts lethal dominance was observed the mutants were re-tested, confirming the growth on YEPD medium at normal and higher temperatures and the failure to grow on a selective medium at elevated temperatures.

In the frogging experiments the appropriate (i.e., carrying the same marker) vectors have been used. The plasmid pMA1189 is derived from pRS317 and pMA1208 from pRS318.

Rich (YEPD), minimal (SD) and α–AA (DL–α–aminoadipic acid) media were as described (6) and (8). When used, the concentration of cycloheximide in the plates was 10 μg/ml. The concentration of ampicillin in LB was 100 μg/ml.

The hydroxylamine solution for mutagenesis was freshly prepared and consisted of 1M hydroxylamine (Sigma Chemical Co., St. Louis Mo),

Table 1

Yeast strains and plasmids used

Strain or plasmid	Genotype
MAY 591	α lys2–801 his3–Δ200 leu2–3112 ura3–52
MAY 2104	α ade2–10 his3–Δ200 leu2–3112 ura3–52 cyh2^R can1^R kipl::HIS3
MAY 2174	a ade2–10 his3–Δ200 leu2–3112 ura 3–52 cyh2^R can1^R cin8::URA3
MAY 2307	a ade2–10 lys2–801 leu2–3112 his3–Δ200 ura3–52 cyh2^R can1^R cin8::URA3 kipl::HIS3 (pMA1189)
pMA1189	CIN8 LYS2 (CEN)
pMA1208	CIN8 LEU2 CYH2 (CEN)

50 mM sodium pyrophosphate (pH 7.0), 10 mM sodium chloride, and 2mM EDTA. The mutagenesis protocol was as described (1). The stock of the target plasmid had a concentration of 1 mg/ml. The reaction was performed using 10 μg CsCl purified plasmid DNA in a total volume of 500 μl at 75°C. The reaction was stopped on ice and the excess of hydroxylamine was removed by dialysis against TE. To assess the degree of mutagenesis, the *E. coli* strain HB101 (leuB$^-$) was used.

The yeast spheroplast high frequency transformation of mutagenized DNA was carried out as described (4), using 200 μl suspension of spheroplasts (starting with an overnight culture of yeast of 3×10^7 cells/ml) and 10 μg of plasmid DNA. After removal of the polyethylene glycol, spheroplasts and DNA were suspended in osmotically stable selective regeneration media (SD–Leu) containing 3% agar and plated on appropriate selective plates. The agar with the embedded colonies was sonicated and from the resulting suspension plate analysis was made.

The lithium acetate procedure (3) was used for the rest of transformations of yeast cells. For all transformations the density of yeast overnight cultures was determined spectrophotometrically by measuring the OD_{600}.

RESULTS AND DISCUSSIONS

The degree of hydroxylamine mutagenesis was titered by removing 100–μl aliquots at several time points (0, 30, 60, 90 and 120 min). After stopping the reaction on ice and removing the excess of hydroxylamine each aliquot contained the 2 μg DNA in 400 μl TE. 10 μl from each time point were transformed into *E. coli* strain HB101 (leuB$^-$) in order to assess the degree of mutagenesis. As a control for the efficiency of transformation, pRS318 was used – in which case about 10^6 cfu/μg DNA were obtained. With the samples corresponding to the five time points of incubation transformants were obtained only in the first two cases: $t_0 = 0$ min, about 5×10^5 cfu/μg DNA and $t_1 = 30$ min, 8×10^3 cfu/μg DNA – as expected, as the treatment could affect other genes, too, including Amp^R. In the rest of the cases the number of transformants

was too little to have statistical meaning for further comparing the growth on a minimal medium. The bacterial transformants grown on rich medium were replica plated to minimal medium that lacks Leu. By comparing the plates with and without Leu in case of t_1 the degree of mutagenesis was estimated ; the auxotrophs were produced (after 30 min of incubation) at a frequency of 7%.

The mutagenized DNA was amplified in bacteria in order to obtain DNA enough to transform yeast. I have collected by scrapping off the LB + Amp plates about 12000 bacterial colonies. This represented the maximum number of individual yeast mutants that can be obtained. Using the CsCl purified plasmid DNA from this pool the yeast strain MAY2104 was transformed by spheroplast transformation. The transformants were selected on SD —Leu top agar. Having plated about 250 transformants per plate, I have then replica plated them on YEPD and SD —Leu plates at 26, 35 and 37°C. I have obtained and screened in this way about 15000 transformants — out of which I have first selected (from the selective plates) 131 transformants that failed to grow on SD-Leu at elevated temperatures, while growing on YEPD at the same temperatures. After a careful frogging the number of ts mutants isolated was reduced to 31. The difference in growth on YEPD versus selective medium is due to the fact that on rich medium no selective pressure exists for maintaining the plasmid bearing the mutant cin8. So, even with KIP1 deleted, a copy of the wild-type CIN8 is sufficient to allow growth at all temperatures tested. Naturally, the dominant lethal effect could only be seen on selective plates ; the mutant allele cin8 is retained together with the LEU plasmid and impedes growth at elevated temperatures, even if a wild-type copy of CIN8 is also present in cells.

The conditionally lethal dominant mutants have also been tested for growth on YEPD at different temperatures after growing them two times on a medium with cycloheximide and compared with the growth of the original strain. Due to the fact that pMA1208 has the gene CYH2 (which confers sensibility to cycloheximide), the cells will preferentially lose the plasmid when cycloheximide is present in the medium. This was another way to rapidly test if the inability to grow at elevated temperatures is the effect of the presence of the plasmid and, consequently, of the mutated cin8. At this stage of experiments, still two possibilities regarding the effect of plasmid existed : the ts phenotype was due either to cin8 allele or to leu2⁻.

The transformants that passed these tests were taken into consideration for the following experiment : re-transforming MAY2104 with the mutagenized plasmid (after amplification in an appropriate bacterial strain), followed by the respective frogging. Six of them had the same phenotype on selective plates at the tested temperatures as in the initial frogging experiments. This was the ultimate proof that the conditional by lethal effect is due to ts dominant mutant cin8 carried by the plasmid. All six mutants showed a similar phenotype ; they grew well at temperatures under 35°C, were obvious sick at 35°C and did not grow at 37°C.

The effect of these cin8 ts alleles has been also tested in different backgrounds ; all of them made the wild-type strain for both CIN8 and

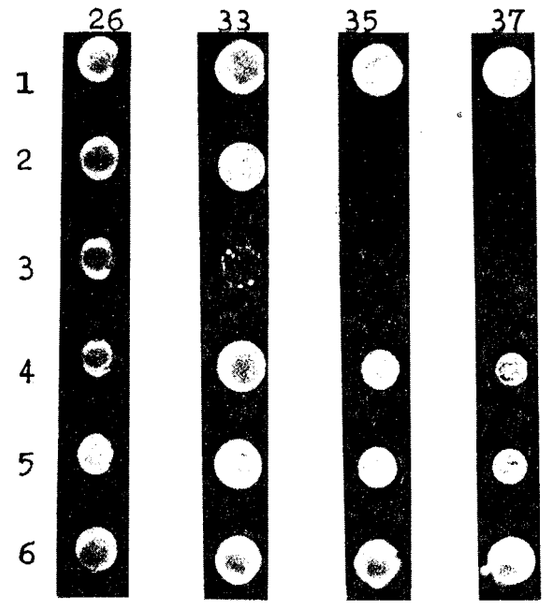


Fig. 1. – Ts conditionally dominant effect of a mutated cin8 on different backgrounds on a selective medium (cin8 on pMA1208*) 1. – MAY2104 + pMA1208; 2. – MAY2104 + pMA1208*; 3. – MAY591 + pMA1208*). 4. – MAY2104 + pRS318; 5. – MAY591 + pRS318; 6. – MAY2307 + pMA1208*. For the complete description of the strains and plasmids used, see Table 1.

KIP1 (MAY591), slightly sick at 33°C, sick at 35°C and out at 37°C ; also, there was no difference among the alleles ; the alleles caused the strain cin8 : URA3 KIP1 (MAY2174) to grow poorly at 33°C, very poorly at 35°C and not to grow at all at 37°C ; finally, there was no observable phenotype on cin8 : : URA3 kipl : : HIS3 strain kept alive by a (CIN8 LYS2 CEN) plasmid (i.e., MAY 2307). In this last case, all transformants grew well at all tested temperatures (from 26 to 37°C). The transformants of the double mutant strain were also tested for growth on αAA medium and all failed to grow. In each set of experiments described herein no obvious difference could be observed among the six alleles — this indicating that the mutations causing the respective phenotypes are, probably, at least in the same functional region of the CIN8 gene (see Figure 1).

A possible explanation of the way in which the mutations induce respective phenotypes should take in consideration the followings : cin8 ts alleles are dominant lethal at nonpermissive temperatures both on CIN8 and KIP1 ; the same effect appears on wild-type strain CIN8 KIP1 ; an excess of Cin8p suppresses the lethal effect ; when a copy of KIP1 is present the effect appears even stronger.

The interpretation is quite difficult as many hypotheses exist to explain the complex process of spindle assembly and function. The mutant molecules cin8p interact both with Cin8p and Kiplp. It should be noticed that this dominance appears only at elevated temperatures. So, the cin8p functions normal (at least without a distinguishable phenotype) or does not cause the arrest at permissive temperatures, but at higher temperatures the domain mutations cause the loss of function. Probably, the mutation(s) blocks some interactions which impede the function of Cin8p/ /Kip1p.

Obviously, the respective alleles are unable to support growth when alone in the cell (with respect to CIN8 and/or KIP1). Somehow paradoxal, but only at first sight, these null-mutations have no dominant effect when CIN8 wild-type is present in many copies ; the CEN plasmids could be present in up to 6 copies per cell (when have a beneficial effect), while the minimal number of copies exists on selective medium when a deleterious effect is associated with its presence. Consequently, the ratio Cin8p : cin8p is, probably, 6 : 1 when the respective genes are carried only by plasmids and on such a background the dominance could not be observed any more. So, the effect of dominant — lethals was suppressible by extra copies of the wild-type gene.

It is very likely that Cin8p does not function as monomer and also, that Cin8p and Kiplp do not act only redundantly, but also interact one with another, and this interaction could be impeded. A mechanism of competing for sites in microtube crosslinking of wild-type and mutant molecules appears also very likely. A simple possibility is that the dominant mutants are caused by the loss of function of one domain, while the other domains interfere with the function of the wild-type form.

Acknowledgements. I wish to thank Dr. Andy Hoyt and his colleagues for their support offered during this work.

REFERENCES

1. Guthrie C., Fink G. R., 1991, *Guide to Yeast Genetics and Molecular Biology*, Methods in Enzymology, Vol. 194.
2. Hoyt M.A., He L., Loo K.K., Saunders W. S., 1992, J. Cell Biol., **118** : 109.
3. Ito H., Funkuda Y., Murata K., Kimura A., 1983., J. Bacteriol., **153** : 163.
4. Lundblad V. 1989, in Current Protocols in Molecular Biology, Vol. I.
5. Roof D. M., Meluh P. B., Rose M.D., 1992, J. Cell Biol., **118** : 95.
6. Rose M. D., Winston F., Hieter P. 1990, *Methods in Yeast Genetics : A laboratory course manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
7. Saunders W. S., Hoyt M. A., 1992, Cell, **70** : 451.
8. Sherman F., Fink G. R., Hicks J. B. 1979, *Methods in Yeast Genetics.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
9. Vale R. D. 1992, Elsevier Science Publ., (UK) : 300.
10. Vale R. D., Reese T. S., Sheetz M. P., 1985., Cell, **42** : 39.
11. Yang Y. T., Saxton W. M., Russell J. S., Raff E.C., Goldstein L.S.B., 1990, Science, **249** : 42.

Received March 28, 1994

University of Bucharest
Faculty of Biology
Aleea Portocalilor nr. 1—3
77206 *Bucharest*

ULTRASTRUCTURAL MODIFICATIONS IN THE CHLOROPLAST EVOLUTION OF *TRITICUM AESTIVUM*

CONSTANTIN CRĂCIUN[1], GABRIEL C. CORNEANU[2], VERONICA CRĂCIUN[1]

The ultrastructural characteristics of the plastids from the mesophyll of mature leaves in wheat, *Triticum aestivum* (at eared) the *erythrospermum* Korn. variety (AM—74 and SM—74 lines) and *milturnum*. Al. variety (Libellula sort), were analyzed. There were described the ultrastructural characteristics of proplastids, the young and mature chloroplasts. It was found the presence of the inner membrane invaginations of proplastids which formed the lamellar system of their stroma. Also, in young chloroplast, there was established the presence of some structures of paracrystalline prolamellar body type (PLB) at the outskirts, which suggests their role in the formation of the chloroplast granal system. Because the plants were maintained for 24 hours before gathering the leaf fragments (for ultrastructural analysis) at a low level of light, it was found the absence (almost in totality) of starch grains. The chloroplast — chromoplast (carotenoidoplast) conversion, through the evidentiation of some intermediary stages, characterized by granal system reduction, the presence of some long thylakoids, ungrouped, at periphery, the formation of numerous vesicles through dilatation of thylakoids, the acumulation of a great number of plastoglobules and other osmophylic components. followed by new membraneous lamellar formations, with a concentric disposition.

The ultrastructure of plastids was intensely studied in numerous species (1—8). The influence of environmental conditions on the plastids ultrastructure (6,7), as well as the interconversion between different types of plastids (5, 8) were analyzed.

In this paper there were described the ultrastructural features of the plastids from the mesophyll of wheat mature leaves (proplastids, young and mature chloroplasts) in the conditions of an insufficient light of the plants time of 24 hours before gathering the leaf fragments for ultrastructural analysis, as well as the chloroplast — chromoplast conversion.

MATERIAL AND METHODS

There were three *Triticum aestivum* (L.) Thell. genotypes, belonging to *erythrospermum* Korn. (the AM—74 and SM—74 lines) and *milturnum* Al. varieties (Libellula sort). The ultrastructural characteristics of the plastids from the foliar mesophyll were established at the mature leaves (at eared). Before gathering the leaf fragments, the plants were maintained for 24 hours under low light (about 400 1x). The prefixing and postfixing of the leaf fragments were performed in a 5% glutaraldehyde solution (2h) and in Millonig fixing (1 1/2 h), the pieces being included in W-vestopal. The ultrafine sections of about 800—900 Å thickness were contrasted with uranyl acetate and lead citrate and examination was effectuated at a TESLA BS—500 electron microscope.

RESULTS AND DISCUSSIONS

(a) THE CHARACTERISTICS OF FOLIAR PARENCHYMATIC CELLS

The mature leaves (at eared) from the three analyzed wheat genotypes were of equifacial type, between the two palisadic parenchyma zones situed near the two epidermis, there are a few cell rows, slightly elongated, without spaces between them, which formed a homogeneous parenchyma (2). The foliar parenchyma cells present a relatively rich cytoplasm with numerous mitochondria and plastids, profils of endoplasmic reticulum, vacuoles, a.o. (Figs. 1, 3, 15). The nucleus presents the heterochromatin zones differently disposed, dependent on genotype and cell metabolic activity. The cells with an enhanced metabolic activity present much cytoplasm and numerous cellular organelles and the heterochromatin disposed in blocks (Fig. 5, AM—line), or in rows (Figs. 2, 6, SM—74 line, respectively Libellula sort). The cells with a reduced metabolic activity present a few cytoplasm and cellular organelles, as well as massive heterochromatin blocks, with very small euchromatin regions with island aspect (Fig. 4, Libellula sort).

(b) CHLOROPLAST CHARACTERISTICS

Young plastids, present an ellipsoidal shape (Fig. 7, AM—74 line) or a round-elongated shape (Fig. 13, SM—74 line). They are found in the mature leaves of the analyzed genotypes maintained at a reduced light level for 24 hours, together with the other plastid forms. It is evident the inner lamellar system which forms through proplastid inner membrane invagination (Fig. 7, arrow). The young plastids have a rich stroma with thylakoids unorganized in grana, and numerous granules similar to ribosomes, as well as plastoglobules in a different number (Figs. 7, 13).

The chloroplast presents the ultrastructural features depending on its ontogenetic development stage. In the juvenile stage, the chloroplast presents the thylakoids membrane unelectronodensified and dilated interthylakoids zones (Figs. 9, 14, arrow). In the mature chloroplasts (Figs. 8, 19), it was evidenced the disappearance of the interthylakoids deposits and the presence of membraneous uniformly and intensely contrasted. These findings are similar with those reported by Casadoro and Rascio (1977) and Rascio (1988) who consider that this process suggests the maturation of the chloroplast.

Previous researches were established that in the C_4 plant chloroplasts, at the stroma periphery, between thylakoids and the chloroplast envelope, there is a system of anastomozed tubes, described as a *paracrystalline prolamellar body* (PLB ; 6, 7). This can be in connection with the thylakoids lamellae (Rascio, 1988). The PLB forming can be considered as a stadium in the building of thylakoids membranes . Although these formations are unusually at the C_3 plants type, they were formerly described in the chloroplasts from wheat leaf mesophyll, as well as in the epidermal cells in *Nymphoides indica* (4). The chloroplast ultrastructure analysis in the three wheat genotypes reveals the presence of some structures similar with the PLB structures type, but having a reduced size (Figs. 8, 14, 19, double arrows).

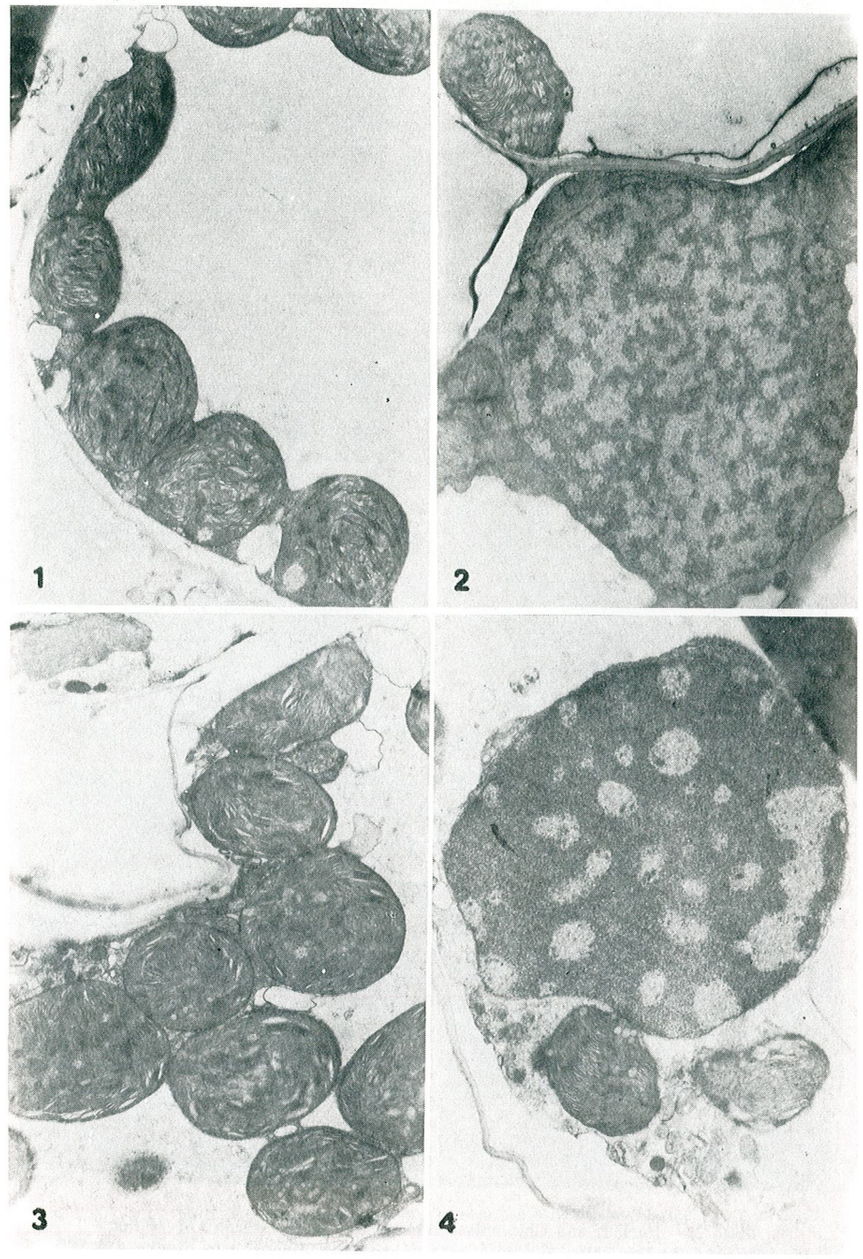


Plate 1. General aspect of the parenchymatous cells from the wheat leaves.
Fig. 1. – AM–74 line. General aspect of a foliar parenchymatic cell. × 5,500.
Fig. 2. – SM–74 line. Nucleus with heterochromatin disposed in rows × 6,000.
Fig. 3. – Libellula sort. General aspect of a folicular parenchymatic cell. × 5,300.
Fig. 4. – Libellula sort. Nucleus with massive blocks of heterochromatin and islands of euchromatin. × 7,200.

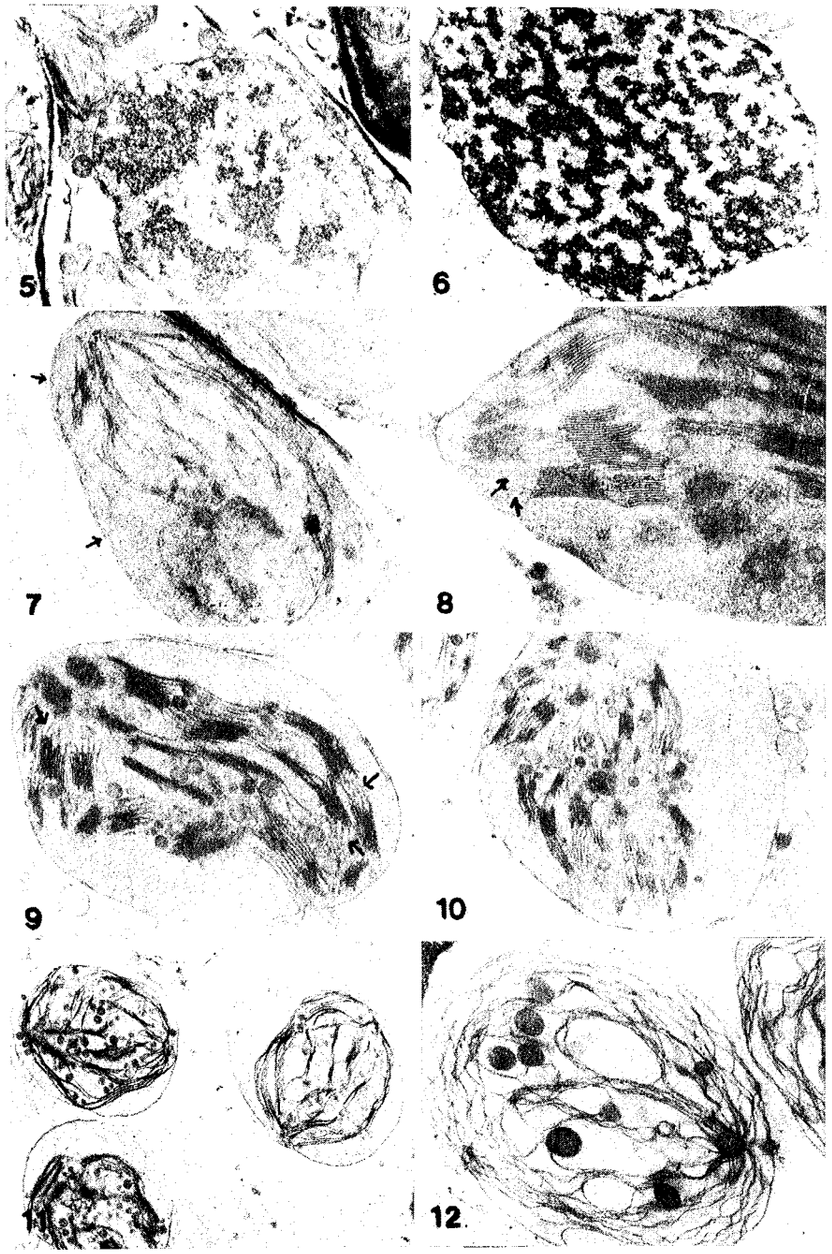


Plate 2. – Nucleus and Chloroplast ultrastructure in the wheat AM–74 line.
Fig. 5.– AM–74 line. Nucleus with heterochromatin disposed in blocks × 4,500. Fig. 6.– Libellula sort. Nucleus with heterochromatin disposed in rows × 6,000. Fig. 7.– Plastid with a bind between the inner membrane and the membranary system (arrows). × 12,000 Fig. 8. – Young chloroplast: the presence of the PLB region type between inner membrane and thylakoids (arrows). × 26,000. Fig. 9. – Young chloroplast with dilatation of thylakoids (arrows). × 19,000. Fig. 10. – Chloroplast – chromoplast conversion: the accumulation of plastoglobules. × 14,000. Fig. 11. – Chloroplast – chromoplast conversion: the accumulation of plastoglobules and the granal system destroy. × 6,200. Fig. 12. – Chromoplast with a typical structure of mielinic type, numerous vesicles and osmiophilic corpuscles. × 19, 000.

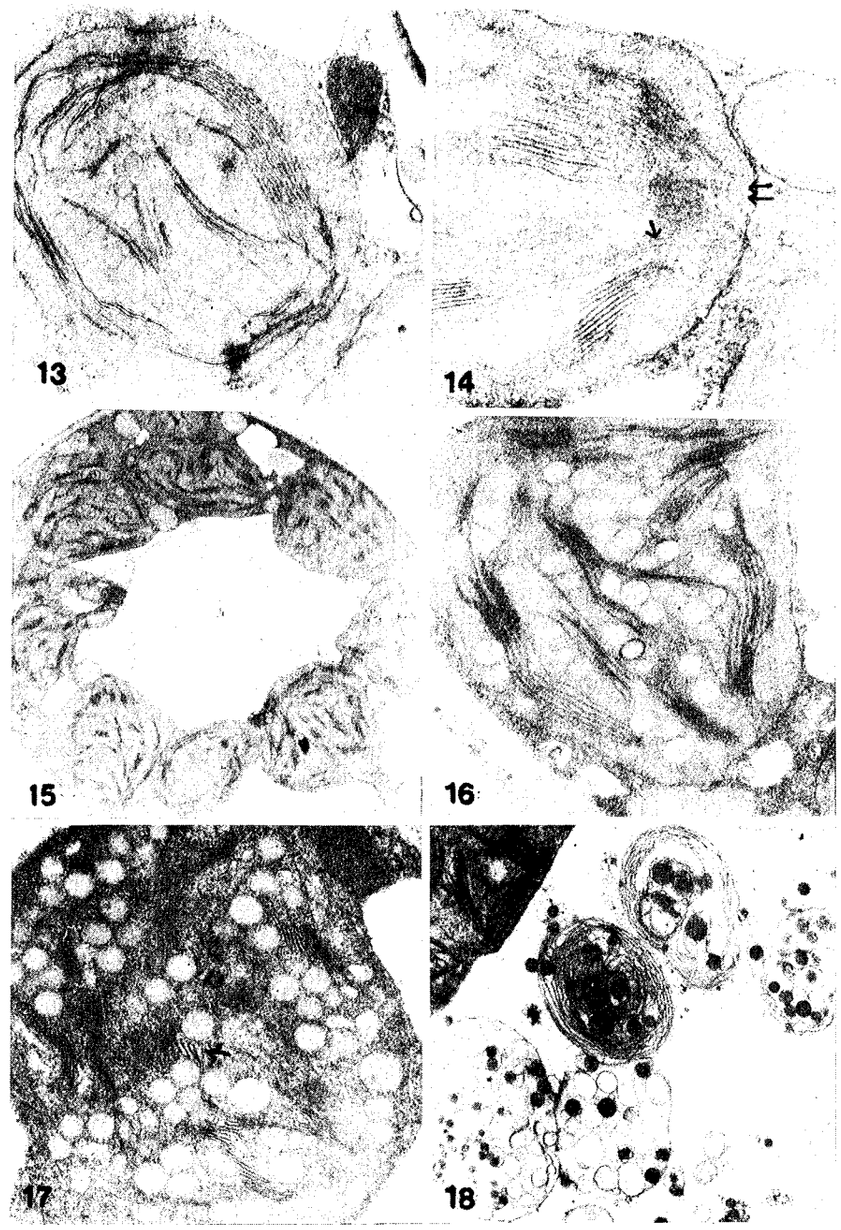


Plate 3. Chloroplast ultrastructure in the wheat SM–74 line.
Fig. 13. – Plastid (proplastid) × 19,000.
Fig. 14. – Young chloroplast with dilatation of thylakoids (single arrow) and PLB type corpuscles (double arrow). × 40,000.
Fig. 15. – General aspect of a cell from foliar parenchyma. × 6,400.
Fig. 16. – Chloroplast – chromoplast conversion: the accumulation of plastoglobules and grana reduction. × 22,000.
Fig. 17. – Chloroplast – chromoplast conversion: the accumulation of plastoglobules, dilatation of thylakoids (arrow) which will form the vesicular system and grana reduction. × 24,000.
Fig. 18. – Chromoplast with a mielinic type structure. × 6,200.

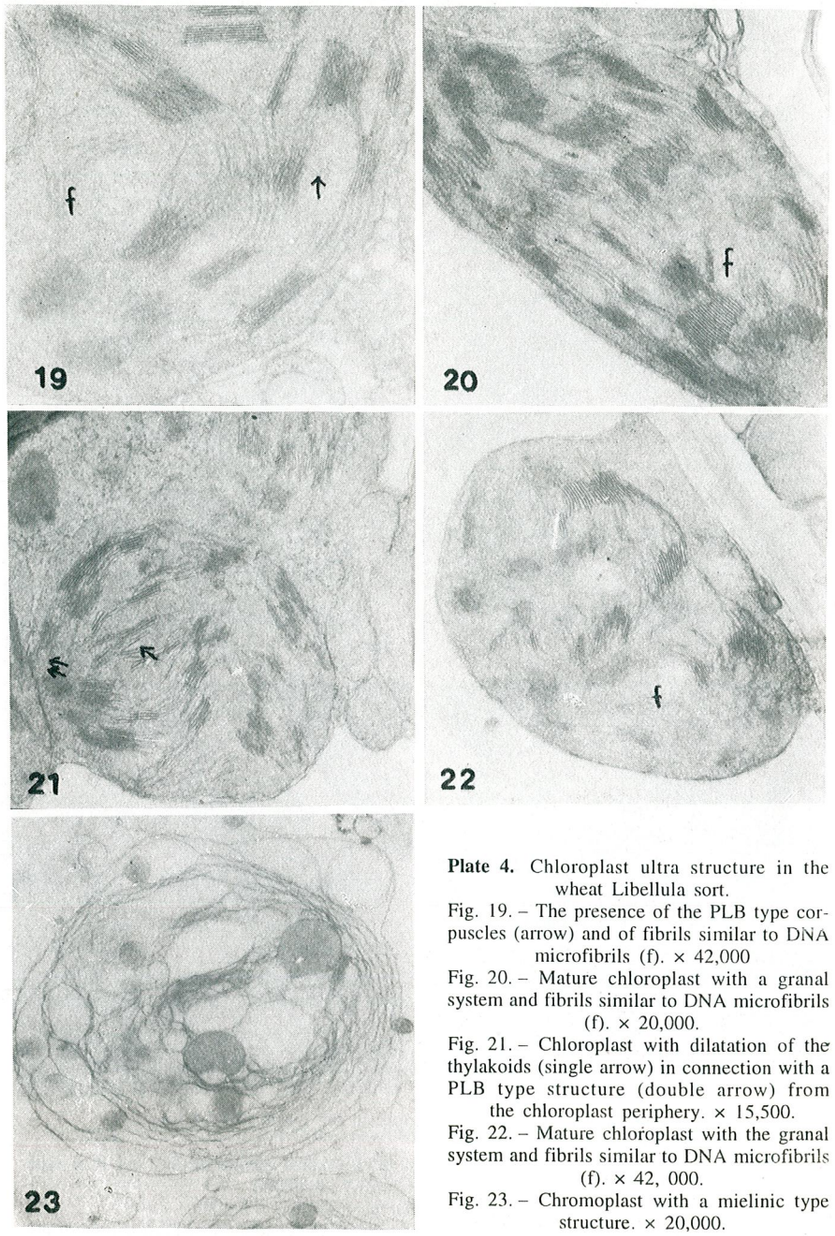


Plate 4. Chloroplast ultra structure in the wheat Libellula sort.

Fig. 19. – The presence of the PLB type corpuscles (arrow) and of fibrils similar to DNA microfibrils (f). × 42,000

Fig. 20. – Mature chloroplast with a granal system and fibrils similar to DNA microfibrils (f). × 20,000.

Fig. 21. – Chloroplast with dilatation of the thylakoids (single arrow) in connection with a PLB type structure (double arrow) from the chloroplast periphery. × 15,500.

Fig. 22. – Mature chloroplast with the granal system and fibrils similar to DNA microfibrils (f). × 42, 000.

Fig. 23. – Chromoplast with a mielinic type structure. × 20,000.

The presence of the PLB structures in closely binding with the dilated regions of thylakoids (Fig. 1, arrow) suggests their implication in the formation of the chloroplast membraneous system, which subsequently, after maturation, becomes thylakoids. In some sections, it was evidenced the presence, in the chloroplast, of some regions with fibrils similar with the DNA fibrils (Figs. 19, 20, 21, *f*). The plastoglobules are present in close association with thylakoids (Figs. 7, 8, 13, 14, 15, 19—22). The amount of plastoglobules per chloroplast is dependent on the genotype, plant age and the ontogenetic development stage of the chloroplast. The absence (or the extremely rare presence) of starch grains in the chloroplast, is determined by the plant maintaining time of 24 hours at a reduced light intensity. Similar findings were reported with other plant species (3, 4).

(c) CHARACTERISTICS OF CHROMOPLASTS (CAROTENOIDOPLASTS)

The chromoplasts are involved in the synthesis of different substances in mature tissue cells, being absent in the meristematic or embryonic tissues. With a view to differentiate the plastids from different pigments, Matienco introduced the *carotenoidoplast* term (plastids with yellow or red pigments), to differ from the chloroplasts which contain chlorophyll mainly (5).

The thylakoidal system from chromoplasts is little developed in comparison with the one from chloroplasts, present a morphologic diversity and has an irregular shape. Dependening on the membrane configuration, the carotenoidoplasts can be of different types : pair-lamellar (canalicular), vesicular, concentric (helicoidal), tubular or of intermediary shapes (5). A chloroplast — chromoplast (carotenoidoplast) conversion, especially in the flower elements (this conversion is responsible was reported for the perianth colour alteration), or in different illuminating conditions. This conversion can be very fast (in the *Orintium aquaticum* flowers), or of long time, when the progressive loss of the inner membrane system took place, followed by *de novo* synthesis of thylakoids (Rascio, 1988).

The analysis of the foliar mesophyll cells ultrastructure, at the three wheat genotypes, reveals the chromoplasts presence in these cells, as well as the chloroplast — chromoplast (carotenoidoplast) conversion stages (Figs. 10—12, AM—74 line; Figs. 16—18, SM—74 line; Fig. 23, Libellula sort). Initially a massive accumulation of plastoglobules took place simultaneously with the reduction and disorganization of the granal system (Figs. 10, 11, AM—74 line, respectively Figs. 16, 17, SM—74 line). Finally there are formed chromoplasts with a monocentric organization, having a similar structure with a mielinic body, at the three wheat genotypes (Figs. 12, 18, 23). They are characterized by the presence of a concentric lamellar system, numerous vacuoles being formed through thylakoids dilatation and numerous plastoglobules (Figs. 12, 18, 123).

CONCLUSIONS

1. In the mature leaves from three *Triticum aesticum* genotypes, maintained for 24 hours at a reduced illumination (400 lx), proplastids were evidenced in the foliar mesophyll. The binding between the plastid inner membrane and the inner lamellar system was distinguished, suggesting this formation through the invagination of the proplastid inner membrane.

2. The presence of the PLB type structures (paracrystalline prolamellar body) was distinguished at the periphery of the young chloroplast, as well as their binding with the dilated lamellar systems from the chloroplast stroma, process which suggests the PLB structure implication in the formation of the chloroplast granal system.

3. The mature chloroplast does not present starch grains as a consequence of the plants maintained at a reduced illumination regime time of 24 hours before the chloroplast ultrastructure analysis.

4. The chloroplast — chromoplast (carotenoidoplast) conversion was found. In the phases of this process, the reduction and destruction of the granal system, the formation of numerous vesicles through thylakoids dilatation, took place, the accumulation of numerous osmophilic corpuscles, followed by the new membranes system organization of monocentric type, similar with a mielinic body.

REFERENCES

1. Casadoro G., Rascio N., 1977, Relationship between plastids with a membrane-boundy body and cell differentiation gradients in Belladonae. Caryologia, **30** (2): 189—198.
2. Corneanu G. C., Crăciun C., Simeanu V. D., 1980, *Caracteristici structurale ale frunzei la grâu*, Analele Universităţii Craiova, Biol. **11**: 51—55.
3. Gunning B.E.S., Steer M.N., 1975, *Ultrastructure and the Biology of Plant Cells*, Edward Arnolds, London, 322 pp.
4. Kirk J.T.O., Tilney-Bassett R.A.E., 1978, *The plastids. Their chemistry, structure, growth and inheritance* (second ed.), Elsevier/North Holland, Biomedical Press, 922 pp.
5. Matienco B. T., Cebanu E. M., 1973, *Ultrastruktura karotenoidoplastov (hromoplastov)*, Izd. „Ştiinţa", Chişinău, 148 pp.
6. Rascio Nicoletta, Orsenigo N., Arboit D.,1976, Prolamellar body transformation with increasing cell age in the maize leaf, Protoplasma, **90**: 255—263.
7. Rascio Nicoletta, Mariani Colombo P., Orsenigo M., 1980, The ultrastructural development of plastids in leaves of maize plants exposed to continuous illumination. Protoplasma, **102**: 131—139.
8. Rascio Nicoletta, 1988, *Chloroplast differentiation :* In : *Cell Interactions and Differentiation* (Gianfranco Chiara, Ed.), pp. 159—174, University of Naples, Italy.

Received March 10, 1994

[1]*Babeş-Bolyai University, Cluj*
Experimental Biology Department
Str. Clinicilor, 5—7
3400 — Cluj-Napoca

[2]*Craiova University*
Genetics Laboratory
Str. Al. I. Cuza, 13
1100—Craiova

WEBER H. (Ed.), 1993, *Allgemeine Mykologie*, Gustav Fischer Verlag Jena — Stuttgart, 541 pp., 206 plates, 66 tables

Drawn up by a team of 12 prestigious authors, widely recognized scientific authorities in the domain, the book 'Allgemeine Mykologie', edited by Professor H. Weber of Jena, fills up a gap in the biological literature in general, and mycological literature, in particular, focusing attention on the significance of this complex botanical science, with real actuality and unexpectedly linked and involved in the life of human communities.

This book is divided into 10 chapters containing 541 pages, of which 474 represent the background of this work (Chapters 2 — 10), supplemented with 206 plates and 66 tables, and ended with a list of fungus genera and species, and an index of scientific terms used.

The classical chapters of this book deal with : fungus cytology and morphology (82 pp., E. Jelke, H. Kreisel), their reproduction and propagation (21 pp., H. Weber, R. W. Arnold), taxonomy (53 pp., N. Dörfelt) and fungus ecology (33 pp., W. Dörfelt); these are completed by updated chapters on molecular biology and fungus genetics (38 pp., H. Weber, W. Künkel), metabolic transport processes (35 pp., W. Roos), etc.

A prominent part of this book (Chapter 8, 143 pp.) is devoted to significance and practical value of fungi, which is reflected in various and essential activities of man's life, and especially in biotechnologies. This chapter tackles the edible mushroom cropping (R. W. Arnold, H. Dörfelt), fungi on consumption goods industry (W. Hirte), albumins from fungi (G. Straube, H. Weber), products of secondary metabolism of fungi and their conversion (D. Gröger), exchange relationships between fungi and metals (G. Staube), fungi in preservation of materials and their biodeterioration (G. Staube), fungi and their role in the biological control of plant pests and diseases (W. Hirte, R. W. Arnold).

The last but one chapter of this book (Chapter 9) reviews the plant pathogenic fungi (M. Schmiedeknecht) as one of the phytopathological mycology concerns, while the last one (Chapter 10) dwells on the problems of medical mycology — human and veterinary — i.e. fungi causing mycoses in man and animals.

'Allgemeine Mykologie' is a model to approach a basic botany science — mycology — in its interrelations with the human activity, in its whole variety, translated into practice as food biotechnologies (bread-making, yeast production, wine-making, brewery, production of ethanol, starch, dairy, meat and fishery foods, fermentation of coffee, tea, cocoa and tobacco, preparing juices, glucose, enzymes, vitamins and aromatic products, food products biodeterioration), biosynthesis of antibiotics, enzymes, vitamins, hormones and other compounds of chemical and drug industry, preservation of materials (wood, paper, leather, plastics, museum exhibits, books, etc.) and their biodeterioration, biotechnologies used in plant protection for the biocontrol of diseases and pests of cropped plants, in the plant, medical and veterinary mycology. All these branches are competently and scrupulously analysed by the authors of this book.

The volume also benefits of an exceptional printing skill, which is also extended to its whole iconography.

This book is intended to specialists working in the field of mycological research, as well as in biological, biochemical, biotechnological, agricultural and medical (human and veterinary) high school teaching, and also to students who prepare for one of these careers. Its value recommends this book as an authoritative achievement in plant biology, in general, and in mycology, in particular.

Dr. Tatiana Eugenia Şesan

AVIS AUX AUTEURS

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

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

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

PRINTED IN ROMANIA