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SCIENTISTS AND PEACE

On 4—5 September 1981 the international symposium "Scientists and peace" developed its proceedings in Bucharest, under the high patronage of the President of the Socialist Republic of Romania, Nicolae Ceaușescu.

The symposium was attended by 68 scientists from 32 countries among whom Nobel Prize winners, presidents of some academies of science, other prestigious scientific personalities, as well as general directors of international organizations.

In the opening session, they read the message of Nicolae Ceaușescu, President of the Socialist Republic of Romania, to the participants in the Symposium.

Expressive of the lofty conception of the Secretary-General of the Romanian Communist Party, the President of the Socialist Republic of Romania, concerning the problems of peace and international security, détente and disarmament, cooperation and understanding among peoples in their efforts to build a new economic order in the world, as well as the scientists' responsibility for the solution of national and international issues by placing the most advanced achievements of the contemporary scientific and technical revolution exclusively in the service of the peoples' peaceful development, President Nicolae Ceaușescu's message made an appeal to scientists all over the world to close their ranks in order to fight the hazards which crises, confrontations and war pose to the future of mankind. The President's message was a guideline for the proceedings of the Symposium.

The participants expressed their support for the leading ideas contained in the message of the President of Romania.

The proceedings closed with an Appeal by the participants addressed to the scientists worldwide. Reflecting the basic ideas contained in President Ceaușescu's message, the appeal suggested the establishing of an Action Committee for the organization of the World Congress "Scientists and Peace".

As an extension of this prestigious international reunion was founded the National Romanian Committee "Scientists and Peace" that unanimously elected as president of the Committee and of the Executive Bureau Academician Elena Ceaușescu, D. Chem. Eng., first vice-prime minister of the Government of the Socialist Republic of Romania, President of the National Council for Science and Technology, illustrious political personality and internationally reputed scientist.

The Romanian National Committee "Scientists and Peace" adopted a comprehensive programme of scientific manifestations, meant to illustrate the contribution of Romanian scientists to the efforts of the Romanian people, alongside all peoples, for safeguarding peace.

The National Romanian Committee "Scientists and Peace" takes action on the international plane for the preparation of the World Congress "Scientists and Peace". The Committee is also represented at the Special Session of the General Assembly of the United Nations Organization devoted to disarmament.

MESSAGE
TO THE PARTICIPANTS IN THE INTERNATIONAL SYMPOSIUM "SCIENTISTS AND PEACE" FROM THE PRESIDENT OF THE SOCIALIST REPUBLIC OF ROMANIA, NICOLAE CEAUŞESCU

It gives me particular pleasure to send to you, the participants in the Symposium "Scientists and Peace" which opens today in Bucharest — distinguished figures in contemporary science and technology — cordial greetings and best wishes for the success of the meeting, so that it may give an impetus to the struggle of progressive forces throughout the world for world progress and peace.

The Socialist Republic of Romania attaches the greatest importance to scientific activities; it gives the achievements of science and culture a place among the very foundations of its efforts to construct the new socialist system, in the belief that they are vital factors for progress and civilization.

We are living in the era of the greatest advances in scientific thought that mankind has ever experienced throughout the ages, in the midst of the most awesome technical and scientific revolution, marked by extraordinary discoveries which are constantly changing man's ideas about nature, society and the universe and influencing all aspects of human existence. We see science directly influencing the never-ending change in the conditons of material production, the discovery of the secrets of matter and the increasingly efficient exploitation of our natural wealth and the development of the creative capacity of peoples.

The development of a nation — both in terms of enhancement of the productive forces of society and as regards thinking and mental creativity — is inconceivable without the input of advanced science and technology. One cannot even envisage the future of mankind without the major accomplishments of scientific thought.

However, we must candidly admit that a great many of the major discoveries of scientific research and technological innovation are currently used for the production of highly sophisticated weapons of mass destruction, from atomic weapons down. We are witnessing a particularly alarming emphasis on the arms race, and a major build-up of military arsenals capable of annihilating the entire planet and endangering the very existence of mankind.

We see tension growing, world-wide, as a result of the imperialist policies of domination, force and *diktat*. A strong tendency to consolidate and divide up spheres of influence is evident, and conflicts between States and groups of States are becoming more and more bitter.

At the same time, peoples throughout the world are asserting with increasing vigour their will to live in freedom, to develop in complete

independence, to put an end for ever to colonialism, neo-colonialism and all forms of oppression, to secure the democratic and progressive renewal of society, to ensure the welfare of the great working masses and to introduce a genuinely new set of relationships on the world scene involving full equality between countries, détente, collaboration and peace.

Socialist Romania is doing all in its power to develop its relations with all States irrespective of their social system. We base our relations with all countries on the principles of full equality of rights, profound respect for national independence and sovereignty, non-interference in internal affairs and non-use of force or threat of force. We believe that every effort must be made to stop the deterioration in the world situation, to ensure that all problems arising between States are settled exclusively by negotiation and to revive and pursue policies of détente and peace.

In the serious international situation which now obtains, scientists bear a tremendous responsibility for the present and future of mankind. No one knows better than the scientist or researcher the destructive power of modern weapons and the danger which the continuing arms race poses to civilization, to the security of peoples and to the very survival of humanity.

The choice between a policy of intensifying the arms race and manufacturing new nuclear weapons of mass destruction and a policy of disarmament, détente and peace is today a question of conscience.

There is no middle course !

It is obvious that scientists, who are very well aware of the destructive power of weapons, especially nuclear weapons, cannot but side with the policy of disarmament and peace. They therefore have a greater duty than ever to speak out and do all they cant to ensure that the amazing attainments of the human spirit are no longer used in the manufacture of weapons of mass destruction, in preparing for war or to serve the policies of aggression, force and domination. The noblest task of scientists and researchers, in all fields and throughout the world, is to see to it that the entire potential of modern science and technology is devoted to the progress, welfare, freedom and independence of peoples and to the preservation of the supreme human right to life and to peace.

We must work with great determination and resolve for an end to the arms race, for disarmament, especially nuclear disarmament, for a cessation of the deployment and development of medium-range missiles in Europe, against the production of the neutron bomb, for a reduction in military budgets and armed forces, for the final abandonment of the use or threat of force in international life and for the creation of a world without weapons and without wars.

Under-development affects most of the population of the earth ; nearly half a billion people suffer from chronic malnutrition. We must therefore establish a new international economic order guaranteeing free access by all peoples, particularly the least advanced, to the amazing achievements of the human spirit — a new international economic order which will guarantee the free flow of knowledge and discoveries and turn science into something that belongs to all mankind.

As the world economic crisis grows worse, science can play a particularly important role in the discovery and development of new sources

of energy and raw materials to be placed at the disposal of people everywhere, making the earth more fertile, increasing its output and solving the great food problem. It has an obligation to contribute to protecting the health of people throughout the world, combating pollution, improving the environment, protecting natural resources and transforming the earth into a verdant garden which can sustain a decent existence for all peoples.

Scientists and specialists in Romania, being profoundly dedicated to the interests of the people, devote all their energies to Romania's economic and social prosperity while at the same time co-operating actively with scientists of other countries in campaigning for progress, for disarmament and for the basic right of all nations to existence, peace and freedom.

Nowadays, the peoples, the masses, throughout the world play an essential role in determining the course of history.

Scientists, vitally involved in the cause of progress and peace, must fight side by side with the peoples for the right to live and work in peace, freely to build their own future without external interference or pressure, to devote their resources and energies to their material and spiritual well-being. Scientists, whatever their philosophical, political and religious views, must close ranks and, together with the peace-loving and anti-imperialistic forces throughout the world, take a stand against the imperialist policy of domination, against war, and for a world of justice, equality and peace.

It is more important than ever to organize a world-wide front of scientists to act and convey their authoritative views to the United Nations, the Committee on Disarmament and other international bodies, concerning disarmament and the establishment of lasting peace on earth.

We are firmly convinced that united action by the men of science and culture, the workers and the progressive forces of all peoples can end the arms race and bring about a move to general disarmament, and in particular nuclear disarmament.

Let us do all we can to ensure for our children and grandchildren, for our generation and future generations, peace, freedom and happiness in a world without war, a more humane, more just and better world !

In keeping with these sentiments, I am convinced that this important meeting in Bucharest will have a great impact on researchers and scientists throughout the world, and I send you my most cordial wishes for much success and satisfaction in your noble work for the advancement of science and for the cause of collaboration, peace and the independence of peoples.

NICOLAE CEAUȘESCU
President of the Socialist Republic
of Romania

Bucharest, 3 September 1981

APPEAL
BY THE PARTICIPANTS IN THE INTERNATIONAL SYMPOSIUM "SCIENTISTS AND PEACE"

Meeting in Bucharest on 4 and 5 September 1981 for the Symposium "Scientists and Peace" in order to discuss, in a wide-ranging and fruitful dialogue, the fundamental issue of the present day — peace, to which all mankind nobly aspires — we, the scientists from many countries of the world and from all continents, aware of the serious hazards which science and its servants may pose to the halting of the arms race, to continuing progress and to the future of the whole world, urgently appeal to scientists, researchers and intellectuals everywhere, and to all peoples, to join forces and co-operate ever more closely in defence of peace, the supreme good of mankind.

The world today is witnessing not only the giant strides of science and technology, as evidenced by awesome discoveries affecting all areas of human existence, but also anachronistic actions which go against the interests of mankind, applying the products of science and technology to destructive ends injurious to the peace and freedom of peoples. Our age is one in which mankind is confronted by highly complex problems, with a new, frenzied arms race, unprecedented growth of military budgets, and the manufacture and development of new means of mass destruction, all of which severely aggravate the international situation, weighing more and more heavily on peoples and increasing the danger of conflagrations which may destroy life everywhere on earth and civilization itself, as it has evolved over the millennia.

Let us, in full awareness of the fact that scientists, faced with the alternative of peace or war, have a duty to defend peace, say a firm NO to war and armaments, this being not only our moral responsibility but essential to the continued existence of all mankind. We call on all scientists, whatever their political, philosophical, religious or other beliefs, to work side by side with the peoples of their countries to halt the deterioration in the international situation, the arms policy, so that we may resume and tirelessly pursue the course towards détente, peace and wide-ranging international collaboration !

Let us act now, before it is too late, now, when we have so great a responsibility for the fate of mankind, to end the arms race, to bring about disarmament, especially nuclear disarmament, to create a world without weapons and without wars and to defend basic right of individuals and peoples — the right to life and to peace.

Let us, as scientists more aware than anyone of the destructive power of modern weapons and the tremendous danger they present to the security of peoples and to the very survival of humanity, join forces more closely and act resolutely against the use of atomic energy for other than

peaceful purposes ! Let us do all we can to ensure that the immense potential of scientific and technical research is not used for weapons production but contributes exclusively to economic development and progress in every country, to the preservation of the finest that the human spirit has accomplished and to the creation of new and important values !

In present circumstances, when there exist numerous economic, social and political problems at the world level, it is our special duty as scientists constantly to increase our contribution to the solution of these problems for the well-being of all nations. Let us use our discoveries to close the great gaps between the rich and poor countries of the world, to eradicate the malnutrition and under-development affecting two thirds of the world population, to eliminate the diseases which continue to claim millions of human lives, and to protect the environment and conserve it for the benefit of future generations ! Let us exert every effort to discover new sources of energy and raw materials, to solve the problems of food, water supply, health, and so forth, on which depend the progress and the future of all mankind ! Let us resolutely oppose any obstacle to the movement of the world's scientific and cultural assets, so that all peoples can derive extensive benefit from the awesome accomplishments of science and technology, so that science may truly become the property of all mankind !

Today, international peace and security provide the most favourable conditions for economic and social progress and for the application of what the human spirit has achieved, the daunting modern technical and scientific revolution, to the benefit of all mankind. Consequently, every effort, every action by scientific and cultural associations, civic organizations and private individuals, or by politicians, Governments and parliaments, that will help to defend and consolidate peace, to promote the cause of peaceful international collaboration based on respect for national independence and sovereignty, equal rights, non-interference in internal affairs and mutual advantage must be appreciated and given determined support, so that the legitimate aspirations of the peoples, of all who are aware of their responsibility for the fate of civilization, may be realized.

We call on scientists, and on their national and international associations, to establish suitable forms of co-operation transcending national, ideological or political differences, to the end that science may be used exclusively in accordance with its humanistic calling.

With this in mind, we have established an International Action Committee to organize scientific activities, to expose the dangers created by the frenzied arms race, particularly the nuclear arms race, to inform public opinion about these dangers and formulate concrete measures to avoid them, and to prepare for a world congress of scientists in the service of peace. We appeal to scientists and intellectuals throughout the world to join the Committee in this noble initiative for peace, to do all in their power to make our views known in the United Nations, in the Committee on Disarmament at Geneva and in all international forums where disarmament, peace and international security and co-operation are discussed.

Let us, in realization of our responsibility to science and to mankind and of the fact that we cannot create an acceptable future without a peace-

ful present, muster our strength of persuasion and the force of our arguments in order to induce the arms enthusiasts to change their approach, in order to influence Governments, parliaments and politicians to promote policies of peace, understanding and collaboration and to abandon entirely the use or threat of force, ensuring that all disputes are settled solely by peaceful means, through negotiation.

Let us do all in our power to ensure that the funds spent on armaments, the enormous military budgets, are used for socio-economic development programmes in each country, for helping the peoples of the developing countries in their struggle for progress, and for creating a more just and better world free from the threat of war !

Let us dedicate ourselves to the noble ideals of peace : let us do our duty to our own consciences, to our contemporaries, to the supreme commandments of mankind ! Let us show mankind a future commensurate to its most cherished aspirations and its creative abilities, let us prove worthy of all the most precious accomplishments of human civilization down through the centuries !

We are firmly convinced that, if we join forces and intensify our co-operation, science will truly become a weapon for living, enabling all peoples to increase their contributions to the heritage of universal knowledge, so that peace, security and collaboration may triumph on earth !

The participants in the
International Symposium
"Scientists and Peace"

Bucharest, 5 September 1981

COENO-ECOLOGICAL GROUPS IN GRASSLANDS OF ROMANIA

BY

A. J. KOVÁCS and G. DIHORU

It is a first attempt to synthesize the ecologic-phytocoenologic informations on the Romanian grasslands. The basic components of the plant communities are considered the main 64 coeno-ecological indicator groups, which are presented on zones and belts of vegetation. Individual species groups are assessed by habitat and coenotaxa characteristics. These groups, real coeno-ecological nuclei of indicator plants, are of major interest in biology, ecology, phytocoenology and utilization of grasslands.

The biotypes collected from a plant community (in investigations about the forage germplasm sources) are an expression of the multiple interactions among species, on the one hand, and the species and the habitat on the other. Therefore, the informations obtained about the nature of "local population" (habitat, community, technology) are of a special interest in evaluating the genetic resources and in establishing the grassland management.

The plant communities containing these useful populations (ecotypes, genoecodemes) are the main important structural components of the grassland ecosystems and are organized in clustered coenopopulations, in various species groups with essential coenological and ecological features. These species gropups, as structural and functional units of the community/ecosystem, can be treated like *coeno-ecological indicator groups* [7, 9]. This concept brings together the species with closed ecological exigencies (light, temperature, humidity, trophicity, soil reaction) and similar coenotic behaviour (frequency and wide coverage, vitality and capacity of competition among the same coenotaxa).

The coeno-ecological species groups of indicator plants from Romanian grasslands have been realized from the statistical analysis of a vast information material and they have a general character, with possibilites of further detailing (regional groups) according to the local parameters.

The synopsis of the coeno-ecological groups (Gr.) has an arrangement on zones and belts of vegetation. The species are displayed alphabetically in three categories *(Poaceae+Cyperaceae+Juncaceae; Fabaceae;* other botanical families), using the taxonomy and nomenclature in Flora Europaea. Those in brackets are considered of a secondary/transgressive importance.

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ENUMERATION OF COENO-ECOLOGICAL GROUPS

I. THE ZONE OF STEPPE - FOREST STEPPE - OAK FORESTS

1. Gr. **Festuca valesiaca :** *Agropyron cristatum* subsp. *pectinatum, Bromus japonicus, Cleistogenes serotina* subsp. *bulgarica, Phleum phleoides, Stipa capillata, S. ucrainica; Astragalus ponticus, A. onobrychis, A. vesicarius, Medicago lupulina, M. minima, M. orbicularis, M. sativa* subsp. *falcata, Onobrychis arenaria, O. gracilis, Oxytropis pilosa; Achillea nobilis* subsp. *neilreichii, A. setacea, Adonis vernalis, A. volgensis, Allium moschatum, Carduus hamulosus, Centaurea orientalis, Cirsium serrulatum, Dianthus membranaceus, D. pallens, Ferulago campestris, Galium humifusum, Linum austriacum, L. hirsutum, Phlomis herba-venti* subsp. *pungens, Plantago argentea, Rumex tuberosus, Salvia nemorosa* subsp. *tesquicola, S. nutans, Taraxacum serotinum, Trinia ramosissima, (Verbascum speciosum), Veronica austriaca, Viola ambigua.*

Continental-xerothermic biotopes, flat areas or sunny slopes ; eubasic and slightly acid-neuter (alkaline) soils. Oligotrophic, xerophilous group. *Festucion rupicolae, Festucetalia valesiacae.*

2. Gr. **Stipa lessingiana :** *(Festuca valesiaca), Stipa pulcherrima; Astragalus austriacus, A. asper; Ajuga laxmannii, Allium albidum, Centaurea trinervia, Cephalaria uralensis, Crambe tataria, Haplophyllum suaveolens, Jurinea mollis* subsp. *transylvanica, Nepeta ucranica, Salvia nutans, Scorzonera hispanica, Teucrium polium.*

Vegetation of primary steppe grasslands, rocky slopes ; dry neuter-alkaline, loamy or with loess soils. Oligotrophic, xerophilous, subthermophilous group. *Stipion lessingianae, Festucetalia valesiacae.*

3. Gr. **Thymus zygioides :** *Agropyron cristatum* subsp. *brandzae, Festuca callieri, Koeleria lobata; Astragalus vesicarius; Convolvulus lineatus, Dianthus nardiformis, D. pseudarmeria, Euphorbia nicaeensis* subsp. *glareosa, Gypsophila glomerata, Paronychia cephalotes, Pimpinella tragium* subsp. *lithophila, Satureja caerulea, Scutellaria orientalis.*

Sunny plateaus and slopes on calcareous substratum in the South-East of the country ; very dry superficial, skeleton, eroded, rocky soils, Oligotrophic, xerophilous, thermophilous group. *Pimpinello-Thymion zygioidi.*

4. Gr. **Festuca pseudovina — Achillea setacea :** *(Festuca rupicola), (F. valesiaca), Koeleria macrantha, Poa angustifolia; Astragalus onobrychis, Medicago lupulina, M sativa* subsp. *falcata, Onobrychis arenaria, Trifolium repens; Achillea collina, (Euphorbia cyparissias), E. salicifolia, Hieracium praealtum* subsp. *bauhinii, Potentilla argentea, P. cinerea Sedum acre, S. sexangulare.*

Dry sunny habitats ; eubasic slightly acide-alkaline soils, Mesotrophic, xeromesophilous group as far as the sessile oak belt. *Festucetalia valesiacae, Festuco-Brometea.*

5. Gr. **Poa augustifolia * :** *Carex praecox, Cynodon dactylon, Dactylis glomerata, (Festuca rupicola), (F. valesiaca); Medicago lupulina, M. sativa* subsp. *falcata, Lotus corniculatus, Trifolium repens; Achillea millefolium, A. setacea, Cichorium intybus, Eryngium campestre, Plantago lanceolata.*

Dry-slightly moist habitats ; eubasic slightly acid-neuter soils, Mesotrophic, mesoxerophilous group, appearing as a successive stage after fertilization. *Festuco-Brometea.*

6. Gr. **Artemisia austriaca — Poa bulbosa :** *(Agropyron cristatum* subsp. *pectinatum), (Dichanthium ischaemum), (Cynodon dactylon); Astragalus onobrychis, Trigonella monspeliaca; (Achillea setacea), Androsace elongata, Ceantaurea diffusa, Ceratocephalus testiculatus, Euphorbia nicaeensis, Galium humifusum, Herniaria incana, Marrubium peregrinum, Verbascum phlomoides.*

Degraded vegetation, ruderal (fallow, pastures) ; eubasic-mesobasic, slightly acid, neuter soils. Mesotrophic, xerophilous, subthermophilous group. *Festucion rupicolae, Festucetalia valesiacae.*

7. Gr. **Dichanthium ischaemum :** *Bromus squarrosus, Cleistogenes serotina, (Cynodon dactylon), Festuca rupicola, F. valesiaca, Stipa capillata; Astragalus asper, A. monspessulanus, Medicago lupulina, M. minima; Aster amellus, Bombycilaena erecta, Centaurea rhenana, Cephalaria transylvanica, Dianthus capitatus, Erysimum diffusum, Euphorbia nicaeensis, E. seguierana, Xeranthemum annuum, X. cylindraceum.*

Degraded, steppizated habitats, rocky slopes ; the soils are dry-slightly moist, sandy, skeleton, with loess, slightly acid-neuter, poor in humus. Oligotrophic, xerophilous, subthermophilous group, especially in the South-East of the country. According to the geographical zone and altitude the nucleus of the species is modified. *Festucetalia valesiacae, Festuco-Brometea.*

8. Gr. **Chrysopogon gryllus :** *(Agrostis capillaris), (Dichanthium ischaemum), Danthonia alpina, (Elymus hispidus), (Festuca pseudovina), (F. rupicola), (F. valesiaca); Coronilla varia, Medicago sativa* subsp. *falcata, Onobrychis arenaria, (Trifolium ochroleucon), Triagonella gladiata; Centaurea salonitana, Inula ensifolia, Jurinea mollis* subsp. *transylvanica, Orlaya grandiflora, Ornithogalum pyramidale, Prunella laciniata, Ranunculus illyricus, (Rhinanthus rumelicus), Salvia transylvanica, Thymus glabrescens, Th. pannonicus, Verbascum banaticum.*

Dry habitats on slight poorly drained slopes ; the soils are eubasic, slightly acid-neuter, sandy, sometimes skeleton, poor in humus. Oligotrophic, xerophilous, subthermophilous group. *Danthonio-Chrysopogonion, Festucetalia valesiacae.*

9. Gr. **Thymus pannonicus – Salvia pratensis :** *Artemisia campestris, A. pontica , Cephalaria radiata, C. uralensis, Euphorbia cyparissias, Fragaria viridis, Potentilla cinerea, Salvia nutans, S. transylvanica, S. verticillata, Teucrium montanum, Thymus glabrescens.*

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* One should not take *Poa angustifolia* L. for *P. pratensis* L. which has other coeno-ecological characteristics (Gr. 12).

Pioneer vegetation on the degraded slopes; mesobasic, eroded, slightly acid-neuter soils. Oligotrophic, mesoxerophilous group. *Festucion rupicolae, Festuco-Brometea.*

II. BELT SESSILE OAK – BEECH

10. Gr. **Lolium perenne — Trifolium repens:** *Festuca pratensis, F. pseudovina, Poa angustifolia, P. pratensis; Lotus corniculatus, Medicago lupulina, Trifolium pratense; Achillea millefolium, Carduus acanthoides, (Eryngium campestre), Inula britannica, Plantago lanceolata, Potentilla argentea, Taraxacum officinale.*

Fertile, slightly moist habitats; eubasic and mesobasic soils. Eutrophic group (sometimes even nitrophilous), mesophilous. *Cynosurion, Arrhenatheretalia.*

11. Gr. **Arrhenatherum elatius — Crepis biennis:** *Dactylis glomerata, Festuca pratensis, (F. rubra), Phleum pratense, Poa pratensis, Trisetum flavescens; Lathyrus pratensis, Trifolium hybridum, T. pratense; Achillea millefolium, Carum carvi, Centaurea jacea, Cichorium intybus, Daucus carota, Heracleum sphondylium, Laserpitium latifolium, Leucanthemum vulgare, Pastinaca sativa, Picris hieracioides, Tragopogon pratensis* subsp. *orientalis.*

Fertile, slightly moist and nondegraded habitats; eubasic-mesobasic, moderately-slightly acid, loose soils. Eutrophic, mesotrophic, mesophilous group. *Arrhenatheretalia.*

12. Gr. **Festuca pratensis:** *Alopecurus pratensis, Bromus commutatus, Carex melanostachya, C. otrubae, Dactylis glomerata, Poa pratensis, P. trivialis; Trifolium dubium, T. hybridum, T. patens, T. pratensis; Cichorium intybus, Galium palustre, Gladiolus imbricatus, Leucanthemum vulgare, Pimpinella saxifraga, Prunella vulgaris, Rhinanthus angustifolius (R. rumelicus), Tragopogon pratensis* subsp. *orientalis.*

Fertile fields (orchads), eubasic, moderate to slightly acid-neuter and fresh soils. Eutrophic mesophilous-mesohygrophilous group. *Agrostion stoloniferae, Molinietalia, Arrhenatheretalia.*

13. Gr. **Cynosurus cristatus:** *(Agrostis capillaris), Festuca pratensis, F. rubra, Dactylis glomerata, Lolium perenne, Phleum pratense, Poa pratensis; Trifolium pratense, T. repens; Bellis perennis, Leontodon autumnalis, (Prunella vulgaris), Ranunculus acris.*

Moderately fertile, slightly moist habitats; eubasic-mesobasic moderate-slight acid, sandy-loam or clay-loam soils. Eutrophic-mezotrophic, mesophilous group. *Cynosurion, Arrhenatheretalia.*

14. Gr. **Plantago major:** *Lolium perenne, Sclerochloa dura; Chamomilla suaveolens, Coronopus procumbens, Poa annua, Polygonum aviculare, Potentilla anserina, Sagina procumbens, Taraxacum officinale (Verbena officinalis).*

Alongside roads, pastures, ploughed fields, trodden lands; eubasic, moderate, slight acid, compact soils. Eutrophic (nitrophilous), mesophilous-mesohygrophilous group. *Polygonion avicularis, Plantaginetalia majoris.*

15. Gr. **Festuca pallens:** *(Helictotrichon decora), Dianthus spiculifolius, Hieracium bifidum, Sedum hispanicum, Silene nutans* subsp. *dubia, Thalictrum foetidum.*

Calcareous rocks, rocky slopes; eubasic, neuter, slightly acid soils. Oligotrophic, saxicolous group. *Stipo-Festucetalia pallentis.*

16. Gr. **Phleum montanum:** *(Festuca rupicola), Melica ciliata; Aster amellus, Campanula sibirica, Centaurea atropurpurea, Erysimum comatum, Galium album, Seseli gracile, S. libanotis, Teucrium chamaedrys.*

Rocky and sunny slopes; eubasic, slightly acid, alkaline soils. Oligotrophic, mesophilous, subthermophilous group. *Thymo-Festucion rupicolae, Stipo-Festucetalia pallentis.*

17. Gr. **Carex humilis — Thymus comosus:** *(Dichanthium ischaemum), Festuca rupicola, Poa badensis; Allium flavum, Alyssum murale, Carduus candicans, Centaurea triumfetti, Cytisus procumbens, Helianthemum canum, Teucrium montanum.*

Rocky biotops, sunny slopes, on limy substrata; eubasic, neuter, alkaline soils. Oligotrophic-mesotrophic, xerophilous-mesoxerophilous group. *Thymo-Festucion rupicolae.*

18. Gr. **Danthonia alpina:** *(Agrostis capillaris), Brachypodium pinnatum, (Festuca rupicola); Astragalus monspessulanus, Cytisus multiflorus, Trifolium montanum; Clematis recta, Hieracium praealtum* subsp. *bauhinii, Inula hirta, I. salicina, Leontodon crispus, Orchis tridentata, Polygala major, Potentilla alba, Veronica austriaca, (Viola hirta).*

Plateaus and dry-slightly moist slopes; eubasic-mesobasic, slight acid-neuter, permeable, often skeleton soils. Oligotrophic, mesoxerophilous, subthermophilous. *Danthonio-Brachypodion, Festucetalia valesiacae, Brometalia.*

19. Gr. **Brachypodium pinnatum—Dorycnium pentaphyllum** subsp. **herbaceum:** *(Carex humilis), (Festuca rupicola), Poa angustifolia, Stipa tirsa; Cytisus multiflorus; Bupleurum falcatum, Cirsium pannonicum, Fragaria viridis, Hypericum elegans, Jurinea mollis* subsp. *transylvanica, Linum flavum, Origanum vulgare, Plantago media, (Polygala major), (Prunella laciniata), Thesium linophyllon.*

Degraded slopes; mesobasic, slightly acid, alkaline, loose, often skeleton soils. Oligotrophic, mesotrophic, xeromesophilous, subthermophilous group. Spread from the forest steppe to the beech belt. *Cirsio-Brachypodion, Brometalia.*

20. Gr. **Festuca rupicola — Onobrychis viciifolia:** *(Agrostis capillaris), Anthoxanthum odoratum, Carex humilis, C. montana, Koeleria macrantha; Lotus corniculatus, Medicago lupulina, M. sativa* subsp. *falcata, Trifolium montanum; Allium albidum, A. oleraceaum, Aster amellus, Asperula cynanchica, Campanula glomerata, C. sibirica, Dorycnium pentaphyllum* subsp. *herbaceum, Linum austriacum, Orchis tridentata, Pulsatilla montana, Salvia austriaca, S. nutans, Stachys recta, Veronica spicata* subsp. *orchidea.*

Moderate slopes, especially exposed to the South and South-West; eubasic-mesobasic, slightly acid-neuter-alkaline soils. Oligotrophic, meso-

xerophilous, xerophilous group (incl. in the zone of common oak). *Festucion rupicolae, Festucetalia valesiacae.*

21. Gr. **Agrostis capillaris:** *Anthoxanthum odoratum, Briza media, Dactylis glomerata, (Festuca rubra), (F. rupicola), Poa pratensis; Coronilla varia, Genista tinctoria, Medicago sativa* subsp. *falcata, Trifolium montanum; Anthericum ramosum, Campanula patula, (Centaurium erythraea), Filipendula vulgaris, Hypericum perforatum, Leucanthemum vulgare, (Moenchia mantica), Pimpinella saxifraga, Polygala comosa, Prunella vulgaris, Rhinanthus minor, Rumex acetosa, Salvia pratensis.*

Dry-slightly moist biotopes, moderately wet; mesobasic, moderately acid, sometimes skeleton or destitute of humus. Mesotrophic (oligotrophic), mesophilous-mesoxerophilous group. *Cynosurion, Arrhenatheretalia.*

III. BELT OF SPRUCE

22. Gr. **Festuca rubra:** *(Agrostis capillaris), Anthoxanthum odoratum, Phleum pratense; Anthylis vulneraria* ?subps. *kerneri, Chamaespartium sagittalis; Achillea distans, Alchemilla vulgaris* agg., *Campanula serrata, Carlina acaulis, Centaurea phrygia* subsp. *melanocalathia, Dactylorhiza cordigera, Gentiana lutescens, Hieracium aurantiacum, Hypericum maculatum, Hypochoeris radicata, Omalotheca sylvatica, (Viola declinata).*

Moderately wet habitats, on plane fields or slopes, in all aspects, at 600—1500 m; oligobasic-eubasic, acid-neuter soils. Oligotrophic-eutrophic, mesophilous (mesohygrophilous) group, in the beech and spruce belts. *Cynosurion, Arrhenatheretalia.*

23. Gr. **Festuca nigrescens:** *Nardus stricta, Phleum alpinum; Chamaespartium sagittalis, Trifolium repens; Campanula patula* subsp. *abietina, Centaurea uniflora* subsp. *nervosa, Hieracium aurantiacum, Hypochoeris uniflora, Scorzonera purpurea* subsp. *rosea, Viola declinata.*

Brown acid soils or podsols, incl. in the belt of mountain pine. Transgressive-mesothermic, mesotrophic group. *Cynosurion, Nardion.*

24. Gr. **Nardus stricta — Viola canina** subsp. **montana:** *Carex pilulifera, Danthonia decumbens, Deschampsia flexuosa, (Festuca rubra); Genista tinctoria* agg.; *Antennaria dioica, Arnica montana, Hieracium argillaceum, H. lactucella, H. pilosella, Potentilla erecta, Polygala vulgaris, Vaccinium myrtillus.*

Habitats with oligobasic, acid-moderately acid, poor in humus, moderately wet or dry-slightly moist (rarely slightly moist — moderately moist) soils. Oligotrophic (mesotrophic) group, sometimes with alternating moisture conditions. Some species appear in the beech belt too. *Nardion strictae, Nardetalia.*

25. Gr. **Deschampsia cespitosa** subsp. **alpina — Carex ovalis:** *Carex canescens, Festuca pratensis* subsp. *apennina, Poa trivialis; Alchemilla glabra, Caltha palustris, (Rumex alpinus), Senecio subalpinus, (Veratrum album).*

In wet biotopes with tendency to bogging up, on hard permeable substrata; mesobasic, slightly acid soils, often in peats. Mesotrophic, mesohygrophilous, hygrophilous group. *Deschampsion cespitosae, Adenostyletalia.*

26. Gr. **Rumex alpinus:** *(Dactylis glomerata), (Deschampsia cespitosa) Poa annua, P. pratensis; Alchemilla vulgaris* agg., *Chenopodium bonus-henricus, Rumex alpestris, R. obtusifolius* subsp. *subalpinus, Senecio subalpinus, Taraxacum officinale, Urtica dioica, (Veronica chamaedrys).*

Round of sheepfolds on nitrificated soils. Megatrophic, mesophilous, mesohygrophilous group. *Rumicion alpini, Adenostyletalia.*

27. Gr. **Calamagrostis arundinacea:** *Festuca arundinacea* subsp. *subalpina, F. rubra, Dactylis glomerata, Poa nemoralis; Campanula glomerata, Cirsium erisithales, Digitalis grandiflora, Origanum vulgare, Picris hieracioides, Tanacetum corymbosum* subsp. *clusii.*

Sunny slopes or forest glades; oligomesobasic, acid, moderately acid soils. Oligotrophic-mesotrophic, mesoxerophilous-mesohygrophilous, group. *Calamagrostion arundinaceae, Adenostyletalia.*

28. Gr. **Epilobium angustifolium:** *Calamagrostis arundinacea, C. epigeios, Dactylis glomerata, Festuca rubra, Poa nemoralis; Chaerophyllum aromaticum, Fragaria vesca, Rubus idaeus, Senecio sylvaticus, S. nemorensis* subsp. *fuchsii.*

Pioneer vegetation in the mountain cutting areas and forest glades with eutrophic (nitrophilous), mesophilous (sometimes mesoxerophilous) character. *Epilobietalia angustifolii.*

29. Gr. **Sesleria rigida:** *Avenula decora, Carex sempervirens, Festuca rupicola* subsp. *saxatilis, Poa nemoralis* (var. *agrostoides*), *Trisetum alpestre; Alyssum petraeum, Athamanta turbith* subsp. *hungarica, Cnidium silaifolium, Ranunculus oreophilus, Seseli libanotis, S. rigidium, Thalictrum foetidum.*

Calcareous rocks. Saxicolous, xerophilous, thermophilous group. *Seslerion rigidae, Seslerietalia.*

IV. BELT OF MOUNTAIN PINE (subalpine belt)

30. Gr. **Nardus stricta:** *Poa media; Genista tinctoria* agg.; *Crepis conyzifolia, Gentiana punctata, Homogyne alpina, Luzula sudetica, Omalotheca norvegica, Plantago atrata, Polygala alpestris, Potentilla aurea* subsp. *chrysocraspeda, Pseudorchis albida, Solidago virgaurea* subsp. *minuta.*

Habitats with excessive water content in spring from the melting of snow. Podsolized soils or acid humus podsols. Mesothermic heliophilous, oligotrophic group. *Nardion, Nardetalia.*

31. Gr. **Festuca airoides:** *Agrostis rupestris, Avenula versicolor, Bellardiochloa violacea, Nardus stricta; Alchemilla flabellata, (Campanula serrata), Euphrasia minima, Gentiana acaulis, Geum montanum, Ligusticum mutellina, Polygonum viviparum, Potentilla aurea* subsp. *chrysocraspeda, Pulsatilla alba, Senecio carpathicus.*

Dry, slightly moist habitats; podsolized brown or humic-silicated-podsolized acid-moderately acid soils with low trophicity. Oligotrophic, mesoxerophilous, oligothermic group. *Caricion curvulae.*

32. Gr. **Festuca amethystina :** *Carex sempervirens, Koeleria macrantha* agg., *Bellardiochloa violacea, (Sesleria rigida); Anthyllis vulneraria, Onobrychis montana; Allium ericetorum, Centaurea kotschyana, Dianthus tenuifolius, Helianthemum nummularium* subsp. *tomentosum, Iris ruthenica, Knautia longifolia, Phyteuma orbiculare, Potentilla crantzii, Thlaspi dacicum.*

Steep slopes in calcareous mountains; oligomesobasic, profound, loose, slightly acid-neuter soils. Heliophilous, thermophilous, mesotrophic, calcicolous group. *Seslerietalia calcariae* s.l.

V. ALPINE BELT

33. Gr. **Carex curvula :** *Agrostis rupestris, Festuca airoides, Juncus trifidus, Luzula alpinopilosa, Oreochloa disticha, Poa media; Anthemis carpatica, Campanula alpina, Dianthus glacialis* subsp. *gelidus Hieracium alpinum, Leucanthemopsis alpina, Omalotheca alpina, Omalotheca supina, Phyteuma confusum, Plantago gentianoides, Primula minima, Ranunculus crenatus, Sedum alpestre, Soldanella pusilla.*

Primary grasslands on siliceous substratum; oligobasic, acid-moderately acid, often skeleton soils. Oligotrophic, oligothermic group. *Caricion curvulae, Juncetea trifidi.*

34. **Sesleria bielzii :** *Carex sempervirens, Festuca picta, Festuca nitida, F. versicolor; Astragalus alpinus, A. frigidus, Hedysarum hedysaroides, Oxytropis carpatica, Trifolium repens* subsp. *ochranthum; Alyssum repens, Bartsia alpina, Biscutella laevigata, Linum perenne* subsp. *extraaxillare, Taraxacum panalpinum, Thymus pulcherrimus, Viola alpina.*

Steep slopes on calcareous substratum; eubasic, alpine rankers, humic-silicated, pararendzinas, rich in humus, moderate-slight acid soils. Oligotrophic, mesoxerophilous, rupicolous, oligothermic group. *Seslerietalia calcariae.*

35. Gr. **Minuartia sedoides :** *Androsace chamaejasme, Cerastium alpinum* subsp. *lanatum, Festuca bucegiensis, Loiseleuria procumbens, Minuartia gerardi, M. recurva, Silene acaulis.*

Habitats with the eolian erosion; skeleton, rocky, humic-silicate soils. Meso-oligotrophic, mesophilous, oligothermic group. *Elynion, Elyno-Seslerietea.*

36. Gr. **Kobresia myosuroides :** *Chamorchis alpina, (Dryas octopetala), Salix reticulata, Silene acaulis.*

Wind-beaten habitats; eubasic, superficial, humic-silicate, moderate acid soils. Oligotrophic, mesophilous-mesoxerophilous, oligothermic group. *Elynion, Elyno-Seslerietalia.*

VI. FLOOD PLAINES AND DEPRESSIONS

37. Gr. **Phragmites australis :** *Agrostis gigantea, Glyceria maxima, Poa palustris, Phalaris arundinacea; Glycyrrhiza echinata; Alisma plantago-aquatica, Butomus umbellatus, Equisetum fluviatile, Iris pseudacorus, Leu-*

canthemella serotina, Lycopus europaeus, Oenanthe aquatica, Rumex hydrolapathum, Scirpus lacustris, S. sylvaticus, Scrophularia umbrosa, Sium latifolium, Sparganium erectum, Stachys palustris, Typha angustifolia, T. latifolia.

Habitats with stagnant or slow flowing water, depressions; temporary or permanent submerged slightly acid — slightly alkaline, fertile soils. Eutrophic-mesotrophic, hygrophilous group. *Phragmitetea.*

38. Gr. **Phalaris arundinacea :** *Poa palustris, P. trivialis; Mentha aquatica, M. longifolia, Ranunculus repens, Rorippa amphibia.*

Wet biotopes near rivers and lakes, depressions; temporary submerged, slightly acid-neuter soils. Eutrophic-mesotrophic, hygrophilous (with alternating conditions). *Phragmitetea.*

39. Gr. **Scirpus maritimus :** *(Aster tripolium* subsp. *pannonicus), (Galium palustre), (Juncus gerardi), Polygonum amphibium, Scirpus lacustris* subsp. *tabernaemontani, (Triglochin maritima).*

Swampy habitats; eubasic-mesobasic, humic gley, ofen salinized (alkaline) soils. Mesotrophic-eutrophic (halophilous) hygrophilous group. *Bolboschoenion (Festuco-Puccinellietalia).*

40. Gr. **Thelypteris palustris :** *Carex elata; Menyanthes trifoliata, Peucedanum palustre, Ranunculus lingua, Salix aurita, S. cinerea.*

Swampy biotopes, peat bogs; mesobasic-eubasic, slightly acid, peaty soils, moistened with stagnant water. Mesotrophic, hygrophilous group. *Magnocaricetalia.*

41. Gr. **Carex rostrata — C. acuta :** *Calamagrostis stricta, Carex acutiformis, C. disticha, C. melanostachya, C. pseudocyperus, C. riparia, C. vesicaria, C. vulpina; Epilobium palustre, Myosotis scorpioides, Senecio paludosus, Teucrium scordium, Veronica scutellata.*

Liable to inundation depressions, swampy grasslands, swamps; oligobasic-eubasic, pseudogleyed, peaty-gleyed, often poor in limestone, moderate-slightly acid soils. Mesotrophic (oligotrophic-eutrophic) hygrophilous group. *Magnocaricion, Magnocaricetalia.*

42. Gr. **Eriophorum latifolium — Carex flava :** *Carex appropinquata, C. davalliana, C. lepidocarpa, Schoenus nigricans, Sesleria caerulea; Galium boreale, Pedicularis palustris, Pinguicula vulgaris, Swertia perennis, (Tofieldia calyculata).*

Swampy habitats and grasslands, fen peats; mesobasic, moderate slightly acid, gleyed or peaty-gleyed soils. Oligotrophic-mesotrophic, hygrophilous (alternating) group. *Caricetalia davallianae.*

43. Gr. **Molinia caerulea :** *Carex hostiana, C. panicea, Juncus conglomeratus; Tetragonolobus maritimus; Achillea ptarmica, Cirsium canum, Dianthus superbus, Epipactis palustris, Gentiana pneumonanthe, Sanguisorba officinalis, Silaum silaus, Succisa pratensis, Thalictrum simplex.*

Swampy (periodically dried) habitats; oligobasic-mesobasic, slightly acid (-neuter), often pseudogleyed, gleyed or peaty with nondecomposed organic substances soils. Oligothrophic-mesotrophic, mesohygrophilous (alternating) group. *Molinion, Molinietalia.*

44. Gr. **Deschampsia cespitosa — Juncus effusus:** *Agrostis canina, A. stolonifera; (A. capillaris), (Festuca rubra), Juncus atratus, J. conglomeratus; Cardamine pratensis, (Lythrum salicaria), Teucrium scordium.*
Wet habitats, swampy grasslands; oligobasic-mesobasic, acid-moderate acid, often pseudogleyed, rich in humus (hydromoder) soils. Oligotrophic, mesotrophic, hygrophilous (in alternating conditions) group. *Agrostion stoloniferae, Molinio-Arrhenatheretea.*

45. Gr. **Cirsium oleraceum:** *(Dactylis glomerata), Festuca arundinaçea, F. pratensis; Angelica sylvestris, Chaerophyllum hirsutum, Filipendula ulmaria, Geranium palustre, Lysimachia vulgaris, Petasites hybridus, (Ranunculus repens), Scirpus sylvaticus.*
Wet biotopes, swampy grasslands, river banks; the soils are eubasic-mesobasic, moderate-acid-neuter, rich in humus, often gleyed or peaty, moisted by the mobile infiltrating water. Mesotrophic-eutrophic, mesohygrophilous-hygrophilous group. *Molinietalia, Filipendulo-Petasition.*

46. Gr. **Agrostis stolonifera — Elymus repens:** *Carex hirta, C. otrubae, Festuca arundinacea, Juncus compressus, Poa trivialis; Lotus corculatus, Trifolium hybridum, T. repens; Fritillaria meleagris, Lepidium latifolium, Oenanthe silaifolia, Plantago altissima, (Potentilla anserina), Rumex confertus, Serratula tinctoria.*
Flooded areas; eubasic-mesobasic, slightly acid-neuter, often compacted and pseudogleyed soils. Mesotrophic-eutrophic, mesohygrophilous (alternating) group. *Agrostion stoloniferae, Molinietalia.*

47. Gr. **Alopecurus pratensis:** *Carex melanostachya, C. otrubae, (Deschampia cespitosa), Festuca arundinacea, F. pratensis, Poa trivialis; (Trifolium angulatum), T. hybridum, T. michelianum; Lychnis flos-cuculi, Oenanthe banatica, Orchis laxiflora* subsp. *elegans, Potentilla reptans, Ranunculus repens, Rumex obtusifolius, Symphytum officinale.*
Wet habitats from flooded plains, with short periods of flooding; eubasic-mesobasic, moderate acid-neuter, loamy or clayey soils. Eutrophic, hygrophilous-mesohygrophilous group. *Agrostion stoloniferae, Molinietea.*

48. Gr. **Poa trivialis** subsp. **sylvicola:** *Agrostis stolonifera, Alopecurus pratensis, Festuca pratensis; Medicago lupulina, Trifolium hybridum, T. pallidum, (T. patens), T. resupinatum; (Galium palustre), Potentilla reptans, Rorippa austriaca, Rumex crispus.*
Slightly-moist habitats in sunny flooded plains; eubasic, slightly acid-neuter, (rich in humus) soils. Eutrophic, mesohygrophilous, subthermophilous group. *Agrostion stoloniferae, Molinietalia* (incl. *Agropyro-Rumicion*).

49. Gr. **Elymus repens — Rumex crispus:** *(Agrostis stolonifera), Dactylis glomerata, Festuca arundinacea, Juncus compressus, Lolium perenne; (Trifolium hybridum), T. repens; Inula britannica, Mentha longifolia, Potentilla anserina, P. reptans, Pulicaria dysenterica, Rorippa sylvestris.*
Wet ruderal depressions, river banks; eubasic, mesobasic, loamy or clayey, often compact, moderately-slightly acid, (sometimes slightly salinized, moistened by phreatic water or by stagnant infiltration water) soils. Pioneer, eutrophic-mesotrophic, mesophilous-mesohygrophilous (alternating) group, spread from the forest-steppe zone as far as the beech belt. *Agropyro-Rumicion, Plantaginetea.*

50. Gr. **Lindernia pyxidaria:** *(Alopecurus aequalis), Eleocharis acicularis, Isolepis supina, Juncus bufonius, (Zingeria pisidica); (Chamomilla recutita), Elatine alsinastrum, Filaginella uliginosa, Lythrum hyssopifolia, Mentha pulegium, (Myosurus minimus), Veronica acinifolia.*
In microdepressions on grey-brown podzolic soil of depression, generally wet, sometimes with stagnant water, rarely slightly salinized. *Nano-Cyperion, Nanocyperetalia.*

VII. SALINIZED AREAS

51. Gr. **Salicornia europaea:** *(Puccinellia festuciformis* subsp. *convoluta); Bassia hirsuta, Salsola soda, Suaeda maritima.*
Inland salt areas; slightly moistened-wet, often in alternating conditions of humidity. Mesohygrophilous, halophilous group, *Thero-Salicornion, Thero-Salicornietalia.*

52. Gr. **Crypsis aculeata:** *Crypsis alopecuroides, C. schoenoides; (Heliotropium supinum), Spergularia marina, S. media, (Suaeda maritima), (Verbena supina).*
Salinised areas with humic gley soil and alternating conditions of humidity. Oligotrophic, mesohygrophilous, halophilous group. *Cypero-Spergularion, Festuco-Puccinellietea.*

53. Gr. **Juncus gerardi:** *(Agrostis stolonifera), Carex distans; Centaurium pulchellum, (Glaux maritima), Lotus tenuis, Peucedanum latifolium, Scorzonera parviflora, Trifolium fragiferum, Triglochin maritima.*
Habitats with salinized, moderately wet, moderately moistened, eubasic, slightly acid, alkaline soils. Mesohygrophilous (alternating), halophilous group. *Juncion gerardi, Festuco-Puccinellietalia.*

54. Gr. **Beckmannia eruciformis:** *(Agrostis stolonifera); Cardamine parviflora, (Mentha pulegium), (Ranunculus sardous), Rorippa sylvestris* subsp. *kerneri, Rumex stenophyllus.*
Marshy grasslands on the river banks; salinized (solonetzs), clayey soils. Mesotrophic, mesohygrophilous (alternating) halophilous group. *Beckmannion, Festuco-Puccinellietalia.*

55. Gr. **Pholiurus pannonicus:** *(Chamomilla recutita), (Hordeum hystrix), Plantago tenuiflora, (Zingeria pisidica).*
Habitats of moderate salt march, flooded, with humic gley soils, poor in humus (solonetzs), under temporary drying. Mesohygrophilous (alternating), halophilous group. *Festuco-Puccinellietalia.*

56. Gr. **Puccinellia festuciformis** subsp. **convoluta:** *Elymus elongatum, E. repens; Lotus tenuis; Lepidium crassifolium, Myosurus minimus, Scorzonera cana, Taraxacum bessarabicum.*
Moderately wet fields or with alternating hydroregime; salinized, neuter-alkaline soils, poor in humus. Mesohygrophilous (alternating), halo-

philous group. *Puccinellion limosae, Festuco-Puccinellietalia.*

57. Gr. **Puccinellia distans** subsp. **limosa :** *Puccinellia festuciformis* subsp. *intermedia; (Artemisia santonicum), Aster tripolium* subsp. *pannonicus, Atriplex littoralis, Limonium gmelinii, Triglochin maritimum (T. palustre).*

Moderate salt habitats; salinized (solonetzs, solonchaks), neuter-alkaline soils. Eutrophic, mesophilous (mesohygrophilous, alternating) group. *Puccinellion limosae, Festuco-Puccinellietalia.*

58. Gr. **Halimione verrucifera :** *(Puccinellia festuciformis* subsp. *convoluta); Halimione pedunculata, (Suaeda maritima).*

Marine and inland salt areas, sulfate-sodium salt marshes, moderately moist-dry soils. Mesohygrophilous, halophilous group. *Festuco-Puccinellietalia.*

59. Gr. **Festuca pseudovina — Artemisia santonicum :** *(Hordeum hystrix), Poa bulbosa; Lotus angustissimus, Trifolium angulatum, T. micranthum, T. strictum, T. subterraneum; Bassia sedoides, Bupleurum tenuissimum, Camphorosma annua, Petrosimonia triandra, Plantago schwarzenbergiana.*

Slightly salt habitats, salinized humic gley soils; dry-moderately moist, neuter soils, poor in humus. Xeromesophilous, halophilous group intermediary between steppe-forest steppe and halophytic vegetation. *Festucion pseudovinae.*

VIII. SANDY AREAS

60. Gr. **Leymus racemosus** subsp. **sabulosus:** *Agrostis gigantea* subsp. *pontica, Aeluropus littoralis; Astragalus varius, Medicago marina, M. orbicularis, Melilotus alba; Achillea clypeolata, Cakile maritima* subsp. *euxina, Carex ligerica, Crambe maritima, Ephedra distachya, Eryngium maritimum, Gypsophila trichotoma.*

Maritime sands and dunes; soils poor in humus. Oligotrophic, xerophilous, psammophilous, subthermophilous group. *Festucetalia vaginatae.*

61. Gr. **Bromus tectorum :** *Digitaria sanguinalis, Secale sylvestre, Tragus racemosus, Vulpia myuros; Medicago minima, Trifolium arvense; Anthemis ruthenica, Ceratocarpus arenarius, Mollugo cerviana, Polygonum arenarium.*

Dry biotopes, continental sands, rocky slopes; eubasic — mesobasic, loamy — sandy, skeleton soils, poor in humus. Oligotrophic—mesotrophic, xerophilous — mesoxerophilous group, of annual pioneer psammophytes. *Bromion tectorum, Festucetalia vaginatae.*

62. Gr. **Festuca vaginata :** *Trigonella monspeliaca; Achillea ochroleuca, Carex stenophylla, Centaurea arenaria, Gypsophila paniculata, Helichrysum arenarium, Kochia laniflora, Minuartia viscosa, Plantago arenaria, (Polygonum arenarium), Rumex tenuifolius, Silene conica.*

Sandy biotopes (forest steppe — sessile oak); sandy or loessoidy, slightly acid — alkaline soils. Oligotrophic, xerophilous, thermophilous group. *Festucion vaginatae, Festucetalia vaginatae.*

IX. BORDER OF FORESTS

63. Gr. **Geranium sanguineum :** *(Dactylis glomerata), (Festuca rupicola) (Coronilla varia), Trifolium alpestra, T. rubens; Clematis recta, Clinopodium vulgare, Dictamnus albus, Fragaria viridis, Galium glaucum, Inula hirta, I. salicina, Peucedanum cervaria, P. oreoselinum, Rosa gallica, Thalictrum minus, Seseli elatum* subsp. *osseum, (Tanacetum corymbosum), (Verbascum lychnitis).*

Dry, dry-moderately moist, basiphilic biotopes eubasic, neuter-alkaline, loose, often superficial, skeleton or rocky soils. Oligotrophic, xerophilous, xeromesophilous, thermophilous group. At the contact area of xerophilous grasslands *(Festuco-Brometea)* and thermophilous *(Quercetea petraeae)* or temperate forests *(Carpinion, Fagion). Geranion sanguinei, Origanetalia.*

64. Gr. **Trifolium medium :** *(Brachypodium pinnatum), Calamagrostis arundinacea, (Dactylis glomerata); Astragalus glycyphyllos; Achillea distans, Agrimonia eupatoria, Gentiana asclepiadea, Laserpitium latifolium, Origanum vulgare, (Pteridium aquilinum).*

At the contact area between mesoxerophilous grasslands and mesophilous forests, on moderately moist soils. *Trifolion medii, Origanetalia.*

REFERENCES

1. Beldie Al., Chiriță C., *Plante indicatoare din pădurile noastre*, Edit. agro-silvică, București, 1967.
2. Cardasol V., *Grupele ecologice de specii de pajiști din Depresiunea Bîrsei în relație cu umiditatea solului și măsurile de sporire a producției*, Doctorat thesis, Agronomic Institute, București, 1980.
3. Csürös Șt. et al., Studia Univ. Babeș-Bolyai-Biol., 1967, **12**, *1*; 1970, **15**, *1*.
4. Dihoru G., Doniță N., *Flora și vegetația podișului Babadag*, Edit. Academiei, București, 1970.
5. Ellenberg H., *Wiese und Weiden und ihre Standortliche Bewertung*, Ulmer Verlaf, Stuttgart, 1952.
6. Ellenberg H., *Vegetation Mitteleuropas mit den Alpen*, Ulmer Verlag, Stuttgart, 1978.
7. Knapp R., *Einführung in die Pflanzen soziologie*, Ulmer Verlag, Stuttgart, 1971.
8. Kovács J. A., *Indicatori biologici, ecologici și economici ai florei pajiștilor*, CMDPA, București, 1979.
9. Kovács J. A., *Cercetări privind germoplasma autohtonă la gramineele perene de pajiști*, Doctorat thesis, Univ. of Iași, 1981.
10. Kovács Margit, Acta Bot, Hung., 1968, **15**, *1—2*.
11. Kruijne A. A. et al., Versl. Landbonwk, Onderz., 1969.
12. Păun M. et al., Vegetația în *"Pajiștile din zona subcarpatică a Olteniei"*, Edit. Scrisul Românesc, Craiova, 1973.
13. Pușcaru Evdochia et al., *Pășunile și fînețele din R.P.R.*, Edit. Academiei, București, 1963.
14. Zólomy B. et al., Fragm. Bot. Mus. Nat., Hung., 1967, **4**.

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CERTAINS ASPECTS DE L'EMBRYOLOGIE CHEZ LE NOYER EN RELATION AVEC LA TAXONOMIE DE LA FAMILLE DES JUGLANDACÉES

PAR

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The analysis of embriological data (microsporogenesis, macrosporogenesis, gametogenesis, pollination) as well as of the ecological-climatological (seasonal) and biological ones in *Juglans regia* L., performed by the authors, provided new evidence as regards the taxonomy and phylogeny of fam. *Juglandaceae* belonging to *Monochlamydeae* as well as to *Angiospermae* in general. The processes of progressive individualisation of the cells of the embryo sacks are pointed out as well as the penetration of the pollinic tube (porogamy), the form of stomates, presence of calcium crystals in the palisade tissue, a.s.o., in comparison with the related units.

Some characters of similitude and differentiation are debated, revealing the progresses obtained in evolution. The value of the derived characters as well as the characters of primitivity are underlined, peculiarities through which the *Juglandales* are related to the *Amentiferae* and to *Terebinthales*, respectively.

Dans la taxonomie des Spermatophytes on utilise aussi de plus en plus de nos jours en dehors des caractères de l'appareil végétatif (structure, consistance et autres), ainsi que ceux de la fleur, du fruit, de la graine — les résultats des recherches de cytologie, d'embryologie, de génétique, d'écologie, de florogenèse.

Les études embryologiques ont une particulière importance, puisque les caractères et les diagnèmes que l'on saisit comme progressions dans le déroulement des divers processus de morphogenèse florale relatifs à la structure des fleurs, des inflorescences, du pollen, des stomates, de même que ceux de microsporogenèse et de macrosporogenèse, y compris de métabolisme, constituent des preuves dans l'établissement des relations taxonomiques phyllogénétiques.

Pour l'explication de l'évolution des *Juglandales*, la connaissance de la structure et de l'organisation florale, en relation avec les facteurs du milieu et des rapports biotiques, constitue une source d'importantes données. Mais l'interprétation de celles-ci diffère, dans la bibliographie, chez différents auteurs quant à la détermination de la valeur des caractères essentiels, deux courants étant connus à cet egard : l'un qui considère primitif le groupe des *Monochlamydeae*, l'autre comme des plantes dérivées, résultats secondaires dans l'évolution. Les présentes recherches ont justement le but d'apporter de nouveaux éléments et de nouvelles interprétations d'embryologie dans ce problème fondamental de l'évolution des certains groupes d'Angiospermes et notamment chez les *Juglandales*.

Chez *Juglans regia* L. l'ovule est orthotrope (Pl. I, fig. 1), (type rencontré chez la plupart des Gymnospermes, chez les Préphanérogames, les

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Polygonacées), basilaire, avec un tégument vascularisé, concrescent avec le nucelle (comme chez les Fagacée, les Salicacée). La vascularisation est plus développée à la base de l'ovule ; le trajet des vaisseaux suit une courbure allant de l'ovaire vers la base de l'ovule.

Le nucelle est volumineux, non vascularisé, concrescent avec le tégument, caractères voisins des Casuarinacée, des Fagacée, des Myricacée, des Salicacée.

L'archespore femelle est multicellulaire, caractère qui attesterait l'ancienneté et la primitivité de ce groupe, caractère rencontré aussi chez les *Polycarpicae*, ce qui constitue un argument pour la ressemblance entre ces groupes ancestraux dans le système des Angiospermes.

Selon certaines considérations (parues) dans la littérature de spécialité (P. M. Jucovschi, 1953, p. 363), l'archespore pluricellulaire de certains groupes plus primitifs (Casuarinacée) ne constituerait pas une preuve de leur primitivité, puisque l'on rencontre aussi un archespore de ce type chez des familles plus jeunes du point de vue évolutif, phylogénétique (Apiacée, Asteracée).

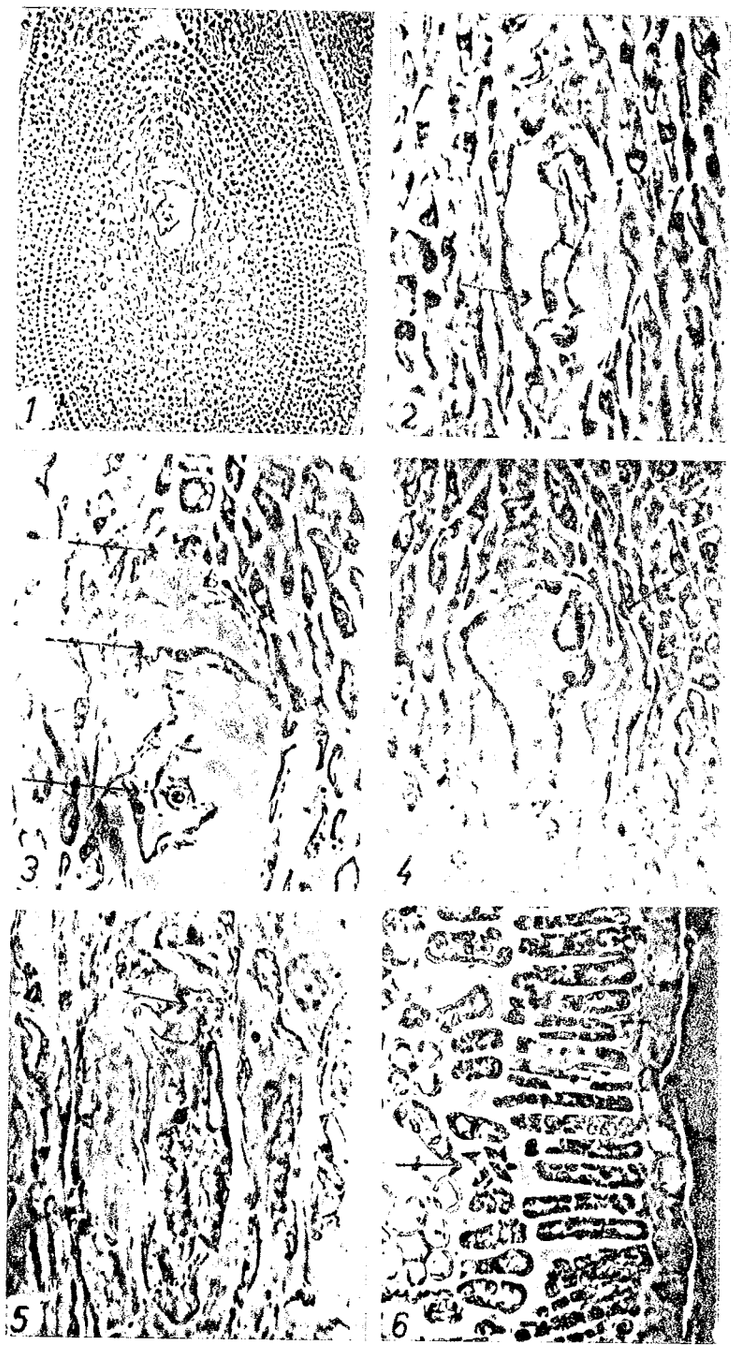
Des recherches sur la gamétogenèse femelle chez *Juglans regia* ont mis en évidence la formation de la cellule-mère des macrospores vers le centre du nucelle massif, formant une tétrade de type linéaire des macrospores. Le sac embryonnaire complet à 8 noyaux se différencie pendant la phénophase de la floraison maximale de la fleur femelle dans la période optimale de fécondation. Après le stade cénocytique octonucléé, le sac embryonnaire subit un processus de cellularisation, qui se déploie dans une certaine succession caractéristique : d'abord se forment les antipodes à grands noyaux et à une cytoplasme intensément colorée (Pl. I, fig. 2), après quoi suit la formation de l'oosphère ayant une grande vacuole et une membrane mince (Pl. I, fig. 3) et ensuite les synergides avec de grandes vacuoles du côté micropylaire du cénocyte. Les noyaux polaires présentent des nucléoles évidents, grands.

L'individualisation progressive dans une certaine succession des cellules du sac embryonnaire constitue un caractère utile en taxonomie et en phylogénie, étant considéré une ressemblance avec l'ontogenèse du gamétophyte chez les plantes archégoniates (J. Bouillot, 1969). En poursuivant la pénétration du tube pollinique dans l'ovule et dans le sac embryonnaire, on observe que celui-ci pénètre par porogamie (Pl. I, fig. 4) et libère ensuite les deux noyaux spermatiques (Pl. I, fig. 5).

Il existe une corrélation entre la conformation de la fleur et la manière dont pénètre, par porogamie, le tube pollinique, stade où la structure anatomique de la fleur est adéquate à ce processus par la présence, dans le canal du style, d'un tissu conducteur rudimentaire, formé de 2—3

Planche I — Aspects de l'embryologie chez *Juglans regia* L. : Fig. 1. — Ovule orthotrope, ayant un intégument et nucelle volumineux, non vascularisé ; Fig. 2. — Stade de la gamétogenèse femelle (formation des antipodes) ; Fig. 3. — Le sac embryonnaire (on observe les synergides, l'oosphère, le noyau secondaire) ; Fig. 4. — Pénétration du tube pollinique dans le sac embryonnaire ; Fig. 5. — Le tube pollinique pénétré dans le sac embryonnaire libérant deux noyaux spermatiques ; Fig. 6. — Section transversale du limbe foliaire (on observe les cristaux d'oxalate de calcium dans le tissu palissadique) (X 100) (orig.).

Planche I



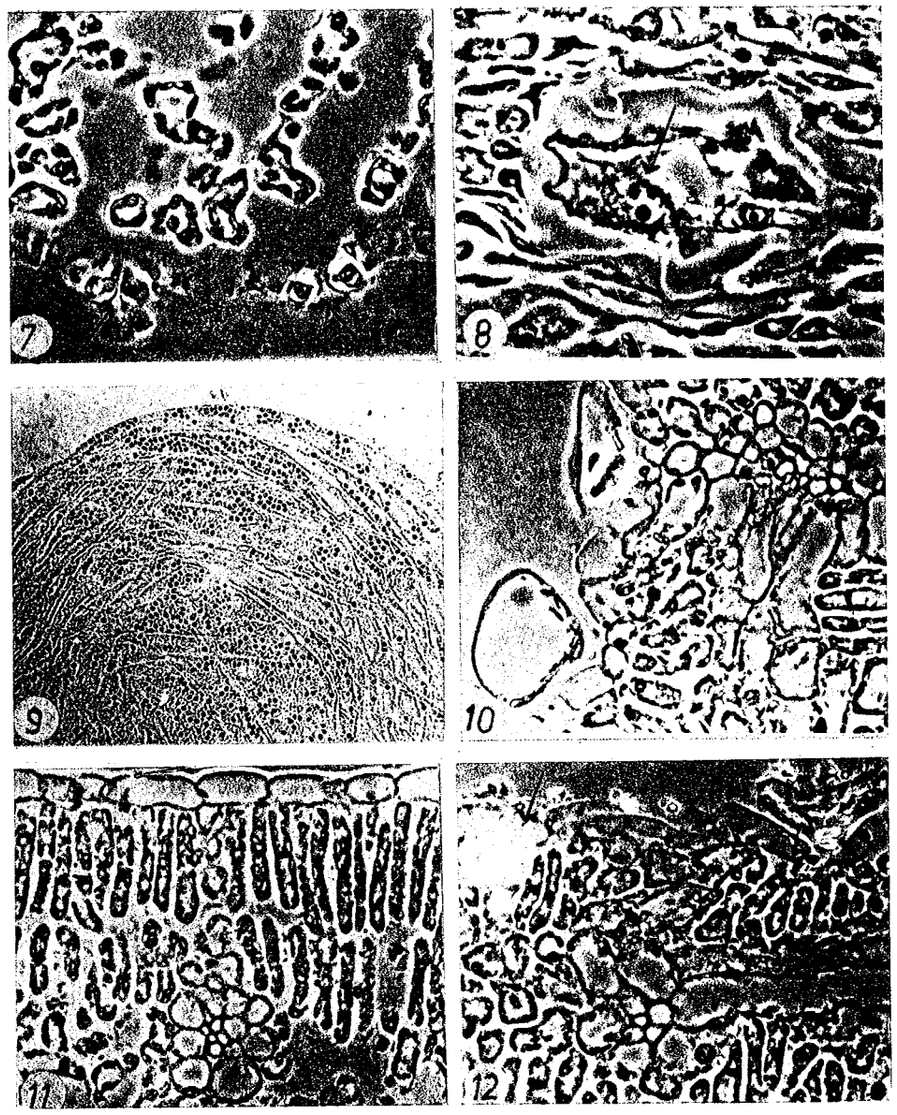


Planche II — Aspects embryologiques et morpho-anatomiques chez *Juglans regia* L. : Fig. 7. — Section transversale du limbe foliaire (le tissu lacunaire, l'épiderme inférieur aux stomates de type anomocytique, renonculé) ; Fig. 8 — Pénétration de deux spermaties près du noyau secondaire du sac embryonnaire ; Fig. 9. — Endosperme nucléaire ; Fig. 10. — Section transversale du limbe foliaire (poils sécréteurs) ; Fig. 11. — La feuille dorsiventrale à deux couches de cellules palissadiques ; Fig. 12. — Section transversale du limbe foliaire (poils glandulaires, cristaux d'oxalate de calcium), (X 100). (orig.)

rangs de cellules sécréteuses, glandulaires. La pénétration du tube pollinique se réalise sur le plan de la concrescence des carpelles, endroit où se forme le tissu conducteur du tube pollinique caractérisé par un riche contenu en lipides.

Sur le mode de pénétration du tube pollinique dans l'ovule chez *Juglans regia*, les opinions des auteurs sont différentes : il y en a quelques-uns qui soutiennent la thèse de l'existence de la chalazogamie (G. G. Nast, 1941 ; S. G. Navașin, 1951), et d'autres qui soutiennent celle de la porogamie (H. Schanderl, 1969). La chalazogamie a été identifiée aussi, outre chez les *Monochlamydeae*, chez d'autres groupes *(Ranales, Cucurbitales)*, constituant l'aporogamie ; cela montre que l'on rencontre ce caractère concernant la pénétration du tube pollinique dans l'ovule, de même chez certains groupes plus spécialisés, plus simplifiés, donc plus évolués, ce qui dénote que ce type dérive d'un type plus primitif, ancestral. La présence de la porogamie chez les Gymnospermes et chez bon nombre d'Angiospermes, ainsi que la présence de l'aporogamie chez les Angiospermes spécialisées et évoluées, tout cela aboutit à la constatation que la porogamie est plus primitive et que l'aporogamie en aurait dérivé. Ainsi, la présence de la porogamie chez le genre *Juglans* représenterait un caractère plus primitif, par lequel il se rapproche de groupes inférieurs d'Angiospermes. D'ailleurs, le type spécifique des fleurs, aussi, est considéré un caractère qui les rapproche du groupe inférieur, celui des Gymnospermes.

Selon la conception de Eichler et de Engler les fleurs apétales, unisexuées, sont primitives et, à partir de cette hypothèse, les réductions au niveau de la fleur ont une importante signification en phylogénie, par exemple le fait de situer l'origine des *Monochlamydeae*, parmi les Gymnospermes (R. Wettstein, 1935 ; A. Engler, 1936), sur la base des caractères suivants : fleurs unisexuées, simplifiées, anémophilie, ovule unitégumenté, intervalle prolongé entre la pollinisation et la fécondation et autres.

Les *Juglandales* ont des caractères communs avec plusieurs unités taxonomiques ; c'est pourquoi leur position systématique est aujourd'hui encore incomplètement clarifiée. Sur la base de certains caractères, les *Juglandales* se rapprochent des *Amentiferae* : inflorescences en aments, fleurs unisexuées, périanthe simple ou absent, étamines épithépales, ovules vascularisés, pollen binucléé, l'archespore femelle pluricellulaire, l'anémophilie, la présence de l'oxalate de calcium dans le tissu palissadique des feuilles (Pl. I, fig. 6), la forme des stomates (Pl. II, fig. 7).

Les études de morphogenèse, portant sur la connaissance du déploiement (visant à connaitre le déploiement) de la méiose et sur l'établissement du nombre fondamental des chromosomes, ont conduit à la conclusion que la famille des Juglandacée serait, du point de vue phylogétique, apparentée à la deuxième section de la famille des Betulacée *(Carpinus. Ostrya, Ostryopsis)*, où le nombre fondamental des chromosomes est 8 comme dans la famille des Juglandacée (R. H. Woodworth, 1930).

Sur la base des caractères ci-haut énumérés, quant à l'origine des *Monochlamydeae* parmi les Gymnospermes, à leur parenté avec les *Amentiferae* (à leur rapprochement des *Amentiferae)*, les *Juglandales* sont insérées différemment dans les systèmes de classification, soit au commen-

cement comme, par exemple, dans le système de Engler, dans la sous-classe des *Archichlamydeae*, soit dans d'autres, comme par exemple, dans le système de Takhtajan (1959) dans le surordre des *Amentiferae*. La position des *Juglandales* varie également dans différents systèmes. Dans le système élaboré par Pulle (1952), les *Juglandales* (comme les *Salicales*, les *Myricales*) sont inscrites dans l'Appendix, et dans de nombreux autres systèmes, elles tiennent diverses places dans le système des Angiospermes, tels les systèmes R. Soó (1953, 1961), Novak (1954), Benson (1954), Takhtajan (1959) chez les *Amentiferae;* Cronquist (1957), Hutchinson (1959), dans la Division 1. *Lignosae;* Zimmermann (1959) insère les *Juglandales* avec les *Casuarinales*, les *Salicales*, les *Fagales*, les *Myricales;* Engler (1964) groupe, dans le cadre de l'ordre des *Juglandales*, les familles des Myricacée et des Juglandacée.

D'après une autre hypothèse, le type unisexué avec le réceptacle non bombé, la gaine florale simple, la disposition verticillée des éléments floraux, l'ovaire infère *(Juglandales)*, le gynécée cénocarpique, l'anatomie du bois à vaisseaux développés et autre, constitue des caractères dérivés en évolution.

Conformément à la théorie strobilaire, la primitivité apparente des *Monochlamydeae* à la gaine simple, aux fleurs unisexuées, l'anémophilie, est le résultat de la réduction et de la simplification des éléments floraux, l'ovaire infère chez le Juglandacée, tout cela constitue des caractères dérivés, évolués, résultés de la différenciation et de l'adaptation supérieure.

L'anémophilie est apparue comme un caractère secondaire dans l'évolution des Angiospermes ligneuses, du fait que celles-ci se constituient dans les foréts dépourvues d'insectes abondants, le périanthe de la fleur s'est réduit et les fleurs se sont rapetissées et agglomérées en inflorescences (aments, épis), sans nectaire ni odeur, mais avec du pollen en grande quantité, léger, de sorte que certains groupes de *Amantiferae* sont redevenus (secondairement) des plantes anémogames. Pendant le développement du gamétophyte, le noyau génératif se divise dans le tube pollinique et cela, à la différence d'autres espèces plus évoluées, chez lesquelles le noyau génératif se divise à l'intérieur du grain de pollen. La cellule générative se divise en deux cellules spermatiques qui participeront au processus de la fécondation. Dans certains cas on a remarqué dans le processus de la fécondation, la présence de deux spermaties à côté du noyau secondaire du sac embryonnaire (Pl. II, fig. 8). Après la fécondation, le zygote accessoire se divise à un rythme intense et forme l'endosperme de type nucléaire (Pl. II, fig. 9).

Quelques traits des *Juglandales* les séparent des *Amentiferae:* feuilles composées, poils sécréteurs, cellules sécréteuses (Pl. II, fig. 11—12), ovule unitégumenté et d'autres, caractères qui les rapprochent des *Terebinthales*, les *Juglandales* étant considérées, par certains auteurs des *Terebinthales* réduites (Emberger, 1960).

La phylogénie basée de plus en plus sur des données paléontologiques explique de manière plus documentée la descendance monophylétique polytope des unités dans le cadre des Angiospermes très anciennes de trois branches indépendantes : *Casuarinales*, *Monochlamydeae*, *Ranales* (Emberger, 1960). Les *Monochlamydeae* constituent un groupe hétérogène

représentant l'extrémité d'une série d'évolution et nullement le commencement, comme voudrait le prouver Wettstein dans son système (M. Guşuleac, 1949, 1956).

Par la simplification, la réduction et la spécialisation dans le processus évolutif des séries d'Angiospermes avec la prédominance du type lignicole, de même que par toute une série de caractères supérieurement différenciés de l'appareil reproducteur, proposés de manière évolutive, les *Juglandales*, comme les *Monochlamydeae*, en général, avec leurs ordres différemment intégrés dans le système constituent des unités taxonomiques perfectionnées du point de vue adaptatif, dans leur organisation et leur évolution (Tr. I. Ştefureac, 1973).

En approfondissant les recherches de biomorphogenèse en utilisant les nouveaux éléments d'embryologie et de cytologie, ainsi que certains aspects concernant les processus microévolutifs, on pourra préciser —à côté de considérations morpho-anatomiques phénoménologiques, y compris certaines données paléontologiques, écologiques, biologiques et géographiques de florogenèse — leur place adéquate dans le système. Le caractère primitif ou dérivé de la valeur de certaines progressions dans l'évolution se référant surtout aux unités taxonomiques de rang supérieur, tel que celui des *Monochlamydeae* et des ordres compris dans leur cadre, justifie la position taxonomique naturelle dans les séries variées du système des Angiospermes dicotylédones.

Les résultats des études actuelles, y compris celles embryologiques considérées dans leur ensemble, sous le rapport morphobiogéographique, contribueront à l'intégration de l'ordre des *Juglandales* à juste titre compte tenu de la valeur des caractères interprétés à la lumière des conceptions modernes de phytotaxonomie phylogénétique.

Les caractères morpho-anatomiques, en corrélation avec ceux embryologiques, cytologiques et génétiques, ainsi que les considérations écologiques, biologiques et phyto-historiques, peuvent constituer, avec d'autres, une ligne directrice dans les recherches taxonomiques et phylogénétiques des *Juglandales*. Ce groupe présente une particulière importance, sous le rapport théorique-scientifique, fondamental, pour faire comprendre l'évolution des Angiospermes ; sous le rapport pratique, elles présentent, par les avantages qu'elles offrent (bois, fruits) un réel intérêt économique.

BIBLIOGRAPHIE

1. Bouillot J., Rev. Cytol. Biol. vég., 1969, **32,** *3—4*, 203—208.
2. Chadefaud M., Emberger L., *Traité de botanique systématique*. Masson éd., Paris, 1960, 524—526.
3. Engler A., *Syllabus der Pflanzenfamilien*, vol. II, ed. 12, Berlin, 1964, 41—42.
4. Guşuleac M., Anal. Rom.-Soviet., ser. Biologie, *1* (28), 1956, 71—91.
5. Guşuleac M., Natura, 1949, *2*, 59—77.
6. Jucovschi P. M., *Botanica*. Ed. de stat pentru literatură ştiinţifică, Bucureşti, 1953, 361—367.
7. Nast G. G., Lilloa, 1941, *6*, 163—205.
8. Navaşhin S. G., Izbr. Trudy, M. L., Izd-vo, A.N., S.S.S.B., Moscva—Leningrad, 1951, 271—286.
9. Schanderl H., Biologisches Zentralblatt, 1964, **83,** *I*, 71—103.
10. Ştefurcac Tr. I., *Evoluţia plantelor oglindită în opere recente de botanică filogenetică*. Ed. didactică şi pedagogică, Bucureşti, 1973, 140—141.

11. Takhtajan A.L., *Die Evolution der Angiospermen*. Jena, 1959, 193—194.
12. Wettstein R., *Handbuch der Systematischen Botanik*, Ed. IV, Leipzig—Wien, 1935, vol. II, 623—625.
13. Woodworth R. H., Bot. Gaz., 1930, **90**, 108—115.
14. Zimmermann W., *Die Phylogenie der Pflanzen*, Stuttgart, 1959, 603—614.

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MODIFICATIONS DES VALEURS OSMOTIQUES DANS LES CONDITIONS DE L'ABSENCE DE L'EAU DANS LE SOL CHEZ LE MAÏS (*ZEA MAYS* L.)

PAR

GEORGETA FABIAN et E. GOMOIU

The behaviour and changes of some physiological processes in plants exposed to drought (water stress) under controlled conditions (phytotron) were studied. The experiments were carried out in order to find some criteria for the selection of plants resistant at drought.
The results point out that osmotic pressure, suction force and cellular sap concentration offer suitable criteria for the estimation of the resistance to drought of genotypes.

Ces dernières décennies le problème de déterminer le nécessaire d'eau des plantes, ainsi que de trouver certains critères de sélection des plantes résistantes à la période de sécheresse a constamment préoccupé les chercheurs. En ce sens les investigations ont été orientées tant vers l'étude du comportement des plantes que vers l'étude des modifications de certains processus physiologiques ayant lieu chez les plantes soumises à des conditions spécifiques à la période de sécheresse.

L'ouvrage a le but d'étudier les modifications de la pression osmotique, de la force de succion et de la concentration du suc cellulaire, en tant que processus qui changent rapidement en fonction de l'intensité et de la durée de la période de sécheresse.

MATÉRIEL ET MÉTHODE

Les études ont porté sur les hybrides HS-218 et HS-275 créés à l'Institut de Recherches pour les Céréales et les Plantes Techniques — Fundulea. La pression osmotique a été déterminée par la méthode de Hugo de Vries, la force de succion par Sardakov (5/11) et la concentration du suc cellulaire par réfractométrie. Les méthodes présentent l'avantage d'être effectuées sur un matériel vivant et à des intervalles de temps relativement courts. Les déterminations ont été effectuées au cours de la période de végétation chez les plantes de contrôle aussi bien que chez les plantes soumises à la sécheresse lors des principales phases, à savoir : croissance, apparition de la panicule, apparition des stigmates et emplissage du grain.

Les conditions d'expérimentation dans le phytotron ont été : l'humidité du sol 75 pour cent de la capacité de champ pour les plantes témoin et 40 pour cent pour les plantes soumises à la sécheresse. L'humidité de l'air a été de 40—45 % tant pour les plantes de contrôle que pour celles

d'expérience, la température de 25°C pendant le jour et de 20°C au cours de la nuit et la lumière 45—50000 lux.

En conditions de stress d'eau, les plantes peuvent ajuster leurs valeurs osmotiques. Telles modifications osmotiques commencent dès l'aube jusque pendant l'après-midi et se produisent dans la plante en fonction des oscillations de l'eau dans le sol. Cette osmorégulation tient de la photosynthèse, de l'accumulation, ainsi que de l'hydrolyse du sucre dans les feuilles.

La comparaison des deux hybrides mis à l'étude, HS-218 et HSF-275, révèle des valeurs de la pression osmotique presque égales chez les plantes témoins et à tendance d'accroissement des valeurs à mesure que les plantes avancent dans les phases de végétation. Toutes autres sont les valeurs osmotiques des plantes soumises à des conditions de sécheresse.

Ainsi, au cas de HS-275, sur le parcours de l'entière période de végétation, les valeurs sont beaucoup plus élevées en comparaison de celles de HS-218 (fig. 1), ce qui montre que HS-275 peut modifier plus aisément les concentrations du suc cellulaire comparativement à l'HS-218. Lors des phases critiques pour le maïs telles celles de l'apparition de la panicule et des stigmates, les valeurs de la pression osmotique enregistrées au cas de HS-218, à savoir : de 10,8 et 13,1 atm., sont moindres que celles de l'HS-275, de 12,6, respectivement 14 atm., ce que l'on peut constater en suivant l'amplitude des deux courbes, celle du témoin et de la plante soumise à la secheresse.

Dans le même sens vont aussi les valeurs de la concentration du suc cellulaire qui sont beaucoup plus élevées au HS-275, notamment 8,6 dans la phase d'apparition de la panicule, 10,0 % dans la phase d'apparition des stigmates et 9,0 % dans la phase d'emplissage du grain contre 8,2 % ; 9 %, respectivement 8,5 % obtenues chez le HS-218 (fig. 2).

Les valeurs de la concentration du suc cellulaire chez les plantes témoins sont moindres chez HS-275 que chez HS-218.

Dans la figure 2 on peut observer qu'au cas de HS-218 dans la phase d'emplissage du grain la concentration du suc cellulaire est de beaucoup inférieure à celle du témoin, ce qui montre que l'osmorégulation chez cet hybride peut être assurée jusqu'à un certain point, celle-ci étant corrélée avec les produits de la photosynthèse. Au delà d'un tel seuil de la période de sécheresse la synthèse d'assimilation est gênée, et le processus d'osmorégulation est plus difficile surtout chez les plantes moins résistantes à la période de sécheresse.

L'apparition de certains déficits d'eau dans la plante détermine des modifications promptes et aisément saisisables des valeurs de la force de succion. A l'intérieur de la plante l'eau circule à partir des zones à force de succion moindre vers une zone à force de succion plus élevée. Ainsi, l'eau est prise des organes plus vieux de la plante (feuilles de la base) et utilisée par les organes à activité plus intense. Plus les valeurs de la force de succion sont grandes d'autant plus le déficit d'eau dans la plante est plus accru. La figure 3 permet d'observer facilement une amplitude des valeurs de la force de succion chez HS-218 de beaucoup supérieure à celle de HS-275, tant dans les phases d'apparition de la panicule et des stigmates que dans celle d'emplissage du grain.

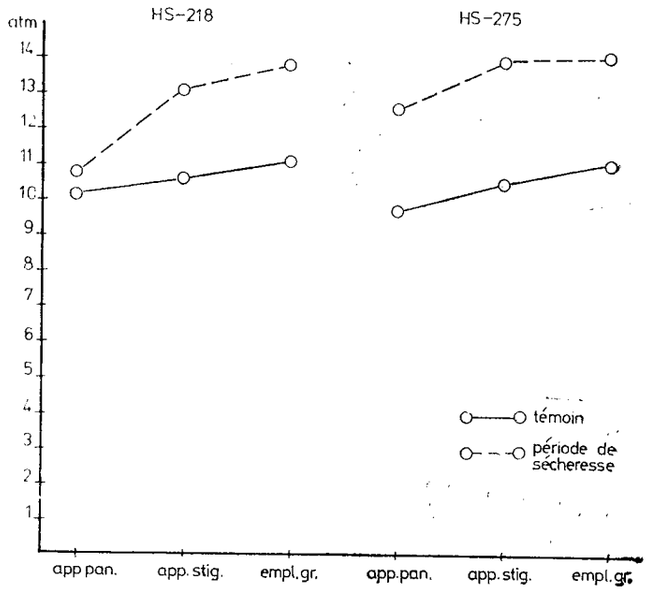

Fig. 1. — Valeurs de la pression osmotique (atm.) chez les hybrides de maïs : HS-218 et HS-275 dans les phases : apparition de la panicule, apparition des stigmates et emplissage du grain.

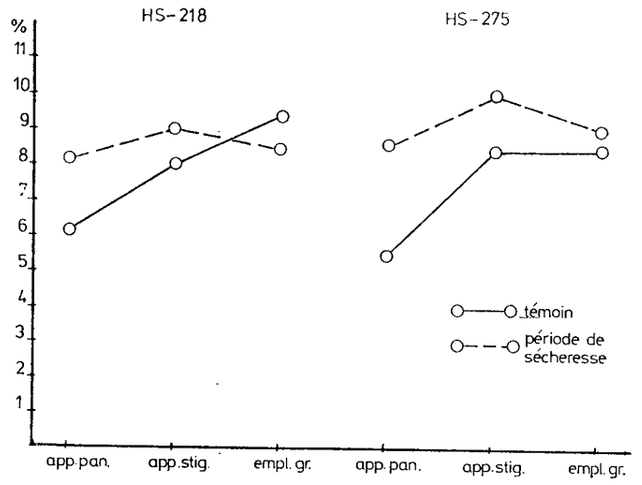

Fig. 2. — Concentration du suc cellulaire (%) chez les hybrides de maïs : HS-218 et HS-275 dans les phases : apparition de la panicule, apparition des stigmates et emplissage du grain.

Cela démontre une moindre résistance du HS-218 à la sécheresse par rapport au HS-275, les déficits en eau étant beaucoup plus grands.

Les valeurs, de 13 atm. de la force de succion montrent que la plante se trouve à proximite du point d'étiolement aussi aux feuilles des étages

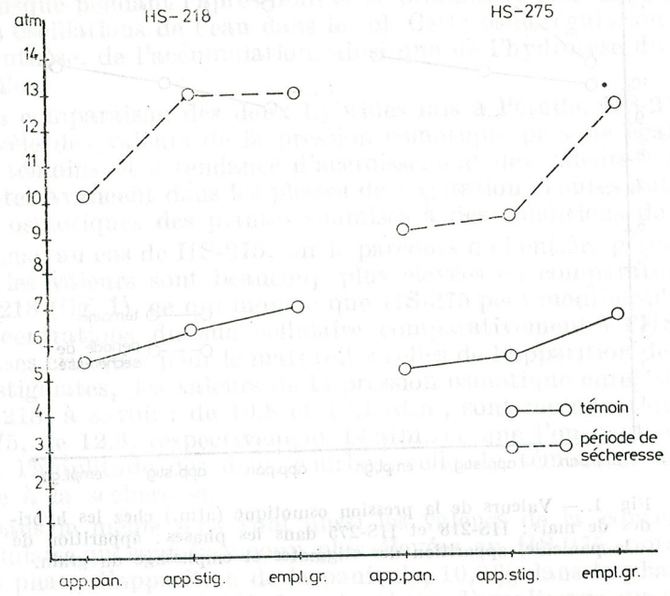

Fig. 3. — Valeurs de la force de succion (atm.) chez les hybrides de maïs HS-218 et HS-275 dans les phases : apparition de la panicule, apparition des stigmates et amplissage du grain.

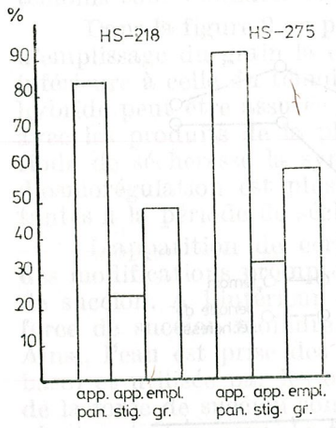

Fig. 4. — Production de grains exprimée en pour cent par rapport au témoin chez les hybrides de maïs HS-218 et HS-275 dans les phases : apparition de la panicule, apparition des stigmates et emplissage du grain.

supérieurs. D'ailleurs, cette valeur de la force de succion est très rapprochée de celle de la pression osmotique, révélant que HS-218 peut passer par une période de sécheresse ayant comme conséquences surtout la diminution de la production de grains.

La figure 4 donne les résultats concernant la production de grains des deux hybrides étudiés. Ainsi, le HS-275, hybride plus résistant à la période de sécheresse fournit dans la succession des phases les valeurs de la production de grains suivantes : (pour cent par rapport au temoin) : 92% dans la phase d'apparition de la panicule et 32% dans la phase d'apparition des stigmates en comparaison de 84%, respectivement 24% obtenues au cas de HS-218.

DISCUSSIONS

En vertu des données que nous avons obtenues on constate que les modifications de la pression osmotique sont déterminées par la synthèse et l'accumulation du sucre aussi longtemps que la photosynthèse n'est pas affectée par le manque d'eau.

La pression osmotique décroît à mesure que l'assimilation ou la transformation CO_2 diminue ou se trouve inhibée. Weatherly [14] et Boyer [3] ont montré que la modification de la pression osmotique, dans les conditions de manque d'eau, a lieu sur la base de la mobilisation et de la concentration des solutions dans les cellules de la feuille. Kramer [7] opine que toute valeur élevée de la pression osmotique confère un avantage en ce qui concerne les espèces soumises à la sécheresse. Stout et Simpson [13] se rallient à cette opinion en montrant que l'osmorégulation a un rôle important du fait qu'elle prépare la plante de supporter le manque d'eau. Brévedon et Hodges [4] ont constaté chez les hybrides moins résistants à la période de sécheresse une tendance de diminution de la concentration des hydrates de carbone dans les tissus assimilateurs, fait que nous avons également constaté au cas de HS-218.

Sălăgeanu [9], Turner [13] et Kramer [7] soutiennent que la force de succion est l'un des indices les plus sûrs qui puissent indiquer l'état de la quantité d'eau dans les plantes. L'allure des courbes des valeurs de la force de succion obtenues chez les hybrides étudiés a montré que la force de succion s'accroît à mesure que l'eau du sol décroît et surtout à mesure que les plantes perdent de l'eau. La période de sécheresse influence beaucoup tant la croissance que le développement des plantes, fait montré, entre autres, par Robins et Domingo [8], Denmed et Shaw [6], Acevedo, Hsiao et Henderson [1].

La période de sécheresse a une influence directe sur la récolte, dont la diminution est fonction des phases de végétation dont les plus sensibles sont : les phases d'apparition des stigmates et d'emplissage du grain, quand la production de grains a été plus réduite que dans la phase d'apparition de la panicule.

En utilisant les indices physiologiques tels que : pression osmotique, concentration du suc cellulaire et force de succion on obtient des indications qui concordent avec les observations et les déterminations effectuées en plein champ.

En conclusion, on peut affirmer que la pression osmotique, la force de succion et la concentration du suc cellulaire utilisées jusqu'à présent surtout pour la détermination du nécessaire d'eau des plantes et pour l'établissement des dates d'arrosage peuvent servir avec succès à la caractérisation des génotypes en vue de la tolérance à la période de sécheresse quand les recherches sont effectuées en conditions contrôlées (phytotron).

BIBLIOGRAPHIE

1. Acevedo E., Hsiao T. C., Henderson D. W., Plant Physiol., 1971, **48,** *5,* 631–636.
2. Akerson C. R., Krieg D. R., Sung R. M., Crop Sci., 1980, **20,** *1,* 10–14.
3. Boyer S. J., Plant Phyisol., 1970, **46,** *2,* 233–235.
4. Brevedan E. R., Hodges H. E., Plant Physiol., 1973, **52,** *5,* 436–439.

5. Cupcea Emilia, Iliescu Emilia, Boldor O., Petrea V., Soare Florica, *Lucrări practice de fiziologia plantelor*, Edit. didactică și pedagogică, București, 1965, p. 43–48.
6. Denmead T. O., Shaw W., Agron. Journal, 1960, **34**, *5*, 218–224.
7. Kramer J. P., Sionit N., Agron. Journal, 1977, **69**, *2*, 274–278.
8. Robins S. J., Domingo C. E., Agron. Journal 1953, **45**, *12*, 618–622.
9. Sălăgeanu N., Galan Georgeta, Bul. St. Soc. St. biol., agron., geol., geogr., 1956, *6*, 6–32.
10. Shmueli E., Proc. 8th. Intern. Congress of Soil Sci., Bucharest, 1964, 6, 423–431.
11. Slavik B., *Methods of studying plant water relations*, Springer Verlag, Berlin, Heidelberg, New York, Acad. St. Ceh. Praga, 1964.
12. Stout G. D., Simpson G. M., Can. J. of Plant Sci., 1978, **51**, *1*, 213–224.
13. Turner C. N., Plant Physiol., 1974, **52**, *3*, 360–365.
14. Weatherly P. E., New Phytol., 1950, **49**, *31*, 87.

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Institut de Recherches pour les Céréales et les Plantes Techniques Fundulea

L'INFLUENCE DE LA KINÉTINE SUR LA CROISSANCE DE L'ALGUE *SPIRULINA PLATENSIS* (GOM) GEITL

PAR

IOANA SPIRESCU, LUCIA POLESCU et L. ATANASIU

The influence of kinetin (conc. 10^{-4}–10^{-7}M) on the culture growth of pluricellular alga *Spirulina platensis* was studied. An obvious stimulation of the algae culture growth, of the photosynthesis (oxygen evolved), of chlorophyl a and b contents and of the value of dry biomass was ascertained.
The most significant effects were observed by using concentrations of 10^{-6}M and 5.10^{-6}M kinetin.

Les effets de la kinétine sur certains aspects de la croissance dans la culture de l'algue *Chlorella* ont été exposés dans un autre ouvrage [1]. Des modifications évidentes ont été enregistrées dans l'évolution du facteur de multiplication des cellules avec le temps et en ce qui concerne l'intensité de la photosynthèse, le contenu en chlorophylle et la biomasse entière.

Cette étude présente des données sur l'influence de la kinétine, en différentes concentrations, sur l'algue pluricellulaire *Spirulina*.

MATÉRIEL ET MÉTHODES

L'algue *Spirulina platensis* (Gom) Geitl. a été cultivée au laboratoire dans un milieu Zarrouk, en système clos, le barbotage étant assuré par un courant d'air, en lumière fluorescente de 8000 lux et à 30°C.

La kinétine a été introduite dans le milieu de culture avant l'ensemencement et en concentrations molaires comprises entre 10^{-4} et 10^{-7} M.

La croissance des algues en culture a été déterminée journellement, pendant l'entier cycle de culture (5 jours) par photoélectrocolorimétrie. L'intensité de la photosynthèse a été déterminée par la méthode Warburg, en utilisant la suspension d'algues traitée et la solution tampon n° 9.

La teneur en chlorophylle a été établie après l'extraction à l'acétone 85%, suivie d'une lecture au spectrophotomètre. L'extinction de la chlorophylle a été mesurée en cuves de 2 cm, à 663 et 645 nm, les chlorophylles a et b étant ensuite calculées selon la formule de Mackinney [6].

La biomasse entière a été récoltée à la fin du cycle de culture, après 5 jours, au moment où la culture avait touché sa densité optimale.

RÉSULTATS ET DISCUSSIONS

Les données obtenues sur l'évolution de la croissance des algues, *Spirulina* en culture sous l'influence de diverses concentrations de la kiné-

tine sont présentées dans la figure 1. Dans toutes les variantes étudiées, il ressort que la présence de la kinétine dans le milieu de culture stimule le développement cellulaire par rapport au témoin. On observe, même

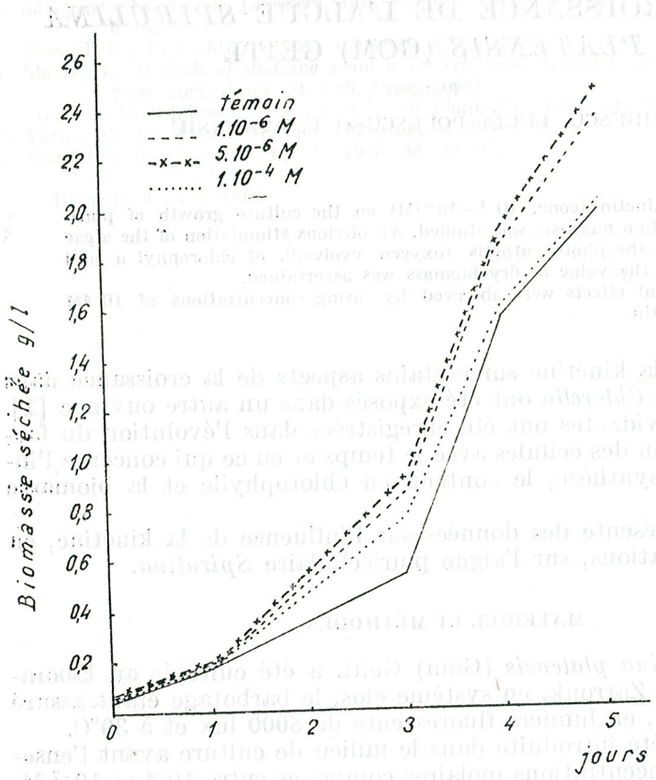

Fig. 1. — Dynamique de l'accumulation de biomasse chez *Spirulina platensis*, cultivée en présence de la kinétine.

le premier jour après l'exposition, que les épreuves traitées se détachent nettement du témoin, la plus forte stimulation étant enregistrée dans la suspension à kinétine en concentration de 510^{-6} M.

L'intensité de la photosynthèse, déterminée selon le dégagement d'oxygène, présente la valeur la plus élevée à la concentration de 5.10^{-6}M, comme l'on peut observer dans la figure 2.

La libération de l'oxygène à des concentrations plus grandes, 10^{-5}M, a montré une diminution par rapport aux concentrations de 10^{-7} et 5.10^{-6} M.

Le contenu en chlorophylle indique une augumentation chez les algues traitées à la kinétine, la concentration optimale étant enregistrée toujours à 5.10^{-6} M, mais les concentrations dépassant 10^{-5} et 10^{-7} M produisent une diminution des chlorophylles a et b, même par rapport à l'épreuve témoin.

La biomasse séchée totale déterminée à la fin du cycle de culture augmente par rapport au témoin à partir des concentrations de 10^{-7},

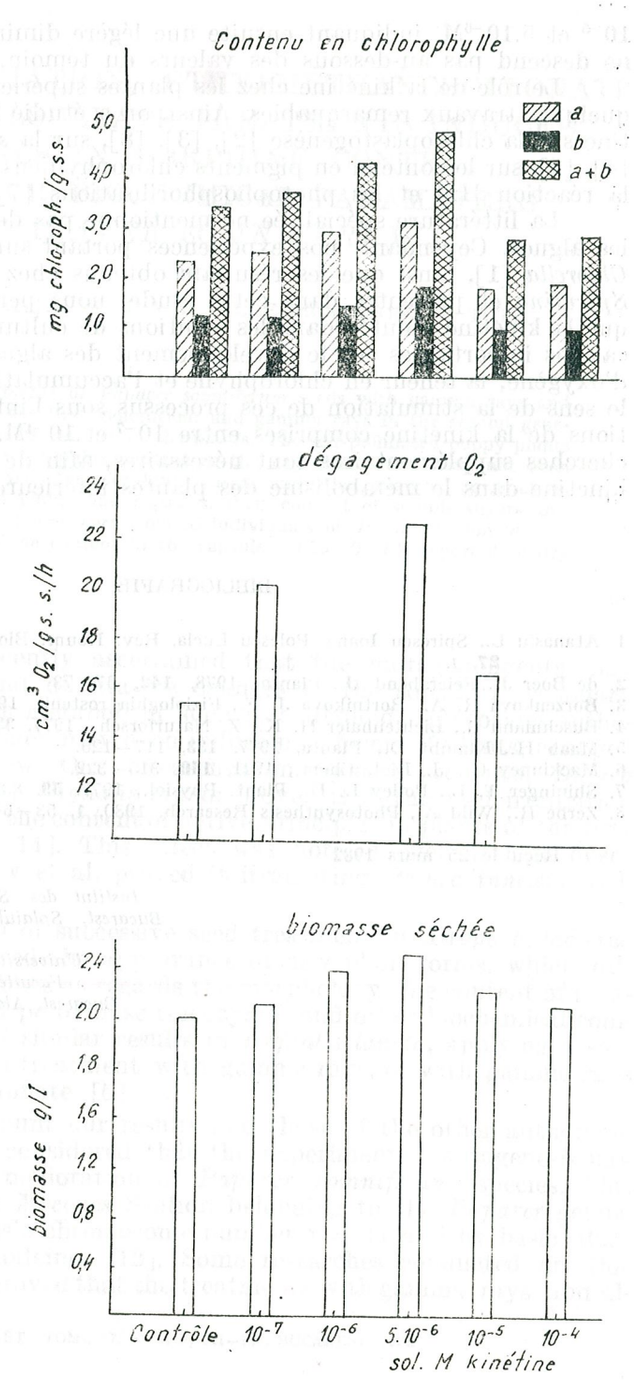

Fig. 2. — Influence de la kinétine sur la teneur en chlorophylle des cellules, le dégagement d'oxygène et la biomasse séchée accumulée.

10^{-6} et 5.10^{-6}M, indiquant ensuite une légère diminution, qui cependant ne descend pas au-dessous des valeurs du témoin.

Le rôle de la kinétine chez les plantes supérieures a été établi dans quelques travaux remarquables. Ainsi, on a étudié l'influence de la kinétine sur la chloroplastogenèse [2], [3], [8], sur la synthèse des protéines [4], [5], sur le contenu en pigments chlorophylliens [4], [8] ainsi que sur la réaction Hill et les photophosphorilisations [7] et [8].

La littérature spécialisée ne mentionne pas des données concernant les algues. Cependant, nos expériences portant sur l'algue unicellulaire *Chlorella* [1], ainsi que les résultats obtenus chez l'algue pluricellulaire *Spirulina* et présentés dans cette étude, nous permettent de constater que la kinétine ajoutée dans les solutions de culture produit des modifications importantes sur le développement des algues, sur le dégagement d'oxygène, la teneur en chlorophylle et l'accumulation de biomasse, dans le sens de la stimulation de ces processus sous l'influence des concentrations de la kinétine comprises entre 10^{-7} et 10^{-4}M. Evidemment, des recherches supplémentaires sont nécessaires, afin de préciser le rôle de la kinétine dans le métabolisme des plantes inférieures.

BIBLIOGRAFIE

1. Atanasiu L., Spirescu Ioana, Polescu Lucia, Rev. Roum. Biol., Série Biol. végét., 1982, **27**, *1*.
2. de Boer J., Feierabend J., Planta, 1978, **142**, 67–73.
3. Borzenkova R. A., Bortnikova J. F., Fiziologhia rostenii, 1977, **25**, 254–261.
4. Buschmann C., Lichtenhaler H. K., Z. Naturforsch., 1977, **32**, 798–802.
5. Maab H., Klambt D., Planta, 1977, **133**, 117–120.
6. Mackinney G., J. Biol. Chem., 1941, **140**, 315–322.
7. Shininger T. L., Polley L. D., Plant. Physiol., 1977, **59**, 831–835.
8. Zerbe R., Wild A., Photosynthesis Research, 1980, **1**, 53–64.

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GAMMA IRRADIATION, ETHYLMETHANESULFONATE AND DIETHYLSULFATE TREATMENTS INDUCED CHANGES IN MORPHINE CONTENT AND OTHER BIOCHEMICAL PARAMETERS IN *PAPAVER SOMNIFERUM* L.

BY

G. I. GHIORGHIȚĂ, ELVIRA V. GILLE, ECATERINA T. TÓTH

The successive treatments of *Papaver somniferum* seeds with gamma rays and alkylating agents in the first generation, and gamma rays in the second generation induced a high variability of the plants. The self-pollinated poppy plants had a clearly higher morphine content in the capsules. There have not been evident relations between the morphine content and other analysed parameters, such as: the sizes of plants and capsules, their content of soluble sugars and free amine nitrogen. There were isolated individuals of *P. somniferum* with a relatively high morphine content in the capsules (0.53–0.54% reported to dry weight).

It has been recently ascertained that the mutagen agents may cause quantitative and qualitative changes of the so-called secondary products of the plant metabolism such as: alkaloids, glycosides,anthocyanes etc. and that they may even induce new forms of plants, changed from this point of view. Our researches on *Cynara scolymus*, *Datura innoxia*, *Vinca minor*, *Digitalis lanata* proved that the seed irradiation resulted in a raising of the content of active principles in plants of the first generation [3], [4], [14]. This effect was noticed even at high doses of irradiation. Tihonov et al. proved it irradiating *Datura innoxia* with small doses [13].

Two generations of successive seed treatment in *Atropa belladonna* and *Vinca minor* caused the appearance of new plant forms, which differed from the initial ones as regards the morphology, the content of alkaloids, the esterase and peroxidase isoenzymes and other biochemical components. We obtained similar results in *Digitalis lanata*, applying a successive two generation treatment with gamma rays, or with gamma rays and ethylmethanesulfonate [5].

Taking into account our results and those of the other authors on medicinal plants, we considered that the experimental mutagenesismay be promising in the melioration of *Papaver somniferum* species. This species is part of the *Mecones* Section belonging to the *Papaver* genus, characterized by a basic chromosome number x = 11 and by basic alkaloids-morphine and codeine, [12]. Some researches conducted on this species have already proved that the treatments with gamma rays and al-

kylating agents determined a change in the content of morphine in the capsules and even the obtaining of mutants valuable from the economic point of view [7]—[11],

MATERIAL AND METHODS

On April 1st, 1980 we treated separately and combined, with gamma rays and alkylating agents — ethylmethanesulfonate (EMS) and diethylsulfate (DES) — *Papaver somniferum* seeds, the variety Extaz. The seeds were received from the Măgurele-Brașov Research Station. We used 10 and 15 kR doses and the concentration of 0.1% for the chemical mutagens. The treatments with EMS and DES lasted for 10 hours. After treatment, the seeds were thoroughly washed and sown on the experimental field of the "Stejarul" Research Station Pîngărați-Neamț. When the plants reached maturity, we determined the total alkaloid content and the morphine content of the capsules (medium test per variant) following a micro-method proposed by Gyéresi and Rácz [6]. The results are presented in Table 1.

Table 1

The influence of the treatment with gamma rays and alkylant mutagens on the total alkaloids and morphine content in *Papaver somniferum* L. (first generation)

No.	The treatment	Total alkaloids % d.m.	Morphine % d.m.
1	Control	0.98	—
2	0.1% EMS	0.80	0.19
3	10 kR	0.88	0.09
4	15 kR	1.06	0.15
5	10 kR + 0.1% EMS	1.18	0.31
6	15 kR + 0.1% EMS	1.02	0.25
7	10 kR + 0.1% DES	1.23	0.18
8	15 kR + 0.1% DES	1.06	0.32

Seeds harvested in 1980 from the plants of each experimental variant were treated with 10 kR gamma rays in the second generation (1981) and then sown in the experimental field. Before the flowering, the capsules of 15—20 plants from each experimental variant were isolated with paper bags to prevent free pollination. We determined the height of the plants at the harvesting moment and the dimensions of the capsules (height and diameter) in order to correlate them with the morphine content. Then we made biochemical analyses of the capsules, consisting of: the morphine content, soluble sugars and free amine nitrogen on average samples from nonisolated (with paper bags) capsules, on average and individual samples from isolated capsules. The morphine content was analysed by the already mentioned method, and the content of the sugars and amine nitrogen by the methods presented in other papers [2]. The results of these investigations are presented in Tables 2 and 3.

RESULTS

In the pedoclimatic conditions of Pingărați, described in a previous article [14], the values of the total alkaloid contents of poppy in the first generation are rather high (Table 1). It may be possible that the Ca^{++} ion, well represented in this soil, favours such a manifestation. When making this statement we consider the results obtained by Costes et al. (1976) showing that the deficit in calcium determines a lowering of the alkaloids content in poppy plants [1]. The separate treatments with mutagen agents has a negative effect on the alkaloid synthesis in the poppy plants. The synthesis of these biochemical compounds was slightly stimulated in the case of combined treatments. The highest value of the total alkaloid content (1.23% d.m.) was recorded by capsules harvested from plants treated with 10 kR and 0.1% DES (see variant 7, Table 1). As table 1 shows, there is no direct correlation between the total alkaloid content and that of morphine in the poppy capsules. Thus, for example, the plants of variant 7 that registered a high level of total alkaloids, present a relatively low morphine content in the capsules (0.18% d.m.), while the plants of variant 8, with a reduced content of total alkaloids (1.06% d.m.), show the highest morphine content (0.32%), (Table 1).

We have to remark also the very low value of the morphine content in the capsules treated wtih 10 kR (0.09% d.m.) as well as the generally higher values of this parameter in the capsules of the plants which received a combined treatment with gamma rays and alkylating agents.

Examining the data of Table 2 we may notice that the successive treatments with physical and chemical mutagen agents induced an inhibition of the growth processes of the poppy plants in the second generation. Their dimensions, as well as those of the capsules are inferior to those of the control plants. In the plants with free pollination, the highest values of morphine was registered by the individuals of the variant treated with 0.1% EMS in the first generation and with 10 kR in the second one —0.44% (variant 2, Table 2). Rather high values of morphine, superior to those of the control were registered by the plants of the variants 6 and 8. They were treated both with gamma rays and alkylating agents in the first generation and with gamma rays in the second.

Our investigations do not show any correlation among the analysed parameters. Even if the height/diameter ratio of the capsule has the highest value (1.95, variant 2), in the case of those with a raised morphine content a value close to this one (1.68) in variant 4 presents a morphine content three times smaller (0.15% d.m.). Although the applied treatments induced changes in the content of soluble sugars and in that of free amine nitrogen, these changes are not in correlation with the morphine content.

Poppy plants are characterized by low values of the soluble sugars in mature capsules. We must still mention the higher values of the content of soluble sugars (total and reducing) and that of free amine nitrogen in the capsules of variant 8 (treated with 15 kR + 0.1% DES in the first generation and with 10 kR in the second).

The values of the investigated biochemical parameters are generally greater in isolated poppy capsules (self-pollination), compared to those free pollinated (Table 2). Referring only to morphine, we may deduce that

Table 2

The values of some morphological and biochemical parameters in *Papaver somniferum* plants treated with gamma rays and alkylant mutagens (second generation, free-pollination)

No.	The treatment: First generation	The treatment: Second generation	Length of plants (cm) $\bar{x} \pm s\bar{x}$	Dimensions of capsules: Height (mm) $\bar{x} \pm s\bar{x}$	Dimensions of capsules: Diameter (mm) $\bar{x} \pm s\bar{x}$	Dimensions of capsules: H/D	Red. sugars % d.m.	Total sugars % d.m.	Free amine nitrogen % d.m.	Morphine % d.m.
1	Control		95.33 2.28	46.80 2.37	29.76 0.56	1.59	1.91	4.14	1.27	0.25
2	0.1 % EMS	10 kR	78.21 0.96	39.92 2.23	20.44 0.15	1.95	1.85	4.95	1.01	0.44
3	10 kR	10 kR	76.55 2.55	39.15 2.89	20.45 5.05	1.91	1.63	4.76	1.09	0.25
4	15 kR	10 kR	84.45 0.29	41.30 0.89	22.11 3.28	1.86	2.05	3.72	1.16	0.15
5	10 kR + 0.1% EMS	10 kR	87.27 1.07	40.44 0.54	21.17 0.33	1.91	2.08	4.59	0.98	0.18
6	15 kR + 0.1% EMS	10 kR	82.81 4.71	40.17 0.31	21.19 1.22	1.89	1.66	4.14	0.89	0.30
7	10 kR + 0.1% DES	10 kR	95.38 3.27	43.77 2.09	23.11 0.88	1.89	1.92	5.76	1.32	0.36
8	15 kR + 0.1% DES	10 kR	92.72 0.74	44.55 0.68	23.29 1.57	1.91	2.61	6.03	1.54	0.29

Table 3

The values of some biochemical parameters in self-pollinated *Papaver somniferum* plants resulted from the treatment with gamma rays and alkylant mutagens

No.	The treatment: First generation	The treatment: Second generation	Medium self-pollinated individuals: Red. sugars % d.m.	Medium self-pollinated individuals: Total sugars % d.m.	Medium self-pollinated individuals: Free amine nitrogen % d.m.	Medium self-pollinated individuals: Morphine % d.m.	Individual analyses: Weight of analysed capsule (g)	Individual analyses: Red. sugars % d.m.	Individual analyses: Total sugars % d.m.	Individual analyses: Free amine nitrogen % d.m.	Individual analyses: Morphine % d.m.
1	Control		1.08	6.19	2.14	0.54	2.16	1.09	5.46	1.36	0.15
2	0.1% EMS	10 kR	1.02	6.27	2.42	0.37	2.08	1.17	4.46	1.12	0.43
3	10 kR	10 kR	1.16	6.99	2.10	0.29	1.60	1.07	3.86	1.19	0.38
4	15 kR	10 kR	1.35	4.96	1.49	0.47	1.21	1.28	3.65	1.14	0.16
5	10 kR + 0.1% EMS	10 kR	1.16	5.79	1.86	0.41	1.12	—	—	—	0.15
6	15 kR + 0.1% EMS	10 kR	—	—	—	—	1.00	—	—	—	0.50
7	10 kR + 0.1% DES	10 kR	1.67	7.03	2.11	0.52	1.00	—	—	—	0.32
8	15 kR + 0.1% DES	10 kR	1.82	7.28	1.67	0.39	1.15	—	—	—	0.53

its level does not lower in any variant under 0.30%, while this value represents a maximum in the case of free-pollinated plants. The analyses reveal also the fact that the applied mutagen treatments had a negative effect on the capacity of plants to synthesize morphine, the isolated capsules of the treated plants showing lower values of the morphine content as compared to the controls. For some experimental variants (var. 4, 5, 7 — Table 3) the morphine content is good. Neither in the case of isolated capsules did we succeed to render evident any correlation between the registered level of morphine and the other investigated parameters.

But, as we have already stated, we selected from the self-pollinated capsules of each variant the biggest one for individual analysis. With three of the 8 variants we succeeded to identify capsules with a high content of morphine, ranging between 0.43 and 0.53% (variants 2,6 and 8). These individual analyses rendered evident once more the inexistence of any correlation between the weight of the capsule and the level reached by morphine content (see variants 1, 6, 8). The individual analyses provided us a valuable material for the melioration of *Papaver somniferum* from the point of view of the morphine content.

CONCLUSIONS

— The single treatments with gamma rays, EMS and DES of the seeds of *Papaver somniferum*, the variety Extaz, had a negative effect on the synthesis of alkaloids in the capsules of the first generation plants. The combined treatments with the two categories of agents stimulated the alkaloid synthesis in plants. There has not been evinced any direct correlation between the total alkaloids in the capsules and their morphine content.

— Irradiation in the second generation of the poppy seeds, that were treated with gamma rays and alkylating agents in the first generation, induced a pronounced variability of plants as to their size at harvesting, the sizes of the capsules and their content of soluble sugars, free amine nitrogen and morphine.

— The poppy plants of second generation forced to self-pollination registered clearly higher values of morphine in the capsules as compared to the free pollinated plants.

— There has not been rendered evident any relation between the morphine content value and the values of other analysed parameters, such as : sizes of plants and capsules, their content of soluble sugars and free amine nitrogen.

— We identified and isolated individuals of *Papaver somniferum* with a relative high morphine content in the capsules (0.53—0.54 %) d.m.) which will be tested as to the stability of this characteristic in the future generations and under different growing conditions.

REFERENCES

1. Costes C., Milhet Y., Caudillon C., Magnier G., Physiol. Plant., 1976, **36**, *2*, 201—207.
2. Ghiorghiță I. G., *Repercusiuni de natură fiziologică ale unor procese genetice declanșate de tratamente cu radiații ionizante și în cîmpuri magnetice la plante de ploidii diferite*. Doctorat thesis, Univ. "Al. I. Cuza"-Iași, 1975, 191 p.

3. Ghiorghiţă I. G., Tóth T. Ecaterina, Gille V. Elvira, Lucr. Staţ. "Stejarul", Ecol. terestră şi genet., 1980, **8**, 157—164.
4. —„— Rév. Roum. Biol.—Biol. Vég., 1981, **26**, *2*, 157—164.
5. Gille V. Elvira, Ghiorghiţă I. G., Rév. Roum. Biol.—Biol. Vég., 1982, 27, 1.
6. Gyéresi Á., Rácz G., Lucr. Staţ. "Stejarul", Ecol. terestră şi genet., 1980, **8**, 299—306.
7. Ilieva S., Mehandjiev A., Matveeva D., Dimitrova S., Herba Pol., 1975, **21**, *4*, 412—419.
8. Ivanova R. M., Ghenetika, 1972, **8**, *1*, 30—37.
9. —„— S-h. Biol., 1975, **10**, *6*, 828—831.
10. Malik S., Akram M., Bibi R., Malik N. A., Pak. J. Sci. Ind. Res., 1977, **20**, *4—5*, 285—287.
11. Popov P., Dimitrov J., Gheorghiev S., Deneva T., Genet. Sel. (Bulg.), 1974, **7**, *4*, 251—257.
12. Preininger V., Novák J., Santavy F., Planta medica, 1981, **41**, 119—123.
13. Tihonov Iu. B., Panarin V. P., Alimova G. V., Mihailova N. B., Morozova S. B., Rastit. resursy, Leningrad, 1979, **16**, 237—242.
14. Tóth T. Ecaterina, Gille V. Elvira, Ghiorghiţă I. G., Lucr. Staţ. "Stejarul", Ecol. terestră şi genet., 1980, **8**, 165—170.
15. Tóth, T. Ecaterina, Ghiorghiţă I. G., Lucr. Staţ. "Stejarul", Ecol. terestră şi genetică, 1980, **8**, 347—352.

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THE EFFECT OF MIXED TREATMENT WITH GLUCOSE, NaF AND DNP ON ROTATIONAL STREAMING

BY

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Glucose (5.10^{-3} M) was supplied to barley *(Hordeum vulgare* L.) root hairs and an increase of the rate of rotational streaming was obtained. With NaF (1.10^{-2} M) and DNP (1.10^{-5} M) two different experiments were performed. Firstly only glucose was added for 30 minutes and then a mixture of glucose and an inhibitory substance was applied. Secondly, the inhibitor only was added for 30 minutes and after that the mixture was applied. The rate of the rotational streaming enhanced by glucose proved to be very sensitive to NaF treatment. The treatment with DNP in contrast caused only little inhibition of the glucose action on rotational streaming. Several patterns of the streaming oscillations after these treatments are also discussed. The authors suggest that the enhancement of rotational streaming after treatment of barely root hairs with glucose may be related to ATP which is produced by glycolysis.

Many experiments [2], [16] prove that the energetical source of the protoplasmic movement is the ATP formed in metabolic processes of the cell. Four main sources of ATP necessary to maintain the intracellular movements are taken into account : the glycolysis [2], the oxidative phosphorylation [12], the photophosphorylation [10], [18], and the ATP supplied in the external medium of the cell [3], [11], [13].

In previous papers the stimulating effect of some monosaccharides on the rotational streaming [6] was tested. The stimulatory effect may be considered as a result of the supplementary synthesis of the ATP in the glycolytic process. The decrease of the rotational streaming speed through hexoses absorption blocking [14] strengthens the above mentioned idea. The use of some inhibitors for oxidative phosphorylation [3], myosin ATP-ase [13] or of ethionine with its "ATP trap-like" action [15], suggests that the protoplasmic streaming depends on ATP synthesis and its further hydrolysis.

In this paper we try to show new aspects about the energetics of the cytoplasmic streaming using in two experimental variants two inhibitors : NaF — an inhibitor of glycolysis and 2,4-dinitrophenol (DNP) — an uncoupler of oxidative phosphorylation.

MATERIAL AND METHOD

The plant materials used in our investigation were the barley *(Hordeum vulgare* L.) root hairs, 750—1000 μm in length. The rate of rotational streaming with the aid of stop-watch was recorded about

three hours, both in control and treated cells. For each treatment five repetitions were made. The obtained data were statistically tested to establish several patterns of streaming oscillations [5].

The treatment consists of a continuous percolation of the root hairs under cover-glass with the following solutions: glucose $5 \cdot 10^{-3}$ M, NaF $1 \cdot 10^{-2}$ M and DNP $1 \cdot 10^{-5}$ M. Besides the concentration for each inhibitor, we performed two kinds of experiments: a) by adding glucose for 30 minutes and then a mixture of glucose and the inhibitor and b) by adding the inhibitor for 30 minutes and after that a mixture of inhibitor and glucose, in equal shares.

RESULTS AND DISCUSSIONS

The continuous treatment with glucose $5 \cdot 10^{-3}$ M by percolation under cover-glass, every 15 minutes, caused the increase of the rate of the rotational streaming in barley root hairs. The ideal curve representing the increase of the streaming rate after glucose supplying (Fig. 1) is similar to an asymptotic function ($y = A - D.10^{-k \cdot x}$) [5]. It suggests that an enzymatic reaction is probably the cause of the increase of the streaming rate. At the end of the experimental period the steady state of the streaming was reached and the speed showed about a constant value.

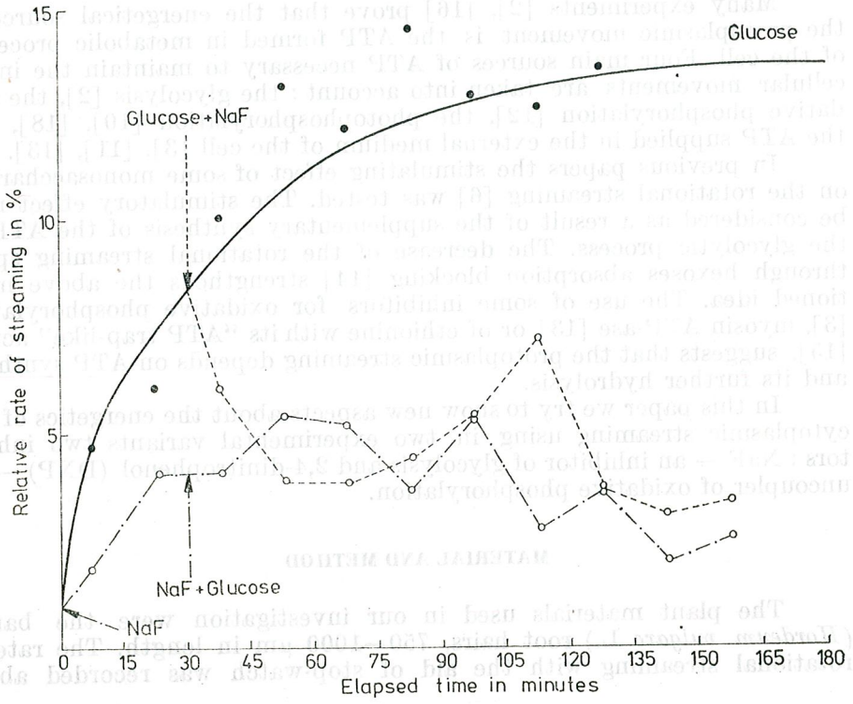

Fig. 1. — The effect of glucose and of mixed treatment with glucose and NaF on the rate of rotational streaming within barley *(Hordeum vulgare* L.) root hairs.

In Fig. 1 is shown the evolution of the rotational streaming speed after supplying NaF in the two experimental variants. The treatment with a mixture of glucose and NaF in the two variants leads to no continuous and constant inhibition of the speed of the rotational streaming, but to a significant decrease of the speed to a certain level (Fig. 1), with some oscillations, which may be explained by the continuous glucose inflow (i.e. the percolation under coverglass every 15 minutes). This result suggests that the NaF "in vivo" might be not a drastical inhibitor of glycolysis as compared to the "in vitro" processes.

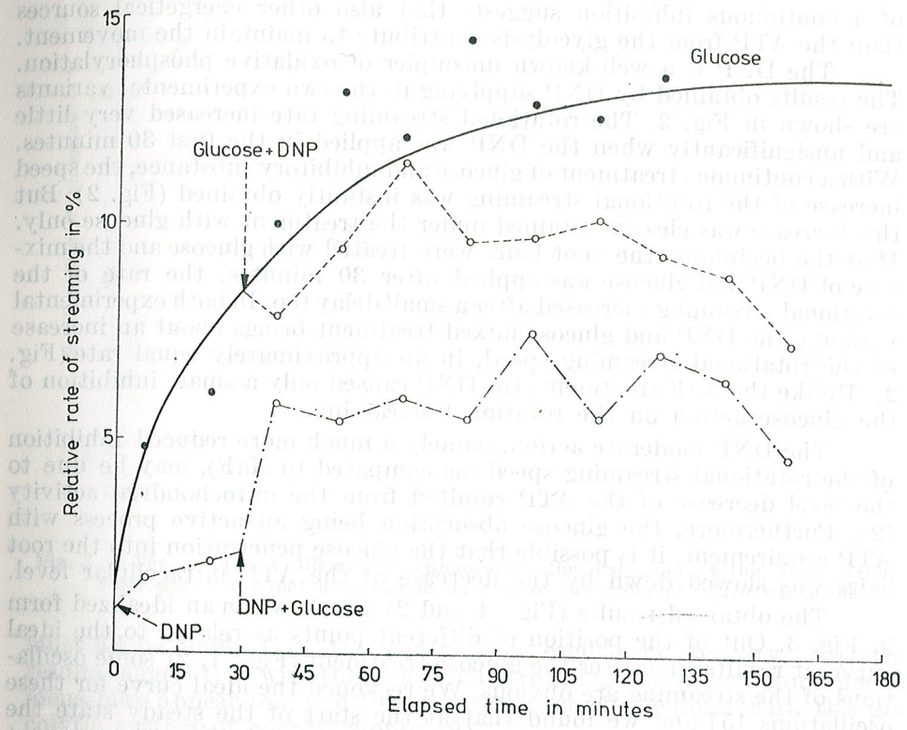

Fig. 2. — The effect of glucose and of mixed treatment with glucose and DNP on the rate of rotational streaming within barley *(Hordeum vulgare* L.) root hairs.

If the NaF was applied in the first 30 minutes, a slight increase of the rotational streaming speed was noticed. It was probably caused by the intensification of the respiratory processes [8]. After that the streaming remained oscillatory at a certain level, when the treatment was continued with the inhibitory substance and glucose mixture (Fig. 1).

It is well known that sugars lead to the increase of the protoplasmic speed flow of different plant specimens [6], [7], [14], probably by the increase of the ATP level originated into the glycolysis. The protecting action of glucose in maintaining and resuming the protoplasmic streaming after UV micro-irradiation [4] was explained in the same way.

The NaF effect on the protoplasmic streaming depends on concentration. With small concentrations it may stimulate the cytoplasmic flow by a favourable effect on respiratory processes [8]. The lowering of the protoplasmic streaming speed or even its stopping by using high concentrations of NaF is due to respiratory inhibition and to the structural and physicochemical modifications of the cytoplasm [8].

Our results (Fig. 1) show the significant lowering of the flow rate, to a certain level, as a consequence of the NaF and glucose simultaneous addition, strengthening the importance of the glycolysis as an energetical source for maintaining the protoplasmic streaming. Besides, the absence of a continuous inhibition suggests that also other energetical sources than the ATP from the glycolysis contribute to maintain the movement.

The DNP is a well-known uncoupler of oxidative phosphorylation. The results obtained by DNP supplying in the two experimental variants are shown in Fig. 2. The rotational streaming rate increased very little and unsignificantly when the DNP was applied in the first 30 minutes. With a continuous treatment of glucose and inhibitory substance, the speed increase of the rotational streaming was instantly obtained (Fig. 2). But this increase was clearly obtained under the treatment with glucose only. If at the beginning the root hairs were treated with glucose and the mixture of DNP and glucose was applied after 30 minutes, the rate of the rotational streaming increased after a small delay too. In both experimental variants, the DNP and glucose mixed treatment brings about an increase of the rotational streaming speed, in an approximately equal rate (Fig. 2). Unlike the NaF treatment, the DNP caused only a small inhibition of the glucose action on the rotational streaming.

The DNP moderate action, namely a much more reduced inhibition of the rotational streaming speed (as compared to NaF), may be due to the level decrease of the ATP resulted from the mitochondrial activity [2]. Furthermore, the glucose absorption being an active process with ATP requirement, it is possible that the glucose penetration into the root hairs was slowed down by the decrease of the ATP intracellular level.

The obtained results (Figs. 1 and 2) are shown in an idealized form in Fig. 3. Out of the position of different points as related to the ideal curve, it results that after the glucose treatment (Figs. 1, 2) some oscillations of the streaming are obvious. We reckoned the ideal curve for these oscillations [5] and we found that at the start of the steady state the glucose has induced at least two major oscillations (Fig. 3). They are not equal because of the continuous influx of glucose into the cell.

The mixed treatment with glucose and inhibitory substances has changed this oscillation pattern of the flow (Fig. 3). If in the first 30 minutes glucose and then glucose and NaF mixture were applied, the oscillation pattern of the flow almost disappeared. When the glucose and NaF mixture was applied after 30 minutes of NaF treatment, the pattern of two oscillations was obtained, but after a significant delay.

The DNP caused the delay of the maximum level of the first oscillation and reduced very strongly the second one. When the mixture of DNP and glucose was added 30 mintes after the DNP action, the first and the second oscillations had a 15—20 minutes delay (Fig. 3).

These oscillation phenomena are observed at different levels of biological organization [1], [9], [17]. With the slime mould *Physarum polycephalum* it is assumed that the cytoplasmic actomyosin, the energetical supply system, the internal deposit of Ca^{2+}, and the plasmalemma

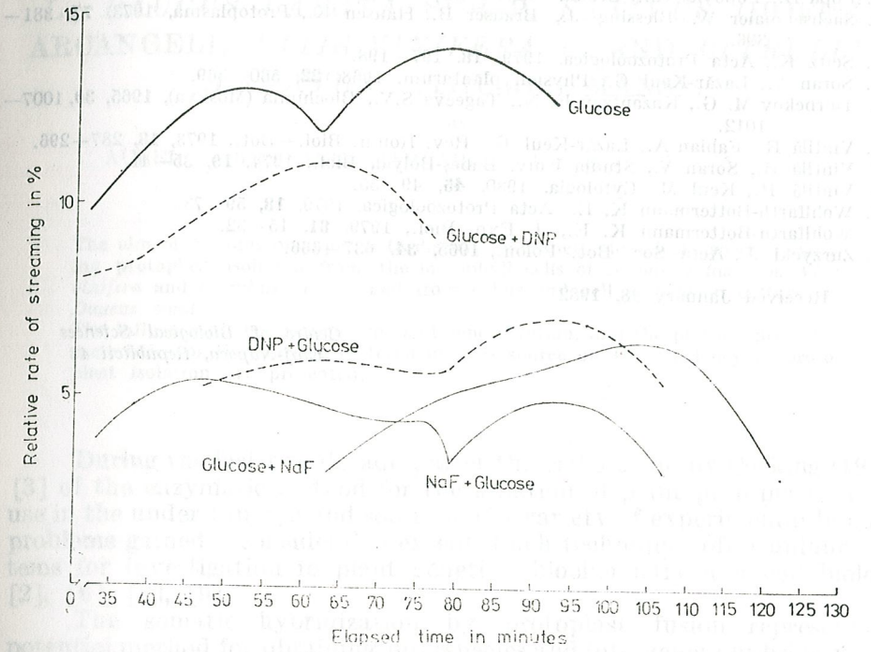

Fig. 3. — The patterns of different oscillations of rotational streaming within barley *(Hordeum vulgare* L.) root hairs caused by glucose and different mixed treatment.

must all be implied in the contraction oscillatory activity [17]. Our results suggest that with the rotational streaming these oscillatory phenomena may appear too, and that a correlation can be done with the glycolytic system of energy supply [9].

The experiments proved that the stimulation of the streaming speed induced by the external supplied glucose is very slightly delayed or slowed down by the DNP treatment. On the contrary, it is very sensitive at the NaF treatment. This underlines that the stimulation of rotational streaming by glucose is a consequence of glycolysis and of the ATP synthetized in this metabolic process.

REFERENCES

1. Geller J., Brenner M., Bioch. Biophys. Res. Comm., 1978, **81**, 814—821.
2. Kamiya N., *Protoplasmic Streaming.* In: *Protoplasmatologia* 8/3a (L. V. Heilbrun and F. Weber, eds.), Vienna, Springer-Verlag, 1959.

3. Lazăr-Keul G., Keul M., Soran V., Biochem. Physiol. Pflanzen (BPP), 1974, **166**, 255—262.
4. Lazăr-Keul, F., Keul M., Vintilă R., Soran V., Cytologia, 1975, **40**, 573—582.
5. Plokhinskii N. A., *Biometrya*, Izd. Mosk. Univ., Moskva, 1970.
6. Pop E., Vintilă R., Soran V., Rev. Roum. Biol.-Bot., 1968, **13**, 181—188.
7. Pop E., Soran V., Vintilă R., Rev. Roum. Biol. —Bot., 1969, **14**, 183—191,
8. Popa D., Popovici Gh., Studia Univ. Babeş-Bolyai, Biol., 1968, **13**, 65—70.
9. Sachsenmaier W., Blessing J., Brauser B., Hansen K., Protoplasma, 1973, **77**, 381—396.
10. Seitz K., Acta Protozoologica, 1979. **18**, 197—198.
11. Soran V., Lazăr-Keul G., Physiol. plantarum, 1968, **22**, 560—569.
12. Tairbekov M. G., Kazantzev E. N., Tageeva S.V., Biochimia (Moskva), 1965, **30**, 1007—1012.
13. Vintilă R., Fabian A., Lazăr-Keul G., Rev. Roum. Biol.—Bot., 1973, **18**, 287—296.
14. Vintilă R., Soran V., Studia Univ. Babeş-Bolyai, Biol., 1974, **19**, 35—41.
15. Vintilă R., Keul M., Cytologia, 1980, **45**, 49—55.
16. Wohlfarth-Bottermann K. E., Acta Protozoologica, 1979, **18**, 59—73.
17. Wohlfarth-Bottermann K. E., J. Exp. Biol., 1979, **81**, 15—32.
18. Zurzycki J., Acta Soc. Bot. Polon., 1965, **34**, 637—666.

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PROTOPLAST ISOLATION BY ENZYMATIC PROCEDURES FROM *NICOTIANA TABACUM* L., *DAUCUS CAROTA* SUBSP. *SATIVUS* (HOFFM.) ARCANGELI, *VITIS VINIFERA* L. AND *CITRULLUS LANATUS* (THUMB.) MANSF.

BY

AURELIA BREZEANU, MARGARETA IORDAN and ANA ROŞU

The aim of the present paper is to discuss some methodological aspects regarding protoplast isolation from the mesophyll cells of *Nicotiana tabacum*, *Vitis vinifera* and *Citrullus lanatus* and from callus and cell suspension cultures of *Daucus carota*.
The influence of the enzyme type and concentration, and the physiological characteristics of the starting material used as source on the efficiency of protoplast isolation are presented.

During the last two decades, after the elaboration by Cocking (1960) [3] of the enzymatic method for the isolation of plant protoplasts, their use in the understanding and solution of a variety of experimental biology problems gained a considerable extent. Such techniques offer unique systems for investigation in plant genetics, biochemistry and cell biology [2], [6], [8], [9].

The somatic hybridization by protoplast fusion represents a potential method for obtaining interspecies and intergenera hybrids which are not feasible by conventional methods. The lack of the cellulosic wall permits the use of protoplasts in transformation experiments by the incorporation of exogen DNA and cell organelles and facilitates the understanding of the transport phenomena through membranes, of the plasma membrane properties during the endocytosis and fusion as well as for the interrelations existing within the cell and between cells.

This kind of research is at the beginning in our country, the present work being an attempt to put under discussion some methodological aspects regarding the possibilities for the isolation of protoplasts from various types of cells belonging to different plant species.

MATERIAL AND METHOD

Leaf mesophyll and cells from "in vitro" cultures (callus and cell suspensions) were used as sources of protoplasts. The chosen species, the type of tissue, its morpho-physiological characteristics and the experimental conditions are presented in Tables 1, 2. We specify that for obtain-

ing protoplasts from the callus, pieces of approximately 1 g were process-
ed after the method described by Power and Cocking [12] for lea
phyll. For cell suspensions the method described by Constabel
used, the incubation taking place under continuous shaking co

Table 1

Biologic material

No.	Species	Cultures conditions	Type of tissue	Age material characteristics
1	*Nicotiana tabacum*	*in situ* (green-house)	mesophyll (peeled of the lower epidermis) pieces of 1 cm² area	Fully expanded leav 20 cm in length) of day-old plants from t of the stem
2	*N. tabacum*	—,,—	—,,—	110 days-old plants ferentiated flower bu Fully expanded lea (cca. 35 cm in lengt
3	*N. tabacum*	laboratory	—,,—	110 days-old plants slow growth; yellow leaves (15—2 length)
4	*Daucus carola*	*in vitro*	callus (small pieces)	3 days from the last on the LS basal me
5	*Daucus carola*	*in vitro*	suspension (sedimented by centrifugation at 500 g for 15 minutes)	2 days from the last on the LS basal me
6	*Vitis vinifera* cv. Dattier	*in vitro*	mesophyll (with lower epidermis) very small pieces	young leaves from lets grown by *in vit* ture
7	*Citrullus lanatus*	*in vitro*	mesophyll (with lower epidermis) very small pieces	young leaves from *in vitro* by meriste
	,,	*in situ* (laboratory)	cotyledon (small pieces from the mesophyll)	plantlets — 10 days germination

of 30 r.p.m. The extent of incubation varied with the experim
tern (4, 6, 16, 18, 20 and 24 hours).

The *N. tabacum* mesophyll was taken from the 80—10
plants grown in the greenhouse or in the laboratory, under na
conditions. In the case of *V. vinifera* and *C. lanatus*, plants ob
"in vitro" bud or meristem cultures represented the protopla

For protoplast isolation from the mesophyll, variants of th
method elaborated by Power and Cocking [12] were used;
laborious sequential method [15] was not experimented.

Table 2

Enzyme mixtures and maceration conditions

Exp. var.	Enzyme mixtures	Concentration	Osmoticum agent	pH	Plant material	Time of maceration	Conditions
1	Cellulase R-10 (*Trichoderma viride*) Macerozyme R-10	0.1% 0.02%	Mannitol 0.7 M	5.5	callus of Daucus mesophyll of Nicotiana	over night	static, dark
2	Cellulase (EC 3.2.1.4) from *Aspergillus niger* Macerozyme R-10 (*Rhizopus* sp.)	0.1% 0.02%	Mannitol 0.7 M	5.5	idem idem	idem idem	idem idem
3	Cellulase R-10 Macerozyme R-10	250 mg% 50 mg%	Salts solution*	5.5	idem idem	idem idem	idem idem
4	Cellulase R-10 Hemicellulase (*Aspergillus niger*)	20 mg/1 ml 10 mg/1 ml	Sorbitol 100 mg/1 ml	5.5	suspensions of Daucus	idem	shaken
5	Cellulase (EC 3.2.1.4) Hemicellulase	40 mg/1 ml 10 mg/1 ml	idem idem	5.5	suspensions of Daucus; callus of Daucus; Vitis, Citrullus and Nicotiana mesophyll	idem idem	shaken dark
6	Macerozyme R-10 Cellulase R-10	0.2% 1.0%	salts sol.*	5.5	idem idem	over night idem	idem idem
7	Snail enzymes	4 ml/25 ml	Sorbitol 0.7 M	5.6	mesophyll of Nicotiana, Vitis, Citrullus	idem	static
8	Cellulase R-10 Hemicellulase	20 mg/1 ml 10 mg/1 ml	Buffer TRIS HCl 0.05M1ml + 1.2M KCl 0.02M $MgSO_4$	5.6	idem	idem	idem
9	Cellulase R-10 Macerozyme R-10	0.1% 0.02%	Sorbitol 0.7M	5.5	mesophyll of Nicotiana from plants grown under physiological stress; plants with flowers	idem	idem
10	Cellulase (EC 3.2.1.4) Macerozyme R-10	0.2% 0.02%	Sorbitol 0.7M	5.5	idem idem	idem idem	idem idem

* Composition of salt solution:

KH_2PO_4	— 27.2 mg/l	$CuSO_4 \times 5H_2O$	— 0.025 mg/l	
$CaCl_2 \times 2H_2O$	— 1,480.0 mg/l	KNO_3	— 101.0 mg/l	
$MgSO_4 \times 7H_2O$	— 246.0 mg/l	KI	— 0.16 mg/l	pH = 5.8

RESULTS AND DISCUSSION

THE PROTOPLAST ISOLATION FROM MESOPHYLL CELLS

The leaf mesophyll of the tested species proved to be the optimal source of protoplasts. The success of the isolation depended on the species, the enzymatic mixture, the physiological state of the plant and the osmotic solution used. As regards the differential receptivity of the experimented species for the digestion conditions, we mention that the best results were recorded for *N. tabacum*. After 16 hours of incubation in the enzymatic solutions 1 and 3 (table 2) almost the whole mesophyll was digested and single cells were released in suspension (Fig. 1). The microscopic

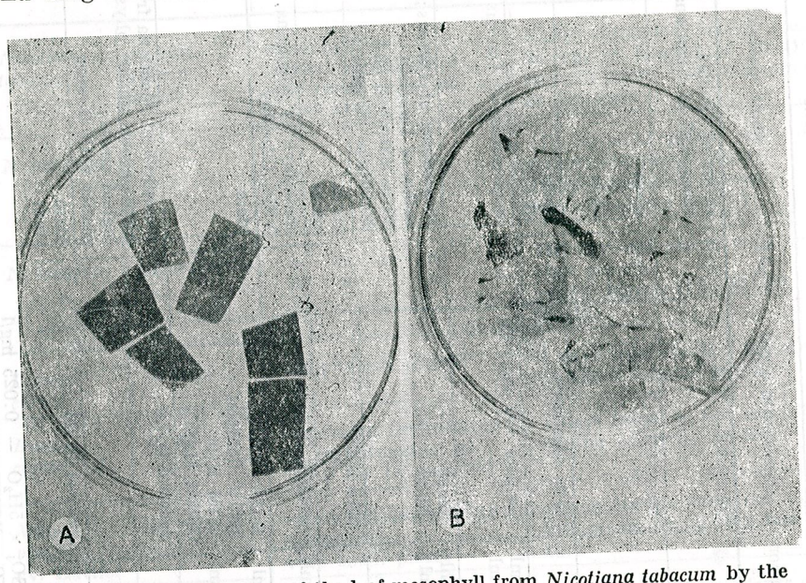

Fig. 1.— The maceration of the leaf mesophyll from *Nicotiana tabacum* by the enzymatic treatment
A — Mesophyll samples in the incipient stage of digestion;
B — The complete maceration of the tissue and protoplast releasing in suspension. after 16 hours of incubation in the enzymatic mixture no. 1.

survey showed dissociated cells, most of them in an advanced state of plasmolysis (Plate I, Figs A, B, C). In the enzymatic mixture predominated round cells deprived of the cell wall (Plate II, Figs. C, D, E) containing chloroplasts with regular parietal distribution. The perfect spherical shape demonstrates the accomplishment of the protoplasting process and the unaltered integrity of the membrane system. Similar quantitative results were obtained in the experimental variant 6 but with a prolonged treatment of about 18—20 hours. The enzymatic mixture no. 5 had a too much hard effect, many cells being destroyed after 10—16 hours of incubation.

For *Vitis vinifera*, the literature indicates the possibility for enzymatic isolation of protoplasts from pericarp callus cells [14]. In our experiments positive results were recorded by the incubation of mesophyll

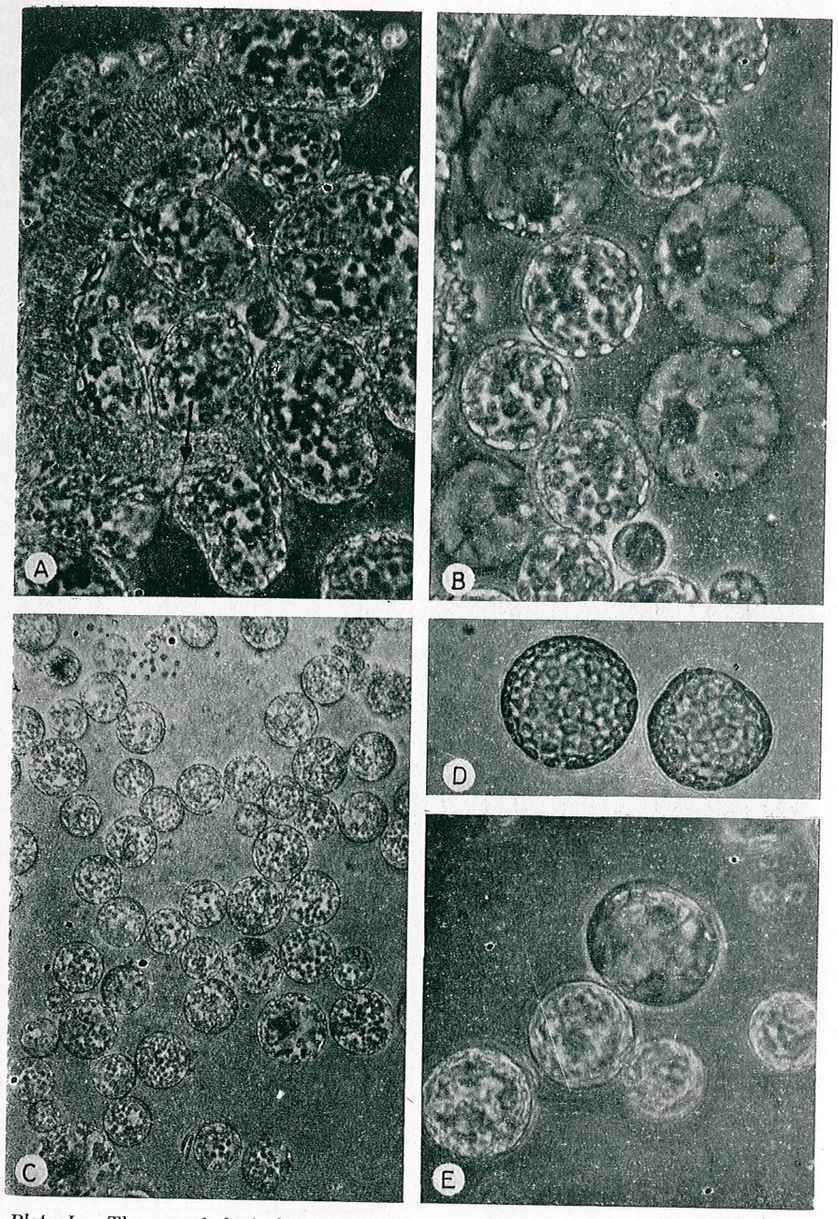

Plate I — The morphological characteristics of the protoplast isolated from the *N. tabacum* mesophyll, by using the enzymatic combinations no. 1 (A, B, C, D) and 3 (E), as they appear in phase contrast microscopy (Fig. A, B, C, D × 188, E ×75). Fig. A shows isolated protoplasts and debris of undigested vascular elements (arrow).

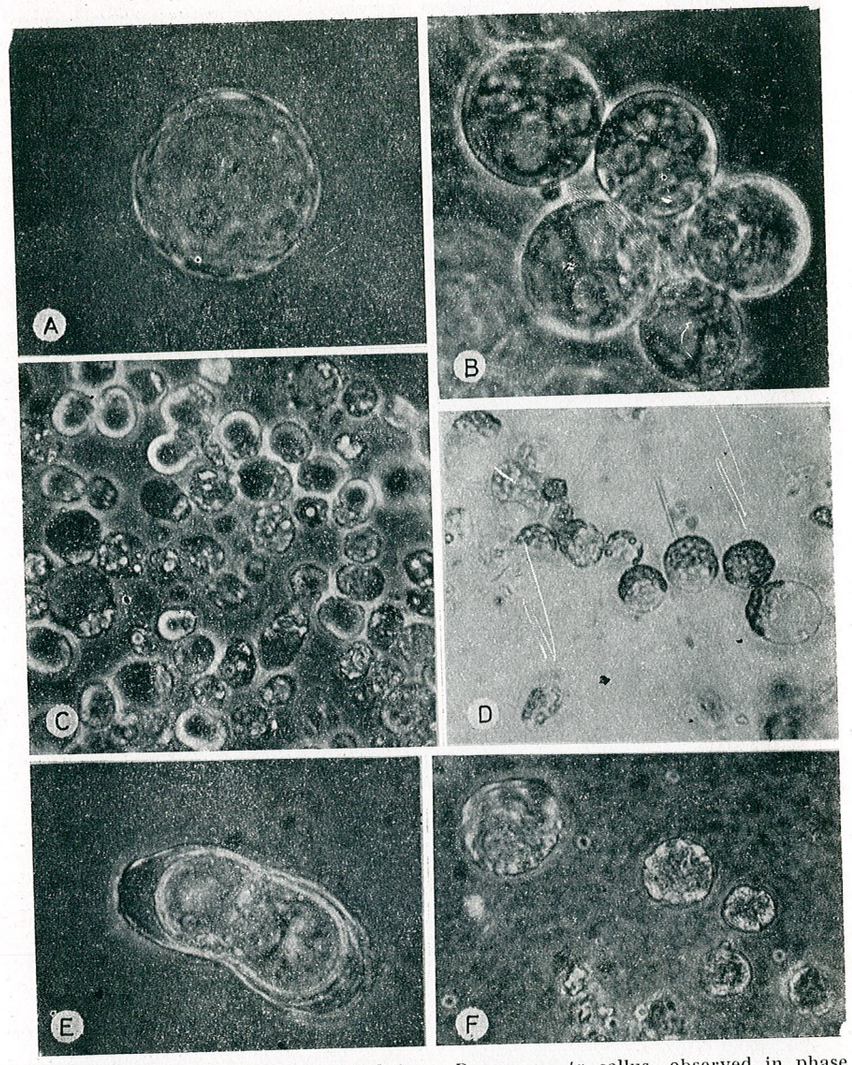

Plate II — A — Protoplast isolated from *Daucus carota* callus, observed in phase contrast microscopy (× 188)

B — Protoplasts isolated from *Daucus carota* cell suspensions cultures, examined in phase contrast microscopy (× 188)

C, D — Protoplasts from *Vitis vinifera* (C) and various degrees of deterioration, as a result of the prolonged enzymatic treatment, examined in phase contrast (C) and direct light (D) microscopy (× 188).

E, F — Successive stages of protoplast obtaining from leaf mesophyll cells of *Citrullus lanatus*, observed in phase contrast microscopy; incipient plasmolysis (E) and the final stage of protoplast isolation (F) (× 188).

pieces in the enzymatic mixture no. 6, for 18—20 hours (Plate II, C). The prolongation of the enzymatic treatment over 20 hours brought about alterations in the cell structure and the agglutination of the whole cytoplasmic content at one of the cell poles (Pl. II, D).

As regards *Citrullus lanatus*, the present knowledge did not offered us direct information concerning the possibilities of obtaining the protoplasts from the genus *Citrullus*, and to a less extent from *C. lanatus* forms with various levels of ploidy. Thereby, excepting the species *Cucumis sativus* and *C. melo*, the representatives from the Cucurbitaceae family were less studied from this point of view [4], [10], [11].

Our experiments proved the possibility of protoplast isolation from the leaf mesophyll of this species, using the enzymatic mixtures no. 6 and 4 (Plate II, E, F). The digestion took place slowly, requiring a prolongation of the treatment to 20—24 hours. The long contact between cells and enzymes resulted in the destruction of protoplasts, the breaking of the plasma membrane and the release of plastids in the medium. We presume that in this case the responsible factor is not an unsuitable enzyme concentration, but rather an osmotic stabilizer.

The cotyledonary tissue did not represent an optimal source of protoplasts. In our experimental conditions we did not notice the beginning of the tissue digestion. Moreno [11] made similar observations on *Cucumis melo*, mentioning a better receptivity of the mesophyll for the isolation conditions in comparison with roots, cotyledon, callus or cell suspension cultures.

Apart from *Nicotiana*, the mesophyll of *Vitis* and *Citrullus* needed a longer time for incubation, and the frequency of the isolated protoplasts was lower. This may be owed not only to the characteristics of specificity but also to the fact that in these cases the lower epidermis was not peeled, and that slowed down the accomplishment of the digestion process.

Along with the specificity and age, the physiological state of the vegetal material used as source significantly influenced the isolation process. Experiments with *N. tabacum* made evident the fact that the optimal material for protoplasts isolation is the leaf mesophyll, during the juvenile stage of the donor plant development (plants about 80—100 day-old, with cca. 7—8 fully expanded leaves), grown in greenhouse conditions (Tabel 1). The obtaining of protoplasts was impossible when mesophyll from plants with mature floral organs was used, as well as in the case of using etiolated mesophyll from plants grown in the laboratory, under unsuitable physiological conditions, despite of the providing with the optimal enzymatic treatment (Table 2, var. 9).

As regards the quality of the experimented enzymes, the best results were given by the digesting media containing Onozuka enzymes (Table 2, var. 1, 3, 4, 6). The enzymes supplied by Flukca, Sigma or Calbiochem companies (Table 2, var. 2, 5, 8, 10) gave less satisfactory results, repeated testing of the concentrations, combinations and of the digestion duration being necessary.

The raw snail gut enzymes did not give positive results in any of the tested variants. It is necessary in this case its purification, the isolation of the most active enzymatic fraction and after that the performing of

a large number of tryings concerning the optimal concentrations and maceration time.

As regards the effect of the osmotic stabilizer, we mention that very good results were obtained by using 0.7 M mannitol and salt solution (Table 3, var. 3, 6). The sorbitol in concentration of 0.7 M, as well as the TRIS buffer in 1.2 M KCl and 0.02 M $MgSO_4$ (Table 2, var, 7, 8 9, 10, 11), did not give satisfactory results.

THE ENZYMATIC ISOLATION OF PROTOPLASTS FROM CELL SUSPENSION CULTURES OF *DAUCUS CAROTA*

The microscopic survey of single cells and cell aggregates in their logarithmic growth phase (3 days since the last subculture) showed, after 4 hours of incubation in the enzymatic mixture 4 and 6 (Table 2), the beginning of the cell wall digestion and the release of protoplasts. After 6 hours of incubation the process advanced, so after 10—18 hours cca. 90% of the initial cells were naked and perfect spherical (Pl. II, B). The event appeared also when cellulase and hemicellulase were employed (Table 2, var. 5).

In some experiments, protoplasts with two or three nuclei are present in suspension, as a result of homoplasmatic fusions occurring between two adjoining cells dring the isolation process.

In our experiments, this kind of fusions were not encountered.

THE ISOLATION OF PROTOPLASTS FROM CALLUS CULTURES OF *DAUCUS CAROTA*

Naked cells were obtained from the *D. carota* callus by the enzymatic mixture 6 (Pl. II, A), but in a lower proportion compared with the cell suspensions; many cells retained their walls undigested even after a longer interval of incubation.

The phenomenon is explicable taking into account that the callus cultures are asynchronous, so at a given time coexist both young cells with thin walls, easily digestible by the enzymatic mixture, and mature or even senescent cells, with thick, resistant walls. Moreover, there is also the inconvenience of obtaining impure protoplast suspensions, due to the debris resulted from the deteriorated cells or dead protoplasts. This requires repeated filtrations, which sometimes have negative repercussions on the cells. Consequently, we consider that the efficiency of the protoplast isolation from callus cells is relatively low, at least in our experimental conditions. One explanation could be furnished by the fact that the callus sources were not represented by primary cultures but by intensely proliferative calli (3 days after the last transfer), previously subjected to repeated subcultures. It is possible that the meristematic cells, the most competent for protoplast isolation, have been present in a relatively low proportion, comparatively with the primary cultures.

Our investigations allow the drawing of the following conclusions:

1. The leaf mesophyll from *Nicotiana tabacum*, *Vitis vinifera* and *Citrullus lanatus* and the cell suspension cultures of *Daucus carota* represent the most efficient cell types for the enzymatic isolation of protoplasts.

2. The success of isolation depended on the species, the physiological state, the age, the enzymatic combinations, the purification degree of enzymes and the incubation conditions.

3. The vegetal material chosen for protoplast isolation must be grown in controlled conditions in order to assure a physiological optimum, essential for achieving repeatable results.

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SELECTIVE REFERENCES

1. Banks S. M., Evans K. P., Plant Science Letters, 1976, **7**, 409—416.
2. Brezeanu A., Roşu A., Iordan M., Vassu-Dimov T., In: "Culturile de ţesuturi — instrument de cercetare în biologia teoretică şi practică", Tipo Agronomia Cluj-Napoca, 1981, p. 57—91.
3. Cocking E. C., Nature, 1960, **187**, p. 927—929.
4. Coutts R. H. A., Wood K. R., Plant Science Letters, 1975, **4**, 189—193.
5. Evans R. P., *Microbial and Plant Protoplasts*, Academic Press, London, New York, San Francisco, 1976, p. 51—69.
6. Ferenczy L., *Advances in protoplasts research*, Ferenczy L., Farkas G. (Ed.), Szeged, Hungary, 1980, p. 55—63.
7. Gamborg O. L., Wetter L. L., *Culture methods*, N.R.C. of Canada, Sashatoon, 1975, p. 11—21.
8. Gamborg O. L., *Plant Tissue Culture, Methods and Applications in Agriculture*, Thorpe A.T. (Ed), Academic Press, New York, London, Toronto, Sydney, San Francisco, 1981, p. 115—153.
9. Gamborg O. L., Bottinoy P., *Advances in Biochemical Engineering*, vol. 19, A. Fiechter (Ed), Springer-Verlag, Berlin, Heidelberg, New York, 1981, p. 239—263.
10. Ingram D. S., Helgeson J. P., In: *Tissue culture methods for plant pathologists*, Blackwell Scientific Publications, Oxford, London, Edinburgh, Boston, Melbourne, 1980.
11. Moreno V., Roig L. A., Garcia B., In: *Application de la culture in vitro à l'amélioration de plantes potagères*, Reunion EUCARPIA, Section "Legumes", Versailles, 1980, p. 166—172.
12. Power J. B., Cocking E. C., J. exp. Bot., 1970, **21**, 64—70.
13. Power J. B., Davey R. M., Laboratory manual, Univ. of Nottingham, 1979.
14. Skene K.G.M., Australian J. Plant Physiol., 1974, **1**, 371—779.
15. Takebe Y., Plant Cell Physiol., 1968, **9**, 115—124.

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DATA ON THE VERTICAL DISTRIBUTION OF PLANKTONIC PRIMARY PRODUCTIVITY IN THE OFFSHORE ZONE OF CONSTANȚA (THE BLACK SEA)

BY

ALEXANDRU S. BOLOGA and PETRE T. FRANGOPOL

The study of the planktonic primary productivity in the offshore zone of Constanța was performed by means of the ^{14}C method, on the water column between 0—50 m. The results obtained in 1980 ranged between 1.7—1938.5 mg C $m^{-3}day^{-1}$. Maximum values were generally obtained in the upper layer (0—10 m).

The study of the planktonic primary productivity, which had been initiated in 1977 by the radiobiological method in the upper water layer [1], [2], [3], was subsequently extended vertically down to the depth of 50 m in 1980.

MATERIAL AND METHOD

Phytoplankton samples were collected every month in the eastward cross-section off Constanța from the same fixed 5 stations [2], [3], from the 0, 10, 25 and 50 m horizons; besides the main environmental factors (Table 1), the total inorganic carbon content was determined with the view of calculating the primary productivity.

The *in situ* "simulated" ^{14}C method [9] was used, by liquid scintillation counting [5]. The transparent (two) and dark (one) bottles containing phytoplankton from each sample were inoculated with 25 μCi (925 kBq) $NaH^{14}CO_3$, introduced into a basin and exposed to natural illumination. The samples from 0 m were maintained at the surface of the basin (100% illumination); those from the other horizons were screened (50% illumination).

The primary productivity was measured in definite conditions of light and temperature (Table 2).

The content of the bottles was filtered at attenuated light, with an in vacuum device, on Millipore membrane filters HA 04700 (Ø = 0.45 μm) without any final washing.

The filters with radioactive phytoplanktonic residues were preserved in Packard type vials (with previously determined background) and ^{14}C activity was measured with the following scintillation mixture: 3 ml dioxan (for filter solubilization) and 5 ml N.E. 233 liquid scintillator. Measurements were performed with a Packard Tri-Carb spectro-

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Table 1

Mean values of the main environmental factors in the Constanța eastward section, stations 1—5 (0—50 m), in 1980 (*) cf. [10])

Month	t°C*)	pH*)	S‰*)	O_2*) cm³/l	O_2*) %	N—NO_2*) (µg/l)	N—NO_3*) (µg/l)	P—PO_4*) (µg/l)	S—SiO_3*) (µg/l)	Total inorganic carbon (mg/l)	Organic matter*) (mg O_2/l)
I	3.64	8.0	17.29	9.01	108.5	3.7	162.7	26.6	620	38.22	1.39
II	4.06	7.9	16.42	8.90	107.5	6.2	94.5	7.5	588	40.76	1.90
III	3.01	8.5	17.01	9.06	109.4	6.7	54.4	14.1	632	40.24	2.05
IV	5.20	8.4	15.95	8.46	106.1	11.1	76.7	8.3	731	38.22	1.42
V	11.16	8.4	15.44	7.73	111.8	7.7	101.1	8.7	846	37.54	2.51
VI	13.74	8.4	15.77	6.90	107.6	4.6	51.3	10.8	699	36.72	2.29
VII	12.96	8.1	17.62	6.02	92.8	21.0	116.8	11.9	805	38.02	1.40
VIII	17.84	8.3	15.98	5.05	85.0	4.6	60.1	15.4	801	37.82	2.16
IX	14.99	8.5	17.56	5.64	87.3	5.2	110.5	5.4	823	36.81	0.99
XI	10.66	8.4	16.29	6.49	93.8	18.9	140.9	8.2	615	40.04	1.53
XII	7.75	8.3	16.19	6.86	82.0	15.3	109.1	11.5	1141	38.78	2.21

Table 2

The experimental conditions (*in situ* "simulated") during the determination of planktonic primary productivity of the Constanța offshore zone in 1980

Date of sampling	Date of determination	Number of stations	Horizons (m)	Time of exposure (hours)	Light ($\bar{x}$ of 5 det.) (lux)	Temperature ($\bar{x}$ of 5 det.) (°C)	$NaH^{14}CO_3$ activity (µCi)	Date of sample measurement
Jan. 26	Jan. 28	3	0	$12-17^{30}$	11,086	1.5	25	Sept.29, 1980
Feb. 09	Feb. 11	4	0	$12-17^{30}$	28,117	12.0	25	,,
Mar. 20	Mar. 21	5	0,10,25,50	$12-18^{30}$	11,245	10.8	25	,,
Apr. 08	Apr. 09	4	0,10,25,50	$12-18^{50}$	9,798	11.3	25	,,
May 26	May 27	5	0,10,25,50	$12-19^{50}$	30,018	16.5	25	,,
June 19	June 23	5	0,10,25,50	$12-20^{00}$	56,476	19.5	25	,,
July 16	July 17	4	0,10,25	$12-19^{55}$	47,741	21.1	25	,,
Aug. 13	Aug. 14	5	0,10,25,50	$12-19^{20}$	5,952	18.5	25	,,
Sept. 19	Sept. 22	5	0,10,25,50	$12-19^{10}$	20,165	20.0	25	,,
Nov. 24	Nov. 25	5	0,10,25,50	$12-16^{40}$	38,321	16.8	25	Jan. 24 1981
Dec. 12	Dec. 13	4	0,10,25	$12-16^{35}$	32,438	13.0	25	,,

meter model 3385. The samples were measured with 96% efficiency. Appropriate background and quenching corrections were applied to the results; each sample was measured 3 times, for 1 minute. For verifying the measurement precision, some samples were re-measured with a Packard Tri-Carb spectrometer model 2425 with 91.5% efficiency; most of the repeated measurement results were close.

Calculation of planktonic primary productivity values was carried out after Burkaltseva's formula [4]. The total inorganic carbon content (CO_2, HCO_3^-, CO_3^{2-}) of the sea water was determined by total carbonic alkalinity analysis (cf. [11]). The measurement values for the dark (control) bottles were neither subtracted from the photosynthetic assimilation values, nor calculated as percentage of the latter, but were considered as such [8]. No light correction was applied to the values of primary productivity for the 10—50 m horizons.

Primary productivity through the water column was calculated, down to the lowest sampling horizon. Phytoplankton P/B annual coefficient = 300 was used for establishing total planktonic production [6], [7]. The results are expressed in mg C m^{-3} day^{-1} and g C m^{-3} $year^{-1}$, respectively. For the estimation of planktonic primary productivity of the entire zone of Constanța, as considered between Chituc (44°20′N) and the southern end of the Romanian coast (43°45′N) — down to the depth of 50 m — a total water volume of 125 km^3 was taken into calculation.

RESULTS AND DISCUSSION

The results on planktonic primary productivity which were obtained in 1980 for the entire water column down to 50 m take values between 4.3—1,938.5 mg C m^{-3} day^{-1} (Table 3).

High average primary productivity values were recorded during this year in February at station 1 at 0 m (579.3 mg C m^{-3} day^{-1}), in July at stations 1 (761.9), 2 (942.9) and 3 (873) and in August at stations 1 (424.5), 3 (639.4), 4 (445.2) and 5 (548) (Table 4): accordingly, like in 1979 (3), higher primary productivity values are found near the shore, within 10 n.m. distance (stations 1—3), as compared to the main sea zone, up to 30 n.m. (stations 4—5) (Table 4), with the insignificant exception of April (station 4).

Seasonally, in contrast to the preceding year, when primary productivity had the usual higher values in spring and early summer [3], in 1980, the maximum productive level was recorded by the radiobiological method during the summer (July-August); this level may be correlated with the yearly maximum salinity (17.62‰) and maximum NO_2 concentrations (21 µg l^{-1}) in July, as well as the yearly maximum temperature (17.8°C) in August (Table 1) — all factors favouring the development of an abundant phytoplankton biomass and, consequently, an important primary productivity.

As concerns the vertical distribution of primary productivity, highest values at 0 m and 10 m horizons and a decrease with depth are generally found (Table 3); exceptions are made by the months of June and July, when maximum primary productivity was determined at stations 2, 3 and 4 and respectively, 2 and 4, at 25 m depth.

The monthly mean values of planktonic primary productivity during the whole year range between 14.2 (December) — 695.0 (July) mg C $m^{-3}day^{-1}$ (Table 4) or for the period March — December between 0.4—14.5 g C $m^{-2}day^{-1}$ (Table 5).

The annual mean values over the stations range between 122.9 (30 n.m.) — 233.5 (10 n.m.) mg C $m^{-3}day^{-1}$ (Table 4) or between 2.4—6.7 g C $m^{-2}day^{-1}$ (Table 5).

Table 3

Planktonic primary productivity in the Constanța offshore zone in 1980 (mg C m^{-3} day^{-1})

Month	Horizons (m)	Stations 1	2	3	4	5
I	0	18.8	112.3	58.1		
II	0	579.3	50.6	100.6	94.5	
III	0	141.1	42.4	67.9	52.9	62.8
	10	19.6	148.3	40.9	4.3	14.2
	25	—	15.9	32.1	23.3	31.6
	50	—	—	—	—	8.3
IV	0	30.9	89.6	85.2	67.8	
	10	90.2	75.4	36.9	61.9	
	25	—	19.5	22.3	64.4	
V	0	71.8	40.7	16.1	79.8	110.7
	10	36.8	25.5	33.8	40.6	99.9
	25	—	17.9	7.9	33.9	11.8
	50	—	—	—	—	2.8
VI	0	33.6	12.9	45.3	76.9	106.9
	10	241.3	395.1	5.7	218.8	106.1
	25	—	452.3	393.9	613.7	52.2
	50	—	—	—	—	18.5
VII	0	432.4	216.5	158.5	185.2	
	10	1089.7	1105.3	1291.5	161.9	
	25	—	1156.3	651.8	284.1	
VIII	0	716.3	96.2	86.7	127.2	179.4
	10	132.6	263.0	1196.0	471.2	1938.5
	25	—	153.6	80.2	613.7	101.4
	50	—	—	—	—	19.6
IX	0	227.0	20.6	200.4	249.1	223.5
	10	184.8	46.2	43.1	13.5	15.3
	25	—	59.8	41.2	17.8	21.6
	50	—	—	—	—	35.0
XI	0	82.7	12.4	76.5	41.6	25.1
	10	755.9	64.5	732.1	776.9	28.2
	25	—	61.6	665.5	48.4	15.6
	50	—	—	—	—	6.1
XII	0	16.6	132.4	6.3	10.8	
	10	15.3	6.0	1.7	3.7	
	25	—	3.7	6.6	6.3	

Table 4

Mean values of the planktonic primary productivity in the Constanța offshore zone in 1980 (mg C $m^{-3}day^{-1}$)

Month	Stations 1 (1 n.m.)	2 (5 n.m.)	3 (10 n.m.)	4 (20 n.m.)	5 (30 n.m.)	Monthly mean
I	18.8	112.2	58.0	—	—	63.0
II	579.3	50.5	100.6	94.5	—	206.2
III	80.4	87.4	43.6	19.7	24.5	51.1
IV	60.6	61.5	42.2	63.8	—	57.0
V	54.3	26.2	22.5	46.4	41.5	38.2
VI	137.5	335.8	130.1	308.9	62.7	195.0
VII	761.9	942.9	873 0	203.2	—	695.0
VIII	424.5	196.8	639.4	445.2	548.0	450.8
IX	205.9	45.2	74.0	61.9	43.6	86.1
XI	419.3	53.2	581 0	411.3	17.3	296.4
XII	16.0	30.6	4.1	5.9	—	14.2
Annual mean	205.7	176.6	233.5	151.0	123.9	

n.m. = nautical mile.

According to the obtained results, the average primary productivity during 1980 was 195.7 mg C $m^{-3}day^{-1}$ or 4.8 g C m^{-2} day^{-1}, comparatively higher than the surface primary productivity of the preceding year (146.9 mg C m^{-3} day^{-1}). Applying the P/B annual coefficient for phytoplankton (300), a total planktonic production of 58.7 g C m^{-3} $year^{-1}$ or 1,455.9 g C m^{-2} $year^{-1}$ was estimated.

Table 5

Values per m^2 of the planktonic primary productivity in the Constanța offshore zone in 1980 (g C m^{-2} day^{-1})

Month	Stations 1 (1 n.m.)	2 (5 n.m.)	3 (10 n.m.)	4 (20 n.m.)	5 (30 n.m.)	Monthly mean
III	0.8	2.2	1.1	0.5	1.2	1.2
IV	0.6	1.5	1.1	1.6	—	1.2
V	0.5	0.7	0.6	1.2	2.1	1.0
VI	1.4	8.4	3.3	7.7	3.1	4.8
VII	7.6	23.7	21.8	5.1	—	14.5
VIII	4.2	4.9	16.0	11.1	27.4	12.7
IX	2.1	1.1	1.8	1.5	2.2	1.7
XI	4.2	1.3	14.5	10.3	0.9	6.2
XII	0.2	0.8	0.1	0.5	—	0.4
Annual mean	2.4	4.9	6.7	4.4	6.1	

The total phytoplanktonic production in the section of Constanța, which is less influenced by the Danube as compared to the northern sections of the Romanian coastal waters, was estimated to be 7,625,000 t C $year^{-1}$.

CONCLUSIONS

1. The application of the ^{14}C method has been extended also on the vertical, through the water column between 0 and 50 m depth.

2. The data obtained indicate, also in 1980, areal and seasonal variability of planktonic primary productivity in the offshore zone of Constanța.

3. The primary productivity ranges between 1.7—1,938.5 mg C m^{-3} day^{-1} or between 0.2—27.4 g C m^{-2} day^{-1}; the total primary production has been estimated to be 7,625,000 t C $year^{-1}$ in the water area between Chituc and the southern end of the Romanian coast.

4. Higher primary productivity values inshore (stations 1—3) and decrease of the productive level offshore (stations 4—5) have been determined, although the decreasing trend is less evident when this level is expressed as per m^2.

5. Vertically, the maximum primary productivity has been established in the 0—10 m water layer, except for June, when the highest values have been found at 25 m.

REFERENCES

1. Bologa A. S., Frangopol P. T., Cercet. mar.—Rech. mar., 1979, **12**, 81—86.
2. — , Frangopol P. T., Frangopol M., Stanef I., Rev. Roum. Biol.—Biol. végét., 1980, **25**, *2*, 129—133.
3. — , Usurelu M., Frangopol P. T., Oceanol. Acta, 1981, **4**, *3*, 343—349.
4. Burkaltseva M. A., in *Sovremennye metody rybohoziaistvennyh morskih gidrokhimitcheskikh issledovanii*, ed. by M. V. Fedosova, Izd. Pisc. promysl., 146—169, 1973,
5. Frangopol P. T., Bologa A. S., 1979, Preprint CSEN/ICEFIZ, RB—**1**, May, Bucharest, 1—39.
6. Kondratyeva T. M., Tr. Sevastop. biol. st., 1968, **10**, 8—26.
7. Morosova-Vodyanitskaya N. V., DAN SSSR., 1950, **23**, *4*.
8. Sournia A., Bull. de l'ét. en comm. de la Médit., Num. spéc. **5**, Monaco, 1973, 1—128.
9. Steemann Nielsen E., J. Cons. perm. int. Explor. Mer, 1952, **18**, 117—140.
10. ***, Anuarul oceanografic (Oceanographic Yearbook), IRCM, Hydrology Laboratory, 1980.
11. * * *, *Protecția calității apelor (Water Quality Control)*, Bibl. Stand., Ser. tehn. A. 1967, **62**, 137—138.

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MICRORGANISMES FILAMENTEUX IMPLIQUÉS DANS LE GONFLAGE DES BOUES ACTIVÉES. ESSAIS DE LES PRÉVENIR OU DE LES COMBATTRE

PAR

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On a décrit et identifié toute une série de microrganismes filamenteux impliqués dans le « bulking » des boues activées formés au cours de l'épuration des eaux usées de l'industrie laitière. On a testé l'influence des conditions d'alimentation, de certaines substances azotées et de micro-éléments sur l'apparition du phénomène de « bulking ».

Les résultats ont démontré qu'une solution complexe de micro-éléments ajoutée à raison de 20 %o aux eaux d'alimentation prévient le gonflage de la boue. L'alimentation discontinue favorise le développement des microrganismes filamenteux.

Parmi les types de « bulking » des boues activées rencontrés dans la pratique de l'épuration des eaux, les plus fréquents sont ceux provoqués par les microrganismes filamenteux. Par cette dénomination on désigne un groupe hétérogène de microrganismes différents comme dimension, structure cellulaire et appartenance taxonomique ayant en commun la morphologie filamenteuse. Le plus souvent, comme il en est aussi pour la bactérie filamenteuse typique — *Sphaerotilus natans* — il ne s'agit pas de vrais filaments, mais de chaînes de cellules envelopées dans une gaine commune qui, à l'examen direct, sur des préparations fraîches, non colorées, cache les cellules faisant paraître l'image d'un filament.

Bien que les boues activées contiennent divers types de microrganismes filamenteux, l'opinion que le gonflage « filamenteux » des boues est déterminé par la bactérie *Sphaerotilus natans* s'est enracinée et c'est pour cette raison qu'on lui a consacré de nombreuses études. Une meilleure connaissance de tous les microrganismes impliqués dans le phénomène, comme des conditions qui favorisent leur multiplication pourrait permettre de prendre des mesures nécessaires susceptibles de les prévenir et de les combattre. C'est ce que nous nous sommes proposé de faire par ce présent rapport.

Le développement des recherches de la microbiologie des boues activées ont conduit — ces dernières années — à la découverte et à la description de nouveaux genres et de nouvelles espèces de microrganismes filamenteux.

Un essai de systématisation et de classification de ceux-ci, compte tenu des caractères morphologiques et structurels, est présenté dans l'ouvrage de D. H. Eikelboom (1975). En utilisant cet ouvrage comme guide et en le complétant avec d'autres (Mulder—1964 ; Lewin—1969 ; Adamse —1968 ; Mandel et al.—1969 ; Cyrus et al.—1970 ; Farquar et al., 1971 ; Ueda et al.—1972 ; Van Veen et al. 1973 a, 1973 b ; Liu et al.—1977)

ainsi que le bien connu : Bergey's manual of determinative bacteriology — — 1974, nous avons élaboré l'étude que nous présentons dans la suite et qui comporte la description et l'identification (là où il en était possible) des microrganismes filamenteux des boues gonflées apparues dans une séries des installations expérimentales d'épuration des eaux usées provenant d'une fabrique de produits laitiers.

MATÉRIAUX ET MÉTHODES

Les échantillons de boue activée ont été prélevés dans des installations de laboratoire en épurant en différentes conditions des eaux résiduelles provenues d'une fabrique de traitement industriel du lait et auxquelles on a ajouté des sels d'azote et une série de micro-éléments (tableau 1).

Tableau 1

Types d'installations expérimentales

No. de la variante	Nom de la variante	Mode d'alimentation	Substances ajoutées aux eaux d'alimentation	
			La formule	La dose en mg/l
Première série				
1	témoin	continue	—	—
2	discontinue	discontinue	—	—
3	NH_4NO_3	continue	NH_4NO_3	200
4	$Ca(NO_3)_2$	,,	$Ca(NO_3)_2$	300
5	micro-éléments	,,	Solution complexe de micro-éléments*	20 ml
Deuxième série				
6	K			5
7	Ca	continue		10
8	Fe	,,		6
9	Mn	,,		0,1
10	Zn	,,		0,1

* La solution complexe des micro-éléments contient : 36 mg $Na_2HPO_4 . 2H_2O$; 624 mg KH_2PO_4 ; 600 mg $FeSO_4 . 7H_2O$; 6 mg $MnCl_2 . 4H_2O$; 13 mg $ZnSO_4 . 7 H_2O$; 12 mg $CoCl_2 . 6 H_2O$; 3,060 mg $MgSO_4 . 7 H_2O$; 780 mg $CaSO_4 . 2H_2O$ dans un litre d'eau distillée.

Leur choix a été fait en tenant compte du fait que la technologie du traitement du lait implique des phénomènes de précipitation, de fermentation et d'autres transformations des composés organiques du lait qui réduisent bien par l'insolubilisation ou la consommation la teneur en calcium et en micro-éléments des eaux usées.

En outre, le lait même représente un substrat non équilibré du point de vue du rapport $CBO_5 : N$ (consommation biochimique d'oxygène par rapport à l'azote). Par l'addition des substances mentionnées dans les eaux d'alimentation on a poursuivi son équilibre et visé à éviter le « bulking » de la boue.

Les expériences ont duré 60 jours étant effectuées en deux séries. Dans la première série on a étudié l'influence des sels d'azote et du complexe de micro-éléments sur l'apparition du phénomène de « bulking ». Après que l'on a constaté l'effet favorable du complexe de micro-éléments, dans la deuxième série ceux-ci ont été testés séparément afin d'en dépister l'un d'entre eux ayant un rôle prépondérant dans la prévention de l'apparition du bulking.

Pour étudier l'influence du mode d'alimentation parallèlement au témoin alimenté continûment a fonctionné une variante où l'alimentation de l'installation s'est effectuée de manière discontinue (une fois par jour).

Les prélèvements de boue ont été examinés et décrits directement sur les préparations microscopiques fraîches et sur des frottis coloriés Gram. Dans certains cas, on a effectué des colorations spéciales pour la gaine (la technique au rouge de Congo) et pour mettre en évidence les inclusions du poly-ß-hydroxybutyrate (technique au Sudan black-Burdon).

RÉSULTATS ET DISCUSSIONS

Première série

L'examen microscopique des biocénoses développées dans les installations expérimentales a mis en évidence l'apparition — tôt ou tard — — des micro-organismes filamenteux dans toutes les variantes, à l'exception de celle dans laquelle on a ajouté le complexe de micro-éléments où la boue a conservé ses qualités de sédimentation, pendant tout le cours de l'expérience (fig. 1). Les échantillons récoltés dans l'installation témoin variante no 1 — ont mis en évidence l'apparition des micro-organismes filamenteux de différents types dès les premiers jours de l'amorce. Vers la fin de l'expérience sont devenus prédominants les microrganismes ayant les caractéristiques suivantes : filaments minces (sous 1 μm) à longueurs variables (20—100 μm), légèrement ondulés, sans gaine, Gram négatifs, attachés par un bout à la surface des flocons (fig. 2). Les caractéristiques correspondent au genre *Pelonema* (Bergey—1974) qui semble être identique au type 1702, I^er^ groupe de la classification de Eikelboom — 1975, caractéristique des eaux à teneur élevée en hydrates de carbone.

Dans l'installation à alimentation discontinue — variante no 2 — les microrganismes filamenteux ont été présents dès le commencement dans des quantités importantes ; mais après environ 50 jours, ils ont complètement disparu, étant remplacés par les bactéries zoogloéales et par celles formatrices de flocons. On a relevé deux types de bactéries fila-

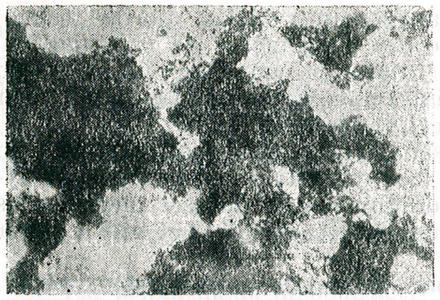


Fig. 1

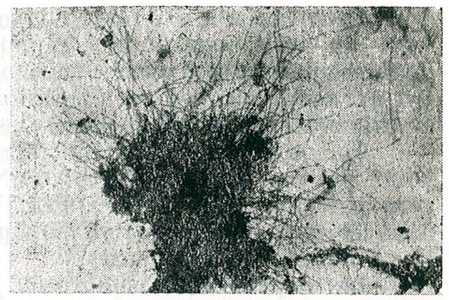
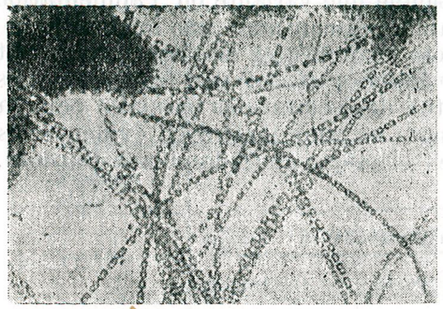


Fig. 2



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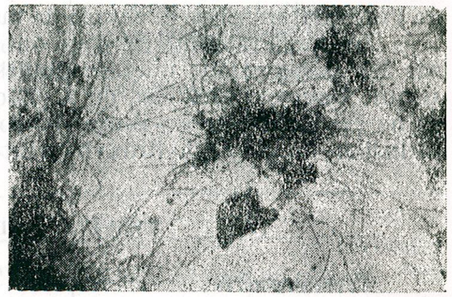

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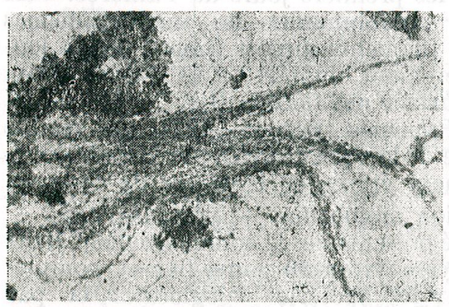

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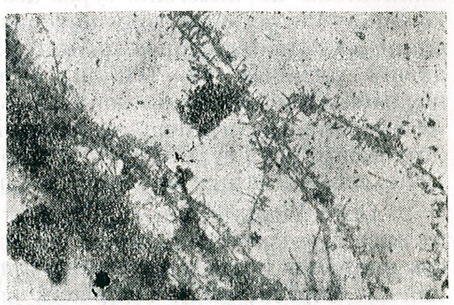

Fig. 6

menteuses : les 30 premiers jours la boue était constituée à raison de près de 80 % de filaments de *Sphaerotilus*. A l'examen microscopique sur des frottis coloriés, ceux-ci sont apparues constitués de chaînes de cellules sphéroïdales (2 × 3 μm), remplis d'inclusions grandes, globuleuses de poly-β-hydroxybutyrate, entourées d'une gaîne épaisse (fig. 3). Successivement, elles ont été remplacées par des micro-organismes filamenteux minces (0,5—0,7 μm en diamètre), droits aux longueurs allant de 10 à 30 μm, enveloppés dans une gaine mince, visible seulement chez certains filaments (fig. 4). Les filaments sont disposés à la surface des flocons pareils à des piques. La description correspond au genre *Haliscomenobacter hydrossis* cité pour la première fois par Van Veen 1973 — qui indique comme excellentes sources de carbone pour cette bactérie : la glucose, la lactose, la saccharose et l'amidon et comme sources d'azote les acides casaminiques, l'acide glutamique ainsi qu'une série de composés inorganiques dont $(NH_4)_2SO_4$, KNO_3, NH_4NO_3. Les vitamines B_{12} et la thiamine sont indispensables à son développement. Ces préférences expliquent sa présence fréquente dans la boue activée développée au cours de l'épuration des eaux usées laitières.

Dans la variante expérimentale no 3 — avec addition de NH_4NO_3 le phénomène de gonflage est apparu beaucoup plus tard, vers la fin de la période d'expérimentation et a été déterminé en premier lieu par la bactérie *Haliscomenobacter hydrosiss* — I^{er} groupe (Eikelboom—1975) identique à *Streptothrix hyalina* (Bergey — 1974) (fig. 5).

Le gonflage de la boue dans la variante no 4 — avec addition de $CaNO_3$ — s'est produit dès les premiers jours, atteignant le maximum après deux semaines. A l'examen microscopique on a relevé divers microrganismes filamenteux parmi lesquels est prédominante une bactérie avec les caractéristiques du genre *Sphaerotilus* mais ayant accrochées à la surface de la gaine de nombreuses bactéries, petites, à l'aspect d'épines (fig. 6—7).

Les filaments groupés en faisceaux, attachés par un bout à la surface des flocons, le bout opposé libre s'amincissant à mesure qu'il s'écarte du flocon comme des queues, donnent au flocon un aspect caractéristique (fig. 8).

La deuxième série

La deuxième série d'expériences a comporté cinq variantes, chacune testant l'influence d'un seul micro-élément. Dans toutes les variantes s'est produit le gonflage de la boue déterminé par divers microrganismes filamenteux, mais dans aucun d'entre eux on n'a pu identifier la bactérie *Sphaerotilus*.

Ainsi, dans les variantes nos 7 et 9, à addition de Ca et respectivement de Mn, le microrganisme prédominant a présenté les caractéristiques du type 0411—VII^{e} groupe (Eikelboom — 1975) ou celles du genre *Peloploca* (Bergey — 1974) c'est-à-dire : chaînes de cellules cylindriques aux bouts droits (0,8—1,0 μm × 4—5 μm) recouvertes d'une gaine mince. Les filaments ondulés sont souvent groupés parallèlement. Sur les frottis coloriés, dans les cellules on remarque de nombreuses vacuoles à gaz (fig. 9).

Dans l'installation à addition de Zn — variante no 10— le bulking de la boue a été déterminé notamment par des bactéries similaires au

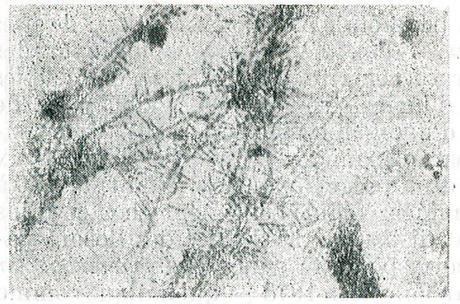

Fig. 7

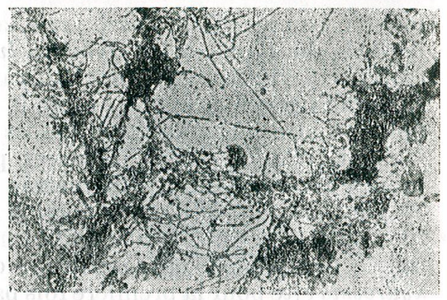

Fig. 8

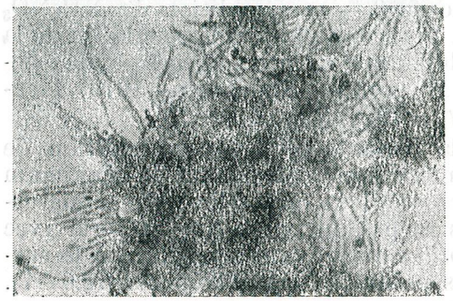

Fig. 9

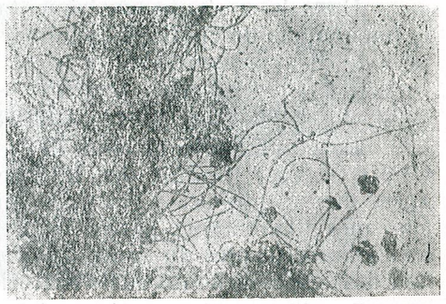

Fig. 10

Fig. 11

Fig. 12

genre *Pelonema* (Bergey — 1974) ou au type 1702 — I^er^ groupe (Eikelboom) décrit ci-dessus (fig. 10).

Dans les autres installations — avec K et Fe — respectivement les variantes nos 6 et 8, la boue a été complètement envahie par une bactérie en forme de filaments courts, ondulés, disposés en groupes parallèles et contenant de nombreuses vacuoles à gaz (fig. 11, 12). Les caractéristiques décrites correspondent à l'espèce. *Peloploca undulata* (Bergey—1974) ou à *Microthrix parvicella* IV^e^ groupe (Eikelboom—1975).

CONCLUSIONS

Des données présentées, il résulte la grande variété des microrganismes filamenteux impliqués dans le phénomène de gonflage de la boue activée. Les possibilités de les identifier sont à présent limitées, d'une part à cause de la pauvreté des données en vue d'une caractérisation complète et d'autre part du fait de leur extraordinaire plasticité qui leur permet l'adaptation rapide et continue aux conditions de l'environnement.

L'implication des bactéries filamenteuses dans le phénomène de gonflage, c'est une certitude.

Ce qui reste à éclaircir c'est le mécanisme par lequel elles réalisent cela car, en dépit des apparences, leur morphologie n'explique pas, mais au contraire, contredit les modifications apparaissant au cours du gonflage. En effet, les chaînes de cellules sont beaucoup plus lourdes que les cellules isolées et, par conséquent, elles ont la tendance à se déposer et non pas a flotter. En outre, on connaît de nombreux types de bactéries filamenteuses qui ne modifient pas les caractéristiques de sédimentation de la boue.

Il y a trois particularités de certaines bactéries aquatiques, rencontrées fréquemment parmi celles filamenteuses qui paraît-il, se trouvent à la base du phénomène.

Il s'agit : de la présence de la gaine gelatineuse, des inclusions lipidiques qui réduisent leur poids spécifique de même que des vacuoles à gaz, dont la signification écologique est justement celle de les maintenir à la surface de l'eau où l'oxygénation est meilleure.

BIBLIOGRAPHIE

1. Bergey's, *Manual of Determinative Bacteriology*, Ed. 8 *th* 1974, The Williams and Wilkins Company, Baltimore, S.U.A.
2. Cyrus Z., Alena Sladka, Hydrobiologia, 1970, **35**, 383—396.
3. Dondero C. N., Adv. Appl. Microbiol., 1961, **3**, 77—105.
4. Eikelboom H. D., Water Research, 1974, **9**, 365—388.
5. Farquhar .I G., Boyle C. W., *Identification of Filamentous Microorganisms in Activated Sludge* J. W. P.C.F., 1971, **43**, 4, 604—622.
6. Lewin A. R., J. Gen. Microbiol, 1969, **58**, 189—206.

7. Mulder G. E., *Principles and Applications in Aquatic Microbiology*, ed. by H. Heukelekian and N. Dondero, New Jersey, 1964, 452 p.
8. Van Veen W. L., Antonie van Leeuwenhoek, 1973, **39**, 189–205.
9. Van Veen W. L., Van Der Kooit, Antonie van Leeuwenhoek, 1973, **39**, 207–216.
10. Adamse A. D., Water Res., 1968, **2**, 715–722.
11. Adamse A. D., Water Res., 1970, **4**, 797–803.

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CONTRIBUTIONS TO THE STUDY OF THE BIOLOGY OF *GLIOCLADIUM ROSEUM* BAINIER

BY

TATIANA ŞESAN

For the growth and sporulation of *Gliocladium roseum*, the most favourable carbon and nitrogen sources were: glucose and mannose, and peptone, DL-asparagine and ammonium nitrate and tartrate, respectively; the least favourable ones were glycerin and the amino acids D-serine, glycocoll and L-cystine. The optimum reaction of the Weindling medium for fungal growth was neutral and slightly alkaline (pH 7.0; 7.5 and 8.5), whereas for sporulation it was slightly alkaline (pH 7.5 and 8.0) and slightly acid (pH 6.0 and 6.4). The least sporulation occurred on media with alkaline and highly alkaline reaction (pH 10.0–13.0).

INTRODUCTION

The antagonistic ability of *Gliocladium roseum* Bainier to some plant parasites is cited in the specialty literature, available to us since 1958 [2]. Among the papers consulted we have found no data on the cultivation of this fungus on different growth media, on media with various carbon and nitrogen sources or with different reactions. Therefore, for the future use of this fungus in the biological control of plant diseases, we considered that some contributions to the study of its biology would be useful.

MATERIALS AND METHODS

Two isolates of *Gliocladium roseum* were used, i.e. Gl_1 — from soybean plants, cvar Merit, sampled from the district of Cluj, and Gl_2 — from pea seedlings, collected from the district of Argeş.

The influence of the same culture media, the same carbon and nitrogen sources and the same ranges of reaction of the Weindling medium on the growth and sporulation of *G. roseum* was followed up by the same method [1], [3]–[5], as in the case of the study of the biology of *Trichoderma viride* Pers. ex Fr. and *Trichothecium roseum* Link.

Each variant was tested in 5 replications. The data were processed statistically by variance analysis according to the PD_2F program in Fortran language.

RESULTS

The most favourable one out of the six culture media tested for the growth of *Gliocladium roseum* (table 1) was the Weindling medium, on which colony diameter was 1.900 cm after 2 days and 8.787 cm after

6 days. Next follows the Czapek-Dox medium, on which after 2 days there was no growth (0.700 cm in diameter), and after 6 days it attained 6.260 cm. A poorer growth was recorded in descending order with the

Table 1

Influence of different culture media on the growth and sporulation of *Gliocladium roseum*, isolate Gl_1

Culture medium	Colony diameter (cm) after 2 days: Diameter	2 days: Difference as to the average	2 days: Significance	6 days: Diameter	6 days: Difference as to the average	6 days: Significance	Sporulation
PDA	1.700	0.270		3.300	−0.850	000	++++
Malt extract	1.380	−0.050		3.820	−0.330		++++
Czapek	1.380	−0.050		3.700	−0.450		+++
Czapek-Dox	0.700	−0.730	000	6.260	2.110	×××	+++
Warcup	1.520	0.090		3.520	−0.630	00	++++
Weindling	1.900	0.470	×××	8.787	4.637	×××	++++
Average growth (check)	1.430	—		4.150	—		
LD 5%	= 0.266			= 0.473			
LD 1%	= 0.352			= 0.626			
LD 0.1%	= 0.456			= 0.810			

++++ = very good sporulation
+++ = good sporulation
++ = medium sporulation
+ = poor sporulation

malt extract media, Czapek and Warcup, on which colony diameter ranged between 1.380 and 1.520 cm and 3.520 and 3.820 cm after 2 and 6 days, respectively. The poorest results were obtained on PDA, on which the diameter of *Gliocladium roseum* colony attained 1.700 cm after 2 days and 3.300 cm after 6 days.

The sporulation was very good on Weindling, Warcup, PDA and malt extract and good on Czapek and Czapek-Dox media.

Among the different carbon sources tested (table 2), the monosaccharides were best assimilated, followed by disaccharides and polysaccharides.

As to the monosaccharides, the best results were obtained with glucose, mannose, fructose and ribose, the growth of the *Gliocladium roseum* colonies determined by them ranging between 1.533 to 2.000 cm after 2 days and 4.067 to 5.300 cm after 6 days. The assimilation of mannite, galactose and arabinose was poorer. On media containing these sugars, colony diameter was 1.033—1.667 cm after 2 days and only 3.233—3.767 cm after 6 days. Sorbose was least assimilated; on the respective medium the colonies attained 1.330 cm and 1.667 cm in diameter after 2 and 6 days, respectively.

Among disaccharides, melibiose and maltose promoted the best growth; in variants containing these sugars colony diameter ranged between 0.967—1.633 cm and 3.533—4.330 cm after 2 and 6 days, respectively. Saccharose was the least assimilated by *Gliocladium roseum* and determined a growth of 1.033 cm after 2 days and 3.000 cm after 6 days.

Table 2

Influence of different carbon sources on the growth and sporulation of *Gliocladium roseum* isolate Gl_1

Carbon source	Colony diameter (cm) after 2 days: Diameter	2 days: Difference as to the average	2 days: Significance	6 days: Diameter	6 days: Difference as to the average	6 days: Significance	Sporulation
MONOSACCHARIDES							
glucose	2.000	0.575	×××	4.467	0.851	×	++++
D-mannose	2.000	0.575	×××	4.067	0.451		++++
mannite	1.667	0.242		3.767	0.151		+++
D-galactose	1.433	0.008		3.467	−0.149		++++
fructose (levulose)	1.600	0.175		5.300	1.684	×××	++++
L-sorbose	1.333	−0.092		1.667	−1.949	000	+
D-ribose	1.533	0.108		4.500	0.884	××	+++
arabinose	1.033	−0.392	00	3.233	−0.383		+++
DISACCHARIDES							
saccharose (sucrose)	1.033	−0.392	00	3.000	−0.616		+
D-maltose	0.967	−0.458	000	3.533	−0.083		+++
cellobiose	1.400	−0.025		3.033	−0.583		+++
lactose	1.167	−0.258	0	3.233	−0.383		+++
melibiose	1.633	0.208		4.333	0.717	×	+++
POLYSACCHARIDES							
cellulose	1.767	0.342	××	4.367	0.751	×	+++
starch	1.500	0.075		4.800	1.184	×××	+++
inulin	1.167	−0.258	0	3.000	−0.616		+++
ALCOHOLS							
glycerine	1.000	−0.425	00	1.700	−1.916	000	+
average growth (check)	1.425	—		3.616	—		
LD 5%	= 0.246			= 0.659			
LD 1%	= 0.327			= 0.877			
LD 0.1%	= 0.426			= 1.141			

Among polysaccharides, starch and cellulose were better assimilated, the colony diameter increasing to 1.500—1.767 cm after 2 days and 4.367—4.800 cm after 6 days. Inulin was less assimilated (1.167 cm in diameter after 2 days and 3.000 cm after 6 days).

It was found that glycerin is a weak source of carbon, fungal growth being poor on the respective medium (1.000 cm in diameter after 2 days and only 1.700 cm after 6 days).

The sporulation of *Gliocladium roseum* was very high on media containing mannose, galactose, fructose; high on media with mannite, arabinose, ribose, maltose, lactose, cellobiose, melibiose, starch, cellulose, inulin and poorest on the variants containing sorbose, saccharose and glycerin; on those latter the lowest growth of the fungus was recorded.

Of the 23 sources of organic and mineral nitrogen (table 3), peptone, followed by amino acids and amides, and then by ammonium salts and nitrates, were most favourable to the growth of *Gliocladium roseum*.

On peptone medium, colony diameter ranged from 2.800 cm after 2 days to 8.737 cm after 6 days.

Among the amino acids tested, the following ones were best assimilated: DL-leucine, DL-citrulline, L-leucine, lysine. On the respective

Table 3

Influence of different nitrogen sources on the growth and sporulation of *Gliocladium roseum*, isolate Gl_1

Nitrogen sources	Colony diameter (cm) after 2 days: Diameter	Difference as to the average	Significance	Colony diameter (cm) after 6 days: Diameter	Difference as to the average	Significance	Sporulation
peptone	2.800	1.497	×××	8.737	5.673	×××	++++
AMINO ACIDS							
glycocoll	0.920	−0.383	000	2.760	−0.304	00	+
L-leucine	1.360	0.057		3.240	0.176		++
DL-leucine	1.520	0.217	×××	4.460	1.396	×××	+++
DL-nor-leucine	1.320	0.017		3.060	−0.004		+
tyrosine	0.960	−0.343	000	3.040	−0.024		++++
D-serine	1.000	−0.303	000	2.860	−0.204		+++
lysine	0.960	−0.343	000	3.160	0.096		++++
tryptophane	1.060	−0.243	000	2.900	−0.164		++++
L-cystine	0.940	−0.363	000	2.340	−0.724	000	+
DL-citrulline	1.540	0.237	000	3.360	0.296	××	+++
L-arginine	1.220	−0.083		3.020	−0.044		++
AMIDES							
DL-asparagine	1.600	0.297	×××	3.660	0.596	×××	++++
urea	1.560	0.257	×××	2.780	−0.284	0	+++
VITAMINS							
riboflavine	1.560	0.257	×××	3.680	0.616	×××	+++
NITRATES							
potassium nitrate	1.440	0.137	×	2.820	−0.244	0	++++
sodium nitrate	1.480	0.177	××	2.980	−0.084		++++
ammonium nitrate	1.920	0.617	×××	3.620	0.556	×××	++++
calcium nitrate	1.340	0.037		2.620	−0.444	000	++++
AMMONIUM SALTS							
ammonium tartrate	1.660	0.357	×××	3.160	0.096		++++
monobasic ammonium phosphate	1.240	−0.063		2.480	−0.584	000	++++
ammonium sulfate	1.120	−0.183	00	2.140	−0.924	000	++++
ammonium carbonate	1.060	−0.243	000	2.040	−1.024	000	++++
ammonium nitrate	1.920	0.617	×××	3.620	0.556	000	++++
average growth (check)	1.303	—	—	3.064	—	—	
LD 5%		= 0.113			= 0.222		
LD 1%		= 0.150			= 0.293		
LD 0.1%		= 0.191			= 0.374		

media, *Gliocladium roseum* grew up to 0.960—1.540 cm after 2 days and 3.160—4.460 cm after 6 days. The amino acids DL-nor-leucine, tyrosine and L-arginine follow in descending order, the growth determined by them was between 0.960—1.320 cm after 2 days and 3.020—3.060 cm after

6 days. D-serine, glycocoll, and L-cystine were least assimilated. In the respective variants, colony diameter ranged from 0.920 to 1.060 cm and 2.340 to 2.900 cm after 2 and 6 days, respectively.

As to the amides, DL-asparagine was better assimilated than urea. On media containing asparagine, the colonies were 1.600 cm in diameter after 2 days and 3.660 cm after 6 days, whereas on media containing urea the diameter recorded after the same time intervals was 1.560 cm and 2.780 cm, respectively.

The assimilation of riboflavine was also good; on the respective medium, the diameter of the *Gliocladium roseum* colonies attained 1.560 cm after 2 days and 3.680 cm after 6 days.

The assimilation of ammonium salts in particular and that of nitrates was fairly good, with a slight superiority of the former.

The most favourable ammonium salts were ammonium nitrate and tartrate, on the respective media the fungal colonies attaining 1.660—1.920 cm and 3.160—3.620 cm in diameter after 2 and 6 days, respectively. Monobasic ammonium phosphate, ammonium sulphate and ammonium carbonate were less assimilated and determined a growth of the colony diameter of 1.060—1.240 cm after 2 days and 2.040—2.480 cm after 6 days.

Among nitrates, the best assimilation was that of ammonium nitrate, and less that of sodium, potassium and calcium nitrates (1.340—1.480 cm and 2.620—2.080 cm after 2 and 6 days, respectively).

Sporulation was very high on media containing peptone, tyrosine, lysine, tryptophan, DL-asparagine, ammonium nitrates and salts; high on DL-leucine, D-serine, DL-citrulline, riboflavine and urea; medium on media with L-leucine, and L-arginine and poor on media with glycocoll, DL-nor-leucine and L-cystine.

As to the growth of *Gliocladium roseum* on the Weindling agar medium with different reactions, it appeared that, as compared to isolate Gl_2, the isolate Gl_1 has wide tolerance limits to the pH values. These range between 5.0 and 13.0 after 6 days' growth. Maximum growth was obtained on a neuter medium (pH 7.0) on which after 6 days the diameter of the colony attained 3.480 cm (table 4).

The limits of the tolerance to the reaction of the medium are narrower in isolate Gl_2 (table 5) as compared to isolate Gl_1, ranging between pH 6.0—8.5 after a 6 day growth. In the colonies of the isolate Gl_2, the maximum diameter was obtained on media with slightly alkaline reaction both after 2 days (2.460 cm in diameter on the medium with pH 8.0) and 6 days of growth (3.420 cm at the pH values of 7.5 and 8.5).

In isolate Gl_1 of *Gliocladium roseum*, the biomass was obtained at narrower pH limits, i.e. between 6.0 and 8.0, and in isolate Gl_2 at wider ones, i.e. between 4.0 and 8.5. Maximum biomass values were recorded on slightly alkaline medium (pH 8.0) with isolate Gl_1 and on slightly acid medium (pH 5.0) with isolate Gl_2 (tables 4 and 5).

The sporulation of *Gliocladium roseum* was very high on slightly alkaline (pH 7.5 and 8.0) and slightly acid (pH 6.0 and 6.4) Weindling medium; high and medium on acid (pH 4.0 and 5.5) and alkaline (pH 8.5 and 9.0) media and poor on highly alkaline media (pH 10.0—13.0).

Table 4

Influence of the reaction of the nutrient medium on the growth and sporulation of *Gliocladium roseum*, isolate Gl_1

Initial pH	Colony diameter (cm) after 2 days: average	2 days: Difference as to the check	2 days: Significance	Colony diameter (cm) after 6 days: average	6 days: Difference as to the check	6 days: Significance	Biomass (cg) after 21 days: average	Biomass: Difference as to the check	Biomass: Significance	Sporulation
1.0							1.898	−14.236	000	
2.0							2.668	−13.466	000	
3.0							8.868	− 7.266	000	
4.0	1.420	−0.120		2.300	0.080		12.556	− 3.578	000	+++
4.5	1.320	−0.220	000	2.280	0.060		12.216	−3.918	000	++
5.0	1.720	0.180	××	2.580	0.360	×××	17.316	1.182	×××	+++
5.5	1.580	0.040		2.380	0.160	××	11.746	− 4.388	000	+++
6.0	1.280	−0.260	000	2.640	0.420	×××	15.844	− 0.290		++++
7.0	1.360	−0.180	00	3.480	1.260	×××	14.060	− 2.074	000	+++
7.5	1.740	−0.200	00	3.160	0.940	×××	17.996	1.862	×××	++++
8.0	1.780	0.240	×××	3.460	1.240	×××	21.070	4.936	×××	++++
8.5	1.700	0.160	××	3.220	1.000	×××	11.494	− 4.640	000	+++
9.0	2.020	0.480	×××	2.760	0.540	×××	12.460	− 3.674	000	++
10.0	1.700	0.160	××	2.540	0.320	×××	11.748	− 4.386	000	+
11.0	1.720	0.180	××	2.320	0.100		15.970	− 0.164		+
12.0	1.760	0.220	×××	2.620	0.400	×××	8.490	− 7.644	000	+
13.0	1.680	0.140	×	2.660	0.440	×××	8.148	− 7.986	000	+
6.4 (check)	1.540	—		2.220	—		16.134	—		++++
	LD 5%	= 0.121			= 0.115			= 0.603		
	LD 1%	= 0.160			= 0.152			= 0.797		
	LD 0.1%	= 0.205			= 0.194			=1.019		

Table 5

Influence of the reaction of the nutrient medium on the growth and sporulation of *Gliocladium roseum*, isolate Gl_2

Initial pH	Colony diameter (cm) after 2 days: average	2 days: Difference as to the check	2 days: Significance	Colony diameter (cm) after 6 days: average	6 days: Difference as to the check	6 days: Significance	Biomass (cg) after 21 days: average	Biomass: Difference as to the check	Biomass: Significance	Sporulation
1.0							1.668	−5.686	000	
2.0							1.950	−5.404	000	
3.0							5.424	−1.930	000	
4.0	1.700	−0.080		2.540	−0.640	000	11.888	4.534	×××	+++
4.5	1.860	0.080		2.620	−0.560	000	10.226	2.872	×××	++
5.0	1.700	−0.080		2.560	−0.620	000	14.792	7.438	×××	+++
5.5	1.460	−0.320	000	2.480	−0.700	000	11.432	4.078	×××	+++
6.0	1.440	−0.340	000	3.200	0.020		10.082	2.728	×××	++++
7.0	1.220	−0.560	000	2.860	−0.320	000	12.408	5.054	×××	+++
7.5	2.080	0.300	×××	3.420	0.240	×××	9.514	2.190	×××	++++
8.0	2.460	0.680	×××	3.320	0.140	×	10.430	3.076	×××	++++
8.5	1.960	0.180	××	3.420	0.240	×××	8.080	0.726	×	++++
9.0	2.000	0.220	×××	3.020	−0.160	00	5.581	−1.770	000	+++
10.0	1.700	−0.080		2.540	−0.640	000	5.816	−1.508	000	+
11.0	1.860	0.080		2.520	−0.660	000	5.970	−1.384	000	+
12.0	1.720	−0.060		2.680	−0.500	000	10.606	3.252	×××	+
13.0	1.680	−0.100		2.640	−0.540	000	12.522	5.168	×××	+
6.4 (check)	1.780	—		3.180	—		7.354	—		++++
	LD 5%	= 0.121			= 0.115			= 0.603		
	LD 1%	= 0.160			= 0.152			= 0.797		
	LD 0.1%	= 0.205			= 0.194			= 1.019		

CONCLUSIONS

1. The fungus *Gliocladium roseum* grew very well on the Weindling medium, and then on Czapek-Dox medium, less so on malt extract, Czapek and Warcup, and very poorly on PDA.

2. Among the carbon sources tested, the monosaccharides were assimilated best, followed by disaccharides and polysaccharides. The following ones were the most favourable : glucose, mannose, ribose (monosaccharides), melibiose and maltose (disaccharides), starch and cellulose (polysaccharides). The poorest assimilation was that of sorbose, saccharose and glycerine.

3. The nitrogen sources supporting the best growth of *Gliocladium roseum* were, in descending order : peptone, amino acids, amides, ammonium salts and nitrates. The best assimilation was that of peptone, amino acids DL-leucine, DL-citrulline, L-leucine, lysine, amide, DL-asparagine, ammonium nitrate and tartrate. Tryptophane, D-serine, glycocoll, L-cystine, calcium and potassium nitrates etc. were least assimilated.

4. The behaviour of *Gliocladium roseum* on Weindling media with different reactions varied in terms of the isolate : the maximum growth of isolate Gl_1 was obtained on media with neutre reaction (pH 7.0), and of isolate Gl_2 on media with slightly alkaline reaction (pH 7.5 and 8.5). The maximum biomass was accumulated by isolate Gl_1 on media with slightly alkaline reaction (pH 8.0) and by isolate Gl_2 on slightly acid media (pH 5.0).

5. The sporulation of *Gliocladium roseum* was very high on Weindling, Warcup, PDA and malt extract agar media ; the values of the reaction of the Weindling medium supporting the best sporulation of the fungus were both the slightly alkaline (pH 7.5 and 8.0) and the slightly acid ones (pH 6.0 and 6.4) ; the alkaline values (pH 10.0—13.0) were the least favourable.

The best sporulation of *Gliocladium roseum* was obtained on the following sources of carbon : glucose, mannose, galactose, fructose, and on the source of nitrogen : peptone, tyrosine, tryptophane, DL-asparagine, ammonium nitrate and salts. The poorest fungal sporulation was recorded on media with sorbose, saccharose and glycerine as carbon sources and on media with glycocoll, DL-nor-leucine and L-leucine as nitrogen sources.

REFERENCES

1. Bontea Vera, Şesan Tatiana, St. cerc. biol.—Biol. Veget., 1980, **32**, *2*, 165—173.
2. Krassilnikov, N. A., 1958, *Antagonismul microbian şi substanţele antibiotice*, Gosudarstvennoe izdatelistvo "Sovetkaia nauka", Translated from Russian, Romanian Academy, Inst. St. Rom.—Sov., 1962.
3. Şesan Tatiana. St. cerc. biol.—biol. veget., 1981, **33**, *1*, 77—84.
4. Şesan Tatiana, St. cerc. biol.—biol. veget., 1982, **34**, *1*, 71—76.
5. Şesan Tatiana, St. cerc. biol.—biol. veget., 1982, **34**, (in press).

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IN VITRO ANTHER AND POLLEN CULTURE IN HAPLOIDS (n = 12) AND TRIPLOIDS (3n = 36) OF *DATURA INNOXIA* MILL.

BY

ELENA BADEA and PETRE RAICU

By experimental androgenesis with haploid and triploid *Datura innoxia*, plants with n, 2n, 3n, 4n and a small number of aneuploids were obtained. The aneuploid spores, resulted following meiosis in the case of plants with an odd number of chromosomes, undergo induction but their chromosome constitution does not allow a normal cytodifferentiation ; that is why high frequency aneuploids are not obtained.

INTRODUCTION

Experimental androgenesis investigations have been performed with haploid plants of *Datura metel* [1] and *Triticum aestivum* [2], haploids and diploids being obtained.

In the case of *Nicotiana tabacum*, an amphidiploid species, the production of plants with a various chromosome number was obtained by anther culture of triploid, tetraploid and chromosome autosubstituted diploid plants [6, 7]. By anther culture in the same species, nullisomics were isolated from monosomic lines [4] and nullihaploids from different genotypes [3, 5]. These results indicated that various types of aneuploid pollen in *Nicotiana tabacum* are able of differentiation into plants by the anther culture technique.

This paper reports experiments aimed at clarifying whether aneuploid pollen of *Datura innoxia* is able of differentiation or the obtaining of aneuploid plants by anther and pollen culture is possible.

MATERIAL AND METHODS

Four haploid (n = 12) and four triploid plants (3n = 36) of *Datura innoxia* produced by in vitro anther culture were used as a source of flower buds.

For the anther culture it was used the basic medium of Nitsch [9] supplemented with 0.1 mg/l of indole-3-acetic acid (IAA).

The anther cultures were kept under light for 16 hours (3000 lux) alternating with 8 hours darkness.

Pollen was cultured after the method of Wernicke and Kohlenbach [11]. Anthers precultured on liquid medium for 2—3 days were split open and by gentle squeezing the microspores were suspended in the cul-

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ture medium. The young embryos were transplanted onto the solid medium for further development.

Androgenetic plants were transplanted for rooting on Murashige-Skoog medium [6] without hormones.

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

RESULTS AND DISCUSSION

By experimental androgenesis with haploid and triploid *Datura innoxia*, there were obtained n, 2n, 3n, 4n plants and a small number of aneuploids (Table 1).

Table 1

Chromosomes number of androgenetic plants produced by anther culture of haploid and triploid plants

Chromosome number of donor plant	No. of plants examined	Type of culture	Number of plants with a various level of ploidy and aneuploidy				
			n	2n	3n	4n	aneuploids
n = 12	51	anther	22	26	1	2	—
n = 12	10	pollen	7	3	—	—	—
3n = 36	34	anther	1	21	2	8	2(34,35)
3n = 36	10	pollen	10	—	—	—	—

Anther productivity was higher in cultures of haploid plants than in those of triploids. A characteristic aspect of the cultures of triploid plants was the presence of a greater number of abnormal plantlets which died in the early development stages so that the efficiency of those cultures was reduced.

Differences concerning the productivity and the response of anthers collected from different triploid plants were noticed. So, from the four triploid plants used as a donor, only one had the anther response higher and more normal plants were obtained.

No differences were noticed as concerns the productivity and the response of anthers from haploid plants.

In the androgenetic descendence obtained by the anthers culture from haploids of *Datura innoxia*, the diploid and haploid plants prevail as in the case of *Datura metel* [1] and *Triticum aestivum* [2].

By the culture of anthers from triploid plants of *Datura innoxia* there were obtained especially diploid plants and a small number of tetraploid and aneuploid plants.

The genetic nonequilibrated spores, resulted after meiosis in the plants with an odd number of chromosomes, either do not undergo induction or the aneuploid embryos are aborted in the competition with those having an equilibrated chromosome number.

Part of the aneuploid spores undergo an induction process, which is proved by the great number of abnormal plants that cannot continue

the development, they probably coming from spores whose chromosome constitution does not allow a normal cytodifferentiation.

The small number of aneuploids obtained could be due to the competition between the embryos in the limited anther space. In order to eliminate this competition, pollen cultures from haploid and triploid plants were made.

The cytogenetic analysis of the plants obtained by the pollen culture evinced that the haploids prevail both in the cultures from haploids and in those from triploid plants, that means in the competition between embryos, the diploid ones have a better chance.

By the culture of pollen from haploid and triploid plants, aneuploids were not obtained.

For a normal cytodifferentiation process in *Datura innoxia* at least a chromosome complement is hence necessary. The aneuploid spores undergo induction but their chromosome constitution does not allow a normal cytodifferentiation.

The aneuploid plants obtained, with a normal growth and development, had 34 and 35 chromosomes [10], that is with *Datura innoxia*, the presence of a great number of genomes is compatible with aneuploidy.

REFERENCES

1. Chandy L. P., Narayanaswamy S., Ind. J. Exper. Biol., 1971, **9**, 472–475.
2. De Buyser J., Henry Y., Theor. Appl. Genet., 1980, **57**, 49–56.
3. Gorenflot R., Raicu P., Pelletier G., Pătrașcu M., C.R. Acad. Sci. Paris, 1975, **281**, series D, 1097–1100.
4. Mattingly C. F., Collins G. B., Chromosoma, 1974, **46**, 29–36.
5. Moore G. A., Collins G. B., Agronomy Abstracts, 1979, p. 70.
6. Murashige T., Skoog F., Physiol. Plantarum, 1962, **15**, 437–494.
7. Niizeki M., Kita F., Japan. J. Breed., 1975, **25**, 258–264.
8. Niizeki M., Kita F., Japan. J. Breed., 1975, 25, 52–58.
9. Nitsch J. P., Phytomorphology, 1969, **19**, 389–404.
10. Raicu P., Badea E., in press.
11. Wernicke W., Kohlenbach H. W., Z. Pflanzenphysiol., 1977, **81**, 330–340.

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I. TARNAVSCHI, GABRIELA ŞERBĂNESCU-JITARIU, NATALIA MITROIU-RĂDULESCU, DIDONA RĂDULESCU, *Morfologia polenului florei din România* (A Monograph of Pollen in the Flora of Romania) vol. 1, Editura Academiei, Bucureşti, 1981, 144 p., 53 pl., 640 figs.

This first volume of a prestigious series analyses the microspores of 30 botanical families with 692 taxa in the Romanian flora, among which the most important are: *Aceraceae*, *Amaranthaceae*, *Boraginaceae*, *Campanulaceae*, *Caryophyllaceae*, *Chenopodiaceae*, *Compositae*, *Convulvulaceae*, *Crassulaceae*. The authors, representatives of the renowned Romanian palynological school, have been studying the microspores for more than two decades offering a very useful reference work. It includes a brief introduction, a historical presentation of researches in the field, the method of investigation, terminology, graphic material, morphological analyses of microspores (97 p.) and an index of actualized Latin names.
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The exquisite graphic illustration was done under the light microscope. Although it hardly reveals details of the pollen grain as the electronmicroscopy does, it nevertheless remains useful for comparison purposes in other fields such as: paleoclimatology, paleogeography, pedology, archaeology, medicine, geology. For each analysed grain, there is a drawing with the respective ornamentation and a partial optical section.

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G. Dihoru

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