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SOMMAIRE

CENOTAXONOMY OF HALOPHYLE PHYTOCOENOSES (*PUCCINELLIO-SALICORNIETEA* CLASS ŢOPA 39) FROM ROMANIA (I)

V. SANDA and A. POPESCU

The paper analyses the coenotic structure, the geographic distribution, the dynamics and tendencies of evolution of halophyle phytocoenoses, 31 in number, placed between *Salicornietalia* Br.—Bl. (28) 33 and *Puccinellietalia* Soó 40.

This vegetation class is characterized by compulsory halophytes, either the common ones (*Aeluropus littoralis*, *Salicornia europaea*, *Suaeda maritima*, *Spergularia marina*, *Plantago maritima*) or the vicarious ones (*Puccinellia limosa*, *Limonium gmelinii*, *Plantago schwarzenbergiana*, *Aster tripolium* var. *pannonicus*, *Artemisia santonicum*) which give the phytocoenoses a peculiar colour (S. Ţopa, 1939)

E. Ţopa (1939), I. Todor (1947, 1948) and I. Şerbănescu (1965) wrote most valuable papers upon the halophyle vegetation from Romania.

SALICORNIETALIA Br.-Bl. (28) 33

It groups together strong salt grounds with excess of humidity during spring time and at the beginning of summer characterized by compulsory halophytes.

Thero-Salicornion Br.-Bl. (30) 33; Pign. 53

The characteristic species both for this alliance and for the *Salicornietalia* order are compulsory halophytes vegetating on strong salt grounds especially with chlorides. These are: *Salicornia europaea*, *Suaeda maritima*, *Aeluropus littoralis*, *Salsola soda*, *Bassia hirsuta*.

1. *Salicornietum europaeae* Wendelbg. 53

The association is characteristic to strong salt grounds with excess of humidity during spring time and at the beginning of summer. It is spread both on maritime sands and on the continental ones being characteristic of the chloride type salt grounds. Owing to the fact that water subsides from these soils late at the end of May the vegetation appears a lot later after the drying of the substratum. With the exception of the prevailing species *Salicornia europaea* there are also never failing species on these soils such as: *Suaeda maritima*, *Spergularia media*, *Puccinellia limosa*, *Plantago maritima*, *Juncus gerardi*, *Centaurium spicatum*, halophyle species well adapted to these conditions. In Transylvania, the association presents differential species such as: *Triglochin maritimus* and *Scorzonera parviflora*.

Owing to the soil drainage the association develops to the *Suaeda maritima* and *Obione pedunculata* ones. On less salted grounds the evolution grows to *Puccinellietum distantis* and *Obionetum verruciferae*.

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2. *Suaedetum maritimae* Soó 27

It develops on strong chloride salt grounds damp mainly during spring time and at the beginning of summer. Together with *Salicornia europaea* or sometimes mixed with it, *Suaeda maritima* is one of the first plants that fixed on strongly salted grounds contributiong thus to the irprocess of formation and of lying fallow. Because of the salt concentration the association is poor in species these being compulsory halophyle with few individuals usually quartered at the edge of the prevailed population by the characteristic species.

Suaeda maritima develops like an enclosure around the excavations on the sands between Mamaia and Năvodari (A. Popescu, V. Sanda ,1973) marking a frontier towards the outside for the *Salicornietum europaeae* association and often growing in mixture in their interference area. When the saltness of the soils is less in quantity the association may be improved by oversowing with *Puccinellia distans*, *Agropyron elongatum* , *Juncus gerardi*, *Aster tripolium* (I. Şerbănescu, 1965).

3. *Aeluropo-Salicornietum* Krausch 65

The association has been described on the halophyle sands from the Danube Delta in Letea (Krausch, 1965). On the sands of the offshore bar representative phytocoenoses have been found in small hallow grounds in the south of Cap Midia (Popescu, Sanda, 1975) where the salt concentration allows the development of the two characteristic species. As part of the association there appear : *Suaeda maritima, Limonium bellidifolium, Spergularia media,* halophyle plants most prevalent on the respective lands.

4. *Salsoletum sodae* Slavnić 39

It is an association in connection with strong chloridic salt grounds, more or less humid, vegetating sometimes in poorer salt grounds. It also develops on maritime dunes higher abundant in humidity especially round the microdepressions where more water is accumulated. From the great number of individuals formed during spring time around the dried shrubs from the previous year some disappear but a rather big number of them reach maturity managing to keep a balance within the vegetation of the moderate wet and salted sands. More frequently met species within the association are : *Suaeda maritima*, *Salicornia europaea*, *Halimione pedunculata*, *Crypsis aculeata* on the continental lands and *Centaurea arenaria*, *Cakile maritima*, *Bromus tectorum*, *Chenopodium glaucum* for the phytocoenoses on maritime sands.

5. *Suaedeto-Kochietum hirsutae* (Br.-Bl. 28) Ţopa 39

(Syn. : *As. de Bassia hirsuta* I. Şerbănescu 65)

The association is developing on very strong and very humid chloridic salt grounds, water excess being an essential element for the existence of these phytocoenoses. From the accompanying species we may mention :

Salicornia europaea, *Aeluropus littoralis*, *Puccinellia limosa*. The association also installs on the halophyle sands at the outskirts of the established *Salicornia europaea* and *Suaeda maritima* phytocoenoses.

The following facies within the association were signaled by E. Ţopa : *Bassisa hirsuta*, *Suaeda maritima*, *Salicornia europaea* and *Aeluropus littoralis*. They are characterized by a certain degree of salinity and humidity

6. *Puccinellio-Salicornietum* Popescu et al. 87

It vegetates on the Stipoc sand bank where the two characteristic species *Salicornia europaea* and *Puccinellia limosa* form phytocoenose with an average covering of 75—85%. From the accompanying species more frequently met we may mention : *Aster tripolium*, *Aeluropus littoralis*, *Spergularia media*, *Trifolium fragiferum*, *Suaeda maritima*, *Bolboschoenus maritimus*, *Juncus gerardi*. They are indicatory and specific for the salted and humid grounds. The association accomplishes the passing from the strong salt grounds phytocoenoses established as *Salicornia europaea* and quartered in humid microdepressions, towards those accomplished by *Puccinellia limosa* quartered on less humid soils with medium salt volume.

PUCCINELLIETALIA Soó 40

Characteristic species : *Artemisia maritima*, *A. santonicum*, *Atriplex littoralis*, *Bupleurum tenuissimum*, *Hordeum hystrix*, *Lepidium perfoliatum*, *Plantago maritima*, *Scorzonera cana*, *Taraxacum bessarabicum*, *Trifolium ornithopodioides*.

Puccinellion peisonis (Wendelbg. 43) Soó 57

Characteristic species : *Lepidium crassifolium*, *Puccinellia intermedia (P. peisonis)*.

7. *Puccinellietum distantis* Soó 37 ; Knapp 48

Puccinellia distans is spread on the salt grounds of the entire country as it is the taxon with the largest ecological exactness. The association is connected with low places with accentuated humidity in the first part of the vegetation season. As a result of the amplitude of soil salt concentration, *Puccinellia distans* phytocenoses are sometimes accompanied by a great number of unhalophyle species. Numerous facies are described within the association : *juncetosum gerardii* Mititelu et al. 67, *plantaginetosum tenuiflorae* Mititelu et al. 67, *polygonetosum aviculare* Mititelu et al. 67, *Scorzonera austriaca*, *Podospermum canum*, *Trigonella procumbens* Răvăruţ et al. 68.

I. Şerbănescu (1965) signals out a number of 20 transitional stages from the strongest halophyle with *Salicornia europaea* to those poor in salt with *Cynodon dactylon*.

8. *Lepidio (crassifolio)-Puccinellietum limosae* (Rapaics 27) Soó 57 (Syn.: *Lepidietum cartilaginei* Rapaics 27, *Lepidietum crassifolii* Ţopa 39, As. prov. de *Lepidium crassifolium* Şerbănescu 65).

Signaled out by E. Ţopa (1939) in the salt grounds from North Romania, the association is also spread in the East of the Romanian Plain where it has been minutely analysed by I. Şerbănescu (1965). The association is connected with old salt grounds with sodium carbonate which can be met both on old valleys and on high plains. The component species are generally compulsorily halophyte. From these we may mention: *Puccinellia distans*, *Camphorosma annua*, *Agropyron elongatum*, *Plantago maritima*, *Spergularia media*, *Dianthus guttatus*. I. Şerbănescu (1959) signals out the subassociation *caricetosum secalinae* Soó 57 in the East of the Romanian Plain.

Puccinellion limosae (Klika 37) Wendelbg. 43, 50

Characteristic species: *Aster tripolium* ssp. *pannonicus*, *Bassia sedoides*, *Camphorosma annua*, *Juncus gerardi*, *Kochia prostrata*, *Lotus tenuis*, *Pholiurus pannonicus*, *Plantago tenuiflora*, *Puccinellia limosa*.

9. *Plantagineto (cornuti)-Agrostetum stoloniferae* Soó et Csűrös 44 corr. 73.

A minute analysis of the stational conditions and of the association structure is made by Margareta Csűrös-Káptalan (1965) who mentions it in the Valley of Aiton where this association vegetates on chlorine sulphated solonchalks, humificated with argillaceous consistence moist and humid during the whole period of the year. The association is characterized by the preponderance of hygro-mesophyll hay species. Among the halophytes constantly met we may mention: *Plantago cornuti*, *Scorzonera parviflora*, *Trifolium fragiferum*, *Aster tripolium*, *Triglochin maritimus* and *Juncus gerardi*.

10. *Funarietum hungaricae* Ştefureac 65

Funaria hungarica is a compulsorily halophyle moss which frequently vegetates in the stations of the *Puccinellietum distantis* and *Festucetum pseudovinae* associations. It is usually less frequent among the groups that are quartered on the strong halophyle lands. A minute study upon the stations with *Funaria hungarica* is accomplished by Tr. Ştefureac (1965).

11. *Staticetum limonii* Borza 66

The association has been described by Al. Borza (1966) in Gălbinaşi—Vasilaţi, Giurgiu county, where it develops on poorer salted grounds. The species comprised in the structure of the phytocoenoses of *Statice limonium* are: *Bupleurum tenuissimum*, *Lotus tenuis*, *Trifolium fragiferum*, *Scorzonera cana*, *Atriplex littoralis*, *Taraxacum bessarabicum*, etc.

Owing to the fact that the characteristic species *Statice limonium* as well as its companions are less halophyle plants, the author of the association himself considers that this can be better appointed to another alliance.

12. *Puccinellietum limosae* Rapaics 27

Vegetates on the solonetzs usually situated in depressions marshed during spring time and dried during summer. The superficial stratum of the soil has a neutral reaction up to basic, the concentration in mineral substances being richer in summer months as a result of water evaporation while the soil dries and splits. The association reaches the developmental climax during spring time and at the beginning of summer forming undersized lawns of 10—15 cm. The most frequent halophytes met in these lawns are: *Juncus gerardi*, *Hordeum hystrix*, *Lotus tenuis*, *Trifolium fragiferum* and *Halimione pedunculata* with which many a times comes to be codominant.

As it is well eaten up by the animals the association suits to the improvement of solonetzs washed beforehand.

The *cynodontetosum* I. Kárpati 59 n.n. subassociation installs preponderantly on higher lands where there is a poor saltness of the ground making thus the passing from halophyle associations to the xeric-mesophyll or xerophyte ones.

The *erysimetosum repandi* (I. Şerbănescu 65) Sanda, Popescu, Doltu 80 (Syn.: As. de *Erysimum repandum* I. Şerbănescu 65) subassociation is connected with more or less argillaceous soils, poor in ground saltness.

The following subassociations are cited: *puccinellietosum* Soó 64, *transsilvanicum* Soó 25, *camphorosmetosum* Slavnić 48 and *scorzoneretosum austriacae-mucronatae* Sanda et al. 78 (Syn.: As. de *Scorzonera austriaca* var. *mucronata* I. Şerbănescu 63).

I. Pop (1968) signals out two facies in Criş Plain: *spergulariosum* and *asterosum*.

13. *Halocnemetum strobilacei* (Kelller 25) Ţopa 39

The association has been described by E. Ţopa (1939) from Sinoe and contains a limited number of species. Beside the prevailing species *Halocnemum strobilaceum* there also vegetate: *Frankenia hirsuta*, *Halimione verrucifera*, *Limonium bellidifolium*, *Petrosimonia oppositifolia*, *Suaeda maritima*, *Salicornia europaea*, *Limonium gmelinii*, *Aeluropus littoralis*, *Lotus tenuis*, *Spergularia media*, *Plantago maritima*.

It vegetates on halophyle, humid soils and it is quartered on the solonchalks around the Sinoe, Goloviţa, Razelm and Smeica lakes.

14. *Aeluropetum littoralis* (Prodan 39) Şerbănescu 65; (Bilik 56) Krausch 65.

The association is spread in the Brăila Plain, the Danube Delta and Dobrogea: it develops on more or less sandy soils, strongly salted, rich in humidity but there where water is not marshy. Within the association we frequently find: *Limonium gmelinii*, *Puccinellia limosa*, *Spergularia me-*

dia, Juncus gerardi, Plantago maritima, Juncus acutus in the coastline phytocoenosy (A. Popescu, V. Sanda, M. I. Doltu, 1980), and *Suaeda maritima, Salicornia europaea, Halimione pedunculata, Aster tripolium, Salsola soda* (I. Şerbănescu, 1965) in the continental ones.

15. *Plantaginetum maritimae* Rapaics 27

In the Romanian Plain the association forms phytocoenoses whose ecology is connected with old saltnesses especially based on sodium carbonate. They form a hard, 20 cm thick, crust at the surface, during spring time. In Transylvania, phytocoenoses of *Plantago maritima* vegetate on constantly humid soils generally chloridic and have differential species *Triglochin maritimus* and *Scorzonera parviflora*. The association has as differential species *Plantago coronopus* in the Danube Delta. The regional subassociation *deltaicum* Sanda et Popescu described on the Sulina sands, prefers small hollowed grounds with constant humidity ensured by rainfall or by the ground-water layer that can be found a few centimeters below.

16. *Agrostetum ponticae* Popescu et Sanda 73

Described on the maritime sands between Mamaia and Năvodari, the association is rather spread in the Danube Delta too; there it also vegetates on sands with different degrees of saltness of the ground. Stretched lawns may be found on Letea sand bank occupying large surfaces between Letea village and the forest. In years with abundant rain-falls the characteristic species develops at large being thus harvested as hay. The fodder is however of mediocre quality because of the very stiff stalk of the species *Agrostis pontica*. The accompanying species are numerous in great majority mesophyll but we may meet here many specific halophyle or psammophyle species.

Evd. Puşcaru-Soroceanu (1966) reminds of the coastline phytocoenoses belonging to this species. They are named As. *Agrostis alba* var. *pontica* but the author does not give any of their floristic composition.

17. *Staticeto-Artemisietum monogynae (santonicum)* Topa 39

It generally installs on solonetzs and solonchalks with poor salinization occupying sometimes sulphate and carbonate salt grounds. It is to be usually found at the edge of strong salt grounds where the soil keeps its humidity. Owing to their requests towards the soil humidity in soil, the characteristic species *Artemisia santonicum* and *Limonium gmelinii* although developing on the same sort of soil, tend to maintain separately. *Limonium gmelinii* occupies the depths of soil where twater exceeds for a good period of time and *Artemisia santonicum* populates the ground elevations a lot drier.

I. Todor (1948) noticed, as result of these conditions, that the association comprises several subassociations in which one of the characteristic species *Puccinellia limosa* is dominant.

staticetosum Todor 48 (Syn.: *limonietosum gmelini* Adelina Pop 77, *Limonio-Artemisietum salinae* Soó 71, As. de *Statice gmelini* I. Şerbănescu

65) subassociation described at Sărate-Turda Springs is also rather frequently spread in Muntenia Plain where it installs on soils with a moderate concentration but rich in humidity.

The *artemisietosum* Sanda, Popescu, Doltu 80 (Syn.: *Artemisietum salinae* auct. rom. non Soó 27) subassociation installs on the positive formations of the microrelief with less salt concentration and lower humidity. Within this subassociation there are many compulsory halophyle species missing such as: *Suaeda maritima, Salicornia europaea* or their presence is absolutely causal.The unhalophyle species are numerous which indicates the passing towards the nonsalted ground vegetation. Thus I. Şerbănescu (1965) indicates the stage with *Artemisia austriaca* where the steppe species abound.

18. *Aeluropo-Puccinellietum limosae* Popescu et Sanda 75

Both *Aeluropus littoralis* and *Puccinellia limosa* are species with large spreading on halophyle sands with a great degree of humidity. Stretched surfaces where the two species are codominant are to be found between Năvodari and Cap Midia. *Aeluropus littoralis* is usually more abundant and owing to its biological particularities of emitting stolons the land is well covered and the development of other competitor species is stopped. The most constant accompanying species are: *Limonium gmelinii, L. bellidifolium, Plantago maritima, Salicornia europaea, Spergularia media, Rumex maritimus*, etc.

19. *Hordeetum maritimi* I. Şerbănescu 65

The association vegetates on poorly salted soils, more humid, well beaten and flooded during autumn and spring time. The association occupies considerable surfaces on the coastline in Cap Midia (Popescu, Sanda, 1975). In Sulina it can be frequently found in courtyards, vacant lands and in the city park where it grows together with: *Plantago coronopus, Juncus gerardi, Lotus tenuis, Lepidium latifolium, Spergularia media, Puccinellia distans*, indicatory plants for the sand salinity.

20. *Hordeetum hystricis* (Soó 33) Wendelbg. 43

It vegetates on more or less solonized solonetzs. *Hordeum hystrix* invades the soils there where secondarily the superior sphere is explored as it is humid in spring but dried during summer time.

In this association along the characteristic species we may more frequently meet the following: *Festuca pseudovina, Poa bulbosa, Cerastium dubium, Scleranthus annuus, Trifolium parviflorum, Scorzonera cana*. The association represents a pionneer coenotaxob characterized by annual species and by the codomination of the hemicryptophytes.

21. *Bassietum sedoidis* (Ubrizsy 49) Soó 64

The association installs at the extremity of salt grounds towards the steppe on soils with sandy structure and poor salinity. It may be found at North-West Sacalin island on humid and salted sands creating a band of *Acorellus pannonicus*. Here, the great number of individuals belonging to the

characteristic species impedes the installation of other species. However, there have been noticed the following, within the association : *Atriplex hastata*, *Acorellus pannonicus*, *Juncus gerardi*, *Salicornia europaea* and *Suaeda maritima*.

The presence of the species *Atriplex tatarica* in the phytocoenoses from the Romanian Plain (I. Şerbănescu, 1965) indicates a poor salinization and a ruderalization of this association through grazing.

In Moldavia (Corbu Nou) the subassociation *atriplicetosum littoralis* Soó 57 is cited.

22. *Agropyretum elongati* I. Şerbănescu 59 ; Vasiu et al. 63

Agropyron elongatum is a species adapted to the salting conditions of the soil, forming circular and compact bushes. It is the reason for which its phytocoenosys may be found in few species with a small number of individuals. Within the association there take part both species with strong salt soils *(Suaeda maritima, Spergularia media, Halimione pedunculata)* and some supporting halophyles as : *Atriples tatarica* and *Cynodon dactylon*. The installation of weed species is done when the association has been used as pasture.

23. *Pholiuro-Plantaginetum tenuiflorae* (Rapaics 27) Wendelbg. 43

(Syn. : As. de *Pholiurus pannonicus*, I. Şerbănescu 65).
The association installs in depressionary places where water stagnates until late in spring. It usually vegetates on sulphatic and carbonated salt grounds. It occupies small surfaces in the Romanian Plain.

The following subassociations are known : *pholiuretosum* Soó 64 ; *plantaginetosum tenuiflorae* Soó 64 (Syn. : *Polygono-Plantaginetum tenuiflorae* I. Pop 68) ; *myosuretosum* Slavnić 48, Grigore 71 ; *polygonetosum avicularis* Wendelbg. 50 and *lepidietosum ruderale* Adelina Pop 77.

24. *Camphorosmetum annuae* (Rapaics 16) Soó 33 corr. Soó 68

(Syn. : *Camphorosmetum ovatae* Rapaics 16).
It was signaled out by E. Ţopa (1939) for the first time in Romania. The association develops on older surfaces of salt grounds belonging to the chloridic salt grounds area. It installs on soils strongly salted and poorly structured. The association claims a humidity increase in the first period of vegetation. During summer time the groups of *Camphorosma annua* distinguish themselves easily owing to the russet colour of the stems. We may mention the following more frequent species that belong to the association structure : *Plantago maritima*, *P. tenuiflora*, *Artemisia santonicum*, *Cynodon dactylon*, *Atriplex tatarica*, etc.

Within the association there are signaled the following subassociations : *festucetosum* Mititelu et al. 67 ; *plantaginetosum maritimae* Wendelbg. 50 ; *puccinellietosum* Mititelu et al. 67 ; *matricarietosum salinae* Grigore 71 ; *matricarietosum* Slavnić 53 and *artemisietosum* Soó (47) 64.

25. *Camphorosmetum monspeliacae* (Ţopa 39) Şerbănescu 65

Camphorosmetum monspeliacae is bound to the calcium carbonate solonetzs but may be found on more or less rich in calcium carbonate loess.

Many halophytes take part in the structure of the phytocoenoses : *Artemisia maritima*, *Limonium gmelini*, *Plantago maritima*, *Halimione verrucifera*, *Puccinellia distans* ; they use the salts from the depth. Other species like : *Lepidium ruderale*, *Trifolium parviflorum*, *Cerastium dubium* and *Hordeum maritimum* indicate a poor salinization. In the process of soil washing the association develops towards the installation of the vegetation of dominant steppe in the initial stages of *Poa bulbosa* passing afterwards towards the installation of phytocoenoses of *Artemisio-Festucetum pseudovinae*.

I. Şerbănescu (1960) described the *camphorosmetosum monspeliacae-ovatae* subassociation in Oltenia.

26. *Petrosimonietum triandrae* Todor 48 ; Şerbănescu 65

The association has been described by I. Todor (1948) in Băile Sărate—Turda. The characteristic species is specific to salt grounds with medium up to high concentration, in the last situation not lacking the species : *Suaeda maritima*, *Salicornia europaea*, *Bassia hirsuta*, etc. The steppe-sizing tendency of these phytocoenoses is indicated with the help of *Cynodon dactylon*, *Polygonum aviculare* and *Artemisia austriaca* installation.

27. *Obionetum verruciferae* (Keller 23) Ţopa 39 ; Prodan 39

The association is bound to argillaceous compact soils with a higher or a lower humidity and saltness accent in depth. When the association is temporarily flooded the salts come to the surface too. Among the compulsory dominant halophytes within the association we may mention : *Puccinellia limosa*, *Suaeda maritima*, *Limonium gmelinii*, *Artemisia maritima*, *Salicornia europaea* species that indicate a strong salinization at soil surface.

In the salt grounds from Caragele (Buzău district) the phytocoenoses of *Halimione verrucifera* are quartered on higher places on rising grounds while *Juncetum gerardii* populates a lot more humid microdepresions.

28. *Obionetum pedunculatae* I. Şerbănescu 65

It is characteristic for the strong salt grounds in the eastern Muntenia Plain. The characteristic species *Halimione pedunculata* develops abundantly stopping the installation of other plants. Among the species most frequently met within the phytocoenoses organized by *Halimione pedunculata* we may mention : *Puccinellia distans*, *Spergularia media*, *Salicornia europaea*, *Juncus gerardi*, etc. The number of the individuals belonging to the species mentioned above may be sometimes most numerous achieving thus well delimited facies. The most spread phytocoenoses with *Halimione pedunculata* can be met in Ialomiţa, Brăila and Buzău counties.

29. As. *Nitraria schoeberi-Obione portulacoides* (Şerbănescu 39)

Borza 60 (Syn. : *Nitrario-Artemisietum maritimae* Mititelu et al. 82).

Nitraria schoeberi may be found at Policiori-Pîclele (Buzău county) vegetating together with numerous halophyle species like : *Halimione*

verrucifera, Artemisia maritima, Lotus angustissimum, Artemisia pontica, Puccinellia limosa, Festuca pseudovina ssp. salina, Limonium gmelinii, Scorzonera cana, Achillea setacea, Matricaria chamomilla. The phytocoenoses vary between 50—90% as surface and may be found on the dried alluvial deposites and marls around Pîcla Mare sout-eastern Pîcla Mică as well as on the valleys of Pîclele rivulet. Owing to the fact that *Nitraria schoeberi* is systematically destroyed by burning and uprooting and the reservation is constantly gazed, protection measures for it are indispensable; the protection must be done with muddy vulcano to preserve the species in a place in the country where it vegetates.

30. *Leuzeetum salinae* (Borza 31 n.n.) Răvăruț 58

(Syn.: *Leuzeeto-Oenanthetum silaifoliae* (Borza 31 n.n.) Țopa 39).
The association corresponds to the hayfields with *Leuzea salina* signaled out by Al. Borza (1931) in Moldavia and named by E. Țopa (1939) as *Leuzeeto-Oenanthetum silifoliae*. The phytocoenoses of *Leuzea salina* Northern Romania are characterized by the presence of the species *Peucedanum latifolium, Iris halophila, Aster sedifolius* and *Scorzonera austriaca* var. *áucronata* all with sinecological affinities gathered together by E. Țopa (1939) in the alliance *Puccinellio-Staticion gmelini*.

From the other accompanying species more frequent are: *Lotus tenuis Taraxacum bessarabicum, Juncus gerardi, Aster tripolium* ssp. *pannonicus, Plantago schwarzenbergiana* and *Limonium gmelinii*.

In the salt ground from Muntenia Plain especially in those from the Călmățui and Buzău valleys phytocoenoses from the *Leuzeetum salinae* association are frequently met. They develop in intensely flooded fields during spring time.

31. *Iridetum halophilae* (Prodan 39 n.n.) I. Șerbănescu 65

The *Iris halophila* association form phytocoenoses extended especially along the Ialomița river and it depends on the alluvial soils with sandy structure, poorly up to very poorly salinized. Within these phytocoenoses a great number of halophyle species take part, such as: *Spergularia media, Camphorosma annua, Juncus gerardi, Puccinellia distans, Atriplex littoralis*, etc. Owing to the intense grazing the phytocoenoses are invaded by numerosus ruderal species.

REFERENCES

1. Borza Al., Contrib. Bot., Cluj, 1966, **2**, 146—162.
2. Csurös-Káptalan Margareta, Contrib. Bot. Cluj, 1965, 221—229.
3. Doltu M. I., Sanda V., Popescu A., Muz. Brukenthal. St. și Comunic. Șt. Nat. Sibiu, 1977, **23**, 197—219.
4. Krausch H. D., Limnologica (Berlin), 1965, **3** (3), 271—313.
5. Mititelu D., Ștefan N., Ciupercă Gh., St și Comunic. Șt. Nat. Bacău, 1982, 99—120.
6. Pop Adelina, *Halofitele din cîmpia joasă a Timișului. Studiu floristic, ecologic și geobotanic.* Summary of the Doctoral Thesis, Cluj-Napoca, 1977.
7. Pop I., *Flora și vegetația Cîmpiei Crișurilor. Interfluviul Crișul Negru—Crișul Repede.* Ed. Academiei, Bucharest, 1968.

8. Popescu A., Sanda V., St. cerc. biol., Seria Bot. 1973, **25** (2), 113—130.
9. Popescu A., Sanda V., Rev. roum, Biol., Sér. Bot., 1975, **20** (1), 7—17.
10. Popescu A., Sanda V., Peuce. St. Comunic. Șt. Nat. Tulcea, **5**, 1976, 193—216.
11. Popescu A., Sanda V., Doltu M. I., Muz. Brukenthal. St. și Comunic. Șt. Nat. Sibiu, 1980, **24**, 147—1314.
12. Popescu A., Sanda V., Fișteag Gabriela, St. cerc. biol., Seria biol. veget., 1987, **30** (1), 25—33.
13. Sanda V., Popescu A., St. cerc. biol., Seria Bot., 1973, **25** (5), 399—424.
14. Șerbănescu I., Com. Geol., St. tehnice și econ. Seria C. Pedol., 1965, **15**, 1—149.
15. Ștefureac Tr., *Studii biologice în unele formațiuni de vegetație din România (sărături, sfagnete, păduri).* Ed. Academiei, Bucharest, 1969.
16. Todor I., Bul. Grăd. Bot. și al Muz. Bot. Cluj, 1947, **27** (1—2), 1—64; 1948, **28** (1—2): 21—175.
17. Țopa E., Bul. Fac. Șt. Cernăuți, **13**: 1—79, 1939.

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PLANT REGENERATION FROM CROWN GALL OF *DATURA INNOXIA*

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Crown galls were induced on *Datura innoxia* with different *Agrobacterium tumefaciens* strains. Some calluses derived from tumours produced by *A. tumefaciens* A1 and K1 were able to regenerate shoots. Only some shoots, those derived from calluses produced by *A. tumefaciens* A1, differentiated roots. The regenerated plants were tested for opine production and resistance to superinfection with. *A. tumefaciens* A1. Some calluses derived from tumours produced by *A. tumefaciens* K1 were resistant to kanamycine.

INTRODUCTION

Crown gall, a disease affecting many dicotyledonous plants, is produced by strains of *Agrobacterium tumefaciens* which carry a tumour-inducing plasmid (Ti) (Zaenen et al.,1974; Van Larebeke et al., 1974; Watson et al., 1975). The Ti plasmid transfers a portion of its DNA (T-DNA) into plant cells (Chilton et al., 1977) where it is integrated into host chromosomal DNA. The integrated T-DNA directs the synthesis of auxin (Inze et al., 1984; Schröeder et al., 1984) and cytokinin (Akiyoshi et al., 1984), which produce tumoral plant cell proliferation and the synthesis of opines, novel compounds catabolized by the inciting bacteria (Petit et al., 1970).

Crown galls induced by different strains of *A. tumefaciens* having unchanged T-DNA are sometimes able to regenerate shoots but never form roots. There is less information about the whole plant regeneration from crown galls: Norton and Towers (1984) reported the formation of rooted shoots after transformation of *Bidens alba* with plasmid pTiT37; Necasek et al. (1988) obtained transformed plants from tumour tissue of *Lycopersicon esculentum* infected with *A. tumefaciens* T37.

In this paper we described the regeneration of plants from crown galls of *Datura innoxia* cv. Laura produced by different strains of *A. tumefaciens*.

MATERIAL AND METHODS

— Bacterial strains used in our experiments are shown in table 1.
— Plants: *Datura innoxia* cf. Laura was used as recipient of T-DNA.
— Isolation of tumours and regeneration of plants: Crown galls were induced on plant stems by *A. tumefaciens* strains. After a month, the tumours were excised, sterilised and transferred on the MS medium (Murashige and Skoog, 1962), without phytohormones and cultivated in light (2000lx, 16 h period) at 25—30°C with periodical subcultivation. For elimination of bacterial cells we used carbenicillin (500 μg/ml). Regenerated shoots were transferred on MS medium without hormones where they formed roots. Regenerated plants were cultivated in pots in a greenhouse.

Table 1

Bacterial strains used in our experiments

Strain	Plasmids	Description
A. tumefaciens A6	pTiA6	ocs$^+$ tim$^+$
A. tumefaciens B6	pTiB6	,,
A. tumefaciens A1	pTiAl	,,
A. tumefaciens K1	pTiA6 : pRD1	ocs$^+$ tum$^+$ Ap^{r}Tc^{i}Km^r

— Superinfection : the regenerated plants were tested for susceptibility to superinfection with strains of *A. tumefaciens*.

— Opine assay : octopine assays were performed on supernatant fraction of centrifuged homogenized plant tissue (Webb, 1986). Aliquots of 20 µl were applied to Whatmann 3 MM paper. The samples were subjected to electrophoresis at 200 V/cm for 1 hour, in an acetic acid/formic acid buffer, pH 1.8 and to paper chromatography. Guanidine compounds were stained with nynhidrine reagent (Seitz and Hochster, 1964).

— Resistance to kanamycine of the transformed calluses was tested on MS medium with Km (100 µg/ml), by comparison with untransformed tissue.

RESULTS AND DISCUSSION

Crown galls were induced on *Datura innoxia* with different *A. tumefaciens* strains : A6, B6, Al and Kl. There were variations in the ability of these strains to induce shoots and roots. Tumoral calluses obtained after the cultivation of tumours on MS medium were maintained for a long time on this medium with periodical subcultivation (Fig. 1). They were positive for the presence of octopine.

Some caluses derived from tumours produced by *A. tumefaciens* Al and *A. tumefaciens* Kl were able to regenerate shoots after a year of cultivation (Fig. 2). Only shoots from calluses produced by *A. tumefaciens* Al differentiated roots on MS medium without hormones. We obtained in this way 7 plants. Only two of them were abnormal morphologically, octopine positive and resistant to superinfection with the same strain of *Agrobacterium* (Fig. 3).

The shoots regenerated on the calluses produced by *A. tumefaciens* Kl were abnormal morphologically (they lost apical dominance) and never form roots.

The caluses obtained from tumours produced by the other two strains of *A. tumefaciens* (A6 and B6) did not regenerate shoots during the same time of cultivation.

Agrobacteria of octopine type can introduce two distinct pieces of T-DNA into dicotyledonous plant cells, namely TL-DNA and TR-DNA (Fig. 5). On the Ti plasmid each of the T-regions is bordered by two 25 bp directly repeated. TL-DNA, which is always present in crown galls harbors the *onc* genes. These consist of gene 1 and 2, together named the

Fig. 1. — Callus obtained from a tumor produced by *Agrobacterium tumefaciens* Al on *Datura innoxia*.

Fig. 2. — Shoot appeared on the tumoral calluses of *Datura innoxia* induced by the infection with *A. tumefaciens* Al.

Fig. 3. — Plant regenerated from tumoral calluses : transformed plant, resistant to reinfection with *A. tumefaciens* Al (left) : normal plant susceptible to reinfection (right).

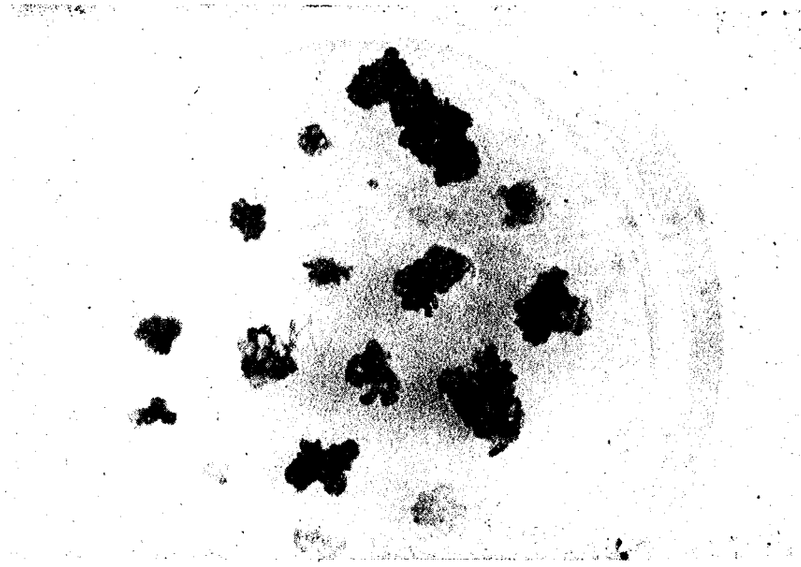

Fig. 4.—Calluses derived from tumors produced by *A. tumefaciens* K1 tested for resistance to kanamycine.

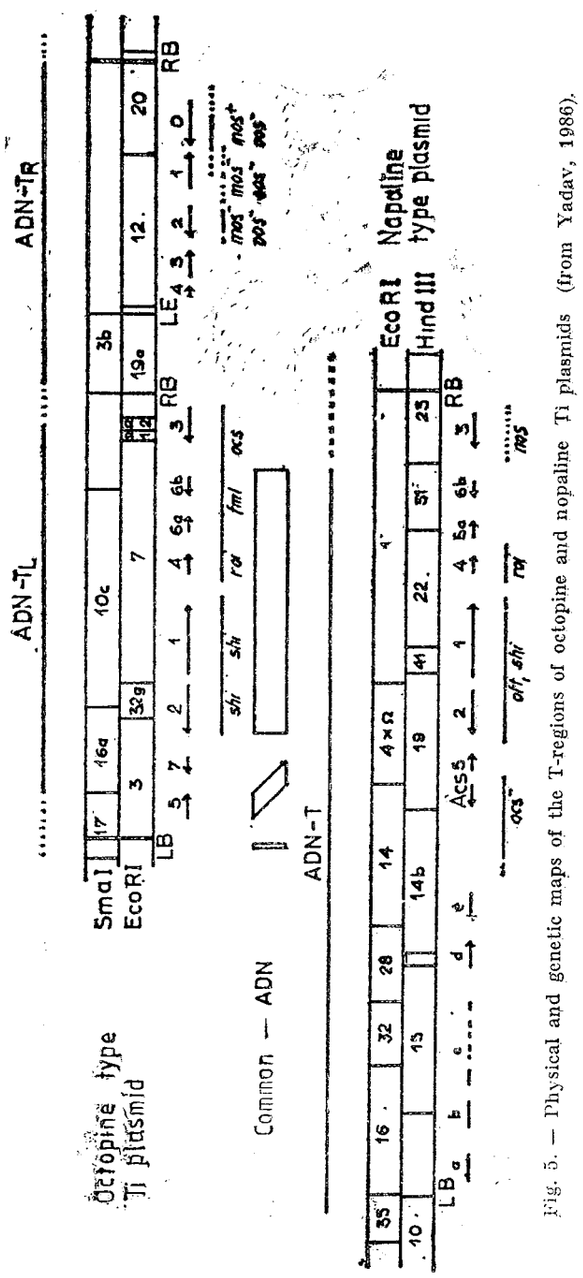

Fig. 5. — Physical and genetic maps of the T-regions of octopine and nopaline Ti plasmids (from Yadav, 1986).

auxin locus, and gene 4, the cytokinin locus. Together the *onc* genes cause a severe disturbance of phytohormone balance at the site of infection, leading to unlimited and undifferentiated proliferation of cells.

If the cytokinin gene is inactivated, tumorous grow slowly and tend to form roots ; if only the auxin locus is inactivated, the transformed tissue spontaneously regenerates shoots (Peerbolte et al., 1986).

Our results with *A. tumefaciens* Al suggest some deletions in TL-DNA which affected the *tmr* or *tms* genes providing shoots only or shoots and roots. The results with *A. tumeaciens* Kl would be produced by the effect of transposition of the transposons from pRD1 (Ap, Tc, Km) in T-DNA of pTiA6. This hypothesis is confirmed by the fact that some calluses derived from tumors produced by *A. tumefaciens* Kl are resistant to Km (100 μg/ml) (fig. 4).

At the same time, our data confirm the affirmation that the tumors represent in fact a mosaic of normal and transformed cells. Thus, the normal plants *(ocs⁻)* susceptible of reinfection) regenerated from tumoral caluses have the origin in the normal cells, while the morphologic abnormal plants *(ocs⁺)* were regenerated from transformed cells.

CONCLUSIONS

The experimental data obtained lead to the following conclusions :

1. All the strains of *A. tumefaciens* tested in our experiments were able to produce tumors on *Datura innoxia.*

2. The caluses derived from tumors produced by *A. tumefaciens* Al differentiated shoots and roots on MS medium.

3. Only two of the regenerated plants were *ocs⁺* and resistant to superinfection with the *A. tumefaciens* Al.

4. Shoots regenerated from the caluses produced by *A. tumefaciens* Kl were abnormal morphologically and never form roots.

5. The caluses produced by the strains *A. tumefaciens* A6 and *A. tumefaciens* B6 did not regenerate shoots.

6. Some of the calluses produced by *A. tumefaciens* Kl were resistant to Km due to the effect of transposition of the transposon for resistance to Km from pRD1 in T-DNA of pTiA6.

REFERENCES

1. Akyioshi, D. E., Klee, H., Amasino, R. M., Nester, E. C., Gordon, M. P., Proc. Natl. Acad. Sci. USA, 1984, **81**, 5994.
2. Chilton, M. D., Drummond, M. H., Merlo, D. J., Sciaky, D., Montoya, A. L., Gordon, M. P., Nester, E. W., Cell, 1977, **11**, 263.
3. Inze, D., Follin, A., Van Lijsebetten, S. M., Simeonsm, C., Genetello, C., Van Montagu, M., Schell, J., Mol. Gen. Genet., 1984, **194**, 265.
4. Murashige, T., Skoog, T., Physiol. Plant., 1962, **15**, 473.
5. Necasek, J., Dusbabkova, J., Pekatkova-Tronckova, E., Biol. Plantarum, 1988, **30**, 1.
6. Norton, R. A., Towers, G. H. N., Canad. J. Bot., 1984, **62**, 408.
7. Peerbolte, R., Leenhouts, K., Hooykaas-Van Slogteren, G. M. S. Hoge, J. H. C., Wullems, G. J., Schilperoort, R. A., Plant Mol. Biol., 1986, **7**, 265.
8. Petit, A., Delhaye, S., Tempe, J., Morel, G., Physiol. Veg., 1970, **8**, 205.
9. Schroeder, G., Waffenschmidt, S., Weiler, E. W., Schroeder, J. Eur. J. Biochem., 1984, **138**, 387.

10. Seitz, E. W., Hochster, R. M., Canad. J. Bot., 1964, **8**, 999.
11. Van Larebeke, M., Engler, G., Holsters, M., Van Den Elsacker, S., Zaenen, I, Schilperoort, R. A., Schell, J., Nature, 1974, **252**, 169.
12. Yadav, N. S., in *Results and Problems in Cell Differentiation*, Springer Verlag, Berlin, Heidelberg, 1986, 109.
13. Watson, B., Currier, T. C., Gordon, M. P., Chilton, M.D., Nester, E., J. Bacteriol., 1975, **123**, 255.
14. Webb, K. J., Theor. Appl.Genet., 1986, **72**, 53.
15. Zaenen, I., Van Larebeke, N., Teuchy, H., Van Montagu, M., Schell, J., J. Mol. Biol., 1974, **86**, 109.

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CONTRIBUTIONS À L'ÉTUDE DU PHYTOPLANCTON DANS UN ÉCOSYSTÈME ANTHROPIQUE

EUGENIA STANCU

Le travail fait une brève présentation de la structure qualitative et quantitative du phytoplancton, dans le lac de Dîmboviţa, au cours de l'année 1989.

Le bassin de Dîmboviţa est un lac d'accumulation construit en 1987, sur l'ancien chénal de la rivière de Dîmboviţa, à l'entrée de Bucarest, zone de Crîngaşi. Il a une surface de 24 ha, une profondeur moyenne de 5,5 m et un volume de 14 millions m.c. L'alimentation se fait par la rivière de Dîmboviţa et par un canal qui amène l'eau de la rivière d'Argeş.

Le présent travail contient une première partie des résultats que nous avons obtenus, concernant la flore alguale de ce lac. Nos recherches ont eu en vue la structure et l'évolution de la cénose alguale dans les conditions d'un biotope anthropique.

MATÉRIELS ET MÉTHODE

En 1989 on a fait l'étude mensuelle du phytoplancton du lac de Dîmboviţa, du point de vue qualitatif, à partir du mois de mars ; le prélèvement des épreuves a été fait de quatre stations situées dans des endroits que nous avons considérés importants dans l'établissement du tableau algofloristique de cet écosystème. Ainsi la première station fut-elle fixée devant le canal qui amène l'eau de l'Argeş et la deuxième à l'entrée de la Dîmboviţa. Nous avons considéré ces deux stations comme nécessaires pour établir les voies de pénétration de certaines espèces alguales. La station « centre »-3- a été fixée aproximativement à la moitié de la distance entre l'entrée de la Dîmboviţa et le barrage, près de l'ancien chénal de Dîmboviţa. Une quatrième station a été fixée dans l'immédiat voisinage du barrage, à la sortie des eaux du lac. Pour l'analyse de la densité numérique on a prélevé des épreuves d'un litre, qui ont été laissées se sédimenter pendant 2 semaines, après quoi on les a concentrées de nouveau et on en a examiné au microscope un volume de 0,05 ml ; les résultats sont exprimés en milliers d'exemplaires/litre. La biomasse du phytoplancton a été estimée par la méthode volumétrique conformément à l'analyse numérique du phytoplancton en utilisant la densité spécifique du contenu cellulaire égale à 1. Les résultats des analyses ont été exprimés en mg substance humide/litre.

RÉSULTATS ET DISCUSSIONS

On a identifié en 1989 dans le lac de Dîmboviţa, 146 taxa et infrataxa d'algues, réparties en 5 groupes taxonomiques : cyanophytes, euglénophytes, pyrophytes, bacilariophytes et chlorophytes.

Du total des taxa déterminés dans le lac au cours de cette année, 47 espèces (représentant 32, 18%) appartiennent aux bacilariophytes, 48 espèces (32,88%) aux chlorophytes et 30 espèces (20,55%) aux euglénophytes. On a déterminé encore 12 espèces (8,22%) pyrophytes et 9 espèces (6,16%) euglénophytes. L'analyse de la composition algofloris-

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tique de cet écosystème nous relève une cénose de type chlorophyte-bacilariophyte-euglénophyte où le rôle d'associé est détenu par les pyrophytes et les euglénophytes.

Le nombre des taxa déterminés mensuellement dans le lac suit une courbe ascendante de mars en mai, de 28 espèces déterminées en mars, 41 en avril, 64 en mai. Au cours des mois d'été on enregistre un maximum d'espèces déterminées (64—65 espèces) pour qu'on enregistre en septembre une baisse à 34 espèces, qui marque l'installation de l'automne. Le mois de juillet se situe en dehors de cette courbe (21 espèces seulement). Cette baisse peut être due à un choc abiotique (déversements toxiques dans le système d'accumulation du lac ou une vidange forcée qui entraîna le phytoplancton aussi).

Au printemps, la cénose est de type diatomées-chlorophytes. Pendant la chaude saison, en été, la cénose alguale du lac enregistre une modification dans le sens de l'augumentation du nombre des espèces de chlorophytes en même tems que la baisse du nombre des diatomées auxquelles s'ajoutent un important nombre de pyrophytes. En automne, on constate un nouveau changement de la composition alguale, la cénose étant de type chlorophytes-cyanophytes.

Sous l'aspect de la constance (tableau 1), on rencontre dans le lac de Dîmboviţa seulement 9 espèces constantes, représentant 6,16% et

Tableau 1

Liste des taxa constants et accessoires du lac de Dîmboviţa en 1989

	Constants	Accessoires
Cyanophyta		
Aphanizomenon flos-aque	—	+
Chroococcus dispersus	—	+
Chroococcus turgidus	—	+
Dactilococcopsis rhaphidioides	+	—
Microcystis flos-aque	—	+
Microcystis pulvereae	—	+
Oscillatoria tenuis	—	+
Dinophyta		
Ceratium hirundinella	+	—
Gymnodinium excavatum	—	+
Peridinium cinctum	+	—
Bacillariophyta = Diatoma		
Asterionella formosa	—	+
Kyclotella kützingiana	+	—
Cocconeis placentula	—	+
Melosira granulata var. *angustissima*	+	—
Nitzschia hantzschiana	—	+
Synedra acus	—	+
Chalorophyta		
Actinastrum hantzschii	—	+
Ankistrodesmus setigerus	—	+
Chlorella vulgaris	—	+
Coelastrum microporum	+	—
Crucigenia rectangularis	—	+
Cocystis naegeli	—	+
Pediastrum duplex var. *reticulatum*	+	—
Scenedesmus quadricauda	+	—
Staurastrum paradoxum	—	+

19 espèces accessoires qui réalisent 13,01%. Le nombre réduit d'espèces constantes et accessoires est caractéristique pour un système jeune — en formation — tel le lac de Dîmboviţa.

Les valeurs de la densité numérique du phytoplancton du lac de Dîmboviţa (tableau 2) se sont situées en 1989 entre centaines de milliers et dizaines de millions d'exemplaires par litre.

Tableau 2

La densité (milliers d'ex./litre) et l'abondance numérique du phytoplancton du lac de Dîmboviţa ee 1989

Mois	Station	Nombre total milliers ex/l	Groupes taxonomiques									
			Cy.		Eugl.		Pyr.		Bacil.		Chl.	
			Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
Mars	1	1082	50	4,62	2	1,85	0	0,00	1018	94,08	12	1,11
	2	716	32	4,47	0	0,00	0	0,00	646	90,22	38	5,31
	3	310	10	3,22	0	0,00	0	0,00	284	91,61	16	5,16
	4	842	12	1,42	0	0,00	0	0,00	814	96,67	16	1,90
Avril	1	238	24	10,08	4	1,68	4	1,68	162	68,08	44	68,49
	2	144	30	20,83	0	0,00	2	1,39	38	26,39	74	51,39
	3	176	34	19,32	2	1,14	0	0,00	108	61,36	32	18,18
	4	182	52	28,57	0	0,00	0	0,00	70	38,46	60	32,97
Mai	1	5576	102	1,83	2	0,04	62	1,11	5136	92,11	274	4,91
	2	10455	459	4,39	0	0,00	16	0,15	9252	88,49	724	6,93
	3	12124	124	1,02	54	0,45	28	0,23	11559	95,34	36	0,30
	4	11930	100	0,84	0	0,00	18	0,15	11058	92,69	754	6,32
Juin	1	435	22	5,06	0	0,00	244	56,09	133	30,58	36	8,28
	2	764	54	7,07	17	2,23	470	61,52	160	20,94	63	8,25
	3	1572	320	20,36	8	0,51	666	42,37	180	11,45	398	25,32
	4	922	40	4,34	2	0,22	792	85,90	52	5,64	36	3,91
Juillet	1	336	8	2,38	0	0,00	268	79,76	0	0,00	60	17,86
	2	670	8	1,19	0	0,00	494	73,73	0	0,00	168	25,08
	3	356	0	0,00	0	0,00	342	96,07	0	0,00	14	3,93
	4	588	4	0,68	0	0,00	238	40,48	36	6,12	310	52,72
Août	1	1830	348	19,02	16	0,87	706	38,58	394	21,53	366	20,00
	2	1438	116	8,07	6	0,42	1086	75,52	54	3,76	176	12,24
	3	1438	242	16,83	22	1,53	616	42,84	110	7,65	448	31,15
	4	1011	244	24,14	0	0,00	454	44,91	82	8,11	232	22,95
Septembre	2	1340	204	15,22	52	3,88	738	55,08	14	1,05	332	24,78
	3	1056	468	44,32	0	0,00	450	42,61	0	0,00	138	13,07
	4	800	482	60,25	0	0,00	130	16,25	40	5,00	148	18,50

Note : Station : 1. entrée de l'Argeş ; 2. entrée de la Dîmboviţa ; 3. centre ; 4. barrage

Dans les associations phytoplanctoniques, les espèces alguales peuvent être dominantes ou dominées. Les espèces dominantes sont celles qui peuvent donner une idée claire sur l'ensemble phytoplanctonique et sur les particularités du biotope dans lequel elles apparaissent. En général, les niches occupées par les espèces dominantes sont plus larges que celles occupées par les espèces rares.

La succession des maximums des différentes espèces alguales phytoplanctoniques reflète, outre une modification du milieu physico-chimique, une intervention des facteurs biotiques. Il y a des auteurs qui considèrent que chaque composante de l'association phytoplanctonique produit une autotoxine qui limite la croissance de sa propre population. De la

sorte, L'*Asterionella* s'inhibe toute seule lorsqu'elle atteint un maximum numérique mais elle stimule le développement de la population de *Synedra*. Les deux diatomées, productrices d'huile comme matière de réserve, sont stimulatrices pour les espèces du genre *Coelastrum* productrices d'amidon (1), (2), (3). Une telle situation se rencontre dans le lac de Dîmboviţa au mois de mai, lorsque la population d'*Asterionella* réalise

Tableau 3

La densité (mg substance humide/litre) et l'abondance (%) de la biomasse du phytoplancton du lac de Dîmboviţa en 1989

Mois	Station	Biomase mg/l	Groupes taxonomiques									
			Cy.		Eugl.		Pyr.		Bacil.		Chl.	
			mg/l	%	mg/l	%	mg/l	%	mg/l	%	mg/l.	%
Mars	1	1,138	0,026	2,28	0,009	0,79	0,000	0,00	0,794	69,77	0,309	27,15
	2	1,369	0,063	4,60	0,000	0,00	0,000	0,00	0,559	40,83	0,747	54,56
	3	0,730	0,001	1,37	0,000	0,00	0,000	0,00	0,206	28,22	0,514	70,41
	4	1,600	0,323	20,19	0,000	0,00	0,000	0,00	0,674	42,12	0,603	37,69
Avril	1	0,846	0,266	31,44	0,008	0,95	0,036	2,01	0,399	22,27	0,137	7,65
	2	0,496	0,005	1,01	0,000	0,00	0,100	20,16	0,181	36,49	0,210	42,34
	3	0,500	0,099	19,80	0,170	34,00	0,000	0,00	0,096	19,20	0,135	27,00
	4	0,370	0,080	21,62	0,000	0,00	0,000	0,00	0,087	23,51	0,203	54,87
Mai	1	9,318	0,405	4,35	0,004	0,04	3,036	32,58	5,289	56,76	0,584	6,27
	2	10,369	1,416	13,66	0,000	0,00	0,800	7,72	7,279	70,20	0,874	8,43
	3	15,585	2,450	15,72	0,243	1,56	1,136	7,29	9,760	62,62	1,997	12,81
	4	13,064	0,300	2,30	0,000	0,00	0,708	5,42	9,610	73,56	2,446	18,72
Juin	1	19,266	0,311	1,61	0,000	0,00	11,424	59,30	7,268	37,72	0,263	1,37
	2	33,699	3,182	9,44	1,130	3,35	23,143	68,68	6,103	18,11	0,141	0,42
	3	34,497	1,532	4,44	0,056	0,16	26,450	76,67	0,636	1,84	5,823	16,88
	4	46,826	0,302	0,65	0,004	0,01	38,849	82,97	3,681	7,86	4,158	8,88
Julliet	1	13,428	0,464	3,46	0,000	0,00	12,545	93,42	0,000	0,00	0,415	3,09
	2	30,687	0,120	0,39	0,000	0,00	25,992	84,70	0,000	0,00	4,575	14,91
	3	17,293	0,000	0,00	0,000	0,00	16,902	98,05	0,000	0,00	0,337	1,96
	4	13,102	0,017	0,13	0,000	0,00	11,564	88,26	0,027	0,21	1,494	11,40
Août	1	50,105	9,312	18,58	0,074	0,15	35,172	70,21	1,812	3,62	3,735	7,45
	2	69,146	2,680	3,88	0,025	0,04	51,710	74,78	0,246	0,36	14,485	20,95
	3	37,479	8,221	21,94	0,154	0,41	28,304	75,52	0,599	1,60	0,201	0,54
	4	41,669	7,566	18,25	0,000	0,00	21,674	22,27	1,390	3,35	10,839	26,14
Septembre	2	50,810	5,364	10,56	0,247	0,49	34,857	68,60	0,042	0,08	10,300	20,27
	3	40,907	15,281	37,36	0,000	0,00	21,415	52,35	0,000	0,00	4,211	10,29
	4	28,356	17,686	62,37	0,000	0,00	6,148	21,68	0,112	0,40	4,410	15,55

Note : Station : 1. entrée de l'Argeş ; 2. entrée de la Dîmboviţa ; 3. centre; 4. carrage

des valeurs élevées sur l'entière surface du lac (jusqu'à 11,488 mille ex./litre dans la station « centre ». Au cours du mois de juin, les valeurs numériques réalisées par le genre *Asterionella* baissent jusqu'à zéro dans les stations 3 et 4. Dans le cas particulier mis en évidence on n'enregistre pas de croissance numérique de la population de *Synedra*, mais on y remarque le développement de la population de *Coelastrum*.

Au cours des mois de juin, juillet, août et septembre, la cénose alguale du lac est dominée de point de vue numérique par la dinophyte *Ceratium hirundinella* qui atteint 1.632 milliers d'exemplaires/litre dans la station 1(entrée de l'Argeş) au mois d'août. La fleuraison réalisée par *Ceratium* au cours de cette période a conduit aussi à la modification de la couleur de l'eau du lac en jaune-brun.

Les mois de mars et avril se situent de point de vue de la biomasse réalisée dans le lac de Dîmboviţa entre les limites de 0,370 mg jusqu'à 1,600 mg substance humide/litre. A partir du mois de mai jusqu'en septembre le poids de la biomasse est réalisé au compte de la dinophite *Ceratium hirundinella*. Au mois de septembre, hormis *Ceratium*, c'est l'algue bleue, philamenteuse, *Aphanizomenon flos-aque* qui est présente dans d'importantes quantités de biomasse. On a estimé jusqu'à 51,6000 mg substance humide/litre en août réalisée par la *Ceratium* toute seule. En juin, juillet et août des pourcentages de la biomasse de 60—98% sont représentés par le groupe de pyrophytes. Au mois de septembre, quoique les pyrophytes atteignent encore des pourcentages de 52—68% de la biomasse dans les stations 2 et 3, dans la station 4 ce sont les cyanophytes qui détiennent le poids de la biomasse (62%).

La grandeur de la biomasse du phytoplancton représente le reflet le plus expressif du degré de sa fonction, de sa capacité d'absortion et d'utilisation des facteurs du milieu (4). Conformément à ces données et considérant que la valeur de 5 mg substance humide/litre représente la limite inférieure étalon d'où se manifeste la processus de la floraison de l'eau (apparition de la couleur, du goût et de l'odeur de l'eau), donc du caractère de biocénose de type eutrophe, ou peut apprécier qu'au moins pendant la période d'été-automne le lac de Dîmboviţa se place dans la catégorie d'un biotope eutrophe.

CONCLUSIONS

1. Conformément aux analyses qualitatives et quantitatives du phytoplancton de cet écosystème, on peut apprécier que le bassin de Dîmboviţa présente le caractère de lac eutrophe, au moins pendant la période été-automne.

2. La fréquence de la floraison des eaux de ce lac, comprenant le changement de l'espèce qui produit ce phénomène à de très courts intervalles de temps (d'un mois à l'autre) et due à l'imput des nutrients.

3. Bien qu'on ait identifié dans le lac un important nombre de taxa, le nombre réduit des espèces constantes et accessoires constatées dans ce lac est caractéristique pour un écosystème jeune, tel le lac de Dîmboviţa. Cette caractéristique peut être maintenue en temps grâce aux manipulations sévères de volumes (la vidange pendant l'hiver) qui ne permettent pas l'installation et l'évolution normale de la cénose alguale.

BIBLIOGRAPHIE

1. Gavrilă, L., 1973, Peuce (Tulcea), 3.
2. Ionescu Al., Gavrilă, L., 1971, St. cerc. biol., Seria botanică, 23, 2.
3. Ionescu Al., Gavrilă L., 1973, St. cerc. biol., Seria botanică, 24, 1.
4. Oltean M., 3 Symposium « Les bases biologiques des processus d'épuration et protection de l'environnement, Argeş, Iaşi, 1985, 230—237.

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THE INDIVIDUAL VARIABILITY OF TREE POPULATIONS IN THE MIXED FIR AND BEECH FOREST BELT IN THE LOTRU MOUNTAINS (VOINEASA)

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The paper presents the populations of beech (*Fagus sylvatica*) and fir (*Abies alba*), the variability of biometrical and structural parameters in the forestry phytocoenosis *Pulmonario (rubro)-Abieti-Fagetum* in the Lotru Mountains in the protection area of the Voineasa district. The density of the beech population is of 665 individuals/ha and the density of the fir population is of 648 individuals/ha, but the mature trees are 184 for the former and 188 for the latter. The variation coefficient (s%) is high for the diameter basic area and the volume of the beech population (75.96—131.42) and somewhat smaller for the fir populations (46.90—76.68). The height has a more reduced variability for both populations (s% = 35.72 for beech and 31.94 for fir). The mixture of populations is very homogeneous. Dendrochronologic newly obtained data show that some fir trees can maintain at the limit stage of growth for many years (until 60—70 years).

The tree populations in the Romanian forests have been frequently studied by silviculturists especially from the biometrical, structural and productive viewpoints at the level of mature tree layer, function of their economic importance. The data obtained representing mean values characteristic for Romania are synthetically given in Dendrometric Tables (1) and many papers issued in scientific journals in the course of years.

Due to their high constancy in time and space the tree populations represent a very interesting topic from the ecologic viewpoint, allowing that the individual variability and the relations specific to this matter organization level — the population — to be studied. In this paper we undertake an analysis of the tree populations characteristics of the fir and beech mixed forests spread widely in the Romanian Carpathians, i.e. in the Lotru Mountains, where they are largely distributed and have a typical structure for entire Romania.

MATERIAL AND METHODS

The populations studied belong to *Abies alba* Mill. and *Fagus sylvatica* L species of the *Pulmonario (rubro) Abieti-Fagetum* (Knapp 42) Soó 62 association. The area investigated was established in the Lotru Mountains in the ecological sector E, in the Voineasa forestry district, on the Cărării brooket, an affluent of the Lotru river. The forest has a protection regime. The soil is exposed E—NE, with a 10—40° slope, at 1250—1280 m altitude. The macroclimate is characterized by temperatures with annual mean values between 4.3 — 5.0°C and precipitations of 980—1060 mm per year. The soil is of brown type, moderately podsolized with skeleton material on the whole soil profile. Soil trophicity is small to medium (V% = 50—60), with reduced acidity (pH = 5.5—6.0 in

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water) moder and moder-mull type humus, wet-moist to wet humidity regime. The forestry massif has a great continuity and is uniform as concerns the tree composition, but not as concerns the age structure, being considered secular forests.

Only 3 tree species *(Abies alba, Fagus sylvatica, Picea abies)* are presented in the area investigated, the diversity estimated by the Simpson—Pielou index being 0.5120 for the effective and 0.4811 for the tree layer biomass. The productivity of stands is medium to under-medium.

The biometrical and structural measurements were made according to the forestry methodology (5), (6) in round working areas of 500 m^2 for mature trees and in 1/4 of the area to make an inventory of the very young trees. The measurement of the heights was made with the Bitterlich relascope and the diameter resulted from the measurement of trunk circumferences at 1.30 m height, with a graduated stiap. The wood density was determined on samples extracted with the Pressler borer.

RESULTS AND DISCUSSIONS

The forests in which there are age differences between the 30 years older trees are called pluriage forests (10) and they exhibit in fact the usual structures of any natural plant community in which there are trees of all stages of growth starting from seedlings up to secular trees. In modern times in case of forests this structure may reflect a natural structure (virgin or quasivirgin) or may result from cultivated forests administered in "gardened woods". Their difference is difficult to be established in the case of a good and consistent management of silviculture in the absence of official data.

The populations investigated include all-age individuals in the case of fir and beech trees except for those under a year, their absence being due to the fructification break in the previous year (Table 1). In case of spruce tree its scarce presence at mature age proves the lack of some conditions favorable for the development of this population. Age measurements were made only to some size categories of trees, the 100—140 years old trees being the oldest.

The effective of analysed populations is large, represented by densities of 648 ind/ha for fir and 665 ind/ha for beech trees. The progressive reduction of a number of individuals at the same time with the increase in size and age is a normal process taking place both with the fir populations and with the beech ones (Table 1). The mature generations, two in case of beech and three in case of fir populations, are prevailing as biomass as well as in the obtaining of the skeleton of all the biocenosis. The formation generations may correspond to the occurrence in the vertical structure of cenosis of some larger free spaces as a result of disappearance of a great number of older trees. They are discontinuously achieved in case of beech and have a continuous distribution in case of fir trees. The diameter categories essential from the productive point of view are 40—56 cm and 16—28 cm, respectively. The intra and inter specific competition, with a gradual reduction of density is recorded at these populations level having an influence on the individual sizes, on the one hand, and on the minimum area necessary for each tree, on the other hand.

Table 1

Distribution of whole tree populations function of the tree diameter

Species/ /Frequency %	Diameter (cm)																			
	0 4	4 8	8 12	12 16	16 20	20 24	24 28	28 32	32 36	36 40	40 44	44 48	48 52	52 56	56 60	60 64	64 68	68 72	72 76	76 80
Fir	83	8	1	0.5	1	0.5	0.5	0.5	0.5	0.5	1	1	0.3	0.3	1	0,3	0.3	0.3		
Beech	86	7	3	—	—	—	1	0.6	0.2	0.5	0.1	0.2	0.1	0.6	0.2					0.1
Spruce fir	—	—	—	—	—	—	—	—	—	—	—	100	—	—	—					

Analysing only the distribution of mature trees in a phytocenosis (trees with diameters larger than 8 cm are investigated) we find that (Table 2) it corresponds to the model of pluriage forests, having the maximum value for young trees. There is however a difference in the distribution of mature fir, much more uniformized numerically as compared to the beech population, for which the progressive reduction of the number is obvious from the young to the old trees. The discontinuities are also more numerous.

Table 2

Distribution of mature trees function of tree diameter

Species/Frequency %	Diameter (cm)																	
	8 12	12 16	16 20	20 24	24 28	28 32	32 36	36 40	40 44	44 48	48 52	52 56	56 60	60 64	64 68	68 72	72 76	76 89
Fir	6	2	7	1	2	1	3	3	3	6	2	3	7	1	1	1	—	—
Beech	21	—	—	1	8	5	2	3	1	2	1	5	—	—	—	1	—	1
Total stand	27	2	7	2	10	6	5	6	4	8	3	8	7	1	1	1	—	1

Biometrically we notice the presence of beech thicker than the fir trees, but having a more reduced frequency; therefore, the mean diameter values of beech (Table 3) are smaller than those of fir trees, while the variation amplitude is greater. Comparing the mean diameter values of these populations with the mean values found in the whole country area (4) we find that the beech trees have mean values and the fir trees higher mean values. The density of adult fir old beech is very low (Table 3) although it also reaches higher values, having variable values all along the phytocenosis area; even its maximum values are reduced for the mean populations in the country. The fir population is a little more numerous than the beech population, but the higher frequency of beech trees of reduced height make the volume of fir wood to be dominant as compared with that of beech. For tree populations the growth in height depends

on the tree diameter, following the specific (uncorrected) curves presented in Fig la for fir trees and in Fig 1b for beech trees. Due to its pluriage structure, the tree population has a height of a few centimeters for seedlings up to 31 m maximum height both for fir and beech trees. We can notice that there is a competition between them. The limits of the mature tree layer range between 11—31 m as a function of the position they succeeded to have as to the neighbouring individuals (Tables 4, 5). In the fir population predominant and dominant individuals are more frequent than in the beech population. As the forestry literature shows (11), in the natural forests the thick trees having 48—50 cm in diameter can be used as standard for the productive possibilities of the whole population, as their growth is steady as compared to the growth of smaller trees; if the tree populations have a little frequency for this category of diameter — similar with our case — we consider as standard trees the last categories whichare more frequent. After this analysis for both tree populations the standard trees show heights small enough as compared to the variability of the species in Romania. A characteristic of this population is a reduced mature number with an undermedium increase in height but with a higher increase in thickness.

Table 3

Mean biometrical characteristics of the populations in the tree layer

Species	Numerical ratio	Volume ratio	Density	Height (m)		Diameter (cm)		Basic area		Volume (m^3)	
			ind /h	mean ± error	variation limits	mean value ± mean error	variation limits	mean value ± mean error	m^2/ha	mean value ± mean error	ε /ha
Fir	50	61	188_{+}^{-} 35	23.7_{+}^{-} 1.1	11—31	38.60_{+}^{-} 2.56	8.3 — 71.3	1409_{+}^{-} 164	25.38	1.744_{+}^{-} 0.192	315
Beech	49	39	184_{+}^{-} 27	20.6_{+}^{-} 1.0	12—31	27.67_{+}^{-} 3.05	8.0—82.0	891_{+}^{-} 155	15.25	1.131_{+}^{-} 0.22	202
Spruce fir	1	0.5									
Total	100 %	100 %	376	22.2	11—31	32.85	8.0—82.0	1150	40.63	1.438	517

The basic area with the variability specific to the tree populations in the resort ranges between the mean values for the stand due to the reduced number of mature individuals in each tree population (Tables 4, 5). We can notice that this parameter has the greatest values of the variation coefficients as compared with the other biometrical parameters both with the beech and the fir trees; the variability is two times greater for beech than for fir trees. The soil covered by trunks is under 0.5% of the total area and the ratio of one population is similar to that of the other population. The wholly more reduced value may be just a result of the struggle of one population for reducing the effective of the other population, as the individuals have very large sizes.

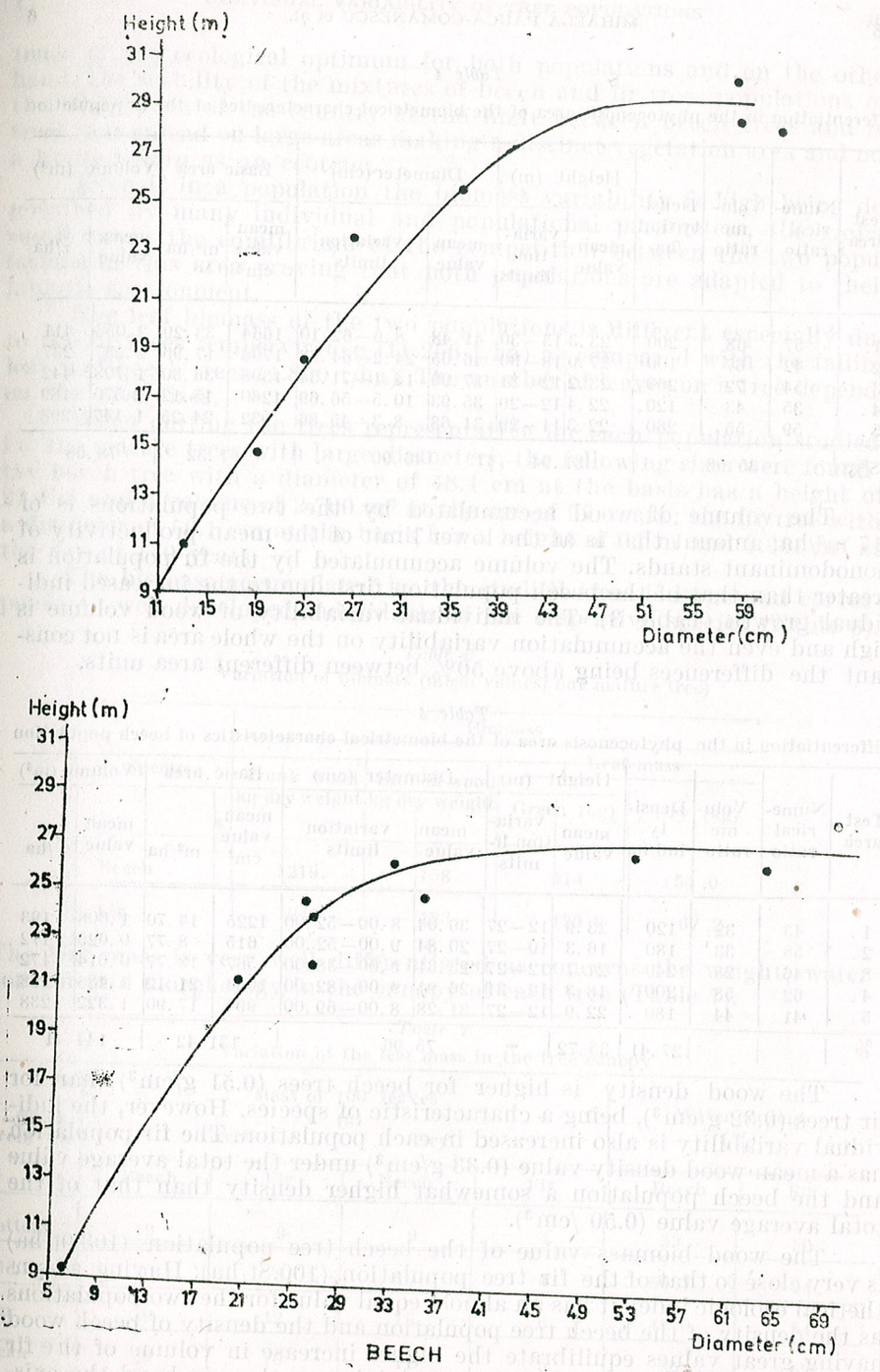

Fig. 1. — The growth curve (uncorrected): a) fir mature population; b) beech mature population.

Table 4

Differentiation in the phytocenosis area of the biometrical characteristics of the fir population

Test area	Numerical ratio	Volume ratio	Density ind/ha	Height (m)		Diameter (cm)		Basic area		Volume (m^3)	
				mean value	variation limits	mean value	variation limits	mean value cm^2	m^2/ha	mean value	ε/ha
1.	57	68	200	23.3	13—30	41.48	8.6—62.10	1664	33.29	2.056	411
2.	42	67	100	27.0	18—30	46.05	24.2—64.33	1796	17.96	2.237	237
3.	54	72	260	23.2	12—31	37.90	14.1—71.34	1396	36.30	1.703	442
4.	35	43	120	22.4	12—29	35.93	10.5—56.69	1260	15.12	1.576	189
5.	59	55	260	22.3	11—29	31.68	8.3—45.86	932	24.25	1.147	298
S%	35.98			31.94		46.90		61.32		78.68	

The volume of wood accumulated by the two populations is of 517 m^2/ha, amount that is at the lower limit of the mean productivity of monodominant stands. The volume accumulated by the fir population is greater than that of the beech population first due to the increased individual growth (Table 3). The individual variability of wood volume is high and even the accumulation variability on the whole area is not constant the differences being above 50% between different area units.

Table 5

Differentiation in the phytocenosis area of the biometrical characteristics of beech population

Test area	Numerical ratio	Volume ratio	Density ind/ha	Height (m)		Diameter (cm)		Basic area		Volume (m^3)	
				mean value	variation limits	mean value	variation limits	mean value cm^2	m^2/ha	mean value	/ha
1.	43	32	120	23.9	12—27	36.04	8.00—52.00	1225	14.70	1.608	193
2.	58	33	180	16.3	10—27	20.84	9.00—52.00	615	8.77	0.625	112
3.	46	28	240	21.7	12—27	23.31	9.00—38.00	567	13.77	0.614	172
4.	62	58	200	18.3	12—31	26.91	9.00—82.00	1056	21.12	1.488	298
5.	41	44	180	22.9	12—27	31.28	8.00—69.00	994	17.90	1.322	238
S %			27.41	35.72		75.96		131.42		144.41	

The wood density is higher for beech trees (0.51 g/cm^3) than for fir trees (0.32 g/cm^3), being a characteristic of species. However, the individual variability is also increased in each population. The fir population has a mean wood density value (0.33 g/cm^3) under the total average value and the beech population a somewhat higher density than that of the total average value (0.50 /cm^3).

The wood biomass value of the beech tree population (103.0t/ha) is very close to that of the fir tree population (100.8t/ha). Having a synthetical ecologic index it has an almost equal value for the two populations as the density of the beech tree population and the density of beech wood having great values equilibrate the higher increase in volume of the fir tree population. These equilibrations explain, on the one hand the existence of the ecological optimum for both populations and, on the other hand, the stability of the mixtures of beech and fir tree populations on the whole area of the country at the limit between beech trees and fir trees, but spread on large areas making a distinct vegetation area and not a limited strip as an ecotone.

Even if in a population the biomass variability is high being determined by many individual and populational parameters, the global result shows the equilibrium of the competition between the two populations in this area proving that both populations are adapted to their labiotic environment.

The leaf biomass of the two populations is different especially due to the fir trees sempervirence (11.256 t/ha) as compared with the falling leaves of beech trees (4.800 t/ha). The number of leaves on a tree depends on the tree sizes.

When cutting the trees representative for each population studied, i.e. the mature trees with large diameters, the following sizes were found: the beech tree with a diameter of 48.4 cm at the basis has a height of 27.4 m and a volume of 2.700 m^3 at the age of 97 years; the fir tree with a diameter of 56.1 cm at the basis has a height of 31 m and 3.901 m^2 at the age of 140 years.

The biomass accumulated by the studied trees is large and corresponds to well developed individuals of the respective species (Table 6).

Table 6

Variation of biomass (mean values/ one mature tree)

Species	Biomass			
	Trunk wood kg dry weight	Branch wood kg dry weight	Leaf mass	
			Green (kg)	Dry (kg)
Beech	1219	158	114	54.0
Fir	1248	123	139.9	67.2

The leaf mass is very well differentiated as concerns the weight, water content and morphology in the canopy of each tree (Table 7).

Table 7

Variation of the leaf mass in the tree canopy

Leaves from the	Mass of 100 leaves (g)				Water content (%)	
	Green		Dry			
	Beech	Fir	Beech	Fir	Beech	Fir
bottom	19	9	9	4	53	50
middle	20	8	10	4	50.7	54
top	29	12	15	6	48.0	54
Mean	23	10	11	5	50	53

Fig. 2. — The cross section of the fir trunk at 1.30 m height.

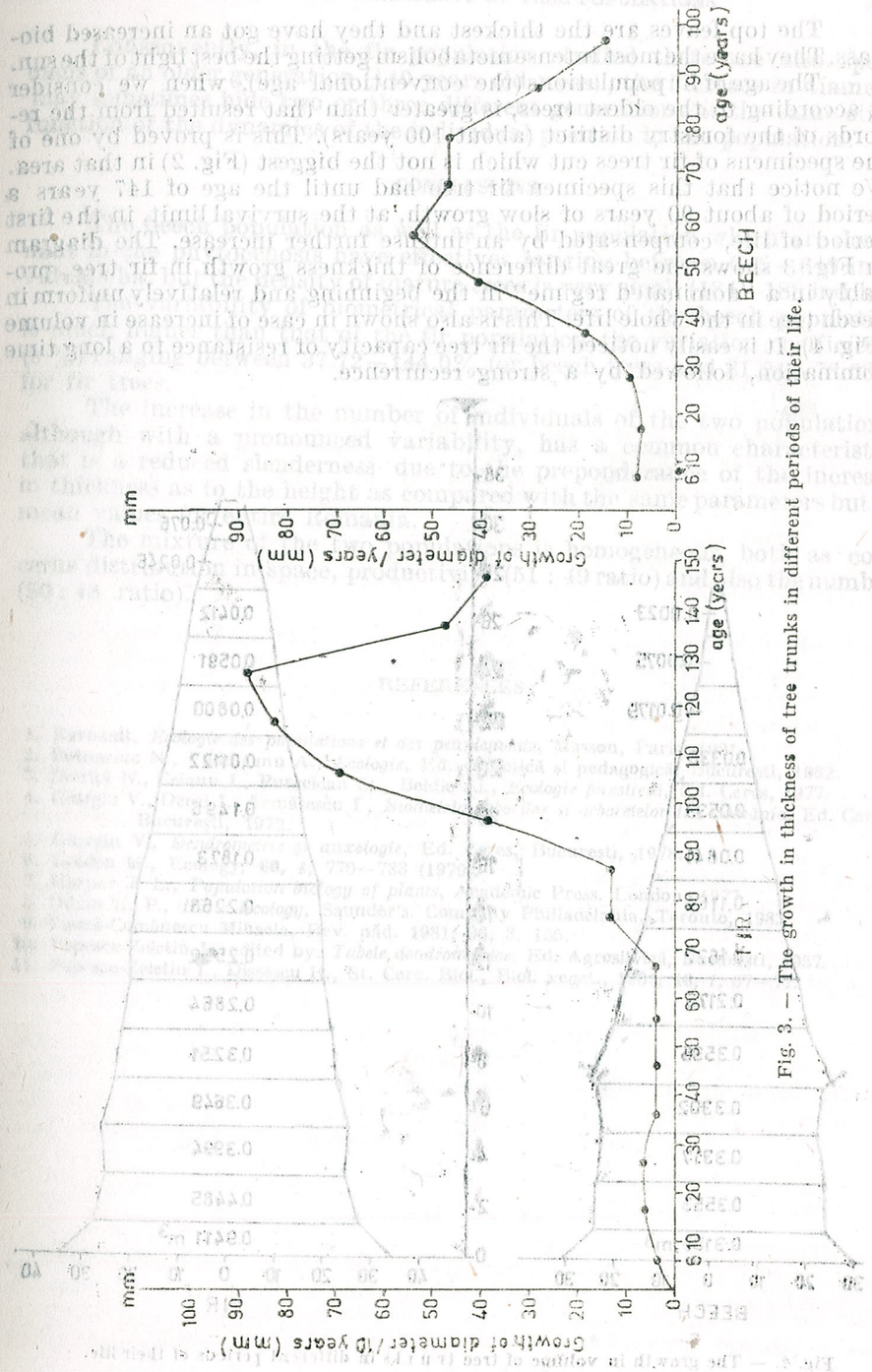

Fig. 3. — The growth in thickness of tree trunks in different periods of their life.

The top leaves are the thickest and they have got an increased biomass. They have the most intense metabolism getting the best light of the sun.

The age of fir populations (the conventional age), when we consider it according to the oldest trees, is greater than that resulted from the records of the forestry district (about 100 years). This is proved by one of the specimens of fir trees cut which is not the biggest (Fig. 2) in that area. We notice that this specimen fir tree had until the age of 147 years a period of about 90 years of slow growth, at the survival limit, in the first period of life, compensated by an intense further increase. The diagram in Fig. 3 shows the great difference of thickness growth in fir tree, probably in a "dominated regime" in the beginning and relatively uniform in beech tree in the whole life. This is also shown in case of increase in volume (Fig. 4). It is easily noticed the fir tree capacity of resistance to a long time domination, followed by a strong recurrence.

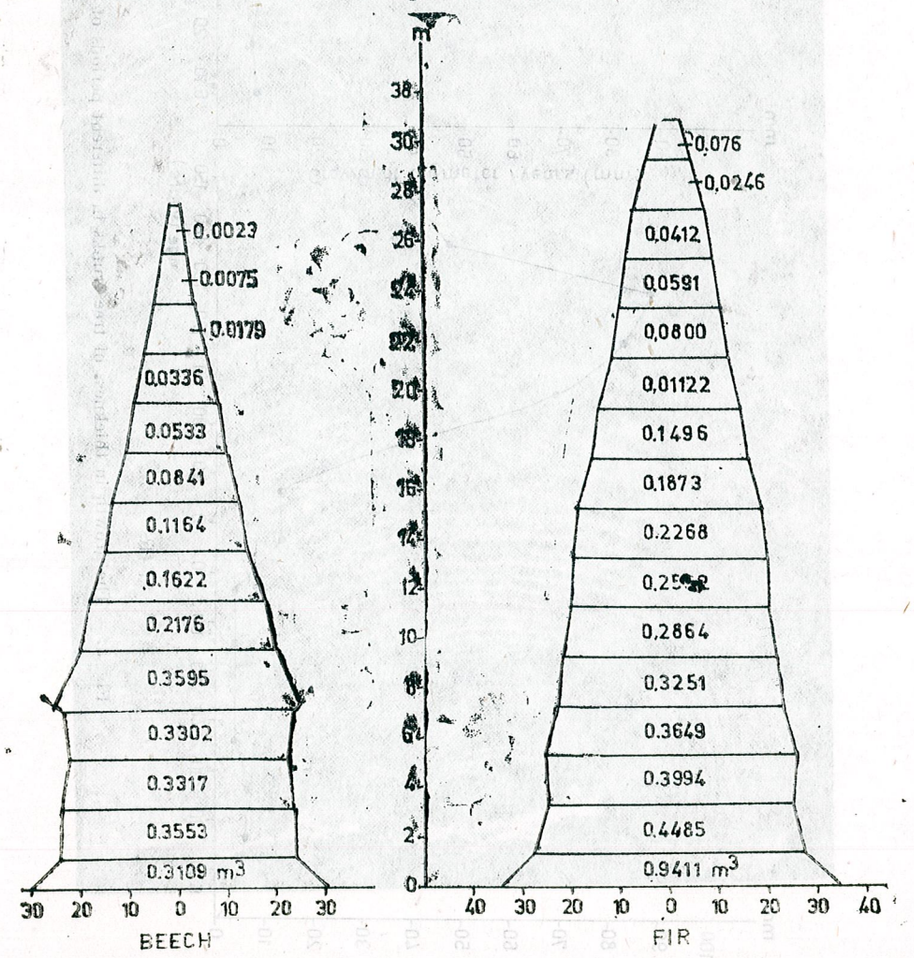

Fig. 4. — The growth in volume of tree trunks in different periods of their life.

Consequently, in the fir population studied there are also specimens of an older generation (140 years old trees); the increase in diameter may sometimes hide two or three different generations at the same sizes, function of the dynamics of the individual position in the population.

CONCLUSIONS

The beech population as well as the fir population which are dominant in the phytocenosis have effectives ranging between 665—648 individuals/ha, but the density of mature trees is very small (184—188 ind/ha).

The variability of biometrical parameters of the beech population is much higher than that of the fir population, the variation coefficients (s %) ranging between 37.72—144.0% for beech trees and 31.94—78.68% for fir trees.

The increase in the number of individuals of the two populations, although with a pronounced variability, has a common characteristic, that is a reduced slenderness due to the preponderance of the increase in thickness as to the height as compared with the same parameters but as mean values for entire Romania.

The mixture of the two populations is homogeneous, both as concerns distribution in space, productivity (51 : 49 ratio) and also the number (50 : 48 ratio).

REFERENCES

1. Barbault, *Ecologie des populations et des peuplements*, Masson, Paris, 1981.
2. Bătnariuc N., Vădineanu A., *Ecologie*, Ed. didactică și pedagogică, București, 1982.
3. Doniță N., Ceianu I., Purcelean St., Beldie Al., *Ecologie forestieră*, Ed. Ceres, 1977.
4. Giurgiu V., Decei I., Armășescu I., *Biometria arborilor și arboretelor din România*, Ed. Ceres, București, 1972.
5. Giurgiu V., *Dendrometrie și auxologie*, Ed. Ceres, București, 1978.
6. Golden M., Ecology, **60**, *4*, 770—783 (1979).
7. Harper J. L., *Population biology of plants*, Academic Press, London, 1977.
8. Odum E. P., *Basic Ecology*, Saunder's. Company Philladelphia, Toronto, 1983.
9. Paucă-Comănescu Mihaela, Rev. păd. 1981, 36, 3, 155.
10. Popescu-Zeletin I., edited by, *Tabele dendrometrice*, Ed. Agrosilvică, București, 1957.
11. Popescu-Zeletin I., Dissescu R., St. Cerc. Biol., Biol. veget., 1961, **16**, *1*, 67—77.

ELECTROFUSION ON YEAST PROTOPLASTS

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The high potentials of electrofusion in yeast protoplasts hybridisation both for the protoplasts of some haploid laboratory yeast strains and also for industrial yeast strains important for the production of ergosterol are reported. The influence of the protoplast isolation quality on the electrofusion process is emphasized. Haploid laboratory strains represent the biological material much easier to handle than the industrial ones. The presence of pronase (1 mg/ml) in the fusion medium represents an important condition for industrial strains especially. The optimal parameters of the electric field for fusion induction are discussed.

Cell fusion has great potential in biotechnological programs. Both somatic hybridisation and genetic engineering experiments offer a way of modifying plant, animal and microorganisms cells. Somatic hybridisation is a promising means of genetic manipulation of yeasts that are of the same mating type. So far, the production of yeasts somatic hybrids by chemical fusion using PEG in the presence of calcium ions, especially, has been reported (2), (8), (19).

Work over the past few years on electric field induced fusion of protoplasts has progressed to the stage where this technique now deserves serious consideration as an effective alternative to PEG (9), (10), (14), (15), (17), (18).

In comparison with the chemical methods, the electric field-induced fusion leads to a very high yield of fused cells and the fusion process occurs synchronously. Most important, only a very small area of the plasma membrane in the contact zone between two adhering cells is subjected to electrical breakdown; most of the membrane surface is not affected by the electric field.

The viability of cells fused with electric field technique should thus be very good as demonstrated for other experimental systems like plant protoplasts or mamalian cells (11), (12), (13), (16), (17), (18).

In spite of these advantages and of the large biotechnological interests, the extended application of the electrofusion in somatic hybridisation of yeasts, met several difficulties of biological and technical nature demanding further thorough investigations. The information in this direction is quite scarce (4), (5), (6), (7), and is focused on *Saccharomyces cerevisiae* especially.

This paper reports some experimental original results concerning the achievement of the electrofusion process in yeasts protoplasts belonging to some haploid laboratory yeast strains and also to industrial strains involved in the production of ergosterol, in order to establish an efficient and reproducible hybridisation technology.

MATERIAL AND METHODS

Two haploid yeast strains of the same mating type (a) were used: *Saccharomyces cerevisiae* a ade6 and *S. cerevisiae* a trp4 kindly supplied

by the Laboratory of Genetics of Microorganisms from the University of Bucharest.

Electrofusion experiments were also carried on two industrial yeast strains producing ergosterol : *S. carlsbergensis* and *S. species*, kindly supplied by the Laboratory of Biosynthesis of the Institute of Chemical and Pharmaceutical Research.

ISOLATION OF YEAST PROTOPLASTS

The yeast cells were harvested in logarithmic phase, after 16—18 hours of cultivation in YEDP medium (yeast extract—1%, glucose —2%, peptona + 1%).

The protoplast isolation was performed in sterile conditions using a method adopted by Anghel and collab. (1), (2). The first step of the isolation protocol was a 30 min incubation in beta-mercaptoethanol 2×10^{-2} M at 30°C under slight stirring. The osmotic stabilizer medium contained — —0.5 M TRIS, pH — 7.5, 0.6 KCl and 0.02 M $MgSO_4$. Usually, cell densities of approx. 10^8 cells/ml appreciated in a Burker-Türk chamber were used. The second and most important step for protoplast isolation is the enzyme treatment of yeast cells in the presence of snail gut juice, 10 mg of dried crude extract per ml of osmotic stabiliser medium. Different time intervals for the incubation of the cell suspension in the enzyme mixture were tested : 1—5 hours at 30°C under slight stirring. Afterwards the protoplasts suspension was washed by three successive centrifugations in a solution of 0.02 M phosphate buffer, 0.6 M KCl and 0.1 M $CaCl_2$, pH—6.0.

Finally, the yeast protoplasts were suspended (10^5—10^6 cell/ml) in a fusion medium of 0.6 M manitol pH—6.4.

In the case of the industrial yeast strains the presence of pronase (0.5—2.0 mg/ml) in the fusion medium was necessary.

Electrofusion protocol. The experimental arrangement for electrofusion induction and visualising used in our investigations consists of :

— a VERSATESTER generator for sinusoidal signals (IEMI—Bucharest);

— a pulse generator and an amplifier (IFIN — Bucharest);

— an optical microscope type MC 3 (IOR — Bucharest);

— an optical microscope with reverse visualisation OPTON type, for sterile processing and cultivation of fusion products;

— fusion chambers with Cr electrodes obtained by deposition on glass by vacuum evaporation; the separation space between electrodes was of about 25—50 μm.

The fusion chambers were sterilised by ethanol washing. The entire experiment was carried in aseptical environment.

A short presentation, of the principles of the electrofusion method was made previously (11).

The protoplasts of two haploid yeast strains (an ade6 and a trp4) were mixed in equal quantities and the cell density was adjusted to 8×10^5 cell/ml. 10 μl of the mixed suspension were deposed on the fusion chamber and the dielectrophoretic alternating field was applied (frequency—2 MHz, intensity—500 V/cm). Shortly after the dielectrophoretic

alignment 3—5 electrical pulses of 6—15 μ sec duration and 3—5 kV/cm intensity were applied on the electrodes at 2 sec. intervals. Subsequent to the pulse, the dielectrophoretic alternating field is automatically replied for seconds. After electrofusion the protoplasts were aseptically transferred in sterile Petri dishes and grown on selective medium.

The efficiency of electrofusion was also expressed by fusion yields determined in the electrofusion chamber by counting of fused cells directly under the microscope or on photographs taken before and after the pulse application. The yield value errors amounted to 15%.

On industrial yeast strains only electrofusion between cells of the same strains was experimented. Different conditions regarding the electric field parameters were tested : dielectrophoretic field frequency 1—2 MHz, intensity 200—600 V/cm, electric pulse intensity 5—10 kV/cm and duration of 10—15 μsec.

CULTIVATION AND VIABILITY TESTING OF FUSION PRODUCTS

The regeneration process of the fused protoplasts was performed only for the hybrids of the two haploid strains of *S. cerevisiae*. Minimal standard medium (yeast nitrogen base without aminoacids, 2% glucose and 0.6 M KCl) was used.

After seven days the number of the colonies was counted. Afterwards the fusion products were isolated and the DNA content was measured by diphenylamine Dische's method (3). The DNA was determined in order to establish the degree of polyploidy.

RESULTS AND DISCUSSION

The development of the electrofusion process is determined by a number of experimental parameters, some referring to the cell suspension (the degree of the cell wall removal, the physiological state of the protoplasts, the cell density, the composition of the fusion medium), while the others characterize the conditions of electrical dielectrophoretic field and electroporative pulse.

A discussion of some of these parameters can be made on the results we obtained from the electrofusion experiments on protoplasts of industrial yeast strains. The interest was focused on finding the optimal electrofusion conditions for each of the two industrial strains ; the optimal conditions had been set by considering the fusion yield values as observed in the fusion chamber on the microscope (the regeneration was not aimed to).

We assume that one of the most important factors in the electrofusion of yeast protoplasts is a proper removel of the cell wall without any damage of the plasma membrane. The quality of the protoplast isolation can be satisfactorily assessed by the electrorotation spectra. We obtained experimental evidence that yeast protoplasts isolated after two hours of incubation in snail gut juice can be fused by chemical fusogenic agents (i.e. PEG) while electrofusion phenomenon was significantly observed only after five hours of enzyme incubation.

In order to achieve a reasonable reproducibility of the electrofusion experiments the cell densities at the moment of incubation in β-mercaptoethanol or in snail gut juice as well as the use the same stock of dried crude snail gut juice for the similar interval of time was carefully observed.

The presence of pronase in the fusion medium represents another important condition for electrofusion induction. The best condition was represented by the quantity of the 1 mg/ml pronase in the fusion medium. In these conditions a dielectrophoretic field of 2 MHz frequency and 400 V/cm intensity was necessary.

The density of the cells in the protoplasts suspension is also an important parameter determining the generation by choice, mainly of bi-cellular or three-cellular fusion products rather than multi-cell bodies.

In figures 1 and 2 the successive stages of the electrofusion process leading to either three-cell products or multi-cell giant bodies are presented. The relaxation time of the yeast fused bodies to a spherical shape is 1—2 min., much shorter than for the plant cell protoplast (cca. 20 min.) (11).

The variation of the electrofusion process as a function of some electric field parameters is presented in Table 1. It can be observed that the optimal electrofusion conditions for the two industrial strains are not dissimilar. As a consequence of electrofusion process, hybrid cells between *S. carlsbergensis* × *S. sp. strains* were produced.

Table 1

Electrofusion yields of industrial yeast strain protoplasts for different electric field parameters

Protoplast service	Alternating electrical field	Fusion electrical pulse		Number of pulses	Maximum fusion yield
		intensity	duration		
S. carlsbergensis mannitol 0.6 M pronase 1mg/ml	2 MHz 400 V/cm	11 kV/cm	10 μs	2	20%
		9 kV/cm	10 μs	2	43%
		9 kV/cm	20 μs	2	70%
		7,5 kV/cm	20 μs	2	65%
		7,5 kV/cm	40 μs	2	40%
S. species mannitol 0.6 M pronase 1mg/ml	2 MHz 400 V/cm	11 kV/cm	10 μs	2	40%
		10 kV/cm	10 μs	2	76%
		9 kV/cm	10 μs	2	75%
		8 kV/cm	10 μs	2	30%
		8 kV/cm	40 μs	2	25%
		6,5 kV/cm	40 μs	2	10%

In the case of the haploid strains, protoplasts we pursued the electrofusion experiments to the stage of hybrid regeneration. The optimal experimental conditions for ad6* a trp* electrofusion products were: 3 electrical pulses of 10 μsec duration and 5kV/cm intensity. The pronase treatment was not necessary in these cases. The fusion yield determined in the electrofusion chamber was of about 40%.

After the regeneration of the fusion products on the selective media the frequency of viable hybrids was estimated to be of 1×10^{-3}, similarly to that reported by Zimmermann et al. (18).

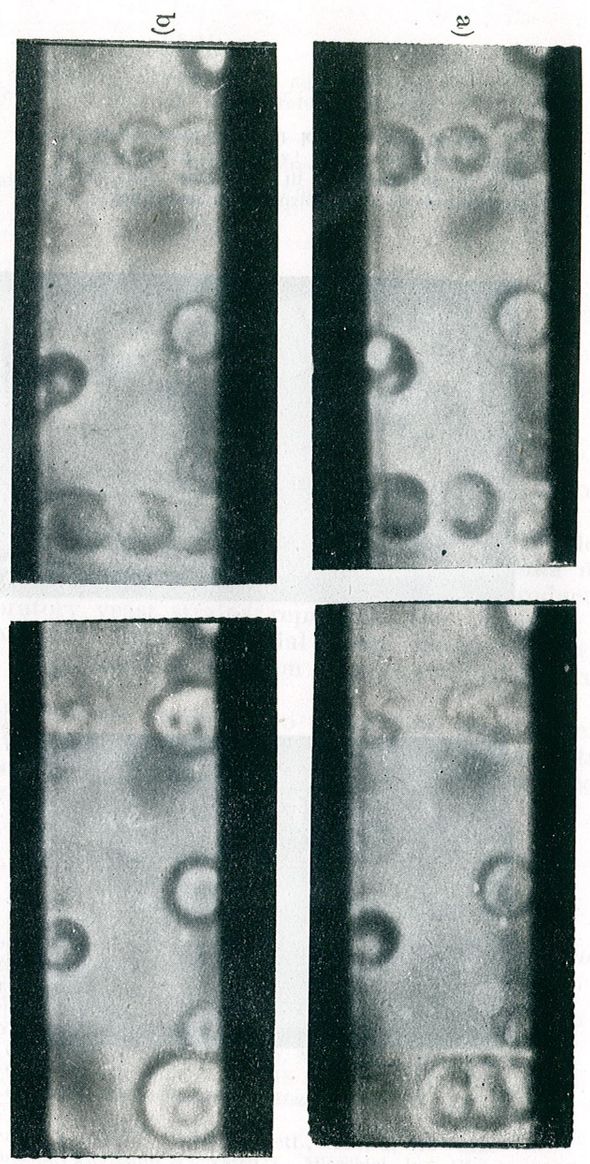


Fig. 1 — Stages of the electrofusion process of s.sp. protoplasts — leading to two- and three-cell fusion products: (a) pearl chain formation in dielectrophoretic field $E_d = 500$ V/cm, f = 2 MHz; (b) 2 sec after 2 electrical pulses of $E_f = 9$ kV/cm, $\tau = 10$ μs; (c) 7 sec after pulse; (d) 2 min after pulse.

Afterwards the polyploidy level of the fusion products was established by DNA content determination. (Table 2). As can be observed, the fusion products had a DNA content corresponding to the haploid, diploid and triploid level.

Table 2

DNA content per cell of haploid a ad 6 and a trp 4 strains and of some of the electrofusion hybrids

Strain	Cell density cells/ml	DNA content 10^{-12}mg/ml per cell	Polyploidy level
a ad 6	1.5 10^8	25	haploid
a trp 4	1.6 10^8	18	haploid
PF 5	1.2 10^8	19	haploid
PF 22	1.0 10^8	18	haploid
PF 29	1.1 10^8	48	diploid
PF 15	1.2 10^8	38	diploid
PF 16	1.0 10^8	38	diploid
PF 20	1.6 10^8	59	triploid

The experimental data obtained allow us to formulate the following conclusions :

— the method of protoplasts isolation is determinative for the degree of the cell wall removal and the physiological state of the plasma membrane and present a significant importance for electrofusion production ;

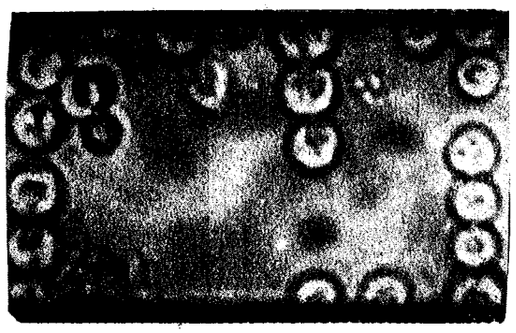
— haploid laboratory yeast strains represents the biological material much easier to handle than industrial yeast strains as regard the reproductibility of protoplasts isolation and electrofusion process induction ;

— futher experiments are necessary to understand more precisely the mechanisms involved in yeast protoplasts electrofusion — for instance to explain the necessity of the pronase presence to induce the electrofusion of industrial yeast strains.

REFERENCES

1. Anghel I., Vassu T., Moisa I., Circiumărescu D., in *Genetica Microorganismelor — Principii și tehnici de laborator*, 1985, Tipografia Univ. Buc., 201.
2. Anghel I., Vassu T., Brezeanu A., Rev. Roum. Biol.– Biol. veget., 1989, **34**, 2, 113–119.
3. Dische Z., in The Nucleic Acids, 1955, Chargoff E., Davidson J. H., Academic Press Inc.
4. Halfmann M. J., Röcken W., Emeis C. C., Zimmermann V., Curr. Genet., 1982, **6**, 25.
5. Halfmann M. J., Emeis C. C., Zimmermann V., Arch. Microbiol., 1983, **134**, 1.
6. Halfmann M. J., Emeis C. C., Zimmermann V., FEMS Microbiol. Lett., **1933**, *20*, 13.
7. Hofmann G. A., Evans G. A., *IEEE Engineering in Medecine and Biology, Magazine*, 1986, 1–20.
8. Maraz A., Kiss M., Ferenczy L., FEMS Microbiol. Lett., 1978, 3, 319–322.
9. Petcu I., Mocanu N., Radu M., Brezeanu A., Vassu T., Microbiol. Ind. Biotehn., 1988, Iași, 1031–1039.
10. Petcu I., Radu M., Mocanu N., Vassu T., Brezeanu A., Bul. Soc. Nat. Biol. Cel., 1988, **15**, 119.

a)



b)

Fig. 2. — Multi-cell electrofusion of S. sp. protoplasts (a) pearl chain formation in dielectrophoretic field E_a = 600 V/cm ; f = 2 MHz ; (b) 15 sec after 2 electrical pulses of E_f = 10 kV/cm, τ = 30 μs.

11. Petcu I., Brezeanu A., Radu M., Rev. Roum. Biol., 1989, **34**, 121.
12. Schnettler R., Zimmermann V., Emeis C. C., FEMS Microbiol. Lett. 1984, **24**, 81.
13. Schnettler R., Zimmermann V., FEMS Microbiol. Lett., 1985, **27**, 195.
14. Tsoneva I., Doinov P., Dimitrov D. S., FEMS Microbiol. Lett., 1989, **60**, 61.
15. Tsoneva I., Acta Microbiol. Bulgarica, 1989, **24**, 53.
16. Zimmermann V., Pilwat G., Pohl N. A., J. Biol Phys., 1988, **10**, 43—50.
17. Zimmermann V., Scheurich P., Planta, 1981, **151**, 26—32.
18. Zimmermann V., Vienken J., Halfmann J., Emeis C. C. Advances in Biotech. Press., 1985, **4**, 79—150.
19. Whittacker P. A., Leach S. M , FEMS Microbiol. Lett., 1978, **4**, 31—34.

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EFFECT OF GAMMA RADIATIONS ON HETEROCHROMATINE DISTRIBUTION UNDER CYSTEAMINE DIFFERENTIATED TREATMENT CONDITIONS

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The radioprotective and radiorepairer effect of cysteamine has been studied in the conditions of irradiation with gamma radiations produced by a Co^{60} source. The effect of irradiation has been considered taking into account the electromicroscopical images of nuclei from the radicular meristem of broad-bean (*Vicia faba* L) during interphase. It was shown that irradiation with gamma radiations caused alteration of the nucleus system, which could have been anoided with a cysteamine pretreatment. This pretreatment determined a relatively normal distribution of heterochromatine in the nucleus. It has been inferred that the cysteamine pretreatment had a clear radioprotecting effect. The cysteamine post-treatment had a somewhat radiorepairer effect, the efficiency of which diminished together with the dose. At a 300 r dose, the cysteamine post-treatment determined a ring-shaped ectopic conjugation of the chromosomes in the interphase nuclei.

The effects of radiations on the cell nucleus, and on the chromosomal apparatus as well, have been minutely studied by means of optic microscopy from a cytogenetical point of view (2),(3), (1), (5).

Numerous biochemical and biophysical researches have been performed on the nucleic acids extracted from the irradiated cells (1), as well as on the effects of some radioprotectors and radiorepairers on the nuclear genetic material (1). There is no information in the literature on the effects of irradiation and of radioprotective or radiorepairer substances on the cell nucleus during interphase.

We have tried, by an adequate electronomicroscopical research, to single out the effect of gamma radiations on the distribution of heterochromatine from the broad-bean radical meristem's nuclei. At the same time, the radioprotective and radiorepairer action of cysteamine over nucleus ultrastructure has been investigated.

MATERIAL AND METHODS

The broad bean (*Vicia faba* L) seeds have been soaked in water for 24 hours. After soaking they have been set to germination in Petri dishes on filter paper which was wetted daily with water.

During the 4 th growing day, when the primary root was 2—3 cm long, the seedlings underwent the following treatments: a) irradiation of the radicular apex with gamma radiation produced by a Co^{60} source, at 100 and 300 r doses; b) pretreatment of a batch with 300 $mg.l^{-1}$ cysteamine for 1 hour, followed by gamma irradiation, in 100 and 300 r doses; c) post-treatment with the same cysteamine concentration after gamma irradiation in the above-mentioned doses.

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After 12—14 hours of irradiation the apex of the primary root was fixed for 1 hour in 2% glutaraldehyde and postfixed for another 1 hour in 1% OsO_4. Fixation and postfixation too place at 7.4 pH, maintained with a buffer solution on phosphates. The interval of time between irradiation and fixation for electron microscopy has been chosen so that root growth relative speed should not be considerably modified by the different irradiation doses (8).

Dehydration of the vegetal material has been accomplished uder high acetone concentration up to absolute acetone. The waterless material has been included in westopal and has been sectioned with the ultramicrotome LKB III. The sections have been doubly coloured with uranyl acetate lead citrate. The electromicroscopical and microphotographical investigations have been accomplished with a BS 613 TESLA electron microscope.

Research has been carried out entirely on 9500 × nucleus microphotographs, which have been subsequently examined qualitatively and quantitatively. For the quantitative determination of heterochromatine distribution, the planimetrical method has been used. By means of this methods the following parameters have been: the total surface of the nucleus section; the area of the nucleus section; the area occupied by heterochromatine on the nucleus surface.

The total chromatine surface per nucleus section has been obtained by subtracting both the nucleol and the heterochromatine surface from the total nucleus surface. The relative quantity of heterochromatine per surface of nuclear section has been expressed in mm^2.

We must mention that in order to obtain some comparable data the measurements have been performed only on the microphotographs showing nuclei with complete nuclear membrane and with nucleole in section.

RESULTS AND DISCUSSION

The results of our investigations and measurements have been included two plates and one table. The effects of irradiations with gamma radiations, as well as the radioprotective and radiorepairer action of cysteamine on the heterochromatine distribution per nucleus section can be deduced by studying the electro-microscopic images and the data included in Table 1.

HETEROCHROMATINE DISTRIBUTION PER NUCLEUS SECTION AT THE CONTROL

Data regarding heterochromatine distribution at the control nuclei are presented in Plate I, Fig. 1 and Table 1.

It was shown that the ultrastructure of a meristematic cell nucleus from the broad bean root apex is the normal one.

The total heterochromatine surface per nucleus section (the relative heterochromatine quantity expressed in mm^2 respectively) stands between

1070 and 2220 mm^2 depending on the cytophysiological and biochemical condition of interphase nuclei. Table 1 presents these values for G_1, S, and G_2 phases.

Table 1

Variation of the relative heterochromatine quantity per section area expressed in mm^2

Type of nucleus	Heterochromatine relative quantity in mm^2
Control	
At phase G_1	1070
At phase S	1650
At phase G_2	2220
Irradiated 100 r	
At phase G_1	580
At phase S	1180
At phase G_2	1890
Irradiated 300 r	
At phase G_1	754
At phase S	1460
At phase G_2	2160

2. HETEROCHROMATINE DISTRIBUTION PER NUCLEUS SECTION AFTER GAMMA RAY IRRADIATION

As presented in table 1 irradiation with gamma rays in 100 and 300 doses led to a lowering of the heterochromatine relative quantity per nucleus section in all the 3 phases of the cellular cycle (G_1, S, and G_2). An interesting thing to notice is the fact that the 100 r gamma ray dose determined a greater reduction of the heterochromatine relative quantity in all the phases of the cellular cycle compared to the 300 r dose. We could interpret this unexpected result as a radiorepairer response, much more increased at high gamma ray doses than at lower doses. Plate 1, Figs 2 and 3 confirm this results; at a 100 r dose greater perturbances are to be noticed in the heterochromatine distribution than when irradiating with a 300 r gamma ray dose.

3. RADIOPROTECTIVE AND RADIOREPAIRER EFFECTS OF CYSTEAMINE

Cysteamine is a well-known radioprotective substance. Plate I, Fig. 4 and Plate II, Figs 1 and 2 clearly reveal the radioprotective ceffets of cysteamine on the ultrastructure of the broad-been radicular meristem. It has been established that the effect of cysteamine pretreatment is greater when irradiating with 300 r gamma ray dose than with the 100 r

dose. This clearly expressed effect of the 300 r dose may be correlated to an association of the cysteamine radioprotective action with the radiorepairer processes which take place in the cells after irradiation. We must mention that although in terms of quality, the heterochromatine distribution seems to be similar to its distribution in the unirradiated nuclei, the heterochromatine relative quantity per nucleus section has been slightly lower.

The radiorepairer effects of cysteamine can be deduced from Plate II, Figs 3 and 4. The radiorepairer effect has been conclusive as a result of 100 r gamma (ray dose) irradiation (Plate II, Fig. 3). In case of irradiation with 300 r gamma ray dose the radiorepairer effect of cysteamine became obvious by deviating from the normal. The nucleole dimensions are double compared to the nucleole in the control nuclei and the heterochromatine distributed itself in a unique ring-shaped body owing to the so-called ectopic conjugation. A minute observation of the microphotograph in Plate II, Fig. 3, indicates that this ring-shaped body has been formed by joining the chromosomes.

This process is not new for the living world, only for *Vicia faba* L. It was noticed in spontaneous conditions (beyond the action of radiations and radiorepairers) with *Crepis capillaris*, by E. B. Vagennaar in 1968 (6) and E. B. Vagenaar, R. S. Sadasivaiah in 1969 (7). In Plate II Fig. 3, it is also to be noticed that the 12 chromosomes of *Vicia faba*, which were to be found in the interphasic nucleus, after cysteamine pretreatment, are in contact with the cell nucleus membrane. This process, too, was observed under normal circumstances, with *Allium fistulosum*, by G. E. Onischenko and Iu. S. Chentsov in 1973 [4]. Consequently, in our opinion, at the 300 r dose gamma irradiation cysteamine had a clear radiorepairer effect. This is true mainly because the chromosomes in the interphasic nucleus, as well as the distribution of heterochromatine in these chromosomes, represent a normal process for other vegetal species, especially in the late telephase. We consider our image to be a late telophase because of cysteamine post-treatment. Gamma irradiation, together with cysteamine post-treatment, could be used in transferring chromosomes from one cell to another, through an appropriate biotechnology.

CONCLUSIONS

1. Gamma radiations produced by a Co^{60} source determine alteration of the broad-bean cell nucleus ultrastructure (*Vicia faba* L) in the radicular meristem.

2. Heterochromatine distribution per nucleus section demonstrates a variation in the heterochromatine relative quantity, depending both on the cell cycle phase and on the damage extent of the irradiated nucleus.

3. Damages are greater at a 100 r dose than at a 300 r dose, probably because of some radiorepairer effects at the latter dose.

4. Cysteamine in a 300 mg. l^{-1} concentration has evident radioprotective and radiorepairer effects.

Plate I

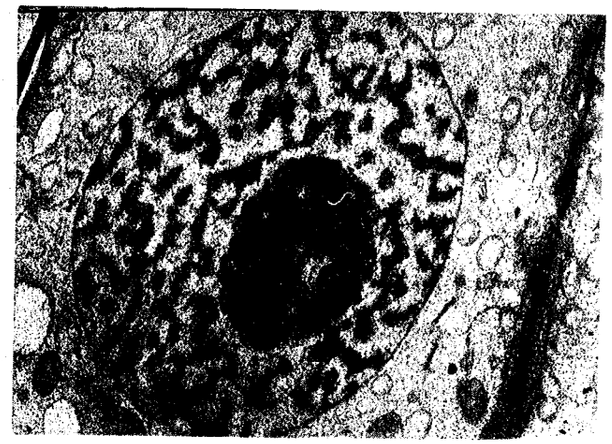


Fig. 1. — Nucleus ultrastructure from the broad-bean (*Vicia faba* L), radicular meristem at control (5.000 ×).

Fig. 2. — Effect of 100 r dose gamma irradiation.

Fig. 3. — Effect of 300 r dose gamma irradiation.

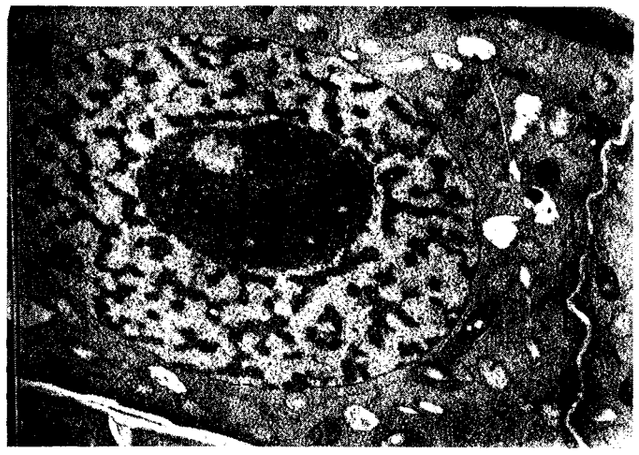
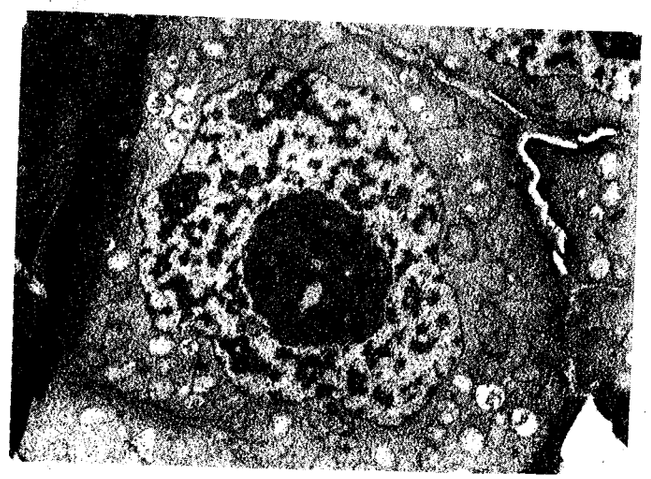


Fig. 4. — Cysteamine radioprotective effect at a 100 r dose gamma irradiation.



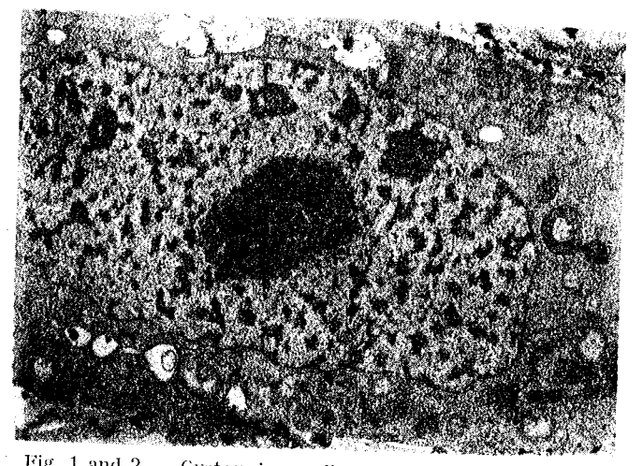


Fig. 1 and 2. — Cysteamine radioprotective effect at a 300 r gamma irradiation.

Fig. 3. — Cysteamine radiorepairer effect after a 100 r dose gamma irradiation.

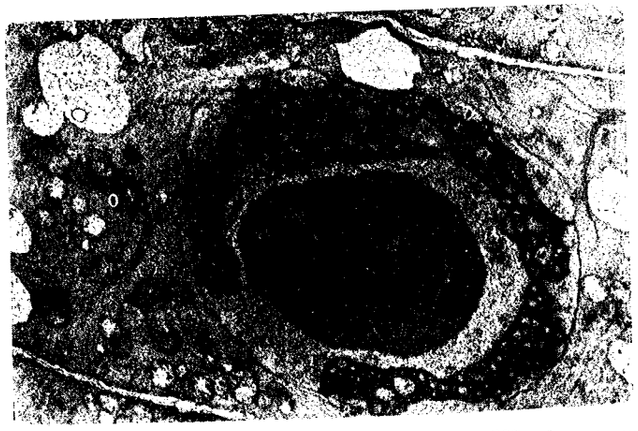


Fig. 4. — Cysteamine radiorepairer effect after a 300 r dose gamma irradiation.

REFERENCES

1. **Auerbach Ch** *Mutation Research. Problems, Results and Perspectives*, Chapman and Hall, London, 1976.
2. **Dalle W. M.**, *Direct and indirect effects of ionizing radiations* in *Encyclopedia of Medical Radiology*, Springer Berlin — Heidelberg — New York, 1966, vol. II, pp 1—38.
3. **Hollander H.**, *Radiation Protection and Recovery*, Pergamon Press, New York, 1960.
4. **Onishchenko I., Iu. S. Chentsov**, Tsitologia, 1973, **15**, pp 643—649.
5. **Prokofieva A. A.**, *Belgovskaja — Geterothromaischeskye raion v khromosom*, Izd.-vo "Nauka", Moskva, 1986.
6. **Wagenaar E. B.**, Chromosome, 1968, **26**, pp 410—426.
7. **Wagenaar E. B., Sadasivaich R. S.**, Can. J. Genet. Cytol., 1969, **11**, p. 403.
8. **Zezina N. V.**, *Formirovanie plecher na krivykh dozo-effekt v khode postradiatsinogo vosstanovlenija meristemy kornye* in *Evristichnost radiobiologii*, Nauka Dumka, Kiev, pp 100—108.

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THE FINDING OUT OF SOME MUTANTS OF *NOCARDIA MEDITERRANNEI* ON SELECTIVE MEDIA

VIORICA COLF, VICENȚIU ȘTEFĂNESCU, RODICA GOLOGAN, ION I. BĂRA

Under UV treatment the *N. mediterrannei* colonies appear with high frequency on the selective media. The higher percent was found on the selective medium with 6 mM Fe^{+2}.

Some connections between the detoxifying mechanisms of heavy metals ions and the biogenetic pathways of the antibiotic, recommend the method as a selection pressor.

1. INTRODUCTION

The efficiency of administering a mutagenous, physical or chemical factor is estimated according to the lethality percentage and to the amplitude of individual variability which a populational sample produces under the incidence of its own action. Another criterion is the estimation of the mutants' frequency on selective media and has the advantage that, by diminishing the number of survivors (ulterior to the treatment with mutagenous factor), the selection activity is considerably reduced.

Taking into account the part played by the mutations in ensuring the best amplitude of variability (field of action for selection), we aimed at inducing and quantifying this process by using the UV radiations and the selective media.

2. MATERIAL AND METHODS

The biological material investigated represented populational samples belonging to *Nocardia mediterrannei* species, R-182 strain from the collection of the Research Center for Antibiotics.

The selective media were prepared by adding 6mM Fe^{+2} or 1.27 mM Cu^{+2} — elements considered as selection pressors — to the Bennett agarised medium (like control variant).

The mutagenous treatment was performed with Philips bactericidal lamp of 30 W for 60″ at a distance of 40 cm.

Both in the treated populational sample and in the control one seriated dilutions were performed. A quantity of 0.2 ml was taken from each of them and put on Petri dishes with selective and control media. The dishes were incubated for 14 days at 28°C (Fig. 1). When the incubation period was over the colonies were counted to estimate the number of surviving individuals. In order to obtain better estimations, the ratio of mutants appearance was computed by using the following formulae: $r_0 = M_0/N_0$ and $r_1 = M_1/N_1$, where N_0, N_1, M_0 and M_1 represent the number of survivors/ml in the conditions listed in Table 1 (2).

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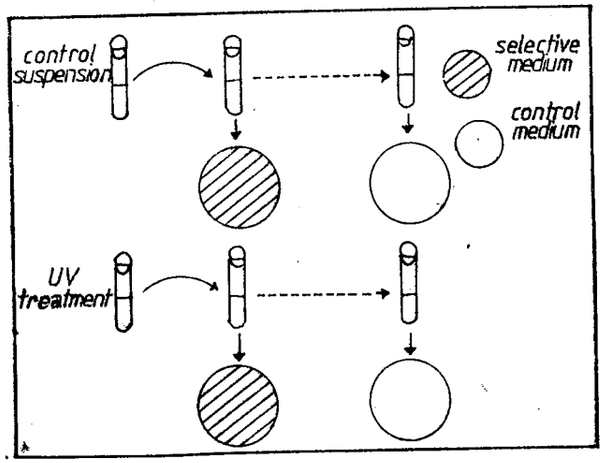

Fig. 1. — Selective media utilization.

Table 1

Vials number on ml suspension

Variants \ Culture media	Control standard	Selective
Control	N_0	M_0
treatment UV	N_1	M_1

$$r_0 = \frac{M_0}{N_0} \qquad r_1 = \frac{M_1}{N_1}$$

3. RESULTS AND DISCUSSIONS

The specialists unanimously agree to the idea that the microorganisms have the highest mutation ratio (1). Therefore, it is to be expected that in any experiment the frequency with which a mutation appears should represent a total of the number of spontaneous mutants and that of the mutants induced through administering a certain treatment. In principle, the control variants reveal the very prevalence of the eventual spontaneous mutations appearing during ca ertain experiment.

On the other hand, it is known that the heavy metals ions, Hg^{+2}, Cu^{+2} and the related organometallic ones, in the presence of β-lactamic

molecules, make up macromolecular complexes, the mutants resistant to metal ions possessing the capacity of increasing the productivity in β-lactamic antibiotics (3). There are some opinions according to which the respective micro-organisms can use the β-lactamic compounds in the process of detoxification by stopping the heavy metals ions linking and the possible interferences with the mercapto groups within certain intercellular structures.

In the present case, by administering the treatment with UV and owing to the selective pressure exerted by the media to which Cu^{+2} and Fe^{+2} have been added there have been obtained sure results which are listed in table 2.

Table 2

Vials number/ ml

Variants \ Culture medium	Bennett (control)	Selective	
		6mM Fe^{++}	1.27 mM Cu^{++}
Control	$13 \cdot 10^{11}$	$8.27 \cdot 10^{8}$	$2.8 \cdot 10^{6}$
UV treatment	$12 \cdot 10^{6}$	$7.11 \cdot 10^{6}$	130^{3}

By applying the above-mentioned formula we est imated the appearance frequency of mutations on both standard medium and on the selective ones (Table 3 and Fig. 2). It was found that, by using the medium

Table 3

Mutation frequency on selective media

Mutation frequency \ Selective media	Fe^{++}	Cu^{++}
$r_0 = \frac{M_0}{N_0}$	$6.3 \cdot 10^{-6}$	$2 \cdot 10^{-6}$
$r_1 = \frac{M_1}{N_1}$	$6 \cdot 10^{-2}$	$1.2 \cdot 10^{-5}$

$r_1 > r_0$

enriched with 6 mM Fe^{+2}, after the treatment with UV we find a mutation ratio by far higher than that of the witness ($r_1 > r_0$). Similar results are obtained by using the medium with 1.27 mM Cu^{+2} as selection factor as well, but the selective efficiency of the medium with Fe^{+2} is by far superior ($r_1 Fe^{+2} > r_1 Cu^{+2}$).

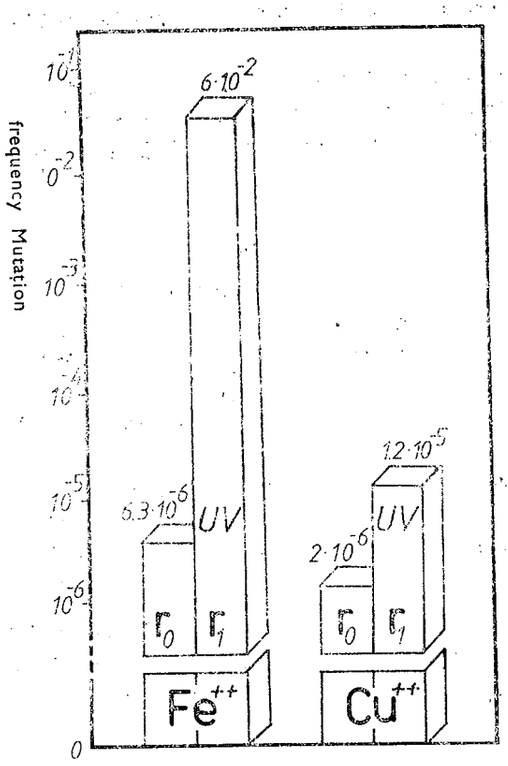

Fig. 2. — Mutation frequency induced by UV treatment on selective media.

The experimental results show the appearance of a high surviving frequency with the UV treatments of the mutants on selective media with Fe^{+2} and Cu^{+2}, which justifies the supposition according to which there are certain detoxifying mechanisms in the case of the R-182 strain of *Nocardia mediterrannei*, too

4. CONCLUSIONS

The high appearance frequency with the colonies on selective media after the treatment with UV radiations proves the efficiency of this mutagenous agent in the case of the R-182 strain.

The selective medium enriched with 6 mM Fe^{+2} proved to be much more efficient with the selection pressor in comparison with the medium containing 1.27 mM Cu^{+2}.

If the next experiments prove the existence of certain links between the detoxifying mechanisms of heavy metals ions and the biogenetic ways of the antibiotic, the method of using them as selection pressors in genotypical ameliorations will be very productive.

REFERENCES

1. Duca E., Duca M., Furtunescu G., *Microbiologie medicală*, Ed. Didactică și Pedagogică, București, 1979.
2. Tudose I., *Genetica microorganismelor*, Ed. Didactică și Pedagogică, București, 1983.
3. Vournakis J. N., Elander R. P., Science, **219**, 704 (1983).

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CHROMOSOMIAL-LEVELED MUTAGENESIS, INDUCED BY A NEW COMPOUND—IASINONE-1 — AT *SECALE CEREALE* L.

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The new synthesized compound, Iasinone-1, is representative for a group of substances of pharmacological importance. Its effect was tested on *Secale cereale* L., at the nuclear, cellular and tissular level. The results obtained confirm the fact that this compound—like the others, previously tested — acts as a modulator of the redox quality of the medium.

A new class of substances, namely Iasinones (A. Gașcaval et al., 1984), offers us, through a representative compound, the occasion of corroborating two of our observations referring to the mutagenesis induced by chemical agents. Compounds belonging to this class possess biostimulative and redox modulating properties (C. V. Zănoagă), alongwith a presumptive corono-dilating action, characterizing several substances with similar structures. Their possible utilizations as drugs and also as phytoregulators determined us to study their eventual mutagenic potential and, thus, to verify our observations upon the mutageneity of the media differing, from the redox viewpoint, from the optimal value (C. V. Zănoagă et al., 1987), as well as the phenomenon of the optimal rH values shifting as dependent on the organization level of the living matter upon which the action is being exercised and/or the observation is performed (I. G. Tudose et al.).

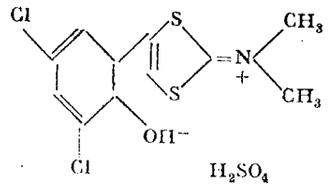
1. MATERIAL AND METHOD

In the elucidation of all these aspects, a method of our own has been employed, involving the experimental procedure described below applied to iasinone-1 sulphate.

Solutions of various concentrations (1/1,000 ... 1/100,000) (Table 1) have been used for moistening the inert substrate on which the rye caryopses (Moara Domnească type) have been germinated, at a temperature of 20°C, at dark.

Table 1

Concentration	H
1/1,000	28.93
1/3,000	29.81
1/5,000	30.33
1/7,000	30.86
1/50,000	34.70
1/100,000	32.53



Iasinone-1 sulphate

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Some of the resulting plantlets have been harvested after 4 days, when the roots' length reached 10 . . . 15 mm, the hypocotyles being fixed in Battaglia fixing bath, followed by conservation in ethyl alcohol 70°. After Feulgen colouring of the apical meristem, on this material there have been microscopically determined the frequency of chromosomial aberrations ($\times$ 400) as marker to the nuclear levelled action, and also the frequency of the cells in mitotic division ($\times$ 200) as marker of the cell-levelled action.

The remaining plantlets have been let to grow in the same conditions, for other 10 days, when their average weight of epicotyles has been determined as marker of the tissular-levelled action.

The redox character of the solutions employed in the treatment has been determined, as quantified by the rH parameter, through a potentiometric method previously established (C. V. Zănoagă et al., 1988), with a view to using these data as reference system in the interpretation of the already mentioned biometrical data, knowing the decisive role played by the medium's redox conditions in ontogenesis and, generally, in plants' physiology (C. V. Zănoagă).

As mere information, Figure 1 presents the biometrical data obtained, as depending upon the concentration of the bioactive substance, in semilogarithmic coordinates ; the interpretation of such a dynamics is less significant — for our investigations — than the rH-dependant one.

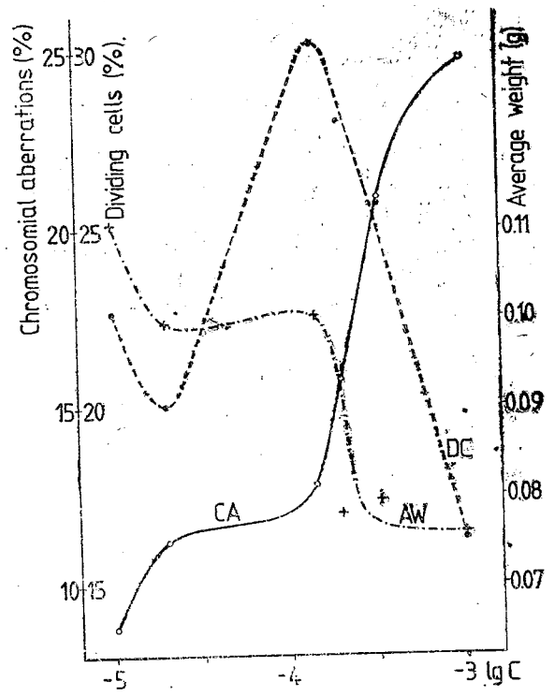

Fig. 1

The results obtained with the three biometrical tests have been graphically represented as a function of the solutions corresponding to each experimental variant (Fig. 2).

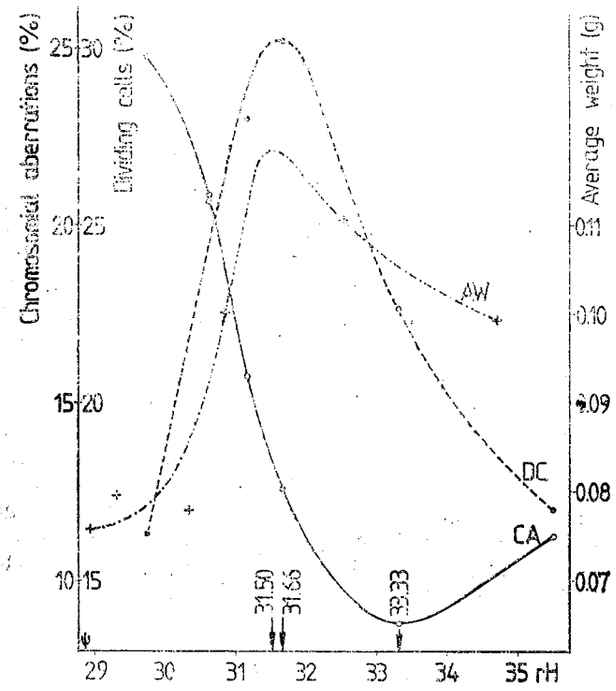

Fig. 2

2. RESULTS AND DISCUSSIONS

The slope of dynamics presented agrees with those characterizing other biologically active substances, previously tested (*C. V. Zănoagă* et al.), namely the Gaussian slope for the epicotyles' weight/height (AW) and for the frequency of the cell in mitotic division (DC), respectively the converse Gauss slope for the frequency of the chromosomial aberrations (CA). This fact confirms our hypothesis regarding the action of the biologically active substances and of the phytohormones by modulating the medium's rH, thus creating proper or improper life conditions to the biological system under study and, respectively, as redundant action — that of the rH at nuclear level, phenotypified in the effect produced at tissular level (*C. V. Zănoagă*).

Mention must be also made of the shifting of the optimum rH values as a function of the level to which the response of the biological system occurs, that is 33.33 at nuclear level, 31.66 at cellular level and 31.50 at tissular level, which supports our previous observations. Certainly, this

may be explained by the existence of some differentiated homeostasis mechanisms at each level, which is actually expected, requiring different redox conditions.

3. CONCLUSIONS

Consequently, one can notice, on the one hand, mutagenic properties of the studied compound within the range of high concentrations, characterized by values different from the optimal ones, usually reducing values (Table 1), which is explained through the differentiated behaviour of the photoautotrophic plants when the rH shifts from the optimum in a reducing or oxidative direction; when shifting towards the former direction, the plants are more drastically affected than in the latter case, as also observed from the abrupt, respectively gentle slopes of the AW curve branches. With lower concentrations, characterized by a rH placed in the optimum range (Table 1), the compound manifests a slightly stimulating effect (*C. V. Zănoagă*).

On the other hand, the difference observed between the optimum rH values may be exploited in the direction of utilizing, generally in chemically-induced mutagenesis, concentrations of the mutagene agent characterized by optimal rH values of the cell-levelled phenomena, which possess a sufficiently high mutageneity degree, implicitly assuring a good survival rate (to an optimal rH al cell level, i.e. 31.66, the ratio of chromosomial aberrations in our situation is double, as compared with that recorded with the nuclear level optimum (33.33) (Fig. 2).

REFERENCES

1. Cașcaval A., Radu C., Romanian Patent 90674, 1984.
2. Zănoagă C. V., *O nouă clasă de inhibitori — Iașinonele. Aspecte redox ale acțiunii lor*, Cercet. agr. în Moldova, in press.
3. Zănoagă C. V., Tudose I. G., Zănoagă Mădălina, *Aspecte ale dinamicii potențialului redox în sisteme biologice sub influența unor agenți chimici. Dinamica aberațiilor cromozomiale la secară — Secale cereale* L. (2n = 14), Culegere de studii și articole de biologie, vol. 3, Univ. Iași, 1987, pp 311–316
4. Tudose I. G., Zănoagă C. V., Păun Camelia, Asaftei Maria, *Cercetări citogenetice la secara tratată cu ape structurate*, St. cerc. biochim, in press.
5. Zănoagă C. V., Neacșu, I., Zănoagă Mădălina, *Considerații asupra tehnicii de determinare a rH-ului unor probe biologice*, St. cer. biochem., **31**, 1, 1988, pp 53–58.
6. Zănoagă C. V., *Un punct de vedere asupra mecanismului de biostimulare indusă de agenții chimici*, Mem. sect. șt. Acad. Rom., in press.
7. Zănoagă C. V., Băra I. I., *Repercusiuni citogenetice ale potențialului redox al apei indus de poluanți chimici*, Paper presented at the Round table "Poluarea ca factor limitativ în acvacultură", Piatra-Neamț, Jan. 1989.

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SOME CONSIDERATIONS UPON MUTAGENESIS INDUCED BY REDOX AGENTS

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Some organic compounds, with very different structures, were investigated to establish their role as a redox modulator. The patterns of this phenomenon were verified by physical ways. There was found a clear dependence of the biological system (at cellular, tissular and nuclear levels) response to different active substances by whole rH values.

Several experiments, performed on plants and aimed at testing some biologically active substances, draw the attention upon an extremely interesting phenomenon — initially neglected or interpreted as an experimental error — namely reaching of different rH values for the maxima of the frequency of the cells in mitotic division or of the plantlets' height, respectively the minimum of the frequency of chromosomial aberrations, as markers of the phenomena occurring at cellular, tissular or nuclear level; in this case, the latter are represented as depending on the rH of the working solutions, the coincidence of these rH values — considered as optimal (Table 1) — being expected.

Table 1

No	optimum rH			
	Tissular (H)	Cellular (DC)	Nuclear (A)	Ref.
1	28.70	29.05	31.00	[1]
2	24.80	—	24.00	[8]
3	33.04	—	31.60	[9]
4	29.50	—	29.40	[6]
5	31.40	32.36	31.00	[7]
6	22.77...22.85	—	25.52	[10]
7	31.00	29.99	29.30	[2]
8	31.50	31.66	33.33	[12]

This phenomenon has been subsequently explained by the different levels of organization of the living matter under investigation, therefore of some differentiated, progressively complex, homeostasis mechanisms at this level and, implicitly, of the necessary existence of some different optimal rH values (I. G. Tudose et al.), having in view that, for example phenomena developing — in the case of plants — in the absence of light are characterized by optimal rH values different from those found in the presence of light (C. V. Zănoagă et al.). Surprised by the absence of a severe order in the succession of the optimal rH values characterizing the three mentioned levels (Table 1), we initiated a standard experiment, aimed at elucidating this aspect, and also at gathering information regarding the whole range of rH variation, as decisive for the above-mentioned phenomenon. In this respect, the dependence on rH of several biotic and abiotic phenomena has been discussed on various occasions, the decisive part played by this parameter being evidenced (*C. V. Zănoagă*).

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1. MATERIAL AND METHOD

Rye caryopses (Moara Domnească type) were employed as biological material — as in the experiments cited in Table 1 — while, for inducing various rH values in the medium, redoxtron was used, known as assuring a rH logarithmic gradient ranging between 0 and 42.4, thus covering the whole range of definition of the notion (C. V. Zănoagă et al., 1987); the rH values thus obtained possess a high degree of reproducibility, in contrast to the situation obtaining from employment of chemical redox modulating agents that due to their much lower redox buffering capacity, lead to quite different optimal rH values (Table 1).

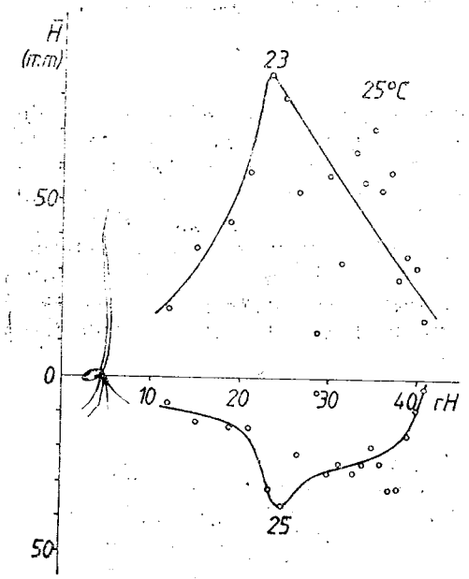

Fig. 1

Caryopses were germinated on redoxtron, in contact with distilled water, to which — in view of the redoxtron device functioning — 0.001 N SO_4^{2-} were added, at a temperature of 25°C, at dark. Six days later, the plantlets were harvested, the determination of the parameters under study being initiated. Thus, there have been determined — as average values — the epycotyles' lengths, then the roots have been fixed with Battaglia fixing solution and conserved in ethylic alcohol 70°. Inthe apical meristem of the roots, there has been determined microscopically, after Feulgen colouring, the frequency of cells in division (× 200) and of the chromosomial aberrations (× 400). The obtained data have been graphically plotted, versus the corresponding rH.

2. RESULTS AND DISCUSSIONS

Figure 1 reveals that the slope obtained, of the Gaussian type, characterizes both the dynamics of the epycotyles' height and that of the average length of the hypocotyles, each of them showing an optimal value, placed at an rH value of 23 for epycotyles and 25, respectively, for hypocotyles. All observations to be further made will employ the hypocotyles' optimal value, that is of the tissue to which the other determinations are also referring.

The frequency of the chromosomial aberrations (Fig. 2) as sum of the following types: bridges, fragments, retarding chromosomes, micro-

nuclei, tri- and tetra- ana- and telophases, dispersed nuclear material, is situated, again as usual, on a converse Gaussian type dynamics, having its minimum at rH 30, which is obviously different from the maximum of roots' development.

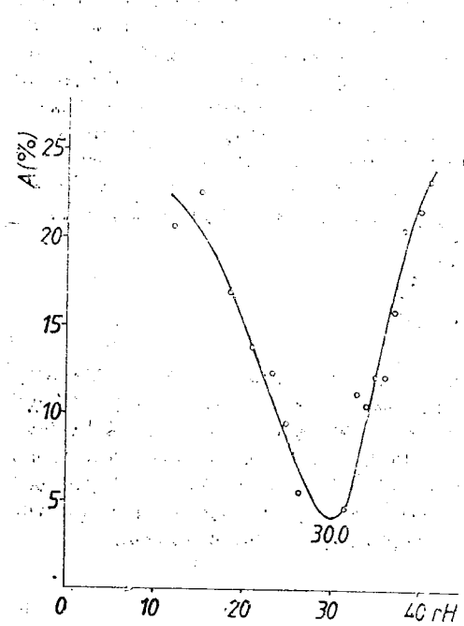

Fig. 2

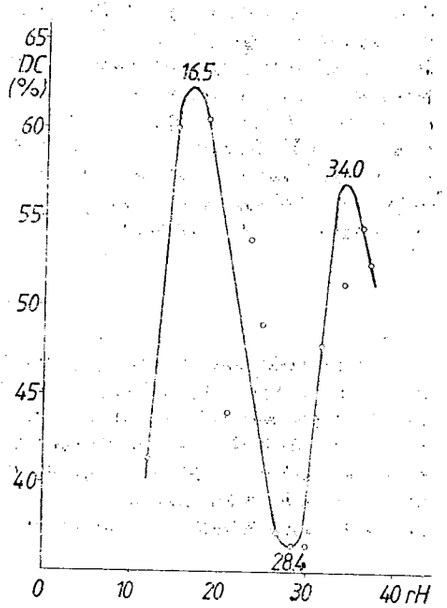

Fig. 3

The frequency of the cells in mitotic division (Fig. 3) as sum of pro-, meta-, ana- and telophases, unlike the situations observed with some restricted rH domains — previously employed (Table 1) — and unlike those expected, shows a minimum at rH 28.4, placed between two maxima (rH 16.5 and 34, respectively), both of them situated at rH values different from the optimal values of the hypocotyles' length and the frequency of the chromosomial aberrations, respectively. This situation explains both the differences observed between the optimal rH values at the three levels — nuclear, cellular and tissular — and the absence of a severe order between these values (the working domain including any one of the two maxima).

Previous interpretation of results (*I. G. Tudose* et al.), according to which the differences between the optimal rH values are due to the presence of some differentiated homeostasis mechanisms, at the three mentioned levels, is still valid, yet on assuming an interdependence between mechanisms. Consequently, the global phenomenon may be presented as such.

At an rH value optimal for the tissue's development, the frequency of the chromosomial aberrations in minimum, which does not request, in compensation, an increased frequency of the cell divisions. Yet, as the

rH of the medium moves off the optimum, the frequency of chromosomial aberrations increases, as a consequence of the mutageneity of the rH values different from the optimum (C. V. Zănoagă et al., 1987), the manifestation of some noxious effects becoming thus possible, a compensatory effect of increasing the frequency of cellular divisions does appear. The compensation introduced is valid only for a relatively low shifting from the optimum of the average rH of medium (16.5 . . .34), after which, in parallel with the increase of the frequency of chromosomial aberrations, a decrease of the frequency of cell division is observed, the cumulated effect being explained by the low development at the tissular level. A similar correlation has been previously observed with sugar beet, between sugar concentration and the roots' weight, the plant's productivity being influenced (*C. V. Zănoagă* et al., 1986).

3. CONCLUSIONS

The existence of the two maxima in the dynamics of the frequency of cell divisions may be exploited in the case of the mutagenesis induced by chemical agents, i.e., in the utilization of concentrations characterized by rH values placed at these maxima. In such situations, a relatively good survival rate is expected, along with a relatively high frequency of the chromosomial aberrations, i.e. a mutagenic effect explained through the compensating effect of increasing the frequency of cell divisions.

REFERENCES

1. Tudose I. G., Zănoagă C. V., Păun Camelia, Asaftei Maria, *Cercetări citogenetice la secara tratată cu ape structurate*, St. cerc. biochim., in press.
2. Tudose I. G., Zănoagă C. V., Filimon M., Cașcaval A., *Efectul citogenetic indus de o substanță nou sintetizată — iașinona — la Secale cereale* L., An. St. Univ. Iași, in press.
3. Zănoagă C. V. Asaftei Maria, Băra I. I., Cruceanu M., *Efecte ale zeoliților asupra dinamicii unor procese biologice la secară, în primele stadii ontogenetice. Corelații cu potențialul redox*, Paper presented at the 6th Technico-Scientific Symposium „Progrese și perspective în fabricarea și prelucrarea fibrelor chimice", May 24—27, 1988, Piatra-Neamț, in press.
4. Zănoagă C. V., *Un punct de vedere asupra mecanismului de biostimulare indusă de agenți chimici*, Mem. sect. șt. Acad. Rom., in press.
5. Tudose I. G., Zănoagă C. V., Cașcaval A., Tăruș C., *Influence of a cumarine derivative upon cell frequency in the mitotic division, chromosome aberrations and plantlet increase with Secale cereale* L. (2n = 14). *Correlation with the rH*, An. St. Univ. Iași, XXXIV, s. II a Biol., 1988, pp 79—82.
6. Tudose I. G., Zănoagă C. V., Cașcaval A., Mihăilă Georgeta, *Efecte citogenetice induse de tratamentul cu un derivat cumarinic la Secale cereale* L., Paper presented at „Simpozionul de genetică și inginerie genetică", Nov. 20, 1987, București, in press.
7. Zănoagă C. V., Tudose I. G., Zănoagă Mădălina, *Aspecte ale dinamicii potențialului redox în sisteme biologice sub influența unor agenți chimici. Dinamica aberațiilor cromozomiale la secară — Secale cereale* L. (2n = 14), Culegere de studii și articole de biologie, vol. 3, Univ. Iași, 1987, pp 311—316.
8. Zănoagă C. V., Băra I. I., Asaftei Maria, Cruceanu M., *Cytogenetic effects of rye germination on zeolite substrate*, An. St. Univ. Iași, in press.
9. Zănoagă C. V., Păun Camelia, Băra I. I., Nicolae Margareta Stan Dana, *Redox aspects regarding biological systems subjected to residual waters*, An. St. Univ. Iași, in press.

10. Tudose I. G., Zănoagă C. V., Drăghici Vasilica, Băra I. I., Cașcaval A., *Mutageneza la nivel cromozomial indusă de un nou compus — iașinona — 1 — la Secale cereale* L., Paper presented at the „Primul simpozion național de inginerie genetică", București, 1989, Rev. roum. biol.—biol. végét., **35**, 2, 1990 pp. 133—136.
11. Zănoagă C. V., Zănoagă Mădălina. Uglea C. V. *The behavior of rye in rH gradient*, Rev. roum, Biochim., **24**, 4, 1987, pp 357—360.
12. Zănoagă C. V., Tudose I. G., Oniscu C., *Considerații privind efectul compușilor ASFA-2 și ASGA-4 asupra greutății rădăcinii și conținutului în zahăr la Beta vulgaris* var. *Saccharifera*, An. St. Univ. Iași, XXXII, s. II a Biol,. 1896, pp 99—100.

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