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Biochemistry

SOME CYTOLOGICAL ASPECTS OF HERBICIDE 2, 4-D TRANSLOCATION IN PLANTS

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(Received January 10, 2005)

Abstract

Herbicide 2, 4-D assimilation and translocation has been studied in cells of maize root apex, stem parenchyma and leaf mesophyll. At 24 h exposition the herbicide does not bind with cell structural elements. The fragmented sites are translocated in the symplast of the parenchyma cells of the cortex via plasmodesmata. The herbicide is degraded in leaf mesophyll, by enzymatic systems of endoplasmic vesicles and cytoplasm.

Key words: pesticide 2, 4-D, localization, root apex, stem parenchyma, leaf mesophyll.

Introduction

Plants are known to be capable of assimilation and biotransformation of xenobiotics [Buadze O., Durmishidze S., et al., 1985; Durmishidze S. 1988; Kakhniashvili Ch. 1988; Korte F., Kvesitadze G. et al., 2000]. The pesticide 2, 4-D penetrates into maize roots after 3 minutes of incubation and after 30 minutes it reaches the leaves [Buadze O., Durmishidze S. et al., 1985]. After more prolonged – 72hour exposition, 2, 4-D undergoes profound oxidative transformations [Durmishidze S., 1988; Kakhniashvili Ch., 1988]. The part of it is reduced to 2, 4-D dichlorphenol and for a certain time is found in the unbound form in the tissues. The final product of 2, 4-D transformation in the plants occurs in the form of phenolpeptide conjugates. Conjugation of 2, 4-D with peptides blocks the functional groups in these compounds, they lose reaction ability and toxic features.

We were aimed at studying some cytophysiological characteristics of assimilation, translocation and localization of the widely applied herbicide 2, 4-D in plants.

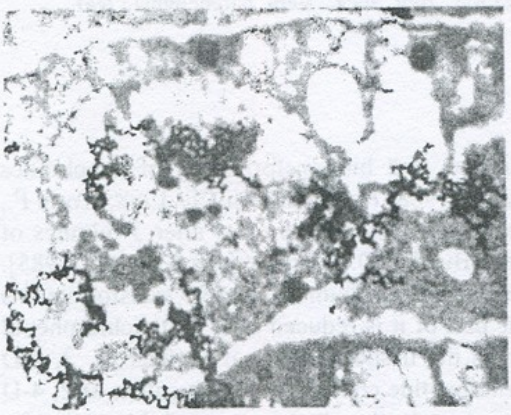
Material and Methods

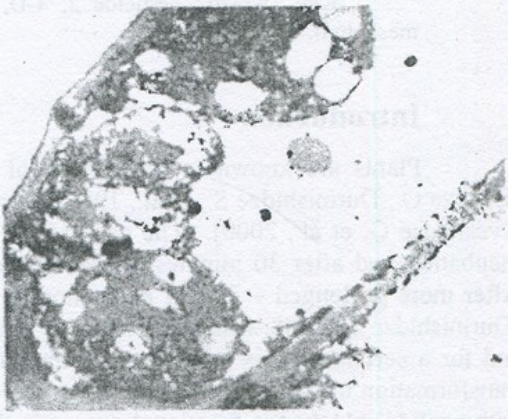
The 7-day maize seedlings served as experimental material. Plants were exposed to 2, 4-D during 24 hours. Experimental plants were placed into solution of herbicide 2, 4-D labeled by $_{14}C$ isotope, specific activity - 5MCi/ml. After 24 hours of incubation the roots were washed by the flowing water and material was taken from root apex, parenchyma of stem cortex and leaf mesophyll. The radioautographic sites (the sites with incorporated radioactive label) were detected using heavy metals [Normandin D. K.,1973].The ultrathin sections were prepared on the ultramicrotom and photographed on the microscope EVM-100L.

Results and Discussion

Figure 1 presents the root cells of 7-day maize seedlings incubated for 24hours on the 2, 4-D containing nutrient medium. The labeled herbicide appears in root tissue in the form of fragments of "twisted threads", which are not incorporated with any organelle of a cell, but cover them. These "threads" cross the cell wall and are scattered in the cell without losing the integrity. It may be supposed, that they are not yet involved into metabolic processes or are partly engaged in them. It is known that plants assimilate xenobiotics with water and nutrients [Zaalishvili G., et al, 2000]. It may be supposed that in root cells of the young seedlings the herbicide 2, 4-D is transported in the form of fragmentary conglomerate being driven by root pressure and osmotic forces.

Figure 2 shows the parenchyma cells of stem cortex of young maize seedling. It unites the systems of ground, vascular and dermal tissues, which have been laid in the process of embryogenesis and at the present stage of the development are presented by the primary meristem - procambium and protoderm. Cells of the primary parenchyma take part in photosynthesis, storage of assimilates and translocation of water and nutrients. The labeled sites here are fragmented and seen as uneven dark spots of spherical shape. The bigger sites of the herbicide are seen in the cell, and the smaller inclusions appear in the intercellular space. Between the cells electronically transparent primary pit fields are seen on the primary wall. These are plasmodesmata and the metabolites of the herbicide are translocated in this way via symplast.



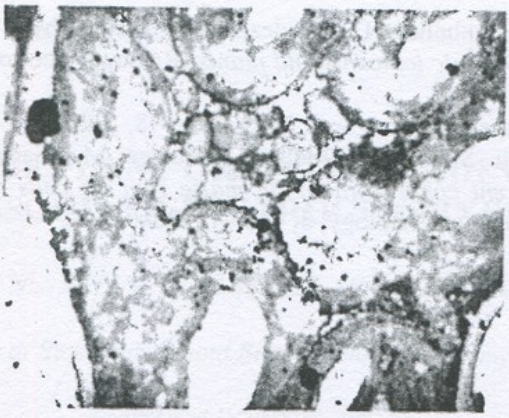


Distribution of herbicide 2, 4-D labeled with ^{14}C isotope in maize seedlings after 24 hours exposition

Fig 1. Cells of root apical zone (magnification ×16000)

Fig 2. Cells of stem parenchyma (magnification ×15000)

Figure 3 shows the fragments of leaf mesophyll cells of maize seedling affected by 2, 4-D. The site of cell wall adjacent to the plasmalemma is clothed by radioactive label or the products of the primary oxidation and the conjugates of the investigated herbicide. The bigger labeled fragments appear between the cell wall and the plasmalemma, presumably indicating the presence of the herbicide conjugated with peptides. Mitochondrial crista are overblown and swollen. Between the crista electronically transparent stroma appears. Dictiosoms of Golji complex are dark. Such structural alterations are characteristic to the cell being in extreme condition. Localization of the investigated herbicide is high in the intercellular spaces. Radioactive label is incorporated into the cell wall as well. Some researchers report about the accumulation of 2, 4-D conjugates in the structure of cell wall [Kakhniashvili Ch., 1988].



Distribution of herbicide 2, 4-D labeled with ^{14}C isotope in maize seedlings after 24 hours exposition
Fig 3. Cells of leaf mesophyll (magnification ×15000)

Also the numerous vacuoles contain the labeled sites. They are accumulated mainly on a tonoplast. The processes of 2, 4-D conjugation seem to take place in the cytosol, with participation of corresponding enzymatic systems and metabolic chains and the formed products penetrate through the tonoplast and attain the vacuolar sap. Some of the 2, 4-D conjugates are far more hydrophyllic than the initial compound [Kakhniashvili Ch., 1988] and are accumulated in the vacuole. Vacuoles are formed from the widened vesicles of endoplasmic reticulum of mesophyll cells of young leaves of the experimental plants. Provacuoles outline the cytoplasmic areas, the membrane fragments are merged isolating the cytoplasmic segments. Enzymatic systems contained in cisterns of endoplasmic reticulum and the cytoplasmic fragments appear in the vacuole. They isolate the alien, toxic substances from the metabolism. Some data available in scientific literature confirm our suggestions. Namely, using the labeled 2, 4-D the localization of its conjugates in the vacuoles of cucumber leaves has been established. The concentration of vacuolar sap changed as a result of these processes, alters the cytophysiological characteristics of a cell - turgor pressure and the processes of osmoregulation.

On the basis of the obtained results it can be supposed, that after 24 hours of exposition the molecules of ^{14}C-2,4-D entered into maize cells undergo oxidative transformation, become conjugated with endogenous peptides and in this way the herbicide is inactivated in the plant.

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მცენარეში ჰერბიციდ 2,4-D-ს გადაადგილების ზოგიერთი ციტოლოგიური ასპექტი

აფაქიძე ა., კახნიაშვილი ქ., ბეჟანიშვილი ქ.

საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახ. ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 10.01.2005)

რეზიუმე

შესწავლილია ჰერბიციდ 2,4-D-ს შეთვისება და გადაადგილება სიმინდის ფესვის აპიკალურ ზონაში, ღეროსა და ფოთლის მეზოფილის უჯრედებში. 24 საათიანი ექსპოზიციის პირობებში ფესვის უჯრედებში, ჰერბიციდი 2,4-D არ უკავშირდება უჯრედის სტრუქტურულ ელემენტებს. ქერქის პარენქიმულ უჯრედებში ფრაგმენტირებული უჯრედები გადაადგილდებიან სიმპლასტურად პირველადი ქერქის პლაზმოდესმებით. ჰერბიციდის გარდაქმნა მიმდინარეობს ფოთლის მეზოფილის უჯრედებში ენდოპლაზმის ბუშტუკებისა და ციტოპლაზმის ფერმენტული სისტემებით.

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Biochemistry

IMPROVEMENT OF PLANT GROWTH AND DEVELOPMENT VIA ASSOCIATIVE NITROGEN FIXERS

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(Received January 12, 2005)

Abstract

The influence of associative nitrogen-fixing microorganisms on development of different varieties of wheat at earlier stages of growth has been studied. Representatives of 13 different species of wheat were used; among them Georgian endemic species and imported hybrid sort " Copper", which at present is widely distributed in Georgia. It was concluded that the inoculation by *Azospirillum brasilense* Г3 has a positive effect on growth and development of plant seedlings and total protein content in roots and upground parts.

Key words: associative nitrogen fixation, inoculation, *Azospirillum brasilense.*

Introduction

Georgia is the breeding ground of ancient wheat cultures and is distinguished by varietal and species diversity of this plant [Naskidashvili,1983]. Genetic variety of Georgian wheat, qualitative indices, immunity against diseases and other characteristics are rich material for modern selection. It enables to suppose that Georgian wheat in future will remain an important and one of the most perspective cultures for Georgia.

At present growth of wheat yield and improvement of qualitative indices by ecologically acceptable means is very actual. In this respect wheat seeds inoculation by associative nitrogen - fixing microorganisms is one of the perspective agronomic means [Bashan,1990]. Among associative nitrogen fixers bacteria of *Azospirillum* genera are well studied.

Azospirilla are active, mobile organisms. They obtain an unique mechanism, which navigates them through soil in search of the plant roots [Okon,1986]. It is well known fact that they are attracted by the signal molecules released by the roots. After that *Azospirilla* form associations on plant roots and define the improvement of plant growth which is stipulated by several factors:

The plant is supplied by fixed atmospheric nitrogen [Shabaev, 1995; Rennie, 1981].

The growth stimulators are excreted by bacteria associated on roots; as a result root system is strengthened, as well as root "branching" and thus root absorbing surface increases, which, in its turn, couses more complete uptake of substrate compounds [Kapulnik, 1981; Lin, 1983].

Growth hormones can increase plant energetic status [Chernyadev, 1993] and indirectly activate nitrogen fixation, as all factors which give rise to plant intensive development and stimulate photosynthesis, promote activation of associative nitrogen fixation [Emtsev, 1985].

Azospirillum can change root cell membrane permeability [Venkateswarlu, 1982; Bashan, 1989] that couses improvement of root absorbtion.

The aim of our work was to study the influence of inoculation by one active strain of *Azospirillum brasilense* on growth and development of different wheat species on earlier stages.

Methods and materials

Isolated strain of *Azospirillum brasilense* Г3 and 12 species of wheat from collection of N. Ketskhoveli Insitute of Botany, Georgian Academy of Sciences and American hybrid sort "Copper" nowadays widely distributed in Georgia were used. The following varieties of wheat have been used in our experiments (Fig. 1):

1. *Tr. Macha v. paleo-imereticum;*
2. *Tr. Durum v. apilicum;*
3. *Tr. Aestivum v. lutescens;*
4. *Tr. Zhukovskyi v. Zhukovskyi;*
5. *Tr. Turgidum v. plinianum;*
6. *Tr. Spelta v. carthlicum;*
7. *Tr. Carthlicum v. stramineum;*
8. *Tr. Paleo-colchicum v. chvamlicum;*
9. *Tr. Timopheevii v.timopheevii;*
10. *Tr. Monococcum v. hohensteinii;*
11. *Tr. Boeticum v. melanorubrum;*
12. *Tr. Dicoccum v. aeroginosum;*
13. Copper.

On the first stage wheat seeds of different species were sterilized by suleme 0.1% solution and put on Petri dishes layed by two layers of filter paper. We added 10ml of sterile water into the control samples, and 10ml of incubation bacteria suspension into the tested samples. The samples were placed into thermostat for incubation, at 28^0C, 2days.

After incubation the germinated seeds were put into polyethylenic boxes with a diameter 10cm and high - 7cm (25 units per box), with sterile soil. The boxes were placed on a light site and watered with tap water. Growth and development of wheat seedlings was being observed during 30 days. The seedlings were collected 30 days later after sowing. The length and mass of plant roots and upground parts were measured. Protein content was estimated in roots and upground parts by Keldal inhanced method [Pleshkov, 1980].

Results and discussion

The quantity of different wheat seedlings and average length of upground parts of seedlings on the 14-th and 21-th days after sowing into the ground are presented in table 1. Only some plants germinated on the 14-th day. Variant Nll, wild variety of wheat, germinated most lately, maximum germination was found with variant N6.

On the 21-th day the number of seedlings sharply increased and didn't change. Among 13 different variants average length of 8 inoculated wheat seedlings exceeded that of control plants, but in 4 cases it was approximately equal. From this point of view most distinguished were N1,6,7,8,12,13 variants of the test (Fig. 2,3,4,5,6,7).

The results of measurements on the 30-th day of sowing are given in table 2. According to the data the inoculation positively effects the growth and development of different species of wheat; in particular, in comparison with the control, from 13 variants the root mass increases in 12 variants, in upground parts - in 10 variants. The results about average length of roots and upground

parts are analogous. The percentage of total protein content is increased in roots of 9 variants of the inoculated plants and upground parts of 10 variants in comparison with the control.

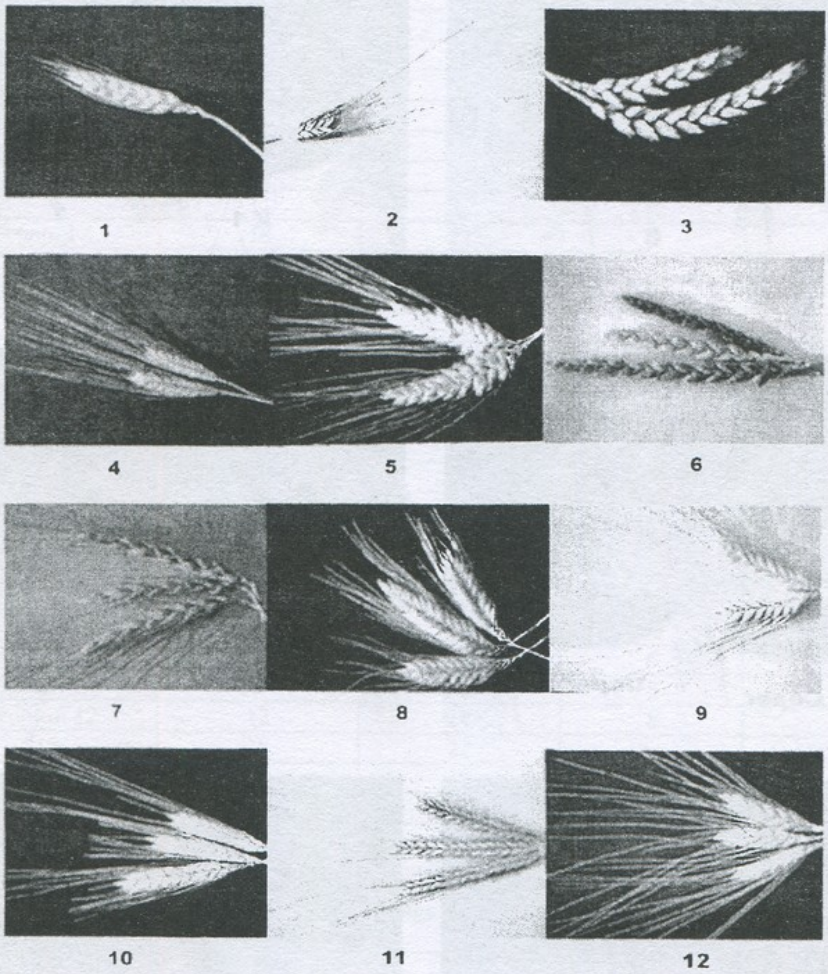

Fig. 1. Wheat varieties used in 1-12 variants.

It must be indicated that the increase of total protein content in plant roots and upground parts is mainly in positive correlation with other indices. Variants N1, N7 and N8 should be pointed out where the roots length significantly increased, in comparison with the control (respectively 32%, 33% and 21%) and root mass (34%, 40% and 33%), as well as the total protein content, both in root (17%,14% and 5%) and upground parts (8%, 12% and 5%).

Inoculation with *Azospirillum brasilense* Г3 had no positive effect in some plant variants; in particular, in variant N4 no increase of root length in comparison with the control, and decrease of all other indices was observed. In variants N5 and N11 only the root length and the mass (respectively 2% and 4%, but in N11 – 3% and 1%) slightly increased in comparison with the control.

Negative results received in variant N3 can be explained by the fact, that in some cases at introduction of *Azospirillum,* they serve as plant commensals or parasites [Volkagon, 2000].

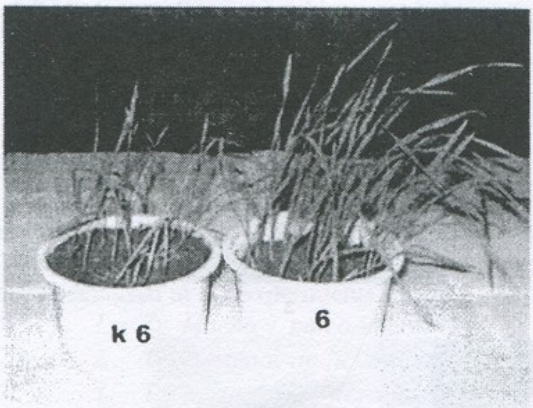

Fig. 2

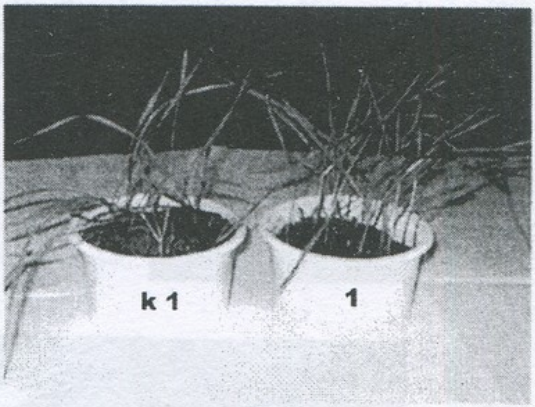

Fig. 3

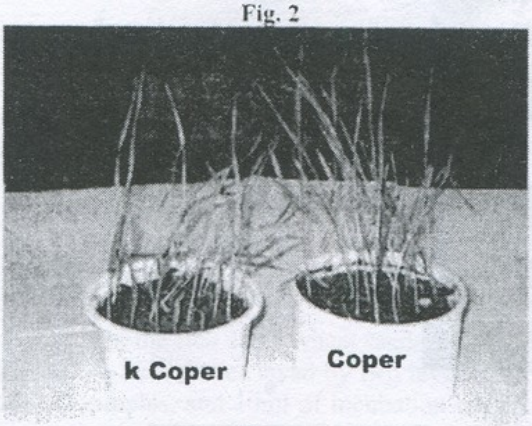

Fig. 4

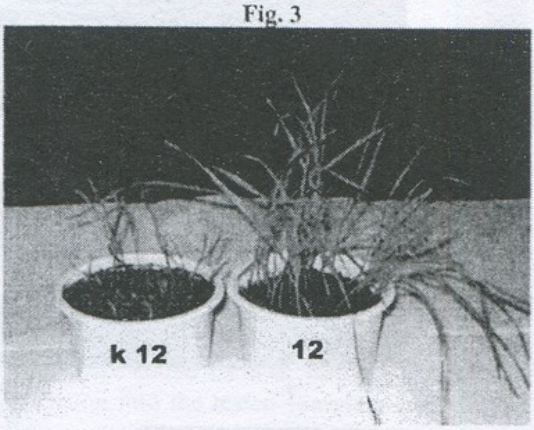

Fig. 5

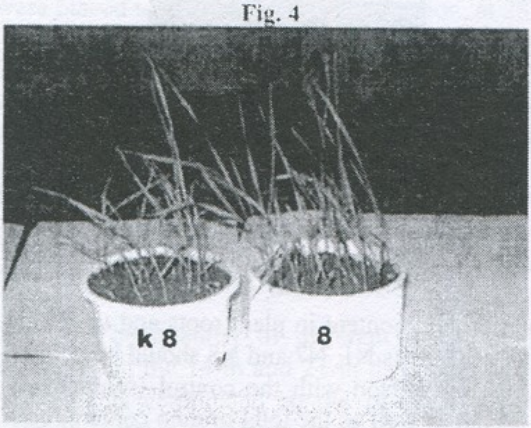

Fig. 6

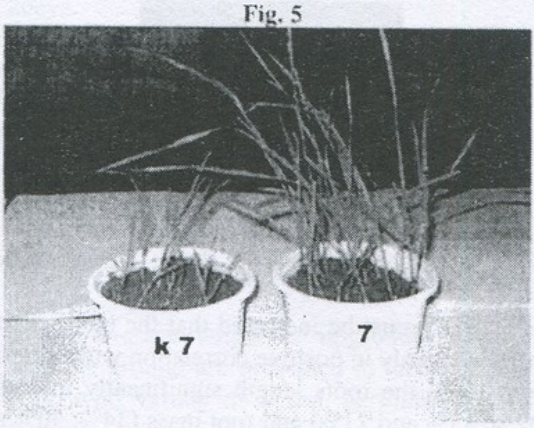

Fig. 7

Fig. 2-7. The effect of inoculation of wheat seedlings on earlier stages.

Thus, wheat seeds inoculation by *Azospirillum brasilence* Г3, in the most cases promoted improvement of plant growth and development and increased the total protein content at earlier stages of development, as a result of plant – microbe successful interaction.

Table 1. Influence of inoculation of wheat seedlings on earlier stages

Variants		Amounts of seedlings		Average lengths of upground parts of seedlings (cm)	
		14th day	21th day	14th day	21th day
1	control 1	8	19	12	16
	1	22	24	16	20
2	control 2	21	23	12	17
	2	18	22	9	17
3	control 3	20	24	11	16
	3	11	21	12	16
4	control 4	18	21	11	15
	4	13	21	14	15
5	control 5	16	22	12	20
	5	15	23	11	16
6	control 6	20	22	13	14
	6	25	25	15	21
7	control 7	13	14	10	12
	7	12	22	14	23
8	control 8	16	18	9	16
	8	18	24	12	19
9	control 9	17	18	8	17
	9	13	23	7	18
10	control 10	7	20	8	16
	10	11	21	13	17
11	control 11	5	18	6	10
	11	3	20	8	12
12	control 12	12	14	8	16
	12	18	22	11	23
13	control13	16	18	12	17
	13	21	22	15	20

Table 2. Influence of inoculation on plant growth and protein content

variant		average dry mass of 1plant mg				average length of 1plant cm				total protein content % per dry mass unit			
				increase %				increase %				increase %	
		root	upground part	root	upground part	root	upground part	root	upground part	root	upground part	root	upground part
1	control 1	12.9	18.4			5.8	19.2			7.6	11.9		
	1	19.6	18.8	34	2	8.5	20.7	32	7	8.9	12.8	17	8
2	control 2	19.6	16.5			17.1	18.1			8.7	9.3		
	2	19.8	17.8	1	7	7.5	18.5	5	2	9.1	9.6	5	3
3	control 3	12.9	16.4			5.8	18.7			7.9	11.5		
	3	17.1	18.1	24	9	7.1	20.1	18	7	8.8	12.0	11	4

4	control 4	10.5	14.8			6.1	17.4			7.1	8.3		
	4	10.5	14.2	-	-	5.9	16.6	-	-	6.6	8.2	-	-
5	control 5	13.6	18.2			5.8	20.1			7.0	11.2		
	5	14.1	13.0	4	-	15.9	17.6	5	-	7.1	10.7	1	-
6	control 6	19.6	19.7			7.7	21.2			6.3	11.6		
	6	23.1	22.1	15	14	9.8	24	21	12	6.6	11.9	5	3
7	control 7	8.7	12.1			4.7	15.1			7.8	12.1		
	7	14.5	18.2	40	34	7	23.3	33	35	8.9	13.6	14	12
8	control 8	13.5	17.1			6.1	17.8			8.6	10.7		
	8	20.0	18.5	33	8	7.7	19.3	21	8	9.0	11.2	5	5
9	control 9	11.1	15.3			6.7	18.1			9.1	11.8		
	9	16.2	16.1	32	5	7.1	19.2	17	6	9.3	12.1	2	3
10	control 10	14.2	17.5			6.3	17.7			7.3	11.2		
	10	19.5	17.8	27	2	7.3	19.4	14	9	7.4	11.6	1	4
11	control 11	10.0	10.2			6.1	13.8			8.1	9.0		
	11	10.1	9.6	1	-	6.3	12.5	3	-	7.9	8.1	-	-
12	control 12	16.2	16.0			6.4	17.2			7.8	12.7		
	12	21.9	19.1	26	16	7.9	23.7	19	27	8.2	13.5	5	6
13	control 13	14.3	15.6			6.9	18.9			8.0	12.9		
	13	18.6	17.9	23	13	8.4	20.7	18	9	8.8	13.1	10	2

Acknowledgment

The authors would like to thank prof. T.Berishvili for delivery of wheat species.

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მცენარის ზრდა-განვითარების გაუმჯობესება ასოციაციური აზოტფიქსატორების მეშვეობით

ბაგალიშვილი მ., კიკვიძე მ., ბასილაშვილი ლ., ნუცუბიძე ნ.

საქართველოს მეცნიერებათა აკადემიის ს, დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 12.01.2005)

რეზიუმე

შესწავლილია ასოციაციურ აზოტმაფიქსირებელ მიკროორგანიზმთა ზეგავლენა სხვადსხვა სახეობის ხორბლის განვითარების ადრეულ ეტაპებზე. საკვლევად გამოყენებულ იქნა ხორბლის 13 სახეობა; მათ შორის ქართული ენდემური სახეობები და აგრეთვე შემოტანილი ჰიბრიდული ჯიში "კოპერი", რომელიც ამჟამად საქართველოში ფართოდაა გავრცელებული. ექსპერიმენტის შედეგად დადგინდა, რომ *Azosoirullum brasilense Г3*-ით ინოკულაცია დადებით გავლენას ახდენს მცენარის აღმონაცენთა ზრდა-განვითარებაზე, აგრეთვე ცილის შემცველობაზე ფესვებსა და მიწისზედა ნაწილებში.

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Biochemistry

AMINO ACIDS VARIATION OF PINK STRONG WINES AT THERMAL TREATMENT PROCESS OF GRAPES HUSKS

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Abstract

It has been established that thermal treatment of husks of grapes at 40^0C before alcoholic fermentation promotes an increase of total concentration of essential amino acids by 35, 9%, aromatic acids – 41, 1% and sulfur-containing acids – 29, 35%. Above Mentioned changes improve organoleptic characteristics and nutrient value of the wines.

Key words: pink strong wines, thermal treatment, husks of grapes, aromatic amino acids, sulphur containing amino acids.

Introduction

Amino acids play an important role in the viticulture technology and at the same time, they increase the value of wine, as the nutrient product. They are the necessary substrates for feeding the yeasts and take active part in the complicated biochemical processes, which are proceeding during the whole development stages of wine.

The quantity and quality contents of the separate amino acids sharply vary depending on the sort of vine, soil, fertilizer, climate conditions, agrotechnics, methods of preparing and type of wine. During the transformation process of amino acids in the wine a number of products, which have strong influence on the taste and bouquet of the wine is formed.

The taste of wine depends on the common content of amino acids in the medium, as well as on the quantity correlation of separate amino acids [Abramov Sh. et al., 1998; Abramov Sh. et al., 2004; Ageeva N., et al., 2002].

The latest studies established that by using the total amino acids concentration, it is possible to judge the naturality and falsification of the grape wines [Ageeva N., 2002]. Reported data concerning amino acids content in the pink wines and influence of the technologies of their preparation on the changing of amino acid composition is limited [Vlasova O., 1977]. The aim of our work was to reveal the changes of amino acid composition in the pink wines during thermal treatment of grapes husks.

Materials and Methods

The researched objects – experimental and control samples of the pink strong wine materials were prepared from the fresh winging out husks of grapes of the red sort grapes "Saperavi" and the must of the white sort grapes "Rkatsiteli".

To prepare the experimental wine materials to the sulphytated (80 mg/dm^3) fresh husks of grapes "Saperavi" the must of "Rkatsiteli" was added . The must volume of "Rkatsiteli" exceeded twice the volume of pressed from the husks of grapes "Saperavi".

Obtained husks heated at 40^{0}C with constant mixing. After cooling below 23^{0}C pure culture of yeasts was added.

Alcohol fermentation and further technological processes were carried out according to the strong wine materials preparing technology. Simultaneously by the same technology, the control samples of wine materials were fermentated without thermal treatment.

Amino acids content in samples of pink strong wines were estimated by the high-performance liquid chromatograph – Pico Tag (Waters Associates Model 441, USA).

Results and discussion

Experimental data concerning the amino acid changing in the pink strong wines are represented in Table.

In the experimental and control samples of the pink strong wines were identified and quantitatively determined the following amino acids: asparagines, glutamine, serine, glycine, histidine, arginine, proline, tyrosine, valine, methionine, cystine, isoleucine + leucine, phenylalanine, lysine.

The results of the Table indicate that the husks thermal treatment promotes the increase of the amino acids total amounts. Increasing of the amino acids quantitative content in the experimental samples can be explain by the hydrolysis intensification of nitrous polymerous compounds (polypeptides, peptides, proteins) caused by the husks fermentative system activity in the thermal treatment process.

It is established that in the experimental samples in comparison with control, the total concentration of the amino acids increased by 26, 5%. The total concentration of the essential amino acids (valine, methionine, isoleucine + leucine, phenylalanine, lysine) increased by 35,9%, as for aromatic amino acids (tyrosine, phenylalanine) – by 41, 1% and for sulphur containing amino acids (methionine, cystine) – by 29, 3%.

It is known that deficiency or absence of the essential amino acids in the food may cause changes in the functioning of the organism [Krichevskaya A., et al., 1983].

It was established that in the experimental samples in comparison with the control ones, among essential amino acids mass concentration of the phenylalanine, valine, lysine and methionine raises the most.

Phenylalanine and tyrosine is believed as precursor of adrenaline and thyroidine hormones secreted from the thyroid gland [Krichevskaya A. A., et al. 1983]. Phenylalanine and tyrosine are aromatic amino acids forming aromatic alcohols – 2-phenyl ethyl and tyrosol – giving to the wine rose aroma [Rodopulo A. K., 1983]. In experimental samples phenylalanine mass concentration increased by 94, 6% and of tyrosine – by 10%.

The most deficient essential amino acid - lysine affects on the nervous system functional activity, regulates the potassium metabolism and hemoglobin synthesis [Krichevskaya A. A., et al. 1983]. The increasing of lysine mass concentration in the experimental samples was 37, 4%.

The essential amino acid - methionine leads to the improvement of carbohydrate, lipid and protein metabolism [Krichevskaya A. A., et al. 1983]. Methionine and cystine are sulphur

containing amino acids. They influence on the free oxygen content in the wine and play significant role in reduction of redox potential in the wine [Rodopulo A. K., 1983]. Methionine mass concentration increased by 35%, and cystine – by 20, 3%. Besides, it has been stated that sulphur containing amino acids are characterized by antiradiation effect [Abramov Sh. A, et al. 2004].

Above mentioned changes of amino acids content in the experimental wine materials favourable effect on the quality of wines and increase their biological value.

Table. Amino acids changing in the pink strong wines during husks thermal treatment

Amino acids (mg/dm^3)	Control	Experiment
Aspartic acid	54,5	64,8
Glutamic acid	75,2	97,3
Serine	25,1	27,3
Glycine	1,1	1,3
Histidine	13,6	26,5
Arginine	67,2	75,8
Proline	431,0	545,5
Tyrosine	15,8	17,4
Valine	6,1	8,8
Methionine	11,7	15,8
Cystine	7,4	8,9
Isoleucine + leucine	55,6	69,5
Phenylalanine	9,3	18,1
Lysine	11,5	15,8
Amino acids total amount	785,1	992,8
Essential	94,2	128
Aromatic	25,1	35,5
Sulphur containing	19,1	24,7

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ამინომჟავების ცვლილება ვარდისფერ შემაგრებულ ღვინოებში დურდოს თერმული დამუშავების პროცესში

ებელაშვილი ნ.

მებაღეობა, მეღვინეობისა და მეღვინეობის სამეცნიერო კვლევითი ინსტიტუტი, საქართველოს სოფლის მეურნეობის აკადემია

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რეზიუმე

დადგენილია, რომ დურდოს თერმული დამუშავებისას $+40^0C$ ალკოჰოლური დუღილის წინ, ვარდისფერ შემაგრებულ ღვინოებში ამინომჟავათა მასური კონცენტრაცია მატულობს 26,5%-ით, შეუცვლელი ამინომჟავების – 35,9%-ით, არომატული ამინომჟავების - 41,1%-ით, გოგირდშემცველი ამინომჟავების – 29,3%-ით. აღნიშნული ცვლილებები აუმჯობესებენ შემაგრებული ღვინოების ორგანოლეპტიკურ მაჩვენებლებს და კვებით ღირებულებას.

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EFFECT OF THE INTRODUCTION OF DIFFERENT DOSES OF AMMONIUM NITRATE IN PODZOLIC SOIL ON MAIZE VAR. AJAMETIS TETRI SEED PROTEIN CONTENT

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(November 17, 2004)

Abstract

The effect of introduction of different doses of ammonium nitrate in podzolic soil in Imereti region on total protein content and protein fraction composition in seeds of maize var. Ajametis Tetri was studied. It has been shown that the change of these doses affects the seed total protein content as well as its fraction composition. Namely, by the increase of the dose of introduced into soil nitrogen fertilizer the protein content in seeds increased. The highest protein content was observed at the introduction of N180kg/ha. However this increase in the total protein content takes place at the expense of seed storage proteins-prolamins and glutelins and thus does not affect its nutritional value. Optimal balance in protein content and its nutritional value is achieved by application of nitrogen fertilizer at following concentration: N60 and N90.

Key words: maize, ammonium nitrate, seed protein, albumins, globulins, prolamins, glutelins.

Introduction

Proteins are the most important compounds among the chemical substances present in a living organism. The role of plant proteins is tremendous not only for its vital activity, but for its nutritional value as well. In a scale of world production of vegetation protein cereals account 68-71% [Konarev, 1980] and provide 50% of nutritional proteins for Earth population and in cattle breeding [Konarev, 1975].

Protein quantitative content in a seed depends on the conditions of plant cultivation and among them on: climatic conditions and type of soils; application of mineral fertilizers, etc. Nitrogen nutrition plays an important role in development and productivity of cereals. In case of proper application of nitrogen fertilizers (doses, vegetation periods, etc.) high yield of seeds with high protein content can be achieved [Methodological Guide, L., 1973].

Mineral fertilizers introduced into soil provide transformations of existed in soil nutrition elements into mobile forms. Hence, the plants uptake and assimilate not only those nutrition substances that are introduced as fertilizers, but nutrition elements found in soil as a stock. As a result the soil becomes poorer and to enrich it the use of fertilizers is necessary. But, an uncontrolled application of fertilizers, namely nitrogen fertilizers arises some problems [Lomsianidze et. al. 2005]. Among them could be mentioned exhaust of soil, as well as

accumulation of nitrates in food products, which negatively affects man's health and causes many serious diseases.

The goal of the present work is to study the effect of introduction of different doses of ammonium nitrate on maize seed total protein and protein fraction composition of maize var. Ajameti Tetri grown in podzolic soils of Imereti region.

Material and Methods

Maize (*Zea mays* L) var. Ajametis Tetri was cultivated in Ajamety field-crop experimental station podzolic soil in 2000-2002. Field tests were conducted in four repetitions in following variants: fertilizer free; using only phosphate-potassium fertilizer - P120-K60; five different doses of ammonium nitrate on the background of constant phosphate-potassium fertilizer - P120-K60-N60; P120-K60-N90; P120-K60-N120 P120-K60-N150; P120-K60-N180; P120-K60-N210. Soil treatment, introduction of fertilizers and plant care was conducted strictly according to the established agricultural rules [Chanishvili, 1973].

Maize seeds were grinded in cold and obtained flour was defatted with dimethylether as a solvent.

Protein content in maize seeds was determined according to Kjeldahl's modified method, using Nesler reagent [Methodological Guide, L., 1973]. For the analysis 100mg of the defatted flour was taken and put into preliminary calibrated biological test tubes. Concentrated sulphuric acid (3ml) and 30% hydrogen peroxide (0.5ml) were added to the samples. The samples were put into a metal block and burned at 400^0C till the complete discoloration. Measure flasks with 50ml volume were half filled with distilled water, added 3 ml of the samples and neutralized with 10% NaOH. After the neutralization 0.5 ml of Nesler reagent was added. Protein total content was determined spectrophotometrically at 413 nm. Nitrogen content was determined from the preliminary constructed calibration curve. Protein concentration was calculated using the so-called protein coefficient [Methodological Guide, L., 1973].

The isolation of seed proteins was conducted from the defatted flour according to Osborne [Osborne 1935], based on protein solubility in water, salt, alcohol and alkaline solutions.

Albumins fraction was obtained by three-fold extraction with 0.1 M phosphate buffer, pH 7.0 (10g flour: 50 ml buffer). The suspension was shaken at 4^0C during 24 hours. After 20 min centrifugation at 3000g, albumins were precipitated from the supernatant by the addition of 50% TCA acid at final concentration 5%. The obtained sediment was washed with ethyl alcohol and ethyl ether.

Isolation of the globulins from 3000g sediment was carried out by the addition of 50ml 0.1 M phosphate buffer, pH 7.0 which contained 1M NaCl. The suspension was shaken at 4^0C during 24 hours and then centrifuged at 3000g. Precipitation of globulins from the obtained supernatants was carried out similar to albumins. The sediment was then washed twice with a distillate, ethyl alcohol and ethyl ether.

Isolation of prolamins was carried out by means of 4-5 times extraction with 70% ethyl alcohol. Samples shaken for 1 h at room temperature were stayed for 16-18 h and then centrifuged at 3000 g, 15 min [Amashukeli et al., 1994]. The obtained supernatants were collected.

Prolamins were precipitated by the addition (1:3) of 0.1 N NaCl, stayed for 24 h at room temperature and then centrifuged. The obtained sediment was washed three times with distilled water.

For the isolation of protein alkali-soluble fractions glutelins 0.05 N NaOH solution was added to the sediment, shaken at room temperature and stayed for 16-18 h. The mixture was centrifuged at 3000g, 15 min. Glutelins were precipitated from the supernatant by the addition of 50% TCA at final concentration 5%. The obtained sediment was washed twice with distillate, once

with each ethyl alcohol and ethyl ether. The obtained fractions were dried in vacuum at 60^0C during 1 h.

Results and discussion

Protein content and composition in a seed define its technological characters [Konarev, 1998]. As it was already mentioned protein quantitative content greatly depends on conditions of plant cultivation, including climate, soil composition and some other factors and among them on nitrogen nutrition.

Biochemical-physiological prerequisitoins for the increase of cereals seeds protein content are: nitrogen content of ripe seed; root capability to absorb nitrogen after blooming during the seed senescence; reutilization of nitrogen substances in vegetative organs during the seed ripening; seed and ear response to protein biosynthesis.

Taking into consideration all these factors, it is obvious importance of soil enrichment with nitrogen fertilizers and the proper selection of optimum agricultural doses.

Table 1 reflects the effect of increasing doses of ammonium nitrate introduced into soil on total protein content in seeds of maize var. Ajametis Tetri. As it is seen from the Table, there is almost no difference between the variants grown without fertilizer and at the introduction of phosphate-potassium fertilizer - P120-K60 at no nitrogen introduction. In case of introduction of ammonium nitrate on the background of P120-K60, the content of the total protein increases. At high N180 dose the total protein content in seed is maximal (10.62%). However, at the tested highest dose N210 the decrease of the protein content in seeds as compared to N180 variant takes place.

Table 1.Total protein content in seeds of maize var. Ajametis Tetri at introduction of different doses of ammonium nitrate on the background of phosphate–potassium fertilizers in podzolic soil

#	Variant of experiment	Total protein in seed, %
1	Fertilizer free	8.94
2	P120-K60	8.96
3	P120-K60 + N60	9.14
4	P120-K60 + N90	9.45
5	P120-K60 + N120	9.47
6	P120-K60 + N150	9.67
7	P120-K60 + N180	10.62
8	P120-K60 + N210	10.13

The results of the effect of introduction of increasing doses of ammonium nitrate into podzolic soil on seed proteins fraction composition is presented in Table 2. Maximum content of protein valuable fractions – albumins and globulins are observed at application of nitrogen fertilizer in doses N60 and N90. Further increase of the concentration of nitrogen fertilizer introduced into soil was accompanied by the decrease of easily soluble proteins: albumins and globulins content in seeds. At the same time the increase of the seed storage proteins - prolamins and glutelins content took place.

Table 2. The protein fraction composition in seeds of maize var. Ajametis Tetri at introduction of different doses of ammonium nitrate on the background of phosphate–potassium fertilizers in podzolic soil

#	Variant of experiment	% from the Total protein			
		Albumins	Globulins	Prolamins	Glutelins
1	Fertilizer free	25.3	10.7	35.0	25.0
2	P120-K60	25.5	10.8	35.3	25.8
3	P120-K60 + N60	26.3	11.0	35.5	26.0
4	P120-K60 + N90	26.8	11.2	35.6	26.3
5	P120-K60 + N120	25.0	10.0	36.0	27.3
6	P120-K60 + N150	24.8	9.0	37.2	27.5
7	P120-K60 + N180	24.5	8.5	38.0	29.0
8	P120-K60 + N210	24.3	8.0	38.2	29.4

Thus, the obtained results enable us to conclude that fertilization of podzolic soil with ammonium nitrate (N180 and N210) in Imereti region increases the seed total protein content. However this increase in the total protein content takes place at the expense of seed storage proteins and thus does not affect its nutritional value [Betsiashvili et al., 2002]. Besides, these doses of nitrogen fertilizer negatively affect the Ajamety podzolic soil properties and cause the decrease of mobile phosphorus and exchangeable potassium [Lomsianidze et al., 2005]. Therefore, application of agricultural doses of nitrogen fertilizer, namely N60 and N90 is reasonable for balanced protein content yield of maize with high nutritional value

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ამონიუმის ნიტრატის გავლენა სიმინდის ჯიშის "აჯამეთის თეთრი" მარცვლის ცილის შემცველობაზე ეწერ ნიადაგში სხვადასხვა დოზით შეტანისას

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საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 17.11.2004)

რეზიუმე

შესწავლილია ამონიუმის ნიტრატის გავლენა სიმინდის ჯიშის "აჯამეთის თეთრი" მარცვლის ჯამური ცილისა და ცილოვანი ფრაქციების შემცველობაზე იმერეთის ეწერ ნიადაგში სხვადასხვა დოზით შეტანისას. ნაჩვენებია, რომ ამ დოზების ცვლილება გავლენას ახდენს როგორც ცილის შემცველობაზე, ისე მის ფრაქციულ შემადგენლობაზე. კერძოდ, ნიადაგში შეტანილი აზოტოვანი სასუქის დოზის გაზრდა იწვევს თესლში ცილის რაოდენობის ზრდას. ცილის ყველაზე მაღალი შემცველობა აღინიშნება N180კგ/ჰა შეტანისას. ამასთან, აღსანიშნავია, რომ ჯამური ცილის შემცველობის ზრდა განპირობებულია მარცვლის სამარაგო ცილების – პროლამინებისა და გლუტელინების ხარჯზე და ამდენად არ მოქმედებს მის კვებით ღირებულებაზე. ცილის შემცველობასა და მის კვებით ღირებულებას შორის ოპტიმალური ბალანსი მიიღწევა აზოტოვანი სასუქის შემდეგი დოზებით: N60 და N90, გამოყენებისას.

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Biochemistry

STUDY OF 2,4,6-TRINITROTOLUENE (TNT) ASSIMILATION ABILITY BY *IN VITRO* CULTURE CELLS OF *YUCCA* GLORIOSA L.

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Abstract

It has been studied 2,4,6-trinitrotoluene (TNT) assimilation potential of cells of *Yucca gloriosa* callus culture. Yucca *in vitro* cells are characterized by high TNT assimilation potential. It is stated the optimal (50 mg/l) and lethal (300mg/l) concentrations for the growth of callus tissue. It has been shown that yucca *in vitro* cells cannot consume TNT as the sole nitrogen or carbon source.

Key words: *Yucca gloriosa*, callus culture, 2,4,6-trinitrotoluene (TNT).

Introduction

Nowadays great attention is paid to cleansing of soils contaminated by military activities (proving grounds, ammunition plants, spots of dislocations). These territories are often contaminated with the organic toxicants, part of which owing to the chemical inertness remain in soil unchanged for long and therefore maintain their toxicity [Rugh et al., 1996]. Among them must be mentioned 2,4,6-trinitrotoluene (TNT).

Based on the researches on animals EPA(Environmental Protection Agency) determined TNT as human carcinogen[EPA, 1991a,b]. Due to its high toxicity cleansing of the contaminated environment is necessary. Selection of plants capable to assimilate the mentioned toxicant and the study of the mechanism of their detoxification are the main tasks of the modern xenobiochemistry.

Plant *in vitro* cells are the best models for the study of biochemical and cytological mechanisms of detoxification. The presented work aimed to study TNT assimilation potential of cells of *Yucca gloriosa* callus culture.

Materials and Methods

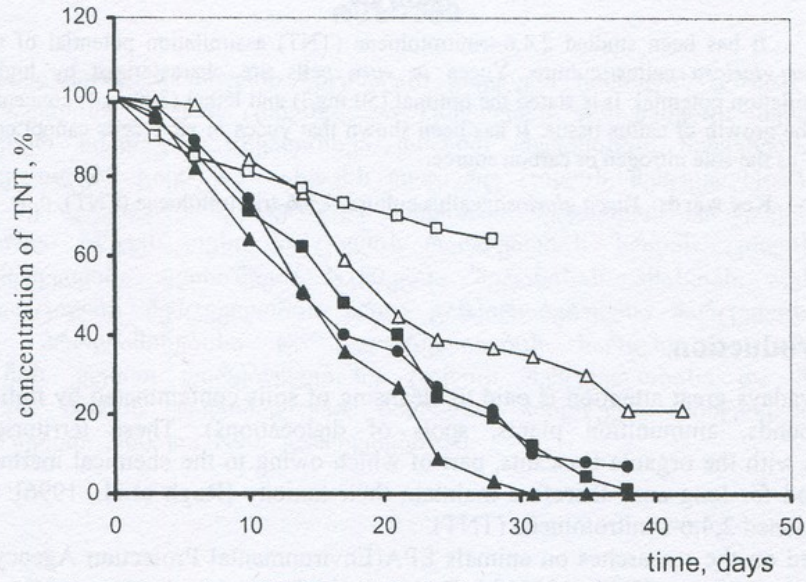
Test object was *Yucca gloriosa* callus culture. Nutrient medium content, cultivation regime and methods of culture growth analysis were described earlier [Gogoberidze et al., 1988].

To state TNT assimilation potential at different phases of growth of *Yucca gloriosa* callus culture 1mg nutrient medium was selected. To control and TNT containing media was added 5ml distillate and was placed on the shaker for extraction. The obtained extract was filtrated and 1ml

1M KOH was added and exposed for 5 min. Absorption spectrum was measured on 447 nm[Oh et al., 2001].

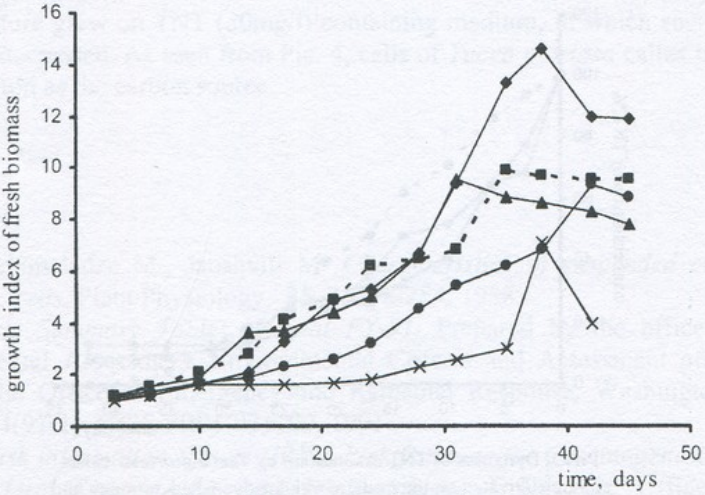
Results and Discussion

To study TNT assimilation by cells of *Yucca gloriosa* callus culture to solid medium different concentrations of the toxicant (25, 50, 100, 200 and 300mg/l) were added; cultivation period - 45 days [Murashige et al., 1962]. Determination of TNT residual amount in the nutrient medium showed that toxicants assimilation started in the exponental phase (cells fast reproduction phase) (Pic.1). At introduction 25mg/l TNT in the nutrient medium in the stationary phase on the 30th day toxicant was totally uptaken. At 50 and 100mg/l concentrations at the end of callus cell growth cycle, TNT content was 10% lower the initial concentration. At 200mg/l concentration TNT content equaled 21%, in case of 300mg/l TNT concentration toxicant assimilation was complicated (Pic. 1). The obtained data point to rather high TNT assimilation potential of the cells of *Yucca gloriosa* callus cultures.



Pic. 1. Dynamics of TNT assimilation by *Yucca gloriosa* callus culture.
TNT concentration in the solid media:
—▲— 25 mg/l TNT —■— 50 mg/l TNT —●— 100 mg/l TNT
—△— 200 mg/l TNT —□— 300 mg/l TNT

Cells of callus tissue revealed the ability to develop and accumulate biomass on the solid nutrient medium at 25, 50, 100 and 200 mg/l TNT concentrations (Pic. 2). The investigation evidenced that toxicants different concentrations indifferently affected the growth of *Yucca gloriosa in vitro* cells: culture grew well at 25, 50 and 100mg/l TNT containing media, but the more was TNT concentration in the nutrient medium the later was revealed the maximum index of fresh biomass accumulation and correspondingly the stationary phase started later.

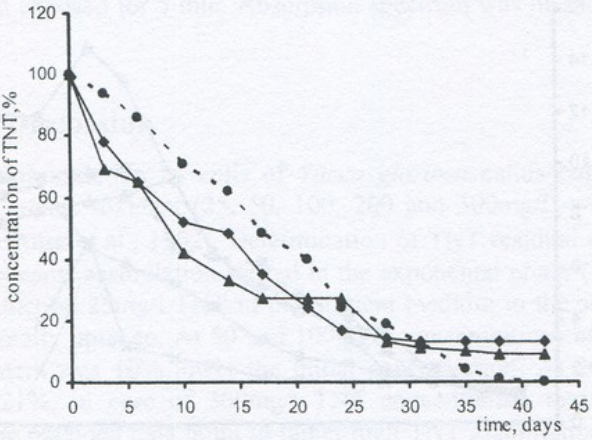


Pic. 2. Effect of TNT on the cell growth of *Yucca gloriosa* callus culture.

At 200mg/l TNT concentration growth compared with control was significantly inhibited. The maximum growth index was received on the 38th day which was followed by cell degradation phase. It should be mentioned that at this moment growth stationary phase wasn't revealed. On 300 mg/l TNT containing medium *in vitro* cells almost lost biomass accumulation ability and after 25 days were necrosed.

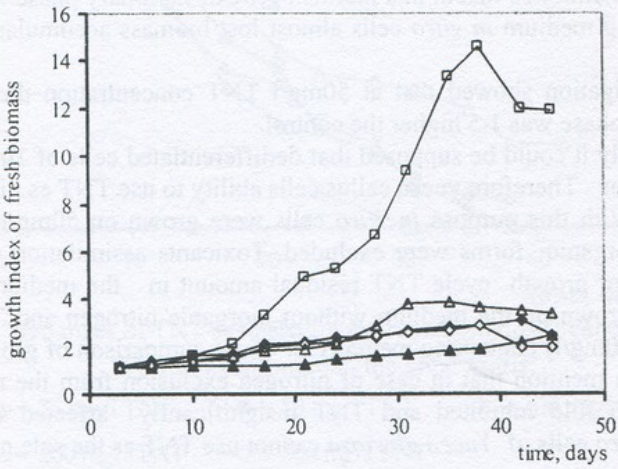
The investigation showed that at 50mg/l TNT concentration the fresh biomass growth index in stationary phase was 1,5 higher the control.

Proceedingly it could be supposed that dedifferentiated cells of *Yucca gloriosa* can utilize TNT detoxified forms. Therefore yucca callus cells ability to use TNT as nitrogen or carbon source has been studied. With this purpose *in vitro* cells were grown on 50mg/l TNT nutrient medium where nitrogen inorganic forms were excluded. Toxicants assimilation started in latent phase (Pic.3), at the end of growth cycle TNT residual amount in the medium was 13%. As growth control were cells grown on the medium without inorganic nitrogen and TNT, and grown on all nutrient and TNT (50mg/l) containing media (Pic. 4). At comparison of growth indices of the three variants, it's easy to mention that in case of nitrogen exclusion from the medium fresh biomass accumulation was 5 fold inhibited and TNT insignificantly affected cell growth. It can be concluded that *in vitro* cells of *Yucca gloriosa* cannot use TNT as the sole nitrogen source.



Pic. 3. Dynamics of TNT assimilation by *Yucca gloriosa* callus culture cultivated on the medium with impoverished nitrogen and carbon sources. TNT concentration in the solid media - 50mg/l.

- control medium
- medium with impoverished carbon sources
- medium without inorganic nitrogen



Pic. 4. Effect of TNT on the cell growth of *Yucca gloriosa* cultivated on the medium with nitrogen and carbon sources.

- TNT (50 mg/l) containing medium without nitrogen
- TNT (50 mg/l) containing medium with impoverished carbon sources
- medium without inorganic nitrogen
- medium with impoverished carbon sources
- TNT (50 mg/l) containing medium

The analogous results were received in case of carbon source deficiency in the nutrient medium. Callus culture grew on TNT (50mg/l) containing medium, in which sucrose and inositol content was by 1/3 decreased. As seen from Pic. 4, cells of *Yucca gloriosa* callus tissue cannot use atoms of TNT skeleton as the carbon source.

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Yucca gloriosa L. *in vitro* კულტურის უჯრედების მიერ ტრინიტროტოლუოლის (TNT) შეთვისების უნარის შესწავლა

რამიშვილი მ., გოგავა მ., ჭელიძე ნ., ღოღობერიძე მ.

საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახ. მცენარეთა ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 05.01.2005)

რეზიუმე

შესწავლილია 2.4.6-ტრინიტროტოლუოლის (TNT) შეთვისების უნარი იუკა დიდებულის კალუსური კულტურის უჯრედები მიერ. იუკას *in vitro* უჯრედები ხასიათდება TNT-ს ასიმილაციის მაღალი უნარით. დადგენილია კალუსური ქსოვილის ზრდისთვის TNT-ს ოპტიმალური კონცენტრაციაა 50 მგ/ლ, ხოლო ლეტალური – 300 მგ/ლ. ნაჩვენებია, რომ იუკას *in vitro* უჯრედებს არ შესწევთ უნარი გამოიყენონ TNT აზოტის ან ნახშირბადის ერთადერთ წყაროდ.

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Biophysics

THE STRUCTURE OF α-ACTININ ROD

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Abstract

For elucidation of structural organization of α-actinin rod the kinetics of rod tripsynolisis have been studied. The molecular weights of obtained polypeptide fragments in native and denaturated conditions have been established. The model of structural organization of α-actinin rod was suggested. The dimer is formed by interaction of rod subunits N-terminals (Mr - 55 kDa). C-terminal part of subunit (Mr –15 kDa) don't participate in the formation of dimer. The polypeptide chains of subunits in α-actinin rod are situated in the way that their C-terminal parts are localized in the form of monomers in both ends of the rod and accordingly in α-actinin molecule beside actin-binding N-domain (Mr – 30 kDa).

Key-words: α-actinin, limited hydrolysis, domain

Introduction

The contractile system of different cells contains minor proteins that have a substantial influence on the properties of the major proteins – myosin and actin. One of these minor proteins is α-actinin which cross-links and bundles actin filament (F-actin). α-Actinin is localized in structures to which actin filaments are anchored. A wide distribution of α-actinin in various organs and tissues, that participate in processes associated with mobility, confirm the fact that α-actinin plays an important role.

α-Actinin is the rod-like antiparallel dimer (subunit Mr – 100 kDa). Using the method of limited hydrolysis it has been shown that at the initial stage of trypsinolysis α-actinin subunit is splitted into two parts forming fragments with Mr 30 kDa and 70 kDa, that embrace nearly the whole α-actinin subunit [Simonidze et al, 1985]. 30 kDa fragment is localized on the N-end and 70 kDa fragment in C-end of subunit. In α-actinin molecule subunits have antiparallel orientation in the way that the C-terminal fragments of these subunits guarantee the dimerization of molecule, and N-terminal ones localized on the opposite ends of α-actinin forming the site of binding of this protein with actin [Simonidze, 1989].

In the native molecule of α-actinin the fragments in terms of the overall packing of their polypeptide chains behave as independent structural units – domains. Fragments of α-actinin were named N- and C-domains according to their localization in polypeptide chain [Simonidze, 1986]. Using the CD method the interaction of N- and C-domains in native α-actinin molecule at the tertiary structure has been shown [Kuridze, 1985].

The purpose of this work was the elucidation of structural organization of C-domain - the "so called" α-actinin rod by physical-chemical methods.

Materials and Methods

A homogeneous preparation of α-actinin was produced from a rabbit skeletal muscle according to the Pinter Method [Pinter et al., 1980].

The limited hydrolysis of α-actinin was carried out in different buffers changing pH, protein concentration, the ratio ferment/substrate and temperature. The kinetics of trypsinolisis have been observed in the following way: in every determined interval of time SDS was added to aliquots, then protein was precipitated by acetone and analyzed using gel-electrophoresis.

Electrophoresis in polyacrylamide gel in the presence of SDS was conducted in a 9-25% polyacrylamide gradient [Leammli, 1970]. The scanning of the gels stained with coomassive blue G-250 was performed on LKB densitometry.

To obtain the native fragments the chromatography and rechromatography of hydrolyzate at analytical high pressure chromatograph were carried out (LKB, Sweden). At first hydrolyzate was applied to column (2,6 x 90 cm) with TSK-HW-55F, equilibrated with 0,05M Tris-HCl, 5mM 2-mercaptoethanol, 1mM EDTA, 0,3M NaCl, pH 8,0 buffer (buffer A). Elution was done with the same buffer at rate 20 mg/hour. Fractions of 7 ml were collected and detected at 200 nm on spectrophotometer SF-4. Then concentrated fractions were applied to column (1,6 x 90 cm) with TSK HW-65F equilibrated with buffer A and eluted with rate 7ml/h. 4 ml fractions were collected and detected at 280 nm on spectrophotometer SF-4.

Equilibrium centrifugation was conducted at analytical centrifuge MOM 3170 (Hungary) the molecular weights of the fragments were calculated according to the formula:

$$M = \frac{2RT(\ln C_2 - \ln C_1)}{\omega^2(1 - \nu p)(r_2^2 - r_1^2)}$$

where ω is the velocity; $1 - \nu p = 0{,}27$. To determine the ratio $\Delta \ln C/\Delta (r^2)$ the dependence of $\ln C(r)$ on r^2 was used.

The aminoacid sequence of N-end was determined by Manual Edman method and the aminoacids were identified using thin layer chromatography [Belenkiy, 1967]. To determine N-end aminoacid sequence the samples were taken from polyacrilamide gels using electroelution [Kelly et al., 1983].

Results and Discussion

Using the method of equilibrium sedimentation we have determined that the N-end (30 kDa) of subunit obtained by limited trypsinolisis removed from α-actinin molecule as a monomer and C-end (15 kDa) extracted as a dimer. Thus, N-domain is a monomer and C-domain is a dimer [Kuridze, 1988]. The last is quite stable and doesn't disintegrate into monomers even while using the chromatographical methods or long-term dialysis.

Generally depending on the conditions of α-actinin limited hydrolysis a number of polypeptides, such as T-1 (98), T-2 (85), T-3 (80), T-5 (64), T-6 (55), T-7 (38), T-8 (15) can be obtained (in brackets - Mr in kDa determined by SDS-electrophoresis). Those polypeptides were disposed in α-actinin subunit molecule by determination of aminoacid sequence of N-end [Simonidze, 1985]. The objects of our studies were C-domain and its fragments T-4, T-5, T-6, T-7 and fragments obtained from the last one by prolonged limited hydrolysis.

The limited hydrolysis of native α-actinin was carried out in conditions described in experimental part; kinetics of disintegration of a-actinin rod (C-domain) has been studied (Fig. 1,2,3). The experiments show that C-terminal part of the rod is easily accessible for proteolytic

ferments and at the beginning of hydrolysis small polypeptides separate from it and fragments T-5 (64 kDa) and T-6 (55 kDa) are formed. T-5 rapidly disintegrates and at the background of its diminish T-6 is accumulated in hydrolyzate.

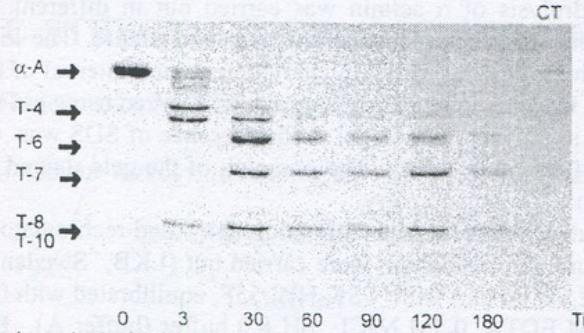

Fig. 1. Electrophoresis in SDS PAGE in the presence of the mixture of products of α–actinin trypsin hydrolysis. The concentration of protein - 1,6 mg/ml; buffer containing 0,5% NH_4NCO_3 (pH 8,1), 1mM EDTA, 2 mM DTT, ratio ferment/substrate 1:50, T – 37^{0}C.

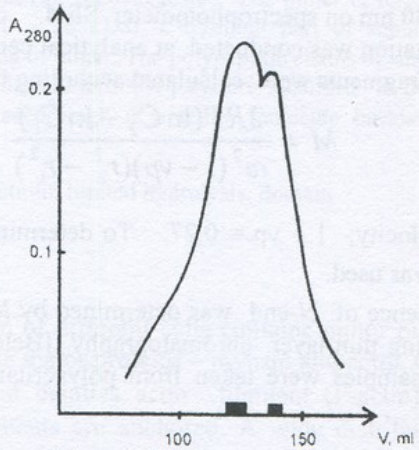

Fig. 2. The change of α-actinin and fragments molar amounts during trypsinolysis (buffer 0,1M NH_4NCO_3, pH 8,1, the ratio ferment/substrate 1:25).

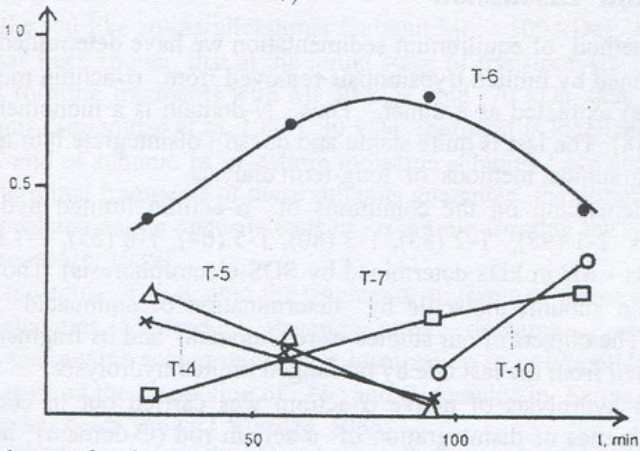

Fig. 3. The change of molar amounts of C-domain and fragments during trypsinolysis (the ratio ferment/substrate 1:50)

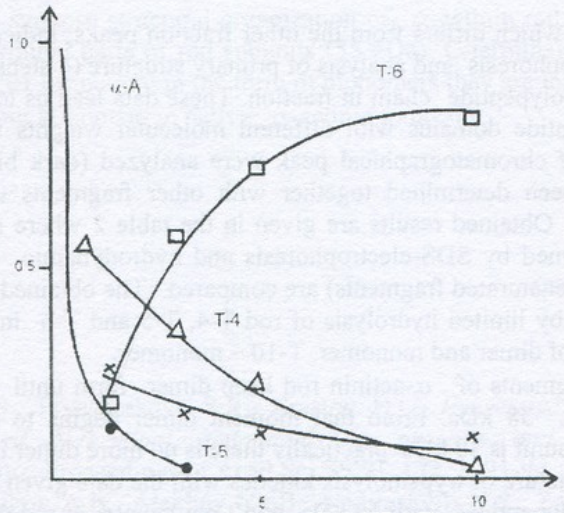

Fig. 4. The rechromatography at the column HW-65F. (The rectangles on the X-axis indicate the limit of combined fractions)

The last splits only in the case of prolonged trypsinolysis and forms T-7(38 kD), T-10(30 kD) and other peptides having small mass. Besides SDS electrophoreses the identification of T-10 was conducted by determination of N-terminal aminoacid sequence. 4 aminoacid residues were fixed: Val–Lei–Ala–Val. It coincides with N-terminal sequence of all other fragments obtained from α-actinin rod. Thus, new fragment T-10 having smaller mass is obtained by diminishing of α-actinin rod from C-end. Table 1 shows the arrangement of fragments obtained by limited proteolysis of α-actinin rod in its polypeptide chain.

Table 1. N-end aminoacid sequence of fragments

T-4	Val-Leu-Ala-Val-Asn-Gln-Glu-Asn-Glu- - -
T-5	Val-Leu-Ala-Val-Asn-Gln-Glu-Asn-Glu-Lis-Leu- - -
T-6	Val–Leu–Ala–Val–Asn-Glu-Asn-Glu-Lis-Leu-Met-Asn-X-Tir- - -
T-7	Val-Leu-Ala-Val- - -
T-10	Val-Leu-Ala-Val- - -

It is known that in α-actinin dimerization 8 SH groups of subunit don't take part, four of such groups are localized in N-domain, 3 – in C-domain and one - in the place of connection of N- and C-domains in the position given below

Arg – Ile – Cis - Liz – Val – Lei – Ala [Jokhadze, 1991]

↑

The arrow shows the beginning of C-domain from N-end. Mentioned SH-groups don't form disulpharic bonds in actinin molecule neither in subunits intrastructure, nor between subunits. We suppose that dimerization of α-actinin rod is caused by hydrogen bonds and electrostatic forces. To clarify rod depolimerization we selected conditions for α-actinin limited hydrolisis (the ratio ferment/substrate, temperature, duration of process). Different chromatographical columns were used to obtain native fragments from hydrolyzate. Rechromatography of fractions gives fractions homogenous according to SDS electrophoresis. The shape of T-7

rechromatographical peak, which differs from the other fraction peaks, indicates its heterogeneity (Fig. 4), while SDS-electrophoresis and analysis of primary structure (4 steps from N-end) shows the existence of only one polypeptide chain in fraction. These data lead us to conclude that in the fraction should be polypeptide domains with different molecular weights in the monomer and dimer forms. Two parts of chromatographical peak were analyzed (dark binds on Fig.4) and molecular weights have been determined together with other fragments using the method of hydrodynamic equilibrium. Obtained results are given in the table 2 where molecular weights of trypsine fragments determined by SDS-electrophoresis and hydrodynamic equilibrium methods (e.g. mol.v. of native and denaturated fragments) are compared. The obtained data obviously show that the fractions obtained by limited hydrolysis of rod T-4, T-5 and T-6 in native condition are dimers, T-7 – the mixture of dimer and monomer, T-10 – monomer.

Thus, trypsin fragments of α-actinin rod keep dimer form until molecular weight of monomer subunit becomes 38 kDa. From that moment dimer begins to dissociate and when molecular weight of rod subunit is 30 kDa practically there is no more dimer in hydrolyzate.

If we collate the picture of trypsinolysis kinetics with the data given in the table 2 we can suppose that C-terminal polypeptides with 15 kDa don't participate in rod dimerization. Besides, taking into consideration that α-actinin subunits have antiparallel arrangement, those "tails" must be localized in the form of monomers in both sides of the rod (Fig. 5a).

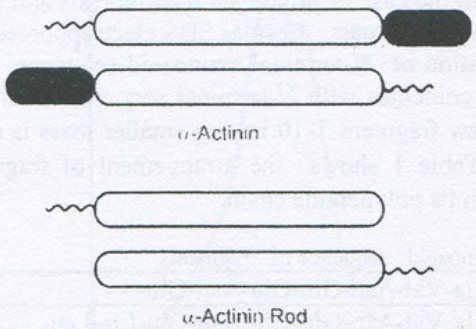

Fig. 5. The structure of α-actinin molecule and α -actinin rod.

Our supposition is in good accordance with the data obtained while studying of the properties of chemically modified α-actinin [Rusia et al., 1989] which showed that bifunctional reagents in α-actinin molecule form intermolecular bonds in C-domain between C-domain unstable C-terminal and N-domain and the distance in α-actinin molecule between N-domain of one subunit and C-terminal of the other subunit of C-domain is ~11,7Å (the length of the bifunctional reagent – dimethylimidate molecule).

Table 2. Molecular weight of α-actinin rod fragments

Fragment	Mr kDa eqv. centr.	Mr kDa SDS-PAGE	State of fragments
T-4	136	70	dimer
T-5	121	64	dimer
T-6	109	55	dimer
T-7	76	38	dimer
T-7'	38	38	monomer
T-10	33	30	monomer

Thus, we can suppose structural organization of α-actinin rod. Dimer is formed directly by interaction of N-terminal parts of rod subunits (55 kDa). C-terminals of rod subunits (15 kD) don't participate in dimer formation; in the form of monomers they are localized at both ends of rod and beside actin-binding N-domain in native α-actinin molecule.

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α-აქტინინის ღეროს სტრუქტურა

სიმონიძე მ., ქურიძე კ., კუპატაძე რ.

საქართველოს მეცნიერებათა აკადემიის მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 18.11.2004)

რეზიუმე

α-აქტინინის ღეროს სტრუქტურული ორგანიზაციის გარკვევის მიზნით შესწავლილია ღეროს შეზღუდული ტრიპსინოლიზის კინეტიკა. დადგენილია პოლიპეპტიდური ფრაგმენტების მოლეკულური მასები ნატიურ და დენატურირებულ მდგომარეობაში. შემოთავაზებულია α-აქტინინის ღეროს სტრუქტურული ორგანიზაციის მოდელი - დიმერი წარმოიქმნება ღეროს სუბერთეულის N-კიდურა ნაწილების (Mr - 55 კდა) ურთიერთქმედებით. ღეროს სუბერთეულის C-კიდურა ნაწილი (Mr - 15 კდა) დიმერის წარმოქმნაში მონაწილეობას არ ღებულობს. დიმერში სუბერთეულების პოლიპეპტიდური ჯაჭვები ლაგდებიან ანტიპარალელურად ისე, რომ მისი C-კიდურა ნაწილები მონომერის სახით თავსდებიან ღეროს ორივე ბოლოში და შესაბამისად α-აქტინინის მოლეკულაში აქტინდამაკავშირებელ N-დომენის (Mr-30კდა) გვერდით.

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Biotechnology

FERMENTATION OF MECHANICALLY PRETREATED CELLULOSIC WASTES WITH *PLEUROTUS OSTREATUS*

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Abstract

Comparative studies on fermentation of composite of mechanically pretreated and non-pretreated cellulosic wastes by the strain of basidial fungus *Pl. ostreatus* had been performed. The stimulating effect of pretreatment method based on combination of chilling and mechanical milling methods on the substrate biodegradation rate was shown. Study of process dynamics showed that on the third day of submerged cultivation of *Pl. ostreatus* on the pretreated cellulosic wastes the maximum amount of soluble sugars -850 γ/ml, was formed. This index is in good correlation with decrease of initial percentage of cellulose. The maximum value of total cellulase activity of the fungus was achieved also on the third day of the fermentation process.

Key words: cellulosic biomass, fermentation, bio-ethanol, cellulase activity, *Pl. ostreatus*

Introduction

Nowadays large scale application of bio-ethanol in fuel blends is considered as one of the effective ways for reduction of green house gases emissions from the transport sector and for fossil fuel saving. Ethanol that is a high octane, non-toxic, biodegradable alcohol produced from renewable resources is usually blended with gasoline as a 10 per cent mix to create a fuel called gasohol. It should be underlined that ethanol blended fuels are approved under the warranties of almost all the automobile manufacturers [Reith et al.,2001]. Approximately 17 million tons/year of fuel ethanol are currently produced from sugar cane and starch crops in Brazil, the USA and some EU countries at a cost of about 0,34 €/liter (16,2 €/GJ) which is 2-fold the price of gasoline (7,3 €/GJ). On the one hand the use of cellulolitic wastes due to its low cost should lead to decrease of bio-ethanol production costs, however the literature survey of international developments show that the estimated production costs of bio-ethanol from lignocellulosic residues are 0, 75-0, 99€/l (34-45 €/GJ), which is considerably higher than the current costs of fuel ethanol from corn starch and gasoline [den Uil et al.,2002].

In general developing technologies that may decrease the cost of bio-ethanol production from different composition cellulosic wastes can be considered in terms of pretreatment, fermentation, alcohol recovery, by-product recovery, and waste treatment. Deriving fermentable

sugars from the hemicellulose and cellulose fractions of lignocellulosic materials via suitable pretreatment and enzymatic cellulose hydrolysis is one of the critical R&D issues [den Uil et al.,2002].

Previous comparative studies on biodegradation of some cellulosic materials such as: newsprint, filter paper, cardboard and sawdust by using *Micromycetes, Basidiomycetes* and *Actinomycetes* performed both in solid phase and submerged cultivation conditions showed that *Pleurotus ostreatus 41* and *Lentinus edodes 1000* had maximum cellulase activity to all above cellulosic substrates both in solid phase and submerged cultivation conditions. It should be mentioned that *Pleurotus ostreatus 41* showed higher growth rate and intensive cellulose bio degradation ability in submerged cultivation conditions compared to other above micro organisms [Chachkhiani et al.,2004]. Next studies devoted to pretreatment of same cellulosic wastes via combination of chilling and mechanical milling methods showed that maximum increase of specific surface area of cellulosic wastes could be reached when the above wastes where chilled at -20^{0}C and milled at once. In addition mechanical milling of frozen cellulosic wastes enhances subsequent breaking of cellulose that leads to further increase of cellulose specific reactionary surface accessible for enzymes. Moreover the mentioned physical pretreatment method does not cause the formation of side products such as organic acids and aromatic compounds and is characterized with low waste production [Chachkhiani et al.,2004].

The present study reports stimulative effect of combined chilling and mechanical milling pretreatment methods on cellulose-to-sugars fermentation by *Pleurotus ostreatus 41* under submerged cultivation conditions.

Materials and methods

The fungus used in this study was *Pleurotus ostreatus 41*. It was obtained from the culture bank of Durmishidze Institute of Biochemistry and Biotechnology. A peace of mycelia of the fungus was cultivated on the following nutrient media (g/l): Na_2HPO_4-0,4; KH_2PO_4- 0,8; $MgSO_4$ – 0,5; NH_4NO_3 – 3,0; yeast extract – 3,0. 1 ml of 1% solution of microelements: $CaCl_2$, $ZnSO_4$, $CuSO_4 \cdot 7H_2O$ and 2 ml of 5% solution of $FeSO_4$ was added to the nutrient media. pH was adjusted to 5, 5. As the source of carbon glucose in amount of 10g/l was used. Sterilization of nutrient media was performed in the autoclave at 0,7 atm during 45 minutes. Afterwards mycelium of *Pleurotus ostreatus 41* was introduced in the flasks. Incubation was done at 28 ^{0}C on the shaker making 180 rotations per minute during 7 days. For further cultivation of the fungus 100 ml of above described nutrient media minus glucose was introduced in 750 ml volume conical flasks where different cellulosic materials were introduced as the carbon sources. For these purposes filter paper, cardboard, newsprint and sawdust have been preliminarily crumbled up in 1 cm pieces and dried at 60^{0}C and were placed in the above flasks in such a way that the concentration of composite of cellulosic wastes in 100 ml of nutrient media was 2%. Cellulosic wastes (filter paper, cardboard, newsprint and sawdust) preliminarily pretreated via combination of chilling at -20 ^{0}C and mechanical milling methods have been introduced in another set of flasks with the same nutrient media as described above. This set of flasks was also autoclaved and the mycelium of *Pleurotus ostreatus 41* was introduced there. Submerged cultivation of the fungus was performed at 28 ^{0}C on the shaker making 180 rot/min during 10 days. Prior to the start of experiments cellulose percentage and concentration of soluble sugars in the substrates were determined. By the end of fermentation process the flacks' contents were centrifuged at 7000 rot/min. In the supernatant pH and total cellulase activity (TCA) were measured. Percentage of crude protein and cellulose were determined in the sediment that was dried at 60 ^{0}C. Determination of percentage of crude protein in biomass was performed according to the Nestler method using 46,8 coefficient [Termkhitarova, Shulga, 1974]. Determination of percentage of cellulose in biomass was conducted according to

Apdegraph method [Apdegraph, 1969] and total cellulolitic activity (TCA) was estimated by using Shomodi-Nelson method.

Results and discussions

The changes in cellulose, soluble sugars and crude protein for the both: non treated and pretreated cellulosic wastes after 4 and 10 days of fermentation with basidial fungus *Pleurotus ostreatus 41* are given in Table 1.

Referring to the results given in table *Pleurotus ostreatus 41* showed ability to biodegradation of both: pretreated and non-pretreated cellulosic substrates. However it is evident that on the 4th day of submerged fermentation process of pretreated cellulosic wastes the amount of accumulated crude protein is 1,7 times more compared to those obtained for non-pretreated ones. Practically the same results have been achieved for decrease of cellulose percentage.

Table 1. Growth and TCA of *Pleurotus ostreatus 41* cultivated on pretreated and non-pretreated cellulosic wastes under submerged fermentation.

Duration of cultivation (days)		Crude protein (%)	Decrease of initial cellulose (%)	Soluble sugars (γ/l)
4	non-pretreated cellulosic wastes	7,12	6,6	104
	pretreated cellulosic wastes	12,20	15,47	720
10	non-pretreated cellulosic wastes	13,10	12,50	80
	pretreated cellulosic wastes	18,21	19,11	335

When pretreated cellulosic wastes were fermented with above mentioned basidial fungus concentration of soluble sugars on the 4th day of fermentation was 7 times more compared to results obtained for non-pretreated cellulosic wastes. Despite the cellulose degradation TCA was zero on the 4th and 10th days of the fermentation process. Due to this fact it became important to study the dynamics of the growth and total cellulase activity of *Pleurotus ostreatus 41* cultivated on the pretreated (via combination of chilling and mechanical milling methods) cellulosic wastes. Results are given in Fig.1, 2, 3 and 4.

Results given in Fig.1 show that *Pleurotus ostreatus 41* starts growth at once as it is introduced in the flask with cellulosic substrate and consequently crude protein content in biomass increases from 5, 1% to 6, 8% after 24 hours of cultivation. Experiments showed that on the 9th day of cultivation the microorganism got into stationary growth phase, however significant increase in crude protein content was not observed.

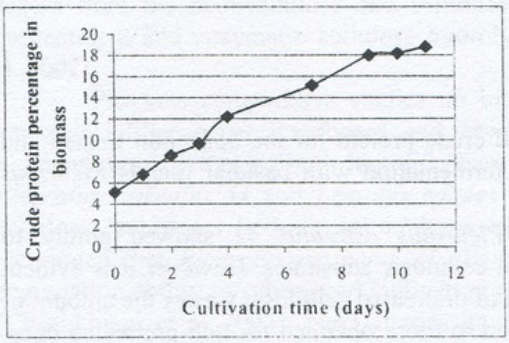

Fig.1

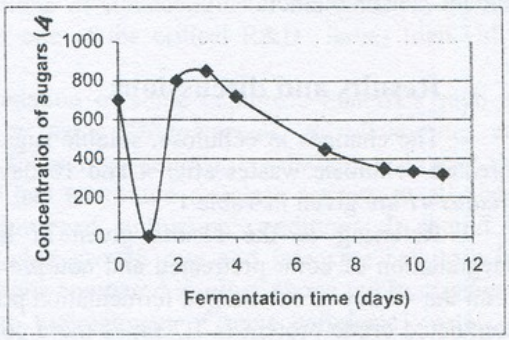

Fig.2

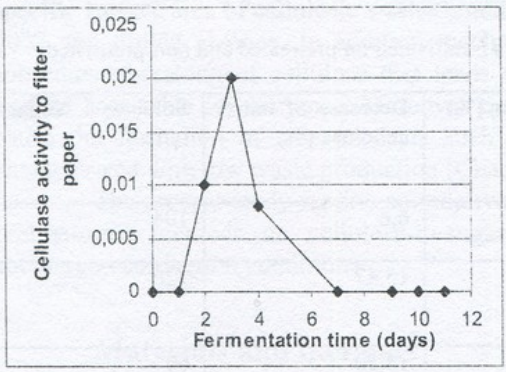

Fig.3

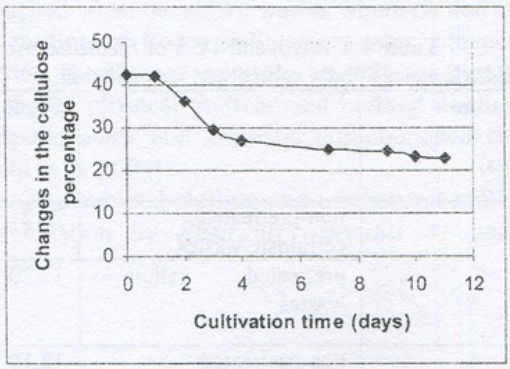

Fig.4

Initial percentage of soluble sugars in pretreated sample of cellulosic wastes was 700 γ/ml, and at the start of the fermentation process *Pleurotus ostreatus 41* fully consumed them for the growth in 24 hours. Following the data given in Fig.2 it is clear that maximum amount of soluble sugars formed as a result of submerged fermentation of pretreated cellulosic wastes are accumulated on the 2nd and 3rd days of the process and their value reach 800 and 850γ/ml correspondingly. On the 4th day of fermentation concentration of soluble sugars starts the gradually decrease. These results are in good correlation with those obtained for changes in cellulose percentage. Fig.3 illustrates that cellulase activity of *Pleurotus ostreatus 41* to filter paper reaches maximum on the 3rd day of fermentation process and then sharply decreases.

Study of cellulose biodegradation dynamics showed that during 24 hours changes in cellulose percentage practically do not take place. Such result could be caused by the fact that at the start the fungus uses the soluble sugars formed under pretreatment process and thus the microorganism is not forced to attack the hardly degradable polymer. After 24 hours of fermentation when the easily assimilated sugars are fully consumed, *Pleurotus ostreatus 41* begins degradation of cellulose and on the 3rd day of the process cellulose percentage decreases by 13%.

So, it could be concluded that *Pleurotus ostreatus 41* is one of the promising microorganisms which ensures saccharification of composite of different cellulosic wastes under submerged fermentation conditions. Pretreatment of cellulosic wastes by combination of chilling and mechanical milling methods enhances the yield of soluble sugars when pretreated cellulosic substrates are fermented by the above mentioned fungus

Acknowledgment

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მექანიკურად წინასწარ დამუშავებული ცელულოზური სუბსტრატების ფერმენტაცია *PLEUROTUS OSTREATUS*-ით

ბერეჟიანი მ.[1], ჩაჩხიანი მ.[1], დუდაური თ.[1], ფარცხალაძე გ.[1], წიკლაური ლ.[1], უგრეხელიძე ვ.[1], ზაქარიაშვილი ნ.[2]

[1] საქართველოს მაღალი ტექნოლოგიების ეროვნული ცენტრი, [2] საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 20.12.2004)

რეზიუმე

შესწავლილია მექანიკურად წინასწარ დამუშავებული და დაუმუშავებელი სხვადასხვა ცელულოზური ნარჩენების შაქრებამდე ბიოდეგრადაციის შესაძლებლობა ბაზიდიალური სოკოს *Pl. ostreatus*–ის გამოყენებით. ნაჩვენებია ცელულოზური სუბსტრატების წინასწარი გაყინვისა და მექანიკური დაფქვის მასტიმულირებელი გავლენა სუბსტრატის ბიოდეგრადაციის სიღრმეზე. პროცესის დინამიკის შესწავლამ აჩვენა, რომ წინასწარ მექანიკურად დამუშავებულ ცელულოზურ ნარჩენებზე *Pl. ostreatus*-ის სიღრმული კულტივირების მესამე დღეს მიიღება ხსნადი შაქრების მაქსიმალური რაოდენობა - 850γ/მლ. ეს მაჩვენებელი კარგ კორელაციაშია ცელულოზის საწყისი კონცენტრაციის შემცირებასთან დროის იგივე მონაკვეთში. სოკოს ფერმენტული აქტივობის მაქსიმუმი ასევე კულტივირების მესამე დღეს დაფიქსირდა.

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Botany

LICHENS OF THE SURROUNDINGS OF LAKE LISI ON THE BACKGROUND OF THE VASCULAR PLANT VEGETATION

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Abstract

The survey of lichens occurring in various communities of vascular plants in the surroundings of Lake Lisi is presented. 81 species of lichens from 33 genera and 20 families were recorded on the studied area. In the presented species list an appropriate geographic element for each species is indicated. A short discussion on morphological, ecological and geographic structure of the lichen flora of the studied area is also provided. No lichens were recorded in the moist habitats at the lakeshore. Lichens occurring in the secondary xerophilous communities which occupy the major part of the studied area refer mainly to species tolerant to a wide range of ecological conditions or adapted to dry habitats; however, a number of broad-leaved forest lichen species still occur in faded forest remnants, shrubbery and tree plantations. The survey has shown that changes in local habitats directed towards xerophytization should lead to further pauperization of the lichen flora.

Key words: lichens, xerophitization, Lake Lisi.

Introduction

The major part of steppe vegetation of East Georgia (200-600 (900) m a. s. l.) has been formed for the last several centuries as a result of deforestation [Ketskhoveli, 1960; Zazanashvili et al., 2000]. Its development was due to a climate change as well as severe human impact. The tendency is pronounced in the surroundings of Lake Lisi, where the present survey was carried out.

Lake Lisi is located in the western part of Tbilisi, on the eroded ridge of the Lisi range located on the right bank of the river Mtkvari, at the boundary between the vertical belts of arid and mountain deciduous forest vegetation. The most part of the studied area is occupied by secondary xerophilous vegetation.

Lichens inhabiting various substrates on the studied area mainly belong to groups of species tolerant to a wide range of ecological conditions or adapted to dry habitats. Communities of moist habitats at the lakeshore have started their development a relatively short time ago and none of the lichen species characteristic to such habitats has established themselves there yet.

Materials and Methods

Several botanical surveys were carried out on the area of approximately 3 x 5 km northwest of Lake Lisi. Vascular plant communities were briefly described and a list of lichen species inhabiting different substrates in these communities was compiled. Besides, several aspects

of the lichen flora (morphological, ecological and geographical) were analysed in order to find out effects of environmental changes on the diversity and structure of the lichen flora.

Lichen nomenclature corresponds to the Guidebook of Lichens [Guidebook of Lichens of the USSR, 1971-1977].

Results and Discussion

Various habitats, several series of ecologically distinct plant communities successively replacing each other and besides, a number of different stages of ecological succession are observed in the surroundings of Lake Lisi [Erkomaishvili, Chelidze, 1987].

Swamp vegetation of the lakeshore is mainly constituted by *Phragmites communis* and several species of the genus *Carex*, which are characteristic to moist habitats (*C. riparia, C. divulsa, C. acuta,* etc.). Meadows following the swamp gradually become more xerophilous and the species of *Carex* mentioned above are substituted by those adapted to relatively dry conditions (such as *C. melanostachys, C. tomentosa*, etc.). Further, the sedge meadows are replaced by forb-grass ones with *Poa trivialis, P. nemoralis, Medicago minima, M. lupulina,* etc. Spots of herbaceous communities dominated by *Stipa spp.* cover dry slopes occurring in the line of the noted meadows. The latter are replaced by steppe.

A small fragment of a deciduous forest *(Quercus iberica – Carpinus caucasica – C. orientalis)* occurs on the investigated area. The forest is gradually falling off towards the periphery of this plot, where *C. orientalis* dominates and the other tree species are rather faded. *Paliurus spina-christi*, a xerophilous shrub, is penetrated into this suppressed forest fragment. More or less dense thickets of *P. spina-christi* (sometimes with admixed *C. orientalis*) and those of more xerophilous *Rhamnus pallassii* occur in a number of places.

Festuca valesiaca and less frequently *Botriochloa ischaemum* are the dominants in the steppe occupying the major part of the studied area. Species of phryganoid plant communities: *Teucrium polium, Salvia sclarea, Stachys lavandulaefolia, Centaurea ovina, Agropyron cristatum, Astragalus microcephalus,* etc. have settled on the most eroded slopes and bare rocks.

81 species of lichens from 33 genera and 20 families have been recorded in the xerophilous plant communities, whereas no lichens have been found in the moist habitats of the lakeshore. Since the communities occurring in the moist habitats are relatively young, none of the lichen species characteristic to such habitats has established themselves there yet.

The species list is presented in the Table. The Diagrams 1, 2 and 3 show the proportion of species inhabiting different substrates, their distribution in various morphological groups and the geographic spectrum [Golubkova, 1983] of the lichen flora of the studied area, respectively. A discussion concerning the structure of lichens inhabiting different substrates is given below.

Saxicolous group. The representatives of the group predominate on the studied area, which is due to deforestation and consequent xerophytization resulted in the lack of substrate and specific microclimate for epiphytic and terricolous species. Namely, 45 saxicolous species of lichens from 21 genera and 14 families have been recorded in the xerophilous communities described above (including the faded forest fragment); 40 of them are crustose and 5 are foliose (*Dermatocarpon miniatum, Collema crispum, C. cristatum, Parmelia pulla, P. stenophylla*). 30 species represent the multizonal geographic element spread over several vegetation zones, species of which are, therefore, tolerant to a wide range of ecological conditions, 10 species refer to the arid, 4 to the mountain and 1 to the arctic-alpine elements. Consequently, the multizonal element creates the background of the saxicolous group, while the arid one (comprising *Verrucaria calciseda, V. lecideoides, Diploschistes calcareus, Acarospora heufleriana, Aspicilia desertorum, A. reticulata, Lecanora frustulosa, Placolecanora alphoplaca, Parmelia pulla, P. stenophylla*) defines its character.

Terricolous group. 17 terricolous species from 8 genera and 8 families have been found in these communities; 3 of them are crustose, 8 are foliose and 6 fruticose. 5 fruticose species are representatives of the genus *Cladonia*. 10 species refer to the multizonal, 6 to the arid and 1 to the arctic-alpine elements. Similar to the previous group, the multizonal element creates the background of the terricolous lichen flora, while the arid one (comprising *Endocarpon adscendens, Toninia coeruleonigricans, Cladonia convoluta. Parmelia ryssolea, P. vagans, Cornicularia steppae*) defines its character.

Epiphytic group. 19 epiphitic species from 11 genera and 7 families have been recorded in forest fragments and scrubs. 8 species are crustose and 11 are foliose. None of the fruticose species, which are characteristic to more or less humid habitats has been recorded. 9 species refer to the nemoral element, 8 to the multizonal and 1 to the arid ones. Thus, the high proportion of the nemoral element is observed in the remains of the forests, which formerly covered the major part of the investigated territory.

The process of xerophytization is in progress in the surroundings of Lake Lisi as well as in a number of other regions of East Georgia resulting in the disappearance of habitats for certain groups of lichens and, consequently, the impoverishment of the lichen flora of the area.

Table. Lichen flora of the surroundings of Lake Lisi.

Groups of species according to substrate type	Species	Geographic element
Saxicolous species	**Verrucariaceae**	
	Verrucaria calciseda D.C.	Arid
	Verrucaria lecideoides Trevis.	Arid
	Verrucaria nigrescens Pers.	Multizonal
	Dermatocarpaceae	
	Dermatocarpon miniatum (L.) Mann.	Multizonal
	Polyblastiaceae	
	Staurothele caesia Arnold	Mountain
	Staurothele rufa (Massal.) Zsch.	Mountain
	Staurothele ventosa Syd.	Mountain
	Diploschistaceae	
	Diploschistes calcareus (Mull. Arg.) Steiner.	Arid
	Diploschistes scruposus (Schreb.) Norm	Multizonal
	Collemataceae	
	Collema crispum (Hads.) Web.	Multizonal
	Collema cristatum (L.) Web.	Multizonal
	Pannariaceae	
	Placynthium nigrum (Huds.) S. Gray	Multizonal
	Lecideaceae	
	Lecidea crustulata (Ach.) Sprgl.	Multizonal
	Lecidea goniophila Floerk.	Multizonal
	Lecidea mosigii (Hepp.) Anzi	Arctic-Alpine
	Rhizocarpon geographicum (L.) DC.	Multizonal
	Rhizocarpon lindsayanum Ras.	Multizonal
	Acarosporaceae	
	Sarcogyne regularis Koerb.	Multizonal
	Acarospora fuscata (Rohl.) Arnold	Multizonal
	Acarospora glaucocarpa (Wahlenb.) Koerb.	Multizonal

	Acarospora heufleriana Koerb.	Arid
	Pertusariaseae	
	Pertusaria lactea (L.) Arnold	Mountain
	Lecanoraceae	
	Aspicilia cinerea (L.) Koerb.	Multizonal
	Aspicilia contorta (Hoffm.) Krempth..	Multizonal
	Aspicilia desertorum (Krempth) Meresche.	Arid
	Aspicilia hoffmannii (Ach.) Flag.	Multizonal
	Aspicilia reticulata Krempth in Arnold	Arid
	Lecanora atra (Huds.)Ach.	Multizonal
	Lecanora dispersa (Pers.) Rohl.	Multizonal
	Lecanora frustulosa (Dicks.) Ach.	Arid
	Placolecanora alphoplaca (Wahlenb.) Ras.	Arid
	Placolecanora muralis (Schreb.) Ras.	Multizonal
	Placolecanora radiosa (Hoffm.) Ras.	Multizonal
	Candelariella aurella (Hoffm.) Zahlbr.	Multizonal
	Candelariella vitellina (Ehrh.) Mull. Arg.	Multizonal
	Parmeliaceae	
	Parmelia pulla Ach.	Arid
	Parmelia stenophylla (Ach.) Heug.	Arid
	Caloplacaceae	
	Caloplaca aurantiaca (Lightfl.) Th. Fr.	Multizonal
	Caloplaca citrina (Hoffm.) Th. Fr.	Multizonal
	Caloplaca flavovirescens (Wulf.) D. Torre et Sarnth.	Multizonal
	Pyrenodesmia chalybeia Mull. Arg.	Multizonal
	Pyrenodesmia variabilis (Pers.) Massal.	Multizonal
	Buelliaceae	
	Diplotomma epipolium Arnold.	Multizonal
	Rinodina bischoffii (Hepp.) Massal.	Multizonal
	Deuterolichenes (Lichenes imperfecti)	
	Lepraria aeruginosa A. L. Sm.	Multizonal
Terricolous species	**Dermatocarpaceae**	
	Endopyrenium cinereum (Pers.) Oxn.	Arctic-Alpine
	Endopyrenium hepaticum (Ach.) Koerb.	Multizonal
	Endopyrenium rufescens (Ach.) Koerb.	Multizonal
	Endocarpaceae	
	Endocarpon adscendens (Anzi.) Mull. Arg.	Arid
	Endocarpon pusillum Hedw.	Multizonal
	Collemataceae	
	Collema tenax (Sw.) Ach.	Multizonal
	Peltigeraceae	
	Peltigera canina (L.) Willd.	Multizonal
	Peltigera rufescens (Weis.) Humb.	Multizonal
	Lecideaceae	
	Toninia coeruleonigricans (Lightfl.) Th. Fr.	Arid
	Cladoniaceae	
	Cladonia chlorophaea (Floerk.) Spreng.	Multizonal
	Cladonia convoluta (Lam.) P.Cout.	Arid
	Cladonia furcata (Huds.) Schrad.	Multizonal
	Cladonia pyxidata (L.) Fr.	Multizonal
	Cladonia rangiformis Hoffm.	Multizonal

	Parmeliaceae	
	Parmelia ryssolea (Ach.) Nyl.	Arid
	Parmelia vagans Nyl.	Arid
	Usneaceae	
	Cornicularia steppae Savicz.	Arid
Epiphitic speices	**Lecideaceae**	
	Lecidea glomerulosa (DC.) Stend.	Multizonal
	Lecanoraceae	
	Lecanora hagenii Ach.	Multizonal
	Lecanora rugosella Zahlbr.	Nemoral
	Lecanora subrugosa Nyl.	Nemoral
	Parmeliaceae	
	Candelaria concolor (Dicks.) Stein.	Nemoral
	Caloplacaceae	
	Caloplaca cerina (Ehrh.) Th. Fr.	Multizonal
	Caloplaca pyracea (Ach.) Th. Fr.	Multizonal
	Teloschistaceae	
	Xanthoria parietina (L.) Beltr.	Multizonal
	Xanthoria substellaris (Ach.) Vain.	Nemoral
	Xanthoria ulophylodes Ras.	Not speciefied
	Buelliaceae	
	Diplotomma alboatrum (Hoffm.) Fw.	Multizonal
	Rinodina pyrina (Ach.) Arn.	Nemoral
	Physciaceae	
	Phaeophyscia orbicularis (Neck.) Moberg.	Multizonal
	Physcia adscendens Oliv.	Nemoral
	Physcia aipolia Hampe	Nemoral
	Physcia biziana (Massal.) Zahlbr.	Arid
	Physcia stellaris (L.) Nyl.	Nemoral
	Physciopsis adglutinata (Flk.) Choisy	Multizonal
	Physconia pulverulenta (Hoffm.) Poelt	Nemoral

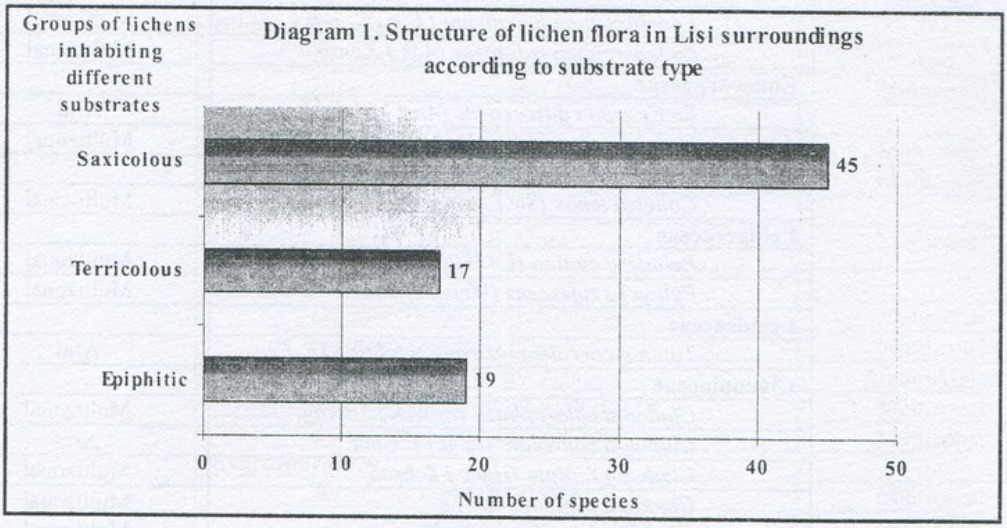


Diagram 1. Structure of lichen flora in Lisi surroundings according to substrate type

Diagram 2. Morphological structure of lichen flora in Lisi surroundings

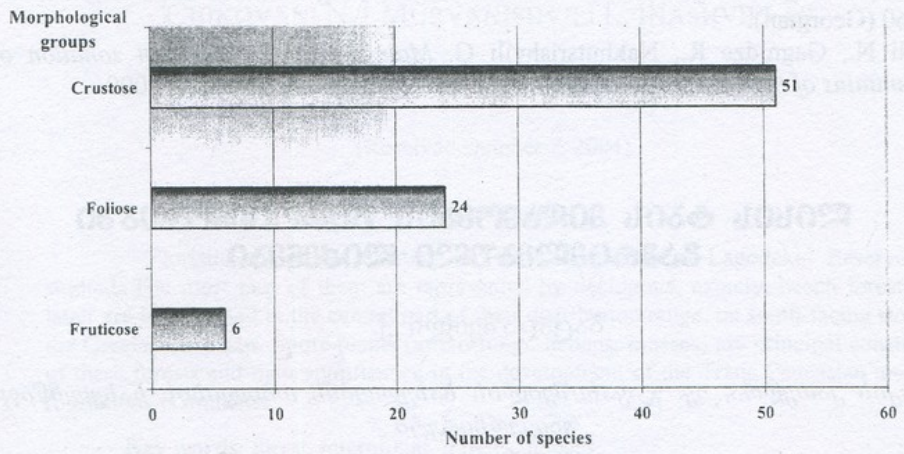
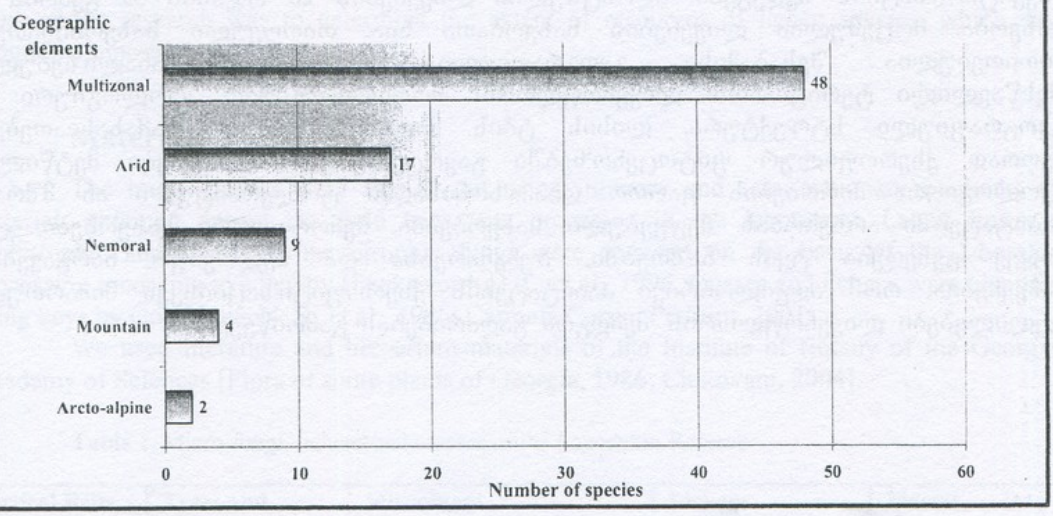


Diagram 3. Geographic structure of lichen flora in Lisi surroundings



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ლისის ტბის მიდამოების ფიტოცენოზებში გავრცელებული ლიქენები

ბაცაცაშვილი ქ.

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(მიღებულია 27.12.2004)

რეზიუმე

წარმოდგენილია ლისის ტბის მიდამოების ფიტოცენოზთა ლიქენოფლორის ინვენტარიზაციის შედეგები. აღრიცხულია ლიქენების 20 ოჯახის 33 გვარის 81 სახეობა. მოცემულია ლიქენების სახეობათა სია. თითოეული სახეობისათვის მითითებულია შესაბამისი გეოგრაფიული ელემენტი. გაანალიზებულია შესწავლილი ტერიტორიის ლიქენოფლორის მორფოლოგიური, ეკოლოგიური და გეოგრაფიული სტრუქტურა. ლისის ტბის მიდამოებისათვის დამახასიათებელ მეორად ქსეროფილურ ფიტოცენოზებში გავრცელებული ლიქენები მეტწილად ეკოლოგიური პირობების ფართო დიაპაზონისადმი ტოლერანტული ან მშრალ ჰაბიტატებში არსებობას შეგუებული სახეობებია. ნემორალური სახეობები ჯერ კიდევ გვხვდება ტყის ნაშთებში, ბუჩქნარებსა და ხელოვნურ ნარგავებში. ნაჩვენებია, რომ ადგილობრივი ჰაბიტატების ქსეროფიტიზაციისკენ მიმართული ცვლილებები ლიქენოფლორის შემდგომ გაღარიბებას გამოიწვევს.

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CRYPTOGAMS AND FUNGI OF FOREST BELT OF LAGODEKHI STATE RESERVE

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Abstract

Floristic complexes existing in forest formations of Lagodekhi Reserve were studied. The most part of them are represented by deciduous, namely, beech forests. The latter are best formed in the central part of their distribution range, on south-facing slopes of the Greater Caucasus. Spore plants (micro-fungi, lichens, mosses) are principal constituents of these forests and their significance in the development of the Trans Caucasian mountain formations is immense.

Key words: forest, microfungi, mosses, lichens.

Introduction

The main purpose of the research was to find out principal and characteristic species of micro-fungi, lichens and mosses occurring on woody plants of the reserve from 400 m to 1800 m a.s.l. We used vertical division systems [Dolukhanov A.G, 1940; Kvachakidze, 1999]. One of the tasks of the research was to determine the extent of resources of lichen species, which have economic importance.

Materials and Methods

The investigations were carried out using itinerary and semi-stationary methods; the material, obtained during the field trips was processed in the laboratory. Latent forms of saprotrophic and biotrophic-necrotrophic fungi were detected on the basis of the laboratory processing according to a key by Hawksworth D. L.,et al., 1996, mosses and lichens were identified using keys by Golubkova N. S., et al., 1996; Carmela Cortini Pedrotti, 2001.

We used literature and herbarium materials of the Institute of Botany of the Georgian Academy of Sciences [Flora of spore plants of Georgia, 1986; Chikovani, 2004].

Table 1. Micro-fungi, lichens and mosses of the Lagodekhi Reserve

Vertical Belts	Trees and Shrubs	Microfungi	Lichens	Mosses
400-100m a.s.l.	*Acer laetum*	*Phyllosticta aceris, P.negundinis, P. pseudoplatani* – on leaves	*Lecanora allophana, Lecidea*	*Amblystegium serpens, A. varium,*

	Cladosporium variabile, Diplodia acerina, Plomopsis protracta – on trunks, branches	*glomerulosa, Nephroma parile, Physcia aipolia, Physconia pulverulenta*	*Anomodon attenuatus*
Alnus barbata	*Cercosporella alni, Microsphaera alni* – on leaves *Hypoxilon coccineum, Stilbospora thelebola* – on dry trunks	*Lecanora carpinea, Parmelia caperata*	*Amblystegiella subtilis, Brachythecium populeum, Metzgeria conjugata*
Carpinus caucasica	*Mamiania fimbriata* – on leaves *Fusicocum macrosporum, Helminthosporium macrocarpum, H.velutinum, Mamiania fimbriata, Melanconium stromaticum, Microsphaeropsis olivacea,* etc. – on dry trunks, branches	*Graphis scripta, Lecanora allophana, L. glabrata, Lecidea glomerulosa, Parmelia caperata,Physcia aipolia, Physconia pulverulenta*	*Leskeela nervosa, Hypnum cupressiforme, Pteryginandrum filiforme*
Cornus mas	*Coryneum corni-albae, Ramularia gracilipes* – on leaves *Colletotrichum corni* – on fruit *Cytospora leucosperma, C. leucostoma* – on dry trunks		*Pteryginandrum filiforme, Leucodon sciuroides, Bryum capillare var. flaccidum*
Corylus avellana	*Gloeosporium coryli* – on leaves *Helminthosporium velutinum, Hypoxylon luridum, Isaria lecaniicola, Taeniolella stilbospora* – on dry branches		*Amblystegiella subtilis, Amblystegium serpens, Brachythecium rivulare*
Crataegus kyrtostyla	*Cercospora confluens, Coryneum foliicalum, Septoria crataegi* – on leaves *Gymnosporangium clavariaeforme* – on shoots, leaves *Camarosporium crataegi, Coniothyrium olivaceum, Diaporthe crataegi, Diplodia crataegi, Podosphaera oxycanthae* – on dry branches	*Anaptychia ciliaris, Caloplaca pyracea, Evernia prunastri, Nephroma parile, Parmelia caperata, P. glabra, P. sulcata, Physcia aipolia, Physconia pulverulenta, Pseudevernia furfuracea*	*Leskeela nervosa, Orthotrichum striatum*
Fraxinus excelsior	*Ascochyta orni, Fusicladium fraxini, Phyllactinia suffulta f. fraxini* – on leaves	*Anaptychia ciliaris, Candelaria concolor, Collema*	*Leucodon sciuroides, Tortella tortuosa*

	Leptosphaeria vagabunda, Melanconium magnum, Microdiplodia fraxini, etc. – on dry branches	*flaccidum, Diploschistes bryophyloides, Nephroma parile, Parmelia caperata, P. glabra, Physconia pulverulenta*	
Juglans regia	*Marssonina juglandis, Microstroma juglandis* – on leaves *Cyclothyrium juglandis, Cytospora leucosperma, Dendrophoma juglandina, Diplodia juglandis, Epicoccum purpurascens Melanconium juglandinum, Microdiplodia juglandis* – on dry trunks, branches	*Lecanora glabrata, Lecidea glomerulosa*	*Leucodon immerses, L. sciurides, Hedwigia ciliate, Orthotrichum montanum*
Lonicera caucasica	*Cercospora periclymeni, Ramularia lonicerae, Septoria xylostei* – on leaves *Camasporium xylostei, Coniothyrium olivaceum, Microdiplodia ascochytula, Microsphaeropsis olivacea* – on dry branches		*Amblistegiella subtilis, Amblystegium serpens, Bryum capillare var. flaccidum*
Populus nigra	*Fusicladium radiosum, Melampsora tremulae, Ramularia uredinis* – on leaves *Cytospora leucostoma, Phoma urens, Tubercularia vulgaris* – on dry branches		*Brachythecium rivulare, B. populeum, B. velutinum, Leucodon sp.*
Quercus iberica	*Cylindrosporium associatum, Gloeosporium quercinum, Microsphaera alphitoides, Oidium alphitoides, Phyllosticta associata, P. quercicola, P. quercus, Septoria quercicola* – on leaves *Bombardia fasciculata, Coryneum depressum, C. umbonatum, Cytospora leucosperma, Diatrypella verruciformis, Stilbospora angustata* – on dry branches, trunks		*Anomodon viticulosus, Leucodon immersus, L. sciuroides, Orthotrichum striatum, Porella platyphylla*
Rosa canina	*Cercospora rosae, Monochaetia depazeoides, Oidium leucoconium, Phragmidium disciflorum,*		*Amblystegiella subtilis, Hypnum cupressiforme, Leskeela nervosa*

		Ph. rosae-pimpinellifoliae – on leaves *Cladosporium fuscum, Cryptostictis cynostabi, Microdiplodia rosarum, Monochaetia compta var. ramulicola, Phoma aculeorum, P. rhodocarpa, Physalospora erratica* – on spines, trunks		– at the base
	Salix excelsa	*Melamspora allii-salicis albae, M. salicina, Ramulaspera salicina* – on leaves *Aposphaeria henryana, Conoithyrium fuligineum, Cytospora salicis, Diplodina salicis, Leastadia carpinea var. salicina, Microdiplodia salicis, Valsa ambiens* – on dry trunks, branches	*Parmelia caperata*	*Amblystegium serpens, Leucodon sciuroides, Mnium stellare, Thuidium philibertii*
	Sambucus nigra	*Septoria sambuciana* – on leaves *Aposphaeria subcorticalis, Diplodia sambucicola, Phoma striaeformis, Phomopsis sambucina, Stagonospora caespitosa* – on dry branches		*Leucodon sp.*
	Sorbus torminalis	*Cytospora rubescens, Diplodia sorbi, Tubercularia vulgaris* – on trunks, branches		
	Thelycrania australis	*Ramularia angustissima, Septoria cornicola* – on leaves *Monochaetia veneta, Sphaerulina intermixta f. corni* – on dry trunks, branches		*Amblystegium varium*
1000-1500m a.s.l.	*Carpinus caucasica*	*Cytospora decorticans, Diatrype stigma, Fusicoccum macrosporum, Melanconium bicolor, Microdiplodia microsporella, Stilbospora angustata, Tubercularia vulgaris, Valsa ambiens* – on dry trunks, branches	*Anaptychia ciliaris, Graphis scripta, Lecanora allophana, L. carpinea, Lecidea glomerulosa, Leptogium saturninum, Parmelia caperata, P. glabra, Physcia aipolia, Ramalina*	*Amblystegium serpens, Leucodon sciuroides, Orthotrichum fastigiatum, Porella platyphylla*

			farinacea	
	Castanea sativa	*Diaporte castaneti, Diatrypella minuta* – on dry branches	*Lecanora carpinea, Opegrapha atra, Phaeophyscia orbicularis*	*Brachythecium populeum, Leucodon sciuroides*
	Corylus iberica	*Dermatea coryli, Isaria lecaniicola, Leptosphaeria avellana, Torula pithyophilum* – on branches		*Amblystegium serpens, Mnium stellare*
	Fagus orientalis	*Ascochyta fagi, Gloeosporium fagicolum, G. fuckelii* – on leaves *Botryosphaeria advena, Diatrype stigma, Hypoxylon coccineum, H. serpens, Phoma antarctida, Tubercularia vulgaris, Xylaria hypoxylon* – on trunks, branches	*Bryoria chalybeiformis, Cetrelia cetrarioides, Collema nigrescens, Graphis scripta, Heterodermia speciosa, Hypogymnia physodes, Leptogium saturninum, Lobaria amplissima, L. pulmonaria, Ochrolechia parella, Parmelia caperata, P. carporhizans, P. perlata, P. sulcata, Pertusaria discoidea, P. pertusa, Pyrenula nitida, Pseudocyphellaria scrobiculata, Ramalina farinacea, R. fraxinea, R. sinensis*	*Anomodon apiculatus, A. attenuatus, A. viticulosus, Brachythecium populeum, Leucodon sciuroides, Neckera crispa, N. besseri, Porella platyphylla, Pteryginandrum filiforme, Radula complanata* – on the bark *Ctenidium molluscum, Isothecium myurum, Lejeunea cavifolia, Schistidium apocarpum, Tortella tortuosa* – on roots
	Rhododendron luteum	*Exobasidium discoideum, E. magnusii* – on leaves *Cladosporium oxycocci, Monochaetia monochaeta, Phoma rhododendri* – on dry branches		*Fissidens cristatus, F. taxifolius, Scapania undulata*
1500-1800 m a.s.l.	*Acer trautvetteri*	*Cercospora acericola* – on leaves *Leptosphaeria mulleri, Steganosporium pyriforme, Tubercularia vulgaris, Valsa ambiens* – on dry	*Alectoria chalybeiformis, A. implexa, Anaptychia solenaria, Cetrelia*	*Brachythecium rutabulum, Leskeela nervosa, Mnium punctatum*

		branches	*cetrarioides, Heterodermia speciosa, Hypogimnia physodes, Leptogium saturninum, Lobaria amplissima, Parmelia carporhizans, Pseudoevernia furfuracea*	
	Betula litwinowii	*Ramularia alnicola, Fusicladium betulae* – on leaves *Hormiscium handelii, Torula handelii* – on the bark	*Anaptychia solenaria, Buellia disciformis, Cetraria pinastri, Heterodermia speciosa, Hypogimnia physodes, H. vittata, Leptogium cyanescens, L. saturninum, Parmelia glabra, Parmeliopsis ambigua, Ramalina farinacea*	*Brachythecium populeum, B. velutinum*
	Quercus macranthera	*Ascochyta quercus, Phyllosticta quercus-ilicus, Ramularia crypta, Septoria dubia, Stigmella dryina* – on leaves *Fusicoccum quercinum, Xylohypha nigrescens* – on branches	*Caloplaca cerina, C. pyracea, Diploschistes bryophyloides, Lecanora allophana, Nephroma parile, Parmelia glabra, P. quercina, Physcia aipolia, Pseudoevernia furfuracea, Ramalina fraxinea*	*Leucodon immersus, L. sciuroides, Orthotrichum montanum, Pteryginandrum filiforme*
	Sorbus caucasigena	*Ramularia sorbi* – on leaves *Nummularia rependa, Tubercularia vulgaris* – on branches	*Physcia aipolia*	*Eurhynchium striatum, Fissidens cristatus, F. taxifolius, Orthotrichum rupestre* – at the base

Results and discussion

On the basis of long-term investigations, we have determined that lignophilous complexes predominate over the microfungi found in the forest belt of the Lagodekhi Reserve. Mycobiota of beech/hornbeam and mixed forests is similar in the middle and upper belts. The same set of species is presented on endemic and relict plants. However, the number of species decreases from the lower belt towards the upper one (owing to the known response of the fungi to the increased solar radiation).

The distribution pattern of mosses is different. Mosses markedly vary according to characteristics of the micro-relief, which is due to their ecological plasticity. On account of the thick litter, excessive shading, etc. there are unfavourable conditions for the distribution of the mosses in the beech forest. However, certain diversity of this group of plants is created by azonal species as well as high-mountain and foothill species in the upper and lower belts, respectively. The dominant and characteristic species occurring on deciduous woody plants are relatively constant in all belts.

The following pattern has been observed in the distribution of lichens: the species richness and abundance of lichens increases parallel to the increasing altitude.

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ლაგოდეხის სახელმწიფო ნაკრძალის ტყის სარტყლის სპოროვანი მცენარეები და სოკოები

ჩიქოვანი ნ., მურვანიშვილი ი., ინაშვილი ც.

საქართველოს მეცნიერებათა აკადემიის ბოტანიკის ინსტიტუტი

(მიღებულია 07.10.2004)

რეზიუმე

სტატიაში მოცემულია ლაგოდეხის სახელმწიფო ნაკრძალის ფართოფოთლოვანი ტყის 25 ძირითად მერქნიან სახეობაზე დამახასიათებელი მიკრომიცეტების, ლიქენებისა და ხავსების გავრცელების კანონზომიერება ზღვის დონიდან სიმაღლეების გათვალისწინებით.

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Cell Biology

SDS INCUBATION TIME DEPENDENT CHANGES OF SEVERAL FUNCTIONAL CHARACTERISTICS OF *11$^{5(1)}$ Y NOCARDIOPHSIS DESSONVILLEY* CELLS

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(Received December 20, 2004)

Abstract

Incubation time dependent effect of detergents (SDS) different concentrations on *11$^{5(1)}$ y Nocardiophsis dessonvillei* viable cells amount, exogenous protein concentration and peroxidation intensity has been investigated. To develop the ways for regulation of exogenous active metabolite content in culture medium along with practical implementation of obtained data, minimal concentration of detergent and optimal incubation time has been established.

Keywords: peroxidation, biodestruction, microorganisms , SDS

Introduction

The ways of using of microorganisms in biodestruction processes is based on their ability to grow on a wide variety of materials or their decomposition products. Characterization of microbial communities and their interaction with various materials or compounds is important for understanding the mechanisms of biodestruction processes [Krilenkov V. et al., 2003, Sokolov B., Ilichov V.1988]. Microbial metabolites such as organic acids, enzymes, active forms of oxygen, nitric oxide, along with mechanical damage, cause considerable changes of physical-chemical and other parameters of materials. Among microbial metabolites the products of oxidative reactions are of great interest. It's established that a high level of these metabolites determine the activity of various strains of microorganisms. Furthermore, various physical and chemical factors cause considerable changes of cell permeability, leading to the disorder of metabolic and functional activity in turn [Dobrecov, 1981]. Causing considerable structural changes of microorganisms membranes, detergents serve as tools for investigation of microbial cells several functional characteristics. The goal of our research was to define metabolic reactions of certain microorganisms. We will discuss dynamic of influence of sodium dodecylsulphate (SDS) different concentrations on cell viability, exogenous protein concentration, activity of acid phosphatase and peroxidation reactions intensity in culture medium of *11$^{5(1)}$ y Nocardiophsis dessonvilley* in order to establish optimal incubation time and minimal concentration of detergent leading to considerable decrease of exogenous protein concentration and viable cell amount.

Materials and Methods

Microorganisms of the collection of N. Ketskhoveli Institute of Botany of Georgian Academy of Sciences were used. Based on our previous experimental data, 9th day old *11$^{5(I)}$ y Nocardiophsis dessonvillei* cells were employed and SDS as detergent was selected in concentrations 10^{-2}M, 10^{-5}M, 10^{-7}M [Gordeziani et al., 2002]. Culture was grown on the medium described by Krassilnikov [Krassilnikov, 1950]. Cells were incubated with SDS for 10, 20, 30 min and all parameters were measured after each incubation period. The quantity of viable cells was calculated according to number of colonies grown on solid medium. Protein concentration was measured by Lowry [Lowry et al., 1951]. Activity of acid phosphatase was assessed quantitatively (amount of inorganic phosphorus split off substrate) [De Duve et al., 1955]. Peroxidation intensity was measured using the production of malonic dialdehyde (MDA) as an indicator for peroxidation intensity [Tong Mak et al.,1983]. Quantity of viable cells, protein concentration, activity of acid phosphatase and concentration of MDA is given in percentage with respect to control (100%). Statistically refined experimental data are given in figures.

Results and Discussion

Effect of various concentrations of SDS on *11$^{5(I)}$ y Nocardiophsis dessonvillei* viable cell amount at different incubation time is given on Fig.1 showing that the SDS highest concentration (10^{-2} M) kills of almost all number of original cells during each incubation period.

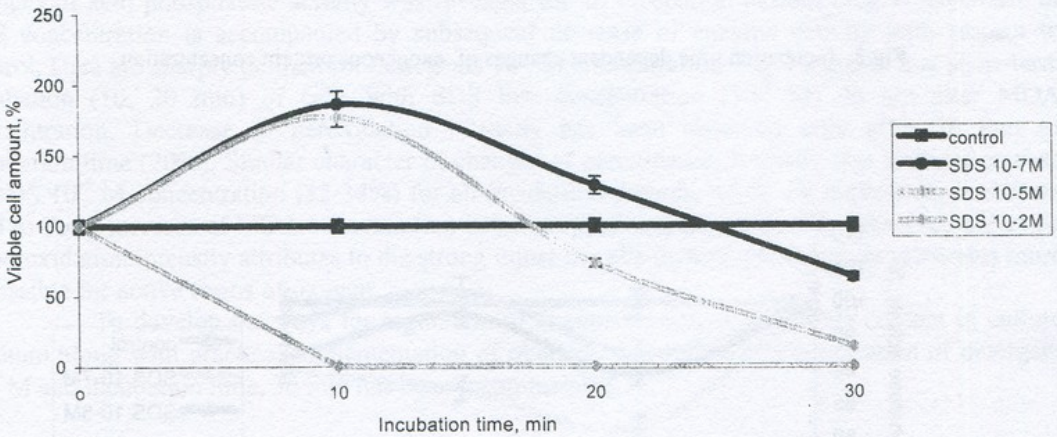

Fig.1. Incubation time dependent changes of viable cell amount.

10 min incubation with SDS 10^{-5} M concentration caused slight stimulation of cells, while sharp decrease of viable cell amount was observed in case of 20 and 30 min incubation - 26.7% and 85.9% respectively. Short-time incubation with SDS minimal employed concentration (10^{-7} M) also revealed stimulation effect on cells and only at 30 min incubation viable cells amount decreased by approximately 68% with respect to control. Fig.2 illustrates incubation time dependent changes of exogenous protein concentration with SDS selected concentrations. Represented data indicate negligible alterations in the exogenous protein concentration after 10 and

20min incubation of *II*$^{5(l)}$ *y Nocardiophsis dessonvillei* cells with given concentrations of SDS. Considerable decrease (44%) of exogenous protein concentration was detected only after 30 min incubation. The result is sharply defined at SDS 10^{-7} M concentration. Obtained data are in compliance with SDS induced alterations of viable cell amount, i.e. decrease of viable cell amount is accompanied by the decrease of exogenous protein quantity in culture medium. According to literature data [Garavito, Ferguson-Miller, 2001] SDS comprises high micelle formation factor, although in case of observed exceeded protein concentration micelle formation seems less efficient.

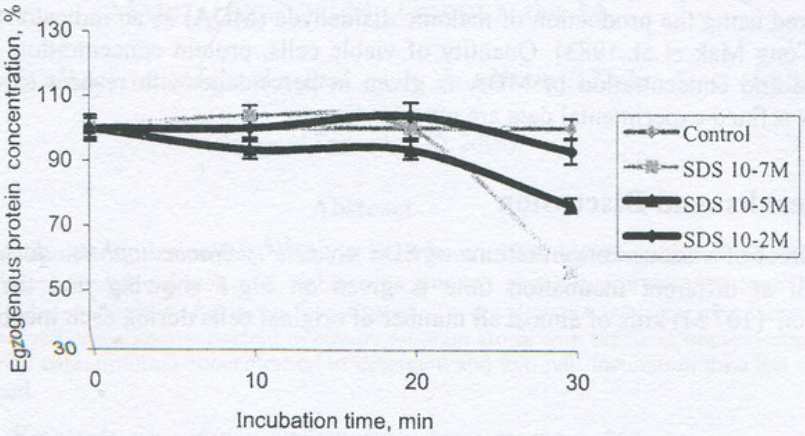

Fig.2. Incubation time dependent changes of exogenous protein concentration

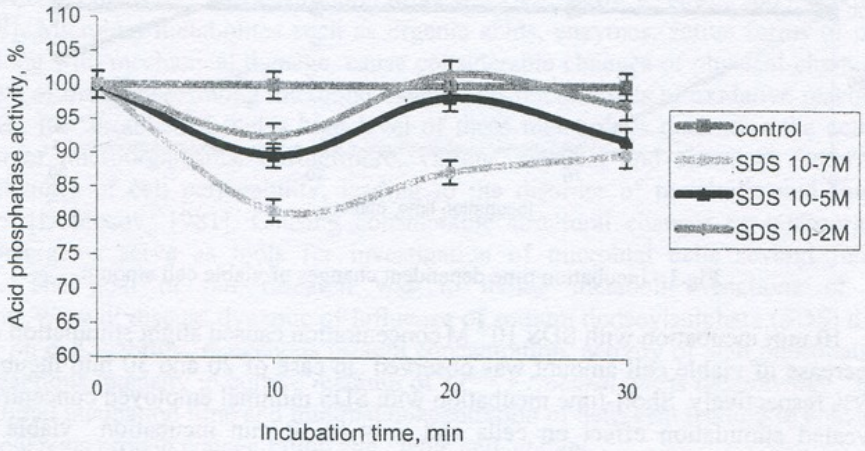

Fig.3. Incubation time dependent changes of acid phosphatase activity

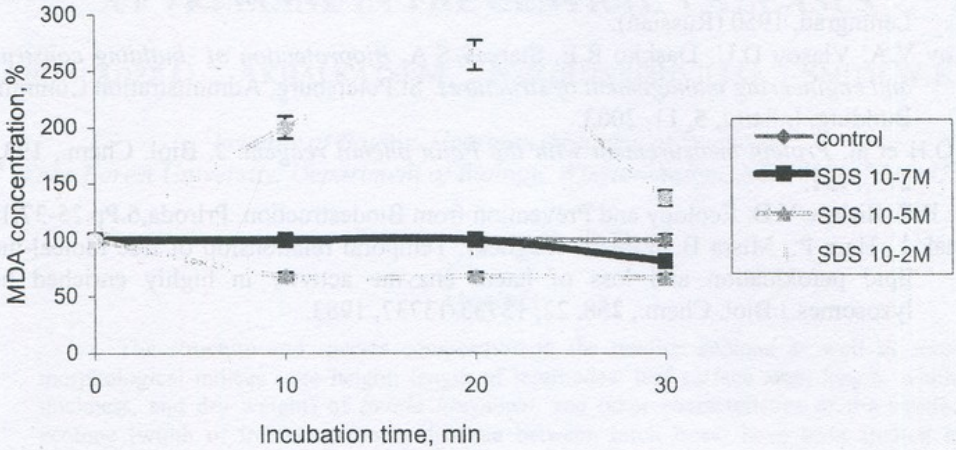

Fig.4. Incubation time dependent changes of MDA concentration

Incubation time dependent changes of acid phosphatase activity as well as peroxidation intensity are represented on Fig. 3 and Fig. 4 respectively. Detergent concentration dependent changes of acid phosphatase activity was revealed for all incubation periods (Fig.3). Decrease of SDS concentration is accompanied by subsequent decrease of enzyme activity with respect to control. Data are sharply defined for detergents 10^{-7} M concentration. Fig.4 indicate that short-term incubation (10, 20 min) of cells with SDS low concentration (10^{-7} M) do not alter MDA concentration. Decrease of peroxidation intensity has been observed only after 30 min of incubation time (20%). Similar character of changes of peroxidation intensity was revealed in case of SDS 10^{-5} M concentration (32-34%) for all incubation periods, while the highest concentration lead to sharp increase of MDA concentration in culture medium, assuming that observed elevation of peroxidation intensity attributes to the strong injure of cells making peroxidation substrates more accessible for active forms of oxygen.

To develop the ways for regulation of exogenous active metabolite content in culture medium along with practical implementation of obtained data, optimal concentration of detergent 10^{-7} M and incubation time, 30 min has been recommended.

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SDS ინკუბაციის დროზე დამოკიდებული 11[5(1)] *y Nocardiophsis dessonvillei*-ის უჯრედების ზოგიერთი ფუნქციონალური მახასიათებლის ცვლილება

ათანელიშვილი ი., ბოჭორიშვილი ნ., ჯიმშიტაშვილი ნ., ტაბატაძე მ., მარდალეიშვილი მ., გორდეზიანი მ.

უჯრედული და მოლეკულური ბიოლოგიის კათედრა, ივ.ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 20.12.2004)

რეზიუმე

შესწავლილია 11[5(1)] *y Nocardiophsis dessonvillei*-ის უჯრედებზე ნატრიუმის დოდეცილსულფატის განსხვავებული კონცენტრაციების მოქმედების დინამიკა. დადგენილია შტამის დამაზიანებელი აქტივობის შემცირებისათვის აუცილებელი დეტერგენტის მინიმალური კონცენტრაცია და უჯრედებზე ზემოქმედების ოპტიმალური დრო.

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Ecology

SOME MORPHOLOGICAL FEATURES OF *BETULA LITWINOWII* AT TREELINE IN THE CENTRAL CAUCASUS

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Abstract

The structure and species composition in the treeline ecotone as well as some morphological indices (tree height; length of internodes; leaf surface area, length, width, thickness, and dry weight) of *Betula litwinowii*, and other characteristics of the treeline ecotone (width of treeline ecotone, distance between birch trees) have been studied in Kazbegi region (the east part of the Central Caucasus). The studies were conducted in the natural (2498-2512 m a. s. l.) and pseudo (caused by the relief configuration) timberlines and treeline ecotones (2155-2164 and 2200-2275 m a. s. l.). The species composition (list of 45 species – trees, shrubs, herbaceous plants) of the natural untouched treeline ecotone is presented. The structure of the natural and pseudo timberlines are very similar. They are represented by 2-3 m tall crook-stem trees. In the treeline ecotone only dwarf birch trees (35-40 cm tall) are scattered exclusively in depressions and eroded ditches on a dense-tussock grass meadow. In the treeline ecotone near the tree species line all the studied morphological indices (except leaf thickness) were significantly decreased compared to the timberline. This regularity was clearly observed in both the natural and pseudo treeline ecotones. It means, that these changes are determined not only by the altitudinal location of the timberline and, accordingly by local climatic conditions, but possibly also other ecophysiological reasons, which strongly limit vertical distribution of the timberline in the east part of the Central Caucasus above 2500 m.

Kay words: *Betula*, Caucasus, timberline, treeline ecotone, tree species line

Introduction

The high altitude limit of forest vegetation is particularly interesting for various aspects of plant life: functional type, adaptation mechanisms, tree seedling establishment, growth limitation, interspecific facilitation and competition, timberline migration, species and biotope diversity, etc. The global climate change phenomenon has especially increased this interest, because human impact on the background of the global warming can have dramatic consequences for life conditions in such a sensitive alpine ecotone as the treeline [Grace, 1989; Grabherr, 1997; Körner, 1998; 2002; Gansert et al., 2002; Smith et al., 2003; Johnson et al., 2004].

The timberline is the name of a nature phenomenon, which forms the most noticeable and important altitudinal boundary in the temperate mountains. The timberline or forest line is a line, above which groups of trees taller than 3m do not occur, i.e. this is the upper limit of a closed mountain forest. The tree species line is a line, beyond which no adult tree species (including

prostrate ones or scrubs) occur (i.e. above this line the treeless alpine zone begins). The treeline ecotone is the transition zone between the timberline and tree species line. There are various reasons for the timberline formation: solar radiation, scarcity of heat and water, overbalance of water, influence of winds, properties of substrate/soil, snow cover, avalanches, mud-streams, relief form, exposition, slope inclination, etc. The ecological importance of the timberline is immense: this rather narrow ecotone is distinguished by high diversity of species and biotopes and regulates ecological balance, particularly, it protects lowlands from avalanches, landslides, mud-flows and debris-flows, and what is especially important, it creates a natural reservoir of fresh water [Körner, 2003; Smith et al., 2003; Burga et al., 2004; Johnson et al., 2004].

In the Central Caucasus the timberline is represented by broadleaved subalpine forest dominated by *Betula litwinowii*. Subalpine forests are located exclusively on the north-facing slopes (in places only single and degraded birch trees are present on the south-facing slopes) with high inclination (> $15\text{-}20^0$), and at the highest distribution limit (2400-2500m a. s. l.) they are mixed with evergreen *Rhododendron caucasicum* shrubbery, which separates crook-stem birch forests and dense-tussock grass meadows and forms 2-3 m tall and usually 7-9 m wide upper timberline, below which 15-18 m tall birch forest is continued. The development of the crook-stem form of *B. litwinowii* is caused by weight of snow covering trees in winter. Such a life form helps woody plants to adjust to severe winter conditions [Dolukhanov, 1978; Nakhutsrishvili, 2004].

The timberline vegetation in the studied region is strongly degraded and descended (on average by 200-400 m) because of long-term human impact: overgrazing, deforestation, etc. [Nakhutsrishvili; 1999]. The treeline ecotone in the Central Caucasus is characterized by a high level of plant diversity and endemism (Kharadze, 1948; Gagnidze, 1974; Sakhokia, Khutsishvili, 1975; Dolukhanov, 1978), as well as by high diversity of biotopes [Abdaladze et al., 2005; Nakhutsrishvili et al., 2005].

Various phytoecological studies of the timberline vegetation in Kazbegi region began two years ago. The first step was to characterize timberline vegetation and biotopes [Nakhutsrishvili et al., 2004a; b; 2005, etc.]. The second step was to characterize some mechanisms (low-temperature photoinhibition and interspesific facilitation) of the timberline stability and reveal the current global warming effect on birch seedling establishment in the treeline ecotone [Akhalkatsi et al., 2004a; b].

The present work is considered in the mentioned context. The objectives of this study were to characterize the treeline ecotone structure and some morphological peculiarities of *B. litwinowii* in the natural and pseudo (caused by relief configuration) timberlines and treeline ecotones in Kazbegi region (the east part of the Central Caucasus).

Materials and Methods

Study Area

The studies have been carried out in Kazbegi region, which is situated on the eastern boundary of the central part of the Greater Caucasus, on its north-facing exposure macro slope.

The climate between 1900 and 2600 m a. s. l. is cool-temperate with annual mean air temperature 4.9°C. Winter is relatively dry and cold and summer is short and more or less cool. The mean temperature of the warmest months (July, August) ranges from 10°C to 14°C (the extreme of heat is 33°C). The mean temperature of the coldest month (January) is -11°C (the extreme of cold is –32.5°C). The number of days with freezing temperatures is 124 per year. Stable snow cover persists for 5-7 months and reaches its maximum depth (115-120 cm) in March. The average annual precipitation is 1000-1200 mm with its peak in May-June, and the summarized precipitation during May-August is about 100 mm. The mean air humidity in summer is 75%. In

this zone, especially in summer fog is frequent (135 foggy days per year). Mountain-gorge winds prevail. The duration of the growing season is 6 months. The mountain-forest peat brown soils of middle depth are predominant [Nakhutsrishvili, 2003].

The flora of Kazbegi region numbers approximately 1100 species of vascular plants [Sakhokia, Khutsishvili, 1975]. The species number in the subalpine zone (1850-2450 m a. s. l. and 245.75 km^2) is 595, from which 198 or 33.2% are endemics to the Caucasus. The total area covered by forest is 8707 ha. About 70% of the total forest area is the subalpine forest, dominated by *B. litwinowii*.

Study Sites

In the Central Caucasus, particularly in the Kazbegi region, the natural timberline is located between 2450 and 2500 m a. s. l. on humid north-facing slopes of various inclination (10-70^0), and with stable and deep snow cover. Its upper distribution limit does not exceed the level of the 11°C-isotherm of August, while in the areas where it is unprotected by snow coat, this level is equal to the 9.5°C-isotherm [Nakhutsrishvili, 2003].

The studies were carried out in different sites situated along the macro transect between village Gergeti (1800 m a. s. l.) and Mt. Mkinvartsveri or Kazbegi (5033 m a. s. l.). Six study sites have been selected and marked (Tab.1). The study plots averaged about 100 m^2 at each site.

Table 1. Characteristics of study sites

Character	Site 1a	Site 1b	Site 2	Site 3a	Site 3b	Site 4
Coordinates	N42°40'01,0" E44°35'49.5"	N42°40'02,0" E44°35'49.4"	N42°39'55,2" E44°36'40.9"	N42°39'56,0" E44°37'04.4"	N42°39'56,9" E44°37'05.7"	N42°40'02,7" E44°37'09.6"
Elevation (m a. s. l.)	2512	2498	2248	2164	2155	2072
Slope exposition (°)	N 12	N 6	N 19	N 22	N 21	N 26
Slope inclination (°)	27 – 28	30 - 34	29 - 30	8 - 9	18 - 20	34 - 35
Plant cover (%)	95	90 - 95	90 - 95	90	85 - 90	70 - 75

The sites 1a and 1b are located at the natural untouched timberline. In site 1a dwarf birch trees are scattered exclusively in depressions and eroded ditches on a dense-tussock grass meadow dominated by *Carex tristis*. Very rarely only single birch trees occur on south-facing slopes in some depressions; however, they always stay dwarf (30-40cm tall). The site 1a represents the natural treeline ecotone (the transition zone between the closed forest and treeless alpine zone) which is very close to the tree species line. The site 1b is a humid and wind sheltered habitat, which is situated somewhat below and north of the site 1a. The site 1b represents the natural timberline (or the forest line). The site 2 is a moderately humid and wind sheltered habitat. Here the timberline is located at 2200 m, above which north-facing slope is expanding to the ridge of 2275 m. This site represents the pseudo treeline ecotone. The site 3a is situated between the pseudo timberline (which is formed owing to a special relief configuration) and a wide plane area covered by a subalpine dense-tussock grass meadow (*Bromopsietum*), developed on the polygonal type of relief with many protuberances and depressions. This is moderately humid and wind exposed habitat. In this site

only dwarf birch trees are present exclusively in the relief depressions, which lack herbaceous vegetation. This is also the pseudo treeline ecotone. The site 3b represents the lower pseudo timberline, which is formed owing to the relief character (a steep north-facing slope is directly followed by a wide plane area). This is also a moderately humid and wind exposed habitat. The site 4 is situated within the subalpine birch forest. This site represents the most optimal ecological conditions for birch trees to grow and develop.

Methods

Co-ordinates and main geographic directions of the study sites were measured using a GPS (*Etrex* Summit, Garmin, Switzerland). The slope inclination was determined by a compass-clinometer (*Recta DP 6*, Switzerland). The distance between the tree species line (the last elfin birch tree) and timberline (i.e. the width of the treeline ecotone), as well as the following indices: distance between trees, tree height, leaf length, leaf width and distance between birch tree internodes were measured using a tape-line. The one side leaf surface area was calculated by a plan meter. The leaf thickness was measured using a micrometer. The leaf dry weight was determined by torsion balance (*WAGA Torsyjna-WT*, Poland). The age of young trees was determined by ring count and the width of the stem and twigs.

The mean values and standard deviations were calculated for every data set. The statistical differences between mean values were determined using paired one-tailed Students t-test ($P < 0.05$).

Results and Discussion

In the study sites the natural timberline is composed by the following characteristic tree species: *Betula litwinowii, B. raddeana, Salix caprea, S. kazbekensis, Sorbus caucasigena* and shrubs: *Daphne glomerata, Empetrum caucasicum, E. hermaphroditum, Rhododendron caucasicum, Rubus saxatilis, Vaccinium myrtillus, V. vitis-idaea*. The following herbaceous plants are recorded both in the timberline and treeline ecotone: *Aconitum nasutum, Agrostis planifolia, Alchemilla oxysepala, Anemone fasciculata, Astrantia ossica, Betonica macrantha, Calamagrostis arundinacea, Campanula latifolia, Carex echinata, Centaurea salicifolia, Cephalanthera longifolia, Cephalaria gigantea, Cerastium hemschinicum, Cicerbita racemosa, Coeloglossum viride, Dolichorrhiza caucasica, D. renifolia, Gentiana schistocalyx, G. septemfida, Geranium silvaticum, Gymnadenia conopsea, Helictotrichon pubescens, Leontodon danubialis, Pedicularis condensata, Poa alpina, Pyrola minor, P. rotundifolia, Ranunculus caucasicus, Senecio caucasigenus, Swertia iberica, Trifolium ambiguum, Veratrum lobelianum*, etc. The high diversity of the species composition of the timberline and treeline ecotone is determined by various ecological factors and peculiar structural properties of the vegetation cover [Dolukhanov, 1978; Nakhutsrishvili, 2004]. In the timberline maximum 29 species (trees, shrubs, herbaceous plants) are found; in *Rhododendron* shrubbery – 38; and in tall herb vegetation – 9 [Nakhutsrishvili et al., 2005]. As it is seen from the above presented list, in the treeline ecotone 44 species were recorded. This comparatively high number is determined by the presence of meadow plants in the treeline ecotone.

On the relatively small area of the treeline ecotone the biotope diversity is also high. More than 15 typical biotopes are found and described [Abdaladze et al., 2005; Nakhutsrishvili et al., 2005]. The ecological state of the timberline can be assessed as normal only on certain massifs. These forests have been protected because of their religious significance; they are called "Holy Forests". According to the degree of naturalness of these fragments, they should be referred to the

first level of hemeroby: natural and close to natural [Nakhutsrishvili et al., 2004a]. Therefore, the treeline ecotone (including elfin birch forests, *Rhododendron* shrubbery and tall herbaceous vegetation) has been referred to the priority habitat type [Nakhutsrishvili et al., 2005].

The natural timberline is determined by an altitudinal limit at 2450-2500 m a. s. l. However, at lower elevations, pseudo timberlines are formed along the ridge; they are formed under the influence of the relief forms: the north-facing slope is broken at the ridge and transformed into plateau and somewhere – directly into south-facing slope. The structure of the natural and pseudo timberlines is very similar. They are represented by 2-3 m tall crook-stem trees having several stems branched already at the soil surface level.

Table 2. Mean values and standard deviation of morphological indices of *Betula litwinowii* at various altitudes (n is number of measurements; in the all data set $p \leq 0{,}001$)

Indices	Site 1a 2512 m	Site 1b 2498 m	Site 2 2248 m	Site 3a 2164 m	Site 3b 2155 m	Site 4 2072 m
Width of treeline ecotone (m)	14.0±3.4 (n = 30)		71.1±15.4 (n = 30)	40.55±12.6 (n = 29)		–
Distance between trees (m)	4.42±1.66 (n = 30)	0.81±0.40 (n = 30)	0.47±0.31 (n = 34)	0.74±0.30 (n = 31)	0.96±0.44 (n = 44)	2.48±1.13 (n = 30)
Tree height (m)	0.35±0.66 (n = 30)	2.41±0.52 (n = 30)	0.39±0.14 (n = 30)	0.40±0.18 (n = 30)	2.65±0.75 (n = 40)	14.20±1.84 (n = 30)
Length of internodes (cm)	2.21±0.90 (n = 30)	10.2±5.8 (n = 44)	4.07±2.3 (n = 66)	3.58±2.4 (n = 43)	9.93±6.0 (n = 54)	–
Leaf surface area (dm^2)	0.077±0.031 (n = 30)	0.097±0.019 (n = 30)	0.116±0.024 (n = 30)	0.100±0.028 (n = 52)	0.170±0.07 (n = 64)	0.183±0.04 (n = 55)
Leaf length (cm)	3.49±0.54 (n = 30)	4.25±0.43 (n = 30)	4.12±0.50 (n = 30)	4.46±0.85 (n = 45)	5.32±1.09 (n = 64)	5.46±0.92 (n = 85)
Leaf width (cm)	3.01±0.64 (n = 30)	3.49±0.40 (n = 30)	3.67±0.43 (n = 30)	3.57±0.76 (n = 42)	4.31±1.05 (n = 64)	4.40±0.82 (n = 85)
Leaf thickness (mm)	0.523±0.003 (n = 30)	0.530±0.003 (n = 30)	0.525±0.005 (n = 30)	0.528±0.006 (n = 30)	0.531±0.008 (n = 30)	0.531±0.005 (n = 88)
Leaf dry weight (mg)	0.051±0.022 (n = 30)	0.080±0.017 (n = 31)	0.075±0.015 (n = 30)	0.066±0.026 (n = 29)	0.096±0.04 (n = 30)	0.096±0.032 (n = 55)

As it is shown in the Tab. 2, in the treeline ecotone near the tree species line the length of the internodes of birch trees was significantly decreased as compared to the timberline. This regularity was pronounced clearly in both the natural (1a and 1b) and pseudo (3a and 3b) timberline habitats. Particularly in the site 1a this index was reduced 4.6 times (by 78%) compared with the site 1b, and in the site 3a 2.8 times (by 64%) compared with the site 3b. In the treeline ecotone of the site 2, where environmental conditions, determined by relief forms and exposition, are much better for the birch tree development and distribution (see the site characteristics), this index was rather higher than in the sites 1a and 3a. In the both natural and pseudo timberline habitats (sites 1b and 3b) examined values were approximately the same.

More or less the same regularities are found for the other studied indices. Leaf surface area in the natural treeline ecotone (site 1a) as compared to the natural timberline (site 1b), is decreased by 21%. Leaf length and leaf width of *B. litwinowii* are also strongly decreased by 18% and 14%, respectively. The most significant change was found in the leaf dry weight index, which is decreased by 36% in the treeline ecotone (site 1a). The same regularities were observed in the pseudo treeline ecotone and timberline. For example, in the site 3a compared to the site 3b, leaf

surface area was reduced by 41%, leaf length – by 16%, leaf width – by 17%, and leaf dry weight – by 31%.

Tree height is also important morphological integral index. As it is seen from obtained data (Tab. 2), this index is strongly reduced (6.6-6.9 times) in the natural treeline ecotone (site 1a), as well as the pseudo treeline ecotone (site 3a), compared to the natural (1b) and pseudo (3b) timberline habitats.

Thus, the results of this study have demonstrated that significant reductions in *B. litwinowii* leaf morphological indices are observed in both (the natural and pseudo) treeline ecotones compared to the timberlines. It means, that these changes are not determined by the altitudinal location of the timberline (along the studied transect the altitudinal difference between the natural and pseudo timberline is ~ 340-350 m).

Only such index as leaf thickness revealed stable character in all studied habitats: no significant changes were observed in the treeline ecotone compared to the timberline. Even in the site 4, which is the optimal habitat for birch tree growing and development, this index was not significantly different than in the other studied plots, including the natural (site 1b) and pseudo (site 3b) timberline sites. Thus, in *B. litwinowii* leaf thickness can be considered as the most conservative morphological index, which does not depend on such important factors as forest structure (real forest, timberline, tree species line), tree age (young – 8-10 years and adult – elder than 20-25 years trees), altitudinal and topographic locations (correspondingly, different ecological conditions).

Such index as distance between trees shows the influence of the ecological conditions on the forest canopy. Distance between trees must be increased in more severe climatic or anthropogenic conditions as well as in the areas with a high level of interspecific competition. Such a situation is observed in the treeline ecotone (site 1a). In this plot the distance between trees is 6-9 times as high as in lower situated pseudo treeline ecotones (site 2 and site 3b) (see also Tab. 2).

In the Tab. 3 the correlation interactions between altitudinal location and some indices of treeline ecotone structure are given. As it is seen, the highest negative correlation between altitude and tree height was revealed. The strong positive correlation between altitude and such index as distance between trees was also observed. It was found out that negative degree of correlation between altitude and width of the treeline ecotone is very small, which indicates that altitude is not the only factor determining the width of the treeline ecotone.

Table 3. Correlation interactions between some indices of treeline ecotone structure and its altitudinal location

Correlation pair	Coefficient of correlation (r)	Coefficient of determination (r^2)
Width of treeline ecotone – altitude	- 0.68	- 0.46
Distance between trees – altitude	0.95	0.90
Tree height – altitude	- 0.99	- 0.98

Our study has shown that the treeline ecotone, which is represented by dwarf birch trees, has rather different width depending on its altitudinal and topographic location (Tab. 2). The natural treeline ecotone (site 1a) is the narrowest from all the investigated treeline ecotones (Tab. 2). The main factors which determines differences between the upper natural treeline ecotone and lower pseudo treeline ones, must be local climatic conditions, which are unfavorable for birch tree and strongly limit its vertical migration above 2500 m a. s. l.

At the same time, main differences between the lower pseudo treeline ecotones (sites 2 and 3a) are the slope inclination and relief form. The steep slope in the site 2 is covered by elfin birch trees according to the distributional pattern of the birch forest growing on north-facing slopes. However, this slope was completely devoid of trees even ten years ago. And, only for the recent years, natural reforestation has been observed in this area. The age of the small and dwarf trees occurring on the slope do not exceed 10 years. In this site formation of a new subalpine forest and a new timberline at a higher altitude is clearly observed. It is rather important, because timberline ascent to higher altitudes is dependent on the new seedling establishment in the treeline ecotone [Smith et al., 2003; Johnson et al., 2004].

In conclusion it might be noted that: 1) In the treeline ecotone near the tree species line all the studied morphological indices (except leaf thickness) were significantly decreased as compared to the timberline; 2) This regularity was clearly observed in both the natural and pseudo treeline ecotones. It means, that these changes are determined not only by the altitudinal location of the timberline and, accordingly by the local climatic conditions, but possibly also other ecophysiological reasons, which strongly limit vertical distribution of the timberline in the east part of the Central Greater Caucasus above 2500 m.

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Betula litwinowii-ის ზოგიერთი მორფოლოგიური ნიშანი ცენტრალური კავკასიონის ტყის ზედა საზღვარზე

აბდალაძე ო.[1], ახალკაცი მ.[1], ნახუცრიშვილი გ.[1], სმიტი ვ.[2]

საქართველოს მეცნიერებათა აკადემიის ნ.კეცხოველის სახ. ბოტანიკის ინსტიტუტი
ბიოლოგიის განყოფილება, კეიკ ფორესტ უნივერსიტეტი, აშშ

(მიღებულია 10.01.2005)

რეზიუმე

ყაზბეგის რეგიონში (ცენტრალური კავკასიონის აღმოსავლეთი ნაწილი) შესწავლილია ტყის ზედა საზღვრის ეკოტონის სტრუქტურა (ეკოტონის სიგანე, ხეებს შორის

მანძილი) და სახეობრივი შემადგენლობა, აგრეთვე *Betula litwinowii*-ის ზოგიერთი მორფოლოგიური მაჩვენებელი (ხის სიმაღლე; მუხლებს შორის მანძილი; ფოთლის ზედაპირის ფართობი, სიგრძე, სიგანე, სისქე და მშრალი წონა). გამოკვლევები ტარდებოდა სოფ. გერგეტი-მყინვარწვერის მაკროტრანსექტზე ბუნებრივ (2498-2512 მ. ზღვის დონიდან) და ფსევდო ტყის ზედა საზღვრებზე და ტყის ზედა საზღვრის ეკოტონებში (2155-2164 და 2200-2275 მ. ზღვის დონიდან). ბუნებრივ და ხელუხლებელი ტყის ზედა საზღვრის ეკოტონში აღწერილია 45 სახეობა (ხეები, ბუჩქები, ბალახოვანი მცენარეები). ბუნებრივი და ფსევდო ტყის ზედა საზღვრის სტრუქტურა ერთმანეთის მსგავსია. ისინი წარმოდგენილია 2-3 მ. სიმაღლის ტანბრეცილი ხეებით. ტყის ზედა საზღვრის ეკოტონში მხოლოდ ჯუჯა (35-40 სმ სიმაღლის) არყის ხეებს ვხვდებით, რომლებიც განვითარებულია მკვრივკორდიანი მდელოს მხოლოდ ეროდირებულ და სხვა მცენარეულობას მოკლებულ ჩაღრმავებებში. ტყის ზედა საზღვართან შედარებით, ტყის ზედა საზღვრის ეკოტონში ჯუჯა ხეების გავრცელების ზედა ზღვართან ახლოს არყის შესწავლილი მორფოლოგიური მაჩვენებლები (გარდა ფოთლის სისქისა) მნიშვნელოვნად დაქვეითებულია. ასეთი კანონზომიერება ნათლად იკვეთება როგორც ბუნებრივ, ასევე ფსევდო ტყის ზედა საზღვარზე. ეს კი ნიშნავს, რომ მორფოლოგიური ცვლილებები განპირობებულია არა მხოლოდ სიმაღლითი სხვაობებით (შესაბამისად, ლოკალური კლიმატური პირობების განსხვავებით), არამედ აგრეთვე ეკოფიზიოლოგიური მიზეზებითაც, რაც ცენტრალური კავკასიონის აღმოსავლეთ ნაწილში მკვეთრად ზღუდავს ტყის ზედა საზღვრის ვერტიკალურ გავრცელებას ზღვის დონიდან 2500 მ. ზემოთ.

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Genetics

STRUCTURAL AND QUANTITATIVE DISORDERS OF CHROMOSOMES OF THE INDIVIDUALS CONTACTED WITH FERROMANGANESE AND SILICOMANGANESE

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Abstract

The cytogenetical studies of the individuals being in contact with ferromanganese and silicomanganese revealed that in their blood cells the frequency of structural and quantitative chromosomal disorders are rather high and vary from 4% to 24% (control index – 1.6±0.4). From chromosomal aberrations occur: single and pair fragments, chromatid and chromosomal translocations, premature separations of centromeres, gaps. Percentage indices of aneuploidy cells (13%-24%) are higher compared to control indices.

Key words: aberration, single fragment, pair fragment, chromatid translocation, chromosomal translocation.

Introduction

Deterioration of ecological situation negatively influences on the human health. To estimate mutagenic effect of the environmental factors it's necessary to carry out genetical monitoring, and first of all of the people leaving in ecologically unsuitable regions or being in contact with harmful chemical and physical factors [Bochkov, 1981]

The studies carried out in industrial towns (Angarski, Chita, Usiol-Sibirsk) of the Baikal Region, Russia, where the parameter of atmosphere pollution is high, children health state is alarming. The amount of the individuals with different chromosomal disorders is 7,9% [Leshchenko et al., 1999].

The complex approach which involves registration of unreliable outcome of pregnancy (spontaneous abortions, prenatal mortality, congenital anomalies) and cytogenetical studies are used for genetical monitoring [Bochkov, Chebotarev, 1989].

The goal of our research was to study structural and quantitative disorders of chromosomes of the individuals being contacted with manganese and silicon compounds, hence they have mutagenic effect.

Materials and Methods

21 clinically healthy workers of Zestaponi non-ferrous factory were examined. We analyzed peripheral blood lymphocytes. They were cultivated using Moorhead's standard method.

The preparations were stained by Gimza-Romanovsky method [Bakton, Evans, 1975]. The results statistically were estimated by Student's method.

Results and Discussion

To determine structural and quantitative disorders of chromosomes 2040 metaphases of 21 individuals were analyzed.

In the studied individuals the frequency of aberrant cells was high and varies from 4% to 24%, which is for sure higher than control index (1.6±0.4). The individuals were grouped by the means of aberrations percentage indices.

In the high frequency group are included 11 individuals. In their cultures number of aberrant metaphases varied from 16% to 24%. in the medium frequency group (8 individuals) metaphases with aberrations varied 8-13%, and in the low frequency group (2 ind.) – 4-6%. The changes are presented on the Fig.1.

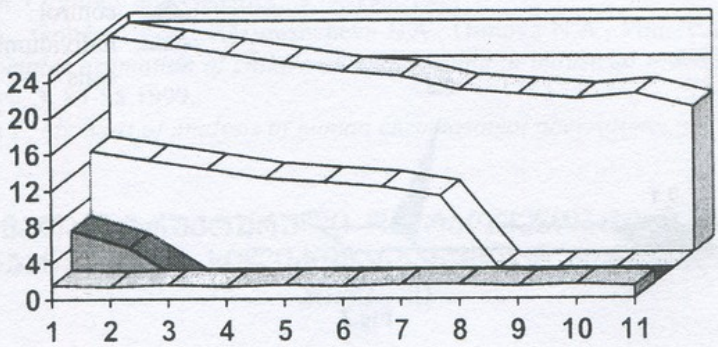

Fig.1. a – The group of high frequency (11 ind.), c – The group of low frequency (2 ind.), b – The group of medium frequency (8 ind.), d – control

In the studied metaphases chromosomal aberrations occur both in single and pair fragments. Chromatid and chromosomal translocations, premature separations of centromeres were revealed. The metaphases with achromatic gaps which frequency is rather higher than control indices were registered (Table).

The analyses of quantitative indices of chromosomes have shown that only in 5 individuals from 21 the high percentage index of aneuploidy cells were occurred (Fig.2). In all other cases it doesn't exceed the control index.

In metaphases of 12 individuals the polyploidy cells occur. Average frequency a little exceed the control (Fig. 3).

Obtained data indicate that in the blood cells of the workers of Zestaponi non-ferrous factory the structural and quantitative disorders frequency of chromosomes is rather high.

So, according to our studies it is necessary to pay proper attention to the genetical monitoring of the individuals working at such enterprises in order to avoid chromosomal disorders, as their frequency increases along with the rise of the influence of environmental pollution factors [Bochkov, 1977].

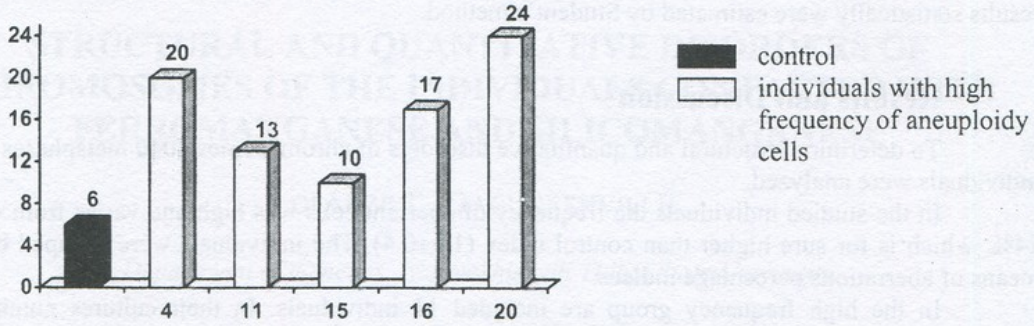

Fig.2.

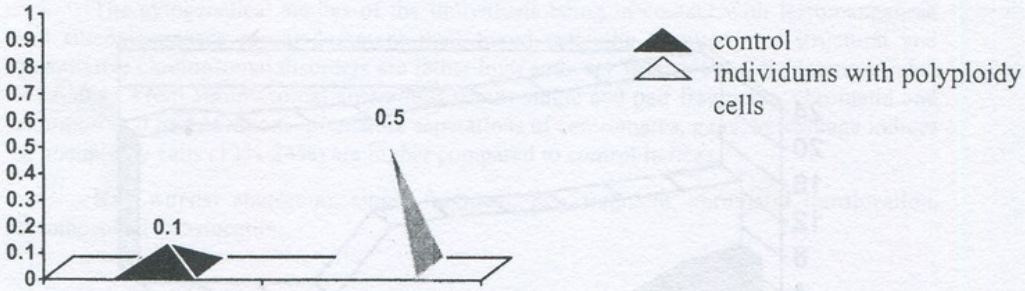

Fig.3.

Table. Structural disorders of chromosomes

Individual s#	Amount of analyzed metaphases	metaphases with aberrations (%±m)	*Structural disorders of chromosomes*				premature separations of centromers	gaps
			single fragments	pair fragments	translocation			
					chromatid	chromosomal		
1.	100	20,0±4,0	14±3,47	6±2,37	1,0±0,99	1,0±0,99	14±3,47	10±3,0
2.	100	24,0±4,27	7,0±2,55	21,0±4,07	-	2,0±1,4	5,0±2,18	13±3,36
3.	95	22,1±4,25	4,21±2,06	17,89±3,93	1,05±1,04	-	8,42±2,85	4,21±2,01
4.	100	21,0±4,07	15,0±3,57	2,0±1,4	6,0±2,37	-	17,0±3,75	7±2,55
5.	90	20,0±4,21	8,89±2,99	6,67±2,63	7,78±2,82	1,1±1,09	5,55±2,41	8,89±2,86
6.	100	19,0±3,92	6,0±2,37	4,0±1,96	10,0±3	1,0±0,99	4,0±1,96	3,0±1,71
7.	100	19,0±3,92	9,0±2,86	12,0±3,25	-	-	8,0±2,71	7±2,55
8.	95	17,9±3,92	6,32±2,49	9,47±3,01	1,05±1,04	3,16±1,79	8,42±2,85	15,79±3,66
9.	100	17,0±3,75	5,0±2,18	13,0±3,36	-	1,0±0,99	11,0±3,13	10±3,0
10.	60	16,67±4,8	10,0±3,81	6,67±3,22	1,67±1,65	-	3,33±2,32	4,21±2,01
11.	100	16,0±3,67	9,0±2,86	5,0±2,18	-	1,0±0,99	16,0±3,67	6±2,37
12	100	13,0±3,36	5,0±2,18	8,0±2,8	-	-	3,0±1,70	8±2,71

13	100	13,0±3,36	3,0±1,71	10,0±3,0	1,0±0,99	-	2,0±1,4	6±2,37
14	100	11,0±3,13	4,0±1,96	7,0±2,55	-	-	3,0±1,70	17±3,76
15	100	10,0±3,0	3,0±1,71	5,0±2,18	1,0±0,99	1,0±0,99	2,0±1,4	6±2,37
16	100	10,0±3,0	5,0±2,18	8,0±2,8	-	-	5,0±2,18	6±2,37
17	100	10,0±3,0	3,0±1,71	4,0±1,96	3,0±1,70	-	3,0±1,70	1±0,99
18	100	10,0±3,0	3,0±1,7	9,0±2,86	-	-	2,0±1,4	6±2,37
19	100	8,0±2,71	3,0±1,7	2,0±1,4	3,0±1,70	1,0±0,99	6,0±2,37	6±2,37
20.	100	6,0±2,37	2,0±1,4	3,0±1,70	1,0±0,99	-	3,0±1,70	-
21.	100	4,0±1,96	2,0±1,4	2,0±1,4	-	-	17,0±3,74	6±2,37
average	2040	14,56±0,78	5,98±0,52	7,69±0,59	1,76±0,29	0,58±0,17	6,57±0,55	7,65±0,61
control index 10 ind.	1000	1,6±0,4	1,1±0,32	0,3±0,17	0	0	0,2±0,14	3,01±0,56

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ქრომოსომათა სტრუქტურული და რაოდენობრივი დარღვევები ფერომანგანუმსა და სილიკომანგანუმთან კონტაქტის მქონე პირებში

ლოლაძე თ., დადუნაშვილი ე.

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(მიღებულია 22.12.2004)

რეზიუმე

ფერომანგანუმსა და სილიკომანგანუმთან უშუალო კონტაქტის მქონე პირების ციტოგენეტიკურმა კვლევამ გვიჩვენა, რომ მათი სისხლის უჯრედებში საკმაოდ მაღალია ქრომოსომთა სტრუქტურული და რაოდენობრივი დარღვევების სიხშირე და მერყეობს 4,0%-დან 24%-მდე (საკონტროლო მაჩვენებელი 1,6±0,4). ქრომოსომული აბერაციებიდან გვხვდება ერთეული და წყვილი ფრაგმენტები, ქრომოსომული და ქრომატიდული ტრანსლოკაციები, ცენტრომერთა ნაადრევი დაცილება, გეპები. საკონტროლო მაჩვენებელთან შედარებით, მაღალია ანეუპლოიდურ უჯრედთა პროცენტული მაჩვენებლები (13%-დან 24%-მდე).

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Genetics

POLYMORPHISM OF STRUCTURAL C-HETEROCHROMATIN IN PATIENTS AFFECTED WITH SENILE DEMENTIA

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Abstract

The variability of C-structural heterochromatin bands on chromosomes 1,9 and 16 in patients affected with senile dementia was investigated. The lymphocyte cultures derived from 10 individuals (aged - 70-90) affected with vascular dementia (VD - 5 patients) and with probable diagnosis on late-onset sporadic form of Alzheimer's disease (AD - 5 patients) have been studied. The heterogeneity of C-bands was determined for chromosomes 1,9 and 16. Generally in the first (VD) group the frequency of small a-variants of C-segments was increased for chromosomes 1,9 and 16 in all patients, while the frequency of large c-variants was decreased on chromosome 16 as compared with healthy elderly controls (aged - 70-90). It is notable that statistically significant increase in small a-variants was determined for all types of chromosomes (1,9 and 16) in the second (AD) group in comparison with control subjects. As for the large c-variants of C-segments, their frequency was increased on chromosomes 1 and 9, however on chromosome 16 it was reduced similar to the VD group.

Key words: Chromosome, heterochromatin, C-bands, dementia.

Introduction

Since the discovery of inherited dementias, their cytogenetic investigation has been intensively started [Petrozzi et al., 2002; Hedera et al., 2003]. However in spite of the numerous scientific data, it is not still possible to distinguish one approach or direction from the other because the experimental results vary within the vast range [Smith et al., 1983; Melargano et al., 1991; da Silva et al., 2000].

AD is an irreversible neurodegenerative disorder of the brain and eventually causes the death [Ritchie et al., 2002; Clark et al., 2003]. It is the most common case of dementia among people over age 65 [Katzman, 2004; Blanchard et al., 2004]. The key neuropathological hallmarks consist of the extracellular senile plaques and intracellular neurofibrillary tangles [Rachakonda et al., 2004]. Certain genetic mutations are identified for late-onset sporadic type of AD [Panza et al., 2002].

In vascular dementia, which is commonly caused by a stroke or a series of small strokes, brain cells are deprived of oxygen and die. It is sometimes difficult to determine whether people have AD or vascular dementia. It is very important to identify the type of dementia, because the treatment for AD and vascular dementia are quite different [Leach et al., 2004]. Various genetic analyses have been carried out in order to classify dementias and to understand the main genetic mechanisms involved in their pathogenesis. It is well-known that demented individuals and

especially patients with late-onset sporadic form of Alzheimer's disease are characterized with genome instability. It has been stated that the special variants of C-band polymorphism correlate with the phenotypic features and pathological state of various abnormalities [Kovalova et al 1983 ; Maligina et al., 1988; Bablishvili et al., 2000;].

Material and Methods

The investigations were carried out on peripheral blood lymphocyte cultures derived from 10 demented individuals (aged - 70-90) and healthy subjects (10 donors) with suitable age. The patients were distributed into two groups according to the clinical diagnosis (diagnosed in the Institute of Neurology): I group with vascular dementia (5 individuals) and II group with probable diagnosis on late-onset Alzheimer's disease (5 individuals). The structural C-heterochromatin has been examined by the method described by Fernandez et al. Four hundred metaphases from 20 lymphocyte cultures (ten donors) were studied. The types of C-segment variants were determined by Patil and Laubs' classification system:

a<0,5x16p; b<0,5-1x16p; c>1,5x16p; d>1,5-2x16p; e>2x16p.

Statistical analysis was performed by Zax formula:

$$\chi^2_{(k-1)} = (n+m)\frac{n}{m}\left\{\sum_{i=1}^{k}\frac{\left(\frac{vi}{n}\right)2}{\frac{vi+\mu i}{n+m}} - 1\right\}$$

Results and Discussion

The quantitative analysis of C-segments on chromosomes 1,9 and 16 was carried out in the individuals affected with two different forms of dementia (vascular dementia and Alzheimer's disease) and healthy elderly controls. The polymorphism towards variants of C-structural heterochromatic bands was revealed among the first (VD) group patients. The frequency of small a-segments was significantly increased on chromosomes 1,9 and 16 while the frequency of large c-variants was decreased on chromosome 16 as compared with healthy elderly controls (aged - 70-90yrs.). (Table 1.)

Table 1. Polymorphism of C-structural heterochromatin in patients affected with vascular dementia.

Chromosomes	Variants of C-segments	v_i	μ_i	v_i/n	$\frac{vi+\mu i}{n+m}$	χ^2
1	a	7	42	0,0693	0,1856	χ^2_3 =11,62 p<0,01
	b	30	49	0,2970	0,2992	
	c	39	43	0,3861	0,3106	
	d	23	29	0,2277	0,1969	
	e	2	0	0,0198	0,0075	
9	a	29	89	0,2566	0,4014	χ^2_3 =13,47
	b	48	69	0,4248	0,3979	
	c	30	22	0,2655	0,1768	
	d	6	1	0,0531	0,0238	

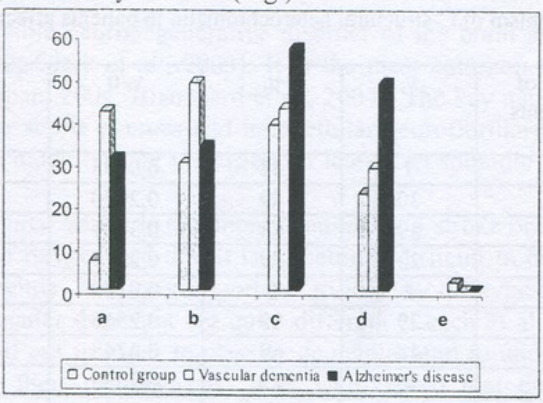
	e	0	0	0	0	p<0,01
16	a	61	115	0,4692	0,5847	χ^2_3 =11,69 p<0,01
	b	55	52	0,4231	0,3554	
	c	15	1	0,1076	0,0498	
	d	0	3	0	0,0099	
	e	0	0	0	0	

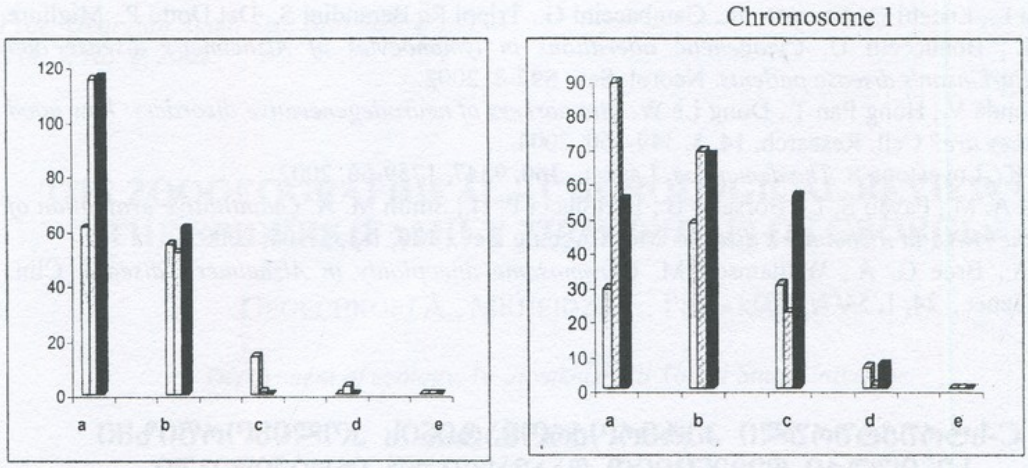
Heteromorphic analysis of various C-segments for each chromosome in the second (AD) group showed that the smallest a-variants were significantly increased for all types of chromosomes (1,9 and 16) in the second (AD) group of patients in comparison with controls. As for the large c-variants of C-segments, their frequency was increased on chromosomes 1 and 9, however it was reduced on chromosome 16 similar to the VD group. (Table 2).

Table 2. Polymorphism of C-structural heterochromatin in patients affected with late-onset form of Alzheimer's disease.

Chromosomes	Variants of C-segments	ν_i	μ_i	ν_i/n	$\frac{vi+\mu i}{n+m}$	χ^2
1	a	7	31	0,0693	0,1397	χ^2_3 =8,05 p<0,01
	b	30	34	0,2970	0,2353	
	c	39	57	0,3861	0,3529	
	d	23	49	0,2277	0,2647	
	e	2	0	0,0198	0,0074	
9	a	29	55	0,2566	0,2809	χ^2_3 =2,96 p<0,01
	b	48	68	0,4248	0,3879	
	c	30	56	0,2655	0,2876	
	d	6	7	0,0531	0,0435	
	e	0	0	0	0	
16	a	61	116	0,4692	0,5765	χ^2_3 =10,75 p<0,01
	b	55	61	0,4231	0,3778	
	c	15	0	0,1076	0,0456	
	d	0	0	0	0	
	e	0	0	0	0	

According to the obtained results we can conclude that the polymorphism of different variants of C-structural heterochromatic bands takes place not only within two different groups of demented individuals but also among separate subjects in each group having the same clinical diagnosis in comparison with elderly controls.(Fig.)





Chromosome 9 Chromosome 16

Fig. Polymorphism of C-bands on chromosomes 1, 9 and 16 in patients with vascular dementia and Alzheimer's disease.

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C-სტრუქტურული ჰეტეროქრომატინის პოლიმორფიზმი სენილური დემენციით დაავადებულ ინდივიდებში.

ტაბატაძე ნ., დადუნაშვილი ე., ლეჟავა თ.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 29.12.2004)

რეზიუმე

C-სტრუქტურული ჰეტეროქრომატინის პოლიმორფიზმი შესწავლილ იქნა სენილური დემენციის ორი ფორმით დაავადებულ ინდივიდთა (ასაკი - 70-90 წლის) პერიფერიული სისხლის ლიმფოციტებში. პირველ ჯგუფში გაერთიანებული იყო ვასკულარული დემენციით დაავადებული პაციენტები (5 ინდივიდი), ხოლო მეორე ჯგუფში პაციენტები ალცჰაიმერის დაავადების გვიან სპორადიულ ფორმაზე სავარაუდო დიაგნოზით (5 ინდივიდი). ჰეტეროგენურობა დაფიქსირდა სამივე წყვილი 1-ელი, მე-9 და მე-16 ქრომოსომებისათვის. C-სეგმენტთა ვარიანტების სიხშირის ვარიაბელობას ადგილი ჰქონდა არა მარტო ჯგუფებს შორის, არამედ ცალკეული ინდივიდების შემთხვევაშიც შესაბამისი ასაკის საკონტროლო ჯგუფის მაჩვენებლებთან შედარებით.

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Zoology

THE ZOOGEOGRAPHICAL – CHOROLOGICAL REVIEW OF THE SPIDERS (FAMILY *THOMISIDAE*) OF GEORGIA

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Abstract

Chorological study of the family *Thomisiade* of Georgia has shown that 3 genera are world tropical, 2 genera – Holarctic, and one by one genus belongs to the following zoogeographical units: Palaearctic – Ethiopian – Oriental – Australian – Neotropical, Holarctic-Neotropical, Palaearctic-Ethiopian-Neotropical, Palaearctic-Ethiopian, Central Eurasian, Ethiopian-Holarctic -Neotropical. It was established that allochtonous element (11genera, 37 species) prevails on autochtonous element (3 genera,13 species). From autochtonous fauna with South Caucasian distribution characterized 3 genera, 10 species, quasi-Caucasian - 2 genera, 3 species. From allochtonous fauna with Holarctic distribution characterized 2 genera, 2 species, with Palaearctic – 8 genera, 25 species, with Palaearctic-Ethiopian – (1 genus, 1 species), with wide Mediterranean – (2 genera, 4 species); with Europe-Siberian (1 genus, 1 species); with Euro-Europe-Siberian (3 genera, 4 species).

Key words: Taxonomy, zoogeography, chorology, *Thomisidae*.

Introduction

11 genera and 50 species of the family *Thomisidae* were registered [Mkheidze, 1992; Mikhailov, 1997].

The family Thomisidae today comprises following genera: Xysticus Koch - 28; Oxyptila Sim.- 7; Synaema Sim.- 4; Tmarus Sim.- 3; Heriaeus Sim.- 2; and one by one species of Rucinia Sim., Pisticus Sim., Diae Thor., Thomisus Walck., Misumena Latr., Misumenops Pick-Cambre [Mkheidze, 1992; Mikhailov, 1997].

Studies of spiders fauna of the family *Thomisidae* in different landscape zones and altitudinal mountain belts in Georgia were carried out from the beginning of 20th century, but in ecological and zoogeographical viewpoint it was not discussed till recent time.

Materials and Methods

Materials have been collected during 2000-2004 in Georgia. To precise the list of species of the family *Thomisidae* with some information about their geographical distribution, scientific sources were used [Mkheidze 1992; Mikhailov 1997].

Results and Discussion

Chorological study of the family *Thomisidae* of Georgia (Table 1) have shown that 3 genera have the world tropical distribution (*Tmarus Sim; Diae Thor; Thomisus Walck.*) [National Science Museum, Tokyo 1988], 2 genera – Holarctic (*Xysticus Koch; Oxyptila Sim.*) [Gertsh, 1953; National Science Museum, Tokyo, 1988], and one by one genus belongs to the following zoogeographical units: Holarctic-Neotropical (*Misumenops Pick-Cambr.*), Palaearctic-Ethiopian-Neotropical (*Synaema Simon*), central-Eurasian (*Pisticus Sim.*), Ethiopian-Holarctic-Neotropical (*Misumena Latr.*), Palaearctic-Ethiopian (*Heriaeus Sim.*), Palaearctic – Ethiopian – Oriental – Australian – Neotropical (*Rucinia Sim.*) [National Science Museum, Tokyo, 1988].

Thus, according to the zoogeographical-chorological studies of species of spiders fauna of the family *Thomisiade*, it was established that allochtonous element (11 genera, 37 species) prevails on autochtonous one distributed in Georgia (3 genera, 13 species).

From autochtonous fauna with South Caucasian distribution characterized 3 genera, 10 species Xysticus koch, abchasicus sub. sp.n. Mkheidze, X. galliscus Sim., batumiensis sub. sp.n. Mkheidze, X.kalandadze, X.caucasicus, X.charitonovi, X.adsharicus, X.nubilus, Ox.mingrelica, Synaema caucasicus, S.richteri) [Mkheidze, 1992; Mikhailov, 1997]; quasi-Caucasian - 2 genera, 3 species (X.umbrinus, X.bacuriensis, Synaema globosum (F) dagestanicum) [Mcheidze, 1992; Mikhailov, 1997; National Science Museum Tokyo, 1988].

From allochtonous fauna with Holarctic distriburion characterized 2 genera, 2 species (*Misumena vatia, Oxyptila praticola*) [Gertsh, 1953; Mcheidze, 1992], with Palaearctic - 8 genera, 25 species (*Xysticus audax, X.cristatus, X.kochi, X.Cambrigei, X.ulmi, X.acerbus, X.luctuosus, X.lineatus, X.kempeleni, X.striatipes, X.ninni, X.sabulosus, X.robustus, X.tristami, Oxyptila lugubris, Ox.conostyla Ox.scarbicula, Synaema globosum, Tmarus piger, Tm.stellio, Tm.horvathi, Heriaeus oblongus, Rucinia lateralis, Diae dorsata, Misumenops tricupsidatus*) [Mkheidze, 1992; Mikhailov, 1997; National Science Museum Tokyo, 1988], with Palaearctic–Ethiopian - 1 genus, 1 species (*Tomisus onustus*), with wide Mediterranean - 2 genus, 4 species (*Xysticus marmoratus, X.cribratus, X.baudueri, Heriaeus hirtus*), with Europe-Siberian - 1 genus 1 species (*Xysticus ukrainicus*), with Euro-Europe-Siberian - 3 genera, 4 species (*Xysticus galliscus, X.lanio, Oxyptila trux, Pisticus truncatus*) [Mcheidze, 1992; Azheganova, 1968; Mikhailov, 1997; Tyshchenko, 1971; Utotchkin, 1989, 1964].

Table 1. Data of Zoogeographical-Chorological Studies of Species of Spiders (Family

		Genera, species	Distribution	Zoogeographical area
1		*Xysticus (Koch* 1835)	Palaearctic (Eurasia, North Africa), North America.	Holarctic
	1	[1]*X.audax (Schrank,*1803) [=*X.pini (Hahn.,* 1831)]	Northern Eurasia, Russia, Carpathians, Estonia, Latvia, Lithuania, Byelorussia, Ukraine, Moldavia, South Caucasus (Georgia), Middle Asia (Uzbekistan, Kirghizia, Tajikistan), Kazakhstan, the Urals, South Siberia, continental Southern Far-East (Amur-Maritime area), Sakhalin and Moneron Islands, Southern Kurile Islands, Japan (Hokaido, Honshu).	Palaearctic
	2	*X.cristatus/(Clerck,1758)* [=*X.viaticus* (C.L.1758)]	North Africa, Europe, Russian, Carpathians, Estonia, Latvia, Lithuania, Byelorussia,	Palaearctic

[1] *X.audax* considered as general species of Neoarctic and Palaearctic fauna [Utotchkin 1964; Mkheidze 1992]

			Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), Middle Asia (Uzbekistan, Kirgizia, Tajikistan), Kazakhstan, South Siberia, the Urals.	
	3	*X.kochi* (Thor., 1972)	Mediterranean countries (Syria, Tunisia), Russia, Carpathians, Estonia, Latvia, Lithuania, Byelorussia, Ukraine, Moldavia, South Caucasus (Armenia, Azerbaijan, Georgia), Middle Asia (Turkmenistan), Kazakhstan, the Urals, South Siberia.	Palaearctic
	4	*X.koch.abchasicus sub. sp.n.* (Mkheidze,Utotshkin, 1971)	Georgia. (endemic of Georgia).	South Caucasian
	5	*X.galliscus* (Sim., 1895)	Asia Minor, France, Switzerland, Russia (North Caucasus), Carpathians, Ukraine, Moldavia, South Caucasus (Georgia), the Urals.	Euro-Europe Siberian
	6	*X.galliscus Sim., betumiensis sub. sp.n.* (Mkheidze et Utotshkin, 1971)	Georgia. (endemic of Georgia).	South Caucasian (According Mkheidze, 1992)
	7	*X.umbrinus* (Utotshkin, 1968)	North Caucasus (Russian), South Caucasus (Georgia) (endemic of Caucasus)	Caucasian
	8	*X.Cambrigei* (Blakw., 1858) [=*X.luctator* (Koch, 1870), (=*X.impavidus* (Thor., 1872)]	Russian, Estonia, Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), the Urals, Middle Asia.	Palaearctic
	9	*X.ulmi* (Hahn., 1831) *[=X.bivitatus (* Westr., 1861)]	Russian, Carpathians, Estonia, Latvia, Lithuania, Byelorussia, Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), Middle Asia (Uzbekistan, Kirgizia), Kazakhstan, South Siberia, the Urals, Sakhalin and Moneron Islands, Japans.	Palaearctic
	10	*X.kalandaze* (Mkheidze, Utotshkin, 1971)	Georgia. (endemic of Georgia).	South Caucasian
	11	*X.ukrainicus (Utotshkin,* 1968)	North Caucasus (Russia) South Caucasus (Georgia), the Urals.	Europe-Siberian
	12	*X.lanio* (Koch, 1835)	Russia, Carpathians, Estonia, Latvia, Lithuania, Byelorussia, Ukraine, Moldavia, South Caucasus (Armenia, Azerbaijan, Georgia), South Siberia , the Urals.	Euro-Europe-Siberian
	13	*X.acerbus* (Thor., 1872)	South Europe, Turkey, Russia, Carpathians, Latvia, Lithuania, Byelorussia, Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), Middle Asia (Uzbekistan, Turkmenistan, Tajikistan), Kazakhstan, Siberia, continental southern Far East (Amur-Maritime area).	Palaearctic
	14	*X.luctuosus* (Blakw., 1836)	Russia, Estonia, Latvia, Byelorussia, Ukraine, Moldavia, South Caucasus (Georgia), Middle Asia (Uzbekistan),	Palaearctic

			Kazakhstan, the Urals, South Siberia, Kamchatka, Sakhalin and Moneron Islands, China.	
	15	*X.lineatus* (Westr., 1851)	Palestine, Russia, Estonia, Latvia, Byelorussia, Moldavia, South Caucasus (Azerbaijan, Georgia), Kazakhstan, the Urals, South Siberia.	Palaearctic
	16	*X.caucasicus* (koch, 1872)	Georgia (endemic of Georgia)	South Caucasian
	17	*X.kempeleni* (Thor., 1872) [=*X.flater* (Herm., 1879)]	Middle Europe, Carpathians, Russia, Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), Middle Asia (Uzbekistan, Turkmenistan), Kazakhstan, the Urals.	Palaearctic
	18	*X.striatipes* (Koch, 1870) [=*X.perogaster* (Thor., 1872)]	Russia, Carpathians, Byelorussia, Ukraine, South Caucasus (Azerbaijan, Georgia), Middle Asia (Uzbekistan, Kirgizia), Kazakhstan, the Urals, South Siberia, China.	Palaearctic
	19	*X.ninni* (Thorell, 1872)	Mediterranean countries (south Europe), Russia, Lithuania, Ukraine, South Caucasus (Armenia, Azerbaijan, Georgia), Middle Asia (Uzbekistan, Turkmenistan), Kazakhstan, the Urals, South Siberia.	Palaearctic
	20	*X.sabulosus* (Hahn., 1831)	Mediterranean countries (Tunisia), Russia, Estonia, Latvia, Lithuania, Byelorussia, Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), Middle Asia, the Urals.	Palaearctic
	21	*X.marmoratus* (Thor., 1875)	Mediterranean countries, Ukraine, South Caucasus (Georgia).	Wide Mediterranean
	22	*X.charitonovi* (Mkheidze, 1971)	Georgia. (endemic of Georgia).	South Caucasian
	23	*X.bacuriensis* (Mkheidze, 1971)	South Caucasus (Georgia), North Caucasus (Russia).	Caucasian
	24	*X.cribratus* (Sim., 1885)	Mediterranean countries (south Europe), South Caucasus (Azerbaijan, Georgia).	Wide Mediterranean
	25	*X.adsharicus* (Mcheidze, 1970)	Georgia (endemic of Georgia).	South Caucasian
	26	*X.robustus* (Hahn., 1831) [=*X.fuscus* (Koch, 1837)]	Mediterranean countries (south Europe, North Africa), Estonia, Latvia, Lithuania, Ukraine, South Caucasus (Georgia), Middle Asia (Uzbekistan), Kazakhstan, the Urals, South Siberia.	Palaearctic
	27	*X.tristami* (Cambr., 1872)	Mediterranean countries (Syria, , Libya, Palestine, Jerusalem), South Caucasus (Azerbaijan, Georgia) Middle Asia (Turkmenistan, Tajikistan, Kirgizia, Uzbekistan), Kazakhstan.	Palaearctic
	28	*X.nubilus* (Simon, 1875)	Georgia.	South Caucasian (According Mkheidze, 1992)
2		*Oxiptila (Sim.,* 1869)		Holarctic

				(According to National Science Museum, Tokyo, 1988)
	29	*Ox.mingrelica* (Mkheidze, 1970)	Georgia (endemic of Georgia)	South Caucasian
	30	*Ox.praticola* (Koch, 1837)	Russia, Estonia, Latvia, Byelorussia, Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), Middle Asia (Uzbekistan, Kirgizia, Tajikistan), Kazakhstan, the Urals, South Siberia, North America (Washington).	Holarctic
	31	*Ox.lugubris* (Croneb., 1875)	Mediterranean countries, Ukraine, South Caucasus (Armenia Azerbaijan, Georgia), Middle Asia (Uzbekistan, Turkmenistan, Kirgizia, Tajikistan) Kazakhstan, west Siberia.	Palaearctic
	32	*Ox.trux* (Blakw., 1846)	Russia, Estonia, Carpathians, Latvia, Lithuania, Byelorussia, Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), the Urals, South Siberia, southern Kurile Islands.	Euro-Europe-Siberian
	33	*Ox.baudueri* (E.S.,1875)	France, Portugal, European countries of the former Soviet Union, South Caucasus (Georgia)	Wide Mediterranean
	34	*Ox.conostyla* (Hippa, Koponen, Oksala, 1986)	Middle Asia (Turkmenistan), Asia Minor South Caucasus (Azerbaijan, Georgia).	Palaearctic
	35	*Ox.scarbicula* (Westring, 1851)	Russia, Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), Middle Asia (Uzbekistan, Kirgizia), Kazakhstan, the Urals, South Siberia	Palaearctic
3		*Synaema* (Simon, 1864)	Eurasia, Africa, South America.	Palaearctic-Ethiopian-Neotropical
	36	*S.caucasum (Utotshkin, 1960)*	Georgia (endemic of Georgia)	South Caucasian
	37	*S.globosum* (Fabr., 1775)	Mediterranean countries (south Europe, Turkey, North Africa, Canary Isl., Spain), Carpathians, Russia, Ukraine, Moldavia, Moldavia, South Caucasus (Azerbaijan, Georgia), Kazakhstan, Middle Asia (Kirgizia, Tajikistan), the Urals, south Siberia, continental south Far East (Amur-Maritime Area), China, Mongolia, Japans (Hokaido, Honshu, Shikoku, Kyushu).	Palaearctic
	38	*S. globousum* (F) *dagestanicus (Utotshkin, 1960)*	North Caucasus (Dagestan), South Caucasus (Georgia)	Caucasian
	39	*S. richteri (Utotshkin, 1960)*	South Caucasus Armenia, Georgia, (endemic of south Caucasus)	South Caucasian
4		*Tmarus (Sim.,* 1875)		World tropical (According to

				National Science Museum, Tokyo, 1988)
	40	*Tm.piger* (Walck., 1802)	Spain, Russia, Carpathians, Ukraine, Moldavia, South Caucasus (Armenia, Azerbaijan, Georgia), Kazakhstan, the Urals, South Siberia, continental southern Far-East (Amur-Maritime area), Japan (Hokaido, Honshu, Kyushu).	Palaearctic
	41	*Tm.stellio* (Simon, 1875)	Mediterranean Countries (South Europe), Central Asia, North Caucasus (Russia), South Caucasus (Georgia), Japan.	Palaearctic
	42	*Tm.horvathi* (Kulcz., 1835)	North Caucasus (Russia), South Caucasus (Azerbaijan, Georgia), Middle Asia (Turkmenistan), continental Southern Far East (Amur-Maritime area).	Palaearctic
5		*Heriaeus (Simon*,1875)	Eurasia, Africa.	Palaearctic-Ethiopian
	43	*H. hirtus* (Latr., 1819) [=*H.sevignyi* (Simon, 1875)]	South Europe, Estonia, Ukraine, North Caucasus (Russia), South Caucasus (Georgia)	Wide Mediterranean
	44	*H.oblongus* (Simon,1918)	Spain, Russia, Carpathians, Ukraine, Moldavia, South Caucasus (Azerbaijan, Georgia), Kazakhstan, Middle Asia (Turkmenistan, Kirgizia, Uzbekistan), the Urals, China, Mongolia.	Palaearctic
6		*Rucinia (Sim.,* 1875)	North and South Africa, South Europe, South Asia, Australia, South America.	Palaearctic-Ethiopian-Oriental-Australian-Neotropical
	45	*R.lateralis* (Koch, 1838)	Mediterranean countries (south Europe, Turkey, North Africa), Russia, Byelorussia, Ukraine, South Caucasus (Armenia, Azerbaijan, Georgia), Kazakhstan, Middle Asia (Turkmenistan, Uzbekistan, Tajikistan), west Siberia, China.	Palaearctic
7		*Pisticus (Sim.,* 1875)	Eurasia.	Central Eurasian
	46	*P. truncatus* (Pallas, 1772)	Carpathians, Ukraine, Moldavia, South Caucasus (Armenia Azerbaijan, Georgia), south Siberia, continental South Far East (Amur-Maritime area).	(According to National Science Museum, Tokyo, 1988) Euro-Europe-Siberian
8		*Misumena (Latr.,*1804)	Africa, Eurasia, North and South America.	Ethiopian-Holarctic-Neotropical
	47	*M. vatia* (Cl., 1757) *(=M.Calyciana L.*,1758*)*	Russia, Carpathians, Latvia, Lithuania, Estonia, Byelorussia, Ukraine, Moldavia, South Caucasus (Armenia, Azerbaijan, Georgia), Kazakhstan, Middle Asia (Kirgizia,	Holarctic

			Tajikistan, Uzbekistan), the Urals, North Siberia, continental South Far East (Amur Maritime) area, Japan (Hokaido and Honshu), Sakchalin and Moneron Islands, South Kurile Islands, North America.	
9		*Diae* (Thor., 1863)		World tropical (According to National Science Museum, Tokyo, 1988)
	48	*D. dorsata* (Fabr., 1777)	Mediterranean countries (Asia Minor), Russia, Estonia, Latvia, Lithuania, Byelorussia, Ukraine, Moldavia, Carpathians, South Caucasus (Armenia Azerbaijan, Georgia), Middle Asia (Turkmenistan, Tajikistan) the Urals, South Siberia.	Palaearctic
10		*Thomisus*(Walck., 1805)		World tropical (According National Science Museum, Tokyo, 1988)
	49	*T.onustus* (Walck., 1805)		Palaearctic-Ethiopian
11		[1]*Misumenops*(Pick-Cambre., 1990)	Palaearctic and equatorial Africa. Eurasia, North and South America.	Holarctic-Neotropical
	50	*M. tricupsidatus* (Fabr., 1775)	Spain, Russia, Carpathians, Latvia, Lithuania, Byelorussia, Ukraine, Moldavia, South Caucasus (Armenia, Azerbaijan, Georgia), Middle Asia (Tajikistan, Uzbekistan), Kazakhstan, the Urals, South Siberia, continental South Far East (Amur-Maritime Area), Sakhalin and Moneron Islands, South Kurile Islands, Japan (Hokaido, Honshu, Shikoku), Mongolia.	Palaearctic

1. *Misumenops Pick-Cambre* - this species is somehow different from typical *Misumenops* species occurring in North America by more developed eyes and very long embolus of male palp (National Science Museum, Tokyo, 1988).

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საქართველოში გავრცელებული ობობების ოჯახ *Thomisidae*-ს ზოოგეოგრაფიულ-ქოროლოგიური მიმოხილვა

გეგეჭკორი ა., მხეიძე თ., ფხაკაძე ვ.

ზოოლოგიის კათედრა, ივ.ჯავახიშვილის სახ.თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 05.01.2005)

რეზიუმე

შესწავლილია საქართველოში გავრცელებული ფეხსახსრიანთა ტიპის, ობობების რიგის (*Aranei*), ოჯახ *Thomisidae*-ს 50 სახეობა, რომელიც მიეკუთვნება 11 გვარს. ზოოგეოგრაფიულ-ქოროლოგიური კვლევის შედეგად დადგინდა, რომ *Thomisidae*-ს ოჯახის 3 გვარი ტროპიკულია, 2–ჰოლარქტიკული; თითო-თითო გვარით წარმოდგენილია შემდეგი ზოოგეოგრაფიული არეალები: პალეარქტიკულ-ეთიოპიურ-ორიენტალურ-ავსტრალიურ-ნეოტროპიკული, ჰოლარქტიკულ-ნეოტროპიკული; პალეარქტიკულ-ეთიოპიურ-ნეოტროპიკული; ცენტრალურ-ევრაზიული, ეთიოპიურ-ჰოლარქტიკულ-ნეოტროპიკული; პალეარქტიკულ-ეთიოპიური. დადგინდა, რომ ფაუნის ალოქტონური ელემენტი (11 გვარი, 37 სახეობა) ჭარბობს ავტოქტონურ ელემენტს (3 გვარი, 13 სახეობა). ავტოქტონური ფაუნიდან სამხრეთ კავკასიურია 3 გვარი, 10 სახეობა; კავკასიური - 2 გვარი, 3 სახეობა. ალოქტონური ფაუნიდან ჰოლარქტიკული გავრცელებით ხასიათდება 2 გვარი, 2 სახეობა, პალეარქტიკულით – 8 გვარი, 25 სახეობა; პალეარქტიკულ-ეთიოპიურით - 1 გვარი, 1 სახეობა; ფართო ხმელთაშუაზღვიურით - 2 გვარი, 4 სახეობა; ევროპა-ციმბირულით - 1 გვარი, 1 სახეობა; ევროპა-ევროპა-ციმბირულით - 3 გვარი, 4 სახეობა.

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Letter to Editor

BIVULVARITY OF *EUDORYLAIMUS SP.* (NEMATODA, DORYLAIMIDA)

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By investigation of soil nematoda populations from restored ecosystems we found a female of ***Eudorylaimus sp.*** with two vulva (sample #2, plot #3, 21. 05. 96) [Kuchava et al.,2000].

The measures of the female are:

1♀ L = 0. 47 mm; a = 24; b = 3.7; c = V_1 =50. 7%; V = 60%.

Description of the female of ***Eudorylaimus sp.*** with two vulva:

Body relatively wide, tail end slightly curved ventrally. Cuticle thin, about 1μm wide, smooth. There are weakly visible longitudinal ribs on the cuticle. The spear wide, about two times wider than cuticle, its length - 19.6μm, orifice equals to 1/3 of its length. Oesophagus widened slighthly behind of the middle part, cardia short, cylindrical. Owaries two, opposite, stright, apperently functioning. Vulva two; distance between them 42μm; each uterus is separated.

Tail is short, equals to body diameter, terminus is finger like. The cuticle of the tail thickened (2μm), on the ventral side bulbed.

The abnormality among Nematodes are not rare, but bivulvarity noted only in a few cases. For the first time this fenomenon was marked by Bütschli for a marine nematode ***Linhomoeus mirabilis.*** Later Poromonov makred for ***Tobrilus gracilis*** [Poromonov, 1926], Cassidy and Mulvey for ***Prionchulus muscarum*** [Mulvey, 1963], Altherr for ***Granonchulus schulzi*** [Altherr, 1958], Andrassy for ***Tobrilus sp.***[Andrassy, 1960] and Mulvey for ***Prionchulus punctatus*** [Mulvey, 1967].

For Dorylaimid Nematode (***Dorylaimus sp.***) this fenomenon till now was marked only by Cassidy [Mulvey, 1963] and second case for ***Eudorylaimus sp***. was found by us.

In contrast to Andrassy [Andrassy, 1960] we suppose that bivulvarity of nematode is result of breach of morphogenesis on the early stage of organogenesis.

On the Fig.1 and Fig.2 drawings and photo of bivulvar ***Eudorilaimus*** are presented.

The material is kept in Laboratory of Soil Zoology of the Institute of Zoology of Academy of Sciences of Georgia.(Prep. #1, Bivulvar *Eudorylaimus*).

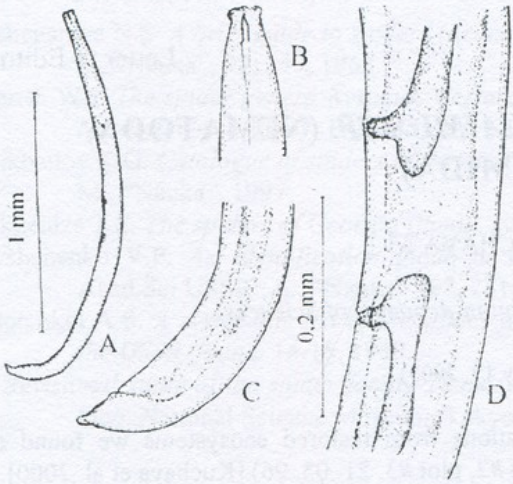

Fig. 1. A - General view of bivulvar ***Eudorylaimus sp.*** B - Had, C – tail region, D – Vulvar region

Fig. 2. Photo of vulvar region of ***Eudorylaimus sp.***

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ორვულვიანი *Eudorylaimus sp.* (Nematoda, Dorylaimida)

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(მიღებულია 17.01.2005)

რეზიუმე

აღდგენილი ეკოსისტემების ნიადაგის ნემატოდების შესწავლისას (თბილისი, ვერეს ხეობა, სინჯი №2, ნაკვეთი 3, 21. 05. 96) ნაპოვნია ორვულვიანი მდედრი. ორვულვიანობა ჩვენი აზრით არის ინდივიდუალური განვითარების ადრეულ სტადიაზე მორფოგენეზის არანორმალური მიმდინარეობის შედეგი.

ინსტრუქცია ავტორთათვის

სამეცნიერო ნაშრომს ჟურნალი ბეჭდავს ინგლისურ ენაზე, მას უნდა დაერთოს რეზიუმე ინგლისურ და ქართულ ენაზე, სამეცნიერო მიმართულება, სათაური, ავტორთა გვარები და მათი სამუშაო დაწესებულების დასახელება, საკვანძო სიტყვათა მოკლე (4-6) სია.

სამეცნიერო წერილის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და 12 გვერდზე მეტი. წერილი უნდა გაფორმდეს შემდეგი რუბრიკაციით: შესავალი და მიზნები (introduction), მასალა და მეთოდები (materials and methods), შედეგები და მათი განხილვა (results and discussion), დამოწმებული ლიტერატურა. უკანასკნელი უნდა იყოს დალაგებული ანბანის მიხედვით, ხოლო ტექსტში წყაროების მითითება უნდა ხდებოდეს ფრჩხილებში ჩასმული ავტორის გვარითა და წლით. ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

ჟურნალის შემთხვევაში:

Carvalho C., Pereira H., Pina C. *Chromosomal G-dark bands determine the spatial organization of centromeric heterochromatin in nucleus.* Mol. Biol. Cell, **12**, 5, 3563-3572, 2001.

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Gentner D., Brush S. *Flowing waters or teeming crowds.* In: Mental Models. D. Gentner (Ed.), Chicago IL., Chicago Press, 865-900, 2001.

ქართული ტექსტისთვის ოპტიმალური ფონტებია AcadNusx და AcadMtavr, ინგლისური ტექსტებისთვის - Times New Roman. შრიფტის ზომა - 12 პუნქტი, ინტერვალი - 1,5. ცხრილებში დასაშვებია უფრო მცირე ზომის შრიფტები. წერილი უნდა დაიბეჭდოს A4 ფორმატით, ზევით და ქვევით - 2,5 სმ., მარცხნივ – 3 სმ. და მარჯვნივ – 2სმ. დაშორებით. ცხრილები, გრაფიკები და დიაგრამები (მხოლოდ შავ-თეთრი) შესაძლებელია დამზადდეს როგორც Microsoft Word-ში, ისე Excel-ში, ფოტოსურათები მიიღება აგრეთვე ორიგინალების (არაელექტრონული) სახითაც. მასალა რედაქციაში წარმოდგენილი უნდა იყოს როგორც ქაღალდზე ამობეჭდილი, ისე კომპიუტერულ დისკეტით ან CD-ზე, წარმოდგენილი წერილი მთლიანად ერთი ფაილით უნდა იყოს შენახული, ხოლო ფაილის სახელწოდება წერილის პირველი ავტორის გვარს უნდა ატარებდეს. ცალკე ფაილად შეიძლება ილუსტრაციების წარმოდგენა.

ჟურნალის ბეჭდვა ავტორთა ხარჯებით ხორციელდება. თანხა რედაქციაში უნდა შემოვიდეს ნაშრომზე დადებითი რეცენზიის მიღებისთანავე. ნაშრომის რეცენზირება ანონიმურია და ავტორს აქვს უფლება მიიღოს ან არ მიიღოს რეცენზენტის შენიშვნები. უკანასკნელ შემთხვევაში ნაშრომი, დამატებით გაეგზავნება სარედაქციო საბჭოს ერთ-ერთ წევრს. მეორე უარყოფითი დასკვნის შემთხვევაში, ნაშრომი არ გამოქვეყნდება.

ნაშრომი ჩაბარება შეიძლება სამუშაო დღეებში, 12-დან 16 საათამდე, შემდეგ მისამართზე: თბილისი, რუსთაველის გამზირი 52, საქართველოს მეცნიერებათა აკადემია, ბიოლოგიის განყოფილება, IV სართული, 429 ოთახი, ტელ: 93-58-92, პასუხისმგებელი მდივანი – მაია გრიგოლავა.