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CONTENTS

**Biochemistry**

Gamtsemlidze E., Tsiklauri G., Zambakhidze N., Simonishvili Sh., Sulaberidze K. **Antioxidant Activity of Phenol Compounds of Persimmon Fruits and their Products.** 1

Gulua L., Omiadze N., Mchedlishvili N., Chachua L., Tsiklauri G., Rekhviashvili M. Torbini V. **Natural Inhibitor of Phenol Oxidases and Peroxidases from Pomegranate Peel.** 6

Lomsianidze I., Sadunishvili T., Dzamukashvili N., Nutsubidze N. **Effect of Systematic Introduction of Ammonium Nitrate on Properties of Ajameti Podzolic Soil.** 11

Tsiklauri N., Kachlishvili E., Kutateladze L., Metreveli E., Asatiani M., Songulashvili G., Aladashvili N. **Basidiomycetes of Georgia – Producers of Lignocellulolytic Enzymes.** 17

**Biophysics**

Gachechiladze N., Melikishvili M., Toriashvili T., Lomidze L., Zaalishvili T., Japaridze N., Zaalishvili M. **Synthesis of Titin in Embryogenesis.** 22

**Biotechnology**

Chachkhiani M., Dudauri T., Kalandadze N., Partskhaladze G., Tsiklauri L., Ugrekhelidze V., Zakariashvili N. **Pretreatment of Cellulosic Wastes by Combination of Chilling and Milling Methods.** 27

**Botany**

Kharazishvili D. **Treeline Vegetation of the River Chirukhistskali (South Colchis, Adjara).** 32

**Cytology**

Ramishvili M., Gogava M., Zaalishvili G., Chelidze N., Gogoberidze M. **Ultrastructural Changes of Yucca Gloriosa Callus Tissues, Leaves and Buds Under 2.4.6-Trinitrotoluene (TNT) Action.** 43

**Ecology**

Tsiklauri Kh., Gurgenidze L., Kvavadze E., Kajaia G. **The Soil Mezofauna of Algethy Reserve Beech Forests.** 49

**Genetics**

Baratashvili N., Chitanava Zh., Shatirishvili A. **Genetic Changes in the Soybean Glycine max (L) Merr Induced by Pesticides (Khomecyn, Ridomil).** 53

Mamulashvili L., Nasyidashvili P., Goginashvili K. **Study of Chromosomal Aberrations Frequency of the Second Cycle Lines of Maize Received on Polluted with Heavy Metals Soils.** 58

Tsilosani N., Alavidze Z., Elpineri L., Chkonia I., Kiknadze M., Zaalishvili I., Dzidzishvili M. **Study on Ecological Aspects of Seawater by Marker Method.** 62

**Molecular Biology**

Akhalkatsi R., Macharadze T., Kharazishvili L. **The Participation of Protein-protein Interaction in the Structural-Functional Organization of Chromatin and Chromatin Nonhistone Proteins.** 67

**Morphology**

Ochigava I., Kalandarishvili L., Zhvania M., Kvesitadse E. **Immunological and Neuromorphological Study of Staphylococcus Aureus Infected Mice.** 71

**Phytopathology**

Gvritishvili M., Kacheishvili-Tavartkiladze K., Churghulia-Shurghaia M., Gotsadze N. **New Data on Microfungi of Georgia.** 77

**Plant Physiology**

Mangaladze N., Oniani J., Oniani T., Gogonaia N., Chankseliani T., Zaalishvili T. **The effect of cobalt on anatomical structure of plant leaves.** 81

**Zoology**

Barjadze Sh., Kvavadze E. **Host Plants and Nutrition Specialization of Aphids (Hemiptera: Sternorrhyncha) Inhabitated in the Rv. Dzama, Tana and Tedzami Ravines.** 85

Gegechkori A., Mkheidze T., Pkhakadze V. **The Zoogeographical-Chorological Peculiarities of the Spiders (Family Dysderidae) of Georgia.** 90

ეროვნული
ბიბლიოთეკა

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Vol. 3, No. 1, 2005.

Biochemistry

# ANTIOXIDANT ACTIVITY OF PHENOL COMPOUNDS OF PERSIMMON FRUITS AND THEIR PRODUCTS

GAMTSEMLIDZE E., TSIKLAURI G., ZAMBAKHIDZE N., SIMONISHVILI SH., SULABERIDZE K.

*S. Durmishidze Institute of Biochemistry and Biotechnology, Georgian Academy of Sciences*

(Received August, 4, 2004)

**Abstract**

Qualitative contents of phenol compounds cultivated in Georgia Japanese persimmon (*Diospyros Kaki*) and Caucasian persimmon (*Diospyros Lotus*) fruits and the products (jam, dried fruits, liqueur) made from the Japanese persimmon fruits and antioxidant activity of the given samples were studied. It has been determined that according to the qualitative contents of phenolic compounds, *D. Kaki and D. Lotus* fruits do not differ substantially from one another, and according to the quantitative contents of phenols and antioxidant activity, *D. Lotus* fruits are distinguished.

**Key words:** flavonoids, fruits, *Diospyros Kaki, Diospyros Lotus.*

## Introduction

Persimmon is a salient specimen of subtropical cultures introduced in Georgia. In Georgia only two specie are spread: Japanese persimmon (*D. Kaki*) and Caucasian persimmon (*D. Lotus*). Their fruits are characterized with a high nutrient value and are used in confectionery and medicine [Martines J., et al., 2001; Gao M., 2001; Minami E., et al., 2000;]. Tannin of the Japanese persimmon is characterized with an anti-hemorrhagic effect against the bite of a snake [Marts W., 1992]. Extract of *D. Kaki* fruits inhibits the growth of human lymphoid leukaemia cells [Achiwa Y., et al., 1997]. Ethanol extract of the Japanese persimmon fruits is distinguished with a high antioxidant activity and is used in Japan as one of the dietetic foodstuffs for the diseased with atherosclerosis [Katsube T., et al., 2004].

Taking into account that phenol compounds are distinguished with versatile medicinal properties and are characterized with high antioxidant activity, persimmon fruits may become a source of biologically active substances from which preparations having antioxidant activity may be obtained.

Purpose of our work was to study the phenol compounds of the both varieties of persimmon fruits cultivated in Georgia and the products (jam, dried fruits, liqueur) made of the Japanese persimmon fruits and their antioxidant activity.

## Materials and methods

Certain quantity (1kg) of ripe and green fruits of the Japanese persimmon (*D. Kaki*)) and Caucasian persimmon (*D. Lotus*), jam made from the fruits of the Japanese persimmon, dried fruits,



საქართველოს
პარლამენტის
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ბიბლიოთეკა

liqueur were taken for analysis. Extraction of flavonoids from the fruits was carried out with 70% of ethanol on boiling bath by backflow condenser three times. Duration of each extraction was 30 minutes. Extracts were combined, filtered in a paper filter and distilled in rotary evaporators at 50°C. Certain quantity (50g) of the jam made of *D. Kaki* fruits was cold extracted with 80% ethanol, settled for 24 hours. Certain quantity (20g) of persimmon dried fruits was extracted 3 times with 70% ethanol on a boiling water bath (duration 30 minutes).

Qualitative contents of each sample was studied by the method of two-sided paper chromatography in the solvent system butanol-acetic acid-water, 4:1:5 (I direction) and 2% acetic acid (II direction), vanillin reagent was used as developer of catechins and proanthocyanidin, and for flavonoids – $ALCl_3$ 1% solution in ethanol. Quantitative determination of phenol compounds of all the samples was performed on the basis of Swain and Hillis methods (Swain I., Hillis W., 1959).

For antioxidant activity test of the sample two alcohol solutions were prepared: 1) working solution of antioxidant in ethanol and 2) DPPH' (2,2-diphenylpicrylhydrazine) working solution in ethanol (60μM), measuring of optical density of this solution and also of incubating medium was performed on "C? 26" at 520 nm wave-length. Seven 10ml test tubes with stoppers were taken, in each test tube 3ml DPPH' solution was placed to which different concentrations of the antioxidant solution of the research object was added. Samples were shaken and settled. Duration of incubation was 30 minutes at room temperature. Further optical density was calculated. According to seven data calibration curve was built by means of which the quantity ($I_{C50}$) of the antioxidant that in reaction medium causes 50% recombination of DPPH' and the time ($TI_{C50}$) required for this recombination was calculated [Pochinok T. et al., 1985]. Per cent of DPPH' inhibition in the reaction medium was calculated by the formula [Satue-Gracia T. M., 1997]:

$$\text{Inhib. \%=?}\ \frac{C-S}{C} \times 100$$

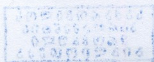
where C is an initial concentration of DPPH' in the reaction medium, S – DPPH' concentration in the reaction medium after adding antioxidant.

The curve shows the quantity (μg) of antioxidant with the highest activity from the given samples that causes in the reaction medium 50% recombination of DPPH'.

## Results and discussion

Results of the qualitative analysis showed that the extracts obtained from both persimmon varieties by chemical composition do not differ much from one another. Each fraction consists of seven substances. Two of them colored in red with vanillin reagent belong to catechins and proanthocyanidins, and three as a result of treatment with $ALCl_3$ acquire greenish and yellow fluorescence in ultraviolet light characteristic for flavonols. Both fractions contain phenolcarboxyl acids, blue fluorescence of which in the ultraviolet light becomes sharper at $NH_3$ vapor treatment. By chromatography it was also determined that products (jam, dried fruits, liqueur) made from *D. Kaki* fruits comprise two flavonols and phenolcarboxyl acid.

By means of quantitative analysis in all samples sum of the phenol compounds, proanthocyanidins and catechins were defined. The results are given in the Table. Sum of the phenol compounds is much more in an unripe fruit of persimmon. Together with ripening of the fruit the quantity of phenolic compounds reduces and this reflected in peculiarity of persimmon fruit taste. In the process of ripening of the fruits metabolism of phenol compounds takes place. According to the literature data the increase of the quantity of soluble pectins in the persimmon fruit is accompanied with reduction of phenol compounds therein which is proved by



disappearance of a tart taste connected with linking of monomer polyphenols with pectin substances [Ito S., 1971].

Caucasian persimmon fruits are characterized with comparably larger quantity of phenol compounds. Among the products made from *D. Kaki* fruits with high quantitative contents of phenolic compounds is distinguished jam. As to the catechins and leucoanthocyanidins, they as labile substances are subjected to transformation during processing of fruit and are in very small quantities.

**Table 1.** Quantitative contents of phenolic compounds of persimmon fruits and the products obtained thereof and antioxidant activity.

| sample | quantitaty of phenol compounds (% in dry extractives) | | | $Ic_{50}$ µg [-] | $Tic_{50}$ minutes [--] |
|---|---|---|---|---|---|
| | Sum of phenol compounds | catechins | proanthocyanidins | | |
| D. K. green fruit | 13,3 | 0,013 | 0,37 | 88 | 25,08 |
| D.K. ripe fruit | 12,4 | 0,0017 | 0,29 | 120 | 23 |
| D.L. green fruit | 23,7 | 0,046 | 0,144 | 23 | 21 |
| Jem | 5,3 | 0,007 | 0,148 | 262 | 45 |
| Dried fruits | 2,92 | 0,099 | | 29200 | 26 |
| Liqueur | 1,79 | 0,001 | 0,08 | 11000 | 22 |
| Quercetin | — | | | 16,9 | 1,30 |
| a-tocopherol | — | — | | 23,5 | 2,15 |

[-] quantity of the antioxidant that in reaction medium causes 50% recombination of DPPH˙.
[--] the time required for 50% recombination of DPPH˙.

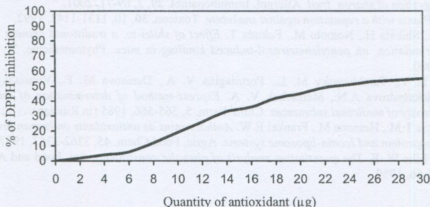



Fig. 1. 50% recombination of DPPH˙ during 30 minutes period of incubation by *D. Lotus* extract.

We have also determined that those samples in which phenol compounds are in larger quantities are characterized with antioxidant activities (Table 1). Activity of this compound is expressed with two peculiarities. Firstly they bind ions of heavy metals that catalyze oxidation and create with them stable components. Secondly, phenol compounds interact by high activity with free radicals that arise at autooxidation. Antioxidant activity of our sample was compared with the

antioxidant activity of quercetin and a-tocopherol. From the given samples Caucasian persimmon (*D. Lotus*) fruits are distinguished with comparably high activity, on the curve (Fig.1) it is clearly seen that 23μg of antioxidant causes 50% recombination of DPPH'.

Data obtained are in full compliance with the literature data [Achiwa Y. et al., 1997; Katsube T. et al., 2004]. According to these data compounds obtained from fruits and leaves of persimmon are characterized with high antioxidant effect.

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# ხურმის ნაყოფების და მათგან მიღებული პროდუქტების ფენოლური ნაერთები და ანტიოქსიდანტური აქტივობა

გამცემლიძე ე., წიკლაური გ., ზამბახიძე ნ., სიმონიშვილი შ., სულაბერიძე ქ.

*საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიის და ბიოტექნოლოგიის ინსტიტუტი*

(მიღებულია 04.08.2004)

## რეზიუმე

შესწავლილია საქართველოში კულტივირებული აღმოსავლური ხურმის (*Diospyros Kaki*) და კავკასიური ხურმის (*Diospyros Lotus*) ნაყოფების და აღმოსავლური ხურმის ნაყოფებიდან დამზადებული პროდუქტების (ჯემი, ჩირი, ლიქიორი) ფენოლური ნაერთების თვისობრივი შედგენილობა, მათი რაოდენობრივი შემცველობა და მოცემული ნიმუშების ანტიოქსიდანტური აქტივობა. დადგენილია, რომ ფენოლური ნაერთების თვისობრივი შედგენილობით *D. Kaki* და *D. Lotus* ნაყოფები ერთმანეთისგან არსებითად არ განსხვავდებიან, ხოლო ფენოლების რაოდენობრივი შემცველობით და ანტიოქსიდანტური აქტივობით გამოირჩევა *D. Lotus* ნაყოფები.

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Biochemistry

# NATURAL INHIBITOR OF PHENOL OXIDASES AND PEROXIDASES FROM POMEGRANATE PEEL

GULUA L. [1], OMIADZE N. [1], MCHEDLISHVILI N. [1], CHACHUA L. [1], TSIKLAURI G. [1], REKHVIASHVILI M. [2], TORBINI V. [3]

[1]*Durmishidze Institute of Biochemistry and Biotechnology, Academy of Sciences of Georgia.*
[2] *Institute of Food Industry*
[3]*Sukhumi Branch of Tbilisi State University*

(Received July 14, 2004)

**Abstract**

Natural inhibitor of phenol oxidase and peroxidase from pomegranate peel have been isolated. The natural inhibitor consisted of flavanoid glycosides and phenol carbonic acids. The effect of the inhibitor on apple phenol oxidase and peroxidase activities have been studied. In the presence of the natural inhibitor maximal rate of apple phenol oxidase reaction decreased 1.6 times ($V_{max}$=12.7 ΔE/mg protein/min, $V_{maxi}$=8.1 ΔE/mg protein/min) while Vmax of peroxidase reaction decreased 2.7 times ($V_{max}$=500 ΔE/mg protein/min, $V_{maxi}$ = 182 ΔE/mg protein/min).

**Key wards:** apple, pomegranate, phenol oxidase, peroxidase, inhibitor.

## Introduction

One of the main factors affecting food products storage process is an enzymatic browning due to the activity of two classes of redox enzymes, polyphenol oxidases or tyrosinase (EC 1.14.18.1) [Mayer 1987] and to lesser extent peroxidases (EC 1.11.1.7) [Mukherejee & Rao, 1993]. As browning in food products is an extremely undesirable process, there has been a constant need in food industry for methods preventing this reaction. [Lee and Whitaker, 1995, Lee and Lee, 1997]. The most commonly used method is blanching. But this method has several limitations. Recently more attention is paid to the use of antioxidants and inhibitors to prevent undesirable browning in food products. Although a large number of polyphenol oxidase and peroxidase inhibitors have been described in the literature [Kubo, 1997; Moon et al., 1999], the search for new natural products and even synthetic compounds is actual. There are several studies on polyphenol oxidases and peroxidases of apple [Espin at al., 1995, Mukharajee, 1993]. We previously described plant polyphenol oxidase and peroxidase role in tea production processes and derived from tea leaves enzymes inhibitors [Pruidze et al., 2003].

The aim of presented study is to isolate natural inhibitor from pomegranate peel and examine its inhibitory effect on the of apple phenol oxidase and peroxidase activities.

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## Materials and methods

Fruits of apple ("Kekhura") and pomegranate (*Punica granatum* L.) peel served as the materials for research. Fruits were market purchased. Protein content was determined by Lowry, *et al.* method [as described by Scopes, 1985]. The polyphenol oxidase activity was determined spectrophotometrically according to [Lanzarini, *et al.*, 1972]. The peroxidase activity was determined according to [Evans and Aldridge, 1965].

**Crude Enzyme preparation**. Fresh apples were washed, dried, frozen in liquid nitrogen, crushed in mortar, adding polyamide powder and homogenized in 0,05 citrate-phosphate buffer (pH 6,5) containing 0,3 M ascorbate. The homogenate was filtrated through two layers of cheesecloth and centrifuged at 3000 g for 40 min. Proteins in the supernatant were precipitated with $(NH_4)_2SO_4$ (90% saturation), stored for 24 h and centrifuged at 10 000 g for 10 min. The pellet was resuspended in distilled water and centrifuged at 10 000 g for 30 min. The obtained supernatant was used as phenoloxidase and peroxidase liquid crude preparation. All steps were performed at 4° C.

**Isolation of inhibitor from pomegranate peel.** 500 g pomegranate peel was powdered in added 2 l boiled water liquid nitrogen, and placed on the water bath at the 80 °C for 30 min. The extract was concentrated up to 200 ml and centrifuged to remove a pellet. 50g polyamide powder was added to the extract and left for two hours. The elution of absorbed inhibitor from the polyamide powder was performed with 60% acetone. Acetone fraction was evaporated under vacuum and the concentrated water extract was analyzed with paper chromatography, which was carried out with the solvent mixture: buthanol – acetic acid - water 4:1:2 (v/v/v) on a filter paper. The spotting of samples was done with vanillin reagent and 1% alcohol solution of aluminum chloride under UV light [Kharborn, 1968].

Presented data are the mean of three replicates ± standard deviation. When necessary, the "least squares" method was used to calculate a straight line that best fitted experimental data. All calculations were performed with Microsoft Excel (Version 4, statistical functions, Microsoft Corp., Redmond, WA, USA).

## Results and discussion.

Paper chromatography analysis of the pomegranate extract showed that the extract did not contain catechins (no reaction with vanillin reagent). By treatment of chromatograms with aluminum chloride 7 spots were detected. Yellow and yellowish fluorescence under UV lights indicated the presence of flavanoid glycosides and phenol carbonic acids in the extract.

The effect of different concentrations of natural inhibitors from pomegranate peel on apple phenoloxidase and peroxidase were studied (Fig. 1).

It was shown that both phenoloxidase and peroxidase activities gradually decreased by increasing of the concentration of natural inhibitor from pomegranate peel. The inhibitor was more effective in case of peroxidase. 50% of inhibition of phenol oxidase and peroxidase achieved at 2.4 mg/ml and 17 μg/ml concentration of the tested inhibitor respectively.

As it can be seen from Fig. 2 dependence of initial rate of phenoloxidase reaction on catechol (substrate) concentration followed Michaelis-Menten equation. Lineweaver-Burk plots with and without natural inhibitor from pomegranate peel were linear.

Maximal rate of enzymatic reaction decreased 1.6 times ($V_{max}$=12.7, $V_{maxi}$=8.1), the affinity towards the substrate decreased too ($K_m$=3.8 mM and $K_{mi}$=4.0 mM) but change in $K_m$ was so insignificant that we could suggest that inhibition of apple phenoloxidase by inhibitor from pomegranate peel was noncompetitive.

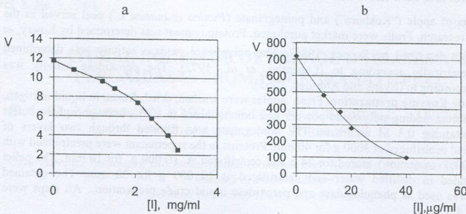


**Fig. 1.** Effect of natural inhibitor from pomegranate peel on apple phenoloxidase (a) and peroxidase (b) activities. V- specific activity ? E/mg protein/min

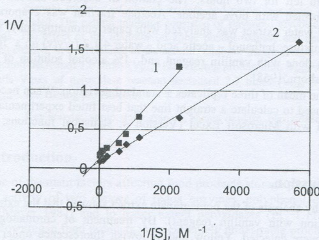


**Fig. 2.** Effect of substrate (catechol) concentration on apple phenoloxidase activity with (1) and without (2) natural inhibitor from pomegranate peel. V- specific activity ? E/mg protein/min

Peroxidase reaction without inhibitor was described by classical kinetics. $V_{max}$ and $K_m$ were calculated from Lineweaver – Burk plots. $V_{max}$=500 ΔE/mg protein/min; $K_m$=100 mM (Fig. 3b). In the presence of natural inhibitor from pomegranate peel the hyperbolic curve of the dependence of initial rate of enzymatic reaction on guaiacol concentration was changed into Sigmoidal one (Fig. 3a). Hill coefficient ($n_H$ ) was found to be 3,2 (Fig. 3c). By expressing the experimental data in $\frac{1}{[S]^{n_H}}, \frac{1}{V}$ coordinates $V_{maxi}$ and $[K]_{0,5}$ were calculated. $V_{maxi}$ was found to be 182 ΔE/mg protein/min and $[K]_{0,5}$ was equal to 167 mM (Fig. 3d). Both maximal rate of the peroxidase reaction and affinity towards the substrate decreased by the action of the inhibitor. This

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fact indicated to the complex manner of the inhibition of peroxidase by natural inhibitor from pomegranate peel.

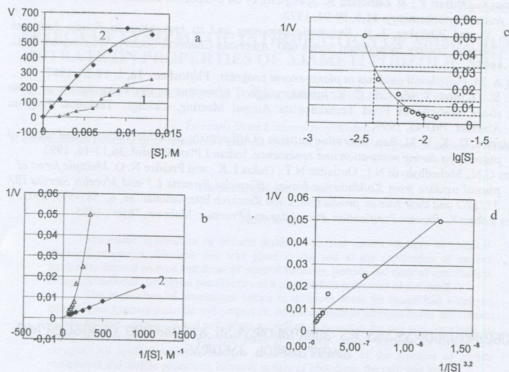


**Fig. 3.** Effect of substrate (guaiacol) concentration on the apple peroxidase activity with (1) and without (2) natural inhibitor from pomegranate peel (a, b) and determination of Hill coefficient (c), $V_{max}$ and $K_{0,5}$ (d).

Based on the obtained data the practical significance of presented studies becomes obvious. The inhibitor from pomegranate peel can be successfully used in apple products as an agent preventing undesirable browning process.

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## ᲤᲔᲜᲝᲚᲝᲥᲡᲘᲓᲐᲖᲐᲡᲐ ᲓᲐ ᲞᲔᲠᲝᲥᲡᲘᲓᲐᲖᲐᲡ ᲑᲣᲜᲔᲑᲠᲘᲕᲘ ᲘᲜᲰᲘᲑᲘᲢᲝᲠᲘ ᲑᲠᲝᲬᲔᲣᲚᲘᲡ ᲙᲐᲜᲘᲓᲐᲜ

[1]გულუა ლ., [1]ომიაძე ნ., [1]მჭედლიშვილი ნ., [1]ჩაჩუა ლ., [1]წიკლაური გ., [2]რეხვიაშვილი მ., [3]ტორბინი ვ.

*[1]საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი*
*[2]კვების მრეწველობის ინსტიტუტი*
*[3]თბილისის სახელმწიფო უნივერსიტეტის სოხუმის ფილიალი*

(მიღებულია 14.07.2004)

### ᲠᲔᲖᲘᲣᲛᲔ

ბროწეულის კანიდან გამოყოფილია ფენოლოქსიდაზასა და პეროქსიდაზას ბუნებრივი ინჰიბიტორი, რომელიც შედგება ფლავანოიდური გლიკოზიდებისაგან და ფენოლკარბოლის მჟავებისაგან. შესწავლილია ინჰიბიტორის გავლენა ვაშლის ფენოლოქსიდაზასა და პეროქსიდაზას აქტივობაზე. ინჰიბიტორის თანაობისას ვაშლის ფენოლოქსიდაზური რეაქციის მაქსიმალური სიჩქარე მცირდება 1.6-ჯერ ($V_{max}$=12.7ΔE/მგ ცილა/წთ, $V_{maxi}$=8.1ΔE/მგ ცილა/წთ), ხოლო პეროქსიდაზური რეაქციის- 2.7-ჯერ ($V_{max}$=500 ΔE/მგ ცილა/წთ, $V_{maxi}$ = 182 ΔE/მგ ცილა/წთ).

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ბიბლიოთეკა

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# EFFECT OF SYSTEMATIC INTRODUCTION OF AMMONIUM NITRATE ON PROPERTIES OF AJAMETI PODZOLIC SOIL

LOMSIANIDZE I.[1], SADUNISHVILI T.[2], DZAMUKASHVILI N[2]., NUTSUBIDZE N.[2]

[1]*Ak. Tsereteli State University of Kutaisi*
[2]*S. Durmishidze Institute of Biochemistry and Biotechnology of the Georgian Academy of Sciences*

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**Abstract**

Systematic application of mineral fertilizers in soil causes changes in physical-chemical properties. Changes that take place in the soil at the application of mineral fertilizers, depend on type and doses of mineral fertilizer, periods and rules of introduction, climatic conditions, biological peculiarities of a plant and on properties of soil itself.

The application of ammonium nitrate in different doses for maize had a definite influence on Ajamety podzolic soil properties. At the increase of nitrogen doses the content of total nitrogen, phosphate and potassium, as well as of humus in the soil was decreased. In spring and autumn low and average doses of nitrogen caused decrease of hydrolyzed nitrogen. All applied doses of nitrogen caused the increase of the amounts of mobile phosphorus and mobile potassium, however in case of high doses the gain was lower.

**Key words:** maize, mineral fertilizers, red soil, podzolic, calcareous, hydrolyzed nitrogen, mobile $P_2O_5$, exchangeable $K_2O$, field test.

## Introduction

The application of mineral fertilizes, besides enrichment of soil by nutrient compounds, causes changes in some physical-chemical characteristics of soil, in result of which plant nutrition conditions and chemical composition of harvest is improved or deteriorated.

Mineral fertilizers introduced into soil, before being utilized by plants undergo transformations, such as their solubilization and complex interactions between soil solid and liquid particles. In the cases of incorrect application of mineral fertilizers deterioration of some soil characteristics is observed, that negatively affects plant development. These changes significantly depend on mineral fertilizers, doses, time and ways of introduction, climatic conditions, biological peculiarities of a plant and soil properties. For example, at systematic introduction of physiologically acid fertilizers an acute increase of acidity of acid podzolic and red soils was observed that hinders the development of some crops and causes drop of harvest.

The principle nutrient elements for plant growth and development are: nitrogen, phosphorus and potassium. Balanced nutrition of a plant is obligatory, otherwise – the application of only one type of a fertilizer will negatively affect both soil and plant [Tsomaya 1998]. Effect of different types and dozes of different mineral fertilizers in different soils have been studied [Kudeyarov, 1988; Bugaev, Osipov, 1968]. The use of phosphorus-potassium fertilizers in soils

under vineyards in Khirsa and Mukhrani regions (Eastern Georgia), increased the content of plant assimilable forms of phosporus and potassium [Nakaidze L., 1965].

At systematic introduction of mineral fertilizers during 3-years (1998-2000) with doses N180-P150-K150 into the soils of Kharagauli Region (village Kitskhi, Western Georgia) with different properties, the content of humus, total nitrogen, phosphorus and potassium almost didn't change [Kelenjeridze N., 2001].

Effect of ammonium nitrate on Imereti podzolic soils properties is poorly studied. There is lack of information in literature that indicates the necessity of further research in this direction. So, the goal of present study was to investigate the effect of systematic introduction of ammonium nitrate on Imereti podzolic soil properties.

## Materials and methods

The field tests were carried out at Ajamety field-crop experimental station podzolic soil in 2000-2002. Ajamety is located in the area (Zemo Imereti) where alluvial, yellow-podzolic, yellow, red, raw humus calcareus, brown, mountain meadow soils are found. Most of yellow podzolic soils are covered by annual (maize, kidney bean) and perennial (vine, tea, fruit trees) agricultural crops [Lortkipanidze, 1997]. Genesis and physical-chemical peculiarities of the soil of Ajamety Field-Crop Experimental Station was studied [Kostava, 1947; Lezhava et al., 2001].

Field tests were conducted in eight variants in four repetitions. Soil treatment, application of fertilizers and plant care was conducted strictly according to the established agricultural rules [Chanishvili Sh., 1973]. Variety of maize *Zea mays,* Ajametis Tetri was used. Soil samples, necessary for testing were collected and treated according to the accepted methods. In spring of every year before introduction of fertilizers, in soil two layers (0-20, 20-40 cm) were determined: total nitrogen – by Kjeldahl method, hydrolizable nitrogen – by Tjurin and Kononova method, mobile $P_2O_5$ and exchangable $K_2O$ – in 0,1 $NH_2SO_4$ extract, by Oniani method. On the first and third years were established: total phosphorus – by Lorenze method, total potassium – on a flame photometer. In autumn, after harvest: hydrolized nitrogen, mobile $P_2O_5$ and exchangable $K_2O$ were determined [Oniani O., Margvelashvili G., 1978, Arinushkina E. 1961].

## Results and discussion

The conducted analyses showed that on the background of phosphate-potassium fertilizers, compared with unfertilized soils the application of increasing doses of ammonium nitrate causes decrease of total nitrogen in soil. This is more evident at the use of high nitrogen dozes N180 and N210.

Analogous changes are observed in content of total phosphorus and total patassium. An increase of introduced ammonium nitrate doses negatively affected total phosphorus and total potassium contents and decreased their amount in soil (Table 1).

Ajamety podzolic soil is characterized by low content of humus. As it is seen from Table 1 the applied dozes of ammonium nitrate only slightly decreased its amount in soil. These changes are more evident at the use of higher dozes of nitrogen that is proved by two last variants indices. In soil samples collected in spring and in autumn the amount of hydrolyzed nitrogen decreased compared to control and background levels. The obtained indices show no conformity between growth of doses and decrease of content, but increased doses of the introduced in spring ammonium nitrate yearly decreased hydrolyzed nitrogen content.

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**Table 1**. Effect of increasing doses of ammonium nitrate on the background of phosphate-potassium fertilizers on total nitrogen, phosphor and potassium content in soil

| # | Variant | Depth of layer, cm | Total nitrogen, % | | | Total $P_2O_5$, % | | Total $K_2O$, % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 2000 | 2001 | 2002 | 2000 | 2002 | 2000 | 2002 |
| 1 | Without fertilizer | 0-20 | 0.049 | 0.0147 | 0.149 | 0.062 | 0.064 | 8.19 | 2.17 |
| | | 20-40 | 0.136 | 0.133 | 0.135 | 0.060 | 0.061 | 2.17 | 2.16 |
| 2 | P120-K60 | 0-20 | 0.144 | 0.142 | 0.140 | 0.063 | 0.068 | 2.18 | 2.20 |
| | | 20-40 | 0.134 | 0.133 | 0.131 | 0.061 | 0.065 | 2.16 | 2.18 |
| 3 | P120-K60+N60 | 0-20 | 0.148 | 0.146 | 0.145 | 0.066 | 0.064 | 2.20 | 2.19 |
| | | 20-40 | 0.134 | 0.132 | 0.130 | 0.062 | 0.060 | 2.18 | 2.17 |
| 4 | P120-K60+N90 | 0-20 | 0.149 | 0.146 | 0.142 | 0.067 | 0.063 | 2.18 | 2.17 |
| | | 20-40 | 0.034 | 0.133 | 0.131 | 0.061 | 0.058 | 2.17 | 2.16 |
| 5 | P120-K60+N120 | 0-20 | 0.145 | 0.141 | 0.137 | 0.069 | 0.064 | 2.19 | 2.17 |
| | | 20-40 | 0.128 | 0.125 | 0.121 | 0.063 | 0.060 | 2.17 | 2.16 |
| 6 | P120-K60+N150 | 0-20 | 0.146 | 0.140 | 0.136 | 0.068 | 0.062 | 2.19 | 2.16 |
| | | 20-40 | 0.134 | 0:130 | 0.126 | 0.064 | 0.060 | 2.17 | 2.15 |
| 7 | P120-K60+N180 | 0-20 | 0.143 | 0.138 | 0.132 | 0.069 | 0.063 | 2.19 | 2.16 |
| | | 20-40 | 0.127 | 0.122 | 0.117 | 0.066 | 0.061 | 2.18 | 2.16 |
| 8 | P120-K60+N210 | 0-20 | 0.148 | 0.141 | 0.133 | 0.068 | 0.061 | 2.20 | 2.16 |
| | | 20-40 | 0.129 | 0.123 | 0.116 | 0.065 | 0.059 | 2.18 | 2.15 |

In soil samples taken in autumn, in most cases of variants these indices yearly decreased, except two latter variants. So, at the use of high doses of nitrogen the content of hydrolyzed nitrogen is higher in autumn, than in spring. It could be explained by the fact that a part of ammonium nitrate applied in excess amount, which remains untaken by a plant, can't be washed out completely (Table 2).

**Table 2.** Effect of increasing doses of ammonium nitrate on the background of phosphate-potassium fertilizers on humus and hydrolyzed nitrogen content in soil

| N | Humus, % | | | Hydrolyzed nitrogen, mg per 100g of soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Before the experiment | | | After harvest | | |
| | 2000 | 2001 | 2002 | 2000 | 2001 | 2002 | 2000 | 2001 | 2002 |
| 1 | 2.88 | 2.78 | 2.85 | 4.06 | 4.03 | 3.99 | 4.02 | 4.03 | 4.06 |
| | 1.71 | 1.68 | 1.72 | 3.72 | 3.70 | 3.68 | 3.66 | 3.64 | 3.65 |
| 2 | 2.17 | 2.65 | 2.58 | 4.08 | 4.10 | 4.06 | 4.06 | 4.08 | 4.09 |
| | 1.74 | 1.69 | 1.63 | 3.70 | 3.72 | 3.69 | 3.60 | 3.68 | 3.69 |
| 3 | 2.88 | 2.80 | 2.70 | 2.40 | 4.18 | 4.13 | 4.18 | 4.15 | 4.09 |
| | 1.71 | 1.65 | 1.58 | 3.72 | 3.71 | 3.67 | 3.70 | 3.68 | 3.62 |
| 4 | 2.84 | 2.75 | 2.65 | 4.18 | 4.14 | 4.06 | 4.16 | 4.11 | 3.01 |
| | 1.69 | 1.62 | 1.54 | 3.83 | 3.79 | 3.72 | 3.80 | 3.76 | 3.68 |
| 5 | 2.89 | 2.80 | 2.69 | 4.29 | 4.23 | 4.12 | 4.25 | 4.18 | 4.07 |
| | 1.73 | 1.65 | 1.56 | 3.94 | 3.84 | 3.78 | 3388 | 3.80 | 3.73 |
| 6 | 2.76 | 2.66 | 2.55 | 4.26 | 4.21 | 4.12 | 4.22 | 4.14 | 4.04 |
| | 1.70 | 1.62 | 1.53 | 4.06 | 3.97 | 3.84 | 3.99 | 3.94 | 3.85 |
| 7 | 2.65 | 2.53 | 2.40 | 4.18 | 4.06 | 3.90 | 4.20 | 4.10 | 3.96 |
| | 1.61 | 1.52 | 1.41 | 3.83 | 3.73 | 3.61 | 3.90 | 3.82 | 3.71 |
| 8 | 2.82 | 2.69 | 2.55 | 4.30 | 4.16 | 3.98 | 4.29 | 4.16 | 4.02 |
| | 1.71 | 1.62 | 1.49 | 3.94 | 3.82 | 3.68 | 3.96 | 3.86 | 3.74 |

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Introduction of increased dozes of ammonium nitrate into soil in spring increases the content of mobile $P_2O_5$. An increase takes place every year of experiment in accordance with the growth of nitrogen doses. High dozes of nitrogen N180 and N210 is the reason for decrease of mobile phosphorus amount.

After harvest the content of mobile phosphorus is higher compared with spring indices and increases every year. High dozes of nitrogen negatively affect the growth of mobile phosphorus content and in samples taken in autumn its decrease is observed (Table 3).

**Table 3**. Effect of increasing doses of ammonium nitrate on the background of phosphate-potassium fertilizers on mobile phosphorus content in soil

| N | Mobile $P_2O_5$, mg per 100g soil | | | | | |
|---|---|---|---|---|---|---|
| | Before the experiment | | | After harvest | | |
| | 2000 | 2001 | 2002 | 2000 | 2001 | 2002 |
| 1 | 1.86 | 2.03 | 1.94 | 1.84 | 1.92 | 1.88 |
| | 1.87 | 1.91 | 1.86 | 1.72 | 1.76 | 1.83 |
| 2 | 1.82 | 1.96 | 2.12 | 2.02 | 2.18 | 2.35 |
| | 1.66 | 1.78 | 1.92 | 1.82 | 1.95 | 2.11 |
| 3 | 1.82 | 1.98 | 2.16 | 2.05 | 2.22 | 2.412.23 |
| | 1.70 | 1.84 | 2.01 | 1.90 | 2.05 | 2.49 |
| 4 | 1.88 | 2.06 | 2.28 | 2.06 | 2.26 | 1.28 |
| | 1.76 | 1.91 | 2.09 | 1.93 | 2.09 | 2.58 |
| 5 | 1.99 | 2.20 | 2.45 | 2.10 | 2.33 | 2.38 |
| | 1.78 | 1.95 | 2.14 | 1.99 | 2.17 | 2.69 |
| 6 | 2.01 | 2.26 | 2.52 | 2.14 | 2.41 | 2.44 |
| | 1.80 | 2.01 | 2.24 | 1.97 | 2.19 | 2.28 |
| 7 | 1.86 | 1.98 | 2.12 | 1.98 | 2.12 | 1.99 |
| | 1.77 | 1.97 | 2.09 | 1.84 | 1.96 | 2.34 |
| 8 | 2.06 | 2.14 | 2.24 | 2.12 | 2.22 | 2.20 |
| | 1.84 | 1.90 | 1.98 | 2.02 | 2.10 | |

**Table 4**. Effect of increasing doses of ammonium nitrate on the background of phosphate-potassium fertilizers on exchangeable potassium content in soil

| N | Exchangeable $K_2O$, mg per 100g soil | | | | | |
|---|---|---|---|---|---|---|
| | Before the experiment | | | After harvest | | |
| | 2000 | 2001 | 2002 | 2000 | 2001 | 2002 |
| 1 | 8.5 | 8.3 | 8.4 | 8.7 | 8.1 | 8.3 |
| | 7.8 | 7.7 | 7.9 | 7.5 | 7.3 | 7.9 |
| 2 | 8.2 | 8.7 | 9.3 | 9.3 | 10.0 | 10.8 |
| | 7.5 | 7.9 | 8.4 | 8.1 | 8.6 | 9.2 |
| 3 | 8.0 | 8.6 | 9.3 | 8.3 | 9.1 | 10.0 |
| | 7.5 | 8.0 | 8.9 | 7.6 | 8.2 | 8.9 |
| 4 | 8.5 | 9.3 | 10.2 | 8.7 | 10.2 | 11.2 |
| | 7.8 | 8.5 | 9.4 | 8.1 | 8.9 | 9.8 |
| 5 | 8.3 | 9.2 | 10.2 | 8.6 | 9.6 | 10.7 |
| | 7.6 | 8.4 | 9.3 | 7.9 | 8.8 | 9.8 |
| 6 | 8.5 | 9.6 | 10.9 | 8.7 | 9.9 | 11.3 |
| | 7.8 | 8.8 | 9.9 | 8.1 | 9.2 | 10.5 |
| 7 | 7.9 | 8.8 | 9.8 | 8.6 | 9.6 | 10.8 |
| | 7.4 | 8.1 | 9.0 | 7.8 | 8.6 | 9.6 |
| 8 | 8.1 | 8.8 | 9.6 | 8.4 | 9.2 | 10.1 |
| | 7.5 | 8.0 | 8.7 | 7.6 | 8.2 | 9.0 |

At introduction of ammonium nitrate into Ajamety podzolic soil the change of exchangeable potassium content is observed. In spring its amount increases at every applied dozes of nitrogen, but in case of high dozes the amount of mobile phosphorus decreases.

After harvest, in soil the content of exchangeable potassium is higher than in spring in case of all applied dozes of nitrogen and every year of observation. High dozes of ammonium nitrate even in this case also negatively affect content of exchangeable potassium and cause its decrease (Table 4).

Thus, application of ammonium nitrate in different doses for maize had affected both total and hydrolyzed nitrogen, mobile phosphorus and potassium as well as humus content in Ajamety podzolic soil.

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# ამონიუმის ნიტრატის სისტემატური შეტანის გავლენა აჯამეთის ეწერი ნიადაგის თვისებებზე

ლომსიანიძე ი.[1], სადუნიშვილი თ.[2], ძამუკაშვილი ნ.[2], ნუცუბიძე ნ.[2]

*[1]ქუთაისის აკ. წერეთლის სახელმწიფო უნივერსიტეტი,*
*[2]საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი*

(მიღებულია 06.09.2004)

## რეზიუმე

მინერალური სასუქების სისტემატური გამოყენებით ნიადაგში ადგილი აქვს ფიზიკურ-ქიმიური ხასიათის ცვლილებებს. ნიადაგისა და სასუქის ურთიერთქმედებისა და მინერალური სასუქების გამოყენების წესების არცოდნის გამო ადგილი აქვს ნიადაგის ზოგიერთი თვისების გაუარესებას, რაც უარყოფითად მოქმედებს სასოფლო-სამეურნეო კულტურების მოსავლიანობაზე. ნიადაგში მომხდარი ცვლილებები დამოკიდებულია მინერალური სასუქის ფორმებზე, დოზებზე, შეტანის ვადებზე, კლიმატურ პირობებზე, კულტურის ბიოლოგიურ თვისებებზე და თვით განსხვავებული ნიადაგის თვისებებზე.

სიმინდის კულტურაზე ამონიუმის ნიტრატის სხვადასხვა დოზებით გამოყენებამ გარკვეული გავლენა მოახდინა აჯამეთის ეწერ ნიადაგის თვისებებზე. აზოტის დოზის ზრდამ შეამცირა ნიადაგში საერთო აზოტის, ფოსფორის, კალიუმის და ჰუმუსის შემცველობა. გაზაფხულზე და შემოდგომით დაბალმა და საშუალო დოზებმა გამოიწვია ჰიდროლიზური აზოტის შემცირება. აზოტის გამოყენებულმა ყველა დოზამ გაზარდა მოძრავი ფოსფორის და კალიუმის რაოდენობა, მაგრამ მაღალი დოზების გამოყენების შემთხვევაში ნამატი შემცირდა.

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ბიბლიოთეკა

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# BASIDIOMYCETES OF GEORGIA – PRODUCERS OF LIGNOCELLULOLYTIC ENZYMES

TSIKLAURI N., KACHLISHVILI E., KUTATELADZE L., METREVELI E., ASATIANI M., SONGULASHVILI G., ALADASHVILI N.

*S. Durmishidze Institute of Biochemistry and Biotechnology of the Georgian Academy of Sciences*

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**Abstract**

Cellulase, xylanase, laccase, and Mn-dependent peroxidase activity of Basidiomycetes spread in the territory of Georgia has been studied. In particular, identified pure strains of fungi have been cultivated on cuttings of grapevine sawdust in solid-state and on mandarin peels in submerged fermentation conditions. Among 15 Basidiomycetes strains, *Trametes versicolor* IBB 13, *Pleurotus dryinus* IBB 93, *Pseudotremella guilbosa* IBB 22 appeared to be the perspective producers of lignocellulolytic enzymes. It has been shown that lignocellulosic growth substrate and method of fungi cultivation are important factors influencing on the yield of aiming enzymes.

**Key words**: basidiomycetes, xylanase, laccase, cellulase, Mn-dependent peroxidase

25920

## Introduction

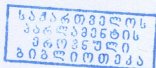
Significant amount of agricultural and industrial wastes, increasingly accumulating in the last years, becomes the reason of environmental pollution. That's why the bioconversion of lignocellulosic raw materials appeared in the center of general attention. Bioconversion of these wastes by higher Basidiomycetes is one of the perspective directions of their application as the value-added products [Breene, 1990; Cohen et al., 2002]. During last years it has been also shown that Basidiomycetes are able to remediate polluted soils and waters [Pointing, 2001].

According to numerous scientific works the white rot Basidiomycetes are the only organisms able to synthesize the extracellular hydrolytic (cellulase and hemicellulase) and oxidizing (ligninolytic) enzymes [Hattaka, 1994; Tekere et al., 2001; Cohen et al., 2002]. By means of these enzymes Basidiomycetes decompose lignocellulosic raw material to low-molecular substances and utilize it as a nutrient source.

Experimental data clear indicate that fungal development, protein accumulation, synthesis of enzymes etc. significantly depend on the fungi growth substrate and method of cultivation [Pirhonen, Hattaka, 1985; Elisashvili et al., 2003].

The goal of the given experimental work was to study the ability of secretion of the lignocellulolytic enzymes (cellulase, xylanase, laccase and Mn-dependent peroxidase) in Basidiomycetes isolated from different ecological niches of Georgia and cultivated on diverse plant substrates. To reveal the perspective producers of the enzymes 15 representatives from different genera of basidiomycetous fungi were studied in plant material bioconversion.





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## Materials and Methods

Basidiomycetes for cultivation were grown in 750 ml flasks on shaker (200 rpm). The nutrient medium composition was (g/l): glucose – 10.0, $NH_4NO_3$ – 2.0, yeast extract – 2.0, $KH_2PO_4$ – 0.6, $Na_2HPO_4$ – 0.2, $MgSO_4 \cdot 7H_2O$ – 0.5, $ZnSO_4 \cdot 7H_2O$ – 0.001, $FeSO_4 \cdot 7H_2O$ – 0.0005, $CaCl_2 \cdot 2H_2O$ – 0.06, pH – 5.8-6.0. Obtained mycelium was homogenized and suspension was used as an inoculum.

One year cuttings of grapevine sawdust (GC) and mandarin peels (MP) were used as cultivating substrates for Basidiomycetes. For screening the fungi submerged cultivation has done in 750 ml flasks, containing 100 ml of above mentioned nutrient medium and 4g of GC or MP. At the end of cultivation the plant-protein product was separated by centrifugation, obtained biomass was dried for measuring the protein content and the cultural liquid was used as a source of enzymes.

The solid-state fermentation (SSF) was done in 100 ml flasks containing 4g of GC (particles size 0.5-2.0 mm) moistured by 12 ml of above mentioned synthetic medium without glucose. After the cultivation was over, part of the biomass, consisting of fungi mycelium, plant substrate and protein was dried at 100°C (to determine the content of protein in the biomass). The residual part of the biomass was washed and extracted for four times with 20 ml of distilled water. The extracts were centrifuged, filtered in a glass filters and used for determining the enzymatic activity. Cultivating temperature of Basidiomycetes was 27°C.

To measure the enzymatic activity the following methods have been used: xylanase and carboximethylcellulase (CMCase) were assayed by standard IUPAC method in 0.05M citrate buffer, pH 5.0 [Ghose, 1987]. Laccase activity was determined by oxidation of syringaldazine [Leonowicz, Grzywnowicz, 1981], and Mn-dependent peroxidase (MnP) activity was measured spectrophotometrically by the oxidation of NADH.

## Results and Discussion

According to the experimental results, it was revealed that basidiomycetous fungi differ by their ability of protein accumulation and enzymes synthesis in solid-state fermentation of GC. Among white rot fungi protein content was accumulated from 5.3 (*F. fomentarius* IBB 16) to 7.5 (*P. dryinus* IBB 903) mg of biomass (Table 1), while in brown rot fungi (*G. saepiarium* IBB 155, *P. aurivella* IBB 43, *D. quercina* IBB 56) the amount of the protein, formed after the substrate bioconversion, made 4.8-5.0 mg.

Citrus peels appeared to be the better substrate for growth and enzymes production by the same fungi (Table 2). The amount of protein in fermented products increased 2 times and more at submerged fermentation of the substrate. Among the studied fungi the best producers of microbial protein appeared to be white rot fungi *T. versicolor* IBB 13 and *P. dryinus* IBB 93.

While studying the enzymes activity, more differences were revealed between white and brown rot fungi. During the solid-state fermentation of GC the activity of CMCase varied from 0.5 U/ml (*D. quercina* IBB 56) to 10.2 U/ml (*T. versicolor* IBB 13), and of xylanase – within the range 0.6 – 12.4 U/ml (Table 1).

From the literature it is known that ligninolytic activity is characteristic only for white rot fungi. In our experiments, among white rot fungi the highest levels of laccase and MnP were accumulated in GC solid-state fermentation by *T. versicolor* IBB 13 followed by *P. dryinus* IBB 93 and *C. hirsutus* IBB 31. As for brown rot fungi, no activity or only traces of these enzymes activity were revealed that coincide with literature data (Table 1).

Basidiomycetes submerged cultivation on mandarin peels appeared to be more convenient for revealing the ability of enzyme synthesis (Table 2). The best producers of CMCase and

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xylanase were - *P. gilbosa* IBB 22, *T. versicolor* IBB 13 and *L. nuda* IBB 72 that accumulated CMC activity, correspondingly, 25.6, 27.8, and 29.4 U/ml. In the same strains secretion of xylanase made 30.0, 40.9, and 35.2 U/ml, correspondingly. Conditions of mandarin peels fermentation were favorable for brown rot fungi too. Accumulation of cellulolytic enzymes in some cultures increased even 10 times compared with the solid-state fermentation of GC.

The study of basidiomycetes ligninolytic enzymes activity in mandarin peels submerged fermentation the highest laccase activity (34.9 nkat/ml) was revealed in *T. versicolor* IBB 13, and Mn-peroxidase activity (10.3 nkat/ml) - in *P. ostreatus* IBB 108. Brown rot fungi didn't synthesize even the traces of these enzymes in the same culture conditions.

So, this study shows that strains belonging to the same genus revealed different abilities of protein accumulation and enzyme production. For example, among four representatives of the genus *Pleurotus* CMCase activity in fungi submerged fermentation varied from 6.4 to 17.7 U/ml, while laccase activity ranged from 0.6 to 6.7 nkat/ml. It is noteworthy that screening of the Basidiomycetes isolated from different ecological niches in bioconversion of plant raw materials has revealed that white rot fungi are distinguished as the best producers of microbial protein and extracellular lignocellulolytic enzymes. In addition, the submerged fermentation is regarded to be more suitable for Basidiomycetes cultivation to receive the highest yield of aiming products.

**Table 1.** Basidiomycetes enzymatic activity after the solid-state fermentation of cuttings of grapevine sawdust

| Strain | Biomass protein mg | CMC U/ml | Xylanase U/ml | Laccase nkat/ml | Mn-peroxidase nkat/ml |
|---|---|---|---|---|---|
| *Pleurotus ostreatus* IBB8 | 5.4 | 5.7 | 6.2 | 0.3 | 0.9 |
| *Pleurotus ostreatus* IBB108 | 6.1 | 4.3 | 4.4 | 0.5 | 1.0 |
| *Pleurotus dryinus* IBB93 | 7.5 | 9.0 | 12.1 | 1.3 | 2.1 |
| *Pleurotus ostreatus* IBB77 | 6.9 | 3.1 | 4.2 | 0.2 | 0.5 |
| *Fomes fomentarius* IBB16 | 5.3 | 1.8 | 2.7 | 0.1 | 0.1 |
| *Coriolus hirsutus* IBB31 | 7.2 | 2.2 | 2.3 | 1.7 | 0.2 |
| *Psudotremella gilbosa* IBB22 | 6.1 | 3.3 | 2.6 | 0.3 | 0.1 |
| *Phelinus robustus* IBB29 | 5.7 | 2.5 | 2.9 | 0.3 | 0.2 |
| *Trametes versicolor* IBB13 | 5.4 | 10.2 | 12.4 | 8.2 | 3.2 |
| *Ganoderma lucidum* IBB47 | 6.0 | 1.6 | 2.1 | 0.3 | 0.2 |
| *Lentinus edodes* IBB123 | 5.9 | 1.1 | 1.5 | 0.4 | 0.3 |
| *Lepista nuda* IBB72 | 4.8 | 2.3 | 4.2 | 0.5 | 1.2 |
| *Gloeophyllum saepiarium* IBB155 | 5.0 | 0.9 | 3.6 | 0.2 | 0 |
| *Pholiota aurivella* IBB43 | 5.0 | 0.8 | 1.5 | 0.1 | 0.2 |
| *Dactolea quercina* IBB56 | 5.0 | 0.5 | 0.6 | 0 | 0.1 |

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**Table 2.** Basidiomycetes enzymatic activity in mandarin peels submerged fermentation

| Strain | Biomass protein mg | CMC U/ml | Xylanase U/ml | Laccase nkat/ml | Mn-peroxidase nkat/ml |
|---|---|---|---|---|---|
| *Pleurotus ostreatus* IBB8 | 11.2 | 17.7 | 9.6 | 0.6 | 3.2 |
| *Pleurotus ostreatus* IBB108 | 11.1 | 6.4 | 5.4 | 2.8 | 10.3 |
| *Pleurotus dryinus* IBB93 | 13.5 | 13.7 | 16.4 | 6.7 | 8.9 |
| *Pleurotus ostreatus* IBB77 | 13.2 | 8.1 | 6.6 | 2.3 | 2.9 |
| *Fomes fomentarius* IBB16 | 11.3 | 3.1 | 4.8 | 1.2 | 0.7 |
| *Coriolus hirsutus* IBB31 | 10.8 | 3.4 | 5.1 | 6.3 | 0.8 |
| *Psudotremella gilbosa* IBB22 | 13.1 | 25.6 | 30.0 | 21.4 | 0.9 |
| *Phelinus robustus* IBB29 | 12.8 | 2.9 | 3.5 | 13 | 2.1 |
| *Trametes versicolor* IBB13 | 13.9 | 27.8 | 40.9 | 34.9 | 7.7 |
| *Ganoderma lucidum* IBB47 | 10.3 | 10.1 | 13.0 | 0.9 | 1.0 |
| *Lentinus edodes* IBB123 | 11.0 | 7.6 | 10.4 | 4.2 | 0.3 |
| *Lepista nuda* 1BB72 | 12.5 | 29.4 | 35.2 | 5.1 | 4.5 |
| *Gloeophyllum saepiarium*1BB155 | 11.7 | 10.3 | 17.3 | 0 | 0 |
| *Pholiota aurivella* IBB43 | 11.9 | 5.3 | 16.2 | 0 | 0 |
| *Dactolea quercina* IBB56 | 11.8 | 0.5 | 1.3 | 0 | 0 |

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# საქართველოს ტერიტორიაზე გავრცელებული ლიგნოცელულაზური ფერმენტების პროდუცენტი ბაზიდიომიცეტები

წიკლაური ნ., ქაჩლიშვილი ე., ქუთათელაძე ლ., მეტრეველი ე., ასათიანი მ., სონღულაშვილი გ., ალადაშვილი ნ.

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

(მიღებულია 26.07.2004)

## რეზიუმე

გამოკვლეულია საქართველოს ტერიტორიაზე გავრცელებულ ბაზიდიომიცეტებში ცელულაზის, ქსილანაზის, ლაკაზის და Mn-პეროქსიდაზის აქტივობა. იდენტიფიცირებული სუფთა კულტურების შესწავლა ხორციელდებოდა ვაზის ანასხლავების ნაჭერხის მყარფაზოვანი და მანდარინის ფქვილის სიღრმული ფერმენტაციის პირობებში. 15 შესწავლილი შტამიდან *Trametes versicolor* IBB13, *Pleurotus dryinus* IBB93, *Pseudotremella guilbosa* IBB22 აღმოჩნდა ლიგნინოცელულაზური ფერმენტების ყველაზე პერსპექტიული პროდუცენტები. ნაჩვენებია, რომ მცენარეული სუბსტრატი და კულტივირების მეთოდი მნიშვნელოვან გავლენას ახდენს ფერმენტის გამოსავლიანობაზე.

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ბიბლიოთეკა

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Biophysics

# SYNTHESIS OF TITIN IN EMBRYOGENESIS

GACHECHILADZE N., MELIKISHVILI M., TORIASHVILI T., LOMIDZE L., ZAALISHVILI T., JAPARIDZE N., ZAALISHVILI M.

*Institute of Molecular Biology and Biological Physics, Georgian Academy of Sciences*

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**Abstract**

During the process of lake frog embryogenesis gel-electrophoresis shows the polypeptide band, as a trace, identical with titin molecular mass motility at fadpole stage, while the nebulin is seen even in an unimpregnate egg. During this process, from unimpregnate egg to fadpole stage, the polypeptide bands corresponding to nebulin and muscle contractile proteins - myosin, actin and tropomyosin are simultaneously synthesised.

The immunoblotting by titin antibody T-11 at the different stages of frog embryogenesis gives the immunoband which does not correspond to titin molecule with its motility and shows identification with polypeptide band having low motility. We can suppose that at early stages of frog embryogenesis at first the short polypeptide chain corresponding to epitope T-11 is formed and then, at the stage when fadpole becomes an adult frog the whole titin molecule is synthesised. Proceeding from this we can conclude, that while biosynthesis of protein that consists of many sites of different nature, the separate sites are synthesised first and then posttranslating combination takes place.

**Key words**: titin, nebulin, unimpregnate egg, fadpole, western blotting.

## Introduction

Besides the main contractile proteins the muscle contains giant proteins with high molecular mass – titin and nebulin. Their size and location within the sarcomere structure determine their important role in the mechanism of muscle elasticity. The giant polypeptide titin is complex multi domain protein [Maruyama, 1994; Wang et al., 1979; Trinick et al.,1984; Zaalishvili et al., 1999]. I-band of titin consists of structurally distinct segments, tandem of immunoglobulin domain chains and unique sequence and PEVK-reach sequence. A-band of titin is mainly composed of Ig-like and FN3-like repeats. It also contains a class of unique sequence insertions: phosphorilation sites, a serine/treonine kinase domain and binding sites for specific muscle protease - calpain. The early appearance of titin in myofibrilogenesis suggests its probable participation in filament alignment during sarcomere assembly [Horowits & Podolsky, 1987; Furst et al., 1989].

A number of data show that the structure of titin determines the structure of sarcomere. After the separation of myosin and actin filaments in the sarcomere the protein skeleton stays, which keeps the properties of elasticity continuously. These properties have been connected with titin and nebulin. Proceeding from this it is obvious that for sarcomere arrangement at first titin and then the other proteins must be synthesized [Horowits & Podolsky, 1987; Furst et al., 1989; Tokuyasu & Maher, 1987].

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ბიბლიოთეკა

Some authors suggest that the alignment of titin and myosin into the sarcomere takes place at the same time [Furst et al., 1989; Tokuyasu & Maher, 1987; Sanger et al., 2002], but the synthesis of titin while myofibrilogenesis is still unclear.

Experiments carried out at the skeletal muscle cells show that the nascent myofibrills are formed from premyofibrills and modified by the addition of titin and muscle myosin filament [Tokuyasu & Maher, 1987].

Recently the protein (D-titin) identical with muscle sarcomere titin has been found in chromosomes of *Drosophilae* embryos. The N-terminal end is homogeneous with muscle titin that is confirmed by immunoblotting [Machado & Andrew, 2000].

Proceeding from abovementioned it is interesting to determine on which step of embryogenesis titin synthesis takes place. We have studied the synthesis of titin on diferent stages of embryogenesis of lake frogs (*Rana Ridibunda*).

## Materials and Methods

The experiments were carried out using the frog eggs; they are the most convenient object for investigation as their development can be observed without any equipment and from one pair of frogs a big amount of impregnate eggs at any season of year (~1000) can be obtained.

The frog mature eggs have been obtained according to Kabakh [Kabakh, 1945]. For the early ripening of oocites the female frogs were injected with hypophisis suspension. After injection they were placed into the vessel with small amount of water. The males were in the same conditions. The spawn from females ready for ovulation was eased by pressing on the bell of a frog. The impregnation was carried out according to the following way: seminal was cut from male frog, minced and diluted by dechlorined water, then the females spawn in this water. The first division could be seen within 2-2,5 hours from impregnation, the second – after 3 hours and so on.

The samples for SDS-gel electrophoresis were taken on the different steps of embryogenesis. The 3 volumes of Hasselbach-Schneider buffer (pH 7, buffer contained inhibitors) was added to eggs at different stages and the mixture was homogenized and centrifugated at 10.000g for 20 min. The upper layer of yolk was removed by pipette and transparent supernatant was used for registration of myofibrillar protein components.

Protein concentration was determined by the burette method. SDS-gel electrophoresis was carried out in 2,5-12% acrylamide gradient according to Laemmly [Laemmly, 1970]. The myofibrills of rabbit and frog skeletal muscles were used as control. The identification of protein having titin-like electrophoretic motility with titin was tested using the western blotting. For that the proteins separated by SDS gel-electrophoresis were transferred onto 0,45 μm nitrocellulose sheets according to Fritz [Fritz et al.,1989], which was specially worked out for high-molecular weight myofibrillar proteins. For titin recognation nitrocellulose sheets were treated with monoclonal antibodies (T II). The binding of antibodies was detected by the horseradish peroxidase conjugated monoclonal anti-goat immunoglobulin [Towbin et al., 1979].

## Results and Discussion

Using SDS-gel-electrophoresis gradient (5-15%) we have shown [Gachechiladze et al., 1987] that in the process of frog embryogenesis, beginning from unimpregnated egg up to definite fadpole, the morphological and structural changes take place. The amount of polypeptide chains with molecular mass 200, 102, 36 kD sufficiently increase. The polypeptide chains with molecular mass 282, 240, 205, 170, 152, 148, 126, 112 and 96 kD appear at the different steps of

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ბიბლიოთეკა

embryogenesis, and 44 kD polypeptide chain doesn't change. But titin was not observed because of its great molecular mass.

In this work we used the SDS-electrophoresis in less acrylamide gradient (2.5-12%).

On the electrophoregrams (Fig.1a,b) of the all stages of frog embryogenesis beginning from unimpregnate eggs up to definite fadpole, the polypeptide bands of nebulin, actin and tropomyosin are observed (Fig.1a,b -3,4,5,6,7 – N, A, TM ). It indicates that nebulin exists together with main muscle contractile proteins, particularly with actin.

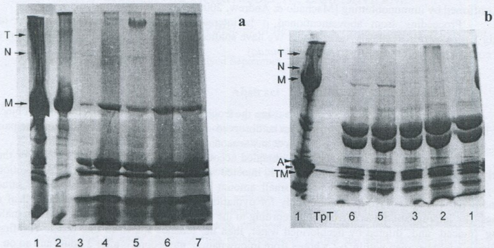


**Fig. 1.** Electroforegram of frog embryons at different stages of embryogenesis.Electrophoresis in 2,5-12% SDS gradient. T – titin, N – nebulin, M – myosin heavy chain, A – actin, Tp T – troponin T, TM - tropomyosin.

1 - frog muscle myofibrills; 2 - rabbit muscle myofibrills; 3- unimpregnate eggs;

4 - 24 h after impregnate; 5 - 18th stage the response on irritation; 6 - 19th stage, fadpole; 7 - fadpole tails.

Using PAGE Loker et al [Loker et al.,1986] have shown the existence of polypeptide band corresponding to titin molecular mass motility in frog fadpole that is in accordance with our data. Namely, on the 19th stage of frog embryogenesis when the fadpoles are already active, PAGE shows titin polypeptide band, as a trace (Fig.1a,b - 6).

PAGE bands of frog and rabbit myofibrilles revealing titin molecular mass motility (Fig.2a-1,2-T) correspond to titin immunobands obtained by immunoblotting (Fig.2b-1,2-T).

Titin immunoblotting (monoclonal antititin-T11) at the different stages of frog embryogenesis (from unimpregnate eggs up to fadpole) gives the band which does not correspond to titin molecular motility and immunoband is seen as polypeptide band with low motility (~100 kd, Fig.2b-3,4,5,6,7). The epitope T11 is situated between the end of myosin protofibrill and PEVK site. We can suppose that at early stages of frog embryogenesis first the short polypeptide chain, which corresponds to epitope T11 is formed and then the whole titin molecule is synthesised at the stage when fadpole becomes an adult frog.

Proceeding from this we can conclude that while biosynthesis of large proteins the molecule of which consists of many sites having different nature, the separate sites are synthesised first and then posttranslating combination takes place.

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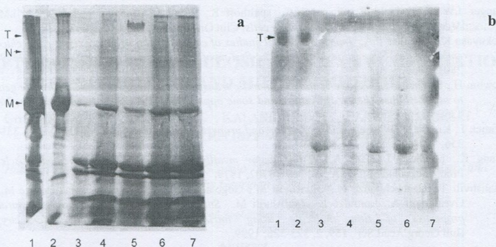


**Fig. 2.** Western-blot of SDS gel at different stages of frog embryogenesis treated with anti-titin (TII)
a - coomassic blue staining of gel strip before transfer conditions the same as in fig. 1.
b - immunostaining with anti-titin of a nitrocellulose strip after transfer
1 - frog muscle myofibrills; 2 - rabbit muscle myofibrills; 3 – unimpregnate eggs; 4- 24h after impregnate; 5 -18$^{th}$ stage the response on irritation; 6 - 19$^{th}$ stage, fadpole; 7 – fadpole tails

The investigation of embryogenesis of a giant protein titin may help us to explain some aspects of mechanism of biosynthesis of macromolecules with many specific motifs and mechanism of precise assembly of myofibrillar proteins and other complex systems. This suggestion needs further investigation.

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გაჩეჩილაძე ნ., მელიქიშვილი მ., ტორიაშვილი თ., ლომიძე ლ.. ზაალიშვილი თ., ჯაფარიძე ნ., ზაალიშვილი მ.

*საქართველოს მეცნიერებათა აკადემიის მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი*

(მიღებულია 20.09.2004)

### რეზიუმე

ბაყაყის ემბრიონალური განვითარების პროცესში, პოლიაკრილამიდის გელ-ელექტროფორეზი, თავკომბალის სტადიაზე, უჩვენებს კვალის სახით ტიტინის მოლეკულური მასის ძვრადობის იდენტურ პოლიპეპტიდურ ზოლს, მაშინ როდესაც ნებულინი ჩანს გაუნაყოფიერებელ კვერცხშიც. ბაყაყის ემბრიონალური განვითარების პროცესში, დაწყებული კვერცხის განაყოფიერებიდან თავკომბალის სტადიამდე, ერთდროულად სინთეზირდება ნებულინისა და კუნთის შეკუმშვადი ცილების - მიოზინის, აქტინის, ტროპომიოზინის მოლეკულური მასის შესაბამისი პოლიპეპტიდური ჯაჭვები.

ბაყაყის ემბრიონალური განვითარების სხვადასხვა სტადიაზე იმუნობლოტინგი ტიტინის ანტისხეულით (T-11) იძლევა იმუნოზოლს, რომელიც თავისი ძვრადობით შეესაბამება დაბალი ძვრადობის მქონე პოლიპეპტიდურ ჯაჭვს. შესაძლებელია ვივარაუდოთ, რომ ბაყაყის ემბრიოგენეზის ადრეულ სტადიებზე ჯერ სინთერზირდება მოკლე პოლიპეპტიდური ჯაჭვი, რომელიც შეესაბამება T-11-ის ეპიტოპს, ხოლო ტიტინის მთლიანი მოლეკულა წარმოიქმნება თავკომბალიდან ზრდასრულ ასაკში გადასვლისას. აქედან გამომდინარე შეიძლება დავუშვათ, რომ რთული, განსხვავებული უბნებისაგან შემდგარი ცილების ბიოსინთეზის დროს, ჯერ ხდება ცალკეული უბნების წარმოქმნა და შემდეგ პოსტტრანსლიაციური შეერთება.

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Biotechnology

# PRETREATMENT OF CELLULOSIC WASTES BY COMBINATION OF CHILLING AND MILLING METHODS

CHACHKHIANI M. [1], DUDAURI T. [1], KALANDADZE N. [1], PARTSKHALADZE G. [1], TSIKLAURI L. [1], UGREKHELIDZE V. [1], ZAKARIASHVILI N. [2]

[1]*National High Technology Center of Georgia*, [2]*Durmishidze Institute of Biochemistry and Biotechnology of the Georgian Academy of Science*

(Received September 6, 2004)

**Abstract**

Cellulose pretreatment method based on combination of chilling and mechanical milling of cellulosic wastes was used for investigation of structural changes of different cellulosic materials. Expansion of frozen water contained in cellulosic wastes causes the mechanical stress that is followed by partial destruction of crystalline cellulose. Cellulose decomposition rate, changes of cellulose polymerization degree and specific surface area of frozen at 0, -20, -70 and -196 $^0$C cellulosic wastes: sawdust, cardboard, newsprint, filter paper and composition of all above mentioned materials have been determined. Studies showed that mechanical milling of frozen cellulosic wastes enhances subsequent breaking of cellulose that leads to further increase of cellulose specific surface accessible for enzymes. It has been shown that maximum increase of specific surface area of cellulosic wastes could be reached when cellulosic wastes were chilled and milled at -20$^0$C.

**Key words**: cellulosic wastes, steam explosion, chilling method.

## Introduction

Biomass encompasses a range of products derived from photosynthesis and is essential chemical storage of solar energy. In some countries it is basically used as a source of thermal energy. On the other hand different forms of biomass that include fuel wood, energy crops, agricultural and forestry residues, food and timber processing residues, municipal solid wastes, sewage etc., can be converted into various carbon-based fuels such as: biogas, ethanol and methanol and thus contribute in partial replacing of fossil fuels in the energy industry. Among the biomass – to – energy conversion technologies biological methods are considered more environmentally friendly ones and improvement of biomass fermentation process becomes one of the topical points for new developments. Among the diverse spectrum of biomass, cellulosic wastes are the most abundant natural organic compounds on the earth and are in general the least expensive carbohydrate source. This is why these wastes attract much attention for bio-fuel (ethanol or biogas) production. In general the types of natural cellulose could be divided in two main species: lignocelluloses such as wood, shrubs, leaves, herbs, algae and pure cellulose - cotton and flax. Both species of cellulose mainly have crystalline structure that makes complicate its biodegradation. Besides, lignin - one of the components of lignocellulose, prevents enzymes' accessibility to cellulose. Studies show that reactivity of cellulosic compounds under enzymatic

fermentation is low and therefore pretreatment of cellulosic wastes becomes one of the topical points. Pretreatment refers to the solubilization and separation of one or more of the four major components of biomass – hemicellulose, cellulose, lignin and extractives – to make the remaining solid biomass more accessible to further biological or chemical treatment. Hydrolysis breaks down the hydrogen bonds in the hemicellulose and cellulose fractions into their sugar components: pentoses and hexoses. These sugars can be fermented into ethanol that commonly is used as a fuel and/or food substance. Apart from this, ethanol is one of the precursors for methane formation when digesting organic wastes are under anaerobic conditions.

Various physical, chemical and biological pretreatment methods which promote the accessibility of polysaccharides in a lignocellulose complex for enzymatic hydrolysis are described [Parisi, 1983; Bjerre, et al., 1996; Zheng, et al., 1995; Olsson, 1996]. Cost-effective pretreatment of cellulosic biomass is major challenge of cellulose to bio-fuel technology research. Developing technologies that may decrease the cost of bio-fuel production from different composition cellulosic wastes can be considered in terms of pretreatment, fermentation, alcohol recovery, by-product recovery, and waste treatment.

Cellulose pretreatment method first introduced here is based on combination of chilling and mechanical milling of cellulosic wastes. The idea lies in application of anomalous characteristic of water; when water temperature is decreased bellow $+4^0$ C the water expansion parameter becomes negative: $\alpha<0$. On water-ice transition phase, specific volume of water increases by 10% [ed. Malkov, 1985]. Under further decrease of ice temperature anomalous specific volume dependence on the temperature remains almost the same. When contained in cellulosic wastes frozen water expands, it causes the mechanical stress that is followed by partial destruction of crystalline cellulose. The mechanical milling of frozen cellulosic wastes enhances subsequent breaking of cellulose that leads to further increase of cellulose specific surface accessible for enzymes. Laboratory experiments showed that frozen cellulosic wastes were reduced to smaller particles in comparison with milling of wet or dry ones.

## Materials and methods

At the initial stage of experiments cellulose and water percentages were determined in the following cellulosic wastes: sawdust, newsprint, cardboard and filter paper. Polymerization degree and specific surface area of non treated sawdust, newsprint, cardboard and filter paper have been determined. 5 gram of each cellulosic waste were impregnated with water. Then analytical samples had been frozen at $0^0$ C, -20 $^0$ C, -70 $^0$ C and -196 $^0$ C and kept at these temperatures during 2 hours. One set of frozen samples have been thawed and afterwards milled. Other ones had been milled at once, in the frozen state. Cellulose hydrolysis rate, change of cellulose polymerization degree and cellulose specific surface area of each analytical sample had been determined after the treatment of analytical samples.

Cellulose hydrolysis rate was determined by percentage reduction of initial cellulose in accordance with the standard Apdegraph method based on determination of optical density of preliminarily treated cellulosic substrate. Treatment was done by the mix of nitric and acetic acids with ratio 1:10. Then analytical samples with mix of above mentioned acids were boiled during ½ hour at the water bath. After cooling the test tubes' contents have been centrifuged at 6 000 rot/min, supernatant was poured out and the sediment was rinsed by distilled water. 72% sulphuric acid was added to the sediment and kept during one hour. Then the preliminarily prepared antron reagent was added to analytical samples, firstly they were kept on the ice bath and than boiled at the water bath during 15 minutes. Cellulose percentage in treated analytical samples was determined on the

Perkin Elmer UV-VIS spectrophotometer LAMBDA EZ 150 at the 625 nm wave-length. Value of obtained extinction was multiplied by the standard factor.

## Results and discussions

Experimental results show that maximum decomposition rate of sawdust and newsprint is reached at -196$^0$C, as for cardboard, filter paper and composite of all above cellulosic wastes, maximum reduction of initial cellulose is obtained at -70$^0$C when the analytical samples are milled in the frozen state (table 1).

**Table 1.** Reduction of initial cellulose of studied cellulosic substrates at different temperatures.

| Cellulosic substrate | Chilling temperature | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | -3 $^0$C | | -20 $^0$C | | -70 $^0$C | | -196 $^0$C | |
| | milled in frozen state | milled after thawing | milled in frozen state | milled after thawing | milled in frozen state | milled after thawing | milled in frozen state | milled after thawing |
| | Reduction of initial cellulose (% weight) | | | | | | | |
| Sawdust | 2,43 | 4,42 | 4,63 | 4,50 | 4,90 | 4,82 | 5,56 | 3,86 |
| Newsprint | 0,81 | 1,80 | 1,0 | 1,8 | 3,85 | 3,50 | 5,05 | 3,57 |
| Cardboard | 3,97 | 5,38 | 3,81 | 3,92 | 6,29 | 5,73 | 4,31 | 5,33 |
| Filter paper | 1,98 | 2,32 | 2,46 | 2,60 | 6,87 | 2,54 | 4,60 | 2,89 |
| Composite | 3,24 | 3,34 | 3,31 | 3,65 | 9,25 | 3,92 | 8,46 | 7,33 |

Cellulose polymerization degree has been determined by the viscosimetry method: Samples of cellulosic wastes were dissolved by coppery-ammonia complex and the viscosities of obtained solutions were measured by using of Ostwald viscosimeter. Degree of polymerization was calculated by the following formula:

$$P= 2000\ \eta_k /[C \times (1+0,29\ \eta_k)]$$

Where C is concentration of cellulose (g/l), $\eta_k$ is the specific viscosity that equals to: $(\tau/\tau_0 -1)$, where $\tau$ - is flow down time of the treated samples, $\tau_0$ is flow down time (sec) of pure dissolvent. Results are given in table 2.

**Table 2.** Plymerization degree of studied cellulosic wastes at different temperatures.

| Cellulosic wastes | Polymerization degree | | | | |
|---|---|---|---|---|---|
| | | Chilling temperature $^0$C | | | |
| | non treated sample | 0 | -20 | -70 | -196 |
| newsprint | 1190 | 733 | 301 | 411 | 360 |
| filter paper | 522 | 461 | 384 | 268 | 244 |
| cardboard | 713 | 687 | 464 | 270 | 252 |
| composite | 670 | 544 | 372 | 260 | 152 |

Data given in the table 2 indicate that polymerization degree of filter paper, cardboard and composite of above cellulosic wastes decrease when they are frozen at – 196 $^0$C and milled at once but newsprint's polymerization degree lessens when it is frozen at -20$^0$C.

For definition of cellulose specific surface area the method based on chemotripsin absorption was used. Samples of cellulosic compounds such as: newsprint, filter paper, cardboard and mix of above substrates were kept in the solution of chemotripsin and the amount of absorbed

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ferment was measured by reduction of ferment activity. Computation of specific surface area was done on assumption that on the analytical sample monomolecular layer of ferment was formed. It is known that one molecule of chemotripsin occupies $10^{-17}$ $m^2$. Calculations have been done by using the following equation:

$$S=S_1 \times a \times N_a / C \ (m^2/g)$$

Where $S_1=10^{-17}$, a - is the amount of absorbed ferment (mol/l), C -is the concentration of cellulose (g/l), $N_a$ - is the Avogadro's number. Results are given in table 3.

**Table 3.** Specific Surface Area of studied cellulosic wastes at different temperatures

| Cellulosic wastes | Specific Surface Area ($m^2$/g) | | | |
|---|---|---|---|---|
| | | Chilling temperature $^0$C | | |
| | non treated sample | -20 | -70 | -196 |
| newsprint | 0,016 | 0,036 | 0,014 | 0,028 |
| filter paper | 0,038 | 0,05 | 0,038 | 0,38 |
| cardboard | 0 | 0,037 | 0,031 | 0,018 |
| composite | 0,002 | 0,032 | 0,026 | 0,017 |

Results obtained show that for all analytical samples maximum increase of specific surface area is achieved when above mentioned cellulosic wastes are frozen at -20 $^0$C and milled in the frozen state.

Taking into consideration the fact that specific surface area determines cellulose fermentation by using of fungi or clostridia species, chilling at -20$^0$C with further milling of frozen cellulosic wastes could be considered one of the promising pretreatment methods.

## Acknowledgment

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# ცელულოზური ნარჩენების წინასწარი დამუშავება გაყინვისა და მექანიკური დაფქვის მეთოდების კომბინაციით

ჩაჩხიანი მ.,[1] დუდაური თ.,[1] ფარცხალაძე გ.,[1] კალანდაძე ნ.,[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 06.09.2004)

## რეზიუმე

წარმოდგენილია სხვადასხვა შემადგენლობის ცელულოზური ნარჩენების წინასწარი დამუშავების მეთოდი, დაფუძნებული აღნიშნული ნარჩენების გაყინვისა და მექანიკური დაფქვის კომბინირებულ გამოყენებაზე. ცელულოზურ ნარჩენების გაყინვის დროს, მათში არსებული წყალი ფართოვდება და იწვევს ცელულოზის ჯაჭვის მექანიკურ სტრესს და შესაბამისად მის ნაწილობრივ დაშლას. შესწავლილია ცალ-ცალკე თითოეული სუბსტრატის: ნახერხის, მუყაოს, გაზეთის ქაღალდის, ფილტრის ქაღალდისა და ყველა ზემოთ ჩამოთვლილი ცელულოზური ნარჩენის ნარევში საწყისი ცელულოზის შემცირების, პოლიმერიზაციის ხარისხისა და ხვედრითი ზედაპირის ცვლილება აღნიშნული ნარჩენების 0, -20, -70 და -196$^0$ C-ზე გაყინვისა და შემდგომი მექანიკური დაფქვის პირობებში. ნაჩვენებია, რომ ცელულოზის კუთრი ზედაპირის მაქსიმალური ზრდა მიიღწევა ცელულოზური ნარჩენების -20$^0$ C-ზე გაყინვისა და დაფქვის დროს.

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# TREELINE VEGETATION OF THE RIVER CHIRUKHISTSKALI (SOUTH COLCHIS, ADJARA)

KHARAZISHVILI D.

*Batumi Botanical Garden of the Georgian Academy of Sciences*

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**Abstract**

It was shown that the major part of the treeline vegetation of the river Chirukhistskali high mountains is under the anthropo- and zoogenic pressure. It is noteworthy, that such undamaged communities as *Festuco-Piceetum, Fago-Betuletum* have remained here. Associations: *Abieto-Piceetum, Rhododendro-Piceetum* are distinguished by diversity of species and fir forests by abundance of endemic species. As for the nature protective measures of this canyon, we consider that protection of the treeline in order to avoid such exogenous events as landslide, mud-stream and avalanche is essential. It is required to allocate protected areas or prohibit overgrazing and deforestation for firewood. It is also necessary to extend haymaking areas at the expense of pastures.

**Key words:** treeline formations, associations.

## Introduction

Chirukhistskali Canyon is located in the southeastern part of the river Adjaristskali (N 41°26'990", E 42°30'795") [Kharazishvili, Memiadze, 2004].

The treeline ecotone of this canyon is located at elevations from 1800 to 2400 m above sea level; it consists of forests, meadows, tall herbaceous vegetation (fragments), shrubberies, peat bogs. The largest areas are occupied by meadows. This part of the area has a form of a plateau and is strongly partitioned as a result of erosion processes. The treeline of this ecotone has descended by about 100 m; however, forest fragments, which have almost not experienced human intrusions, have also remained here.

The treeline is very sensitive to the human impact in general and on the territory of Adjara, in particular. The local population did not use the treeline near villages. Unfortunately, this tradition has been broken for many years and the treeline areas are now more or less deforested in a number of places. Heavy precipitation and deforested steep slopes caused landslides in some high-mountainous regions (Khulo, Shuakhevi), which compelled the local population to move to other regions of Georgia.

The rarefication of the treeline is observed at elevations from 1800 m to 2400 m; individual trees reach 2500-2550 m. The treeline is mainly represented by fir forests, rarely by beech ones with admixed *Abies nordmanniana, Populus tremula, Salix caucasica.*

It is worth mentioning that evergreen shrubbery made up of *Ilex colchica, Rhododendron ponticum, Daphne pontica* forms the understory here.

The high mountain vegetation is poorly studied in Colchis unlike the vegetation of the lowland and mountains [Dolukhanov, 1942, 1980; Mandjavidze, 1982]. Our task was to investigate the floristic composition, structure, ecological characteristics of the Chirukhistskali treeline phytocoenoses and elaborate measures of their protection.

## Materials and Methods

We used conventional techniques implemented by Russian and Caucasian botanists. We distinguished the vegetation sintaxa (formations, associations) according to the principle of plant dominance [Dolukhanov, 1989; Kvachakidze, 1979]. Further, we used a 6-graded cover-abundance scale by Braun-Blanquet [Wilmans, 1999; Grabherr, Mucina, 1993; Dolukhanov, 2003; Box et al., 2000]. Stages of hemeroby were assessed on the basis of the naturalness of the phytocoenoses [Pott, 1996]. Namely, a coenosis evaluated as natural or close to natural was referred to the 1st stage of hemeroby and a coenosis evaluated as semi-natural and experiencing human intrusions (hay making, grazing, deforestation) was referred to the 2nd stage.

Vegetation records (relevés) were made in 1999-2004 on randomly selected plots. The areas of the plots were 10 x 10 m and 25 x 25 m. Vegetation was described on more than 200 plots, the total area of which was 80 ha. The syntaxa were named according to Pott [Pott, 1996].

## Results and Discussion

Forest vegetation of the treeline is considered according to separate formations and associations.

Formation - ***Fagion orientale.***

Beech forests represent the most widespread forest formations of Georgia. These forests occupy almost a half of the territory covered by forests [Dolukhanov, 1989]. In the mountains of West Georgia, where precipitation is heavy above the beech (or dark coniferous) forests in winter, a narrow line of subalpine beech forests is formed in some places. Its formation is due to strong and stable snow cover. Beech clumps occur on mountain forest brown and calcareous soils.

Association - ***Arctostaphylo-Piceo-Fagetum.***

The community has been formed in moderately humid conditions. It is referred to the 2nd stage of hemeroby. The following species are constant to this community: *Festuca montana, Valeriana alliariifolia.* Despite the human impact, self-renewal of the community is observed. Particularly, good renewal is characteristic to beech trees. One of the relevés is presented below as an example (List 1):

| List 1 | | | Chirukhistkali Canyon, Mt. Chirukhi |
|---|---|---|---|
| T1 12 m | 40% | | 2110 m, 15° N |
| T2 8 m | 20% | | |
| S 2.5 m | 10% | | |
| H 1 m | 95% | | 25 x 25 m |
| T1 | *Fagus orientalis* | 3 | |
| | *Picea orientalis* | 2 | |
| | *Salix caucasica* | 2 | |
| | *Populus tremula* | 2 | |
| | *Acer trautvetteri* | + | |
| T2 | *Fagus orientalis* | 2 | |
| | *Picea orientalis* | + | |
| | *Salix caucasica* | + | |

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|---|---|---|
| | *Populus tremula* | + |
| S | *Vaccinium arctostaphylos* | 2 |
| | *Viburnum opulus* | + |
| | *Sorbus boissieri* | + |
| H | *Trifolium ambiguum* | 4 |
| | *Ranunculus repens* | 3 |
| | *Pyrola minor* | 3 |
| | *Festuca montana* | 2 |
| | *Valeriana alliariifolia* | 2 |
| | *Pedicularis nordmanniana* | 2 |
| | *Prenanthes purpurea* | 1 |
| | *Polygonatum verticillatum* | 1 |
| | *Polygala alpicola* | 1 |
| | *Anemone ranunculoides* | 1 |
| | *Veronica filiformis* | + |
| | ***Draba hispida*** | + |

Association - ***Arctostaphylo-Fagetum***.

This forest association makes up 3.5% of the beech forests of our country. It mostly occurs on steep slopes (25-35°) of different aspects in West Georgia [Bakhsoliani, 2002].

The community described by us (List 2) is quite rich in shrubs: *Vaccinium arctostaphylos, Daphne pontica, Rubus buschii.* The following elements of tall herbaceous vegetation are also noteworthy: *Valeriana alliariifolia, Senecio rhombifolius, Kemulariella caucasica.* The latter two species are endemics. The coenosis is referred to the 2nd stage of hemeroby.

**List 2** — Chirukhistkali Canyon, Mt. Chirukhi

| | | |
|---|---|---|
| **T** 8 m | 40% | 2120 m, 5° NW |
| **S** 2 m | 20% | 10 x 10 m |
| **H** 1 m | 80% | |

| | | |
|---|---|---|
| T | *Fagus orientalis* | 3 |
| S | *Vaccinium arctostaphylos* | 2 |
| | *Viburnum opulus* | + |
| | *Sorbus boissieri* | + |
| | *Daphne pontica* | + |
| | *Rubus buschii* | + |
| | *Smilax excelsa* | + |
| H | *Ranunculus repens* | 3 |
| | *Festuca montana* | 3 |
| | *Aruncus vulgare* | 3 |
| | *Valeriana alliariifolia* | 2 |
| | *Galium albumi* | 2 |
| | *Trifolium ambiguum* | 2 |
| | *Senecio rhombifolius* | 1 |
| | *Kemulariella caucasica* | + |
| | *Prenanthes purpurea* | + |
| | *Polygonatum verticillatum* | + |
| | *Polygala alpicola* | + |
| | *Anemone ranunculoides* | + |
| | *Veronica filiformis* | + |

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|---|---|---|
| | *Pyrola minor* | + |
| | *Draba hispida* | + |

Association - ***Lonicero-Fagetum***.

The community occupies small areas and is, in general, rare in the high mountains of Adjara. It is referred to the 2nd stage of hemeroby. The community is poor in species. It's principal characteristic species are: *Aruncus vulgare, Mycelis muralis* (List 3).

| List 3 | | Chirukhistkali Canyon, Mt. Chirukhi |
|---|---|---|
| T 10 m | 80% | 2190 m, 10° NE |
| S 2 m | 15% | 10 x 10 m |
| H 0.8 m | 15% | |

| | | |
|---|---|---|
| T | *Fagus orientalis* | 4 |
| | *Picea orientalis* | 1 |
| S | *Lonicera caucasica* | 2 |
| H | *Aruncus vulgare* | 4 |
| | *Mycelis muralis* | 3 |
| | *Valeriana alliariifolia* | 3 |
| | *Festuca montana* | + |
| | ***Draba hispida*** | + |
| | *Prunella vulgaris* | + |
| | *Bellis perennis* | + |
| | *Alchemilla retinervis* | + |

Formation - ***Piceion orientale.***

Dark coniferous forests dominated by oriental fir (*Picea orientalis*) (5.6% of the area covered by forests in Georgia) and Nordmann's silver fir (*Abies nordmanniana*) (10%) are widespread in the mountains of Colchis (900-2150 m). They are formed mainly on mountain forest brown acid soils. In Colchis these forests reach their optimum at 1400-1750 m [Dolukhanov, 1989].

Association - ***Abieto-Piceetum***.

The coenoses of this association are quite widespread not only in this canyon, but also in the whole Colchis. 15.6% of Georgia's woodlands fall on these forests. They are widespread from 900 to 2200 m and rarely occur at altitudes of 200-300 m and 2300-2500 m. Mainly they are formed on slopes of different degrees of inclination covered with acid soils [Dolukhanov, 1989; Mandjavidze, 1982]. The community is distinguished by diversity of shrubs as well as herbs (List 4). Understory is constituted by such Colchic elements as *Daphne pontica*, *Vaccinium arctostaphylos*. The following species of the subalpine meadows are worth mentioning: *Pyrethrum roseum, Alchemilla retinervis, Ranunculus ampelophyllus, Lilium spp., Geranium psilostemon, Dactylorhiza flavescens*, etc. Representatives of the tall herbaceous vegetation occurring in coenoses of the association are *Senecio propinquus, Athyrium filix femina, Grossheimia polyphylla, Heracleum cyclocarpum, Gentiana schistocalyx*. The following endemics can be found in this community: *Senecio propinquus, Gadellia lactiflora, Symphytum asperum* (Caucasian), *Grossheimia polyphylla, Lilium kesselringianum* (Colchic). The community is characterized by good renewal and is referred to the 2nd stage of hemeroby.

| List 4 | | Chirukhistkali Canyon, Mt. Chirukhi |
|---|---|---|
| T1 22-25 m | 70% | 1990 m, 20° N |
| T2 10-12 m | 20% | 10 x 10 m |
| S 2.5 m | 60% | |
| H 2 m | 80% | |

ეროვნული
ბიბლიოთეკა

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|---|---|---|
| **T1** | *Abies nordmanniana* | 4 |
| | *Picea orientalis* | 3 |
| | *Acer trautvetteri* | + |
| **T2** | *Abies nordmanniana* | 2 |
| | *Picea orientalis* | + |
| | *Acer trautvetteri* | + |
| **S** | *Vacciniumm arctostaphylos* | 3 |
| | *Lonicera caucasica* | 2 |
| | *Rubus buschii* | 2 |
| | *Viburnum opulus* | + |
| | *Daphne pontica* | + |
| **H** | *Senecio propinquus* | 3 |
| | *Athyrium filix femina* | 3 |
| | *Pyrola minor* | 3 |
| | *Valeriana alliariifolia* | 3 |
| | *Festuca montana* | 2 |
| | *Lilium kesselringianum* | 1 |
| | *L. szowitsianum* | 1 |
| | *Taraxacum litwinowii* | 1 |
| | *Dactylorhiza flavescens* | 1 |
| | *Gadellia lactiflora* | 1 |
| | *Silene wallichiana* | + |
| | *Geranium psilostemon* | + |
| | *Veratrum lobelianum* | + |
| | *Polygonatum verticillatum* | + |
| | *Trifolium pratense* | + |
| | *Alchemilla retinervis* | + |
| | *Plantago lanceolata* | + |
| | *Prunella vulgaris* | + |
| | *Pyrethrum roseum* | + |
| | *Ranunculus repens* | + |
| | *Luzula forsteri* | + |
| | *Fragaria vesca* | + |
| | *Tussilago farfara* | + |
| | *Petasites albus* | + |
| | *Hesperis matronalis* | + |
| | *Grossheimia polyphylla* | + |
| | *Knautia involucrata* | + |
| | *Galium album* | + |
| | *Centaurea nigrofimbria* | + |
| | *Scrophularia chlorantha* | + |
| | *Potentilla recta* | + |
| | *Heracleum cyclocarpum* | + |
| | *Pyrethrum marcophyllum* | + |
| | *Aruncus vulgare* | + |
| | *Sanicula europaea* | + |
| | *Gentiana shistocalyx* | + |
| | *Rumex acetosella* | + |
| | *Carex capitellata* | + |

| | | |
|---|---|---|
| | *Cirsium kosmelii* | + |
| | *Sedum tenellum* | + |
| | *Symphytum asperum* | + |
| | *Paris incompleta* | + |
| | *Inula orientalis* | + |
| | *Oxalis acetosella* | + |

Association - ***Arctostaphylo-Piceetum.***

The coenoses of the association are spread on northeast-facing slopes covered with acid soils from 2100 to 2300 m. They occupy quite large areas. Human impact on coenoses of this association is particularly significant, which is proved by widespread *Nardus, Ajuga, Prunella*. The community is referred to the 2nd stage of hemeroby. The following endemics are noteworthy: *Viola orthoceras* (Colchic) and *Euphorbia macroceras* (Caucasian) (List 5).

**List 5** — Chirukhistkali Canyon, Mt. Lelovani, 2270 m, 10° NE

| | | |
|---|---|---|
| T 4 m | 70% | |
| S 1.5 m | 40% | |
| H 0.5 m | 100% | |
| T | *Picea orientalis* | 3 |
| | *Fagus orientalis* | 1 |
| | *Acer trautvetteri* | 1 |
| S | *Vaccinium arctostaphylos* | 3 |
| | *Rhododendron caucasicum* | 1 |
| | *Sorbus boissieri* | 1 |
| | *Daphne glomerata* | 1 |
| H | *Phleum pratense* | 4 |
| | *Luzula forsterii* | 2 |
| | *Coronilla balansae* | 2 |
| | *Ajuga orientalis* | 2 |
| | *Prunella vulgaris* | 2 |
| | *Betonica macrantha* | + |
| | *Euphorbia macroceras* | + |
| | *Achillea latiloba* | + |
| | *Viola orthoceras* | + |
| | *Geranium psilostemon* | + |

Association - ***Rhododendreto-Piceetum.***

The association is quite characteristic to Colchis. Its coenoses are distributed mainly from 1800 to 2100 m. The following woody plants are worth mentioning: *Euonymus latifolia, Populus tremula, Ilex colchica*. Noteworthy elements of the tall herbaceous vegetation are: *Lilium kesselringianum, Verbascum adzharicum, Doronicum macrophyllum*. The following plants are endemics: *Aconitum nasutum* (Caucasian), *Lilium kesselringianum* (Colchic) (List 6). The community is referred to the 2nd stage of hemeroby.

**List 6** — Chirukhistkali Canyon, Mt. Lelovani, 1850 m, 10° SW, 25 x 25 m

| | | |
|---|---|---|
| T 12 m | 80% | |
| S 3 m | 60% | |
| H 0.5 m | 90% | |
| T | *Picea orientalis* | 4 |
| | *Populus tremula* | 2 |
| | *Euonymus latifolia* | + |
| S | *Rhododendron ponticum* | 4 |

| | | |
|---|---|---|
| | *Ilex colchica* | 2 |
| | *Vaccinium arctostaphylos* | 2 |
| **H** | *Petasites albus* | 3 |
| | *Pteridium tauricum* | 3 |
| | *Heracleum cyclocarpum* | 3 |
| | *Achillea latiloba* | 3 |
| | *Campanula latifolia* | 2 |
| | *Aconitum nasutum* | 2 |
| | *Kemulariella caucasica* | 1 |
| | *Veratrum lobelianum* | 1 |
| | *Hypericum montanum* | 1 |
| | *Rhynchocorys elephas* | + |
| | *Betonica macrantha* | + |
| | *Lilium kesselringianum* | + |
| | *Solidago virgaurea* | + |
| | *Digitalis ferruginea* | + |
| | *Verbascum adzharicum* | + |
| | *Primula auriculata* | + |
| | *Centaurea cianus* | + |
| | *Psoralea acaulis* | + |
| | *Fragaria vesca* | + |
| | *Prunella vulgaris* | + |
| | *Trifolium ambiguum* | + |
| | *T. repens* | + |
| | *T. pratense* | + |
| | *Lotus caucasicus* | + |
| | *Hedera colchica* | + |
| | *Silene ruprechtii* | + |
| | *S. italica* | + |
| | *S. compacta* | + |
| | *Doronicum macrophyllum* | + |
| | *Veronica gentianoides* | + |
| | *Stachys iberica* | + |
| | *Pimpinella rhodantha* | + |
| | *Briza elatior* | + |

<u>Association - ***Rubo-Piceetum***</u>.

The community is subjected to strong human impact. The only shrub occurring in this community is *Rubus*. The coenoses of this type are distributed from 1850 to 2050 m. The following herbaceous plants are noteworthy: *Inula orientalis, Geranium psilostemon, G. platypetalum* and the following are endemics: *Euphorbia macroceras, Potentilla brachypetala* (Caucasian) (List 7). The community is referred to the 2nd stage of hemeroby.

**List 7** — Chirukhistkali Canyon, Mt. Lelovani

| | | | |
|---|---|---|---|
| **T** 10 m | 60% | | 1900 m, 15° N-NW |
| **S** 1.5 m | 60% | | |
| **H** 0.5 m | 90% | | 25 x 25 m |
| **T** | *Picea orientalis* | 3 | |
| **S** | *Rubus hirtus* | 4 | |
| **H** | *Pteridium tauricum* | 3 | |

ეროვნული
ბიბლიოთეკა

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| *Bellis perennis* | 3 |
| *Potentilla brachypetala* | 2 |
| *Dactylorhiza triphylla* | 1 |
| *Campanula alpigena* | 1 |
| *Veratrum lobelianum* | 1 |
| *Euphorbia macroceras* | 1 |
| *Inula orientalis* | + |
| *Senecio platyphylloides* | + |
| *Ranunculus ampelophyllus* | + |
| *Poa pratensis* | + |
| *Prunella vulgaris* | + |
| *Draba hispida* | + |
| *Betonica macrantha* | + |
| *Veronica filiformis* | + |
| *Geranium platypetalum* | + |
| *Petasites albus* | + |
| *Cirsium kosmelii* | + |
| *Achillea latiloba* | + |
| *Ficaria popovii* | + |
| *Carum carvi* | + |

Association *- **Festuco-Piceetum***.

The coenoses of this association are undisturbed, which is rare in Adjara as well as other regions. They occur at 2000-2200 m. The community is still primary, because the forest has always been thought cursed. Fir is the monodominant in the community and the grass cover is constituted only by *Festuca montana* and separate plants of *Cephalanthera grandiflora*. The understory is absent. The community is floristically poor (List 8). It is referred to the 1st stage of hemeroby.

**List 8**

Chirukhistkali Canyon, Mt. Tbethi
2080 m, 40° W-SW

T 25-35 m 85%
N 41°33'574"
H 0.4 m 70%
E 42°10'463"

| | | |
|---|---|---|
| T | *Picea orientalis* | 5 |
| H | *Festuca montana* | 4 |
| | *Cephalanthera grandiflora* | + |

Association *- **Rhododendreto-Piceetum***.

The coenoses of this association are located 200 m below the coenoses of the previous association. They are also undisturbed and contain the understory made up of *Rhododendron ponticum.* Separate beech trees occur in this forest. The association is floristically poor (List 9). It is referred to the 1st stage of hemeroby.

**List 9**

Chirukhistkali Canyon, Mt. Tbethi
1900 m, 25° W-SW
10 x 10 m

T 25-35 m 80%
S 2-3 m 25%
H 0.5 m 60%

| | | |
|---|---|---|
| T | *Picea orientalis* | 5 |
| | *Fagus orientalis* | + |
| S | *Rhododendron ponticum* | 2 |

ეროვნული
ბიბლიოთეკა

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|---|---|---|
| H | *Festuca montana* | 3 |
| | *Cephalanthera grandiflora* | + |

Association - ***Betulion medwedewii***.

*Betula medwedewii* and forests dominated by this species are quite widespread in the subalpine belt of the seaside part of southern Colchis (Guria, Adjara, Lazistan of Turkey) [Dolukhanov, 1980]. As reported by Dolukhanov, *B. medwedewii* is a conservative relict of the ancient flora. It is referred to a section *Costatae*, other representatives of which are mainly distributed in southern and south-eastern Asia. A number of its species occur in the Himalayas and mountains of the North America. According to this author, Colchis is the only place throughout the vast areas of the west Eurasia, where two representatives of this section, namely, *B. medwedewii* and *B. megrelica* have remained. The frequency of the latter species is lower and it occurs in northern Colchis. *B. medwedewii* represents the isolated, vegetatively motile, semi-prostrate life form, which is well adapted to highly humid (perhumid) climate [Dolukhanov, 1980].

Association - ***Fago-Betuletum***.

These are elfin forests. The coenoses of such composition, characterized by the elfin form, such distribution pattern and, first of all, not subjected to human intrusions is rare. These coenoses are formed on very steep slopes, at quite high altitudes. They are primary owing to the high inclination degree of slopes. Woody plants are diverse. The maximum tallness of the trees is 3m, some trees have the elfin form (beech), others do not exceed shrubs (birch, willow, poplar). *Gypsophila tenuifolia* is a noteworthy endemic (Caucasian) (List 10). The community is referred to the 1st stage of hemeroby.

**List 10** Chirukhistkali Canyon, Mt. Tbethi

**T** 3 m 85% 2380 m, 60° NE N 41°32'640"

**S** 1.5 m 60% 10 x 10 m

**H** 0.8 m 90% E 42°11'512"

| | | |
|---|---|---|
| **T** | *Betula medwedewii* | 4 |
| | *Fagus orientalis* | 4 |
| | *Picea orientalis* | + |
| | *Salix caucasica* | + |
| | *Populus tremula* | + |
| **S** | *Rhododendron luteum* | 3 |
| | *Vaccinium arctostaphylos* | 2 |
| | *V. uliginosum* | 2 |
| | *Juniperus pigmaea* | 2 |
| | *Sorbus boissieri* | 2 |
| | *Rubus hirtus* | + |
| **H** | *Polystichum lobatum* | 3 |
| | *Gypsophila tenuifolia* | 2 |
| | *Pyrola minor* | 2 |
| | *Achillea millefolium* | 2 |
| | *Alchemilla retinervis* | + |
| | *Dentaria quinquefolia* | + |
| | *Valeriana alliariifolia* | + |
| | *Draba hispida* | + |
| | *Phleum pratense* | + |
| | *Gentiana schistocalyx* | + |

ეროვნული
ბიბლიოთეკა

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| *Senecio platyphylloides* | + |
| *Aruncus vulgare* | + |
| *Veratrum lobelianum* | + |
| *Geranium psilostemon* | + |

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ბიბლიოთეკა

# მდინარე ჭირუხისწყლის (სამხრეთ კოლხეთი, აჭარა) ტყის ზედა საზღვრის ტყის მცენარეულობა

ხარაზიშვილი დ.

*საქართველოს მეცნიერებათა აკადემიის ბათუმის ბოტანიკური ბაღი*

(მიღებულია 11.11.2004)

## რეზიუმე

მდ. ჭირუხისწყლის ტყის ზედა საზღვრის მცენარეულობის ძირითადი ნაწილი ანთროპოგენურ-ზოოგენური დატვირთვის ქვეშაა. ამასთან, აქ შემორჩენილია ისეთი ხელუხლებელი ფიტოცენოზები, როგორებიცაა *Festuco-Piceetum, Fago-Betuletum*. სახეობათა მრავალფეროვნებით გამოირჩევა ასოციაციები: *Abieto-Piceetum, Rhododendro-Piceetum*, ხოლო ენდემური სახეობების სიმრავლით – ნაძვნარები. უცილებელია ამ ხეობის ტყის ზედა საზღვრის ზოლისათვის დაცული ტერიტორიის სტატუსის მინიჭება ან ამ ზოლში შეშის მოპოვებისა და ძოვების აკრძალვა, აგრეთვე საძოვრების ხარჯზე სათიბების ფართის გაზრდა.

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Cytology

# ULTRASTRUCTURAL CHANGES OF *YUCCA GLORIOSA* CALLUS TISSUES, LEAVES AND BUDS UNDER 2.4.6-TRINITROTOLUENE (TNT) ACTION

RAMISHVILI M., GOGAVA M ., ZAALISHVILI G., CHELIDZE N., GOGOBERIDZE M .

*Durmishidze Institute of Biochemistry and Biotecnology, Academy of Sciences of Ceorgia*

(Received July, 26, 2004)

**Abstract**

The intracellular distribution of assimilated (1-$^{14}$C) TNT in *Yucca gloriosa* callus tissues and intact plant leaves has been studied. In vacuoles, plastids, mitochondria, endoplasmic reticulum and cytoplasm radioactive label was exposed. By electron-microscopic analysis was stated that in callus tissues, compared with the intact plant, toxicants in larger amount are located in vacuoles. Correspondingly, dedifferentiated cell ultrastructure is less destructed.

**Key words:** *Yucca gloriosa*, callus tissue, 2.4.6- trinitrotoluene (TNT), cell ultrastructure.

## Introduction

Grand scale contamination of soil and ground waters with explosives due to the military activities is a key problem. Among explosives 2.4.6-trinitrotoluene (TNT) is one of the most toxic and stable toxicant which affect on water and land species [Hughes et al., 1997]. In human organism TNT penetrates through digestive tract, skin and lungs and further accumulates in liver, kidneys and fatty tissues [Opresko, 1998]. It causes chronic diseases. According to EPA (Environmental Protection Agency) classification TNT is a C-group carcinogenic substrate [EPA, 1991a,b]. Lately great effort was made for revelation of TNT-detoxifying plants.

Plant detoxificational potential is determined by cell ability to uptake and transform toxicants different concentrations and maintain metabolic features. Literary data show that penetrated into the cell xenobiotics induce ultrastructural and metabolic deviations [Allnuff et al., 1991]. The best elucidation of cell ultrastructural changes is organelles pathology. Sequence and nature of cell organelles destruction depends on xenobiotic concentration, period of action, structure, cell sensibility, adaptation rate etc.

Exploiting of plant tissue cultures enables us to study the changes proceeding at different levels of plant cell differentiation in the process of xenobiotic detoxification.

The presented work aimed to study localization of TNT in *Yucca gloriosa* callus tissue and intact plant (leaf, bud) cells and its influence on ultrastructure.

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## Materials and Methods

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

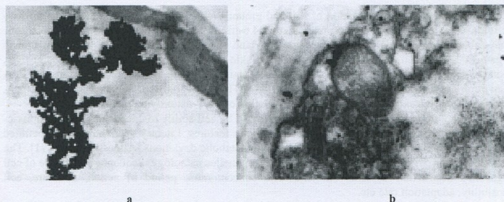
To study cell ultrastucture the material was prepared due to the standard method [Heyer, 1974]. Intracellular distribution of TNT was studied by the method of microscopic radio-autography.

Callus tissue was cultivated on labeled with (1-$^{14}$C) TNT (50, 100, 200 mg/l) containing MS nutrient medium. Leaves and young buddy stems were sterilized in 1% sublimate solution during 5 min and rinsed 5-fold in sterile water; Then callus was incubated for 5 days in sterile water solution containing labeled with (1-$^{14}$C) TNT, in sterile chamber. After 5 days the material was prepared by standard electron-microscopic method. Golden cuts of moulded in epoxide resin material were placed on sieve covered with forewarn layer. After exposition of cuts on $NiCl_2$ ($10^{-4}$M) solution drop for 2x30sec, the samples were shifted to hydroquinon's 1% water solution drop at pH - 9.0, exposure time was 2x40sec. Finally the samples were analyzed on electron microscopy [Buadze et al., 1985].

## Results and Discussion

By the method of electron-microscopic radioautography the intracellular distribution of assimilated (1-$^{14}$C) TNT in *Yucca gloriosa* callus tissue and intact plant leaf cells has been studied.

In callus cells radioactive label was manifested in vacuoles (Fig.1a), plastids, endoplasmic reticulum and cytoplasm. On the Fig.2b the label is in the contact place of plasmalemma and mitochondria.



a b

**Fig.1.** TNT (100mg/l) localization in *Yucca gloriosa* callus cells. a)Label in vacuole, b) Label in the contact place of mitochondria and plasmalemma , x 30 000.

In leaf cells lebel $^{14}$C is manifested in globules and was detected in vacuoles (Fig.2a), chloroplasts and mitochondria (Fig. 2b). Radioactive label compared with the callus cells more deeply penetrates in cell and is detected in nucleus (Fig.2c).

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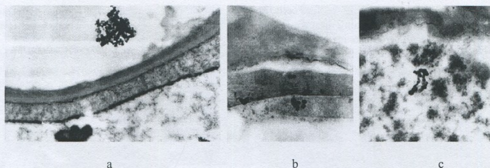


a b c

**Fig.2.** TNT (100mg/l) localization in *Yucca gloriosa* intact plant leaf cells.
a)Label in vacuole, x 25 000; b)Label in chloroplast and mitochondria, x 25000; c)Label in nucleus, x 30 000.

Electron microscopic analysis showed, that structures of *Yucca gloriosa* callus tissue and intact plant (leaf, bud) cell mitochondria and nucleus are similar. The main difference is that in callus cells plastid structure is not formed.

It is of great importance to state the correlation between xenobiotic concentration and pathological changes in plant cells. Therefore on *Yucca gloriosa* callus tissue and plant (leaf, bud) cells three concentrations of toxicants (50, 100, 200mg/l) were tested.

At 50mg/l TNT action on callus cells, the most part of endoplasmic reticulum is smooth and fragmentized, cisterns are widened. Mitochondria, having swollen cristae, are in close contact with endoplasmic reticulum. Plastids matrix is not dense and is electron light. Tonoplast wholeness is damaged, there are globular derivatives. Many osmiophilic insertions are in vacuoles.

At 100mg/l TNT action on callus cells invagination of nuclear layer occurred; intra membrane structures amount in plastids were enhanced but no grana were detected. In vacuoles electron dense insertions were seen. The most of endoplasmic reticulum was agranulated, cisterns were widened and were in contact with mitochondria swolling saturated cristae. Also tight contacts between mitochondria and plastids were observed (Fig.3).

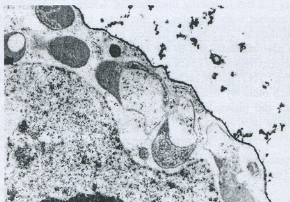
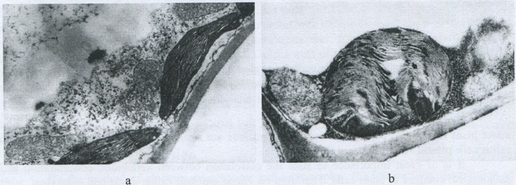


**Fig. 3.** TNT (100mg/l) action on *Yucca gloriosa* callus cells. Nucleus is invaginated. In plastid matrix are many membranes, in vacuoles are osmiophilic insertions, endoplasmic reticulum is widened, x 25000.

At 50mg/l TNT action on leaf cells, chloroplasts matrix is darkened, tyllacoid cisterns are widened. Structure maintaining lameles are located along chloroplasts. Tight contact between mitochondria and chloroplasts is observed. Nucleus is partially destructed (Fig.4a).

At 100mg/l TNT action the leaf cell destruction degree is increased. Beside the mentioned changes, chloroplasts are swollen, electron dense globules are increased, which represent lipoid drops formed in the result of membrane structure destruction. Tonoplasts wholeness is damaged and exocytozic secretion is observed (vacuole type vesicle contact with plasmalemma) (Fig.4b).



a b

**Fig.4.** 50 mg/l (a) and 100 mg/l (b) TNT action on *Yucca gloriosa* intact plant leaf cells. a) in chloroplasts are widened tyllacoid cisterns, contacts between mitochondria and chloroplasts, x 20 000. b) swollen chloroplasts, vesicle contact with plasmalemma, x 20 000.

At action of 50mg/l TNT on bud cells great amount of mitochondria was observed, cisterns of endoplasmic reticulum are widened and are in contact with mitochondria (Fig. 5a). Cells with mitochondria displaced symmetrically on the periphery and which were in contact with plasmalemma were detected. Plasmalemma owing to active exocytoze is extremely invaginated. Very large vacuoles with osmiophylic insertions, many vacuoles and little bubbles are observed.

Electronograms analysis allows us to suppose that cellular excretion of toxicant and/or its metabolites occurs via the following mechanisms: widened agranulated cisterns of endoplasmic reticulum are in contact with vacuoles, at this time transfer of vacuole intestines to cisterns of endoplasmic reticulum is supposed to happen, leading to fragmentation of cisterns. Formed little vesicles move to plasmalemma and in the result of coupling of their membranes, the release of vesicle interiors into extracellular space occurs.

At 100 mg/l TNT action on bud cells cytoplasm is electron dense. Large mitochondria are often linked to each other by widened cisterns of endoplasmic reticulum. Many small bubbles are seen in cytoplasm and plasmalemma. Nucleus is invaginated and "chromatin coagulation" is observed, pointing to destruction of DNA synthesis. Membranes of nucleus and endoplasmic reticulum are widened. Destruction of mitochondria is started, some of them are conjoined (Fig.5b)

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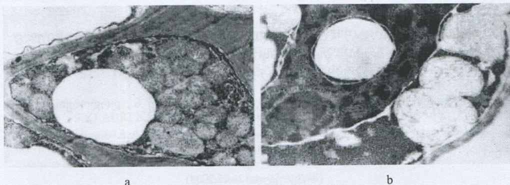

a b

**Fig.5.** 50 (a) and 100 mg/l (b) TNT action on *Yucca gloriosa* intact plant bud cells.
a) great amount of mitochondria, winded endoplasmic reticulum and their contacts with mitochondria, x 20 000; b) swollen lysed mitochondria, electron dense cytoplasma, x 28 000.

At 200 mg/l TNT action full destruction of callus, as well as intact plant cells was observed.

As plant differentiated, so callus cell ultrastructural changes are in correlation with the increase of TNT concentration.

As a result of our studies it has been stated that at the same (100 mg/l) TNT concentration in the nutrient medium, destruction degree of intact plant bud and tissue culture cells are different. Dedifferentiated cells ultrastructural wholeness is less damaged. On the basis of comparison of obtained electronogrames it can be supposed, that the above mentioned is the result of compartmentization of most of TNT conjugates in vacuoles by callus cells. In plant differentiated cells owing to the active degradation processes, cell destruction is more expressed.

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ბიბლიოთეკა

# 2.4.6-ტრინიტროტოლუოლის (TNT) ზეგავლენით გამოწვეული *Yucca gloriosa L.* კალუსური ქსოვილის, კოკრისა და ფოთლის ულტრასტრუქტურული ცვლილებები

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

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

(მიღებულია 26.07.2004)

## რეზიუმე

შესწავლილია შეთვისებული (1-$^{14}$C) TNT-ს შიდაუჯრედული განაწილება იუკა დიდებულის კალუსური ქსოვილისა და ინტაქტური მცენარის ფოთლის უჯრედებში. რადიოაქტიური ნიშანი მჟღავნდება ვაკუოლებში, პლასტიდებში, მიტოქონდრიებში, ენდოპლაზმურ ბადესა და ციტოპლაზმაში.

ელექტრონულ-მიკროსკოპული ანალიზით დადგენილია, რომ კალუსურ უჯრედებში, ინტაქტურ მცენარესთან შედარებით, ტოქსიკანტი გაცილებით დიდი რაოდენობითაა ლოკალიზებული ვაკუოლებში. შესაბამისად, დედიფერენცირებული უჯრედების ულტრასტრუქტურული მთლიანობა ნაკლებადაა დარღვეული.

ბიბლიოთეკა

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Ecology

# THE SOIL MEZOFAUNA OF ALGETHY RESERVE BEECH FORESTS

TSIKLAURI KH., GURGENIDZE L., KVAVADZE E., KAJAIA G.

*Institute of Zoology of the Georgian Academy of Sciences*

(Received July 07, 2004)

**Abstract**

Composition, inhabitance density and quantitative dynamics of 6 associations of mesofauna of the Algethy Reserve beech forests have been studied. The complete composition of main groups of mesofauna is found in *Fagetum poosum (Poa nemoralis)* and *Fagetum oxolidosum* phytocenosis. The maximal density ( 118,6 specimen/ m$^2$) was found in *Fagetum dryopterosum* and minimal ( 10,9 specimen/m$^2$ ) – in the association of *Fagetum asperulosum.* It was revealed that the growth is maximal in spring and autumn and minimal in winter periods.

**Key words:** Algethy Reserve, Quantitative dynamics, Beech forest, Trialeti Range, Mezofauna .

## Introduction

The largest part of the Algety Reserve (6042 ha) is covered by forests. One third of these forest are represented by beech forests (2255 ha). It should be noticed that the beech forests are mainly distributed in shadowy gorges. The main groups of mesofauna of the coniferous forests of the Algethy Reserve have already been identified and studied. The main characteristics of the quantitative dynamics of the mezofauna of coniferous biotopes of Algethy Reserve has been identified [Tsiklauri Kh., et al., 2004], while the invertebrates of the deciduous forests soils and particularly of beech forests soils has not been studied yet .

## Material and Methods

To the study the soil mezofauna route and stationary methods were carried out in 2001-2003 years. Route methods were used in different types of beech forests (1200-1900 m. a.s.l.). For the stationary method we chose beech forest on the right bank of the river Algethy (1200 m a.s.l.). We took three samples from the same plot in succession. Researches were carried out according to the accepted methods in soil zoology [Giliarov, 1964; Krebs, 1989]. 6 types of beech forests has been studied:

1 – *Fagetum poosum (Poa nemoralis)* – Rigth bank of the river Algethy. Stationary site. (1100 m a.s.l.)
2 – *Fagetum dryopterosum* – Gokhnari. (1200 m. a.s.l.)
3 – *Fagetum nudum* – Chinchriani. (1600 m a.s.l.)

4 – Fagetum festucosum (Festuca drymeja) – Ukhmara. (1500 m. a.s.l.)
5 – *Fagetum asperulosum* – Arkhoti. (1400 m. a.s.l.)
6 – *Fagetum oxalidosum* – Ugudeti. (1900 m. a.s.l.)

## Results and Discussion

The soil mezofauna of the beech forests are presented by the following groups: *Mermitidae, Lumbricidae, Enchitreidae, Oniscoidea , Aranei, Diplopoda, Insecta, Molluska*. As it is shown in the Table, more or less complete composition of mezofauna is identified in the stationary plot (1100 m.a.s.l.) and in the upper altitude of beech distribution (Ugudeti.1900 m.a.s.l.). Unlike the soil of coniferous biotops, the mezofauna of beech forests is distinguished by the high amount of *Myriapoda* [Tsiklauri, 2004]. From the main groups of mezofauna, on stationary site *Insecta, Myriapoda, Enchitreida* and *Lumbricidae* are dominant ones. In other beech forests in many occasions predominate insects (Tab. 1), except Ugudeti forest where *Enchitreidae* and *Lumbricidae* are dominant. Beech forest in Gokhnari area is of a special interest. In this forest the maximal amount of mezofauna is found (118 ,6 specimen/m$^2$), that is generally a very high value. The picture of quantitative monthly dynamics of the whole mezofauna, observed for two years is presented on the Fig.1.

**Table 1.** Composition and density (specimen/m$^2$ ) of mezofauna in beech forests of Algethi Reserve

| Groups | Plots | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1.*Mermitidae* | 0,9 | - | - | 1,3 | - | 2,7 |
| 2.*Lumbricidae* | 17,3 | 46,7 | 1,3 | 6,7 | 4 | 13,3 |
| 3.*Enchitridae* | 7,1 | 17,3 | - | - | - | 13,3 |
| 4.*Oniscoidea* | 1 | - | - | - | - | - |
| 5.*Aranei* | 2,7 | - | 2,7 | 1,3 | 1,3 | 2,7 |
| 6.*Myriapoda* | 7,4 | 5,3 | 1,3 | 4 | 8 | 2,7 |
| 7.*Insecta* | 20 | 49,3 | 5,3 | 26,7 | 10,7 | 6,7 |
| 8.*Mollusca* | 0,5 | - | - | - | - | 1,3 |
| Total density | 56,9 | 118,6 | 10,6 | 40 | 24 | 61,3 |

The diagrams shows a tendency of alternative variations of the quantities. In winter period, namely in December, the quantity is minimal and in spring – very high. Fluctuation of quantity can be explained by climatic changes. For mezofauna in spring due to temperature rise good living conditions occur. Therefore in April of 2003 the margin was highest for the quantity of mezofauna. A great number of mezofauna in summer and autumn can also be explained by temperature changes.

In the beech forests only 7 Order of insects are identified, two of them are noteworthy for their diversity: *Coleoptera* and *Diptera*. Number of their families is almost equal. The Order of *Coleoptera* is noteworthy for the families: *Carabidae* and *Elateridae*. In family *Carabidae* genera *Notiophilus* and *Carabus* and in family *Elateridae* – one of the unknown species of worms from the genus of *Cardiophorus* are interesting. It should be mentioned that the worms of this genus were found in coniferous forests as well [Tsiklauri, 2004]. Unlike to the coniferous forests, in the beech forests the order of *Diptera* is presented by a small amount of families. However, this order in both types of forests is distinguished by the diversity and density of species (*Tipulidae* and *Bibionidae*). The quantitative dynamics of insects is similar to the dynamics of the whole mezofauna, with just insignificant difference. In particular, from the July to the October of 2001 the quantity of insects remained unchanged, while the dynamics of mezofauna fluctuated (Fig.1)

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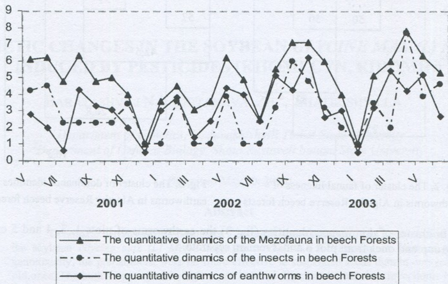


**Pic.1.** The quantitative dinamics of the Mezofauna in beech Forests

As for the earthworms, 8 species of earthworms has been found in the beech forests of the Algethy Reserve, among them dominating species are: *Dendrobaena surbiensis, D. tellermanica, Allolobophora jassiensis* and *Eisenia rosea*. Other species of earthworms are not numerous, except *Omodeioa alpina alpina*, which is distinguished by high density (10,7 specimen/m$^2$ ) (Ugudeti forest). The highest density of earthworms (46,7 specimen/m$^2$) was registered in Gokhnari, minimal – (1,3 specimen/m$^2$ ) in Arkhoti and average in stationary plot (17,2 specimen/m$^2$).

It should be noticed that the coefficient of faunal resemblance is very high among them: between the points 1 and 2 – 42 % and between 4,5 and 6 – 50 % (tab. 2).

**Table 2.** Coefficients of faunal likeness (upper site) and dominance identities (lower site) of earthworms in Algethy Reserve beech forests (%) .

| plots \ plots | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | | 42 | 14 | 25 | 42 | 25 |
| 2 | 43 | - | 33 | 20 | 20 | 20 |
| 3 | 46 | 17 | - | 33 | 33 | 33 |
| 4 | 56 | 17 | 59 | - | 50 | 50 |
| 5 | 57 | 17 | 33 | 52 | - | 50 |
| 6 | 19 | 9 | 9 | 37 | 42 | - |

There is some succession in the quantitative dynamics of the earthworms. Their maximal density was observed in October 2002, minimal – in July, December and January 2003 when the humidity level was low. It is interesting that after the decrease of density, in the winter 2002-2003, their number increases. In these three years the tops of increases are observed in the end of the spring. The earthworms of nearby ecosystems created one faunal cluster; namely, one cluster with 1 and 2 ecosystems and another with 4, 5 and 6 ecosystems (Fig. 2).

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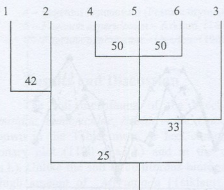


**Fig. 2.** The cluster of faunal likeness of earthworms in Algethy Reserve beech forests

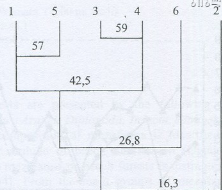


**Fig. 3.** The cluster of dominance identities of earthworms in Algethy Reserve beech forests

In cluster of dominance identities (fig. 3) the earthworms of plots 1, 3, 4 and 5 create a separate group and ones from plot 6 and 2 remain separate.

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## ალგეთის ნაკრძალის წიფლნარების ნიადაგის მეზოფაუნა

წიკლაური ხ., გურგენიძე ლ., ყვავაძე ე., ქაჯაია გ.

*საქართველოს მეცნიერებათა აკადემიის ზოოლოგიის ინსტიტუტი*

(მიღებულია 07.07.2004)

### რეზიუმე

შესწავლილია ალგეთის ნაკრძალის წიფლნართა 6 ასოციაციის მეზოფაუნის შემადგენლობა, დასახლების სიმჭიდროვე და რიცხოვნობის დინამიკა. მეზოფაუნის ძირითადი ჯგუფების სრული შემადგენლობა დაფიქსირებულია *Fagetum poosum (Poa nemoralis)*-ის და *Fagetum oxalidozum* - ის ფიტოცენოზებში. მისი მაქსიმალური სიმჭიდროვე – 118,6 ეგზ/მ$^2$ აღინიშნა *Fagetum dryopterosum*-ში, მინიმალური – 10,9 ეგზ/მ$^2$ კი – *Fagetum asperulosum*-ში. მეზოფაუნის რიცხოვნობის დინამიკის შესწავლით დადგინდა, რომ იგი პიკს აღწევს გაზაფხულსა და შემოდგომაზე, ხოლო მინიმალურია ზამთარში.

ბიოლოგიური
ბიბლიოთეკა

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Genetics

# GENETIC CHANGES IN THE SOYBEAN *GLYCINE MAX (L) MERR* INDUCED BY PESTICIDES (KHOMECYN, RIDOMIL)

BARATASHVILI N.[1], CHITANAVA ZH.[2], SHATIRISHVILI A.[1]

[1]*Department of Genetics, Iv. Javakhishvili Tbilisi State University*
[2]*Department of General Biology, Shota Rustaveli batumi State University*

(Received May 10, 2004)

**Abstract**

Genotoxic activity of the pesticides - Ridomil and Khomecyn, has been studied in the soybean *Glycine max (L) Merr* using the worked out test-system. In view of the genotoxicity, the preparations were far more effective in suspended seeds than in one-year-old ones. The both preparations in higher rate induced direct mutations than reversions. No significant difference was found between the two agents by the spectrum of induced mutations.

**Key words:** soybean, pesticide, mutation, somatic mosaicism

## Introduction

Use of chemical methods against fungous diseases of vermin at the present time still remains one of the actual problems in protection of agricultural crops. The assortment of pesticides grows every year due to the induction of resistant forms among the vermin and parasite Fungi. Pesticides take the first place among environmental pollutants [Dubinin, 2000]. Most pesticides are characterised by genotoxicity [Alfonso et al., 2001, Baratashvili et al., 2003, Chitanava., 2003]. Genetically active xenobiotics raise mutational press on populations that, on one hand, favours induction of resistant forms. In the other hand, wide use of such pesticides are followed by their insertion into food chains and accumulation in individuals, that put all living beings, and the mankind among them, in danger [Dubinin, 2000, Alfonso et al., 2001]. The genetoxic agents reveal different activities in individuals belonging not only to different species, but the same populations as well. The induction of mutation depends on the age and physiological state of an individual [Dubinin, 2000].

Nowadays, to reveal genetoxic pesticides, to study the mechanisms of their influence on genetic apparatus and to seek the ways to avoid their harmful effect on genetic apparatus of living systems are the goals of primary importance [Dubinin, 2000, Charries et al., 2000, Alfonso et al., 2001].

## Material and Methods

The genetic line of soybean *Glycine max (L) merr*, generated by the American scientists B. Vig and E. Paddok [Vig, Paddok, 1970] was used in our experiments. The origin of the line was described in previous publications [Baratashvili et al., 2003, Chitanava et al., 2003]. The

experiment was conducted on the seeds obtained from heterozygous plants $Y_{11}y_{11}$ (the allele $Y_{11}$ is semi-dominant. It controls chlorophyll synthesis). Sprouts of three phenotype classes developed from the seeds: Green ($Y_{11}Y_{11}$), light-green ($Y_{11}y_{11}$) and yellow ($y_{11}y_{11}$) in the ratio of 1:2:1. Since the synthesis of the chlorophyll in the plants with yellow phenotype is blocked, the shoots perish at the two-leave-stage.

The one-year-old seeds were treated with the pesticides dissolved in distilled water (0.02, 0.04, 0.06, 0.08 and 0.1% solutions). 100 air-dried seeds were treated with the preparation of corresponding concentration for 24 hours. After the procedure the seeds were washed in running water for 4 hours and sowed in wooden boxes (black sand an soil, intermixed in equal amount). The seeds destined for control were placed in distilled water for 24 hours and then planted.

The analysis of obtained results was performed according to the method of assessment of patches on upper surfaces of the first two simple- and the third complex-leaves [Vig, Paddok, 1970, Vig, 1985]. The analysis of induced patches was conducted under the microscope (MBC-9, at 10x2 magnification). The obtained results were statistically estimated.

## Results and Discussion

The pesticide-induced genetic alterations in somatic cells of the homozygous ($Y_{11}Y_{11}$) sprout leaves of plants with green phenotype are given in Table 1. Yellow patches as well as light-green areas were observed on leaf surfaces. Appearance of light-green patches is caused by the mutation of one of the semi-dominant allele pair. Yellow patches arise only in case if the both alleles are mutated. Khomecyn and Ridomil cause formation of light-green patches more often than yellow ones indicating that the induction of a single mutation is more frequent compared with double mutations. 0.1% concentration of Ridomil induces formation of 0.53 light-green and 0.09 yellow patches per leaf on average. The corresponding values for Khomecyn were 0.55 and 0.07 respectively. The both preparations in equal rates caused induction of mutations and the dose-dependent correlation was observed.

**Table 1.** Genetic changes induced by pesticides in the leaves of homozygous green plants

| compound, concentration % | total number of analyzed leaves | total number of spots | average number of spots per leaf | | |
|---|---|---|---|---|---|
| | | | yellow | light-green | in all |
| Ridomil | | | | | |
| 0,02 | 80 | 28 | 0.03±0.007 | 0.32±0.05 | 0.35±0.06 |
| 0,04 | 75 | 30 | 0.05±0.006 | 0.35±0.04 | 0.40±0.06 |
| 0,06 | 65 | 35 | 0.07±0.008 | 0.46±0.08 | 0.53±0.09 |
| 0,08 | 55 | 26 | 0.08±0.009 | 0.40±0.06 | 0.48±0.07 |
| 0,1 | 60 | 37 | 0.09±0.01 | 0.53±0.06 | 0.62±0.07 |
| Khomecyn | | | | | |
| 0,02 | 80 | 14 | 0.04±0.008 | 0.13±0.04 | 0.17±0.05 |
| 0,04 | 85 | 21 | 0.04±0.007 | 0.21±0.02 | 0.25±0.03 |
| 0,06 | 70 | 29 | 0.05±0.008 | 0.36±0.03 | 0.41±0.04 |
| 0,08 | 65 | 30 | 0.05±0.008 | 0.41±0.09 | 0.46±0.10 |
| 0,1 | 50 | 31 | 0.07±0.01 | 0.55±0.09 | 0.52±0.10 |
| Control | 100 | 18 | 0.03±0.02 | 0.15±0.03 | 0.18±0.05 |

Influence of Khomecyn and Ridomil on suspended seeds has also been studied. The age of seeds markedly affect induction of mutations. The latter is far more intensive in suspended that in one-year-old seeds.

The tested pesticides caused induction of only simple (green and yellow) patches in somatic cells of light-green ($Y_{11}y_{11}$) sprout leaves. Such alterations are due to different genetic disorders, such as point mutations, deletions, chromosome non-disjunction, etc. The results are shown in Table 2. The preparations do not differ from each other by the rate and spectrum of induced mutations. The two preparations induced direct mutations more often than reversions. The dose-effect phenomenon was also registered.

**Table 2.** Genetic changes induced by pesticides in the leaves of heterozygous light-green plants

| compound, concentration % | total number of analyzed leaves | total number of spots | average number of spots per leaf | | | in all |
|---|---|---|---|---|---|---|
| | | | yellow | green | double | |
| Ridomil | | | | | | |
| 0,02 | 105 | 96 | 0.58±0.09 | 0.33±0.03 | 0 | 0.91±0.12 |
| 0,04 | 90 | 93 | 0.64±0.06 | 0.39±0.05 | 0 | 1.03±0.11 |
| 0,06 | 95 | 104 | 0.70±0.08 | 0.39±0.02 | 0 | 1.09±0.10 |
| 0,08 | 80 | 88 | 0.71±0.07 | 0.40±0.06 | 0 | 1.10±0.13 |
| 0,1 | 85 | 124 | 1.18±0.13 | 0.28±0.02 | 0 | 1.46±0.16 |
| Khomecyn | | | | | | |
| 0,02 | 100 | 72 | 0.51±0.05 | 0.21±0.02 | 0 | 0.72±0.07 |
| 0,04 | 110 | 84 | 0.55±0.04 | 0.21±0.03 | 0 | 0.76±0.07 |
| 0,06 | 95 | 80 | 0.62±0.05 | 0.22±0.04 | 0 | 0.84±0.09 |
| 0,08 | 85 | 83 | 0.81±0.04 | 0.17±0.03 | 0 | 0.98±0.07 |
| 0,1 | 80 | 111 | 1.27±0.04 | 0.12±0.02 | 0 | 1.39±0.06 |
| Control | 120 | 38 | 0.22±0.03 | 0.10±0.02 | 0 | 0.32±0.05 |

In the same test system for suspended seeds the following effect was observed: Ridomil and Khomecyn more frequently induced mutations. In the plants, grown from suspended seeds when treated with 0.1% concentration of the preparations, the total number of patches per leaf was on average 2.58 for Khomecyn and 3.40 for Ridomil, but in one-year-old seeds they were 1.39 and 1.46 respectively. Our results correspond to the data obtained by other authors in plants belonging to other species [Dubinin, 2000].

Pesticide-treatment in the case of homozygous yellow ($y_{11}y_{11}$) shoots caused appearance of only light-green patches on the surface of leaves (see Table 3). Such process in somatic cells may be caused by the mutation occurred only in one of the allele pair. In our experiment no occurrence of simultaneous mutations in both loci was registered. It would induce formation of green patches. The effect of Ridomil in low concentrations almost did not differ from spontaneous level, while Khomecyn in high concentration caused significant elevation of reversion rates. As compared with one-year-old seeds, in the plants with yellow phenotype developed from the suspended seeds, the two preparations were more effective to cause reversions [Baratashvili et al., 2003]. For instance, when treating suspended seeds with 0.1% solution of Khomecyn, on average, 0.81 light-green patch per leaf was developed. In the case of Ridomil this number is 0.62. When one-year-old seeds were exposed to the same concentrations of agents these indices were 0.22 and 0.27 respectively.

Table 3. Pesticide-induced genetic changes in the leaves of homozygous yellow plants

| compound, concentration % | total number of analyzed leaves | total number of light-green spots | total number of spots per leaf |
|---|---|---|---|
| Ridomil | | | |
| 0,02 | 32 | 2 | 0.06±0.02 |
| 0,04 | 33 | 2 | 0.06±0.02 |
| 0,06 | 30 | 4 | 0.13±0.08 |
| 0,08 | 28 | 7 | 0.25±0.08 |
| 0,1 | 30 | 8 | 0.27±0.07 |
| Khomecyn | | | |
| 0,02 | 31 | 3 | 0.10±0.03 |
| 0,04 | 26 | 2 | 0.08±0.05 |
| 0,06 | 20 | 3 | 0.15±0.06 |
| 0,08 | 22 | 3 | 0.14±0.05 |
| 0,1 | 18 | 4 | 0.22±0.06 |
| Control | 50 | 1 | 0.02±0.008 |

The same test-system has been used for testing the influence of Mikol and Kerozat on suspended seeds. High genetoxicity of these preparations were detected [Chitanava et al., 2003]. Genetoxic activities of other pesticides widely used in Georgia were studied as well. Keltan, Epidor, Hexathiuram and Heptathiuram were found to have high rate of mutagenic and recombinogenic activities. The same genetic line of soybean was used for studying the effect of some physical mutagenes: X-rays, γ-radiation, neutrones [Vig, 1985; Zakharov, Davronov, 1985]. The effect of low doses of these mutagenes significantly exceed the genetic effect of the mutagenes we have tested.

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# პესტიციდებით (ხომეცინი, რიდომილი) გამოწვეული გენეტიკური ცვლილებები სოიაში *(GLYCINE MAX (L) MERR)*

ბარათაშვილი ნ.[1], ჭითანავა ჟ.[2], შათირიშვილი ა.[1]

*[1]გენეტიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი*

*[2]ზოგადი ბიოლოგიის კათედრა, შოთა რუსთაველის სახ. ბათუმის სახელმწიფო უნივერსიტეტი*

(მიღებულია 10.05.2004)

## რეზიუმე

სოიაში *(Glycine max (L) Merr)* შემუშავებული ტესტ-სისტემის გამოყენებით შესწავლილია პესტიციდების, რიდომილის და ხომეცინის გენოტოქსიკური მოქმედება. პრეპარატები დაყოვნებულ თესლებზე გაცილებით მაღალ გენოტოქსიკურობას ავლენენ დაუყოვნებელთან (ერთწლიანი) შედარებით. ორივე პრეპარატი მაღალი სიხშირით იწვევს პირდაპირი მუტაციის ინდუქციას, ვიდრე რევერსიებს. მუტაციის ინდუქციის სპექტრით პრეპარატები ერთმანეთისაგან უმნიშვნელოდ განსხვავდებიან.

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Genetics

# STUDY OF CHROMOSOMAL ABERRATIONS FREQUENCY OF THE SECOND CYCLE LINES OF MAIZE RECEIVED ON POLLUTED WITH HEAVY METALS SOILS

MAMULASHVILI L. NASYIDASHVILI P., GOGINASHVILI K.

*Department of Genetics and Selection, Georgian State Agricultural University*

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**Abstract**

Chromosomal aberrations of meristemal cells of maize's second cycle lines: "Georgian 9" $i_5$ 15/36 and "Enguri" $i_5$ 11/28, grown on ecologically pure soils (Mtskheta region), as well as on polluted with heavy metals soils (Bolnisi region) were studied. It was shown that frequency of chromosomal aberrations of maize's second cycle lines of Bolnisi region is increased compared to Mtskheta region: this parameter for "Georgian 9" $i_5$ 15/36 of Bolnisi region is 26,87±4% and of Mtskheta region – 2,4±0,7%; for "Enguri" $i_5$ 11/28 of Bolnisi region is 11,57±2% and of Mtskheta region 0,2±0,56%.

**Key Words:** maize's hybrid, heavy metals, chromosomal aberrations

## Introduction

The increase of ecologically pure agricultural products, as well as the decrease of the negative influence of the anthropogenic factors for conducting of biosphere's natural cycle is nowadays argent problem. The gravest danger for natural cycle of biosphere is the industrial and consumption waste as they cause the negative changes of natural processes. Among these processes the most important is to reveal the mutagenic influence of heavy, toxic metals on the plant cultures [Ilin V., 1991].

For the plant defence it is very important to study mutagenic activity induced by the heavy metals, estimate the frequency of chromosomal aberrations of meristemal cells of maize's new lines.

## Materials and Methods

Meristemal cells of maize's heterosised hybrids of second cycle lines: "Georgian 9" $i_5$ 15/36 and "Enguri" $i_5$ 11/28, grown on ecologically pure soils of village Tserovani, Mtskheta Region (experimental plot of the Agriculture University), as well as on polluted with heavy metals soils of village Khatissopeli, Bolnisi Region plot, were studied [Mamulashvili L. et al., 2004].

Microscopic analysis were carried on "? ??-3" and "? ??-6" microscopes using photo films "Mikrat-200" and "Mikrat 300". For microscopical studies of meristemal cells accepted cytological methods were used [Pausheva, 1988].

Percentage of aberrations frequency was calculated.

The standard deviation formula of chromosomal aberrations is:

$$m = \pm\sqrt{\frac{(100-n)n}{N}},$$

where n – is the percentage of cells with chromosomal disorders, N – the amount of studied mitoses.

## Results and Discussion

In the meristemal cells of second cycle lines of "Georgian 9" $i_5$ 15/36 of Bolnisi (total amount – 117) the anaphases with bridges and fragments (17), sticky and circle chromosomes (13), backward and asymmetrical anaphases (11) are occurred more often (Fig.1). The aneuploidy and polyploidy cells are occurred in considerable less amount – 2. In this line chromosomal aberrations frequency is 26,88 ± 4,00%, while this parameter of 530 meristemal cells of the same line of Mtskheta Region is 2,4 ± 0,66% (Fig.2).

**Table 1.** The frequency of chromosomal aberrations of the meristemal cells of second cycle lines of maize ("Georgian9", "Enguri") of polluted and unpolluted regions

| № | The lines of maize | Region | The total amount of studied normal cells | Anaphases with the bridges and fragments | sticky and circle chromosomes | Backward and asymmetrical anaphases | aneuploidy and polyploidy cells | total amount of studied mitoses | The total amount of aberrations | The frequency of aberrations % |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | "Georgian 9" $i_5$15/36 | Mtskheta | 530 | 8 | 2 | 3 | - | 543 | 13 | 2,394 ± 0,66 |
| 2 | "Georgian 9" $i_5$15/36 | Bolnisi | 117 | 17 | 13 | 11 | 2 | 160 | 43 | 26,87 ± 4,00 |
| 3 | "Enguri" $i_5$ 11/28 | Mtskheta | 605 | 10 | - | 1 | 1 | 617 | 12 | 0,194 ± 0,56 |
| 4 | "Enguri" $i_5$ 11/28 | Bolnisi | 237 | 14 | 11 | 4 | 2 | 268 | 31 | 11,567 ± 2,07 |

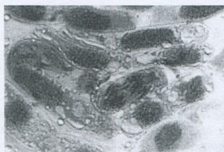

**Fig.1.** Anaphase with bridges (two-bridged) of "Georgian 9" $i_5$ 15/36 (Bolnisi r.)

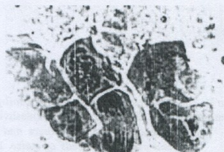

**Fig.2.** asymmetrical anaphase "Georgian 9" $i_5$ 15/36 (Mtskheta r.)

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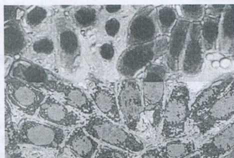

**Fig.3.** anaphase with bridge; backward chromosomes "Enguri" $i_5$ 11/28 (Bolnisi r.)

**Fig.4.** Telophase with bridge."Enguri" $i_5$ 11/28 (Mtskheta r.)

As for meristemal cells of second cycle lines of "Enguri" $i_5$ 11/28, the chromosomal aberrations are less compared to "Georgian 9" $i_5$ 15/36. In Bolnisi region from studied 327 normal cells the anaphases with bridges and fragments were only 14 (Fig.3). More often were occurred backward chromosomes. The amount of sticky and circle chromosomes was 11, of backward and asymmetrical anaphases – 4, of aneuploidy and polyploidy cells –2. Chromosomal aberrations frequency was 11,57 ± 2,07% and for 605 studied cells of the same line of Mtskheta region – 0,2 ± 0,56% (Fig.4).

Thus, the mutagenic influence of heavy metals on the maize's lines is clear. The decrease of the total amount of meristemal cells and increase of separate cells size of received in Bolnisi region lines was observed. The chromosomal aberrations of Bolnisi lines were expressed stronger compared to Mtskheta lines.

It was revealed that the frequency of chromosomal aberrations of meristemal cells of "Georgian 9" $i_5$ 15/36 is higher than of "Enguri" $i_5$ 11/28 in both, polluted and unpolluted regions. The frequency of chromosomal aberrations of meristemal cells of received in Bolnisi "Georgian 9" $i_5$ 15/36 is by 24,48% more compared to the ones of Mtskheta region. The frequency of chromosomal aberrations of meristemal cells of received in Bolnisi "Enguri" $i_5$ 11/28 is by 11,37% more compared to the ones of Mtskheta region.

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# მძიმე მეტალებით დაბინძურებულ ნიადაგზე მიღებული სიმინდის მეორე ციკლის ხაზების მერისტემული უჯრედების ქრომოსომული დარღვევების სიხშირის შესწავლა

მამულაშვილი ლ., ნასყიდაშვილი პ., გოგინაშვილი ქ.

*გენეტიკისა და სელექცია-მეთესლეობის კათედრა, საქართველოს სახელმწიფო აგრარული უნივერსიტეტი*

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## რეზიუმე

შესწავლილია, როგორც მცხეთის რაიონის ეკოლოგიურად სუფთა ნიადაგზე, ასევე ბოლნისის რაიონის მძიმე მეტალებით დაბინძურებულ ნიადაგზე მიღებული სიმინდის მეორე ციკლის ხაზების: "ქართული 9" $i_5$ 15/36-ისა და "ენგური" $i_5$ 11/28–ის მერისტემული უჯრედების ქრომოსომული აბერაციები. დადგინდა, რომ ბოლნისის რაიონში მიღებული სიმინდის მეორე ციკლის ხაზებში მცხეთის რაიონთან შედარებით საგრძნობლად არის მომატებული ქრომოსომული აბერაციების სიხშირე: ბოლნისის რაიონში "ქართული 9" $i_5$ 15/36 ხაზის მერისტემული უჯრედების ქრომოსომული აბერაციების სიხშირე 26,87±4%-ია. მცხეთის რაიონის ანალოგიური ხაზის ქრომოსომული აბერაციების სიხშირე 2,4±0,7%-ს შეადგენს. "ენგურის" $i_5$ 11/28–ში ქრომოსომული აბერაციების სიხშირე ბოლნისის რაიონში 11,57±2%-ია, მცხეთის რაიონში კი 0,2±0,56%-ს შეადგენს.

ეროვნული
ბიბლიოთეკა

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Genetics

# STUDY ON ECOLOGICAL ASPECTS OF SEAWATER BY MARKER METHOD

TSILOSANI N., ALAVIDZE Z., ELPINERI L., CHKONIA I., KIKNADZE M., ZAALISHVILI I., DZIDZISHVILI M.

*Department of Genetics, Iv. Javakhishvili Tbilisi State University*

(Received May 10, 2004)

**ABSTRACT**

The methods for modeling of spreading the infection agents through water are considered. The data on improving selective methods for biological tracer-bacteriophages are presented. It has been shown, that standard nonspecific bacteriophages may be tested on their fitness for tracing by plating them on the mixture of permissive and non-permissive bacterial strains. The method is expected to be convenient in terms of its simplicity.

**Key words:** tracer-bacteriophages, permissive bacterial cultures, phage-resistant mutant.

## Introduction

Water plays an essential role in spreading intestinal, bacterial and viral diseases. It is obvious that we should carry out sanitary-epidemiological investigations [Melnik, et al. 1990], especially it is important to study the sources and spreading ways of infections to plan and perform appropriate measures [Khazenson; et al. 1975].

In recent years bacteriophages and nonpathogenic bacteria have been widely used as tracers because of their biological similarity to infectious agents - viruses and bacteria [Chkonia, 1997].

The purpose of the present paper was to work out a simple method for identification of tracer bacteriophages. The following activities were conducted: 1. selection of non-specific phage clones with high reproduction capacity and with high resistance to environmental factors; 2. determination of the methods for their detection and cultivation that would facilitate further tracer selection and introduction in to the practice.

## Material and methods

The resistance of bacterial strains towards phages was studied by the method of Ledenberg [Ledenberg J., Lederberg E., 1952]. Phages were isolated by water sample enrichment method. The concentration of active phages was determined by the method of Graigie. The neutralization reaction was used to study the serological features; the latent period and average productivity were registered by the method of Elice and Delbriuke.

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ბიბლიოთეკა

## Results and Discussion

*E.coli*-specific bacteriophages were isolated on standard laboratory strains of *E.coli* - CR-63 and k-12(?) cultures (26 phages). Polyvalent preparations "intestiphage" and high-specific phage M17 were obtained from starter strain of the "colibacterium" by means of UV-irradiation.

13 phages were cloned and classified into two main groups according to the negative colonies. 3 phages were selected for further studies, in particular FC-1 (from the 1st group), FC-2 (2nd group) and high-specific phage FM-17 (as a control).

It was shown that the phage FC-1 by serological features is close to DDIV and DDV group phages and according to Akerman classification system belongs to Syphoviridae [Ackermann, 1995]. As to phage FC-2, it appears to be close to T, even DDVI phages and it belongs to the Family of Myoviridae. The bacteriophage FM-17 was different from the above mentioned group in terms of serological and morphological relationship. The main taxonomic characteristics of the mentioned bacteriophagrs are given in Table 1.

**Table 1.** General taxonomic characteristics of FC-1, FC-2 and Fm-17 bacteriophages.

| | Morphology of negative colonies | Morphology of nucleocapsides | Serological group | Phases of host cell relationship | | Resistance | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | to the high temperature | | | | |
| | | | | of maximum adsorption | average harvester cell | low limit | high limit | to the UV rays (compared with T unit) | chlorous lime mg/g | NaCl high concentration |
| FC-1 | Diameter 3.0-4.0 mm with clear centre and incomplete lysis | Syphoviridae | I- related to the DDIV and DDV phages | 84 | 140-150 | 58°C – 90.6 % | 68°C – 0.3 % | 4.5 | 0.45 | 15 – 17% |
| FC-2 | Diameter 3.0-4.0 mm with clear centre and incomplete lysis | Myoroviridae | II related to T pair phages | 74 | 90-100 | 58°C – 96.6 % | 74°C – 0.5 % | 1 | 0.6 | 20% and more |
| FM-17 | Diameter 3.0-4.0 mm with clear centre and incomplete lysis | Syporoviridae | Specific | 80 | 190-200 | 58°C – 96 % | 68°C – 0.35 % | 2.55 | 0.3 | 15-17 % |

The investigated bacteriophages differed from each other by the following features: the capacity to form negative colonies; the morphology of nucleocapsides and the serological relationship. Besides, the studied phages are to be dependent on the influence of lime chloride, ultraviolet light, high temperature and salinity.

To create a favorable method for bacteriophage identification, phages were plated on permissive and non-permissive bacterial cultures and their mixture. The following cultures were

taken as permissive cultures: *E.coli* K12(?) and E.*coli* M17 and their mutants resistant to phages, *E.coli* K12 (?)/FC-1, *E.coli* k12(?) / FC-2, *E.coli* M17 / FC-17 obtained by indirect selection according to Lederberg's method. Plating efficiency indices of selected bacteriophages on permissive, non-permissive and mixed bacterial cultures, are given in Table 2.

It was shown that the tested phages do not reproduce on the phage-resistant mutants (or their reproduction is very limited), while the efficiency of phage plating on the mixed cultures containing both the permissive and non-permissive strains seems to be in close proximity to the values obtained on the initial strains (Table 2). Only negative colonies have different morphology: they are clear on the wild cultures, but turbid on the mixed cultures. In addition, the mutants do not express cross-resistance. All this indicates the specificity of the method applied for the identification of the tracer-bacteriophages.

**Table 2**. The plating efficiency indices of bacteriophages

| Bacterial strains | Bacteriophages, morphology of negative colonies | | |
|---|---|---|---|
| | FC-1 | FC-2 | FM-17 |
| 1 | 2 | 3 | 4 |
| *E.coli* K-12λ | $2*10^9$ | $3*10^9$ | 0 |
| *E.coli* K-12 / FC-1 *E.coli* K-12 λ + | 0 | $3*10^9$ | 0 |
| K-12 λ / FC-1 | $5*10^9$ | $3*10^9$ | 0 |
| *E.coli* K-12 / FC-2 *E.coli* K-12 λ + | $1*10^9$ | 0 | 0 |
| K-12 λ / FC-2 | $4*10^9$ | $2.7*10^9$ | 0 |
| *E.coli* M-17 | $8*10^8$ | $8.2*10^8$ | $3*10^9$ |
| *E.coli* M-17 / FM-17 *E.coli* K-17 λ + | $8*10^8$ | $8.2*10^9$ | 0 |
| M-17 / FM-2 | $8*10^8$ | $8.2*10^8$ | $3*10^9$ |

For recent years water quality of the Black Sea has been significantly deteriorated resulting in seawater pollution in the resort regions. Seawater has been contaminated with various infectious viruses and pathogenic bacteria.

Modeling of the spreading infectious material was conducted in the coastline zone of the Black Sea (Kobuleti, Gagra and the regions neighboring Pitsunda).

200 ml of each sample containing the tracer-bacteriophages ($2\text{-}5\times10^{11}$ infectious unit per ml) were poured into the seawater at different distances from the seacoast. In particular, the sample with phage FC-1 was tested in Kobuleti near the mouth of the Natanebi river. The phages FM-17 were poured into water at 500 m distance from the Pitsunda seashore, and the phage FC-2 samples – in Gagra near the water collector in 20 m distance from the shore.

The water sample were taken from different places (at 200, 450, 600 m and 15 km distances from the shore) at the fixed time (1, 2, 8, 12 and 22 h after starting the experiment) from various depths – 0.5; 2.5; 5.0; 10 and 20 m from a water surface.

In Kobuleti the phage FC-1 was found on the water surface after 1 h at a distance of 200 m, 2h later it moved to the depth (5-10 m) far from the seashore (640 m). After 8 h they were found in low quantities only at the place they had been poured.

The spread of FC-2 tracer was observed only in surface layers moving away from and towards the seashore. 8 h later only a small quantity of phages was observed at 500-700 m distance from the sea-front, both in surface layers and at 2.5-5 m depth as well.

In Gagra the presence of FC-2 tracers has been observed at a rather long distance (15 km) on the water surface and at 10 m depths for a quite long period of time (22 h). Distribution of tracers mostly depends on wind and underwater currents. We suppose that the sewage pouring from the collector-cleaner in Gagra could affect the phages causing them to move away from the shore. Viability of the phage can be explained by the relatively high resistance of the phage FC-2 to environmental conditions. Above all, it should be mentioned, that rather large amount of free phages has been observed in the water, pouring from the collector, and the method applied allowed us to reveal the tracers via registration of turbid negative colonies.

The data obtained enable us to suggest that any phages, even a nonspecific bacteriophage, which is easy to select, multiply and concentrate, can be used as a tracer. Furthermore, the method provides recognition of the tracer-phages from the variety of free phages.

The method is simple and economic. Special qualification is not needed for preparing and practical application of the tracers. It can be used also for studying the process of self-cleaning dynamics of water reservoirs and the work efficiency of plants waste treatment.

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წილოსანი ნ. ალავიძე ზ. ელპინერი ლ. ჭყონია ი. კიკნაძე მ.
ზაალიშვილი ი. ძიძიშვილი მ.

*გენეტიკის კათედრა, ივ. ჯავახიშვილის სახელობის*
*თბილისის სახელმწიფო უნივერსიტეტი*

(მიღებულია 10.05.2004)

## ᲠᲔᲖᲘᲣᲛᲔ

ტრასირებისათვის შესაძლებელია გამოყენებული იქნეს როგორც მაღალსპეციფიკური, ისე ნებისმიერი ბაქტერიოფაგი, რომელთა სელექცია, გამრავლება, კონცენტრირება სიძნელეს არ წარმოადგენს. ამასთან ერთად, შემუშავებული მეთოდი უზრუნველყოფს ტრასერი ფაგების გამოვლენას სისტემაში არსებული თავისუფალი ფაგების ფონზე. აღნიშნული მეთოდი ხელმისაწვდომია და არ მოითხოვს დიდ ხარჯებს ტრასერების მომზადებისა და მათი პრაქტიკული გამოყენებისათვის. ფაგებით ტრასირება შეიძლება გამოყენებულ იქნეს ასევე წყალსატევების თვითგაწმენდის დინამიკისა და გამწმენდი ნაგებობების მუშაობის ეფექტურობის შესასწავლადაც.

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Molecular biology

# THE PARTICIPATION OF PROTEIN-PROTEIN INTERACTION IN THE STRUCTURAL-FUNCTIONAL ORGANIZATION OF CHROMATIN AND CHROMATIN NONHISTONE PROTEINS

AKHALKATSI R., MACHARADZE T., KHARAZISHVILI L.

*Department of Biochemistry and Biotechnology, Iv. Javakhishvili Tbilisi State University*

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**Abstract**

The existence of glycoproteins on the surface of calf brain chromatin with following terminal carbohydrates: Man, GlcNAc, GalNAc, Gal and less Fuc was revealed. The chromatin nonhistone proteins are glycoproteins. Unlike the intact chromatin they have a lot of terminal carbohydrates: GlcNAc, Man, Gal and less Fuc. The Most of glycoprotein carbohydrates are directed inside the chromatin, which may have part in the realization of their biological function. The method worked out and GlcNAc-, Gal-, Man- specific lectins were isolated from chromatin nonhistone proteins (by affine chromatography). Unlike the nuclear membrane lectins with the same specificity, they reveal low specific activity and a high content of carbohydrates. It was concluded that protein-protein interaction take place in the structural-functional organization of chromatin and chromatin nonhistone proteins, which is realized by means of protein-carbohydrate interaction.

**Key words:** calf brain, chromatin, nonhistone proteins, lectins, glycoproteins

## Introduction:

Cytosole, nuclear membrane and nuclear matrix lectins were isolated and characterized in order to study of lectins biosynthesis, transport and their possible function in nuclei [Akhalkatsi et al., 1996; Kharazishvili et al., 1999; Kharazishvili et al. 2000]. On the one hand, our aim was to investigate the terminal carbohydrates of calf brain chromatin and nonhistone proteins and on the other hand, to isolate of the proteins with lectin activity from nonhistone proteins.

## Materials and methods

Calf brain cell nuclei were isolated by the method of Chauveau [Chauveau et al., 1956]. Chromatin was prepared by Huang and Huang [Huang R.C. et al., 1969] and nonhistone proteins – by Wang [Wang T., 1967].The lectin activity was measured by hemagglutination of trypsinized rabbit erythrocytes. The lectin-binding activity of chromatin nonhistone proteins was determined by the minimal protein concentration which inhibited the agglutination of trypsinized rabbit erythrocytes. In experiments plant lectins from "Diagnosticum Lvov" were used. The proteins with lectin activity were isolated by affine chromatography with carbohydrates (GlcNAc, Gal, Man) immobilized on tris-acryle. Carbohydrates concentration in lectins were measured by the method of Colb and Kamishnikov [Kolb V. et al., 1982], the proteins by Lowry et al., [Lowry O. et al., 1951]

and DNA by Dische [Dische E., 1955]. The data presented below are the average result obtained in five or six independent experiments ($p<0.05$).

## Results and discussion

In the first series of experiments chromatin lectin activity which was measured after detecting the presence of carbohydrates on the surface of chromatin by the happen inhibitory method was not revealed [Akhalkatsi R. et al., 1996]. The chromatin plant lectin binding ability was established in two cases: 1. Plant lectins of various specificity with constant concentration (using the titre 8) were added to titred chromatin and hemagglutination activity was measured after 30 min of preincubation. 2. Chromatin was in constant concentration and the plant lectins were titred (Table 1). Both cases of experiments indicate that the hemagglutination activity caused by galactose specific lectin SBA and mannose (glucose) specific lectins PSL, Con A and LCL was inhibited well by chromatin and less caused by fucose specific lectin LAL. So, on the surface of chromatin there are specific sites for plant lectins - glycoproteins with following terminal carbohydrates: mannose, N-acetyl-D-glucosamine, N-acetylgalactosamine, galactose and less fucose.

**Table 1**. The influence of calf brain chromatin on hemagglutination activity of plant lectins

| *The sources of the lectins* | Lectins | The carbohydrate specificity | The number of wells | | |
|---|---|---|---|---|---|
| | | | 1 | 2 | 3 |
| *Laburnum anagiroides* | LAL | Fucoso specific a L Fuc | + | - | - |
| | | Galactoso specific | | | |
| *Arachis hipogeae* | PNA | ß D Gal | + | + | - |
| *Glicine max* | SBA | a D GalNAc | + | - | - |
| *Sambucus nigra* | SNA | D Lac, D Gal | + | + | + |
| | | Mannoso-(Glucoso) specific | | | |
| *Pisum sativum* | PSL | a D Man >D Glc> D GlcNAc | + | - | - |
| *Canavalia ensiformis* | Con A | a D Man >D Glc> D GlcNAc | + | - | - |
| *Triticum vulgaris* | WGA | $(D\ GlcNAc)_n$, n=1,2,3, NANA | - | - | - |
| *Lens culinaris* | LCL | a D Man > a D Glc> D GlcNAc | - | - | - |

"+"- hemagglutination; "-" no hemagglutination.

The plant lectins were titred $T^{-1}=8$ + 67.5 mkg/50mkl chromatin in PBS + 50 mkl of 2% trypsinized rabbit erythrocytes.

In the next series of experiments the plant lectin binding activity of calf brain nonhistone proteins of chromatin was established (Table 2). In these conditions of experiments nonhistone proteins do not reveal the hemagglutination (lectin) activity. The affinity of nonhistone proteins to plant lectins was asserted by the ratio of lectin/lectin binding protein (Table 2). LAL reveals especially high affinity and less – WGA (LAL>PNA>SNA>LCL>PSL>Con A>SBA>WGA). The same picture was obtained with nonhistone proteins of rat brain chromatin, but with different specificity [Akhalkatsi R. et al., 1996]. So, isolated nonhistone proteins have a higher level of glycoproteins than those on the surface of chromatin, with following terminal carbohydrates Fuc>Gal>Man>GlcNAc. Our experiments suggest that they are directed inside the intact chromatin and they may take part in the regulation of biological processes (genome activation-inhibition) and in the structural organization of chromatin in protein-protein interaction by protein-carbohydrate connection.

It is known, that the protein-carbohydrate connection is carried out by a lectin [Gabius H., 1997]. Therefore, it may be admitted, that there are lectins between nonhistone proteins of

ეროვნული
ბიბლიოთეკა

chromatin. The method for isolation the proteins with lectin activity from nonhistone proteins of calf brain chromatin was worked out.

**Table 2.** The plant lectin binding capacity of calf brain chromatin nonhistone proteins

| The sources of the lectins | Lectins | The carbohydrate specificity | Lectin/lectin-binding Protein (nonhistone) |
|---|---|---|---|
| *Laburnum anagiroides* | LAL | Fucoso specific a L Fuc | $43.2 \times 10^{-2}$ |
| | | Galactoso specific | |
| *Arachis hipogeae* | PNA | ß D Gal | $1.88 \times 10^{-2}$ |
| *Glicine max* | SBA | a D GalNAc | $3.05 \times 10^{-5}$ |
| *Sambucus nigra* | SNA | D Lac, D Gal | $8.12 \times 10^{-3}$ |
| *Pisum sativum* | | Mannoso-(Glucoso-) specific | |
| *Canavalia esiformis* | PSL | a D Man > a D Glc> D GlcNAc | $4,47 \times 10^{-4}$ |
| *Triticum vulgaris* | Con A | a D Man > a D Glc> D GlcNAc | $4.04 \times 10^{-4}$ |
| *Lens culinaris* | WGA | $(D\ GlcNAc)_n$, n=1,2,3, NANA | $1.36 \times 10^{-5}$ |
| | LCL | a D Man > a D Glc> D GlcNAc | $3.15 \times 10^{-3}$ |

The nonhistone proteins were titred (20 mkg/ 100 mkl of PBS) + 50 mkl PBS which contain plant lectins $T^{-1} = 8 + 50$ mkl 2% of trypsinized rabbit erythrocytes.

The nonhistone proteins were dialyzed in PBS (0.9% NaCl+40mM potassium phosphate buffer pH 7.4). This protein revealed no hemagglutination activity. In PBS dialyzed nonhistone proteins were applied to GlcNAc, Gal, Man affine column (carbohydrates immobilized on tris-acryle) (3-4 mg protein to column with volume 3 ml). The unbound proteins were eluted by PBS. The specifically bound proteins were eluted by 0.25 M glicyne-HCl buffer (pH 2.5), then they were dialyzed in PBS, concentrated and the hemagglutination activity was measured (Table 3). Unlike the calf brain nuclear membrane lectins with the same specificity, they reveal low specific activity [Kharazishvili L., et al., 2000].

**Table 3.** The hemagglutination activity and carbohydrate content of chromatin nonhistone proteins GlcNAc-, Gal-, Man- specific lectins

| E# | Protein fraction | Protein concentration mg/ml | Titer $T^{-1}$ | Specific activity | Carbohydrates content mkg/mkg protein |
|---|---|---|---|---|---|
| 1 | GlcNAc-specific | 0.011 | 4 | 58.8 | 25 |
| 2 | Gal- specific | 0.007 | 4 | 142.6 | 14.2 |
| 3 | Man- specific | 0.009 | 2 | 222 | 31.25 |

It is known, that most of animal lectins are glycoproteins. The lectin of chromatin nonhistone proteins are also glycoproteins, as they contain carbohydrates (Table 3). The low level of specific activity is the reason of the high content of carbohydrates.

Thus, the lectins which take part in the protein-protein interaction by protein-carbohydrate connection were isolated from nonhistone proteins of chromatine. This suggests the participation of protein-protein interaction in the structural-functional organization of chromatin and chromatine nonhistone proteins which is realized by means of protein-carbohydrate interaction.

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## ქრომატინისა და ქრომატინის არაჰისტონური ცილების სტრუქტურულ-ფუნქციურ ორგანიზაციაში ცილა-ცილოვანი ურთიერთქმედების მონაწილეობის შესახებ

ახალკაცი რ., მაჭარაძე თ., ხარაზიშვილი ლ.

*ბიოქიმიისა და ბიოტექნოლოგიის კათედრა, ივ.ჯავახიშვილის სახ.თბილისის სახელმწიფო უნივერსიტეტი*

(მიღებულია 23.02.2004)

### რეზიუმე

ხბოს თავის ტვინის ქრომატინის ზედაპირზე გამოვლენილია არსებობა გლიკოპროტეინებისა შემდეგი ტერმინალური ნახშირწყლებით: Man, GlcNAc, GalNAc, Gal და მცირედ Fuc. ქრომატინის არაჰისტონური ცილები ასევე წარმოადგენენ გლიკოპროტეინებს. ინტაქტური ქრომატინისაგან განსხვავებით მათ ჭარბად აქვთ შემდეგი ტერმინალური ნახშირწყლები: GlcNAc, Man, Gal და მცირედ Fuc. გლიკოპროტეინების ნახშირწყლების უმეტესობა მიმართულია ქრომატინის შიგნით, რასაც მნიშვნელობა უნდა ჰქონდეს მისი ბიოლოგიური ფუნქციის გამოვლენაში. შემუშავებულია მეთოდი და არაჰისტონური ცილებიდან აფინური ქრომატოგრაფიით გამოყოფილია GlcNAc-, Man-, Gal-სპეციფიკური ლექტინები, ბირთვის მემბრანის ამავე სპეციფიკურობის ლექტინებისაგან განსხვავებით, დაბალი სპეციფიკური აქტივობითა და ნახშირწყლების მაღალი შემცველობით. გაკეთებულია დასკვნა, რომ ქრომატინისა და არაჰისტონური ცილების სტრუქტურულ-ფუნქციურ ორგანიზაციაში ადგილი აქვს ცილა-ცილოვან ურთიერთქმედებას, რომელიც ცილა-ნახშირწყლოვანი ურთიერთქმედებით ხორციელდება.

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Morphology

# IMMUNOLOGICAL AND NEUROMORPHOLOGICAL STUDY OF *STAPHYLOCOCCUS AUREUS* INFECTED MICE

OCHIGAVA I.[1] KALANDARISHVILI L.[2], ZHVANIA M.[3], KVESITADSE E.[1]

[1]*Georgian State Technical University;* [2]*Pharmaceutical Company "Biopharm L" LtD;* [3]*Georgian Academy of Science, Institute of Physiology*

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**Abstract**

Antibacterial features of *Saccharomycs cerevisiae* (*S.cerevisae*) was studied in vivo. Comparative analysis of the effect of antibacterial (Staphylcoccal bacteriophage, gentamicine ) and immunostimulating preparations (*S.cerevisiae*, Glucan) on various groups of animals suffering from *Staphylococcus aureus* (*S. aureus*) was carried out. The effect of *S. aureus* on the number of neurons and glial cells in different areas of mice hippocampus and the effects of those preparations on such possible changes of hippocampus was elucidated. Immunostimulating action of the yeasts manifested by high titers of antibacterial and anti-toxic antibodies was observed in animals treated with *S.cerevisiae*. There were statistically true changes in the total number of glial elements in CA 1 area of hippocampus in the animals treated with *S. cerevisiae*.

**Key words**: *Saccharomyces cerevisiae*; staphylococcal infection; Passive Hemoglutation Test; CA1 Field of Hippocampus.

## Introduction

Host defense presents one of the most critical physiological challenges for an animal. The ability to prevail against infection with pathogenic microorganisms requires first that animals possess a sensory system capable of detecting pathogens [Besedovsky et al. 1996]. Second, the animals must be able to remove or inactivate the pathogens once detected. In vertebrates, specialized immune cells (macrophages, granulocytes and certain T cells) and blood - borne molecules (complement) directly destroy pathogens. These efforts are supported by endocrine, metabolic, and cardiovascular changes that are coordinated by autonomic and neuroendocrine areas of brain.

Immune-derived mediators, including cytokines, released following detection of pathogens initiate the activation of a constellation of brain regions collectively involved in homeostatic regulation, including host defense [Elmquest et. al. 1996]. Brain mediated host defense mechanisms, components of the "acute phase response" to infection, include fever, somnolence, and elevations of plasma corticosteroids, as well as behavioral alternations such as social withdrawal and hypophagia. In this way, the immune system is able to recruit a wide variety of body-wide defense mechanisms in response to infection. [Goehler et. al. 2001 ].

In addition to T cell - dependent immune sensory mechanisms, many immune cells, including dendrite cells and macrophages, express specific receptors for bacterial cell wall constituents or viral proteins [Medzitov et. al. 1997]. Lipopolysaccharide (LPS) are believed to be

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the most important marker for gram-negative bacteria, whereas peptidoglican byproducts such as muramyl dipeptides (MDP) serve as salient stimuli from gram positive bacteria [Pabst et. al.1999]. Immune cells activated by binding of those substances release hormone – like mediators such as cytokines and chemokines that activate other immune cells and can also serve to signal the brain. Thus both T cell - dependent and –independent mechanisms of pathogen detection lead to the realize of mediators coordinating both the immune and nervous system.

Whereas both types of immune - sensory transduction mechanisms lead to production of mediators capable to signaling the brain, most of them derives from T cell - independent stimuli such as LPS or MDP. However, it was shown that T cell activation by a superantigen derived from gram – positive bacteria [staphylococcal enterotoxin B (SEB)] activates the hypothalamus-pytuitary – adrenal (HPA) axis and initiates anxiety – like behavior in mice [Shurin et al. 1997]. Those findings raise interesting issues regarding the specificity of neuronal responses to immune stimuli. For this reason it is important to study the influence some antibacterial substances closely with changes in CNS and immunsystem.

The present investigation is focused on antibacterial features of *Saccharomycs cerevisiae* (*S.cerevisae*) in vivo. The following goals were set: 1. Comparative analysis of the effect of antibacterial (Staphylococcal bacteriophage, gentamicine ) and immunostimulating preparations (*S.cerevisiae*, Glucan) on various groups of animals suffering from *Staphylococcus aureus* (*S. aureus*). 2. Elucidate: a). The effect of *S. aureus* on the number of neurons and glial cells in different areas of mice hippocampus and b) if those preparations have some effects on such possible changes of hippocampus.

## Material and Methods

*Animals.* Four weeks old male mice were used in this work. Animals were housing in flexible plastic isolators and handled according to the institutional guidance for animal welfare. The different groups of animals were used (total number of animals – 30; 5 – animals in each group).

*Immunological and Histological studies*. To initiate infection the mice were challenged hypodermically with $4x10^9$ cfu (colony forming units) of *S.aureus* strains. At 72h postinfection mice were treated with different antibacterial and immunostimulant drugs. Group 3 mice were treated with 1ml of $10^9$ pfu $ml^{-1}$ (plague forming units) of staphylococcal bacteriophage daily; group 4 mice were challenged with 0.5 ml of $10^7$ cfu $ml^{-1}$ of *S. cerevisiae* daily; group 5 mice were challenged with 1.2g glucan daily; group 6 mice were treated with 4% solution of gentamicine-0.25ml (daily dose). groups 1 and 2 were controls.

After 10 days treatment blood was collected from intraorbital vein. Mice were killed by ether anoxia and brain was removed from each.

Antibacterial and antitoxic antibodies and staphylococcal α-toxin were detected using the modified method of passive hemagglutination test [Solovjov et. al 1980]. The antibacterial and antitoxic erythrocyte diagnosticums were prepared in the Laboratory of immunology of the G. Eliava Institute of Microbiology, Virusology and Bacteriophage of Georgian Academy of Sciences. Brains for histological analysis were placed into 10% buffered formalin and embedded in paraffin. 20 μm sections were stained with Thionin. The quantitative analysis of total number of neurons, glial cells and satellites was performed in all layers and all subdivisions (CA1-CA 4, fascia dentate) of hippocampus of the mice from all experimental groups according the method of West. The counts were made with the optical detector from a systematic random sample of the sections. The sections were selected at uniform intervals along the entire length of each region with a random start position.

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*Fever measurement.* Body temperatures were measurement daily using standard biothelemetry procedures.

*Statistical analysis.* Data were analyzed by statistical software- MINITAB Release 13.1. In all tests p value below 0.05 was taken as an indication of statistical significance. All values are reported as means ± standard errors of the means.

## Results and Discussion

Immunological results are summarized in the Fig 1. There were statistically true changes in the total number of antibacterial antibodies in the infected animals (53±19; p=0.04) and in the animals infected and treated with yeast (53±19; p=0.04); Surprisingly, there were no statistical changes in the number of antitoxic antibodies in the group of infected animals, since the statistically significant results were received in the group 4 (16±0.1; p=0.00) and in the group 6 (9±6; p=0.2).

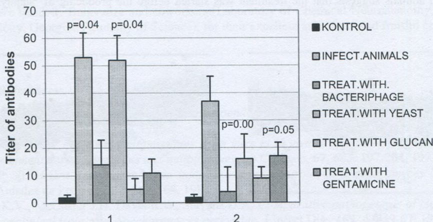


**Fig. 1.** Passive Hemagglutination Test Response: 1. Change of antibacterial antibodies; 2. Change of antitoxic antibodies.

Body temperatures were statistically true changed at the acute phase of infection (39± 0.9; p = 0.007) and at the duration of treatment (38 ±.6; P = 0.007).

The total number of neurons and glia were unchanged in mice from group 2 (infected by *S. aureus*), group 3 (infected by *S. aureus* and treated with phage), group 5 (infected by *S. aureus* and treated with gucane) and group 6 (infected by *S. aureus* and treated with gentamicine). As to group 4 (infected by *S. aureus* and treated with *S. cerevisae*) there were statistically true changes in the total number of glial elements in CA 1 area of hippocampus. In particular, if in the intact animals this number was 761±181, and in the animals infected with *S. aureus* it was-649±157; p=0.5, in the animals infected and treated with *S. cerevisae,* the total number of glial elements was statistically increased: 1710±392; p=0.05. (Fig. 1.B) Surprisingly, there were no statistical changes in the number of satellites in the mice of this group: no in this field, nor in others – such changes aren't found.

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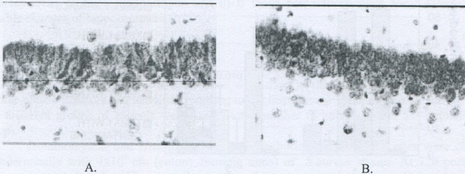
Because of a long association with human food and drink, the yeast *S. cerevisiae* has been considered to be harmless saphropite. The scientists began to study the pharmacological spectrum of *Saccharomyces* yeasts from 1940. It was determined, that at the presence of quantitative contents of B-group vitamins in the yeasts a single injection with *Saccharomyces boulardii* was enough to cure rats having a deficit of B1 vitamin [Brunnel et. al. 1972 ]. Further evaluation suggests that the passive or negative effect of these yeasts depends on the dosage. Later the positive effect of application of *S. boulardii* in the cases of acute diarrhea infections and diarrhea and colits caused by antibiotic therapy was shown [Chapoy et. al. 1987].

In the experiments the staphylococcal infectious was chosen because of the pathogenesis of *S.aureus*. This is the important human pathogen, which pathogenesis was not diminished by the application of antibiotics and there are no literature data about the influence of *S.cerevisiae* on *S.aureus* in vivo.

Study of changes of antibacterial and antitoxic antibody enables us to detect development of the infection (based on the statistical significant results of antibacterial antibodies in the group of infected animals), but the absence of statistical changes of antitoxic antibodies in the group of infected animals suggests that the treatment was started before the producing of staphylococcal toxins.



A. B.

**Fig. 1.** A) CA1 field of hippocampus – control (40 × 4.3); B) CA1 field of hippocampus – *S. cervisiae* treated animals (40 × 4.3).

As a first step of our research of structural changes we decided to estimate the total number of neurons and glia in different levels and subdivisions of hippocampus – one of the major structures of limbic system. The hippocampal formation is believed to play important role in the learning and memory, as well as in the control of emotional behaviors and neuroendocrine functions. In mice it was shown, that T-cell dependent immune stimuli, including the bacterial superantigen staphylococcal enterotoxin B (SEB), can activate the hypothalamic pituitary adrenal axis, elevates interleucin-2 and provoke c-fos expression in the paraventricular nucleus of hypothalamus and amygdala. Consistent with the notion that central corticotrophin releasing hormone (CRH) alterations induced by SEB may affect emotionality, SEB challenge augmented appetitive neophobia in a context-dependent manner, being marked in a novel and stressful environment. Correspondingly it is interesting to investigate the structures of limbic system which are actively involved in these processes; especially interesting is the investigation of possible structural changes provoked by staphylococcus in these regions.

According to our quantitative data, true changes in the CNS were found exclusively in the mice infected and treated with the *S. cerevisae*. However *S. aureus* itself doesn't provoke any

modifications in the cytoarchitectonics of studied areas. So, it is possible to suggest that the true increase of the number of glia is the result of the action of *S. cerevisae* itself. It was shown that some clinical isolates of *S. cerevisiae* can proliferate and resist clearance in vivo that suggests the potential of *S. cerevisiae* to cause the clinical disease; at the same time, it was shown the increase of the number of glial cells in the brain by clinical and nonclinical isolates of *S. cerevisiae* [Clemons et. al. 1994].

Though according to our data we can't explain the mechanism involved in these changes, we have some doubts about the ways of using the living cells of *S. cerevisae* in medicine. This question should be investigated in more details, and at first, it is necessary to determine exact dose/s and duration of treatment with this probiotic.

## Acknowledgments:

Authors express gratitude to Dr. M. Bubashvili and coworkers (Laboratory of immunology, G. Eliava Institute of Bacteriology, Bacteriophage and Virology) for their help in the study of immunological changes.

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Solovjov L.B., Georgadze U.A., Bubashvili M.A., Natadze M.M., Rigvava S.A., Kiladze L.V. *Lymes-gemolyse (Lh) and passive hemagglutation (Phg) reactions against humans staphylococcal infectious.* Abstractsbook "Viral and Bacterial Preparations", Tomsk, **29**, 168-172,1980 (in Russian).

ეროვნული ბიბლიოთეკა

# იმუნოლოგიური და ნეირომორფოლოგიური ცვლილებების შესწავლა *Staphylococcus aureus* - ით ინფიცირებული თაგვების მკურნალობისას

ოჩიგავა ი.[1], კალანდარიშვილი ლ.[2]; ჟვანია მ.[3], კვესიტაძე ე.[1]

*[1]საქართველოს ტექნიკური უნივერსიტეტი,*
*[2]ფარმაცევტული კომპანია, შპს "ბიოფარმ L",*
*[3]საქართველოს მეცნიერებათა აკადემიის ფიზიოლოგიის ინსტიტუტი*

(მიღებულია 10.10.2004)

## რეზიუმე

შესწავლილია *S. crevisiae*–ს ანტიმიკრობული თვისებები in vivo. ჩატარებულია ანტიბაქტერიული (სტაფილოკოკური ბაქტერიოფაგი; გენტამიცინი) და იმუნომასტიმულირებელი (*S.cerevisiae*, გლუკანი) პრეპარატების ქმედითუნარიანობის შედარებითი ანალიზი *S. aureus*-ით ინფიცირებულ ცხოველთა ჯგუფებში. განსაზღვრულია, ახდენენ თუ არა ეს პრეპარატები ზეგავლენას *S. aureus* – ით გამოწვეულ ცვლილებებზე ცნს-ის ზოგიერთ უბანში (ჰიპოკამპალური ფორმაცია). გამოვლენილია *S. cerevisiae*-ით ნამკურნალევ ცხოველთა ჯგუფში იმუნური სისტემის მოდულაცია (ანტიტოქსიკური და ანტიმიკრობული ანტისხეულების მაღალი ტიტრი სხვა ჯგუფებთან შედარებით). *S. cerevisiae*-ით ნამკურნალევ ცხოველთა ჯგუფში სტატისტიკურად სარწმუნო ცვლილება იქნა გამოვლენილი ჰიპოკამპის CA1 ველის გლიურ ელემენტთა საერთო რიცხვში.

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*Proc. Georgian Acad. Sci., Biol. Ser. B*
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Phytopathology

# NEW DATA ON MICROFUNGI OF GEORGIA

GVRITISHVILI M. [1], KACHEISHVILI-TAVARTKILADZE K. [1], CHURGHULIA-SHURGHAIA M. [2], GOTSADZE N. [3]

[1] *Tbilisi Botanical Garden Georgian Academy of Sciences*
[2] *Sukhumi Branch of Iv. Javakhishvili Tbilisi State University,*
[3] *Department of Botany, Iv. Javakhishvili Tbilisi State University*

(Received September 14, 2004)

**Abstract**

*Diaporthe pulla* Nitschke and its anamorph *Phomopsis pulla* (Sacc.) Traverso on *Hedera helix*, *Phomopsis picea* (Pers.) Höhn. on *Hypericum sp.* are found for the first time in Georgia. Additional host-plants and new localities are reported for eight further species of microfungi from Georgia: *Cytospora chrysosperma* (Pers.) Fr., *Diatrypella favacea* (Fr.) Ces. & De Not., *Disculina vulgaris* (Fr.) B.Sutton, *Eutypella ailanthi* (Sacc.) Sacc., *E.scoparia* (Schwein.) Ellis & Everh., *Hysterographium fraxini* (Pers.) De Not., *Stigmina obtecta* (Petrak & Esfandiari) M.B.Ellis, *Valsa ambiens* (Pers.) Fr.

**Key words** : mycobiotic complexes, fire injured plants, necrotrophic micromycetes.

## Introduction

This paper continuing a contribution to the mycobiotic diversity of Georgia provides brief information concerning new records of necrotrophic micromycetes (NM) and new fungus-host combination from Georgia. More interesting from the viewpoint of fungus-host specificity and pathogenecity of NM are mycobiotic complexes (MC) abundantly appeared in/on fire injured plants [Gvritishvili, 1982] which are considered to be the model object for studying plant-necrotrophic fungi interaction, fungal life strategy and formation of MC as well as to evaluate their role in woody plants pathogenesis in relation to the known conception of predisposition, stress and diseases [Schoeneweiss, 1975].

## Materials and Methods

The materials for investigation have been collected during field observations conducted mainly in Tbilisi and the surrounding area and in some other regions of Georgia. The specimens collected contain necrotrophic micromycetes associated mainly with woody plants including fire injured trees and shrubs. It is postulated that places after fire are considered as an accidental experimental plots with artificial plantations or natural stands damaged from fire to a different extent. At the same time uninjured plants or their uninjured parts can be considered as a control variants. Routine light microscopic method has been used for identification of collected specimens on the base of macro and micromorphological features.

## Results and Discussion

The list of the host plants of necrotrophic micromycetes identified on the base of macro and micromorphological features and their localities are presented:

***Cytospora chrysosperma*** **(Pers.) Fr.** [Gvritishvili, 1982; Hayova & Minter, 1998]

*Alnus barbata*, on fire injured stems and branches. Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 20.06.2004.

*Cedrus deodara*, on fire injured branches. Tbilisi, Saburtalo, Djikia street, 05.06.2001.

*Corylus avellana*, on fire injured plants. Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 13.06.2004.

*Crataegus sp.*, on fire injured plants. Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 13.06.2004.

*Jasminum fruticans*, on fire injured plants. Tbilisi environs, Vere river gorge, Delisi, 07.05.2002.

*Pyracantha coccinea*, on fire injured stems. Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 20.06.2004.

*Quercus pedunculiflora*, on fire injured stems and branches. Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 13.06.2004.

*Robinia pseudoacacia*, see *Stigmina obtecta*

*Thuja orientalis*, on fire injured plants. Tbilisi, mountain Mtatsminda, 15.09.2002.

***Diaporthe pulla*** **Nitschke** together with anamorph, *Phomopsis pulla* (Sacc.) Traverso [Wehmeyer, 1933; Uecker, 1988].

*Hedera helix*, on dead stems. Tbilisi Botanical Garden, 16.02.2001.

***Diaterypella favacea*** **(Fr.) Ces. & De Not.** (*Diatrypella verruciformis* (Ehrenb.) Nitschke)

*Betula litwinowii*, on dead stems and branches. Tetritskaro distr., source of Vere river gorge, approach to Didgori mountain, 1660 m, 11.08.2004.

This is the first record of *B.litwinowii* as a host plant for *D.favacea* in Georgia.

***Disculina vulgaris*** **(Fr.) B. Sutton** *(Cryptosporium neesii* Corda*)*

Teleomorph: *Winterella suffusa* (Fr.) Kuntze *(Cryptospora suffusa* (Fr.) Tul. & C.Tul.*)*

Conidia 40-68 x 4-5 µm (25-45 x 4-4,5 in [Sutton, 1980] ).

*Alnus barbata*, on fire injured stems. Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 13.06.2004.

In Georgia the fungus is known (as *Cryptospora suffusa*) on *Alnus glutinosa* from Batsara State Reserve [Kanchaveli, Gvritishvili 1961] and Gori and on *Betula pendula* from Bacuriani Highmountain Botanic Garden and Tbilisi Dendropark (as *Cryptosporium neesii*).

***Eutypella ailanthi*** **(Sacc.) Sacc.**

*Robinia pseudoacacia*, on fire injured stems in association with *Camarosporium robiniae* (Westend.) Sacc., *Cytospra chrysosperma* (Pers.) Fr., *Cytosporina ludibunda* Sacc., *Diplodia profusa* De Not., *Phomopsis pseudoacaciae* Traverso, *Tubercularia vulgaris* Tode. Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 07.08.2004.

***Eutypella prunastri*** **(Pers.) Sacc.**

*Cerasus mahaleb* (*Prunus mahaleb*), on dead stems and branches. Tbilisi environs, Vere river gorge, Delisi, 06.04.2003.

***Eutypella scoparia*** **(Schwein.) Ellis & Everh.**

*Albizia julibrissin*, on dead stems. Tbilisi, Saburtalo, 25.01.2003.

*Artemisia annua*, on the base part of dead stems. Tbilisi, between the Library of the Georgian Academy of Sciences and WWF offise, 26.11.2003.

*Caesalpinia gilliesii*, on dead stems and branches. Tbilisi Botanical Garden, 05.12.2003.

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*Ficus carica*, on the base part of dead trunk. Tbilisi, Saburtalo, Mgaloblishvili Street, 01.02.2003.

*Smilax excelsa*, on dead stems. Tbilisi environs, Vere river gorge, Delisi, 25.03.1989.

*Tilia sp.*, on dead stems. Tbilisi Botanical Garden, 07.07.1989.

All of the above mentioned plants are first host records in Georgia for *E.scoparia* referred to as *Eutypa heteracantha* (Sacc.) Sacc., *Eutypella exigua* Ellis & Everh. ex Berl., *Peroneutypa heteracantha* (Sacc.) Berl., on different woody plants from the genera *Ailanthus, Broussonetia, Caragana, Cercis, Gleditsia, Hybiscus, Juglans, Laburnum, Morus, Robinia,* etc.

***Hysterographium fraxini* (Pers.) De Not.**

*Olea europaea*, on dead twigs in association with *Camarosporium dulcamarae* Died., *Coniothyrium fuckelii* Sacc., *Cytospora pruinosa* (Fr.) Sacc., *Diplodia oleae* Pegl., Tbilisi, park Vake, 05.04.2003.

*H.fraxini* and other fungi listed, except *D.oleae*, are recorded for the first time on *Olea europaea* in Georgia.

***Phomopsis picea* (Pers.) Höhn.** [Uecker,1988].

*Hypericum sp.*, on dead stems and branches in association with *Dichomera sp., Diplodia sp., Macrophoma sp.*, Tbilisi Botanical Garden, 30.04.2003.

***Stigmina obtecta* (Petrak & Esfandiari) M.B.Ellis** [Ellis, 1976].

*Robinia pseudoacacia*, on fire injured stems in association with *Camarosporium robiniae* (Westend.) Sacc., *Cytospora chrysosperma* (Pers.) Fr., *Cytosporina ludibunda* Sacc., *Diplodia profusa* De Not., *Phomopsis pseudoacaciae* Traverso, *Tubercularia vulgaris* Tode. Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 07.08.2004.

***Valsa ambiens* (Pers.) Fr. *subsp.* ambiens** (together with anamorph *Cytospora leucosperma* (Pers.) Fr.) [Gvritishvili, 1982; Hayova&Minter, 1998].

*Acer divergens*, on dead stems and twigs in association with *Coniothyrium fuckelii* Sacc., *Microdiplodia perpusila* (Desm.) Tassi (*M.subtecta* Allesch.) and *Massaria inquinans* (Tode) Ces. & De Not. Tbilisi Botanical Garden, 27.06.2003.

*Alnus cordata*, on dead stems and twigs in association with *Botryosphaeria dothidea* (Moug. ex. Fr.) Ces. & De Not., *Coniothyrium olivaceum* Bonord., *Diplodia alni* Fuckel. Tbilisi Botanical Garden, 24.09.1998.

So far *A.divergens* and *A.cordata* were not listed among the host plants of fungi of Georgia.

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# ახალი მონაცემები საქართველოს მიკობიოტის შესახებ

გვრიტიშვილი მ.[1], ყაჭეიშვილი-თავართქილაძე ქ.[1], ჭურღულია-შურღაია მ.[2], გოცაძე ნ.[3]

[1]საქართველოს მეცნიერებათა აკადემიის თბილისის ბოტანიკური ბაღი
[2]სოხუმის ფილიალი, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი
[3]ბოტანიკის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 14.09.2004)

## რეზიუმე

წარმოდგენილია თბილისის შემოგარენსა და საქართველოს ზოგიერთ რეგიონში მოპოვებული მიკოლოგიური მასალის იდენტიფიკაციის შედეგად მიღებული ახალი მონაცემები, რომლებიც ეხება ძირითადად ხეებისა და ბუჩქების, მათ შორის ხანძრების დროს დაზიანებულ მცენარეთა მიკობიოტურ კომპლექსებს. სიახლეები მოიცავს უპირატესად იმ მიკრომიცეტებს, რომლებიც ამა თუ იმ მცენარეზე აქამდე არ იყო ცნობილი.

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Plant Physiology

# THE EFFECT OF COBALT ON ANATOMICAL STRUCTURE OF PLANT LEAVES

MANGALADZE N.[1], ONIANI J.[2], ONIANI T.[2], GOGONAIA N.[2], CHANKSELIANI T.[2], ZAALISHVILI T.[3]

[1]*Kutaisi A.Tsereteli Universiti*, [2]*Iv. Javakhishvili Tbilisi State University*,
[3]Telavi I.Gogebashvili University

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**Abstract**

Cobalt deficiency in nutrient solution caused decrease in the height and volume of columnar parenchymatous cells of the leaves in *Allium cepa*. Presumably, these effects are due to the alterations of metabolic activity in chloroplasts. Excess of cobalt lead to the marked decrease in the cell height and length, as well as in the width and volume. Evidently, excessive cobalt turned into poisonous agent for cells, affecting the normal development of leaves in *Allium cepa* specimen. At the same time, structure of parenchymatous cells in *Solanum melongena* was not affected under the conditions of excessive cobalt and cobalt deprivation.

**Key words:** leaves, cells, size, cobalt

## Introduction

The deficit of the metal molecules in developing plant is considered a major cause of the inhibition of a normal growth of vegetative and reproductive organs in several plant species [Bruce, 1984]. The leaves represent a target organ of iron deprivation in plants [Platt-Aloia &Thompson, 1983; Terry Abadia, 1986].

At the same time, excess of some metals either in soil or in watery culture may lead to the serious damage to plants [Pereligin&Plugin, 1990; Popov&Soloviov, 1991; Saakadze,1992]. Little is known, however, about the effects of excessive cobalt on the development of tissue in plant vegetative organs, in particular, in leaves.

Present study was aimed at comparative study of structural alterations in leaves of *Allium cepa* and *Solanum melongena* specimen, grown under conditions of cobalt deficiency and cobalt excess.

## Material and metods

*Allium cepa* and *Solanum melongena* specimen were treated in nutrient solution, enriched with all non-organic components, necessary for plant development and reproduction. Experimental plants were grown under the conditions of cobalt deficiency (specimen CD) and cobalt excess (specimen CE). Normal content of cobalt was retained in nutrient solution for control plants (C).

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Anatomical structure of columnar parenchymatous cells of leaves was studied in microscopic sections under the apparatus MBY-3.

The height and width, as well as the length and the volume of columnar parenchymatous cells was registered in the end of May and April.

## Results and discussion

Cobalt deficiency was shown to have no significant effect on the development of leave tissue. In particular, the size of columnar parenchymatous cells of *Allium cepa* specimen was not significantly altered in experimental plant leaves as compared to control specimen (see Table 1).

**Table 1.** The size of columnar parenchymatous cells under the conditions of cobalt deficiency

| Leaves of *Allium cepa* specimen | Height (µm) | Width (µm) | Length (µm) | Volume (µm x 10) |
|---|---|---|---|---|
| Control Average of the circles 3-5 | 41.0 | 13.1 | 13.0 | 15.4 |
| Experimental (the average of each circle) | | | | |
| • Circle 3 | 41.2 | 12.0 | 11.4 | 14.0 |
| • Circle 4 | 42.0 | 13.4 | 12.3 | 14.0 |
| • Circle 5 | 32.0 | 11.1 | 13.4 | 13.4 |

Thus, cobalt deficiency in nutrient solution did not affect the development of plant columnar parenchymatous tissue. The height and volume, as well as the length and width of columnar parenchymatous cells did not change significantly as compared to the same parameters of tissue in control specimen.

As it is shown in the Table 2, excess of cobalt in nutrient solution lead to the marked changes in the size of leaves.

**Table 2.** The size of columnar parenchymatous cells under the conditions of cobalt excess

| Leaves of *Allium cepa* specimen | Height (µm) | Width (µm) | Length (µm) | Volume (µm x 10) |
|---|---|---|---|---|
| Control Average of the circles 3-5 | 41.0 | 13.1 | 13.0 | 15.4 |
| Experimental (the average of each circle) | | | | |
| • Circle 3 | 40.0 | 12.1 | 10.0 | 13.0 |
| • Circle 4 | 42.0 | 11.1 | 7.1 | 14.2 |
| • Circle 5 | 31.0 | 11.0 | 5.0 | 13.1 |

In particular, in comparison to control specimen, length parameters of the parenchymatous cells were significantly reduced in experimental plants.

In sum, cobalt deficiency in nutrient solution did not affect the normal development of leaves. Presumably, cobalt does not play a significant role in the development of parenchymatous cells in plant leaves.

At the same time, cobalt excess is likely to inhibit the growth of parenchymatous cells in length. It is suggested, however, that the inhibitory effect of cobalt is related rather to the poisonous action of this element on plant cell development, than to the specific role of cobalt in the growth of parenchymatous cell in length. Presumably cobalt affects the structure of cell membrane [Mushkambarov & Kuznecov, 2003].

Plant species may differ in their capability to resist the inhibitory influence of metals presented in environment. The effects of cobalt on development of leaves in *Solanum melongena* provide further evidence for the specific resistance of plants to excessive cobalt.

As it is shown in the Table 3, parenchymatous cells of the leaves in *Solanum melongena* retained the same developmental characteristics as compared with control specimen. Presumably, leaves of *Solanum melongena* specimen are cobalt-resistant and excess of this metal does not affect the development of parenchymatous cells.

**Table 3.** The size of columnar parenchymatous cells under the conditions of cobalt excess in *Solanum melongena* specimen

| Leaves of *Solanum melongena* specimen | Height (µm) | Width (µm) | Length (µm) | Volume (µm x 10) |
|---|---|---|---|---|
| Control Average of the circles 3-5 | 39.0 | 15.1 | 14.2 | 12.0 |
| Experimental (the average of each circle) | | | | |
| • Circle 3 | | | | |
| • Circle 4 | 38.3 | 14.4 | 14.2 | 12.2 |
| • Circle 5 | 39.4 | 13.4 | 13.3 | 13.1 |
| | 37.3 | 13.1 | 14.0 | 12.3 |

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მანგალაძე ნ.[1], ონიანი ჯ.[2], ონიანი თ.[2], გოგონაია თ.[2], ჩანქსელიანი თ.[2], ზაალიშვილი თ.[3]

*[1]ქუთაისის ა. წერეთლის სახ. უნივერსიტეტი,*
*[2]ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი,*
*[3]თელავის ი. გოგებაშვილის სახ. უნივერსიტეტი*

(მიღებულია 26.07.2004)

### ᲠᲔᲖᲘᲣᲛᲔ

შესწავლილია კობალტის ზეგავლენა ფოთლის ქსოვილების ჩამოყალიბებაზე საკვებ არეში კობალტის სიმცირის და სიუხვის პირობებში. ხახვის წყლის კულტურის ფოთლის ანათლებში განსაზღვრულია პარენქიმული უჯრედების სიგრძე, სიმაღლე, სიგანე და მოცულობა. ნაჩვენებია, რომ საკვებ არეში კობალტის მცირე რაოდენობით შეტანის შემთხვევაში, პარენქიმული უჯრედების სიმაღლე, სიგრძე, სიგანე და მოცულობა ისეთივეა, როგორც საკონტროლო ქსოვილში, მაგრამ როდესაც საკვებ არეში კობალტი დიდი რაოდენობითაა, ფერხდება უჯრედების ზრდა სიგრძეში. სავარაუდოა, რომ კობალტს არ აქვს სპეციფიკური მნიშვნელობა ხახვის ფოთლის პარენქიმული უჯრედების განვითარებისათვის და მისი ცდაში გამოვლენილი ეფექტი უფრო ზოგად-ტოქსიკური ხასიათისაა. იმავდროულად, საკვებ არეში კობალტის ზედმეტ რაოდენობას არანაირი ზეგავლენა არ მოუხდენია ბადრიჯნის ფოთლის განვითარებაზე. როგორც ჩანს, ბადრიჯნის ფოთლის ქსოვილი მედეგია კობალტის ტოქსიკური ზეგავლენის მიმართ.

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Zoology

# HOST PLANTS AND NUTRITION SPECIALIZATION OF APHIDS *(HEMIPTERA: STERNORRHYNCHA)* INHABITATED IN THE RV. DZAMA, TANA AND TEDZAMI RAVINES

BARJADZE SH., KVAVADZE E.

*Institute of Zoology, Georgian Academy of Sciences*

(Received October 14, 2004)

**Abstract**

The distribution of aphids of the Rv. Dzama, Tana and Tedzami ravines and the specialization of their nutrition is discussed. 113 species of aphids are registered on the mentioned River ravines, that are united within the 54 genera and 3 families. The aphids are inhabitants of 112 species of host plants, belonging to the 44 families. The following representatives of plant families are distinguished by the maximum quantity of aphid species: *Rosaceae* – 22 species, *Compositae* – 13 species, *Salicaceae* – 8 species. Aphids are divided into 3 groups on the base of nutrition specialization: 46 species of aphids are monophagous, 20 species of aphids – oligophagous, 33 species of aphids – polyphagous, whereas the nutrition specialization of 14 species was not identified.

**Key words:** Aphids, host plant, nutrition specialization, Shida kartli.

## Introduction

The whole area of the Rv. Dzama, Tana and Tedzami ravines composes 1598 km$^2$. The rivers represent right branch of Rv. Mtkvari in Shida kartli region. The aphids of the Rv. Dzama, Tana and Tedzami ravines have not been the special subject of research before. However 34 species of these aphids are serious pests of agricultural plants on the above mentioned territory.

The aim of our research was to determine the distribution characteristics of aphids on the host plant of the mentioned territory and to study their nutrition specialization.

## Material and Methods

We have been collecting the faunistic material of aphids during 2000-2003 years. The investigation involved all the plant belts of the Rv. Dzama, Tana and Tedzami ravines. The preparation of aphids slides, identification of aphid species and the study on their nutrition specialization were performed according to the methodology widely used in Aphidology [Shaposhnikov 1964; Blackman, Eastop 2000; Jibladze 1967; Holman 1974].

## Results and Discussion

113 species of aphids were registered on the mentioned river ravines, that are united within the 54 genera and 3 families. The maximum quantity of aphid species was registered in

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family *Aphididae* – 109 species and 3 species of aphids – in family *Adelgidae.* The minimum quantity of aphid species (1 species) was registered in family *Phylloxeridae.*

113 species of aphids are inhabitants of 112 species of host plants, belonging to the 44 families: *Pinaceae, Cupressaceae, Aceraceae, Araliaceae, Berberidaceae, Betulaceae, Boraginaceae, Campanulaceae, Cannabaceae, Capripholiaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cornaceae, Corylaceae, Crassulaceae, Cruciferae, Cucurbitaceae, Dipsacaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fagaceae, Gramineae, Helleboraceae, Juglandaceae, Labiatae, Leguminosae, Malvaceae, Oleaceae, Onagraceae, Polygonaceae, Punicaceae, Rhamnaceae, Rosaceae, Rubiaceae, Salicaceae, Scrophulariaceae, Solanaceae, Tiliaceae, Ulmaceae, Umbeliferae, Urticaceae, Valerianaceae.*

The following representatives of plant families were distinguished by the maximum quantity of aphid species: *Rosaceae* – 22 species, *Compositae* – 13 species, *Salicaceae* – 8 species. The minimum quantity of aphid species was registered in the the representatives of the other families.

Aphids are divided into 3 groups on the base of their nutrition specialization: 46 species of aphids are monophagous, 20 species of aphids – oligophagous, 33 species of aphids – polyphagous, whereas the nutrition specialization of 14 species was not identified.

**Table 1.** Host plants and nutrition specialization of aphids *(Hemiptera: Sternorrhyncha)* inhabitated in the Rv. Dzama, Tana and Tedzami ravines.

| № | Aphid species | Plant species | Nutrition specialization |
|---|---|---|---|
| 1. | *Pineus orientalis* (Dreyf., 1889) | *Picea orientalis* (L.) Link | oligophagous |
| 2. | *P. sp.* | *Pinus sosnowskyi* Nakai | ? |
| 3. | *Dreyfusia nordmannianae* (Eckch., 1890) | *Abies nordmanniana* (Stev.) Spach | oligophagous |
| 4. | *Moritziella sp.* | *Quercus iberica* Stev. | ? |
| 5. | *Prociphilus fraxini* (F., 1777) | *Fraxinus oxycarpa* Willd. | polyphagous |
| 6. | *Thecabius affinis* (Kalt., 1843) | *Populus sosnowskyi* (Grossh.) Makashvili | polyphagous |
| 7. | *Pemphigus borealis* Tullgr., 1909 | *Populus pyramidalis* (Roz.) Celak. | monophagous |
| 8. | *P. bursarius* (L., 1758) | *Populus pyramidalis* (Roz.) Celak. | monophagous |
| 9. | *P. spirothecae* Pass., 1856 | *Populus pyramidalis* (Roz.) Celak. | monophagous |
| 10. | *Eriosoma lanigerum* (Hausm., 1802) | *Malus orientalis* Uglitzk., *M. domestica* Borkh. | polyphagous |
| 11. | *E. laniginosum* (Hart., 1841) | *Ulmus suberosa* Moench | polyphagous |
| 12. | *E. ulmi* (L., 1758) | *Ulmus suberosa* Moench | polyphagous |
| 13. | *E. patchiae* (C. B. et Blunck, 1916) | *Ulmus suberosa* Moench | monophagous |
| 14. | *Tetraneura ulmi* (L., 1758) | *Ulmus suberosa* Moench | polyphagous |
| 15. | *Cinara juniperi* (Deg., 1773) | *Juniperus oblonga* Bieb. | monophagous |
| 16. | *C. pini* (L., 1758) | *Pinus sosnowskyi* Nakai | monophagous |
| 17. | *Schizolachnus pineti* (F., 1781) | *Pinus sosnowskyi* Nakai | monophagous |
| 18. | *Pterochloroides persicae* (Chol., 1899) | *Persica vulgaris* Mill. | oligophagous |
| 19. | *Lachnus roboris* (L., 1758) | *Quercus iberica* Stev. | oligophagous |
| 20. | *Anoecia corni* (F., 1775) | *Swida australis* (C.A.Mey.) Pojark. ex Grossh. | polyphagous |
| 21. | *Thelaxes driophila* (Schrk., 1801) | *Quercus iberica* Stev. | monophagous |
| 22. | *Phyllaphis fagi* (L., 1767) | *Fagus orientalis* Lipsky | monophagous |
| 23. | *Betulaphis quadrituberculata* | *Betula litwinowii* Doluch. | monophagous |

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| | (Kalt., 1843) | | |
| 24. | *Callaphis juglandis* (Goeze., 1778) | *Juglans regia* L. | monophagous |
| 25. | *Chromaphis Iuglandicola* (Kalt., 1843) | *Juglans regia* L. | monophagous |
| 26. | *Eucallipterus tiliae* (L., 1758) | *Tilia caucasica* Rupr. | monophagous |
| 27. | *Euceraphis punctipennis* (Zett., 1828) | *Betula pendula* Roth. | monophagous |
| 28. | *Myzocallis coryli* (Goeze.,1778) | *Corylus avellana* L., *C. iberica* Wittmt. ex Kem.-Nath. | monophagous |
| 29. | *M. carpini* (Koch., 1855) | *Carpinus caucasica* Grossh., *Carpinus orientalis* Mill. | monophagous |
| 30. | *Tuberculatus annulatus* (Hart., 1841) | *Quercus iberica* Stev. | monophagous |
| 31. | *Pterocallis alni* (Deg.,1773) | *Alnus barbata* C.A.Mey. | monophagous |
| 32. | *Chaitophorus albus* Mordv., 1901 | *Populus hybrida* Bieb. | monophagous |
| 33. | *Ch. vitellinae* (Schrk., 1801) | *Salix alba* L. | monophagous |
| 34. | *Periphillus lyropictus* (Kessl., 1886) | *Acer platanoides* L. | monophagous |
| 35. | *Sipha maydis* Pass.,1860 | *Zea mays* L. | oligophagous |
| 36. | *Pterocomma populeum* (Kalt., 1843) | *Salix sp*., *Populus nigra* L. | oligophagous |
| 37. | *Rhopalosiphum maidis* (Fitch., 1856) | *Zea mays* L. | oligophagous |
| 38. | *Hyalopterus pruni* (Geoffr., 1762) | Stone fruits, *Phragmites australis* (Cav.) Trin. ex Steud. | polyphagous |
| 39. | *Aphis sambuci* L., 1758 | *Sambucus ebulus* L., *S. nigra* L. | polyphagous |
| 40. | *A. fabae* Scop., 1763 | *Phaseolus vulgaris* L., *Beta vulgaris* L., *Galium* sp., *Rumex sp*., *Seseli transcaucasicum* (Schischk.) M. Pimen. & Sdobnina , *Cirsium arvense* (L.) Scop., *Cytisus caucasicus* Grossh. | polyphagous |
| 41. | *A. hederae* Kalt., 1843 | *Hedera helix* L. | monophagous |
| 42. | *A. craccae* L., 1758 | *Vicia sp.* | monophagous |
| 43. | *A. sp.*[I] | *Euphorbia iberica* Boiss. | ? |
| 44. | *A. craccivora* Koch., 1854 | *Glycyrrhiza glabra* L., *Phaseolus vulgaris* L., *Trigonella procumbens* (Bess.) Reichenb., *Onobrychis radiate* (desf.) Bieb., *O. sp.*, *Robinia pseudoacacia* L. | polyphagous |
| 45. | *A. cytisorum* Hart., 1841 | *Cytisus caucasicus* Grossh. | monophagous |
| 46. | *A. capsellae* Kalt., 1843 | *Capsella bursa-pastoris* (L.) Medik. | monophagous |
| 47. | *A. intybi* Koch., 1855 | *Cichorium intybus* L. | monophagous |
| 48. | *A. farinosa* Gmel., 1790 | *Salix caprea* L., *S. viminalis* L. | monophagous |
| 49. | *A. pomi* Deg., 1773 | *Malus orientalis* Uglutzk., *M. domestica* Borkh., *Crataegus sp.*, *Cydonia oblonga* Mill., *Mespilus germanica* L. | oligophagous |
| 50. | *A. idaei* Goot., 1912 | *Rubus ideus* L., *Rubus sp. sp.* | oligophagous |
| 51. | *A.. paliuri* ( Licht., 1885) | *Paliurus spina-christi* Mill. | monophagous |
| 52. | *A. punicae* (Pass., 1863) | *Punica granatum* L. | monophagous |
| 53. | *A. umbrella* (C. B., 1950) | *Lavatera thuringiaca* L., *Malva sylvestris* L. | oligophagous |
| 54. | *A. praeterita* Walk., 1849 | *Chamaenerium angustifolium* (L.) Scop. | oligophagous |
| 55. | *A. urticata* Gmel., 1790 | *Urtica dioica* L. | monophagous |
| 56. | *A. affinis* Guerc., 1911 | *Menta longifolia* (L.) Huds. | monophagous |
| 57. | *A. sp.*[II] | *Nepeta pannonica* L. | ? |
| 58. | *A. . sedi* Kalt., 1843 | *Sedum caucasicum* (Grossh.) Boriss. | monophagous |
| 59. | *A. sp.*[III] | *Rubia transcaucasica* Grossh. | ? |
| 60. | *A. sp.*[IV] | *Cephalaria gigantea* (Ledeb.) Bobr. | ? |
| 61. | *A. gossypii* Glov., 1854 | *Citrullus vulgaris* Schrad., *Cucumis sativus* L., *Cucurbita sp.*, *Scrophularia alata* Gilib. | polyphagous |
| 62. | *A. alexandrae* ( Nevs., 1928) | *Centaurea iberica* Trev. ex Spreng. | monophagous |
| 63. | *Dysaphis devecta* (Walk., 1849) | *Malus orientalis* Uglitzk., *M. domestica* Borkh. | monophagous |
| 64. | *D. affinis* (Mordv., 1929) | *Malus orientalis* Uglitzk., *M. domestica* Borkh. | monophagous |
| 65. | *D. mali* (Ferr., 1872) | *M. domestica* Borkh., *Malus orientalis* Uglitzk. | polyphagous |
| 66. | *D. pyri* (B. d. F., 1841) | *Pyrus caucasica* Fed., *P. communis* L.-cult. | polyphagous |

ეროვნული
ბიბლიოთეკა

| 67. | *D. reaumuri* (Mordv., 1929) | *Pyrus caucasica* Fed., *P. communis* L.-cult. | monophagous |
|---|---|---|---|
| 68. | *D. aucupariae* (Buckt., 1879) | *Sorbus torminalis* (L.) Crantz. | polyphagous |
| 69. | *Brachycaudus divaricatae* (Shap., 1953) | Stone fruits | polyphagous |
| 70. | *B. cardui* (L., 1758) | Stone fruits, *Cirsium arvense* (L.) Scop., *C. caucasicum* (Adams) Petrak, *Onopordum acantium* L., *Symphytum asperum* Lepech. | polyphagous |
| 71. | *B. helichrysi* (Kalt., 1843) | *Prunus spinosa* L. | polyphagous |
| 72. | *B. spiraeae* (C.B., 1932) | *Spiraea hypericifolia* L. | monophagous |
| 73. | *B. persicae* (Pass., 1860) | *Persica vulgaris* Mill. | oligophagous |
| 74. | *Acaudinum sp.* | unknown | ? |
| 75. | *Liosomaphis berberidis* (Kalt., 1843) | *Berberis vulgaris* L. | monophagous |
| 76. | *Brevicoryne brassicae* (L., 1758) | *Brassica oleracea* L. var. capitata L | oligophagous |
| 77. | *Cavariella aegopodii* (Scop., 1763) | *Coriandrum sativum* L., *Anethum graveolens* L. | polyphagous |
| 78. | *Hyadaphis foeniculii* (Pass., 1860) | *Heracleum sosnowskyi* Manden. | polyphagous |
| 79. | *Semiaphis dauci* (F., 1775) | *Daucus carota* L. | monophagous |
| 80. | *Neanuraphis rhamni* B. d. F.,1841 | *Swida australis* (C.A.Mey.) Pojark. ex. Grossh. | polyphagous |
| 81. | *Ovatus insitus* (Walk., 1849) | *Cydonia oblonga* Mill., *Mespilus germanica* L., *Malus domestica* Borkh. | polyphagous |
| 82. | *Phorodon humuli* (Schrk., 1801) | Stone fruits, *Humulus lupulus* L. | polyphagous |
| 83. | *Ph. cannabis* Pass., 1860 | *Cannabis sativa* L. | monophagous |
| 84. | *Myzus cerasi* (F., 1775) | *Cerasus incana* (Pall.) Spach , *C. avium* (L.) Moench, *C. vulgaris* Mill. | polyphagous |
| 85. | *M. persicae* (Sulz., 1776) | Stone fruits, *Solanum lycopersicum* L. | polyphagous |
| 86. | *Aulacorthum solani* (Kalt., 1843) | *Convolvulus arvensis* L. | polyphagous |
| 87. | *Microlophium evansi* (Theob., 1923) | *Urtica dioica* L. | monophagous |
| 88. | *Metopolophium dirhodum* (Walk., 1849) | *Hordeum leporinum* Link | polyphagous |
| 89. | *Acirthosiphon pisum* (Harr., 1776) | *Vicia peregrina* L. | oligophagous |
| 90. | *Hyperomyzus lactucae* (L., 1758) | *Sonchus oleraceus* L. | polyphagous |
| 91. | *H. sp.* | *Scrophularia alata* Gilib. | ? |
| 92. | *Cryptomyzus galeopsidis* (Kalt., 1843) | *Galeopsis bifida* Boenn. | polyphagous |
| 93. | *C. alboapicalis* (Theob., 1916) | *Lamium album* L. | oligophagous |
| 94. | *Capitophorus sp.* | *Elaeagnus angustifolia* L. | ? |
| 95. | *C. vandergooti* H.R.L., 1947 | *Inula helenium* L. | polyphagous |
| 96. | *Corylobium avellanae* (Schrk., 1801) | *Corylus avellana* L., *C. iberica* Wittmt. ex Kem-Nath. | monophagous |
| 97. | *Macrosiphum rosae* (L., 1758) | *Rosa sp. sp.* | polyphagous |
| 98. | *M. melampyri* Mordv., 1919 | *Digitalis feriginea* L. | oligophagous |
| 99. | *M.* sp.[I] | *Symphytum asperum* Lepech. | ? |
| 100. | *M.* sp.[II] | *Helleborus caucasicus* A. Br. | ? |
| 101. | *M. sp.*[III] | *Valeriana tiliifolia* Troitzk. | ? |
| 102. | *M. euphorbiae* (Thom., 1878) | *Euphorbia iberica* Boiss. | polyphagous |
| 103. | *Sitobion fragariae* (Walk., 1848) | *Rubus ideus* L., *R.. sp.sp.* | polyphagous |
| 104. | *S. avenae* (Fabr., 1775) | *Triticum sp.* | oligophagous |
| 105. | *Masonaphis sp.* | *Rhododendron caucasicum* Pall. | ? |
| 106. | *Uroleucon chondrillae* (Nevs., 1929) | *Chondrilla juncea* L. | monophagous |
| 107. | *U. sonchi* (L., 1767) | *Sonchus oleraceus* L. | oligophagous |
| 108. | *U. cichorii* (Koch., 1855) | *Cichorium intybus* L. | oligophagous |
| 109. | *U. jaceae* (L., 1758) | *Serratula quinquefolia* Bieb. ex Willd. | oligophagous |
| 110. | *U. sp.* | *Cirsium hydrophiloides* Charadze | ? |
| 111. | *U. campanulae* (Kalt., 1843) | *Campanula hohenackeri* Fisch. & C.A.Mey. | monophagous |
| 112. | *Macrosiphoniella artemisiae* (B.d. F., 1841) | *Artemisia vulgaris* L. | monophagous |
| 113. | *M. sanborni* (Gill., 1908) | *Chrysanthemum indicum* L. | monophagous |

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## მდინარეების ძამას, ტანასა და თეძამის აუზების ბუგრების *(Hemiptera: Sternorrhyncha)* მასპინძელი მცენარეები და კვებითი სპეციალიზაცია

ბარჯაძე შ., ყვავაძე ე.

*ზოოლოგიის ინსტიტუტი, საქართველოს მეცნიერებათა აკადემია*

(მიღებულია 14.10.2004)

**რეზიუმე**

მდინარეების ძამას, ტანასა და თეძამის აუზებში აღრიცხულია ბუგრების 113 სახეობა, რომლებიც გაერთიანებული არიან 54 გვარსა და 3 ოჯახში. ბუგრების 113 სახეობა ბინადრობს 112 სახეობის მასპინძელ მცენარეზე, რომლებიც მიეკუთვნებიან 44 ოჯახს. ბუგრების სახეობათა მაქსიმალური რაოდენობით გამოირჩევიან შემდეგი ოჯახების წარმომადგენლები: *Rosaceae* – 22 სახეობა, *Compositae* – 13 სახეობა, *Salicaceae* – 8 სახეობა.

კვების სპეციალიზაციის მიხედვით ბუგრები 3 ჯგუფად იყოფა: ბუგრების 46 სახეობა მონოფაგია, 20 სახეობა – ოლიგოფაგი, 33 სახეობა – პოლიფაგი, ხოლო 14 სახეობის კვების სპეციალიზაცია დაუდგენელია.

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Zoology

# THE ZOOGEOGRAPHICAL-CHOROLOGICAL PECULIARITIES OF THE SPIDERS (FAMILY *DYSDERIDAE)* OF GEORGIA

GEGECHKORI A., MKHEIDZE T., PKHAKADZE V.

*Department of Zoology, Iv. Javakhishvili Tbilisi State University*

(Received September 6, 2004)

**Abstract**

The zoogeographical-chorological review of the family *Dysderidae*'s spiders of Georgia has shown that distribution of this group belongs to Palaearctic type in general. It was established that autochtonous element (4 Genera, 31 species) prevails on allochtonous element (2 genera, 10 species). From allochtonous fauna with Palaearctic distribution characterized 1 genus, 1 species; with South European – 2 genera, 6 species; with Wide Mediterranean – 1 genus, 3 species.

**Key words:** taxonomy, zoogeography, chorology, *Dysderidae.*

## Introduction

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

4 genus and 41 species of the family *Dysderidae* were registered [Mkheidze, 1992; Mikhailov 1997]

The family *Dysderidae* today comprises following genera: *Desdera Latreie* – 24 species, *Harpactea Bristowei* – 10 species, *Hygrocrates Deeleman-Reinold* – 4 species, *Cryptoparachtes Dunin* – 3 species.

## Materials and Methods

Material has been collected during 2000-2004 in Georgia. To precise the list of species of the family *Dysderidae* scientific sources were used [Mkheidze, 1972, 1972a, 1979, 1979a, 1992; Dunin, 1992,1992a; Mikhailov, 1997; Kharitonov, 1956].

## Results and Discussion

Chorological study of spiders of the family Dysderidae of Georgia has shown that 27 species are South-Caucasian (D.spassky, D.atra, D.tkibuliensis, D.armenica, D.tbilisiensis, D.imeretiensis, D.iberica, D.meschetiensis, D.chritonovi, D.richteri, D.bogatschevi, D.gmelini, D. inopinata, Hygrocrates georgicus, H.caucasicus, H.bristowei, H.trialetiensis, Cr.fedotovi, Cr.adzharicus, Ch.charitonov, Harpactea zaitzevi, H.chritonovi, H.camenarium, H.eskovi, H.mcheidzae, H.mithridatis, H.paradoxa [Mkheidze, 1972, 1972a, 1979, 1979a, 1992; Dunin,

ეროვნული ბიბლიოთეკა

1992,1992a; Mikhailov, 1997]; 4 species are Quasi-Caucasian (D.ạzerbaijanica, D.martensi, Harpactea caucasica, H.logunovi) [Mkheidze, 1992; Mikhailov, 1997; Kharitonov, 1956]; 6 species – South European (D.hungarica, D. erythrina, D.ukrainensis, D.dunin, D.lata, Harpactea rubicunda) [Mkheidze, 1992; Mikhailov, 1997]; 3 species – wide Mediterranean (D.westring, D.punctata, D.cribrata,) [Mkheidze, 1992,1979a]; 1 species – Palaearctic (D.croceta) [Mkheidze, 1972a,1992].

Thus, according to the zoogeographical-chorological studies of species of the family *Dysderidae*'s spiders fauna, it was established that autochtonous element (4 genera, 31 species) prevails on allochtonous element distributed in Georgia (2 genera, 10 species).

From allochtonous fauna with Palaearctic distribution characterized 1 genus, 1 species, with South European – 2 genera, 6 species, with wide Mediterranean – 1 genus, 3 species.

**Table 1.** Data of zoogeographical-chorological studies of species of spiders (family *Dysderidae*) fauna of Georgia

| N | | Genera, species | Distribution | Zoogeographical area |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1 | | *Dysdera latr* 1804 | | |
| | 1 | *D.spassky* Charit 1956 | Georgia | South Caucasian |
| | 2 | *D.crocata* C.L.koch, 1838 | Mediterranean countries, Crimea, Carpathians, Russia, Ukraine, Azerbaijan, Middle Asia, Georgia | Palaearctic |
| | 3 | *D.westringi* Pick-Cambr, 1972 | Spain, Corsica, Algiers, Greece, Mesopotamia, Syria, Crimea, Ukraine, Georgia | Wide Mediterranean |
| | 4 | *D.punctata* C.L.koch, 1838 | Mediterranean countries, Crimea, Ukraine, Georgia | Wide Mediterranean |
| | 5 | *D.cribrata* Sim, 1882 | Mediterranean countries [with Canary Islands], European countries of the former Soviet Union; Georgia | Wide Mediterranean |
| | 6 | *D.hungarica* Kulcz, 1897 | Crimea, Ukraine, Moldavia, Azerbaijan, Georgia | South European |
| | 7 | *D.atra* Mcheidze, 1979 | Georgia | South Caucasian |
| | 8 | *D.tkibuliensis* Mcheidze, 1979 | Georgia | South Caucasian |
| | 9 | *D.erythrina* Walck, 1838 | Crimea, Ukraine, Russia (North Caucasus), Georgia | south European |
| | 10 | *D.azerbaijanica.* Charit, 1956 | Russia [North Caucasus], Georgia, Azerbaijan | Caucasian |
| | 11 | *D.armenica* Charit, 1956 | Armenia, Georgia | South Caucasian |
| | 12 | *D.tbilisiensis* Mcheidze, 1979 | Georgia | South Caucasian |
| | 13 | *D.imeretiensis* Mcheidze, 1979 | Georgia | South Caucasian |
| | 14 | *D.iberica* Mcheidze, 1979 | Georgia | South Caucasian |
| | 15 | *D.meschetiensis* Mcheidze, 1979 | Georgia | South Caucasian |

ეროვნული
ბიბლიოთეკა

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| | 16 | *D.charitonovi* Mcheidze, 1979 | Georgia | South Caucasian |
| | 17 | *D.richteri* Charit, 1956 | Armenia, Azerbaijan, Georgia | South Caucasian |
| | 18 | *D.ukrainesis* Charit, 1956 | Russia [North Caucasus], Ukraine, Georgia | South European |
| | 19 | *D.bogatschevi* Dunin, 1990 | Azerbaijan, Georgia | South Caucasian |
| | 20 | *D.gmelini* Dunin, 1991 | Georgia | South Caucasian |
| | 21 | *D.inopinata* Dunin, 1991 | Georgia | South Caucasian |
| | 22 | *D.Dunin* Deeleman-Reinhold, 1988 | Russia [North Caucasus], Ukraine, Azerbaijan, Georgia | South European |
| | 23 | *D.martensi* Dunin, 1991 | Russia [North Caucasus], Georgia | Caucasian |
| | 24 | *D.lata* Wider, 1834 | Crimea, Russia [North Caucasus], Ukraine, Moldavia, Georgia | South European |
| 2 | | *Harpactea* Bristowe, 1939 | | |
| | 25 | *H.Caucasica* Kulcz, 1895 | Russia [North Caucasus], Georgia | Caucasian |
| | 26 | *H.zaitzevi* Charit, 1956 | Georgia | South Caucasian |
| | 27 | *H.Charitonovi* Mcheidze, 1972 | Georgia | South Caucasian |
| | 28 | *H.camenarium* Brignoli, 1977 | Georgia | South Caucasian |
| | 29 | *H.eskovi* Dunin, 1989 | Armenia, Georgia | South Caucasian |
| | 30 | *H.logunovi* Dunin, 1992, | Russia [North Caucasus], Georgia | Caucasian |
| | 31 | *H.Mcheidze* Dunin, 1992 | Georgia | South Caucasian |
| | 32 | *H.mithridatis* Brignoli, 1979 | Georgia | South Caucasian |
| | 33 | *H.paradoxa* Dunin, 1992 | Georgia | South Caucasian |
| | 34 | *H.rubicunda* C.L.Koch, 1938 | Carpathians, Crimea, Russia [North Caucasus], Lithuania, Ukraine, Moldavia, Georgia | South European |
| 3 | | *Hygrocrates* Deeleman-Reinold, 1988 | | |
| | 35 | *H.georgicus* Mcheidze, 1972 | Georgia | South Caucasian |
| | 36 | *H.caucasicus* Dunin, 1994 | Georgia | South Caucasian |
| | 37 | *H.bristowei* Charit, Mcheidze, 1964 | Georgia | South Caucasian |
| | 38 | *H.trialetiensis sp.n* Mcheidze, 1939 | Georgia | South Caucasian |

ეროვნული
ბიბლიოთეკა

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| 4 | | *Cryptoparachtes* Dunin, 1992 (Harpactocrates Sim, 1914) | | |
| | 39 | Cryptoparachtes *adzharicus* Dunin, 1992 | Georgia | South Caucasian |
| | 40 | *Cryp.charitonovi* (*H.charitonovi*) Mcheidze, 1972 | Georgia | South Caucasian |
| | 41 | *Cryp.fedotovi* (*H.fedotovi*) Charit, 1956 | Georgia, Azerbaijan | South Caucasian |

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