ISSN 1512-2123

საქართველოს მეცნიერებათა აკადემიის

მაცნე

ბიოლოგიის სერია B
Biological series

2006
No. 4
Vol. 4

PROCEEDINGS

of the Georgian Academy of Sciences

ISSN 1512 – 2123

საქართველოს მეცნიერებათა აკადემიის

მაცნე

ბიოლოგიის სერია B
Biological series

2006
No. 4
Vol. 4

PROCEEDINGS

of the Georgian Academy of Sciences

Tbilisi. Georgia
2006

To order your copies, please send to:

Georgian Academy of Sciences,
Department of Biology
52, Rustaveli Avenue, Tbilisi, 0108, Georgia
Tel. + 995-32 93-58-92
Fax: + 995-32 93-58-92
E-mail: bio@gw.acnet.ge
www.acnet.ge/matsne/biology

Journal founded in 2001

ISSN 1512 – 2123

Published by NGO "Biomed", 14, L. Gotua st., Tbilisi 0160, Georgia

CONTENTS

Biochemistry

Biophysics

Biotechnology

Botany

Ecology

Morphology

Mycology

Plant Physiology

Zoology

Letter to editor

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

წერილის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და 12 გვერდზე მეტი. წერილი უნდა გაფორმდეს შემდეგი რუბრიკაციით: შესავალი და მიზნები (Introduction), მასალა და მეთოდები (Materials and Methods), შედეგები და მათი განხილვა (Results and Discussion), დამოწმებული ლიტერატურა. უკანასკნელი უნდა იყოს დალაგებული ანბანის მიხედვით, ხოლო ტექსტში წყაროების მითითება უნდა ხდებოდეს ფრჩხილებში ჩასმული ავტორის გვარითა და წლით [Lernmark, Hagglof 1981].

მითითებული ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

ჟურნალის შემთხვევაში

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

წიგნის შემთხვევაში

Kuhn T.S. *The structure of scientific revolutions.* Chicago, IL, Chicago Press, 2000.

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

მასალა რედაქციაში წარმოდგენილი უნდა იყოს ქაღალდზე ამობეჭდილი და დისკეტით (ან CD-ით). წერილი ერთი ფაილით უნდა იყოს შენახული (ცალკე ფაილად შეიძლება ილუსტრაციების წარმოდგენა), ხოლო ფაილის სახელწოდება წერილის პირველი ავტორის გვარს უნდა ატარებდეს.

ქართული ტექსტისთვის ოპტიმალური ფონტებია AcadNusx და AcadMtavr, ინგლისური ტექსტებისთვის - Times New Roman. შრიფტის ზომა - 12 პუნქტი, ინტერვალი - 1,5. ცხრილებში დასაშვებია უფრო მცირე ზომის შრიფტები. წერილი უნდა დაიბეჭდოს A4 ფორმატით, ზევით და ქვევით - 2,5 სმ., მარცხნივ – 3 სმ. და მარჯვნივ – 2სმ. დაშორებით. ცხრილები, გრაფიკები და დიაგრამები (მხოლოდ შავ-თეთრი) შესაძლებელია დამზადდეს როგორც Microsoft Word-ში, ისე Excel-ში, ფოტოსურათები მიიღება აგრეთვე ორიგინალების (არაელექტრონული) სახითაც.

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

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

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

Biochemistry

TNT EFFECT ON THE NITROREDUCTASE, PEROXIDASE AND PHENOLOXIDASE ACTIVITIES *IN VITRO* CULTURES OF *YUCCA GLORIOSA* AND *GLYCINE MAX.*

GOGAVA M., RAMISHVILI M., GHOGHOBERIDZE M., KURASHVILI M., PRUIDZE M., CHELIDZE N.

Durmishidze Institute of Biochemistry and Biotechnology

(Received February 6, 2006)

Abstract

Changes of the nitroreductase, peroxidase and phenoloxidase activities of *Yucca gloriosa* and *Glycine max in vitro* tissues cultivated on the MS solid medium with and without TNT at the different growth phases have been studied. It has been shown that nitroreductase is activated in the cells of *Glycine max*, and peroxidase – in the cells of *Yucca gloriosa*. As for phenoloxidase, it has no significant role in intracellural conversion of TNT. It has been stated, that in both *Yucca gloriosa* and *Glycine max* callus cultures the detoxication of TNT occurs by activation of different enzymatic systems depending on the growth phase of cultures.

Key words: xenobiotic, phytoremediation, callus tissue, spectrophotometric method

Introduction

As plant callus and suspension culture generally enables the study of degradation of xenobiotics under well-controlled conditions (temperature, light, medium composition) during the experiment and the study of the pathway of metabolite formation independent from the microorganisms such as bacteria, fungi or yeasts consortia; this *in vitro* systems represent the best models for the investigation of biochemical and cytological mechanisms of detoxification.

Nepovim et al. [Nepovim et al., 2004] reported *in vitro* degradation of 2,4,6-trinitrotoluene (TNT) using plant tissue cultures of *Salanum aviculare* and *Rheum palmatum*. The different concentrations of degradation products in each culture indicated that the metabolism of TNT is controlled by different enzymatic systems. It was concluded that studying different species for TNT degradation is necessary for the search of most suitable candidates for TNT phytoremediation.

Two pathways of degradation of TNT were found in suspension culture of *Rheum palmatum* [Nepovim et al., 2003]. In the first case the mixture of aminodinitrotoluenes is a product of reduction on nitro groups of TNT. The other one leads to the formation of trinitrotobenzene probably by oxidation of methyl group.

The enzymes that catalyze the reduction of nitro groups of the explosives, such as TNT, are known as nonspecific NAD(P)H-dependent nitroreductase. It has been shown, that complete reduction of nitro group to amino group significantly decreases mutagenic effect of given compounds and thier toxicity, respectively [Cash, 1998].

Reactoin catalized by peroxidases leads to the producion of free radicals ($^{\cdot}OH, R^{\cdot}$) which are able to oxidize other compounds, xenobiotics among them. According to some data the purified horse-radish preparation oxidizes mythyl group of (C^3H_3)-TNT [Khatisashvili et al., 2004].

The products of oxidative reactoin catalizing by phenoloxidase - semiquinons and quinons have a high redox potential, which enables enzyme to perform oxidization of xenobiotics indirectly by mechanism of co-oxidation. Likewise enzymatic preparation of phenoloxidase received from tea leaves oxidizes mythyl group of (C^3H_3)-TNT.

Materials and Methods

Callus cultures: Callus tissue of *Yucca gloriosa* was induced from the flower buds of the plant. The composition of nutrient medium, the regime of cultivation and the methods of growth analysis were described earlier [Gogoberidze et al., 1988].

Glycine max callus culture was produced from soybean leaf grown on Knop sterile nutrient medium and was cultivated on MS solid *in vitro* medium [Murashige & Skoog, 1962] with different additives.

Enzyme assay: Nitroreductase activity was determined according to the rate of TNT reduction. Untransformed TNT was measured by a spectrophotometric method using 447 nm illumination in a highly alkaline environment (pH >12.2) [Oh et al., 2000].

Peroxidase activity was determined spectrophotometrically using 470 nm illumination according to the rate of H_2O_2-dependent oxidation of guaiacol [Gregory, 1966].

Phenoloxidase activity was determined spectrophotometrically using 420 nm illumination by observing the rate of catechol oxidation [Lanzarini et al., 1972].

Protein determination: Protein was measured according to Bradford [Bradford, 1974].

Results and Discussion

We have studied TNT effect on nitroreductase, peroxidase and phenoloxidase activities at different phases of growth cycle of *Yucca gloriosa* and *Glycine max* calluss cultures. With this purpose callus tissues were cultivated on the 100 mg/l TNT-containing MS nutrient medium.

In vitro cultures growth phases have been stated earlier: latent growth phase of *Yucca gloriosa* callus culture lasts for seven days, active exponential growth phase – from the 14th to the 35th day, followed by stationary growth phase, which takes for 8-9 days, later cells pass to degradation phase; latent growth phase of *Glycine max* callus culture also lasts for 7 days, active exponential growth phase – from the 14th to 32nd day, after which growth of the culture is slowed down, this corresponds to stationary growth phase, after this short stationary growth phase cells degradation phase begins.

Changes in the activity of the above mentioned enzymes at latent growth phase (7th day) of both *Yucca gloriosa* and *Glycine max in vitro* cultures with or without TNT (control variant) in the nutrient medium are shown in Figure 1.

These results showed that at latent growth phase specific activity of all the three enzymes was low both in cells of *Yucca gloriosa* and *Glycine max*. No significant effect of TNT was observed.

At exponential growth phase (21st day) of the cells of *Yucca gloriosa* callus culture, as compared to latent growth phase nitroreductase activity increased both at TNT presence in the nutrient medium and in the control variant as well; likewise peroxidase activity increased as compared to latent growth phase as well as compared to the control variant (2.6 fold); phenoloxidase activity underwent insignificant changes (Fig.2a).

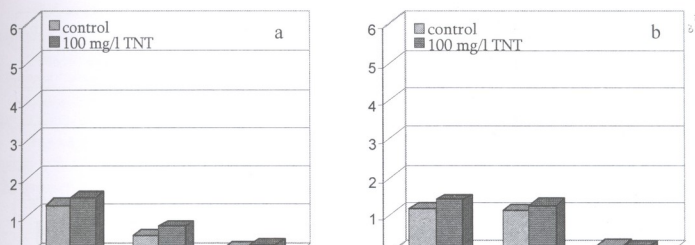


Fig.1. TNT effect on the nitroreductase, peroxidase and phenoloxidase activities in callus culture of *Yucca gloriosa* (a) and *Glycine max* (b) at latent growth phase. 1. Nitroreductase activity, nmol/h per mg protein; 2. Peroxidase activity, ΔA_{470}/min per mg protein; 3. Phenoloxidase activity, ΔA_{420}/min per mg protein (x 10).

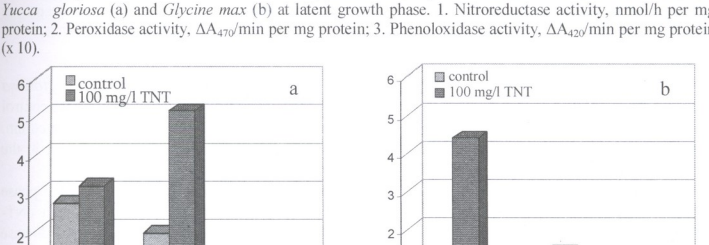


Fig. 2. TNT effect on the nitroreductase, peroxidase and phenoloxidase activities in callus culture of *Yucca gloriosa* (a) and *Glycine max* (b) at exponential growth phase. 1. Nitroreductase activity, nmol/h per mg protein; 2. Peroxidase activity, ΔA_{470}/min per mg protein; 3. Phenoloxidase activity, ΔA_{420}/min per mg protein (x 10).

At exponential growth phase of *Glycine max* callus culture nitroreductase activity increased by 3.5 under the effect of TNT; peroxidase activity underwent insignificant changes both in the phases and in the control variant as well; phenoloxidase activity increased only a little (Fig. 2b).

At exponential growth phase of *Glycine max in vitro* cells nitroreductase activity has a sharply expressed inductive character – the enzyme activity is significantly increased with tissue cultivation on TNT-containing nutrient medium, whereas at this phase of growth cycle of *Yucca gloriosa* cells peroxidase is activated.

Changes in the activity of the above mentioned enzymes at stationary growth phase of both *Yucca gloriosa* and *Glycine max in vitro* cultures with or without TNT (control variant) in the nutrient medium are shown in Figure 3.

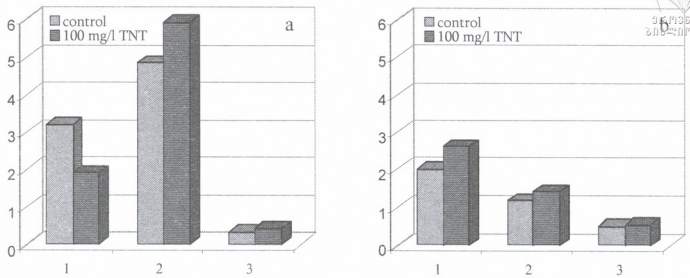


Fig.3. TNT effect on the nitroreductase, peroxidase and phenoloxidase activites in callus culture of *Yucca gloriosa* (a) and *Glycine max* (b) at stationary growth phase. 1. Nitroreductase activity, nmol/h per mg protein; 2. Peroxidase activity, ΔA_{470}/min per mg protein; 3. Phenoloxidase activity, ΔA_{420}/min per mg protein (x 10).

At stationary growth phase (42nd and 35th day, respectively) of cells of *Yucca gloriosa* nitroreductase activity decreased by 1.9 under the effect of TNT, and increased by 1.2 in the control variant; peroxidase activity increased both with and without TNT in the nutrient medium. Although, it should be pointed out, that increasing peroxidase activity is more significant in the control variant (2.5 fold), than at TNT presence in the nutrient medium.

At stationary growth phase of cells of *Glycine max* compared to exponential growth phase nitroreductase activity increased only a little, and decreased by 1.5 in the tissue cultivated on TNT-containing nutrient medium. At stationary growth phase TNT has no effect on nitroreductase activity; peroxidase and phenoloxidase activities underwent insignificant changes only.

While comparing inductive degree of nitroreductase, peroxidase and phenoloxidase activities in the dynamics of cell growth both of *Yucca gloriosa* and *Glycine max* cultivated on the TNT- containing nutrient medium it appears, that in cells of *Glycine max* reduction enzyme is more activated, and peroxidase – in the cells of *Yucca gloriosa*. As for phenoloxidase, its activity insignificantly increases in both *Yucca gloriosa* and *Glycine max* callus cultures. These results revealed that the abovementioned enzyme has no significant role in intracellular conversion of TNT.

This conclusion contradicts the idea of some researchers [Ugrekhelidze et al., 1974] according to which the decisive role in the detoxication processes of the cells belongs to phenoloxidase. Also it is known, that phenoloxidase is localized in chloroplasts. Low phenoloxidase activity in both *Yucca gloriosa* and *Glycine max* callus cultures may be explained by the fact, that plant *in vitro* cultures represent heterotrophic cell population with undeveloped chloroplasts. Though, studies conducted at the Institute of Biochemistry and Biotechnology showed low phenoloxidase activity in the cells of intact plant of *Glycine max* [Khatisashvili et al., 2004].

The results lead us to suppose that, the detoxication of TNT in callus cultures of *Yucca gloriosa* and *Glycine max* occurs by activation of different enzymatic systems dependent on the growth phase of cultures and their environmental conditions. This coincides with the conclusions of Nepovim et. al [Nepovim et al., 2004], that the study of different species for TNT degradation is necessary to find out the most suitable candidates for TNT phytoremediation.

References:

Bradford M.M. *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the trinciple of protein dye binding.* Anal.Biochem. **59**, 277-282, 1974.

Cash G.G. *Prediction of chemical toxicity to aquatic microorganism: ECOSAR vs. Microtoc assay.* Environ. Toxical. Water Qual., **132**, 211-216, 1998.

Gogoberidze M., Mamaladze M., Jaoshvili M., et al. *Characterization of suspension cell culture.* Plant Physiol., **35**, 2, 278-284, 1988 (in Russian).

Gregory, R.P.F. *A rapid assay for peroxidaze activity.* Biochem. J., **101,** 582-583, 1966.

Khatisashvili G., Kvesitadze G., Adamia G., et al.. *Bioremediation of contaminated soil on the former military locations and proving grounds in Georgia.* Journal of Biological Physics and Chemistry, **4**, 162-168, 2004.

Lanzarini G., Pifferi P., Zamorani A. *Specificity of an o-diphenol oxidase from Prunus avium fruits.* Phyrochemistry, **11,** 89-94, 1972.

Murashige T., Skoog F. *A revised medium for rapid growth and bioassays with tobacco tissue cultures.* Physiol. Plant., **15**, 473-497, 1962.

Nepovim A., Hubalek M., Vanek T., *Enzymatic Degradation of 2,4,6-TNT by Cell Suspension Culture of Rheum palmatum.* Eng. Life Sci., **3**, 1, 39-43, 2003.

Nepovim A., Hubalek M., Podlipna R., Zeman S., and Vanek T. *In vitro Degradation of 2,4,6-Trinitrotoluene Using Plant Tissue Cultures of Solanium aviculare and Rheum palmatum.* Eng. Life Sci., **4**, 1, 46-49, 2004.

Oh B., Sarath G., Drijber R.A. & Comfort S.D. *Rapid spectrophotometric determination of 2,4,6 - trinitrotoluene in a Pseudomonas enzyme assay.* Microbiol. Methods, **42,** 149-158, 2000.

Ugrekhelidze D., Chrikishvili D. Participation of o-diphenoloxidase in the process of oxidation of benzol in plants. Bulletin of Acad. Sci. of Georgian SSR, **62**, 701-704, 1974 (in Russian).

TNT-ს გავლენა იუკა დიდებულისა და სოიას კალუსურ კულტურების უჯრედებში ნიტრორედუქტაზულ, პეროქსიდაზულ და ფენოლოქსიდაზურ აქტივობებზე

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

ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 06.02.2006)

რეზიუმე

შესწავლილია ნიტრორედუქტაზული, პეროქსიდაზული და ფენოლოქსიდაზური აქტივობების ცვლილებები იუკა დიდებულისა და სოიას კალუსური კულტურების ზრდის ციკლის სხვადასხვა ფაზაში როგორც საკვებ არეში TNT-ს თანაობისას, ასევე TNT-ს გარეშე. ნაჩვენებია, რომ სოიას უჯრედებში უფრო აქტიურდება ნიტრორედუქტაზა, ხოლო იუკას უჯრედებში – პეროქსიდაზა; რაც შეეხება ფენოლოქსიდაზას, იგი TNT-ს შიდაუჯრედულ გარდაქმნებში მნიშვნელოვან როლს არ ასრულებს. დადგენილია, რომ იუკა დიდებულისა და სოიას კალუსური კულტურებში TNT-ს დეტოქსიკაცია უჯრედთა სასიცოცხლო ფაზებზე დამოკიდებულებით სხვადასხვა ფერმენტული სისტემების გააქტიურებით მიმდინარეობს.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

MICROFLORA AND ANTIMICROBIAL ACTIVITY OF GREEN TEA EXTRACT

GULUA L., PATARAIA D., GURIELIDZE M., KHUTSIDZE T., CHOLOKAVA N., ABUTIDZE M., SALIA G.

Durmishidze Institute of Biochemistry and Biotechnology
0159, Agmashenebeli alley 10 km, Tbilisi, Georgia. Tel/Fax: (99532) 528317
E-mail: chai@gol.ge

(Received April 17, 2006)

Abstract

Microflora of green tea extract was studied. 34 000 colonies of mesophilic bacteria per 1 gram of the extract were found. Fungi and *Esherichia coli* bacteria do not occur in the extract. Antimicrobial activity of green tea extract was tested against microorganisms: Gram positive bacteria – *Staphylococcus aureus, Rhodococcus* sp., Gram negative bacteria – *Esherichia coli, Pseudomonas aeroginasa*; Yeasts – *Candida utilis, Saccharomyces fragilis, Sacharomyces cerevisiae, Torulopsis* sp., Pathogenic fungi – *Fusarium solani, Rhizoctonia* sp. Green tea extract exerted antimicrobial activity against pathogenic bacteria: *Staphylococcus aureus, Esherichia coli,* and yeast – *Candida utilis*. Green tea extract in combination with other medications can be used as antimicrobial agent for stimulation of healing process of infectious wounds in humans.

Key words: pathogenic bacteria, polyphenols, yeast, phytochemicals, flavanoids, *Staphylococcus aureus*

Introduction

Ecological concerns in recent years have accelerated the usage and searching of preparations derived from plants. Phytochemicals are supposed to substitute some chemicals in treatment of various diseases and infections. Useful antimicrobial phytochemicals can be divided in several categories, such as phenolics, terpenoids, essential oils, alkaloids, lectins and polypeptides, polyacetylenes [Cowan, 1999]. Plants are rich in a wide variety of secondary metabolites, such as flavonoids, which have been found to have antimicrobial properties *in vitro*. Green tea consists of up to 20% flavanoids and as a result has been reported to possess high antimicrobial activity. Toda et al. noticed that green tea exerted antimicrobial activity [Toda et al., 1992]. Tea catechins inhibited in vitro *Vibrio cholerae* O1 [Boris, 1996], *Streptococcus mutans* [Batista et al., 1994, Sakanaka et al., 1989], *Shigella* [Vijaya et al., 1995], and other bacteria and microorganisms [Sakanaka et al., 1992].

As we reported earlier [Khutsidze et al., 2006] green tea extract was found to stimulate healing process of infectious wounds in humans. One of the mechanisms of that was presumably an antimicrobial activity of green tea extract.

The objective of the present work was to test *in vitro* green tea extract antimicrobial activity against pathogenic bacteria "yellow stapilococus"- *Staphylococcus aureus* which is main pathogen of infectious wounds, as well as against some other pathogenic bacteria, such as Gram positive bacteria – *Rhodococcus* sp., Gram negative bacteria – *Esherichia coli, Pseudomonas aeroginasa*; Yeasts – *Candida utilis, Saccharomyces fragilis, Saccharomyces cerevisiae, Torulopsis* sp., phytopathogenic fungi – *Fusarium solani, Rhizoctonia* sp.

Materials and Methods

Green tea extract. Green tea extract was commercially produced at the JS "Kolkheti-93" (Tsalenjikha, Georgia). Green tea leaves were subjected to the high temperature (100°C) treatment for 5 min, as a result, enzymes present in the leaves were inactivated and the chemical composition was fixed. Next, the leaves were undergone extraction process with 70°C water, the extract was filtered, concentrated and spray dried.

Antimicrobial tests. For antimicrobial tests of green tea extract a method of diffusion of the testing material (extract) in agar was used [Krasilnikov, 1966]. The method is based on the ability of the material to get diffused into the agar medium and detain growth of microorganisms. The following microorganisms were used as tests in our experiments: Gram positive bacteria – *Staphylococcus aureus, Rhodococcus* sp., Gram negative bacteria – *Esherichia coli, Pseudomonas aeroginasa;* Yeasts – *Candida utilis, Saccharomyces fragilis, Sacharomyces cerevisiae, Torulopsis* sp., Pathogenic fungi – *Fusarium solani, Rhizoctonia* sp.

Quantitative determination of antimicrobial activity of the extract. A subsequent dilution method was used to determine quantitatively an antimicrobial activity of the green tea extract against *S. aureus, E-coli* and *Candida utilities.* 1 ml nutrient mediums of these test microorganisms were placed into sterile tubes. 10g of green tea extract was dissolved in 90g sterile water. Then 0.5 ml green tea extract solution was added into the first tube; 0.5 ml of the solution from the first tube was added to the second tube and so on, so that the dilution numbers were 1:3, 1:9, 1:27, 1:81, 1:243 and 1:729. After dilution one drop of the test microorganisms were added to each tube. The tubes were placed into thermostat at the 30°C for 48 h. Growth of the test microorganisms was monitored. Mean value of the dilution numbers in the tube wherein the microorganism was grown and in the tube just before this one was applied as the titre number of the green tea extract against test microorganism in the tubes. The titre number represents the minimal quantity of the antimicrobial material in 1 ml of the testing solution capable to detain the growth of the test microorganism. 1 unit of the titre number is equivalent of 1 microgram of the active antimicrobial substances [Egorov, 1965].

Study of microflora of the green tea extract. Agar meat-peptone nutrient medium was used to study existence of mesophilic bacteria, Saburo medium was used to study fungi, and Kessler liquid medium was used for E-coli bacteria.

Results and Discussions

Microflora of green tea extract was studied. It was found 34 000 colonies of mesophilic bacteria per 1 gram of the extract. There were not any fungi and *E. coli* bacteria in the extract (Fig.1).

The green tea extract inhibited growth of pathogenic bacteria: *Staphylococcus aureus* (Fig. 2), *Esherichia coli,* and yeast – *Candida utilis*, but it did not affect other pathogenic microorganisms (Table 1).

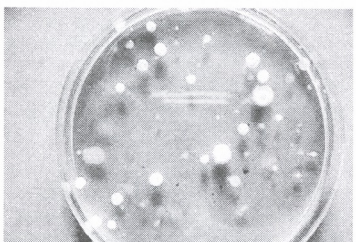


Fig.1. Colonies of mesopilic bacteria of green tea extract

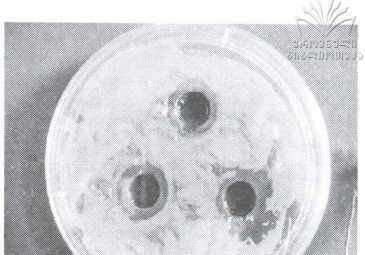


Fig. 2. Antagonistic activity of green tea extract against *Staphylococcus aureus* (light zones around colonies indicate on detention of growth of bacteria)

Table 1. Effect of green tea extract on pathogenic microorganisms*

Microorganisms:	Effect
Gram positive bactria	
Staphylococcus aureus	+
Rhodococcus sp.	-
Gram negative bacteria	
Esherichia coli	+
Pseudomonas aeroginasa	-
Yeasts	
Candida utilis Saccharomyces fragilis	+
Sacharomyces cerevisiae	-
Torulopsis sp.	-
Pathogenic fungi	
Fusarium solani,	-
Rhizoctonia sp.	-

*Remark: "+" sign denotes an effect, "-" sign denotes no effect

The titre number of the green tea extract against *Staphylococcus aureus* was equal to162 units. This number against *Esherichia coli,* and *Candida utilis* was equal to 18 units. Thus, the green tea extract showed a high antimicrobial activity against *Staphylococcus aureus,* which is one of the pathogenic bacteria of human wounds. We suggest that the green tea extract can be successfully used in medicine along with other medications in order to heal infectious wounds.

Acknowledgement: This work was carried out with the financial assistance of STCU within the framework of the project No: GG-122 "Creating plant bioconcentrates with antiradiation and anticancerogenic action on the base of polyphenols and working out industrial technology for their production".

References:

Batista O., Duarte A., Nascimento J., Simones M.F. *Structure and antimicrobial activity of diterpenes from the roots of Plectranthus hereroensis.* J. Nat. Prod., **57**, 858–861, 1994.

Borris R.P. *Natural products research: perspectives from a major pharmaceutical company.* J. Ethnopharmacol., **51,** 29–38, 1996.

Cowan M. *Plant products as antimicrobial agents.* Clin. Microbiol Rev., **12**, 4, 564–582, 1999.

Egorov N.S. *Microbes the antagonists and the biological methods of determination of antibiotic activity.* Moscow, "Visshaja Shkola", p.152, 1965 (in Russian).

Khutsidze T., Gulua L., Mchedlishvili N., Kvesitadze E., Omiadze N., Akvlediani K., Abutidze M., Losaberidze P. *Green tea extract as a stimulator of human wounds recovery process.* Proceedings of the Georgian Academy of Sciences, Biol. Ser. B, **4**, 3, 7-11, 2006.

Krasilnikov N. A. (editor). *The methods of study of soil microorganisms and their metabolites.* Moscow State University, 153-156, 1966 (in Russian).

Sakanaka S., Kim M., Taniguchi M., Yamamoto T. *Antibacterial substances in Japanese green tea extract against Streptococcus mutans, a cariogenic bacterium.* Agric. Biol. Chem., **53**, 2307–2311, 1989.

Sakanaka S., Shimura N., Aizawa M., Kim M., Yamamoto T. *Preventive effect of green tea polyphenols against dental caries in conventional rats.* Biosci Biotechnol Biochem. **56**, 592–594, 1992.

Toda M., Okubo S., Ikigai H., Suzuki T., Suzuki Y., Hara Y., Shimamura T. T*he protective activity of tea catechins against experimental infection by Vibrio cholerae O1.* Microbiol. Immunol. **36**, 999–1001, 1992.

მწვანე ჩაის ექსტრაქტის მიკროფლორა და ანტიმიკრობული აქტივობა

გულუა ლ., პატარაია დ., გურიელიძე მ., ხუციძე თ., ჭოლოკავა ნ., აბუთიძე მ., სალია გ.

ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 17.04.2006)

რეზიუმე

შესწავლილია მწვანე ჩაის ექსტრაქტის მიკროფლორა. გამოვლენილია მეზოფილური ბაქტერიები – 34 000 კოლონიის წარმომქნელი ერთეული 1 გრამ ექსტრაქტზე. სოკოები და ნაწლავის ჩხირის ბაქტერიები მწვანე ჩაის ექსტრაქტში არ აღმოჩნდა. გამოკვლეულია ანტიმიკრობული აქტივობა ისეთი მიკროორგანიზმების წინააღმდეგ, როგორიცაა: გრამ-დადებითი ბაქტერიები – *Staphylococcus aureus, Rhodococcus* sp., გრამ-უარყოფითი ბაქტერიები - *Esherichia coli, Pseudomonas aeroginasa*; საფუვრები - *Candida utilis, Saccharomyces fragilis, Saccharomyces cerevisiae, Torulopsis* sp., ფიტოპათოგენური სოკოები - *Fusarium solani, Rhizoctonia* sp. მწვანე ჩაი ავლენდა აქტივობას *Staphylococcus aureus, Esherichia coli* და *Candida utilis* მიმართ. მწვანე ჩაის ექსტრაქტი, როგორც ანტიმიკრობული პრეპარატი, სხვა მედიკამენტებთან ერთად შეიძლება გამოყენებულ იქნას ინფექციური ჭრილობების სამკურნალოდ.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

CHANGES IN CONTENT OF NITROGEN, PHOSPHORUS AND POTASSIUM IN LEAVES OF SOME GEORGIAN SORTS OF HAZELNUT DURING DIFFERENT VEGETATION PERIODS

VADACHKORIA N. [1], MCHEDLISHVILI N. [2], GAKHOKIDZE R. [1]

[1] *Ivane Javakhishvili Tbilisi State University*
[2] *Durmishidze Institute of Biochemistry and Biotechnology*

(Received March 15, 2006)

Abstract

Changes in content of nitrogen, phosphorus and potassium in leaves of some Georgian sorts of hazelnut (Gulshishvela, Shveliskura, Chkhikvistava and Khazarula) during different periods of vegetation were detected. Total nitrogen content in young leaves of hazelnut varied from 0.9 to 5.4% and in old leaves – from 0.3- to 2.45%. Phosphorus content in both young and old leaves varied from 0.26 to 0.41 % while content of potassium ranged from 0.79 to 1.43 %. Leaves of sort of Chkhikvistava were distinguished by the highest content of these macroelements.

Key words: macroelements, volumetric method, spectrophotometrical method.

Introduction

Determining the nutrient status of P, N and K of hazelnut plant is very important. The plant analysis gives a benefit to prevent the plant nutritional disorders. Analyzing the leaf samples is one of the most useful methods for determination of the plant nutrition requirements. [Horuz, Korkmaz, 2000]. Leaf analysis indicates which elements are present in adequate, deficient or excess amounts. According to the literature data sufficient amount of nitrogen in the leaves of hazelnut is 2.5-3.50%, that of phosphorous is 0.15-0.4% and potassium - 0.6-0.8 % [Horuz, Korkmaz, 2000].

The aim of our work was to determine content of nitrogen, potassium and phosphorus in leaves of various Georgian sorts of hazelnut grown on the same plot during different vegetation periods.

Materials and Methods

Young and old leaves of Georgian sorts of hazelnuts, particularly Gulshishvela, Khazarula, Shveliskura, Chkhvistava grown on the plot of Research Institute of Tea and Subtropical Cultures (Ozurgeti, Anaseuli, Guria) were served as materials for our investigation. The leaf samples were washed with distilled water, dried and ground in a mill. To 2g of ground leaves of each sort of hazelnut was added 25 ml concentrated sulfuric acid and boiled for 5-6h. Obtained homogeneous mixture was diluted with water up to 200 ml in the volumetric flasks and

analyzed for N, P and K. Content of phosphorus in this solution was determined spectrophotometrically according to the method modified by Levitsky [Oniani, Margvelashvili, 1978] and of potassium was determined by cobalt nitrite volumetric method as described in [Oniani, Margvelashvili, 1978]. The total nitrogen content was determined using the standard Kjeldahl method as described in [Tkemaladze, Kvesitadze, 1975].

Results and Discussions

As a result of our investigations some changes in content of nitrogen, potassium and phosphorus in the leaves of all tested sorts of hazelnut during different vegetation periods (from 25 July to 20 September) were observed.

As is seen from Table 1 the content of nitrogen significantly changes during the vegetation period. In all sorts of hazelnut the content of nitrogen in young leaves is higher than in old ones. Total N content in young leaves of hazelnut varies from 0.9 to 5.4% and in old leaves from 0.3- to 2.45%. The highest content of nitrogen was detected in young leaves of sort Chkhikvistava (average content of this element during the vegetation periods - 3.69% and maximal content - at the end of vegetation period in September - 5.4%). It should be mentioned that both young and old leaves of this sort of hazelnut were distinguished by high content of this macroelement. In young leaves its amount remained sufficient during the periods of vegetation.

Phosphorus content in all tested sorts of hazelnut leaves varied slightly from 0.26 to 0.41% and remained sufficient during all vegetation period. The highest amount of phosphorus was found in young leaves of Khazarula (average content 0.37% during the different periods of vegetation) (Table 2) and in old leaves of Chkhikvistava (average content 0.36% during different periods of vegetation period). The content of potassium in the hazelnut leaves varied from 0.79 % to 1.43 %, but it remained sufficient in all the sorts of hazelnut during the different periods of vegetation). In young leaves the highest amount of potassium was detected in sort of Sveliskura (1.43%) and in old leaves in sort of Chkhikvistava (1.37%).

Table 1. Changes in content of N in various sorts of hazelnut leaves during different vegetation periods.

Sort of hazelnut	Data	Content of N, %	
		Young leaves	Old leaves
Gulshishvela	25 July	2,32 ± 0,01	1,97 ± 0,01
	30 August	2,95 ± 0,02	2,45 ± 0,02
	20 September	1,25 ± 0,02	0,4 ± 0,00
Sveliskura	25 July	2,17 ± 0,01	1,84 ± 0,02
	30 August	2,85 ± 0,01	2,2 ± 0,01
	20 September	0,9 ± 0,00	0,3 ± 0,01
Chkhikvistava	25 July	2,61 ± 0,01	2,22 ± 0,01
	30 August	3,05 ± 0,02	2,21 ± 0,03
	20 September	5,4 ± 0,01	1,25 ± 0,03
Khazarula	25 July	2,15 ± 0,02	1,97 ± 0,01
	30 August	2,85 ± 0,01	2,4 ± 0,03
	20 September	3,9 ± 0,01	0,9 ± 0,01

Table 2. Changes in content of P in various sorts of hazelnut leaves during different vegetation periods

Sort of hazelnut	Data	Content of P, %	
		Young leaves	Old leaves
Gulshishvela	25 July	0,31 ± 0,01	0,29 ± 0,02
	30 August	0,32 ± 0,02	0,30 ± 0,01
	20 September	0,37 ± 0,02	0,26 ± 0,01
Sveliskura	25 July	0,27 ± 0,01	0,26 ± 0,00
	30 August	0,31 ± 0,01	0,30 ± 0,01
	20 September	0,35 ± 0,01	0,26 ± 0,01
Chkhikvistava	25 July	0,31 ± 0,01	0,35 ± 0,00
	30 August	0,29 ± 0,01	0, 41 ± 0,01
	20 September	0,39 ± 0,01	0,31 ± 0,01
Khazarula	25 July	0,36 ± 0,01	0,35 ± 0,00
	30 August	0,35 ± 0,01	0,31 ± 0,01
	20 September	0,39 ± 0,01	0,31 ± 0,01

Table 3. Changes in content of K in various sorts of hazelnut leaves during different vegetation periods

Sort of hazelnut	Data	Content of K, %	
		Young leaves	Old leaves
Gulshishvela	25 July	1,25 ± 0,01	1,05 ± 0,01
	30 August	1,20 ± 0,02	1,04 ± 0,02
	20 September	1,49 ± 0,00	0,83 ± 0,01
Sveliskura	25 July	1,43 ± 0,01	1,04 ± 0,01
	30 August	1,33 ± 0,00	0,99 ± 0,00
	20 September	1,12 ± 0,01	0,83 ± 0,01
Chkhikvistava	25 July	1,18 ± 0,01	0,87 ± 0,01
	30 August	1,14 ± 0,01	1,37 ± 0,01
	20 September	0,99 ± 0,01	0,83 ± 0,01
Khazarula	25 July	0,97 ± 0,01	0,71 ± 0,02
	30 August	1,14 ± 0,02	1,14 ± 0,01
	20 September	1,16 ± 0,01	0,79 ± 0,01

Thus, the content of nitrogen, phosphorus and potassium in leaves of various sorts of hazelnut, particularly in Gulshishvela, Sveliskura, Chkhikvistava and Khazarula during different vegetation periods remained sufficient. At the same time leaves of sort of Chkhikvistava was distinguished by the highest content of these macroelements during the periods of vegetation.

References:

Horuz A., Korkmaz A. *Determination of nutrient status in hazelnut leaves sampled from Terme and Unye regions.* International Symposium on Desertification, 2000.

Oniani O.G., Margvelashvili B.N. *Chemical analysis of plant.* Tbilisi, "Ganatleba", 415, 1978.

Tkemaladze G., Kvesitadze G. *Practical Ensymology.* Tbilisi, "Metsniereba", 299, 1975.

თხილის ზოგიერთი ქართული ჯიშის ფოთლებში აზოტის, ფოსფორისა და კალიუმის შემცველობის ცვლილება ვეგეტაციის სხვადასხვა პერიოდში

ვადაჭკორია ნ.[1], მჭედლიშვილი ნ.[2], გახოკიძე რ.[1]

[1]ივ. ჯავახიშვილის თბილისის სახელმწიფო უნივერსიტეტი
[2]ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგის ინსტიტუტი

(მიღებულია 15.03.2006)

რეზიუმე

თხილის ზოგიერთი ქართული ჯიშის (გულშიშველა, შველისყურა, ჩხიკვისთავა და ხაზარულა) ფოთლებში შემჩნეულია აზოტის, ფოსფორისა და კალიუმის შემცველობის ცვლილება ვეგეტაციის სხვადასხვა პერიოდში. აზოტის საერთო შემცველობა ახალგაზრდა ფოთლებში მერყეობდა 0.9-დან 5.4%-მდე, ხოლო ძველ ფოთლებში – 0.3-დან 2.45%-მდე. ფოსფორის რაოდენობა, როგორც ახალგაზრდა, ისე ძველ ფოთლებში იცვლებოდა 0.26-დან 0.41 %-მდე, კალიუმის შემცველობა კი - 0.79-დან 1.43 %-მდე. ჯიში ჩხიკვისთავას ფოთლები გამოირჩეოდა ამ მაკროელემენტების ყველაზე მაღალი შემცველობით.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

Biophysics

PURIFICATION AND SOME PHYSICAL - CHEMICAL PROPERTIES OF TITIN

ZAALISHVILI T., GACHECHILADZE N., GRIGOLAVA M., TOIDZE P., ESIPOVA N., ZAALISHVILI M.

Institute of Molecular Biology and Biological Physics

(Received April 24, 2006)

Abstract

The method of obtaining of native pure titin was worked out. Some physical – chemical properties of titin were studied. Analysis of sedimentation and electrophoresis of pure preparation show one asymmetric pick and one band, correspondingly. Coefficient of sedimentation $S^0_{20,w} = 13.7S$. While molecular ellipticity of unpurified preparation is -800, this value for pure preparation equals to -3000. According to the circular dichroism curves phase transition occurs at 55-60°C. X-ray analysis revealed that titin molecule consists of polyprolin II (PPII) type left-handed helix structure. Experimental data obtained enable us to conclude that titin molecule is mainly presented by β-structure with left-handed PPII structures involved in it.

Key words: electrophoresis, sedimentation, circular dichroism (CD), x-ray study.

Introduction

In 50-s of 20th century it was already known that an elastic component exist in muscle fibers. It has been assumed that this component is responsible for their elastic properties, especially during passive stretch. The first indication of the existence of a structural lattice in striated muscle came along with the discovery of the actin-myosin-based sliding filament mechanism of contraction. It was demonstrated that total extraction of myosin from myofibrils yielded "ghost myofibrils" being both, continuous and extensible [Haxley, Hanson, 1954]. Elastic protein responsible for continuity of myofibrils was isolated in 70s [Maruyama, 1976; Wang, McClure 1979] and called as "connectin" and further - titin.

Titin (connectin) is a giant elastic protein with molecular mass 3-5 MDa [Maruyama, 1976; Maruyama et al., 1981; Wang et al., 1984]. Titin is complex multi-domain protein. Each titin molecule spans half a sarcomere from the M line to the Z line, associates with myosin thick filaments, and connects thick filaments to the Z line via an extensible I band segment. The extension of this elastic connection in the I band generates the restoring force when resting muscle is stretched [Wang et al., 1993]. In A-band region titin is mainly composed by immunoglobulin-like domains (Ig G) and/or fibronectin (FN-3), and a class of unique insertions: phosphorilating sites of serine/treonine domain and binding sites for specific muscle protease – ealpain. In I-band region, where titin extends during passive force development, it is composed of serial-connected motifs of three types: (i) the folded 100-residue modular repeats of immunoglobulin G (Ig G) domains, (ii) a unique proline rich tandem repeats of ~ 28-residue PEVK motif, which consists of

70% proline (P), glutamate (E), valine (V) and lysine (K), and (iii) the N2A or N2B insert [Trinick, 1992; Trinick, Tskhovrebova, 1999].

According to the data [Labeits, Kolomerer, 1995] elastic properties of titin is due to two main structurally distinct motifs of I-band: (i) tandemly arranged of ~ 100-residue immunoglobulin (IgG) and (ii) PEVK-motif. Tissue- and species specific alternative slicing alters the expression of these motifs [Freiburg et al., 2000], and correspondingly changes composition and mass of titin, mainly of immunoglobulin and PEVK-motifs. This, in turn gives unique passive force-extension curves.

Two major reasons prevent to obtain pure native titin. First, they are extremely sensitive to any proteases. Second, this protein is not very soluble in aqueous solution without denaturants. Methods occurred in scientific literature [Trinick et al., 1984; Maruyama, 1994;] do not allow to isolate ultra-pure preparation of titin necessary to determine the physical-chemical characteristics of this giant protein. Therefore, the goal of our study was to work out modified method of isolation of pure titin and to study its some properties.

Materials and Methods

Preparation of pure titin

Rabbit psoas muscle, removed immediately postmortem, was passed through cooled chopper twice. To 100 g muscle 3 volume of an ice-cold solution I (50 mM KCl, 5 mM EGTA, 1 mM $NaHCO_3$, 5 μM E64c, pH7) was added and homogenized for 60s at 5000 turn per min. All procedures were carried out at 4°C. Suspension of myofibrils was washed 2 times by centrifugation at 2000g for 10 min followed by resuspension in 3 vol. of solution I, which didn't contain E64c. Suspension of fresh myofibrils was centrifuged for 10 min at 5000g and the supernatant removed carefully. To the pellet twice volume of an ice-cold extracting solution II (0.9 M KCl, 2 mM $MgCl_2$, 10mM imidazol, 2 mM EGTA, 1 mM PMSF, 10 μg/ml tripsin inhibitor, 0.5 mM DTT and 5 μM E64c, pH 7) was added. After dispersing the pallet with a glass rod, and stirring for 5 min, suspension was centrifuged for 30 min at 1500g. The clarified supernatant was removed carefully, diluted with 3 vol. distilled water, stored for 40 min, following which precipitated myosin was removed by centrifugation at 15 000 g for 30 min. The supernatant was diluted with 4 vol. water, stored for 40 min and centrifuged at 11 000 g for 30 min. Crude pellet of titin was diluted in solution III (0.6 M KCl, 30 mM K-phosphate buffer, pH 7) and was dialyzed against this solution during 12 h. After centrifugation at 25 000 g for 20 min, clear supernatant (v ~ 10-12 ml, c = 10-15 mg/ml) was applicated on a top of the bed of the column (1.5 cm x 90 cm) containing Sepharose CL2B equilibrated in this buffer. The flow rate was 6 $ml \cdot cm^{-2} \cdot h^{-1}$. Fractions (3 ml each) are collected, and 0.2 ml aliquot of each fraction is analyzed by gel electrophoresis on gradient stab gels (3.5–10% acrylamide, 0.5% bisacrilamide). Titin containing fractions was pooled (v ~ 30 ml, c = 0.18 - 0.20 mg/ml) and concentrated by polyethylenglycol (M=4000-8000). After centrifugation and concentration volume and concentration of solution were ~ 12 ml and 0.4 mg/ml correspondingly. Concentrated titin solution was again passed through a gel filtration CL2B column (1.6 cm x 10 cm) equilibrated in solution III and run at a rate 5 $ml \cdot cm^{-2} \cdot h^{-1}$. Titin containing fractions were pooled, centrifuged by polyethylenglycol (M = 4000–8000) and dialyzed overnight against the same solution. Volume and concentration of pure titin solution were ~2 ml and 0.96-1.00 mg/ml, correspondingly.

The native titin solution was dialyzed against a salt solution containing 0.1 m KCl and Tris-buffer (pH 7.5).

Circular dichroism (CD)

CD spectra of titin were obtained by Jasco spectropolarimeter, J-715. Buffer spectra were subtracted. A quartz cell of 0.2 mm of path length was used; spectra were recorded with a 1 nm band width over the wavelength range 185 – 250 nm.

Sedimentation analysis was carried out by the analytical centrifuge MOM-3180

X-ray investigations

Lyophilized from water solutions titin samples were investigated by X-ray using diffractometer CAD4. Diffraction was recorded within the range of scattering angle θ - 3°-17°. Step of changing of θ was 0.25°. Diffraction at the given angle θ was registered during 1 min. Monochromatic characteristic radiation CuKα was used. Interplanar spacings were calculated according to Wolf-Bragg expression: $n\lambda=2d\sin\theta$.

Results and Discussions

We obtained pure preparation of titin according to the method described above. Data of electrophoresis in the gradient of SDS polyacrilamide gel (2.5-15%) are presented on Fig. 1.

Sedimentation analysis of titin pure preparation revealed one asymmetric pick (Fig. 2.) Coefficient of sedimentation $S^{0}_{20,w} = 13.7S$

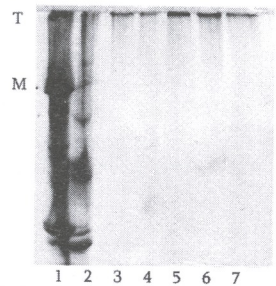


Fig. 1. Electrophoresis in the gradient of SDS polyacrilamide gel (2.5-15%). Electrophoregram: 1 – myofibrils, 2 – not purified titin, 3, 4, 5, 6, 7 – pure titin.

CD spectra of titin shows strong negative band at 216 nm. While molecular ellipticity of unpurified preparation is -800, this value for pure preparation equals to -3000 (Fig. 3). The relationship between the CD-spectra of titin and the temperature (Fig. 4) shows that denaturation of protein takes place at 55-60°C. As a result of destruction of β-structure a negative mean residue ellipticity becomes lower and CD-curves shift towards long-wave ultraviolet light.

CD spectra of titin display similar characteristics as PPII helix spectra, which reveal strong negative band near 200 nm.

Conformational studies by circular dichroism (CD) and Nuclear magnetic resonance spectroscopy (NMR) indicate the presence of PPII left-handed helices in PEVK domain in titin [Kan Ma et al., 2001; 2003]. Further examination of titin PEVK sequences showed the presence of numerous PXXP motifs (e.g., PVAP) that are frequently found in PPII left-handed helices. Analysis suggests that PPII may be a major structural motif of PEVK in titin [Greaser, 2001; Gutierrez-Cruz et al., 2001].

To prove realization of conformation of polyproline II left-handed helix, samples lyophilized from titin water solution were studied. X-ray reflection with interplanar spacing of ~ 0.3 nm was revealed on diffractograms (Fig. 5). Among energetically stable helix structures of polypeptide single chains constructed on the basis of one amino acid only left-handed PPII helix could have the projection of residue on helix axis - 0.3 nm and correspondingly, interference described by the Bessel function of zeroth order should be accounted for such interplanar spacing. Earlier we have shown the existence of collagen-like structure in the molecule of titin by x-ray investigation [Kobakhidze et al., 1984]. Data of x-ray diffraction pattern obtained in 1984 and data of titin pure preparation are presented in Table 1. Interplanar spacings of observed reflections of titin (connectin) and corresponding spacings of PPII are compared. Similarity between diffraction patterns of titin and PPII within researched range of scattering angle is clear.

Table 1. Observed interplanar spacings (nm) of titin (connectin) and PPII.

Interplanar spacings (nm) of corresponding reflections for PPII	Observed interplanar spacings (nm) of connectin (titin) (1984)	Observed interplanar spacings (nm) of pure titin
0.49	0.47	0.46
0.36	0.37	0.38
0.31	0.29	0.31
0.29		0.29
0.19	0.18	-

One of the principal problems of muscle contraction is the problem of tension transition from myofibril to tendons and bones. Obviously, in molecular viewpoint, the protein transmitting the tension to non-stretched elements of organism should contain such non-stretched thermostable structures. It was shown that near Z-disk titin binds to actin, that makes this region of titin functionally stiff [Linke et al., 1997; Trombitas & Granzier, 1997]. The relatively weak actin-PEVK interaction gives rise to a viscous force component opposing filament sliding. Actin association with titin filaments is not a new proposal. Interaction of actin with whole titin filaments was described by Maruyama [Maruyama et al., 1987]. It is likely that those parts of PEVK domain of the titin molecule, which have the PPII left-handed structure should be related with actin thin filaments connecting them with membrane or extracellular elements of the same (PPII) structure.

The basic characteristic of PPII structure is its non-stretchability related with fixed angle φ in proline. So, functional role of titin, which consists of such structures in PEVK domain, first of all, could be signed in fixation of limit length of muscle cell, i.e. in stabilization of relaxation of muscle fiber. Titin should constrain the extent of every sarcomere in entire myofibril.

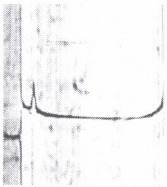


Fig. 2. Sedimentation curve of pure titin

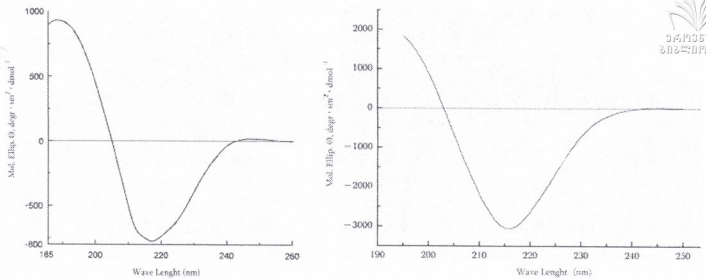


Fig. 3. CD-spectra of a) unpurified titin (mol.ellip. -800); b) pure titin (mol.ellip. -3000)

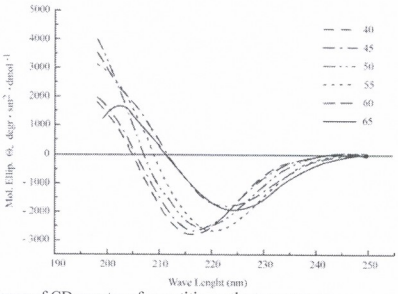


Fig. 4. Dependence of CD-spectra of pure titin on the temperature

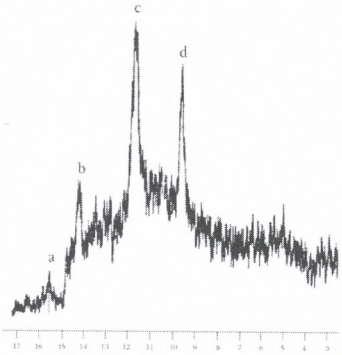


Fig. 5. Diffraction pattern of lyophilized titin received by diffractometer. a) Θ=15.6°; d=0.286 nm. b) Θ=14.25°; d=0.313 nm. c) Θ=11.75°; d=0.378 nm. d) Θ=9.6°; d=0.459 nm.

References:

Freiburg A., Trombitas K., Hell W., et al. *Series of exon-skipping events in the elastic spring region of titin as the structural basis for myofibrillar elastic diversity.* Circ. Res., **86**, 1114-1121, 2000.

Greaser M. *Identification of new repeating motifs in titin.* Proteins, **43**, 145-149, 2001

Gutierrez-Cruz G., van Heerden A.H., Wang K. *Modular motif, structural folds and affinity profiles of the PEVK segment of human fetal skeletal muscle titin.* J. Biol. Chem., **276**, 7442-7449, 2001.

Haxley H., Hanson J. *Changes in the cross striations of muscle during contraction and stretch and their structural interpretation.* Nature, **173**, 971-973, 1954.

Kan Ma, Lou-sing Kan, Kuan Wang. *Polyproline II helix is a Key structural motif of the elastic PEVK segment of titin.* Biochemistry, **40**, 3427-3438, 2001.

Kan Ma, Kuan Wang. *Malleable conformation of the elastic PEVK segment of titin: non-co-operative interconversion of polyproline II helix, β-turn and unordered structures. Biochem.* J., **374**, 687-695, 2003.

Kobakhidze G., Grigolava M., Zaaloshvili T., Rogulenkova V., Zaalishvili M., Esipova N. *Muscle cell connectin is collagen-like protein.* Biophosysics, **29**, 2, 314-314, 1984.

Labeit S., Kolomerer S. *Titins, giant proteins in charge of muscle ultrastructure and elasticity.* Science, **270**, 293-296, 1995.

Linke W., Ivemeyer M., Labeit S., Hinssen H., Ruegg J. Gautel M. *Actin-titin interaction in cardiac myofibrils: probing a physiological role.* Biophys. J., **73**, 905-919, 1997.

Maruyama K. Natori R., Nonomura Y. *New elastic protein from muscle.* Nature, **262**, 58-60, 1976.

Maruyama K., Kimura S., Ohashi K., Kuwano Y. *Connectin an elastic protein of muscle. Identification of 'titin" with connectin.* J.Biochem., **89**, 701-709, 1981.

Maruyama K., Hu D., Suzuki T., Kimura S. *Binding of actin filaments to connectin.* J Biochem (Tokyo), **101**, 1339-1348, 1987.

Trinic J., Knight P., Whiting. *Purification and properties of native titin.* J.Mol.Biol., **180**, 331-356, 1984.

Trinick J. *Understanding the functions of titin and nebulin.* FEBS, **307**, 1, 44-48, 1992.

Trinick J., Tskhovrebova L. *Titin: a molecular control freak.* Trends Cell Biol., **9**, 377-380, 1999.

Trombitas K., Granzier H. *Actin removal from cardiac myocytes shows that near the Z-line titin attaches to actin while under tension.* Am. J. Physiol., **273**, C662-C670, 1997.

Wang K., McClure J., Tu A. *Titin: major myofibrilar component of striated muscle.* PNAS USA, **76**, 8, 3698-3702, 1979.

Wang K., Ramirez-Mitchell R., Palter D. *Titin is an extraordinarily long, flexible and slender myofibrilar protein.* PNAS USA, **81**, 3685-3689, 1984.

Wang K., McCarter R., Wright J., Beverly J., Ramirez-Mitchell R. *Viscoelasticity of the sarcomere matrix of skeletal muscle - the titin-myosin composite filament is a dual-stage molecular spring.* Biophys. J. **64**, 1161-1177, 1993.

ნატიური სუფთა ტიტინის მიღება და მისი ზოგიერთი ფიზიკური-ქიმიური თვისება

ზაალიშვილი თ., გაჩეჩილაძე ნ., გრიგოლავა მ., თოიძე პ., ესიპოვა ნ., *ზაალიშვილი მ.*

მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 24.04.2006)

რეზიუმე

დამუშავებულია ნატიური სუფთა ტიტინის მიღების მეთოდი და შესწავლილია მისი ზოგიერთი ფიზიკურ-ქიმიური თვისება. სუფთა პრეპარატის სედიმენტაციური და ელექტროფორეზის ანალიზის შედეგად ვლინდება, შესაბამისად, ერთი ასიმეტრიული პიკი და ერთი ზოლი. სედიმენტაციის კოეფიციენტია $S^{0}_{20,w} = 13.7S$. იმ დროს, როდესაც ჭუჭყიანი პრეპარატის ელიფსუროზა არის -800, სუფთა პრეპარატისთვის ეს მნიშვნელობა -3000 ტოლია. წრიული დიქროიზმის მრუდების განხილვის შედეგად ნაჩვენებია, რომ ფაზურ გადასვლას ადგილი აქვს 55-60°C–ზე. რენტგენოსტრუქტურული ანალიზის საშუალებით ნაჩვენებია, რომ ტიტინის მოლეკულა შეიცავს პოლი-პროლინ II ტიპის მარცხენა სპირალურ სტრუქტურას. მიღებული ექსპერიმენტული მონაცემები საშუალებას გვაძლევს დავასკვნათ, რომ ტიტინის მოლეკულა ძირითადად წარმოდგენილია β-სტრუქტურით, რომელშიც ჩართულია მარცხენა სპირალური სტრუქტურა.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

STUDY OF BACTERIOPHAGES AGAINST THE TOMATO WITH SOME BACTERIAL ETIOLOGIES

GHUDUMIDZE N.[1], CHKONIA I.[2], SHAPOVALOVA N.[1], SADUNISHVILI T.[1], MEIPARIANI A.[2]

[1] *S. Durmishidze Institute of Biochemistry and Biotechnology*
[2] *G. Eliava Institute of Bacteriophages, Microbiology and Virology*

(Received April 3, 2006)

Abstract

The paper deals with the application of bacteriophages or phages as an alternative way in controlling of diseases of tomato seeds and all aboveground parts of the plants, caused by *Xanthomonas vesicatoria* and *Clavibacter michiganensis*. 6 phages were isolated as a result of the multiple inoculation of the bacterial strains, previously checked on lysogenecity, from sewage water or soil samples taken from diseased area and damaged tomato materials. The phage existence was determined by their lysis ability of the bacterial strains. Lysis by avirulent bacteriocins with phage-like action was eliminated. The studies showed that *in vitro* lysis of bacteria was conducted by phages.

Keywords: *Xanthomonas vesicatoria*., *Clavibacter michiganensis*, disease control, bacteriophage, isolation.

Introduction

Bacteriophages are the integral part of ecosystems and therefore regulate population and spiecies composition of bacteria within biocenoses [Polishuk et al., 2005]. Bacteriophages are found in the representatives of over 100 bacterial genera and many different habitats [Ackerman, 1996]. Phages multiply in the case of contact with the bacteria causing the diseases of humans, animals, plants and actinomycetes.

Alterations of the phage population by their artificial introduction into environment or through natural way have not been properly studied yet [Polishuk et all, 2005]. Via artificial introduction the phages may be purposefully used in controlling the plants diseases caused by phytopathogenic bacteria. Application of bacteriophages is particularly significant in dealing with bacteria, which damage the agricultural plants. One of the important agricultural plants is tomato (*Lycopercicon).*

The yield level of tomato crops is very important, which is hindered by various diseases challenged both by fungi, viruses, nematodes and the phytopathogenic bacteria like *Xanthomonas*, Pseudomonas, *Clavibacter*. These bacteria produce the lesions on seeds and all aboveground parts of the plants. In addition, bacteria accumulate in the soil where they can survive within a year. All kind of water movement may spread bacteria from sick plant to healthy one and cause diseases on a large area leading to the reduction of crops.

The wet conditions in the plant canopy create a suitable environment for bacteria to growth. Each pathogen is characterized by particular temperature range, in which it reaches its peak growth rate and ability of infection [Leboeuf et al., 2005]. In Georgia mainly found tomato bacterial diseases are: *Bacterial spot* caused by *Xanthomonas vesicatoria* (OP.T.24-30°C) and *bacterial canker*, caused by *Clavibacter michiganensis* (OP.T.24-30°C).

In order to control these bacterial diseases the chemical substances are used. One of the encouraging alternative ways against tomato bacterial diseases is an application of bacteriophages for treatment and preventive purposes. After the suppression of all bacteria, phages are gradually eliminated from the individual and the environment [Kutter, 1997]. No adverse effects to humans, wildlife or the environment are expected from phages. For treatment of tomato plants specific polyvalent phage cocktails against various bacterial strains and species are created, and in case of resistance development new ones are selected [Kapanadze, 2005].

The aim of our work was obtaining the phages against *Xanthomonas vesicatoria* and *Clavibacter michiganensa* pathogenic bacteria isolated from various infected organs of tomato.

Materials and Methods

As materials for phage isolation were used: tomato damaged fruits, tomato damaged leaves, soil samples taken from the zones of diseases; sewage water. As nutrient media were used - 2% potatoes agar; 0.7% semiliquid potatoes agar, LB Broth. MILLER and LB Agar MILLER, [1-800-557-4367. www. emdchemicals. com. manufactured for EMD. chemicals Inc. An. Affilite of merck kGat. Dermstads. Germany; Usa 1-800-222-0342]. Bacterial strains were isolated and checked on pathogenecity by staff from phytopathogenic department of L. kanchavely Institute of Plant Protection.

Phages from sewage water were isolated using the method of Adams. Phages existence was tested by their titration on 2% agar with adding 0.7% semiliquid agar using Gratia method [Adams, 1959].

Results and Discussion

Each bacterial strain was tested on lysogenicity. The lycogenic (temperate) phages can actually integrate their DNA into the host DNA, leading to virtually permanent association as a prophage with a specific bacterium and with its progeny. Bacterium multiplies together with prophage for many generations, protecting the host cell from other unrelated lytic phages [Kutter, 1997]. In the cases of lysogenicity the lysogenical conversion phenomenon frequently occurs among the bacteria. Sometimes prophage escapes from prophage-bacterium association that by transduction often can lead to transfer to new host genes involved in bacterial pathogenecity and inducing resistance. To test the lysogenicity the strains were washed with 2.5-2.5ml of broth and each bacterial culture was centrifuged at 5000xg for 20min. The supernatants were separately filtrated through single millipore membrane filters with pore sizes of 0.22μm. The filtrates were dropped on the bacterial lawn plates. After incubation in thermostat at 27°C for 4.6 and 24 hours, lysis was not observed i.e. none of strains was lysogenic.

Phage isolation was performed by inoculation of the bacterial cultures into sewage or inoculation of the plant material in the broth together with bacterial cultures. After incubation at 27°C for 24 hours the growths were centrifuged at 5000xg for 35min. The supernatants were filtrated through single millipore membrane filters and checked on the phage presence on the bacterial lawn plate with 2% agar by dropping or by titration on 2% agar with adding 0.7%

semiliquid agar. As a result of the multiple inoculation 6 various phages were isolated. The results of action of filtrates on bacterial lawns are shown in the table.

It was found out, that lysis was not caused by bacteriocins, the avirulent (non-disease producing) variants of the bacterial pathogens with the phage-like action. Bacteriocins are substances produced by pathogenic bacteria that antagonize other closely related or unrelated bacteria. They adsorb on the specific receptors and disturb their processes of metabolism.

Bacteriocins are specific and do not lose this ability [Adams, 1959], the bacterium are mostly characterized by immunity to those bacteriocins which are produced by itself and after their production it dies itself. One bacterium is able to produce one or more kind of bacteriocin. Each filtrate to be tested was titrated up to dilution 10^{4}-10^{-5}, using Gratia method.

If lysis was caused by bacteriocins, the lysis level on each plate must have been identical, since amount of the bacterial cultures in each dilution was equal (0.1ml), only phage concentration was decreased. After titration it turned out that lysis was the most powerful in the initial dilution showing that the phage existed in the filtrates and lysis was caused by phage action.

Table 1. Sensitivity of bacterial strains to filtrates.

Strain #	Strain Isolation source	Filtrate					
		I From T. f.	II From T. f.	III From T. f.	IV From T. f.	V From S. w.	VI From S.w.
1.	T.l.	-	-	-	-	±	-
2.	T.l.	-	-	-	-	-	-
3.	T.l.	-	-	-	-	-	±
4.	T.l.	-	-	-	-	-	-
5.	T.l.	-	-	-	-	±	-
6.	T.l.	-	-	-	-	±	-
7.	T.f.	4+	4+	4+	4+	4+	-
8.	T.f.	4+	4+	3+	4+	4+	2+
9.	T.l.	-	-	-	-	-	±
10.	T.f.	-	-	-	-	-	-
11.	T.f.	1+	3+	±	2+	3+	4+
12.	T.f.	-	-	-	-	-	+
13.	T.f.	±	-	-	-	-	±
14.	T.f.	±	±	±	±	±	-
15.	T.l.	+	±	±	+	+	-
16.	T.f.	-	4+	4+	4+	-	2+

T.l. – Tomato leaves, T.f.—Tomato fruit, S.w.—Sewage water

The mixture of filtrates (phages) gives round plaques of three different sizes on the plate with 2% potatoes agar after titration: the large plaques, the midsize plaques and the small plaques. Clarification of whether they are modifications of one phage race or they are several phages and study of their properties and activity *in vivo* are the objectives of our further work.

Acknowledgment: This study was supported by ISTC grant G-1129.

References:

Adams H. M. *Bacteriophages*. INC. New York. Interscience Publishers, 1959.

Ackerman H. W. *Bacteriophages.* 1996

Kapanadze A. *Phagology from the position of holistic approach to human organism.* 1st. International seminar of JSC Biochempharm and Eliava Institute of Bacteriophages, Microbiology and Virology. Tbilisi, Georgia, 61-62, 2005.

Kutter E. *Phage therapy! Bacteriophages as Antibiotics.* Evergreen state college. Olympia. WA 98505, 1997.

Leboeuf J., Cuppels D., Dick J., Pitblado R., Poewen St., Celetti M. *Bacterial diseases of tomato; Bacterial Spot, Bacterial Speck, Bacterial Canker*. Queen's Printer for Ontario. Factsheet ISSN 1198-712X, 363-365, 2005.

Polischuk V. P., Semchuk L. I., Romashev S.A., Andriychuk E.N. *Perspectives and problems of using of Bacteriophages for the population regulation of microorganism in biocenoses.* 1st International seminar of JSC Biochempharm and Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia, 72, 2005.

ბაქტერიოფაგების გამოქვინება პამიდორის ბაქტერიული დაავადებების წინააღმდეგ საბრძოლველად

ღუდუმიძე ნ.[1], ჭყონია ი.[2], შაპოვალოვა ნ.[1], სადუნიშვილი თ.[1], მეიფარიანი ა.[2]

[1]ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი
[2]გ. ელიავას ბაქტერიოფაგიის, მიკრობიოლოგიისა და ვირუსოლოგიის ინსტიტუტი

(მიღებულია 03.04.2006)

რეზიუმე

შესწავლილია ბაქტერიოფაგების გამოყენება *Xanthomonas vesicatoria* და *Clavibaqter michiganensis*-ით გამოწვეული პამიდორის თესლისა და მიწისზედა ორგანოების ბაქტერიული დაავადებების წინააღმდეგ საბრძოლველად. 6 ფაგი, წინასწარ ლიზოგენურობაზე შემოწმებული ბაქტერიული შტამების მრავალი ჩათესვის შედეგად, გამოიყო ჩამდინარე წყლებიდან, ან დაზიანების კერიდან აღებული მიწის ან პამიდორის დაზიანებულ მასალებიდან. ფაგების არსებობა დადგინდა მათ მიერ ბაქტერიების ლიზისის უნარით. გამოირიცხა ლიზისი ფაგის მსგავსი მოქმედების, არავირულენტური ბაქტერიოცინების მიერ. გამოკვლევებმა აჩვენეს, რომ *in vitro* ბაქტერიების ლიზისს ახდენენ ფაგები.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

DIVERSITY OF THE FAMILY *ORCHIDACEAE* IN THE FLORA OF IMERETI (WEST GEORGIA)

CHEISHVILI T.[1], GAGNIDZE R.[2]

[1] *A. Tsereteli Kutaisi State University*
[2] *N. Ketskhoveli Institute of Botany*

(Received April 11, 2006)

Abstract

Diversity of the family *Orchidaceae* in the flora of Imereti (West Georgia) was studied. 32 species and 15 genera of the family are represented on the studied area. The paper presents botanical-geographic analysis of the family. Taxonomic, geographic and ecotopological structure of orchids occurring in Imereti is considered.

Key words: *Orchidaceae*, botanical-geographic analysis, taxonomic structure, ecotopological structure.

Introduction

Imereti is a western part of a botanical-geographic province of Colchis [Kolakovsky, 1961; Kharadze, 1966; Gagnidze, 1974, 1997, 2004; Gagnidze et al., 2000, 2002]. Its flora is not studied comprehensively. According to the data reported by Kuthatheladze, the flora of the region numbers approx. 1100 species, while Gagnidze [Gagnidze, Davitadze, 2000] indicates another number, namely, approx. 900 species. The region is rich in endemic species [Kuthatheladze, 1961, 1962; Cheishvili, Churadze, 2006]: 121 endemic species and 5 endemic subspecies are presented there; 69 of them are endemic to the Caucasus, 52 – to Georgia and 14 species are locally endemic to Imereti.

The family *Orchidaceae* is one of the characteristic families of Imereti as well as Colchis in general. 23 species and 15 genera of orchids are represented in the flora of the region. Only one endemic subspecies of the family is distributed in Imereti: *Ophrys caucasica* subsp. *caucasica* [Gagnidze, 2005]. The species is endemic to the Caucasus. It was described by Woronow on the basis of materials collected in different parts of the Caucasus, namely, Dagestan, Georgia (Abkhazeti), Azerbaijan (*Ophrys caucasica,* 1928). In Georgia this subspecies is distributed in: Abkhazeti - 1, Svaneti - 2, Imereti - 5, Kartli - 9, Trialeti - 16, Kvemo Kartli - 17; also western parts of the North and South Caucasus.

As reported by Averianov [1994], *O. caucasica* is also represented by another subspecies in Georgian flora, namely, *O. caucasica* subsp. *cyclocheila* Aver. It is described from Azerbaijan and in Georgia is distributed in Kartli - 9, Trialeti - 16, Kvemo Kartli - 17. This subspecies is also endemic to the Caucasus. *O. caucasica* is a close relative of *O. transhyrcana*, Mediterranean and West Asian species.

Materials and Methods

The survey was conducted using itinerary, classic morphological-geographic and arealogical methods. Materials of the Herbaria of Ketskhoveli Institute of Botany, State Museum of Georgia, Tbilisi State University, Kutaisi State University. Sense and status of species follows Averyanov [1994], Czerepanov [1995], Gagnidze [2005].

Results and Discussion

Taxonomic structure. The family *Orchidaceae* is represented by approx. 800 genera and 18000-25000 species worldwide [Takhtajan, 1987; Mabberley, 1997]. The Caucasus is rich in orchids with 60 species and 21 genera occurring in the region [Averyanov, 1994]. In Georgia 48 genera from 20 families are distributed; 32 species from 15 genera occur in Imereti.

Orchidaceae is a cosmopolite family. The majority of the species are found in paleotropical (South-East Asia) and neotropical (Central and South America) kingdoms. These are perennial mycotrophic, mostly epiphytic and terricolous plants. Species occurring in Georgia and, particularly, in Imereti are perennial terricolous mostly green plants; however, orchids lacking chlorophyll such as *Neottia, Limodorum* are also found in the region.

Orchids occurring in Imereti as well as in Georgia in general have "disperse" composition, i.e. they are represented by species from many different genera. The number of participating genera is large; the genera are mono-, oligo- and polytopic. The number of species pertaining to the polytopic genera is 9. This phenomenon is similar to so-called "disperse endemism" [Gagnidze, Margalitadze, 1981; Gagnidze, 2005]. The following are monotypic genera of orchids of Imereti: *Anacamptis* (a monotypic genus in general), *Gymnadenia, Coeloglossum, Limodorum, Neottia, Platanthera, Serapias, Spiranthes, Traunsteinera.*

The following genera are represented by 2 species each: *Epipactis, Listera*; by 3 species: *Cephalanthera, Ophrys*; by 4 species – *Dactylorhiza*; by 9 species – *Orchis.*

The orchids of Imereti belong to 2 subfamilies (Table 1)

Table1. Orchids of Imereti

Subfamily **Neottioideae:**	
genera	species
1. Cephalanthera	1. *C. damasonium* (Mill.) Druce 2. *C. longifolia* (L.) Fritsch (*C.lonehophyllum* L.fill.) 3. *C. rubra* (L.) Rich.
2. Epipactis	4. *E. palustris* (L.) Crantz. 5. *E. helleborine* (L.) Crantz (*E.latifolia* (L.) All.)
3. Limodorum	6. *L. abortivum* (L.) Boehm. (*Centrosis abortive* (L.) Sw.)
4. Listera	7. *L. cordata* (L.) Br. 8. *L. ovata* (L.) Br.
5. Neottia	9. *N. nidus-avis* (L.) Rich.
6. Spiranthes	10. *S. spiralis* (L.) Chevall.
Orchidoideae	
7. Anacamptis	11. *A. pyramidalis* (L.) Rich.
8. Coeloglossum	12. *C. viride* (L.) Haztm.
9. Dactylorhiza	13. *D. euxind* (Nevski) Czer. (*Orchis caucasica* (Klinge) Medw. 14. *D. flavescens* (C. Kach.) Holub 15. *D. salina* (Turcz. ex Lindl.) Soó (*Orchis sanasunitensis* Duct.) 16. *D. urvilleana* (Steudel) Baumann et Küenkele (*Orchis amblyoloba* Nevsky; O. trifilla C.Koch)

10. Gymnadenia	17. *G. conopsed* L.
11. Ophrys	18. *Oph. Apifera* Huds. 19. *Oph.caucasica* Woronow ex Grossh. subsp. caucasica 20. *Oph. oestifera* Bieb.
12. Orchis	21. *O. coryophora* L. 22. *O. maclura* (L.) L. 23. *O. morio L.* (O.morio subsp. picta (Loisel.) K.Risht. 24. *O. militaris* L. 25. *O. pallens* L. 26. *O. palustris* Jacq. 27. *O. purpurea* Huds. 28. *O. provincialis* Balb. ex Dc. 29. *O. ustulata* L.
13. Serapias	30. *S. vomeracea* (Burm. Fil.) Briq.
14.Traunsteinera	31. *T. spaerica* (Bieb.) Schlechter
15. Platanthera	32. *P. chlorantha* (Custor) Reichenb.

Geographic structure. Colchis is distinguished by diversity of orchids in the Caucasus [Grossheim, 1949; Kolakovsky, 1961]. Along with other parts of the botanical-geographic province of Colchis, Imereti is a region with remarkable generic and specific diversity. Colchis together with Asia Minor is a center of diversity of orchids. In Asia Minor the family is represented by 24 genera and about 140 species. The following genera are especially diverse: *Ophrys* (45 species), *Orchis* (31 species), *Dactylorhiza* (25 species) [Renz, Taubenheim, 1984].

In general, orchids including their representatives occurring in Imereti are characterized by wide amplitude of their geographic distribution. Among them are European-Mediterranean monotypic genera *Anacamptis, Limodorum* spread in the Caucasus and West Asia. *Coeloglossum* is a Holarctic genus. *Epipactis helleborine* also has the distribution range of the Holarctic type; *Epipactis palutris, Gymnadenia conopsea, Orchis mascula* are Palearctic. European-Mediterranean, Minor and West Asian species spread in the Caucasus predominate. They belong to the most of the genera of orchids: *Cephalanthera, Dactylorhiza, Orchis, Traunsteinera, Serapias, Ophrys* (23 species) [Butter, 1986; Baumann, Kunkele, 1988; Averyanov, 1994] (types of the distribution ranges follow Gagnidze, 2004). The spectrum is similar too that of Colchis [Kolakovsky, 1961] and the North-Western Caucasus [Litvinskaya, 1988; Perebora, 2003].

Some of the species are rare and pertain to "dying relicts", e.g., *Listera cordata* and *L. ovata, Neottia nidus-avis, Orchis militarius*, etc. Small populations of the majority of the species remain principally on calcareous ecotopes of Okriba and Nakerala.

The most part of the species should be evaluated as Critically Endangered (CR), Endangered (EN), Vulnerable (VU) and Near Threatened (NT) according to IUCN Categories and Criteria.

Ecotopological structure. As mentioned above, orchidss of the Caucasus, Georgia and Imereti are terricolous. Area of their ecitopological distribution is limited. They are mainly restricted to moist, swampy habitats and forests: broad-leaved (zelkova, oak, chestnut, beech forests) and light coniferous, on soils covered by mosses, wet and mesophilous primary meadows and shrubbery of shibliak type of the forest ecosystem.

These are ephemeroid tuberiferous and rhizomatous plants. Many of them are characterized by wide amplitude of ecological distribution and form large populations in various forest (e.g., *Gymnadenia conopsea, Platanthera chlorantha, Dactylorhiza flavescens*) and meadow (e.g., *Transteinera spearica*) bioms of lower, middle, upper forest as well as subalpine and alpine belts.

Results of the bioecological analysis (habitats) are given in the table below (Table 2).

Table 2. Habitats of orchid species of the flora of Imereti.

1. Moist meadows, mixed deciduous and dark coniferous forest openings, shrubbery of the lower and middle mountain belt	*Cephalanthera damasonium* *C. grandiflora* *C. longifolia* *C. rubra* *Epipactis helleborina* *Limonium abortivum* *Listera ovata* *Orchis coriophora* *O. maculra* *O. Mario* *O. militaris* *O. provincialis* *O. ustulata* *Ophrys aestrifera* *Oph. caucasica* *Platanthera chlorantha* *Spiranthes spiralis*
2. Dense mixed deciduous and dark coniferous forests of the middle and upper mountain belt	*Listera cordata* *Neottia nidus-avis* *Ophrys apifera*
3. Broad-leaved forests of the middle forest belt	*Anacamptis pyramidalis* *Cephalanthera rubra* *Dactylorhiza flavescens* *D. urvilleana* *Orchis pallens* *O. purpurea*
4. Forest openings of the middle and upper mountain belt, mesophilous forb meadows of the subalpine and alpine belts	*Coeloglossum viride* *Dactylorhiza euxina* *D. salina* *D. urvilleana* *Gymnodenia conopsea* *Serapias vomeracea* *Traunsteinera sphaerica*
5. Peat soils, moist meadows and swampy biotopes	*Epipactis palustris* *Orchis palustris*

References:

Averyanov L. *Review species of Orchidaceae of the flora of Caucasus.* Bot. Journ., **79**, 10, 109-126, 1994.

Baumann B., Künkele S. *Die Orchideen Europas.* Franckhische Verlagshandlung. Stuttgart, 1988.

Butter K.P. *Orchideen.* Mosaik Verlag Gmbh. München, 1986.

Cheishvli T., Churadze M. *Diversity of endemic flora of Jmereti (West Georgia).* Proc. Georg. Acad. Sci., Biol. Ser. B, **4**, 2, 40-48, 2006.

Cherepanov S. *Vascular Plants of Russia and Adjacent States (the former USSR).* Cambridge University Press. Cambridge, 1995.

Gagnidze R. *Botanical and geographic analysis of tall herbaceous vegetation of the Caucasus.* Tbilisi, 1974 (in Russ.).
Gagnidze R. *Arealogical reviw of Colchic evergreen broadleaved mesophyllous dendrophlora species.* In: Recent shifts in vegetation boundaries of deciduous forests. Basel-Boston, Berlin, 209-216, 1999.
Gagnidze R. *Up to date problems and tasks of botanical geography of the Caucasus.* Notulae Systematicae ac Geographice Jnstituti Botanici Botanici Thbilissiensis, 44-45, 8-52, 2004 (in Russ.).
Gagnidze R., Davitadze M. *Local flora (Plant life of Georgia).* Batumi, 2000 (in Georg.).
Gagnidze R., Gviniashvili Ts., Shetekauri Sh., Margalitadze N. *Endemic genera in the Caucasian flora.* Feddes Repertorium, **113**, 7-8, 616-630, 2002.
Gagnidze R., Margalitadze N. *Concerning the South-Eastern Boundary of the Mediterranean Phytogeographical Region.* Bull. of the Acad. Sci., **102**, 1, 125-128 1981(in Russ.).
Kharadze A. *For botanical geographic division of high-mountainous regions of the Great Caucasus.* The Problems of Botany, Moscow-Leningrad, **8**, 75-89, 1966 (in Russ.).
Kolakovsky A. *Flora and vegetation of Colchis.* Moscow, 1961 (in Russ.).
Kutateladze A. *Plantae in montibus calcareis.* Notulae Systematicae ac Geographicae Jnstituti Botanici Thbilissiensis, **22**, 36-42, 1961 (in Georg.).
Kutateladze A. Georgia's endemics in the flora of calcareous Hassifs of Jmereti. Bull. of the Georg. Botanical Society, **1**, 21-39, 1962.
Litvinskaya S.A. *Orchidaceae of the natural flora of north-west Caucasus.* Bull. GBS AN SSSR, **150**, 64-68, 1988 (in Russ.).
Ophrys Caucasica. Ed. Woronow ex Grosh. Fl., **I**, p. 261, 1928.
Perebora E.A. *Distribution of Orchids (Orchidaceae) in the North-Western Caucasus.* Bull. Journ. **88**, 9, 109-116, 2003 (in Russ.).
Renz J., Taubenheim G. *Orchidaxeae in Davis.* Flora of Turkey and the East Aegean Islands, **8**, Edinburgh, University Press, 450-552, 1984.
Taktajan A. *Systema Magnoliphytorum.* Leningrad, "Nauka", 1987.

იმერეთის ფლორის ჯადვარისებრთა ოჯახის მრავალფეროვნება

ჭეიშვილი თ.[1], გაგნიძე რ.[2]

[1]ა. წერეთლის ქუთაისის სახელმწიფო უნივერსიტეტი
[2]ნ. კეცხოველის ბოტანიკის ინსტიტუტი.

(მიღებულია 11.04.2006)

რეზიუმე

შესწავლილია იმერეთის ფლორის ჯადვარისებრთა ოჯახის მრავალფეროვნება. იგი ითვლის 32 სახეობასა და 15 გვარს. ჩატარებულია ოჯახის ბოტანიკურ-გეოგრაფიული ანალიზი, განხილულია იმერეთის ჯადვარისებრთა სისტემატიკური, გეოგრაფიული და ეკოტოპოლოგიური სტრუქტურა.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

"FLORESCENCE" OF CONIFEROUS PLANTS IN TBILISI BOTANIC GARDEN

GRIGOLIA I.

Faculty of Natural Sciences of Sokhumi State University Branch of Iv. Javakhishvili Tbilisi State University

(Received July 18, 2006)

Abstract

Dynamics of "florescence" of 35 endemic and introduced coniferous plant species grown in Tbilisi Botanic Garden were studied. Phenological observations of insemination-development and fertilization of plantlets and elaboration of obtained data were carried out. It was found out that transition into generational maturity phase of studied exotic plants in the earliest age occurs for subtropical plants of middle and south latitudes of northern hemisphere, then come plants of cold climatic zone. Micro- and mega-sporangium were revealed in June-July of vegetation year preceding fertilization, or in fertilization year (species of genus Cedrus). Species of genus Cedrus have the shortest period from occurrence of generational organs to fertilization. Fertilization of studied plants takes place from September up to the end of May. Pollen spraying was observed in winter, which is ancestral feature. In studied exots duration of fertilization lasts 6-26 days and depends on temperature and moisture of air.

Key words: introduced plants, fertilization, ancestral feature, phenological observation.

Introduction

During the process of adjustment to environmental conditions plant develops the rhythm of seasonal development in its natural habitat, which is hereditary. But, while introduction in new environment development of new ontogenesis rhythm of viability should be changed.

The aim of our research is to study "florescence" dynamics of 3 endemic and 32 introduced coniferous plants in eastern Georgia.

Materials and Methods

Phenological observations of insemination-development and fertilization were carried out in Tbilisi Botanic garden during 1996-1999 via the known method [Phenological observations of coniferous, 1973]. We have studied insemination and development of generative organs after every 3 days during the vegetation period, and phases of beginning and ending of fertilization – everyday during the "florescence". Data of phonological observations were worked out by special manual [Manual of mathematical treatment of data of phenological studies, 1972].

Results and Discussion

Insemination of generative organs of studied species occurs in vegetative period preceding fertilization, but insemination of male strobiles of genus Cedrus species happens in 2nd half of vegetation of fertilization year. The shortest period (3-4 months) from appearance of mega- and micro-sporangium up to fertilization have species of genus Cedrus. Strobiles of species of genera Chamaecyparis, Cephalotaxus, Caleocedrus, Sequoia, Platycladus, Thuia, Taxus, mature for 6-9 months. Maturing period of species of genera Pinus, Abies, Pseudotsuga, Picea, Juniperus, is longer (10-12 months), generative organs of which inseminate in June-July, and fertilization takes place in April-May.

Insemination of male strobiles on the crown at the end of shoots occurs in the following species: *Sequoia sempervirens, Cupressus arisonica, C.goveniana, C.sempervirens, C.torulosa, C.duclouxiana, C.lusitanica, Thuia occidentalis, Platycladus orientalis, Juniperus polycarpos, J.procumbens, Chamaecyparis lawsoniana, Ch. funebris*; For some species *(Taxus baccata, Cedrus deodara, Pinus pallasiana, P.sosnowskyi, P.bungeana, Cupressus sempervirens)* male strobiles are placed on the lower part of the main crown, or on the whole crown.

Female strobiles of the following species: *Taxus baccata, Cupressus sempervirens, Chamaecyparis lawsoniana, Ch. funebris, Platycladus orientalis, Thuia occidentalis, Pinus griffithii, P. sosnowskyi, P. pallasiana, Juniperus* and of the species of genus Cedrus, are placed on the whole crown; and of species: *Sequoia sempervirens, Pinus bungeana, P. armandii, Cupressus duclouxiana, C. torulosa, C. lusitanica, C. arisonica, C.goveniana, Callocedrus decurrens, Abies, Pseudotsuga, Tsuga* and of the species of genus Picea - on the upper part of crown.

Fertilization of conifers in Tbilisi Botanic garden occurs from September to the end of May (Table 1). Species: *Cedrus deodara, C. atlantica, C. libani,* "florescence" in autumn, species: *Cupressus arisonica, C.lusitanica, Callocedrus decurrens, Thuia occidentalis, Platycladus orientalis, Sequoia semperuirens*, spray pollen in winter, which is ancestral feature. Although those species, which generally spray pollen in spring, reveal tendency towards fertilization in winter. This phenomenon was observed in 1999 for *Taxus baccata*, which could be explained by warm winter and biological characteristics of this species. In 1999 pollen spraying (variation coefficient – 29-65%) of the species: *Cupressus sempervirens, Juniperus polycarpos, J.procumbens* "florescencing" usually in March-April, took place 10-18 days earlier, which is caused by the same reasons.

Species of the genera: Abies, Picea, Pseudotsuga, Tsuga, Pinus, Chamaecyparis, are characterized by the stable regime of fertilization; their organic rest phase is not impaired up to spring even at high temperature conditions. These species evolutionary need low temperature for maturing of generative organs, which accelerates "florescence", as well as could change photoperiod [Leopold, 1968].

Table 1. Fertilization of coniferous plants in Tbilisi Botanic Garden.

№	Species	beginning				ending				duration
		M	n	±2m	V%	M	n	±2m	V%	
1	*Taxus baccata*	4.03	20.02	4	8	15.03	4.03	10	68	11
2	*Cephalotaxus fortunei*	7.04	3.04	3	8	13.04	11.04	2	5	7
3	*Sequoia sempervirens*	7.02	30.01	6	2	13.02	5.02	6	2	7
4	*Abies nordmanniana*	21.04	12.04	6	11	27.04	17.04	7	13	7
5	*A.numidica*	20.04	16.04	3	6	29.04	20.04	6	10	10
6	*A.cephalonica*	22.04	15.04	7	12	2.05	28.04	7	11	11

7	*A.Pinsapo*	26.04	19.04	6	10	3.05	22.04	7	9	8
8	*A.cilicica*	22.04	14.04	8	15	28.04	19.04	10	16	8
9	*A.concolor*	1.05	26.04	4	7	9.05	2.05	6	9	9
10	*Pseudotsuga glauca*	19.04	12.04	5	9	29.04	22.04	6	11	8
11	*P.menziesii*	22.04	12.04	7	13	29.04	15.04	8	14	8
12	*Tsuga canadensis*	27.04	18.04	7	13	2.05	21.04	9	14	6
13	*Picea abies*	22.04	19.04	5	10	28.04	22.04	4	7	7
14	*P.pungens*	29.04	22.04	5	8	9.05	5.05	5	6	10
15	*Cedrus atlantica*	23.09	18.09	5	2	14.10	10.10	4	2	21
16	*C.libani*	20.09	17.09	4	2	16.10	14.10	3	1	26
17	*C.deodara*	21.10	15.10	4	2	12.11	5.11	5	2	21
18	*Pinus pallasiana*	6.05	1.05	4	6	11.05	5.05	4	5	6
19	*P.griffithii*	15.05	10.05	4	5	24.05	18.05	6	7	9
20	*P.bungeana*	12.05	3.05	7	9	21.05	16.05	5	6	9
21	*P.sosnowskyi*	9.05	5.05	3	4	19.05	15.05	3	4	10
22	*P.armandii*	18.05	15.05	2	3	27.05	22.05	2	2	9
23	*Cupressus sempervirens*	28.03	10.03	9	29	14.04	23.03	14	33	16
24	*C.torulosa*	8.04	2.04	6	15	21.04	10.04	8	16	14
25	*C.lusitanica*	21.02	2.02	12	3	13.03	3.03	8	59	21
26	*C.arisonica*	13.02	27.01	12	3	22.02	10.02	7	2	10
27	*C.duclouxiana*	3.04	28.03	3	10	11.04	5.04	3	8	9
28	*C.govenina*	19.03	1.03	7	65	27.03	12.03	13	46	19
29	*Juniperus polycarpos*	11.03	1.03	4	38	5.04	10.03	5	13	25
30	*J.procumbens*	27.03	12.03	10	38	12.04	28.03	4	12	15
31	*Chameacypamris lawsoniana*	3.04	30.03	6	18	13.04	12.04	4	9	10
32	*C.funebris*	8.04	4.04	3	9	14.04	10.04	4	8	7
33	*Callocedrus decurrens*	11.02	15.01	18	5	20.02	30.01	14	4	9
34	*Thuia occidentalis*	6.02	25.01	8	2	12.02	28.01	9	3	7
35	*Platycladus orientalis*	24.02	13.02	3	1	10.03	01.03	6	63	17

M- arithmetic mean; n – the earliest time of beginning and ending; ±2m – deviation from arithmetic mean in both directions (days); V% - variation coefficient.

Except *Abies concolor*, species of the genera: Picea, Tsuga, Pseudotsuga, spray pollen in 20-29 April; fertilization of non-fluctuating phenotype pines introduced from various floristic regions was noted in 6-12 May.

"Florescence" of coniferous plants proceeds 6-26 days in average, and duration of this period decreases by the effect of high temperature and low relative humidity. Such situation does not occur in case of plants growing in sea coast line of Ajara in the conditions of high relative humidity.

With abundant fertilization are characterized the following species: *Taxus baccata, Abies nordmanniana, A. numidica, A. pinsapo, Pseudotsuga menziesii, Cedrus deodara, Pinus griffithii, P. sosnowskyi, P. pallasiana, Cupressus sempervirens, C. lisitanica, C. goveniana, Chamaecyparis funebris, Platycladus orientalis.*

References:

Leopold A. *Plant Growth and Development.* Moscow, "Mir", 227-263, 1968.

Manual of mathematical treatment of data of phonological studies. Council of Botanical Gardens. Academy of Sciences of USSR, Moscow, 1972.

Phenological observations of coniferous. Methodological guideline. Academy of Sciences of USSR, Yalta, p.42, 1973.

ᲕᲘᲕᲕᲝᲕᲐᲜ ᲛᲪᲔᲜᲐᲠᲔᲗᲐ "ᲥᲕᲐᲕᲘᲚᲝᲑᲐ" ᲗᲑᲘᲚᲘᲡᲘᲡ ᲑᲝᲢᲐᲜᲘᲙᲣᲠ ᲑᲐᲦᲨᲘ

გრიგოლია ი.

ივ. ჯავახიშვილის თბილისის სახელმწიფო უნივერსიტეტის სოხუმის ფილიალის ზუსტ და საბუნებისმეტყველო მეცნიერებათა ფაკულტეტი

(მიღებულია 18.07.2006)

ᲠᲔᲖᲘᲣᲛᲔ

კვლევის მიზანს წარმოადგენდა თბილისის ბოტანიკურ ბაღში მოზარდი 35 სახეობის ადგილობრივი და ინტროდუცირებული წიწვოვანი მცენარის „ქვავილობის“ დინამიკის შესწავლა. „ქვავილე“ კვირტების ნასახვა-განვითარებაზე და დამტვერიანებაზე ჩატარდა ფენოლოგიური დაკვირვება და მონაცემები დამუშავდა სპეციალური ცნობარით. გამოკვლევების შედეგად ადმოჩნდა, რომ შესწავლილი ეგზოტების გენერაციულ სიმწიფეში გადასვლის ფაზა ყველაზე ადრეულ ხნოვანებაში ჩრდილოეთ ნახევარსფეროს შუა და სამხრეთ განედის სუბტროპიკულ, უფრო გვიან კი ცივი კლიმატის მცენარეებს ეწყებათ. მიკრო – და მეგასპორანგიუმების გამოჩენა ხდება დამტვერვის წინა საქვეტაციო წელიწადს ივნის-ივლისში, ან დამტვერვის წელიწადს (Cedrus-ის გვარის სახეობები). გენერაციული ორგანოების გამოჩენიდან დამტვერიანებამდე ყველაზე ხანმოკლე პერიოდი აქვთ Cedrus-ის გვარის სახეობებს. შესწავლილ მცენარეთა დამტვერიანება მიმდინარეობს სექტემბრიდან მაისის ბოლომდე. აღინიშნა ზამთარში მტვრის გაჰნევა, რაც ანცესტრალური ნიშანია. შესწავლილ ეგზოტებში დამტვერვის ხანგრძლივობა 6-დან 26 დღეს მოიცავს და დამოკიდებულია ჰაერის ტემპერატურასა და ტენიანობაზე.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

THE DEVELOPMENT OF THE MICROSTROBILUS AND MALE GAMETOPHYTE OF ENGLISH YEW - *TAXUS BACCATA* L.

SHAKARISHVILI N.

N. Ketskhoveli Institute of Botany, Plant Reproduction Research Laboratory

(Received March 27, 2006)

Abstract

The development of the male reproductive structures has been studied in English Yew *Taxus baccata* L. natural population in the Nichbisi forest. The timing and duration of special phenological phases has been established. Microstrobili and ovules reveal synchronicity during the development of primordia and formation of sporocytes. Slight developmental delay was observed in postdormant reproductive organs of both male and female. Protandry up to 15 days was observed. Possible influence of ontogenetic retardation on pollination and reproduction success is discussed.

Key words: microstrobilus development, pollination, male gametophyte, phenology, *Taxus baccata* L.

Introduction

English yew –*Taxus baccata* L. is dioecius conifer known to be rare in many parts of Europe. In spite of conservation status [Georgia Red Data Book, 1982] yew populations over the Georgia are in acute danger. Populations are mainly fragmented, small size and seed propagation is not efficient.

Nichbisi population (41°48′20″ N, 44°31′59″ E) is located in the mixed broadleaf-coniferous forest at 1300 m a.s.l. The main source of population growth is vegetative expansion via shoot rooting. This might have negative effect on population viability due to loss of genetic diversity, lack of new gene recombination. The last is expected to reflect on population decline and on increased probability of extinction in long-term perspective [Yong, 1996]. Sexual reproduction as a unique source of new genetic recombination allowed keeping genetic diversity and might offset the effects of fragmentation reducing the loss of genetic diversity and the danger of extinction.

It is obvious that success of the sexual reproduction is entirely depended on the development of male and female gametophytes. Despite the importance of data concerned reproductive biology in sustainable management programs for yew, few studies are available to date on yew reproductive biology in Georgia [Dolukhanov, 1956, Shakarishvili, 2004; Shakarishvili, Gachechiladze, 2005]. General structure and developmental morphology of male reproductive organs is well documented at light and electron microscope levels [Chamberlain, 1935; Favre-Duchartre, 1960; Chira, 1964; Pennell, Bell, 1985, 1986a, 1986b, 1988; Kuprianova, Gumbatov, 1988, Kirzo, Korinkova, 1989].

Our objectives were to study the morphology of microstrobilus, pollination and the development of male gametophyte, determine the timing of critical events in the phenology of microsporogenesis in continuing of our previous study concerned ovule development in yew.

Materials and Methods

Microstrobili were collected at principle stages of the development in 2003-2005. Material was fixed in FAA solution (5 parts of 40% formaldehyde : 10 parts of glacial acetic acid : 90 parts of 70% ethyl alcohol). After treatment according to the standard microtechnique protocol microstrobili were embedded in paraffin, sectioned (6-8 μm) and stained with Hemalaun solution [Berlin, Mikshe, 1976]. Preparations were observed under light microscope Polyvar [Reichart, Austria] equipped with Nikon CoolPix 5000 digital fotocamera.

Results and Discussion

Microstrobili are formed in the leaf axils of one year old branchlets. Primordial protuberances become visible in July-August of the year preceding pollination. Under the light microscope observation primordia consist of meristematic cells about 15 μm in diameter, with deeply stained nuclei. Central part of the primordial meristem gives rise to sporogenous tissue while peripheral differentiates into tapetum. Growing microstrobilus is formed of the axis with radially arranged microsporophylls. Overlapping budscales are 2 ranked: outer ones are imbricate and smaller than inner. The last are semitransparent, larger and located adjacent to epidermal cells of the microsporophyll. The number of microsporophylls varies from 7 to 12. The last forms globular structure. Peltate sporophylls vary in shape from triangular to octahedral. Epidermal cells seemed to reveal photosynthetic ability; thus adaxial surface of intact microsporophylli is bright green at globular stage (Fig. 1-a). From 3 to 8 pollen sacs are formed on the abaxial side of microsporophylls. It seems, they loss photosynthetic ability later in ontogeny becoming pale yellowish at dormant period. Phenology of microsporogenesis starts in the late autumn preceding pollination.

The date for a special phonological stage varies with temperature. Shortly before winter dormancy megaspore mother cell undergoes meiosis, after which microstrobili pass the winter at the haploid microspores stage. During the whole period of winter dormancy microstrobili are sessile or shortly pedicellate (pedicilla ca 1 mm), microsporophylls are entirely covered by decussate budscales (Fig. 1-b).

Winter dormancy is finished at the end of March - first decade of April. This is the time when prepollinated microstrobili begin to grow, pushing the microsporophylls out of the covering budscales. Longitudinal sections of prepollinated microstrobili show that meiosis is completed, microspores maturation occurs centripetally.

Taxus baccata L. is wind pollinated species. Ripe pollen grain is non saccate. Before pollination starts, microspores have irregular shape and small size (15 μm, long axis). A quite intensive pollination continues usually about two weeks. In most cases the synchronicity in the maturation of microspores is obvious (Fig. 1-c); however we observed the strobili with traits of ontogenetic retardation (Fig. 1-d). In such cases developmental delay was revealed on cytological level by the presence of undifferentiated sporogenous tissue at pollination stage, while the common pool of microstrobili was presented by empty pollen sacs after mature pollen release (Fig. 1-e, Fig. 1-f).

At the time of pollination tapetal layer is completely collapsed and pollen extrusion is due to rupture of the pollen sac wall. Pollination occurs in March-April, its duration is about 3 weeks

depending on actual temperature. Ovule receptivity is determined by the presence of pollination drop on the micropyle. We observed ovular secretion up to 2 weeks after the pollination starts and stopped within 10 days. Thus anthesis continued overlapping the period of actual female receptivity. Persistence of the pollination drop seems to be depended on humidity conditions. We did not observe ovular secretion at all in 2003 when humidity was <70%. In contrast in 2004 secretion was quiet persistent under high humidity.

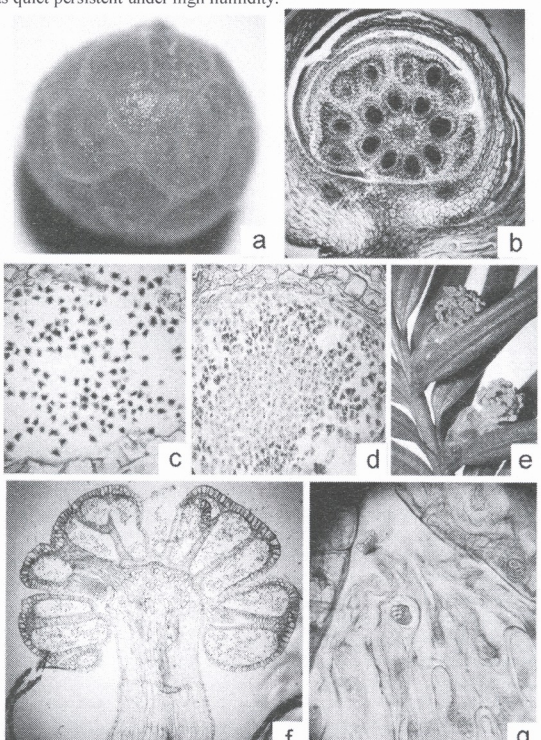


Fig. 1. - Globular microstrobilus X 37; 2 - microstobilus at predormant stage X70; 3 – mature microspores X 100; 4 – undifferentiated sporogenous tissue X 40; 5 – prepollinated microstrobili, 6 – longitudinal section of mature microstrobilus at pollination stage X 20; 7 – male gametophyte development inside the ovule X 230.

Soon after the pollen has been taken into the micropylar channel it changes the shape from irregular to spherical. Unicellular microspore divides forming the male gametophyte. First mitosis gives rise to a large tube cell and smaller antheridial cell. Pollen tube elongates toward the

archegonium (Fig.1-g). We observed collapse of the nucellar cells in close proximity of growing pollen tube. Before fertilization occurs microspore undergoes 3 mitosis forming 4 cellular male gametophyte. The last contains tube cell, sterile cell and 2 sperms [Fernando et al, 2005]. An interval between pollination and fertilization is over 1.5-2 month. We observed free nuclear proembryo in mid August.

As an integral part of the sexual reproduction the development of reproductive structures is of particular importance in rare *Taxus baccata* L. species. Developmental coherence between male and female gametophytes was observed at the all reproduction cycle, especially in predormant period. Thus we revealed synchronicity in the occurrence of the primordial structures. At this time male and female reproductive initials show similar histology. In both archesporium is differentiated and meiocytes formed before winter dormancy. A slight developmental delay was observed in ovules when pollination starts. Haploid microspores inside the nucellus were germinated but deeply in nucellar tissue occurred megaspore mother cell. Sporogenous tissue in the pollen sacs instead of mature haploid microspores was also observed in a small pool of microstrobili, while major pool was involved in pollination. Such ontogenetic retardation may have negative influence on the pollination success. As it was reported for *Taxus brevifolia* [DiFazio et al, 1998] positive relationship is established between pollen availability and pollination success.

Before being shed, microspores have an irregular counter that seems to be a normal condition for mature microspores caused by dehydration. In conifers dehydration level vary slightly and water content of prepollinated microspores ranges from 10 to 30% [Fernando et al, 2005]. For non-saccate pollen of *Taxus baccata* L. high level of dehydration may be important to achieve remarkable buoyancy at pollination. Captured microspores are round shaped when occur on nucellar surface. Obviously, the hydration starts on the micropyle and secrete of the pollination drop seems to involve in the pollen-ovule interactions from the very beginning. Observation showed that the timing of pollination drops was aimed to coincide with anthesis. However it depends on meteorological conditions such as moisture and temperature. The duration of the anthesis was slightly prolonged as compared with the effective period of ovule receptivity. Such overlapping was observed during all study period. In general, anthesis continued about 3 weeks. Data for England [Pennell, Bell, 1986 b] show that anthesis ranged from late February to early March. According to data recording for *Taxus brevifolia* [DiFazio et al, 1998] species pollinated in March - April. It seems, between factors limiting the anthesis temperature plays rather big role. The pollination phenology may be altered by environmental conditions, such as temperature, that may change the actual date of pollination as well as its length. However, the duration of pollination is quite predictable, if the timing of precursory phenological phase is known.

First mitosis of male gametophyte gives rise to the tube cell and generative cell. We observe bicellular male gametophyte 10 days after the pollination. This event occurs in vitro after 3-4 days of growing in culture [Pennel, Bell, 1986 b]. Moving toward the archegonium pollen tube is in close contact with the nucellar cells. We observed the degeneration of these cells. Pollen tube tip triggers the collapse of the nucellar cells filling the space left by this cells. As it was reported for lodgepole pine nucellar cells undergo programmed cell death initiated by pollen tubes from which the cell death signal diffuses into surrounding nucellus [Owens et al, 2005]. It seems that mechanism underlying the lysis of the nucellar cells in *Taxus baccata* L. is similar to described above.

Fertilization seems to occur from mid July to the first decade of August. Free nuclear proembryo was observed in mid August. Obviously postfertilization events have a quiet high developmental rate.

To conclude, it must be noted that the development of male reproductive structures in *Taxus baccata* L. can be divided on two groups of events; first – starts with the formation of the primordia and finishes by pollination. These events take place on the male tree and are in general

strictly synchronized with the ovule formation in the female tree. The second group of events occurs inside the ovule, are less prolonged, characterized with a slight developmental delay and are rather predictable in phenology of concrete phases. Apparently, cytologicaly revealed ontogenetic retardation can affect as pollination efficiency as an entire reproductive success.

References:

Anderson E., Owens J. *Microsporogenesis pollination, pollen germination and male gametophyte development in Taxus brevifolia.* Ann. Bot. **86**, 1033-1042, 2000.

Berlin G., Mikshe J. *Botanical microtechniqe and cytochemistry*. Iowa State Univ. Press, Ames, Iowa USA, 1976.

Chamberlain Ch. *Gymnosperms Structure and Evolution.* 1936.

Chira E. *Effect of heat on the course of meiosis in Taxus baccata pollen mother cells.* Biologia, Bratislava, **19**, 4, 235-244, 1964.

DiFazio S., Wilson M., Vance N. *Factors limiting seed production of Taxus brevifolia (Taxaceae) In western Oregon.* Am. J. Bot. **85**, 910-918, 1998.

Dolukhanov A. *Taxus baccata* L. In: Cacasus dendroflora. Tbilisi, 17-36, 1956.

Favre-Duchartre M. *Contribution a l'etude des spermatozoïdes de Taxus baccata.* Rev. Cyt. Biol. Veget. **21**, 4, 329-337, 1960

Fernando D., Lazzaro M., Owens J. *Growth and development of conifer pollen tubes.* Sex. Pl. Repr. **18**, 149-162, 2005.

Gelbart G., von Aderkas P. *Ovular secretion as part of pollination mechanism in Conifers.* Ann. For. Sci. **59**, 345-357, 2002.

Georgia Red Data Book p.82, 256, 1982.

Kirzo M., Korinekova M. *Microsporogenesis of the yew (Taxus baccata L.) under conditions of Central Slovakia.* Biol. (Bratis). **44,** 21-26, 1989.

Kuprianova L., Gumbatov Z. *Morphology of Taxus baccata pollen grains.* Bot. Zhur. **73**, 5, 661-665, 1988.

Owens J., Bennett J., L'Hirondelle S. *Pollination and cone morphology affect cone and seed production in lodgepole pine seed orchards.* Can. J. For. Res. **35**, 383-400, 2005.

Pennel R., Bell P. *Microsporogenesis in Taxus baccata L: the development of the archesporium.* Ann. Bot. **56,** 415-427, 1985.

Pennel R., Bell P. *Microsporogenesis in Taxus baccata L.: the formation of the tetrad and development of the microspores.* Ann. Bot. **57,** 545-555, 1986 a.

Pennel R., Bell P. *The development of the male gametophyte and spermatozoids in Taxus baccata L.* Proc. Roy. Soc. Lond. **228**, 86-96, 1986 b.

Pennel R., Bell P. *Insemination of the archegonium and fertilization in Taxus baccata L.* J. Cell. Sci. **89**, 551-559, 1988.

Shakarishvili N. *Reproductive biology of English Yew – Taxus baccata L. In Georgia: Ovule morphology.* Bull. Georg. Acad. Sc., **170,** 1, 130-133, 2004.

Shakarishvili N., Gachechiladze M. *Relationship between ovule development and seed Efficiency in English yew Taxus baccata L.* Bull.Georg. Acad. Sci. **171,** 3, 506-509, 2005.

Yong A., Boyle T., Brown T. *The population genetic consequences of habitat fragmentation for plants.* Trends in Ecol. Evol. **11**, 413-418, 1996.

უთხოვრის *Taxus baccata* L. მიკროსტრობილისა და მამრობითი გამეტოფიტის განვითარება საქართველოში

შაქარიშვილი ნ.

ნ. კეცხოველის ბოტანიკის ინსტიტუტი, მცენარეთა რეპროდუქციის კვლევის ლაბორატორია

(მიღებულია 27.03.2006)

რეზიუმე

შესწავლილია უთხოვრის *Taxus baccata* L. მამრობითი გენერაციული სფეროს განვითარება. დადგენილია ცალკეული ფენოლოგიური ფაზების ვადები და ხანგრძლივობა საქართველოს კლიმატური პირობებისთვის. გამოვლენილია მიკროსტრობილის ანომალური განვითარების ციტოლოგიური საფუძვლები. განხილულია განვითარების შეფერხების შესაძლო ზეგავლენა რეპროდუქციულ წარმადობაზე.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

PLANT INVASION IN SOUTH COLCHIS BOGS

DAVITADZE M., JAKELI E., DAVITADZE R.

Sh. Rustaveli Batumi State University

(Received March 13, 2006)

Abstract

44 adventive (feral) species were revealed in already degraded bogs of south Colchis. Those species are divided according to the time of their invasion and of inhabitation-establishment within vegetation.

Key words: relict, kenophyte, neophyte, euneophyte.

Introduction

One of the most important biome spread on the wide area of Colchis refugium of Tertiary period of western Georgia is bog. By habitats diversity it is still very favorable living environment for species spread in the past geological periods and remained up today. Worm Atlantic, as well as glacial relicts, occur and are revealed as dominants here.

Peculiarities of Colchis primary, oldest habitats cause establishment of unique, favorable living environment for biodiversity. Various animal species, migrant birds of wide range, trees, shrubs, lianas, and also herbage of various living forms inhabited here when on Colchis lowland bog went alongside the sea coast sandy vegetation. Flora and vegetation of that time of Kobuleti lowland bogs are described by Flerov [Flerov, 1936; 1951]. The features of distribution of some species are given in scientific literature [Dmitrieva, 1960; Gagnidze & Davitadze, 2000; Davitadze, 2001].

Materials and Methods

To study plant invasion into the contemporary vegetation of south Colchis bogs we investigated bogs of Kobuleti and Kakhaberi lowlands.

For floristic research known route method was used.

Results and Discussion

In the 20th century sharp antropogenic factors caused wide changes in vegetation of Colchis lowland. Natural habitat of many relict species decreased drastically and many of them even disappeared [The Red Book of Georgia, 1982; Dacitadze & Diasamidze, 2005]. In the beginning of 20th century bogs of Kobuleti surroundings occupy up to 900 hectare [Ketskhoveli, 1960]. *Pterocarya pterocarpa, Qurcus hartwissiana, Carpinus caucasica, Ruscus hypophyllum, Ilex colchica, Buxus colchica, Vaccinium arctostaphylos, Rhododendron ponticum, Rh. luteum, Alnus barbata, Smilax excelsa, Hedera colchica, Periploca grace, Hibiscus ponticus, Nuphar lutea,*

Nymphaea colchica, Trapa maleevii, T. colchica, Drosera rotundifolia, Osmunda regalis, Ericaarborea, Carex lasiocarpa, C. acutiformis, etc. existed along with bog mosses (*Sphagnum imbricatum, Sph. papillosum, Sph. acutifolium*). Today this unique biome, due to unsystematized interference, is degraded, the primary form of its biodiversity is destroyed, biotope is changed. For invasion-naturalization and colonization of both, aboriginal and foreign (with high potential of adaptation) species favorable conditions established on secondary habitats.

Invasion of exotic plants and their establishment in Colchis proceeded from ancient period. This process was insignificant earlier due to smallness of migrant species. At that time Colchis vegetation was comparatively virgin and for invasiants there was no place. Number of migrant plants was also small. In Colchis appearance of the first, so called "plant refugees of free will" is connected with introduction of southern subtropical cultural plants, such as: rice, millet, *Setaria viridis*, etc. In Ajara flora they are presented by 9 species [Davitadze, 2001]. The ancient invasiant-archeophytes of the flora of southern Colchis are the following: *Acorus calamus, Arthraxon hispidum, Cyperus difformis, C. esculentus, Hydrocotile ramiflora, Oplismenus undulatifolius, Schoenoplectus juncoides, Eleusine indica, Dichrocephala bicolor.* Today these species are distributed within modified secondary vegetation, in bogs and strongly humid biotopes. Invasiants inhabited on habitats of species oppressed and displaced by them, such as: *Juncus bufonius, J. articulatus, Mentha aquatica, Iris pseudacorus, Solidago turfosa, Osmunda regalis, Typha latifolia, Sparganium neglectum, S. erectum, Drosera rotundifolia, Menyanthes trifoliate, Alisma plantago-aqutica, Nasturtium officinale*, etc. Adventive, exotic plants, such as: *Kyllinga gracillima, Paspalum digitaria, Hydrocotyle ranunculoides, H. ramiflora, Polygonum thunbergii, P. alatum,* developed phytocenosis. Those changes of vegetation on bog and strongly humid lowlands greatly oppressed areal and made it rare to many species, such as: *Iris pseudacorus, I.lazica, Primula megaseifolia, P. sibthorpii, Staphylea colchica, S. pinnata, Berberis vulgaris, Cistus salvifolius, Osmanthus decorus,* etc.

There are 8 species of kenophytes (old ones, penetrated in 16-19th century) which were distributed-inhabited on bogs and strongly humid places of degraded lowlands of sea coast; they are: *Cardamine hirsute, Cyperus badius, Leonurus quinquelobatus, Ludwigia palustris, Polygonum minor, Ranunculus chius, R. muricatus, Veronica polita.*

There are 19 species of neophytes *(Hemarthia altissima, Paspalum paspaloides, Pycreus korschinsky, Polygonum posumbu, P. thunbergii, P. multiflorum, P. alatum, Ranunculus seleratus, Hydrocotyle ramiflora, H. vulgaris, H. ranunculoides, Hypericum mutilum, Kyllinga squamutala, Sagittaria platifilla, Cyperus glabra, Artemisia vulgaris, Onagra bienis, Bacharis halimifolia)*, new ones - penetrated during the end of 19th century up to 50s of 20th century, which distributed within the water-bog biotopes.

Euneophytes, the newest ones – penetrated from 50s of 20th century up today, which are distributed on boggy habitats - are presented by the following 8 species*: Pinus pinaster, Juncus teumus, Althaea officinalis, Bidens bipinnata, Ammania verticilata, Elodea Canadensis, Artemisia annua, Physalis peruviana.*

Among 44 adventive species established by invasion-natiralization in boggy and strong humid places the most abundant are neophytes (19 species). Their penetration and distribution within degraded vegetation of south Colchis (on boggy and strong humid lowlands) coincide with drainage of bogs and extirpation of Colchis liana forests during socialist agriculture.

Destruction of primary habitats narrowed the areal of relicts having conservative characteristics. Developed ecological "niche" was filled by plastic invasiants.

Thus, invasion-naturalization of exotic plants within natural ecosystems (bogs, forests, etc.) caused depletion of autochthonous species, loss of structure and originality of aboriginal flora and vegetation.

References:

Dmitrieva A.A. *Guide-book of Ajara plants*. Tbilisi, Publ. of Georgian Academy of Sciences, 1960 (in Russian).

Davitadze M., Diasamidze I. *Flora of Batumi coast*. Proceedings of Batumi State University, **V**, 79-87, 2005 (in Georgian).

Davitadze M. *Adventive flora of Ajara*. Batumi, Publ. of Batumi University, 2001 (in Georgian).

Flerov A.F. *Vegetation of Kobuleti bogs*. Soil Science, M.-L., **2**, 38-47, 1936 (in Russian).

Flerov A.F. *Vegetation of Colchis lowland*. Bulletin of the Institute of Tea and Subtropical Cultures, Makharadze, **1**, 54-86, 1951.

Gagnidze R., Davitadze M. *Native Flora*. Batumi, "Ajara", p.268, 2000 (in Georgian).

Ketskhoveli N. *Vegetation of Georgia*. Tbilisi, Publ. of Georgian Academy of Sciences, 1960 (in Georgian).

The Red Book of Georgia. Tbilisi, "Soviet Georgia", 1982 (in Georgia).

მცენარეთა ინვაზია სამხრეთ კოლხეთის ჭაობებში

დავითაძე მ., ჯაყელი ე., დავითაძე რ.

შ. რუსთაველის ბათუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 13.03.2006)

რეზიუმე

სამხრეთ კოლხეთის უკვე დეგრადირებულ ჭაობებზე გამოვლენილია 44 ადვენტური (გზადმოყოლილი და გაველურებული) სახეობა. ეს სახეობები დაყოფილია კოლხეთში შემოჭრისა და მცენარეულობაში განსახლება-დამკვიდრების დროის მიხედვით.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

ESTIMATION OF BROWN BEAR ABUNDANCE AND POPULATION STRUCTURE IN BORJOM-KHARAGAULI NATIONAL PARK WITH MOLECULAR-GENETIC METHODS

MURTSKHVALADZE M.[1], TARKHNISHVILI D.[2]

[1]*NACRES - Centre for Biodiversity Conservation and Research*
PO Box 20; 0179 Tbilisi, Georgia (CIS); Web: http://www.nacres.org
Tel: +99532 537125 E-mail: mari.murtskhvaladze@nacres.org
[2]*I. Chavachavadze Tbilisi State University.*
http://www.geocities.com/dtarkhnishvili/
Tel: (995 77) 794424 E-mail: t@gol.ge

(Received February 6, 2006)

Abstract

The study is based on the molecular genetic analysis of 32 faecal samples of brown bear (*Ursus arctos*), collected from the area covering 2,113 km^2 in Central Georgia. 60 faecal samples were collected from the Borjomi-Kharagauli National Park, and 42 from the western part of the Trialeti Range. Each DNA sample was screened for 2 Mitochondrial and 6 Nuclear Microsatellite loci, although only 32 successfully identified genotypes were used for the further analysis. We conclude that at least 28 bears were present in the study area from September to the end of November 2004. The analysis reveals that the bears were more or less evenly distributed throughout the area. Judging from the repeated records of faeces of the same bear at the same geographic location, some animals remained at the same location for 4-5 days. In two cases, faeces of supposedly the same bear were found within a month at the distance of approximately 20 km that indicates approximate movement range of an individual in Autumn. Several bears were commonly recorded at the same location or in the same gorge simultaneously.

The genetic analysis of samples collected from the both sides of rivers Mtkvari basin does not support the hypothesis that heavily urbanized zone along the river is an important barrier for bear dispersal. In all parts of the study area, the same alleles are found in similar proportion, Fst does not differ from zero significantly, and the studied population could be considered panmictic.

Key words: *Ursus arctos*, DNA, microsatellite markers, genetic diversity, Borjomi-Kharagauli National Park.

Introduction

Since early 1990-s, molecular genetic studies became an important component of the species conservation-oriented studies. Genetic studies help to identify the closest relatives of a target population and their geographic location; to measure intensity of gene flow between the populations; to find out how diverse is gene pool of a population and to recognize individuals using samples containing DNA.

Among the molecular genetic techniques, sequencing of mitochondrial and nuclear genes is most common applied [Moore, 1995], along with scoring genotypes by microsatellite markers [e.g. Hills et al., 1997]. Microsatellites are tandem repeats of short DNA segments, typically each repeat include 1-5 nucleotide base pairs [e.g. Hillis et al., 1977]. Such repeats are found in many regions across the genome of most living species. Microsatellites are assumed to be evolutionary neutral (adding or deleting AT repeats do not affect morphological or physiological characters), and microsatellite loci are commonly highly polymorphic and, consequently, highly informative from the population genetic point of view. They combine high variability, with nuclear co-dominant inheritance and they can be typed following non-invasive sampling. These advantages have made microsatellites a favorite tool in the various studies on the population level [Frankhal at al., 2000].

The main objective of our study was estimation of the brown bear population throughout a target area. Within the framework of the project **Ecosystems and Species Conservation in Georgia: Brown Bear, (Phase 1)**, NACRES team have collected 102 samples of bear faeces. For part of these samples, DNA was screened for 2 Mitochondrial and 6 Nuclear Loci.

Materials and Methods

NACRES team collected 102 faecal samples in the field from a limited area in central Georgia. Among 102 faecal samples, 60 were from the Borjomi-Kharagauli National Park (the eastern part of the Meskheti Range, 1,175km^2), and 42 from the western part of the Trialeti Range (938km^2) (Map 1). These two parts of the study area are adjacent but separated by river Mtkvari. The field study covered period from the beginning of September through the end of November 2004. Each faecal sample was picked up with a stick of wood and put in a 50 ml bottle. For each sample, a sampling date, a geographical location scored with GPS, and weather conditions were recorded. The samples were preserved in 95% alcohol until DNA extraction [Murphy et al., 2000].

We carry out the molecular genetic study at the laboratory of Conservation genetics, College of Natural Resources, Idaho State University, under supervision of Dr. Cort Anderson.

We extracted DNA from faecal samples in the laboratory equipped for the non-invasive and ancient DNA samples. We extracted a small amount of ground faeces (0.1–0.2 mL) using a QIAamp™ stool kit (Qiagen™) protocol, especially designed for this type of material. All extractions were carried out in a room dedicated only to processing hairs and faeces. The final volume of the DNA extract was 150µL.

Each group of DNA extractions and PCR's contained one to three negative controls (reagents only) to monitor for exogenous DNA contamination [Waits et al., 2000].

The amplification of DNA extracted from non-invasive samples is often problematic because the DNA is usually degraded or present in low quantity. This can lead to scoring errors such as allelic dropout or false alleles and often leads to incorrect genotypes scoring. These problems are now well understood, and different methods have been proposed to limit genotyping errors and their impacts on the subsequent analyses. We combined a two-step PCR approach or 'seminested PCR' with the newly defined 'multiplex preamplification method' to amplify DNA from brown bear (*Ursus arctos*) faecal samples, with the aim to increase the quality of the multilocus genotypes.

Each DNA extract was first used for amplification of a 146 bp mtDNA segment for species identification (Species ID using fragment analysis). PCR conditions were as follows: 20 µL reactions containing a final concentration of 0.9× Amplitaq buffer, 2.3 mm $MgCl_2$, 0.2 µ m each primer (SIDL 5'-/56-FAM/TCT ATT TAA ACT ATT CCC TGG-3' H16145 5'-GGG CAC GCC ATT AAT GCA CG-3'), 0.05 mM each dNTP, 1.5 µL template, and 1 U Amplitaq gold DNA polymerase. PCR was performed on Tetraid thermocycler with the following conditions: initial 10

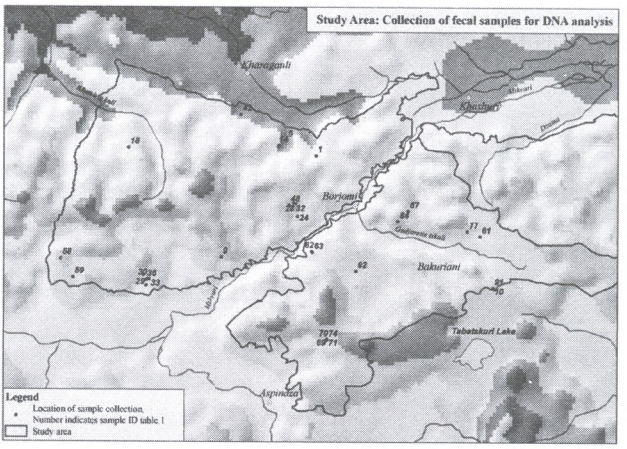
min at 95°C, then 44 replicates of the following cycle: 30 s at 95°C, 30 s at 48°C, 40 s at 72°C, and a 2 min extension at 72°C after cycles were complete [Murphy et al., 2000; Sambrook et al., 1989; Hillis et al., 1997]. Success rate for this reaction was 75.7% (69/110). For the successful 69 samples, we amplified six nuclear microsattelite loci: G10P; G10D; UarMu23; UarMu50; UarMu15; UarMu59 [Waits et al., 2000; Bellemain & Taberlet, 2004; Marta De Barba, unpublished Data].

PCR preamplifications for screening of microsatellite loci were prepared in 25 μL volume containing 5μL template DNA, 0.1 mM of each dNTPs, 0.01μm of each forward and reverse primers, 2 mm $MgCl_2$, 0.5 U Amplitaq Gold Polymerase (Applied Biosystems) and 1 × Taq buffer (containing 100 mm Tris-HCl, pH 8.3, 500 ml KCl, according to the manufacturer's specifications; Applied Biosystems). Amplifications were performed in a Tetraid PCR Thermocycler with the following conditions: 10 min at 95°C, 24 cycles for the, composed of 30 s denaturing at 95°C, 30 s annealing at 60°C, 1-min extension at 72°C, and as a final extension step, 7 min at 72°C. Aliquots from the preamplification were then used as templates in another round of PCRs. In this second step, amplifications were performed in a Tetraid PCR Thermocycler with the following conditions: 10 min at 95°C, and 44 cycles for the second amplification step, composed of 30 s denaturing at 95°C, 30 s annealing at 60°C, 1-min extension at 72°C, and as a final extension step, 7 min at 72°C.

PCR products were fluorescently labelled (TET for X and 6-FAM for Y) and 1.0 μL of undiluted product was added to a mix containing 0.25 μL GS350 Tamra standard (PE Applied Biosystems), 0.30 μL loading dye and 1.45 μL formamide. Loading mixture was denatured at 96°C for 2 min, loaded in a 6% long-ranger acrylamide gel on an ABI Prism 377 DNA sequencer (PE Applied Biosystems) and run at 2400 V for 3 Hours.

Gels were analysed with Geenscan 2.1 Genotyper 3.2.1, (Appliyed Biosystems) and Genepop 32 (http://www.ualberta.ca/~fyeh/fyeh).

To minimize the impact of stochastic pipeting error each amplification ware attempted at least two times.



Results and Discussion

PCR amplification success rates. DNA from faecal samples was amplified successfully in 35% PCR attempts. None of the negative controls (reagents only) for faecal DNA extraction or PCR amplification produced positive product, and all positive controls amplified the target locus. As expected, PCR amplification success was greater overall for mtDNA (75.7%: 69/110) than for nuclear DNA (35%: 38/110). However, for the individual screening we used only four most successfully amplified loci: G10D, G10P, MU23, and MU50. The list of 31 individuals, successfully screened for at least one of these loci (and consequently discussed in the further sections) is given in Table 1.

Table1. Successfully amplified samples' origin and screened microsatellite loci

Sample ID	Geographic coordinates		Number of basepairs in microsatelite loci			
	East	North	G10D**	G10P**	Mu23**	Mu50**
1	43°19'29''	41°54'36''	114-116	95-97	?	102-102
5	43°16'9''	41°55'49	114-116	95-97	?	96-98
9	43°9'18''	41°46'10''	104-108	?	153-159	98-104
10	43°39'6''	41°44'6''	104-108	95-97	153-159	98-104
14	43°15'20''	41°55'20''	102-114	97-101	157-159	106-106
25	43°0'49''	41°44'35''	102-104	97-101	157-159	106-106
18	42°58'49''	41°54'54''	?	?	157-157	102-104
24	43°17'33''	41°49'39''	102-102	?	153-159	100-102
28	43°17'19	41°50'40''	102-102	103-105	153-159	96-98
29	43°1'7''	41°43'42''	102-102	103-105	149-159	104-106
30	43°1'16''	41°44'15''	?	?	149-149	100-106
32	43°17'9''	41°50'42''	?	?	?	96-98
33	43°1'25''	41°44'13''	116-118	95-97	153-159	106-106
36	43°1'3''	41°44'9''	116-116	95-97	149-149	100-106
42	43°11'5''	41°57'48''	104-116	101-101	159-159	100-106
48	43°16'35''	41°50'32''	114-114	?	157-159	102-104
58	42°51'41''	41°45'42''	108-114	101-101	157-157	102-104
59	42°53'6''	41°44'13''	114-114	?	149-149	102-104
61	43°37'36''	41°48'16''	102-104	?	153-159	100-106
77	43°36'11''	41°48'39''	102-104	?	153-159	100-106
62	43°19'13''	41°46'46''	100-102	?	159-159	106-106
63	43°19'17''	41°46'42''	114-116	101-103	157-159	104-106
67	43°21'3''	41°39'40''	114-114	?	153-159	102-106
69	43°21'7''	41°39'41''	114-114	?	149-159	100-104
70	43°21'6''	41°39'40	114-114	?	149-159	100-104
71	43°21'5''	41°39'39''	104-108	95-97	?	102-106
74	43°21'4''	41°39'38''	114-114	101-101	?	104-106
84	43°28'34''	41°49'24''	102-102	?	149-153	102-106
87	43°29'40''	41°50'16''	102-102	103-105	149-153	104-106
91	43°39'6''	41°44'6''	114-116	101-103	153-155	96-98
92	43°24'5''	41°45'15''	114-116	101-103	153-155	100-106

? – Unknown number of nucleotides

G10D, G10P, Mu23, Mu50 amplified loci, numbers of nucleotides in allele.

High lined are samples from the same individual

Genetic variation of the target population

Summary of genetic variation statistics for all loci are shown in Table 2.

Table 2. Summary of genetic variation statistics for all loci [See Nei (1987) Molecular Evolutionary Genetics (p. 176-187)] of heterozygosity statistics for all loci

Locus	Sample size	na[1]	ne[2]	I[3]	Obs. homoz.	Obs. heteroz.	Exp. homoz.[4]	Exp. heteroz.[4]	* Nei[5]	Ave. heteroz.
G10D	44	7	4.03	1.5676	0.5	0.5	0.2304	0.7696	0.7521	0.7521
10P	18	8	6	1.9231	0.2222	0.7778	0.1176	0.8824	0.8333	0.8333
Mu23	38	7	4.98	1.7380	0.0526	0.9474	0.1792	0.8208	0.7992	0.7992
Mu50	46	9	6.83	2.0164	0.1304	0.8696	0.1275	0.8725	0.8535	0.8535
Mean		7.75	5.46	1.8113	0.2263	0.7737	0.1637	0.8363	0.8095	0.8095
Standard deviation		0.96	1.21	0.1995	0.1952	0.1952	0.052	0.052	0.0444	0.0444

[1] na = Observed number of alleles

[2] ne = Effective number of alleles [Kimura and Crow, 1964]

[3] I = Shannon's Information index [Lewontin, 1972][4] Expected homozygosity and heterozygosity were computed using Levene (1949)

[5] Nei's (1973) expected heterozygosity

Genotype diversity and the number of bears throughout the study area

The analysis of the screened loci in 31 study samples revealed 28 genotypes differing from each other with at least one locus. One should conclude that from beginning of September through the end of November at least 28 bears presented in the study area. Among them, 17 were in Borjomi-Kharagauli National Park (the eastern part of the Meskheti Range, 1,175km^2), and 11 from the western part of the Trialeti Range (938km^2) map 1. This figure however could not be interpreted as absolute numbers of bears throughout the area, because the data does not land themselves to the application of the statistical models for the calculation of more precise population size (for example based on mark-recapture model).

The available information is insufficient for establishing territorial and migrating bears were among those we identified, for studying the population structure, or for establishing the number of reproductive individuals.

Some preliminary conclusions could be drawn, however genotypisation of the samples collected in some parts of the study area could not be completed successfully (Map 1, Table 2). The analysis of the microsatellite genotypes shows that:

1. Two faecal samples from Tsagvei and Qvibistskali (ID 84; 87) probably belong to two different bears, although the age of the faeces differ in ca 20 days.
2. The samples from Tsinubani (ID 61, 77) belong to the same individual that did not move from the study location for at least 5 days.
3. The samples near the river Mtkvari (Chobiskhevi) (ID 62; 63) belong to two different bears, which simultaneously appeared at the same place.
4. The samples from mt Kvadjvari and Sabatkne (ID 10; 91) belong to two different individuals, they were not a mother and cub. They visited the same location 2-3 days one after another.

5. The samples from riv. Oshora (ID 69; 70; 71; 74) belong to three bears, one of that (samples 69, 70) remained at the same place for 5 days, and two others visited the site at the same time.
6. The two samples from Marelisi (ID 5; 14) belong to two bears, which were at the location simultaneously; one of them (ID 14) 25 days earlier visited the Tsinubnistkali gorge.
7. The four samples from Likani Valley (Borjomi Gorge; ID 24; 28; 32; 48) belong to 3 or 4 bears, two of that were in the location simultaneously. Two samples (ID 24; 28) are identical at two loci, G10D and Mu23; therefore, they also may belong to the same individual.
8. The 5 faecal samples from Bagebis Gele (ID 25; 29; 30; 33; 36) belong to five different bears. Two bears visited the location within the same day, and other three bears visited the area 1-8 days earlier or later. One of the bears (ID 25) 25 days later joined the Marelisi group.
9. Samples with ID 1; 9; 18; 42; 58; 59 belong to the six different individuals, separated from each other with a distance of 7-14 km of mountainous landscape.

Conclusions:

- During the observation period, brown bears were more or less evenly distributed throughout the study area. Samples collected from distant locations usually belonged to different bears.
- Individual bears remained at the same location up to 4-5 days. Within a month they sometimes moved to a location at least 20 km away.
- Several bears from different family groups are commonly found at the same location or in the same gorge at the same time.

Our genetic data allow us to develop some preliminary ideas on the population structure of bears in the study area. The studied loci are unlinked, and three out of four studied loci were in Hardy-Weinberg equilibrium (Table 3). One locus (G10D) showed significant deviation from Hardy-Weinberg equilibrium ($P< 0.04$), as a result of the excess of homozygotes 102-102 and 114-114. The observed excess may be the result of presence of a small group of closely related individuals in the sample rather than of any kind of selective mating (Table 3).

Table 3. Hardy-Weinberg disequilibrium – heterozygosity deficiency in each loci

Locus	Hardy-Wainberg disequilibrium			likelihood ratio test			common genotype	rare genotype
	χ^2	degree of freedom (df)	probability P	G^2	degree of freedom (df)	probabiliy P		
G10D	29.4	21	0.1	33.5	21	0.04	102-102 114-114	104-116
G10P	35.7	28	0.15	22.97	28	0.73	101-101	97-101
Mu23	28.22	21	0.13	29.96	21	0.09	153-159	157-157
Mu50	34.77	36	0.53	31.12	36	0.7	100-106	98-104

Comparative study of the samples collected from the left and right sides of the river Mtkvari, do not support the hypothesis that heavily urbanized zone along the river is an important barrier for bear dispersal. In all parts of the study area, the same alleles are found with similar proportion. Fst counted for three sub-populations (left side of Mtkvari, right side of Mtkvari/southern slopes of Meskheti Mountains), did not differ from zero significantly. Therefore, in spite of the deviation from Hardy-Weinberg equilibrium at a single locus, the population could be considered panmictic. The conclusions given here are preliminary ones. So far, we could estimate only the minimal population size in the study area. To obtain more exact figures and to

study family structure of the population, individual movements of bears and related questions, it is important to continue genetic study of the brown bear in Georgia.

Acknowledgments: Cort Anderson, Dave O'norato, Marta de Barba, Paulina Viteri, Liset Waits provided a valuable assistance during the work in the laboratory of Conservation Genetics, University of Idaho.

We appreciate Drs Djuro Huber and Jon Swenson trainings in sample collection and their useful advises and comments.

We wish to thank NACRES team: Levan Butkhuzi, Irakli Shavgulidze, Bejan Lortkipanidze, Natia Kopaliani, Mariam Shikhashvili, Giorgi Gorgadze, Our special thank to volunteers Vano Skhirtladze, Nato Avaliani, Giorgi Sulamanidze, Tamar Chunashvili, Tatia Chochua, Levan Mumladze, Giorgi Midelashvili, Tamuna Khardziani and Borjom-Kharagauli National Park administration for the assistance in the field work.

References:

Bellemain E., Taberlet P. *Improved noninvasive genotyping method: application to brown bear (Ursus arctos) faeces.* Molecular Ecology Notes, **4**, 519–522, 2004.

Brian F., Manly J. *The Statistics of Natural Selection.* p.272-282, 1985.

Frankham R., Ballou J., Briscoe D. *Introduction to Conservation Genetics.* Cambridge University Press. p.53-57, 2000.

Gooseens B., Chikhi L., Utami S.S., Ruiter J.D., Bruford M.W. *A multi-samples, multi extracts approach for microsatellite analysis of faecal samples in an arboreal ape.* Conservation genetics **1**, 157-162, 2000.

Levene H. In: *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*, I. Olkin et al., Stanford University Press, p. 278-292, 1960.

Lisette Waits, Pierre Taberlet, Jon E. Swenson, Finn Sandergen. *Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian Brown Bear (Usus Arctos).* Molecular Ecology, **19**, 421-431, 2000.

Masatoshi Nei. *Molecular Evolutionary Genetics.* p.176-187, 1987.

Melanie A. Murphy, Lisette Waits and Katherine C. Kendall. *The influence of diet on faecal DNA amplification and sex identification in brown bear (Ursus arctos).* Molecular Ecology **12**, 2261-2265, 2003.

Murphy M.A., Waits L.P., Kendall K.C. *Quantitative evaluation of faecal drying methods for brown bear DNA analysis.* Wildlife Society Bulletin, **28**, 951-957, 2000.

Sambrook J., Fritsch E.F. and Maniatis T. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Press, Cold Spring Harbor, New York, 1989.

Weir B.S. and Cockerham C.C. *Estimation of linkage disequilibrium in randomly mating populations.* Heredity, **42**, 105-111, 1979.

მურა დათვის (*Ursus arctos*) გენეტიკური კვლევა ბორჯომ-ხარაგაულის ეროვნულ პარკსა და მის მიმდებარე ტერიტორიაზე

მურცხვალაძე მ.[1], თარხნიშვილი დ.[2]

[1]*სახეობათა კონსერვაციის ცენტრი (NACRES)*
[2] *ი. ჭავჭავაძის თბილისის სახელმწიფო უნივერსიტეტი*

(მიღებულია 06.02.2006)

რეზიუმე

შესწავლილია მურა დათვის 31 ინდივიდის მიკროსატელიტური გენოტიპი ექსკრემენტის სინჯებიდან გამოყოფილი დნმ-ის საფუძველზე. ექსკრემენტები მოგროვებული იყო 2004 წლის სექტემბერ-ნოემბერში ბორჯომ-ხარაგაულის ეროვნული პარკიდან და დაბა ბაკურიანის მიდამოებიდან. ექსკრემენტის სინჯებში 4 G10P; G10D; UarMu23; UarMu50 მიკროსატელიტური ლოკუსის შესწავლამ გამოავლინა 28 გენოტიპი, რომლებიც ერთმანეთისაგან ერთი ლოკუსით მაინც განსხვავებოდა. შეიძლება ვივარაუდოთ რომ კვლევის პერიოდში საკვლევ ტერიტორიაზე იკვებებოდა მინიმუმ 28 (17 ბორჯომ-ხარაგაულის ეროვნულ პარკში და 11 ორიალეთის ქედის დასავლეთ ნაწილში) მურა დათვი. დადგენილია, რომ მურა დათვები კვლევის პერიოდში შემთხვევითად იყვნენ განაწილებულნი მთელ შესწავლილ ტერიტორიაზე. დაშორებულ ადგილებში შეგროვებული ექსკრემენტები, როგორც წესი, განსხვავებულ ინდივიდებს ეკუთვნოდა. ცალკული ინდივიდები ერთსა და იმავე ადგილას ზოგჯერ 4-5 დღე რჩებოდნენ, ამავე დროს ერთი თვის განმავლობაში ზოგიერთი დათვი გადაინაცვლებდა ახალ ადგილას, რომელიც 20 კმ მთის ლანდშაფთით იყო დაშორებული. ერთ ხეობაში ხშირად ერთდროულად რამოდენიმე დათვი იკვებება, ეს ცხოველები უმრავლეს შემთხვევაში არ არიან ერთი ოჯახიდან. მტკვრის მარცხენა და მარჯვენა მხარეზე შესწავლილი გენოტიპების შედარება არ ადასტურებს მტკვრის გასწვრივ მდებარე ურბანული ხოლის როლს მიგრაციის შეხღუდვაში, ორივე ნაპირზე ერთიდაიგივე ალელები მსგავსი სიხშირით გვხვდება, ხოლო პოპულაციის მსგავსების მაჩვენებელი სტატისტიკა (Fst) ნულისგან სარწმუნოდ არ განსხვავდება. პოპულაცია სავარაუდოდ პანმიქტურია.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

BIOINDICATION OF SEMIDESERT, STEPPE AND LIGHT FOREST ORIBATID MITE (ACARI, ORIBATIDA) COMMUNITIES VIA ISOVALENT SPECIES GROUPS

MURVANIDZE M., KVAVADZE E.

Institute of Zoology

(Received February 28, 2006)

Abstract

The oribatid mites of semideserts, steppes and light forests were studied in Eastern Georgia. 56 species of oribatid mites were registered on the studied territory. Two of them – *Jacotella ornata* and *Austrocarabodes foliaceisetus* are new for fauna of Caucasus. For ecological classification the method of "isovalent species groups" was used. This technique allows evaluating the fauna in regard to environmental factors. Basing on this experience and on cluster analysis (excluding species with no indicator qualities) the characteristic species for semidesert, steppe and light forest biocenoses were established.

Key words. Georgia, isovalent groups, *Oribatida*, bioindication.

Introduction

Isovalent species groups include the species with similar ecological valency. Usage of these groups for bioindication is more comfortable than the same with single indicator species. Indication by means of single species requires presence of this species in all studied sites. Isovalent species group includes wide spectrum of species that can be used in indication of environmental conditions.

Researches on isovalent species groups were provided only in Europe [Knölle 1957; Weigmann, 1973, 1991, 1997 a, b]. Our work is a first attempt to provide such kind of researches in Caucasus area.

The goal of our researches is to establish the isovalent species groups for semideserts, steppes and light forests of Eastern Georgia.

The semideserts in Eastern Georgia are distributed on altitudes of 150-300 m a.s.l. In vegetation predominate *Anabasis aphylla, Salsola errcoides, S. dendroides, S. glaura. Gamanthus pilosus, Aellania glauca* and *Kalidium easpicom.* The ediphicators of this vegetation are floristically connected with Turan-Anterior Asia and Eastern Mediterranean floristic centers [Sakhokia, 1958].

The steppe vegetation is largely distributed on the formation of foothill landscapes (300-700 m a.s.l.). Current expansion of steppes is due to the anthropogenic influence on forest, arid light forest and even on secondary shrubwoods [Sakhokia, 1958]. The dominant species of steppe vegetation – *Bothriochloa* is widely distributed in both hemispheres [Nakhutsrishvili, 1999].

Because of dry climate of Eastern Georgia, the arid open woodlands are found among the steppe and desert vegetation. The xerophytic woody plants with drought-resistant grass cover make

these communities. The following types of communities of open woodland can be distinguished: pistachio-woodland and juniper open woodland with predominance of *Pyrus* and *Celtis* species [Nakhutsrishvili, 1999].

Material and Methods

Our investigations were provided in November 2005. From each plot three soil samples (10 cm^3) were taken and animals were extracted by a Tullgren–apparatus. For the determination of the oribatid mites mainly Ghilarov & Krivolutski [1975] and other special papers reported by Balogh and Mahunka [1983] and Weigmann and Kratz [1981] were used.

As coefficient of faunal likeness between different plots, indicating species identity, Jaccard's coefficient was calculated.

The calculation of community likeness was based on Renkonen's coefficient, which expresses the dominance identity of the site's fauna derived by summing up the lesser of the dominance values of each species occurring in the two sites compared [Krebs, 1989].

In this investigation only the adult mites were identified and counted.

The method of isovalent species groups (IVG) was elaborated by Knülle [1957] and evaluated by Weigmann [1991, 1997a, b]. The IVGs are created in each concrete study to establish reactions of oribatid mites along the ecological transect. The first step is creating of possible IVGs. Then, according to the ecological data, the species are assigned to these IVGs.

In our case we use the method to establish the indicator groups of species for semideserts, steppes and light forests. We created three IVGs. The wide distributed, eurytopic, ubiquitous species have no indicating value and are assembled in IVG 0.

The ecological characteristic of the sites is as follows:

IVG I. Semideserts.

Sd1. riv. Pantishara ravine at the boarder to Azerbaijan. H = 262m.

Sd 2. Kajiri Mountain. Gorge with badland. Southern exposition. Foothill semidesert.

Sd 3. Kajiri Mountain. Gorge with badland. Northern exposition.

Sd 4. Jikuberi Lake. Valley.

Sd 5. Jikuberi Lake bottom. Salty soil.

Sd 8. Tetritskaro district. *Salsola ericoides*. H = 510m.

IVG II. Steppes.

St 6. David Gareji. Astragaluses, hazelnut, almond.

St 7. David Gareji. Gorge. Xerophyl shrub and steppe complex.

St 9. Tetritskaro district. *Stipa - Andropogon ischaemum - Paliurus spina christi* steppe.

St 10. Tetritskaro district. *Andropogon ischaemum – Artemisia phyllostachys* steppe.

IVG III. Light forests.

Lf 11. Tetritskaro district. *Ligustrum vulgare* grows.

Lf 12. Tetritskaro district. *Lactuca serriola*. H = 510m.

Lf 13. Vashlovani Reserve. Juniperus open woodland. H = 462m

Lf 14. Vashlovani Reserve. Pistachio woodland. H = 462m.

Results and Discussion

56 species of oribatid mites were registered on the studied territory. Two of them – *Jacotella ornata* and *Austrocarabodes foliaceisetus* are new for fauna of Caucasus. The number of species was highest in *Stipa-Andropogon-Paliurus* steppe (20 species), number was high also in *Ligustrum vulgare* growth (16 species), *Salsola ericoides* association and xerophyt shrubs in David

Gareji (14-14 species). As the poorest site appeared the bottom of Jikuberi Lake where only one species was found (Tab. 1).

Table 1. The list of oribatid mites of semideserts, steppes and light forests and their dominance values (%) (+: Dom. < 1%.)

#	species	Sd 1	Sd 2	Sd 3	Sd 4	Sd 5	Sd 8	St 6	St 7	St 9	St10	Lfl1	Lfl2	Lfl3	Lf 14
	IVG I. semideserts														
1	*Camisia biverrucata*						2								
2	*Neoliodes theleproctus*	3					3								
3	*Liacarus brevilamellatus*	5		8						1		4			
4	*Xenillus ibericus*						1								
5	*Austrocarabodes foliaceisetus*	+		8											
6	*Scutovertex minutus*		10												
7	*Trichoribates caucasicus*	3													
8	*T. trimaculatus*			8											
9	*Scheloribates distinctus*		10												
10	*Lucoppia burovsi*			33			7			3			4		
11	*Zygoribatula exarata*		30												
	IVG II. steppes														
12	*Liochthonius lapponicus*								6						
13	*Licnobelba alestenensis*									7		4		4	
14	*Scutovertex serratus*									2					
15	*Eupelops occultus*								1						
16	*Latilamellobates nalischiki*							14							
17	*Peloptulus phaenotus*						6				14		4		
18	*Oribatula pallida*								1	1					
19	*Zygoribatula cognata*				33					6	14		1	13	
	IVG III. light forests														
20	*Phthiracarus lanatus*													13	
21	*Sphaerochthonius splendidus*											2			
22	*Aleurodamaeus setosus*	16		8			1		7	1				4	17
23	*Jacotella ornata*													9	
24	*Amerus troisii*													9	
25	*Xenillus tegeocranus*						+								
26	*Oppiella unicarnata*														7
27	*Ramusella clavipectinata*								1	8		2		13	
28	*Eupelops acromios*											2			

29	*Pilogalumna crassiclava*	16							4				4		
30	*Liebstadia pannonica*	1								44	14		3		
31	*Dometorina plantivaga*														3
IVG 0.															
32	*Steganacarus carinatus*											2			
33	*Rhyzotritia ardua*							5				4			
34	*Trhipochthonius tectorum*	3					+								
35	*Camisia horrida*	16							1					13	
36	*Hermanniella granulata*						+								
37	*Hermanniella punctulata*								1						
38	*Metabelba pulverulenta*											5			
39	*Damaeolus ornatissimus*								1						
40	*Doricranosus splendens*			8			2				1	11			
41	*Ceratoppia bipilis*	5		8					3						17
42	*C. quadridentata*										2		2		
43	*Tectocepheus sarekensis*	3						5		1		5			
44	*T. velatus velatus*						2		17	3	22		28	13	
45	*Oppiella fallax*			8					1					4	
46	*Oppiella hygrophila*			33						1					3
47	*Oppiella subpectinata*							5				2			
48	*Quadroppia michaeli*				33										
49	*Suctobelbella subcornigera*			8								2			
50	*Parachipteria nicoleti*									4					
51	*Minunthozetes pseudofusiger*						19			1	7		38		
52	*Punctoribates punctum*		10						1						
53	*Liebstadia longior*							5				2			
54	*Scheloribates laevigatus*	8					1	64		6	14	4	1	9	53
55	*Sch. latipes*	16	30						1	1			1	4	
56	*Oribatula tibialis*		10			100			3			50			
total		13	6	9	2	1	14	7	14	20	7	16	10	13	6

In IVG I (sites Sd 1, Sd 2, Sd 3, Sd 4, Sd 5, Sd 8) are united semidesert species (11 species). A high dominance value shows *Lucoppia burowsi* (33%), but with lower indexes it is found in steppes and light forests as well. A high dominance shows *Zygoribatula exarata* too (30%). Other species are characterized by low dominance indexes.

IVG II (sites St 6, St 7, St 9, St 10) unites the steppe species (8 species), where coefficients of dominance are lower. By 14 % of dominance were distinguished *Peloptulus phaenotus, Latilamellobates naltschiki* and *Zygoribatula cognata.*

IVG III (sites Lf 11, Lf 12, Lf 13, Lf 14) unites the light forest species (11 species). Coefficients of dominance are as low as in steppe biocenoses. *Phthiracarus (A) laudatus* predominates with 13% and *Jacotella ornata* and *Amerus troisii* – with 9-9%.

The great part of fauna is presented by eurytopic species – 25 species. They are united in IVG 0. Dominance indexes here are much more high (Tab.1).

According to these data the cluster of dominance identities was build. The results show no evident correlation between the sampling sites (Fig. 1). In the second cluster (Fig. 2) the species of IVG 0 were omitted because they have no discriminating value [Weigmann 1991, 1997b]. Without species of IVG 0 the dominance identities are much lower, but the received diagram shows that groups of semidesert, steppe and light forest species are separated from each other. In the first group the semidesert species (Sd 1, Sd 3) grouped together with steppe oribatids (St 7). Grouping of mites of Lf 14 seems unusual here, but this likeness is provided by *Aleurodamaeus setosus*, which is the forest element but in high dominance (16%) was presented in semidesert too. The second group is composed by steppe and light forest species (St 9, St 10, Lf 13) and the third group was made by dominant species of light forests (Lf 12, Lf 11) (Fig. 2). Presence of semidesert species in the second and the third group is provided by entrance of light forest elements in steppe and semidesert vegetation [Nakhutsrishvili, 1999].

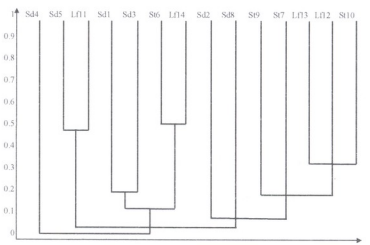


Fig. 1. Cluster of dominance identities of oribatid mites of semideserts, steppes and light forests

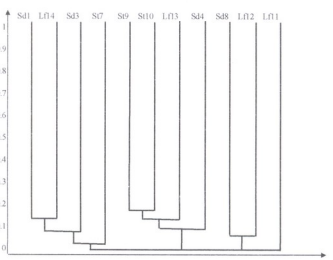


Fig. 2. Cluster of dominance identities excluding the species of IVG 0

The analysis of oribatid mites of semiderets, steppes and light forest shows that in fauna predominate the wide distributed, eurytopic species that show high dominance compared to the

indicator species. The identified oribatid mites were divided in four isovalent groups. Species, which are distinguished by high dominance, are indicators for these groups.

The presented method is very comfortable for indicating the biotope qualities and may be used within the studies of environment pollution, nature protective and succession processes.

References:

Balogh J., Mahunka S. *Primitive Oribatis of Palaearctic Region.* Akademiai Kiado, Budapest, 372, 1983.

Gilarhov M.S., Krivolutski D.A. (ed.) *Opredelitel obitaiushchikh v pochve kleshchei. Sarcoptiformes.* (*The Identification Keys of Soil Inhabiting Mites. Sarcoptiformes*) Moscow, "Nauka", 375, 1975. (in Russian).

Knülle W. *Die Verteilung der Acari: Oribatei im Boden.* Z. Morph. skol. Tiere **46**, 397-432, 1957. (in German)

Krebs Ch.J. *Ecological Methodology.* New York, Harper & Row Publishers, 654, 1989.

Nakhutsrishvili G. *The vegetation of Georgia (Caucasus).* Braun-Blanquetia 15, Camerino, 74, 1999.

Sakhokia M.F. (ed) *Botanikuri exkursiebi saqartveloshi (Botanical excursions over Georgia).* Izd. AN Gruzinskoi SSR. Tbilisi, 60, 1958.

Weigmann G. *Oribatid Communities in Transects from Bogs to Forests in Berlin Indicating the Biotope qualities.* Modern Acarology. **1,** 259-364, 1991.

Weigmann G. *Bioindication by Means of Isovalent Species Groups.* Abh. Ber. Naturkundemus. Gerlitz **69, 2**, 59-65,1997a.

Weigmann G. *Die Hornmilben-Fauna (Acari, Oribatida) in Auenboden des Unteren Odertals.* Faun. skol. Mitt. **7,** 319-333, 1997 b (in German).

ნახევრადწდაბნოს, სტეპისა და ნათელი ტყის ინდიკატორი ორიბატიდული თანასაზოგადოებების გამოვლენა იზოვალენტური ჯგუფების მეშვეობით

მურვანიძე მ., ყვავაძე ე.

ზოოლოგიის ინსტიტუტი

(მიღებულია 28.02.2006)

რეზიუმე

აღმოსავლეთ საქართველოში შესწავლილი იქნა ნახევრადწდაბნოს, სტეპისა და ნათელი ტყის ორიბატიდები. გამოკვლეულ ტერიტორიაზე რეგისტრირებული იქნა ჯავშნიანი ტკიპების 56 სახეობა. მათგან ორი – *Jacotella ornata* და *Austrocarabodes foliaceisetus* პირველად აღინიშნება კავკასიის ფაუნისათვის. ეკოლოგიური კლასიფიკაციისათვის გამოყენებული იქნა "იზოვალენტური სახეობების ჯგუფები". ეს ტექნიკა საშუალებას იძლევა დავახარისხოთ ფაუნა გარემოს ფაქტორების მიხედვით. ამ მეთოდისა და კლასტერული ანალიზის საშუალებით (ინდიკატორული ღირებულების არმქონე სახეობების გამორიცხვით) გამოვლინდა ნახევრადწდაბნოს, სტეპისა და ნათელი ტყეების ბიოცენოზებისათვის დამახასიათებელი სახეობები.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

DISTRIBUTION, TODAY'S STATE AND CONSERVATION PROBLEMS OF SEA-BUCKTHORN (*HIPPOPHAË RHAMNOIDES* L.) IN GEORGIA

TODUA V.[1], CHKHETIANI I.[2], NATROSHVILI I.[3]

[1] *N. Ketskhoveli Institute of Botany*
[2]*Sokhumi Branch of Iv. Javakhishvili Tbilisi State University*
[3]*Institute of Soil Sciences*

(Received May 16, 2006)

Abstract

Distribution area and ecological state of sea-buckthorn were established. During 1960-2003 occupancy of sea-buckthorn shrubs decreased from 30 000 to 2 900 hectare caused by intensive gathering of its fruits for medicinal and nutritious purposes and also by reduction of forest-meadows. Methods of sea-buckthorn reproduction in its distribution area and vegetative propagation via grafting were studied and examined in field conditions. Recommendations for total regeneration and normal development of sea-buckthorn were worked out.

Key words: zoning, grafting, wild form, cultivated form, raw material supply

Introduction

At present problems of registration, utilization and regulation of sea-buckthorn shrub-land resources are uncontrolled in Georgia. Sea-buckthorn is not yet considered as natural resource, economical mechanisms of its utilization, issues of renovation and reproduction in its distribution area are not worked out.

The goal of our work was to study today's state, distribution area, reasons of degeneration and possibilities of conservation of sea-buckthorn. Methods of receiving of sowing material via grafting and its usage in horticultural farms were also examined.

Materials and Methods

During field expeditions distribution area, extent and interspecies variations of sea-buckthorn in Georgia were established [Todua, 2000; 2004; 2005a; 2005b]. Morphological description of wild forms, quantitative registration, measuring, comparison of obtained data were carried out according to the known methods [Kondrashov, 1977].

Vegetative propagation was conducted by grafting. As a mother plant previously chosen promising forms were used. For better grafting conditions we used green shoots. Grafting was performed in April, July and August. meadow-brown soils of the river Mtkvari bank of 0.35-0.45 cm horizon on Eastern Georgia and subtropical red soils of the same horizon of Western Georgia were used as substrates. Coarse send and ground was taken from the both above mentioned places

and mixed in proportion 2 portion of soil/1 portion of sand. Nitrogen-fixation process occurring in mycorrhizal nodules of sea-buckthorn roots in the presence of actinomycetes influences positively rootage of cuttings and its development.

Soil mixed with sand was put in polyethylene bags of 20 cm height and of 15 cm width. Cuttings were placed inclined in polyethylene bags, in a way that part of cuttings with 3 leaves was under ground. Due to "activity" of leaves green cuttings produce roots. During 2-3 weeks before rootage and appearance of new shoots on cuttings turgor must be retained, which mainly is realized by frequent watering. At summer grafting in such conditions at the end of vegetative period, several spread roots are produced, and from the leaf blade sprouts underground shoots of 10-15 cm were grown. Rootaged cuttings were hibernated in closed sheds, but they were grown in open ground.

The plots for sea-buckthorn growing were chosen. Before sowing holes of the sizes of 30 x 35 cm were dug out. Polyethylene bags were removed from saplings and the plant with the soil clod was put in the holes, in a way that upper part of the clod should be 3-4 cm lower from the ground surface. Clods were covered with loose soil and trample down. Saplings were transplanted in autumn (October and November) and spring (March and April).

Results and Discussion

Wild sea-buckthorn is distributed almost everywhere in Georgia from sea level up to 2000 m a.s.l. It is characterized by high viability and has stronger adaptation potential to environmental conditions than other medicinal plants of Georgian flora.

According to the obtained data distribution area of sea-buckthorn (Fig. 1), supply of row material and outlooks of its reproduction (Table 1) were established.

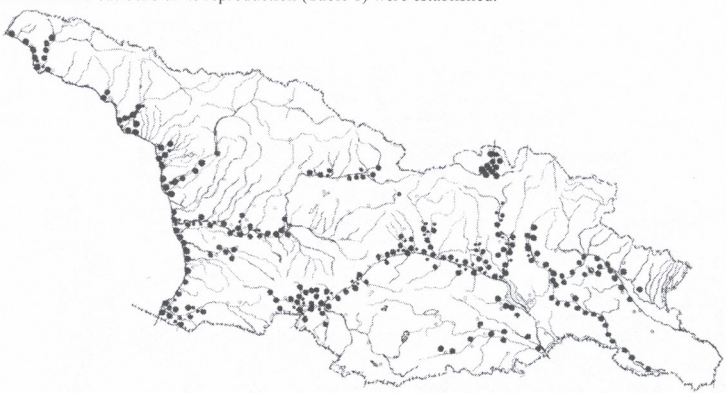


Fig. 1. Sea-buckthorn distribution area in Georgia.

Uncontrolled gathering of sea-buckthorn fruits destroyed mating correlation between plants: number of male plants increased and number of mother plants (fructiferous plants) decreased.

In 1960 sea-buckthorn occupied 30 000 hectare [Kikava et al., 1999] in Georgia and according to our data, in 2003 its occurrence composed 2910 he. So, we consider to introduce and

keep rule of pattern gathering with conservation of plants in the patterns, which means gathering of fruits but not cutting the branches, without injuring young sowings and shoots. Next year fruit gathering should be carried out from another pattern, i.e. the plant from which fruit was gathered in previous year should rest and branch off. Due to such order of gathering plant develops new fruiter shoots and the harvest increases. This method favours spreading of patterns into the shrubbery, reproduction of natural shrub tracts within the limits of given population and gathering of more fruits.

Table 1. Natural distribution of sea-buckthorn and outlooks of its reproduction in Georgia

Distribution area	Occupied with sea-buckthorn area in 2003		Planed enlargement of sea-buckthorn plots (hectare)		Planning of production of sea-buckthorn fruit 10 years after (tone)
	Zones (hectare)	% of the total area	Natural conditions	Cultivated	
1. Apkhazeti	225, II	7,7	10	2	1,8
2. Samegrelo	75	2,5	5	-	0,7
3. Guria	10	0,3	-	-	-
4. Ajara	350, III	12,1	10	5	2,2
5. Imereti	80	2,7	-	-	-
6. Racha-Lechkhumi	60	2,1	-	-	-
7. Kazbegi	860, I	29,6	20	10	4,5
8. Borjomi	15, IV	0,5	3	1	0,6
9. Khashuri	150, IV	5,2	10	2	1,8
10. Kareli	23, IV	0,8	5	-	0,7
11. Samachablo	17, IV	0,6	5	-	0,7
12. Gori	20, IV	0,7	-	-	-
13. Kaspi	30, IV	1,0	10	3	1,9
14. Mtskheta	40, IV	1,4	10	3	1,9
15. Dusheti	20, IV	0,7	3	-	-
16. Tianeti	100, V	3,4	10	-	1,5
17. Surroundings of Tbilisi	33	1,1	-	-	-
18. Gorge of the river Iori	750, V	25,8	20	5	3,7
19. Telavi	37, V	1,3	5	3	1,2
20. Dmanisi	12	0,4	-	2	0,3
21. Bolnisi	3	0,1	-	41	24,2
Total	2910x2kg=582t.	100	126	41	24,2

Note: amount of fructiferous plants per 1he composed 1000 shrubs, and productivity – 2 kg fruit per 1 shrub.

As is seen from table 1, sea-buckthorn shrubbery in Georgia is still abundant to realize its reproduction up to 30 000 he which should be approached by: 1) carrying out reproduction and renovation of existed natural sea-buckthorn shrubberies and 2) development of special horticultural farms.

High productive forms selected from natural habitats of sea-buckthorn should be used for development of special horticultural farms. We received such forms of sea-buckthorn and they are grown on the experimental plot of Institute of Botany. According to our data the best result was received using vegetative reproduction of cuttings from mother plant. This method for sea-

buckthorn is used for the first time in Georgia and obtained saplings satisfy standard demands. The following data are in favour of these facts: while using coarse sand (2 portions of soil per 1 portion of sand) composing actinomycetes high percentage of rootage of cuttings (96%) was obtained in spring; this parameter is lower (63%) when using ordinary soil as substrate. Those parameters for saplings transplanted in natural habitats in autumn and spring were 99% and 92% correspondingly.

Zoning

1st zone of sea-buckthorn distribution is shrubberies of the rivers Dariali and Sno (especially villages Achkhoti, Sno, Tsikhisdziri), area of interflow of the rivers Tergi and Sno, area of central park in Kazbegi region.

Sea-buckthorn is better preserved in the gorge environment (Kazbegi region), but young saplings and shoots are partially extirpated due to usage of this area as pastures and grasslands.

2nd zone is the part of territory of Abkhazeti – meadows near the lower parts of the rivers Bzipi, Kodori, Okumi, and also sea coastal line.

3rd zone is Ajara – shores of the rivers Chorokhi and Ajaristskali, sea coastal line near village Akhalsopeli.

4th zone - begins in Akhaltsikhe gorge and comes along shore of the river Mtkvari near villages Aspindza, Adigeni, Akhaltsikhe, then along mountains to north-west up to the towns Borjomi, Khashuri, Gori and Mtskheta, then goes to the north in the gorges of the rivers Aragi and Ksani. Everywhere in this zone sea-buckthorn is under the impact of antropo-zoogenic factors.

5th zone is the shore line of the river Iori from Tianeti to Sagarejo region, shore line of the river Kisikhevi up to highway. Sapling material for plantations located on Dmanisi plateau was taken just from the shores of the river Iori.

Recommendation:

1. For regeneration and normal development of sea-buckthorn usage of its distribution area as pastures and grasslands should be stopped. Plots of mother plants could be enclosed for 5 years. During this period selecting and reserving of high productive plants, weeding out not perspective plants should be finished. Distances between male and female plants should not exceed 10-15 m.

2. To keep the rule of pattern gathering with conservation of plants in the patterns means gathering fruits without injuring young sowings and shoots but not cutting the branches. Next year fruit gathering should be carried out from another pattern, i.e. the plant from which fruit was gathered in previous year should rest and branch off.

3. Within the limits of sea-buckthorn populations reproductive and rehabilitation measures should be carried out (maximal reservation of plants in the patterns; weeding out of the diseased, dried up specimens; to keep fertilizing plants (1-2) in the patterns).

4. Horticultural special sapling farms should be organized within the limits of sea-buckthorn distribution area, where vegetative propagation via grafting of recommended forms of high productivity should be carried out.

References:

Kikava G., Chkhetiani I., Todua V. *Wild Fruits*. Tbilisi, p.196, 1999.

Kondrashov V.T. *Methods of description of wild forms of sea-buckthorn.* Plant Resources, *13*, I, 354-361, 1977.

Todua V. *Sea-buckthorn in Abkhazeti and Samegrelo.* Proceedings of Georgian Academy of Sciences, Biol.Ser., **26**, 1-3, 93-104, 2000.

Todua V. *Data of sea-buckthorn distribution in Georgia.* Proceedings of the Institute of Botany, **94**, 91-95, 2004.

Todua V., Mchedlishvili M. *Sea-buckthorn (Hippophaë rhamnoides L.) in the surroundings of the river Kisiskhevi.* Proceedings of the Institute of Botany, **95**, 156-158, 2005.

Todua V. *Morphological peculiarities of the root system of sea-buckthorn, (Hippophaë rhamnoides L.) and ultrastructure of mycorhizal nodules.* Proc. Georg. Acad. Sci. Biol. Ser. B, **3**, 3, 23-29, 2005.

ქაცვის ***Hippophaë rhamnoides*** **L.** გავრცელება, დღევანდელი მდგომარეობა და კონსერვაციის პრობლემები საქართველოში

თოდუა ვ.[1], ჩხეტიანი ი.[2], ნატროშვილი ი.[3]

[1]ნ. კეცხოველის ბოტანიკის ინსტიტუტი,
[2]ივ. ჯავახიშვილის თბილისის სახელმწიფო უნივერსიტეტის სოხუმის ფილიალი,
[3]ნიადაგმცოდნეობის ინსტიტუტი

(მიღებულია 16.05.2006)

რეზიუმე

დადგენილია ქაცვის გავრცელების არეალი და ეკოლოგიური მდგომარეობა. 1960 წლის მონაცემებით ქაცვნარებს დაკავებული ჰქონდა 30 000 ჰა ფართობი. 2002 წლისათვის მისმა ფართობმა შეადგინა 2 900 ჰა. ქაცვნარებით დაკავებული ტერიტორიების შემცირება ძირითადად გამოწვეულია ქაცვის სამკურნალო და სხვადასხვა სამომხმარებლო მიზნით მისი ნაყოფების ინტენსიური კრეფის და აგრეთვე ჭალის ტყის შემცირების გამო. შესწავლილია და საველე პირობებში გამოცდილია ქაცვის გავრცელების არეალშივე მისი აღდგენისა და დაკალმების წესით გამრავლების მეთოდიკა. ქაცვის სრული რეგენერაციისა და ნორმალური განვითარებისათვის შემუშავებულია სათანადო რეკომენდაციები.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

SOIL POPULATION OF NEMATODES OF AJARA

ZHGENTI L.

Sh. Rustaveli Batumi State Universiti

(Received June 15, 2006)

Abstract

Soil nematodes from subalpine and mountain-forest belts of Ajara were studied. 138 forms of nematodes have been recorded on studied plot, among them species *Trachactinolaimus montanus* is described for the first time in the scientific literature, and *Eudorylaimus longicardius* Thorne, 1974 – for Georgian fauna.

Key words: Dominant species, hydrobiont, edaphic biont, Dorylaimida

Introduction

Data about soil nematodes of Ajara is scanty. In 1960s several species of nematodes have been recorded in Batumi environs [Brzeski, 1961], in 1970s, while studying citrus nematodes tens of species were established from the soil round the roots [Tskitishvili, 1971; 1973]. Complex relief of Ajara, high humidity, plant diversity, high mountain ridges, complex structure of soil zones [Palavandishvili, 2003] cause the interest for studying of nematodes, one of the main component of soil microfauna.

During 2001-2005 we have studied threadworms – nematodes, as well as bladder worms – lumbricides. In the given paper we examine only nematodes, which are the most diverse among soil metazoan animals. They are distributed in all types of soil and represent the principal taxocene of soil microfauna. Hence, they take important place in processes occurring in soil, but also have a diagnostic significance.

Materials and Methods

Soils of coastal and montane regions of Ajara according to natural zones are divided into mountain and lowland soils [Palavandishvili, 2003]. Mountain soil area is divided into 3 zones: alpine, subalpine and mountain-forest zones. Material was collected from subalpine and mountain-forest zones.

Soil samples were taken by route method on the mountain Mtirala, Gorges of the rivers Kintrishi and Ajaristskali according to plant belts. From each point 3 samples were taken at the depth of 15 cm from soil surface. On the mountain Mtirala material was collected in 5 points, in the Gorge of river Kintrishi – in 12 points, and in the gorge of river Ajaristskali – in 18 points.

Isolation from soil, preparation and identification of nematodes was carried out in Tbilisi Institute of Zoology.

Results and Discussion

The results of identification of Ajara nematodofauna is presented in Table 1. The list of established species of the studied regions is presented separately. By species diversity the gorge of river Kintrishi is distinguished, which is caused by the fact that material from this region was picked up thrice, but from other regions only once.

Table 1. Soil nematodes of Ajara

#	Nematodes	Mount Mtirala	Riv. Kintrishi gorge	Riv. Ajaristskali gorge		Nematodes	Mount Mtirala	Riv. Kintrishi gorge	Riv. Ajaristskali gorge
I	II	III	IV	V	I	II	III	IV	V
1.	*Tripyla glomerans*	+	+	+	70.	*D. major*	+	+	
2.	*T. longicaudata*	+	+		71.	*Epidorilaimus lugdunensis*			+
3.	*T. setifera*		+		72.	*Aporcelaimellus krigeri*	+	+	+
4.	*Tripyla sp.*		+		73.	*A. obscuroides*		+	
5.	*Tripylina arenicola*	+	+		74.	*A. obscurus*		+	+
6.	*Tripylina sp.*		+		75.	*A. obtusicaudatus*	+	+	+
7.	*Paratripyla sp*		+		76.	*A. paraconicandotus*	+	+	+
8.	*Tobrilus abberans*		+		77.	*A. paraobtusicaudatus*		+	+
9.	*T. gracilis*	+	+		78.	*A. simplex*		+	
10.	*Tobrilus sp.*		+	+	79.	*A. simus*			+
11.	*Alaimus primitivus*	+	+		80.	*A. trilici*	+	+	
12.	*Alaimus sp.*		+	+	81.	*A vanderlaani*		+	
13.	*Bastiania gracilis*	+	+		82.	*Aporcelaimellus sp.a*	+	+	+
14.	*Monhystera vulgaris*	+	+		83.	*Aporcelaimellus sp.b*			+
15.	*Plectus annulatus*	+	+		84.	*Paratonchium striatum*		+	+
16.	*P. elongatus*		+	+	85.	*Sectonema ventrale*		+	
17.	*P. longicaudatus*	+			86.	*Dorydorella pratensis*		+	+
18.	*P. parietinus*	+	+	+	87.	*Pungentus engadinensis*		+	+
19.	*Plectus sp*	+		+	88.	*P. imonohistera*			+
20.	*Ceratoplectus assimilis*			+	89.	*P. silvestris*	+	+	
21.	*Anaplectus granulosus*	+	+	+	90.	*Pungentus sp.*		+	+
22.	*A. submersus*			+	91.	*Enchodelus altherri*		+	
23.	*Paravulvus hartingii*			+	92.	*E. macrodorus*			+
24.	*Nygolaimus sp*		+	+	93.	*Enchodelus sp.*		+	+
25.	*Mesodorylaimus bastiani*	+	+	+	94.	*Longidorus laevicapitatus*		+	
26.	*M. filicaudatus*		+	+	95.	*Xiphinema brevicolle*	+	+	+
27.	*M. flagellatus*	+	+	+	96.	*X. diversicaudatus*		+	+
28	*M. mesonyctius*	+	+	+	97.	*Xiphinema sp.*	+	+	
29.	*M. subtiloides*		+		98.	*Trachactinolaimus montanus n. sp.*		+	
30.	*Mesodorylaimus sp. a*	+	+	+	99.	*Tylencholaimus crassus*			+
31.	*Mesodorylaimus sp. b*		+		100.	*T. minimus*	+	+	
32	*Dorylaimus montanus*	+	+	+	101.	*T. mirabilis*		+	
33.	*D. hernei*			+	102.	*T. nanus*		+	
34.	*Dorylaimus sp*	+			103.	*T. obscurus*		+	

35.	*Eudorylaimus acuticauda*	+	+	+	104.	*T. stecki*	+	+	
36.	*E. acutus*		+		105.	*Tylecholaimus sp.*	+	+	+
37.	*E. altherri*		+		106.	*Leptonchidae g. sp.*			+
38.	*E. arcus*			+	107.	*Belondira clava*		+	
39.	*E. bombilectus*		+	+	108.	*B. cylindrica*	+		+
40.	*E. carteri*	+	+	+	109.	*Belondira sp.*	+		
41.	*E. centrocercus*	+	+	+	110.	*Oxydirus oxycephalus*	+	+	
42.	*E. franzi*			+	111.	*Oxydirus sp.*		+	
43.	*E. georgiensis*		+		112.	*Axobchium siddiqi*		+	
44.	*E. humilis*		+		113.	*Axonchium sp.*		+	+
45.	*E. iners*	+			114.	*Diphtherophora communis*			+
46.	*E. kirjanovae*		+		115.	*D. kirjanovae*			+
47.	*E. leuckarti*		+		116.	*Clarcus papillatus*	+	+	+
48.	*E. leptosoma*		+		117.	*Clarcus sp.*			+
49.	*E. lindbergi*			+	118.	*Coommansus sp.*	+	+	
50.	*E. longicardius*		+		119.	*Prionchulus muscorum*		+	+
51.	*E. maritus*			+	120.	*Prionchulus sp.*		+	+
52.	*E. paramonovi*		+		121.	*Miconchus sp.*	+	+	
53.	*E. pectinatus*		+		122.	*Mylonchulus brachyuris*		+	+
54.	*E. spauli*			+	123.	*Mylonchulus sp.*			+
55.	*E. subdigitalis*			+	124.	*Anatonchus tridentatus*			+
56.	*E. vestibulifer*			+	125.	*Jotonchus sp.*		+	
57.	*Eudorylaimus sp.a*	+	+	+	126.	*Cephalobus parvus*	+	+	
58.	*Eudorylaimus sp.b*		+		127.	*C. persegnis*		+	
59.	*Allodorylaimus diadematus*			+	128.	*Eucephalobus mucronatus*		+	+
60.	*A. digiturus*			+	129.	*Heterocephalobus elongatus*		+	+
61.	*A. granuliferis*	+	+		130	*H. nanus*		+	
62.	*A. holdemani*		+		131.	*Tylenchus davainei*		+	
63.	*Takamangai ettersbergensis*	+			132.	*Tylenchorhynchus sp.*	+	+	
64.	*T. laticollis*		+		133.	*Helicolenchus crenacauda*		+	
65.	*T. lautus*			+	134.	*H. digonicus*	+	+	+
66.	*Microdorylaimus longicollis*		+		135.	*Helicotylenchus sp.*			+
67.	*Labronemella georgiensis*		+		136.	*Aphelenchus avenae*		+	+
68.	*Labronemella sp.*		+		137.	*Aphelenchoides composticola*	+		
69.	*Discolaimus levinae*		+		138.	*A. subtenuis*			+

As is seen from the table 138 forms of nematodes are registered, among them 111 are identified up to species. Mentioned nematodes belong to 8 order and 26 families. One species from Kintrishi gorge – *Trachactinolaimus montanus* – is for the first time identified in scientific literature and its description is in press. Species – *Eudorylaimus longicardius* Thorne, 1974 – is for the first time observed in Georgian fauna.

Taxonomic structure of nematodes is characterized with domination of one group of typical edaphic bionts. This dominant group is the order Dorylaimida. 60% of registered forms belong to this order. Such asymmetric structure is the common phenomenon in the natural ecosystem. The rest species are united in 7 orders (Enoplida, Monhysterida, Areolaimida, Mononchida, Rhabditida, Tylenchida, Aphelenchida).

Similarity coefficients of fauna [Chernov, 1975] are quite low (Fig. 1), which is caused by distant situation of studied areas and by ecological differences.

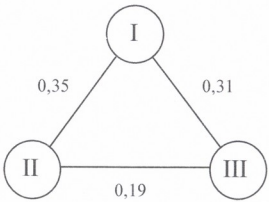


Fig. 1. Similarity coefficient of fauna. (I - mount Mtirala, II – River Kintrishi gorge, III – River Ajaristskali gorge)

Geographical and ecological ubiquists are represented greatly in the obtained material. They form dominant groups and are representatives of the families Eudorylaimus, Aporcelaimellus, Clarcus Plectus.

It should be especially mentioned existence of fresh water nematode *Tobrilus abberans* in the ground waters flown up on the ground surface in Kintrishi gorge (Khino).

Dorylaimus montanus was recorded in every point of material collection. It is typical hydrobiont, and as *T. abberans,* it comes with ground waters in case of water setting at high level.

In studied regions dominant species mainly belong to the representatives of the orders: Dorylaimida, Areolaimida and Monochida. In the material obtained from mount Mtirala dominants are: *Plectus parietinus, Mesodorylaimus mesonyctius, Eudorylaimus carteri* and *Aporcelaimellus obtusicaudatus*. In Kintrishi gorge besides those species to the dominant species belong *Eudorylaimus carteri* and *Clarcus papillatus*.

Typical edaphic bionts form the main body of fauna, which are related trophically with the inhabited in the soil organisms and detritus. Diversity of polyphages from Dorylaimida order is significant. They are fed with detritus, soil algae, chlorophyll composed parts of plants [Nielsen, 1949; Wood, 1973].

The number of plant true parasites and forms fed with fungus hypae (mycohelminths) from Tylenchus and Aphelinidae orders was insignificant, which should be caused by the fact that material was picked up only in the natural ecosystems.

References:

Brzeski M. *Przyezyuek do poznania nicieni (Nematoda) mcolubnyc Gruzii.* Przeglad zoologiczny, **2**, 137-139, 1961 (in Polish).

Chernov Iu.I. *The principal synecological characteristics of soil invertebrates and methods of their analysis.* In: Method of soil-zoological studies. Moscow, "Nauka", 160-216, 1975.

Nielsen C.O. *Studies on the soil Microfauna. II. The soil inhabiting Nematodes.* Natura Jutlandica. Naturhistorisk Museum, Aarhus. Danmark. 2 Bind, 1-113.1949.

Palavandishvili Sh. *Geography of Ajara soils and their agroindustrial usage.* Batumi, p.201, 2003.

Tskitishvili T. *Study of mandarin nematode fauna of Ajara.* In: Articles in parasitology, **II**, Georgian Academy of Sciences. Tbilisi, "Metsniereba", 149-153, 1971.

Tskitishvili T. *Study of lemon and orange nematode fauna of Ajara.* In: Articles in parasitology, **III**, Georgian Academy of Sciences. Tbilisi, "Metsniereba", 155-158, 1973.

Wood F. H. *Nematode feeding relationships of soil dwelling nematodes.* Soil Biol. Biochem., 5. 593-601, 1973.

აჭარის ნიადაგების ნემატოდების შესწავლისათვის

ჟღენტი ლ.

შ. რუსთაველის ბათუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 05.06.2006)

რეზიუმე

შესწავლილია აჭარის ნიადაგების ნემატოდები. დადგენილია ნემატოდების 138 ფორმა, რომელთა შორისაა მეცნიერებისათვის ახალი სახეობა *Trachactinolaimus montanus* და საქართველოს ფაუნისათვის პირველი *Eudorylaimus longicardius* Thorne, 1974.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

Entomology

NATURAL ENEMY COMPLEXES ASSOCIATED WITH THE RUSSIAN WHEAT APHID (THE RWA) - *DIURAPHIS NOXIA* (MORDVILKO) IN EAST GEORGIA

ALEXIDZE G.[1], *BARJADZE SH.[2]

[1] *Georgian academy of Agricultural Sciences,*
[2] *Institute of Zoology*
E-mail: shalva1980@yahoo.com

(Received March 3, 2006)

Abstract

Extensiveness of wheat plant damage by the RWA and its natural enemies were investigated in 8 localities (Kumisi, Marneuli, Tetritskaro, Tserovani, Sagarejo, Sartichala, Satskhenisi, surroundings of Lake Lisi) of Kartli and Kakheti regions. 12 species of natural enemies were registered on the investigated territory, from which 10 belong to predators and 2 species are parasitoids. High extensiveness of plant damage was registered in Kumisi and Satskhenisi - 80-90% and 50-60% respectively, whereas the minimum percentage of plant damage - in Tserovani (2-5%).

Key words: the RWA, predators, parasitoids, extensiveness of plant damage, East Georgia

Introduction

The native range of the RWA is considered the area between the Caucasus Mountains and the Tian Shan [González et al., 1990]. It was first described in 1913 by Kurdjumov, when a major outbreak took place in Moldova and Ukraine. It was accidentally introduced into South Africa (1978), North and South America in 1980 and 1987 respectively and Middle East (1984).

The RWA is a pest of cereals causing serious damage to wheat and barley. This species is distributed over Georgia, but high level of plant damage is registered only in East Georgia [Jibladze, 1975]. Important data about biology and control measures against the RWA are given by A. Abashidze [Abashidze, 1956]. It hibernates on winter wheat and barley and produces 15-18 generations.

It must be noted that biological enemies of the RWA have not been subject of special research before. The aim of our research was to determine predator and parasitoid complexes associated with the RWA on the above mentioned territory.

Material and Methods

Materials for investigation were collected in June 2000, from 8 localities (Kumisi, Marneuli, Tetritskaro, Tserovani, Sagarejo, Sartichala, Satskhenisi, surroundings of Lake Lisi) of Kartli and Kakheti regions. Collection of aphids and its natural enemies and their identification

were performed according to the methodology well-known in entomology [Blackman & Eastop, 2000; Jibladze, 1975; Kuznetsov, 1993; Nikolskaya & Yasnosh, 1966; Quednau, 2003; Rojo et al., 2002; Shaposhnikov, 1964].

Results and Discussion

12 species of natural enemies were registered on the investigated territory, from which 10 are predators (5 species belong to the family *Coccinellidae,* while the families *Cecidomyiidae, Chamaemyiidae, Chrysopidae, Syrphidae* and *Trombidiidae* are represented by one species each) and 2 species - parasitoids (*Aphelinus asychis* Walker, 1839 and *Aphelinus sp.*). Natural enemies of the RWA are listed in Table.

Table 1. List of natural enemies and extensiveness of plant damage in Georgia

Orders	Families	species	Extensiveness of plant damage in							
			Kumisi	Marneuli	Tetritskaro	Tserovani	Sagarejo	Sartichala	Satskhenisi	Surroundings of Lake Lisi
Acari	*Trombidiidae*	*Allothrombium fuliginosum* (Hermann, 1804)	80-90	3-6%	4-5%	2-5%	23%	20-25%	50-60%	5-6%
Coleoptera	*Coccinellidae*	*Adalia bipunctata* Linnaeus, 1758								
Coleoptera	*Coccinellidae*	*A. decempunctata* Linnaeus, 1758								
Coleoptera	*Coccinellidae*	*Coccinella septempunctata* Linnaeus, 1758								
Coleoptera	*Coccinellidae*	*Hippodamia undecimnotata* (Schneider, 1792)								
Coleoptera	*Coccinellidae*	*H. variegata* (Goeze, 1777)								
Diptera	*Cecidomyiidae*	*Aphidoletes aphidimyza* (Rondani, 1847)								
Diptera	*Chamaemyiidae*	*Leucopis glyphinivora* Tanasijtshuk, 1958								
Diptera	*Syrphidae*	*Episyrphus balteatus* (de Geer, 1776)								
Hymenoptera	*Aphelinidae*	*Aphelinus asychis* Walker, 1839								
Hymenoptera	*Aphelinidae*	*Aphelinus sp.*								
Neuroptera	*Chrysopidae*	*Chrysoperla carnea* (Stephens, 1836)								

High extensiveness of plant damage was registered in Kumisi and Satskhenisi - 80-90% and 50-60% respectively, while the minimum percentage of plant damage - in Tserovani (2-5%).

References:

Abashidze A. *Biology of Barley Aphid and modern control methods against it.* Proc. Georg. Inst. Plant Prot. **11**, 73-88, 1956 (in Georgian).

Blackman R., Eastop V. *Aphids on the World's Crops. An Identification and Information Guide.* Chichester, "JOHNWILEY & SONS", 466, 2000.

González D., Gilstrap F., Zhang G., Zhang J., Zareh N., Wang R., Dijkstra E., McKinnon L., Starý P., Wooley J. *Foreign Exploration for Natural Enemies of Russian Wheat Aphid in China, Iran, Turkey and the Netherlands.* Proc. Fourth the RWA Workshop, 154-165, 1990.

Jibladze A. *Aphids of Agricultural Plants of Georgia.* Tbilisi, "Metsniereba", 242, 1975 (in Georgian).

Kuznetsov V. *Coccinellids (Coleoptera, Coccinellidae) of the Russian Far East.* Vladivostok, "Dalnauka", 334, 1993 (in Russian).

Nikolskaya M., Yasnosh V. *Aphelinids of the European Part of the USSR and the Caucasus.* M. - L., "Nauka", 294, 1966 (in Russian).

Quednau F. *Atlas of the Drepanosiphinae Aphids of the World. Part II: Panaphidini Oestlund, 1923 - Panaphidina Oestlund, 1923 (Hemiptera: Aphididae: Callaphidinae).* Mem. Amer. Entomol. Inst., **72,** 2003.

Rojo S., Gilbert F., Marcos M., Nieto J., Mier P. *Revísion Mundial de los Sírfidos Depredadores y Sus Presas (Diptera, Syrphidae).* Alicante, " Publicationes de la Universidad de Alicante", 282, 2002.

Shaposhnikov G., Aphidinea Kh. *Clasification keys to the insects of the European Part of the USSR.* Ed. Bey-Bienko G. Y., M., "Nauka", 489-616, 1964 (in Russian).

ქერის ბუგრის ***Diuraphis noxia*** **(Mordvilko)** ბუნებრივი მტრები აღმოსავლეთ საქართველოში

ალექსიძე გ.[1], ბარჯაძე შ.[2]

[1] საქართველოს სოფლის მეურნეობის მეცნიერებათა აკადემია
[2] ზოოლოგიის ინსტიტუტი

(მიღებულია 03.03.2006)

რეზიუმე

ნაშრომი ეხება ქერის ბუგრის *Diuraphis noxia* (Mordvilko) მიერ ხორბლის დაზიანების ექსტენსივობასა და მავნებლის ბუნებრივი მტრების გამოკვლევის შედეგებს ქართლისა და კახეთის რეგიონებში (კუმისი, მარნეული, თეთრიწყარო, წეროვანი, საგარეჯო, სართიჭალა, საცხენისი, ლისის ტბის შემოგარენი). საკვლევ ტერიტორიაზე რეგისტრირებული ქერის ბუგრის ბუნებრივი მტრების 12 სახეობიდან 10 მიეკუთვნება მტაცებლებს, ხოლო 2 პარაზიტოიდებს. ბუგრის მიერ მცენარის დაზიანების მაღალი ექსტენსივობა რეგისტრირებულ იქნა კუმისსა და საცხენისში - შესაბამისად 80-90% და 50-60%, მცენარის დაზიანების დაბალი ექსტენსივობა კი წეროვანში (2-5%).

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

PREDICTION OF VIABILITY OF BARK BEETLE (*IPS TYPOGRAPHUS* L.) POPULATIONS AND WAYS OF THEIR CONTROL

CHKOIDZE M., KOBAKHIDZE L., SKHIRTLADZE I.

V. Gulisashvili Institute of Mountain Forestry

(Received May 8, 2006)

Abstract

The paper deals with classification of blood elements of dangerous pest, bark beetle (*Ips typographus* L.) of oriental spruce (*Picea orientalis Link*). It was established that physiological state and fertility of typographus should be determined by quantitative, morphological and structural changes of hematocytes caused by entomopathogenic microorganisms. Hence, we can forecast the alterations in the number dynamics. Ecological measures of regulation of the number of typographus based on hematocyte analysis of infectious pathologies are worked out.

Key word: leukogram, hemolymph, mycosis, virus and bacterial infections

Introduction

For the last years oriental spruce in Borjomi gorge is subjected to invasion of bark beetles. The density of pest population is very high in some places.

Data about mass reproduction of *Ips typographus* based on estimation of pest physiological state carried out by hematological express method were presented in 1999 [Chkoidze, 1999]. In current use as a control measure against typographus is considered the pheromones. Efficiency of pheromones depends on bioecological characteristics and physiological conditions of the pest [Ozols et al., 1987; Shcherbakov, 2001]. By hematological parameters the level of viability of pest population in particular biotope was determined, and according to those data the number of pheromone traps was established. Hematological express method provides reduction of expenses for conducting the control measures during mass reproduction [Chkoidze, Kvinikhidze, 2001; Choidze, 2002].

The aim of our research is to work out ecological technology for protection of Borjomi gorge spruce from bark beetles.

Materials and Methods

Visual and microscopical studies of typographus larvae and beetles of different age were carried out. Diagnostics of entomopathogens was conducted according to proper methods of insect pathology [Sirotina, 1965; Evlakhova, Shvetsova, 1965]. Typographus hemolymph smears were fixed in 96% alcohol for 30 min. Fixed preparations were dyed according to Gimza-Romanovski method. Dyed preparations were analyzed microscopically. Leukogram was calculated by

leukocytometer. To study a complete picture of disease hemolymph preparations of typographus larvae and beetles taken at different heights (800-1000 and 1400-1800 m a.s.l.) were obtained. Composition of Hemolymph was determined, and according to the pest development stages hemograms were compiled. By hematological diagnostics percentage of infested individuals was established.

Results and Discussion

Hematological investigations carried out in Borjomi gorge during 1999-2003 revealed that typographus were infected with various microorganisms: fungi, viruses, bacteria, unicellular organism of sporoza class (Table 1).

It was found out that typographus is mainly diseased by mycoses (20-30%); then comes mixed infections (10-20%), viruses (7-10%), bacteriosis (7-10%). Invasion with sporozoa causing protozoonosis was also recorded.

As a result of hemolymph analysis it was revealed that vitalities of various populations of typographus in Borjomi gorge differ from each other. Typographus populations of "Mzetamze" and "Tsagveri" are distinguished by higher vitality compared with populations of "Borjomi" and "Bakuriani". Hemolymphs of pests from "Mzetamze" and "Tsagveri" populations consist of less number of dead and pathological cells and a great number of protective cells – phagocytes.

Based on the number dynamics and hematological parameters of typographus we worked out complex system of control, which should be conducted in spring and summer: to carry out pathological and physiological studies at various heights in April, right away when larvae and adult beetles are appeared; visual estimation of number dynamics; to reveal infective and invasive diseases; their diagnostics; determination of percentage of diseased beetles; establishment of the reasons of pest death-rate by hematological analysis.

Determination of bioecological characteristics and hematological parameters of vitality enable us to define the number of pheromone traps needed for particular area of the forest.

To determine flight beginning and flight dynamics of pest in April when air temperature rises up to 15-16°C one pheromone trap per 10 hectare should be hung at different heights (800-1000 and 1400-1800 m a.s.l.). Before flight beginning of imago the traps must be examined every day. But further, recording of the traps should be carried out once a week. As two primary, and often two-daughter generations of typographus occur in Borjomi gorge, flight of pests take place from second half of April up to the end of September. The pheromone traps should be used for both, flight signaling and pest control during the whole summer. For attraction of hibernated pests hanging of pheromone traps by vertical belts should be finished in April-May, and in case of summer population – in June-July. Traps must be hung on deciduous trees, pest-damaged spruces, dried up trees at the height of 1.3-1.4 m in the forest outskirts.

In Borjomi gorge forests nidus of weak, medium and strong intensity occur. According to the kind of measures, necessary for the particular population – preventive, number regulating and control measures, from 10 up to 20 traps per hectare should be used. Number of pheromone traps is determined by percentage of infested individuals, hematological parameters of population, density of population, forest density, exposition and vertical zoning.

During the mass flight (April-May-June), when total intensity of various diseases is not higher than 7-10%, population is considered as viable. At the same time, when increased number of typographus is noted 20-30 traps/1 he should be hung. When the disease intensity is less than 20-30% 15-20 pheromone traps are hung, and at the intensity less than 40-50% - 10-12 traps.

For diagnostics of disease and determination of resistance of new generation hematological analysis during various phenophases of development of typographus must be carried out in summer and autumn. Vertical zoning should be also taken into account.

Table 1. Hemograms of larvae and beetle of typographus in norm and pathology

Composition of hemocytes (%)	healthy		mycosis		virus infections		bacterial infections		protozoonoses	
	1*	2*	1	2	1	2	1	2	1	2
proleucocyte	8.9	2.9	10.2	7.0	5.2	1.1	6.1	1.0	2.3	1.2
macronucleocyte	26.6	11.6	30.2	22.1	31.0	19.0	15.3	3.2	6.0	3.1
micronucleocyte	38.1	45.9	81.1	11.0	6.0	15.2	12.2	18.0	50.2	52.0
Fusiform phagocyte	4.3	5.9	21.1	23.1	25.2	25.0	30.1	31.0	20.2	17.1
Non-fusiform phagocyte	9.4	10.8	3.0	2.4	3.1	2.3	2.2	2.3	2.1	2.0
eosinocyte	5.2	3.5	1.2	1.0	3.2	1.1	3.0	2.2	1.2	1.1
oenocytonde	5.1	3.0	-	1.1	3.1	1.0	2.0	1.2	1.0	1.1
granulocyte	-	12.3	-	4.2	-	4.1	-	10.1	-	4.2
Dead and patho-logical cells	2.4	4.1	25.1	28.1	23.2	31.2	29.0	30.0	17.0	18.2

* 1- larvae; 2 – beetle

References:

Chkoidze M. *Hematological diagnostics of oriental spruce (Picea orientalis Link) pest entomofauna, diseases and forecasting of its propagation.* Bull of Georg. Acad Sci., **160**, 3, 1999.

Chkoidze M., Kvinikhidze G. *Role of complex control measures against the harmful pests in conservation of forests of Georgia.* Proceedings of the Institute of Zoology of Georg. Acad. Sci. 364-374, 2001.

Sirotina M. *Analysis of pest hemolymph.* Moscow, p.135-170, 1965.

Evlakhova A., Shevtsova O. *Diseases of harmful insects.* Moscow, p.50, 1965.

Ozols G., et al. *Usage of pheromone against bark beetle Ips typographus for spruce forest control and conservation.* Moscow, "Gosleskhoz", USSR, p.1-16, 1987

Scherbakov A. *Nidus of bark beetle Ips typographus in spruce forests of Losini Island.* Eastern Palaearctic Section of Int. Org. of Biol.Control. Pushchino, p.48-50, 2001.

Chkoidze M. *Hematological diagnostics of the harmful insect diseases and its use for forest conservation.* Proceedings of V. Gulisashvili Institute of forestry, Tbilisi, "Metsniereba", **XXXVIII**, 197-203, 2002.

ქერქიჭამია ტიპოგრაფის (*Ips typographus* L.) პოპულაციათა სიცოცხლისუნარიანობის პროგნოზირება და მასთან ბრძოლის ხერხები

ჭყოიძე მ., კობახიძე ლ., სხირტლაძე ი.

ვ. გულისაშვილის სატყეო ინსტიტუტი

(მიღებულია 08.05.2006)

რეზიუმე

შესწავლილია აღმოსავლეთის ნაძვის (*Picea orientalis* Link) განსაკუთრებით საშიში მწერის ქერქიჭამია ტიპოგრაფის (*Ips typographus* L.) სისხლის ფორმიანი ელემენტები. ენტომოპათოგენური მიკროორგანიზმებით გამოწვეული ჰემოციტების რაოდენობრივი, მორფოლოგიური და სტრუქტურული ცვლილებების მიხედვით შეგვიძლია დავადგინოთ ქერქიჭამია ტიპოგრაფის ფიზიოლოგიური მდგომარეობა, მისი ნაყოფიერება და აქედან გამომდინარე გავაკეთოთ პროგნოზი მავნებლის რიცხოვნობის დინამიკაში მოსალოდნელი ცვლილებების შესახებ. ინფექციური პათოლოგიის ჰემატოლოგიური ანალიზის საფუძველზე დამუშავებულია ტიპოგრაფის რიცხოვნობის რეგულირების ეკოლოგიური ღონისძიებანი.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

STRAINS RESISTANT TO HEAVY METAL IONS IN THE NATURAL POPULATION OF WINE YEAST

MACHAVARIANI N.[1], SHATIRISHVILI A.[2], JORBENADZE TS.[1]

[1] *Iv. Javakhishvili Tbilisi State University*
[2] *I. Chavchavadze Tbilisi State University*

(Received March 20, 2006)

Abstract

Resistance to some heavy metal (Cu, Mn and Cd) ions has been studied in Shilda (Kvareli region, Kakheti) population of wine yeast. By resistance, the population was found to be polymorphic – within the constituent micropopulations different frequencies of heavy metal-resistant or -sensitive strains were revealed. The micropopulations are suggested to be grouped into three categories: 'resistant', 'mild' and 'sensitive' strains.

Key Words: wine yeast, natural population, resistance to heavy metals

Introduction

For rather a long period the biosphere has been exposed to the influence of heavy metals. At first it had local character, but later in the era of scientific-technical progress it acquired global scales. Processing of heavy metal-containing ores in parallel to landscape changes induced insertion of the metals into the process of biogenic migration [Dubinin, 2000; Shatirishvili, Chuchulashvili, 2000].

Certain heavy metals represent microelements. Some of them are enzyme cofactors that play a remarkable role in cell metabolism. Deficiency in them induces destructions in essential vital processes. For every organism very significant role is attributed to metals in the maintenance of homeostasis. Living organisms must get optimal amount of needed metals from surrounding environment. Deviations from normal values (either increase or decrease) are followed by dramatic consequences. Sharp increase in the concentration of heavy metal ions in the environment causes its contamination. The organism adequately reacts on the stressful effect. Natural selection and other microevolutionary factors drive formation and accumulation of heavy metal-resistant strains in natural populations [Avery et al., 2000; Shatirishvili, Chuchulashvili, 2000; Cohen et al., 2000].

Materials and Methods

Shilda (Kvareli region, Kakheti)), one of the most developed vine-farming regions of Georgia has been chosen in order to study wine yeast natural populations.

In Saharomyces, and in the wine yeast in particular, at the present time no characteristics are worked out for such interspecies ranges as are the populations. The area of reproductive activity of the wine yeast vastly depends on drosophila, and therefore, it is rather small. For this reason, the wine yeast forms that are spread over villages and the surrounding areas were considered as

populations. Material (wine sediment) was collected from 10 remote private cellars (so called micropopulations). Besides, the vineyards belonging to the private farmers were not near. The samples of wine sediment were obtained from large clay vessel buried in ground. Wine fermentation in such permanent conditions continues spontaneously.

Standard technologies were used for isolation of pure cultures and strains from the population. The strains were specified (identifying their specific status) according to the proposed standard method [Kreger Van Rij 1969; Kvasnikov, Shelokova 1991]. Detailed description of the method for yeast cell incubation and preparation of the nutrient media, which are applied in various investigations, are proposed in the methodological textbook [Zakharov et al., 1984].

Solutions of copper sulphate, magnesium sulphate and cadmium chloride have been tested. Wine yeast strains isolated from the population were transferring to the metal-containing and metal-free (standard) culture media using the replicator for detecting identity of cultures (whether they were resistant or sensitive forms). Different amounts of heavy metal ions were preliminary added to the solid media. Culture incubation lasted 4 days at 30° C.

Results and Discussion

As we have learnt from the data [Zakharov et al, 1980], presence of manganese ions in the environment causes enhanced heavy metal resistance. For this reason, manganese salts have not been added to the culture media. It has also been found, that resistance of strains to heavy metal ions depends on composition of the medium. In relatively poor, copper ion-containing media (perfect medium that contains salts) resistance of the strains fluctuated within the range of concentration from 0.5 to 0.8 mM/ml while in perfect peptone-containing media it varied from 8 to 18 mM/ml. For testing we applied the medium containing salts.

Copper is the most common metal in nature. Copper-containing preparations are widely used in agricultural practice to fight against pathogenic fungi. 250 wine yeast strains of 10 micropopulations derived from Shilda population were transferred to the solid medium containing 1-8 mM/ml of copper sulphate. The results obtained are presented in Table 1. It is clear from the results, that copper ion-resistant strains were revealed in the V and VII micropopulations. In I, II and X micropopulations copper ion-sensitive strains exceeded others. In the other micropopulations copper-resistant strains were met at different rates.

It was accepted, that in the Saccharomyces resistance to copper ions is determined by two dominant genes – MTHI and MTH2. They are not linked and are located in chromosomes 8 and 16 accordingly. Nowadays it has already been established that resistance to metals is under the control of SMF genes belonging to the SMF family. Their polymeric activity causes enhanced copper and other heavy metal resistance [Cohen et al., 2000].

All the strains from ten studied micropopulations showed high sensitivity to cadmium ions. The norm of reaction was rather limited: it ranged within the indices of 0.5-2.5 mM/ml (Table 2). No strain could grow over the concentration of 2.5 mM/ml. Relatively high resistance to cadmium ions revealed the strains from the micropopulations IX and X, while the strains belonging to the I micropopulation were remarkably sensitive. Such interrelations between the yeast cells and cadmium ions are likely to be explained by high toxicity of $CdCl_2$. The latter can cause breath retention. The strains grown in cadmium-containing medium had a brownish-red phenotype instead of white. It is thought, that such alterations of the phenotype is due to the changes occurring in mitochondria [Welch et al., 1983].

Table 1. Relation of population – composing strains to cupper ions

Micropopulation	number of strains studies	mM	1			2			3			4			5			6		
		growth	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-
I	25	№	16	2	7	4	12	9	0	0	25	0	0	25	0	0	25	0	0	25
		%	64	8	28	12	48	36	0	0	25	0	0	100	0	0	100	0	0	100
II	25	№	18	2	5	11	8	7	1	3	21	0	0	25	0	0	25	0	0	25
		%	72	8	20	44	32	28	4	12	84	0	0	100	0	0	100	0	0	100
III	25	№	23	2	0	17	3	5	11	10	4	8	12	5	4	14	7	0	2	23
		%	92	8	0	68	12	20	44	40	16	32	48	20	16	56	28	0	8	92
IV	25	№	24	1	0	20	10	4	16	5	4	10	9	6	4	7	9	0	3	22
		%	96	4	0	80	40	16	64	20	16	40	36	24	16	28	36	0	12	88
V	25	№	25	0	0	25	0	0	25	0	0	24	1	0	11	9	5	1	3	21
		%	100	0	0	100	0	0	100	0	0	96	4	0	44	36	20	4	12	84
VI	25	№	25	0	0	19	4	2	13	6	6	9	9	7	0	4	21	0	1	24
		%	100	0	0	76	8	4	52	24	24	36	36	28	0	16	84	0	4	96
VII	25	№	25	0	0	24	1	0	19	5	1	12	12	1	9	10	6	1	1	23
		%	100	0	0	96	4	0	76	20	4	48	48	4	36	40	24	4	4	92
VIII	25	№	23	2	0	17	3	5	5	12	8	6	13	6	2	7	11	0	1	24
		%	92	8	0	68	12	20	20	48	32	24	52	24	8	28	44	0	4	96
IX	25	№	19	5	1	19	4	2	10	11	4	1	10	14	3	6	16	0	0	25
		%	76	0	4	96	16	8	40	44	16	4	40	56	12	24	64	0	0	100
X	25	№	21	4	0	11	13	1	9	12	4	7	7	11	2	9	14	0	0	25
		%	84	16	0	44	52	4	36	48	16	28	28	44	18	36	56	0	0	100

notice: + high growth in medium
± low growth in medium
- no growth in medium

The micropopulations were found to be far more resistant to the manganese ions than to the copper and cadmium ions. Critical doses varied within the range of 40-90 mM/ml (Table 3). The resistant forms are more frequently met in micropopulations IX and X. In I and VIII micropopulations sensitive strains prevail. As it proceeds from the results, Cd- and Mn-resistant strains at high rates are met in the IX and X micropopulations. Such a positive correlation indicates that the resistance to cadmium and manganese ions is determined by the genes grouped in the SMF family. For today, it is ascertained that the genes SMF1 and SMF2, members of this family, are responsible for resistance to cobalt and manganese ions [Cohen et al., 2000].

Proceeding from the obtained results, we suggest, that the population studied is highly polymorphic including three groups of strains – 'sensitive', 'mild' and 'resistant' forms. The maintenance of intrapopulational polymorphism is encouraged and supported by natural selection.

Table 2. Relation of population-composing strains to cadmium ions

Micropopu-lation	number of strains studies	mM	0.5			1			1.5			2			2.5		
		growth	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-
I	25	№	0	6	19	0	0	25	0	0	25	0	0	25	0	0	25
		%															
II	25	№	16	4	5	5	2	17	2	1	22	0	0	25	0	0	25
		%															
III	25	№	3	3	19	0	0	25	0	0	25	0	0	25	0	0	25
		%															
IV	25	№	13	1	11	12	0	13	1	2	22	0	4	21	0	0	25
		%															
V	25	№	25	0	0	25	0	0	1	24	0	0	6	19	0	0	25
		%															
VI	25	№	21	3	1	11	13	1	2	22	1	5	11	9	0	0	25
		%															
VII	25	№	23	2	0	13	9	3	11	5	9	6	5	14	0	1	24
		%															
VIII	25	№	25	0	0	16	6	3	13	4	8	7	8	10	0	0	25
		%															
IX	25	№	25	0	0	22	1	2	22	1	2	21	1	3	1	23	1
		%															
X	25	№	25	0	0	25	0	0	23	1	1	22	0	3	12	12	1
		%															

notice: + high growth in medium; ± low growth in medium; - no growth in medium

Table 3. Relation of population-composing strains to manganese ions

Micropopu-lation	number of strains studies	mM	40			50			60			70			80			90		
		growth	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-
I	25	№	23	2	0	20	4	1	14	4	7	4	9	12	0	3	22	0	0	25
		%																		
II	25	№	25	0	0	23	2	0	15	4	6	14	5	6	5	11	9	0	0	25
		%																		
III	25	№	25	0	0	19	1	5	16	2	7	11	6	8	4	4	17	0	0	25
		%																		
IV	25	№	25	0	0	22	3	0	16	9	0	6	19	0	0	1	24	0	7	18
		%				88	12	0												
V	25	№	25	0	0	25	0	0	25	0	0	6	17	2	4	11	10	0	4	21
		%																		
VI	25	№	25	0	0	14	11	0	7	7	11	3	20	2	2	21	2	0	3	22
		%																		
VII	25	№	24	1	0	16	8	1	8	2	14	3	3	19	0	1	24	0	0	25
		%																		
VIII	25	№	23	2	0	1	19	5	0	15	10	0	11	14	0	0	25	0	0	25
		%																		
IX	25	№	25	0	0	25	0	0	22	2	1	16	8	1	6	14	5	2	1	22
		%																		
X	25	№	25	0	0	25	0	0	20	3	2	11	14	3	4	15	6	1	1	23
		%																		

notice: + high growth in medium; ± low growth in medium; - no growth in medium

References:

Avery S.V., Malkapuram S., Mateus C., Babb K.S. *Copper/Zinc-Superoxide Dismutase Is Required for Oxytetracycline Resistance of Saccharomyces cerevisiae* J. Bacteriol., **182**, 1, 76 - 80, 2000.

Cohen A., Nelson H., Nelson N. *The Family of SMF Metal Ion Transporters in Yeast Cells.* J. Biol. Chem., **275**, 43, 33388 - 33394, 2000.

Dubinin N. *Radioactive and chemical mutagenesis.* Selected Works, **2**, M.,"Nauka", 2000 (Russian).

Kreger-van Rij N.F. W. *Classification of yeasts.* In: The Yeasts. London etc., Acad. Press, v.**1**, 5-61, 1986.

Kvasnikov E., Shcholkova I. *The Yeast, biology and ways of reproduction.* Kiev, "Naukovo Dumka", 1991 (Russian).

Shatirishvili A., Chuchulashvili I. *The alteration of natural population of yeast induced by the action of heavy metals.* In: The actual problems of modern Biology. Tbilisi, Univ. Publ., 198-216, 2000.

Zakharov I., Kozhin S., Kozhina T., Fegorova I. Complete Works in Methodology of Genetics of Yeast - Saccharomyces. M., "Nauka", 1984 (Russian).

Welch I., Fogel S., Cathala G., Karin M. *Industrial yeast display tandem gene iteration of the region.* J. Mol. Cell. Biol., **3**, 8, 1353-1361, 1983.

მძიმე მეტალების იონებისადმი მდგრადი შტამების სიხშირის განსაზღვრა საფუარის ბუნებრივ პოპულაციებში

მაჭავარიანი ნ.[1], შათირიშვილი ა.[2], ჯორბენაძე ც.[1]

[1]ივ. ჯავახიშვილის თბილისის სახელმწიფო უნივერსიტეტი
[2]ი. ჭავჭავაძის სახელმწიფო უნივერსიტეტი

(მიღებულია 20.03.2006)

რეზიუმე

შილდის (კახეთი) ღვინის საფუარის ადგილობრივ პოპულაციიდან გამოყოფილ 250 შტამში შესწავლილია მძიმე მეტალების იონებისადმი (Cn, Mn და Cd) მდგრადობა. პოპულაცია, ამ ნიშნის მიხედვით, პოლიმორფული აღმოჩნდა. მასში გამოვლენილია "მგრძნობიარე", "საშუალო" და "მდგრადი" მორფები. მიკროპოპულაციებში მათი შეხვედრის სიხშირე არაერთგვაროვანი აღმოჩნდა.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

ERYTHROCYTIC PHENOTYPE FREQUENCY DISTRIBUTION IN POPULATION OF KEDA REGION (AJARA)

*NAGERVADZE M., DIASAMIDZE A., AKHVLEDIANI L., DUMBADZE G.

Sh. Rustaveli Batumi State University, Laboratory of Immunogenetics
**E-mail: mari-na@nm.ru*

(Received January 16, 2006)

Abstract

While studying erythrocytic phenotypes all four phenotype groups of *ABO* system: *O(I), A(II), B(III), AB(IV)*, were revealed within Keda population. Among them *O(I)* group occur with high frequency (58%), then comes *A(II)* group (32%). Frequency distribution of *B(III)* and *AB(IV)* groups is low – 8% and 2% correspondingly. Antigen frequency distribution of RH system was established as follows: *e* – 99%, *c* – 87%, *D* – 78%, *C* – 51%, *E* – 20%, C^w and D^u antigens were not revealed at all. 78% of Keda population is RH-positive. 44% of population is carrier of *M*, 41% - of *MN*, 14% - of *N* blood group. *K* antigen was not registered in Keda population.

Key words: blood group, genotype, erythrocyte, immunoserological method.

Introduction

Today 25 erythrocytic group systems involving up to 300 antigens are singled out [System of immunogenetical polymorphism, 2000]. Within world population blood group antigens are distributed non-uniformly [Nasidze, 1995; Salamatina, Nasidze, 1993; Varsahr et al., 2003; Schmidt, Scheil, 2003; Shneider et al., 2002; Kucher et al., 2000; Babu, Naidu, 1999; Udoh et al., 1999]. For forecasting of allosensitization caused by group antigens is significant to establish group antigen frequencies and peculiarities of erythricytic phenotypes.

The goal of our work was to study characteristics of erythrocytic antigens and, therefore, their phenotyping within the population of Keda region (Ajara).

Materials and Methods

Keda population (100 individuals) was researched on *ABO, Rh-Hr, Kell, MN* erythrocytic group systems by immunoserological methods. In particular, 13 erythrocytic group antigens were investigated: *A* an *B* antigens of *ABO* system; *C, c,* C^w*, D,* D^u*, E, e* antigens of *Rh* system; *K* and *k* antigens of *Kell* system; and *M* and *N* antigens of *MN* system. For this aim the standard serum of I, II, III and IV groups, standard erythrocytes (Hemostandard, Russia), and the following monoclonal antibodies: anti - AB, - B, - A, - M, - N, - K, - k, -C, -c, - D, - E, - e, - C^w, - D^u, were used.

Statistical treatment of the obtained data was carried out by Urbakh method [Urbakh, 1975].

Results and Discussion

Population polymorphism according to *ABO, Rh-Hr, Kell* and *MN* systems was recorded. Among above mentioned 13 antigens three antigens – C^w, D^u and *K*, do not appear at all in studied population. The rest 10 ones are characterized by different frequency distributions.

The all four phenotypic groups of *ABO* system – *O(I), A(II), B(III), AB(IV),* were recorded in Keda region. Among them *O(I)* group occur with highest frequency (58%), then comes *A(II)* group (32%). Frequency distribution of *B(III)* and *AB(IV)* groups is low – 8% and 2% correspondingly (Fig. 1).

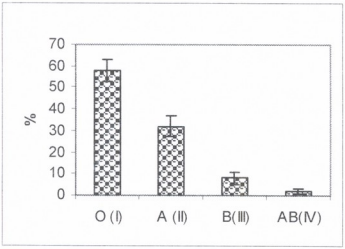


Fig. 1. Frequency distribution of ABO system phenotypes within Keda region population.

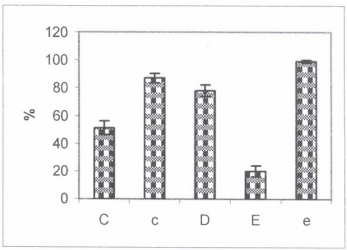


Fig. 2. Frequency distribution of RH antigens within Keda region population.

Among *Rh-Hr* system antigens almost every studied individual is carrier of *e* antigen (99%); frequency distributions of *c, D* and *C* antigens are the following: *c* – 87%, *D* – 78%, *C* – 51%, and of *E* antigen is very low – 20%, C^w and D^u antigens were not revealed at all (Fig. 2). According to *RH* system 78% of Keda population is *RH*-positive and 22% - *RH*-negative (Fig. 3). It means that theoretical expectance of allosensitization caused by *D* antigens is significant in this area.

While studying *MN* system antigens it was shown that 44% of population is carrier of *M* blood group, 41% - of *MN* group, 14% - of *N* group (Fig. 4).

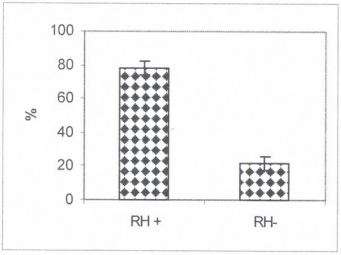


Fig. 3. Frequency distribution of RH+ and RH- phenotypes within Keda region population.

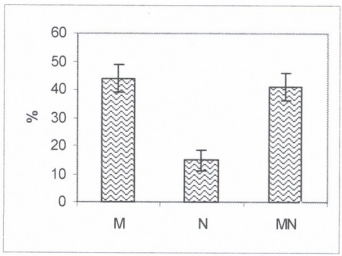


Fig. 4. Frequency distribution of M, N and MN groups within Keda region population.

According to studies by blood *Kell* group system, *K* antigen was never registered in Keda population, which means that every researched individual is carrier of *K*-negative phenotype (Fig.5).

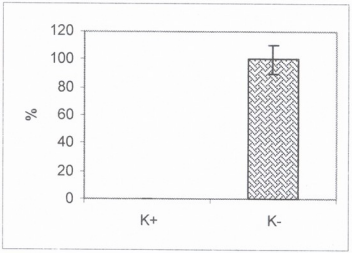


Fig. 5. Frequency distribution of K+ and K- groups within Keda region population.

Thus, according to the data obtained it was established that by erythrocytic antigenic systems population of Keda region is polymorphous. In particular, *O(I)* and *A(II)* erythrocytic phenotypes as distinct from *B(III)* and *AB(IV)* groups are distributed with high frequencies (58% and 32%, correspondingly). The frequencies of *Rh*-positive (78%), and *M* and *MN* phenotypes (44% and 41% correspondingly) of *MN*-system are also high. *K* antigen was not registered at all in researched population.

References:

Babu BV., Naidu JM. *Genetic variability of blood and saliva antigens and serum proteins among subtribes of Mali from Andhra Pradesh, India.* Anthropol Anz., **57**, 2, 105-110, 1999.

Kucher AN., Puzyrev VP., Chernetsov DB., Erdynieva LS., Sanchat NO. *Polymorphism of immunological and biochemical marker genes in rural populations of the Tuva Republic.* Genetika, **36**, 4, 562-9, 2000.

Nasidze IS. *Genetic polymorphisms of the Caucasus ethnic groups: Distribution of some serum protein and red cell enzyme genetic markers.* Gene Geogr., **9**, 2, 91-116, 1995.

Salamatina NM., Nasidze IS. *Genetic polimorfisms in a rural Osetian community.* Gene Geogr., **7**, 3, :251-5, 1993.

Schmidt HD., Scheil HG. *Blood group frequencies in Romania: microregional and ethnic differences. Anthropol.* Anz. **61**, 4, 381-93, 2003.

Shneider IuV., Shilnikova IN, Zhukova OV. *Materials for studying the gene pools of Russia and neighboring countries: the Russian population of the Pskov region.* Genetika, **38**, 11, 1561-5, 2002.

Systems of immynogenetical polymorphism. In:"Gene pool and gene geography of population." Saint-Petersburg, "Nauka", **1**, 611, 2000.

Udoh EA., Usanga EA., Emeribe AO. *Frequency of MNSs blood groups in south eastern Nigeria.* East Afr. Med. J. **74**, 7, 442-3, 1997.

Urbakh VIu. *Statistical analysis in biological and medical studies.* Moscow, "Medicine", p.153, 1975 (in Russian).

Varsahr AM., Dubova NA., Kutuvev IA. *Serological researches in the south of Moldavia in connection with the problem of the Gagauzes, the Moldavians and Bulgarians.* Anthropol. Anz. **61**, 4, 395-411, 2003.

ერითროციტური ფენოტიპების გავრცელების სიხშირე ქედის რაიონში

ნაგერვაძე მ., დიასამიძე ა., ახვლედიანი ლ., დუმბაძე გ.

შ. რუსთაველის ბათუმის სახელმწიფო უნივერსიტეტი, იმუნოგენეტიკის ლაბორატორია

(მიღებულია 16.01.2006)

რეზიუმე

ქედის რაიონის მოსახლეობაში ერითროციტური ფენოტიპების კვლევისას გამოვლინდა *ABO* სისტემის ოთხივე *O(I), A(II), B(III), AB(IV)* ფენოტიპური ჯგუფი. მათგან მაღალი სიხშირით (58%) გვხვდება *O(I)* ჯგუფი, შემდეგ – *A(II)* ჯგუფი (32%), დაბალია *B(III)* და *AB(IV)* ჯგუფების სიხშირე (8 და 2%). დადგინდა RH-სისტემის ანტიგენების გავრცელების შემდეგი სიხშირეები: *e* - 99, *c* - 87, *D* - 78, *C* - 51%, დანარჩენ ანტიგენებთან შედარებით დაბალია *E*-ს მაჩვენებელი (20%), C^w და D^u ანტიგენები კი საერთოდ არ გამოვლინდა. ქედის მოსახლეობის 78% RH-დადებითია, 22% კი – RH-უარყოფითი. მოსახლეობის 44% სისხლის *M*, 41% – *MN*, ხოლო 14% - *N* ჯგუფის მატარებელია. პოპულაციაში K ანტიგენი საერთოდ არ დაფიქსირდა.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

Genetics

STUDY OF HETEROMORPHISM OF HETEROCHROMATIN AT PARANOIAC SCHIZOPHRENIA

TALASHVILI T.[1], BABLISHVILI N.[2], TADUMADZE N.[2], ZURABASHVILI ZIG.[1]

[1] *M. Asatiani Tbilisi Institute of Psychiatry*
[2] *Iv. Javakhishvili Tbilisi State University, Department of Genetics*

(Received June 12, 2006)

Abstract

Polymorphism of centromeric C-structural heterochromatin was studied in the patients with paranoiac schizophrenia. Heterogeneity was registered for 1st and 9th chromosomes, whereas for 16th chromosome stability was noted. At the same time polymorphism for 1st and 9th chromosomes was evoked by increasing of large size segments.

Introduction

It is known that chromatin is composed of different functional domains. Heterochromatin involves facultative heterochromatin and constitutional heterochromatin, which is entirely constructed by sequences of noncoding satellite DNA. At the metaphase of mitosis C-bands localized in centromeric regions of chromosomes are revealed [Carvalho, et al., 2001].

The data about heteromorphism of C-structural heterochromatin in the cases of some mental diseases (senile dementia, oligophrenia) were recently presented [Dadunashvili, et al., 2004; Tabatadze, et al., 2005].

The aim of our research was to estimate heteromorphism of C-structural heterochromatin in the case of paranoiac schizophrenia.

Materials and Methods

Heteromorphism of C-structural heterochromatin was studied in 10 patients with paranoiac schizophrenia and 10 healthy individuals by modified method of Fernandez [Fernandez, et al., 2002]. 200 metaphases from individuals with schizophrenia and 200 metaphases from healthy controls were analyzed. The types of C-segment variants were determined using Patil and Lubs' classification system, which singles out 5 variants: a<0,5x16p; 0,5x16p<a<1x16p; 1x16p<c<1,5x16p; 1,5x16p<d<2x16p; e>2x16p. Statistical analysis was performed by Zax formula:

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

where η – is an amount of certain variants (a, b, c, d) in the control group; μ - is an amount of certain variants (a, b, c, d) in the group diseased with paranoiac schizophrenia; n – is an amount of all variants of C-segments in control group; m – is an amount of all variants of C-segments in the group with paranoiac schizophrenia.

Results and Discussion

At the first stage of research comparative analysis of C-segments of 1st, 9th and 16th chromosomes was carried out by summary parameters. The data obtained for individuals with paranoiac schizophrenia were compared with the data of control group. Results of analysis are presented in Table 1.

Table 1. Heteromorphism of C-segments for the individuals with paranoiac schizophrenia

Variants of C-segments	η_1	μ_1	η_1/n	$\frac{\eta_1+\mu_1}{n+m}$	χ^2
a	290	338	0,2504	0,2666	χ^2_3=44.4
b	546	440	0.4715	0.4185	
c	274	303	0.2366	0.2449	p<0.001
d	48	117	0.0415	0.0700	

As is seen from the table the frequency of occurrence of large size (c, d) C-heterochromatin segments is higher in the diseased group. Consequently, statistical valid difference between studied groups was revealed: $\chi^2_3 = 44.4$, $p < 0.001$.

Table 2. Polymorphism of C-segments of 1st, 9th and 16th chromosomes of the individuals with paranoiac schizophrenia

Chro-mosome	variants of C-segments	η_1	μ_1	η_1/n	$\frac{\eta_1+\mu_1}{n+m}$	χ^2
1st	a	34	31	0,0872	0,0823	χ^2_3=27,96 p<0,001
	b	158	125	0,4051	0,3582	
	c	160	150	0,4103	0,3924	
	d	38	94	0,0974	0,1671	
9th	a	78	95	0,2021	0,2207	χ^2_3=17,86 p<0,001
	b	198	149	0,5130	0,4426	
	c	100	131	0,2591	0,2946	
	d	10	23	0,0259	0,0421	
16th	a	178	212	0,4660	0,4987	χ^2_3=5,97 p>0,05
	b	190	166	0,4974	0,45552	
	c	14	22	0,0366	0,0460	
	d	-	-	-	-	

While studying polymorphism of C-segments by separate chromosomes (1st, 9th, 16th) heterochromatic nature of 1st and 9th chromosomes was shown (Table 2). In the 1st chromosome of individuals with paranoiac schizophrenia the frequency of large segments (c, d) increased with statistical validity: $\chi^2_3 = 27.06$, $p < 0.001$. Analogous result was found out for 9th chromosome - $\chi^2_3 = 17.86$, $p < 0.001$. As distinct from those chromosomes 16th chromosome maintained stability - $\chi^2_3 = 5.97$, $p < 0.05$. It should be mentioned that decrease of the frequency of small size b variant was registered in all three chromosomes of diseased individuals.

The obtained results are in agreement with the scientific data. Some authors noted the heteromorphism of C-heterochromatin. Variability of C-segments of 9th chromosome was also

mentioned [Kuznetsova, et al., 1996; Haaf & Schmid, 2000]. Variability of 9th chromosome was found in the case of some pathologies [Narayan, et al., 1998].

Polymorphism of C-heterochromatin for all three pairs of chromosomes was recorded in patients with Alzheimer's disease, vascular dementia and oligophrenia [Tabatadze et al., 2005]; Dadunashvili et al., 2004].

References:

Carvalho C., Pereira H., Ferreira J., Pina C., Mendonca D., Rosa A., Carmo-Fosneca M. *Chromosomal C-dark bands determine the spatial organization of centromeric heterochromatin in the nucleus.* Mol. Biol. Cell, **12**, 3563-3572, 2001.

Dadunashvili E., Jokhadze T., Bablishvili N., Tadumadze N., Didebulidze D. *The study on polymorphism of C-heterochromatin in patients with undifferentiated from of oligophrenia.* Proc. Georg. Acad. Sci. Biol. Ser.B, **2**, 3-4, 61-64, 2004.

Fernandez R., Barragas M., Bullejos A., Marchal I., Diaz L., Sanchez A. *New C-band protocol by heat denaturation in the presence of formamide.* Hereditas, **137**, 145-148, 2002.

Haaf T., Schmid M. *Experimental condensation inhibition in constitutive and facultative heterochromatin of mammalian Chromosome.* Cytogen. Cell. Genet., **91**, 113-123, 2000.

Kuznietsova S., Kuranova N., Kalashvikov M. *Chromosomal polymorphism, the biological and medical aspects.* Cytol. Genet. **30**, 2, 67-74, 1996 (in Rus.).

Narayan A., Tsien F., Sawyer T., Erliech M. *Heterochromatin decondensation and DNA hypomethylation in cultured cells from patients with ICF.* Tulane Health Research, **2**, 12-16, 1998.

Tabatadze N., Dadunashvili E., Lezhava T. Polymorphism of structural C-heterochromatin in patients affected with senile dementia. Proc. Georg. Acad. Sci. Biol. Ser.B, **3**, 2, 70-77, 2005.

C-სტრუქტურული ჰეტეროქრომატინის ჰეტერომორფიზმის შესწავლა პარანოიდული შიზოფრენიის შემთხვევებში

თალაშვილი თ.[1], ბაბლიშვილი ნ.[2], თადუმაძე ნ.[2], ზურაბაშვილი ზიგ.[1]

[1]მ. ასათიანის თბილისის ფსიქიატრიის ინსტიტუტი
[2]ივ. ჯავახიშვილის თბილისის სახელმწიფო უნივერსიტეტის გენეტიკის კათედრა

(მიღებულია 12.06.2006)

შესწავლილია ცენტრომერული C-სტრუქტურული ჰეტეროქრომატინის პოლიმორფიზმი პარანოიდული შიზოფრენიით დაავდებულ ინდივიდებში. ჰეტეროგენულობა დაფიქსირდა 1-ლი და მე-9 ქრომოსომებისათვის მაშინ, როცა აღინიშნა მე-16 ქრომოსომის სტაბილურობა. ამასთან, 1-ლი და მე-9 ქრომოსომების პოლიმორფულობა, ძირითადად, განპირობებული იყო დიდი ზომის სეგმენტების გაზრდით.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

BIODEGRADATION OF OIL PRODUCTS BY MICROSCOPIC FUNGI

KHAREBASHVILI M., KHOKHASHVILI I., *KUTATELADZE L.

S. Durmishidze Institute of Biochemistry and Biotechnology
**E-mail: kutateladze@geomail.ge*

(Received April 5, 2006)

Abstract

The possibility of restoration of soils polluted with oil products by microbiological means has been studied. The pure cultures of microscopic fungi belonging to different genera were isolated from soils polluted with oil products, in particular: *Trichoderma viride N 1-9, Aspergillus niger N 2-2, Trichotecium S1-6, Mucor K 1-1* and *Aspergillus N 3-5.* Ability of carbohydrate assimilation by the fungi has been investigated. Experiments were set both in sterile and unsterile - field conditions. It was established that selected microorganisms possess ability of assimilation of oil products and may be successfully used for restoration of polluted soils.

Key words: bioremediation, mazut, biotransforming activity, *Aspergillus.*

Introduction

Oil products are considered as one of the main pollutants of environment today. After they appear in soil and water basins, they reveal poisonous effect on living organisms, attenuate their vital abilities [Belousova et al., 2002]. Releasing the water basins and soil from oil products is considered as one of the main tasks of biotechnology and environment protection. Bioremediation appears to be one of the perspective methods of soil restoration [Alexsander, 1994]. Detoxifyng properties of microorganisms are studied intensively in many countries. Number of investigators has revealed detoxifying strains but those were mainly bacteria and basidial fungi [Eggen, 1999, Stabnikova et al., 1996]. Microscopic fungi have been less investigated from this point of view. According to literature data of recent years some of them possess this ability.

The purpose of the given study was to isolate the pure cultures of detoxifying strains of microscopic fungi from polluted soils, also to investigate destructing abilities of the strains both in sterile and unsterile soils.

Materials and Methods

Soils samples for isolation microorganisms were got from territories polluted with oil products, in particular, from surrounding territory of Patardzeuli oil well. Soil samples were sowed on solid nutrient medium using Waksman's diluting method [Waksman, 1916]. After sowing Petri dishes with testing material were placed in thermostat at 30°C. 3, 5, 7 and 10 days later experimental samples were observed and described.

For studying the detoxifying ability, pure cultures, isolated from polluted soil, were grown at Chapek-Docks's modified medium with adding black oil. Assimilation of black oil was evaluated visually, according to cultural and morphological features.

The biotransforming activity of strains was studied under submerged cultivation adding to the nutrient medium different concentrations of black oil as the only source of carbon. 10 days-old suspensions of cultures were used as sowing material.

Submerged cultivation was done on thermostated shaker (180rot/min), at 25°-30°C during 3-4 days. Two variants were used as a control: a) nutrient medium with experimental culture without adding black oil, and b) nutrient medium with black oil, without experimental culture. Extent of oil biotransformation was evaluated following the amount of residual black oil, using gravimetric method. Black oil was extracted by means of chloroform. The biotransforming activity was calculated following the formula:

$$A_t\ (\%) = (P_c - P_t)/P_t \cdot 100$$

Where, A_t is the biotransforming activity (%), P_t is the residual weight of sample (g), P_c is the residual weight of the control sample (g).

In field experiment as models were used unstrile black and red soils, preliminary polluted with determined concentration (3%) of oil. Sterile soils, contaminated with the same concentration of oil were used as control.

The cultural liquid, obtained by means of submerged cultivation, together with biomass was added to both, sterile and unsterile soils in equal amounts.

Results and Discussion

It is known that soil contamination with different toxicants changes its biochemical balance, in particular, microorganisms able to degrade contaminating toxins are activated.

Experimentally it was corroborated that as more is the extent of soil pollution, as more but monotonous are the microorganisms there. Among the isolated cultures prevailed genera: *Aspergillus, Mucor, Rizophus, Trichoderma, Penicillium, Trichotecium, Fuzarium.*

About 40 species of microorganisms were tested on detoxifying ability. Cultures were grown at different concentrations of black oil both, at solid and liquid nutrient mediums. Biodegrading ability of cultures grown at a solid medium was evaluated using three-mark system, following their cultural and morphological features.

According to the experimental data 16 cultures developed well at low concentration of mazut (10mg/l), 9 cultures – at 20mg/l and only 5 cultures developed at high concentration (30mg/l) of mazut. At higher concentrations of black oil increment of fungi was low.

By means of our work 5 strains of microscopic fungi, able to grow at high concentration (30mg/l) of oil were selected: *Trichoderma viride N 1-9, Aspergillus niger N 2-2, Trichotecium S1-6, Mucor K 1-1, Aspergillus N 3-5* (Table1).

Further observations were done on those 5 cultures. The optimal conditions for cultivation of studied cultures were determined and optimization of the nutrient medium was done for the purpose to increase the biotransforming activity of cultures.

As the temperature is one of the important factors for growth regulation and physiological activity of microorganisms, its influence on biotransforming activity of the cultures have been primarily studied. The submerged cultivation was done at different temperatures from 20° to 55°C, with 5°C interval. The amount of residual mazut was determined in a cultural liquid. As control were used: a) nutrient medium with sowed culture without mazut and b) medium with mazut, without culture. Experimental data are demonstrated on the Fig. 1.

Table 1. Development of microscopic fungi on different nutrient mediums and at different concentrations of mazut

Nutrient medium	Culture	Growth intensity at a given concentrations of mazut			
		10g/l	20g/l	30g/l	50g/l
Universal	*Mucor K1-1*	+++	+++	+++	++
	MucorSh 6-3	+++	+++	++	++
	Trichoderma viride N1-9	+++	+++	+++	++
	Trichoderma N2-3	+++	+++	++	++
	Trichotecium S1-6	+++	+++	+++	+
	Penicillium N-2	++	++	++	+
	A.niger N2-2	+++	+++	+++	++
	Aspergillus N3-5	+++	+++	+++	++
	Fusarium mon S2-6	++	++	++	+
Chapek's	*Mucor K1-1*	+++	+++	+++	++
	MucorSh 6-3	+++	+++	+++	++
	Trichoderma viride N1-9	+++	+++	+++	++
	Trichoderma N2-3	+++	+++	+++	++
	Trichotecium S1-6	+++	+++	+++	+
	Penicillium N-2	++	++	++	+
	A.niger N2-2	+++	+++	+++	++
	Aspergillus N3-5	+++	+++	+++	++
	Fusarium mon S2-6	++	++	++	+

Physiological activity of microorganisms significantly depends on pH of the medium too. To determine the optimal pH, the selected destructor strains were grown on a liquid nutrient mediua with varying pH from 2 to 9 (Table 2).

Table 2. Influence of different meanings of pH on assimilation of mazut by the microscopic fungi of high detoxifying activity

Culture	Mazut residuals (%) at different meanings of pH of the incubating medium		
	pH 2.0	pH6.0	pH9.0
Trichoderma viride S1-9	45.5	11.0	15.0
Aspergillus niger N2-2	20.0	15.0	12.0
Trichotecium sp. S1-6	40.0	21.0	40.0
Mucor sp. K1-1	32.0	0	19.0
Aspergillus sp. N3-5	38.0	17.0	25.0

Obtained data show that the strains of microscopic fungi *Trichoderma viride N 1-9* and *Mucor K 1-1* revealed the maximal ability of degrading of oil products at pH6, while cultures *Aspergillus niger N 2-2* and *Aspergillus N3-5* were mostly effective at pH9.

The optimal duration of cultivation was also established (Fig. 2). From the picture it is clear that for microscopic fungi *Aspergillus niger N 2-2* and *Trichoderma viride N 1-9* optimal was 72h of cultivation, while for another three strains: *Trichotecium S1-6, Mucor K 1-1* and *Aspergillus N 3-5* – 96h did (Fig. 2).

On the next step of investigation the biotransforming properties of selected cultures Aspergillus niger N 2-2 and Trichoderma viride N 1-9 were tested by means of their inoculation in unstrile, contaminated with mazut red and black soils. The same types of sterile soils were taken as control.

After 40 and 100 days of incubation in each sample the amounts of residual mazut and colonies per gram of dry soil was calculated. Results are demonstrated on Fig. 3.

From the figure it is clear that contamination of soils with oil products causes activation of aboriginal microorganisms. Moreover, the extant of biodegradation of mineral oil makes 20%, and for inoculated strains this index reaches 60-70%.

Among the tested cultures microscopic fungi *Trichoderma viride N 1-9* appeared to be especially active.

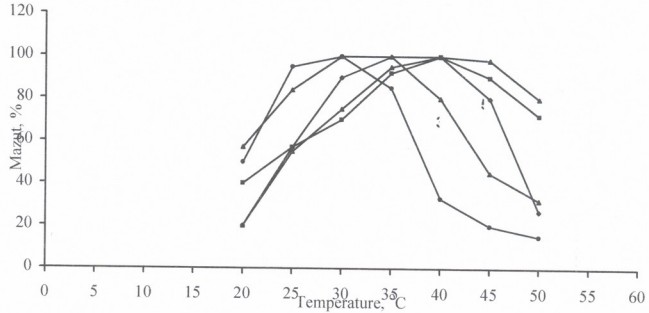


Fig. 1. Influence of temperature on assimilation of oil products by microscopic fungi. **1**-Aspergilus niger N2-2; **2**-Trichoderma viride N1-9; **3**-Aspergillus N3-5; **4**-Mucor K1-1; **5**-Trichotecium N1-6.

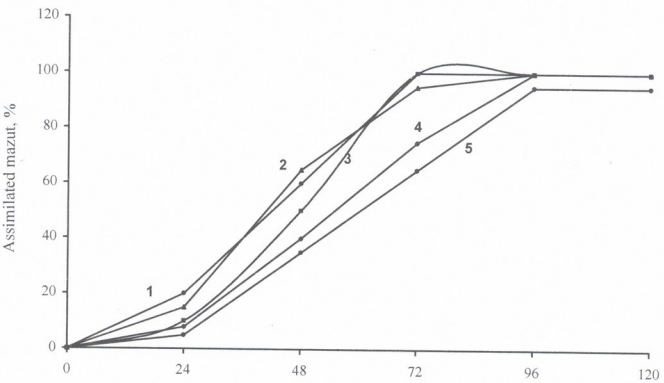


Fig. 2. Influence of duration of biodegradation on the extent of mazut biodegradation. 1. *Mucor* K1-1; 2. *Trichoderma viride* N1-9; 3. *Trichothecium* N1-6; 4. *Aspergilus* N3-5; 5.*Aspergillus niger* N2-2. Mazut concentration 30mg/l

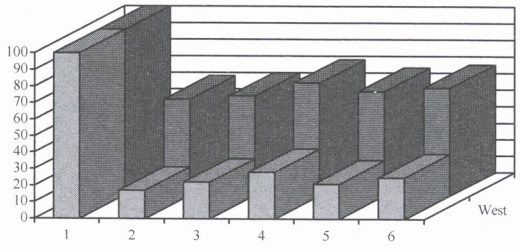


Fig.3. Biodegradation of oil products in sterile (front) and unsterile soils by the microscopic fungi (back). **1.** Control, **2.** *Trixoderma viride N1-9,* **3.** *Aspergillus nigeri N2-2,* **4.** *Aspergillus N3-5,* **5.** *Mucor K 1-1,* **6.** *Trixotecium S1-6*

References:

Alexsander M. *Biodegradation and Bioremediation.* Acad. Press, San Diego, Calif., 1994.

Belousova N. I., Barishnikova L. M., Shkidchenko A. N. *Selection of microorganisms able to destruct oil and oil products at low temperatures.* Appl. Biochem. Microbiol., **38,** 5, 513-517, 2002 (Russian).

Eggen T. Application of fungal substrate from commercial mushroom production – Pleurotus ostreatus – for bioremediation of creosote contaminated soil. Int. Biodeter Biodegr., **44**, 117-126, 1999.

Stabnikova E. V., Seleznova M. V., Dulgerov A. I., Ivanov V. N. *Application of biopreparation "lestan" for soil remediation polluted with oil carbohydrates.* Appl. Biochem. Microbiol., **32**, 2, 219-223, 1996.

Waksman S.A. *Soil fungi and their activities.* – Soil., Sci., **2,** 1,103-105,1916.

ნავთობპროდუქტების ბიოდეგრადაცია მიკროსკოპული სოკოებით

ხარებაშვილი მ., ხოხაშვილი ი., ქუთათელაძე ლ.

ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 05.04.2006)

რეზიუმე

შესწავლილია ნავთობპროდუქტების დაბინძურებული ნიადაგების გასუფთავების შესაძლებლობა მიკრობიოლოგიური საშუალებებით. ამ მიზნით დაბინძურებული ნიადაგებიდან გამოყოფილია სხვადასხვა გვარის მიკროსკოპული სოკოების სუფთა კულტურები *Trichoderma viride N 1-9, Aspergillus niger N 2-2, Trichotecium S1-6, Mucor K 1-1 and Aspergillus N 3-5* და გამოკვლეულია მათ მიერ ნახშირწყალბადების ასიმილაციის უნარი. გამოკვლევები ჩატარებულია როგორც სტერილურ, ასევე არასტერილურ, საველე პირობებში. შესწავლილია ნავთობპროდუქტების საწყისი და ნარჩენი რაოდენობები დაბინძურებულ ნიადაგებში. დადგენილია, რომ შერჩეულ მიკროორგანიზმებს გააჩნიათ ნავთობპროდუქტების ასიმილაციის მაღალი უნარი და ისინი წარმატებით შეიძლება გამოვიყენოთ დაბინძურებული ნიადაგების გასასუფთავებლად.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

MICROSCOPIC FUNGI, PRODUCERS OF HYDROLITIC ENZYMES, FROM VARIOUS REGIONS OF GEORGIA

*KUTATELADZE L., URUSHADZE T., CHVEDELIDZE R., CHKARTISHVILI D., DAUSHVILI L., BURDULI T., KVESITADZE E.

S. Durmishidze Institute of Biochemistry and Biotechnology
E-mail: kutateladze@geomail.ge

(Received November 14, 2005)

Abstract

146 cultures - the producers of enzymes were selected from 351 microscopic fungi cultures isolated from various ecological niches of southern slops of Caucasus. Their biosynthetic potential, focused on intensive biosynthesis of biologically active materials, especially enzymes, was studied. 37 strains were selected as α-amylase producers, 47- producers of glucoamylase, 55 - of cellulase, 16 - of proteinase. Certain number of cultures is producer of both α-amylase and glucoamelase. Degree of extremophility of selected producers was established.

Key words: producers, extremophilisity, α-amylase, glucoamylase, cellulase

Introduction

In recent 20 years microbiological synthesis has become the most intensively developing trends. At present, microbial synthesis actively competes with chemical synthesis in obtaining low molecular compounds, including secondary metabolites in industrial scales. As for the synthesis of high molecular compounds, in particular, enzyme microbial synthesis has no analogies [Niehaus et al., 1999].

In order to work out qualitatively new technologies based on the enzymes isolated from microorganisms selection of microorganisms living under extreme conditions (high temperature, acid and alkaline conditions) seems extremely important [Schafer et al., 2000].

From the viewpoint of selection of extremophilic mycelial fungi, great interest has been focused on Caucasian region, including almost all kinds of extreme conditions. Creation of collection characteristic for Caucasus mycelial fungi made possible to search for the producers of target enzymes (α-amylase, glucoamylase, cellulase, proteinase) among the mycelial fungi cultures, to select highly active strains, to obtain enzyme preparations, to estimate their stability against extreme conditions.

Materials and Methods

Among the mycelial microscopic fungi collection isolated from various ecologic niches of Caucasus screening of producers of enzymes was carried out. In order to reveal the target enzymes deep cultivation of microscopic fungi was accomplished in 750 ml Erlinmayer conical flasks on

thermostatic shaker (150-200t/min) at 30°C for 72 hours, as a carbon source were selected the substrates where inductive synthesis of enzymes supposed to take place.

To obtain amylases, deep cultivation was carried out in a liquid medium supplemented with (%): starch-6.0, $NaNO_3$-0.91; KH_2PO_4-0.1; $MgSO_4 \cdot 7H_2O$-0.05, KCl-0.05, $FeSO_4$-0.0002, malt spate-3.0 per 100ml.

In case of cellulase, deep cultivation was carried out in a liquid medium supplemented with (%): microcrystalline cellulose-0.1; $NaNO_3$-0.3; KH_2PO_4-0.2; $MgSO_4 \cdot 7H_2O$-0.05; maize extract-1.5.

To obtain proteinase, deep cultivation was performed in a liquid supplemented with (%): KNO_3-0.1; KH_2PO_4-0.1; $MgSO_4 \cdot 7H_2O$-0.0007; KCl-0.05. $FeSO_4$-0.005; east extract-0.5; casein-0.1.

Total cellulase activity was determined by method based on potential of cellulase to perform hydrolysis on insoluble substrate (in particular, filter paper) to monosaccharides and oligosaccharides [Ghose, 1987]. Glucoamylase activity was estimated according to the amount of glucose yielded as a result of enzyme operation and was determined by [Dalgvist, 1961]. Estimation of α-amylase was performed according to [Ruchliadeva et al., 1960]. For proteinase activity assessment the modified method of Anson [Mosolov, 1971] was used.

Results and Discussion

The analyses of obtained results suggest that, among isolated microscopic fungi cultures, 84 are amylase producers; 47 - of glucoamylse and 37 - of α-amylase, 55 - cellulase producers; that of proteinase-16 (Tab. 1).

The most active amylase producers are black Aspergillus. Two representatives of the genus Mortierella are of interest, since in literature it is not mentioned as the producer of amylase.

Among cellulase producers, as it was expected, Aspergillus and Penicilium genera dominate, though it should be mentioned, that cellulase producers are 6 cultures of Chaetomium and 2 cultures of Sporotrichum. From the results of studies is apparent, that representatives of the genera Aspergillus and Mucor are active producers of proteinases.

Fungi of various genera reacted differently on temperature changes. Generally thermostability of microorganisms exceeds the ranges of biokinetic zone. If the potential of thermophiles to develop at high temperature is genetically determined property, mesophiles ability to grow at high temperature is conditioned by the adaptation of microorganisms to unfavorable environment, at that, vitality or non-vitality of microorganism is determined by thermostability of cell compounds (especially enzymes).

According to literature data [Cooney et al., 1964] thermophilic microscopic fungi are considered cultures, which maximum rate of growth is above 50°C; to thermotolerant cultures can be related those cultures whose maximal growth temperature is around 50°C and minimal not below 20°C. Facultative phsychrophiles develop at low temperature, but their maximal temperature is far more higher than that of any obligative phsychrophiles. They are called frost resistant or phsychrotolerant.

In order to estimate the optimal temperature afore mentioned cultures (enzyme producers) were grown under temperature regime within 5°-55° C increased by 5° of interval. The obtained data suggest that among α-amylase producers 4 cultures were thermophiles, 2 phsychrophiles, 3 cultures were phsychrotolerant and 4 were mesophiles. Among thermophiles prevail the representatives of Mucor genus (Tab. 2).

Among cellulase producers, as compared with other enzyme producers, prevail any thermophile cultures (Tab. 3)

Producers of glucoamilase and proteinase extremophilic fungi are shown in Table 4 and Table 5.

Fungi of various genera at high and low pH develop differently. Though it should be mentioned, that most of microscopic fungi cultures grow within wide pH range, in particular between pH 2,0-10,0. Alkalinity and acidity of the media are the parameters determining spreading of microorganisms under extreme conditions. The subject of study - strains of microscopic fungi has been isolated from the regions were the soils are characterized by increased acidity and alkalinity, so the probability of isolation of acido- and akcaliphilic microscopic fungi was high.

To estimate the optimal pH of growth, the experimental cultures were grown within pH-2.0 -10.0 with intervals of pH-0.5 in the initial medium.

Among α-amylase producers 6 cultures appeared to be acidophile, 3 - alcaliphile. Others were well developing at a wide range of pH (Tab. 2). Among cellulase producers only 3 producers appeared to be alkaliphiles (Tab. 3). Among the glucoamylase producers prevail alcaliphile producers (Tab. 4).

Great attention is focused on mycelial fungi growing under such extreme condition as high salt concentration. Enzymes of microorganisms living under salty conditions represent a special group of proteins able to operate in critical physiological conditions. Therefore, revealing of halophiles within the mycelial fungi collection was important issue. For this aim, the cultures were grown on following universal medium: 0.5 l molasses, 0.5 l running water, 20g agar-agar (pH 5.5-6.0)/liter of nutrient medium; NaCl was administered in various concentrations from 0.5M up to 4.0M (2,93%-23,2% as appropriate). Cultivation on the solid surface was done at 30°C. In experiments carried out at 25°C, cultures of microorganisms able to grow in 4M NaCl are classified as highly tolerant against salt, whereas at 20°C it can be considered as a moderate halophile. As it follows from the data among enzyme producer - microscopic fungi cultures 8 strains appeared to be extreme halophiles, they were grown on the medium with: NaCl from 1M(16%) to 4M (24%) and optimal development was achieved in presence of 3M (18%) NaCl. Halophilic cultures were noted having low rate of reproduction and low density of population. 11 cultures have been considered as moderate halophiles, they grow up from 3M to 5M of NaCl concentration with growth optimum 2.5M of NaCl. The results of these experiments are presented in Tables 2, 3, 4, 5.

Summarizing we can say that, among mycelial fungi cultures isolated from various ecologic niches of Caucasus 25% are extremophiles. At present collection of extremophilic mycelial fungi has been already created. It does not cover all extreme conditions of Caucasus, but presents the existing diversity of mycelial fungi.

Table1. Microscophic fungi-producers of hydrolitic enzymes

#	cellulase producers	glucoamylase producers	α-iamylase producers	proteinase producers
1	*Aspergillis* sp. S51	*Aspergillis* sp. S51	*Aspergillis* sp. S53	*Aspergillus niger* T22
2	*Aspergillus* sp. S 60	*Aspergillis* sp. S52	*Aspergillis* sp. S54	*Aspergillus* sp. S54'
3	*Aspergillus* sp. S71	*Aspergillis* sp. S53	*A. versicolor* S83	*Aspergillus* sp. S71
4	*Aspergillus* sp. T2	*Aspergillis* sp. S58	*Aspergillis* sp. S63	*Aspergillus niger* S73
5	*Aspergillus* sp. T5	*Aspergillus* sp. S 60	*Aspergillis* sp. S71	Aspergillus niger S64
6	*Aspergillus* sp. T6	*Aspergillis niger* S64	*Aspergillis* sp. T10	*Aspergillus niger* T14
7	*Aspergillus* sp. T9	*Aspergillis niger* S65	*Aspergillis* sp. R5	*Aspergillus* sp.S66
8	*Aspergillus* sp. T10	*Aspergillis* sp. S71	*Aspergillis* sp. S54'	*Aspergillus* sp. P5
9	*Aspergillus* sp. T20	*Aspergillis* sp. S73	*Aspergillus* sp. S 16	*Aspergillus* sp. S55
10	*Aspergillus* sp. T55	*Aspergillis* sp. T2	*Aspergillis niger B47*	*Mucor* sp. T37
11	*Aspergillis versicolor* S83	*Aspergillis* sp. T10	*Aspergillis niger B80*	*Mucor* sp. T44
12	*Aspergillis terreus* T39	*Aspergillis* sp. T31	*Aspergillis. oryzae* S27	*Mucor* sp. T54

13	*Aspergillis batatae* B50	*Aspergillis* sp. S2	*Fusarium* sp. S56	*Aspergillus Tamarii* T53
14	*Chaetomium* sp. S67	*Aspergillis* sp. K2	*Fusarium* sp. R16	*Aspergillus nidulans*
15	*Chaetomium* sp. S77	*Aspergillis.niger* M 8	*Fusarium* sp. R20	Mucor plumbus B17
16	*Chaetomium* sp. S5	*Aspergillis awamori* S11	*Fusarium* sp. S27	*Rhizopus* sp. K20
17	*Chaetomium* sp. S26	*Aspergillis awamori* T23	*Fusarium* sp. R29	
18	*Chaetomium* sp. P36	*Aspergillis niger* 68B	*Rhizopus* sp. S75	
19	*Chaetomium* sp. T35	*Aspergillis niger* 75B	*Rhizopus* sp. S76	
20	*Trichoderma* sp. S78	*Aspergillis* sp. S51'	*Rhizopus* sp. T33	
21	*Trichoderma* sp. P10	*Penicillium* sp. P5	*Trichoderma* sp. S79	
22	*Penicillium* sp. P11	*Penicillium* sp. S48	*Trichoderma* sp. P10	
23	*Penicillium* sp. S80	*Penicillium* sp. S80	*Trichoderma* sp. P11	
24	*Penicillium* sp. S84	*Penicillium* sp. T21	*Penicillium* sp. S80	
25	*Penicillium* sp. T21	*Penicillium* sp. T25	*Penicillium* sp. T21	
26	*Penicillium* sp. T25	*Penicillium* sp. S32	*Penicillium* sp. T25	
27	*Penicillium* sp. S4	*Penicillium* sp. S35	*Penicillium* sp. T26	
28	*Penicillium* sp. S10	*Penicillium* sp. S46	*Penicillium* sp. R15	
29	*Penicillium* sp. S29	*Penicillium* sp. T57	*Penicillium* sp. S46	
30	*Penicillium* sp. S35	*Penicillium* sp. K17	*Penicillium* sp. T52	
31	*Penicillium* sp. S60'	*Penicillium* sp. K28	*Penicillium* sp. T57	
32	*Penicillium* sp. T53	*Mucor* sp. S57	*Mucor* sp. T37	
33	*Penicillium* sp. K17	*Mucor* sp. K32	*Mucor* sp. R33	
34	*Penicillium* sp. K19'	*Mucor* sp. K55	*Mucor* sp. K22	
35	*Penicillium* sp. K36	*Chaetomium* sp. S67	*Mucor* sp. T18	
36	*Cladosporium* sp. T27	*Chaetomium* sp. S77	*Cladosporium* sp. T48	
37	*Cladosporium* sp. T48	*Chaetomium* sp. P36	*Mortierella* sp. S34	
38	*Fusarium* sp. T30	*Chaetomium* sp. S'48		
39	*Fusarium* sp. R8	*Cladosporium* sp. T38		
40	*Fusarium* sp. R20	*Cladosporium* sp. T48		
41	*Fusarium* sp. R27	*Rhizopus* sp. R1		
42	*Fusarium* sp. R28	*Rhizopus* sp. R22		
43	*Fusarium* sp. S10	*Rhizopus* sp. S33		
44	*Fusarium* sp. S61'	*Rhizopus* sp. S13		
45	*Fusarium* sp. P4	*Rhizopus* sp. P39		
46	*Rizhopus* sp. R22	*Trichoderma* sp. S57'		
47	*Rizhopus* sp. R26	*Trichoderma* sp. P7		
48	*Rizhopus* sp. P39	*Trichoderma* sp. P10		
49	*Absidia* sp. K61	*Trichoderma* sp. P11		
50	*Absidia* sp. K69	*Mortierella* sp. K64		
51	*Absidia* sp. K68	*Mortierella* sp. K33		
52	*Mucor* sp. K70			
53	*Mucor* sp.T54			
54	*Sp. pulverulentum* S7			
55	*Sp. pulverulentum* S43			

Table. 2 Extremophility of a-amylase producers

#	culture	description of culture			
	α-amylase producers				activity un/ml
1	Aspergillus sp. S54	hermophile	alcaliphile	extr. halophile	1,2
2	*Aspergillus* sp. R 5	mesophile	acidophile		2.5
3	*Aspergillus* sp. S16	mesophile	alcaliphile	moderate halophile	3.0
4	*Mucor* sp. T 18	thermophile	acidophile		4.5
5	*A.Oryzae*	phsychrotolerant		moderate halophile	9.0
6	*Mucor* sp. T 37	phsychrotolerant			2.0
7	*Rhizopus* sp. S 75	mesophile	acidophile		4.5
8	*Penicillum* sp. S 80	mesophile	acidophile	moderate halophile	2.0
9	*Penicillum* sp. T 52	phsychrophile		extr. halophile	0.8
10	*Mortierella* sp. S 34	phsychrophile	acidophile		0.6
11	*Mucor* sp. K 22	thermophile	acidophile		4.2
12	*Mucor* sp. R 33	thermophile	alcaliphile		6.5

Table.3 Extremophility of cellulase producers

#	culture	description of culture			
	cellase producers				activity un/ml
1	*Aspergillus* sp. S 60	thermotollerant		moderate halophile	0.5
2	*A. versicolor* sp. 83	thermophile			1.2
3	*A. terreus* sp. 39	thermophile			0.4
4	*Chaetomium* sp. S 77	thermophile			0.3
5	*Sporotrichum purverulentum* 7	thermophile			0.3
6	*Sporotrichum purverulentum* 43	thermophile			0.4
7	*Mucor* sp. T 54	thermotollerant	alcaliphile		0.3
8	*Absidia* sp. K 61	thermotollerant	alcaliphile	extr. halophile	0.65
9	*Penicillum* sp. S 4	thermophile			0.2
10	*Penicillum* sp. S 10	phsychrophile		extr. halophile	0.15
11	*Penicillum* sp. T 25	mesophile		moderate halophile	0.5
12	*Rizhopus* sp. R 22	mesophile		moderate halophile	0.75
13	*Chaetomium* sp. P36	mesophile	alcaliphile		0.3

Table.4. Extremophility of glucoamylase producers

#	culture	description of culture			
	glucoamylase producers				activity un/ml
1	*Aspergillus* sp. S 60	mesophile	acidophile	moderate halophile	3.3
2	*Chaetomium* sp. S 48	thermophile			4.5
3	*Chaetomium* sp. S 77	thermophile	alcaliphile		14.6
4	*Aspergillus* sp. T 31	thermophile	alcaliphile	extr. halophile	7.5
5	*Penicilium* sp. S35	phsychrophile		extr. halophile	8.3
6	*Trichoderma* sp. P 7	mesophile			12.0
7	*Trichoderma* sp. P 10	mesophile	alcaliphile		11.2
8	*Mortierella* sp. K 33	phsychrophile			3.75
9	*Cladosporium* sp. T 48	thermophile	alcaliphile		15.0
10	*A. awamori* S 11	mesophile		moderate halophile	15.0
11	*A. awamori* T 23	mesophile		moderate halophile	25.0
12	*Penicillum* sp. P 5	mesophile			2.5
13	*Aspergillus niger* 8	mesophile	alcaliphile	extr. halophile	14.5

Table.5. Extremophility of proteinase producers

#	culture	description of culture			
	proteinase producers				activity un/ml .
1	*A. Tamar* 53	mesophile		moderate halophile	0.52
2	*Penicillum* sp.T 53	thermophile			0.34
3	*Fusarium* sp.T 51	phsychrophile			0.4
4	*Mucor* sp.T 44	thermotollerant	alcaliphile		0.6
5	*Penicillum* sp.K 28	mesophile			0.46
6	*Mucor plumbeus* 17	B mesophile	acidophile	moderate halophile	0.35
7	*Chaetomium sp.* E-1	thermophile		.extr. halophile	0.30

References:

Cooney D. Emerson. *Thermophilic Fungi. An Account of their Biology, Activities and Classification.* W.N. Freeman, San Francisco and London, 1964.

Dalgvist A. *Determination of maltase and isomaltase activities with glucose-oxidase reagent.* J.Biochem. **80**, 547, 1961.

Ghose T.K. *Measurement of cellulase activities.* Pure Appl.Chem., **59**, 257-268, 1987.

Kutateladze L., Iashvili T., Zakariashvili N., Alexidze T., Sabashvili N., Aplakov V., Khokhashvili I., Jobava M. *Isolation and identification of miroscophic fungi from some soil-climatic zones of Caucasus.* Proc. Georgian Acad. Sci., Biol. Ser. B, **2**, 5-6, 2004.

Mosolov V.V. *Proteolitic Enzymes.* M."Nauka", 414, 1971.
Niehaus F., Bertolodo C., Kahler M., Antranikian G. *Extremophiles as a source of novel enzymes for industrial application.* Appl.Microbiol. Biotechnol., **51**, 711-729, 1995.
Ruchliadeva A., Goriacheva M. *Determination of amilolytic activities of enzymes.* In: "Enzymes and Alcohol production" M., "Pishch. Prom.", 1-9,1960.
Shafer T., Duffer F., Borchet T. *Extremophilic enzymes in industry, screening, protein engineering and application.* Proceedings of 3rd International Congress on Extremophiles. Hamburg, Germany, 306-307, 2000.

საქართველოს სხვადასხვა რეგიონიდან გამოყოფილი ჰიდროლიზური ფერმენტების პროდუცენტი მიცელიარული სოკოები

ქუთათელაძე ლ., ურუშაძე თ., ხვედელიძე რ., ჩხარტიშვილი დ., დაუშვილი ლ., ბურდული თ., კვესიტაძე ე.

ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 14.11.2005)

რეზიუმე

ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტში ჩატარებული კვლევების შედეგად, საქართველოს სხვადასხვა ეკოლოგიური ნიშიდან გამოყოფილი მიცელიარული სოკოების 351 კულტურიდან შერჩეულია ჰიდროლიზური ფერმენტების პროდუცენტი 146 შტამი. შესწავლილია ამ შტამების ბიოსინთეზური პოტენციალი. 37 შტამი შერჩეულია როგორც α-ამილაზას პროდუცენტი, 47-გლუკოამილაზას, 55-ცელულაზას, 16 პროტეაზას პროდუცენტი. დადგენილია შერჩეული პროდუცენტების ექსტრემოფილობის ხარისხი.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

TOPOGRAPHY OF STATOCYST SENSORY CELLS OF LUNG GROUND SNAIL *HELIX LUCORUM*

BUKIA R., KALANDARISHVILI E., TAKTAKISHVILI A., DAVITASHVILI M., GELASHVILI N.

Iv. Javakhishvili Tbilisi State University, Morpho-Physiological laboratory

(Received April 11, 2006)

Abstract

On the inner surface of statocyst of *Helix lucorum* 13 sensory cells were revealed by reconstruction method on paraffin sections. Each of them is surrounded with 5-6 supporting cells. Sensory cells are arranged by certain regularity: on frontal sections one starlike cell is placed at front pole of statocyst; the rest 12 polygonal cells form 3 belts, composed of 4 by 4 cells, on the inner surface of statocyst: front (cranial), middle (equatorial) and back (caudal) belts. Cell construction of every belt is like of brickwork. Existence of sensory cell of starlike form at front pole of statocyst and absence of such cell at the opposite back pole set up polarization of this structure. Size parameters of right and left statocysts of both, young and adult snails are similar. Length and width of sensory cells are less in young snails, but the height is similar. Equatorial ventral and dorsal cells appeared to be the biggest ones. Arrangement of sensory cells lengthwise coincides with meridian direction of sphere, and along the width - with parallel direction. Localizations of cells are counted in degrees.

Key words: morphometry, sensory cells, supporting cell, statocyst belt.

Introduction

In mollusks statocysts are the analogous of vestibular sensory system of higher animals. Statocysts in snails are presented by transparent vesicle, inner surface of which is composed with numerous small supporting cells and some large sensory cells with typical kinocilium. Cavity of statocyst is filled with viscous liquid – statolymph and statoconia [Geuze, 1968; Dijkgraaf, Hessels, 1969; Wolf, 1970; 1973; Vinnikov et al., 1971; Tsirulis, 1974; Zaitseva, 1990; 1992; 1999]. Investigations of the last years have shown that *Helix lucorum* is promising modeling object for studying the structural-functional organization of gravity-sensitive forms in norm and in the conditions of alternation of gravitational field (imponderability and hyperponderability). It was shown that imponderability stimulates the growth of statoconia. Increase of morphological parameters of statoconia, reduction of the number of "silent" cells occur in the snails exposed in imponderability. Vice versa, exposition of animals in the conditions of increased gravity causes destruction and elimination of statoconia [Gorgiladze, 2001; 2002; Gorgiladze et al., 2006]. Meanwhile, existed in scientific literature experimental data do not allow to judge about absolute number and morphological parameters of sensory cells, and also about their localization on the inner surface of statocyst.

Materials and Methods

Experiments were carried out on young and adult specimens of *Helix lucorum var. taur.* Kryn. Weight of young snails was 2.0±0.08 g, diameter of shell 18.8±0.2 mm. The same parameters for adult ones were 13.0±0.06 g and 35.7±0.3 mm, accordingly (the snail considered as adult which edges of shell near shell openings are unbend behind, making small node called a lip. In this state shell loses growth ability) [Javelidze, 1949; Likharev, Rammelmeiser, 1952]. Snails were collected in the park "Mziuti" (Tbilisi). Snail body was extracted from the shell, by entomological pins fastened on the preparative table. Body on the dorsal side was sectioned through middle line, peripharyngeal ganglia ring was bared. Using binocular hypopharyngeal ganglia complex with statocysts were excised. Material was fixed in Carnua liquid and after dehydration was placed in paraffin. Frontal, sagittal and horizontal serial sections of the 5-7 μm were dyed by iron hematoxylin according to Heidenhain, by iron hematoxylin with further dyeing by cresylviolet [Mepisashvili, 1973], and by cresylviolet according to Nissl. Sections were researched using optical microscope "MICMED-2" (LOMO, Russia).

Results and Discussion

Statocysts of *Helix lucorum* are paired structures of sphere shape. They are located on dorsolateral surface of pedal ganglia of hypopharyngeal ganglia complex. On the outer side statocyst is covered with two connective tissular membranes: inner – dense and homogenous, and outer – loose, containing smooth muscle and collagen fibers.

Reconstruction of statocyst via serial sectioning enables us to establish existence of 13 sensory cells located identically and every of them surrounded with 5-6 supporting cells-satellites on its inner surface of both, adult and young snails. Position and sizes of every sensory cell on inner wall of statocyst of young and adult snails are presented in Table 1. In the present work topography of every cell is given by the example of adult helix. One sensory cell of starlike form is placed on the front pole of statocyst due to the great number of cytoplasmic appendices coming out of it. The most part of this cell is localized on dorsolateral surface of statocyst, getting partially over on its ventral surface. From the total length (77μm) of this cell 47μm is located on the lateral side of statocyst, and 30μm – on the medial side. 38μm of the width of cell (the total width is 55μm) is located on the dorsal surface, and the rest, 17μm get over the side of ventral surface of statocyst. Cytoplasmic appendices of star shaped cell reach 15-25μm lengthwise. Thus the length and width of the central region of this cell are 52 and 39μm, correspondingly. The rest 12 polygonal cells constitute 3 belts on the inner wall of statocyst: front belt (edge zone), middle belt (equatorial zone) and back belt (caudal zone). 4 cells occur in every belt. On the front belt 2 cells out of those 4 are placed on ventral and 2 – on dorsal surface of statocyst. Lateral side of ventral surface is occupied with a second cell, and alongside, medial side is occupied with the third cell. Next to the third cell on the dorsal surface of statocyst medial side is occupied by the forth cell, and lateral – by the fifth cell.

In middle (equatorial) belt of statocyst one cell out of 4 is placed on its ventral surface (6^{th} cell), and another – on dorsal (7^{th} cell), lateral and medial regions between them are occupied by the 8^{th} and 9^{th} cells, correspondingly. It should be mentioned that these cells turned out to be shifted: part of the 6^{th} cell is shifted towards the medial side of statocyst, and part of 7^{th} cell – towards the lateral side. Namely: out of the total length of 6^{th} cell (112μm) smaller part, 36μm, is located on lateral side of statocyst, and the larger part (76μm) gets over medial side. Out of the total length of 7^{th} length (108μm) larger part, 64μm, is located on lateral side of statocyst, and the smaller part (44μm) gets over the medial side. Thus, those cells present mirror reflection of each other. From the rest two cells of equatorial belt (8^{th} and 9^{th} cells) part of the 8^{th} cell (45μm) out of

the total length (98μm) is located on dorsal surface of statocyst, and the rest, 53μm, – goes over ventral surface. Part of 9th cell (44μm) out of the total length (98μm) occurs on ventral surface of statocyst, and the rest, 55μm, goes over dorsal surface of statocyst. Hence, like 6th and 7th cells, they present mirror reflection of each other.

On the back belt (caudal) of statocyst, like the front belt, 2 cells are placed on ventral surface, and 2 – on dorsal. Hence, on ventral surface of statocyst lateral region is occupied by 10th cell, and medial – by 11th cell. Next to the 11th cell on the dorsal surface of statocyst, medial region is occupied with the 12th cell, and alongside this cell the lateral region is taken up with the 13th cell. All sensory cells are oriented parallel to the frontal plane of statocyst by their longitudinal axis. Except for the first star-form cell located on the front pole of statocyst, four cells of every belt have unidirectional orientation: they are pulled one by another via their longitudinal axis forming closed circular zone, and surrounding the whole inner perimeter of statocyst.

Perimeter of the front belt, i.e. the length of the circle ($2\pi R$) is 398.8μm, that is actually equal to the sum of all 4 sensory cells of this belt altogether with the supporting cells (on frontal section of statocyst between the neighbor sensory cells 2 supporting cells are observed, both of the length of about 7-7.5μm. Thus, total sum of lengths of supporting cells is equal to ~60μm). Sizes of circles and the summary composition of cells of middle and back belts are presented in Table 2.

As the statocyst has a sphere shape, cells on its inner wall should be placed along the length according to meridian, and along the width – according to parallel. Distance between the meridians from the equator to poles is decreased gradually. Hence, at the equal sizes of cells value of degree is higher on poles, than on equator. Data about lengths and widths of sensory cells and their location on the surface of statocyst (in degrees) are presented in Table 3.

Table 1. Sizes (M±m) and location of sensory cells on inner wall of statocyst of adult (13.0±0.04 g) (1) and young (2.0±0.08) (2) *Helix lucorum*

Statocyst zones	№ of cell	location of sensory cells on inner wall of statocyst	Sizes of cells, μm					
			1	2	1	2	1	2
cranial belt (front zone)	1.	around cranial pole	77.0±0.7	74.0±0.7	55.2±1.6	51.0±0.7	10.0	10.0
	2.	ventrolateral	81.0±1,6	81.0±1,0	66.0±1.2	63.0±1.2	85.0±0.3	8.0±0.2
	3.	ventromedial	99.0±1.1	91.0±1.1	70.8±2.0	73.0±1.4	10.4±0.4	10.0±0.3
	4.	dorsomedial	78.0±0.7	76.0±0.5	66.6±1.6	63.0±0.4	8.0±0.2	8.0±0.2
	5.	dorsolateral	79.0±0.5	78.0±0.5	66.2±1.6	63.0±0.4	8.0±0.3	8.0±0.2
equatorial belt (middle zone)	6.	ventral	112.0±1.1	101.0±1.2	94.5±2.7	85.0±1.0	10.0±0.3	9.0±0.2
	7.	dorsal	108.0±1.4	103.0±1.5	87.2± 2.2	86.0±1.4	9.0±0.25	9.0± 0.2
	8.	lateral	98.0±0.8	90.0±0.7	80.9±2.2	75.0±0.8	10.3±0.4	9.0± 0.2
	9.	medial	98.0±0.8	91.0±0.8	80.9±1.6	75.0±0.8	10.2±0.4	9.0± 0.2
caudal belt (back zone)	10.	ventrolateral	76.0±1,3	71.0±0.6	59.9±1.6	63.0±0.5	10.0±0.2	10.0±0.1
	11.	ventromedial	75.0±0.6	71.0±0.3	59.9±1.6	63.0±0.5	10.0	10.0±0.3
	12.	dorsomedial	80.0±0,9	78.0±1.0	67.1±2.0	66.0±0.5	10.0	10.0±0.3
	13.	dorsolateral	79.0±0.8	73.0±1.0	66.0±1.0	60.0±0.8	10.0	10.0±0.3

Note: M - mean arithmetic; m - mean standard error $p>0.05$

Thus, investigations carried out have shown that epithelial lining of statocyst of *Helix lucorum* is composed of 13 sensory cells, each surrounded with 5-6 cells-satellites. According to the literature data [Zaitsev, 1990; 1992] statocysts of *Helix vulgaris*, as well as *Helix pomatia*, snails of one family – *Helix lucorum*, comprise from 10 to 13 sensory cells. Unlikely, that the number of sensory cells of the snails of one and the same species should differ so. Sensory cells are

described as round saucer-shaped cells on fixed preparations [Zaitsev, 1990; 1992]. Morphometry of sensory cells of *Helix lucorum* has revealed that the length of cells exceed their width by 15-30%. Distribution of all 13 sensory cells in both, left and right statocysts represent the mirror symmetry of each other. It should be mentioned that the number and localization of sensory cells in the statocysts of adult and young snails are not actually distinguished from each other. Sizes of lengths and widths of sensory cells of young snails are less than of adult ones. Ventral and dorsal cells of equatorial belt of statocyst turned out to be the largest ones.

Sensory cells organizing front and beck belts appeared to be shifted towards the cells of equatorial belt, and as a result, this cellular construction reminds of brickwork. Such localization of sensory cells at various level of statocyst was found in sea mollusk *Aplyisia limacina* [Dijkgraaf, Hessels, 1968]. Original distribution of sensory cells on the inner surface of statocyst sets up structural polarization of this formation. Existence of a single sensory cell on the front pole of statocyst distinguished by its starlike form, and also, absence of such cell on the opposite back belt, is an evidence of this fact.

Table 2. Circle perimeter and sum of lengths of sensory and supporting cells of front (cranial), middle (equatorial) and back (caudal) belts of statocyst of adult *Helix lucorum* (13.0±0.04 g).

	front belt	middle belt	back belt
Circle diameter, μm	128	152	118
Circle perimeter, μm	398.8	477	370
Sum of lengths, μm	398	477	369

Table 3. Sizes and location of sensory cells on inner wall of statocyst of adult *Helix lucorum* in degrees*

№ of cell	Sizes of sensory cells, μm		Sizes of sensory cells in degrees		location of sensory cells along the length in degrees		location of sensory cells throughout the width in degrees	
	length	width	length	width	dorsal surface	ventral surface	dorsal surface	ventral surface
1	77	55	75	39	0-23	0-52	0-27	0-12
2	81	66	73.6	47.1		183.5-257.3		15-62
3	93	70.3	90	50.2		270-360**		16-66
4	78	66	70.9	47.1	13.6-84.5		29-76	
5	79	66	71.8	47.1	98.1-169.9		30-77	
6	112	94.5	84.8	67.5		231-315.5		82-149
7	108	87	81.8	62.1	52.9-134.4		72-134	
8	98	81	74.2	57.7	145.7 -	219.7	70 -	127
9	99	81	75	57.7	(337-360**) -	(0-41.6)	77 -	134
10	76	60	73.8	42.8		101-175.6		131-173
11	75	60	72.8	42.8		14.5-87.3		130-174
12	80	67.5	77.6	48.2	280.3-357.9**		132-180***	
13	79	66	75.7	47.1	190-265.8		132-179***	

*Zero point of count corresponds to intersection of prime meridian and parallels

**Count of the lengths of sensory cells was carried out according to sphere meridians from 0° to 360°

***Count of the widths of sensory cells was carried out according to parallels of hemisphere from 0° to 180°.

References:

Dijkgraaf S., Hessels H.G.A. *Uber Ban und Funktion der Statocyste bei der Schnecke Aplysia limacine.* Z.Vvergl.Physiol. **62**, 38-60, 1969.

Geuze J.J. *Observations on the structure of the Statocysts of Limnaea stagnalis (L).* Neth.J. of Zool., **18,** 2, 155-204, 1968.

Gorgiladze G.I. *Stimulating effect of imponderability on the growth of statoconia (experiments on automatic spacecrafts and man-tended orbital complex "MIR").* Georgian engineering news, **4**, 113-119, 2001 (in Rus).

Gorgiladze G.I. *Structural-functional characteristics of statocyst of Helix lucorum.* In: Orbital station "Mir", **2**, 6, 366-382, 2002 (in Rus).

Gorgiladze G.I., Bukia R., Davitashvili M., et al. *Destructive effect of increased gravity on inertia mass in statocysts of Helix lucorum.* Proccedings of Rus. Acad. Sci., **406**, 3, 416-418, 2006 (in Rus).

Javelidze G. *Materials for bioecological study of Helix lucorum var. taurica Kryn.* Proccedings of Tbilisi State University, **33a**, 163-170, 1949 (in Georgian).

Likharev I.M., Rammelmeier E.S. *Land molluscs of USSR fauna.* Moscow-Leningrad, 1952.

Mepisashvili I.S. *PhD Thesis*, Tbilisi, 1973 (in Rus.).

Tsirulis T.P. *The fine Structure of the Statocyst in the Gastropod Mollusc Clione limacine.* J. of Evol. Biology and Physiology, **10**, 2, 181- 188, 1974 (in Rus).

Vinnikov Ia.I., Gazenko O.G., Titova L.K. et al., *Receptor of gravity.* Series "Problems of Space Biology", Leningrad, "Nauka", **XII**, 1971 (in Rus.).

Wolff H.G. *Einige Ergebnisse der Ultrastruktur der Statocysten von Limax maximus, Limax flauns and Arion empiricorum (Pulmonate).* Z. vergl.physiol. **69**, 326-366, 1970.

Wolff H.G. *Statische Orientierung bei Mollusken.* Fortschritte der Zoologie, **21**, 2/3, 80-99, 1973.

Zaitseva O. V. *Structural organization of the sensoriy system of the land snail Helix lucorum.* J.Evolut.Bioch.Physiol., **26**, 1, 105-111, 1990 (in Rus.).

Zaitseva O. V. *Structural organization of the sensoriy system of the land snail.* J. of Higher Nervous Activity, **2**, 6, 1132-1149, 1992 (in Rus.).

Zaitseva O. V. *Structural Organization of Statocystes in Prosobranchial Mollusks.* Sensory Systems, 13, 2, 99-109, 1999 (in Rus.).

ხმელეთის ფილტვიანი ლოკოკინა *Helix lucorum*-ის სტატოცისტის მგრძნობიარე უჯრედების ტოპოგრაფია

ბუკია რ., კალანდარიშვილი ე., თაქთაქიშვილი ა., დავითაშვილი მ., გელაშვილი ნ.

ივ. ჯავახიშვილის თბილისის სახელმწიფო უნივერსიტეტის მორფო-ფიზიოლოგიის ლაბორატორია

(მიღებულია 11.04.2006)

რეზიუმე

პარაფინიანი ანათლებზე რეკონსტრუქციის მეთოდით ლოკოკინა *Helix lucorum*-ის სტატოცისტის შიდა კედელზე გამოვლინდა 13 მგრძნობიარე უჯრედი. თითოეული მათგანი გარშემორტყმულია 5-6 საყრდენი უჯრედით. მგრძნობიარე უჯრედები გარკვეული კანონზომიერებით არიან განლაგებული: ფრონტალურ ანათლებზე ერთი ვარსკვლავისებური ფორმის უჯრედი მოთავსებულია სტატოცისტის წინა პოლუსზე; დანარჩენი 12 პოლიგონალური უჯრედი ქმნის 44 უჯრედისაგან შემდგარ სამ სარტყელს სტატოცისტის შიდა ზედაპირზე: წინას (კრანიალური), შუა (ეკვატორული) და უკანას (კაუდალური). ყოველი სარტყლის უჯრედები აგურისებურად არიან განლაგებული ერთმანეთის მიმართ. სტატოცისტის წინა პოლუსზე ვარსკვლავისებური მგრძნობიარე უჯრედის არსებობა და ასეთის არ არსებობა საწინააღმდეგო უკანა პოლუსზე ქმნის ამ სტრუქტურის პოლარობას. მგრძნობიარე უჯრედის ზომები სიგრძის და სიგანის მიხედვით მზარდ ლოკოკინებში ნაკლებია, სიმაღლე კი ორივე ჯგუფის ცხოველებში ერთნაირია. აღნიშნული პარამეტრები იდენტურია როგორც მზარდი, ისე ზრდასრული ლოკოკინას მარჯვენა და მარცხენა სტატოცისტებში. ყველაზე დიდი აღმოჩნდა ეკვატორის ვენტრალური და დორსალური უჯრედები. მგრძნობიარე უჯრედების განლაგება სიგრძეში ემთხვევა სფეროს მერიდიანის მიმართულებას, სიგანეში კი სფეროს პარალელის მიმართულებას. შესაბამისად გამოთვლილია უჯრედების ლოკალიზაცია გრადუსებში.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

FUNGI ASSOCIATED WITH BARK BEETLES OF ORIENTAL SPRUCE IN GEORGIA

BURJANADZE M.

V. Gulisashvili Institute of Forestry, Tbilisi, Georgia
E-mail: medeabu@yahoo.com

(Received June 5, 2006)

Abstract

The spruce bark beetle, *Ips typographus* L. (*Coleoptera: Scolitidae*) causes considerable damage in stands of oriental spruce trees (*Picea orientalis* Link) and is very common throughout Borjomi gorge forests in Georgia. An investigation of the bark beetle populations was carried out with the focus on the natural occurrence of insect pathogenic fungi. During 2002-2005 in different forests of Georgia, the following fungi were isolated from *Ips typographus and Ips acuminatus,* viz., *Beauveria bassiana, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Fusarium sp.*

Key words: pathogenic fungi, *Ips typographus, Ips acuminatus, Beauveria bassiana, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum.*

Introduction

The spruce bark beetle, *Ips typographus* L. (*Coleoptera: Scolitidae*) causes considerable damage to stands of oriental spruce (*Picea orientalis* Link) and is very common throughout Borjomi gorge forests of Georgia. The outbreak of this pest insect is often developed after cutting, wind or snow breaks, followed by warm and dry climatic conditions.

In the last years numerous investigations were focused on the complex of pathogens of bark beetle and their influence on insect population dynamics. The pathogens possess biological control agents of a great potential due to their ability to develop strong epizootics that result in natural regulation of *Ips typographus* populations.

Information on natural occurrence of specific fungal pathogens associated with *Ips typographus* populations is well known at present [Balazy, 1966; Kreutz et al., 2004; Matha, Weiser, 1985; Wegensteiner, 1992; 1996; 2000; Weiser et al., 2003]. Unfortunately, very limited information is available in respect to this group of fungi on the bark beetles in Georgia, while *Beauveria bassiana* was reported on European spruce beetle - *Dendroctonus micans* [Isarlishvili, 1968, Tsilosani et al., 1972].

Consequently, the first step towards studying natural biocontrol agents against *Ips typographus* is to reveal species spectrum of fungi associated with this pest insect.

Material and methods

The survey for fungal pathogens associated with *Ips typographus* and *Ips acuminatus* was carried out during 2002-2005. The larvae, pupae and adults of bark beetles were sampled from two locations in Borjomi and Bakuriani regions (1000-1200 m a.s l.) and from one location of Shovi resort (Caucasian mountain, 1200-1400 m a.s.l.).

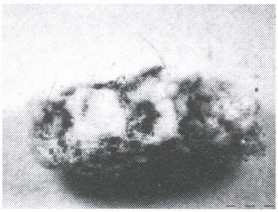
Investigations on the populations of bark beetles were carried out with the focus on the natural occurrence of insect pathogenic fungi. The basic survey procedure was to inspect visually bark beetle habitats under spruce bark or collected by pheromon trap. Adult beetle cadavers have apparent mycosis symptoms, such as internal mummification or fungal growth. Infected and uninfected, dead or moribund specimens of insects were collected and investigated in the laboratory environment. The material was studied using accepted methods in insect fungal pathology [Weiser, Briggs, 1970; Hoog, 1972; Humber, 1997]. The fungi were identified via microscopic preparations of mycelium developed on dead bark beetles.

Fungal isolates were derived from collected, infected deletions according to standard methodology [Evlakhova, Velickaia, 1961; Gerlach, Nirenberg, 1982; Poinar & Thomas, 1984] and cultivated on three isolation media, malt extract agar (MEA), potato extract agar (PEA), Beauveria Selectivmedium (BSM), in Petri dishes (diameter 10 sm), for 14 days at 25°C, until they develop feature permitting their identification up to genus or species.

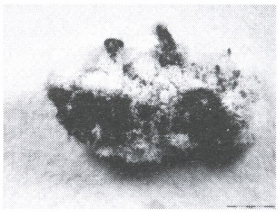
Results and Discussion

Adult bark beetles infected by entomophatogenic fungi were found under trees bark on the gallery or from pheromon trap. The fungi were identified with the help of microscopic preparations made directly from mycelium developed on dead bark beetle. The following fungi from the class Hyphomicetes - *Beauveria bassiana* (Bals) Vuill, (Fig.1,2,3) *Aspergillus niger* v. Tieg, *Aspergillus flavus* Linkex Fr., *Fusarium oxysporum* Schlecht. em. Snyd. et Hans., *Fusarium sp.* (Fig.4, 5) - have been revealed on *Ips typographus* and *Ips acuminatus* in different regions of Georgia (Tab.1).

The fungi were isolated from the samples for further investigation and cultivated on the different media. Microscopical observation revealed pathogen species belonging presumably to the Class of *Hyphomycetes*, the Order - *Moniliales.*

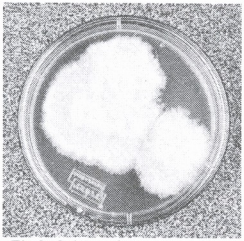


A



B

Fig. 1. *Beauvaria bassiana* on the *Ips typographus* (*A* - Caucasia, Shovi population B - Borjomi, Tsagveri population)



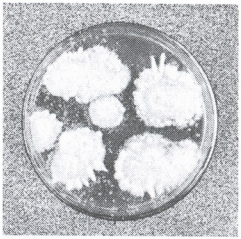
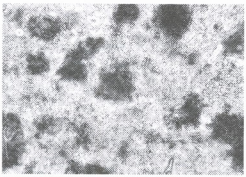


Fig.2. Colony of *Beauveria bassiana* (Bals) Vuill.



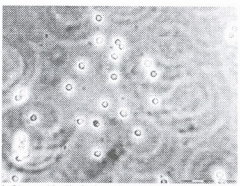


Fig.3. Conidia of *Beauveria bassiana:* (1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5(3.0) μm

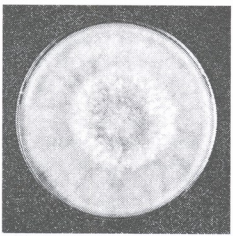


Fig.4. Colony of *Fusarium sp.*

Fig.5. Conidia of *Fusarium sp.*

The species list of pathogenic fungi isolated from bark beetles in different region of Georgia is presented in Table 1.

Table 1. Natural occurrence of pathogenic fungi *Hyhpomicetes* on bark beetles in Georgia

Region	Bark beetles	Fungus species	year
Borjomi, Tsagveri 1000-1200 m a.s.l	*Ips typographus*	*Beauveria bassiana*	2004-2005
		Aspergillus niger	2003
		Aspergillus flavus	2003
		Fusarium oxysporum	2004
		Fusarium sp.	2005
	Ips acuminatus	*Beauveria bassiana*	2005
Caucasus, Racha, Shovi 1200-1400 m a.s.l.	*Ips typographus*	*Beauveria bassiana*	2003

The study on entomopathogenic fungi aimed at carrying out bark beetles biocontrol measures is in progress.

Acknowledgment: The work has been supported by DAAD Exchange Service, Dr. Prof. Jürg Huber, Dr. Gisbert Zimmermann, Dr. Regina G. Kleespies (Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany)

References:

Balazy S. *Living organisms as regulators of population density of bark beetles in spruce forest with special reference to entomogenous fungi.* I. Poznan. Tow. Przyj. Nauk Wydz. Nauk Roln. Lesn., Pr. Kom. Nauk **21**, 3-50, 1966.

Evlakhova A., Velickaia S. *Methodology for biopreparation from entomopathogenic fungi for using against pests.* Research Institute of Plant Protection, Leningrad, p.25, 1976.

Gerlach W., Nirenberg H. *The Genus Fusarium – Pictorial Atlas.* Federal Biological Research Centre for Agriculture and Forestry, Institute of Microbiology, Berlin-Dahlem, p.40, 1982.

Hoog G.S. *The Beauveria, Isaria, Tritirachium and Acrodontium Gen. Nov.* Institute The Roial Netherlands Academy of Sciencesand Letters, Studies in Mycology, **1**, 42, 1972.

Humber R.A. *Fungi Identification.* In: Manual of Techniques in Insect Pathology. Ed. Lawrence, A. Lacey, Acad. Press, 153-185, 1997.

Isarlishvili S. *Entomoparasitic fungi on the pests in Georgia.* Proceedings of the Institute of Plant Protection, Tbilisi, **XX**, 177-182, 1968.

Kreutz J., Zimmermann G., Vaupel O. *Horizontal transmission of the entomopathogenic fungus Beauveria bassiana among the spruce bark beetle, Ips typographus (Col., Scolytidae) in the laboratory and under field conditions.* Biocontrol Science and Technology, **14**, 8, 837-848, 2004.

Matha V., Weiser J. *Effect of the fungus Beauveria bassiana on adualt bark beetles Ips typographus.* Conf. Biol. Biotechn. Contr. Forest Pests 1985, Tabor (CSFR), 1985.

Poinar G.O. & Thomas M.G. *Laboratory guide to insect pathogens and parasites.* Plenum Press, New York, 89-91, 1984.

Tsilosani G., Imnadze T., Buadze N. *Investigation of European spruce beetle microflora (Dendroctonus micans Kugel) in Georgia.* Proceedings of the Institute of Plant Protection, Tbilisi, **XXIV**, 192-196, 1972.

Wegensteiner R. *Untersuchunge zur Wirkung von Beauveria-Arten auf Ips Typographus (Col., Scolytidae).* Mitt. Dtsch. Ges. allg. angew. Ent., **8**, 104-106, 1992.

Wegensteiner R. *Laboratory evaluatin of Beauveria bassiana (Bals.) Vuill. against the Bark beetle, Ips typographus (L) (Coleoptera, Scolytidae).* Proceedings of the first joint meeting, IOBC/wprs Bulletin, Bulletin OILB/srop, **19**, 9, 186-189, 1996.

Wegensteiner R. Laboratory evaluation of *Beauveria bassiana* (Bals.) Vuill. and *Beauveria brongniatii* (Sacc.) against the four eyed spruce bark beetle, Polygraphus polygraphus (L.) (Coleoptera, Scolytidae). IOBC/wprs Bulletin, **23**, 2, 161-166, 2000.

Weiser J., Wegensteiner R., Handel U., Zizka Z. *Infections whith the Ascomycetes Fungus Metchnikowia typographi sp. Nov. in the bark beetles Ips typographus and Ips amitinus (Coleoptera, Scolytidae).* Folia Microbiol., **48**, 5, 611-618, 2003.

აღმოსავლური ნაძვის ძერძიჭამიებთან ასოცირებული სოკოები საქართველოში

ბურჯანაძე მ.

ვ. გულისაშვილის სატყეო ინსტიტუტი

(მიღებულია 05.06.2006)

რეზიუმე

საქართველოს წიწვოვან ტყეებში გავრცელებულ დეროს მავნებლებს შორის განსაკუთრებით უარყოფითი სამეურნეო მნიშვნელობით გამოირჩევა მბეჭდავი ქერქიჭამია *Ips typographus* L. (*Coleoptera: Scolitidae*), რომლის მასობრივი გამრავლება უკანასკნელ წლებში აღინიშნებოდა ბორჯომის ხეობაში აღმოსავლურ ნაძვზე (*Picea orientalis* Link). აღნიშნული მავნებლის პოპულაციებში გამოვლენილი პათოგენური მიკროორგანიზმებიდან კვლევის ობიექტად არჩეულ იქნა პათოგენური სოკოები. 2002-2005 წლებში საქართველოში გავრცელებული მბეჭდავი ქერქიჭამიას პოპულაციებში გამოვლენილია პათოგენური სოკოები: *Beauveria bassiana, Aspergilus niger, Aspergilus Flavus, Fusarium oxysporum, Fusarium sp.,* რომლებიც *Hyphomycetes* კლასს და *Moniliales* რიგს მიეკუთვნება. ბორჯომის ხეობაში აღმოსავლურ ნაძვზე გვხვდება კენწეროს ქერქიჭამიაც *Ips acuminatus* (*Coleoptera: Scolitidae*), რომლის პოპულაციებშიც ასევე ნაპოვნია პათოგენური სოკო *Beauveria bassiana.*

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

PRELIMINARY DATA ON FUNGAL ANTAGONISTS OF FALSE HELLEBORE (*VERATRUM ALBUM* SSP. *LOBELIANUM*) IN GEORGIA AND THEIR POTENTIAL AS EFFECTIVE BIOCONTROL AGENTS

GVRITISHVILI M.[1], KIKODZE D.[2], HEINZ MÜLLER-SCHÄRER[3]

[1]*Tbilisi Botanic Garden. E-mail: mirian_550@yahoo.com*
[2]*Ketskhoveli Institute of Botany*
[3]*Department of Biology, University of Fribourg, Perolles, CH-1700 Fribourg. E-mail: heinz.mueller@unifr.ch*

(Received April 17, 2006)

Abstract

Among pathogenic fungi (antagonists) occurring in Georgia on False Hellebore *Cylindrosporium veratrinum*, *Marssonina veratri* and *Botrytis elliptica* are considered to be potential antagonists for biological control of this noxious weedy herbaceous plant of mountain pastures and haylands. Besides 12 species of fungi associated with False Hellebore are first recorded in Georgia.

Key words: Georgia, *Veratrum*, fungal antagonists, weed biocontrol.

Introduction

False Hellebore is among the most noxious weedy herbaceous plants of mountain pastures and haylands in Georgia. Traditional mechanical and chemical methods of control available are time consuming and economically and ecologically undesirable.

Though importance of biological control of weeds with plant pathogenic fungi has been understood for a long time, the use of mycoherbicides is relatively recent concept and a number of products are already available on the market [Hawksworth et al., 1996].

Materials and Methods

The material investigated includes specimens collected during field observations carried out in east (Gudauri environs) and west (Shuakhevi district, Ajara) Georgia in 2002-2004. Populations of False Hellebore were examined at 8 sites utilizing the methodology described below.

A transect was made through the population (straight line or W–shaped) of 50 x 2m = 100m^2. Numbers of *Veratrum* plants were counted, and every x^{th} plant was calculated to result in 50 regularly spaced plants over transect. Damage level has been assessed on a 0-5 scale (0: healthy, no signs of damage, 5: >80% defoliated or infected). Plant leaves infected by pathogens were collected and identified. All 50 plants were uprooted and the root and root crown were inspected for pathogen damage.

Results and discussion

According to literature sources [Badridze, 1969; Imerlishvili, 1968; Kanchaveli, 1971; Murvanishvili, 1966; Vassilevsky, Karakulin, 1950] 11 fungal species are referred to as associated with False Hellebore in Georgia. As a result of identification of all the fungal complexes that inhabit the weed under the consideration, their total number is equal to 25 (see list below). Among them *Cylindrosporium veratrinum*, *Marssonina veratri* and *Botrytis elliptica* seem to be most perspective biocontrol agents.

Cylindrosporium veratrinum. The fungus is very common leaf-spotting pathogen of False Hellebore in Georgia. The disease first appears at the end of June and reaches its maximum in August-September. Spots are brown, linear, limited by the veins, often confluent and covering nearly the whole of the leaf surface (Fig. 1). *C. veratrinum* produces conidiomata with grey to rose conidial mass predominantly on upper leaf surface. This indicates that during the growing season spread of conidia and infection of plants take places in rainy weather (by splash and by wind blown mist). Ways and means of mycelium (inoculum) survival in dormance period are not known. However, using analogy with similar (allied) parasitic fungi from Coelomycetes the fungus survival can be assumed by mycelium existing in the rhizomes.

Table 1. List of pathogenic and other fungi associated with *Veratrum album* ssp. *lobelianum* in Georgia. Abbreviations: C - Coelomycetes; H - Hyphomycetes; M - Mycelia sterilia (Agonomycetales); P - Pyrenomycetes (in broad sense); U - Uredinales.

Species	Parasitic	Saprobic	Sources	Host range
Alternaria alternata (Fr.) Keissl. (H)		+		Plurivorous
Ascochyta veratri Cav. (C)	+		[1,5]	Specific
Botrytis elliptica (Berk.) Cooke (H)	+		[2,3]	Known on *Lilium* spp.
Cercosporella veratri Peck (H)	+		[6,7]	Specific
Coleophoma sp. (C)		+		Specific ?
Cylindrosporium veratrinum Sacc. & G.Winter (C)	+		[5,9]	Specific
Didymella sp.-1 (P)		+		?
Didymella sp.-2 (P)		+		Plurivorous
Epicoccum nigrum Link (H)		+		Plurivorous
Fusoma veratri Allesch. (C)	+		[7]	=*Marssonina veratri*
Leptosphaeria modesta (Desm.) P.Karst. (P)		+		Plurivorous
L. veratri Earle (P)		+		Specific?
Leptosphaeria sp. (P)		+		?
Marssonina veratri (Ellis & Everh.) Magnus (C)	+		[5]	Specific
Phyllosticta albina Bub. & Káb. (C)	+			Specific
P. melanoplaca Thüm. (C)	+		[1,7]	Specific
Pleospora sp. (P)		+		?
Puccinia veratri Duby (U)	+		[5,7]	Specific
Rosellinia sp. (P)		+		?
Sclerotium sp. (M)	+?			?
Septogloeum veratri (Allesch.) Wollenw.	+		[1,5]	=*Marssonina veratri*
Septoria sp.	+		[1]	Specific
Uromyces veratri (DC.) J.Schröt (U)	+		[1,5,7]	Specific
Discom. gen. indet.-1		+		?
Discom. gen. indet.-2		+		?

Marssonina veratri. The fungus causes necrotic brown spots on leaves, rarely on vaginae and stems. Spots are elliptical, oblong-elliptical with darker brown margins and somewhat pointed at both ends. Conidiomata with greyish mass of hyaline conidia develop on upper leaf surfaces in the centre of the spots (Fig. 2). In Georgia *M. veratri* occurs throughout the regions observed but in comparison with *C. veratrium*, rarely and less intensively.

Botrytis elliptica. The fungus is a widespread pathogen of lilies in Europe and North America [Gould, 1953]. In Georgia it is known on *Lilium candidum* [Bagaturia, 1980]. It has been first found on False Hellebore in east Georgia in July 2004 as a causative agent of grey-brown leaf spot up to 13 cm in diameter or more depending on the size of leaf blades (Fig. 3). Sporulation occurs on the lower leaf surface. It differs from known plurivorous fungus *B. cinerea* Pers. by its large conidia that measure 15-30x13-25 μm.

Fig. 1. *Cylindrosporium veratrinum.*

Fig.2. *Marssonina veratri.*

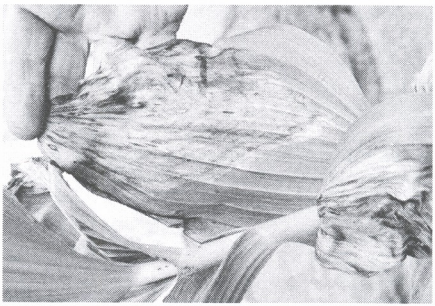


Fig. 3. *Botrytis elliptica.*

Acknowledgement: Financial support was provided through SCOPES, an international research programme of the Swiss National Foundation (grant N 7 GEPJ065726 and N IB76AO-106455 to HMS).

References:

Badridze M.A. *Mycoflora of the Iori river valley.* Cand. dissert. Tbilisi, 1969 (Georg.).

Bagaturiya V.Ya. *Diseases of herbal ornamental plants and preventative measures in eastern Georgia.* In: "Ornamental plant protection from diseases in Caucasus environment" Tbilisi, **1**, 7-36, 1980 (Russ.).

Gould C.J. *Decay of lily and tulip.* In: Plant Diseases. M., 574-579, 1956, USDA, Washington, 1953 (Russ.).

Hawksworth D.L., Kirk P.M., Sutton B.C., Pegler D.N. *Ainsworth & Bisby's Dictionary of the Fungi.* 8th ed IMI CAB Int., Cambridge, 1996.

Imerlishvili V.I. *Mycoflora of the middle part of Kakheti Caucasioni.* Cand. dissert. Tbilisi, 1968 (Georg.).

Kanchaveli L.A., Melia M.S. *Species of the genus Cercosporella in Georgia.* Trans. Georg. Inst. Plant Prot. **23**, 312-315, 1971 (Georg.).

Murvanishvili I.K. *Mycoflora of the Aragvi river valley.* Cand. dissert. Tbilisi, 1966 (Georg.).

Schaffner U., Kleijn D., Brown V., Müller-Schärer H. *Veratrum album in montane grasslands: A model system for implementing biological control in land management practices for high biodiversity habitats.* Biocontrol News and Information, **22**, 19-28, 2001.

Vassilevsky N.I., Karakulin B.P. *Parasitic imperfect fungi.* **2**, M.-L., 1950 (Russ.).

ᲕᲘᲜᲐᲡᲬᲐᲠᲘ ᲛᲝᲜᲐᲪᲔᲛᲔᲑᲘ ᲨᲮᲐᲛᲐᲡ, ***Veratrum album*** **ssp.** ***lobelianum*****,** ᲡᲝᲙᲝᲕᲐᲜᲘ ᲐᲜᲢᲐᲒᲝᲜᲘᲡᲢᲔᲑᲘᲡᲐ ᲓᲐ ᲛᲐᲗᲘ, ᲠᲝᲒᲝᲠᲪ ᲑᲘᲝᲙᲝᲜᲢᲠᲝᲚᲘᲡ ᲐᲑᲔᲜᲢᲔᲑᲘᲡ, ᲞᲝᲢᲔᲜᲪᲘᲣᲠᲘ ᲔᲤᲔᲥᲢᲣᲠᲝᲑᲘᲡ ᲨᲔᲡᲐᲮᲔᲑ

გვრიტიშვილი მ.[1], ქიქოძე დ.[2], ჰაინც მიულერ-შერერ.[3]

[1]თბილისის ბოტანიკური ბაღი
[2]ნ. კეცხოველის ბოტანიკის ინსტიტუტი
[3]ფრაიბურგის უნივერსიტეტი

(მიღებულია 17.04.2006)

ᲠᲔᲖᲘᲣᲛᲔ

საქართველოში შხამაზე გავრცელებული პათოგენური სოკოებიდან *Cylindrosporium veratrinum, Marssonina veratri* და *Botrytis elliptica* განიხილება, როგორც პოტენციური ანტაგონისტები ალპური სათიბ-საძოვრების ამ აბეზარა სარეველის ბიოკონტროლის მიზნით გამოყენებისათვის. გარდა ამისა, შხამასთან ასოცირებული 12 სახეობა პირველად არის მოყვანილი საქართველოს მიკობიოტისათვის.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

EFFECT OF STERILIZING SUBSTANCES ON DEVELOPMENT OF GROUNDSEL (*SENECIO PLATYPHYLLUS*) IN VITRO CULTURES

MANJGALADZE S., ZARNADZE N., ALASANIA N.

Batumi Institute of Agrarian Biotechnology and Business

(Received May 15, 2006)

Abstract

Effect of various sterilizing substances: diocide, chlorine solution, ethanol and hydrogen peroxide, on the yield of aseptic and viable buds of groundsel was studied. The best result was achieved using 0.2% diocide solution at 20 min exposition. Obtained cultures are characterized with normal growth and development and are subjected to further stage of micro-propagation.

Key words: aseptic cultures, apical buds, explants, diocide.

Introduction

Groundsel (*Senecio platyphyllus*) is a perennial plant pertinent to Compositae family. It is composed of alkaloids, such as: platyphyllin, saracen, sanatiphyllin. In medicine they are used as spasmolytics at various diseases [Khidasheli, Papunidze, 1985].

It is significant to work out the ways of in vitro propagation of plants containing bioactive compounds, as on a small experimental area a big amount of biomass containing various substances should be received [Oledzka et al., 1991; Alasania et al., 2006; Manjgaladze et al., 2003].

The goal of our work was to obtain sterile cultures of groundsel and to work out convenient conditions for further cultivating.

Materials and Methods

The material of our research was the groundsel collected on subalpine and alpine belts of Ajara at 1000-1400 m a.s.l.

As an explants for introduction in culture rosette-like apical buds of young vegetated sprouts were used. As sterilizing substances the following water solutions were examined: 1) 0.2-0.5% of diocide; 2) 25-50% of chlorine solution; 3) sequential action of 70% ethanol and 10-15% of hydrogen peroxide.

To chlorine and hydrogen peroxide solutions some drops of Twin-80 was added as surface sterilizing activating agent.

Explants were washed with soap under flowing water, with further washing out several times in distilled water. Then experiments were carried out in aseptic conditions, in laminar boxes. Exposition time of explants in sterilizing substances was chosen experimentally. After finishing sterilization material was washed in sterile water 3-4 times; in the last water portion it was stayed

for 20-30 min. Such procedure facilitates for washing out the chemical compounds from plant tissues.

Sterilized explants were sown on nutrient medium. Nutrient medium contained mineral salts and vitamins according to Gamborg formula [Gamborg, Willer, 1968]. Besides, medium contained agar and sucrose in concentrations of 0.7% and 3% correspondingly. At that stage of micro-propagation cytokinin - benzilaminopurin (BAP) and auxin - naphthyl acetic acid (NAA) were added. pH of nutrient medium was 5.8-5.9. Sterilization was conducted in autoclaves for 20-25 min. Cultivation of explants was carried out in phytotron, at lightening of 2-3 klux, 16/8 phytoperiod and 25°C. Sub-cultivation was taken place every 25 days.

Recording of the effectiveness of sterilizing substances was carried out regularly. Degree of infection and viability of explants were estimated according to statistical method [Dospekhov, 1985].

Results and Discussion

Sterilizing substances used for obtaining the aseptic cultures have different effect. They appeared less effective for the given material. It is caused not by various degree of chemical activity of sterilizing substances, but by anatomical-morphological structure of explants. The plant is perennial, at the first year of development rosette-like sprouts are formed, growth cone is introduced deeply between leaf vagina. At the same time, buds on root collar are deepened in soil, and hence, microflora is abundant there. Surface sterilization of such material is difficult. Usage of severe conditions causes poisoning of tissue and necroses. But slighter conditions can not provide for asepsis and degree of infection is high. It should be mentioned that upper epidermis of groundsel leaf is setulose that decreases the effect of sterilization considerably.

The effect of solutions of sterilizing substances on the yield of aseptic cultures and viability of explants are presented in Table 1.

Table 1. The effect of sterilizing substances on the yield of aseptic cultures and viability of explants.

Sterilizing substance	Concentration (%)	Exposition (min)	Amount of uninfected explants (%)	Amount of viable explants (%)
Diocide	0.2	10-20	25.0-40.0	52.7-70.1
Diocide	0.5	10	56.6	48.2
Chlorine solution	25	15-20	12.4-19.3	40.0-45.0
Chlorine solution	50	15-20	20.1-24.0	54.0-60.6
Ethanol/hydrogen peroxide	70/7	1-10	10.5-15.2	30.0-36.0
Ethanol/hydrogen peroxide	70/15	1-15	14.0-18.0	34.0-48.0

As is seen from the table 0.2% solution of diocide at 10-12 min exposition was more effective. In case of 0.2% diocide solution degree of uninfected explants is noticeably less (25-40%) compared to 0.5% diocide solution (56.6%), but yield of viable explants is higher (52.7-70.1%). Diocide is strongly toxic compound, so, increase of concentration and exposition time cause decrease of a number of viable explants.

While using chlorine water solution (25-50%) at exposition time of 15-20 min, amount of uninfected material was greatly lower due to its weak action. In spite of this, amount of viable explants is near to the result when diocide is used. This indicates to less toxic nature of this sterilizer.

The sequential usage of 70% ethanol and 10-15% hydrogen peroxide showed even lower effect of the yield of aseptic cultures. 15% hydrogen peroxide solution was more effective, than 7% solution, but the yield of viable explants was nearly similar. Increase of concentration causes the decease of the tissues.

Activation of viable buds began 4-5 days after. Rosette-like arranged buds grew in height, apical domination was restricted. Growth of the main sprout was realized by adding the rosette leaves in a way that growth in height was delayed. After 20-25 days micro-sprouts of 15-20 mm were subjected to the further stage of micro-propagation.

Thus, to receive aseptic cultures of groundsel, 0.2% solution of diocide at exposition of 20 min turned out to be the best sterilizing substance. Obtained cultures are characterized with good ability of development and high morphogenetical potential.

References:

Alasania N., Zarnadze N., Lomtatidze N., Dumbadze G., Japaridze Z. *Effect of phytohormones on development of nut buds (Corylus avellana L.) culture.* Proc.Georg.Acad.Sci. Ser.Biol.B, **4**, 1, 81-83, 2006.

Dospekhov B. A. *Methods of field experiments*. Moscow, "Mir", 1985 (in Russian).

Gamborg O.Z. Willer R.A. *Nutrient regiment of suspension cultures of soybean root cells*. Exp.Cell Res., **50**, 1, 151-158, 1968.

Khidasheli Sh., Papunidze V. *Medicinal plants of forests of Georgia.* Tbilisi, "Metsniereba", 1985 (in Georgian).

Manjgaladze S., Zarnadze N., Alasania N., Lomtatidze N. *Regulation of microclonal propagation via changing of the ratio of growth regulators.* Bulletin of Batumi Botanical Garden, **32**, 2003 (in Georgian).

Oledzka N., Iuzerovich E., Furmanov M. *Effect of various phytohormones on development of sprouts from axillary buds and top shoots of Catarantus roseus.* In: Biology of cultivating cells and biotechnology of plants. Moscow, "Nauka", 1991 (in Russian).

მასტერილებელი ნივთიერებების გავლენა ხარიშუბლას (*Senecio platyphyllus*) განვითარებაზე *in vitro* კულტურაში

მანჯგალაძე ს., ზარნაძე ნ., ალასანია ნ.

ბათუმის ბოტანიკური ბიოტექნოლოგიებისა და ბიზნესის ინსტიტუტი

(მიღებულია 15.05.2006)

რეზიუმე

შესწავლილია სხვადასხვა მასტერილებელი ნივთიერებების: დიოციდის, ქლორიანი წყალხსნარის, ეთანოლისა და წყალბადის ზეჟანგის მოქმედება ხარიშუბლას ასეპტიკური და სიცოცხლისუნარიანი კვირტების მისაღებად. საუკეთესო შედეგი მიღებულია დიოციდის 0,2% წყალხსნარის გამოყენებისას 20 წუთის ექსპოზიციით. მიღებული კულტურები ხასიათდებიან ნორმალური ზრდა-განვითარებით და ქქვემდებარებიან მიკროგამრავლების შემდგომ ეტაპს.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

METABOLISM OF 2-^{14}C-MEVALONIC AND 2-^{14}C-ABSCISIC ACIDS IN THE FLESHES OF TEA PLANT

MARGVELASHVILI N.

Ak. Tsereteli Kutaisi State University

(Received February 10, 2006)

Abstract

Metabolism of 2-^{14}C-mevalonic and 2-^{14}C-abscisic acids has been studied in the fleshes of tea plant. It is shown that tea plant fleshes have ability to convert labeled mevalonate - the precursor of biosynthesis of abscisic acid, into compound having similar to abscisic acid mobility in the system of solvents chloroform-methanol-water. However, during metabolism of 2-^{14}C-abscisic acid the high radioactivity is fixed in the chromatogram zones corresponding to glucose ether of abscisic acid and phaseic acid.

Key words: phytohormones, abscisic acid, flesh, mevalonic acid, phaseic acid

Introduction

Regulation of the process of plant growth and development is realized with participation of multicomponent hormonal system. One of the significant components of this system is phytohormone abscisic acid, which has polyfunctional type of action. Abscisic acid plays an important role in providing of dormancy, regulation of aging processes, abscission and stomatal movement. Abscisic acid inhibits chlorophyll formation, takes part in the processes of ripening of fruits, activates tuberization in conditions of unfavorable photoperiod, participates in the morphogenesis of leaves [Ho et al., 1983, Kefeli, et al. 1989].

From the results of current data we can suppose that direct synthesis from mevalonic acid is the main way of biosynthesis of abscisic acid in plants, which can proceed both, in overground plant organs and in roots [Kuznetsov, Dmitrieva, 2005].

Due to the fact that there is a big quantity of abscisic acid [Vlasov, Margvelashvili, 1986] in young leaves of tea plant we aimed to study metabolism of 2-^{14}C-mevalonic and 2-^{14}C-abscisic acids in fleshes of tea plant.

Materials and Methods

Fleshes of tea plant were submerged into solutions of (3 ml) of 2-^{14}C-mevalonic acid ($4.8 \cdot 10^6$ imp/min) as well as 2-^{14}C-abscisic acid ($2.6 \cdot 10^6$ imp/min) for 72 h, then they were washed out several times with distilled water and fixed with boiled methanol. Methanol extract was evaporated in vacuum evaporator till dryness. The dry residue was solved in 5 ml of methanol. On plates with thin layer of silica gel (Silufol UV-254) 200 µl of extract was put. Mevalonic and abscisic acids were used as standard substances. Fractionation was performed in the system of solvents chloroform-methanol-water – 70:12:0.5.

After viewing through UV (λ254) the plates were cut into sections and eluted with 5 ml of methanol for 12 h. Then the eluates were poured into scintillation flasks and added by 10 ml of scintillation liquid (50 ml of 1,4-di-(5-phenyl –2) of oxazoilbenzole and 4 g of 2,5 diphenyloxazole were solved in 1l of chemically pure toluol). The radioactivity was measured on liquid scintillation system 6892. The histograms were made according to the obtained data.

Hydrolysis of glucose ether of abscisic acid was conducted in the following way: chromatogram zone with the value *Rf* of glucose ether was scrapped off from the plates; the powder was placed in the flask with distilled water (volume 5 ml), which was alkalized with 3 N KOH up to pH10. The obtained hydrolyzate was filtered through the glass filter and alkalized by 1 N HCl up to pH3. Fractionation was conducted with sulfur ether (1:1) three times. The united ether fraction was evaporated till dryness. The dry residue was solved in a small amount of methanol and was put as a strip on the plate with thin layer of silica gel. Fractionation was conducted in the system of solvents chloroform-methanol-water – 70:12:0.5. The rechromatography of phaseic acid was performed in the following way: zone with the value of Rf of phaseic acid was scrapped off, eluted with methanol, then filtered by glass filter. The filtered eluate was evaporated till dryness. The dry residue was solved in a small amount of methanol and as a the strip was put on the plate with thin layer of silica gel. Chromatographic fractionation was conducted in the system of solvents chloroform-methanol-water – 70:22:3. After radioisotope analysis histograms were made.

Results and Discussion

After chromatography in the plates with thin layer of silica gel methanol extract of fleshes incubated in the solution of 2-^{14}C-mevalonic acid was viewed through UV. After this each zone of chromatogram (out of 11 zones detected) was cut, eluted with scintillation liquid and its radioactivity was measured. The distribution of radioactivity in various chromatogram zones is illustrated on Fig.1. As is seen from the figure the fleshes are able to convert the labeled mevalonate into compound having mobility similar to abscisic acid during fractionation of substances in the thin layer of silica gel in the system of solvents chloroform-methanol-water.

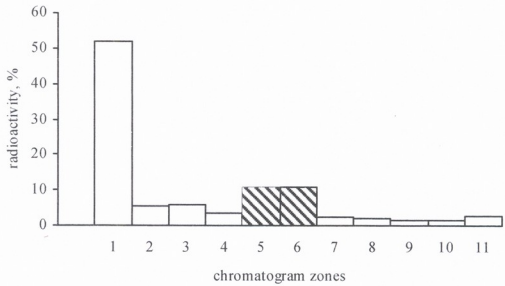


Fig.1. Distribution of radioactivity in various chromatogram zones after incubation of fleshes in the solvent containing 2-^{14}C-mevalonic acid.

Not only synthesis of abscisic acid takes place in plant tissues but its inactivation too. The inactivation can proceed by means of decay or conjugation. In numerous experiments it was shown that exogenous abscisic acid quickly transforms into ester with glucose, phaseic, hydrophaseic and 4-epidihydrophaseic acids [Lehmann,1984].

Study of metabolism of abscisic acid in tea plant fleshes included the extraction of plant material with methanol and chromatography on the plates with thin layer of silica gel in the system of solvents chloroform-methanol-water – 70:12.05.

After chromatographic fractionation of methanol extract and radioisotope analysis of each chromatogram zone it was shown that the most part of the activity was fixed in the zones 1, 2 (*Rf* – 0 - 0.09); 6, 7 (*Rf* – 0.3 - 0.4) and 9, 10 (*Rf* – 0.5 - 0.7), see Fig.2.

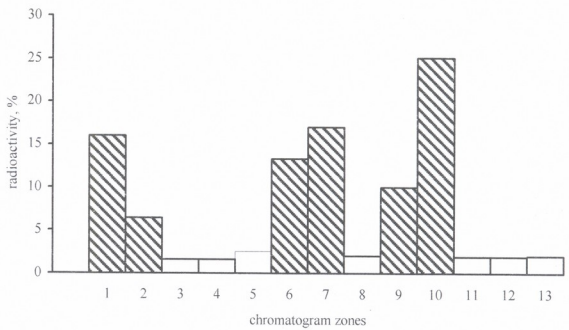


Fig.2. Distribution of radioactivity in various chromatogram zones after incubation of fleshes in the solution containing 2-^{14}C-abscisic acids.

The zone with *Rf* 0-0.09 by its mobility in the given system of solvents corresponds to glucose ether of abscisic acid. Glucose ether can easily hydrolyze and let the abscisic acid out. Thus for more reliable proposition on the formation of glucose ether in the fleshes of tea plant during metabolism of 2-^{14}C-abscisic acid the chromatogram zone with the value of (*Rf* 0-0.09) was cut and subjected to alkaline hydrolysis. After rechromatography of this zone radioisotopic analysis was done. The histogram presented in Fig. 3b shows that maximal radioactivity was detected in the zone which corresponds to abscisic acid. Zones 6 and 7 were also rechromatographed (chloroform-methanol-water 75:22:3). The data of radioisotopic analysis of chromatogram are presented on Fig. 3a. As is seen from the histogram high level of radioactivity is displayed by the compound, which has mobility similar to phaseic acid [Lehmann, 1984].

Thus during incubation of tea plant fleshes in the solution of 2-^{14}C-mevalonic acid there is formed a compound, which has similar to abscisic acid mobility at chromatography in the system of solvents chloroform-methanol-water – 70:12:0.5; at introduction of 2-^{14}C -abscisic acid two metabolites, having similar mobility to glucose ether of abscisic acid and phaseic acid, are formed.

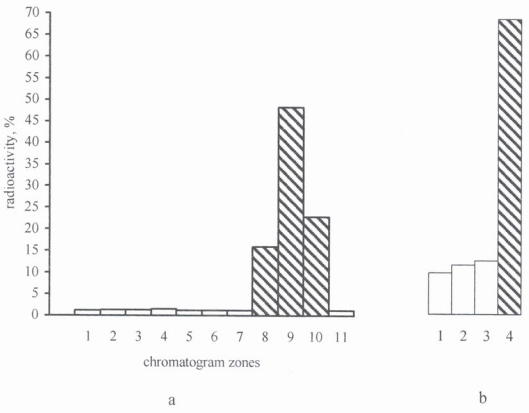


Fig. 3 Radioactivity of chromatogram zones with *Rf* value of phaseic (a) and abscisic (b) acids.

References:

Alekhina N.D., Balnokin Yu.V., et al. *Plant Physiology.* M., Publ. Acad. Sci., 2005 (Russian).

Kefeli V.I., et al. *Natural growth inhibitors – abscisic acids.* M., 1989 (Russian).

Kuznetsov V.V., Dmitrieva G.A. *Plant Physiology.* M., 2005 (Russian).

Margvelashvil N.Z., Vlasov P.V. J. Subtropical cultures, **4**, 1986 (Russian).

Ho L.C., Sjui V., Hoad G.V. *The effect of assimilate supply on fruit growth and hormone levels in tomato plants.* J. Plant Growth Regul., **1**, 3, 1982/83.

Lehmann H. *Metabolism of (±)2-^{14} C-abscisic acid in Lactuca sativa aches.* Physiol. Plant. **62**, 1984.

2-^{14}C-მევალონისა და 2-^{14}C-აბსციზის მჟავების მეტაბოლიზმი ჩაის მცენარის დუყებში

მარგველაშვილი ნ.

აკ. წერეთლის ქუთაისის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.02.2006)

რეზიუმე

შესწავლილია 2-^{14}C-მევალონისა და 2-^{14}C-აბსციზის მჟავების მეტაბოლიზმი ჩაის მცენარის დუყებში. ნაჩვენებია, რომ ჩაის მცენარის დუყებში მონიშნული მევალონის მჟავა გარდაიქმნება ნაერთად, რომელსაც აბსციზის მჟავის მსგავსი ძვრადობა ახასიათებს გამხსნელთა სისტემაში ქლოროფორმი - მეთანოლი - წყალი, ხოლო 2-^{14}C-აბსციზის მჟავების მეტაბოლოზმის დროს მაღალი რადიოაქტიურობა დაფიქსირდა ქრომატოგრამის იმ ზონებში, რომლებიც შეესაბამება აბსციზის მჟავის გლუკოზის ეთერსა და ფაზეის მჟავას.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

Zoology

THE FALL WEBWORM (*HYPHANTRIA CUNEA*) IN WESTERN GEORGIA

JAPOSHVILI G.[1], NIKOLAISHVILI A.[2], DZNELADZE N.[2], GOGUADZE L.[3]

[1]*Institute of Zoology, Chavchavadze ave. 31, 0179, Tbilisi, Georgia, E-mail: giorgij70@yahoo.com*
[2]*Scientific-producing Unity of Subtropical cultures and Tee products, Anaseuli, Georgia*
[3]*Ozurgeti Plant Protection Service, Ozurgeti, Georgia*

(Received April 10, 2006)

Abstract

The American fall webworm (AFW) *Hyphantria cunea* Drur (Lepidoptera: Arctidae) was first recorded in the Western Georgia in 1976. The AFW has since become a common species and a harmful pest in western Georgia attacking more than 300 species of ornamental plants, forest and trees. This is the first complete study on the phenology, degree of plant damage and biology of this species for the region. Possible biocontrol mechanisms are suggested. Observations and material collected from 1980-2005 revealed that the degree of damage by AFW was variable over the years. In 2000, mortality during high temperatures was very high (almost 90-95%), but declined as temperature decreased. Parasitism by chalcidoid complexes did not differ significantly among the AFW populations. Collection of the species *Chouioia cunea* Yang (Hymenoptera: Eulophidae), a parasitoid of *Hyphantria cunea*, represents a new record from the Caucasus. The primary regulating factor of AFW was high temperature during the investigation.

Key words: *Hyphantria cunea,* Georgia, Pest, Dynamic of number

Introduction

The American white butterfly (*Hyphantria cunea*) was first recorded in the Western Georgia in 1976, from wood material from the Ukraine. This invasive pest infests fruit and other deciduous hardwood trees. In 1988, the biology of the pest was studied in city Poti. At this time, AFW had not been recorded from Guria Region (South-Western Georgia), but from 1990, this pest slowly spread into this region (Lanchkhuti, Ozurgeti). From 1995, population numbers of this pest and damage to plants has been substantial. It's spread into Samegrelo, Guria, Achara, Abkhazia, and part of the Imereti Regions up to the Tsipa pass. There are 2 generations a year, sometimes - 3rd facultative generation. Its places of chief occurrence include parks and inhabited areas where chemicals cannot be used. The AFW is a common species and harmful attacking almost 300 species of subtropical, ornamental, bush, field, horticultural, fruit and other plants in western Georgia.

AFW is controlled by a large number of parasitoids and predators, which play an important role in regulating this pest in Eastern European countries [Sarov and Izhevskii 1987; Zerova and Shvedova 1988; Isik and Yanilmaz 1992; Barbagallo 1995; Bolshakova 1997]. We initiated a study on the phenology of AFW in Western Georgia. Our objective was to clarify the

population dynamics during long-term observations and evaluate those potential factors for this pest control.

Integrated Pest Management (IPM) is now recognized as the preferred strategy to achieve sustainable agricultural production [Yasnosh et al. 1996], because alternatives such as the use of pesticides have – a variety of negative effects on both the environment and human health.

Material and Methods

Surveys were carried out in different locations in South-western Georgia, in 1980-2005 to evaluate the population dynamic of the *H. cunea* and potential biocontrol mechanisms. In Poti during the 1980's, there was an emergency situation resulting in attempts to spray plants using aviation spraying, but this proved impractical and dangerous from an ecological point of view. For determination of distribution of AFW route methods were used in Western Georgia.

Four sites were chosen for observation plus laboratory investigations. The sites selected were: 1. Poti green plantation *Platanus* trees. 2. Anaseuli experimental territory with *Morus*, *Platanus*, *Tilia*, *Corylus* species. 3. Ozurgeti private orchards (almost 100) with *Morus*, *Corylus*, *Juglans*, *Malus*, *Pyrus*, *Prunus*, and *Cydonia* species. 4. Chokhatauri forest sides with *Quercus* and *Castanea.*

The phenology of the AFW was studied by recording average developmental stage of 100 insects on the plant. In city Poti, specimens (eggs and larvae) were collected weekly (taking account quarantine), taken to Anaseuli laboratory and observations made on the morphology and bioecology of the specimens. Systematic phytosanitary monitoring was conducted to determine feeding site preference on plants. Also, observations were made on their feeding behavior in nature on citrus plants, *Thea*, *Laurus*, *Corylus*, *Feijoa*, *Morus* and *Platanus*.

Pesticides were examined in larval stage by spraying, and mortality was recorded in the laboratory. We used the nematodobacterial (NMC) complex created by Dr G. Kakulia and preparations "Nimatsili" and "Phitoverm" from plant extracts. "Bacterine" preparation made in Khanchaveli Plant Protection Institute was examined as well.

The samples were taken to the laboratory to breed out the parasitoids in glass bottles covered with a fine mesh. A wet filter paper was put inside each bottle to maintain the humidity and this was changed every day. At the same time, the percentage of parasitism and the species of parasitoids were identified and counted.

Density of the plant damage was determined visually, as much as plants were quite high: 0 - when butterfly and caterpillars were not registered; 1 - when single leaves were infested; 2 - when 1-2 leaves were infested; 3 - when single cobwebs were noticed; 4 – each plant had 10-20 cobwebs; 5 – each tier was with cobwebs and larvae.

Investigation time in months was divided into three decades each 10 days.

Results and Discussion

AFW was recorded on Morus, Malus Pyrus, Prunus, Cydonia, Cerasus, Corylus, Juglans, Salix, Acer, Persica, Vitis, Fraxinus, Platanus, Citrus, Castanea, Acacia, Tilia, Quercus, Carpinus and other broadleaf trees.

AFW adults emerge in spring soon after the temperature reaches 10°C. After copulation they begin to lay eggs. After 15-20 days, larvae hatch and begin to feed. They molt up to 7 times before pupation. Their phenology on different plants is different (Table 1).

Table 1. a) Phenology of AFW on *Malus* in Ozurgeti; b) Phenology of AFW on *Platanus* in Ozurgeti; c) Phenology of AFW on *Morus* in Anaseuli.

Month	I			II			III			IV			V			VI			VII			VIII			IX		X			XI			XII		
Decade	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
a) Phenology of AFW on *Malus* in Ozurgeti																																				
Owerwintering II generation	•	•	•	•	•	•	•	•	•	•	•	• ♂♀	♂♀	♂♀	♂♀	♂♀	♂♀	♂♀	♂♀	♂♀																
I generation															∘ -	∘ -	∘ -	∘ -	∘ - • ♂♀	∘ - • ♂♀	- • ♂♀	• ♂♀	• ♂♀	• ♂♀	♂♀												
II generation																			∘ -	∘ -	∘ -	∘ -	∘ -	∘ - •	∘ - •	- •	•	•	•	•	•	•	•	•	•	•	•
b) Phenology of AFW on *Platanus* in Ozurgeti																																				
Owerwintering II generation	•	•	•	•	•	•	•	•	•	• ♂♀	• ♂♀	• ♂♀	♂♀	♂♀	♂♀	♂♀	♂♀	♂♀																			
I generation												∘ -	∘ -	∘ -	∘ -	∘ -	∘ -	- • ♂♀	- • ♂♀	- • ♂♀	- • ♂♀	• ♂♀	• ♂♀	♂♀												
II generation																			∘ -	∘ -	∘ -	∘ -	∘ -	∘ - •	- •	•	•	•	•	•	•	•	•	•	•	•	•
c) Phenology of AFW on *Morus* in Anaseuli																																				
Owerwintering II generation	•	•	•	•	•	•	•	•	•	•	• ♂♀	• ♂♀	♂♀	♂♀	♂♀	♂♀	♂♀	♂♀																			
I generation													∘	∘ -	∘ -	∘ -	∘ -	∘ -	∘ - •	• ♂♀	• ♂♀	• ♂♀	• ♂♀	♂♀													
II generation																			∘ -	∘ -	∘ -	∘ -	∘ -	∘ - •	∘ - •	- •	•	•	•	•	•	•	•	•	•	•	•

♀ - Adult female; ♂ - Adult male; ∘ - egg; - - larvae; • - pupae

Damage on *Malus* by first generation was extensive during III decade of IV month until II decade of VII month and by the second generation during II decade of VII month until II decade of IX month.

Damage on *Platanus* by first generation was considerable and high during III decade of IV month until III decade of VII month and by second generation during I decade of VII month until I decade of IX month.

Damage on *Morus* by first generation was considerable and high during II decade of V month until I decade of VII month and by second generation during II decade of VII month until II decade of IX month.

From 1995-2004, population density was different (Table 1b). On July 30, 2000, temperature was 60° and mortality of AFW was 90-95%. After 2001, as our statistical observation shows, the population density begin to increase in 2005, and while high, it was not more than 3.

Chouioia cunea is reported from the Caucasus for the first time, the eulophid was found parasitizing pupae of *H. cunea* in Ozurgeti (Georgia) in 2000. *C. cunea* was previously known from China and Japan, then it was found in Italy [Boriani 1991]. This parasitoid was primarily responsible for the mortality of 70% to 80% of the overwintering pupae of AFW in some places. From the biological and chemical pesticides tested in different sites the nematobacterial complex was responsible for almost 100% mortality in the laboratory, but it could not be tested in the field due to its expense. Nimacyl in the laboratory killed over 66.6% of the crawlers (crawlers and first instars are the same), but only 8.7% of fifth-stage larvae. Herbal preparations of Phytoverm and Bacterin created by Plant Protection Institute of Georgia were also tested in the lab resulting in a mortality rate of 35-100% and 5.1-14.7% respectively. Unfortunately we were unable to test these preparations under field conditions due to their expenses.

Thus, in the laboratory, the bio-preparations were effective against I-II stages of larvae and may also be effective in the field. AFW populations are maintained under damaging levels by their natural enemies in their native country, but no one had been recorded this species in Europe. As such, introduction and mass-rearing of its natural enemies may well constitute an effective biological control tactics for suppression of this invasive pest.

Table 2. Population density on the plants in the Guria region in 1995-2004.

Plants	Years									
	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Platanus	5	5	5	5	5	5	1	0	1	1
Morus	5	5	5	5	5	5	0	0	0	1
Corylus	3	3	4	4	3	4	0	1	1	2
Juglans	5	5	5	5	4	4	0	0	0	1
Malus	5	5	5	5	5	3	0	0	0	0
Pyrus	3	3	3	3	3	3	0	0	0	0
Prunus	4	3	3	3	3	3	0	0	0	1
Cydonia	3	2	2	3	2	2	0	0	0	0
Quercus	3	3	3	3	2	2	0	0	0	1
Castanea	2	2	2	2	2	2	0	0	0	0

References:

Barbagallo S. *Recent acquisitions of biological control in Italy*. Recenti applicazioni di lotta biologica. Informatore-Fitopatologico. **45**, 7-8, 13-17, 1995.

Bolshakova V.N. *Natural enemies of the American white butterfly*. Zashchita-i-Karantin-Rasteni. **5**, 30-31, 1997 (Russian).

Boriani M. *Chouioia cunea Yang (Hymenoptera, Euliohidae), parasitoid of Hyphantria cunea (Drury) (Lepidoptera, Arctiidae), new for Europe.* Bollettino-di-Zoologia-Agraria-e-di-Bachicoltura. **23**, 2, 193-196, 1991.

Isik M., Yanilmaz A.F. *Studies on the natural enemies and control measures of the fall webworm (Hyphantria cunea Drury. Lep.: Arctiidae) in hazelnut plantation in Samsun.* Zirai-Mucadele-Arastirma-Yilligi. No. 22-23, 55-58, 1992 (Turkish).

Sharov A.A., Izhevskii S.S. *The complex of parasitoids of the American white butterfly Hyphantria cunea Drury (Lepidoptera, Arctiidae) in the south of the European part of the USSR.* Entomologicheskoe-Obozrenie. **66**, 2, 290-298; 22 ref. 1987 (Russian).

Zerova M.D., Shvedova R.I. *Chalcids (Hymenoptera) as parasites of the fall web-worm moth in the southern European USSR.* Vestnik Zoologii, **0**(5), 3-10, 1988 (Russian).

Yasnosh V., Chkhaidze L., Tabatadze E. *Ecologisation of citrus protection.* J. Quarantine Pl. Prot., Moscow, **8**, 12-14, 1996 (Russian).

ამერიკული თეთრი პეპელა (*Hyphantria cunea*) დასავლეთ საქართველოში

ჯაფოშვილი გ.[1], ნიკოლაიშვილი ა.[2], ძნელაძე ნ.[2], გოგუაძე ლ.[3]

[1]*ზოოლოგიის ინსტიტუტი*
[2]*ანასეულის სუბტროპიკული კულტურებისა და ჩაის პროდუქტების სამეურნეო-საწარმოო გაერთიანება*
[3]*ოზურგეთის მცენარეთა დაცვის სამსახური*

(მიღებულია 10.04.2006)

რეზიუმე

ამერიკული თეთრი პეპელა (ათპ) *Hyphantria cunea* Drur (Lepidoptera: Arctidae) პირველად დასავლეთ საქართველოში რეგისტრირებულ იქნა 1976 წ. და მას შემდეგ იქცა ჩვეულებრივ სახეობად. ათპ ახიანებს 300-ზე მეტი სახეობის დეკორატიულ, ტყის და ხეხილოვან მცენარეს. ნაშრომი წარმოადგენს პირველ მცდელობას ათპ-ს ფენოლოგიის, მის მიერ მცენარეთა დაზიანების და ბიოლოგიის სრულყოფილი შესწავლისა რეგიონში. შესწავლილია ათპ-ს ფენოლოგია და შემოთავაზებულია შესაძლო ბიოკონტროლის მექანიზმები. კვლევები მიმდინარეობდა 1980-2005 წლებში. ნაჩვენებია, რომ ზიანის ხარისხი სხვადასხვა წლებში სხვადასხვა იყო. 2000 წლის 30 ივლისს, როდესაც ტემპერატურამ 60°-ს მიაღწია მწერის სიკვდილიანობა ძალზედ მაღალი იყო (90-95%). სიკვდილიანობა შემცირდა ტემპერატურის დაწევასთან ერთად. პარაზიტოიდების ზემოქმედება ათპ-ს პოპულაციაზე მნიშვნელოვნად არ განსხვავდებოდა. ათპ-ს პარაზიტი *Chouioia cunea* Yang (Hymenoptera: Eulophidae) ჩვენს მიერ პირველადაა რეგისტრირებული კავკასიისთვის. ათპ-ს რეგულაციის უპირველესი ფაქტორი დასავლეთ საქართველოში იყო მაღალი ტემპერატურა.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

REPRODUCTION OF *PSEUDORASBORA PARVA* (SHLEG.) AND ITS PRACTICAL IMPORTANCE

SHONIA L., KOKOSADZE T.

Institute of Zoology

(Received January 23, 2006)

Abstract

Representatives of both sexes of small Pseudorasbora reach puberty at the age of 1 year. Life span is also similar for male and female specimens, which in Bazaleti Lake don't exceed 3 years. By choosing of spawning area it belongs to lithophile. Pseudorasbora spawns on clean hard substrate occurred on the bottom of the lake at the depth of 60-70 cm. Among distributed fish in Georgia Pseudorasbora is the only one whose males protect spawn and newborn larvae of 2-3 days from other fish. Spawning proceeds in three stages with the interval of 10-11 days, at 16-17°C temperature of water. Individual fertility varies from 839 to 2816 eggs. Pseudorasbora is used as avian, fish and human food. It should be considered as alternative of *Gambuzia holbruk*.

Key words: lithophilous, spawning, fertilization, maturation.

Introduction

Among inhibited fish in Bazaleti Lake small Pseudorasbora belongs to lithophilous ones. Its male protects impregnated spawn and newborn larvae. Duration of incubation period varies according to temperature regime, and spawning goes at the optimal water temperature conditions. For entire formation of embryo 9-13 days are needed. During this period Pseudorasbora carries out its "protective" functions and then it spawns again. Pseudorasbora is used in poultry and fish farming as food component of high quality.

Materials and Methods

During 1999-2002 Pseudorasbora was caught in Bazaleti Lake according to seasons and 5 chosen stations: I and IV stations are rock-sandy biotopes resembling biocoenosis rich in vegetation. II and III stations are small fractioned rock-and-sandy, vegetation lacked biotopes. V station is muddy, placed between vegetation zones, here and there rocky biotope (Fig. 1). We used netting gear of length of 6 m, height – 1 m, cell size – 3 mm. Obtained material was treated in the laboratory of hydrobiology and ichthyology by common charts of biological analysis used in ichthyology [Chugunova, 1952]. Sex and stage of sexual maturity were determined for 641 specimens from obtained material (83.2%).

Results and Discussion

Pseudorasbora parva (Shleg.), representative of the family Gobioninae belongs to small size-weight fish group. Both, male and female reach sexual maturity in the second spring at 1^+ age. Absolute length doesn't exceed 30 mm. Maximal age of male and female is similar and is not more than 3^+. In Bazaleti Lake spawning begins at the end of May and lasts up to August; it is related to temperature regime of water. Spawning period varies with ecological environment. For example, in Japan (near Tokyo) spawning goes in May, in the river Amur basin – from May to August [Banarescu, 1990]. Spawn of three generations (1.04 mm, 0.76 mm, 0.47 mm) occur in gonads. Pseudorasbora spawns three times in separate portions. Amount of eggs is in average 451, which depends on ecological state of reservoir and existence of predatory. It has good adoptive potential. During the whole period of reproduction spawners spawn 978-2017 eggs in average (Table 1).

Table 1. Fertility of Pseudorasbora in Bazaleti Lake

Age	Number	Body length, mm		Body weight, mg		Fertility (number of eggs)	
		variation	average	variation	ave.	variation	ave. (%)
1^+	103	31-38	36	450-1000	748	751-1365	978 (23.5)
2^+	103	45-50	48	500-1880	1146	842-1367	1165 (28.0)
3^+	103	56-60	58	1800-2760	1983	1531-2446	2017 (48.5)

Egg number by generations									Egg diameter by generations		
big			small			middle					
variation	ave.	%	variation	ave.	%	variation	ave.	%	big	small	middle
205-372	267	23.4	251-457	327	23.4	269-488	384	23.4	0.08-1.01	0.30-0.46	0.59-0.72
232-376	321	28.1	278-451	384	28.1	345-561	460	28.0	0.88-1.03	0.30-0.47	0.59-0.74
421-673	555	48.5	505-807	665	48.5	605-978	797	48.6	0.90-1.07	0.30-0.49	0.60-0.82

Fertility of Pseudorasbora of the river Danube is 610-3200 eggs, of the river Amur - 1400 and more, in Japan waters – only 300 [Banarescu, 1990]. So, fertility of one and the same species varies according to ecological conditions. Data about Pseudorasbora fertility in accordance with the growth rate in the reservoir "Oteska" of Rumania are presented in Table 2 [Giurca & Angelescu, 1971].

Comparison of tables 1 and 2 shows that fertility of Pseudorasbora of "Oteska" is higher than of Bazaleti lake caused by artificial feeding of fish in Rumanian reservoir. In Basaleti lake Pseudorasbora depends only on natural food.

Table 2. Fertility of Pseudorasbora in "Oteska" reservoir.

Age	Length, mm	Weight, g	Fertility (number)	Average number
1+	65-73	5,0-8,0	839-1520	1092
2+	76-85	8,8-11,4	949-1539	1312
3+	87-95	10,6-14,4	1760-2816	2324

Reproduction period of Pseudorasbora is summer, but in warm spring conditions spawning should begin in May. Spawning areas of Pseudorasbora occur in north-eastern part of Bazaleti Lake, on rock-and-sand actic zones at the depth of 60-70 cm. Males clean up areas of the

diameter of 130-310 mm from organic precipitations and lower algae just before spawning trying to attract females. Female spawns in the middle of cleaned up area, then male fertilizes it and protects from other fish. Adhesive is produced after 2-15 min from fertilization, which causes strong sticking of spawn to the substrate [Ivanov, 1988; Koshelev, 1981]. During the reproduction process physiological and behavioral changes occur in males revealed in sharp aggression and morphological changes – asperities above eye and round upper lip appear. Embryonic development of spawn proceeds about 7-10 days in 7 stages. At the 8th stage of development embryo of about 4.10-4.45 mm diameter leaves spawn membrane. Embryo has negative reaction towards light and sticks substrate strongly for 2-3 days, then comes up to the upper layers.

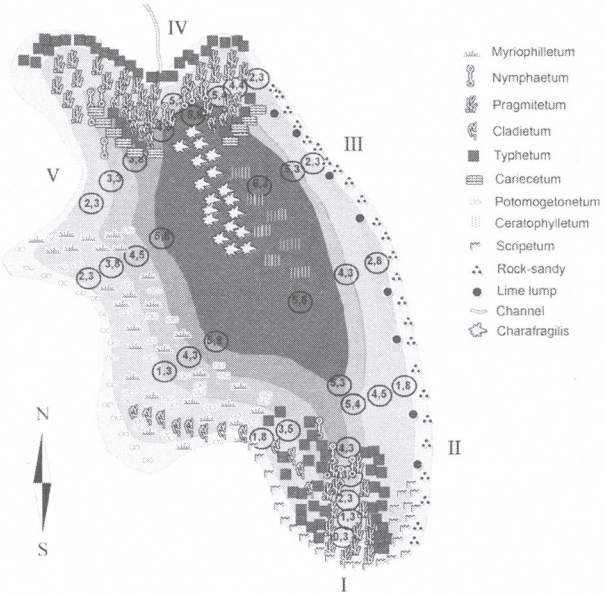


Fig.1. Stations (I, II, III, IV, V) on Bazaleti Lake

Stages of maturation and development are as follow: 1st juvenile stage – establishment of sex is impossible even microscopically. Before spawning of 1st portion gonads are of sharpened triangular form and dense. Along with mature spawn grains immature semi-transparent grains occur also. To distinguish small and coarse size spawn of second generation is possible. Before spawning of 2nd portion gonads are round shaped and soft. Along with mature spawn grains immature grains of similar size occur. Before spawning of 3rd portion gonads have elongated form, among mature spawn grains immature ones do not occur. Maturation period between portions lasts 10-11 days.

Pseudorasbora should be used in poultry, fish farming and also as human food. It consists A vitamin in a big amount. Flour of high quality should be received from pseudorasbora. It should be considered as alternative of *Gambuzia holbruk* and could be used against malaria mosquito - Anopheles in damp climatic regions of Georgia.

References:

Ivanov A. *Fishing in natural reservoirs.* Moscow, "Agropromizdat", 14-30, 1988 (in Russian).

Koshelov B. *Investigations of fish reproduction.* Methodological manual. Moscow, "Nauka", 5-16, 1981 (in Russian).

Chugunova N.I. *Methods of study of age and growth of fish.* Moscow, "Sovetskaia Nauka", p.69, 1952 (in Russian).

Banaresccu P. *Zur Ausbretungsgeschichte von Psevdorasbora parva in Sudes- teuropa (Pisces, Gyprynidae).* Re Roum Biol. Amin., **35**, 1, 13-16, 1990.

***Pseudorasbora parva* (Shleg.) - ს გამრავლება და პრაქტიკული მნიშვნელობა**

შონია ლ., ქოქოსაძე თ.

ზოოლოგიის ინსტიტუტი

(მიღებულია 23.01.2006)

რეზიუმე

მცირე ფსევდორასბორას ორივე სქესის წარმომადგენლები სქესობრივ სიმწიფეს აღწევენ 1 წლის ასაკში. ერთნაირია მათი სიცოცხლის ხანგრძლივობაც, რომელიც ბახალეთის ტბის პირობებში არ აღემატება 3 წელს. სატოფე სუბსტრატის არჩევითობის მიხედვით იგი ლითოფილია, ქვირითს ყრის 60-70 სმ-ის სიღრმეზე ფსკერზე განფენილ ნებისმიერ მყარ, სუფთა საგანზე. საქართველოში გავრცელებულ თევზებს შორის იგი ერთადერთია, რომლის მამრებიც იცავენ ქვირითს და ახლად გამოჩეკილი ლარვის 2-3 დღიან ფორმებს მათთან მიახლოების მსურველი თევზებისაგან. ქვირითობა სამჯერადად, 10-11 დღიანი ინტერვალით მიმდინარეობს. ტოფობს წყლის 16-17°C ტემპერატურის დადგომისას. ინდივიდუალური ნაყოფიერება მერყეობს 839-2816 ცალ ქვირითამდე. ფსევდორასბორას გამოყენება შესაძლებელია ფრინველის, თევზის და ადამიანის საკვებად. მისგან შესაძლებელია მაღალი ხარისხის ფქვილის დამზადება და იგი შეიძლება განვიხილოთ *Ggambuzia holbruk*.-ის ალტერნატივად.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 4, 2006.

Letter to Editor

NEMATODOFAUNA OF SOME VEGETABLE CULTURE PESTS OF KVEMO KARTLI

VARDIASHVILI E.

Institute of Zoology

(Received April 6, 2006)

Abstract

The paper deals with nematodofauna of some vegetable culture pests registered in Kvemo Kartli region. 31 forms of nematodes were revealed. Among them 30 ones were ascertained up to species, and *Steinernema sp.* – up to genus due to insufficiency of data. Representative of the family Museidae, species - *Stomachorhabelitis georgica* Vardiashvili, 2006 - is described for the first time.

Among natural enemies of pests entomopathogenic nematodes take an important place. To reveal and use them for pest control is the basic problem of entomonematodology. To study nematodes associated with pests and reveal among them pathogenic forms we have studied the following pests of vegetable cultures in Kvemo Kartli: *Baratra brassicae, Thytometra gamma, Agriotes sputator, Melolontha pectoralis, Pieris napi, Amphimallon solstitialis, Leptinotaesa decemlineata, Pieris beassicae, Poliphilla olivieri, Gryllotulpa grillotalpa, Muscidae g.sp.*

Imago and worm material of insects were collected during May-October of 2003-2005 in three regions of Kvemo Kartli: Tetri Tskaro (1), Gardabani (2), Marneuli (3) (in the table numbers 1, 2, 3 indicate corresponding places).

Section of insects was carried out in laboratory environment according to the known methods applied in entomonematodology. Nematodes were fixed in 4% formalin solution.

During research period 31 forms of nematodes were established within transected 11 species of insects.

List of nematodes, their distribution according to the hosts, and a place of occurrence are presented in the table.

N	Host insect / Nematode	Baratra beassicae	Pieris beassicae	Poliphilla olivieri	Gryllotalpa grillotalpa	Trytometra gamma	Agriotes sputator	Melolontha Pectoralis	Pieris napi	Amphimalla on Istitialis	Leptinotarsa decemlinata	Muscidae g.sp.
1	Protorhabditis elaphri			$+_{(2)}$								
2	Cenorhabditis elegans						$+_{(1)}$					
3	Parasitorhablitis malii						$+_{(1)}$					
4	Stomachorhabditis georgica sp.now*											$+_{(1)}$
5	Heterorhabditis bacteriophara							$+_{(2)}$				

6	Protorhabditis holophila	$+_{(1)}$											
7	Pelodera teres			$+_{(1)}$									
8	Mesorhabditis quercophila		$+_{(1,2)}$										
9	Protodiplogasteroides Saperdae								$+_{(3)}$				
10	Eudiplogaster leptospiculum		$+_{(2)}$										
11	Steinernema sp.	$+_{(2)}$											
12	Panagrolaemus rigidus	$+_{(1)}$											
13	Panagrobelus coranatus				$+_{(1)}$								
14	Cephalobellus melolonthae						$+_{(1)}$						
15	Cephalobellus leuskarti									$+_{(2,3)}$			
16	Diploscapter coronata			$+_{(1,3)}$									
17	Hexamermis albicans	$+_{(2)}$											
18	Mesomermis Korsakovi							$+_{(1)}$					
19	Amphimermis elegans										$+_{(1)}$		
20	Skriabinomermis apiculiformis							$+_{(2,3)}$					
21	Camponotimermis bifidus						$+_{(1)}$						
22	Thelastoma macraamphidus							$+_{(1)}$					
23	Thelastoma Leuckarti							$+_{(1)}$					
24	Thelastoma cuspidatum			$+_{(1)}$									
25	Severianoia gracilis			$+_{(1)}$									
26	Severianoia glomeridis				$+_{(3)}$								
27	Talpicola pseudornatum							$+_{(1)}$					
28	Gryllonema bispiculata				$+_{(1)}$								
29	Thelastoma Korsakovi					$+_{(1)}$							
30	Bursaphelendus Eucarpus			$+_{(2)}$									
31	Tylaphelenchus leichinicola												

Thus, researched pest nematodes belong to 4 order (Oxyurida, Mermithida, Rhabditida, Aphelenchida) and 11 families (Rhabditidae, Heterorabditidae, Doploscapteridae, Steinernematidae, Stomachorhabditidae, Diplogasteridae, Panagrolaimidae, Oxyuridae, Thelastomathidae, Aphelenehoididae, Mermithidae). Among them 30 ones are ascertained up to spacies, *Steinernema sp.* - up to genus. We could not identify it up to species because of insufficiency of material. Representatives of this genus belong to hard pathogens of insects. Species

- *Stomachorhabditis georgica* Vardiashvili, 2006 from the family Museidae - is described for the first time (in the table it is denoted by *) [Vardiashvili, 2006]. Majority of registered nematodes was earlier recorded in Georgia by Kakulia [Kakulia, 1989].

References:

Vardiashvili E. *New species Stomachorhabditis georgica N.sp. (Nematoda, Rhabditidae) from eastern Georgia.* Proceedings of the Georgian Academy of Sciences, **4**, 1, 98-99, 2006.

Kakulia R. *Parasite nematodes of insects (Rhabditida, Talenchida) and biological method of control.* Tbilisi, "Metsniereba", p.209, 1989 (in Russian).

ქვემო ქართლის ბოსტნეული კულტურების ზოგიერთი მავნე მწერის ნემატოდოფაუნა

ვარდიაშვილი ე.

ზოოლოგიის ინსტიტუტი

(მიღებულია 06.04.2006)

რეზიუმე

მოცემულია ქვემო ქართლის რეგიონში მოპოვებული ბოსტნეული კულტურების ზოგიერთი მავნე მწერის ნემატოდოფაუნა. გამოვლენილია ნემატოდების 31 ფორმა. აქედან სახეობამდე გარკვეულია 30, ხოლო გვარამდე *Steinernema sp.* მისი სახეობამდე გარკვევა მასალის სიმცირის გამო არ მოხერხდა. *Stomachorhabelitis georgica* Vardiashvili, 2006 ჩვენს მიერ აღწერილია როგორც მეცნიერებისთვის ახალი სახეობა ოჯახი Museidae-ს წარმომადგენლებიდან.