ISSN 1512-2123

საქართველოს მეცნიერებათა აკადემიის

მაცნე

ბიოლოგიის სერია B

Biological series

2006
No. 1
Vol. 4

PROCEEDINGS

of the Georgian Academy of Sciences

ISSN 1512 – 2123

საქართველოს მეცნიერებათა აკადემიის

მაცნე

ბიოლოგიის სერია B

2006
No. 1
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

CONTENTS

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

Biochemistry

DISTRIBUTION OF MANNOSE AND MALTOSE-BINDING LECTINS IN DIOSCOREACEAE SPECIES

GAIDAMASHVILI M.[1]*, OGAWA T.[2], MURAMOTO K.[2]

[1]*Department of Plant Physiology, Iv. Javakhishvili Tbilisi State University.*
[2] *Department of Biomolecular Sciences, Tohoku University, Sendai, Japan.*
**E-mail: mari_gaid@yahoo.com*

(Received July 4, 2005)

Abstract

The distribution of mannose- and maltose-binding lectins (DB1 and DB3) in different Dioscoreaceae species was analyzed by immunoblot analysis using anti-DB anybodies. DB1 was detected in all yam species except *D. alata*. DB3 was detected in *D. batatas, D. japonica* and *D. alata*, but not in *D. bulbifera* tubers. Tissue-specific distribution of yam lectins showed that germinating shoots of *D. bulbifera* contained DB1, while mature leaves and stems of *D. batata, D. japonica* and *D. alata* were lacking both DB1 and DB3. The possible defensive role of yam tuber lectins is discussed.

Key words: *Dioscoreaceae,* immunoblot analysis, yam lectins.

Abbreviations: DBs - *Dioscorea batatas* lectins; HPLC - high-performance liquid chromatography; SDS-PAGE - sodium dodecylsulfate-polyacrylamide gel electrophoresis.

Introduction

Yam is the one of the principal nutrient source of many people in the tropics and Asia. It's a common name of about 500 species of the genus Dioscorea of the Dioscoreaceae family. There are great variations among different cultivars of yams in relation to their leaf morphology, vine characteristics, growing season, size and shape of tubers. Yam tubers are basically made up of carbohydrates but also constitute an important source of proteins accounting for 1-3% of the fresh tubers [Shewry P., 2003]. Over 80% of the tuber protein represents the storage protein known as Dioscorin [Harvey P., Boulter D., 1983]. Apart from the storage proteins plant tubers are known to contain other, defense-related proteins such as chitinase and lectins [Araki T., et al., 1995].

In the previous work we described tuber proteins from typical Japanese yam *Dioscorea batatas* Decne, and demonstrated that mannose- and maltose-binding lectins presented in significant amounts [Gaidamashvili M., et al., 2004]. Moreover, dioscorin-like storage protein itself proved to be maltose-binding lectin. Meanwhile, the presence of lectins in other yam species of Dioscoreaceae family has not been revealed yet.

In the present work we detected the presence of mannose- and maltose-binding lectins in various yam types and demonstrated that distribution of DBs differ in the tubers and vegetative parts of *Dioscoreaceae* species.

Materials and Methods

4 yam species *Dioscorea batatas, Dioscorea japonica, Dioscorea alata* and *Dioscorea bulbifera* were obtained in Japan and stored at 4°C until use except *D. bulbifera* which was stored at room temperature. For the isolation of yam lectins whole tubers, steam and leaves were used.

Experimental procedures used for the isolation and purification of *Dioscorea batatas* lectins were developed as before [Gaidamashvili M. et al., 2004]. Briefly, proteins were extracted from whole tubers using 50 mM sodium acetate buffer pH 4.0 and applied to hydrophobic chromatography on a Phenyl-Toyopearl 650 M column (3.5 × 20 cm) (Tosoh, Tokyo, Japan). DB1 and DB3 were purified by ion-exchange chromatography on a Hi-Trap Q column (5 ml) (Amersham Pharmacia Biotech. Uppsala, Sweden). The proteins from tubers and vegetative parts of *Dioscorea japonica, Dioscorea alata* and *Dioscorea bulbifera* were isolated and purified using the same protocol.

Lectin activity was detected by the hemagglutination assay using 2% rabbit erythrocytes. Sugar-binding specificity was determined by hapten-inhibition assay and affinity chromatography on maltose-sepharose 4B. Molecular mass of yam lectins was determined by SDS-PAGE as described by Laemmli [Laemmli U., 1976].

Anti-DB antiserum was raised in rabbits by repeated injection of *D. batatas* agglutinins emulsified with Freund's complete adjuvant. The titer and specificity of antibodies were estimated by double immunodiffusion analysis (Ouchterlony test) on agar gels [Munoz, J., 1971] and enzyme-linked immunosorbent assay (ELISA). After three booster injections, serum was collected and stored at -20°C.

Purified *D. batatas* agglutinin was transferred electrophoretically to Trans-Blot PVDF membrane (BIO-RAD, CA) by the method of Laurriere (1993). Membrane was incubated in the blocking buffer containing 50 mM Tris-HCl, 0. 15 M NaCl, 5 % skim milk (pH 7.5) for 40 min at 37°C. The membrane was then incubated with anti-DB3 antiserum at the 1:10 000 dilution for 90 min at 37°C, washed three times with TBS containing 0.05 % Tween 20 and were subsequently incubated with goat peroxidase-conjugated anti-rabbit IgG (Jacson Immunoresearch Laboratories, West Baltimore, PA) at the dilution 1:1 000 for 90 min at 37°C. Blots were developed with 3,3'-diaminobenzidine and H_2O_2 as substrate in 50 mM Tris-HCl buffer (pH 7.5).

Results and Discussion

The tuber proteins were successfully isolated and purified from *D. batatas, D. japonica, D. alata* and *D. bulbifera* tubers, leaves and stem by hydrophobic chromatography and ion-exchange chromatography. The tuber protein fractions of all yam species gave several polypeptide bands upon SDS-PAGE without 2-ME treatment, among those a major polypeptide band of MW 31 000 Da represented *ca* 75 % of the total protein in *D. batatas, D. japonica, D. alata* tubers as well as MW 66 000 Da minor bands corresponding to DB3 subunits (Fig. 1). In the *D. bulbifera* tubers DB3 subunits have not been observed, suggesting the possible lack of storage proteins. Two polypeptide bands corresponding to DB1 of MW 20 000 Da and 10 000 Da was found in *D. batatas* and *D. japonica,* but only one band of MW 10 000 Da in *D. bulbifera*. No bands were observed in *D. alata* tuber proteins.

Antiserum raised against DB1 and DB3 gave corresponding bands upon immunoblot analysis when reacted with tuber proteins of *D. batatas* thus indicating antiserum was monospecific (data not shown). No cross reactions were detected between anti-DB1 and anti-DB3 and specific proteins. Similarly, no bands were detected when reacted either with other tuber proteins such as yam chitinase. Similar results were obtained by using Double Diffusion (Ouchterlony) method, where cross-reacting antiserum and specific protein gives clear diffusion lines. Protein fractions

obtained from tubers of *D. japonica, D. alata* and *D. bulbifera* as well as mature leaves and stems of *D. batatas* were then electroblotted and reacted with anti-DB1 and anti-DB3. DB1 was expressed in all yam species except *D. alata*. Interestingly, DB1 was presented as a single polypeptide of MW 10 000 Da in *D. bulbifera* tubers lacking MW 20 000 Da polypeptide (Fig. 2, A). DB3 was found in *D. japonica* and *D. alata* tubers where it was presented as double bands corresponding to DB3L and DB3S subunits of maltose-binding lectin, However, DB3 subunits have not been detected in *D. bulbifera* tubers (Fig. 2, B).

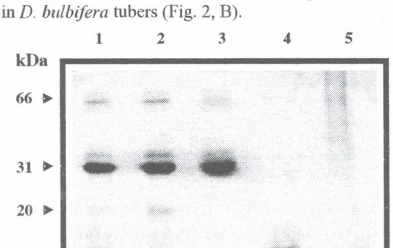


Fig. 1. SDS-PAGE of yam tuber proteins in 15 % acrylamide gel. Unreduced tuber proteins from *D. japonica,* (lane 1), *D. batata* (lane 2), *D. alata* (lane 3) *and D. bulbifera* (lane 4). Lane 5, young shoots of *D. bulbifera.*

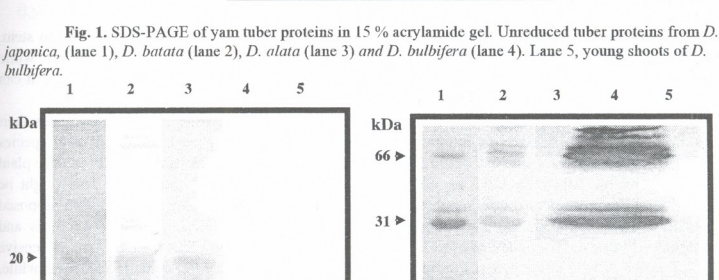


Fig. 2. Western-blot analysis of DBs in different Dioscoreaceae species. Tuber proteins of *D. batatas, D. japonica, D. bulbifera* and *D. alata* were subjected to SDS-PAGE and semi-dry blotting onto PVDF membranes. The membranes were immunostained with anti-DB1 (A) or anti-DB3 (B). Lane 1, DB1 and DB3 stained with CBB; Lanes 2-5, *D. batatas, D. japonica, D. alata* and *D. bulbifera* tuber proteins.

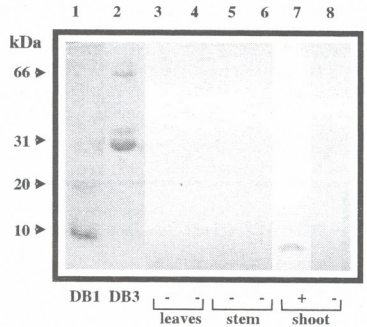


Fig. 3. Western-blot analysis of DBs in vegetative parts of Dioscoreaceae species. Proteins isolated from leaves and stem of *D. batatas* and shoot of *D. bulbifera* were subjected to SDS-PAGE and semi-dry blotting onto PVDF membranes. The membranes were immunostained with anti-DB1 (lanes 3, 5, 7) or anti-DB3 (lanes 4, 6, 8). Lanes 1 and 2 DBs stained with CBB. Lanes 3 and 4, *D. batatas* leaves; Lanes 5 and 6, *D. batatas* stem; Lanes 7 and 8, two weeks old shoot of *D. bulbifera*.

For tissue-specific distribution of yam lectins immunoblot analysis was applied to stem, leaves and young shoots of yam plants. Germinating shoots of *D. bulbifera* contained DB1, while none of DBs were detected in mature leaves and stems of other yam species (Fig. 3). Thus, it can be concluded that yam lectin-like proteins predominantly reside in the plant storage organs.

The presence of mannose- and maltose-binding lectins in true yam types indicates their common distribution features in monocotyledonous family of Dioscoreaceae. In all yam species both DB1 and DB3 were located in the tubers. Neither DB1 nor DB3 do not occur in mature plant leaves and stem. However, DB1 occurs in the young seedlings of *D. bulbifera*. This might be related with the biological functions of plant lectins accumulated in the storage parts with proposed defensive role as anti-insect, anti-fungal, anti-microbial, as well as being toxic to birds and mammals [Peumans W., Van Damme E., 1995]. We found DB1 is GNA-like protein, defensive role of which is well documented in monocotyledonous plants [Hilder V., et al. 1995]. Meanwhile, DB3 is homologous to dioscorins, the major storage protein of Dioscoreaceae and its proposed biological role is to participate in the interactions between the protein bodies and starch grains. Interestingly, we do not detect DB3 in aerial tubers of *D. bulbifera*, which is wild yam type and is of less importance because of low nutritional value.

References:

Araki T., Kuramoto M., Torikata T. *Purification of three acidic chitinases from yam aerial tuber. Application of quaternary ammonium ion detergent for the separation of yam chitinase from viscous tissue exstract.* Biosci. Biotech. Biochem., **59**, 430-434, 1995.

Gaidamashvili M., Ohizumi Y., Iijima Sh., Takayama T., Ogawa T., Muramoto K. *Characterization of the yam tuber storage proteins from Dioscorea batatas exhibiting unique lectin activities.* J. Biol. Chem., **279,** 26028-26035, 2004.

Harvey P., Boulter D. *Isolation and characterization of the storage proteins of yam tubers (Dioscorea rotundata).* Phytochemistry, **22**, 1687-1693, 1983.

Hilder V., Powell K., Gatehouse A., Gatehouse J., Gatehouse L., Shi Y., Hamilton W., Merryweather A., Newell C., Timans J., Peumans W., van Damme E., Boulter D. *Expression of snowdrop lectin in transgenic tobacco plants results in added protection against aphids.* Transgenic Res., **4**, 18-25, 1995.

Laemmli U. *Clevage of structural proteins during the assembly of the head of the bacteriophage T4.* Nature. **227,** 680-685, 1976.

Lauriere M. *Asemidry electroblotting system efficiently transfers both high- and low-molecular-weight proteins separated by SDS-PAGE.* Anal. Biochem. **212,** 206-211, 1993.

Munoz J. *Double diffusion in plates.* In: Methods in immunology and immunochemistry Williams C.A. (Ed), volume III, 146-160, 1971.

Peumans W., Van Damme E., *Lectins as plant defense proteins.* Plant Physiol. **109,** 347-352, 1995.

მანოზა- და მალტოზა-დამკავშირებელი ლექტინების განაწილება DIOSCOREACEAE სახეობებში

გაიდამაშვილი მ.[1], ოგავა ტ.[2], მურამოტო კ.[2]

[1] ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი.
[2] ტოჰოკუს უნივერსიტეტი, სენდაი, იაპონია.

(მიღებულია 04.07.2005)

რეზიუმე

შესწავლილია მანოზა- და მალტოზა-დამკავშირებელი ლექტინების (DB1 და DB3) განაწილება Dioscoreaceae ოჯახის სახეობებში იმუნობლოტინგით ანალიზისა და ანტი-DB ანტისხეულების გამოყენებით. DB1 ვლინდებოდა Dioscoreaceae ოჯახის ყველა სახეობაში გარდა *D. alata* ტუბერებისა. DB3 ვლინდებოდა *D. batatas, D. japonica* და *D. alata* ტუბერებში, მაგრამ არ ვლინდებოდა *D. bulbifera*-ში. ლექტინების განაწილების შესწავლამ ვეგეტატიურ ქსოვილებში აჩვენა, რომ *D. batata, D. japonica* და *D. alata*-ს ზრდასრული ღერო და ფოთლები არ შეიცავდა ლექტინებს, მაშინ როცა *D. bulbifera*-ს ორკვირიან აღმონაცენში ვლინდებოდა მანოზა-დამკავშირებელი ლექტინი. განხილულია მალტოზა-დამკავშირებელი ლექტინების სავარაუდო დამცველობითი როლი Dioscoreaceae ტუბერებში.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

TNT ASSIMILATION ABILITY OF SOYBEAN (*GLYCINE MAX*) AND EPIGENETIC PECULIARITIES OF INTRACELLULAR DISTRIBUTION

RAMISHVILI M., GHOGHOBERIDZE M., GOGAVA M., ZAALISHVILI G., CHRIKISHVILI D., CHELIDZE N., BATSIKADZE G.

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

(Received September 20, 2005)

Abstract

Soybean (Glycine max) *in vitro* tissue culture was produced. It has been studied the effect of 2,4,6-trinitrotoluene (TNT) different concentrations on the growth index of dedifferentiated cells of the fresh biomass. At starting of a stationary phase, after TNT deficiency in the nutrient medium, the biomass accumulation potential increases at 150%. The optimum (50mg/l) and lethal (200mg/l) concentrations for soybean *in vitro* tissue cells have been stated. (1-^{14}C)TNT localization in cells of soybean leaf and callus culture was analyzed by electron-microscopic radioautographic method. Radioactive label in differentiated as well as in dedifferentiated tissue cells was revealed basically on the cell membrane structures.

Key words: callus culture, remediation potential, radio-autography, phytotoxicity.

Introduction

2,4,6-Trinitrotoluene (TNT) is one of the widely distributed explosive. TNT and its metabolites have toxic and mutagenic potential. TNT is classified as a possible human carcinogen, with a Drinking Water Equivalent Level (DWEL) of 20 μg/l and a lifetime health advisory of 2 μg/l [Mueller et al., 1999].

Excavation and thermal processes are currently the most common remediation technologies, but are costly. Therefore it's necessary to perfect bio- and phytoremediation technologies of this compound, which is actively investigated recently [Mueller et al., 2004].

Plants differ due to TNT remediation potential. Monocotyledonous plants are less sensitive to TNT than dicotyledonous plants [Gong et al., 1999]. At the Institute of Biochemistry and Biotechnology, Georgian Academy of sciences on TNT assimilation potential were tested the following cultural plants: soybean (*Glycine max*), barley (*Hordeum sativum*), pea (*Cicer arietinum*), garden pea (*Pissum sativum*), sunflower (*Helianthus annuus*) and maize (*Zea mays*). Privilege to cultural plants was given owing to the biomass regulation availability via the known agro-technical methods. Among the studied cultures soybean was distinguished by high TNT assimilation ability [Khatisashvili et al., 2004].

TNT degradation ability of *in vitro* cultures of *Salanum aviculare* and *Rheum palmatum* was studied [Nepovim et al., 2004]. The different concentrations of degradation products in each culture were an indication that the metabolism of TNT is controlled by different enzymatic

systems. Therefore, studying different species for TNT degradation is necessary for the search of the most suitable objects for TNT phytoremediation.

Plant *in vitro* systems generally enables us to study xenobiotics degradation under well-controlled conditions (temperature, light, composition of the nutrient medium) during the experiment and the pathway of metabolite formation independent on the microorganisms such as bacteria, fungi or yeasts consortia. Moreover, in this case each cell has contact with the contaminant and problems connected with the uptake of contaminant from soil via the root system can be avoided. As a result, this arrangement speeds up the degradation to the maximum and allows us to study and characterize TNT degradation in the plant system only.

Materials and Methods

Glycine max callus culture was produced from soybean leaf grown on Knop sterile nutrient medium and was cultivated on MS *in vitro* medium [Murashige, Skoog, 1962] with different additives. Growth index of fresh biomass of isolated cultures was calculated due to the ratio between inoculums fresh weight (N_1) and inoculums initial weight (No).

In order to state TNT assimilation by soybean callus culture and its toxic effect on cells, the dedifferentiated tissue was grown on above mentioned medium, in which different concentrations of TNT (50, 100, 150, 200 mg/l) were introduced. Samples of the TNT-containing medium on which the tissues grew were taken every 4 days and the concentration of TNT was determined spectrophotometrically using 447nm illumination in a highly alkaline environment [Oh et al., 2001].

Radioactive (1-^{14}C) TNT was synthesized by (1-^{14}C) toluene. (1-^{14}C)TNT specific activity equaled 500 Bq/mg. Intracellular distribution of (1-^{14}C) TNT in soybean intact plant and callus tissue was studied by microscopic radio-autography method. Soybean leaf callus tissue was grown on MS nutrient medium containing labeled (1-^{14}C) TNT (50mg/l). Soybean 7 day old seedlings were shifted to (1-^{14}C) TNT (50mg/l) water solution. 5 Days later, material was prepared by the standard electron microscopic method. Golden cuts of moulded in epoxide resin material were placed on sieve covered with forewarm layer. After exposition of cuts on $NiCl_2$ (10^{-4}M) solution drop for 2x30sec, the samples were shifted to hydroquinon's 1% water solution drop at pH - 9.0, exposure time was 2x40sec. Finally the samples were analyzed on electron microscopy [Buadze et al, 1998].

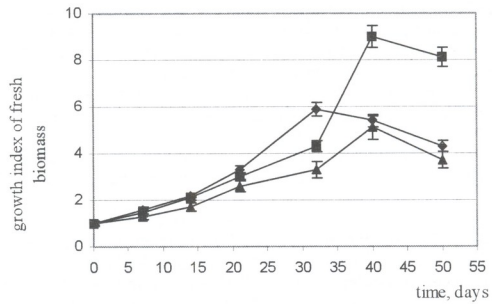
Results and Disscussion

Plants grown in soil, as a rule are infected significantly and contain as superficial so intracellular infections, which complicates the production of *in vitro* cultures. Therefore, to produce soybean tissue culture, sterile seedlings were cultivated. Soybean seeds were sterilized in 1% sublimate ($HgCl_2$) solution for 3-4 min, rinsed 4-5 fold in sterile distillate, placed in sterile tubes each containing 5ml of sterile Knop solution. Covered samples were placed for a week in thermostat at 25°±1°C. Tubes with germinated seeds were placed in light. Leaves of sterile soybean seedlings (in laminar box-LKB) were cut in 2-2,5 cm pieces and placed on the sterile nutrient medium. 1/3 of 0.8-1.0cm pieces of stem was placed on the apical side of segment in the same medium. MS modified area served as a nutrient medium[Murashige, Skoog, 1962].

The best callus tissues were produced when into MS medium were introduced 2,4 dichlorphenoacetic acid (2,4D) (1ml/l), Casein hydrolyzate (100mg/l) and Nicotinic acid-(0,5mg/l), sucrose (30g/l) and agar (8g/l). Callus was grown at 26°±1 °C in thermostate and sub-cultivation

occurred in every 40 day. For further investigation soybean callus tissue was used. It was stated the fresh biomass accumulation ability in growth cycle.

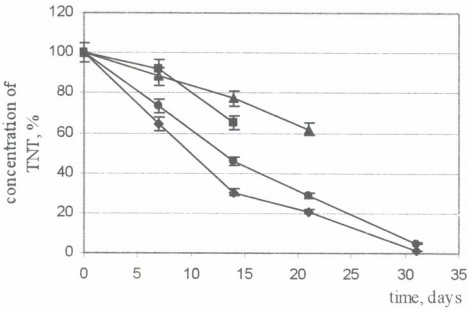
Growth curve of fresh biomass (Pic.1) shows, that latent phase of freshly produced soybean callus tissue lasts for about seven days, active exponential phase from the 14th to 32nd day, after which short stationery phase is followed by a cell degradation phase. In order to study TNT assimilation ability of cells of soybean *in vitro* culture and phytotoxicity of this compound, callus was placed on the nutrient medium containing the toxicant in 50, 100, 150, 200 mg/l concentrations. As seen in picture 1, 150 and 200 mg/l concentrations of TNT appeared toxic for cells of soybean *in vitro* culture at the 21st and 14th days of the growth cycle. At 50 mg/l concentration of TNT in the nutrient medium cells of soybean callus culture at starting the stationary phase (33rd day) almost totally assimilated toxicant (Pic.2), and in case of 100 mg/l TNT its amount didn't prevail 5%.



Pic.1. Effect of TNT on the cell growth of *Glycine max* callus culture

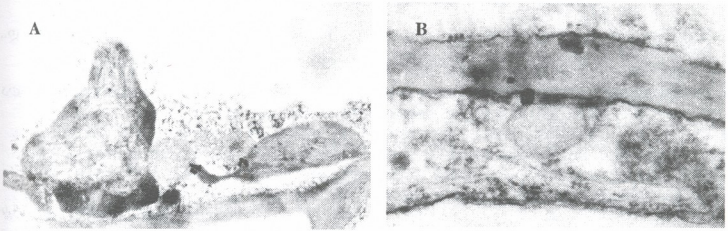
kontrol medium — 50 mg/l — 100 mg/l

Further, the effect of 50 and 100 mg/l concentrations of TNT on the growth cycle of soybean callus cells was investigated. It was stated, that up to the 21st day, at 50 mg/l TNT concentration the curve of fresh biomass growth index of callus tissue cells was almost similar the control, but in case of 100mg/l TNT concentration, dropped behind insignificantly (Pic.1). In case of 50 mg/l TNT content in the nutrient medium after TNT deficiency in the stationary phase (33rd day), unlike the control, fast acceleration of the growth index of soybean callus tissue biomass was observed. As compared with the control, the *in vitro* culture cells didn't undergo degradation, on the contrary the biomass accumulation ability was increased at 150%. In case of 100 mg/l TNT content in the nutrient medium at the 33rd day starts the growth of biomass accumulation, though on the 40th day fresh biomass accumulation proceeds insignificantly slower the control. The received results point to the fact, that at 50-100mg/l TNT concentrations cells of soybean *in vitro* culture consume all cell energetics for toxicant assimilation. After the defficience of this compound in the nutrient medium cell metabolism basically serves to activate cell proliferation.



Pic. 2. Dynamics of TNT assimilation by *Glycine max* callus culture. TNT concentratoin in the solid media:

Electron-microscopic radio autographic method was applied to study (1-14C)TNT localization in cells of soybean leaf and callus culture (Pic.3A,B). These pictures show that radioactive label in differentiated (Pic.3A) as well as in dedifferentiated (Pic.3B) tissue cells revealed basically on the cell membrane structures (plasmalemma, chloroplasts, mitochondria). The mentioned phenomenon can be explained by the fact that TNT transformation in plant cells generally proceeds by NAD(P)H nonspecific nitroreductase, which is localized generally on cell membrane structures [Khatisashvili et al., 2004].



Pic. 3. A.(1-^{14}C)TNT (100mg/l) localization in cells of soybean intact plant. Radioactive label at the contact side of mitochondria and chloroplast. x 30 000. B. (1-^{14}C)TNT (100mg/l) localization in cells of soybean leaf callus culture. Radioactive label on the contact side of mitochondria and plasmalemma. x 30 000.

It should be mentioned, that unlike the earlier investigation [Ramishvili et al., 2005] of *Yucca gloriosa* L. intact plant cells, the soybean (*Glycine max*) leaf cell destruction due to TNT action is less expressed which doesn't coincide with the data that monocotyledonous plants are less sensitive to TNT than dicotyledonous plants [Gong et al., 1999].

References:

Bernd W., Shoenmuth and Wilfried Pestemer. *Dendroremediation of Trinitrotoluene(TNT). Part 1:Litereture Overview and Research Concept.* ESPR-Environ. Sci & Pollut.Ress, **11**, 4, 273-278, 2004.

Buadze, O.A., Durmishidze, S.V., Apakidze, A.V. *Ultrastructural aspects of herbicide 2,4-D movement and localization in plant cells.* Bull. Georg. Acad. Sci., **119**, 4009-411, .1985.

Gong P., Wilke B.M., Fleischmann S. *Soil-based Phytotoxicity of 2,4,6-trinitrotoluene(TNT) to terestrial higher plant.* Arch. Environ. Cont. Toxicol., **36**, 152-157, 1999.

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.

Mueller W.F., Bedell G.W., Shojaee S and Jackson P.J. *Bioremediation of TNT wastes by higher plants*. Proceedings of the 10th Annual Conference on Hazardous Waste Research, 222-230,1999.

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., 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.-T., Sarath G. Shea P. J. *TNT nitroreductase from a Pseudomonas aeruginosa strain isolated from TNT-contaminated soil.* Soil. Boil. Biochem., **33**, 875-881, 2001.

Ramishvili M., Gogava M., Chelidze N., Gogoberidze M. *Study of 2,4,6-trinitrotoluene (TNT) assimilation ability by in vitro culture cells of Yucca gloriosa L.* Proceedings of the Georgian Academy of Sciences, B, **3**, 2,21-25,2005.

სოიას (*Glycine max*) TNT-ს შთანთქმის უნარი და შიდაუჯრედული განაწილების ეპიბენეფიკური თავისებურებები

რამიშვილი მ., ღოღობერიძე მ., გოგავა მ., ზაალიშვილი გ., ჭრიკიშვილი დ., ჭელიძე ნ., ბაციკაძე გ.

საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიის და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 20.09.2005)

რეზიუმე

მიღებულია სოიას *in vitro* ქსოვილური კულტურა. შესწავლილია 2,4,6-ტრინიტროტოლუოლის სხვადასხვა კონცენტრაციის გავლენა დედიფერენცირებული უჯრედების ნედლი ბიომასის ზრდის ინდექსზე. სტაციონალური ფაზის დადგომისას, საკვებ არეში TNT-ს გამოლევის შემდგომ, ბიომასის დაგროვება იზრდება 150%-მდე. დადგენილია სოიას *in vitro* ქსოვილის უჯრედებისთვის ოპტიმალური (50მგ/ლ) და ლეტალური (200მგ/ლ) კონცენტრაციები. ელექტრონულ-მიკროსკოპული რადიოავტოგრაფიის მეთოდით შესწავლილია (1-^{14}C) TNT-ს ლოკალიზაცია სოიას ფოთლის და კალუსური კულტურის უჯრედებში. როგორც დიფერენცირებულ, ასევე დედიფერენცირებულ უჯრედებში რადიოაქტიური ნიშანი ძირითადად გამოვლინდა უჯრედების მემბრანულ სტრუქტურებზე.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

Biochemistry

BIOLOGICAL ACTIVE COMPOUNDS OF RHODODENDRON (*RHODODRNDRON CAUCASICUM PALL.*) LEAVES

SHALAMBERIDZE M.[1], MELKADZE R.[2], OMIADZE N.[3]

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

(Received September 8, 2005)

Abstract

Biologically active compounds of rhododendron (*Rhododendron caucasicum Pall.*) leaves have been studied. Localization of tanning substances, catechins, albutin, flavonoids, phloroglucine and resorcinol derivatives was established by qualitative reaction in a leaf tissue. A simplified method of arbutin content determination based on arbutin specific reaction with natrium sulphacyl and nitrate in alcaline medium was developed. It has been shown that the complex coloring is stable for several hours. Precision of analyses by this method exceeds that of State Pharmacopoeia iodometric method. At the same time it excludes the use of toxic solvents in a big amount. Comparative statistical indices of the known and developed methods for arbutin determination have been presented.

Key words: tanning substances, arbutin, catechins, flavonoids, localization,

Introduction

Rhododendron (*Rhododendron caucasicum Pall.*) is an evergreen, 1,5 m high shrub, grown in high mountain zone, at 3000 m a.s.l., where it forms brushwood, consisting completely of rhododendron. All parts of the plant are rich in various biologically active compounds, vitamins C and P [Kezeli & Chrelashvili, 1947], phenols and their derivatives – arbutin [Zolotitskaya, 1958], catechines [Durmishidze et al., 1960], tanning substances, flavonoids are found in leaves. The plant is widely used in homoeopathy: against hydrargyrism, at mucous membrane diseases and headache. The preparation received from leaves has high P-vitamin activity; rhododendron leaves infusion is widely used [Melkadze].

Rhododendron is known as arbutine containing plant. For identification of this compounds physical and chemical methods are used. The iodometric titration via arbutin acidic hydrolysis is defined as a chemical method [State Pharmacopoeia, 1987]. It is rather volumetric and long: it takes 4 hours for one analysis. The other method of arbutin determination is based on its reaction with 4-amonoantipyrine in alkaline medium, at the presence of potassium ferricyanide as an oxidant. The colored complex was extracted by chloroform and optical density was defined. The method is specific, time-consuming and a big amount of toxic organic solvents is requested.

Material and Methods

The mixture of 2nd and 3rd leaves of rhododendron gathered in Imereti highland zone (Khoni Region, village Gordi) was used as object of the research to localize some classes of bioactive compounds by characteristic histochemical reactions.

To determine arbutin content we used photoelectrocalorimetric method developed by us: 10 g of rhododendron leaves were cut into small pieces to pass through 1 mm diameter sieve cells. Approximately 0,5 g of this mass was placed into 100 ml flask, 50 ml water was added and boiled over the water bath for 30 min. The hot extract was filtered by cotton into 100 ml graduated flask. The cotton with raw material was put into the flask again, 25 ml water was added to wash out the raw material from the funnel. The raw material was extracted again for 30 min. Then filtration using cotton filter in the graduated flask was carried out. The raw material left on the filter was washed twice with 10 ml of hot water. 6 ml of saturated solution of lead acetate was added to filtrate in the graduated flask, shaken and filled with water up to the grade mark. Then it was moved into a conic flask, placed over the boiled water-bath and stayed for 10 min till complete coagulation of a precipitate. The precipitate was filtered by goffered filter, 0.8 ml natrium sulphate was added and the obtained precipitation was again filtered through the goffered filter, removing the first portion of filtrate.

4 mg of 0.02% natrium nitrate and 4 mg of 0.08% natrium sulphate solutions were introduced into 10 ml graduated flask and stayed for 3 min; to 1 ml filtrate 0.08 ml of 10 % natrium hydroxide solution was added and filled with water up to a grade mark. Received solution was put into a water-bath at 45-50^{0}C for 1 min; then stayed at a room temperature for 20 min.

Optical density of the obtained solution was measured photoelectrocalorimetrically in 10 mm thick cuvette, at 490 nm wave length; as a control solution was used water.

The calculation of arbutin content on absolutely dry raw material (in %) was carried out by formula:

$$x = \frac{D \cdot 0{,}938 \cdot 10 \cdot 100 \cdot 100}{E^{1\%} \cdot m \cdot a \cdot (100 - w)} = \frac{D \cdot 846{,}95}{m \cdot (100 - w)}$$

where: D – optical density of the studied solution; 0,938 – calculation coefficient for water-free arbutin; $E^{1\%}$ - specific index of arbutin absorption on 490 nm wave length; m – raw mass (g); a – extract mass (ml); w – moisture content of a raw material (%).

Results and Discussion

Table 1 presents the results of histochemical reactions which establish localization of some classes of biologically active compounds in rhododendron leaves. This reactions confirm the data about the presence of bioactive compounds in rhododendron leaves.

Table 2 presents statistical data on arbutin quantitative content received by known and developed by us methods. According to the obtained results it can be concluded that arbutin estimation by our method has some advantages over the known methods, in particular, in duration of the analysis and decreased amount of toxic organic solvents as well as high precision of analysis (3,4% error in comparison with 4,8%).

The suggested method can be used for studying of arbutin content in other plant raw materials.

Table 1. Localization of bioactive compounds in leaves of rhododendron.

Class of natural compounds	Reaction	Localization of bioactive compounds
Tanning	with ferric oxide chloride, with molybdenic acid ammonia (Gardiner' s method)	on a film of upper epidermis, hair, in phloem elements, xylem radial parenchyma, wood fibril layers; weak coloring of epidermis and nerve main tissues, phoem, parenchyma, xylem, hypodermis.
catechines	Riva's method	Red coloring of parenchyma layers around mechanical tissues, weak- in phloem, fragmentary – in hair, collenchymas and hypodermis of side nerves.
arbutine	ammonia and 10% phosphor-molybdenic acid with HCl solutions	Blue-green coloring of peripheral main parenchyma around longitudinal bundles, weak coloring of parenchyma xylem and hypodermis, gradual coloring of mesophyll.
flavonoids	Mg and concentrated HCl.	Weak coloring of parenchyma xylem, hair and epidermis get red-brownish coloring.
phloroglucine and resorcine detivatives, catechines	1% alcoholic solution of vaniline and concentrated HCl	Acutely expressed coloring of lower epidermis in multicellular hair alveoli

Table 2. Statistical data of quantitative contents of rhododendron leaves received by known and our method

Method	X	$X_{average}$	S	α (%)	t (α)	ΔX	ε
iodometric (State Pharmacopeia)	1,87 2,03 1,79 1,94 1,81 1,96	1,90	0,093	95	2,57	0,0975	4,8
photoelectro-calorimetric	1,48 1,37 1,36 1,44 1,38 1,50	1,42	0,0602	95	2,57	0,063	3,4

References:

Durmishidze S.V., Shalashvili A.G., Ushakova M.P. *New sources of bioflavonoids.* Bull. Georg. Acad. Sci., **25**, 6, 673-676, 1960.

Kezeli T.A., Chrelashvili M.N. *Ascorbic acid and catalase in rhododendron leaves connected with vertical zonality.* Bull. Acad. Sci. SSSR., **8**, 6, 13-416, 1947.

Melkadze R. *Method of tea substrate production.* Patent of Georgia GE U2002 887U.

State Pharmacopoeia of SSSR. General methods of analysis. Ministry of Health of SSSR X1 ed., **1**, Medicine, 336,1987.

Zolotitskaya S.Ya. *Medical resources of flora of Armenia.* Erevan, vol. **1**, 327, 1958.

კავკასიური დეკას (*Rhododendron caucasicum Pall.*) ფოთლების ბიოლოგიურად აქტიური ნივთიერებები

შალამბერიძე მ.[1], მელქაძე რ.[2], ომიაძე ნ.[3]

[1]ქუთაისის ა. წერეთლის სახელობის სახელმწიფო უნივერსიტეტი
[2]საქართველოს მეცნიერებათა აკადემიის ქუთაისის სამეცნიერო ცენტრი
[3]ს. დურმიშიძის სახ. ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, საქართველოს მეცნიერებათა აკადემია

(მიღებულია 8.09.2005)

რეზიუმე

შესწავლილია კავკასიური დეკას ფოთლების ბიოლოგიურად აქტიური ნივთიერებები. ნაჩვენებია, რომ ფოთლის ქსოვილში ლოკალიზებულია მთრთოლავი ნივთიერებები, კატექინები, არბუთინი, ფლავონოიდები, ფლუოროგლუცინისა და რეზორცინის წარმოებულები. შემუშავებულია არბუთინის შემცველობის განსაზღვრის გამარტივებული მეთოდი, რომელსაც საფუძვლად უდევს არბუთინის სპეციფიკური რეაქცია ნატრიუმის სულფაცილთან და ნიტრიტთან ტუტე არეში. ნაჩვენებია, რომ კომპლექსის შეფერილობა მედეგია რამდენიმე საათის განმავლობაში. წარმოდგენილია არბუთინის განსაზღვრის ცნობილი და შემოთავაზებული მეთოდების შედეგების შედარებითი სტატისტიკური მახასიათებლები.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

FLAVONOID COMPOUNDS OF DIFFERENT PEACH CULTIVARS (*PERSICA VULGARIS MILL.*) FRUITS

BLIADZE G.[1], CHUBINIDZE V.[2], TSIKLAURI G.[2]

[1]*Research Institute of Gardening, Viticulture and Winemaking, Georgian Academy of Agriculture*
[2]*S. Durmishidze Institute of Biochemistry and Biotechnology, Georgian Academy of Sciences*

(Received May 16, 2005)

Abstract

Qualitative and quantitative analyses of flavonoid (flavonols, catechines and leukoanthocyanidins) compounds of the fruits of different peach cultivars were carried out. It was established that different cultivars, as well as peels and pulps of the same cultivar differ by contents of flavonoid compounds.

Introduction

Flavonoid compounds are widely spread and they are discovered nearly in all plants [Geissman, 1960]. These compounds are characterized by high biological activity and they are broadly used in medicine for prophylactic of several diseases [Vekhov, et al., 1978; Choi Myhang Hee, et al., 2000; Shi Jingin, et al., 2000]. Flavonoid compounds take an important role in technological processes of food industry, they increase nutritive value of food products [Durmishidze, 1955; Fiodorova, 1965].

Peach is widely spread in Georgia as significant food stuff. Furthermore, characterized with good flavour and aroma, peach fruit is used as raw material, so its juice, jam, dried fruit. It also contains big amount of biological active compounds. So, the goal of our research was to study flavonoid compounds of the fruits of different peach cultivars.

Material and Methods

Researched material was collected on experimental plot of the Institute of Gardening, Viticulture and Winemaking, in Skra. Peels and pulps of the fruit were separated and extracted by 70% methanol. Extracts were combined, filtrated on paper filter and evaporated to small volumes on rotational evaporator in vacuum, at 40-50°C. To determine composition of received samples bidirectional chromatography was carried out in the following solvent systems: 1) butanol : acetic acid : water in the ratio 4 : 1 : 5 (1st direction) and 2) 2% acetic acid (2nd direction). Chromatograms were dried in drying box and amount of the spots were marked on chemoscope (UV- lightening).

For quantitative analysis from exact weights of raw material thrice-repeated extraction of flavonoid compounds by 70% methanol was carried out. Received extracts were evaporated in rotational vacuum evaporator in above mentioned conditions up to exact volume, from which required amount of liquid was obtained and determined quantitatively. For quantitative analysis of

flavonoid compounds Wilson's method with modification was used [Wilson, 1939]; for catechines and leucoanthocyanidins determination – Swain & Hills' method [Swain & Hills, 1959].

Results and Discussions

Results of bidirectional chromatography analysis of flavonoid compounds qualitative composition of peach fruits peels and pulps are presented in Table 1. Received data show that the number of spots varies in different samples which indicate to the differences of qualitative composition of flavonoid compounds according to cultivars. It is also seen from the table that by qualitative composition fruits peels are richer than pulps in which some compounds were not revealed.

Data of determination of quantitative contents of some groups of flavonoid compounds (flavonols, catechines and leukoanthocyanidins) are presented in Table 2. Quantitative analysis of these compounds was based on color reaction between flavonoids and different chemical reagents.

Namely, quantitative determination of flavonols is based on color reaction of flavonols with boric acid. In this case flavonols in dry acetone get yellow coloration, and at adding of citric acid coloration becomes more intensive and stable. Absorption intensity of received yellow coloration was measured on ФЭК-56, with N3 light-filter and СВД-120А lamp, in the cuvette with absorption layer width of 20 mm. As a control fresh reagent of citric boric acid was used. By optical density quantity of flavonols (mg) per 1 g of crude material was counted using preliminarily built up standard curve.

Analogous quantitative determination of catechines was based on color reaction of catechins with vanillin reagent. To the definite amount of analyzed solution needed amount of vanillin reagent was added. Composite was stirred and put in dark place, at 25^0C during 15 minutes. As a control distillate and vanillin reagent composite was used. Absorption intensity of received reddish coloration was measured on СФ-4А spectrophotometer at 500 nm wavelength in the cuvette with absorption width of 10 mm. By optical density quantity of catechines (mg) per 1 g of crude material was counted according to standard curve.

Quantitative determination of leukoanthocyanidins was based on their ability to produce corresponding anthocyanidins while heating with mineral acids. 1 ml of analyzing extract was brought in two conic flasks. 1 ml of distilled water and 8 ml leukoanthocyanidin reactive (25 ml concentrated HCl and 475 ml butanol) were added to each flask. Flasks were stirred and one flask was heated up to 40^0 for 3 min without cover, then it was closed and heating was continued for 40 min; then the flask was cooled by flowing water. Intensity of absorption of received solution of pink-red coloration was measured on СФ-4А spectrophotometer at 550 nm wavelength. By optical density quantity of leukoanthocyanidins (mg) per 1 g of crude material was counted according to standard curve.

Analysis of received data (Table 2) shows that quantitative contents of flavonols and leukoanthocyanidins dominate in fruit peels, and catechines contents are higher in fruit pulps, except the cultivar "Krimchak" in which catechines were fixed as a trace. By quantitative content differences between cultivars were noticed mainly in the case of leukoanthocyanidins. Flavonols contents in all studied cultivars are practically identical; small differences were noticed in catechines contents.

Thus, received data show that peach fruits consist every group of flavonoid compounds in more or less amounts that increase their nutritive value.

Table 1. Qualitative analysis of flavonoids of peach fruit; solvents: I direction - butanol-acetic acid-water (4:1:5); II direction - 2% acetic acid.

Cultivars	Analyzing material	Fluorescence, R_F								Amount of spots
		Light yellow		Dark yellow		turquoise		violet		
		solvents								
		I	II	I	II	I	II	I	II	
Krimchak	peel	0.75	0.32	0.65	0.48	0.32	0.39	0.11	0.51	4
	pulp	0.77	0.70	0.41	0.63	0.51	0.21	-	-	3
Alberta	peel	0.84	0.63	0.77	0.58	047	0.82	0.18	0,72	4
	pulp	0.25	0.35	0.36	0.41	0.59	0.57	0.10	0.12	4
Bestavashvili	peel	0.62	0.38	0.59	0.38	0.31	0.41	0.18	0.24	4
	pulp	-	0.41	0.34	0.57	0.48	-	-	0.31	3
Bel	peel	0.18	0.25	-	0.37	0.23	0.21	0.19	0.31	3
	pulp	-	0.13	-	0.51	0.11	0.14	-	0.15	2
Geokchai	peel	0.34	0.25	0.38	0.21	0.12	0.48	-	-	3
	pulp	0.29	0.36	0.21	-	-	-	0.17	-	2
Vezhuri	peel	0.93	0.43	0.81	0.63	0.53	0.88	0.29	0.81	4
	pulp	0.50	0.27	0.52	0.49	0.67	0.63	0.23	0.29	5
Eristavis vardisperi	Peel	0.85	0.87	0.93	0.95	0.69	0.95	0.32	0.98	5
	pulp	0.81	062	0.40	0.57	0.82	0.83	0.19	0.27	5

Table 2. Total quantity of flavonoid compounds in the peel and pulp of peach fruit (mg/g crude mass)

Cultivar	flavonols		catechines		leukoanthocyanidins	
	Peel	pulp	peel	pulp	peel	pulp
Krimchak	1.25	0.55	0.50	-	1.8	1.08
Alberta	1.32	0.52	0.55	1.10	6.8	2.00
Bestavashvili	1.35	0.63	0.59	1.15	7.5	2.50
Bel	1.30	0.57	0.43	0.75	4.8	2.05
Geokchai	1.27	0.52	0.37	0.90	4.5	1.09
Vazhuri	1.37	0.55	0.7	1.20	7.3	2.10
Eristavis vardisperi	1.38	0.52	0.58	1.30	7.5	2.00

References:

Choi Myhang Hee, Choi Huun-Suk, Shin Kilman, Jung Soon-Teck, Kim Kyong-Sun, Hice Myung Yue. J.Food Sci.Nutz., **5**, 1, 37, 2000.

Durmishidze S. V. *Tanning agents and anthocyanidins of grapevine and vine.* M., "Nauka", 1955.

Fiodorova G.A. *Natural staining agents in berry fruitage raw material.* M., "Nauka", 1965.

Geisman T. *Biochemical methods of plant analysis.* M., "Inostr.lit.", 453, 1960.

Shi Jingin, Huang Shaohua Meli, Jhung Jinming. Chemical Abstracts, **133**, 1, 3876, 2000.

Swain T., Hills W.E. *The phenolic constituents of Prunes domestica. The quantitative analysis of phenolic constituents.* J.Sci.Food Agric., **10**, 63, 1959.

Vekhov V.N., Gubanov I.A., Lebedeva G.F. *Cultivated plants of USSR.* M., "Misl", 252, 1978.

Wilson C.W. *A study of the boric acid color reaction of flavonoid derivatives.* J.Amer.Chem.Soc., **61**, 1939.

სხვადასხვა ჯიშის ატმის ნაყოფის ფლავონოიდური ნაერთები

ბლიაძე გ.[1], წუბინიძე ვ.[2], წიკლაური გ.[2]

[1]მებაღეობის მევენახეობისა და მღვინეობის სამეცნიერო კვლევითი ინსტიტუტი, საქართველოს სოფლის მეურნეობის აკადემია
[2]ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, საქართველოს მეცნიერებათა აკადემია

(მიღებულია 16.05.05)

რეზიუმე

შესწავლილია სხვადასხვა ჯიშის ატმის ნაყოფის ფლავონოიდური ნაერთების თვისობრივი შემადგენლობა და რაოდენობრივი შემცველობა. დადგენილია თვისობრივი შემადგენლობის ჯიშობრივი განსხვავება და მათი რაოდენობრივი განაწილება ერთი და იგივე ჯიშის ნაყოფის კანსა და რბილობში.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

Biophysics

THE COMPARATIVE ANALYSIS OF LOCUST FUNCTIONALLY DISTINGUISHED FLIGHT MUSCLES MYOFIBRILLAR PROTEINS

PAPIDZE G.[1], GACHECHILADZE N.[2], TORIASHVILI T.[2], CHUBINIDZE A.[2]

[1]*Department of Citology, Hystology and Development Biology, Iv. Javakhishvili Tbilisi State University*
[2]*Institute of Molecular Biology and Biological Physics, Georgian Academy of Sciences*

(Received September 7, 2005)

Abstract

Contents of Locust (*Locusta migratoria migratorioides R.F.*) functionally different monofunctional (MOF) and bifunctional (BIF) flight muscles' (Longus dorsati-112, tergosternal –113, tergocoxal – 119, 120) myofibrillar proteins have been studied. It has been shown that in studied muscles the percentage of myosin and other protein components (with molecular mass 118, 110, 90, 56, 39,37, 33 kDa) are almost the same. The amount of actin in MOF muscles practically doesn't differ (in muscle 112 amount of actin is 17,36%, in 113 - 18,21%). In BIF muscles the content of actin is bigger than in MOF muslces and is 21,12% in muscle - 119 and 26,17% in muscle - 120. The difference between amount of actin in MOF and BIF flight muscles should be caused by their structural and functional characteristics.

Key-words: flight muscles, monofunctional muscle, bifunctional muscle, Locust.

Introduction

The flight muscles, according to their functions, are divided into MOF and BIF muscles [Wilson, 1962]. BIF muscles participate in the movement of wings and extremities and MOF muscle - only in wings movement. For present the ultrastructure [Mandelshtam et al., 1986; Mandelshtam et al., 1987; Papidze et al., 1987; Papidze, 1988], histochemistry [Shumova, 1973; Shumova et al., 1974; Shumova, 1976; Shumova et al., 1982; Mandelshtam, 1983], physiology [Grigorev, 1980], the cation consistence [Leontyev et al., 1990], morphometry [Papidze, 2003; Papidze, 2004] of MOF and BIF flight muscles are investigated almost completely. It has been shown that these muscles differ from each other with some parametres (ultrastructure, histochemistry, physiology, morphometry). In this work we intended to study the quantitative content of myofibrillar proteins of Locust MOF and BIF flight muscles.

Materials and Methods

The experiments were conducted using the laboratorial culture of Locust (*Locusta migratoria migratorioides R.F.*). The whole year insects were placed into the hothouse at 28-30^{0}C with eternal photoperiod (12 hours light and 12 hours darkness). In such conditions the

culture develops well during the whole year. For investigation we used Longus dorsati (112) and tergosternal (113) muslces from MOF muscles and tergocoxal (119, 120) muscles from BIF muscles. The nomenclature of muscles is given according to Snodgrass [Snodgrass, 1935]. The muscle was prepared using the stereoscopic microscope (МБС-9), gathered in cold china mortar and freeze. The freeze muscle was powdered to the homogeneous mass. To 1 g of muscle 3 volumes of Hasselbach-Schneider buffer (0,6M KCl; 0,1M KH_2PO_4; 10mM $Na_4P_2O_7 10H_2O$; 1mM $MgCl_2$; pH 7.0) was added, extracted during 15 min and centrifuged at 10 000 g for 20 min.

Protein concentration was determined by the Burette method [Bailey, 1965], SDS-gel electrophoresis was carried out in 10% acrylamide according to modified Laemmly method [Laemmly, 1965]. The electrophoregrams of MOF and BIF muscles myofibrillar proteins were scanned by densitometer LKB-2202, λ-600nm.

The proteins on the electrophoregramms of Locust MOF and BIF muscles were identified by the densitometry of the protein bands.

Results and Discussion

It is known that the ratio of actin and myosin threads in muscle myofibrills of insects distinct from vertebrates (2:1) fluctuates from 3:1 up to 6:1. The minimal ratio 3:1 is characteristic for insect fast flight muscles and maximal 6:1 - for muscles among segments [Hoyle, 1983; Elder, 1979].

It must be mentioned that from studied muscles the Longus dorsati (112), tergosternal (113) and tergocoxal (119) muscles consist of structurally homogeneous fibers. Tergocoxal muscle (120) is not homogeneous and consists of three types of fibers – phasic, tonic and transitory [Mandelshtam et al., 1986; Mandelshtam et al., 1987; Papidze et al., 1987; Sumova,1973; Mandelshtam,1983].

In MOF muscles (112, 113) actin-myosin ratio is 3:1, in BIF muscles (119) this ratio is >3:1, in phasic fibres (120) it is >4:1, in tonic fibres – >5:1 [Mandelshtam et al., 1986; Mandelshtam et al., 1987; Papidze et al., 1987]. The ratio actin/myosin is the important morphological sign that characterizes muscle functional specialization. Basing on generalization of morphological and functional data the direct dependence of the contraction speed on the sarcomere length actin/myosin ratio has been determined [Hoyle, 1983].

While investigating the quantitative consistence of myofibrillar proteins via actin/myosin ratio our attention was mainly addressed to the actin quantitative content.

Table 1. The myofibrillar protein contents (%) of Locust MOF and BIF flight muscle

N	The object of investigation and name of muscle	Myofibrillar proteins (KDa)								
		MHC 200	118	110	90	56	A 45	39	37	TM 33
1	Longus dorsati muscle (MOF) – 112 (%)	31,35	3,98	3,71	7,8	3,94	17,36	3,54	4,4	2,7
2	Tergosternal muscle (MOF) - 113 (%)	32,25	3,78	3,52	6,98	5,98	18,21	3,47	5,07	2,75
3	Tergocoxal muscle (BIF) - 119 (%)	33,22	4,32	3,61	5,91	4,10	21,12	3,44	5,14	,65
4	Tergocoxal muscle (BIF) – 120 (%)	31,54	4,85	3,65	7,16	4,34	26,17	4,13	4,13	3,1

MHC – myosin heavy chain, A – actin, TM-tropomyosin

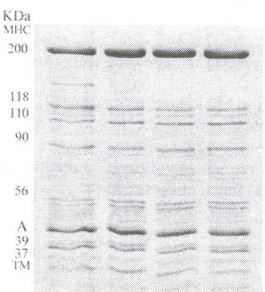


Fig. 1. The electrophoregrams of Locust MOF (112, 113) and BIF (119, 120) flight muscles preparations. SDS electrophoresis has been carried out in 10% PAGE. A, B, C, D - Locust flight muscles - 112,113,119,120, accordingly. MHC - myosin heavy chain; A - actin; TM - tropomyosin; protein concentration in each sample - 30μg.

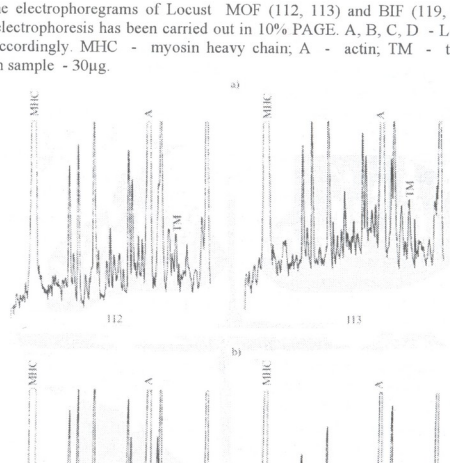


Fig. 2. a) Densitograms corresponding to Locust MOF flight muscles (112,113) electrophoregrams (fig. 1 A, B); **b)** Densitograms corresponding to Locust BIF flight muscles (119,120) electrophoregrams (fig. 1 C, D).

Fig. 1. Shows the MOF and BIF muscle miofibrillar protein content (Fig. 1 A, B, C, D). The densitometry (Fig.2 a,b) of electrophoregrams practically doesn't show the difference in MOF muscles actin (Table 1, muscle 112 – 17,36%, muscle 113 – 18,21%). The amounts of actin in BIF muscles are bigger than in MOF and equal to 21,12% and 26,17% for (119) and (120) correspondingly (Fig. 2 - a,b; Table 1, A).

The content of myosin in MOF and BIF muscles is almost the same (Table 1, MHC). In the protein components with molecular mass 118, 110, 90, 56, 39, 37, 33 kDa the difference is insignificant (Table 1).

Thus, the difference between myofibrillar proteins (actin) of MOf and BIF muscles is stipulated by their structural and functional properties.

References:

Bailey J. *Methods of protein chemistry.* M., "Nauka", p.165,1967.

Elder H. Y. *Muscle structure (Insect muscle).* London, N.Y., p.1-75, 1975.

Grigorev V.V. *Physiological features of wing fiber of the locust-Locusta migratoria migratorioides R.F.* Zh.Evol.Biochimii Fiziol., **16**, 148-153, 1980 (in Russian)

Hoyle G. *Muscle and their neural control.* N. Y. p.690, 1983.

Laemmly U.K. *Cleavege of structural proteins, during assembly of the head bacteriophage T-4.* Nature, **227**, 680, 1970.

Leontiev V.G., Papidze G. P., MandelshtamYu. E. *Cation content in the wing and leg muscles of the locust.* Bull. Georg. SSR Acad. Sci. **137**,145-148, 1990 (in Russian).

Mandelshtam Yu.E. *Insect neuron and muscle.* Leningrad, "Nauka", p.168 , 1983 (in Russian).

Mandelshtam Yu. E., Marshanskii V.F., Papidze G.P. *Structural differentiation of wing muscles in the Locust.* Zh. Evol.Bikhimii Fiziol., **22**, 211-213,1986 (in Russian).

Mandelshtam Yu.E., Papidze G. P. *Ultrastructure of mono- and bifunctional wing muscle in larval and imago Locusta migratoria.* Zh.Evol.Bikhimii Fiziol., **23**, 780-785 1987 (in Russian).

Papidze G.P., Mandelshtam Yu. E. *Ultrastructure of wing muscles of Locust.* Bull. Georg.SSR Acad. Sci., **126**,169-172, 1987 (in Russian).

Papidze G.P. *Ultrastructure of monofunctional and bifunctional flight muscles of Locust.* Abstracts of IV International Congress of Cell Biology. Montreal, Canada, 120, 1988.

Papidze G.P. *Morphometric study of locust wing muscles in ontogenesis.* Proc.Georgian Acad. Sci. Biol.ser.A 29, 705-711, 2003 (in Russian)

Papidze G.P. *Morphometric study of phasic and tonic fibers of tergocoxal muscle in a course of locust development.* Proc. Georg. Acad. Sci. Biol. ser.A, **30**, 257-262, 2004 (in Russian).

Shumova I.A. *Histochemical investigation of bifunctional flight muscles of asian locusta Locusta migratoria.* Zh. Evol.Biochimii Fiziol., **9**, 306, 1973 (in Russian).

Shumova I.A., Mandelshtam Yu.E. *Localization of succindehydrogenase, lipids, glicogens in locusta muscle.* Arch. Anat. Histol. Embr., **67**, 121-125, 1974 (in Russian).

Shumova I. A., *Histochemical investigation of dorsoventral flight muscles of locusta Locusta migratoria.* Zh. Evol.Biochimii Fiziol., **12**, 475- 476, 1976 (in Russian).

Shumova I.A., Mandelshtam Yu.E., Grigorev V.V. *Histochemistry of flight muscles of Locuste.* Cytology, **24**, 647-651, 1982 (in Russian).

Snodgrass R.F. *Principles of insect morphology.* N.Y. p. 667, 1935.

Wilson D.M. *Bifunctional muscles in the thorax of grasshoppers.* J.Expr.Biol., **39**, 669-677, 1962.

კალიის ფუნქციურად განსხვავებული საფრენი კუნთების მიოფიბრილური ცილების შედარებითი ანალიზი

პაპიძე გ.[1], გაჩეჩილაძე ნ.[2], ტორიაშვილი თ.[2], ჩუბინიძე ა.[2]

[1]ციტოლოგიის, ჰისტოლოგიისა და განვითარების ბიოლოგიის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი
[2]საქართველოს მეცნიერებათა აკადემიის მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 07.09.2005)

რეზიუმე

შესწავლილია კალიის ფუნქციურად განსხვავებული – მონოფუნქციური (მოფ) და ბიფუნქციური (ბიფ) საფრენი კუნთების (ზურგის სიგრძივი - 112, ტერგოსტერნული - 113, ტერგოკოკსული - 119, 120) მიოფიბრილური ცილების პროცენტული შემცველობა. ნაჩვენებია, რომ შესწავლილ კუნთებში მიოზინისა და დანარჩენი ცილოვანი კომპონენტების (მოლეკულური მასით - 118, 110, 90, 56, 39, 37, 33 kDa) პროცენტული შემცველობა, თითქმის ერთნაირია. მოფ კუნთებში აქტინის რაოდენობა პრაქტიკულად არ განსხვავდება (კუნთ 112-ში არის 17,36% , ხოლო 113-ში–18,21%). ბიფ კუნთებში აქტინის შემცველობა, მოფ კუნთებთან შედარებით მეტია, კერძოდ, კუნთ 119-ში არის 21,12%, ხოლო 120-ში –26,17%. მოფ და ბიფ საფრენ კუნთებში აქტინის რაოდენობრივ შემცველობაში განსხვავება გაპირობებულია მათი სტრუქტურული და ფუნქციური თავისებურებებით.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

NATURAL RESTORATION OF FOREST AND PROCESS OF SPECIES REPLACEMENT AT THE EAST EDGE OF THE CAUCASIAN FIR (*ABIES NORDMANNIANA*) HABITAT

DZOTSENIDZE N.

Department of Botany, Kutaisi A. Tsereteli State University

(Received July 11, 2005)

Abstract

At the very east edge of the Caucasian fir (*Abies nordmanniana*) distribution area the process of species replacement was studied. Replacement of Caucasian fir with more xerophilous species, mainly with Oriental beech (*Fagus orientalis*), pine (*Pinus kolchiana*), Alpine oak (*Quarcus macranthera*), Caucasian hornbeam (*Carpinus caucasica*) and other species in general is stipulated by lack of moisture and low relative humidity.

Key words: pleistocene, holocene, habitat, xerophyte.

Introduction

Natural restoration of forest and the process of species replacement was studied in the Algeti Reserve, west part of which is the east edge of fir and spruce habitat. East edge of Caucasian fir and Oriental spruce habitat on the Central Caucasus is the river Khevsuretis Aragvi and on the Small Caucasus - gorge of the river Algeti [Flora of Georgia, 1992; Makhatadze, 1966; Dolukhanov, 1989].

On the Central Caucasus fir distribution area reaches to the river Liakhvi and on the Small Caucasus by Trialeti ridge - to the river Tedzamy gorge. Spruce is distributed up to Tbilisi (village Bevreti, Mtskheta region) [Gulisashvili, 1952]. Due to lack of moisture and low relative humidity fir and spruce are not distributed eastern (Outer Kakheti, Lower Kartli). But in some regions of Inner Kakheti atmospheric precipitates and relative humidity is favourable for more moisture-liking species (*Zelcova, Castanea, Acer velutinum* etc.). Hence the reason of absence of fir and spruce in Kakheti is the climatic peculiarities of these regions in ancient geological epochs. In particular, fir and spruce disappeared in Kakheti valley in glacial age (Pleistocene) or after glacial age (Holocene). According to the data of some authors fir and spruce disappearance apparently occurred after glacial age in one of the xerothermal climatic periods [Palbin, 1936; Berg, 1947; Chochieva, 1975; Shatilova, Ramishvili, 1990]. Though in some regions of the East Caucasus damp climatic conditions were recovered, fir and spruce were not distributed any more due to long distance from the principal habitat [Gulisashvili, 1952; Dolukhanov, 1989].

Materials and Methods

The process of species replacement and restoration of forest was studied on the north and south slopes of the r.Algeti gorge in Algeti State Reserve. The researches were carried out on north-west exposition with slope inclination 10-15° at 1600 m.a.s.l. and on south-west exposition with slope inclination 15-20° at 1700 m.a.s.l. Dynamics of natural restoration and replacement of fir and spruce species with other species was established. Closure of cover and composition of forest, average diameter and height of tree, age of seedlings and undergrowths were determined.

Results and Discussion

The process of species replacement and fir natural restoration was studied on the north and south slopes of the r. Algeti gorge in Algeti State Reserve which differ from each other by floristic composition. East part of Trialeti mountain differ from west part which is rich in evergreen (fir, spruce, pine) and deciduous dendroflora. Among the shrubs it is worth mentioning both evergreen (*Rhododendron, Lauracerasus*), and deciduous (*Ruscus, Vaccinium arctosta phylos*) species. In the central and west parts of Trialeti (Nichbisi and Algeti gorges), where the climate is dry compared to the west part, in hornbeam-oak forest single specimens of yew (*Taxus baccata*) are found. Due to continental climate in some places hemixerophilous juniper is developed [Gagnidze, Davitadze, 2000].

In the reserve from 1400 to 2100-2200 m. a.s.l. dark coniferous forest belt (fir, spruce) is dominated with some occurrence of beech, pine and alpine oak. In the central part of Trialeti occurrence of pine is considerably decreased (basins of the rivers Tana, Tedzami, Kavturi). For these places mixed forest with spruce and beech domination is more characteristic which to the east reaches up to the Algeti gorge. As fir occurs rarely, this area is considered as the east edge of fir distribution.

On the middle-dry soil of north-west exposition of the Algeti gorge with slope inclination - $10\text{-}15^0$, at 1600 m. a.s.l. beech - hornbeam mixed forest occurs, in the main storey of which Oriental beech (*Fagus orientalis*), blend with *Picea orientalis* and *Carpinus caucasica*, is dominant and edificator. Single specimen of *Abies nordmanniana* occur. *Corylus avellana* and *Rosa canina* are characteristic for understorey. Characteristic species for the subforest are *Corylus avellana* and *Rosa canina*. In the covering *Festuca Montana* is dominant, *Asperula odorata, Brachypodium silvaticum, Rubus hirtus* etc. were also met.

Characteristics of the forest at the north exposition are following: closure of cover – 0.6-0.7, average diameter of the tree – 42 cm, average height – 28 m. composition – 7 beech, 2 spruce, 1 hornbeam + fir. In this forest natural restoration takes place mainly via beech; seedling-undergrowth of spruce and hornbeam occur in small amount. As for seedling-undergrowths of fir, their amount is negligible under the cover and in small sized openings. In the middle sized openings restoration is mainly takes place via beech and hornbeam, amount of spruce is small and fir occurs rarely. These data indicate that on middle-dry soils, in spite of optimal light conditions, low air relative humidity is the hindering factor for spruce and especially fir restoration.

Natural restoration of the forest and species replacement issue were studied on middle-dry and dry soils of south-west exposition of Trialeti mountain with slope inclination - $15\text{-}20^0$, at 1700 m. a.s.l. In the principal understorey of the forest the dominant and edificator is Caucasian pine (*Pinus Kolchiana*) blend with Oriental spruce, Alpine oak (*Quercus macranthera*), *Populus tremula.* In the understorey as a single specimens *Rhododendron luteum, Jonicera caucasica, Rosa canina, Vaccinium myrtillus, Rubus idaeu* occur. In the covering *Calamagrostis arundinaceae* dominates. *Achillea biserrata, Geranium ibericum, Festuca montana, Campanula rapunculoides* etc. were also met.

Afforestation valuation of above mentioned forest is the following: closure of cover 0.6-0.7, average diameter of the tree – 36 cm, average height – 28 m. composition – 7 pines, 2 oaks, 1 spruce + elm.

As is seen from our studies on the south slopes of the utmost east edge of the dark coniferous forests natural restoration mainly takes place in favour of pine and oak; restoration of fir doesn't happen at all, undergrowth of 0.5 m height in the middle sized openings at north slopes solely occur. As for big openings, in spite of optimal conditions of lightening, seedling-undergrowth of fir isn't observed there which is caused by hard drying of soil. Besides, effect of solar radiation is strong here that negatively influences seedling-undergrowth of spruce and especially fir. It is worth mentioning that middle dry and dry soils of south slopes of the Algeti gorge are distinguished by low relative humidity of air which is inhibiting factor of fir and spruce restoration. Thus, east edge of spruce and especially fir habitat is just the Algeti gorge. According to literature data [Gulisashvili, 1952; Eradze, 2002] single specimens of spruce are discovered near Tbilisi in the village Bevreti indicating that spruce is less demanding species of moisture than Caucasian fir.

Dynamics of natural restoration of spruce and fir at the north and south expositions of the utmost east edge of habitat is given in Table 1.

So, according to our researches we can conclude that fir and spruce species replacement mainly by beech, pine, oak, hornbeam and other species is generally caused by lack of moisture and low air relative humidity. This reason stipulates for replacement of fir and spruce with more xerophilous species.

Table 1. Dynamics of natural restoration of spruce and fir at the utmost east edge of habitat

Cover closure of the forest	Exposition inclination	Age of seedling under-growth (year)	Amount of seedling-undergrowth per 1 hectare ($x10^3$)				Total amount of seedling-undergrowh per 1 ha ($x10^3$)
			beech	spruce	hornbeam	fir	
0.7-0.8	north-west	1-2	5.2±0.6	2.1±0.1	0.9±0.2	0.20±0.05	8.40±0.25
		3-5	3.1±0.4	1.1±0.1	0.3±0.1	-	4.5±0.2
	10-12°	6-10	2.0±0.2	0.50±0.08	-	0.10±0.05	2.6±0.1
		>11	1.2±0.2	0.10±0.05	-	-	1.3±0.1
		total	11.5±0.4	3.8±0.1	1.2±0.1	0.30±0.05	16.8±0.2
Opening (D=18-20m)	north-west	1-2	6.0±0.7	0.8±0.1	0.8±0.2	-	7.6±0.4
		3-5	3.9±0.5	-	0.5±0.1	-	4.4±0.2
	12-15°	6-10	2.9±0.3	0.10±0.05	-	0.010±0.001	3.01±0.10
		>11	2.0±0.2	0.10±0.05	0.10±0.03	0.010±0.001	2.21±0.05
		total	14.8±0.4	1.00±0.07	1.4±0.1	0.020±0.001	17.22±0.18
			pine	oak	spruce	fir	
0.6-0.7	south-west	1-2	10.3±0.6	2.2±0.2	0.30±0.02	0.010±0.001	12.81±0.20
		3-5	8.5±0.5	1.2±0.1	-	-	9.7±0.3
	15-20°	6-10	6.1±0.3	0.40±0.01	0.10±0.01	-	6.6±0.2
		>11	2.4±0.1	0.10±0.01	0.10±0.01	0.010±0.001	2.61±0.03
		total	27.3±0.2	3.90±0.08	0.50±0.01	0.020±0.001	31.72±0.18
	south-east	1-2	8.1±0.5	1.9±0.2	0.40±0.02	-	10.4±0.2
		3-5	5.3±0.3	1.2±0.1	-	-	6.5±0.2
	15-18°	6-10	2.1±0.1	-	-	-	2.1±0.1
		>11	1.3±0.1	0.10±0.01	0.20±0.01	0.010±0.001	1.61±0.03
		total	16.8±0.2	3.2±0.1	0.60±0.01	0.010±0.001	20.61±0.08

References:

Berg L. *Natural Regions of USSR*. **I**, M., "OGIZ", 1947 (in Russian).

Chochieva K. *For the History of Dark Coniferous Forests of Georgia*. Bull. Acad. Sci. Georg. SSR, **80**, 2, 1975 (in Russian).

Dolukhanov A. *Forest Vegetation of Georgia*. Tbilisi, "Metsniereba", 1989 (in Russian.).

Eradze N. *Status of Flora of Tbilisi Surroundings in the Past and Today*. Tbilisi, "Metsniereba", 2002 (in Georgian).

Flora of Georgia. **XIV**, Tbilisi, "Metsniereba", 1992 (in Georgian).

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

Gulisashvili V. *Forest Vegetation and Regularity of Its Distribution*. Proceedings of The Institute of Forestry, **IV**, "Metsniereba", Tbilisi, 1952 (in Georgian).

Makhatadze L. Dark *Coniferous Forests of the Caucasus*. M., "Lesn. prom.", 1966 (in Russian).

Palibin I. *Steps of Development of Near Caspian Countries Flora of Cretaceous Period*. M.-L., "Nauka", 1936 (in Russian).

Shatilova I., Ramishvili I. *Materials of the History of Georgian Flora*. Tbilisi, "Metsniereba", 1990 (in Russian).

ტყის ბუნებრივი განახლება და სახეობათა ცვლის პროცესი სოჭის არეალის აღმოსავლეთ საზღვარზე

ძოწენიძე ნ.

ბოტანიკის კათედრა, ქუთაისის აკ. წერეთლის სახ. სახელმწიფო უნივერსიტეტი

(მიღებულია 11.07.2005)

რეზიუმე

კავკასიური სოჭის (*Abies normanniana*) არეალის უკიდურეს აღმოსავლეთ საზღვარზე შესწავლილ იქნა სახეობათა ცვლის პროცესი. სოჭის ცვლა უმთავრესად წიფლით (*Fagus orientalis*), ფიჭვით (*Pinus kolchiana*), მუხით (*Quercus marcanthera*), რცხილით (*Carpinus caucasica*) და სხვა სახეობებით ძირითადად განპირობებულია ტენის ნაკლებობით და ჰაერის დაბალი შეფარდებითი ტენიანობით. ეს კი განაპირობებს სოჭის ცვლას უფრო ქსეროფილური სახეობებით.

Afforestation valuation of above mentioned forest is the following: closure of cover 0.6-0.7, average diameter of the tree – 36 cm, average height – 28 m. composition – 7 pines, 2 oaks, 1 spruce + elm.

As is seen from our studies on the south slopes of the utmost east edge of the dark coniferous forests natural restoration mainly takes place in favour of pine and oak; restoration of fir doesn't happen at all, undergrowth of 0.5 m height in the middle sized openings at north slopes solely occur. As for big openings, in spite of optimal conditions of lightening, seedling-undergrowth of fir isn't observed there which is caused by hard drying of soil. Besides, effect of solar radiation is strong here that negatively influences seedling-undergrowth of spruce and especially fir. It is worth mentioning that middle dry and dry soils of south slopes of the Algeti gorge are distinguished by low relative humidity of air which is inhibiting factor of fir and spruce restoration. Thus, east edge of spruce and especially fir habitat is just the Algeti gorge. According to literature data [Gulisashvili, 1952; Eradze, 2002] single specimens of spruce are discovered near Tbilisi in the village Bevreti indicating that spruce is less demanding species of moisture than Caucasian fir.

Dynamics of natural restoration of spruce and fir at the north and south expositions of the utmost east edge of habitat is given in Table 1.

So, according to our researches we can conclude that fir and spruce species replacement mainly by beech, pine, oak, hornbeam and other species is generally caused by lack of moisture and low air relative humidity. This reason stipulates for replacement of fir and spruce with more xerophilous species.

Table 1. Dynamics of natural restoration of spruce and fir at the utmost east edge of habitat

Cover closure of the forest	Exposition inclination	Age of seedling under-growth (year)	Amount of seedling-undergrowth per 1 hectare ($x10^3$)				Total amount of seedling-undergrowh per 1 ha ($x10^3$)
			beech	spruce	hornbeam	fir	
0.7-0.8	north-west	1-2	5.2±0.6	2.1±0.1	0.9±0.2	0.20±0.05	8.40±0.25
		3-5	3.1±0.4	1.1±0.1	0.3±0.1	-	4.5±0.2
	10-12°	6-10	2.0±0.2	0.50±0.08	-	0.10±0.05	2.6±0.1
		>11	1.2±0.2	0.10±0.05	-	-	1.3±0.1
		total	11.5±0.4	3.8±0.1	1.2±0.1	0.30±0.05	16.8±0.2
Opening (D=18-20m)	north-west	1-2	6.0±0.7	0.8±0.1	0.8±0.2	-	7.6±0.4
		3-5	3.9±0.5	-	0.5±0.1	-	4.4±0.2
	12-15°	6-10	2.9±0.3	0.10±0.05	-	0.010±0.001	3.01±0.10
		>11	2.0±0.2	0.10±0.05	0.10±0.03	0.010±0.001	2.21±0.05
		total	14.8±0.4	1.00±0.07	1.4±0.1	0.020±0.001	17.22±0.18
			pine	oak	spruce	fir	
0.6-0.7	south-west	1-2	10.3±0.6	2.2±0.2	0.30±0.02	0.010±0.001	12.81±0.20
		3-5	8.5±0.5	1.2±0.1	-	-	9.7±0.3
	15-20°	6-10	6.1±0.3	0.40±0.01	0.10±0.01	-	6.6±0.2
		>11	2.4±0.1	0.10±0.01	0.10±0.01	0.010±0.001	2.61±0.03
		total	27.3±0.2	3.90±0.08	0.50±0.01	0.020±0.001	31.72±0.18
	south-east	1-2	8.1±0.5	1.9±0.2	0.40±0.02	-	10.4±0.2
		3-5	5.3±0.3	1.2±0.1	-	-	6.5±0.2
	15-18°	6-10	2.1±0.1	-	-	-	2.1±0.1
		>11	1.3±0.1	0.10±0.01	0.20±0.01	0.010±0.001	1.61±0.03
		total	16.8±0.2	3.2±0.1	0.60±0.01	0.010±0.001	20.61±0.08

References:

Berg L. *Natural Regions of USSR*. **I**, M., "OGIZ", 1947 (in Russian).

Chochieva K. *For the History of Dark Coniferous Forests of Georgia*. Bull. Acad. Sci. Georg. SSR, **80**, 2, 1975 (in Russian).

Dolukhanov A. *Forest Vegetation of Georgia*. Tbilisi, "Metsniereba", 1989 (in Russian.).

Eradze N. *Status of Flora of Tbilisi Surroundings in the Past and Today*. Tbilisi, "Metsniereba", 2002 (in Georgian).

Flora of Georgia. **XIV**, Tbilisi, "Metsniereba", 1992 (in Georgian).

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

Gulisashvili V. *Forest Vegetation and Regularity of Its Distribution*. Proceedings of The Institute of Forestry, **IV**, "Metsniereba", Tbilisi, 1952 (in Georgian).

Makhatadze L. Dark *Coniferous Forests of the Caucasus*. M., "Lesn. prom.", 1966 (in Russian).

Palibin I. *Steps of Development of Near Caspian Countries Flora of Cretaceous Period*. M.-L., "Nauka", 1936 (in Russian).

Shatilova I., Ramishvili I. *Materials of the History of Georgian Flora*. Tbilisi, "Metsniereba", 1990 (in Russian).

ტყის ბუნებრივი განახლება და სახეობათა ცვლის პროცესი სოჭის არეალის აღმოსავლეთ საზღვარზე

ძოწენიძე ნ.

ბოტანიკის კათედრა, ქუთაისის აკ. წერეთლის სახ. სახელმწიფო უნივერსიტეტი

(მიღებულია 11.07.2005)

რეზიუმე

კავკასიური სოჭის (*Abies normanniana*) არეალის უკიდურეს აღმოსავლეთ საზღვარზე შესწავლილ იქნა სახეობათა ცვლის პროცესი. სოჭის ცვლა უმთავრესად წიფლით (*Fagus orientalis*), ფიჭვით (*Pinus kolchiana*), მუხით (*Quercus marcanthera*), რცხილით (*Carpinus caucasica*) და სხვა სახეობებით ძირითადად განპირობებულია ტენის ნაკლებობით და ჰაერის დაბალი შეფარდებითი ტენიანობით. ეს კი განაპირობებს სოჭის ცვლას უფრო ქსეროფილური სახეობებით.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

Botany

SHRUBBERY VEGETATION OF CHIRUKHISTSKALI GORGE (SOUTH COLCHIS, ADJARA)

KHARAZISHVILI D.

Batumi Botanical Garden of the Georgian Academy of Sciences

(Received September 7, 2005)

Abstract

Shrubbery of the Chirukhistskali gorge is mainly represented by communities of *Rhododendron caucasicum*. On account of anthropogenic pressure these communities are thinned on significant areas, though impassable primary communities also occur. Plant diversity of *Rh. caucasicum* communities is not high (25-30 species per 25 m^2). The second widespread formation of the studied area is Junipereta, which is poorer in species (15-20 species per 25 m^2) than Rhododendreta caucasicum. Rhododendreta pontici, Rododendreta luteum, Vaccineta occur in the subalpine understory in a form of separate fragments. In all shrub formations forb species are abundant.

Key words: shrub formations, associations, *Rhododendron caucasicum*, *Juniperetа pigmaea*.

Introduction

The Chirukhistskali gorge is located in the south-eastern part of the river Adjaristskali basin (N 41°26'330"; E 42°30'795") [Kharazishvili, Memiadze, 2004].

Shrubberies, particularly, those which constituted by *Rhododendron caucasicum*, have an important part in creating the high mountain plant cover of the Chirukhistskali gorge. The major part of these shrubberies is of secondary origin, which is caused by strong exploitation of the subalpine forests. However, primary thickets of *Rh. caucasicum* occur on very steep slopes and hardly accessible places.

Beside thickets of *Rh. caucasicum, Junipereta pigmaea* is widespread in high-mountainous parts of the gorge. *Rh. caucaiscum* and *Juniperus pigmaea* form monodominant as well as polydominant communities at 2100-2500 m in high-mountainous parts of the Chirukhistskali gorge. Separate shrubs of these species frequently occur at lower (1800 m) and higher (2650 m) altitudes too. Separate fragments of Rhododendreta pontici, Rhododendreta luteum and Vaccineta are mainly parts of the understory. In the studied gorge principal limits of distribution of Rhododendreta pontici, Rhododendreta luteum and Vaccineta pass between 1800-2100 m, 1800-2500 m and 1800-2600 m, respectively.

In contrast to quite well studied lowland and mountain vegetation of Colchis, its high mountain vegetation is poorly investigated [Dolukhanov, 1942, 1980; Manjavidze, 1982; Papunidze, 1990].

Our task was to study floristic composition, structure and ecological characteristics of the shrubberies distributed in high-mountainous parts of the Chirukhistskali gorge.

Materials and Methods

During our research we in the main followed conventional techniques used by Russian and Caucasian botanists. Particularly, to distinguish the vegetation syntaxa (formation, association) we applied the principle of plant dominance [Dolukhanov, 1989; Kvachakidze, 1979; Ketskhoveli, 1959]; further, to compile lists we used the 6-sign scheme of abundance-coverage by Braun-Blanquet [Willmanns, 1999; Grabherr, Mucina, 1993; Dolukhanov, 2003; Box et al., 2000]. According to the degree of human impact on a community, we distinguished the following stages of hemeroby [Pott, 1996]: I stage – communities which are natural or close to natural; II stage – communities which are semi-natural and experience anthropogenic pressure (hay making, grazing, felling).

Descriptions were made in 1999-2004 on randomly chosen plots. The areas of the plots were either 10 x 10 m, or 25 x 25 m depending on the whole area of a slope. More than 100 descriptions were made over about 20 ha. Spelling of the syntaxa corresponds to Pott (1995), species names are given according to Dmitrieva (1990), Flora of Georgia (1971-2003).

Results and Discussion

The shrubbery vegetation is considered below according to separate formations and associations.

Formation Rhododendreta

Rhododendreta is an independent formation of high mountain shrubberies. It is widespread almost throughout the high mountains of the Caucasus. In Georgia the extreme limits of the distribution of Rhododendreta are: Mt. Obteni to the west, Zakatala to the east and Mt. Miskhani to the south. Confinement of this community to the north-facing slopes, i.e. to conditions of ecotopes where the snow cover is deep and stable, is its ecological characteristic [Kikava, 1970].

According to the degree of coverage, G. Kikava (1970) distinguishes 5 categories of Rhododendreta. In compliance with this scheme we distinguished 2 main types of the formation in the Chirukhistskali gorge: impassable (compact) and thinned. As reported by Kikava (1972), impassability is typical of Rhododendreta. It is characterized by high density of *Rh. caucasicum* shrubs, totally covered ground surface, floristic poorness, intensive peat accumulation process, high soil acidity. These reasons have called forth the originality of the formation, coenological contacts of which to other high mountain plant formations are very limited [Kikava, 1972]. Just these types of thickets were considered by Grossheim (1948), when he mentioned that Rhododendreta presents the conformed picture during the whole time of their development and almost cannot be divided into associations. Our research in the Chirukhistskali gorge has shown that these communities do not always present the conformed picture and quite diverse plant species are frequently found there. In this regard thinned thickets of *Rh. caucasicum* rather enriched with species invaded from other high mountain communities are especially worth mentioning.

According to the classification scheme by G. Kikava, we distinguish 2 classes of associations: community of *Rh. caucasicum* with the predominance of shrubs (Rhododendreta fruticosa) and community of *Rh. caucasicum* with the predominance of herbs (Rhododendreta herbosa).

Class of associations – Rhododendreta fruticosa

Communities of *Rh. caucasicum* with shrubs occur on north-, northwest- and west-facing slopes with 2-30° angle of inclination. Beside *Rh. caucasicum,* the following shrubs constitute the

community with different frequency: *Vaccinium myrtillus, Daphne glomerata, Sorbus boissieri, Juniperus pigmaea, Rubus buschii, Rosa boissieri, Vaccinium arctostaphylos* (List 1). One of these communities – community of *Rh. caucasicum* with *Juniperus pigmaea* – is presented below as an example. The association belongs to the II group of hemeroby.

List 1. Border segment of the observation post "Chirukhi" (12.07.2003)

S	0.5 m	60%	2190 m, 2-5°, W
H	0.4 m	40%	25 x 25 m

S	*Rhododendron caucasicum*	3
	Juniperus pigmaea	2
	Vaccinium arctostaphylos	+
	Daphne glomerata	+
	Rosa boissieri	+
H	*Anemone fasciculata*	3
	Pyrethrum roseum	3
	Lotus caucasicus	3
	Poa pratensis	2
	Ranunculus repens	2
	Trifolium ambiguum	2
	Cerastium purpurascens	2
	Carum carvi	2
	Pedicularis nordmanniana	1
	Aquilegia caucasica	+
	Kemulariella caucasica	+
	Geranium psilostemon	+
	Polygonum carneum	+

The number of species in this association is 18; five of them are shrubs. One of the herbaceous species is a grass, 2 are legumes and 10 forbs.

Class of associations – Rhododendreta herbosa

Communities of *Rh. caucasicum* with herbs occur on north-, northwest- and rarely northeast-facing slopes with 2-25° angle of inclination. According to the degree of thinning, the frequency of occurrence of plant species in this community is different. The following herbaceous plants are characteristic to Rhododendreta herbosa of the Chirukhistskali gorge: *Anemone fasciculata, Aquilegia caucasica, Polygonum carneum, Geranium psilostemon, Pyrethrum roseum, Poa pratensis, Festuca montana, Nardus grabriculmis,* etc. One of these communities – community of *Rh. caucasicum* with *Nardus grabriculmis* – is presented below as an example. The association belongs to the II group of hemeroby.

List 2. Border segment of the observation post "Chirukhi" (14.07.2003)

S	0.8 m	60%	2170 m, 10-12°, W
H	0.6 m	35%	25 x 25 m

S	*Rhododendron caucasicum*	4
	Vaccinium myrtillus	2
	Juniperus pigmaea	+
H	*Poa pratensis*	4
	Festuca montana	3
	Nardus glabriculmis	3
	Carum carvi	3

Geranium psilostemon	3
Potentilla recta	3
Myosotis sylvatica	2
Ranunculus repens	2
Pyrethrum roseum	2
Aquilegia caucasica	1
Polygonum carneum	1
Trifolium pratensis	+
Gentiana cruciata	+
Kemulariella caucasica	+
Lotus caucasicus	+
Pimpinella rhodantha	+
Anemone fasciculata	+
Achillea latiloba	+

The number of species in this association is 21; three of them are shrubs. Tree of the herbaceous species are grasses, 2 are legumes and 13 forbs.

Formation Junipereta

High mountain juniper thickets of the Chirukhistskali gorge are constituted by *Juniperus pigmaea*. Rarely *J. sabina* take part in the juniper thickets in a form of separate individuals as well as micro-communities.

Unlike Rhododendreta, Junipereta do not form impassable thickets. They are mostly represented by complexes with subalpine shrubberies and herbaceous vegetation.

Formations of this type are spread on west-, northwest and rarely north-facing slopes with 2-25° angle of inclination.

A description of an association – juniper thickets with *Rh. caucasicum* – is considered below as an example.

List 3. Border segment of the observation post "Chirukhi" (12.07.2003)

S	0.6 m	75%	2200 m, 5°, NW
H	0.5 m	25%	10 x 10 m

S	*Juniperus pigmaea*	4
	Rhododendron caucasicum	2
	Daphne glomerata	+
	Vaccinium arctostaphylos	+
H	*Poa pratensis*	2
	Anemone fasciculata	2
	Lotus caucasicus	+
	Trifolium ambiguum	+
	Polygonum carneum	+
	Pyrethrum roseum	+
	Ranunculus repens	+
	Bupleurum polyphyllum	+
	Carum carvi	+
	C. meifolium	+
	Aquilegia caucasica	+
	Myosotis sylvatica	+
	Pedicularis nordmanniana	+

The number of species in this association is 17; four of them are shrubs. One of the herbaceous species is a grass, 2 are legumes and 10 forbs.

Junipereta belong to the II stage of hemeroby.

Beside the listed species, the following herbaccous plants also participate in the described formation: *Rhynchocorus elephas, Senecio othonnae, Coronilla balansae, Nardus glabriculmis, Draba hispida, Aster caucasicus, Dactylorhiza flavescens, Macrotomia echioides, Silene ruprechtii, Gentiana cruciata, Senecio propinquus, S. pseudoelatior,* etc.

Separate shrubs and micro-communities of *Rhododendron ponticum, Rh. luteum, Vaccinium uliginosum, V. myrtillus, V. arctostaphylos* are in the main represented in a form of components of the subalpine understory or shrubbery. The principal limit of distribution of *Rh. ponticum* passes between 1800-2100 m and that of *Rh. luteum* and *Vaccinium spp.* between 1800-2600 m.

Rh. ponticum is mainly characteristic to formations of fir, whereas communities of *Rh. luteum* and *Vaccinium spp.* occur in formations of beech, fir and birch.

References:

Dolukhanov, A. G. *Colchic understory*. "Metsniereba", Tbilisi, 1980.

Dolukhanov A. G. *Forest vegetation of Georgia*. In: Vegetation of Georgia, Part 1. Tbilisi, 1989.

Dolukhanov, A. G., Sakhokia, M. F., Kharadze, A. L. *Concerning the question on high mountain vegetation belts of the Caucasus*, Works of the Tbilisi Institute of Botany, vol. **8**, 1942.

Kharazishvili, D., Memiadze, N. *Analysis of the flora of the subalpine belt in the Chirukhistskali Canyon.* Proc. of the Georg. Acad. Sci., **2**, 1-2, 42-54, 2004.

Box, E., Fujiwara, K., Nakhutsrishvili, G., Zazanashvili, N., Liebermann, R., Miuawaki, A. *Vegetation and landscapes of Georgia (Caucasus) as a basis for landscape restoration.* Bull. of the Institute of Environmental Science and Technology, Yokohama National University, Yokohama, **26**, 1, 69-102, 2000.

Grabherr, G., Mucina, L. (Hrsg.) *Die pflanzengesellschaften Osterreichs*. vol. **II.** Gustav Fischer Verlag Jena-Stuttgart-New York, 1993.

Kikava, G. *Floristic composition of Rhododendron caucasicum communities.* Collection of works of young scientific workers, vol. **VI**, p.21-26, 1970 (in Georgian).

Kikava, G. *Distribution pattern of Rhododendron caucasicum communities.* Collection of works of young scientific workers, vol. **VI**, p.51-55, 1970 (in Georgian).

Kikava, G. *For study of subalpine forest with understory constituted by Rhododendron caucasicum and typology of Rh. caucasicum communities.* Bull. Georgian Botanical Society, vol. **V**, Tbilisi, „Metsniereba", p. 74-78, 1972 (in Georgian).

Kikava, G. *For study of some principal shrub formations in Aragvi gorge. Botany*. Tbilisi, „Metsniereba", p. 81-91, 1972 (in Georgian).

Pott, R. *Biotoptypen Schutzanworte Lebersraume Deutchlands und Angrenzender Regionen.* Verlag Eugen Ulmer, Stuttgart (Hohenheim), p. 43, 1996.

Wilmans, O. *Okologische pflantensoziologie.* Quelle Meyer Verlag, Heidelberg-Wiesbaden, 1993.

მდინარე ჩირუხისწყლის ხეობის (სამხრეთ კოლხეთი, აჭარა) ბუჩქნარების მცენარეულობა

ხარაზიშვილი დ.

საქართველოს მეცნიერებათა აკადემიის ბათუმის ბოტანიკური ბაღი

(მიღებულია 07.09.2005)

რეზიუმე

მდ. ჩირუხისწყლის ბუჩქნარი მცენარეულობა ძირითადად დეკიანითაა წარმოდგენილი. მისი მნიშვნელოვანი ფართობები გამეჩხრებულია, რაც ანთროპოგენური ზემოქმედებითაა გამოწვეული. ამავე დროს აღსანიშნავია პირველადი ფიტოცენოზებიც. დეკიანის მცენარეთა მრავალფეროვნება არ არის დიდი (25-30 სახეობა 25 $მ^2$-ზე). დეკიანის შემდეგ ფართოდაა გავრცელებული ღვიიანის ფორმაცია, რომელიც კიდევ უფრო ღარიბია სახეობრივი შემადგენლობით (15-20 სახეობა 25 $მ^2$-ზე). შქერიანები, იელიანები და მოცვიანები გვხვდება სუბალპურ ქვეტყეში ცალკეული ფრაგმენტების სახით. ყველა ბუჩქოვან ფიტოცენოზში სჭარბობს ნაირბალახოვან მცენარეთა სახეობები.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

FLORISTIC COMPOSITION OF ARGILLACEOUS BADLAND ECOSYSTEMS OF IORI PLATEAU (EAST GEORGIA)

LACHASHVILI N.*, LACHASHVILI J.

N. Ketskoveli Institute of Botany of the Georgian Academy of Sciences
**E-mail: botanins@gw.acnet.ge*

(Received July 11, 2005)

abstract

The floristic composition of argillaceous badland ecosystems of Iori plateau was studied for the first time. Flora of these ecosystems is poor – 138 species of 109 genera and 34 families of vascular plants are recorded. The leading families are: *Asteraceae* – 19 species (13.8%), *Chenopodiaceae* – 14 (10.1%), *Poaceae* – 13 (9.4%), *Boraginaceae* – 11 (8%), *Fabaceae* – 10 (7.3%), *Apiaceae* – 9 (6.5%), *Brassicaceae* – 8 (5.8%), *Lamiaceae, Liliaceae, Linaceae* – 4-4 (2.9-2.9%). These 10 families embrace 69.6% (96 species) of the whole number of species. Analysis of the systematic structure and geographic elements of the flora has shown that the Irano-Thuranian, Minor Asian-Front Asian and Caucasian floristic centres played the main role in its formation. Compared to the mentioned centers, the Mediterranean influence is somewhat weak. The number of Caucasian endemic species is 16 (11.6%). The existing variety of plant life forms is considered. The complete floristic list is given.

Key words: South Caucasus, arid region, flora, geographic element.

Introduction

In the South Caucasus the ecosystems of argillaceous badlands mostly occur in arid and semiarid regions. They are widespread in the central and eastern parts of the South Caucasus as well as the southern part of the South Caucasus. In the central and eastern South Caucasus these ecosystems are mostly represented in the southern part of Iori plateau (East Georgia), Sheki plateau (Azerbaijan) and Kabistan foothills (Azerbaijan) [Grossheim and Sakhokia, 1931; Sakhokia, 1931, 1958; Grossheim, 1946; Prilipko, 1970, 1980]. Argillaceous badland ecosystems of these regions have common origin and constitute a united system.

Ecosystems of argillaceous badlands of the central-eastern part of the South Caucasus have direct contact with ecosystems of arid forests *(Junipereta, J. foetidissima, J. polycarpos; Pistacieta, P. mutica)* and steppes *(Bothriochloeta, B. ischaemum; Stipeta, S. lessingiana, S. capillata, S. pulcherrima)* as well as desert ecosystems of Mtkvari-Arax lowland. They are characterized by lack of real soil cover and presence of eroded and partitioned steep slopes. In respect to lithological structure, stratifications of sea clays, clay sand and easily crumbling sandstone of Apsheron and Aghchagil age are presented within the mentioned badland ecosystems [Sakhokia, 1931; Tsereteli, 1971]. The substrate is salinized and often contains gypsum. Average annual temperature is 14°, annual precipitation about 250-400 mm, evaporation 1000 mm, moistening coefficient 0,3-0,4 [Atlas of Azerbaijan SSR, 1963; Atlas of Georgian SSR, 1964].

Floristic structure of the argillaceous badlands present in the above mentioned regions of the central and eastern parts of the South Caucasus is poorly studied till now [Lachashvili, 2004a, 2004b, 2005]. No more than a few principal characteristic species (8-10) as belonging to so-called "argillaceous complex" were mentioned [Grossheim & Sakhokia, 1931; Sakhokia, 1931; 1958].

The aim of our research was to ascertain the floristic composition of the designated ecosystems within their principal distribution range in Georgia – in the south-eastern part of Iori plateau; to determine the systematic and ecobiomorphic structure and find out geographic elements of the flora; to ascertain the florogenetic relation of argillaceous badland ecosystems of Iori plateau on the basis of their analysis.

Materials and Methods

The floristic material was collected during 1984-1991 and 2003-2004 by route methods.

Life forms are distinguished according to classification systems of Raunkyer C. [Raunkyer, 1934] and Serebriakov I. [Serebriakov, 1964].

Names of the plants correspond to Czerepanov S. [Czerepanov, 1995] and Gagnidze R. [Gagnidze, 2005].

Results and discussion

Ecosystems of argillaceous badlands of Iori plateau are widespread in the south-eastern part of the area. They are distributed on Kotsakhura, Chobandaghi, Kaladara, "Patara chrdili", "Didi chrdili", Kumro, Dusdaghi and other monoclinic low ranges of the region, in Vashlovani depression and on massifs of Mijniskure and Usakhelo-Mta, etc.

As a consequence of the floristic-geobotanical investigation of this region it was determined that mainly phytocenoses of phryganoid vegetation *(Reaumurietum alternifoliae, Atraphaxietum spinosae, Caraganetum grandiflorae)* and foothill desert vegetation *(Artemisietum lerhianae, Salsoletum nodulosae,* also *Gamanthetum pilosae* and *Atriplexetum canae)* are formed within the distribution range of the argillaceous badland ecosystems. "Dead" plots completely devoid of vegetation are not rare on the area.

Floristically the argillaceous badland ecosystems of Iori plateau are not rich. 138 species of 109 genera and 37 families of vascular plants are recorded. 133 of the species are angiosperms (107 (77.5%) – dicotyledonous and 26 (18.9%) – monocotyledonous plants), while 5 species (3.6%) – are gymnosperms. According to the generic composition, the 1-9 leading families comprise 74 genera (67.9%) (Table 1), while the rest 28 families – only 35 genera (32.1%).

Table 1. Numbers of genera of the 1-9 leading families in the flora of the argillaceous badland ecosystems of Iori plateau.

Family	Number of genera	%
1. *Asteraceae*	15	13.7
2. *Poaceae*	10	9.2
3. *Chenopodiaceae*	9	8.3
4. *Boraginaceae*	9	8.3
5. *Apiaceae*	9	8.3
6. *Fabaceae*	8	7.3
7. *Brassicaceae*	7	6.4
8. *Lamiaceae*	4	3.7
9. *Caryophyllaceae*	3	2.7
Total	74	67.9

Species are disproportionately distributed among the families too. Families 1-5 comprise almost a half of the whole number of species – 48.5% (67 species), while families 1-10 embrace 69.6% (96 species) of the whole number of species (Table 2). The other 42 species (30.4%) are distributed to 27 families.

Table 2. Number of species of the 1-10 leading families in the flora of the argillaceous badland ecosystems of Iori plateau.

Family	Number of species	%
1. *Asteraceae*	19	13.8
2. *Chenopodiaceae*	14	10.1
3. *Poaceae*	13	9.4
4. *Boraginaceae*	11	8
5. *Fabaceae*	10	7.3
6. *Apiaceae*	9	6.5
7. *Brassicaceae*	8	5.8
8. *Lamiaceae*	4	2.9
9. *Liliaceae*	4	2.9
10. *Linaceae*	4	2.9
Total	96	69.6

High position of families *Chenopodiaceae* and *Boraginaceae* in the floristic spectra refers to strong influence of Irano-Thuranian deserts [Bikov, 1978; Kamelin et al., 1989]. The proportion of Mediterranean families [Tolmachev, 1986; Gagnidze, 2005] is decreased on the above background (*Brassicaceae* – only 7th position, *Caryophyllaceae* (3 species) and *Scrophulariaceae* (2 species) – doesn't fall info the first 10 families). Position of the family *Lamiaceae* which is typical of stony and skeleton biotopes of argillaceous badland ecosystems among the first ten families is unexpected; though in the designated ecosystems representatives of this family (*Thymus tiflisiensis, Teucrium polium, Stachys fruticulosa* and *Scutellaria orientalis*) basically occur on the substrate comprising argillo-arenaceus and sandstone scree and do not belong to the constant species except *Stachys fruticulosa*. We suppose that family *Linaceae* position among the first ten families is accidental: only *Linum orientale* subsp. *armenum* belongs to the relatively typical plants, the rest 3 species occur rarely in a form of separate individuals.

Study of the geographic elements of the flora of the argillaceous badland ecosystems existing on Iori plateau has shown that the Irano-Thuranian geographic element is represented by the largest number of species – 16 (11.6%). Wide representation of the species (12 species – 8.7%) distribution range of which comprises Irano-Thuranian and Mediterranean regions is noticeable. We refer them to the following geographic elements:

1. East Mediterranean – Irano-Thuranian – 6 species (4.4%)
2. Mediterranean – Irano-Thuranian – 5 (3.6%)
3. South European – Irano-Thuranian – 1 (0.7%)

A group of geographic elements with distribution ranges connected to the Mediterranean area and Minor Asia – Front Asia is also important. These geographic elements are:

1. Mediterranean – Front Asian – 9 species (6.5%)
2. East Mediterranean – Front Asian – 5 species (3.6%)
3. South European – Front Asian – 1 species (0.7%)

The Minor Asian – Front Asain geographic element is represented by 5 species (3.6%), while the Minor Asian – Irano-Thuranian element is represented by 2 species (1.5%).

A group of geographic elements, with distribution ranges comprising the Caucasus and Minor Asia – Front Asia (21 species – 15.2%) is particularly noteworthy. The following geographic elements are distinguished:

1. Caucasian – Front Asian – 9 species (6.5%)
2. East Caucasian – Front Asian – 3 species (2.2%)
3. East Transcaucasian* – Front Asian – 6 species (4.4%)
4. Caucasian – Minor Asian – 1 species (0.7%)
5. East Caucasian – Minor Asian – 1 species (0.7%)
6. South Transcaucasian* – Minor Asian – 1 species (0.7%)

Beside these geographic elements, we discuss species (18 species – 13%) with distribution ranges comprising mainly the Caucasus with only a small penetration into Minor Asia – Front Asia (North-East Anatolia, Armenia-Kurdistan and Northern and North-West Iran). A part of them may be tentatively considered as Caucasian subendemics (some of the species have been considered the Caucasian endemics so far). We refer them to the following geographic elements:

1. Caucasian – 2 species (≈ 1.5%)
    a) with North Iranian irradiation – 1 species (0.7%)
    b) with Armenia-Kurdistanian irradiation – 1 species (0.7%)
2. East Caucasian – 8 species (5.8%)
    a) with North Iranian irradiation – 5 species (3.6%)
    b) with North-East Anatolian irradiation – 3 species (2.2%)
3. East Transcaucasian – 8 species (5.8%)
    a) with North Iranian irradiation – 4 species (2.9%)
    b) with North-East Anatolian irradiation – 2 species (1.45%)
    c) with Armenia-Kurdistanian irradiation – 2 species (1.45%)

In the flora of the considered ecosystems the number of real Caucasian endemic species in not small: 16 endemic species are recorded (11.6%). If the above listed geographic elements (with distribution ranges comprising mainly the Caucasus) are taken into account, the role of the Caucasian floristic centre will be more obvious. Nine of the 16 Caucasian endemic species are East Caucasian endemics.

The rest geographic elements are represented by small numbers of species. However, participation of geographic elements with distribution ranges including Europe along with the Mediterranean area, Minor Asia-Front Asia and Thuran must also be mentioned. Particularly, we have distinguished following geographic elements:

1. European – Mediterranean – Irano-Thuranian – 3 species (2.2%)
2. Middle European – Mediterranean – Irano-Thuranian – 1 species (0.7%)
3. Middle European – East Mediterranean – Irano-Thuranian – 1 species (0.7%)
4. European – Mediterranean – Front Asian – 2 species (1.5%)
5. Middle European – Mediterranean – Front Asian – 1 species (0.7%)
6. Middle European – Irano-Thuranian – 4 species (2.9%)

Species, with distribution ranges extend eastwards (including Siberia, Central Asia, East Asia) are close to the above listed geographic elements.

Participation of the Mediterranean (2 species – 1.5%) and Palearctic (3 species – 2.2%) species is insignificant.

Analysis of systematic structure and geographic elements has shown that floristic centres of Irano-Thuran, Minor Asia – Front Asia and the Caucasus play the main role in the formation of the flora of the argillaceous badland ecosystems on Iori plateau. The Mediterranean influence is relatively weak on the described background. Although the number of species with distribution

---

* East Transcaucasus referrers to the eastern and southern part of the South Caucasus – arid and semiarid regions.

In order to simplify the names of the geographic elements, the old term "Transcaucasus" is used herein instead of the modern term "South Caucasus".

ranges comprising the Mediterranean area is not small, the participation of the Mediterranean species themselves is insignificant. The same can be said of the European and Palearctic species.

Peculiarities of the ecological conditions are reflected in the ecobiomorphic composition of the flora. Life form spectrum of the flora of the studied ecosystems is interesting and original. According to the classification system of Raunkyaer [1934], the local flora comprises: phanerophytes – 14 species (10.1%), chamaephytes – 22 species (16%), hemicryptophytes – 34 species (24.7%), geophytes – 18 species (13%), terrophytes species (including biennial plants) – 50 species (36.2%). 11 species of phanerophytes are shrubs and only 3 species are trees *(Pistacia mutica, Juniperus foetidissima, J. polycarpos)*. The viability of these tree species within the ecosystem distribution range is low and they are mostly represented by faded individuals. The majority of the chamaephytes belongs to desert semi-shrubs and dwarf semi-shrubs (*Salsola nodulosa, S. ericioides, S. dendroides, Kochia prostrata, Noaea mucronata, Aellenia glauca, Reaumuria alternifolia, Capparis herbacea, Camphorosma monspeliaca, Artemisia lerchiana* and others) [Rachkovskaia, 1957]. Dwarf semi-shrubs of a biomorph completely different from that of the mentioned species are not numerous. These are: *Thymus tiflisiensis, Scutellaria orientalis, Teucrium polium, Astragalus xiphidum, Linum orientale* subsp. *armenum*. The listed plants are rare and do not belong to the species typical of the ecosystems except *Linum orientale* subsp. *armenum*. The majority of the terrophytes is ephemeral (40 species). The number of annuals with a long growing period (4 species – *Gamanthus pilosus, Petrosimonia brachiata, Salsola soda, Atriplex calothea*) as well as that of biennials (6 species – *Onosma armeniaca, Lappula barbata, Amberboa glauca, Melilotus albus, Trinia leiogona, Reseda lutea*) are small.

Table 3. The number of life forms (%) by [Raunkyaer, 1934].

Life forms	Argillaceous badland ecosystems of Iori plateau	Vashlovani State reserve	Eldari lowland
1. *Phanerophytes*	10.1	12.7	8.8
2. *Chamaephytes*	16	4.3	8.8
3. *Hemicryptophytes*	24.7	29.4	16.7
4. *Geophytes*	13	6.4	6
5. *Terrophytes*	36.2	47.2	59.7

Compared with the corresponding spectra of the neighboring Eldari lowland as well as the Vashlovani State Reserve* [Lachashvili J., et al., 2004] a proportion of the terrophytes is markedly decreased (Table 3). At the same time the number of chamaephytes and geophytes is increased. However, it is noteworthy that only a small part of geophytes *(Podospermum cannum, Bongardia chrysogonum, Iris iberica, Eremurus spectabilis, Tulipa eichleri)* belong to comparatively constant (characteristic) species; the rest 13 species are very rare. Such important changes of life forms in almost the same climatic conditions are caused by topographic-edaphic factors: annual plants (especially ephemers having weak root system) cannot establish themselves on erosive steep slopes devoid of soil cover, which is characteristic to badlands. Since the majority of the ephemers is glycophytic, salinization of the substrate also negatively affects their establishment. Consequently, the majority of the terrophytes (38 species) occurs in the ecosystem in a form of single plants and is rare. Only a small part them – 12 species *(Bupleurum wittmannii, Lagoseris sancta, Bromus japonicus, Eremopyron orientale, E. distans, Astrodaucus orientalis, Nonea caspia, Torularia eldarica, Amberboa glauca, Lappula barbata, Gamanthus pilosus, Petrosimonia brachiata)* belong to the characteristic species (the core of the constant ones). At the same time the role of life forms with comparatively strong root system (phanerophytes, chamaephytes, hemicryptophytes)

* On the territory of the Vashlovani State Reserve the argillaceous badland ecosystems are presented beside arid forest ones.

increases. Their total number exceeds the half of the total number of species (52.1%), while the situation is almost opposite on the Eldari lowland and in Vashlovani State Reserve. Therefore, 36.2% for terrophytes is not low, but quite high index for the argillaceous badlands.

The list of 138 vascular plant species from 109 genera and 37 families recorded by us in the argillaceous badland ecosystems of Iori plateau is presented below.

Caucasuan endemics are designated by – ◎

Gymnospermae

Cupressaceae

Juniperus foetidissima Willd.
J. oxycedrus L.
J. polycarpos C. Koch

Ephedraceae

Ephedra distachya L.
E. procera Fisch. et. C.A. Mey.

Angiospermae
Dycotyledones

Anacardiaceae

Pistacia mutica Fisch. et. C.A. Mey.

Apiaceae

Astrodaucus orientalis (L.) Drude
◎ *Bupleurum wittmannii* Stev.
Daucus carota L.
Ferula szowitsiana DC.
Ferulago setifolia C. Koch
Malabaila dasiantha (C. koch) Grossh.
Prangos ferulacea (L.) Lindl.
Scandix stellata Banks & Soland
Trinia leiogona (C.A. Mey.) B. Fedtsch.

Asteraceae

Amberboa glauca (Willd.) Grossh.
A. moschata (L.) DC.
Artemisia caucasica Willd.
A. lerchiana Web.
A. scoparia Waldst. et kit.
Centaurea ovina Pall. ex Willd.
Crinitaria villosa (L.) Cass.
Crupina vulgaris Cass.
Galatella dracunculoides (L.) Nees.
◎ *Jurinea blanda* (Bieb.) C.A. Mey.
J. elegans (Stev.) DC.
Koelpinia linearis Pall.
Pterotheca sancta (L.) C. Koch
Podospermum canum C.A. Mey.
Senecio vernalis Walldst. et Kit.
◎ *Sosnowskya ambliolepis* (Ledeb.) Takht.
Steptorhamphus petraceus (Fisch. et C.A. Mey.) Grossh.
Stizolopus coronopifolius (Lam.) Cass.
◎ *Tragopogon tuberosus* C. Koch

Berberidaceae

Bongardia chrysogonum (L.) Spach

Boraginaceae

Arnebia decumbens (Vent.) Coss. et Kral.
Caccinia rauwolfii C. Koch
Heterocarium rigidum A. DC.
H. szowitsianum (Fisch. et C.A. Mey.) A. DC.
Lappula barbata (Bieb.) Guerke
Lycopsis orientalis L.
Moltkia coerulea (Willd.) Lehm.
Nonea caspica (Willd.) G. Don fil.
N. lutea (Desr.) DC.
Onosma armeniaca Klok. ex M. Pop.
Suchtelenia calycina (C.A. Mey.) A. DC.

Brassicaceae

Chorispora tenella (Pall.) DC.
Erysimum leptophyllum (Bieb.) Andrz.
Lepidium vesicarium L.
Leptaleum filifolium (Willd.) DC.
Matthiola odoratissima (Bieb.) R. Br.
Strigosella africana (L.) Botsch.
Torularia contortuplicata (Steph.) O.E. Shulz.
◎ *T. eldarica* Grossh.

Capparaceae

Capparis herbacea Willd.

Caryophyllaceae

Dianthus crinitus Smith.
◎ *Gypsophila stevenii* Fisch. ex Schrank
Silene chlorifolia Smith.

Chenopodiaceae

Aellemia glauca (Bieb.) Aell.
Atriplex cana C.A. Mey.
A. calotheca (Rafn) Fries
Camphorosma monspeliaca L.
Gamanthus pilosus (Pall.) Bunge
Kochia prostrata (L.) Schrad.
Noaea mucronata (Forssk.) Aschers et Schweinf.
Petrosimonia brachiata (Pall.) Bunge
Salsola dendroides Pall.
S. ericoides Bieb.
S. nodulosa (Moq.) Iljin
S. soda L.
Suaeda dendroides (C.A. Mey.) Mog.
S. microphylla Pall.

Cistaceae

Helianthemum salicifolium (L.) Mill.

Dipsacaceae

Scabiosa micrantha Desf.

Euphorbiaceae

Andrachne rotundifolia C.A. Mey.
Euphorbia seguierana Neck

Fabaceae

Alhagi persarum Boiss. et Buhze
Astragalus stevenianus DC.
A. xiphidium Bunge
Caragana grandiflora (Bieb.) DC.
Colutea orientalis Mill.
Hedysarum formosum Fisch. et C.A. Mey. ex Basin.
H. ibericum bieb.
Medicago coerulea Less ex Ledeb.
Melilotus albus Medik.
◎ *Onobrychis komarovii* Grossh.

Geraniaceae

Erodium cicutarium (L.) L'Her.

Helleboraceae

Delphinium cyphoplectrum Boiss.

Lamiaceae

Scutellaria orientalis L.
Stachys fruticulosa C. Koch
Teucrium polium L.
◎ *Thymus tiflisiensis* Klok et Schost.

Limoniaceae

◎ *Acantholimon fominii* Kusn.
Limonium meyeri (Boiss.) O. Kuntze

Linaceae

Linum austriacum L.
L. liburnicum Scop.
L. nodiflorum L.
L. orientale (Boiss. et Heldr.) Boiss. subsp. *armenum* Bordz.

Nitrariaceae

Nitraria schoberi L.

Polygonaceae

Atraphaxis caucasica (Hoffm.) Pavl.
A. spinosa L.

Resedaceae

◎ *Reseda globulosa* Fisch. et C.A. Mey.
R. lutea L.

Rubiaceae

Galium verum L.
◎ *Rubia transcaucasica* Grossh.

Rutaceae

Haplophyllum armenum Spach

Santalaceae

Thesium arvense Horvatovsky

Scrophulariaceae

Linaria simplex (Willd.) DC.
Veronica multifida L.

Solanaceae

Lycium ruthenicum Murr.

Tamaricaceae

Reaumuria alternifolia (Labill.) britten

Zygophyllaceae

Zygophyllum falago L.

Monocotiledones

Alliaceae

Allium rubellum Bieb.

Asparagaceae

◎ *Asparagus caspius* Schult. ex Schult. fil.

Asphodelaceae

Asphodeline dendroides (Hoffm.) Woronow
Eremurus spectabilis Bieb.

Hyacinthaceae

Bellevalia speciosa Woronow
◎ *B. montana* (C. Koch) Boiss.

Iridaceae

◎ *Iris iberica* Hoffm.
I. pumila L.
Iuno caucasica (Hoffm.) klatt

Liliaceae

◎ *Gagea caroli-kochii* Grossh.
G. chlorantha (Bieb.) Shult. et Schult. fil.
G. commutata C. Koch

◎ *Tulipa eichleri* Regel

Poaceae

Aegilops cylindrica Host
Agropyron pectinatum (Bieb.) Beauv.
Anisantha rubens (L.) Nevski
Bothriochloa ischaemum (L.) Keng.
Bromus japonicus Thunb.
Eremopyrum distans (C. Koch) Nevski
E. orientale (L.) Jaub. et Schpach
Lolium rigidum Gaudin
Melica transsilvanica Schur
Poa crispa Thuill.
Stipa caspia C. Koch
S. lessingiana Tin. et Rupr.
Trachynia distachya (L.) Link.

References:

Atlas of Azerbaijan SSR. Baku-Moscow, p. 49, 1963 (in Russian).

Atlas of Georgian SSR. Tbilisi-Moscow, p. 269, 1964 (in Russian).

Bikov B. A. *General analysis of flora of the deserts of the Turanian lowland*. In: Structure and productivity of the desert zone vegetation of Kazakhstan. Alma-Ata. pp.3-8, 1978 (in Russian).

Czerepanov S. K. *Vascular Plants of Russia and adjacent states (the former USSR)*. Cambridge. p. 516, 1995.

Gagnidze R. *Vascular plants of Georgia. A nomenclatural checklist*. Tbilisi. p. 247, 2005.

Grossheim A. A. *Vegetation cover of the Caucasus*. Moscow. p. 265, 1946 (in Russian).

Grossheim A. A. and Sakhokia M.F. *Description of the vegetation of Kabristan. Worsk on geobotanical investigation of winter pastures of Azerbaijan SSR*. Ser. A. Winter pastures, Fascicle 7, Baku, p.102, 1931.

Kamelin R. V., Bochantsev V. I., Gorelova T. G. *Short characteristic of the flora of Badkhiz; vascular plants*. In: Pistacietum of Badkhiz, Leningrad, pp. 7-21, 1989 (in Russian).

Lachashvili J., Lachashvili N., Khachidze M. *Synopsis of Flora of the Vashlovani State Reserve*. Tbilisi, p. 157, 2004.

Lachashvili N. Phytocenoses of *Reaumuria alternifolia* in the Vashlovani State Reserve (East Georgia). Bull. Georg. Acad. Sci. **170**, 1, 122-124, 2004a.

Lachashvili N. *Rare phytocenoses of Atraphaxis spinosa in the Vashlovani State Reserve (East Georgia)*. Bull. Georg. Acad. Sci. **170**, 2, 330-333, 2004b.

Lachashvili N. *New data on Reaumuria alternifolia formation (Reaumurieta alternifoliae)*. Bull. Georg. Acad. Sci. **171**, 3, 503-505, 2005.

Prilipko L.I. *Vegetation cover of Azerbaijan*. Baku. p.169, 1970 (in Russian).

Prilipko L.I. *Mountain xerophyte vegetation*. In.: Vegetation of the European part of the USSR. Leningrad, pp. 277-280, 1980 (in Russian).

Rachkovskaia E. I. *For biology of desert dwarf semi-shrubs*. In: Geobotany, fascicle 11, Moscow-Leningrad, pp. 5-87 (in Russian).

Raunkyaer C. *Life forms of plants and statistical plants geography*. Oxford, p.105, 1934.

Sakhokia M.F. *Description of the vegetation of winter pastures of Sheki plateau. Works on geobotanical investigation of winter pastures of Azerbaijan SSR*. Series A. Winter pastures. Fascicle 9. Baku. p. 30, 1931 (in Russian.)

Sakhokia M.F. *Botanical description of the Tbilisi surroundings along the itinerary: Tbilisi - Shiraki plateau*. In: Botanical excursions through Georgia. Tbilisi, pp. 7-30, 1958 (in Russian).

Serebriakov I. G. *Life forms of higher plants and their investigation*. Field geobotany, vol. III. Moscow-Leningrad, pp. 145-205, 1964 (in Russian).

Tolmachev A. I. *Methods of comparative florogenetics and problems of florogenesis*. Novosibirsk, 1996 (in Russian).

Tsereteli D. V. *Iverian part of intermountain*. In: Geomorphology of Georgia. Tbilisi, pp. 282-309, 1971 (in Russian).

ივრის ზეგნის (აღმოსავლეთ საქართველო) თიხიანი ბედლენდების ეკოსისტემების ფლორისტული შემადგენლობა

ლაჩაშვილი ნ., ლაჩაშვილი ი.

საქართველოს მეცნიერებათა აკადემიის ნ. კეცხოველის სახელობის ბოტანიკის ინსტიტუტი

(მიღებულია 11.07.2005)

რეზიუმე

პირველადაა შესწავლილი ივრის ზეგნის თიხიანი ბედლენდების ეკოსისტემების ფლორისტული შემადგენლობა. აღნიშნული ეკოსისტემების ფლორა ღარიბია – აღირიცხა ჭურჭლიან მცენარეთა 37 ოჯახის 109 გვარის 138 სახეობა. სახეობათა შემცველობით წამყვანი ოჯახებია: *Asteraceae* – 19 სახეობა (13,8%), *Chenopodiaceae* – 14 (10,1%), *Poaceae* – 13 (9,4%), *Boraginaceae* – 11 (8%), *Fabaceae* – 10 (7,3%), *Apiaceae* – 9 (6,5%), *Brassicaceae* – 8 (5,8%), *Lamiaceae, Liliaceae, Linaceae* – 4-4 (2,9-2,9%). ამ 10 ოჯახის წილად მოდის სახეობათა საერთო რაოდენობის 69,6% (96 სახეობა). ფლორის სისტემატიკური სტრუქტურისა და გეორგაფიული ელემენტების ანალიზმა აჩვენა, რომ მის ჩამოყალიბებაში განმსაზღვრელი როლი ირან-თურანის, მცირე აზია-წინა აზიისა და კავკასიის ფლორისტულმა ცენტრებმა შეასრულეს. მათ ფონზე შესუსტებულია ხმელთაშუაზღვისპირეთის ზეგავლენა. კავკასიის ენდემების რაოდენობა 16 სახეობას (11,6%) შეადგენს; განხილულია ფლორის სასიცოცხლო ფორმების შემადგენლობა. მოცემულია ფლორისტული შემადგენლობის სრული სია.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

PREDICTION TECHNOLOGY OF *LOBESIA BOTRANA DEN. & SCHIFF.* (LEPIDOPTERA: TORTRICIDAE) DEVELOPMENT

ABASHIDZE E.

Department of Ecology, Iv. Javakhishvili Tbilisi State University
E-mail: elena@gacor.org.ge

(Received September 19, 2005)

Abstract

The technology of prediction of grape berry moth - *Lobesia botrana* worked out. Special program for calculating of many years` data on pest phenology and meteorological information was created. As a result of analysis of bioecological peculiarities of vine pest the prognostic predictors and vulnerable phase to pesticides were established. On the base of mathematical method of the heat phenology the temperature-phenological nomograms of pest and vine development in Sagarejo area was constructed.The proposed technology of predicting is universal and can be used for other crop pests.

Key words: phenological forecasting, grape berry moth, mathematical methods, temperature-phenological nomogram.

Introduction

Intensive use of chemical pesticides for plant protection created serious threat to human health and environment, promoted reducing of biodiversity and development of resistant population of phytophages. As a result further extension of pesticides range is observed. There were few ones who thought about results of uncontrolled use of pesticides. When the threat became real, new attitude and approaches started to develop. Necessity of reduction of pesticide press and ecologization of plant protection needs transition to new system that is based on forecasting. The purpose of forecasting is to predict in advance breeding, harmfulness of pest insect and the optimal date of safety control measures [Abashidze, 1991, 1994]. The grape berry moth is a very important pest of vineyards of Georgia.

Damage caused by the grape berry moth annually is rather considerable [Abashidze,1994]. High harmfulness is stipulated by polivoltinity of species, secret mode of its life and imperfection of existed system of pest control in Georgia. The main viticulture massive of Georgia are located in area, having average annual temperature from 9.5 C to 16 C [Davitaya, 1968]. They are located in the same areas that are pointed for grape berry moth. Inspite of it, that *Lobesia botrana* occurred in all vine growing regions it injured vineyards in East Georgia, mostly in Kakheti region. *Lobesia botrana* has three generations in year. It causes damage to vine in phase of larvae. Because of bioecological peculiarities the grape berry moth vulnerable phase to pesticides has short-term period. Our studies have focused on the development of predicting technology of pest vulnerable phase appearance for safety control.

Material and methods

The target object of our investigations is important pest of vineyard *Lobesia botrana*. Location of investigations is Sagarejo region. Technology of investigations - mathematical methods of prediction of grape berry moth development [Gabel & el, 1986]. The mathematical prediction technology needs in good quality information about bioecology of target object [Knight & el., 1995]. The many years field trips and laboratory research on bioecology of grape berry moth were carried out. The climatic net of Sagarejo region was developed on the base of meteorological information for 15 years. Meteorological information of Sagarejo was received from local meteorological station. We developed the special computer program for calculation. The method of heat phenologhy for phenological forecasting was applied. For development of prediction technology were used the method of temperature-phenological nomograms. They are based on graphical comparison of the heat quantity required for phytophages development and area thermal resources [Podolsky, 1974].

As a result of study of the grape berry moth and vine bioecological peculiarities phenological curves of pest and vine were constructed. The phenological lines of nomogram were calculated by regressive equations. For developing of regressive equations was used statistical program SPSS-8.

The temperature-phenological nomogram of *Lobesia botrana* for Sagarejo region was constructed by combining the phenological curves of pest and meteorological net of region. Use of temperature-phenological nomogram permit to predict the vulnerable phase of pest and optimal date for effective control [Podolsky, 1974]. Precision of prediction was calculated by Polyakov (1984). Identity of predicting results estimated by retroprognosis.

Results and Discussion

The date of appearance of *Lobesia botrana* larva varied depending on meteorological conditions of year. Period of larva appearance decreased with temperature rising. Investigations on phenological peculiarities of pest and effect of meteorological factors on its development revealed that main forecasting predictor of grape berry moth larva appearance is temperature that exerts as direct so indirect influence on pest development.

On the base of our investigations and regressive analysis the temperature-phenological nomograms of grape berry moth and vine for Sagarejo area were constructed (Fig.1 and 2).

Forecasting of phenology of grape berry moth by use technology of temperature-phenological nomograms showed high results. Accuracy of calculation varied within the limits of 89 –95%. There was little difference between calculated and actual data- 3-5 days. Forecasting indices were higher if prediction period is expected dry with temperature within 27-30^{0}C and moderate precipitation. Application of prediction in pest control (1988-1989) decreased the volume of pesticide use (30%) and promoted conservation of useful fauna. Survey of vineyard fauna in 1989-1990 showed generally increasing of number of biological agents. The phenoprognostic calendars of *Lobesia botrana* and vine for Sagarejo were worked out on the base of nomograms by use of extrapolation method (Table 1 and 2). They give approximate dates of grape berry moth and vine phenophase development, that needs correction from time to time depending on actual meteorological information.

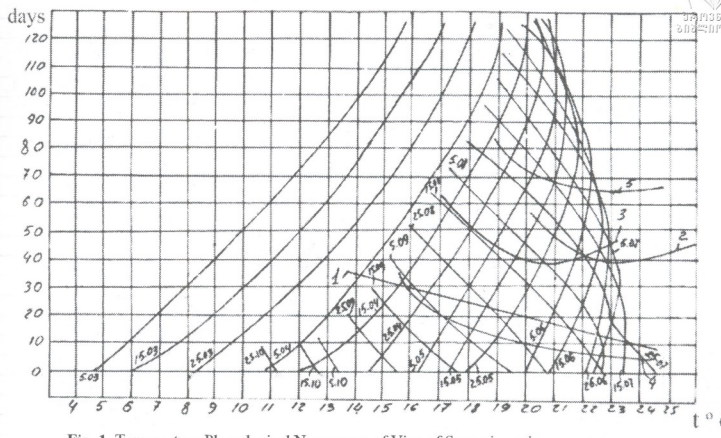


Fig. 1. Temperature-Phenological Nomogram of Vine of Sagarejo region.

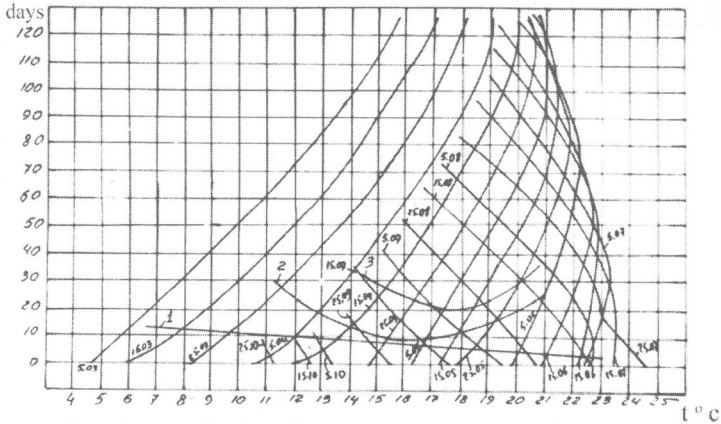


Fig. 2. Temperature-Phenological Nomogram of *Lobesia Botrana* of Sagarejo region.

Table 1. Phenoprognostic calendar of vine

N	Dates			
	Moth flight	Appearance of I generation larva	Appearance of II generation larva	Appearance of III generation larva
1	15.04	11.05	24.06	05.05
2	16.04	12.05	25.06	06.08
3	17.04	12.05	25.06	06.08
4	18.04	12.05	25.06	06.08
5	19.04	13.05	25.06	06.08
6	20.04	13.05	25.06	06.08
7	21.04	14.05	25.06	06.08
8	22.04	14.05	25.06	06.08
9	23.04	15.05	26.06	07.08
10	24.04	15.05	26.06	07.08
11	25.04	16.05	27.06	08.08
12	26.04	16.05	27.06	08.08
13	27.04	17.05	28.06	09.08
14	28.04	18.05	28.06	09.08
15	29.04	18.05	28.06	09.08
16	30.04	18.05	28.06	09.08
17	01.05	19.05	29.06	10.08
18	02.05	19.05	29.06	10.08
19	06.05	20.05	30.06	11.08
20	07.05	20.05	30.06	11.08
21	08.05	20.05	30.06	11.08
22	09.05	21.05	01.07	12.08
23	10.05	22.05	02.07	13.08
24	11.05	23.05	03.07	14.08

Table 2. Phenoprognostic calendar of lobesia botrana larvae appearance

N	Dates					
	Bud swelling	Appearance of I leaf	Appearance of III leaf	Beginning of blossom	Ending of blossom	Ripenning
1	2	3	4	5	6	7
1	20.03	02.04	30.04	17.05	01.06	15.08
2	21.03	03.04	29.04	18.05	02.06	16.08
3	22.03	04.04	29.04	19.05	02.06	17.08
4	23.03	05.04	29.04	19.05	03.06	18.08
5	24.03	06.04	29.04	20.05	03.06	19.08
6	25.03	06.04	30.04	21.05	04.06	20.08
7	26.03	07.04	01.05	21.05	05.06	21.08
8	27.03	08.04	02.05	22.05	05.06	22.08
9	28.03	09.04	04.05	23.05	06.06	23.08
10	29.03	10.04	05.05	23.05	06.06	24.08
11	30.03	11.04	07.05	24.05	06.06	25.08
12	31.03	12.04	08.05	24.05	07.06	26.08
13	01.04	13.04	10.05	25.05	07.06	27.08
14	02.04	14.04	13.05	25.05	08.06	28.08
15	03.04	15.04	14.05	26.05	09.06	29.08
16	04.04	16.04	15.05	27.05	09.06	30.08
17	05.04	16.04	16.05	28.05	10.06	31.08
18	06.04	17.04	17.05	29.05	11.06	01.09

19	07.04	18.04	18.05	31.05	12.06	02.09
20	08.04	19.04	19.05	01.06	13.06	03.09
21	09.04	20.04	20.05	03.06	13.06	04.09
23	10.04	21.04	21.05	04.06	14.06	05.09
24	11.04	22.04	22.05	06.06	15.06	06.09
24	12.04	23.04	23.05	06.06	16.06	07.09
26	13.04	24.04	24.05	07.06	17.06	08.09
27	14.04	25.04	26.05	09.06	17.06	09.09

References:

Abashidze E. *Comparative assessment of entomophenological forecasting methods*. Ecology, **1,** 72-77, 1991 (in Russian).

Abashidze E. *Use of ecological forecasting in plant protection.* Proceedings of VII International Congress of Ecology, 44, Manchester, UK, 1994.

Davitaya F. *Vine growing zones of Georgia*. Moscow, 1968 (in Russian).

Gabel B. and Mocko V. *A functional simulation of European vine moth Lobesia botrana population development*. Appl. Ent., **52**, 121-127, 1986.

Knight A.L. and Croft B.A. *Modeling and prediction technology in Tortricid*. World Crop Pests, **5,** 234-239, Elsevier, Amsterdam, 1995.

Podolsky. A. *Phenological forecasting (mathematical forecasting in ecology).* Moscow, "Kolos", 219- 312, 1974.

Polyakov I. et al. *Prediction of crop pests and diseases,* Leningrad, "Kolos", 318, 1984.

Lobesia botrana Den. & Schiff. (Lepidoptera: Tortricidae) პროგნოზის ტექნოლოგიის შემუშავება

ე. აბაშიძე

ეკოლოგიის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი
E-mail: elena@gacor.org.ge

(მიღებულია 19.09.2005)

რეზიუმე

შესწავლილია ვაზის საშიში მავნებლის *Lobesia botrana*-ს ბიოეკოლოგიური თავისებურებანი, რის საფუძველზე შემუშავებულია მავნებლის განვითარების პროგნოზირების ტექნოლოგია. სპეციალური კომპიუტერული პროგრამის გამოყენებით დამუშავებულია *Lobesia botrana*-ს და ვაზის ტემპერატურულ-ფენოლოგიური ნომოგრამები. რეგრესიული ანალიზისათვის გამოყენებული იყო სტატისტიკური პროგრამა SPSS-8.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

DISTRIBUTION OF RH-HR ANTIGENS IN ADJARA POPULATION

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

Immunogenetic laboratory, Sh. Rustaveli Batumi State Univerity
**E-mail: mari-na@nm.ru*

(Received May, 16, 2005)

Abstract

The frequency of distribution of rhesus system D, C, c, E, e antigens of Adjara population was studied (274 individuals). To express Rh-Hr antigens several immunoserological methods were used. It was shown that frequencies of antigens are distributed in the following way: c antigen – 86.13%, D – 75.18%, e – 68.25%, E – 48.18% and C – 47.45%. There also was displayed Du antigen (1.01%). In Adjara population 9 Rh-phenotypes were fixed which have different frequencies of spreading: CcDe – 27%, cde – 18.24%, cDe – 14.23%, CDe – 10.58%, cDEe – 7.2%, CcDEe – 6.93%, cDE – 3.2%, CDEe and Ccddee – 1.5%. From researched individuals 31.02% were Rh-negative and 67.51% - Rh-positive.

Key words: immunoserological methods, Rh-phenotypes, rhesus system antigens.

Introduction

There are a lot of investigations about blood group systems, role of Rh system antigens and their gene geography [Ragimov, 2004; Gene pool and gene geography of population, 2000]. In Adjara population there is not much information about this issue. The studies were carried out only over D antigen.

The goal of our research was to reveal D, C, c, E, e antigens in Adjara population from different regions (247 individuals) and study frequency of their distribution.

Materials and Methods

To express Rh-Hr antigens several immunoserological methods were used. Namely, express-method with the use of monoclonal antibodies; colloidal solution method with the use of polyglycon; saline method - using physiological solution, gelatinization method – using 10% gelatin, and also Cumbs direct and indirect methods with the use of antiglobulin serum [Gide-book on blood transfusion and blood substitutes, 1982; Donskov et al., 1998; Kosiakov, 1974] Anti- D, C, c, E, e monoclonal antibodies were used ("Hemostandard", Russia).

Results and Discussion

We studied blood of individuals from different regions of Adjara – Khelvachauri, Keda, Suakhevi, Kobuleti, Khulo. According to our data from rhesus system antigens c-antigen has the

highest frequency of distribution (86.13%); then comes D-antigen (75.18%). As for the rest of rhesus system antigens their frequency of distribution are: e-antigen – 68.25%, E-antigen – 48.18% and C-antigen – 47.45% (Fig.1).

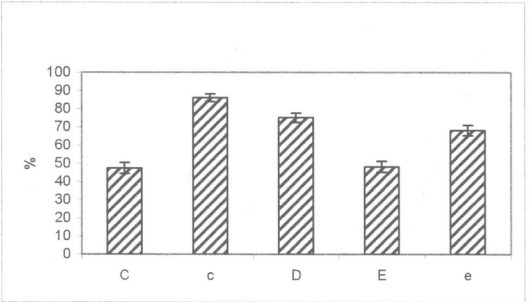


Fig. 1. Frequency of rhesus antigens distribution

We also revealed Du-antigen. The characteristic feature of this antigen is the following: it doesn't often respond to anti-D antibodies or gives very week reaction. This antigen is displayed by Qumbsy reaction. For the studied population frequency of Du-antigen is very low – 1.09% (Fig.2).

In studied population 9 Rh-phenotypes were fixed which have following frequencies of spreading: CcDe – 27%, cde – 18.24%, cDe – 14.23%, CDe – 10.58%, cDEe – 7.2%, CcDEe – 6.93%, cDE – 3.2%, CDEe and Ccddee – 1.5% (Fig. 3).

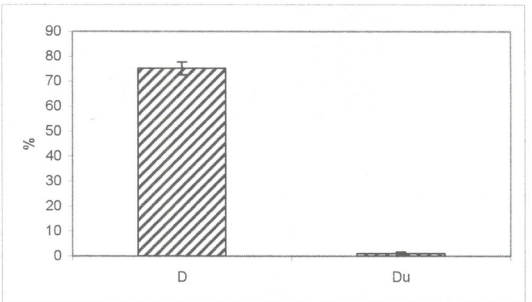


Fig.2. Frequency of D and Du antigens distribution

It is known from scientific literature [Margni, 1977] that there exist such phenotypes which doesn't consist any of Rh-system antigens; they are called Rhnull. The above mentioned phenotype was not revealed in our studies, but we displayed one phenotype - -D- -, where C, c, E, e antigens were not met at all.

By the content of rhesus system antigens two phenotypes of individuals are distinguished: Rh-positive and Rh-negative. In studied population 31.02% of individuals are negative phenotype carriers (Fig.4).

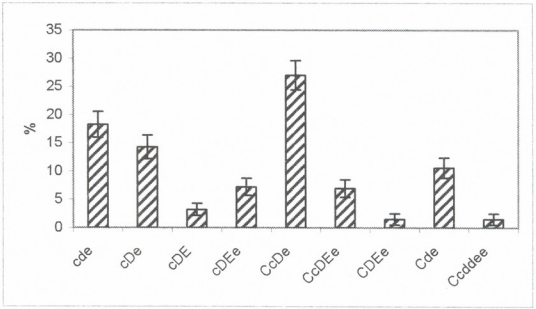


Fig. 3. Frequency of Rh-phenotypes occurrence

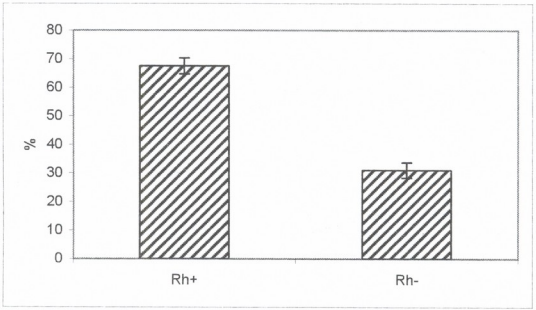


Fig. 4. Frequency of Rh+ and Rh- phenotypes occurrence

Thus, according to our investigations it was established polymorphism of Adjara population by the content of Rh-Hr antigens. In particular, c-antigen has the highest frequency of distribution, then comes D, e, E, C antigens. It was also revealed Du-antigen with low frequency. In the studied population with different frequencies of occurrence 9 phenotypes were fixed: CcDe, cde, cDe, Cde, cDEe, CcDEe, cDE, CDEe and Ccddee. 31.02% of studies individuals are Rh-negative.

References:

Donskov S., Cherviakov V., Paskunova T. *Ways of Immunological Security of Erythrocytes Transfusion*. In: "New Ways of Transfusion", **21** issue, 35, 1998 (in Rus.).

Gene Pool and Gene Geography of Population. ed. Richkov Iu., St. Petersburg, "Nauka", **1**, 98, 2000 (in Rus.).

Guide-book on Blood Transfusion and Blood Substitutes. ed. Gavrilov O., M., "Medicina", 304, 1982 (in Rus.).

Kosiakov P. *Iso-antigens and Iso-antibodies of Human in the Norm and Pathology*. M., "Nauka", 178, 1974 (in Rus.).

Margni R.A., Leoni J., Bazzurro M. *The incomplete anti-Rh antibody agglutination mechanism of trypsinized ORh+ red cells*. Immunology, **33**, 2, 53-60, 1977.

Ragimov A., Dashkova N. *Basis of Transfusional Immunology*. M., "Medical-Informational. Agency", p.68, 2004 (in Rus.).

Rh-Hr ანტიგენების გავრცელება აჭარის პოპულაციაში

ნაგერვაძე მ., ახვლედიანი გ., დუმბაძე ქ., ბაგრატიონი ქ., დიასამიძე ა.

იმუნოგენეტიკის ლაბორატორია, შ. რუსთაველის სახელობის ბათუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 16.05.05)

რეზიუმე

შესწავლილია რეზუს სისტემის D, C, c, E, e ანტიგენების გავრცელების სიხშირე აჭარის მოსახლეობაში (274 ადამიანი). Rh სისტემის ჯგუფური ანტიგენების გამოსავლენად გამოყენებულ იქნა იმუნოსეროლოგიური მეთოდები. დადგენილია c ანტიგენის გავრცელების მაღალი სიხშირე (86.13%), შემდეგ მოდის D ანტიგენი (75.18%). დანარჩენი ანტიგენებიდან e-ს გავრცელების სიხშირე შეადგენს 68.25%-ს, E – 48.18%, ხოლო C – 47.45%. გამოვლენილ იქნა აგრეთვე Du ანტიგენის დაბალი სიხშირე (1.01%). გამოკვლეულ პოპულაციაში დაფიქსირებულ იქნა 9 Rh-ფენოტიპი, რომლებიც გვხვდებიან შემდეგი სიხშირით: CcDe – 27%, cde – 18.24%, cDe – 14.23%, Cde – 10.58%, cDEe – 7.2%, CcDEe – 6.93%, cDE – 3.2%, CDEe და Ccddee – 1.5%. გამოკვლეული ორგანიზმების 31.02% Rh-უარყოფითია, ხოლო Rh–დადებითი ფენოტიპის მატარებლის სიხშირე 67.51%-ია.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

AVIDITY OF THE ANTIBODIES PRODUCED AGAINST MUTANT RECOMBINANT hCGβ

CHIKADZE N., JANIKASHVILI N., BURJANADZE L., GOLIADZE E., SHELEGIA K., GACHECHILADZE N., OSADZE M., PORAKISHVILI N.

Department of Immunology, Iv. Javakhishvili Tbilisi State University

(Received September 20, 2005)

Abstract

The vaccine prototype based upon the recombinant form of the human chorionic gonadotropin (hCG) β chain BAChCGβ R68E containing an arginine to glutamic acid replacement at a position 68, is designed to elicit an effective and safe immune response against native hCG. The majority of antibodies in the mutant sera are specific for C-terminal peptide of hCG β (hCG β CTP) and do not bind to the human luteinizing hormone. Since the neutralizing capacities of the antibodies depend on their concentration as well as their avidity. We have determined the binding avidity of the mutant sera with the native hCG αβ, the mutant hCG β and the hCG β CTP using a direct binding ELISA after ammonium thiocyanate elution. Received data demonstrate that the sera of the rabbits immunized with BAChCG β-R68E are able to bind to native hCG with high avidity that enables us to conclude that the BAChCG β -R68E is an effective and safe prototype of the anti-hCG vaccine characterized by a high degree of immunogenicity.

Key words: human chorionic gonadotropin (hCG), vaccine, human luteinizing hormone (hLH).

Introduction

Human chorionic gonadotropin (hCG) is a glycoprotein hormone which belongs to the cystine knot growth factor families [Pierce & Parsons, 1981]. In its role as a pregnancy hormone hCG is produced by the trophectoderm of the pre-implantation embryo within a few days of fertilization and has corpus luteum-maintaining action ensuring continued production of progesterone. Since progesterone is needed for the successful completion of implantation of the blastocyst, in case when the production or function of hCG is inhibited immunologically, the corpus luteum would regress, its production of progesterone would decline and menstruation would occur at or about the expected time, thus mimicking the events that occur naturally in a non-conceptual cycle. Therefore hCG is considered an anti-fertility vaccine candidate [Stevens, 1999, Talwar, 1999].

hCG also is a biochemical marker of malignancy associated with all the major types of cancer. The tumor cells not only secret hCG, but it is also expressed on their surface. hCG may act at several different levels to facilitate cancer progression, as a transforming growth factor, an immunosuppressive agent, an inducer of metastasis, and/or as an angiogenic factor [Acevedo & Hartsock, 1996]. Thus, immunization against hCG may result in increasing the capability of

humoral and/or cellular immune effectors to directly lyse tumor cells expressing hCG. In addition, neutralization of soluble hCG with antibody may abrogate hCG-mediated tumor growth signals, angiogenesis, and immune escape phenomena [Acevedo et al., 1992; Triozzi et al.,1994].

While creating the vaccine based upon hCG, the problem emerged from its structural similarity with the other members of glycoprotein hormone family (the luteinizing hormone (hLH), follicle–stimulating hormone (FSH) and thyroid-stimulating hormone (TSH). These hormones are the α/β heterodimers containing the same α chain, with a certain percentage of similarity among the β -chains. The closest 85% homology is between hCG- β and hLH- β. As a result the antibodies syntesized in response to the native hCG cross-react with hLH [Lapthorn et al., 1994]. A reduction of the hLH would affect negatively the ovary functioning.

In order to avert the problem, the method of constructing the epitop-specific vaccines was developed at the Immunology Department of University College London (UCL). Selected amino acid residues at presumed LH cross-reactive epitope regions have been substituted [Chiesa et al., 2001; Jackson et al., 1996]. One of these mutants, hCG β -R68E with arginine^{68} substituted with glutamic acid, failed to bind all tested LH-cross reactive monoclonal antibodies [Jackson et al., 1996].

We have previously shown that baculovirus-produced hCGβ-R68E (BAChCG β-R68E) elicited antibodies with minimal LH cross-reactivity in both mice and rabbits. The antibodies produced were predominantly directed against C-terminal peptide of the hCGβ (hCG β CTP) [Porakishvili et al., 2002; Chiesa et al., 2001].

In this study we demonstrate that antibodies formed through immunization of rabbits with the mutant molecule hCG β -R68E, bind with a high affinity to the native hCG, the mutant hCG and the hCG β CTP. This finding is important in developing of anti-hCG vaccine.

Materials and Methods

Immunization of rabbits:

The immunization of rabbits has been carried out at the animal house of University College London. Two rabbits were immunized with the baculovirus-produced mutant molecule BAChCG β-R68E, two with the wild type hCG β -chain - BAChCG β and one with the native hCG$\alpha\beta$. Rabbits were primed by intramuscular injection with 100μg of hormone-tetanus toxoid (TT) conjugate mixed (1:1/v:v) with 100μl Ribi Adjuvant System (Sigma, Poole, Dorset, UK) and boosted by intramuscular injection with an equal amount of conjugate three weeks later. Sera were collected after a repeated immunization and were preserved in aliquots at -20° C for subsequent checking at the Department of Immunology at Tbilisi State University.

Assessment of the sera avidity:

The avidity of the sera were determined using a chaotropic agent ammonium thiocyanate (ATC) in ELISA assays. Nunc Maxisorp C96-well flat-bottomed microtiter plates were coated at 4° C overnight with 50μg per well of native hCG$\alpha\beta$, mutant BAChCGβ-R68E or hCGβCTP at 1.0 μg/ml in 0.05M carbonate-bicarbonate buffer, pH 9.6 (CBB). The plates were washed three times with PBS containing 0.05% Tween 20 (PBS-T), followed by blocking with 200 μl/well of 2% dried skimmed milk powder in CBB overnight at 4° C.

After washing three times with PBS-T, 50μl serum from immunized rabbits diluted in PBS-T with 2% bovine serum albumine (PBS-T-BSA) up to the concentration, correspondent to 75% of plateau binding, previously determined by us [Porakishvili et al., 2002] was added and incubated for 2hrs at 37° C. The plates were washed three times with PBS-T and 100 μl of ATC (Sigma) in PBS was added for 15min at room temperature (RT). The ATC dissociates antibody-

antigen binding in molarity-dependent manner and was used at 0.031125M-8M and all measurements were performed in duplicate.

The plates were washed three times with PBS-T before a goat anti-rabbit IgG alkaline phosphatase-conjugated antibody (Sigma, Poole, Dorset, UK) was added for 2hrs at 37^0 C. Following further three washes with PBS-T-BSA and one wash with CBB, the substrate p-nitrophenylphosphate (Sigma) in CBB containing 2mM $MgCl_2$ was added, the plates left for 15-20 min at RT, and then read at A_{405} using an Titertek Multiskan MCC. Resistance to thiocyanate elution was utilized as the measure of avidity and an index representing 50% of effective antibody binding was used to compare different sera.

Results and Discussion

Among the characteristics of the anti-hCG antibodies, affinity is one of the important determinants of their biological efficacy. The binding of an antibody and its corresponding antigen could be disrupted by thiocyanate solution of different concentrations, depending upon the affinity of the antibody and molarity of the ATC [Wang et al., 2004].

As our data have shown the anti-sera, resulting from a BAChCG β-R68E immunization, are marked with a high-level avidity binding to hCG. In order to inhibit the binding ability by 50% 1M and 2M concentrations of ATC were required (Fig. 1). The binding strength nearly equaled the avidity of the binding of the sera to the immunogen itself, since to inhibit it by 50% 2M of ATC has been used. The avidity of binding of those sera to the hCG β CTP appeared to be high too and required 2M and 3.7M concentrations of ATC for 50% inhibition. This signifies an ability of mutant sera to react with the native hCG and hence, indicates that the mutant hCG β has indeed a potential of an effective vaccine.

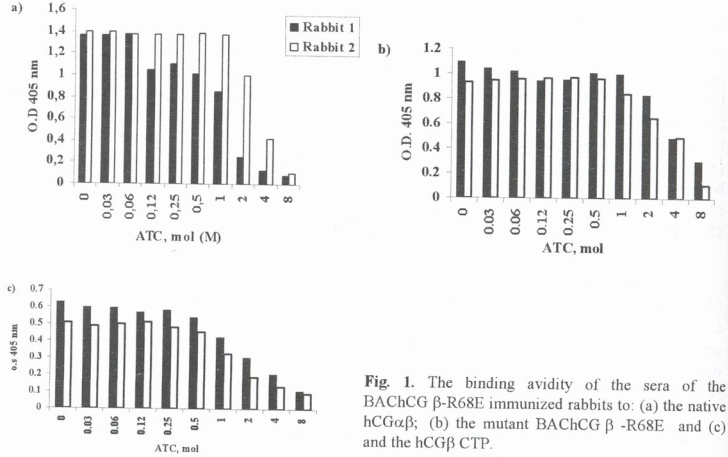


Fig. 1. The binding avidity of the sera of the BAChCG β-R68E immunized rabbits to: (a) the native hCGαβ; (b) the mutant BAChCG β -R68E and (c) and the hCGβ CTP.

Our previous data has shown that in both the native hCGαβ and the wild type hCGβ molecules the epitope groups cross-reactive with hLH are immunodominant [Porakishvili et al., 2002]. In the current study we found that the avidity of anti-hCGαβ and anti-BAChCGβ sera to hLH is similar to that to hCG. The percentage of inhibition of the binding of these sera to both hCG and hLH by the different concentration of ATC is nearly equal. Moreover, in order to inhibit the anti-hCGαβ binding to the native hCG and/or hLH by 50% even a very high concentration of 8M ATC is not enough (Fig. 2). Therefore, if the hCGαβ or the hCGβ chain is used for immunization, they will be equally potent for either hCG or hLH immunoneutralization and this would have negative clinical implications.

An outcome of that part of the experiment has convinced us in the validity of our concept and may be regarded as the evidence that in a formation of an hCG-based vaccine, an application of hCG analogue is favorable, since the synthesized antibodies reveal a high level avidity in binding to the target antigen solely. In this case, there is more guarantee of keeping a hormone balance in the female body intact.

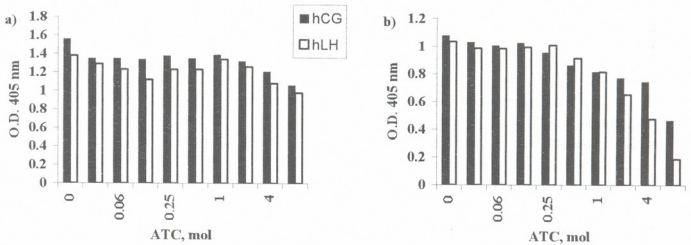


Fig. 2. The avidity of the sera of the rabbits immunized with a) hCGαβ and b) BAChCG β to hCG and hLH

We have recently shown that a single arginine[68] to glutamic acid substitution in hCG β molecule dramatically alters its antigenicity and immunogenicity. Immunogenicity studies with baculovirus-derived hCG β-R68E in both mice [Chiesa et al., 2001] and rabbits [Porakishvili et al., 2002] showed that in the mutant molecule the immune response is refocused toward epitope(s) present on the CTP, which is missing in LHβ and the mutant sera fail to bind to LH. It is well known, the hCG βCTP is highly mobile as a free entropy-rich peptide segment and, therefore, the B-cell epitopes on the C-terminal region are low-immunogenic. Also, the anti- hCGβCTP monoclonal antibodies directed towards the CTP are generally of low affinity (typically with a Kd of approximately 10^{-8} mol/l) [Berger et al., 2002]. We suggest that the point mutation in hCGβ-R68E molecule has caused a hCGβ–CTP mobility constraint. The glutamic acid[68] in the mutant forms a salt bridge with one or more of the basic amino acid residues present in the hCG β CTP [Charrel-dennis et al., 2005]. Only by reducing the entropy of the hCGβ–CTP, it is possible to trigger a relatively substantial primary immune response, which can then undergo affinity maturation to generate the high affinity antibodies necessary to bind effectively to hCGβ-CTP on the native molecule.

The fact that the antibodies formed through immunization of the mutant molecule bind with a high affinity to the native hCG, may serve as a basis for an effective phagocytosis of the created complexes (investigations currently underway). Indeed, binding affinity is not merely a matter of theoretical interest, as affinity and avidity affect the properties of antibodies. A high-affinity antibody is superior to a low-affinity antibody in the immune elimination of an antigen. In experimental animals, antigen-antibody complexes containing low-affinity antibodies persist in the circulation, localize on the glomerular basement membrane of the kidney and impair renal function. High-affinity complexes are more rapidly removed from the circulation and do not have any affect on renal function [Roit, 1994].

Based upon the results of these and previous experiments we may conclude that the BAChCG β-R68E molecule is a good potential candidate for the creation of the hCG-based vaccine.

Acknowledgement - Our work is supported by grant from the Ministry of Education and Science of Georgia №19

References:

Acevedo HF, Krichevsky A, Campbell-Acevedo EA, Galyon JC, Buffo MJ, Hartsock RJ. *Expression of membrane-associated human chorionic gonadotropin, its subunits and fragments by cultured human cancer cells.* Cancer, **69**, 1829-42, 1992.

Acevedo HF, Hartsock RJ. *Metastatic phenotype correlates with high expression of membrane-associated complete b-human chorionic gonadotropin in vivo.* Cancer, **78**, 2388-99, 1996.

Berger P., Sturgeon C., Bidart JM., Paus E., Gerth R., Niang M. et al. *The ISOBM TD-7. Workshop on hCG and related molecules. Towards user-oriented standardization of pregnancy and tumor diagnosis: assignment of epitopes to the three-dimensional structure of diagnostically and commercially relevant monoclonal antibodies directed against human chorionic gonadotropin and derivatives.* Tumor Biol., **23**, 1-38, 2002.

Charrel-dennis M., Terrazzini N., McBride JD, Kaye P. et al. *The hCGβ arginine[68] to glutamic acid substitution fixes the conformation of the C-terminal peptide.* Molecular Endocrinology, 2005;

Chiesa MD, Martensen PM, Simmons C, et al. *Refocusing of B-cell responses following a single amino acid substitution in an antigen.* Immunology, **103**, 172-83, 2001.

Jackson AM, Klonisch T, Lapthorn AJ, et al. *Identification and selective destruction of shared epitopes in human chorionic gonadotropin beta subunit.* J. Reprod. Immunol. **31**, 21-36, 1996.

Lapthorn AJ, Harris DC, Littlejohn A, et al. *Crystal structure of human chorionic gonadotropin.* Nature, **369**, 455-461, 1994.

Pierce J. G. & Parsons T. F. *Glycoprotein hormones: structure and function.* Annual Review of Biochemistry, **50**, 465-395, 1981.

Porakishvili N, Chiesa MD, Chikadze N, et al. *Elimination of luteinizing hormone cross-reactive epitopes from human chorionic gonadotropin.* Vaccine, **20**, 2053-9, 2002.

Roit IM. *Essential Immunology.* 8th Edn, Blackwell Scientific Publications, Oxford, 1994.

Stevens VC. *Antifertility vaccine.* In: Perlmann P, Wigzell H (eds) Handbook of experimental pharmacology. Spriger-Verlag, pp 443 – 461, 1999.

Talwar GP. *Vaccines and passive immunological approaches for the control of fertility and hormone-dependent cancers.* Immunol. Rev., **171**, 173-92, 1999.

Triozzi PL, Gochnour D, Martin EW, Aldrich W, Powell J, Kim JA, Young DC, Lombardi J. *Clinical and immunologic effects of a synthetic b-human chorionic gonadotropin vaccine.* Int. J. Oncol., **5**, 1447-53, 1994.

Wang G, Wang Y, Li CY, Gao TW, Liu YF. *Thiocyanate elution measurement of relative affinity of phage antibodies.* Sheng Wu Gong Cheng Xue Bao, **20**, 3, 429-33, 2004.

მუტანტური, რეკომბინანტული აქბβ-ს საპასუხოდ სინთეზებული ანტისხეულების ავიდობა

ჭიკაძე ნ., ჯანიკაშვილი ნ., ბურჯანაძე ლ., გოლიაძე ე., შელეგია ქ., გაჩეჩილაძე ნ., ოსაძე მ., ფორაქიშვილი ნ.

იმუნოლოგიის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 20.09.2005)

რეზიუმე

ადამიანის ქორიონული გონადოტროპინის აქბβ-ჯაჭვის რეკომბინანტულ ფორმაზე დაფუძნებული ვაქცინის პროტოტიპი BAChCGβ R68E, რომელიც შეიცავს ერთ ჩანაცვლებულ ამინომჟავას (არგინინი[68] ჩანაცვლებულია გლუტამინის მჟავით) შექმნილია იმისათვის, რათა აღძრას ეფექტური და უსაფრთხო იმუნური პასუხი ნატიური აქბ-ს წინააღმდეგ. განსაზღვრულია მუტანტური შრატის დაკავშირების ავიდობა ნატიურ აქბαβ, მუტანტურ აქბβ და აქბβ-ჯაჭვის კარბოქსი-ტერმინალურ პეპტიდთან. გამოყენებულია არაპირდაპირი იმუნოფერმენტული ანალიზი ამონიუმ-თიოციანიტის მონაწილეობით. ნაჩვენებია, რომ მუტანტური შრატები ნატიურ აქბ-ს მაღალი ავიდობით უკავშირდებიან. რაც გვაძლევს საშუალებას დავასკვნათ, რომ BAChCGβ R68E არის ანტი-აქბβ ვაქცინის უსაფრთხო და ეფექტური პროტოტიპი, რომელიც ხასიათდება იმუნოგენურობის მაღალი ხარისხით.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

EFFECT OF ANTIBIOTICS ON VITAL ACTIVITY OF BACTERIOPHAGES

ANTIA I., BALANCHIVADZE M., NATIDZE M., NATIDZE T., GABISONIA T.

Department of microbiology and Virology, Zootechnical Veterinary University of Georgia

(Received September 12, 2005

Abstract

The effect of widely used in the poultry against gastrointestinal infections antibiotics: enrophloxacin, vil-phlox and enrophlone, on staphylococcic bacteriophage, colibacteriophage and salmonellosis bacteriophage was studied. It was shown that these antibiotics at 6 and 18 hours exposure with bacteriophages practically don't have on influence on them. Their negative colonies amount before and after antibiotics action are identical. According to received data for growing therapeutic efficiency combined use of antibiotics and bacteriophages is expedient.

Key words: staphylococcic bacteriophage, colibacteriophage, salmonellosis bacteriophage, poultry.

Introduction

Gastrointestinal bacterial infections cure and prophylactics are based on the use of antibiotics. Today tetracycline, kanamycin, enrophloxacin, vil-phlox, enrophlone, TNF-600 etc. are widely used [Kisiliova, 1975; Borisenkova et al., 2002; Lagutin, 2004].

In the recent years data about adverse reaction of antibiotics are revealed. Organisms microflora have had significant changes, the amount of available microbes in the digestive tract decreased, the process of digestion was impaired, dysbacteriosis were set on. Systematic use of antibiotics has changed nature of microbes and resistant strains against antibiotics were generated.

Working out of antibiotics alternative preparations is carried out intensively. In this viewpoint the use of bacteriophages is perspective [Samadashvili, 1969; Natidze et al., 2004]. In medicine they are used widely, but in veterinary less. With the combination of bacteriophages the use of less amount of antibiotics for proper medicinal and prophylactic effect considerably eliminate development of adverse reactions. We have to elucidate the question – can antibiotic inhibit bacteriophage that should decrease its activity?

The goal of our research is to study the effect of widely used in poultry against gastrointestinal infections (staphylococcosis, colibacillosis, salmonellosis) antibiotics: enrophloxacin, vil-phlox and enrophlone, on intestinal bacteriophages - colibacteriophage, staphylococcic bacteriophage and salmonellosis bacteriophage.

Materials and Methods

Keeping standard conditions we worked out original method: antibiotics were diluted in meat-pepton broth with the ratio 1:1. At the same time in this ratio it was taken into account that contents of antibiotics per 1 ml were 100, 50 and 25 μg. 4.5 ml of these composites were brought into the flasks. In the control flask (without antibiotics) was poured 4.5 ml of 0.9% NaCl isotonic solution. To the test and control flasks ware added 0.5 ml of corresponding phage. After 10^7 titration the flasks were put in thermostat at 37^0C. After 6 and 18 hours incubation flasks composites were diluted with thratio 1:10 000. The amount of phages particles per 1 ml was determined and by received data effect of antibiotics on bacteriophages was ascertained.

Results and Discussion

Received data showed that antibiotics: enrophloxacin, vil-phlox and enrophlone, at 6 and 18 hours exposure don't influence intestinal bacteriophages constituents - colibacteriophage, staphylococcic bacteriophage and salmonellosis bacteriophage (Table 1, Table 2). The amount of phage particles in test and control flasks were practically identical.

Table 1. Indexes of antibiotics effect on bacteriophages; incubation 6 hours

#	phages	antibiotics											
		enrophloxacin (μg)				vil-phlox (μg)				enrophlone (μg)			
		25	100	50	control	25	50	100	control	25	50	100	control
1	colibacteriophage	128 n/c	126 n/c	130 n/c	127 n/c	186 n/c	182 n/c	186 n/c	188 n/c	157 n/c	156 n/c	153 n/c	159 n/c
2	salmonellosis bacteriophage	217 n/c	215 n/c	211 n/c	217 n/c	170 n/c	169 n/c	169 n/c	170 n/c	98 n/c	98 n/c	96 n/c	98 n/c
3	staphylococcic bacteriophage	176 n/c	116 n/c	175 n/c	114 n/c	207 n/c	206 n/c	203 n/c	202 n/c	113 n/c	113 n/c	110 n/c	114 n/c

n/c – negative colonies

Table 2. Indexes of antibiotics effect on bacteriophages; incubation 18 hours

#	phages	antibiotics											
		enrophloxacin (μg)				vil-phlox (μg)				enrophlone (μg)			
		25	50	100	control	25	50	100	control	25	50	100	control
1	coli-bacteriophage	130 n/c	130 n/c	127 n/c	131 n/c	184 n/c	182 n/c	182 n/c	184 n/c	161 n/c	160 n/c	158 n/c	163 n/c
2	salmonellosis bacteriophage	230 n/c	226 n/c	228 n/c	226 n/c	167 n/c	166 n/c	166 n/c	169 n/c	105 n/c	102 n/c	102 n/c	100 n/c
3	staphylococcic bacteriophage	181 n/c	180 n/c	180 n/c	183 n/c	163 n/c	162 n/c	160 n/c	163 n/c	141 n/c	139 n/c	135 n/c	142 n/c

n/c – negative colonies

Results of experiment show that after 6 and 18 hours exposure salmonellosis bacteriophage is still active with respect to enrophloxacin and the amount of negative colonies is more than 200; while exposure with enrophlone the amount of negative colonies is about 100.

Staphylococcic bacteriophage after exposure with antibiotics gives high amount of negative colonies and is the most active with respect to vil-phlox after 6 hours exposure.

Colibacteriophage reveal high indexes at 6 hours exposure with vil-phlox, which are slightly decreased at 18 hours exposure.

It is worth to mention that antibiotics concentrations have no differences. 25, 50 and 100 μg are identically effected by phages.

So, according to our data antibiotics: enrophloxacin, vil-phlox and enrophlone, at 6 and 18 hours exposure don't influence intestinal bacteriophages constituents activities, and it is reasonable to use decreased doses of antibiotics with combination of bacteriophages.

References:

Berisenkova A.N., Rojdenstvenskaia T.I. Novikova A.N. Eliseeva E.N. *Determination of enrophlone activity at avian bacterial diseases.* Veterinaria, **6**, 15, 2002.

Kisiliova M.N. *Antimicrobial and medicinal and prophylactic features of kanamycin at typhoid fever of chicken.* Abstract of PhD, Leningrad, 1975.

Lagutin N. *Some aspects of chemotherapy of infectious diseases.* Poultry Keeping, **10**, 2004.

Natidze M., Rigvava S., Toklikishvili T. *Study of medical and prophylactic effectiveness of intestinal bacteriophages at enteric fever of chicken.* Materials of the conference, Erevan, 179, 2004.

Samadashvili M.S. *Effectiveness of combined effect of specific bacteriophage and some antibiotics at enteric fever of chicken.* Materials of XVII conference GZVUII, Tbilisi, 199, 1969.

ანტიბიოტიკების გავლენა ბაქტერიოფაგის ცხოველმყოფელობაზე

ანთია ი., ბალანჩივაძე მ., ნათიძე მ., ნათიძე ტ., გაბისონია ტ.

მიკრობიოლოგიის კათედრა, საქართველოს ზოოტექნიკურ-ვეტერინარო უნივერსიტეტი

(მიღებულია 12.09.05)

რეზიუმე

შესწავლილია მეფრინველეობაში ფართოდ გამოყენებული ანტიბიოტიკების: ენროფლოქსაცინის, ვილ-ფლოქსის და ენროფლონის ზემოქმედება სტაფილოფაგზე, კოლიფაგსა და სალმონელას ფაგზე. დადგენილია, რომ აღნიშნული ანტიბიოტიკები ბაქტერიოფაგებთან 6 და 18 საათიანი კონტაქტის პირობებში პრაქტიკულად გავლენას არ ახდენს ბაქტერიოფაგზე. მათი ნეგატიური კოლონიების რაოდენობა ანტიბიოტიკების მოქმედებამდე და მის შემდეგ ერთმანეთის ტოლია. მიღებული შედეგებიდან გამომდინარე ანტიბიოტიკების და ბაქტერიოფაგების კომბინირებული გამოყენება მიზანშეწონილია, რაც ზრდის მათ სამკურნალო ეფექტურობას.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

Microbiology

DETERMINATION OF SENSITIVITY/RESISTANCE OF BACTERIA ISOLATED FROM RESPIRATORY SYSTEM

DZAGNIDZE M.[1], KERESELIDZE M.[2], KIKACHEISHVILI E.[1], KHAREBAVA SH.[2], APRIDONIDZE K.[1]

[1]*Georgian State Medical Academy*
[2]*Georgian & Swiss Diagnostic Center "CITO"*

(Received September 20, 2005)

Abstract

Sensitivity of microbes was determined by the use of ATB identification system. The studies showed that *S.aireus* isolated during respiratory tract infections was sensitive to Vankomicin, Teikoplanin, Kotrimoxazol, Pristinamicin (100%), moderately sensitive to - Fuzidin, Phosphomicin, Nitrofurantoin, Oxacillin, Tobracin, Gentamicin. and resistant to - Penicillin. *S.epidermidis* appeared sensitive to Vankomicin, Monociclin, Tobramicin, Pristinamicin, Fuzidin (100%), moderately sensitive to Oxacillin, Kanamicin, Nitrofurantoin, Phosphomicin, Kotrimoxazol, Pefloxacin; and resistant - to Erythromicin, Lincomicin. *P.mirabilis* was sensitive to Amoxicillin+Clav, Piperacillin+Tazobactam, Cefotaxim, Pefloxacin, Ciprofloxacin (100%), resistant to - Amoxicillin, Tikarcillin, Cefalotin, Cefoxitin. *K.pneumoniae* appeared sensitive to Imipinem (100%), moderately sensitive to Piperacillin+Tazobactam, Amicacinm Nethilmicin, Ciprofloxacin, Pefloxacin, Cefotaxim, Amoxicillin+Clav and resistant to - Gentamicin, Amoxicillin, Tikarcillin, Cefalotin, Cefoxitin.

Key words: ATB system, microecology, S.aureus, S.epidermidis, Proteus mirabilis, K.pneumoniae, C.albicans.

Introduction

Infections of respiratory system are the urgent problems of modern medicine. These diseases are directly connected with environmental factors. Ways of passing of infections are mainly conditioned by aerial-dust and aerosol-drop ways and are known for high contagiousness [Kiknadze & Imnadze, 1999; Polrovski & Pozdnev, 1998]. Micromorphology of respiratory system, with the practical point of view is problematic. If taking materials from the upper layers is relatively easy (nose, mouth), study of the bronchols and alveola from the lower fragments is technically difficult, and practically possible at tracheostomy. Therefore complete picture about microecology of respiratory system, due to variable factors is less studied yet. The correct laboratory diagnostics of infectious pathologies of various fragments of respiratory system is especially important. In the first place it is connected with adequate taking of the material to be studied, when the contamination with the accidental flora should be excluded, which is the principal cause of diagnostic errors. Quality and standards of nutrient media, quality of "disks" of antibiotic sensitivity and study of tinctorial properties of isolated cultures should be paid specific attention. In the Medical Diagnostic Center "Cito" the international technologies - Api and ATB

systems ("BioMerieux", France) were introduced [Clinical Bacteriology, 2001; Baquero &Moreno, 1984]. Within 2000-2004 unique material was accumulated in the Center dealing with respiratory system micro ecology. The aim of our work was to determine microbial sensitivity of upper and lower respiratory tracts to antimicrobial preparations by the use of ATB system.

Materials and Methods

We have studied 1305 bacterial cultures, 471 – from the nose, 457 -from the mouth, 184 from sputum, 194 –from trachea aspirate. Sensitivity of isolated microorganisms was studied against 36 various anti microbial preparations: Penicillin, Oxacillin, Kanamicin, Tobramicin, Gentamicin, Tetracicline, Monociclin, Erythromycin, Lincomicin, Pristinamicin, Phosphomicin, Nitrofurantoin, Pefloxacin, Rifampicin, Acide Fuzidin, Vancomicin, Teicoplanin, Kotrimoxazol, Amoxicillin, Amoxicillin+Clav, Tikarcilin, Pyperracilin+ Tazobactam, Pyperaciline, Imipenem, Cepelotin, Cepoxitin, Cepotaxim, Ceptazidim-l, Ceptazidim, Cefpirom, Tobramicin, Amikacin, Nethylmicin, Pefloxacin, Ciprofloxacin.

ATB strips are composed by 16 pair recesses. The first pair doesn't compose antibiotics and it is used as a control. 14 pair recesses contain antibiotics with common or doubled concentrations. The last ones are empty recesses for such case when adding of antibiotics is needed.

Studied bacteria were placed in distilled water or in saline, then they were brought in growth promoting medium and were inoculated in strips. After 18-24 hours of incubation the growth was visually observed. Received data were classified as sensitive, week sensitive and resistant.

Results and Discussion

S.aureus (35,06%) and *S.epidermidis* (25,5%) were isolated from the material taken from the nose. *S.aureus* turned out sensitive to Vankomicin, Teicoplanin, Cotrimoxazol, Pristinamicin, in 100%. 92% of the material was sensitive to Monociclin, 84% – to Rifampicin and Fuzidin, 76% - to Phosphomicin, Nitorfurantoin, Pefloxacin. 70% - to Lincomicin, 61% - to Oxacilin, Tobramicin, Gentamicin. Material turned out resistant to Penicilin. *S.epidermitis* turned out sensitive to Tobramicin, Monociclin, Pristinamicin, Acide fuzidin – in 100%, 88% of the material appeared sensitive to Oxacilin, Kanamicin, Pefloxacin, Rifampicin, Vankomicin, Teikoplanim, 77% - to Cotrimexazol, Nitrofurantoin, 66% - to Phosphomicin. The material was resistant to Erythromicin and Lincomicin.

S.aureus (51,3%), *S.epidenmidis* (34,4%) and *Proteus mirabilis* (14,3%) were isolated from the material taken from the mouth. *S.Aureus* was sensitive to Teicoplan, Monociclin, Vankomicin in 100%; 89% to - Nitrofurantoin, Acide fuzidin, Pristinamicin, 80% to - Kanamicin, Linkomicin, Phosphomicin, Rifampicin, 58% - to Erythromicin, Kotrimoxazol. It appeared resistant to Pefloxacin, Tetraciclin, Tobramicin, Gentamicin. *S.epidetmidis* was sensitive in 100% to Vankomicin, in 99% to Monociclin, Pristinamicin, in 76% to Tobramicin, Gentamicin, Acide fuzidin, in 70% to - Oxacillin, Rimfampicillin, Kotrimoxazol, in 64% - to Kanamicin, Tetracicllin, in 58% – to Pefloxacin, Teikoplanin; it appeared resistant to Vankomicin, Linkomicin, Erythromicin. *Proteus mirabilis* was sensitive in 100% to Amoxicilin+Clav, Piperacil+Tazobactam, Cefotaxim, Ceftazidim, Ceftazidim-1, Cefapim, Cefapirom, Tobramicin, Amicacin, Gentamicin, Nethylmicin, Pefloxsacin, Ciprofloxacin. It was resistant to Amoxicilin, Tikarcilin+Clav, Cefalotin, Cefoxitin.

Table 1. Sensitivity of bacteria isolated in 2000-2004 from respiratory system to anti microbial preparations (minimum suppressing concentration and sensitivity in percents). P < 0,001

Anti microbial preparations	mg/l	*S.aureus*	*S.epidermidis*
Penicillin	0-25	40-R	10-R
Oxsacillin	2	61-I	88-S
Kanamicin	8-16	80-S	88-S
Tobramicin	4-8	61-I	100-S
Gentamicin	4-8	61-I	76-I
Tetraciclin	4	21-R	64-I
Minociclin	4	92-S	100-S
Erythromicin	1-4	21-R	10-R
Linkomicin	2-8	70-I	10-R
Pristinamicin	2	100-S	100-S
Fosfomicin	32	36-R	66-I
Nitrofurantoin	25-100	36-R	89-S
Pefloxacin	1-4	84-S	88-S
Rifampicin	0.25-16	15-R	88-S
Acide fuzidin	2-16	15-R	100-S
Vankomicin	4	100-S	100-S
Teikoplanin	4	100-S	100-S
Kotrimoxazol	2/38-8/152	100-S	100-S

Table 2. Sensitivity of bacteria isolated in 2000-2004 from respiratory system to anti microbial preparations (minimum suppressing concentration and sensitivity in percents). P < 0,001

Anti microbial preparations	mg/l	*K.pneumoniae*	*P.mirabilis*
Amoxicillin	4-16	100-R	100-R
Amoxicillin+Clav	4/2-16/2	34- I	100-S
Tikarcillin	16	100-R	100-R
Tikarcillin+ac.Clav	16/2	84- R	100-R
Piperacillin+Tazobactam	8/4-64/4	50-S	100-S
Piperacillin	8	50-I	40-I
Imipenem	4	100-S	40-I
Cefalotin	8	100-R	100-R
Cefoxitin	8	84-R	100-R
Cefotaxim	4-32	33-I	100-S
Ceftazidim-1	1	100-R	100-S
Ceftazidim	4	100-R	100-S
Cefepim	4	100-R	100-S
Cefpirom	4	100-R	100-S
Tobramicin	4	84-R	100-S
Amikacin	8	50-S	100-S
Gentamicin	4	100-R	100-S
Nethilmicin	4	50-S	100-S
Kotrimoqsazol	2/38	100-R	50-I
Vankomicin	8	84-R	50-I
Pefloxacin	1-4	17-I	100-S
Ciprofloxacini	1-2	50-S	100-S

K.pneumoniae (50%) and *C.albicans* (50%) were isolated from the material obtained from sputum. K.pneumoniae was sensitive in 100% to Imipenem, in 50% - to Piperacilin+Tazobactam, Amikacin, Nethylmicin, Ciprofloxacinm. It turned out resistant to

Amoxicilin,Ticarcilin, Cefalotin, Ceftazidim, Ceftazidim-1, Cefepim, Cefpirom, Gentamicin, Kortimoxsazol, Tobramicin. *C.albicans* was sensitive in 100% to Econazol, Ketokonazol, in 76% to 5-Flucitozin, Nistatin; it turned out resistant to Amfotericin-B, Miconazol, Econazol, Amfotericin.

S.aureus (56,4%) and *K.pneumoniae* (43,6%) were isolated from the material obtained from trachea aspirate. *K.pneumoniae* was sensitive in 100% to Imipinem, Amoxicilin, in 90% – to Cefoxitin, Gentamicin, Amikacin, in 80% to - Ticarcilion+Clav, Pipecilin, Pefloxacin, in 70% to - cefoxitin, Nethylmicin, Kotrimoxczzol, in 60% - to Amoxicilin+Clav; it appeared resistant to Cefotaxim, Ceftazidim, Ceftazidim-1, Cefepim, Cefprom. *S.aureus* was sensitive in 100% toImipenem, Fosfomicin; it appeared resistant to Tikarcilin, Ceftazidim, Piperacilin, Tobramicin, Amicacin, Gentamicin, Kanamicin, Kotrimoxazol.

The results of our studies for the assessment of sensitivity/resistance of micro flora of upper and lower respiratory ways to anti microbial preparations are very important, with the ecogenic, as well as practical point of view.

References:

Baquero F., Moreno F. *The microbes*. Microbiol. Lett., **23**, 117-124, 1984.

Kereselidze M. *Clinical Bacteriology*. "EURO", Ed. G. Katsitadze, Tbilisi, 16-21, 2001.

Kiknadze G., Imnadze P. *Control of some infections in hospitals* (Methodological Instructions), Tbilisi, 1999.

Polrovski V.P., Pozdnev O.K. *Medical Microbiology*, M., "Nauka", 590-595, 1998.

სასუნთქი სისტემიდან იზოლირებული ბაქტერიების მგრძნობელობა/რეზისტენტობის განსაზღვრა

ძაგნიძე მ.[1], კერესელიძე მ.[2], კიკაჩეიშვილი ე.[1], ხარებავა შ.[2], აფრიდონიძე კ.[1]

[1]საქართველოს სახელმწიფო სამედიცინო აკადემია
[2]ქართულ-შვეიცარული დიაგნოსტიკური ცენტრი "ციტო"

(მიღებულია 20.09.05)

რეზიუმე

განსაზღვრულია მიკრობთა მგრძნობელობა საიდენტიფიკაციო ATB სისტემების გამოყენებით. დადგენილია, რომ სასუნთქი სისტემის ინფექციების დროს იზოლირებული *S.aureus* მგრძნობიარე აღმოჩნდა ვანკომიცინის, ტეიკოპლანინის, კოტრიმოქსაზოლის, პრისტინამიცინის მიმართ (100%), საშუალოდ

მგრძნობიარე - ფუზიდინის, ფოსფომიცინის, ნიტროფურანტოინის, ოქსაცილინის, ტობრამიცინის, გენტამიცინის მიმართ, პენიცილინის მიმართ - რეზისტენტული. *S.epidermidis* მგრძნობიარე აღმოჩნდა ვანკომიცინის, მონოციკლინის, ტობრამიცინის, პრისტინამიცინის, ფუზიდინის მიმართ (100%), საშუალოდ მგრძნობიარე - ოქსაცილინის, კანამიცინის, ნიტროფურანტოინის, ფოსფომიცინის, კოტრიმოქსაზოლის, პეფლოქსაცინის მიმართ; ერითრომიცინის, ლინკომიცინის მიმართ რეზისტენტული. *P.mirabilis* მგრძნობიარე აღმოჩნდა ამოქსიცილინ+კლავის, პიპერაცილინ.+ტაზობაქტამის, ცეფოტაქსიმის, პეფლოქსაცინის, ციპროფლოქსაცინის მიმართ (100%), ამოქსიცილინის, ტიკარცილინის, ცეფალოტინის, ცეფოქსიტინის მიმართ - რეზისტენტული. *K.pneumoniae* მგრძნობიარე აღმოჩნდა იმიპენემის მიმართ (100%), საშუალოდ მგრძნობიარე პიპერაცილინ+ტაზობაქტამის, ამიკაცინის, ნეთილმიცინის, ციპროფლოქსაცინის, პეფლოქსაცინის, ცეფოტაქსიმის, ამოქსიცილინ+კლავის მიმართ; რეზისტენტული - გენტამიცინის, ამოქსიცილინის, ტიკარცილინის, ცეფალოტინის, ცეფოქსიტინის, მიმართ.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

CHANGES OF *ACTINOSPORANGIUM VIOLACEAE* CELL WALL ULTRASTRUCTURE DURING CULTURE DEVELOPMENT

KOTIA N., LOMTATIDZE Z., SHENGELIA M.

Chair of Microbiology and Virology, Iv Javakhishvili Tbilisi State University

(Received June 23, 2005)

Abstract

Change in chemical content of cell wall of *Actinosporangium violaceae* has been studied in the dynamics of culture growth. It has been established that according to growth phases a mass of cell wall of actynomycetes changes. It is maximal in logarithmic phase of growth and is minimal in stationary phase. An insignificant increase of cell wall mass was observed in the phase of dying. Qualitative changes of its constituents - peptidoglycane, teihoic acid and their monomers, amino acids and monosaccharides were not found. But quantitative ratios of separate components were changed.

Key words: peptidoglycane, teihoic acid, amino acids, monosaccharides

Introduction

Among diagnostic features used in the systematization of actynomycetes, ratio of chemical composition of cell wall appears to be very important, in particular lipid, amino acidic and monosaccharide content [Gauze et al., 1983; Krasilnikov, 1970]. Peptidoglycane and teihoic acid appearing main components of procaryotic cell wall are complex compositions, containing amino acids and monosaccharides and they may be used in the systematization of actynomycetes as one of the additional chemotaxonomic signs.

Material and methods

Actinosporangium violaceae obtained from the collection of microorganisms of Microbiology Department of N. Ketskhoveli Institute of Biology, Georgian Academy of Sciences was used as the subject of the investigation.

The culture was grown on Krasilnikov synthetic medium. Morphology was studied using methods of B. Krasilnikov and V. Kuznetsov [Gauze et al., 1983; Krasilnikov, 1970]. Cell wall was obtained according to L.N. Robson and Baddiley [Gerkhardt, 1984].

Peptidoglycane and teihoic acid were obtained using the method of Streshinskaia [Gerkhardt, 1984; Streshinskaia, et al., 1996]. Qualitative and quantitative analysis of amino acids and monosaccharides was carried out using thin-layer chromatography and densitometry method [Practical work of biochemistry, 2001].

Actinosporangium violaceae (synthetic medium of Krasilnikov) creates colonies with rough surfaces having 0,5-1 mm diameter, as well as with weakly developed grayish-violet coloured aerial mycelium. Substrate mycelium is of brown colour. Dynamics of cultivation is

determined; complete growth of culture is 168 h, logarithmic phase – 0-48 h, exponential phase – 48-100 h, stationary phase – 100-144 h. Then 144 h later the phase of dying occurs (Fig.1).

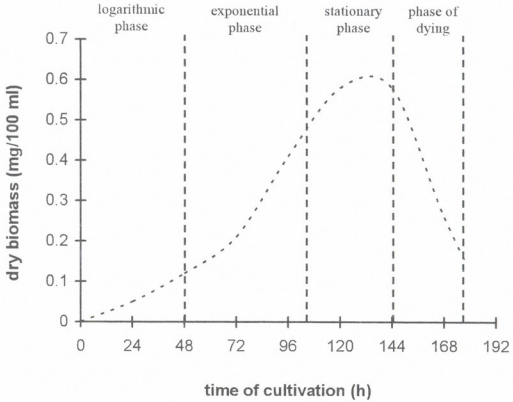


Fig. 1. Dynamics of *Actinosporangium violaceae* growth

As is shown from the scheme, cell wall of *Actinosporangium violaceae* consists 14-17% of dry biomass. During growth and development of the culture, its mass changes. It is maximal in logarithmic phase and minimal – in stationary phase. In the phase of dying cell wall mass insignificantly increases. The amount of main component determining cell wall mass – peptide-glycane varies within the limits of 55-65%, while the amount of teihoic acid – approximately within the limits of 19-29% (in 1 g of dry cell wall). Change in peptidoglycane mass depends on the change of cell wall mass. It is maximal in logarithmic phase and minimal – in stationary phase. The mass of teihoic acid reduces from logarithmic to the phase of dying.

Table 1. Quantitative changes in *Actinosporangium violaceae* cell wall and its main components in dynamics of culture growth

Phases of growth	Mass of cell wall and its components, mg/g		
	cell wall in 1 g of dry biomass	peptide-glycane in 1 g of cell wall	teihoic acid in 1 g of cell wall
Logarithmic	170,8	655,15	291,8
Exponential	167,15	597,8	267,3
Stationary	148,7	555,5	215,44
Phase of dying	150,31	557,8	198,79

Quantitative and qualitative analysis of *Actinosporangium violaceae* peptidoglycane was performed. The results are given in Table 2.

As is shown from Table 2, main skeleton of *Actinosporangium violaceae* cell wall peptidoglycane appears to be a glycane fraction, consisting 59-63%, while peptide fraction consists 25-30%. The amount of monosaccharides is maximal in logarithmic phase, minimal – in dying phase of . The amount of amino acids is maximal in logarithmic phase, minimal – in stationary phase. In the phase of dying in peptide fraction of peptidoglycane the amount of amino acids increases.

Table 2. Quantitative ratio of peptidoglycane components of *Actinosporangium violaceae* (%)

Phases of growth	Mass of chemical components in 1 g of peptide-glycane (%)	
	Monosaccharides	Amino acids
Logarithmic	63,8	30,08
Exponential	62,5	28,57
Stationary	61,0	25,08
Phase of dying	59,3	21,01

Results of qualitative analysis of peptidoglycane are given in Fig. 2 and 3. In Fig. 2 it was shown that quantitative ratio of amino acids in peptidoglycane of investigated culture changes according to growth phases.

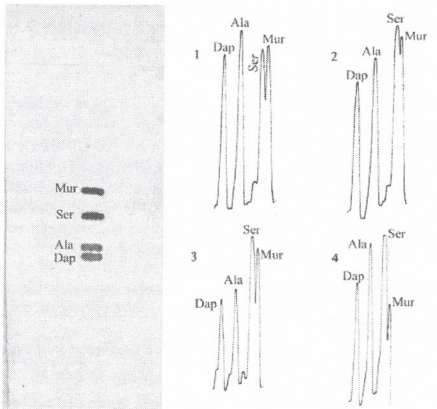


Fig. 2. Amino acidic content of peptidoglycane of *Actinosporangium violaceae*: diaminopimelic acid; alanine; serine, murami acid. Densitograms: 1. Logarithmic phase. 2. Exponential phase. 3. Stationary phase. 4. Phase of dying

Glycane fraction of peptidoglycane is presented by three monosaccharides, quantitative ratio of which appears to be changeable in dynamics of culture growth (Fig. 3).

Analysis of our investigations has shown that glycerin-teihoic acid of cell wall of *Actinosporangium violaceae* appears to be quantitatively changeable value and depends on conditions of development of the culture.

As is shown in Fig. 4,.5, alanine, glycine and lysine are identified in the content of teihoic acid of *Actinosporangium violaceae,* while from monosaccharides - glucosamine, galactose.

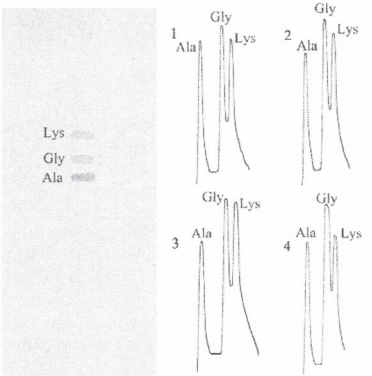


Fig. 3. Amino acidic content of teihoic acid of *Actinosporangium violaceae*: alanine, glycine, lysine. Densitograms: 1. Logarithmic phase. 2. Exponential phase. 3. Stationary phase. 4. Phase of dying .

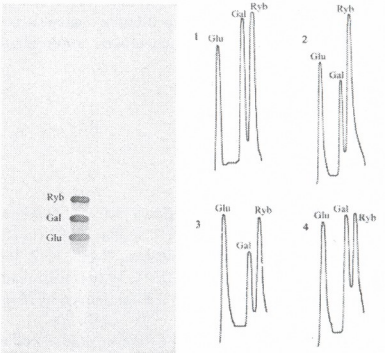


Fig. 4. Monosaccharide content of *Actinosporangium violaceae* peptidoglycane. Glucosamine, galactosamine, ribose. Densitograms: 1. Logarithmic phase. 2. Exponential phase. 3. Stationary phase. 4. Phase of dying

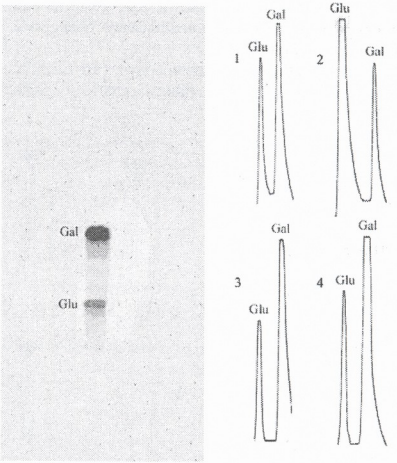


Fig. 5. Amino acidic content of teihoic acid of *Actinosporangium violaceae:* glucosamine, galactose. Densitograms: 1. Logarithmic phase. 2. Exponential phase. 3. Stationary phase. 4. Phase of dying

So, *Actinosporangium violaceae* has a cell wall containing peptidoglycane and glycerin-teihoic acid that is characteristic for typical gram-positive procaryotes. Quantitative ratio of monomers of peptidoglycane and teihoic acid - amino acids and monosaccharides - changes according to growth phases, but change in their qualitative content is not observed Proceeding from this, it may be used as one of the additional chemotaxonomic signs in the systematisation of actynomycetes.

References:

Gauze G.F., Preobrazhenskaia R.P., Sveshnikova M.A., Terekhova L.P., Maximova R.S. *Determinant of actynomycetes.* Moskva, "Nauka", 1983 (in Russian).

Gerkhardt F. *Methods of general bacteriology.* Moskva, "Mir", v. **2,** 1984 (in Russian).

Krasilnikov N.A. *Ray fungi.* Moskva, "Nauka", 25-37, 1970 (in Russian).

Practical work of biochemistry (Ed. N.P. Elinov). Publ. House of Moscow State University, 2001 (in Russian).

Streshinskaia G.M., Kozlova H.I., Evtushenko L.I. *Teihoic acid of cell wall of Nocardiopsis B.K.M. Ac. 1451 A.* Biochemistry, **61**, 2, 28-32, 1996 (in Russian).

ctinosporangium violaceae-ს უჯრედის კედლის ულტრასტრუქტურის ცვლილება კულტურის ზრდა-განვითარების პიოროგებში

კოტია ნ., ლომთათიძე ზ., შენგელია მ.

მიკრობიოლოგიისა და ვირუსოლოგიის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტის

(მიღებულია 23.06.05)

რეზიუმე

შესწავლილია *Actinosporangium violaceae* –ს უჯრედის კედლის ქიმიური შემადგენლობის ცვლილება კულტურის ზრდის დინამიკაში. დადგენილია, რომ ზრდის ფაზების მიხედვით იცვლება აქტინომიცეტის უჯრედის კედლის მასა. იგი მაქსიმალურია ზრდის ლოგარითმულ ფაზაში, მინიმალური – სტაციონარულ ფაზაში. კვდომის ფაზაში შეიმჩნევა უჯრედის კედლის მასის უმნიშვნელო მატება. მის შემადგენლობაში შემავალი პეპტიდოგლიკანის, თეიხოის მჟავისა და მათი მონომერების, ამინომჟავების და მონოსაქარიდების თვისობრივი ცვლილება არ აღინიშნება, იცვლება ცალკეული კომპონენტების რაოდენობრივი თანაფარდობა.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

ISOLATION AND IDENTIFICATION OF MICROSCOPIC FUNGI FROM SALTY SOILS OF KAKHETI (ALAZANI) VALLEY

LASKHISHVILI M., ZAKHARIASHVILI N., KUTATELADZE L.*, ALEKSIDZE T., DAUSHVILI L., KVESITADZE E.

S. Durmishidze Institute of Biochemistry and Biotechnology of the Georgian Academy of Sciences. David Agmashenebeli Alley 10km, 0059 Tbilisi, Georgia
**E-mail: kutateladzel@geomail.ge*

(Received June 27, 2005)

Abstract

44 different species of microscopic fungi have been isolated and identified to genus from low, middle and high salty soils of the left bank of river Alazani (Kakheti valley, Georgia), for the purpose to reveal halophils. Belonging to Ascomycetes, Zygomycetes and Deiteromycetes classes 8 genera were identified, and the frequency of their distribution have been determined. It was manifested that in all types of Kakheti valley soils genera *Aspergillus* and *Penicillium* are widely spread, representing the first range dominants of this region. Microflora of soils with different salinization was compared and the influence of salinization on distribution regularities of particular genus has been demonstrated. 12 weak, 12 middle and 20 extreme halophils were revealed.

Key words: pure culture, halophils, aspergillus, penicillium

Introduction

The special group of extremophils – halophilic microorganisms are adapted to exist at high concentrations of salt, due to mighty antioxidative and other effective systems [Vladimirov, 1998]. Resistance against the damaging agents, the unique chemical composition and lack of morbidness made possible to use halophils as producers of biologically active substances [Gavicchioli & Thomas, 2003]. Discovery of still unknown substances-adaptogens is supposed to be made among those matters, which are responsible for the adaptation to critical environmental conditions of extreme halophils [Gai, et al., 1998].

The halophilic collections of the world are presented mainly by the bacteria and so, searching of halophils among the microscopic fungi is of great interest. From this point of view, attention must be paid to the salty soils of Georgia, where all conditions for halophils' developing are presented. At the same time the micro flora of these saline lands is practically not studied.

The territory between hills and river bank in the south-east part of the right bank of Alazani is rich of salty soils. At this territory soils of different salinity are replaced by solonez soils [Sabashvili G., 1965].

Soil samples were taken from geographical distant saline-lands of Kakheti plane: 1) from the middle, elevated place of low-land and Alazani river bank (high salinity); 2) from saline-solonez lands of the surroundings of villages Chatma, Badiauri and Lakbe (middle salinity); and 3)

from the peripheral places of salinated soils of the plane (low salinity). The aim of the work was to find different groups of halophilic micromycetes among the micro flora of the diverse quality salinated soils.

Materials and Methods

10 g of averaged soil samples were picked from geographical distant saline territories of the left bank of river Alazani, for the purpose to reveal halophils [Fomin G., et al., 2001].

Micromycetes were removed at nutrient mediums with different composition. Most abundant and diverse micro flora was characteristic for the universal (with agar) nutrient medium, where all isolated microscopic fungi developed well. Therefore the mentioned nutrient medium in further experiments was considered to be reasonable.

To obtain the homogenous suspension, containing microorganisms as separately and freely moving cells, the samples were previously treated, using the method of soil aggregates dispersing [Zvyagintsev, 1980]. Treated material was sowed on a sterile Petri glasses by Waksman's method of soil dilution [Waksman, 1916] and direct sowing method [Warcup, 1950]. For this purpose suspensions, with following dilution were prepared: 10^1, 10^2, 10^3 and 10^4.

The microscopic fungi were isolated on the following nutrient mediums: (g/l) 1) The universal medium - 0.5l 7°B wort, 0.5l tap water, 20g agar, pH – 5.5-6.0. 2) Chapek's acidified medium (for inhibition of bacteria) – $NaNO_3$-9.1, KH_2PO_4-1.0, $MgSO_4$- 0.5, KCl-0.5, $FeSO_4$-0.02, glucose-40.0, agar-20.0, pH-3.5 – 4.2. 3) Selective nutrient medium – $NaNO_3$-3.2, KH_2PO_4-2.0, $MgSO_4$-0.5, yeast extract-10.0, microcrystal cellulose-1%, agar-20.0, pH-5.5 – 6.0. 4) Chapek's modified medium – $NaNO_3$-9.1, KH_2PO_4-1.0, $MgSO_4$-0.5, KCl-0.5, $FeSO_4$-0.02, starch-20.0, agar-20.0, pH-5.5 – 6.0. 5) Chapek-Dox's nutrient medium – $NaNO_3$-2.0, KH_2PO_4-1.0, $MgSO_4 \times 7H_2O$-0.5, KCl-0.5, $FeSO_4 \times H_2O$-0.02, sucrose-30.0, agar-20.0. Nutrient mediums were sterilized under 0.6 atm for 45 min, in autoclaves.

Incubation of the microscopic fungi, obtained from the soil samples was performed at 30°C in thermostat. Plate cultures were observed on the 3rd, 5th, 7th and 10th days. On the 10th day the separate colonies of Petri cultures were sowed once more and cultivated at 30°C for 10 days. If the pure culture developed after this procedure, the piece of mycelium or a small portion of spores from plate culture were placed in the test-tube containing sterilized universal medium. Test-tubes were placed into the thermostat at the same temperature as for releasing pure culture, for 10 days.

Using the soil dilution method for purifying the microscopic fungi, the frequency of detection of particular species was determined following the ratio: $FD = \frac{IS}{TS}$ (FD – frequency of species detection, IS – amount of the investigated sample, TS – total amount of the sample).

During identification, using the light microscopy, morphological and cultural peculiarities of the cultures were taken into account. Also different guides were used [Pidoplichko N., 1967; Bilaiy V., 1988; Malloch D., 1981]. At first plate cultures were generally observed at a small magnification, after - the preparations were made. In some cases the dry optical system microscopy was used.

To establish the quality of halophily the cultures were grown at selected universal nutrient medium (with agar), adding different concentrations of NaCl (1.0-3.5M). Intensity of growth of micromycetes was evaluated following the 3 mark system. Those microscopic fungi, which could not develop at more than1.5M concentrations of NaCl, were considered as weak halophils. Cultures growing at 1.0 – 3M concentrations of NaCl, with optimal level of growth at 2M of NaCl, were regarded as moderate halophils. Micromycetes, growing at NaCl medium ranging from 1.0 up to 4M, with optimal growth at 3M NaCl, were considered as extreme frontier halophils.

Results and discussion

During experimental work 44 different species of microscopic fungi has been isolated and identified till genus. Among them 20 were from high salinated soils, 12 - from middle and low salinated ones. In Table 1 genera of microscopic fungi, discovered in saline lands of Alazani plane and frequency of their distribution are demonstrated. It is clear that this region of Kakheti soils is rich of genera from Deiteromycetec class. Four genera of the mentioned class were found here: *Trichoderma, fusarium, Cladosporium* and *Allescheria*. But the frequency of their distribution was significantly lower compared with the representatives of Ascomycetes class. The microflora of Alazani valley is poor with representatives of Zygomycetes class. The only genus of this class – *Mucor* was identified at the middle, elevated place of the plane (Table 2).

Different quality of salination of plane soils significantly affects the composition of micro flora of Alazani saline-lands (Tables 2, 3). Genera *Aspergillus* and *Penicillium* are widely spread in all types of soils (high, middle and low saline) of this region.

Table 1. Microscopic fungi distributed in saline soils of Kakheti (Alazani) valley

Microscopic fungi			Frequency of distribution (%)
Genus		Class	
1.	*Aspergillus*	Ascomycetes	32.08
2.	*Penicillium*		29.27
3.	*Chaetomium*		9.1
4.	*Trichoderma*	Deoteromycetes	6.8
5.	*Fusarium*		9.1
6.	*Cladosporium*		4.55
7.	*Allescheria*		4.55
8.	*Mucor*	Zygomycetes	4.55

Genus *Aspergillus* is especially distributed in elevated middle part of the plane. It must be mentioned that this genus is the first range dominant through the whole Alazani valley, except the middle saline soils, where the absolute dominant is *Penicillium* (Tables 2, 3). The third representative of Ascomycetes class – genus *Chaetomium*, is characteristic only for high saline soils (the bank of Alazani and middle, elevated part of the plane) (Table 2). It deserves to mention that geographical distant soils differ by their specific genus, e.g. genus *Trichoderma* is spread in very salinated zone, *Allescheria* – in low salinated, while *Cladosporium* and *Mucor* are met in soils with middle salinity (Tables 2,3).

Table 2. Microscopic fungi distributed in high salinated soils of Alazani valley

Mycromycete		Frequency of distribution (%)	
Genus	Class	River bank	Middle place of valley
1.Aspergillus	Ascomycetes	25.00	50
2.Penicillium		16.67	25
3.Chaetomium		25.00	12.50
4.Trichoderma	Deiteromycetes	16.67	12.50
5.Fusarium		16.67	-

To determine the extent of micromycetes halophility they were grown at agar nutrient mediums with different concentrations of NaCl.

Table 3. Microscopic fungi distributed in low and middle saline soils of Alazani valley

Microscopic fungi		Frequency of distribution (%)	
Genus	Class	Low salination	Middle salination
1.Aspergillus	Ascomycetes	33.33	16.67
2.Penicillium		16.67	50.00
3.Fusarium		33.33	-
4.Cladosporium	Deoteromycetes	-	16.67
5.Allescheria		16.67	-
6.Mucor	Zygomycetes	-	16.67

20 extreme, 12 middle and 12 weak halophils were revealed. Extreme halophils were obtained from high saline zone (river bank and middle of the plane). The moderate halophils were removed from middle saline soils (saline-lands and solonetzs), while weak halophils represented the micoflora of low saline soils.

References:

Bilay V. I., Koval E. Z. *Aspergillii.* Kiev, "Naukova dumka", 1988 (in Russian).

Fomin G. S. , Fomin A. G. *The soil-control, quality and ecological security according to the international standards.* Moscow, 2001 (in Russian).

Gai F., Hasson K. C., et al. *Chemical dynamics in proteins: thr photoizomerization of retinal in bacterio rodopsin.* Science. **279**, 1886-1891, 1998.

Gavicchioli R., and Thomas T. *Extremophiles.* In: The Encyclopedia of Microbiology. Acad. Press, San Diego. 2003.

Malloch D., Moulds T. *Micromycetes, their isolation, cultivation and identification.* University of Toronto-press, Toronto-Buffalo-London, 1981.

Pidoplichko N. M., Milko A. A. *Atlas of mucoral fungi.* Kiev, 1971(in Russian).

Sabashvili G. *Soils of Georgia.* Tbilisi, "Metsniereba", 1965 (in Georgian).

Vladimirov I. A. *Free radicals and antioxidants.* Proceedings of Russian Med. Acad., **7,** 43-51, 1998 (in Russian).

Waksman S. A., Severson G. M., Goering K. I. *Fungi and their activities.* Science, **2,** 1, 103-105, 1916.

Warcup I. H. *The soil-plate method for isolation of fungi from soil.* Nature, **166**, 4502, 117-119, 1950.

Zvyagintsev D. G. et al. *Methods of soil microbiology and biochemistry.* Moscow, 1980. 12p.(in Russian).

კახეთის (ალაზნის) ვაკის დამლაშებული ნიადაგების მიკროსკოპული სიკოების გამოყოფა და იდენტიფიკაცია

ლასხიშვილი მ., ზაქარიაშვილი ნ., ქუთათელაძე ლ., ალექსიძე თ.
დაუშვილი ლ., კვესიტაძე ე.

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

(მიღებულია 27.06. 2005)

რეზიუმე

ჰალოფილების ძიების მიზნით, ალაზნის ვაკის მარჯვენა ნაპირის სუსტი, საშუალო და ძლიერი დამლაშების ნიადაგებიდან გამოყოფილია და გვარამდე იდენტიფიცირებულია 44 განსხვავებული სახეობის მიკროსკოპული სოკო. გარკვეულია 8 გვარი, რომლებიც მიეკუთვნებიან Ascomycetes, Zygomycetes და Deiteromycetes კლასებს. დადგენილია ალაზნის ვაკის მარილიან ნიადაგებში გავრცელებული მიკროსკოპული სოკოების გვარები და განსაზღვრულია მათი შეხვედრის სიხშირე. ნაჩვენებია, რომ კახეთის ველის ყველა ტიპის ნიადაგში ფართოდაა გავრცელებული *Aspergillus* –ისა და *Penicillium*-ის გვარები, რომლებიც ამ რეგიონის პირველი რანგის დომინანტებია. შედარებულია განსხვავებული ხარისხის დამლაშების ნიადაგების მიკოფლორა და ნაჩვენებია დამლაშების გავლენა ცალკეული გვარის გავრცელების კანონზომიერებაზე. გამოვლენილია 12 სუსტი, 12 ზომიერი და 20 ზღვრულად ექსტრემალური ჰალოფილი.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

PRELIMINARY DATA ON THE INSECTIVORE SKELETON (SORICIDAE) FROM THE LATE MIOCENE OF SOUTHERN GEORGIA, CAUCASUS

VANISHVILI N.

L. Davitashvili Institute of Paleobiology, Georgian Academy of Science, Niagvari str. 4, Tbilisi 0108, Georgia. E-mail: vanishvili@mail.ru

(Received September 12, 2005)

ABSTRACT

Description of the complete skeleton of shrew from the Late Miocenian (Pontian) deposits of the South Georgia (Kisatibi locality), is given. It is determined as *Sorex* cf. *minutus*. This is one of the oldest representative of this species, which points out that this species may be arose on the territory of Eastern Paratethyan region.

Key words: Insectivore, Sorex, Miocene, Paratethys, Georgia, Caucasus.

Introduction

Some years ago, a near-complete skeleton of a fossil shrew was discovered in Miocene (Pontian) diatomite deposits near Kisatibi village (Akhaltsikhe region, southern Georgia, Caucasus). The fossil vertebrates preserved in these deposits have been studied by many scientists since the end of the nineteenth century [Abich, 1882; Bogachev, 1927, 1938; Gabunia, 1955, 1959; Gabunia and Lazarashvili, 1962; Gabelaia, 1976; Wangenheim, et al., 1989].

The remains of *Hipparion* described by Gabunia [Wangenheim, et al., 1989] from the Kisatibi deposits appear to be middle or late Turolian representatives of this genus from the poorly developed preorbital fossa and the weakly plicated masticatory surface of the upper molars. Paleomagnetic and paleobotanic data from this location [Wangenheim, et al., 1989; Baranov, 1959] confirm its pontian (MN 13) age.

In the Caucasus, fossil shrew remains have not yet been found, except for one incisor (I1) from Dmanisi [Vekua, 1995]. In general, shrew fossils are very rare because of the animal's small size and fragile bones. For this reason, the paleobiological history of this group of mammals is not well known.

Systematic Paleontology

Order: Isectivora Bowdich, 1821
Family: Soricidae Grey, 1821
Subfamily: Soricinae Murray, 1866
Tribe: Soricini Fisher, 1817
Genus: *Sorex* L., 1758
Sorex cf. *Minutus* L., 1766

Distribution - To date, the oldest remains of *S. minutus* are those from the central European Ruscinian (MN 14) localities. Today *Sorex minutus* has a nearly pan-Eurasian distribution.

Material - A complete skeleton without mandible. Collection of the L. Davitashvili Institute of Paleobiology (IPB), specimen no. ki-1(Fig.1).

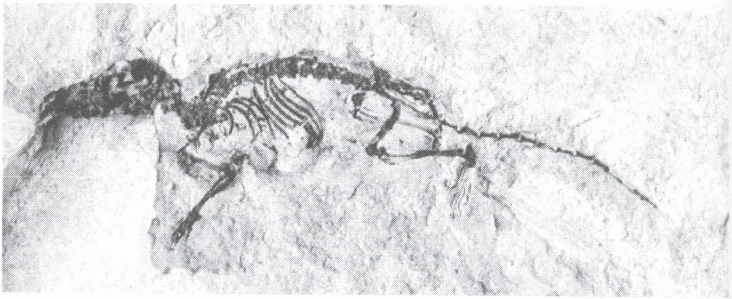


Fig. 1. The piece of diatomite containing the remains of the Kisatibi fossil shrew.

Geological age and location - Late Miocene (MN 13). Environs of Kisatibi village, Akhaltsikhe region, Georgia, Caucasus. Diatomite deposits, of a former freshwater basin.

Description - The skeleton lies on its right side, and all bones are in their natural anatomical articulation. Unfortunately, the diagnostic characteristics of the skull are not well preserved: only an imprint of I1 and "W" like cobs of molars are left. The snout is narrow and somewhat elongated. The limbs are slightly bent, and the right forelimb is completely preserved, whilst the left one is not preserved at all. The right hind limb is represented only by the distal part of the femur and by the tibia-fibula. The tail is longer then half the body and consists of 15 vertebrae.

Results and Discussion

The morphological characteristics of this specimen, i.e. its small body size, and narrow slightly elongated snout, indicate that it is most likely a member of the tribe Soricini. This tribe contains three genera: *Microsorex* – known only from North America (Pleistocene-recent), *Blarinella* – distributed throughout Europe, South China and North Burma (Miocene-recent), and *Sorex* – of nearly pan-Eurasian distribution (Miocene-recent). The following complex of morphological characteristics indicate its affinities to the genus *Sorex*: the tail is longer then half the body, the limbs are slim and long (typical of *Sorex*), and the manus and pes are relatively short [Gureev, 1971].

Among the representatives of the genus *Sorex*, the shrew from Kisatibi has several features in common with *S. minutus*. Specifically these include: (1) a very high brain case (in *S. minutus* the brain case is twice as high as its rostral part on the level of P4 [Gureev, 1971]; and (2)

a comparatively long tail (the skeleton measurements of the Kisatibi shrew are closest to *S. minutus* – see Table 1).

Based on these skeletal characteristics, we assign the Kisatibi shrew to *S.* cf. *minutus*. A more definitive identification is precluded by the lack of suitable diagnostic features (i.e. the complete lack of teeth). The presence of *S. minutus* during the Late Miocene would indicate the existence of forest-steppe landscapes in southern Georgia at that time.

The oldest fossil remains of *S. minutus*, according to the "Neogene of the old World" database (www.helsinki.fi/science/now), are known from central European localities: Podlesice (Poland MN 14) and Dranic 0 (Romania MN 14 – 15). Consequently, the occurrence of *S.* cf. *minutus* in Kisatibi (Late Miocene time MN 13) could be the oldest remains of *S. minutus* thus far discovered in the world. If so, this could indicate that the origin of this species may be took place in the Eastern Paratethyan region.

Acknowledgments

I express my sincere gratitude to the following persons for their help during preparation of this paper: G. Mchedlize, A. Vekua (L. Davitashvili Institute of Paleobiology. Tbilisi, Georgia). J. Agusti (M. Crusafont Institute of Paleontology, Sabadell, Spain). M. Bukhsianidze (Department of Geology and Paleontology, National Museum of Georgia. Tbilisi, Georgia).

Table 1. Main measurements (lengths unless otherwise stated) of some representatives of the genus *Sorex* (in mm). A: *S.* cf. *minutus* from Kisatibi; B: *S. minutus*; C: *S. raddei*; D: *S. araneus*; E: *S. isodon*; F: *S. minutisimus* (D, E, and F Are taken from Gureev, 1971; Yudin, 1971).

	A	B	C	D	E	F
Skull	17.5	15–16.5	20–21	18–21.7	18–20	12–14
Facial part	9.5	8.5	11.7	4.6–5.7	4–5.8	2–3.6
Cranial part	8	7.5	10	10–11.7	10–12	6.7–8
Skull (height)	7.5	4.5	6	5–6.6	5.6–7	3–4.5
Humerus	7	6.8	8	—	—	—
Antebrachium	8	7.9	11	—	—	—
Femur	7.8	8	9	—	—	—
Tibia-fibula	11.5	11	15	—	—	—
Manus	5.8	6	7	—	—	—
Pes	10	10.5	13.5	12–14	12–15	12–15
Pelvis	10	10.4	14	—	—	—
Tail	38	35–40	42.5	33–49	39–5	17–33
Vertebral						
column						
without tail	40	40	37	—	—	—

References:

Abich, H. *Geologische Forschungen in den Kaukasischen Ländern*. Westhalfte, Wien, 478pp., 1882.

Baranov, V. I. *Stages in the development of flora and vegetation during the Tertiary Period on the territory of USSR*. "Vishaia skola" Press, Moscow, 364pp., 1959 (in Russian).

Bogachev, V. V. *Fauna of the diatomitian deposits of the Akhaltsikhe basin.* Izv. Azerb. Gos. Universiteta **6**, 121–133, 1927 (in Russian).
Bogachev, V. V. *Fauna of The diatomite Pliocene deposits of the Transcaucasus.* Trudi Azerb. filiala Akademii Nauk, Geol. seria, **19**, 61–89, 1938 (in[Russian).
Gabelaia, C. D. *Fishes of The Pliocene deposits of Georgia.* "Metsniereba" Press. Tbilisi, 112pp., 1976 (in Russian).
Gabunia, L. K. *The Discovery of Hipparion remains in the Kisatibi diatomite.* Dokl. Acad.Nauk. SSSR, **102**, 5, 997–998, 1955 (in Russian).
Gabunia, L. K. *The history of Hipparions.* Publishing House of the USSR Academy of Science, Moscow, 565pp., 1959 (in Russian).
Gabunia, L. K., and T. A. Lazarashvili. *New data on the geological age of tufogenous deposits of southern Georgia.* Soobshenie Acad.Nauk. Georgian SSR, **26**, 1, 53–55, 1962 (in Russian).
Gureev, A. A. *Shrews (Soricidae) fauna of the world.* "Nauka", Leningrad, 242pp., 1971 (in Russian).
Wangenheim, E. A., Gabunia L. K., Pevzner M. A., Ciskarishvili G. V. *Stratigraphic position of the Localities of Hipparion fauna In the Transcaucasus in the light of magnetostratigraphy.* Izv. Acad Nauk. SSSR Geol. seria, **8**, 70–77, 1989 (in Russia).
Vekua, A. K. *Dir Wirbeltier fauna des Villafranchium von Dmanisi und ihre biostratigraphische Bedeutnung.* Jahrbuch des Romisch-Germanischen Zentral-Muzeums **42**, 77-180, 1995.
Yudin, B. S. *The Insectivorous Mammals of Siberia.* "Nauka", Nowosibirsk, 168pp., 1971 (in Russian).

წინასწარი მონაცემები გვიანმიოცენური მწერიჭამიის (SORICIDAE) შესახებ სამხრეთ საქართველოდან

ვანიშვილი ნ.

ლ. დავითაშვილის სახელობის პალეობიოლოგიის ინსტიტუტი, საქართველოს მეცნიერებათა აკადემია, ნიაღვრის ქ. 4, თბილისი 0108, საქართველო.

(მიღებულია 12.09.2005)

რეზიუმე

მოცემულია სამხრეთ საქართველოს გვიანმიოცენურ ნალექებში (სოფელი ქისათიბი) აღმოჩენილი ბიგის სრული ჩონჩხის აღწერა. იგი პირობითად განისაზღვრა როგორც *Sorex* cf. *minutus*. სამხრეთ საქართველოს გვიანმიოცენურ (პონტურ) ნალექებში *Sorex* cf. *minutus*-ის აღმოჩენა მიგვანიშნებს, რომ შესაძლოა ეს სახეობა სათავეს იღებდეს აღმოსავლეთ პარატეთისის ტერიტორიიდან, ვინაიდან *S.* cf. *minutus*-ის ნაშთები სამხრეთ საქართველოდან დღეისათვის მსოფლიოში ცნობილ ამ სახეობის ნაშთებს შორის უძველესია.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

EFFECT OF PHYTOHORMONES ON DEVELOPMENT OF NUT BUDS (*CORYLUS AVELLANA L.*) CULTURE

ALASANIA N.[1], ZARNADZE N.[1], LOMTATIDZE N.[2], DUMBADZE G.[2]*, JAPARIDZE Z.[2]

[1]*Batumi Institute of Agrarian Biotechnologies and Business*
[2]*Sh. Rustaveli Batumi University*
**E-mail: anachi@nm.ru*

(Received September 10, 2005)

Abstract

The process of microclonal reproduction of nut buds *in vitro* cultures was studied. It was established that this process depends on trophic factors and phytohormones. Namely, bringing in nutrient medium different concentrations of 6-benzilaminopurin (BAP) – naphthyl-acetic acid (NAA) and also BAP – indol-butyric acid (IBA), induced intensive blastogenesis in nut cultures. The optimal ratio of cytokinin - auxin concentrations was 10:2 μM. Rootage takes place on modified Murashige-Skoog (MS) nutrient medium in the presence of 8 μM IBA.

Key words: microclonal reproduction, benzilaminopurin, indol-butiric and naphthyl-acetic acids.

Introduction

The role of phytohormones influence on regulation of plant morphogenesis is well known [Schestibratov, et al., 2003; Sun, et al., 2004]. *In vitro* cultures plant organs formation *de novo* is carried out by the expression of genes which control morphogenesis induction, and the necessary inductors along with nutrient medium components are plant growth regulators [Wei Tang, et al., 2004].

The main goal of our research was to reveal optimal potential of microclonal reproduction at the effect of phytohormones.

Materials and Methods

The object of our investigation was nut (*Corylus avellana L.*). As an explant apical and axillary buds of young vegetated sprout were used. To study microclonal reproduction to MS nutrient medium different concentrations and different ratios of phytohormones were added. Namely it was added 2, 5, 10, 15 μM of BAP; 5, 10, 15 μM of kinetin; 20 μM of NAA and 0.5-2 μM of IBA. pH of nutrient medium was 5.8-6. Incubation of cultures was carried out at 16/8 hours phytoperiod; Lightening -2-3 klux; temperature - 26 ± 1^{0}C; subcultivation occurred every 25-30 days.

Results and Discussion

According to our studies bringing in and cultivation of nut buds *in vitro* systems was hardly subdued. In the first subcultivation process significant morphogenetic process was not noticed, only self development of the primary explant took place. On the further stages of cultivation buds proliferation already occurred. Necessary inductors for this process were phytohormones. At the same time the intensity of buds *de novo* formation depended on their concentrations and ratios in the nutrient medium.

The results of our study are presented in the Table 1.

Table 1. Effect of phytohormones on nut buds proliferation *in vitro* cultures

Phytohormones (μM)				Average height of buds (mm)	Average amount of adventitious buds	callusogenesis
BAP	NAA	IBA	kinetin			
*-	-	-	-	5.0	1.0	-
2	-	-	-	37.6	3.1	-
5	-	-	-	31.2	3.8	-
10	-	-	-	27.1	4.9	-
15	-	-	-	22.0	4.9	-
5	0.5	-	-	20.0	13.3	+
10	0.5	-	-	21.2	12.1	-
5	2	-	-	23.1	16.3	-
10	2	-	-	24.1	10.4	++
5	-	0.5	-	21.1	19.1	+++
10	-	0.5	-	20.0	15.0	+
5	-	2	-	18.4	20.1	+++
10	-	2	-	17.7	16.3	++
-	-	-	5	13.8	10.1	++
-	-	-	10	13.1	18.4	--
-	-	-	15	12.0	14.6	--
-	-	-	20	10.0	18.0	--

*control

As is seen from the table for nut buds proliferation *in vitro* cultures kinetin is less effective than BAP. This fact is clearly seen on intensity of adventitious buds production and on the height of apical dominated sprouts. At the presence of kinetin in the nutrient medium for the growth of buds height long period is needed; development phase of newly formed buds is also prolonged. At the same time it was established that less than 10 μM of kinetin concentration is not effective for bud induction. Namely, meristemization of axillary buds was observed, but lateral buds growth did not occur. While adding to the nutrient medium of 15 μM concentration of above mentioned phytohormone this process proceeded comparatively sharper.

Using different concentrations of BAP it was determined that minimal concentration (2-5 μM) of this phytorormone promoted the height growth of the main sprouts. Basipetal regions came out from a state of rest . In some cases additional buds development occurred, the height of which at the end of subcultivation came to the half of the main sprouts height.

Increase of BAP concentration up to 10 μM proper at the reproduction stage provoked adventitious blastogenesis along with apical domination of the primary explant with relatively low intensity; increase of concentration up to 15 μM caused intensification of adventitious buds induction and decrease of height growth of apical sprouts. With the increase of amount of subcultivars quantitative decrease of dominated sprouts and increase of apexes of newly formed buds took place, which further development required reduction of cytokinin concentration in the nutrient medium. Even greater increase of BAP concentration (20 μM) caused sprouts height stunt and in basal part buds collection with calluslike tissue structure produced.

To produce callusogenesis from buds basal part adding NAA and IBA up to 0.5-2 μM concentrations to the nutrient medium along with BAP was effective. At the presence of these auxins decrease of the main sprouts growth, stem thickening occurred, axillary buds activation was inhibited. In return, as a result of dedifferentiation process of callus which is formed in hypocotyl of sprouts, from embryonic structures developed a lot of adventitious buds. Such potential of plant enables us to use studied auxins along with BAP to increase the coefficient of reproduction .

On the next stage of reproduction subcultivation of callus fragments containing meristemal regions occurred; further from these fragments primary leaves were formed, later – buds. At that time the explant represented fascicles containing small buds. Partition of these fascicles on the new nutrient medium caused development of apical dominated sprouts.

Buds and sprouts of 20-22 mm of height for rootage were sowed on modified MS nutrient medium, to which for root induction 8 μM of IBA was added. Rootage coefficient was 90%. Rooted plant-regenerates for acclimatization were placed in unsterile conditions. After 20-25 days already acclimatized plants were easily grown in soil.

Thus, described method of microclonal reproduction enables us to receive intensively plant-regenerates of nut *in vitro* cultures.

References:

Schestibratov K.A., Mikhailov R.V., Dolgov S.V. *Plantlet regeneration from subculturable nodular callus of Pinus Radiata.* Plant Cell, Tissue and Organ Culture, **72**, 2, 139-146, 2003.

Sun Y., Jin S., Liang S., Nie Y. *Somatic embryogenesis and plant regeneration in wild cotton (Gossipium Klotzschianum).* Plant Cell, Tissue and Organ Culture, **76**, 2, 189-192, 2004.

Wei Tang, Harris L., Quthavong V. *The effect of different plant growth regulators on adventitious shoot formation from Virginia Pine (Pinus Virginia) zygotic embrio explants.* Plant Cell, Tissue and Organ Culture, **75**, 2, 247-253, 2004.

ფიტოჰორმონების გავლენა თხილის (*Corylus avellana L.*) კვირტების ზრდა-განვითარებაზე in vitro კულტურაში

ალასანია ნ.[1], ზარნაძე ნ.[1], ლომთათიძე ნ.[2], დუმბაძე გ.[2], ჯაფარიძე ზ.[2]

[1] ბათუმის აგრარული ბიოტექნოლოგიებისა და ბიზნესის ინსტიტუტი
[2] შ. რუსთაველის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.09.2005)

რეზიუმე

შემუშავებულია თხილის მიკროკლონური გამრავლების პროცესი in vitro კულტურაში. დადგენილია, რომ აღნიშნული პროცესი დამოკიდებულია საკვებ არის ტროფიკულ ფაქტორებსა და ფიტოჰორმონებზე. ფიტოჰორმონების, კერძოდ, 6-ბენზილამინოპურინი - ნაფტილძმარმჟავას და 6-ბენზილამინოპურინი - ინდოლ-ერბომჟავას სხვადასხვა კონცენტრაციათა შეტანა საკვებ არეში იწვევდა მასიური კვირტწარმოქმნის ინდუქციას თხილის კულტურაში. ციტოკინინ-აუქსინის კონცენტრაციათა ოპტიმალური თანაფარდობა იყო 10 : 2 მკ მოლი. დაფესვიანება წარმოებდა მოდიფიცირებულ MS საკვებ არეზე 8 მკ მოლი ინდოლერბომჟავას თანაობისას.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

THE EFFECT OF MICROELEMENTS ON THE ACTIVITY OF ENZYMES IN VEGETABLES.

MANGALADZE N., CHICHINADZE A., BOJGUA N., MERKVILADZE S.

Department of Botany and Ecology, Kutaisi A. Tsereteli State University

(Received July 11, 2005)

Abstract

Copper produced negative effect on the activity of phenoloxidase, caused the decrease in the activity of amylase $\alpha\beta$ and did not influence the activity of amylase α in *Raphanus sativus L.*, while influenced positively the activity of polyphenoloxidase in all plant species under examination. Activity of peroxidase was decreased under the influence of boron in *Raphanus sativus L.* and *Capsicum annuum L.*, whereas the same nutrient produced positive effect on the activity of polyphenoloxidase and amylase. Boron, copper and iodine produced positive influence on the ascorbinoxidase, while the effect of cobalt and zinc was predominantly negative.

Key words: Raphanus sativus L., Capsikum annuum L., enzyms, microelements.

Introduction

It is well established that microelements are crucial for the development of plants. In the beginning of 20th century scientists pointed to the significance of fertilizers for the increasing of crop capacity [Bagdasarishvili, 1965; Giorgadze, 1960; Aleksandrov, 1954; Alekseev & Gorelova, 1954]. Modern authors confirm, that microelement deficiency may lead to so called alimentary diseases in plants with concomitant disorders of seed germination, underdevelopment of vegetative organs and delayed fruitage [Hensen, 1979; Lutts et al., 2004; Marschner, 1995; Murray et al., 2004; Obrador et al., 2003; Outten & O'Halloran, 2001; Pai et al., 2002; Rausch & Busher, 2002; Salt, 2004; Williams, 2001]

Present work was aimed to examine the role of microelements in the development of vegetable plant species.

Material and methods

Plant species as *Coriandrum sativum L., Capsicum annuum L., Raphanus sativus L.* has been examined. Seeds were processed in 0.02 % water solution of $ZnSO_4$ $CuSO_4$, H_3BO_3, $CoCl_2$, KI, $KMnO_4$ for 24 hrs and sawed in soil, kept in experimental drawers. Control seeds were set apart in pure water for 24 hrs and sawed in the same conditions. Widely known cytochemical methods [Chernavina, 1979] were used to measure the activity of plant cellular enzymes as peroxidase, polyphenoloxidase, amylase and ascorbinoxidase as well as vitamin C content in the young sprouts of plant species under examination in experimental and control material.

Results and Discussion

Boron, zinc and copper produced positive effect on the activity of polyphenoloxidase. As compared to control material, activity of polyphenoloxidase was significantly increased young sprouts, processed in water solution of $ZnSO_4$ CuSO4, H_3BO_3 (Table 1). The effect of copper and boron was stronger as compared to zinc in all plant species under examination. At the same time, activity of peroxidase was decreased under the influence of boron in *Raphanus sativus L.* and *Capsicum annuum L.* Negative effect of copper on the activity of peroxidase was obvious in the same plant species, whereas no effect of microelements on the activity of peroxidase was revealed in *Coriandrum sativum L.*

Table 1. The effect of microelements on the activity of polyphenoloxidase (1) and peroxidase (2)

Plant species	Control		H_3BO_3		$ZnSO_4$		$CuSo_4$	
	1	2	1	2	1	2	1	2
Coriandrum sativum L.	6,15	12,0	6,71	12,0	12,0	24,0	15,1	12,0
Raphanus sativus L.	11,.0	26,0	15,0	5,1	29,0	27,0	31,5	20,1
Capsicum annuum L.	5,0	20,0	6,0	16,0	10,5	9,0	10,5	9,0

Copper produced negative effect on the activity of amylase αβ in *Raphanus sativus L.* and did not influence the activity of amylase α of the same plant species (Table 2). In all the rest cases microelements produce positive effect on the activity of amylase.

Table 2. The effect of microelements on the activity of amylase α and αβ

Plant species	Control		H_3BO_3		$ZnSO_4$		$CuSo_4$	
	α	αβ	α	αβ	α	αβ	α	αβ
Coriandrum sativum L.	3,7	4,8	6,7	9,4	8,2	8,4	6,9	10,4
Raphanus sativus L.	1,3	19,2	4,6	24,0	4,6	24,0	1,3	11,2
Capsicum annuum L.	3,66	7,2	9,6	14,4	3,8	14,0	6,9	14,6

As for the effect of microelements on the activity of ascorbinoxidase (Table 3), boron, copper and iodine produced positive influence in *Coriandrum sativum L.* and *Capsicum annuum L.* during vegetation and flowering, whereas cobalt influenced negatively vegetation and flowering of plant species under examination. Zinc had positive effect on the flowering of *Capsicum annuum L.*, did not influence the flowering of *Coriandrum sativum L.* and caused the delay in vegetation of *Coriandrum sativum L.*

Table 3. The effect of microelements on the activity of ascorbinoxidase

Plant species	Control	H_3BO_3	$ZnSO_4$	$CuSo_4$	$CoCl_2$	KI
Coriandrum sativum L. (vegetation)	1,61	1,68	0,42	2,40	1,32	2,42
Coriandrum sativum L. (flowering)	0,16	0,30	0,16	1,7	0,05	0,17
Capsicum annuum L. (vegetation)	0,5	0,5	0,55	0,95	0,05	1,4
Capsicum annuum L. (flowering)	1,0	1,3	1,60	2,15	1,0	1,28

In sum, microelements as boron, zinc, copper, cobalt and iodine produced species-specific effects on the activity of enzymes in vegetable plants. Copper produced negative effect on the activity of phenoloxidase, caused the decrease in the activity of amylase $\alpha\beta$ and did not influence the activity of amylase α in *Raphanus sativus L.*, while influenced positivelly the activity of polyphenoloxidase in all plant species under examination. Activity of peroxidase was decreased under the influence of boron in *Raphanus sativus L.* and *Capsicum annuum L.*, whereas the same nutrient produced positive effect on the activity of polyphenoloxidase and amylase. Boron, copper and iodine produced positive influence on the ascorbinoxidase, while the effect of cobalt and zinc was predominantly negative.

References:

Aleksandrov V.G. *Plant Anatomy*. Moscow, "Mir", 1954 (in Russian)

Alekseev A.M. Gorelova Z.P. *Effect of mineral nutrition level on water regime of plant.* Proc. Acad. Sciences of USSS, **LXVII**, 1, 1949 (in Russian).

Bagdasarishvili Z. *Importance and Usage of Microelements in Winemaking.* Tbilisi, "Metsniereba", 1965 (in Georgian).

Chernavina I.A. *Practical Work in Plant Physiology*. Moscow, "Prosveshchenie", 1979.

Giorgadze L. *Treatment of maize seeds before sawing in potassium bromide solution and its influence on crop-producing.* Bulletin of Academy of Sciences of Georgian SSR, **24**, 16, 1960 (in Georgian).

Hansen V. W., Israelsen D. W., Stringham Q. E. *Irrigation principle and practices*. 4th ed. John Wiley and Sons, New York. 1979.

Lutts S., Lefevre I., Delperee C., Kivits S., Dechamps C., Robledo A., Correa E. J. *Heavy metal accumulation by the halophyte species Mediterranean Saltbush*. Environ. Qual., **33**, 1271-1279, 2004.

Marschner H. *Mineral nutrition of higher plants*. ed. 2nd, Academic press, London, 1995.

Murray S., Rogers D., Kaufman M. *Heavy metals in urban watershed in Southeastern Michigan.* J. Environ. Qual., **33**, 163-172. 2004.

Obrador A., Novillo J., Alvarez J. M. *Mobility and availability to plants of two Zinc sources applied to a Calcareous soil.* Soil Science of America Journal, **67**, 564-572, 2003.

Outten C. E., O'Halloran T. V. *Fentomolar sensitivity of metaloregulatory proteins controlling Zinc homeostasis.* Science, **292**, 2488-2492, 2001.

Pai T. G. Ohkimoto K., Sakatibara Y. Manganese stimulation and stereospecificity of the Dopa (3,4-dyhydroxephenylalamine)/tyrosine-sulfating activity of human monoamine-from phenol sulfotransferase. J. Biol. Chem., **277**, 46, 43318-43820, 2002.

Rausch C., Busher M. *Molecular mechanisms of phosphate transport in plants.* Planta, **216**, 23-37, 2002.

Salt D. E. *Update of Plant Ionomics*. Plant Physiology, **136**, 2451-2456, 2004.

Williamss R. J. P. *Chemical selection of elements by cells*. Coordin. Chem. Rev., **216-217**, 583-595, 2001.

მიკროელემენტების ზეგავლენა ზოგიერთი ფერმენტის აქტიურობაზე ბოსტნეულ მცენარეებში

მანგალაძე ნ., ჭიჭინაძე ა., ბოჯგუა ნ., მერკვილაძე ს.

ბოტანიკისა და ეკოლოგიის კათედრა, ქუთაისის აკ. წერეთლის სახელობის უნივერსიტეტი

(მიღებულია 11.07.2005)

რეზიუმე

ნაჩვენებია, რომ სპილენძმა შეამცირა ფენოლოქსიდაზას აქტიობა ოხრახუშში, ქინძსა და ბოლოკში, უარყოფითად იმოქმედა αβ ამილაზის აქტიობაზე და ზეგავლენა არ მოუხდენია α ამილაზაზე ბოლოკში; გაზარდა პეროზიდაზის აქტივობა ყველა საკვლევ მცენარეში. ბორმა შეამცირა პეროქსიდაზას აქტიობა ბოლოკში და ოხრახუშში, მაგრამ დადებითად იმოქმედა პოლიფენოლოქსიდაზას და ამილაზას აქტიობაზე ყველა საკვლევ მცენარეში. ბორმა, სპილენძმა და იოდმა გამოიწვიეს ასკორბინოქსიდაზის აქტიობის გაზრდა, ხოლო კობალტის და ცინკის ეფექტი ამ მხრივ ძირითადად უარყოფითი იყო.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

MODIFICATION OF TRACHEAL CELLS UNDER THE EFFECT OF ANTHELMINTH REMEDY "K" IN HENS INFECTED BY *SYNGAMOS SKRYABINOMORPHA*

BOEVA L.

Institute of Zoology of The Georgian Academy of Sciences

(Received September 20, 2005)

Abstract

The structure and ultrastructure of diseased with *Syngamos* hens tracheal cells under the effect of new anthelminth remedy "K" was investigated. It was shown that haemorrhage and destruction of mucous and under-mucous membranes of hen trachea occurred as a result of invasion by helminth *Syngamus skryabinomorpha*. The invasion of anhelminth caused death of helminth but did not substantially change the structure of the mucous and under-mucous cells of hen trachea. The changes remained localized and didn't influence vital functions of the birds.

Key Words: *Syngamus skryabinomorpha*, Helminth, Anthelminth.

Introduction

It was shown that synthesized in Georgia anthelminth remedy "K" causes destruction of covering, intestine and ovary cells of helminth *Syngamus skryabinomorpha* [Goderdzishvili et al., 1977; Boeva, 2002]. These changes of cells structure of the studied organs rouse death of helminth. In this viewpoint it is interesting to determine capability of the use of preparation "K" against *Syngamus skryabinomorpha* without causing any damages of the cells of hostess. So, the aim of our work was to study the effect of the anthelminth on hen tracheal cells.

Materials and Methods

Preparation "K" was introduced to 2.5 month chicks which were spontaneously invasioned by helminth *Syngamus skryabinomorpha*. 100 mg of Anthelminth per kg of chick weight was non-permanently introduced via food. Chicks were killed after 3 hours. Cells were fixed in 2.5% of Glutar – Aldehid and 1% OsO_4 for electron microscopic studies. Dehydration and embedding in to epoxide pitch, Epon – 812 was conducted by known method [Novikov, 1963].

Results and Discussion

Experiments showed that hen trachea is a tube with a diameter of 5-6 mm. Its walls consist of cartilage half circles and spaces between them are connected with a loose connective tissue.

Mucous of hen trachea as well as of other vertebrae is presented by multiserial ciliated epithelium, gobletlike mucoid and ciliary cells [Kurashvili & Boeva, 1988].

Stem cells form mucoid and ciliary cells. During the intensive secretion mucoid cells are characterized by possession of a great number of big granules and small round nucleus, strictly lined chromosomes located at nuclei membrane. On the apical surface of ciliary cells filaments occur on which basis there are a great number of microtubules. Microfibers of these cells form brush like edging (Fig. 1a). At the basis of the ciliary cells there are basal corpuscles presenting centriole and basal feet which keep basal corpuscles on the place of its localization.

In the ciliary cells endoplasmic reticulum is well developed and there are also a lot of polysomes, great round nuclei with a big nucleolus. Nucleolus has a granular structure (Fig.1a).

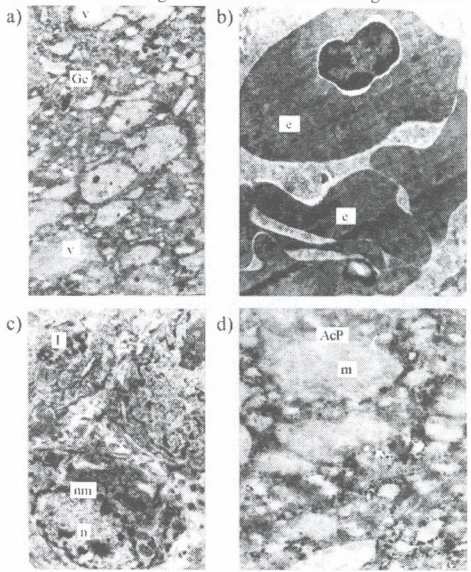


Fig 1. Electronogrammes of the chick trachea cells after the invasion with the helminth *Syngamus skrjabinomorpha* and after the influence of the preparation "K". a) Gc- Golgi complex; v-vacuole; b) e- erythrocytes between the tracheal cells; c) l - lysosome; n- nucleus; nm-nuclear membrane; d) m - mitochondria; AcP - acid phosphatase; destruction of mitochondrial cristas. Fixing in 6% OsO4 ; embing in Epon-812, x: a - 20 000; b - 15 000; c - 20 000; d-25 000.

In the under-mucous membrane there is a great number of collagen fibers connecting the ends of cartilage half circles of hen trachea.

Destruction of mucoid and ciliary cells occur in the area of connection of helminth. In these places punctate haemorrhages are observed. In the intercellular space, as a result of destruction of capillary membranes, a great number of erythrocytes are found (Fig 1b). Cytoplasm

forms finger like appendixes (branches) and invagination (Fig 1b). In the cells which are far from the area of invasion any changes in morphology or ultrastructure of cells are not observed.

After 3 hours of introducing of anthelminth helminth dies. Plasmatic membranes of trachea wall cells end endoplasmic cell membranes where helminth was fixed are destructed, as well as mitochondrial cristae (Fig.1. c, d). Nucleus is oval, nucleolus - not big, with dense structure and hardly distinguishable fibrillar and granular components. In the cytoplasm there are free, rosette-form polysomes. In the undermucous trachea there are rather well kept collagen fibers which connect the ends of cartilage half circles.

The results of observation show that invasion by helminth causes haemorrhage and destruction of mucous and undermucous cells membranes. But invasion of anthelminth preparation causes helminth's death, although it doesn't substantially change the structure and ultrastructure of tracheal cells. Some ultrastructural changes of cells which are close to the place of helminth attachment to the walls of trachea are observed just locally.

References:

Boeva L. *Electronocytochemical research of chick liver and trachea cells after the action of the anthelminth preparation "K".* In: Proceedings of the Institute of Zoology, **XXI**, 457-459, 2002.

Goderdzishvili I., Gugunishvili N., Korkotashvili N., Shengelia E.. Tiabendazol – effective preparation at syngamose of chicken. In: Proc."Achievements of veterinarian helmintology in practice", Tbilisi, "Sabchota Sakartvelo", 1977 (in Russian).

Kurashvili T., Boeva L. *Pathomorphological changes of tissues and organs of Syngamus trachea after effect of anthelminth.* In: Vertebrates eyes cells reaction on environmental factors. Tbilisi, "Metsniereba", 163, 1988 (in Russian).

Novikov A. *Lysosomes in the physiology and pathology of cells.* In: Giba Found Symp. on Lisosomes, Churchil, Ltd, London, 36-73, 1963.

სინგამოზიანი ქათმის ტრაქეის უჯრედების ცვლილება ანტჰელმინთურ პრეპარატ „K“-ს მოქმედებისას

ბოევა ლ.

საქართველოს მეცნიერებათა აკადემიის ზოოლოგიის ინსტიტუტი

(მიღებულია 20.09.05)

რეზიუმე

შესწავლილია საქართველოში სინთეზირებული ანტჰელმინთური პრეპარატი „k“-ს მოქმედება სინგამოზით დაავადებული ქათმის ტრაქეის უჯრედების სტრუქტურასა და ულტრასტრუქტურაზე. ნაჩვენებია, რომ ჰელმინთ *Syngamus Skryabinomorpha* –თი დაინვაზირების შედეგად ხდება სისხლჩაქცევა და ქათმის ტრაქეის ლორწოვანი და ლორწოსქვეშა გარსების დარღვევა. ანტჰელმინთ "k"-ს შეყვანა იწვევს ჰელმინთის დაღუპვას, მაგრამ მნიშვნელოვან გავლენას არ ახდენს ტრაქეის გარსების ლორწოვან და ლორწოსქვეშა უჯრედების სტრუქტურასა და ულტრასტრუქტურაზე, შენარჩუნებულია ქსოვილებისა და უჯრედების მეტნაკლებად "ნორმალური" სტრუქტურა. ეს ცვლილებები ატარებენ ლოკალურ ხასიათს და არ მოქმედებენ ფრინველის ცხოველმყოფელობაზე.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

Zoology

DETERMINING THE DESICCATION TOLERANCE OF ENTOMOPATHOGENIC NEMATODES FROM THE GENUS HETERORHABDITIS AND STEINERNEMA

MIKAIA N.

Biological Department of Sokhumi University Branch of Iv.Javakhishvili Tbilisi State University
E-mail: Skarabidae@mail.ru

(Received 27.09.2005)

Abstract

The desiccation tolerance of entomopathogenic nematodes from the genus Heterorhabditis and Steinernema was determined. At the effect of desiccators (K_2SO_4, KNO_3, KCl) *Heterorhabditis bacteriophora (HP88)* and its strain HIS-Drova are characterized with high viability: 66%-79% and 65%-78% correspondingly, which is caused by slow deprivation of water from the nematode body. The study of *Steinernema feltiae (SFG)* and its strains revealed that at the effect of desiccators viability for SFG varies from 78% to 88%, for the strain SIS-6 viz. 52% - 85% and for SIS-2 – 44%-58%. Low viability of the strain SIS-2 is caused by fast loss of water.

Key words: biological control agents, insect pests, *Steinernema feltiae, Heterorhabditis bacteriophora*

Introduction

Entomopathogenic nematodes from the families Heterorhabditis and Steinernematidae are effective biological control agents of various insect pests. The 3-rd stage infective juveniles penetrate insect hosts through natural body openings and localize in the middle intestine. Invading the host they feed with its hemolymph and release symbiotic bacteria, which proliferate and kill the insect host [Kaya & Gaugler 1993]. After decomposition of hemolymph and adipose tissue of the host the juvenile nematodes left infected cadavers and continue their life cycle in other host organizm. Their survival depends on environmental conditions [Glazer et al., 1991; Glazer, 1996; Kaya, 1990]. Like all other nematodes, Heterorhabditis and Steinernematidae are aquatic organisms and for motility and survival they require film of water to surround their bodies. Under desiccating conditions nematodes pass to "anhydrobiosis" state [Barrett, 1991], lose up to 95-98% of their body water, but some of them survive.

Marerials and methods

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) are isolated from wide variety of ecosystems of Israel, ranging from sub-Arctic to arid and tropical climates. It is expected that natural populations of these nematodes will be pre-adapted to specific ecological

conditions in their environments. Heterorhabditidae and Steinernematidae nematode strains were reared in the last instars wax moth larvae (*Galleria mellonella L.)* as described by Kaya and Stock [Kaya & Stock 1997]. The emerging juvenile nematodes were stored in distilled water in 250 ml flasks for 2-3 weeks prior the use.

Each strain of nematodes from aqueous suspensions (10 ml) which consist of 40 000 nematodes after vacuum filtration by Buckner device was concentrated on a filter paper (Whatman N1) of 5cm diameter [Liu & Glazer, 2000]. The juvenile nematodes were exposed in desiccators - K_2SO_4, KNO_3 and KCl for 72 h, 48 h and 24 h at different relative humidities: 93%, 97% and 85%. Further nematodes were placed in Petri dishes with distilled water for 24 hours, at room temperature. Viable nematodes were calculated by microscope [Glazer, 1992]. Experiments with all desiccators were repeated three times.

Results and Discussion

Desiccation tolerance of entomopathogenic nematodes from the genus Heterorhabditis and Steinernema was studied. The most suitable desiccation conditions that lead to induction of the anhydrobiotic state for HP88 (*Heterorhabditis bacteriophora*) and its strain HIS-9-Dvora, SFG (*Steinernema Feltiae*) and its strain SIS-1-Holit were determined.

At the exposure in K_2SO_4 for 72 h at 97% relative humidity, survival for HP88 was 79%; and for HIS-9 - 78%. At the exposure in KNO_3 for 24 h at 93% relative humidity, survival for HP88 was 77%, and for HIS-9 - 75%. At the exposure in KNO_3 for 48 h at 93% relative humidity, survival for HP88 was 72%, for HIS-9 was - 66%. At the exposure in KNO_3 at 93% relative humidity for 72 h survival for HP88 was 66%, for HIS-9 - 65%.

According to our data at the effect of desiccators HP88 and it isolates show high viability: for HP88 it viz.: 66%-79%; for HIS-9 viability viz.: 65%-78%. Such high viability is caused by slow loss of water from the nematode organism.

At the exposure in K_2SO_4 for 72 h at 97% relative humidity, survival for SFG was 88%, and for SIS-1 - 78%. At the exposure in KNO_3 for 24 h, 48 h and 72 h at 93% relative humidity survival for SFG was 78%-85% , and SIS-1 - 74%-68% (Fig.1).

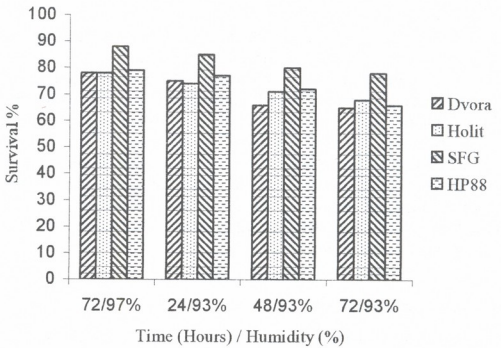


Fig. 1. Desiccation survival of nematodes HP88, SFG, HIS-9-Dvora and SIS-1-Holit.

We also studied desiccation survival of entomopathogenic nematodes HP88 and SFG and their strains Zeelim (SIS-6), HP Grofit (HIS-10) and SFG Besor (SIS-2). At the Exposure in K_2SO_4 for 72 h at 97% relative humidity survival for SIS-6 was 85%, for SIS-2 - 79%, for HIS-10 - 74%, for HP88 - 78%, At the exposure in KCl for 24 h at 85% humidity, survival for SIS-6 was 60%, for SIS-2 - 58%, for HIS-10 - 38%, for HP88 - 55%. At the exposure in KCl for 48 h at 85% humidity, survival for SIS-6 was 55%, for SIS-2 - 49%, for HIS-10 - 34%, for HP88 - 52%. At the exposure in KCl for 72 h at 85% relative humidity survival SIS-6 was 52%, SIS-2 - 44%, for HIS-10 - 31%, for HP88 - 50% (Fig.2).

At examining of SFG, HP88 and it isolates in desiccators K_2SO_4 at 97% of relative humidity high viability possesses strain SIS-6 (52%-85%). In desiccators KCl at 85% humidity low viability show SIS-6 (52%-60%), SIS-2 (44%-58%) and HIS-10 (31-38%). The low viability of the strains SIS-2 and HIS10 is caused by fast water loss from the nematode body. As for HP88, its high viability (65%-79%) in desiccators K_2SO_4 at 97% of relative humidity is reasoned by slow water loss from the nematode.

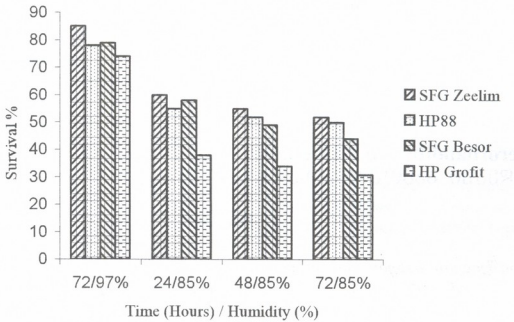


Fig.2. Desiccation survival SFG Zeelim (SIS-6), SFG Besor (SIS-2), HP88, and HP Grofit (HIS-10).

Acknowledgements

Work is executed in laboratory Entomonematology of the Institute of a Plant, Protection, Israel under the project: "Entomopathogenic nematodes as pest control agents in Georgia: Application and genetic improvement of beneficial traits for enhancement effectiveness" Project № CA22-007.

References:

Barret J. *Anhydrobiotic nematodes*. Agriv. Zool. Rev.**4,** 161-176, 1991.

Glazer I, Liran, N. & Steinberger Y. A *survey of entomopathogeni Nematodes (Rhabditida) in the Negev desert.* Phytoparasitica, **19**, 291-300, 1991.

Glazer I. *Survival and efficacy of Steinernema carpocapsae in an exposed environment.* Biocontrol Sci.Technol. **2**, 101-107, 1992.

Glazer I. Survival mechanisms of entomopathogenic nematodes. Biocontrol Sci. Technol., **6**, 373-378, 1996.

Kaya H. K. *Soil ecology*. In: *Entomopathognic nematodes in Biological Control*. Gaugler, R. and Kaya H. K. (Eds), CRC Press, Boca,1990.

Kaya, H.K. & Gaugler R. *Entomopathogenic nematodes*. Annu.Rev. Entomol. **38**,181-206.1993.

Kaya H.K. & Stock S.P. *Technique in insect nematology*. In: *Manual of Techniques in Insect Pathology*. Lacey L. (Ed), Academic Press, Ltd, NY, pp.303-305, 1997.

Liu Q. Z. & Glazer I. *Desiccation survival of entomopathogenic nematodes of the genus Heterorhabditis*. Phytoparasitica, **28**, 331-340, 2000.

Heterorhabditis და **Steinernema** გვარის ენტომოპათოგენურ ნემატოდებში გამოშრობისადმი ტოლერანტულობის განსაზღვრა

მიქაია ნ.

ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტის სოხუმის უნივერსიტეტის ფილიალის ბიოლოგიის ფაკულტეტი

(მიღებულია 27.09.2005)

რეზიუმე

შესწავლილია Heterorhabditis-ს და Steinernema-ს გვარის ენტომოპათოგენური ნემატოდების შტამების ტოლერანტულობა დესიკატორების (K_2SO_4, KNO_3, KCl) მიმართ. *Heterorhabditis bacteriophora (HP88)* და მისი შტამების გამოცდისას, ყველაზე მაღალი სიცოცხლისუნარიანობით გამოირჩეოდა HP88 (66%-79%) და მისი შტამი HIS-9 (65%-78%). *Steinernema feltiae (SFG)* და მისი შტამების გამოცდისას ნაჩვენები იყო, რომ K_2SO_4 დესიკატორის ზემოქმედებისას, 97% ტენიანობის პირობებში, შედარებით მაღალი სიცოცხლისუნარიანობით ხასიათდება SIS-6 (52%-85%). დაბალი სიცოცხლისუნარიანობა შეინიშნებოდა KCl-თან ურთიერთქმედებისას, 85% ტენიანობის პირობებში, SIS-6 (52%-60%) და SIS-2 (44%-58%) შტამების შემთხვევაში. დაბალი სიცოცხლისუნარიანობა SIS-2-თვის გამოწვეული იყო ამ ნემატოდის სხეულიდან წყლის სწრაფი დაკარგვით. რაც შეეხება HP88-ს მათი მაღალი სიცოცხლისუნარიანობა გამოწვეული იყო ამ ნემატოდების სხეულიდან წყლის ნელი დაკარგვით.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

Letter to editor

RADIOACTIVE NUCLIDES CONTENT OF BLACK SEA MUSSELS (*RAPANA THOMASIANA GROSSE*) BODIES

PALAVANDISHVILI N.

Department of Zoology and Water Bioresources, Sh. Rustaveli Batumi State University

(Received September 23, 2005)

Abstract

Pollution of Georgia (Adjara) costline Black Sea mussels with radioactive elements after Chernobyl accident was studied. Radioactivities of ^{40}K and ^{137}Cs nuclides were determined. According to the radiation safety norms of Georgia these radionuclides radiation level is permissible.

After Chernobyl atomic power-station wreck Georgian coastline of Black Sea was rather damaged, amount of ^{90}Sr and ^{137}Cs significantly increased in surface waters that have had negative effect on Black Sea hydrobiont.

Dynamics of accumulation of radioactive elements (phosphor, yttrium, thorium) in different organs of different marine organisms (green aqueous plants, sea anemones, rock mussels bodies, mud mussels bodies and sells, see lettuce) was studied [Polikarpov, 1960; Topcuoglu et al., 1993].

^{137}Cs Radionuclide activity was quite different at water surface and at the bottom. At the surface it was 0.398 Bq/l, and at the bottom - 0.042 Bq/l, on the depth of 60 m its concentration sharply decreased (0.090 Bq/l) that was connected with temperature, nitrates and nitrites concentration. At the same time on 1600 m depth ^{137}Cs activity increased (0.310 Bq/l) [Portakal et al., 1991].

Studies of radioactive nuclides carried out on *Rapana thomasiana* mussels caught in Bosporus and different regions of Black Sea showed that activities of ^{134}Cs, ^{137}Cs and ^{106}Ru were higher in east than in west region of Black Sea and Bosporus [Bulut et al., 1990].

It is known that *Rapana thomasiana* is a prolific extremely versatile species, tolerating low salinities, water pollution and oxygen deficient waters [Mann & Harding, 2003]. So, the aim of our work was to determine the quantity of radioactive elements got to Black Sea mussels after Chernobyl accident.

Mussels were collected in October, 2003 from 15-18 m of depth at Batumi promontory and from 10-20 m of depth at Kobuleti coastline. Alive mussels were put in distilled water at constant supply of oxygen, without feeding, for 24 hours.

Mussels were divided into three size classes: I class – mussels with 50 mm of length, II class – 60 mm of length, and III class – 70 mm of length. As the amount of caught mussels at Batumi promontory area was a little we united mussels of all three size classes.

Mussels bodies were homogenized and dehydrated in the cryogenic drier. Dried specimens were analyzed by shielded gamma-spectrometry, at 24 hour exposition. Radioactivities of primordial radionuclide ^{40}K and technogenic radionuclide ^{137}Cs were determined [Mukhin, 1974; Debertin & Helmer, 1988].

As it is seen from the table 1 in Kobuleti mussels amount of ^{137}Cs is more in the II size class (60 mm of length) and it equals 3.2±1.0 Bq/kg. In I and III size classes (50 and 70 mm of length correspondingly) it varies slightly – 2.7±0.07 – 2.9±1.5 Bq/kg. ^{40}K is more in I class and it equals 244±32 Bq/kg and in the II and III classes amounts to 190±18 and 198±14 Bq/kg. Total amount of ^{40}K is higher in mussels of Batumi promontory and of ^{137}Cs - in Kobuleti Region mussels. According to the radiation safety norms of the Georgian Health and Social Care Ministry above mentioned radionuclides radiation level is permissible [Radiation Safety Norms, 2000].

Table 1. Radioactive nuclides concentrations in mussels from Batumi and Kobuleti

Samples	Size class	Mass of sample (g)	Activity of dry mass (BC/kg)	
			^{40}K	^{137}Cs
Batumi	total	70.17	232±16	2.5±0.7
Kobuleti	I	32.82	244±32	2.9±1.5
Kobuleti	II	62.30	190±18	3.2±1.0
Kobuleti	III	80.36	198±14	2.7±0.7
Kobuleti	total	58.49	210±21	2.9±1.0

Acknowledgments

Radioactivities of nuclides were determined in the department of ^{14}C and low radioactivity of Iv. Javakhishvili Tbilisi State University. Special thanks to Prof. S. Pagava.

References:

Bulut A.M. Topcuoglu S., Sezginer N., Sonmerz M. *The radioactivity levels in Rapana thomasiana from the Bosphorus and Black Sea after the Chernobyl accident.* Comm.Int.Mer.Medit., Monaco, **32**, 1, 1993.

Debertin K., Helmer G. *Gamma- and X-ray spectrometry with semiconductor defectors.* North Holland, Amsterdam. Oxford-New-York-Tokyo, 1988.

Mann R., Harding J.M. *Salinity tolerance of larval Rapana venosa: implication for disperse and establishment of an invading predatory gastropod on the North American Atlantic.* Biol.Bull., **204**, 161-177, 2003.

Mukhin K. *Experimental nuclear physics. vol.* **I**, M., "Atomizdat", 1974 (in Russian).

Polikarpov G. *Absorption of radioactivity by marine organisms.* Wildlife, **1**, 104-105, 1960 (in Russian).

Portakal S., Varinlioglu A., Pala F., Seddigh E. *^{137}Cs and $^{239,240}Pu$ Concentrations in the Black Sea.* NATO Publication. Luwer Academic Press. Black Sea Oceanography, 302-324, 1991.

Topcuoglu S., Kut D., Esen N. *The thorium concentration in some marine organisms and thorium bio kinetic.* VI National Nuclear Science Congress, Izmir, Turkey, 1993.

Radiation Safety Norms. Georgian Health and Social Care Ministry, Tbilisi, 2000.

შავი ზღვის მოლუსკ რაპანაში (*Rapana thomasiana Grosse*) რადიონუკლიდების შემცველობა

ფალავანდიშვილი ნ.

(მიღებულია 23.09.2005)

ზოოლოგიისა და წყლის ბიორესურსების კათედრა, შ. რუსთაველის სახელობის ბათუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 23.09.05)

რეზიუმე

შესწავლილია შავი ზღვის მოლუსკ რაპანაში რადიოაქტიური ელემენტების შემცველობა დაბინძურების ხარისხის დასადგენად. გამა-სპექტროსკოპული ანალიზით დადგენილია რადიონუკლიდების - ^{137}Cs და ^{40}K-ის შემცველობები ქობულეთისა და ბათუმის კონცხის მიმდებარე ტერიტორიის მოლუსკ რაპანებში.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

Letter to Editor

NEW SPECIES *STOMACHORHABDITIS GEORGICA N. SP.* (NEMATODA, BHABDITIDAE) FROM ESTERN GEORGIA

VARDIASHVILI E.

Institute of Zoology of the Georgian Academy of Sciences

(Received September 5, 2005)

New species *Stomachorhaditis georgica n. sp.* from tomato pest fly worms (*Muscidae g. sp*) is described (East Georgia, Kvemo Kartli). Description, measures of male and female specimen, differential diagnosis and pictures are given.

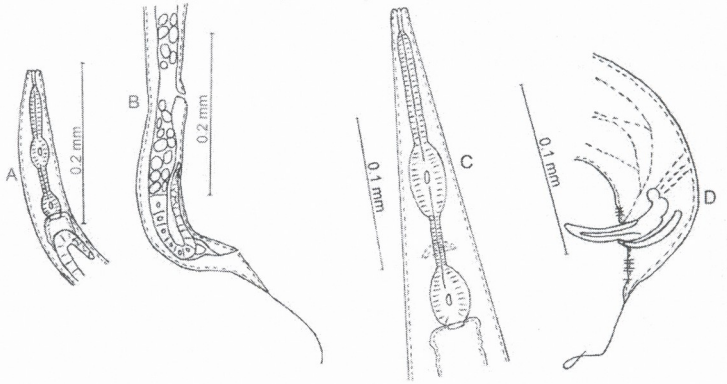


Fig. 1. Stomachorhabditis georgica: A - fore part of female; B – back of female; C – fore part of male; D – back of male.

Stomachorhabditis georgica n. sp.

Holotipe ♀: L = 0,9 μm; a = 23; b = 7,1; c = 4,8; V = 42, 8%; stoma = 16,5 μm.

Allotipe ♂: L = 0,89 μm; a = 20; b = 4,9; c = 4,5; stoma = 20 μ m; spicule = 45 μm; gubernaculums – 27, 5 μm;

Body spindle – shaped. Cuticle smooth. Head not set of from body contour, lips rounded, papillae not distinct, amphids is not visible. Oesophagus typical rhabditoid, metacorpal and cardial

bulbs well developed, muscular. Distance between them 56 μm. Stoma rather wide (4,5 μm), length nearly four times biggen them width (16, 5 μm), twice longer than the width of the head; cardia is cylindrical. Small onch is located at the dorsall wall of metastom. Female gonads amphidelphic bend to the vulva; anterior gonad reaches the cardia. Vulva preequatorial. Tail long, conical in the beginning, then narrowed to filiform. Its length is 9 times bigger than the anal diameter, terminus sharpen.

Male gonad - single, continues up to the cardiac bulb. Bursa not developed. 3 short ribs situated before the cloacae and 5 behind it. Spicule weakly bent, 1,5 times bigger then cloacal diameter, but length of gubernaculums is equal to cloacal diameter.

Paratypes 2 ♀: L = 0,70 – 0, 76 μm; a = 16-20; b = 4,0-7,3; c = 4,3-7,1;
V = 44-52%; stoma 17,6 = 22 μm;

Differential diagnosis: *Stomachorhabditis georgica n. sp.* is close to the *Stomachorhabditis vietnamica Andrassy*, 1970 [Andrassy, 1983] by its morphological features, but differs by following signs: 1) Cuticle is smooth. 2) Tail of female is shorter. 3) Spicule of male is noticeably bent (by *S. vietnamica* is straight). 4) Spicule much longer (of *St. vietnamica* is only 27-28 μm).

The materials are collected in the East Georgia, Kvemo Kartli, (Tetritskaro) and are kept in Entomonematology laboratory of the Institute of Zoology of the Georgian Academy of Sciences.

References:

Andrassy I. *A Taxonomic review of the Suborder Rhabditina (Nematoda: Secernentia)*, Paris, 241, 1983.

ახალი სახეობა *Stomachorhaditis georgica n. sp.* (Nematoda, Bhabditidae) აღმოსავლეთ საქართველოდან

ვარდიაშვილი ე.

ზოოლოგიის ინსტიტუტი, საქართველოს მეცნიერებათა აკადემია

(მიღებულია 5.09.2005)

რეზიუმე

აღწერილია ნემატოდას ახალი სახეობა ***Stomachorhaditis georgica n. sp.*** პამიდვრის მავნებელი ბუზის (*Muscidae g. sp.*) მატლებიდან (აღმოსავლეთ საქართველო, ქვემო ქართლი, თეთრიწყაროს რაიონი). მოცემულია მისი ზომები, აღწერა, დიფერენციალური დიაგნოზი.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 4, No. 1, 2006.

Letter to Editor

STUDY OF GEORGIAN SOUTH-EAST COSTLINE OF THE BLACK SEA MACROZOOBENTHOS

VARSHANIDZE M. *, MIKASHAVIDZE E.

Scientific-Research Institute of Marine Ecology and Fishery of Georgia
**E-mail: mefri@gol.ge*

(Received September 20, 2005)

Abstract

Composition, abundance of some species and biomass of macrobenthos of south-east costline of the Black Sea was studied. Dominant species of benthos are bivalve mussels which takes an important role in trophic series of ecosystems and has commercial value.

It is known that recently the Black Sea and in particular, its south-east costline is under significant antropogenic impact that caused significant changes in species composition, quantitative parameters and biomass of hydrobiont. The aim of our research was to receive general data about structure and some parameters of macrozoobenthos.

For this purpose material was collected in 2003-2004 in south-east part of the Black Sea at 16 stations – from village Kvariati up to the river Korolistskali. I, II, XIV-XVI stations were in Kvariati region; III-VI and IX-XIII stations – at entries of the rivers Chorokhi and Korolistskali; and VII-VIII in the area of Batumi port. The specimen were taken from 5-20 m depths. Material was collected by special benthos fishing nets with 0.045 m^2 of grabbing area [Zhadin, 1949]. Material was fixed in 4% formaldehyde. Species density (ind/m^2) and biomass (g/m^2) were determined.

At the studied aquatic area 65 species of macrozoobenthos were revealed. Among them 27 species (41%) were Bivalve mussels, 18 (28%) – Crustacea, and 20 species (31%) – Polychaeta (Fig. 1).

Dominant species consisting the main mass of benthos were revealed. They are: from Polychaeta – *Nephthus longicornis* and *Melinna palmate*; from Bivalve mussels – *Chamelea gallina, Rapana thomasiana, Lentidium mediterraneum, Cuclope donovani*; and from Crustacea – *Balanus improvisus*.

In the researched aquatic area two, new for the region, species were also observed – *Anadara inaequivalvis* and *Mnemiopsis Leidy* [Gogmachadze & Mikashavidze, 2005]. First one at Bulgarian costline was found in 1981 [Mikashavidze, 1981] and at north costline of the Black Sea - in 1960-1970 [Alekseev & Sinegub, 1992].

By species diversity Kvariati region was distinguished (13 species of mussels) and by poorness of species - Batumi port (2 species of mussels).

Though species abundance in spring and summer was higher, data about seasonal dynamics of macrozoobenthos did not reveal somewhat evident regularity, which is probably caused by undercurrents, different levels of pollution by seasons etc. (Fig.2).

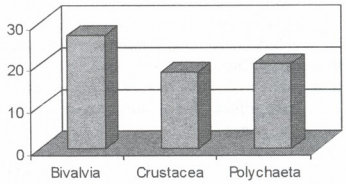


Fig. 1. Number of macrobenthic groups.

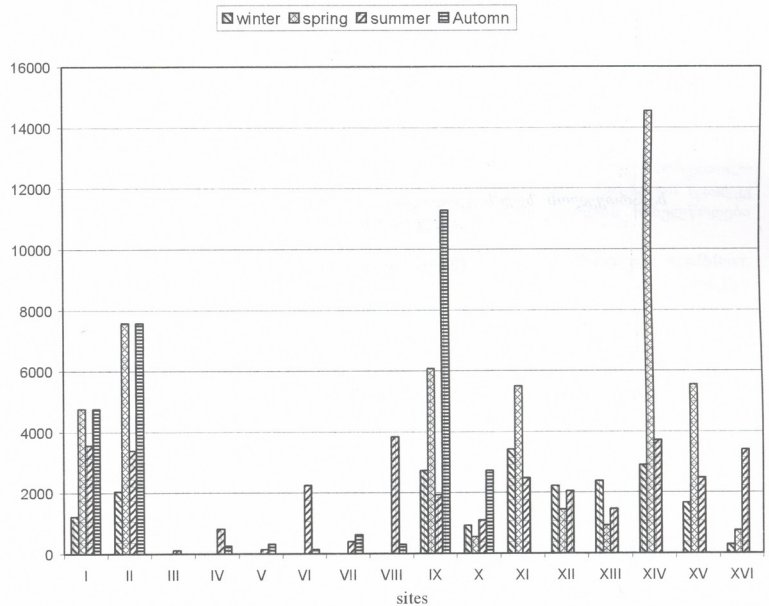


Fig. 2. Seasonal variation of abundance ind/sq.m by individual sites.

References:

Alekseev R.P. Sinegub I.A. *Macrozoobenthos and bottom biocenosis of the Black Sea on the Caucasus, Crimea and Bulgarian shelf.* Ecology of the Black Sea coastal zone. VNIRO, Moscow, 212-234, 1992 (in Russian).

Gogmachadze T.M. Mikashavidze E.V. *New dominant hydrobiont Cunaeca cornea (reeve) of Georgian costline of the Black Sea.* Collection of scientific works: Proceedings in Biology, 12-14, 2005 (in Georgian).

Mikashavidze E.V. *New species of polychaeta, mussels and Crustacea at south-east coast line of the Black Sea.* Zoological J., **X**, 9, 1415-1418, 1981 (in Russian).

Zhadin V.I. *Life of fish waters.* **IV**, Moscow-Leningrad, Publ.AN USSR, 34-50, 1949(in Russian).

შავი ზღვის საქართველოს სამხრეთ-აღმოსავლეთ სანაპიროს მაკროზოობენთოსის შესწავლისთვის

ვარშანიძე მ., მიქაშავიძე ე.

საქართველოს ზღვის ეკოლოგიისა და თევზის მეურნეობის სამეცნიერო-კვლევითი ინსტიტუტი

(მიღებულია 20.09.2005)

რეზიუმე

შესწავლილია შავი ზღვის სამხრეთ-აღმოსავლეთ ნაწილის მაკრობენთოსის თანამედროვე შემადგენლობა, ცალკეული სახეობების სიმჭიდროვე და ბიომასა. გამოვლენილია ბენთოსის დომინანტური წარმომადგენელი – ორსაგდულიანი მოლუსკი, რომელიც მნიშვნელოვან როლს ასრულებს ეკოსისტემის კვებით ჯაჭვებში.

ინსტრუქცია ავტორთათვის

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.

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