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Biochemistry

SOME PROBLEMS OF IDENTIFICATION AND DETERMINATION OF HUMUS ACID STRUCTURES

GARUCHAVA M., TKEMALADZE G., MENTESHASHVILI M., CHIABERASHVILI L.

Georgian Agricultural State University

(Received December 5, 2005)

Abstract

From black earth soils of eastern Georgia humus acids have been isolated and studied. The advantage of extraction method by turbidity point was shown. Elemental composition and content of acidic groups of fulvic acids was established. Occurrence of resonance signals of methylate group, carboxylic acid methyl ether methyl, amino acetate group and bonded with it methyl protons, with corresponding chemical bias, was registered in nuclear magnetic resonance spectrum.

Key words: humic acid, fulvic acid, humate, NMR.

Introduction

Humus acids are polyfunctional, polydispersed components of ecosystems [Djorobekova, 1977; Trubetskoy, 2001]. They compose more than half of soil organic carbon and affect on quantitative and qualitative characteristics of soil composition [Almendors et al., 1998].

Humus compounds, like all other organic fertilizers, are especially significant for soil reproduction. Humates biogenic compounds improve nutrient balance, and as a result a great amount of saprophyte microflora get into soil, activating soil reproduction process [Lopes-de Brinas et al., 2001]. Usage of humates provides development of root system, their effective nutrition, accelerates passing through phonological phase and increases productivity. It is significant that single treatment with potassium humate of polluted with ^{137}Cs and ^{40}K soil, content of radionuclides in agricultural products is decreased by 2-3 times [Kondrashov, 2000].

Study of complex compounds of humus acids with metal cations is actual problem. Transport of complex compounds, as well as its constituent minerals, are depended on the solubility of these compounds [Shinkarev, Gnevashov, 2001].

In humic and fulvic acids ash, isolated from organic-mineral constituent of humus acids, was found: kaolin, quartz, hydro-strata, etc. Purification of humus acids from these compounds, and hence, determination of their nature by different physical and chemical methods is difficult [Orlov, 1990]. At present there is not necessary theoretical and experimental basis for precise estimation of chemical composition of humus compounds. Consequently, investigation of humus acids considers methods of isolation, fractioning, and determination of dependence to solvents.

The data about effect of nitrogenous fertilizers on humus mineralization, composition of fulvic acids and existence of functional groups are dissimilar [Orel, 1999]. Chemical and ecological estimation and prediction of soils is complicated. The aim of our work was: to isolate from black-

earth soils of eastern Georgia humus acids, to study their composition and to determine existence of different functional groups by nuclear magnetic resonance (NMR).

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Materials and Methods

Soil samples were picked up from upper humus horizons of black earth of eastern Georgia lowlands. Their storage and further treatment was carried out according to [Garuchava, Buachidze, 2002].

Isolation of humus acids was hold by [Orlov, Grishina, 1968]. 100 ml 0.1N NaOH was added to the flasks with soil samples of 5 g and mixed. After 3-4 hours 50 g of $Na_2SO_4 \cdot 10H_2O$ was added to the flasks and mixed up to full dilution. It enables us to coagulate and precipitate entirely mineral kolloids. Further, flask content was filtrated. If filtrate was not enough clear filtration was repeated and centrifuged. Extraction of humus acids from filtrate by small amounts of alkali was carried on until filtrate becomes colorless.

Then humic and fulvic acids precipitation and separation was hold on. Humic acid (from alkali extract) was precipitated by concentrated sulphuric acid at pH 2-3. After 24 hours supernatant was separated from precipitate by filtration.

Occurring in precipitate humic acid via decantation was washed 2-3 times by water, acidified with sulphuric acid, pH 2-3. Consisting humic acids precipitate was solved in 0.1N NaOH; then placed in 100 ml volumetric flask and filled up to top by 0.1N NaOH. 10-50 ml solution was taken from volumetric flask, placed in 100 ml conic flask and evaporated up to drying. In obtained small values humic acid was determined by Turin's method [Arinushkina, 1962].

Spectrophotometrical study was carried out on 590 nm wavelength in 5 cm cuvette.

Acid solution containing fulvic acids was evaporated on sand basin. At very moment of turbidity appearance, evaporation process was stopped. Concentrate was placed in volumetric flask, aliquot fraction was taken for further determination of fulvic acids.

Results and Discussion

Extraction method by turbidity point was used for concentration of fulvic acids from soil water solutions. The method considers changes of different chemical and physical parameters, causing separation of studied solution into two isotropic layers. In that case hydrophobic compounds, having potential of solubility, are concentrated in one (lower) layer. Temperature range, when concentration degree increases by 2-3 times, was 70-90°C.

According to some researchers usage of extraction method at spectrophotometrical determination of fulvic acids is not always expedient [Calvet, 1984; Teit, 1991], as along with fulvic acids other colored organic compounds (humic acids, polyphenols) should concentrate in the lower layer of solution. We consider that purity of fulvic acids isolated from soils is sufficient for spectrophotometrical measurements.

We have studied isolated from black earth soils fulvic acids elemental composition and acidic groups contents (Table 1).

As is seen from the table fulvic acids, isolated from one and the same horizons and depths, by elemental composition, as well as by acidic-alkaline characteristics almost don't differ from each other. The differences are seen only in elemental composition of strata located at different depths. This variation in the case of carbon for A_1 (0-18 cm) – B_2 (56-96 cm) depths typical black-earth soils equals to 1%, for black-earth soil – 0.97%. Hence, we consider that formation of humus acids in studied soils occurs mainly in the range of A-horizon.

Table 1. Elemental composition of fulvic acids and acidic groups contents.

horizon	Depth, cm	pH of water extract	Total content, %		C:N atomic	mg. equi./g	
			Organic C	N		COOH	phen-OH
Typical black-earth soils							
A_1	0-18	6.2	1.25	0.11	13.3	4.0	2.1
A_2	18-27	5.8	0.47	0.05	10.8	3.8	2.0
B_1	27-56	5.7	0.43	0.03	16.7	3.4	2.0
B_2	56-96	5.4	0.25	0.02	14.6	3.3	1.8
Black-earth soil							
A_1	0-18	6.1	1.20	0.11	13.0	4.1	2.2
A_2	18-27	5.6	0.45	0.03	9.8	3.5	2.0
B_1	27-56	5.5	0.43	0.03	15.6	3.2	2.1
B_2	56-96	5.4	0.23	0.02	14.0	3.0	1.8

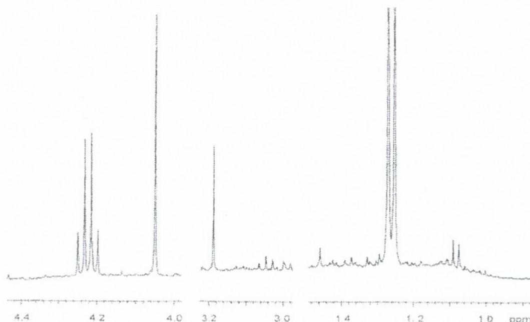

Fig. 1. NMR spectrum of fulvic acids

Isolated humus acids were studied by NMR spectroscopy. Registration of spectra of researched compounds was carried out on impulsive spectrometer (BC-567, "TECLA"). Study of NMR spectra showed that fulvic acids, isolated from different soils, by regions of chemical bias actually don't differ from each other, only intensities of peaks vary. Fig.1 presents NMR spectra of the fulvic acids, which have high intensity of resonance signals. As is seen from the table there is 1-5 ppm region of chemical bias. Doublet bind with amino acetate group, characteristic for methyl protons (H_3C-CH_2-COO^-), with center at 1.26-1.27 ppm, is clearly observed in this region.

Resonance signal occurs at δ=3.2 ppm, which is characteristic for protons of methylate group (CH_3-Oalk). As for chemical bias at 4.03 ppm, it corresponds to protons of carboxylic acid methyl ether methyl (H_3C-OCOR).

It is significant that at determination of relaxation time, quartet signal, at δ=4.2-4.3 ppm, characteristic for methyl protons (H_3C-CH_2-COO^-) of amino acetate group appeared in NMR

spectrum. Existence of identified groups in fulvic acids is very important, as via these groups they participate in complex-production reactions occurring in the soil. In spite of numerous investigations, data about stability of complex compounds of fulvic acids are not similar. We suppose that such stability is mainly caused by the degree of ionization of fulvic acids, as well as by molecular weights of their associations.

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გარუჩავა მ., ტყემალაძე გ., მენთეშაშვილი მ., ჭიაბერაშვილი ლ.

საქართველოს სახელმწიფო სასოფლო-სამეურნეო უნივერსიტეტი

(მიღებულია 05.12.2005)

რეზიუმე

აღმოსავლეთ საქართველოს შავმიწა ნიადაგებიდან გამოყოფილი და შესწავლილია ჰუმუსოვანი მჟავები. სიმღვრივის წერტილის მიხედვით ნაჩვენებია, ექსტრაქციის მეთოდის უპირატესობა. დადგენილია ფულვომჟავების ელემენტური შედგენილობა და მჟავური ჯგუფების შემცველობა. ბმრ სპექტრში დაფიქსირებულია მეთოქსი ჯგუფის, კარბონმჟავა მეთილის ეთერის მეთილის, ამინოაცეტატური ჯგუფის, მასთან მიერთებული მეთილის პროტონების რეზონანსული სიგნალების არსებობა, შესაბამისი ქიმიური წანაცვლებებით.

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Biochemistry

THE STUDY OF DIVALENT CATIONS INFLUENCE ON THE HEMAGGLUTINATION ACTIVITY OF LECTIN FROM SUBCELLULAR FRACTIONS OF A PROSTATE IN VARIOUS PATHOLOGIES

KVITSINADZE N.[1], MEGRELISHVILI I.[1], DAVITASHVILI E.[1], SOLOMONIA R.[1], ALEKSIDZE N.[1], KARAZANASHVILI G.[2]

[1] *Iv. Javakhishvili Tbilisi State University, Department of Biochemistry and Biotechnology*
[2] *Tbilisi State University, Medical Diagnostic Center, Urology Service Office*

(Received December 13, 2005)

Abstract

The influence of divalent cations (Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, Ca^{2+}, Pb^{2+}) on the hemagglutination activity of lectins from cytoplasmic and plasma membrane fractions of prostate fiber-muscular tissue was studied at different pathological conditions. The minimal concentration of Zn^{2+} required for agglutination inhibition is increased in cytoplasmic fraction in parallel with the severity of pathological condition. The minimal concentration of Ca^{2+}-ions, required for plasma membrane lectin agglutination inhibition raises in parallel of disease severity, but the opposite is observed for Mg^{2+}- and Mn^{2+}-ions. The minimal concentration of Fe^{2+}- and Mg^{2+}-ions required for cytoplasmic lectins activity inhibition decrease with malignancy. It was concluded, that changes in ions levels in pathologies are in the functional relationship with lectins activity.

Key words: lectin activity, ions, cytoplasm, plasma membrane, human prostate

Introduction

Hemagglutination activity and structure of some lectins are affected by the number of divalent cations [Lutzik, 1981]. Therefore it is interesting to study the lectins properties of those structures, which are characterised by the high level of divalent cations. It is well known, that cells of prostatic tissue posses high level of magnesium and calcium ions [Tauber & Zaneveld, 1976], but the most specific features of these cells is the high content of zinc ions [Leslie et al., 1997]. Considerable portion of the zinc in the prostate appears to be bound to unique proteins (enzymes, metallothionein and other) [Suzuki et al, 1994; Suzuki et al., 1995]. In contrast to the high zinc content of normal human prostate, prostate cancer (adenocarcinoma) tissue contains very low zinc levels [Leslie et al., 1997]. Consequently it is evident, that ions play an important role in the disease [Costello & Franklin, 1998].

Take into consideration all above mentioned we investigated influence of divalent cations on the hemagglutination activity of cytoplasmic and plasma membrane lectins from prostate fiber-muscular tissue of different pathological conditions.

Materials and methods

Prostate post-operation fiber-muscular tissues with different diagnosis were investigated. Tissue samples were obtained from following pathological prostates: 1) BPH - benign prostatic hyperplasia; 2) BPH+PING 2-3 (prostatic intraepithelial neoplasia); 3) BPH+AAH (atipic adenomatoze hyperplasia).

The cytoplasmic and plasma membrane fractions of prostate tissue cells were obtained by differential centrifugation. The isolation of lectins from plasma membrane were carried out by detergent 0.2% Triton X 100 solution. Lectin activity was determined by hemagglutination test on 2% trypsin-treated rabbit erythrocytes suspension by serial 2-fold dilution of extract in microtitter-U-plates [Lutzik et al., 1983]. The relation between lectins and divalent ions has been investigated by hapten-inhibitory technique in hemagglutination area [Lutzik et al., 1983]. The following ions have been tested: Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, Ca^{2+}da Pb^{2+}. Minimal concentration required for inhibition of agglutination activity was determined.

In control experiments testified ions concentrations had no hemagglutination activity in 2% trypsin-treated rabbit erythrocytes suspension.

Results and Discussion

Results of experiments are summarised in Table 1. The effect of most ions on lectin hemagglutination activity changes in dependence on pathological conditions. The most striking effects are observed for Zn^{2+}. The minimal concentration of Zn^{2+} required agglutination inhibition is increased in cytoplasmic fraction in parallel with the severity of pathological condition. These results could indicate the low saturation of cytoplasmic lectins with Zn^{2+}, which in turn may be the effect of low Zn^{2+} content in malignant prostatic tissue. The minimal concentration of Ca^{2+} required for agglutination inhibition is decreased for plasma membrane lectins with worsening of pathology, but the opposite picture is observed for Mg^{2+} with the same membrane lectins (Table 1).

In conclusion, with the complication of disease the minimal concentration of several ions required for agglutination inhibition by cytoplasmic and plasma membrane lectins changes. These changes may be accounted by the altered saturation of prostatic lectins with these ions and/or by the changes of their properties. Changes in ions levels in pathological prostate gland could also be involved in the above outlined changes.

Table 1. The effects of divalent cations on the hemagglutination activity of lectin from prostate fiber-muscular tissue cells cytoplasmic and plasma membrane fractions with state of pathology

Divalent cations	BPH		BPH + PING 2-3		BPH+AAH	
	minimal concentration of ions required for agglutination inhibition					
	cytoplasm	plasma membrane	cytoplasm	plasma membrane	cytoplasm	plasma membrane
Zn^{2+}	50 nM	100 nM	200 nM	25 nM	19.5 μM	6.25 nM
Mg^{2+}	1.25 mM	0.625 mM	0.625 mM	1.25 mM	156 μM	2.5 mM
Ca^{2+}	2.5 mM	2.5 mM	2.5 mM	1.25 mM	2.5 mM	0.625 mM
Mn^{2+}	1.25 mM	39 μM	1.25 mM	0.312 mM	0.675 mM	0.312 mM
Fe^{2+}	1.25 mM	0.625 mM	0.625 mM	0.625 mM	4.25 μM	0.625 mM
Pb^{2+}	no effect	10 mM	no effect	10 mM	no effect	no effect

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ორვალენტიანი კათიონების გავლენა პროსტატის უჯრედების სუბუჯრედული ფრაქციების ლექტინების ჰემაგლუტინაციურ აქტივობაზე სხვადასხვა პათოლოგიებისას

კვიწინაძე ნ.[1], მეგრელიშვილი ი.[1], დავითაშვილი ე.[1], სოლომონია რ.[1], ალექსიძე ნ.[1], ქარაზანაშვილი გ. [2]

[1] *ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, ბიოქიმიისა და ბიოტექნოლოგიის კათედრა*
თბილისის სახელმწიფო უნივერსიტეტი, სამედიცინო დიაგნოსტიკის ცენტრი

(მიღებულია 13.12.2005)

რეზიუმე

შესწავლილია ორვალენტიანი კათიონების გავლენა პროსტატის სხვადასხვა პათოლოგიების ფიბრო-მუსკულარული უბნის ციტოპლაზმისა და პლაზმური მემბრანის ლექტინების ჰემაგლუტინაციურ აქტივობაზე. განსაკუთრებით მაღალი მგრძნობელობით გამოირჩევა თუთიის იონები როგორც ციტოპლაზმის, ასევე პლაზმური მემბრანის ლექტინების მიმართ. დაავადების გართულებასთან ერთად ძლიერდება პლაზმური მემბრანის ლექტინების სპეციფიკურობა Ca^{2+}-ის მართ, ხოლო კლებულობს Mn^{2+}-სა და Mg^{2+}-ის მიმართ. ციტოპლაზმის ლექტინები კი მაღალ მგრძნობელობას ავლენენ Fe^{2+}-სა და Mg^{2+}-სადმი ქსოვილის გადაგვარებასთან ერთად. გაკეთებულია დასკვნა, რომ ლექტინებისა და ორვალენტიანი კათიონების ფუნქციური ურთიერთკავშირი დამოკიდებულია პროსტატის დაავადების ფორმაზე.

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BASELINE PARAMETERS OF METABOLIC DISBALLANCE IN OBESE PATIENTS

MERKVILADZE N., KESHELAVA M., TUSHURASHVILI P.

Department of Medical Chemistry, Tbilisi State Medical University

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Abstract

In the patients with different level of obesity the lipid and carbohydrate metabolism was evaluated. The levels of plasma triacylglycerols (TG), cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), the oral glucose tolerance test (OGTT) and glycosylated hemoglobin (HbA_{IC}) were examined. It was shown that 12% of obese patients appeared to be ill with diabetes mellitus and 44% - with boarder line diabetes. HbA_{1C} measurement may allow refinement of diabetic risk and provide more precise identification of candidates for lifestyle intervention.

Key words: obesity, diabetes mellitus 2 type, HbA_{IC}, OGTT.

Introduction

General practitioners are being increasingly asked to play a key role in the shared care of obese people, but data concerning the effects of this in Georgia are still scarce. It widely known, that obesity is quite serious risk of developing obesity-related diseases, such as atherosclerosis, cardiovascular diseases, type 2 diabetes mellitus, hypertonic disease [Chan W.B., 2004; Skrha J., 2005].

The aim of this research is complex relationships between lipid and carbohydrates metabolism in obese patients. Obesity is often associated with type 2 (non insulin-dependent) diabetes. A growing body of evidence support the hypothesis that these two diseases share a common pathogenesis. Nevertheless, experience derived from clinical observation on type 2 diabetic patients indicates that reduction of body weight is not always accompanied by an improvement in metabolic control and that a good metabolic control is often obtained without influencing body composition [Fumelli P., 2000].

Materials and Methods.

65 patients with various level of obesity were investigated: control group (14 male and female individuals), overweight patients with Body Mass Index (BMI) 25 to 30, and obese patients. Obese patients we divided into two groups: BMI viz. 30-35 and BMI>35. To evaluate the lipid metabolism we examined the levels of plasma triacylglycerols (TG), cholesterol, low density lipoproteins (LDL) and high density lipoproteins (HDL) (Table 1). Furthermore we had determined the glycemic control of obese patients by measuring oral glucose tolerance test (OGTT) and glycosylated hemoglobin (HbA_{IC}) (Table 2).

Results and discussion

Disbalance in lipid metabolism, shown in the Table 1, indicated atherogenic type changes in the blood: concentration of cholesterol is moderately increased in obese patients, there are elevated levels of TG-s and LOL, but decreased HDL.

Investigation of carbohydrate profile showed poor correlation between BMI and HbA_{IC}. These results are in agreement with the scientific data [Aldebesi S.A., 2003]. However in obese patients with type 2 diabetes mellitus the level of HbA_{IC} was between 8-9%, but in the control group at was not above 5.5%.

Our investigation confirmed serious disorders in metabolic processes in obese patients: 12% of obese patients appeared to be ill with diabetes mellitus and 44% - with boarder line diabetes.

These results matched with others about BMI and HbA_{IC} are the only significant predictors of new onset diabetes, with HbA_{IC} heaving a greater effect than BMI [Edelman D., 2004]. Glycosylated hemoglobin HbA_{IC} provides practical assessment of long-term glycemic control on obese patients [Pettitt D.Y., 2004]. HbA_{IC} values would allow risk stratification for patients, likelihood of developing diabetes. Patients, with BMI over 27.5 and high normal HbA_{IC} had a modestly increased incidence of diabetes and may also merit closer attention and more frequent periodic screening than patients of normal weight [Edelman D., 2004].

Table 1. Parameters of lipid metabolism in obese patients

Parameters of lipid metabolism	Control group female n=6	Control group male n=5	Female and male obese patients n=36	Male obese patients n=29
Total Cholesterol	185±10 mg/dl	190±10 mg/dl	210±10 mg/dl P<0,005	224±10 mg/dl P<0,005
TG	170±10 mg/dl	177±10 mg/dl	220±20 mg/dl P<0,005	231±20 mg/dl P<0,005
LDL	2,1±0,005 g/l	2,1±0,005 g/l	2,9±0,01 g/l P<0,001	2,6±0,01 g/l P<0,001
HDL	83±0,5 ng/l	82±1 ng/l	75±6 ng/dl P<0,005	76±7 ng/dl P<0,005

Table 2. Glycosylated hemoglobin in obese patients

Investigated individuals	HbA_{IC}
Control group n=14 BMI<25	4,5%±0,5 P<0,01
Obese patients BMI 25-30 n=13	6,3%±0,5 P<0,01
Obese patients BMI 30-35 n=18	6,4%±0,5 P<0,01
Obese patients BMI>35	7,2±0,5 P<0,01

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მეტაბოლური დარღვევების ძირითადი მაჩვენებლები სიმსუქნის დროს

მერკვილაძე ნ., კეშელავა მ., თუშურაშვილი პ.

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი

(მიღებულია 05.12.2005)

სიმსუქნის სხვადასხვა ხარისხის პაციენტებში შესწავლილია ლიპიდური და ნახშირწყლოვანი ცვლების მაჩვენებლები: საერთო ქოლესტეროლი, ტრიაცილგლიცეროლები, HDL და LDL, გლუკოზის ტოლერანტობის პერორალური ტესტი (OGTT), გლიკოზილირებული ჰემოგლობინი HbA_{IC}. მიღებული მონაცემებით დადგენილია დადებითი კორელაცია ლიპიდური და ნახშირწყლოვანი ცვლების მაჩვენებლებს შორის სიმსუქნის დროს; რითაც დასტურდება, რომ სიმსუქნე წარმოადგენს რისკ ფაქტორს შაქრიანი დიაბეტის 2 ტიპის განვითარებისათვის. სიმსუქნით დაავადებულთა 12%-ში აღინიშნა 2 ტიპის შაქრიანი დიაბეტი, ხოლო 44%-ში დიაბეტის მოსაზღვრე ფორმა. ყველა შემთხვევაში მგრძნობიარე და შედარებით ხელმისაწვდომ სადიაგნოსტიკო ტესტს წარმოადგენდა HbA_{IC}-ს განსაზღვრა სისხლში.

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ეროვნული ბიბლიოთეკა

SATELLITE DNA BENDING DEPENDENCE ON SODIUM ION CONCENTRATION

PIPIA I.

S. Durmishidze Institute of Biochemistry and Biotechnology of the Georgian Academy of Sciences
E-mail: pipiaia@yahoo.co.uk

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Abstract

The mobility of three different satellite DNA monomers (*Citrus limon*, *Poncirus trifoliata* and *Mus musculus*) in polyacrylamide gel as a temperature function in the presence 0.015 M and 0.15 M NaCl was investigated. The dependences of K-factor on the temperature of mouse satellite DNA monomer at different ionic strength don't differ from each other and from the curves in the absence of NaCl. They have chair-like form. In the presence of 0.015 M NaCl two components are observed in the case of *Poncirus trifoliata* satellite DNA at the temperature interval 5 – 45°C. At 55°C only one component is observed. In the presence of 0.15 M NaCl the monomer splitting is not observed. In the case of lemon satellite DNA monomer splitting is not observable at the presence of NaCl at all. In the case of mouse satellite DNA monomer the presence of NaCl does not influence monomer splitting.

Key words: electrophoresis, mouse, Citrus limon, Poncirus trifoliata, sodium ion.

Introduction

In our earlier studies the mobility of three different satellite DNA monomers (*Citrus limon*, *Poncirus trifoliata* and *Mus musculus*) in polyacrylamide gel (PAAG) as a temperature function has been studied. K-factor (the ratio of apparent length to the actual length) dependence on temperature for both – mouse monomer and dimer has chair-like form [Pipia, 2004; Pipia, 2005].

The specific form of the curve was explained on the basis of CDH-form (Coiled double helix) characteristic for satellite DNA. It was also found that satellite DNA monomers of *C.limon* and *P.trifoliata* in the solution exist in two – bent and straight forms at low ionic strength in the temperature interval 5-35°C [Pipia, 2005].

In this paper the dependence of satellite DNA monomer mobility on temperature at different ionic strength – in the presence 0.015 M and 0.15 M NaCl was investigated.

Materials and Methods

Nuclear DNA isolation from mouse liver and its partial hydrolysis by restriction endonuclease *Sau96 I* was described earlier [Pipia, 2004]. Nuclear DNA isolation from *C.limon* and *P.trifoliata* leaves was performed according to Beridze et al. [Beridze et al., 1992]. Partial hydrolysis of nuclear DNA of these species by restriction endonuclease StyI was carried out by the

recommendations of manufacturer (Promega). Agarose and polyacrylamide gel electrophoresis procedures were described earlier [Pipia, 2004]. For the investigation of Na^+ influence on monomer mobility NaCl was added directly to electrophoresis buffer and was also included in the polyacrylamide gel during its preparation.

Results and Discussion

It was earlier shown that in the case of lemon and *P.trifoliata* monomers were split into two components at 5°C [Pipia, 2005]. The lengths of the retarded components are 184 bp and 185 bp. The length of fast moving components of both plants is 181 bp, in accordance with actual length of monomers in agarose gel. At the temperature elevation the observed two components are united. Usually the content of the retarded component is about 10 %. In the case of mouse satellite DNA monomer the splitting can be observed at high temperatures - 45°C and 55°C.

In the table 1 the NaCl concentration dependence on the splitting of satellite DNA monomers in 5% PAAG at different temperature and different NaCl concentration are presented.

Table 1. NaCl concentration dependence of mouse, *P.trifoliata* and lemon satellite DNA monomer splitting in 5% PAAG at different temperatures. 1 – monomer is visible as one component; 2 – monomer is split into two components.

Tempera-ture	Mus musculus			Poncirus trifoliata			Citrus lemon	
	0	0,015M NaCl	0,15M NaCl	0	0,015M NaCl	0,15M NaCl	0	0,015M NaCl
5°C	1	1	1	2	2	1	2	1
15°C	1	1	1	2	2	1	2	1
25°C	1	1	1	2	2	1	1	1
35°C	1	1	1	2	2	1	1	1
45°C	2	2	2	1	2	1	1	1
55°C	2	2	2	1	1	1	1	1

In the Fig.1 and 2 the dependence of K-factor on the temperature of mouse satellite DNA monomer at different ionic strength – 0.015M and 0.15M NaCl are given. Generally the forms of the curves don't differ from each other and from the curves in the absence of NaCl. They have chair-like form in both cases. In the case of mouse satellite DNA monomer the presence of NaCl does not influence the monomer splitting.

In the presence of 0.015 M NaCl two components are observed in the case of *Poncirus trifoliata* satellite DNA within the temperature interval 5 – 45°C (Fig.3). Only at 55°C one component is observed. In the presence of 0.15 M NaCl the monomer splitting is not observed (Fig.4). In the case of lemon satellite DNA monomer splitting is not observable at the presence of NaCl at all.

The $MgCl_2$ and NaCl dependence on the gel migration anomaly for DNA molecules was measured [Diekmann, 1987; Diekmann, 1987]. For all sequences analyzed, at the addition of NaCl to the gel, the running buffer decreases migration anomaly. The increasing amounts of NaCl might affect the curved DNA structure itself; in addition, it influences the DNA flexibility. The gel migration anomaly is considerably increased when normal DNA sequences are presented on both ends of the fragment up to about the persistence length. Since NaCl reduces the persistence length, the contribution of the ends to the effect is reduced. In the measured NaCl concentration range the

reduction of the persistence length is small; indeed, the reduction of the migration anomaly due to additional NaCl is small.

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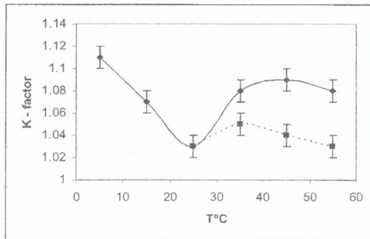

Fig.1. The temperature dependence of K-factor of mouse satellite DNA monomer in the presence 0.015 M NaCl. Full line shows the retarded component of stDNA, punctuated line shows the main component of satellite DNA.

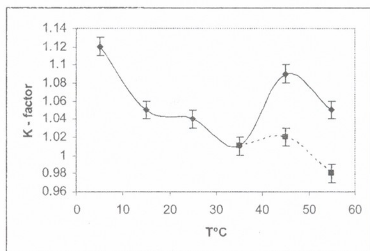

Fig. 2. The temperature dependence of K-factor of mouse satellite DNA monomer in the presence 0.15 M NaCl. Full line shows the retarded component of stDNA, punctuated line shows the main component of satellite DNA.

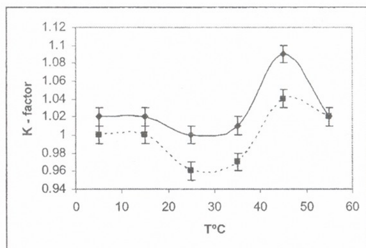

Fig.3. The temperature dependence of K-factor of *Poncirus trifoliata* satellite DNA monomer in the presence 0.015 M NaCl. Full line shows the retarded component of stDNA, punctuated line shows the main component of satellite DNA.

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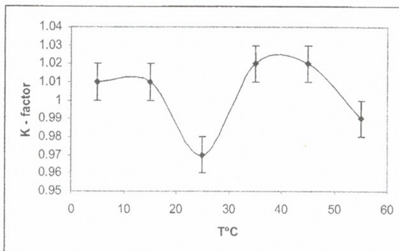

Fig.4. The temperature dependence of K-factor of *Poncirus trifoliata* satellite DNA monomer in the presence 0.15 M NaCl.

A different picture is obtained for the $MgCl_2$ dependence; the $MgCl_2$ influence on the migration anomaly is sequence dependent. For the kinetoplast DNA fragment and its parts as well as for most of the fragments analyzed by Diekmann, the addition of $MgCl_2$ increases the anomaly. Obviously, the effect of the salt on the DNA persistence length does not play a dominant role for the $MgCl_2$ dependence on the migration anomaly.

In general it can be concluded that the addition of NaCl to the gel and the running buffer decreases migration anomaly of lemon and *P.trifoliata*, but not the mouse.

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ნატრიუმის იონების ზეგავლენა სატელიტური დნმ-ის გადაღუნვებზე

ფიფია ი.

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

(მიღებულია 12.12.2005)

რეზიუმე

შესწავლილი იყო სამი განსხვავებული სატელიტური დნმ-ის (*Citrus limon*, *Poncirus trifoliata* და *Mus musculus*) მონომერების ძვრადობა პოლიაკრილამიდის გელში როგორც ტემპერატურის ფუნქცია 0.015 M და 0.15M NaCl თანაობისას. თაგვის სატელიტური დნმ-ის მონომერის K-ფაქტორის ტემპერატურაზე დამოკიდებულების მრუდები როგორც NaCl-ის თანაობისას, ასევე მის გარეშე არ განსხვავდებიან ერთმანეთისაგან. მათ სავარძლისებური ფორმა აქვთ. 0.015M NaCl–ს თანაობისას *Poncirus trifoliata*-ს სატელიტური დნმ 5–45°C ტემპერატურულ ინტერვალში იყოფა ორ კომპონენტად. 55°C–ზე მხოლოდ ერთი კომპონენტი შეიმჩნევა. 0,15M NaCl–ს თანაობისას *Poncirus trifoliata*-ს მონომერის გაყოფა არ ხდება. ლიმონის მონომერი NaCl-ს თანაობისას საერთოდ არ იყოფა. NaCl გავლენას არ ახდენს მონომერების დაყოფაზე თაგვის სატელიტური დნმ-ის შემთხვევაშიც.

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ეროვნული ბიბლიოთეკა

EFFECT OF NITROBENZENE ON AMMONIA ASSIMILATION ENZYMES AND CELL ULTRASTRUCTURE IN MAIZE AND SOYBEAN

*SADUNISHVILI T.[1], KUPRAVA N.[1], APLAKOV V.[1], BETSIASHVILI M.[1], ZAALISHVILI G.[1], MITHAISHVILI T.[2], KVESITADZE E[1].

**E-mail: sadunishvili@hotmail.com*
[1]S.Durmishidze Institute of Biochemistry and Biotechnology
[2]Georgian State Agricultural University

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Abstract

Changes in the activities of enzymes of ammonia assimilation were studied in roots of maize and soybean exposed to different concentrations of nitrobenzene at different time of exposure. It was demonstrated that nitrobenzene at concentration 0.015mM did not cause any noticeable changes, while significant increase of glutamate dehydrogenase and slight inhibition of glutamine synthetase activities were observed under the influence of 0.15 mM nitrobenzene. Simultaneously, substantial reorganization of plant cell ultrastructure takes place, expressed in slightly brightened mitochondria as well as increase of the ribosome quantity and contacts of endoplasmic reticulum with mitochondria and plasmalemma. High concentration of nitrobenzene caused inhibition of both enzymes intensified with prolongation of exposure. After the termination of the influence of nitrobenzene, the return of enzyme activities to initial levels was observed in case of effective 0.15mM but not highly toxic 1.5 mM concentration of nitrobenzene.

Key words: Glutamate dehydrogenase, glutamine synthetase, γ-glutamylhydroxamic acid, spectrophotometrical method.

Introduction

Plants are capable to absorb and detoxify xenobiotics – environmental contaminants. Main part of hydrocarbons, absorbed by plants is transformed by conjugation with endogenous compounds after their hydroxylation. Hydroxylation of aromatic ring is an important step in the process of transformation of many arene derivatives. One of the most important pathways of detoxification is deep oxidation, which in case of aromatic compounds proceeds by aromatic ring splitting and forming typical cell metabolites [Korte et al., 2000]. This is the way of transformation in plants of highly toxic compound nitrobenzene, distinguished by stability and inability of autooxidation [Mithaishvili et al., 2005].

Enzymes participating in detoxification process, catalyzing conjugation and oxidation reactions are revealed [Kvesitadze et al., 2005, 2006]. Their induction with xenobiotics has also been demonstrated [Khatisashvili et al., 1997]. These data indicate on plant response to

contaminated environment in which induction of enzymes responsible for transformation of xenobiotics to avoid their harmful effect is of primary importance.

Presumably, in the multi step process of deep transformation of toxic compounds enzymes are indirectly involved which provide the plant cell with energy and are important for defense reactions by the provision of necessary endogenous compounds and secondary metabolites [Kvesitadze et al., 2001, Chrikishvili et al., 2006]. However there is little data indicating on participation of main metabolic enzymes in the detoxification process.

The aim of the present work is study of the effect of different concentrations of nitrobenzene at different exposure time on key enzymes of ammonia assimilation, such as glutamate dehydrogenase and glutamine synthetase as well as cell ultrastructural organization in maize and soybean seedlings roots.

Materials and methods

Experiments were carried out on 7-days old water cultures of maize (*Zea mays*) and soybean (*Glycine max*). Plant seedlings were exposed to 0.015mM, 0.15mM and 1.5mM nitrobenzene containing Knop's solution, exposure time 1, 24 and 72 hours. Control plants were exposed to Knop's solution. After 72 hour the plants were transferred to Knop's solution lacking nitrobenzene for 24 and 48 hours. After each exposition plants roots were washed and homogenized in 50 mM Tris-HCl buffer, pH 7.5. In the supernatant after centrifugation at 22000g activities of enzymes and protein amount were determined.

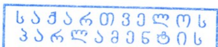
Glutamate dehydrogenase amination activity was determined spectrophotometrically at 340nm according to the rate of NADH oxidation in reaction mixture described earlier [Sadunishvili et al., 1993]. Glutamine synthetase activity was determined by a colorimetric method, according to the amount of γ-glutamylhydroxamic acid (γ-GHA) formed in a transferase reaction in mixture described earlier [Sadunishvili et al., 1996]. Specific activities of enzymes were expressed in micrograms of oxidized NADH or formed γ-GHA per mg of protein.

Protein was determined according to Bredford [Bradford et al., 1974].

For study of cell ultrastructure root tips were excised and $1mm^3$ samples fixed in a 2.5% solution of glutaraldehyde with postfixation in 1% osmium tetroxide. After dehydration in graded series of ethanol solutions the samples were embedded in Epon-Araldite resin (1.5:1.0) and poured into gelatin capsules. Ultrathin sections were made using Reichert Ultramicrotome, stained with uranyl acetate and examined in a Tesla BS 500 electron microscope.

Results and discussion

Results of action of nitrobenzene on nitrogen metabolism enzymes in maize and soybean are presented in Tables 1 and 2. As it is seen from Table 1, there is correlative dependence between the concentration of nitrobenzene, time of exposure and changes in the activities of studied enzymes. Namely, in case of maize at short term, one hour exposure to low nitrobenzene concentration – 0.015mM, there is practically no effect on either glutamate dehydrogenase or glutamine synthetase activities. No deviations from norm were revealed in the ultrastructural organization of a cell of plants exposed to this concentration of nitrobenzene. However at higher nitrobenzene concentration – 0.15mM, there is 25-50% stimulation of glutamate dehydrogenase, which is maximal at 24 h exposure. The highest tested concentration of nitrobenzene – 1.5mM caused time dependent inhibition of the enzyme activity with maximum at 72 h exposition. Glutamine synthetase appeared to be less sensitive to nitrobenzene, except the case of high concentration – 1.5mM, when 60% inhibition, even more than in case of glutamate dehydrogenase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at prolonged exposure was observed. At lower concentrations of nitrobenzene there was slight inhibition of this enzyme.

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Table 1. Glutamine synthetase and glutamate dehydrogenase activities in roots of maize seedlings exposed to nitrobenzene

Exposure time, h	Nitrobenzene concentration, mM	Protein, mg/ml	Glutamine synthetase activity, μM γ-GHA*/min x mg protein	Glutamate dehydrogenase activity, μM NADH/min x mg protein
1	0	0.20	10.0	0.060
	0.015	0.19	10.0	0.068
	0.15	0.24	9.2	0.090
	1.5	0.18	8.6	0.050
24	0	0.20	9.6	0.048
	0.015	0.20	7.8	0.030
	0.15	0.25	7.3	0.068
	1.5	0.18	4.6	0.032
72	0	0.19	4.1	0.032
	0.015	0.18	3.5	0.034
	0.15	0.20	2.7	0.040
	1.5	0.14	1.6	0.019
72 followed by 24 h incubation on nitrobenzene-free medium	0	0.20	4.7	0.040
	0.015	0.20	4.1	0.040
	0.15	0.21	3.7	0.044
	1.5	0.16	2.2	0.028
72 followed by 48 h incubation on nitrobenzene-free medium	0	0.20	2.4	0.040
	0.015	0.18	2.2	0.040
	0.15	0.18	1.9	0.038
	1.5	0.16	1.2	0.028

*γ-Glutamylhydroxamic acid

Slight increase in protein content in experimental plant roots under the influence of nitrobenzene, except the highest dose was observed. On the increase of protein synthesis also indicates the increase in ribosome quantity in maize root cortical cells under the influence of 0.15mM nitrobenzene (Fig.2,3).

Similar results were observed for soybean; however the inductive effect of nitrobenzene on glutamate dehydrogenase in this plant was expressed at less extent (Table 2). It could be stated that soybean is more resistant to the nitrobenzene action than maize.

Based on these results, it could be suggested that there is concentration and exposure dependent response of nitrogen metabolism enzymes to nitrobenzene: low concentration 0.015mM does not cause any significant changes; higher concentration 0.15mM revealed the induction of glutamate dehydrogenase activity, however the highest concentration caused the inhibition of the enzyme. These results indicate that glutamate dehydrogenase is indirectly involved in the process of detoxification of nitrobenzene. Stimulation of amination activity could be related to increased protein synthesis which often takes place under toxicity stress, needed for increased synthesis of oxidation enzymes as well as of both substrate and enzyme for conjugation [Zaalishvili et al., 2000; Kvesitadze et al., 2006]. The increase of activities of enzymes, participating in energy and nitrogen metabolism was reported in plants, exposed to benzidine [Chrikishvili et al., 2006]. Inhibition of glutamine synthetase up to 50% and simultaneous stimulation of glutamate dehydrogenase took place in alfalfa as a result of phosphinotricine exposure [Bataynen et al., 1986].

Table 2. Glutamine synthetase and glutamate dehydrogenase activities in roots of soybean seedlings exposed to nitrobenzene

Exposure time, h	Nitrobenzene concentration, mM	Protein, mg/ml	Glutamine synthetase activity, μM γ-GHA*/min.mg protein	Glutamate dehydrogenase activity, μM NADH/min.mg protein
1	0	0.66	12.6	0.09
	0.015	0.70	12.7	0.10
	0.15	0.72	10.8	0.11
	1.5	0.58	5.8	0.08
24	0	0.44	8.3	0.07
	0.015	0.44	7.6	0.09
	0.15	0.46	7.2	0.09
	1.5	0.40	3.2	0.06
72	0	0.34	6.4	0.07
	0.015	0.34	5.8	0.07
	0.15	0.36	4.61	0.08
	1.5	0.28	2.8	0.04
72 followed by 24 h incubation on nitrobenzene-free medium	0	0.28	4.8	0.06
	0.015	0.26	4.8	0.06
	0.15	0.26	4.6	0.07
	1.5	0.18	2.8	0.04
72 followed by 48 h incubation on nitrobenzene-free medium	0	0.20	3.2	0.07
	0.015	0.20	3.0	0.07
	0.15	0.21	3.4	0.07
	1.5	0.18	1.7	0.05

*γ-Glutamylhydroxamic acid

It is important to determine whether the changes in plants caused by toxic compounds are reversible or not and what are the limits of reversibility? Some answers on this question gave the data of Tables 1 and 2 obtained in experiments where plants exposed to nitrobenzene during 72 hours were transferred to nitrobenzene free medium, i.e. the action of this xenobiotic was terminated. As it is seen from tables, there is a tendency of return of enzyme activities to initial levels. However, in case of plant exposure to high concentration of nitrobenzene (1.5 mM), there is no restoration of initial activities, inhibition of glutamate dehydrogenase and glutamine synthetase is irreversible, indicating on toxicity of nitrobenzene at this concentration to plants.

Evidence of the changes in plant under the influence of 0.15mM nitrobenzene was obtained by electron microscopy of cell ultrastructure (Fig.1-3). Several deviations under the influence of nitrobenzene as compared to control were revealed and first of all expressed in the increase of quantity of ribosome and multiple contacts of endoplasmic reticulum with mitochondria and plasmalemma. This is typical reorganization of cell ultrastructure taking place during the transformation of xenobiotics to overcome their toxicity [Kvesitadze et al., 2001, 2006; Zaalishvili et al., 2002], indication on which is mitochondria with damaged cristae, and formation of gigantic vacuoles with depositions of cell degradation structures in it. The increase in ribosome quantity directly indicates on the increase of protein synthesis in cell. Such picture was not observed in soybean which is known to contain high protein in vegetative organs itself.

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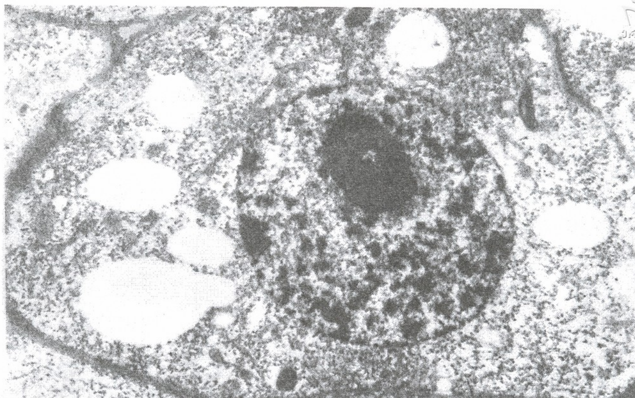

Fig.1. Maize roots cortical cell. Control variant. ×20000.

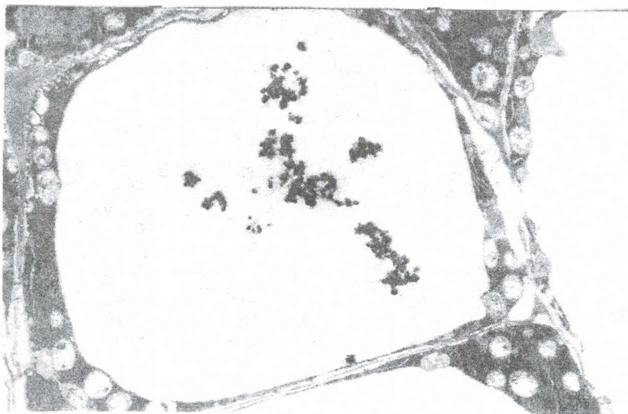

Fig.2. Cortical cell of roots of maize exposed to 0.15mM nitrobenzene, exposure time 24 hours. Gigantic vacuoles with osmiophilic insertions, brightened mitochondria. ×8000.

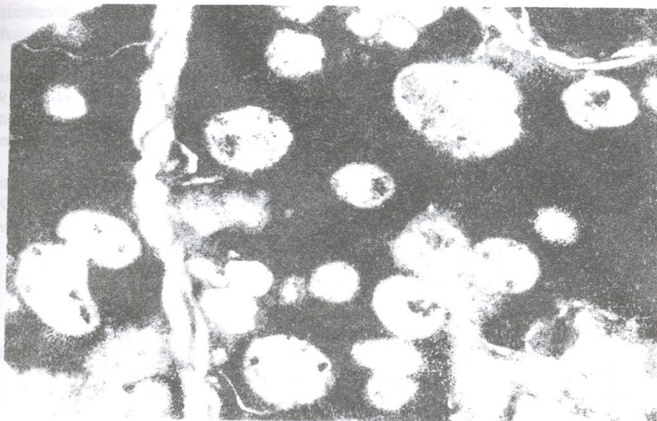

Fig. 3. Fragment of cortical cell of roots of maize exposed to 0.15mM nitrobenzene, exposure time 24 hours. Big quantity of ribosomes in cytoplasm multiple contacts of endoplasmic reticulum with mitochondria and plasmalemma. ×28000.

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ნიტრობენზოლის გავლენა სიმინდისა და სოიას ფესვებში ამონიუმის ასიმილაციის ფერმენტებსა და უჯრედის ულტრასტრუქტურაზე

სადუნიშვილი თ. [1], კუპრავა ნ. [1], აპლაკოვი ვ. [1], ბეციაშვილი მ. [1], ზაალიშვილი გ. [1], მითაიშვილი თ. [2], კვესიტაძე ე. [1]

[1]*დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი*
[2]*საქართველოს სახელმწიფო აგრარული უნივერსიტეტი*

(მიღებულია 09.01.2006)

რეზიუმე

შესწავლილია ნიტრობენზოლის სხვადასხვა კონცენტრაციისა და ექსპოზიციის ხანგრძლივობის გავლენა სიმინდისა და სოიას ფესვებში ამონიუმის ასიმილაციის ფერმენტების აქტივობებსა და უჯრედის ულტრასტრუქტურაზე. ნაჩვენებია, რომ ნიტრობენზოლი 0.015mM კონცენტრაციით არ იწვევს რაიმე შესამჩნევ ცვლილებას, მაშინ როდესაც გლუტამატდეჰიდროგენაზის აქტივობის მნიშვნელოვანი მომატება და გლუტამინსინთეტაზის მცირე ინჰიბირება შეიმჩნევა ნიტრობენზოლის 0.15mM კონცენტრაციის დროს. ამავე დროს აღინიშნება უჯრედის ულტრასტრუქტურის მნიშვნელოვანი რეორგანიზაცია, რაც გამოისახება ოდნავ განათებული მიტოქონდრიებისა და ენდოპლაზმური ბადის მრავლობითი კონტაქტების არსებობით მიტოქონდრიებსა და პლაზმალემასთან. ამ ნივთიერების მაღალი კონცენტრაცია იწვევს ორივე შესწავლილი ფერმენტის აქტივობის ინჰიბირებას, რომელიც ძლიერდება ექსპოზიციის ხანგრძლივობის გაზრდასთან ერთად. ნიტრობენზოლთან ინკუბაციის შეწყვეტის შემდეგ – მცენარის გადატანისას მისგან თავისუფალ არეზე აღინიშნება ფერმენტების აქტივობის საწყისი დონის აღდგენა 0.15mM ნიტრობენზოლის შემთხვევაში, მაგრამ არა ძლიერ ტოქსიკური 1.5mM კონცენტრაციის დროს.

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ეროვნული ბიბლიოთეკა

Biochemistry

ANTIRADICAL EFFICIENCY OF SOME FLAVONOID STANDARDS

SHALASHVILI A., ZAMBAKHIDZE N., TARGAMADZE I., SIMONISHVILI SH., PAPUNIDZE S., UGREKHELIDZE D.

S. Durmishidze Institute of Biochemistry and Biotechnology

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Abstract

Antiradical efficiency of 23 standard flavonoids in a radical-forming system containing 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) is investigated. The findings show that flavonoids are potential scavengers of free radicals, and antiradical efficiency with respect to DPPH is defined by chemical structure of flavonoid. Catechins and flavonols possess the strongest antiradical properties among flavonoids.

Key words: 1,1-diphenyl-2-picrylhydrazyl, free radical; structure-activity relationship.

Introduction

Flavonoids are a group of natural benzo-γ-pyrane derivatives ubiquitous in the plant kingdom [Zaprometov, 1993]. Flavonoids possess wide spectra of pharmacological activity including cardioprotective, spasmolytic, anti-inflammatory, radioprotective, antiallergic, hepatoprotective, antisclerotic, diuretic and other kinds of action [Middleton, 2000; Youdin, 2002; Shalashvili, 2002]. We have shown earlier the antioxidant activity of some standard flavonoids and grape bioflavonoids in hydrophylic and lipophilic systems [Shalashvili, 2002]. According to these data, in hydrophylic as well as in lipophylic systems the antioxidant activity of the flavonoids increased with the increase of the number of hydroxyl groups and was dependent on the position of hydroxyl groups.

The purpose of our work was study of antiradical efficiency of some standard flavonoids with the help of a free radical - 1,1-diphenyl-2-picrylhydrazyl [Sanchez-Moreno, 2002].

Materials and methods

23 Standard flavonoids have been studied in this experiment; from these compounds (-)-epicatechin-3-gallate, (-)-epigallocatechin-3-gallate, cyanidin, luteolin, astragallin, robinetin, myricetin, myricitrin and hesperetin have been received from professor M. Zaprometov (Moscow, Timirjazev Institute of Plants Physiology). (+)-Catechin was supplied by Theodor Schuchard (Munich); pelargonidin, dihydroquercetin and fisetin from Austrowaren (Vienna); malvidin and hesperidin from Gee Lawson Chemical (London); naringin from Loba-Chemical (Vienna); eriocitrin and eriodictyol were isolated from the peels of lemon fruit sp. "Dioscuria" [Tsiklauri, 1991]; apigenin from Serva (Heidelberg); morin from Fezak (Berlin); quercetin and rutin from

Chemapol (Prague); α-tocopherol from Sigma; avicularin was isolated from the leaves of *Rhododendron ponticum* [Durmishidze, 1981].

The solution (40 μM) of 1,1-diphenyl-2-picrylhydrazyl ($DPPH^•$) in ethanol has been prepared. To three milliliters of this solution 5 different concentrations of investigated flavonoid were added. After mixing solutions, samples were incubated for 30 minutes at room temperature. After incubation the optical density of samples was determined at 520 nm, with the spectrophotometer СФ-26 (Russia) [Sanchez-Moreno, 1998; Pochinok, 1985]. Each experimental variant has been repeated three times. Experimental data have been processed statistically by computer program "MS Excel". Antiradical efficiency (*AE*) has been calculated under the formula $AE = \frac{1}{EC_{50} \cdot T_{EC50}}$, where EC_{50} shows the amount of an antioxidant sufficient for reduction by 50 % of optical density of initial alcoholic solution of $DPPH^•$; for each sample this amount is calculated with the help calibration curve and expressed as grams of an antioxidant per kg of $DPPH^•$; T_{EC50} is time in minutes during which full decoloration of a solution is achieved.

Results and discussion

As shown in table 1, of the tested flavonoids the highest antiradical efficiency possess catechins: (-)-epigallocatechin-3-gallate, (-)-epicatechin-3-gallate and (+)-catechin, which are 10 times more effective than α-tocopherol. Interesting regularity is observed by comparison of antiradical efficiency of flavonoids possessing of 3, 5, 7, 3', 4'-pentahydroxy-structure: (+)-catechin, quercetin, cyanidin, dihydroqercetin. According to the data of Table 1, antiradical efficiency of flavonoids possessing of 3, 5, 7, 3', 4'-pentahydroxy-structure, decreases in a following line: (+)-catechin > quercetin > cyanidine = dihydroqercetin. In this case it is necessary to note considerably higher activity of (+)-catechin which is 11 times more effective, than quercetin, in spite of the fact that the last includes 2,3-double bond and 4-keto functional group which raise radical-binding capacity owing to delocalization of electron from ring B [Sanchez-Moreno, 2002]. In LDL oxidation system, Teissedre et al showed that catechin, which lacks a keto group in the 4-position, was more inhibitory than the flavonol myricetin, which was more active than quercetin, both having a keto group in the 4-position [Teissedre, 1996]. It is also necessary to note that on antioxidizing efficiency, equivalent to Trolox, quercetin exceeds catechin two times [Rice-Evans, 1996]. Such difference in activity of these two compounds can be explained to that quercetin reacts with $DPPH^•$ more slowly [Gordienko, 1988].

In separate groups of flavonoids antiradical efficiency decreases as follows: in anthocyanins - cyanidin > pelargonidin > malvidin; in flavanols – dihydroquercetin > eriodictyol > eriocitrin > hesperetin > naringin > hesperidin; in flavons – luteolin > morin > apigenin; in flavonols – myricetin > myricitrin > quercetin > robinetin = fisetin > rutin > avicularin > astragallin. These data show that in all classes of flavonoids antiradical efficiency is directly connected to quantity of hydroxyl groups and their disposition in a molecule.

It is known that glycosylation of flavonoids reduces their antioxidant activity, in comparison with corresponding aglycons [Rice-Evans, 1996]. According to the data in Table 1, antiradical activity of quercetin, myricetin and eriodictyol decreases after glycosylation of their ring C in the third position. In case of quercetin, this parameter decreases almost twice (for avicularin AE = 0.11, and for rutin AE = 0.14), in case of myricetin this parameter decreases 1.6 times (for myricitrin AE = 0.33), and in a case of eriodictyol – 1.8 times (for eriocitrin AE = 0.05).

Effect of ortho-diphenol structures on antiradical activity is well visible on an example of quercetin and morin (Table 1). Antiradical efficiency of morin (in ring B the hydroxyl groups are

situated to each other in a meta-position) is 3 times less of quercetin efficiency. If we compare antiradical efficiency of myricetin and robinetin, we shall see that robinetin (AE=0.23) is less effective, almost in 2 times, than myricetin (AE=0.52). In contrast to myricetin, robinetin has no hydroxyl group in the fifth position of a ring A. Apparently, on antiradical efficiency of flavonols an essential influence renders the presence of hydroxyl group in the fifth position.

Table 1. Antiradical efficiency of the standard flavonoids

Standard flavonoid	EC_{50} (g antioxidant per kg of DPPH$^{\bullet}$)	$T_{EC50,}$ (min)	Antiradical efficiency, AE
(+)-Catechin	212 ± 4.3	1.5	3.1
(-)-Epicatechin-3-gallate	59 ± 0.6	5	3.4
(-)-Epigallocatechin-3-gallate	55 ± 0.4	6	3
Pelargonidin	684 ± 1.5	9	0.16
Cyanidin	368 ± 2.5	13	0.21
Malvidin	777 ± 3.1	25	0.05
Naringin	> 5000	-	-
Eriodictyol	528 ± 3.8	20	0.09
Eriocitrin	948 ± 3.9	20	0.05
Hesperetin	> 5000	-	-
Hesperidin	> 5000	-	-
Dihydroquercetin	239 ± 1.5	21	0.2
Apigenin	1399 ± 9.0	27	0.02
Luteolin	495 ± 8.0	15	0.13
Morin	510 ± 12.8	25	0.08
Fisetin	169 ± 2.2	25	0.23
Astragallin	824 ± 0.4	14	0.08
Quercetin	140 ± 2.5	26	0.27
Avicularin	453 ± 4.8	20	0.11
Rutin	253 ± 5.4	28	0.14
Robinetin	162 ± 2.0	27	0.23
Myricetin	130 ± 1.9	15	0.52
Myricitrin	334 ± 3.7	9	0.33
α-Tocopherol	345 ± 3.3	9	0.32

On the basis of T_{EC50} values, Sanchez-Moreno at al. classified kinetics of actions of antioxidant compounds as follows: < 5 min (fast); 5 - 30 min (average) and > 5 min (slow) [Sanchez-Moreno, 1998]. According to the data in the Table 1, from the flavonoids under study, (+)-catechin and (-)-epicatechin-3-gallate belong to the fast reacting compounds, and other compounds make intermediate group where T_{EC50} varies within the ranges of 6-28 minutes (Table 1). Thus, catechins and flavonols are distinguished among flavonoids by their antiradical efficiency.

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ზოგიერთი სტანდარტული ფლავონოიდის ანტირადიკალური ეფექტურობა

შალაშვილი ა., ზამბახიძე ნ., თარგამაძე ი., სიმონიშვილი შ., პაპუნიძე ს., უგრეხელიძე დ.

ს. დურმიშიძის სახ. ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 12.01.2006)

რეზიუმე

შესწავლილია 23 სტანდარტული ფლავონოიდის ანტირადიკალური ეფექტურობა 1,1-დიფენილ-2-პიკრილჰიდრაზილის (DPPH•) რადიკალის წარმომქმნელ სისტემაში. მიღებული შედეგები მიუთითებს, რომ ფლავონოიდური ნაერთები თავისუფალი რადიკალების პოტენციური შემბოჭველებია და DPPH•-ის რადიკალის მიმართ აქტიურობა გაპირობებულია მათი მოლეკულების ქიმიური სტრუქტურით. ფლავონოიდებიდან ანტირადიკალური ეფექტურობით გამოირჩევა კატეხინები და ფლავონოლები.

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ეროვნული ბიბლიოთეკა

Biophysics

STUDY OF TEMPERATURE AND PH EFFECT ON STRUCTURAL PROPERTIES OF α-ACTININ MOLECULE USING INTRINSIC FLUORESCENCE METHOD

*ZAALISHVILI T., GACHECHILADZE N., LOMIDZE L., TORIASHVILI T., GAMKRELIDZE TS., ZAALISHVILI M.

Institute of Molecular Biology and Biological Physics, Georgian Academy of Sciences
**E-mail: tzaalishvili@yahoo.com*

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Abstract

The influence of temperature and pH on the structural properties of frog skeletal muscle α-actinin has been studied by means of intrinsic fluorescence method. It has been shown that at 15°C the alkaline denaturation of α-actinin starts above pH 9 and sour environment doesn't cause unfolding of structure but only protein aggregation occurs. The transition of α-actinin from native into denaturated state in 42-70°C temperature range is determined. Besides, one more weak transition can be noticed in 18-30°C temperature range.

Key words: α-actinin, fluorescence intensity, quantum yield.

Introduction

The investigation of physical–chemical and biophisical parameters of contractile muscles of separate representatives of amphibia has essential importance for their confrontation with their physiological indexes. All this is also significant in order to extend comparative-biological researches.

At the same time with main concractile proteins the essential attention must be paid to the study of physical-chemical properties of frog skeletal muscle minor protein α-actinin. α-Actinin is an F-actin cross-linking protein that is found in stress fibers and adhesion plaques of nonmuscle cells, as well as in Z-discs and their homologues in muscle cells [Blanchard et al., 1988]. Its function in the cell is not clear, but its subcellular distribution suggests that it may be important for the attachment of cytoskeletal structures to the membrane. α-actinin is homodimer, which contains subunits with molecular mass ~100 000 D. The content of α-helices in 45-75%, isoeletric point is located within pH 4.7–6.5.

Earlier we have studied biological activity and molecular parameters of frog (Rana Ridibunda) skeletal muscle α-actinin and by means of calorimetric method also investigated its melting process [Zaalishvili et al., 1983; Intskirveli et al., 1991; Zaalishvili et al., 1991]. In given work frog skeletal muscle protein α-actinin changes caused by variation of temperature and pH of reaction area has been studied by means of intrinsic fluorescence method for more complete characterizing of α-actinin structural properties.

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Materials and Methods

α-Actinin was isolated from frog skeletal muscle according to Goll et al. [Goll et al., 1972]. The purity of preparations was examined by means of Laemli method [Laemli et al., 1970]. The fluorescence spectra were measured with RF-5000 "Shimadsu" spectrofluorometer. Spectra position and maximum intensity were determined automatically. The rate of cuvette heating was of the order 1-2° K/min.

Results and Discussion

Earlier, while investigation of the melting process of frog skeletal muscle α-actinin we have shown that the transition of macromolecules from native into denaturated state occurs in the 42 – 47°C temperature interval, that is up to 42°C the structural changes of α-actinin aren't noticed. It is interesting to study α-actinin structural changes occurring in molecule by means of intrinsic fluorescence method, sensible for their registration.

Practically all proteins maintain natural fluorophores such as tyrosine and tryptophan. The fluoroscence of the most proteins is mostly stipulated by the tryptophan amino acid residues, the indole groups of which are particularly sensitive and complex fluorophores. α-actinin molecule contains ~30 tryptophan residues. As a rule the protein fluoroscence gets exited at absorption maximum - 280nm or higher wave length. The changes in any of luminescence parameters are recorded on the basis of registering the fluoroscent radiation intensity. The flouroscent spectra and yield are especially sensitive. As a rule the quantum yield is discussed according to the fluoroscent intensity measured at spectrum maximum. Spectrum shifts occur only with a change in protein conformation, whereas quantum yield undergoes change with a disruption of the protein secondary and tertiary structures. As fluoroscent parameters are essentially dependent on the environmental conditions, proceeding from abovementioned, the use of intrinsic fluorescence method and study of the influence of temperature and pH of reaction area on α-actinin molecule allows us to judge about the protein properties.

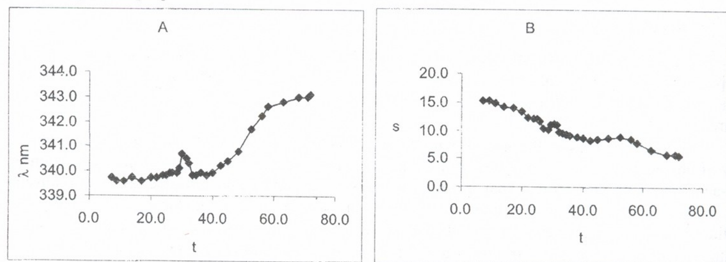

Fig.1. The dependence of frog skeletal α-actinin ($3 \cdot 10^{-5}$ M) fluorescence spectrum position A and the relative quantum yield B on temperature, pH 8, excitation wave length 280 nm.

Fig.1 shows the dependence of α-actinin fluoroscence parameters on temperature. As is seen from the figure, α-actinin thermal denaturation takes place within a 42 – 47°C temperature range, that is manifested in luminescence spectrum shift to the longer wave length region (~3nm) and increase of relative yield of radiation. Besides, in 18–30°C interval the curve is slightly

crooked that indicates to the existence of thermal transition. It must be mentioned, that the thermal transition in this interval is recorded by intrinsic fluorescence and other methods while investigating the rabbit and carp α-actinin [Permyakov et al., 1988; Toriashvili et al., 1991; Lomidze et al., 1994], and though in mentioned interval rabbit α-actinin only slightly changes, in the case of fish and frog the structural changes are more essential. Some internal tryptophans relocate to the surface of protein molecule which must be caused by its partial unfolding.

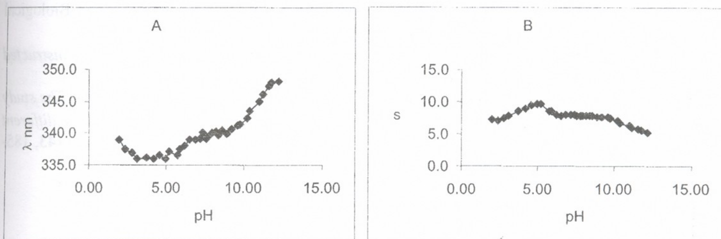

Fig.2. The dependence of frog skeletal α-actinin ($3 \cdot 10^{-5}$ M, 20 mM Tris-Acetate, 15°C) fluorescence spectrum position A and the relative quantum yield B on pH. Excitation wave length 280 nm.

The data of frog skeletal muscle fluorescence spectrum position and quantum yield dependence on pH (Fig.2) show that while pH decreases from 7 to 5 the fluorescence spectrum of α-actinin shifts towards short wave length range (by ~3nm) and the relative quantum yield of radiation increases, which is caused by isoelectric aggregation of protein. At the lower pH the fluorescence spectrum relocates to longer wave length region (by ~3nm) and quantum yield decreases. Above pH 9 the relative quantum yield gradually decreases with the fluorescence spectrum shift to long length wave region. Data obtained for frog skeletal muscle α-actinin are in good accordance with the data obtained for rabbit and carp α-actinins [Permyakov et al., 1988]. So we can suppose, that above pH 9 the protein structure unfolding, due to the denaturation, in frog α-actinin also takes place.

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ტემპერატურისა და pH-ის გავლენის შესწავლა ბაყაყის α-აქტინინის სტრუქტურულ ცვლილებებზე საკუთარი ფლუორესცენციის მეთოდით

ზაალიშვილი თ., გაჩეჩილაძე ნ., ლომიძე ლ., ტორიაშვილი თ., გამყრელიძე ც., ზაალიშვილი მ.

საქართველოს მეცნიერებათა აკადემიის მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 15.11.2005)

რეზიუმე

საკუთარი ფლუორესცენციის მეთოდით შესწავლილია pH-ისა და ტემპერატურის გავლენა ბაყაყის ჩონჩხის კუნთის α-აქტინინის სტრუქტურულ თვისებებზე. ნაჩვენებია, რომ 15°C-ზე α-აქტინინის ტუტე დენატურაცია იწყება pH 9-ის შემდეგ, ხოლო მჟავე არე არ იწვევს სტრუქტურის გაშლას და ადგილი აქვს მხოლოდ ცილის აგრეგაციას. დადგენილია, რომ α-აქტინინის გადასვლა ნატიურიდან დენატურირებულ მდგომარეობაში მიმდინარეობს 42-70°C ტემპერატურულ ინტერვალში. ამასთან, აღნიშნულის გარდა, 18-30°C ინტერვალში შეინიშნება კიდევ ერთი სუსტი გადასვლა.

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ეროვნული ბიბლიოთეკა

OBTAINING OF ENZYMATIC HYDROLYSATES FROM WHEAT FLOUR USING THERMOPHILIC MICROORGANISMS CELLULASES

ABASHIDZE M., KHOKHASHVILI I., *KUTATELADZE L., CHESHMARITASHVILI M.

Durmishidze Institute of Biochemistry and Biotechnology, Academy of Sciences of Georgia
E-mail: kutateladze@geomail.ge

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Abstract

The possibility of obtaining of sugared hydrolysates from the flour of low quality has been revealed. The technical preparations of high cellulase activity have been obtained. The content of carbohydrates in different sorts of wheat flour has been investigated. The optimal conditions of enzymatic hydrolysis has been determined. The syrup containing soluble sugars has been obtained. This syrup can be used to improve bread quality.

Key words: sugared hydrolysate, cellulase activity, thermophilic micromycete

Introduction

One of the ways to improve bread quality is the production of sugared enzymatic hydrolysates by using of non- traditional raw materials.

It was established that use of sugared enzymatic hydrolysates activates yeasts, decreasing their expenditure by 20-30%, intensifies the process of making dough by 1-2 h, improves the quality of bread [Polandova et al.,1977].

The perspective raw materials for hydrolysates producing are cellulose-containing wastes of fruit and vegetables, wheat flour of low quality, flour of damaged wheat, etc. [Silagadze,1990]

It was established that during the preparation of high-sugared semiproducts the right choice of enzymatic preparations is of great importance [Paschenko et al.,1974; Kislukhina, 2002]. Amylase preparations are mainly used to prepare hydrolysates, but for hydrolysis of the cellulose- and hemicellulose containing wastes cellulase preparations of high activity are used [Toshev, 2003].

Materials and Methods

The flour obtained from different sorts of wheat with bran containing both starch and cellulose fractions and extremophilic cultures *Abzidia* sp. K69, *Chaetomium* sp. S77 and *Penicillium* sp. S29 from the microbiological collection of Durmishidze Institute of Biochemistry and Biotechnology, were used in our investigations.

The submerged cultivation of these strains was carried out in a 750-ml flasks on a thermostatic rotary shaker (180-200rpm) at 40^0 for 96 h in the medium of the following

composition (%): micro-crystalline cellulose-1.0, $NaNO_3$-0.3; KH_2PO_4-0.2; $MgSO_4$ x $7H_2O$-0.05; maize extract-1.5.

The seed culture was the suspension of 10-day conidia cultures.

Technical preparations of cellulase was obtained by the precipitation of culture filtrates with ethanol and freeze dried. Cellulase activity was determined towards filter paper. The degree of hydrolysis was evaluated according to the quantity of glucose and reducing sugars.

Results and Discussion

In order to use enzymatic hydrolysis for industrial purposes a number of problems such as selection of substrate, obtaining of highly active cellulase complex and determination of optimal conditions for hydrolysis must be solved.

The content of starch, cellulose and hemicellulose of flour obtained from different sorts of wheat was determined. As it can be seen from Table 1 the total quantity of cellulose and hemicellulose in each sort of wheat is about 10%. It is well known that these components significantly reduce the flour quality and extra technological charges are needed to remove them. That's why it is necessary to convert these biopolymers into soluble sugars to improve the quality of flour and at the same time to reduce the industrial expenditure of sugar

The activities of technical preparations from the culture filtrates of *Abzidia* sp. K69 *Penicillium* sp. S29 and *Chaetomium* sp. S77 obtained by the precipitation with ethanol are given in Table 2.

Table 1. The content of carbohydrates in of different sorts of wheat

Substrate	Starch, %	Cellulose, %	Hemicellulose, %
wheat "Mtsketa"	69.1	4.5	6.3
wheat "Upkho"	65.1	5.1	6.0

Table 2. Cellulase activity of enzyme preparations

Cellulase preparation	Cellulase activity, unit /mg filter paper	Protein, mg/ml	Specific activity, unit/mg protein
Abzidia sp. K69	0.035	0.09	0.38
Penicillium sp. S29	0.028	0.078	0.35
Chaetomium sp. S77	0.02	0.07	0.28

In order to optimize the hydrolysis process, the duration of maximal decomposition of wheat cellulase fraction, optimal temperature of the action of the cellulase preparations and optimal ratio of substrate and enzyme concentrations have been studied. From the reaction mixture samples were taken every hour and content of both reducing sugars and glucose were determined in them (Fig.1). As it can be seen from Fig.1 after 6 h the formation of both glucose and reducing sugars are stopped, i.e. 6 h are needed for maximal decomposition of wheat cellulase fraction. To determine temperature optimum for the action of the cellulase preparations hydrolysis of cellulose was carried out in the temperature range 20-60^0 C. Optimal temperature of all the investigated cellulase preparations was shown to be at 50^0 C. Optimal ratio of substrate and enzyme concentrations was found to be 1:10. The hydrolysis of flour of different sorts of wheat was carried out by the obtained cellulase preparations under the optimal conditions. After 6-hour hydrolysis in the solution the

quantity of sugars was determined and in the residue the content of cellulose was estimated. The results of the investigations are shown in Table 3.

ეროვნული ბიბლიოთეკა

Table 3. Obtaining of sugared hydrolysates from flour of different sort wheat

Wheat sort	Cellulase preparation	Initial cellulose, %	Reducing sugars,%	Glucuse,%	Residual cellulose, %
"Mtsketa"	*Abzidia* sp. K69	6.5	50	40	3.0
"Upkho"		7.1	60	57	4.5
"Mtsketa"	*Penicillium* sp. S29	6.5	70	55	2.0
"Upkho"		7.1	75	60	1.8
"Mtsketa"	*Chaetomium* sp. S77	6.5	40	20	2.7
"Upkho"		7.1	45	26	3.0

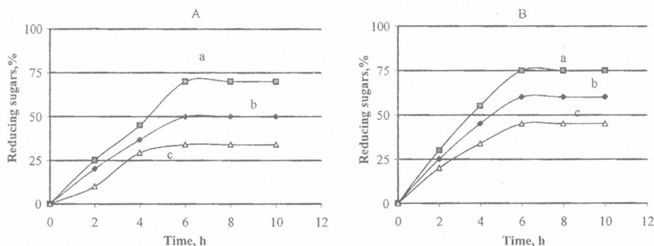

Fig.1. Kinetic curves of enzymatic hydrolysis

Substrates: A - wheat "Mtsketa", B - wheat "Upkho". Enzyme preparations: a - *Abzidia* sp. K69, b - *Penicillium* sp. S29, c - *Chaetomium*

Thus, the data of Table 3 show that by use of *Abzidia* sp. K69, *Penicillium* sp. S29 and *Chaetomium* sp. S77 cellulase preparations a large amount of wheat cellulose is decomposed and the soluble sugars are obtained. These soluble sugars further may successfully be used in the baking of bread to improve its quality.

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ფერმენტული ჰიდროლიზატების მიღება ხორბლის ფქვილისაგან თერმოფილური მიკროორგანიზმების ცელულაზების გამოყენებით

აბაშიძე მ., ხოხაშვილი ი., ქუთათელაძე ლ., ჭეშმარიტაშვილი მ.

საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 05.12.2005)

რეზიუმე

დადგენილია დაბალი ხარისხის ფქვილისაგან დაშაქრებული ჰიდროლიზატების მიღების შესაძლებლობა ცელულაზური პრეპარატების გამოყენებით. მიღებულია მაღალი ცელულაზური აქტივობის მქონე ტექნიკური პრეპარატები. გამოკვლეულია სხვადასხვა ჯიშის ხორბლის ფქვილში ნახშირწყლების შემცველობა. დადგენილია ხორბლის ფქვილში ფერმენტული ჰიდროლიზის ოპტიმალური პირობები. მიღებულია ხსნადი შაქრების შემცველი სიროფი, რომელიც შეიძლება გამოყენებულ იქნას პურის ხარისხის გასაუმჯობესებლად.

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Biotechnology

BIOCONVERSION OF NEGATIVE VALUE SOLID FOOD WASTES TO HYDROGEN AND SINGLE-CELL PROTEIN

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

[1]*National High Technology Center of Georgia.*
[2]*Durmishidze Institute of Biochemistry and Biotechnology*

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Abstract

This study demonstrated that combination of anaerobic hydrogen and aerobic fungal fermentation technologies makes single cell protein microbial production economically sound process. Typical kitchen wastes composed mainly of solid food wastes, paper and some other cellulosic materials were studied for chemical composition. Under the dark hydrogen fermentation process easily degradable fraction of these organic wastes were converted into hydrogen biogas (56% H_2 and 34% CO_2) by using mixed cultures of anaerobic cellulolitic and saccharolytic bacteria isolated from fresh cattle manure. Under thermophilic conditions hydrogen cumulative production came to 140 ml/g substrate. Analyses showed that percentage of weakly degradable bio-polymers such as cellulose, lignin and hemicellulose did not significantly decrease under anaerobic fermentation when hydraulic retention time was 24 hours. Bioconversion of residual lignocellulosic fraction of the above wastes was followed by aerobic fermentation using white rot fungi strain - *Pleurotus ostreatus* 41. Under submerged cultivation conditions *Pleurotus ostreatus* 41 revealed ability to degrade 42% of cellulose, 58% of lignin and 22% of hemi cellulose when using anaerobically pretreated lignocellulosics as the sole source of carbon. Row protein percentage in obtained biomass made 30.9%.

Key words: hydrogen fermentation, cellulolitic bacteria, hydraulic retention time, fungal fermentation, *Pleurotus ostreatus* 41, submerged cultivation.

Introduction

In most countries, the major disposal method of solid food wastes, such as foodstuffs manufacturing wastes, fruits and vegetables processing wastes and market residues is the landfill (about 95%) and only 5% is recycled as animal feed or fertilizers through composting. When not utilized, these putrescent organic wastes damage to environment through pollution of soil, underground waters and air. On the other hand, solid food wastes generated in huge amounts everywhere are regarded as a widely available and cheap renewable biomass feedstock for production of "green" energy carrier such as hydrogen using anaerobic fermentation technology [Claassen et al., 1999]. Considering that solid food wastes contain both easily degradable compounds and ligno-cellulosic fraction composed mainly of cellulose, lignin, xylan etc. that are

weakly degradable bio-polymers, biodegradation of ligno-cellulosic substrate residuary after completion of dark hydrogen fermentation, can be followed by aerobic fungal fermentation resulting in single- cell protein and carbohydrates reach biomass production. These products can be used as a high quality additive to forage. In general ligno-cellulosic wastes based single-cell protein production is considered as one of the most effective ways for improvement nutritive values of these residues. The major problem that hampers implementation of this biotechnology in developing countries is associated with high cost of both heat and electric energy [Howard et al., 2003]. Estimations show that integration of hydrogen ("green" energy carrier chemical energy of which could be converted in electricity by using highly effective hydrogen fuel cell) and single cell protein production from solid food wastes will allow reducing single-cell protein production costs.

This paper describes that combination of anaerobic hydrogen and aerobic fungal fermentation technologies makes single cell protein microbial production economically sound process.

Materials and Methods

The mixed culture of anaerobic thermophilic cellulolitic and saccharolytic bacteria, isolated from cattle manure was used to ferment typical kitchen wastes composed of onion, potato and carrot peels, residuals of bread, cabbage, green, filter paper, cardboard and sawdust to hydrogen. Initially the wastes were studied for moisture content, percentage of total solids, total volatile solids, total carbon, ammonia, protein, soluble sugars, cellulose, hemicellulose and lignin. Standard methods were used for conducting above described analyses [Apdegtaph, 1969; Katkevich et al., 1982; Klesova et al., 1980; Termkhitarova, Shulga, 1974; Zadrazhil, 1977]. Afterwards organic wastes, including lignocellulosic fraction, were pretreated by CO_2 based freeze explosion method. Frozen wastes were milled and diluted with tap water in such a way that percentage of total organic solids came to 5%. This substrate was charged in 3 1 temperature-controlled bioreactor equipped with online pH and gas flow meters, bio-mass mixer and contact thermometer. Prior to the start of experiment forced nitrogen flow was used to achieve an anaerobic environment in bioreactor. The mixed culture of anaerobic cellulolitic and saccharolytic bacteria were isolated from fresh cattle manure by using Pervozvanski selective Media [Egorova et al., 1984]. Cultivation was performed in anaerobic conditions at 55° C. Bacteria inoculum in amount of 10 ml was introduced in the above bioreactor. Hydrogen production started in 6 hours and was completed in 24 hours. Hydrogen and carbon dioxide concentration in produced biogas was determined by gas chromatograph with thermal detector. A methane impurity in produced biohydrogen was measured by Perkin Elmer gas chromatograph with flame ionization detector. When hydrogen production stopped, the residual substrate was discharged from bioreactor and was studied for cellulose, hemicellulose, lignin, protein and soluble sugars concentration. Solid biomass was separated from the liquid substrate and was dried in the thermostat at 60° C. Brought to the dry weight; the residual solid biomass was used as the substrate for aerobic fungal fermentation resulting in single-cell protein and carbohydrates-rich biomass. Basidial fungi – *Pleurotus ostreatus* 41, obtained from the collection of Durmishidze Institute of Plant biochemistry and Biotechnology (Tbilisi, Georgia) was used for these purposes.

The submerged cultivation of *Pl. ostreatus* was performed in 750ml conic flasks where 2g of residual lignocellulosic substrate and 100ml of nutrient medium were introduced. The flasks were autoclaved at 0, 7 atm during 45 min. Inoculum of *Pleurotus ostreatus* 41 was prepared following to the protocol described earlier [Chachkhiani et al., 2005] and introduced in the flasks which where placed on the shaker making 120 rot/min. Cultivation temperature and period were 20°C and 12 days correspondingly. At the end of the process, flasks contents were centrifuged, the

sediment was dried and studied for raw protein, cellulose, lignin and hemicellulose percentage. Concentration of soluble sugars was measured in the supernatant.

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Results and Discussion

The purpose of this study was to evaluate combined hydrogen and single-cell protein output from solid food wastes. At the first stage of the experiment hydrogen was produced under dark fermentation of food wastes by using mixed culture of hydrogen producing bacteria. At thermophilic conditions when hydraulic retention time was 24 hours, hydrogen productivity from the above described composition solid food wastes came to 140 ml/g substrate. Data describing changes in cellulose, hemicellulose, lignin, soluble sugars and protein content under anaerobic treatment of solid food wastes and lignocellulosics are given in Table 1.

Table 1. Changes in cellulose, hemicellulose, lignin, soluble sugars and protein content under anaerobic treatment of solid food wastes and lignocellulosics

	Raw protein, %	Cellulose, %	Hemi cellulose, %	Lignin, %	Soluble sugars, γ/ml
Raw substrate	15.54	21.17	53	9.6	2500
substrate residuary after anaerobic fermentation	18.2	18.00	49.2	9.6	70

Comparing initial and final figures given in table 1, it becomes clear that under hydrogen fermentation when hydraulic retention time is 24 hours, cellulose and hemicellulose content in the substrate decreases only by 3% and 4% correspondingly while the concentration of lignin remains the same. As for soluble sugars, they were almost fully transformed in hydrogen biogas (mix of 56% H_2 and 44 % CO_2) and metabolites of hydrogen fermentation (fatty acids, alcohol) by the mixed culture of cellulolitic and saccharolytic bacteria. Based on results it could be concluded that anaerobic cellulolitic and saccharolytic bacteria mostly convert easily metabolizing sugars to hydrogen biogas. Thus after hydrogen fermentation completion about 21% of cellulose, 49% of hemicellulose and 9% of lignin remained in the substrate.

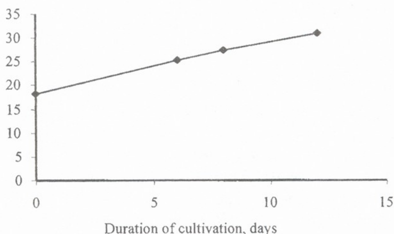

Fig.1. Dynamics of protein accumulation by *Pl. ostreatus* under submerged cultivation conditions

At the next stage of this work basidial fungus strain *Pleurotus ostreatus* 41 which possess both cellulose and lignin degrading enzymes, was used to produce single-cell protein from the ligno-cellulosic substrate residuary after dark hydrogen fermentation completion. Study of protein

accumulation dynamics under submerged cultivation at 20°C showed that *Pleurotus ostreatus* 41 successfully utilized all three biopolymers: cellulose, lignin and hemicellulose for growth. Fig. 1 demonstrates that maximum percentage of raw protein accumulated in fungal biomass on the 12th day of cultivation came to 30, 9%.

Fig. 2 shows cellulose, hemicellulose and lignin degradation dynamics by basidial fungus *Pl. ostreatus* under submerged cultivation conditions. During 12 days of cultivation the fungus has destructed 42% of the initial amount of cellulose, 58% of lignin and 22% of hemi cellulose.

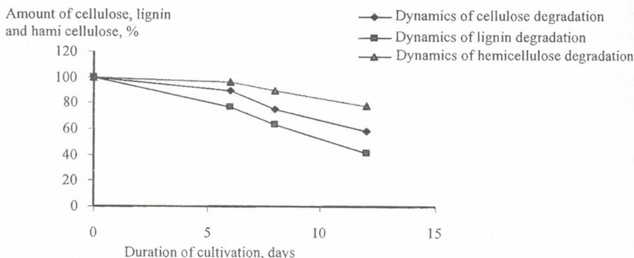

Fig.2. Dynamics of cellulose, hemicellulose and lignin degradation under submerged cultivation of *Pl. ostreatus*

Based on results it could be concluded that combination of anaerobic hydrogen and aerobic fungal fermentation technologies applied for reprocessing negative value solid food wastes is profitable both in energy and economic ranges.

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მყარი საკვების ნარჩენების ბიოკონვერსიით წყალბადისა და ცილით მდიდარი ბიომასის ერთდროული მიღება

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

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

(მიღებულია 11.11.2005)

რეზიუმე

წარმოდგენილია წყალბადისა და ცილით მდიდარი ბიომასის ერთობლივად მიღების შესაძლებლობა ანაერობული წყალბადის ფერმენტაციისა და აერობული სოკოებით ფერმენტაციის ტექნოლოგიების ინტეგრირებით. შესწავლილია ქიმიური შემადგენლობა ტიპიური საოჯახო ნარჩენებისა. ცელულოლიტიკური და საქაროლიტიკური ბაქტერიების შერეული კულტურის გამოყენებით აღნიშნული კომპოზიტის ადვილად დეგრადირებადი ფრაქციის ანაერობული წყალბადური ფერმენტაციის საფუძველზე მიღებულია ბიოწყალბადი (56% H_2; 34% CO_2). თერმოფილურ პირობებში წყალბადის გამოსავალი შეადგენდა 140 მლ/გ სუბსტრატი. ნაჩვენებია, რომ ანაერობული ფერმენტაციის პირობებში ცელულოზის, ლიგნინისა და ჰემიცელულოზის შემცველობა სუბსტრატში მცირე რაოდენობით იცვლება. ანაერობული ფერმენტაციის დამთავრების შემდეგ დარჩენილი ლიგნო-ცელულოზური ფრაქციის ბიოკონვერსია . განხორციელდა ბაზიდიალური სოკოს -*Pleurotus ostreatus* 41 – გამოყენებით. სუბსტრატში არსებული ცელულოზის საწყისი შემცველობის 42%-ს, ლიგნინის 58%-ს და ჰემიცელულოზის 22%-ს დეგრადაციის ხარჯზე მიღებულია ბიომასა, რომელიც 30.9% ნედლ ცილას შეიცავდა.

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ეროვნული
ბიბლიოთეკა

DIVERSITY OF ENDEMIC FLORA OF IMERETI (WEST GEORGIA)

CHEISHVILI T.[1], CHURADZE M.[2]

[1]*Department of Biology of A.Tsereteli Kutaisi State University*
[2]*Department of Botany of I. Javakhishvili Tbilisi State University*

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Abstract

The paper deals with diversity of the flora of Imereti, where 121 species and 5 subspecies endemic to the Caucasus and Georgia were distinguished as a result of the accomplished investigation; 69 of them are endemic to the Caucasus and 52 to Georgia. The number of the local endemics of Imereti is 14. Peculiarities and diversity of endemic taxa confined to calcareous ecotopes are discussed. It is concluded that formation of the endemic flora of calcareous ecotopes is a result of geographic isolation.

Key words: Colchis, Caucasus, Compositae, Leguminosae, Leguminosae.

Introduction

Imereti is one of the ethnographic-floristic regions of West Georgia and a part of the botanical-geographic province of Colchis. Imereti encompasses Okriba, foothills of northern Imereti with irregular karst topography. The region is characterized by floristic complexes of the broad-leaved forest, evergreen shrubbery and calciphilous species, chestnut forests at 800m a.s.l also occur. An important part of the relief in Imereti is represented by plain-mountainous karst massif of the old Zemo Imereti plateau with floristic complexes of broad-leaved forest and secondary meadows as well as with rather large number of calciphilous species. To the north the region verges on a segment of the middle- and high-mountainous Racha range and south-facing slopes of the Nakerala range with floristic complexes of dark coniferous forest and high mountain meadows. This segment is also rich in calciphilous species. The relief of southern Imereti is formed by low hilly landscape and massifs of southern Imereti foothills. These foothills border the Colchic lowland and consist of low massifs located at north-facing slopes of the Adjara-Imereti range; they are covered by broad-leaved forest complexes.

Okriba, Zemo Imereti plateau, the segments of the Nakerala and Racha ranges are distinguished as parts of the Transcaucasian calcareous sub-province of the province of Colchis [Kolakovsky, 1961; Kharadze, 1966; Gagnidze, 1974; Gagnidze et al., 2000, 2002; Sokhadze, 1981]. Several species new to Georgia and the Caucasus were first described in this sub-province.

Materials and methods

The present paper is based on results of field-itinerary and comparative floristic studies of the endemic flora of Imereti, an ethnographic-floristic region of West Georgia. The study was carried out according to methodological program [Jurtsev, ed., 1987]. Materials kept at the herbaria of Tbilisi Institute of Botany, the State Museum of Georgia, Tbilisi State University and Kutaisi State University were analyzed during the study. Taxonomy and nomenclature of the species follows the 2nd edition of "Flora of Georgia" [Ketskhoveli et al., eds., 1971-2003] and "Vascular plants of Georgia. A nomenclatural checklist" [Gagnidze, 2005].

Results and Discussion

According to floristic and phytolandscape schemes, Imereti belongs to the botanico-geographic province of Colchis [Kolakovsky, 1961; Sokhadze, 1968; Gagnidze, 1974, 2000, 2004]. Flora of Imereti numbers about 900 species, i.e. almost a quarter of Georgian flora, which includes 4130 species [Gagnidze, 2005]. Despite comparatively small number of present taxa, Imereti is distinguished by relatively high level of endemism. 121 endemic species and subspecies occur in the region (approx. 8.5% of its flora); 69 of them are endemic to the Caucasus and 52 to Georgia. The number of local endemics is 14.

In the flora of Imereti dicots are represented by 115 species and monocots by 8 species.

As to the endemic genera, *Paederotella* is a Caucasian lithophylous genus, *Grossheimia* – a genus pertaining to the Caucasian subendemic tall herbaceous vegetation, *Polylophium* – a calciphilous disjunctive genus of Colchis/West Asia [Gagnidze et al., 2002].

The following families are leading in the flora of Imereti: *Compositae* – 26 species, *Leguminosae* – 10 species, *Rosaceae* – 10 species, *Campanulaceae* – 8 species, *Umbelliferae* – 8 species, *Cruciferae* – 6 species, *Boraginaceae* – 5 species, *Scrophulariaceae* – 5 species, *Helleboraceae* – 4 species, *Paeoniaceae* – 4 species.

Almost the same species dominate in the flora of Georgia; therefore, the family spectrum of the flora of Imereti is similar to that of the whole country, i.e. Mediterranean-Euxinian-South European.

In the flora of Imereti the proportion of species of *Compositae* endemic to the Caucasus and Georgia is 17/9. Genera: *Anthemis* and *Hieracium* are leading according to the number of present endemic species; representatives of these genera are constituents of meadow ecosystems. Tall herbaceous vegetation is made up of species of *Grossheimia, Cirsium, Inula, Tephroseris.*

In the family *Leguminosae* the proportion of species endemic to the Caucasus and Georgia is 8/2. Endemic species mainly are constituents of meadow ecosystems.

The family *Rosaceae* is represented by 10 species. The proportion of species endemic to the Caucasus and Georgia is 6/4. Calciphytes (representatives of *Potentilla, Dryas*), species of forest and meadow ecosystems are noteworthy among the endemics.

From the representatives of the family *Campanulaceae* species endemic to Georgia predominate – the above proportion is 1/7. Seven of the 8 endemic species belong to the genus *Campanula*; the most part is calciphilous with distribution areas confined to calcareous orographic systems.

The family *Umbelliferae* is represented by 8 endemic species. The proportion of species endemic to the Caucasus and Georgia is 4/4. The endemic species are components of tall herbaceous vegetation (species of the genus *Heracleum*) and calciphilous flora (species of *Astrantia, Anthriscus, Cnidium*). A representative of the Colchic/West Asian calciphilous disjunctive genus *Polylophium* – *P. panjiutinii* is worth mentioning from the endemic species.

The family *Cruciferae* is represented by 6 species. The proportion of species endemic to the Caucasus and Georgia is 4/2. All the 6 species are confined to the calcareous ecotopes.

Five endemic species from the family *Boraginaceae* occur in Imereti. The proportion of species endemic to the Caucasus and Georgia is 4/1. All the 5 species are confined to secondary meadow ecosystems.

The family *Scrophulariaceae* is represented by 5 endemic species and 1 variety. The proportion of species endemic to the Caucasus and Georgia is 3/2. All the species grow on the calcareous ecotopes. *Paederotella pontica*, a calciphilous species belonging to the Colchic/ West Asian genus, is a representative of this family.

Four endemic species of the family *Helleboraceae* occur in Imereti. Two of them are endemic to the Caucasus and the other 2 to Georgia. Participation of strictly local species: *Aquilegia colchica* and *Helleborus abchasicus* in the flora of the calcareous ecotopes is noteworthy.

The family *Paeoniaceae* is represented by 4 endemic species. The proportion of species endemic to the Caucasus and Georgia is 1/3. Thus, the Georgian endemics predominate. All the 4 species belong to the genus *Paeonia*.

Special type of endemism is characteristic to the calcareous orographic units of Imereti: the Nakerala and Racha ranges, Dzirula karst massif, Chiatura plateau [Kutateladze, 1961, 1962]. These orographic units are extensions of the West Transcaucasian calcareous massifs located almost parallel to the Greater Caucasus. As it is mentioned above, the Transcaucasian calcareous massifs with abundance of calciphilous species are distinguished as a sub-province of the botanico-geographic province of Colchis [Kolakovsky, 1961; Kharadze, 1968; Gagnidze, 1974, 2005].

Biodiversity of Imereti as well as that of the Transcaucasian calcareous massifs, is characterized by dispersed endemism [Gagnidze, 2005]. Many species of various genera are presented in the endemic flora of these ecotopes. The number of participating genera is large (e.g. *Campanula, Dianthus, Senecio, Draba, Sisymbrium, Leptopus, Aquilegia, Cyclamen, Veronica*). Genera of the calcareous biotope flora are frequently represented by rare species with local distribution ranges (e.g. *Campanula imeretina, C. irinae, Sisymbrium praetermissum, Aquilegia colchica, Thymus ladjanuricus, Potentilla imerethica, Polylophium panjutinii*) [Kolakovsky, 1961; Kutateladze, 1961, 1962; Gagnidze, 1983, 2005; Adzinba, 2000].

Age of the species of the calcareous biotope flora is different and groups of young and old species can be clearly distinguished. Rare occurrence and strictly local distribution of a species must be due to ecological, historical and genetic factors, including species geographic isolation. Calcareous rock-skeleton ecotopes create diverse habitats for plants. Species of different ecology, i.e. those with different requirements to environmental conditions, settle on these ecotopes. Ecological diversity of calcareous habitats underlies their floristic richness. It can be concluded that during periods of historical climatic changes species survived mainly owing to presence of the calcareous ecotopes [Kolakovsky, 1961]. Furthermore, these ecotopes served as areas of species formation.

The calcareous massifs of Imereti may be considered as a local centre of species formation on the background of the Transcaucasian calcareous areas. Twenty-five new species were described on the calcareous ecotopes of Imereti; particularly, the following species were first described in Okriba, calcareous foothills of North Imereti: *Astragalus kemulariae, Centaurea bella* subsp. *nathadze, Dianthus imereticus, Draba imeretica, Iris colchica, Ornithogalum imereticum, Veronica galathica*.

The following species are described on the Zemo Imereti plateau: *Aquilegia colchica, Campanula imeretina, C. kemulariae, Corylus imeretica, Paeonia ruprechtiana, Potentilla imerethica, Quercus imeretina, Scrophularia imerethica, Thesium laxiflorum.*

The following species are described on south-facing slopes of the Nakerala range: *Campanula irinae, Euphrasia kemulariae, Genista sachokiana, Paracynoglossum imeretinum, Veronica imerethica (=V. kemulariae).*

The following species are local endemics of Imereti: *Campanulaceae: Campanula irinae, C.kemulariae; Compositae: Centaurea bella subsp. nathadze; Helleboraceae: Aquilegia colchica; Hyacinthaceae: Ornithogalum imereticum; Rosaceae: Potentilla kemulariae, P. imerethica; Scrophulariaceae: Euphrasi, kemulariae, Scrophularia imerethica, Veronica galathica, V. imerethica (=V. kemulariae).*

List of endemic species of the flora of Imereti

◎ – endemic to the Caucasus O – endemic to Georgia

A n g i o s p e r m a e

Dicotyledoneae

1. Boraginaceae

◎ *Nonea decurrens* (C. A. Mey.) G. Gon fil.
◎ *N. intermedia* Ledeb.
◎ *N. setosa* (Lehm.) Roem. et Schult.
O *Paracynoglossum imeretinum* (Kusn.) M. Pop.
◎ *Symphytum caucasicum* Bieb.

2. Campanulaceae

◎ *Asyneuma campanuloides* (Bieb. ex Sims) Bornm.
O *Campanula imeretina* Rupr.
O *C. irinae* A. Kutateladze
◎ *C. grossheimii* Charadze
O *C. kemulariae* Fomin
O *C. letschumensis* Kem.-Nath.
O *C. makaschvili* E. Busch
O *C. radchensis* Charadze
Symphyandra pendula (Bieb.) A. DC Ovar. *transcaucasica* Somm. et Levier

3. Caryophyllaceae

◎ *Dianthus imereticus* (Rupr.) Schischk. (*D. montanus* Bieb. f. *imeretica* Rupr.)

4. Celastraceae

◎ *Euonymus leiophloea* Stev.

5. Compositae

Asteroideae

◎ *Achillea grizeo-virens* Albov
◎ *Anthemis macroglossa* Somm. et Levier
O *A. schischikiniana* Fed.
◎ *A. sosnovskyana* Fed.
◎ *A. woronowii* Sosn.

Centaurea bella Trautv. ○ subsp. *nathadzeae* (Sosn.) Djindjolia

○ *Cirsium imereticum* Boiss.

○ *C. kemulariae* Charadze

◎ *Grossheimia polyphylla* (Ledeb.) Holub [*Centaurea polyphylla* Ledeb.; *Grossheimia ossica* (C. Koch) Sosn. et Takht.]

◎ *Inula magnifica* Lipsky

○ *Petasites georgicus* Manden.

○ *Psephellus colchicus* Sosn.

○ *Pyrethrum chamaemelifolium* (Somm. et Levier) Sosn. [*P. roseum* (Adams) Bieb. var. *chamaemelifolium* Somm. et Levier; *Tanacetum coccineum* (Willd.) Grierson subsp. *chamaemelifolium* (Somm. et Levier) Grierson]

○ *P. peucedanifolium* (Sosn.) Manden. (*P. parthenifolium* Willd. var. *peucedanifolia* Sosn.; *P. svanicum* M. Pop.)

◎ *Senecio massagetovii* Schischk.

◎ *S. rhombifolius* (Adams) Sch. Bip. [*Cacalia rhombifolia* Adams; *Adenostyles rhombifolia* (Adams) M. Pimen.; *Pojarkovia macrophylla* (Bieb.) Asker.; *Caucasalia macrophylla* (Bieb.) B. Nordenstam]

◎ *Tephroseris cladobotrys* (Ledeb.) Griseb. et Schenk (*Senecio cladobotrys* Ledeb.)

◎ *Tripleurospermum colchicum* (Manden.) Pobed. [*Chamaemelum colchicum* Manden.; *Matricaria colchica* (Manden.) Rauschert]

Cichoroideae

◎ *Cicerbita prenanthoides* (Bieb.) Beauverd (*Sonchus prenanthoides* Bieb.)

○ *Hieracium x abakurae* Shelk. et Zahn

◎ *H. caucasicum* Naeg. et Peter

◎ *H. elisabethae* Kem.-Nath.

◎ *H. x pseudosvaneticum* Peter

◎ *H. x raddeanum* Zahn.

◎ *Lapsana pinnatisecta* (Somm. et Levier) Ter.-Chatsch. (*L. grandiflora* Bieb. f. *pinnatisecta* Somm. et Levier)

◎ *T. confusum* Schischk.

◎ *Taraxacum grossheimii* Schischk.

6. Corylaceae

◎ *Corylus iberica* Wittm. ex Kem.-Nath. (*C. colurna* L.)

○ *C. imeretica* Kem.-Nath.

7. Cruciferae

◎ *Draba bryoides* DC.

○ *D. imeretica* (Rupr.) Rupr.

○ *D. mingrelica* Schischk.

◎ *Erysimum aureum* Bieb.

◎ *E. ibericum* (Adams) DC.

◎ *Sisymbrium praetermissum* T. Mardalejshvili

8. Dipsacaceae

◎ *Cephalaria gigantea* (Ledeb.) Bobr.

◎ *S. georgica* Sulak.

○ *Scabiosa imeretica* (Somm. et Levier) Sulak.

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9. Euphorbiaceae

◎ *Euphorbia macroceras* Fisch. et C. A. Mey.

◎ *E. scripta* Somm. et Levier

○ *Leptopus colchicus* (Fisch. et C. A. Mey. ex Boiss.) Pojark. (*Andrachne colchica* Fisch. et C. A. Mey.)

10. Fagaceae

○ *Quercus imeretina* Stev. ex Woronow [*Q. robus* L. subsp. *imeretina* (Stev. ex Woronow) Menitsky]

11. Gentianaceae

◎ *Gentiana kolakovskyi* Doluch.

12. Helleboraceae

◎ *Aquilegia caucasica* (Ledeb.) Rupr.

○ *A. colchica* Kem.-Nath.

○ *Helleborus abchasicus* A. Br. (*H. orientalis* Lam. p. p.)

◎ *H. caucasicus* A. Br. (*H. orientalis* Lam. p. p.)

13. Labiatae

○ *Thymus caucasicus* Willd. ex Ronn

◎ *Th. collinus* Bieb.

○ *Th. ladjanuricus* Kem.-Nath.

14. Leguminosae

◎ *Anthyllis irenae* Juz.

◎ *A. lachnophora* Juz.

○ *Astragalus kemulariae* Grossh. [*A. raddeanus* Regel var. *kemulariae* (Grossh.) Grossh.]

◎ *Cytisus caucasus* Grossh.

◎ *Galega orientalis* Lam.

◎ *Genista patula* Bieb.

○ *G. sachokiana* A. Kuthatheladze

◎ *Lotus caucasicus* Kuprian. ex Juz.

◎ *Vicia antiqua* Grossh.

◎ *V. ciliatula* Lipsky

15. Malvaceae

◎ *Alcea transcaucasica* (Iljin) Iljin

16. Paeoniaceae

◎ *Paeonia caucasica* (Schipcz.) Schipz.

○ *P. macrophylla* (Albov) Lomak.

○ *P. ruprechtiana* Kem.-Nath.

○ *P. wittmanniana* Hartwiss ex Lindl.

17. Polygalaceae

◎ *Polygala caucasica* Rupr.

○ *P. makaschwilii* Kem.-Nath. (*P. kemulariae* Tamamsch.)

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18. Primulaceae

○ *Cyclamen colchicum* (Albov) Albov
◎ *Primula woronowii* Losinsk.

19. Rhamnaceae

○ *Rhamnus cordata* Medw.

20. Rosaceae

○ *Alchemilla kozlowskii* Juz.
◎ *A. undecimloba* Juz.
○ *A. woronowii* Juz.
◎ *Dryas caucasica* Juz.
○ *Potentilla imerethica* Gagnidze et M. Sochadze
○ *P. kemulariae* Kapell. et A. Kuthatheladze
◎ *Pyrus brachypetala* Fisch. et C. A. Mey.
◎ *P. caucasica* Fed.
◎ *Rubus moschus* Juz.
◎ *R. ponticus* (Focke) Juz.

21. Santalaceae

○ *Thesium laxiflorum* Trautv.

22. Scrophulariaceae

○ *Euphrasia kemulariae* Juz.
◎ *Paederotella pontica* (Rupr. ex Boiss.) Kem.-Nath. [*Paederota pontica* Rupr. ex Boiss.; *Paederotella teberdensis* Kem.-Nath.]
○ *Scrophularia imerethica* Kem.-Nath.
◎ *Veronica galathica* Boiss.
◎ *V. imerethica* Kem.-Nath. (*V. kemulariae* A. Kuthatheladze)
V. serpyllifolia L. ○ var. *pumila* Kem.-Nath.

23. Thymelaeaceae

◎ *Daphne axilliflora* (Keissl.) Pobed.

24. Umbelliferae

◎ *Anthriscus schmalhausenii* (Albov) K.-Pol.
○ *Astrantia colchica* Albov
◎ *Chaerophyllum roseum* Bieb.
○ *Cnidium grossheimii* Manden.
◎ *Heracleum chorodanum* (Hoffm.) DC.
○ *H. grossheimii* Manden.
◎ *H. mandenovae* Satzyperova
○ *Polylophium panjutinii* Manden. et Schisck.

25. Urticaceae

○ *Parietaria kemulariae* Schchian

26. Valerianaceae

◎ *Valeriana colchica* Utkin

◎ *V. jelenevskyi* P. Smirn.
◎ *V. tiliifolia* Troitzk.

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Monocotyledoneae

27. Alliaceae

○ *Allium gracilescens* Somm. et Levier
◎ *A. karsianum* Fomin

28. Amaryllidaceae

○ *Galanthus schaoricus* Kem.-Nath.

29. Convallariaceae

◎ *Convallaria transcaucasica* Utkin ex Grossh.

30. Hyacinthaceae

○ *Muscari alpanicum* Schchian
○ *Ornithogalum imereticum* Sosn. [*O. woronowii* Krasch. var. *imereticum* (Sosn.) Grossh.]

31. Iridaceae

◎ *Iris colchica* Kem.-Nath.
Crocus speciosus Bieb. ○ var. *imereticus* Kem.-Nath.

32. Liliaceae

◎ *Erythronium caucasicum* Woronow

33. Orchidaceae

Ophrys caucasica Woronow ex Grossh. ◎ subsp. *caucasica*

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We are grateful to Dr. R. Gagnidze for editing our paper.

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იმერეთის ენდემური ფლორის მრავალფეროვნება

ჭეიშვილი თ.[1], ჭურაძე მ.[2]

[1] ა. წერეთლის სახელობის ქუთაისის სახელმწიფო უნივერსიტეტის ბიოლოგიის დეპარტამენტი
[2] ი. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტის ბოტანიკის კათედრა

(მიღებულია 10.12.2005)

რეზიუმე

შესწავლილია იმერეთის ფლორის მრავალფეროვნება. იმერეთის ფლორის შემადგენლობაში გამოვლენილია კავკასიისა და საქართველოს ენდემური 121 სახეობა და 5 ქვესახეობა. მათ შორის კავკასიის ენდემია 69, საქართველოს ენდემი – 52 სახეობა. იმერეთის ლოკალური ენდემების რიცხვი 14-ია. აღნიშნულია კირქვიანი ეკოტოპების ენდემების თავისებურება და მრავალფეროვნება. გამოტანილია დასკვნა, რომ კირქვიანი ეკოტოპების ენდემური ფლორის ჩამოყალიბება გეოგრაფიული იზოლაციის შედეგია.

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Botany

PALYNOLOGICAL STUDY OF THE CAUCASIAN SPECIES OF THE GENUS SERRATULA L. (COMPOSITAE) IN VIEW OF THEIR SYSTEMATICS

JUGELI T.

Institute of Botany of the Georgian Academy of Sciences
E-mail: 15Djugeli @rambler.ru

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Abstract

Morphological characteristics of pollen of multyspecific genus Serratula distributed in the Caucasus were studied microscopically. Pollen of these genera is stenopolynous. Their sizes are given. 5 subtypes of exine by reticulum structure were determined and distinguished. Morphological peculiarities of pollen grains revealed pollen dimorphism of some species and especially of section Klasea.

Key words: exine reticulum, pollen dimorphism, Klasea, *S. nudicaulis*,

Introduction

Genus Serratula distributed in Eurasia and North Africa counts tens of species. Representatives of genus are permanent grasses. In the Caucasus occur 8 genera [Jugeli, 2005]. In scientific literature there are a lot of data about pollen analysis of composite family but about morphological peculiarities of pollen of genus Serratula species are rather poor [Aleshina, 1972; Canto, 1984; Bovina 2004; Meier-Melikian, Bovina, 2004].

The aim of our work was to study pollen morphological characteristics of genus Serratula of the Caucasian species in the view of the systematics, ecology and floristic genetic affinities.

Materials and Methods

We have studied the following species: *S.erucifolia* (L.) Boriss. (section Piptochaete), *S.radiata* (Waldst. et Kit.) Bieb. (section Klasea), *S.nudicaulis* (L.) DC.subsp. transcaucasica (Bornm.) Djugeli (section Klasea), *S.coriacea* Fisch. et C.A.Mey. (section.Klasea), *S.quinquefolia* Bieb. ex Willd. (section Klasea), *S.serratuloides* (Fisch. et C.A.Mey.) Takht. (section Leuzeopsis), *S.caucasica* Boiss. (section Demetria).

As a researching material we used collection of herbarium of The Institute of Botany and private collection. Electron microscopic analysis was carried on JMS-35C. Pollen was previously treated with 70% ethanol and then shadowed in vacuum camera. Studies were also carried out on light microscope via acetolysis method.

Results and Discussion

Morphological studies of Serratula pollen grains showed that they varied by sizes, apertures, and reticulum structure of exine. Pollen of studied genera is stenopolynous; It is 3-colporate, oblate spheroidal, radiosymmetrical. In the polar position it has 3-rounded-loptate (fossaperturate pollen grains) shape, and in equatorial – rounded or ellipsoid outline. Maximal amb of oblate pollen grains do not coincide with equator. At the breaking of colpus membrane pollen grains took ellipsoid form.

Pollen sizes usually do not depend on flower sizes and in average it is 50 μm. Species of *S.radiata* have pollen grains of minimal sizes (Fig.1.a) and species of *S.quinquefolia* – grains of maximal sizes (Fig.1.b).

Aperture of pollen grains is diorite; furrows (sulci) – meridional pontoperculate orate [Punt et al., 1993], more or less long (20-26 μm) with mucronated, not reaching up to the poles ends. Furrows membrane is granulated. Species of *S. radiata, S.quinquefolia* and *S.caucasica* have strongly convex geniculum. Sizes of apocolpium and mezocolpium are 22-32μm and 32-42μm correspondingly. Colpus – equadorial; colpus membrane, as well as furrow membrane are formed by exine; surface – pleated or smooth, with coarse rare sporopollenin granules. Sporoderm is composed from exine and intine. Exine is thickened, in its turn - composed from inner and outer parts, stratification type – astral [Vezey, 1994]. Endoexine is consists of more fine bacula, interfluent with the part of sexine – tectum. Species differ from each other by the character of exine reticulum, which is of spicular type. We distinguished 5 subtypes of exine: spicular-lacunary (Fig.1.c); spicular-conicolate-lacunar-like (Fig.1.d); tuberous-lacunar-like (apomorphic spicular state) [Jeffrey, 2002] (Fig.1.e); spicular-costate-lacunary (Fig. 1. f); spicular-costate-lacunar-like (Fig.1.g).

It is worth to mention that according to the morphological peculiarities of pollen grains, pollen dimorphism of some species, and especially of species Klasea was revealed. Dimorphism encloses existence of round and oval pollen in one and the same flower. Same dimorphism was found by Canto (Canto, 1984) for the section Klasea, species – *S.nudicaulis*.

According to the obtained data we can conclude that pollen grain features of the genus Serratula are one of the criteria for determination of species specificity and identification one or another taxon. In this viewpoint pollen grain sizes and type of exine reticulum may be considered as a significant diagnostic feature. Differences of pollen grains sizes of the species of genus Serratula and their dimorphism should be taken into account at the paleopalynological, as well as at paleoecological studies.

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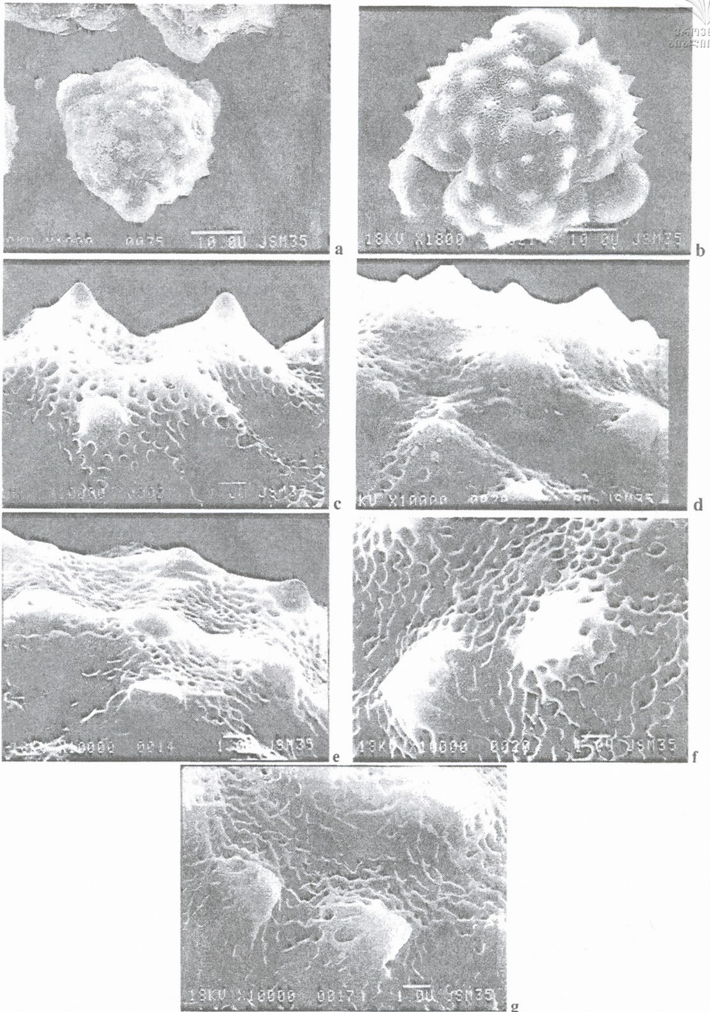

Fig. 1. a - S. radiata; b - S. quinquefolia; c - S. quinquefolia, S.coriacea; d - S. radiata; e - S. nudicaulis, S. erucifolia; f - S. caucasica; g - S.serratuloides.

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გვარი **Serratula L. (Compositae)** -ს კავკასიის სახეობების პალინოლოგიური შესწავლა

ჯუღელი თ.

საქართველოს მეცნიერებათა აკადემიის ბოტანიკის ინსტიტუტი

(მიღებულია 20.09.2005)

რეზიუმე

შესწავლილია კავკასიაში გავრცელებული მულტისპეციფიკური გვარი Serratula L. (Compositae) –ს მტვრის მორფოლოგიური მახასიათებლები. მოცემულია მტვრის ზომები. მტვრის მარცვლები პალინოლოგიურად ერთტიპურია. რეტიკულუმის სტრუქტურის მიხედვით განსაზღვრული და გამოყოფილია ეკზინის 5 ქვეტიპი. მორფოლოგიური თავისებურებების მიხედვით გამოვლინილია ზოგიერთი სახეობის მტვრის მარცვლის დიმორფიზმი, განსაკუთრებით Klasea–ს სექციაში. Serratula–ს მტვრის მორფოლოგიური მახასიათებლების შესწავლა მნიშვნელოვანია სისტემატიკის, პალეოეკოლოგიის საკითხების გადასაჭრელად, და ასევე ფლორათა გენეტიკური კავშირების დასადგენად.

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ეროვნული ბიბლიოთეკა

STRUCTURE AND FUNCTION OF THE HYPERTROPHIC SYNERGID IN SOME SPECIES OF GENUS *ALLIUM* L.

NADIRASHVILI N., GVALADZE G., AKHALKATSI M.

Institute of Botany, Georgian Academy of Sciences

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Abstract

In some species of genus *Allium* L. mainly one and sometimes both of the synergid cells enlarge in size, undergo endopolyploidization and become hypertrophic. We have studied structure of the synergid cells of *Allium atroviolaceum* Boiss., *A. rotundum* L., *A. fistulosum* L. and *A. cepa* L. and determined DNA amount (C value) in the synergid cells of *A. atroviolaceum*. Cytophotometric study of various Feulgen-stained synergid and integument nuclei revealed clear difference in DNA content both in different types of the cells and in different stages of synergid development. The amount of DNA measured in newly formed synergid was already equal to 2C value found in the integument cell nucleus. At the time of fertilization the DNA amount in synergid is 4C. At the stage of proembryo it is already 6C and before degeneration at the late globular stage of embryo development 8C. No sign of mitotic cell division or formation of metaphase plate have been observed in any of investigated materials. It is assumed that endopolyploidization might determine longer persistent of intact synergid and increase of its trophic function.

Key Words: Embryology, ovule, synergid, endopolyploidy

Introduction

The egg cell in the embryo sac of flowering plants is generally accompanied by two symmetrical cells, called synergid cells, which usually contains haploid nucleus like as the other cells of the female gametophyte. However, in some species of genus *Allium* L. (*Allium cepa, A. nutans, A. rotundum, A. schoenoprasum, A. uniflorum, A. ursinum* etc.) mainly one and sometimes both of the synergid cells enlarge in size, undergo endopolyploidization and become hypertrophic [Weber, 1929; Tschermak-Woess, 1950; Hasitschka-Jenschke, 1957; Gvaladze, 1962,1976; Sokolov, 1968; Batygina, 1990]. The mechanism and the role of this phenomenon, however, are not known until now.

The general role of the synergids in the embryo sac is assumed to be cooperation with egg and central cells to accomplish double fertilization. This cooperation is of crucial importance in the attraction and acceptance of the pollen tube [Higashiyama, 2002]. The last develops from the pollen grain after germination on the stigma and carries two male gametes through the maternal reproductive tissues to the embryo sac, which contains two female gametes, egg and central cells. The sperm cell of a flowering plant cannot migrate unaided and it must be transported by the pollen tube before successful fertilization can occur. The mechanism of guidance of the pollen tube from stigma to the embryo sac has been studied for more than a century. Nowadays, it is determined that

synergids play most significant role in this process attracting pollen tube due to chemotropic and diffusible signals [Higashiyama, 2002].

The pollen tube penetrates into one of the synergid cells and releases its two male gametes leading to the degeneration of the synergid cell. The second persistent synergid remains intact during some period after fertilization and degenerates gradually. Two male pronuclei enter the egg and the central cells and accomplish syngamy (fusion of sperm nucleus with egg nucleus) and triple fusion (unification of the sperm and two polar nuclei). These two processes represent consistent steps of double fertilization determining formation of embryo and endosperm correspondently.

The function of synergids determines their structure. They develop distinct filiform apparatus, some kind of cell wall protuberances at the micropylar end enlarging plasmalemma surface and playing a role in reception of pollen tube. The cytoplasm contains abundant organelles, such as mitochondria, endoplasmic reticulum, and plastids, which indicate that they are metabolically active cells. No cell wall is present at the chalazal part of a synergid, and there are some flocculent materials and vesicles in the spaces of cytoplasm membranes among synergid, egg cell and central cell in embryo sacs. It is assumed that synergids besides participation in double fertilization have trophic role supplying embryo sac with nutritive substances [Higashiyama, 2002].

In the most flowering plants, both synergids show similar structure before fertilization and contain haploid nuclei. The fact of synergid proliferation in some species of genus *Allium*, however, should be indicative of different functional role, which it might play during seed formation. So far, not much is known about a special role of such giant synergid. According to literature data, synergids of *A. ursinum* undergo endomitosis direct after cell formation in the embryo sac and become polyploid. At the fertilization time, the degenerating synergid receiving pollen tube contains 4n, and the nucleus of persistent synergid 16n set of chromosomes [Hasitschka-Jenschke, 1957]. Endomitosis in one of the synergid of *A. nutans* lead to formation of polytene chromosomes and cause proliferation of the nucleus, while the second synergid remains haploid. Histochemical studies have revealed that the cytoplasm of the proliferated synergid before fertilization compare with the egg cell contains more storage substance, such as polysaccharides, rRNA, proteins, fats [Batygina, 1990]. The nucleus is more intensively stained for DNA and RNA. During endosperm development the storage substances disappear from the synergid, which according to some authors might be indicative of trophic function of them [Batygina, 1990]. In spite of these data, in general information about factors determining synergid proliferation in the genus *Allium* is scarce and it is not known what the function of this phenomenon is.

Throughout growth and development, eukaryotic organisms must co-ordinate DNA synthesis, chromosome segregation, and cell division in order to generate differentiated cells with the proper complement of chromosomes. The amount of DNA in plant nuclei has been possible to estimate for over 50 years. Work on plants has played a leading part in research to describe and understand the origin, extent and effects of variation in the DNA amount in the unreplicated haploid nuclear chromosome complement defined as the 1C-value. Nowadays, C-values are determined for over 4100 species of angiosperms. They vary c. 1000-fold from c. 0.11 pg in *Fragaria viridis* to 127.4 pg in *Fritillaria assyriaca* [Bennett, Leitch, 1995]. Polyploids are expected to have larger C-values than their diploid progenitors, increasing in direct proportion with ploidy. Similarly, basic genome size is predicted to be the same at all levels of ploidy. Endopolyploidization, i.e. the existence of different ploidy levels (labelled as 2C, 4C, 8C…) in adjacent cells of a species, is a common phenomenon in seed plants. Nevertheless, the biological significance of endopolyploidy is not yet clear.

In the present study we addressed the following questions: 1) What is the nature of synergid proliferation in species of genus *Allium*; 2) what is biological function of synergid proliferation; 3) what is DNA C-value in the nucleus of proliferated synergid.

Materials and Methods

The following species of genus *Allium* L. have been used in this study: *Allium atroviolaceum* Boiss., *A. rotundum* L. (genus *Allium* L., subg. *Allium*, sect. *Allium*), *A. fistulosum* L. (genus *Allium* L., subg. *Rhizirideum* (G.Don ex Koch) Wendelbo, sect. *Cepa* (Mill.) Prokh., subsect. *Phyllodolon* (Salisb.) Kamelinand) and *A. cepa* L. (genus *Allium* L., subg. Rhizirideum (G.Don ex Koch) Wendelbo, sect. *Cepa* (Mill.) Prokh., subsect. *Cepa* (Mill.) Stearn).

For light microscopy, buds, flowers and fruits have been collected at different stages of development, fixed in FAA (formalin, acetic acid, 70% ethanol, 5:5:90) and embedded in paraffin. 10-12 μm thick sections were prepared on microtome Reichert, Austria, and stained in hematoxylin according to known method by Meier. Examination was carried out using light microscope Polivar, Reichert, Austria.

The investigation of endopolyploidization pattern in the nucleus of giant synergid of *A. atroviolaceum* (2n=16) was carried out by cytophotometric measurements of DNA content. Investigation was carried out in the Laboratory of Cytology at the Institute of Zoology of the Georgian Academy of Sciences. DNA content was determined by scanning cytophotometer, Reichert, Austria, with one-wave cytophotometric method (wave length was 550 μm) on sections after Feulgen staining. Probe diameter was 0,63 μm. All nuclei were scanned with the same magnification (ocular x15, objective x40). DNA content was determined using the formula C=VA, where C is DNA content in a single nucleus, V – volume of a nucleus, A – DNA concentration on the section of a nucleus. Error of the method is 5%. Obtained data were processed statistically [Brodskii, 1956]. DNA content of the integument cell nucleus (number of measured nuclei x=30) in the ovule of *A. atroviolaceum* was used as reference for species-specific diploid DNA content. DNA content in synergid nuclei was measured at different stages after cell formation in the embryo sac up to late globular stage of the embryo development (x=50).

Results and Discussion

The ovule in all studied species is ortho-campylotropous, crassinucellate, with funicular obturator covering micropyle (Fig. 1 A, B, C). It is bitegmic. Embryo sac in investigated species develops according to the bisporic Allium type. The megaspore nuclei in the diade undergo two successive mitotic divisions and form 8-nucleate embryo sac with 3-celled egg apparatus, containing egg cell and two symmetrically located synergids, the central cell with two polar nuclei, and 3 antipodals. During maturation of the embryo sac, one of the synergids enlarges in size considerably and becomes giant (Fig.1 B, D, E, F). It has much bigger sizes than the second synergid (Fig.1 G, H, I). Filiform apparatus persists in both bigger and smaller cells, but, it is more prominent in the hypertrophic one. Polar nuclei in the central cell do not fuse before fertilization. However, they form close contact with each other (Fig.1 D). Antipodals, as usual, are ephemeral and degenerate during maturation of the embryo sac.

The morphology of the giant synergid changes in different developmental stages. Soon after formation it contains larger nucleus than the egg cell and the second synergid (Fig.1 D). The form of the nucleus is lobed and irregular. At early stages of the development, the synergid nucleus possess heavily condensed chromatin region, which by position and structure corresponds to polytene chromosomes. The condensation increases during fertilization and afterwards during embryogenesis (Fig.1 H, I). Simultaneously enlarges cell volumes of the synergid. In *A. cepa* we have observed that the second synergid, which receives the pollen tube, enlarges together with the giant synergid. It might have lesser size or in some cases be equal to the giant one (Fig.1 I).

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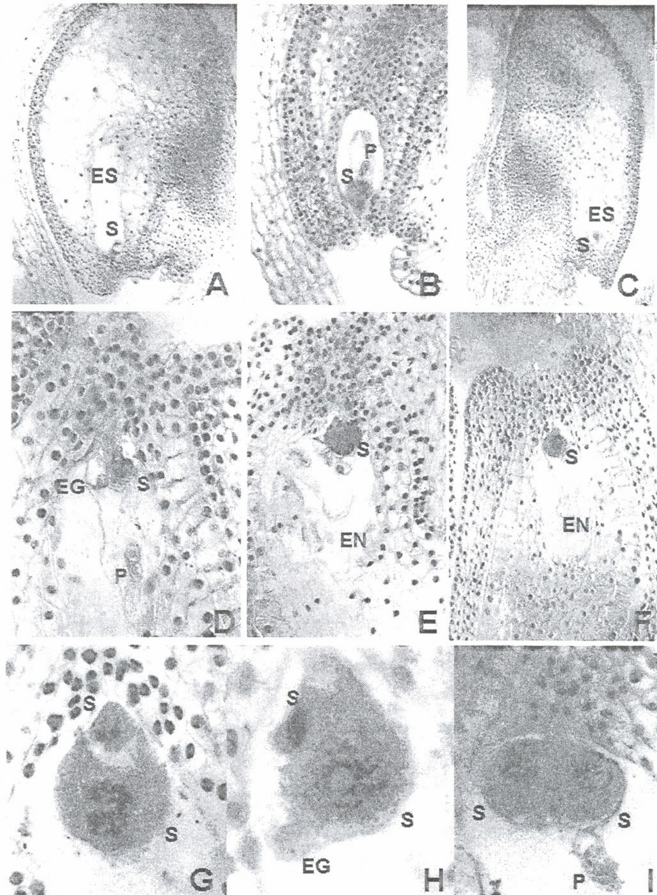

Fig. 1. A – Ovule and embryo sac of *Allium fistulosum*, x200; B – Embryo sac with giant synergid in the ovule of *A. cepa*, x 250; C – Ovule of *A. rotundum*, x 180; D – Giant synergid, egg cell and polar nuclei before fertilization in *A. cepa*, x 390; E – Giant synergid at the moment of fertilization in *A. atroviolaceum*, x 410; F – Giant synergid and nuclear endosperm in *A. fistulosum*, x380; G – Condensed chromatin in the polyploid nucleus of hypertrophic synergid in *A. atroviolaceum*, x 550; Structure of nucleus of the giant synergid, degenerating synergid and egg cell in *A. fistulosum* before fertilization, x 550; I- Proliferation of both synergids in *A. cepa*, x 510. EG- egg cell; EN – endospem nucleus; ES – embryo sac; P – polar nucleus; S – synergid.

Before fertilization both synergis are intact. Shortly before fertilization, cytoplasm and nuclear content of the smaller synergid become more condensed. The pollen tube penetrates into the smaller synergid cell and releases its contents. After this process the synergid degenerates. The persistent giant synergid remains intact until later stages and degenerates at late globular stage when the embryo becomes pear shaped.

Cytologically, the structure of the somatic interphase nuclei of the integument tissue is euchromatic and contains more or less uniformly distributed chromocenters, which should correspond to the constitutive heterochromatin. The chromatin is condensed and distributed uniformly in the nucleus.

Cytophotometric study of various Feulgen-stained synergid and integument nuclei revealed clear difference in DNA content both in different types of the cells and in different stages of synergid development. The amount of DNA measured in newly formed synergid was already equal to 2C value found in the integument cell nucleus. The increase of DNA content in the synergid nucleus starts before fertilization and continues until late globular stage of embryo development, when degeneration processes start (Fig. 2). The following dynamic was observed. Newly formed synergid in *A. atroviolaceum* contains 2C DNA. At the time of fertilization this amounts to 4C. At the stage of proembryo it is already 6C and at the late globular stage of embryo development 8C. No sign of mitotic cell division or formation of metaphase plate have been observed in any of investigated materials. Intermediate amounts of DNA were found only in tissues presumably undergoing an interphase synthesis of DNA preceding endoreduplication.

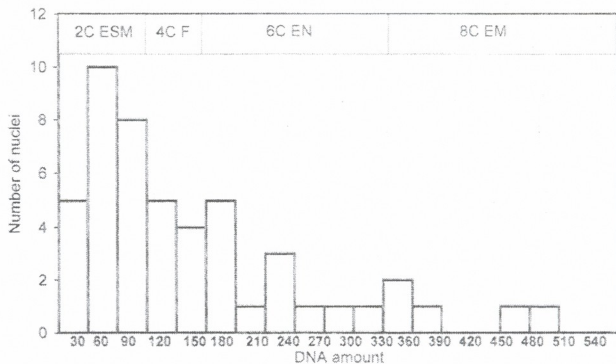

Fig. 2. DNA amount (C) in conditional optical units and number of nuclei measured in the giant synergid of *A. atroviolaceum* at different stages of development. The DNA amount, 2C, 4C, 6C and 8C corresponding to the optical units are shown at the top of the graphic together with the stages of development: EMS – embryo sac maturation, F – fertilization, EN – nuclear endosperm formation, EM – embryogenesis.

Nuclear polyploidy is commonly encountered in eukaryotic tissues, and its occurrence in plants and animals is well reviewed [Leitch, 2000]. Nuclear polyploidy can hugely increase the DNA content of a cell. Nagl [1978] reviewed maximum levels of polyploidy and reported values as high as 8,192C in the suspensor cells of the plant *Phaseolus coccineus* and 524,288C (i.e., 219C) in silk glands of the insect *Bombyx mori*. There are several mechanisms that give rise to polyploidy. The first is endoreduplication, where genomes replicate without cell division. In many organisms

the chromatids remain tightly associated, forming polytene chromosomes, and these have been found in a diverse range of tissues and taxa in vascular plants, they regularly occur in synergid and tapetal cells [Hasitschka-Jenske, 1957; Leitch, 2000]. Another mechanism is endomitosis, where replicated chromosomes condense as if entering mitosis but then do not segregate; instead, they remain together within a single nucleus. Polyploidy may also be accompanied by genome reorganization via DNA splicing, as occurs in the ciliate *Oxytricha* and by polytene chromosome breakage, as occurs in some cells of *Drosophila melanogaster* [Leitch, 2000].

The results of the present study have revealed that the nucleus of the synergid cell in *A. atroviolaceum* undergoes endoreduplication up to 8C. The increase in DNA amount occurs by DNA doubling as it is usual in polyteny (1C, 2C, 4C, 8C) [Leitch, 2000]. The volume of the nucleus increases considerably and changes in patterns of nuclear morphology. Thereafter, the nucleus becomes lobed and fragmented, and amount and sizes of dense chromatin masses increase considerably. These changes have to be indicative that nuclear polyploidy is involved in cell development. Furthermore, once DNA replication is complete, the nucleus can continue to change and undergo further differentiation.

It is generally assumed that polyploidy occurs to amplify genes without the energetically demanding process of cell division [Leitch, 2000]. Thus, many secretory cell types are polyploid. A gene amplification-expression argument to explain polyploidy does not explain the several types of polyploidy found in a single organism or why polyploid nuclei themselves undergo developmental changes. It is possible that the polyploidization process in the synergid of genus *Allium* increases metabolic activity of the cell, which might be involved in the nutrition of the embryo in early stages of endosperm development.

There are other potential roles for polyploidy. In polyploid nuclei, chromosome arms might be able to associate in a manner that is impossible without multiple copies of each chromosome. Such interactions might be important for chromosome *trans*-sensing [Leitch, 2000]. Alternatively, nuclear polyploidy could amplify the genetic component of a cell which is destined to be long-lived and perhaps vulnerable to mutation. In so doing, nuclear polyploidy might extend the duration of cell viability. The full significance of polyploidy is unknown. However, it could play roles in gene amplification, genome restructuring, chromosome interactions, and cell longevity. It is not excluded that the polyploidization process in the persistent synergid in genus *Allium* determines longer functioning of this cell degenerating usually soon after fertilization, i.e. after completion of the main task of this type of cell. However the trophic function might be good reason for organism to maintain a structure participating in crucial developmental process, such as supply the embryo with nutrients.

One interesting point is the negative correlation between genome size and extent of endopolyploidization in animals and seed plants [Nagl, 1978]. It is assumed that occurrence of endopolyploidy in seed plants is determined genetically. Moreover, endopolyploidization behaviour seems to be typical for some seed plant families [Tschermak-Woess 1950; Nagl 1978]. In some taxonomic groups many species contain highly endopolyploidized tissues whereas this is not the case in other taxa. The degree of endopolyploidization differs between the different organs of a species. Additionally, endopolyploidization is related to life cycle. Endopolyploidy is very frequently found in annual and biennial species and also in some perennial herbs whereas it seems to be absent in wooden species. At the same time, it was assumed that a minimum amount of nuclear DNA in species with small genomes is frequently realized by endoreduplication, to be required to maintain the regulatory and functional state of certain cells [Kasahara et al., 2005]. All these data are indicative of importance of endopolyploidization for maintenance of successful life conditions for living organisms. As well, it seems that some taxonomic groups are more depending on the increase of genome size than others.

Variation in DNA content is common for vegetative parts of different species of genus *Allium*. It was estimated [Ohri et al., 1998] that 4C DNA amounts of 86 species from genus *Allium* show a 8.35-fold difference ranging from 35.60 pg (*A. ledebourianum*, 2n = 16) to 297.13 pg (*A. validum*, 2n = 56). At diploid level the difference was 3.57-fold between *A. ledebourianum* (35.60 pg) and *A. ursinum* (127.14 pg). Strong variation in genome size has been obtained for 28 species and altogether 57 accessions or cultivars by different authors [Baranyi, Greilhuber, 1999]. These results have shown that a significant loss and/or gain of DNA have to occur during evolution of these taxa. Therefore, it should be expected that the genome size variation might occur within different cell types and at different developmental stages in the species of this genus.

Acknowledgement

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Allium-ის გვარის ზოგიერთი სახეობის ჰიპერტროფული სინერგიდას სტრუქტურა და ფუნქცია

ნადირაშვილი ნ., ღვალაძე გ., ახალკაცი მ.

საქართველოს მეცნიერებათა აკადემიის ბოტანიკის ინსტიტუტი

(მიღებულია 02.12.2005)

რეზიუმე

ყვავილოვანი მცენარეების კვერცხუჯრედი ჩვეულებრივ გარშემორტყმულია ორი სიმეტრიულად განლაგებული სინერგიდათი, რომლებიც, როგორც წესი, ჰაპლოიდურ ბირთვს შეიცავენ. თუმცა, *Allium*-ის გვარის ზოგიერთ სახეობაში ერთი ან ზოგჯერ ორივე სინერგიდა იმატებს ზომაში, განიცდის ენდოპოლიპლოიდიზაციას და ხდება ჰიპერტროფული. ჩვენ შევისწავლეთ სინერგიდების სტრუქტურა *Allium*-ის გვარის შემდეგ სახეობებში *Allium atroviolaceum* Boiss., *A. rotundum* L., *A. fistulosum* L. და *A. cepa* L. და განვსაზღვრეთ დნმ-ის რაოდენობა *A.atroviolaceum*-ის სინერგიდას ბირთვში. ჩატარდა ფიოლგენით შეღებილი სინერგიდას და ინტეგუმენტის ბირთვების ციტოფოტომეტრიული შესწავლა, რომელმაც გამოავლინა მკვეთრი სხვაობა დნმ-ის რაოდენობას შორის, როგორც განსხვავებული ტიპის ბირთვებში, ისე სინერგიდას განვითარების სხვადასხვა სტადიაზე. დნმ-ის რაოდენობა სინერგიდას ფორმირებისთანავე შეადგენდა 2C–ს. განაყოფიერების წინ იგი უდრიდა 4C-ს. პროემბრიონის სტადიაზე 6C–ს, ხოლო დეგენერაციის წინ, ჩანასახის განვითარების გვიან გლობულარულ სტადიაზე, 8C-ს. ნავარაუდებია, რომ ენდოპოლიპლოიდიზაცია განაპირობებს სინერგიდას არსებობის გახანგრძლივებას და მისი ტროფიკული ფუნქციის გაზრდას.

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Botany

ECOCOENOTIC DISTRIBUTION OF BRYOFLORA IN LOWER FOREST BELT OF LAGODEKHI RESERVE

TIGISHVILI K.

N. Ketskhoveli Institute of Botany of Georgian Academy of Sciences

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Abstract

The pattern of bryoflora distribution has been studied in three contrasting types of forest coenoses in the lower forest belt of Lagodekhi Reserve. The humid forests developed on proluvial terraces of narrow river ravines turned out to be the richest in bryoflora species - 120 species. Then follow hornbeam forests and Fagetum nudum forests with 98 and 80 moss species correspondingly. Table of dominant species distribution according to ecotopes is compiled for each forest type.

Key words: ecotope, Hepaticeae, epylithic bryoflora, epigeal mosses.

Introduction

Forests of lower belt of Lagodekhi Reserve, especially those developed on the proluvial lower fluvial terraces deserve great interest as natural monuments. Such forests have not been preserved in untouched state at present in East Georgia [Dolukhanov, 1942]. Forests of lower mountain belt are contiguous with populated areas and thus are subjected to the severe anthropogenic load [Dolukhanov, 1941; Kvachakdze, 1999]. In this viewpoint to reveal complete taxonomic structure of bryoflora and establishing its ecocoenotic distribution seems to be very important.

Materials and Methods

Ecocoenotic distribution of bryoflora in lower forest belt of Lagodekhi Reserve was studied in forest coenoses of three contrasting types: a) in the depth of ravines, in the mixed forests formed on river proluvial terraces with bramble, fern and ivy cover; b) in the forests developed on well illuminated and steep slopes of S and SE expositions with hornbeam domination; c) in Fagetum nudum forests developed on the slopes of medium inclination of N and NW exposition [Dolukhanov, 1941]. Nine ecotopes have been singled out in each ecosystem: 1. newly formed bare soils; 2. formed soils; 3. stony and rocky complexes; 4. decaying wood; 5. water ecotopes; 6. tree basis and epirhizum; 7. tree stem; 8. large branches; 9: small branches. Ecocoenotic distribution of mosses was studied using itinerary and semistationary methods. Material was collected according to the geobotanical method.

Results and Discussion

Fig. 1 illustrates quantitative distribution of bryoflora in different ecotopes. Distribution of bryoflora species in ecosystems is presented in Fig. 2 and Tables 1, 2, 3.

Distribution of mosses in different forest types is subjected to the certain regularity. It is connected with main types of ecosystems on the one hand and with the variety of ecotopes on the other. Humid forests of narrow river ravines are distinguished by the greatest taxonomic variety and high biomass of bryoflora. Such ecotopes are less windy, more cloud. Relative air humidity is high, fluctuation of temperature is less. These conditions are optimal for the development of Bryophytes. Total of 120 species of Bryopsida were stated in this ecosystem [Tigishvili, Chikovani, 1983]. Great variety of Hepaticeae (liwerworts) is also evident. Distribution of species by ecosystems is as follows: 1- 15, 2 -30, 3 - 80, 4 - 18, 5 - 1, 6 - 25, 7 - 10, 8 - 5, 9 - 2 (Table 1). Richness of epylithic bryoflora is conditioned by the variety of stony-rocky ecotopes. Especially should be noted large stones of 2-3 m dimensions completely covered with mosses, found on shaded riverside terraces. *Thamnium alopecurum, Mnium undulatum, Mnium punctatum, Ctenidium molluscum, Brachythecium rivulare, Dichodontium peluicidum* and *Racomitrium aciculare* make the core of dominant epilythic flora. Permanently moistened rocks are also characteristic to the mentioned ecosystem. The peculiar group is comprised here by the following species of mosses: *Neckera crispa, Pleuropus euchleouron, Racomitrium aquaticum, Calliergonella cuspidata, Philonotis fontana.* The mentioned complexes of mosses are unique for the given ecosystem. In stony dippings, situated on the well illuminated areas of riversides not covered with forest, small dense patches, formed with mosses *Grimmia pulvinata, Schistidium apocarpum* and *Hedwigia ciliata* are found.

Small areas of bare soils are rarely met in humid forest ecosystems (1st ecotope). Specific composition of bryoflora of the given ecotope is rather scanty and is represented by the following taxons: *Atrichum undulatum, Atrichum angustatum, Tortula subulata, Eurhynchium speciosum, Brachythecium rutabulum.* It should be mentioned, that in all three contrasting forest types synusia of mosses, characteristic to the first ecotope is nearly identical. In general, soil coverage with mosses is insignificant and it does not have regular character, but specific composition of bryoflora is rich. Complex of epigeal mosses is not of specific character and it partly resembles the composition of epilythic flora: *Mnium undulatum, Mnium punctatum, Fissidens cristatus, Fissidens taxifolius, Euhynchium zetterstedtii.* The process of wood decay and decomposition goes quickly in humid forests. Such substrate is very common here and it is almost completely covered by epixylic flora, where the following species dominate: *Bracyithecium sallebrosum, Brachythecium rutabulum, Brachythecium velutinum, Mnium cuspidatum, Hypnum cupressiforme.* In the flowing water and on the surfaces of stones *Rhynchostegium riparioides* can be found.

Epiphytic flora of tree stems and epirhizum is less diverse as compared with epilyths. Mainly the following species have been stated here: *Brachythecium populeum, Plagiothecium neglectum, Hypnum cupressiforme, Mnium undulatum.* For tree stems *Ulota crispa, Pylaisia polyantha* and *Hypnum cupressiforme* were characteristic. The typical epiphyte *Leucodon sciuroides* is less common here. Large and small branches of trees are inhabited by small number of species. Bryoflora here is presented by the thin cover of *Orthotrichum speciosum, Ulota crispa* and *Leucodon sciuroides.* Comparison of bryoflora species of different ecotopes makes clear the mode of migration of mosses, characteristic for undestructed phytocoenoses - the first row: stem and branhces of a tree → tree base → decaying wood → forest soil; the second row: bare soil → forest soil with litter [Bardunov, 1961, Malysheva, 1991].

In the second type of studied ecosystems, developed on the well illuminated slopes of South and South-East expositions, bryoflora is represented by epilythic and epigeal mosses. Quantitative distribution of species according to ecotopes is as follows: 1 - 5, 2 - 35, 3- 40, 4 - 15,

5- 1, 6 - 28, 7 - 16, 8 - 9, 9 - 4 (Table 2). *Hypnum cupressiforme, Thuidium philibertii, Mnium cuspidatum, Dicranum scopiarium, Bracyithecium populeum, Bracyithecium rutabulum, Anomodon viticulosus* are the dominant species of epilythic grouping. Epigeal mosses do not form regular cover and steady synusia in hornbeam forest, but their specific composition is rather diverse. Fragments of mossy cover (of 5-10 m^2 area) are found on comparatively illuminated plain areas, where the main species are: *Dicranum scoparium, Hypnum cupressiforme, Polytrichum formosum, Mnium undulatum, Thuidium philibertii. Racomitrium conescens* and *Tortella tortuosa* make mossy cover on small areas in comparatively open and dry places. *Tortula subulata, Atrichum undulatum, Cynodontium polycarpum, Weisia controversa* make mossy cover usual for bare soils. As to the epixylic flora, it does not differ from species characteristic for analogous ecosystems of other forest types and it is presented by the following species: *Brachythecium sallebrosum, Mnium cuspidatum, Brachythecium velutinum, Hypnum cupressiforme*. Epiphytic flora is presented mainly by xeromesophyte and mesophyte species. *Anomodon viticulosus, Neckera besseri* and *Leucodon sciuroides* are characteristic for the tree base and roots. Nearly all surface of tree stem is covered by *Leucodon sciuroides*, *Hypnum cupressiforme*, *Neckera besseri* and *Radula complanata*. Sporadic occurrence of *Leucodon sciuroides* and *Orthotrichum* species is characteristic for small branches.

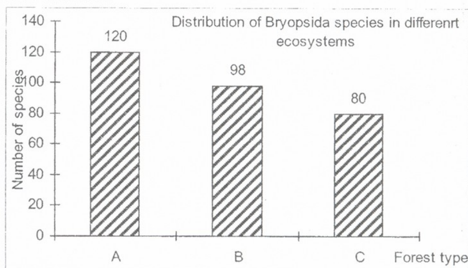

Fig. 1. A - Humid forests developed on proluvial terraces of narrow river ravines; B - Hornbeam forest; C - Fagetum nudum.

Landscape and phytocoenotic structure of Fagetum nudum is rather uniform. Less developed moss synusia is also evident. The species are distributed in different ecotopes in the following mode: 1 - 13, 2 - 14, 3 - 19, 4 - 14, 6 - 28, 7 - 15, 8 - 12, 9 - 5 (Table 3). Mosses of beech base and epirhizum determine the appearance of bryoflora. Specific diversity and the degree of coverage in this ecotope are higher than in other ecological niches. Dominant and characteristic group is composed by the following moss species: *Isothecium myurum*, *Brachythecium populeum*, *Ctenidium molluscum*, *Anomodon attenuatum*, *Pterigynandrum filiforme*, *Neckera besseri*. Epigeal mosses are slightly developed. Small microgroupings of mosses are spread only on small dense soil hillocks. Their specific diversity is rather limited and partly repeats the species of the previous ecotopes: *Ctenidium molluscum*, *Brachythecium rutabulum*, *Isothecium myurum*, *Eurhynchium speciosum*. The primary synusia of mosses settled on bare soils, formed on the places of trees eradicated by the wind is characteristic for Fagetum nudum: *Atrichium undulatum*, *Fissidens bryoides*, *Eurhynchium speciosum*, *Brachythecium rutabululm*, *Tortulla subulata*, *Dicranella heteromalla*. The following complexes of moss species are found on small stones and rocky remains: *Brachythecium populeum*, *Brachythecium rutabulum*, *Isothecium myurum*, *Schistidium apocarpum*, *Ctenidium molluscum*, *Hypnum cupressiforme*.

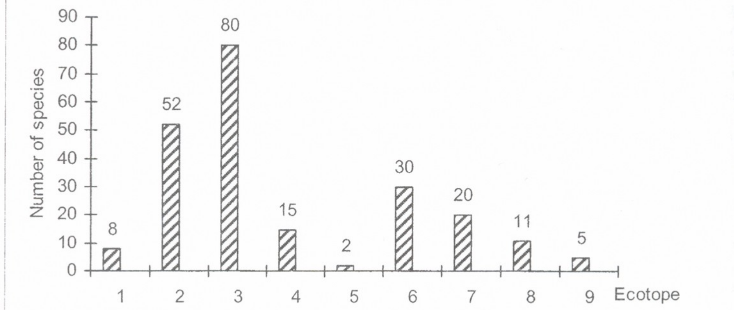

Fig. 2. 1 - newly formed bare soils; 2 - formed soils; 3 - stones and rocky complexes; 4 - decaying wood; 5 – aquatic ecotopes; 6 - tree basis and epirhizum; 7 - tree stem; 8 - large branches; 9 - small branches.

Table 1.

	Bryoflora species	Ecotopes								
		1	2	3	4	5	6	7	8	9
1.	*Atrichum angustatum*	+								
2.	*Atrichum undulatum*	+								
3.	*Brachythecium rutabulum*			+						
4.	*Brachythecium populeum*			+			+			
5.	*Brachythecium sallebnosum*				+					
6.	*Brachythecium rivulare*			+		+				
7.	*Ctenidium molluscum*			+						
8.	*Mnium undulatum*		+	+	+					
9.	*Mnium cuspidatum*		+	+	+					
10.	*Mnium punctatum*		+		+		+			
11.	*Fissidens cristatus*		+	+						
12.	*Plagiothecium neglectum*		+				+			
13.	*Isothecium myurum*		+	+						
14.	*Fissidens taxifolius*		+	+						
15.	*Eurhinchium speciosum*	+								
16.	*Hypnum cupressiforme*		+	+	+		+			
17.	*Ulota crispa*							+	+	+
18.	*Tortula sabulata*	+								
19.	*Neckera crispa*			+						
20.	*Leucodon spec.*						+	+	+	+
21.	*Radula complanata*							+		
22.	*Pleuropus euchleoron*			+						
23.	*Racomitrium acciculare*			+						
24.	*Rhynchostegium ripariodes*					+				
25.	*Thamnium alopecurum*			+						
26.	*Dichodontium pellucidum*			+						
27.	*Racomitrium aquaticum*			+						
28.	*Brachythecium velutinum*		+	+	+					

Table 2.

	Bryoflora species	Ecotopes								
		1	2	3	4	5	6	7	8	9
1.	*Atrichum angustatum*	+	+							
2.	*Atrichum undulatum*	+	+							
3.	*Brachythecium rutabulum*	+	+	+	+	+				
4.	*Dicranella heteromalla*	+								
5.	*Brachythecium velutinum*	+	+	+	+	+				
6.	*Brachythecium populeum*		+	+		+	+			
7.	*Fissidens bryoides*	+								
8.	*Tortula subulata*	+								
9	*Ctenidium molluscum*	+	+	+		+				
10.	*Isothecium myurum*		+	+	+	+				
11.	*Hypnum cupressiforme*	+	+	+	+	+	+	+	+	
12.	*Mnium affine*			+						
13.	*Schistidium apocarpum*			+						
14.	*Brachythecium sallebrosum*				+					
15.	*Leucodon spec.*				+	+	+	+	+	+
16.	*Pylaisia polyantha*	+		+	+	+	+	+	+	
17.	*Anomodon attenuatus*			+	+	+				
18.	*Neckera besseri*				+	+	+	+		
19.	*Pterigynandrum filiforme*			+	+	+	+	+	+	
20.	*Frulania dilatata*							+	+	
21.	*Orthotrichum striatum*								+	+
22.	*Porella platiphylla*						+	+	+	

Table 3.

	Bryoflora species	Ecotopes								
		1	2	3	4	5	6	7	8	9
1.	*Atrichum hauskrechtii*	+	+							
2.	*Amblystegiella subtilis*						+	+		
3.	*Brachythecium rutabulum*			+	+					
4.	*Brachythecium sallebrosum*		+		+					
5.	*Anomodon viticulosus*			+	+		+	+		
6.	*Neckera besseri*			+	+		+	+		
7.	*Mnium cuspidatum*		+	+	+					
8.	*Brachythecium populeum*			+						
9.	*Breidleria arcuata*					+				
10.	*Thuidium philibertii*		+	+						
11.	*Dicraium scoparium*		+	+			+			
12.	*Hypnum cupressiforme*		+	+	+		+	+		
13.	*Tortula subulata*	+								
14.	*Weisia controversa*	+								
15.	*Racomitrium canescens*		+							
16.	*Tortella tortuosa*		+							
17.	*Fissidens taxifolius*		+							
18.	*Pylaisia polyantha*							+		
19.	*Leucodon spee*			+	+		+	+	+	+
20.	*Thamnium alopecurum*			+						
21.	*Leskeela nervosa*			+		+	+			
22.	*Polytrichum formosum*		+							
23.	*Orthotrichum spec.*								+	+
24.	*Porella platyphylla*			+				+		
25.	*Ryinchostegium riparioides*					+				
26.	*Mnium undulatum*		+							

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ბრიოფლორის ეკოცენოლოგიური განაწილება ლაგოდეხის ნაკრძალის ტყის ქვედა სართულში

ტიგიშვილი ქ.

საქართველოს მეცნიერებათა აკადემიის ბოტანიკის ინსტიტუტი

(მიღებულია 13.12.2005)

რეზიუმე

შესწავლილია ლაგოდეხის ნაკრძალის ტყის ქვედა სართულის ბრიოფლორის ეკოცენოლოგიური განაწილების კანონზომიერება ტყის თანასაზოგადოების სამ კონტრასტულ ტიპში. ბრიოფლორის სახეობებით ყველაზე მდიდარია მდინარეთა ვიწრო ხეობების პროლოვიალურ ტერასებზე განვითარებული ტენიანი ტყეები - 120 სახეობა, შემდეგ რცხილნარები - 98 სახეობა და ბოლოს მკვდარსაფრიანი წიფლნარები - 80 სახეობა. ტყის თითოეული ტიპისათვის მოცემულია დომინანტი სახეობების განაწილების ცხრილი ეკოტოპების მიხედვით.

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ეროვნული ბიბლიოთეკა

Cytology

INFLUENCE OF A-VITAMIN DEFICIENCY ON THE ULTRASTRUCTURE OF THE EYE RETINA PHOTORECEPTORS OF WHITE MICE

DJANELIDZE KH., KVINIXIDZE G., GRATIASHVILI N.

Institute of Zoology, Georgian Academy of Sciences

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Abstract

It was shown, that A vitamin deficiency causes important ultrastructural changes in photoreceptors of retina of adult white mice: the membrane discs lose their usual strict orientation and destroy. The mitochondria of cones ellipsoid change their configuration and crista are disoriented and obliterated. This fact suggests that deficiency of A vitamin is the reason of photoreceptors membrane discs destruction, because the oxide of A vitamin – retinal is the structural component of visual membranes. Obliteration of the ultrastructure of mitochondria is secondary process, which is caused by destroy of molecular mechanism of the vision.

Key words: membrane discs, mitochondria, cones, ellipsoid.

Introduction

It is known, that A vitamin deficiency causes pathological changes of the vertebrates' retina after which the disease "the blindness of hen" takes place. The investigation was hold to study morphology and physiology of this disease. The first experiments on white rats were carried out in 1961 [Dowling, Gibbons, 1961], and later on the hens [Janelidze, 1978; Janelidze 2000].

As visual pigment - rhodopsin contains A-vitamin oxide - retinal [Vinnicov, 1971; Kvinikhidze et al., 2002], it would be interesting to find out what kind of pathological and physiological changes are shown in the photoreceptors during quite long period of A vitamin starvation. Study of these questions, has theoretical, as well as practical importance.

Materials and Methods

The investigations were carried out on adult white mice. The animals were divided into two groups: control (10 specimen) and experimental (10 specimen). Different food was given to the control animals, but the experimental mice were fed without vitamin-A for 4 months.

4 months later the retina of experimental and control mice were treated with the methods used in electronic microscopy: the material was fixed in 5% glutaraldehyde, pH 7.4; then it was fixed in 2% OsO_4 solution for 3 hours. The material was embedding in 812 epon epoxide. Ultrathin slides were made on the UM-2 ultratom (Reichert), the electrograms were made on the electronic microscope JM-100 B.

RESULT AND DISCUSSION

The results of investigations showed that the photoreceptors of retina of white mice are presented by rods: their external segments are represented by the folds of the plasmatic membrane, which is produced from the cilia's of embryonic photoreceptor cell membrane folds (Fig.1a).

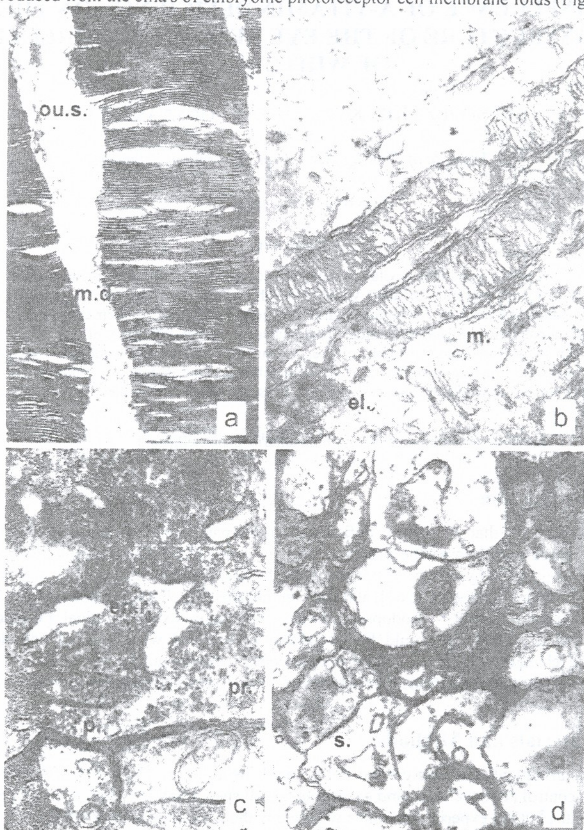

Fig.1 The fragments of photoreceptor cells of normal adult white mice, embeding in epon 812, X 45000. a) ou.s.- outer segment, m.d. – membrane discs: b) el. –ellipsoide, m. – mitochondria, c) p.-paraboloid, en.r.- endoplasmatic reticulum, pr.- polyribosoms; d) s- synapses between the photoreceptors and bipolar cells.

The ellipsoid is placed in the middle of the photoreceptor, which contains thick mitochondria heap and they have ellipsoid shape. These mitochondria have well expressed dual

plasmatic membrane and long crista, which are oriented perpendicular (Fig.1b). Under the ellipsoid there is the paraboloid, which is represented with well expressed granular and smooth endoplasmic reticulum. There are many complexes of ribosome in cytoplasm (Fig.1c).

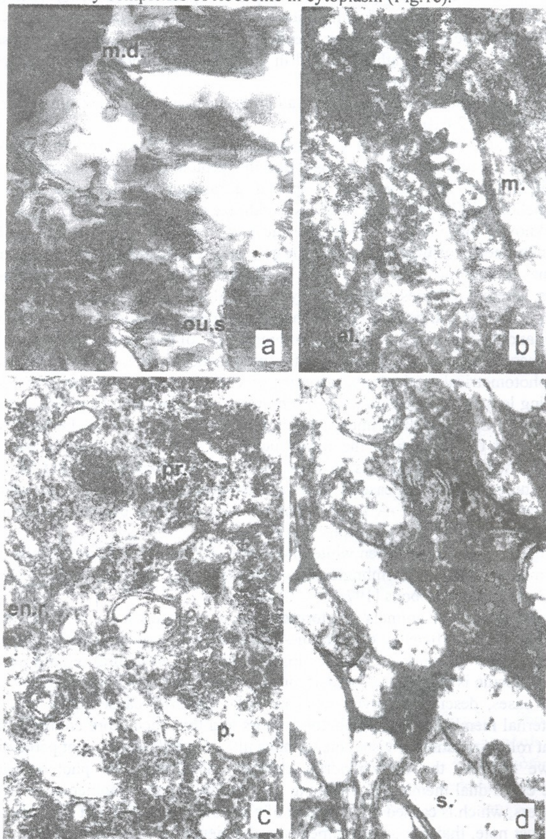

Fig.2 The fragments of photoreceptor cells of adult white mice at 4 months defeciency of vitamin A. Embeding in epon 812, X 45000. a) ou.s.- outer segment, m.d.–membrane discs; b) el.–ellipsoide, m.–mitochondria, c) p.-paraboloid, en.r.- endoplasmatic reticulum, pr.- polyribosoms; d) s.- synapses between the photoreceptors and bipolar cells.

The nucleus of the photoreceptor is of ellipsoidal shape with well expressed nucleolus, in which the fibrillar and granular components are well seen. In the nucleoplasma the grains of

cromatin in the shape of small heaps and thread are clearly seen. The nucleoplasma is surrounded by well expressed double membrane, which has numerous holes. Among the retina's photoreceptors and bipolar cells we can see strongly marked multiple synapses (Fig.1d).After 4 months deficiency of vitamin-A the membrane discs of white mice photoreceptors external segments were disrupted: parallel orientation of the membrane discs was destroyed and the membranes were separated (Fig. 2a). The quantity of crista was rather reduced in the ellipsoid mitochondria, their fragments were clearly seen in mitochondrial matrix (Fig.2 b).

But in paraboloid of photoreceptors, as in the nucleus, important ultra structural changes were not observed. (Fig.2 c,d).

No doubt that these changes were caused by the deficiency of vitamin-A, as it is the component of the membrane discs. Along with the protein - opsin, it forms visual pigment-rhodopsin, which is at the same time a special ultrastructural molecular component of the membrane [Janelidze, 1978; Janelidze, 1986; Kvinikhidze et al., 2002].

It is known, that vitamin-A gets into the body via food and is stored in the liver cells. From the liver by means of blood circulation it gets into retina. Vitamin-A is accumulated in the retina pigment epithelium cells by vessel capillaries. From the cells vitamin-A gets into photoreceptors, where it oxidizes, and as aldehyde, retinal with opsin forms rhodopsin.

Thus, it is clear that deficiency of vitamin-A impedes producing the complex molecule of visual pigment and the ultrastructure of the membrane discs of rod photoreceptors.

Our conclusion is confirmed with biochemical data, in which by means of microspekcrophotometrical methods the quantity of vitamin-A was calculated in adult hens liver and retina during long A vitaminstarvation (5,6 months). Vitamin-A in liver was reduced from 100 mg % (norm) to 18 mg % (in experiment), but in retina from 80 mg % to 14 mg % (at the end of the experiment). At the same time the ultrastructure of rods' external discs was changed, the membrane discs were disoriented as it took place in our experiment and at last the discs disappeared [Vinnicov, 1971]. In case of white rats when they were fed without vitamin-A for 1,5 months, the quantity of vitamin -A in rods was reduced by 50%, but the membrane discs were separated into corpuscle [Dowling, Gibsons, 1978].

During our experiment, when white mice were on the diet and didn't accept vitamin-A for more then 4 months, the rod membrane discs ultrastructural changes differed from the cases, described by Dowling and Gibbons [1978].

As it is shown, all animals' photoreceptoric membranes have different reaction to the medium. Namely, at the deficiency of vitamin-A different ultrastructural changes are seen. One is common, namely, photoreceptor membranes lose special ultra structural organization, because of the rhodopsin molecule is decomposed and accordingly they lose functional activity.

In all cases, described above, deficiency of vitamin-A causes quite strong destructive changes in external membrane disc of photoreceptors and this once more confirms, that it has the most important role in creation of visual membranes ultrastructure and in light perception.

As we noticed, the mitochondrial changes in ellipsoid of photoreceptors (namely, destroying the crista dual membrane, changing orientation and the fragmentation of the crista) is secondary process, which is caused by reduction of physiological activity of the body.

We must note, that in our experiment the deficiency of vitamin-A doesn't reduce protein synthesis, because the granular endoplasmic reticulum in paraboloid polyribosome complexes is not changed noticeably.

Thus obtained result showed that the aldehyde of vitamin-A is the structural component of rods external membrane discs of mice photoreceptors and its deficiency is one of the main reasons of their degeneration changes.

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A ვიტამინის დეფიციტის გავლენა თეთრი თაგვების თვალის ბადურის ფოტორეცეპტორების ულტრასტრუქტურაზე

ჯანელიძე ხ., კვინიხიძე გ., გრატიაშვილი ნ.

საქართველოს მეცნიერებათა აკადემიის ზოოლოგიის ინსტიტუტი

(მიღებულია 05.12.2005)

რეზიუმე

ნაჩვენებია, რომ A ვიტამინის დეფიციტი იწვევს მნიშვნელოვან ულტრასტრუქტურულ ცვლილებებს მოზრდილი თეთრი თაგვების ბადურის ფოტორეცეპტორებში: მემბრანების დისკოები კარგავს ჩვეულებრივ მკაცრ ორიენტაციას და იშლება. კოლბების ელიფსოიდის მიტოქონდრიები იცვლის კონფიგურაციას, კრისტები ხდება დეზორიენტირებული და იშლება. ეს ფაქტი მეტყველებს იმის შესახებ, რომ A-ვიტამინის ნაკლებობა წარმოადგენს ფოტორეცეპტორების მემბრანული დისკოების დაშლის მიზეზს, ვინაიდან A-ვიტამინის ოქსიდი – რეტინალი არის მხედველობის მემბრანების სტრუქტურული კომპონენტი. მიტოქონდრიების ულტრასტრუქტურის დაშლა მეორადი პროცესია, რომელსაც იწვევს მხედველობის მოლეკულური მექანიზმის მოშლა.

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Ecology

ASSESSMENT OF POLLUTION OF SOUTH-EAST COASTAL ZONE OF GEORGIA

ZOBOVA N.[1], VILSON J.[2], JAVELIDZE E.[1], LURSMANASHVILI L.[1]

[1]*Batumi Institute of Sea Ecology and Fishery, Georgia*
[2]*Trinity College, Zoology Department, Ireland, Dublin*

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Abstract

Three biotopes distinguished by the level of pollution and located at the Black Sea coastal zone of Georgia from the village Kvariati to the mouth of the river Korolistskali were studied. In the samples of the blanket of the ground depositions total amounts of organic compounds, nitrogen, phosphorus, heavy metals - Zn, Cr, and Cd were determined. According to the obtained results individual pollution load index (PLI) scores by every pollutant and also pollution indexes of separate stations, biotopes and studied regions as a whole were calculated. It was shown that PLI values enables to estimate the level of pollution of sea medium by ten-score scale.

Key words: heavy metals, biotopes, pollution load index.

Introduction

Sea costal zones and especially hydrofront zones due to carrying out to sea of great amount of suspended and soluble materials, among them heavy and transient metals are specifically distinguished. As a result of environmental changes (increasing of salinity and alkalinity of the water) contact zones of the sea and fresh waters serve as a kind of "natural filters" on the way of migration of elements to ocean. Hydrobionts, as well as abiotic components of coastal ecosystems are characterized with higher contents of several heavy and transient metals [Morozov & Patin, 1977].

It is significant to note that data about heavy metals content in the ground depositions of the Black Sea is rather confined. In the blanket of the ground depositions of Sevastopol Bay concentrations of Cu, Zn, Mn [Ovsianii et al., 2003], in the main ports of Ukraine concentrations of Cr, Cd, Ni, Co, Zn, Mn [Mikhailov, 1999], on the shelf between the city of Poti and the river Rioni concentrations of Mn, Zn, Cu, Ni, Co, Y, Cr, Mo, Pb ["Terminal-2000", 1999] – were determined. The goal of our work was to study ecological conditions and assessment of pollution level with heavy metals of ground depositions of Batumi Bay and neighboring regions.

Materials and Methods

In the studied area of costal zone three biotopes were distinguished: I – village Kvariati area considered as conditionally pure, II – sea sublittoral area from the mouth of the r. Chorokhi to the mouth of the r. korolistskali, III – the most polluted ragion – Batumi port. Samples of the blanket of the ground depositions were collected in 2003 seasonally (February-March, May-June,

August-November) from the shipboard in 16 points at the depth of 4-18m by bottom-grab with the capture area of 0.045 m^2. Points 1,2,14,15,16 concerned as I biotope, 3-6 and 9-13 as II biotope, 7-8 as III biotope.

Right away of gathering in the samples of ground depositions total amounts of organic compounds were determined via the loss in the weight at ground pricking with the particle size of less than 1mm (percentage from air-dry weight) during 3 hours (t=500°C); total nitrogen was determined by the Keldal's method [Zamiatina, 1975]; total phosphorus by Truoge-Meyer's method (modification of Denije-Atkins's method) [Ginsburg, 1975]. Determination of heavy metals – Zn, Cr and Cd – was carried out by the method of decomposition of samples with concentrated nitric acid and further measuring of the concentration of metals by atomic-absorption method [Bock, 1984].

Assessment of the ground depositions was carried out by the pollution indexes [Jefrey et al., 1985]. Individual pollution index by every pollutant was calculated with the formula:

$$PLI=10^{(1-(C-B]/\{T-B\})},$$

where PLI -- Pollution Load index, C – pollutant concentration, B – baseline concentration (μg/g), T – threshold concentration (μg/g).

Pollution Load index permits to estimate pollution by ten-score scale: in the polluted region when C=B, that is PLI=10; at C=T, that is PLI=1; in the high polluted regions PLI may approach to 0.

Pollution index of the separate station is determined as a mean geometric of the individual values of indexes by pollutants:

$$PLI_{site}=(PLI_1 x PLI_2 x \ldots PLI_n)^{1/n},$$

where n – number of determined pollutants on this station.

Analogous, pollution index of the studied region is determined as mean geometric of the pollution indexes of every station of this region.

Results and Discussion

According to the obtained results concentration of total nitrogen and total phosphorus in the three biotopes were correspondingly, 152-1-84 μg/g and 81-193 μg/g dry weight (Figure 1). Our data are in good accordance with the scientific data about Caucasian costal zone [Kiriukhina, 1975]. These values are less than corresponding values obtained in north-west costal zone of the Black Sea which includes mouths of such huge rivers as the Danube, the Dnepr, the Dnestr. By maximal values of the concentrations of total nitrogen and total phosphorus is characteristic for III biotope.

Total amount of organic carbon in the I and II biotopes was varied in the range of 2.8-4.4%, with exclusion only the point located in the mouth of the r.Chorokhi and equaled to 9%. In Kvariati biotope absolute values of concentrations were the least; in the ground depositions of the III biotope content of organic compounds was the largest – 8.2-13.8% of dry compound.

Concentrations of metals in three biotopes follow the scheme: Zn>Cr>Cd. Adjacent members differ by one order: correspondingly hundreds, tens and units of μg/g dry weight. Higher levels of Zn and Cd were registered in the III biotope and Cr – in the II biotope.

According to received data PLI were calculated by separate six biotopes.

In the I biotope maximal PLI were recorded for nitrogen and phosphorus: 9.62-10 and 7.83-10, correspondingly. For carbon PLIs were less – 4.90-5.25. Although for metals minimal PLI were noted in this conditionally pure region, even they were about thresholds values: concentrations of Cr and Cd were on the threshold level and a bit less, concentration of Zn – higher than thresholds. For Zn PLI varies from 0.57 to 0.95. Calculated pollution indexes of stations in the I biotope viz.: 2.65 (1st station) – 3.45 (16th station).

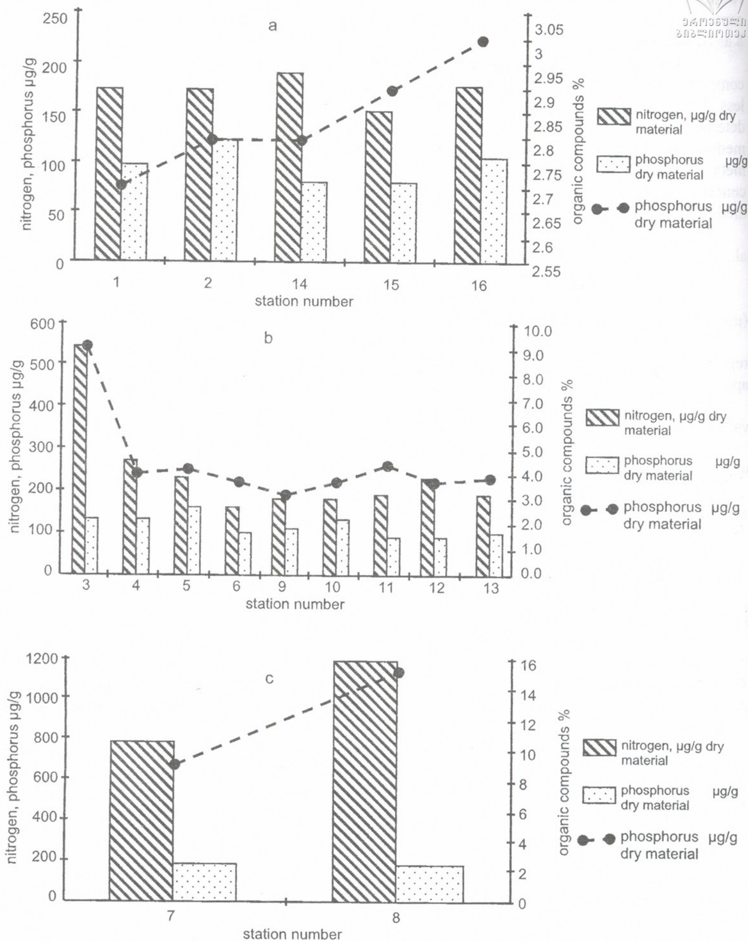

Fig.1. Total amount of organic compounds, nitrogen, phosphorus in the blanket of the ground depositions of Georgian coastal zone.

In the II biotope compared to the I, PLI values somewhat decreased and for N it equaled 6.92-9.89, for P – 6.25-9.35 and for C – 0.59-4.47. Concentrations of Cr of the ground depositions in the stations 3, 5, 9 and 11 were higher threshold: PLI(3 station)=0.74; PLI(5 station)=0.89; PLI(9 station)=0.66; PLI(11 station)=0.44. In the stations 6 and 10 PLI for Cd were 0.5 and 0.79; besides, in most stations of this biotope pollution index for Zn also <1. Consequently range of PLI values of stations viz.: 1.54-3.03.

Based on the values of PLI the most polluted turned out the III biotope. In this biotope sharp decrease of pollution indexes values by all toxicants was registered: minimal value of PLI for nitrogen was 4.00; for phosphorus – 5.83; for carbon – 0.11. The following values of pollution indexes for metals were found: PLI (Zn) = 0.016; PLI (Cd) = 0.1; PLI (Cr) = 0.91. Values of indexes of this biotope by stations were varied from 0.39 to 1.43.

Mathematical calculation by corresponding pollution indexes of separate stations gives the following results:

$$PLI_{\text{I biotope}} = 3.04$$

$$PLI_{\text{II biotope}} = 2.15$$

$$PLI_{\text{III biotope}} = 0.75$$

Pollution index of overall costal zone equals:

$$PLI_{\text{Overall Bay}} = 2.1$$

Obtained data enables us to refer Batumi Bay to moderate polluted areas.

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შავი ზღვის სამხრეთ-აღმოსავლეთ საქართველოს სანაპიროს დაბინძურების შეფასება

ზობოვა ნ.[1], ვილსონ ჯ.[2], ჯაველიძე ე.[1], ლურსმანაშვილი ლ.[1]

[1]ზღვის ეკოლოგიისა და თევზის მეურნეობის სამეცნიერო-კვლევითი ინსტიტუტი
[2]ზოოლოგიის განყოფილება, ტრინიტის კოლეჯი, დუბლინი, ირლანდია

(მიღებულია 07.09. 2005)

რეზიუმე

შესწავლილია საქართველოს შავი ზღვისპირა რაიონის, კვარიათსა და მდ. ყოროლისწყალის შესართავამდე, სამი ბიოტოპის დაბინძურების ხარისხი. ზედაპირული ფსკრული ნალექების სინჯებში (აღებული 16 სადგურიდან) განსაზღვრულია ორგანული ნივთიერების, აზოტის, ფოსფორის და მძიმე მეტალების - Zn, Cr, და Cd, საერთო რაოდენობა. განსაზღვრულია თითოეული დამაბინძურებლის, ცალკეული სადგურის, ბიოტოპისა და მთლიანი შესწავლილი რაიონის დაბინძურების ინდექსი (PLI). ნაჩვენებია, რომ PLI სიდიდეები საშუალებას იძლევა ათბალიანი შკალით ობიექტურად იქნეს შეფასებული ზღვის დაბინძურების ხარისხი.

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ეროვნული ბიბლიოთეკა

Genetics

STRUCTURAL-NUMERICAL AND FUNCTIONAL CHARACTERISTICS OF CHROMOSOMES IN PATIENTS WITH PARANOID SCHIZOPHRENIA

BABLISHVILI N.[1], TADUMADZE N.[1], TALIASHVILI T.[2], ZURABASHVILI D.[2]

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

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Abstract

Structural and numerical characteristics and transcriptional activity of nucleolus organizer regions (NORs) of acrocentric chromosomes in the cultivated lymphocytes of peripheral blood of the patients with paranoid schizophrenia were studied. Similarity of frequencies of structural disorders of chromosomes with the control group was established. According to the obtained results in the patients with paranoid schizophrenia transcriptional activity of ribosomal cistrons and the frequency of polyploidy cells were increased.

Key words: aberration, aneuploidy, acrocentric chromosomes, transcription.

Introduction

Schizophrenia is the most dramatic psychic disorder and serious medical and social problem. Hereditary of this disease was established. There are data about definite correlation of risk of schizophrenia with some chromosomal syndromes [Steven & Hyman, 2001; Murphy & Owen, 2001].

As it is known that structural, numerical and functional characteristics are essential for the estimation of pathological condition [Peresunko et al., 2001; Toyota et al., 2001; Demirham & Tastemir, 2003], the aim of our work was to study main cytogenetic parameters in the patients with paranoid schizophrenia (PS).

Material and Methods

The studies were carried out on peripheral blood blast-transformed small lymphocytes short-time cultures of 10 individuals diseased with paranoid schizophrenia. Material was taken from the patients of M. Asatiani Tbilisi Institute of Psychiatry.

The following test-systems were used: estimation of structural and numerical disorders of chromosomes by International System of Cytogenetic Nomenclature [ISCN, 1985]; determination of transcriptional activity of acrocentric chromosomes by Ag-NOR test [Fernandes et al., 2002].

Results and Discussion

Structural and numerical characteristics of chromosomes in the patients with PS are presented on the Figure 1. The frequency of aberrant chromosome containing cells in PS group in average equaled 2.1% that is approximately in the same range as the corresponding value in control group (1.6%). Only chromatid aberrations involving single and paired fragments were registered. By the frequency of aberrant cells individual variability was 1-5%. The obtained results are in agreement with scientific data according to which frequency of chromosomal disorders of PS group compared to control group was insignificantly increased [Demirhan & Tastemir, 2003].

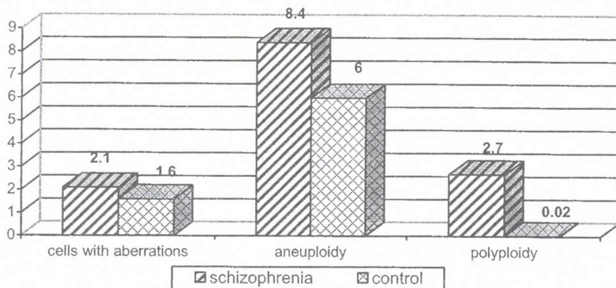

Fig. 1. Structural and numerical characteristics in the cases of paranoid schizophrenia

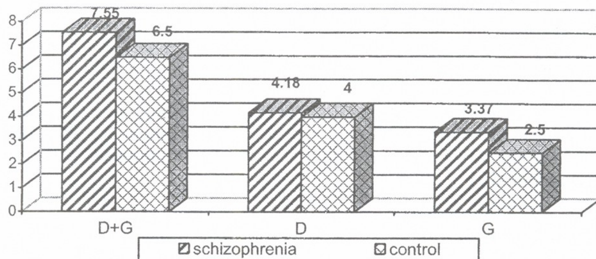

Fig. 2. The frequency of Ag-positive acrocentric chromosomes in the cases of paranoid schizophrenia

Parameter of aneuploidy mainly due to hypodiploid cells was slightly higher in PS group than in controls. The average frequency of aneuploidy cells was 8.4%, which statistically did not differ from the corresponding value of control group (6.5%). By individuals this parameter varies in 6-15% range. The frequency of polyploidy cells in average equaled 2.7%, which statistically was significantly higher than the parameter of the control group – 0.02%.

Parameters of transcriptional activity of nucleolus organizer regions (NORs) in the case of PS are presented on the Figure 2. The tendency of numerical growth of Ag-positive acrocentric chromosomes was revealed. Among individuals this parameter was slightly varied. The average number of Ag-positive chromosomes per one cell equaled to 7.55. Corresponding parameter for controls was 6.50 Ag^{+}-NOR/1cell. At the same time, increasing of acrocentric chromosomes taking part in satellite associations compared to control index was registered (Fig.2). Average percentage index of associations consisting cells was higher than corresponding control index (52%) and equaled 59.2%.

Obtained data enables us to conclude that structural characteristics of chromosomes in the patients with PS are stable and slightly differ from the indices of control group. Thus chromosomal disorders should not be the specific prerequisite of the risk of schizophrenia. As for the increase of somatic polyploidization, we consider that the effect of medicines for schizophrenia should be excluded. So, to continue investigations in this way might be interesting.

We consider that the tendency of increasing of NORs transcriptional activity of acrocentric chromosomes in the case of schizophrenia which indicates to the activity of synthesizing processes in the cells of studied individuals, is significant. It is known that human ribosomal genes are localized in the secondary constrictions (NORs) of acrocentric chromosomes. Transcriptional activity of these genes indicates to increasing of cell synthesizing processes and correspondingly of immune system functioning [Lezhava et al., 2004]. From the scientific data it is known that schizophrenia compared to other diseases has some priority in view of activating state of immune system [Crocker, 1990; Fridlund & Raisberg, 2001; Peresunko et al., 2001].

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ქრომოსომათა სტრუქტურულ-რაოდენობრივი და ფუნქციური მახასიათებლები პარანოიდული შიზოფრენიის შემთხვევებში

ბაბლიშვილი ნ.[1], თადუმაძე ნ.[1], თალიაშვილი თ.[2], ზურაბაშვილი დ.[2]

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

(მიღებულია 03.10.2005)

რეზიუმე

შესწავლილია ქრომოსომათა სტრუქტურულ-რაოდენობრივი მახასიათებლები და აკროცენტრული ქრომოსომების ბირთვაკ-მაორგანიზებელი უბნების ტრანსკრიფციული აქტივობა პარანოიდული შიზოფრენიით დაავადებული პაციენტების პერიფერიული სისხლის კულტივირებულ ლიმფოციტებში. დადგენილია ქრომოსომათა სტრუქტურული დარღვევების სიხშირის მსგავსება საკონტროლო ჯგუფთან. მიღებულ შედეგები მიუთითებენ, რომ პარანოიდული შიზოფრენიით დაავადებულ ინდივიდებში მომატებულია რიბოსომული ცისტრონების ტრანსკრიფციული აქტივობა, რაც უჯრედის სინთეზური პროცესების გაზრდილ აქტივობაზე მიუთითებს.

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ეროვნული ბიბლიოთეკა

STUDY OF ISOENZYMATIC SYSTEMS IN THE LEAVES OF THE TANGERINE (COVANE VACE)

KORIDZE M.[1], KHUKHUNAISHVILI R.[1], ALASANIA N.1, BERULAVA I.[2]

[1]*Biological Department, Sh. Rustaveli Batumi State University*
[2]*Biological Department, Sokhumi State University*
E-mail: mkoridze@mail.ru

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Abstract

Electrophoretic studies of isoenzymes of spontaneous and induced mutants of tangerine (Covane Vace) revealed that at the beginning of vegetation, process of genes differentiated expression occurs intensively which is expressed on the protein markers level and displayed by different isoelectrophoregrams. According to peroxidase the individual differences should be isoenzymatic variations produced by allelomorphs of one structural gene. Resemblance-distinction of mutant plants via isoenzymatic composition was recorded.

Key words: peroxidase, electrophoresis, zymogram, marker.

Introduction

In the studies of different plant species ontogenetic and evolution problems isoenzymes, as phylogenetic markers, are widely used [Konarev, 1983; Korochkin, 1976; Button & Spiegel, 1976]. Although, today isoenzymes are well researched, molecular mechanisms of isoenzymes encoding by separate and multiallelic genes are still unclear. Isoenzymes polymorphism is determined by electrophoretic method [Kenny, 1974; Truveler & Nefyodov, 1974; Khukhunaishvili et all., 1988]. The main reason of polymorphism are polymorphous locus which cause genesis of various isoenzymes, as well as structural mutations, or formation of separate isoenzymes controlled by different alleles of one and the same gene.

To reveal the loci which are responsible for the isoenzymes synthesis enables us to use isoenzymes as genetical markers. In this viewpoint investigations on some subtropical plants were already carried out [Khukhunaishvili et al., 1988; Khukhunaishvili & Diasamidze, 2004; Kapanadze, 1985].

Materials and Methods

To detect genetical potential of experimental material electrophoretic spectra - zymograms of isoenzymes of spontaneous and induced mutants were studied.

Experiment was carried on plantlet leaves of one row. Leaves space position (east, west, south, north) was preserved. Seasonal and climatic conditions during samples collection was taken into account. To reveal the degree of endogenous changes comparative studies of electrophoretic

spectra of the identical exposition of tangerine leaves, up and down parts of crone leaves, were conducted.

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Materials and Methods

Data obtained by electrophoretic spectrum of peroxidase according to seasonal activity, as well as diversity of molecular forms, in various cases were distinct. Namely, analysis of tangerine mutants showed that isoenzymatic composition of peroxidase is not varied within one form (Fig.1). Electrophoretic spectra for samples taken from different parts of plant, as well as samples taken in different day-time were identical. Though, the differences in electrophoregrams of various plants were detected. By isoenzymatic activity on the electrophoregrams of leaves which were collected from the cephalic south part of tangerine bush small differences were recorded (Fig. 2).

To study daily dynamics of electrophoregrams of peroxidase samples were collected every 3 hours. Only insignificant changes in enzymes activities of separate forms were observed. These changes do not influence the distribution of the zones of enzymatic activities spectra.

Electrophoregrams of studied mutants at different periods of plant vegetation revealed much variable. It is worthy to note that electrophoregrams of peroxidase are more stable at winter period.

Comparatively high activity of peroxidase, and also polymorphism was found in spring and autumn. From analyzed 70 samples zymograms of 5 types were detected (Fig. 3, Tab. 1); among them 2 are of penta-loci (I, IV), 3 – tetra-loci (II, III, V). I locus is characteristic for all five spectra, II locus – for all spectra except V spectrum; III locus is not characteristic for II and III spectra. At the same time III locus in I spectrum is expressed more actively. IV locus is detected in all spectra and in I, III and IV spectra it is more active than in II and IV spectra. V locus is characteristic for all spectra. This locus in III and IV spectra is less active than in the rest ones. I and II loci is in minor state for all spectra. III locus is active only for I spectrum, in II and III spectra it doesn't appear at all, and in IV and V spectra it is in minor state. IV locus is intensive in I, III and IV spectra, in II and V spectra – minor. V locus in I, II and V spectra is actively expressed and in III and IV spectra is in minor state. It is significant that in the plants of I spectrum peroxidase is appeared more intensive than in the plants of the rest four spectra.

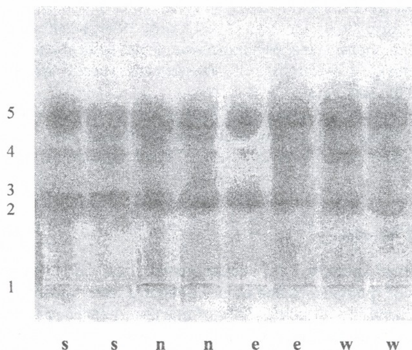

Fig. 1. Electrophoretic spectra of peroxidase in the tangerine leaves (s-south, n-north, e-east, w-west. 1,2,3,4,5, - numbers of loci)

Heterogeneity of peroxidase changes according to vegetation periods. At the beginning of the first vegetation the sharp increase of peroxidase activity begins. At the end of the first vegetation peroxidase activity is somehow decreased; at the beginning of the second vegetation peroxidase activity is also low. At the end of the second vegetation gradual increase of enzyme activity begins. Its heterogeneity sharply increases and remains high almost up to the beginning of cold weather. Though peroxidase is functionally labile enzyme and depends on the effect of various factors, received data of experiments conducted during 3 years are rather stable. This fact indicates that peroxidase zymograms should be used for mutants genetical analysis.

At the same time using of peroxidase as genetical marker is possible only at particular steps of plant ontogenesis – at the beginning and at the end of vegetation.

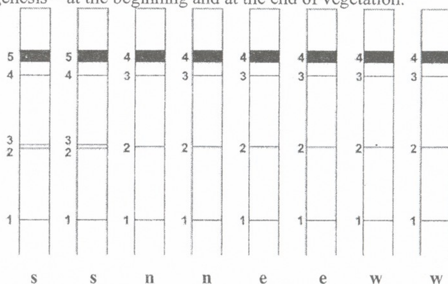

Fig. 2. Scheme of electrophoretic spectra of peroxidase in the tangerine leaves (s-south, n-north, e-east, w-west. 1,2,3,4,5, - numbers of loci)

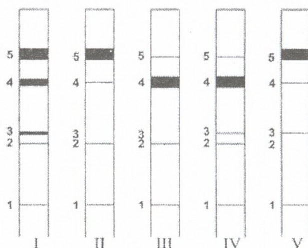

Fig. 3. Isoenzymatic spectra of peroxidase in different mutants leaves of tangerine. I, II, III, IV, V – types of electrophoretic spectra (corresponding mutant forms are presented in Tab.1)

Table 1. Structure of electrophoregram spectra of peroxidase (+ - existence of active locus; **- -** not existence of active locus; ± - minor locus).

Spectra of peroxidase	Loci of spectrum				
	1	2	3	4	5
I	±	±	+	+	+
II	±	±	-	±	+
III	±	±	-	+	±
IV	±	±	±	+	±
V	±	-	±	±	+

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იზოფერმენტული სისტემების შესწავლა მანდარინ კოვანო ვასეს ფოთლებში

ქორიძე მ.[1], ხუხუნაიშვილი რ.[1], ალასანია მ.[1], ბერულავა ი.[2]

[1]ბიოლოგიის დეპარტამენტი, შ. რუსთაველის სახელობის ბათუმის უნივერსიტეტი
[2]სოხუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 17.1.2005)

რეზიუმე

მანდარინ კოვანო ვასეს სპონტანური და ინდუცირებული მუტანტების იზოფერმენტების ელექტროფორეზული სპექტრების შესწავლის შედეგად დადგენილია, რომ ვეგეტაციის დასწყისში აქტიურად მიმდინარეობს გენების დიფერენცირებული ექსპრესიის პროცესი, რაც აისახება ცილოვანი მარკერების დონეზე და ვლინდება ფერმენტების განსხვავებული იზოელექტროფორეგრამების სახით. პეროქსიდაზას მიხედვით მცენარეთა ინდივიდუალური განსხვავებანი წარმოადგენს ერთი სტრუქტურული გენის ალელოფორმების მიერ პროდუცირებულ იზოფერმენტულ ვარიაციებს. დაფიქსირებულია იზოფერმენტული შემადგენლობით მუტანტურ მცენარეთა მსგავსება-განსხვავება.

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ეროვნული ბიბლიოთეკა

BLOOD GROUP ANTIGENS CORRELATION WITH PULMONARY TUBERCULOSIS IN ADJARA POPULATION

NAGERVADZE M., AKHVLEDIANI L., GOGITIDZE T., DUMBADZE G., DIASAMIDZE A., TSETSKHLADZE D.

Immunogenetic laboratory, Sh. Rustaveli Batumi state University
E-mail: mari-na@nm.ru

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Abstract

Correlation between blood group antigens - ABO, MN and Kell, with pulmonary tuberculosis (PT) was studied. To express blood group antigens immunoserological study was carried out on 50 patients with PT and 50 healthy controls. It was revealed that tuberculosis somehow correlates with H and B antigens. O(I) and B(III) blood group individuals are more sensitive to this disease, but A(II) group individuals are less subdued to tuberculosis. Some correlation was also found out against MN group antigens. In patients with PT carriers of M antigens significantly prevailed which is presumably caused by high sensitivity of M antigen against tuberculosis. As for Kell system antigens, in Adjara population these antigens occurred rare and their correlation with PT was not recorded.

Key words: ABO, Kell, MN system antigens, immunoserological method.

Introduction

In scientific literature there are a lot of data about the correlation of erythrocyte group antigens with infectious and noninfectious diseases. Connection of blood group antigens with children infectious diseases, such as paratyphoid, measles, scarlet fever, coli-infection, was shown [Artemev et al., 1983].

Children with A – blood group can not elaborate immunity against plague even at secondary vaccination [Bogkov, 1978]. It was also shown that A-blood group carriers are often diseased with infectious hepatitis, and O-group carriers are less resistant against influenza virus [Komorovich &Rimkov, 1966]. It was also reported ABO system antigens correlation with such diseases, as diabetes, cancer, cirrhosis, pneumonia, bronchitis, tuberculosis etc. From above mentioned diseases tuberculosis arouses interest as it is rather spread in Adjara region. Probably, along with immunogenetical peculiarities of Adjara population high frequency of this disease is evoked by environmental and economical conditions.

Every year 20 million cases of tuberculosis are revealed and 3-8 million die. About 1/3 of humans are infected with *M.tuberculosis*. Among them only 10% is clinically revealed and majority are bacteria carriers [Gackett et al., 1988; Dolin et al., 1994; Comstock, 1982; Avidienko et al., 2003].

Material and Methods

Studies have been carried out on blood erythrocytes of 50 individuals with PT and 50 healthy controls. Experiments were carried out on blood samples of the patients from Batumi Pneumonic-Pulmonary Clinic. To reveal blood group antigens immunoserological methods were used.

In our experiments anti- AB, B, A, M, N, K, k monoclonal antibodies and I, II, III, IV group standard serums were used ("Hemostandard", Russia).

Results and Discussion

Obtained data show correlation of PT with erythrocytic group antigens. Results of experiments are presented in Figure 1. As is seen from the figure frequency of O(I) and B(III) blood group individuals in patients is higher compared to control group; frequency of A blood group individuals in patients is lower compared to control group. Obtained results are in good accordance with scientific data [Platonova, 1999; Viskum, 1975].

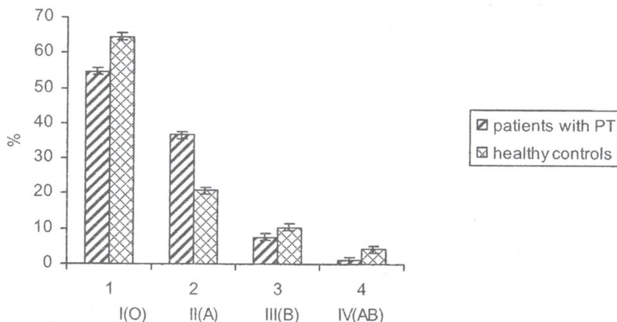

Fig.1. Frequency of ABO system antigens distribution in patients with PT and in healthy controls.

According to the obtained results we can say that tuberculosis correlates with H and B antigens. O(I) and B(III) blood group individuals are more sensitive to this disease, but A(II) group individuals are less subdued to tuberculosis. They are characterized with some immune resistance.

Some correlation was also found out against MN group antigens (Fig.2). As is seen from the figure in patients with PT carriers of M antigens significantly prevailed which is presumably caused by high sensitivity of M antigen against tuberculosis.

As for Kell system antigens, in Adjara population these antigens occurred in single instances and their correlation with PT was not recorded. Almost all studied individuals by Kell system antigens are recessive homozygotes – kk (Fig.3).

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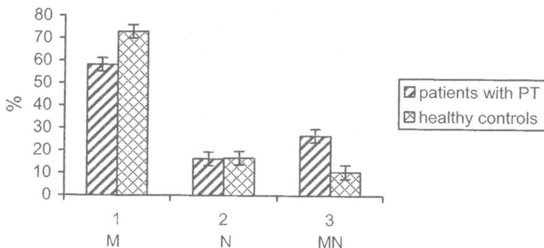

Fig. 2. Frequency of MN system antigens in patients with PT and in healthy controls.

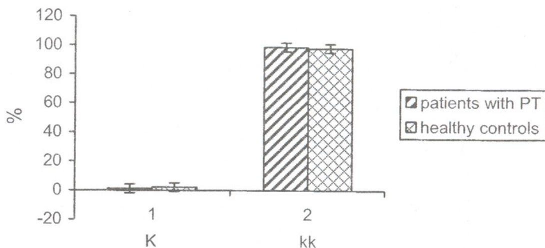

Fig.3. Frequency of Kell system antigens in patients with PT and in healthy controls.

Thus our investigations revealed erythrocytic antigens correlation with tuberculosis. Based on our research it is possible to stand out high risk group and carry out preventive arrangements on this group.

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სისხლის ჯგუფური ანტიგენების (ABO, Kell, MN) კორელაცია ტუბერკულოზთან

ნაგერვაძე მ., ახვლედიანი ლ., გოგიტიძე თ., დუმბაძე გ., დიასამიძე ა., ცეცხლაძე დ.

იმუნოგენეტიკის ლაბორატორია, შ. რუსთაველის სახელობის ბათუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 16.05.2005)

რეზიუმე

გამოკვლეულ იქნა ფილტვის ტუბერკულოზით დაავადებული 50 პაციენტის და 50 ჯანსაღი დონორის სისხლი. შესწავლილი იქნა აღნიშნული დაავადების კორელაცია ABO, MN და Kell სისტემის ანტიგენებთან. გამოვლენილია ტუბერკულოზის გარკვეული კორელირება H და B ანტიგენებთან. 0(I) და B (III) ჯგუფის სისხლის მატარებელი ადამიანები უფრო მგრძნობიარენი არიან აღნიშნული დაავადების მიმართ, ხოლო A (II) ჯგუფის მატარებლები შედარებით ნაკლებად ექვემდებარებიან აღნიშნულ დაავადებას. გარკვეული კორელაციაა შემჩნეული MN ჯგუფის ანტიგენების მიმართაც. ტუბერკულოზით დაავადებულებში მნიშვნელოვნად სჭარბობენ M ანტიგენების მატარებლები, რაც ამ დაავადების მიმართ აღნიშნული ანტიგენის მგრძნობელობითაა განპირობებული.

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ACIDOPHILIC AND ALKALIPHILIC MICROSCOPIC FUNGI ISOLATED FROM SOILS OF DIFFERENT REGIONS OF GEORGIA

DAUSHVILI L., *KUTATELADZE L., BURDULI T., JOBAVA M., KHVEDELIDZE R.

*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

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Abstract

Attitude to acid and alkali medium of 145 cultures of microscopic fungi from 14 genera has been studied. The optimal and boundary meanings of pH were established. 7 acidophilic and 6 alkaliphilic cultures were revealed, belonging to genera: *Aspergillus, Penicillium, Fusarium, Rhizopus* and *Trichoderma*. It has been established that acidophilic and alkaliphilic properties of micromycetes are significantly affected by the soil type.

Key words: micromycetes, *Aspergillus, Penicillium*, soil-climatic zones.

Introduction

Studying the peculiarities of exremophilic microorganisms is one of the popular directions of microbiological studies. Extremophilic microorganisms are adapted to survive in ecological niches such as at high temperatures, extremes of pH, high salt concentrations and high pressure. Extremophilic microscopic fungi are of special interest as they are able to produce the enzymes resistant to different critical conditions. This group of microorganisms, also their biologically active substances, as a result of their metabolic activity, make possible to elaborate a new-quality high effective technologies [Niehaus F. et al., 1999; Kvesitadze G., 1990; Ventosa A. et al., 1998].

The goal of the given study was to reveal the acidophilic and alkaliphilic microscopic fungi among the collection of micromycetes isolated from different soil-climatic zones of Georgia and to determine their extent of extremophilicy.

Materials and Methods

The cultures from the collection of microscopic fungi, isolated from different soil-climatic zones of Georgia served as objects for investigation [Daushvili et al., 2004].

Microscopic fungi were grown on the universal nutrient medium with different pH: wort (content of sugar 7.0%)-1.0l, agar-20.0. The pH of the medium changed from 2.0 till 10.0, with 0.5 intervals. Surface cultivation of micromycetes was performed on Petri dishes at the optimal growth temperature.

The growth of microscopic fungi was determined by means of measuring two parameters – the diameter of the colony in two perpendicular directions after 3fold cultivation, 3, 5 and 7 days later. On the other hand the density of hyphae of the developing colonies in different parts was measured. The final sum of both parameters was appreciated by means of three-mark system.

Results and Discussion

Experiments were done on 145 cultures of microscopic fungi from 14 genera. For grouping micromycetes following their attitude to acid or alkali medium, the velocity of their radial growth has been studied at different pH of the nutrient medium. Using this approach the optimal and boundary (limiting) meanings of pH were established.

In the group of acidophilic micromycetes the fungi with optimal growth at pH3.0 to 4.5 were united. Micromycetes with optimal growth above pH 8.0 were considered as alkaliphils. Acid tolerant fungi developed at low pH of the medium too, but the neutral pH seemed to be optimal for them. Alkali tolerant micromycetes embraced the cultures growing at the neutral pH, but they were able to grow at strong alkalic (pH8-pH9) conditions too.

Analysis of the obtained data has revealed that soils of three different regions of Georgia were clearly diverse with both, quantitative and general composition of acidophilic and alkaliphilic micromycetes.

Majority of micromycetes isolated from Sighnaghi soils (60%) were acid tolerant. The pure acidophils (13%) have been also revealed in the soils of this region (Fig. 1). Among the micromycetes of Telavi soils pure alkaliphils and alkali tolerant cultures prevailed. This was expectable, because of the alkali reaction of saline solonetz soils of Telavi. Other authors also have mentioned about the alkaliphilic properties of this type of soils [Horikoshi K., 1999; Mahdy HM., 1996].

Among the 14 studied genera of micromycetes real acidophilic and alkaliphilic properties were revealed only in representatives of 6 genera (Table 1). Great deal of extremophilic microscopic fungi, isolated from Sighnaghi and Telavi regions belonged to genera: *Aspergillus* and *Penicilliunm*, while in Oni region the species from genera *Fusarium* and *Rhizopus* have been mentioned.

From the acid tolerant group of micromycetes separate cultures of *Aspergillus, Penicillium* and *Fusarium* must be distinguished, which have revealed the tolerance to concentration of hydrogen ions in a wide range of pH (from pH3.0 to pH10.0). But the limiting meaning of pH caused significant morphological changes, mainly reflected on color, consistence of colonies and appearing the laces.

Table 1. Genera of acidophilic and alkaliphilic micromycetes isolated from different regions

	Genus of the fungus		
	Telavi	Sighnaghi	Oni
Acidophiles	*Aspergillus*	*Aspergillus*	*Fusarium*
		Penicillium	
		Trichoderma	
Alkaliphiles	*Aspergillus*	*Aspergillus*	*Rhizopus*
	Penicillium	*Penicillium*	*Fusarium*
	Fusarium		

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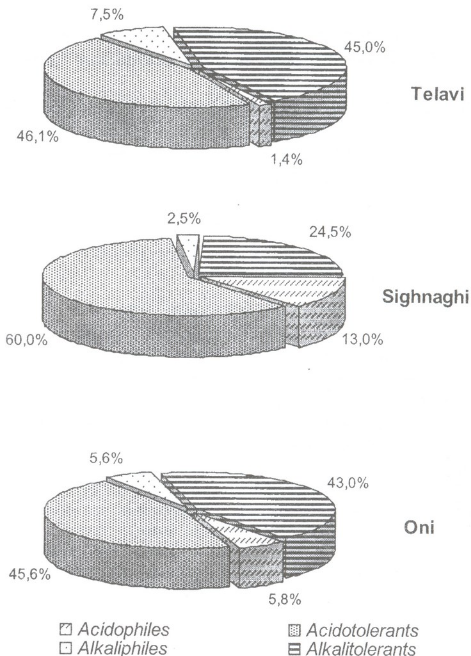

Fig. 1. Acidophilic and alkaliphilic micromycetes from different regions of Georgia (%).

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საქართველოს სხვადასხვა რეგიონის ნიადაგებიდან გამოყოფილი აციდოფილური და ალკალიფილური მიკროსკოპული სოკოები

დაუშვილი ლ., ქუთათელაძე ლ., ბურდული თ., ჯობავა მ., ხვედელიძე რ.

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

(მიღებულია 14.11.2005)

რეზიუმე

შესწავლილია 14 გვარის მიკროსკოპული სოკოების 145 კულტურა მათი მჟავე და ტუტე გარემოს მიმართ დამოკიდებულების მიხედვით. დადგენილია pH-ოპტიმუმები და pH-ის ზღვრული მნიშვნელობები. გამოვლენილია 7 აციდოფილური და 6 ალკალიფილური კულტურა, რომლებიც *Aspergillus, Penicillium, Fusarium, Rhizopus* და *Trichoderma*-ს გვარებს მიეკუთვნებიან. დადგენილია, რომ მიკრომიცეტების აციდოფილურ და ალკალიფილურ თვისებებზე მნიშვნელოვანწილად მოქმედებს ნიადაგის ტიპი.

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ეროვნული ბიბლიოთეკა

Zoology

MEDICAL PLANT APHIDS *(HEMIPTERA: APHIDOIDEA)* IN THE RIV. DZAMA, TANA AND TEDZAMI GORGES (EASTERN GEORGIA)

ALEXIDZE G.[1], *BARJADZE SH.[2], GIGOLASHVILI S.[1], KHAIKASHVILI KH.[3]

[1] *Academy of Agricultural Sciences of Georgia,*
[2] *Institute of Zoology, Georgian Academy of Sciences,*
[3] *Tbilisi Botanical Garden, Georgian Academy of Sciences*
**E-mail: shalva1980@yahoo.com*

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Abstract

The peculiarity of vertical-zonal distribution of medical plant aphids and the rate of host plant damage by them are discussed in the riv. Dzama, Tana and Tedzami gorges. 78 species of medical plant aphids were registered on the above mentioned territory that are united in 3 families and 42 genera. The majority of species (58 species) are registered in the steppes, arid forests and hemixerophilous grass-shrub plant belt. Their quantity is sharply decreased in the direction of alpine belt. Aphids are divided into three groups on the base of the degree of damage on the medical plants: 35 species of aphids cause high rate of plant damage, 24 species - middle rate and 19 species - low rate of damage.

Key words: Aphids, host plant, rate of plant damage, plant belts.

Introduction

The whole area of the riv. Dzama, Tana and Tedzami gorges composes about 1598 km^2 [Petriashvili, 1961]. The rivers represent right branches of the riv. Mtkvari in Shida kartli region of Georgia.

The folowing plant belts are represented on the area: steppes, arid forests and hemixerophilous grass-shrub (500-900m), broad-leaved forests (700-1400, 1500m), coniferous forests (1400-1900m), subalpine (1800-2500m) and alpine (from 2500m) [Gagnidze, Davitadze, 2000].

The medical plant aphids of this territory have not been the special subject of research before.

Material and Methods

We have collected the faunistic material of aphids during 2000-2003. The investigation comprised all the vertical plant belts of the above mentioned river gorges. The preparation and identification of aphid species were carried out according to the widely used methods in Aphidology [Börner, Heinze, 1957; Shaposhnikov, 1963; Jibladze, 1975; Blackman, Eastop, 2000; Quednau, 2003].

Results and Discussion

The aphids on the territory investigated are represented with 113 species [Barjadze, Kvavadze, 2005], from which 78 species are registered on the medical plants. They belong to 3 families and 42 genera. The majority of the aphids (76 species) belong to the family *Aphididae*. The families *Adelgidae* and *Phylloxeridae* are represented with 1 species each.

The maximum quantity of aphids (6 species) were populated on *Malus domestica*.

The aphids and their host plants of the territory studied are given in Table 1. 78 species of aphids are inhabitants of 76 species of host plants, belonging to the 38 families.

Aphids are divided into three groups on the base of the degree of damage on the medical plants: 35 species of aphids cause high rate of plant damage, 24 species - middle rate and 19 species - low rate of damage. The list of the aphid species and the rate of damage caused by them are given in Table 1.

Table 1. Aphids species, their host plants and the rate of plants damage.

#	Aphids species	Plant species	Rate of plant damage		
			High	Middle	Low
1.	*Acirtosiphon pisum*	*Vicia peregrina*		+	
2.	*Aphis affinis*	*Menta longifolia*	+		
3.	*A. craccae*	*Vicia sp.*	+		
4.	*A. craccivora*	*Glycyrrhiza glabra, Phaseolus vulgaris*	+		
5.	*A. fabae*	*Phaseolus vulgaris, Galium sp.*	+		
6.	*A. farinosa*	*Salix caprea, S. viminalis*		+	
7.	*A. gossypii*	*Citrullus vulgaris, Cucumis sativus, Cucurbita sp.*	+		
8.	*A. hederae*	*Hedera helix*			+
9.	*A. idaei*	*Rubus ideus, Rubus sp. sp.*		+	
10.	*A. intybi*	*Cichorium intybus*	+		
11.	*A. oxytropis*	*Paliurus spina-christi*		+	
12.	*A. pomi*	*Malus orientalis, M. domestica, Crataegus sp.* *Cydonia oblonga, Mespilus germanica*	+		
13.	*A. praeterita*	*Chamaenerium angustifolium*		+	
14.	*A. punicae*	*Punica granatum*	+		
15.	*A. sambuci*	*Sambucus ebulus, S. nigra*			+
16.	*A. sedi*	*Sedum caucasicum*		+	
17.	*A. umbrella*	*Lavatera thuringiaca, Malva sylvestris*	+		
18.	*A. urticata*	*Urtica dioica*			+
19.	*A. sp.*[I]	*Euphorbia iberica*		+	
20.	*A. sp.*[III]	*Rubia transcaucasica*	+		
21.	*Aulacorthum solani*	*Convolvulus arvensis*		+	
22.	*Betulaphis quadrituberculata*	*Betula litwinowii*			+
23.	*Brachycaudus cardui*	*Symphytum asperum*	+		
24.	*B. divaricatae*	*Stone fruits*	+		
25.	*B. helichrysi*	*Prunus spinosa*	+		
26.	*B. persicae*	*Persica vulgaris*	+		
27.	*B. spiraeae*	*Spiraea hypericifolia*	+		
28.	*Capitophorus pakansus*	*Inula helenium*			+
29.	*C. sp.*	*Elaeagnus angustifolia*		+	
30.	*Cavariella aegopodii*	*Coriandrum sativum*	+		
31.	*Chaitophorus vitellinae*	*Salix alba*	+		
32.	*Chromaphis juglandicola*	*Juglans regia*			+

33.	*Cinara juniperi*	*Juniperus oblonga*			+
34.	*C. pini*	*Pinus sosnowskyi*			+
35.	*Corylobium avellanae*	*Corylus avellana, C. iberica*		+	
36.	*Cryptomyzus alboapicalis*	*Lamium album*			+
37.	*Dysaphis affinis*	*Malus domestica, M. orientalis*	+		
38.	*D. aucupariae*	*Sorbus torminalis*	+		
39.	*D. devecta*	*Malus domestica, M. orientalis*	+		
40.	*D. plantaginea*	*Malus domestica, M. orientalis*	+		
41.	*D. pyri*	*Pyrus caucasica, P. communis* -cult.	+		
42.	*D. reaumuri*	*Pyrus caucasica, P. communis.*-cult.	+		
43.	*Eriosoma lanigerum*	*Malus domestica, M. orientalis*	+		
44.	*Eucallipterus tiliae*	*Tilia caucasica*			+
45.	*Euceraphis punctipennis*	*Betula pendula*			+
46.	*Hyadaphis foeniculi*	*Heracleum sosnowskyi*		+	
47.	*Illinoia sp.*	*Rhododendron caucasicum*			+
48.	*Lachnus roboris*	*Quercus iberica*		+	
49.	*Lìosomaphis berberidis*	*Berberis vulgaris*		+	
50.	*Macrosiphoniella artemisiae*	*Artemisia vulgaris*	+		
51.	*Macrosiphum euphorbiae*	*Euphorbia iberica*		+	
52.	*M. melampyri*	*Digitalis feriginea*			+
53.	*M. rosae*	*Rosa sp. sp.*	+		
54.	*M. sp.*[I]	*Symphytum asperum*		+	
55.	*M. sp.*[II]	*Helleborus caucasicus*		+	
56.	*M. sp.*[III]	*Valeriana tiliifolia*			+
57.	*Microlophium carnosum*	*Urtica dioica*		+	
58.	*Moritziella sp.*	*Quercus iberica*			+
59.	*Myzocallis coryli*	*Corylus avellana, C. iberica*		+	
60.	*Myzus cerasi*	*Cerasus incana, C. avium, C. vulgaris*	+		
61.	*M. persicae*	*Solanum lycopersicum*	+		
62.	*Ovatus insitus*	*Cydonia oblonga, Mespilus germanica, Malus omestica*	+		
63.	*Panaphis juglandis*	*Juglans regia*		+	
64.	*Periphillus lyropictus*	*Acer platanoides*			+
65.	*Phorodon humuli*	Stone fruits, *Humulus lupulus*	+		
66.	*Pineus sp.*	*Pinus sosnowskyi*			+
67.	*Prociphilus fraxini*	*Fraxinus oxycarpa*		+	
68.	*Pterocallis alni*	*Alnus barbata*	+		
69.	*Pterochloroides persicae*	*Persica vulgaris*	+		
70.	*Pterocomma populeum*	*Populus nigra*		+	
71.	*Rhopalosiphum maidis*	*Zea mays*	+		
72.	*Schizolachnus pineti*	*Pinus sosnowskyi*			+
73.	*Semiaphis dauci*	*Daucus carota*	+		
74.	*Sipha maydis*	*Zea mays*	+		
75.	*Sitobion fragariae*	*Rubus sp.sp.*		+	
76.	*Thelaxes driophila*	*Quercus iberica*		+	
77.	*Tuberculatus annulatus*	*Quercus iberica*			+
78.	*Uroleucon cichorii*	*Cichorium intybus*		+	
		Total	35	24	19

The aphids are distributed through the plant belts according to the following way: 59 species of aphids are represented in the steppes, arid forests and hemixerophilous grass-shrub(I), 52 species - in the broad-leaved forests (II), 18 species - in the coniferous forests (III), 10 species - in the subalpine (IV) and 2 species of aphids - in the alpine (V) one. The aphid distribution through the plant belts is given in Table 2.

Table 2. Aphids distribution to the plant belts in the riv. Dzama, Tana and Tedzami gorges.

#	Aphids Species	Plant belts				
		I belt	II belt	III belt	IV belt	V belt
1.	*Acirtosiphon pisum*	+				
2.	*Aphis affinis*	+	+			
3.	*A. craccae*	+				
4.	*A. craccivora*	+	+	+	+	+
5.	*A. fabae*	+	+	+		
6.	*A. farinosa*	+	+	+		
7.	*A. gossypii*	+	+			
8.	*A. hederae*	+	+			
9.	*A. idaei*	+	+	+	+	
10.	*A. intybi*	+				
11.	*A. oxytropis*	+				
12.	*A. pomi*	+	+	+		
13.	*A. praeterita*		+			
14.	*A. punicae*	+	+			
15.	*A. sambuci*	+	+	+	+	
16.	*A. sedi*		+			
17.	*A. umbrella*	+	+			
18.	*A. urticata*	+	+			
19.	*A. sp.*[I]	+				
20.	*A. sp.*[III]	+				
21.	*Aulacorthum solani*	+				
22.	*Betulaphis quadrituberculata*				+	
23.	*Brachycaudus cardui*	+	+	+	+	+
24.	*B. divaricatae*	+				
25.	*B. helichrysi*		+			
26.	*B. persicae*	+				
27.	*B. spiraeae*	+				
28.	*Capitophorus pakansus*	+	+			
29.	*C. sp.*	+				
30.	*Cavariella aegopodii*	+				
31.	*Chaitophorus vitellinae*	+				
32.	*Chromaphis juglandicola*	+	+			
33.	*Cinara juniperi*		+	+		
34.	*C. pini*		+	+		
35.	*Corylobium avellanae*	+	+	+		
36.	*Cryptomyzus alboapicalis*	+				
37.	*Dysaphis affinis*	+	+			
38.	*D. aucupariae*		+			
39.	*D. devecta*	+	+			
40.	*D. plantaginea*	+	+			
41.	*D. pyri*	+	+			

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42.	*D. reaumuri*	+	+			
43.	*Eriosoma lanigerum*	+	+			
44.	*Eucallipterus tiliae*		+			
45.	*Euceraphis punctipennis*			+		
46.	*Hyadaphis foeniculi*		+			
47.	*Illinoia sp.*				+	
48.	*Lachnus roboris*	+	+			
49.	*Liosomaphis berberidis*	+				
50.	*Macrosiphoniella artemisiae*	+	+			
51.	*Macrosiphum euphorbiae*	+				
52.	*M. melampyri*		+			
53.	*M. rosae*	+	+	+	+	
54.	*M. sp.[I]*	+	+			
55.	*M. sp.[II]*		+	+	+	
56.	*M. sp.[III]*		+			
57.	*Microlophium carnosum*	+	+			
58.	*Moritziella sp.*	+	+			
59.	*Myzocallis coryli*	+	+	+		
60.	*Myzus cerasi*	+	+			
61.	*M. persicae*	+				
62.	*Ovatus insitus*	+	+			
63.	*Panaphis juglandis*	+	+			
64.	*Periphillus lyropictus*		+	+		
65.	*Phorodon humuli*	+				
66.	*Pineus sp.*		+	+	+	
67.	*Prociphilus fraxini*		+			
68.	*Pterocallis alni*		+			
69.	*Pterochloroides persicae*	+				
70.	*Pterocomma populeum*	+	+			
71.	*Rhopalosiphum maidis*	+				
72.	*Schizolachnus pineti*		+	+		
73.	*Semiaphis dauci*	+				
74.	*Sipha maydis*	+				
75.	*Sitobion fragariae*	+	+	+	+	
76.	*Thelaxes driophila*	+	+			
77.	*Tuberculatus annulatus*	+				
78.	*Uroleucon cichorii*	+	+			
Total		59	52	18	10	2

Therefore, the usage of control measures against these aphids is advisable because they represent a serious pests for the medical plants of the above mentioned territory.

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მდინარეების ძამას, ტანასა და თეძამის აუზების სამკურნალო მცენარეების ბუგრები *(Hemiptera: Aphidoidea)*

ალექსიძე გ.[1], ბარჯაძე შ.[2], გიგოლაშვილი ს.[1], ხაიკაშვილი ხ.[3]

[1] საქართველოს სოფლის მეურნეობის მეცნიერებათა აკადემია
[2] ზოოლოგიის ინსტიტუტი, საქართველოს მეცნიერებათა აკადემია
[3] თბილისის ბოტანიკური ბაღი, საქართველოს მეცნიერებათა აკადემია

(მიღებულია 11.11.2005)

რეზიუმე

შესწავლილია მდინარეების ძამას, ტანასა და თეძამის აუზების სამკურნალო მცენარეების ბუგრების ვერტიკალურ-ზონალური გავრცელების კანონზომიერებები და შეფასებულია მათ მიერ მასპინძელი მცენარის დაზიანების ხარისხი. საკვლევ ტერიტორიაზე რეგისტრირებულია სამკურნალო მცენარეების ბუგრების 78 სახეობა, რომლებიც გაერთიანებულნი არიან 3 ოჯახსა და 42 გვარში. სახეობათა უმრავლესობა (58 სახეობა) რეგისტრირებულია სტეპის, არიდული ტყეებისა და ჰემიქსეროფილურ ბალახოვან-ბუჩქნარ მცენარეულობის სარტყელში. ბუგრების სახეობათა რაოდენობა ალპური სარტყელის მიმართულებით შემცირებას განიცდის. სამკურნალო მცენარეების დაზიანების ხარისხის მიხედვით ბუგრები 3 ჯგუფად იყოფა: 35 სახეობა იწვევს მასპინძელ მცენარეთა მაღალი ხარისხის დაზიანებას, 24 - საშუალო ხარისხის დაზიანებას, 19 სახეობა კი დაბალი ხარისხის დაზიანებას.

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Zoology

PRODORILAIMUS LONGICAUDATUS (BÜTSCHLI, 1874) ANDRASSY, 1959 FIRST FOUND IN GEORGIA

ELIAVA I., TSKITISHVILI E.

Institute of Zoology of the Georgian Academy of Sciences

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Abstract

By study of soil nematodes of Gombori mountain ridge (Western Georgia), the species of *Dorilaimida* nematode was found, which is new for Georgian fauna. Measurements, pictures and key for the species of genus are given.

Key words: cuticle, spear, spicula, supplement.

Prodorylaimus longicaudatus (Bütschli, 1874) Andrassy, 1959 [1,2]

Measurement: (Gombori population)
Females (4): L=2,5 mm; a=25-34; b=5.5-5.9; c=4.0-5.,5; c^1=13.5 v=36-39%
Males (2): L=2.3-2.4 mm; a=28; b=5.8-6.1; c=5.9-7.9; c^1=6.9;
spic.=57.8-58.8 mm; Suppl. = 26-27

Body slender. Had slightly set off from body contour. Kuticle thick, with weak longitudinal ribs and many pores. The guiding ring double. Length of spear about 25 μm or at least 1.1 times more than the head width, and a bit distort. Orifice equals to 1/3 of spear length. Amfids goblet shaped, its width more than ½ of had diameter.

Oesofagus enlarged by its middle, cardia conical. Nerve ring just in front of the middle of oesofagus. Vagina sclerotized, its width equals to ½ of body diameter. Ovaries paired, out stretched and reflexed. In uterus of one of the four females is elongated egg (109-64 μm). Prerectum of males - 1.3-1.6 times more than rectum. Prerectum of males slightly shorter. Males have 26-27 contiguous supplements. Spicula broad, 57.8-58.8 μm long.

The tail of both sexes of filiform.

Key to the species of *Prodorylaimus* Andrassy, 1959

1(4) The tail very long, equal to 1/3 – ¼ of body length (c=2.8-3.4).
2(3) Oesofagus expanding in the middle, the orifise equal 2/5 of spear length, suppl. =16--**4. *P. dolichurus* (loos)**
3(2) Oesofagus expanding just before the middle, orifice equal 1/3 of spear length, suppl. = 14-15 --**6. *P. ensis* Kleinhans**
4(1) The tail more or less short (c more than 5)
5(8) The tail very short (c more than 20)

6(7) Small species, body length under 1,5mm; suppl. = 13-14; spicula about 26-30μm long---2. *P. braziliensis* **(Meyl)**

7(6) Larger species, body length about 3mm; suppl.= 17-18; spicula 60 μm long; --14. *P. rionenesis* **(Gerlach)**

8(5) The tail not so long; c under 20

9(10) The row of supplements divide into the two groups -----**7.** ***P. filarum*** **Andrassy**

10(9) The row is not divide

11(20) The number of supplements in the row more than 20

12(15) The spear very short, less than 40 μm long

13(14) The number of supplements in the row about 21-22; spear 30- 33μm long-- **12.** ***P. longicaudatoides*** **Alterr**

14(13) Suppl=23-31; spear about 25 μm long; -------------- **1.** ***P.longicaudatus*** **(Bütschli)**

15(12) Spear longer, more than 40 μm.

16(17) The female has two paravulvar glands, sometime they are polymerized---**11.** ***P. kukuy*** **Tsalolichin**

17(16) Paravulvar glands absence.

18(19) The tail of female 11-16 times more than anal diameter. The cardia cone shaped---**10.** ***P. kralli*** **Tsalolichin**

19(18) The tail of female 6,5 times more than anal diameter; tail of males 3,5 times more than anal diameter; cardia is rounded --------------------**5.** ***P. eliavai*** **Tsalolichin**

20(11) In the row 14-20 suppl.

21(22) Body length under 2mm; suppl.=20 -----------------------**8.** ***P. gurvitschi*** **Eliava**

22(21) Body length more than 2mm;

23(26) Body very slender (more than 40)

24(25) The tale is sharp at the end. Spear is very short (10 μm long)--- **9.** ***P. kazakstanicus*** **Sagitov**

25(24) Tail is slightly rounded at the end. Spear longer (35μm)----------- **3.** ***P.dahli*** **(Alterr)**

26(23) Body not so slender (more than 35)-----------**13.** ***P. paralongicaudatus*** **(Mikoletzky)**

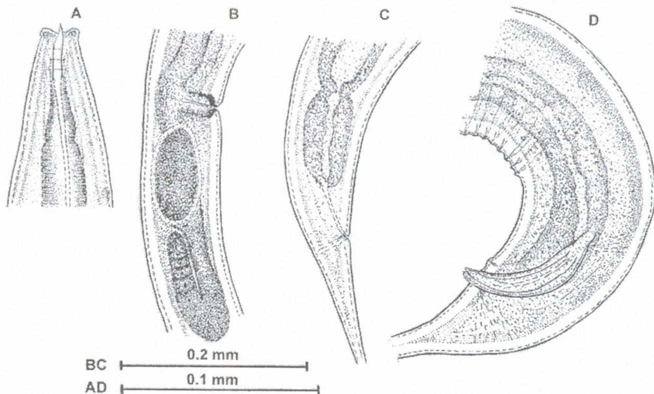

Fig. 1. ***Prodorylaimus longicaudatus*** **(Butschli, 1874) Andrassy, 1959.** A - Female had (x90); B - Vulva region (x40); C - Female tail (x40); D - Spicules and supplements (x90).

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საქართველოს ფაუნისათვის ახალი სახეობა *Prodorylaimus longicaudatus* (Bütschli, 1874) Andrassy, 1959

ელიავა ი. ცქიტიშვილი ე.

საქართველოს მეცნიერებათა აკადემიის ზოოლოგიის ინსტიტუტი

(მიღებულია 19.12.2005)

რეზიუმე

აღწერილია საქართველოს ფაუნისათვის ახალი ნემატოდა *Prodorilaimus longicaudatus* აღმოსავლეთ საქართველოდან. მოცემულია მისი განაზომები, სურათები და გვარის სახეობათა სარკვევი.

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ეროვნული ბიბლიოთეკა

STUDY OF PSYLLIDS (HEMIPTERA, PSYLLOIDEA) OF THE RIVER MEJUDA GORGE (SHIDA KARTLI, GEORGIA)

GEGECHKORI ARN., GINTURI Z., CHUNASHVILI T.

Department of Zoology, Iv. Javakhishvili Tbilisi State University

(Received October 10, 2005)

Abstract

The paper deals with psyllid fauna of hitherto not studied region. 49 species of psyllids were collected in Mejuda gorge. Investigated region represents 3 natural belts. The forest belt is rather rich and consists of 31 species, subalpine – 27 and alpine -12. 4 polyzonal species are distributed in every three zones. 14 species were recorded in agrocenosis. The last one contains some harmful pests of agricultural arboreal plants and herbs.

Introduction

Psyllids are of small size (1-5 mm) insects with limited mobility. They occur throughout the globe, more than 200 species are recorded in the Caucasus [Gegechkori, 1984; Gegechkori & Loginiva, 1990]. Psyllid fauna of the river Mejuda gorge, Shida Kartli, East Georgia was not investigated till now. Floristic data of the river Mejuda gorge was given by Ketskhoveli [Ketskhoveli, 1960]. The aim of our work was to study Psyllid fauna in above mentioned area by vertical belts – forest, subalpine and alpine belts.

Material and Methods

Series of special field studies in the r. Mejuda Gorge was carried out in 2000-2003. Psylloidea of ordo Hemiptera, Insecta class was investigated seasonally during the whole vegetation period of food plant. In the studied region three vertical zones (belts) were distinguished - forest, subalpine and alpine zones.

Results and Discussion

Data of our research is presented in the Table 1. According to the table psyllid fauna of the r. Mejuda gorge consists of 49 species. By feeding habits 31 species consider as dendrophilous forms, 18 species – as horthophilous forms, 19 species are feed on tree plants, 17 – on shrubs, 1- on semishrub, 13 – on perennial herbs, 5 species – on annual herbs.

According to vertical belts distribution of psyllid fauna gives the following picture: in the forest zone 31 species are specialized, in subalpine zone – 27 species, in alpine – 12 species, in agrocenosis – 14 species. 4 species are polyzonal – they are distributed in every three zones.

In the agrocenosis psyllids feed on agricultures and cause plant damages of different kind. Particularly: *Cyamophila medicaginis* is distributed in mountain places, on lucerne plantations; *Psylla mali* feeds on wild and cultural forms of apple, *P. pruni* – on plums, *P. pyri, P. pyrisuga, P. permixta* and *P. bidens* – on pear cultures, *Homotoma ficus* – on fig tree, *Trioza nigricornis* – on vegetables, *T.brassicae* – on anion sowings, *T. daucus* – on carrot sowings. The trophic web of psyllid species with the weeds of agricultural plantations was recorded in the following way: *Trioza urticae* - nettle, *Heterotrioza obliqua* – Atriplex hortensis.

Thus, in the viewpoint of trophic links for psyllid fauna of the r.Mejuda gorge is highly favorable dendroflora; in the viewpoint of vertical belts – forest belt is favorable. Only in agrocenosis were recorded: *Homotoma ficus* (on fig tree), Trioza brassicae (on anion sowings); simultaneously on different cultures of agrocenosis and biocenosis were developed *Cyamophila medicaginis* – on lucerne, *Psylla mali* – on apple, *P. pyri, P. permixta, P. bidens, P. pyrisuga* – on pear, *Trioza nigricornis* – on different herbal vegetables, *T.apacalis* – on carrot.

N	Name of different taxons	Host plants	Species distribution by plant forms					Species distribution by vertical belts and agrocenosis			
			Tree	Shrub	Semishrub	perennial herbs	Annual herbs	Forest belt	Subalpine belt	Alpine belt	Agrocenosis
1	2	3	4	5	6	7	8	9	10	11	12
	Family Aphalaridae										
1	Rhinocola aceris (L.)	Acer ssp.	+	-	-	-	-	-	+	-	-
2	Camarotoscena speciosa flor	Populus nigra	+	-	-	-	-	+	-	-	-
3	Aphalara polygoni Frst.	Polygonum ssp.	-	-	-	-	+	+	+	+	-
4	A.maculipennis (Low)	Polygonum ssp.	-	-	-	-	+	+	+	+	-
5	Craspedolepta sonchi (Frst.)	Leontodon spp.	-	-	-	-	+	-	+	-	-
6	C.pontica Dobr.et Man.	Achillea spp.	-	-	-	-	+	+	+	+	-
7	C.nervosa (Frst.)	Achillea spp.	-	-	-	-	+	+	+	+	-
8	C.malachitica (Dahlb.)	Artemisia absinthium	-	-	+	-	-	+	-	-	+
	Family Psyllidae										
9	Cyamophila medicaginis (Andr.)	Medicago sp.	-	-	-	+	-	-	+	-	+
10	C.caucasica Bajeva	?Vicia sp.	-	-	-	+	-	-	+	+	-
11	Psyllopsis discrepans flor	Fraxinus excelsior	+	-	-	-	-	+	-	-	-
12	Ps. fraxinicola Frst.	Fraxinus excelsior	+	-	-	-	-	+	-	-	-
13	Psylla alni (L.)	Alnus barbata	+	-	-	-	-	+	+	-	-
14	P.foersteri Flor	Alnus barbata	+	-	-	-	-	+	+	-	-
15	P.mali Schmdbdug.	Malus spp.	+	-	-	-	-	+	-	-	+
16	P.peregrina Frst.	Crataegus spp.	-	+	-	-	-	+	-	-	-

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17	P.melanoneura Frst.	Crataegus spp.	-	+	-	-	-	+	+	-	-
18	P.crataegi (Schrnk.)	Crataegus spp.	-	+	-	-	-	+	-	[illegible]	[illegible]
19	P.pyri Frst.	Pyrus spp.	+	-	-	-	-	+	-	-	+
20	P.pruni Scop.	Prunus spp.	+	+	-	-	-	+	-	-	+
21	P.bidens Sulc.	Pyrus spp.	+	-	-	-	-	+	-	-	+
22	P.permixta Burck. et Holdk.	Pyrus spp	+	-	-	-	-	+	-	-	+
23	P.pyrisuga Frst.	Pyrus spp.	+	-	-	-	-	+	-	-	+
24	P.viburni Löw	Viburnum	-	+	-	-	-	+	+	-	-
25	P.hippophaes Frst.	Hippophae rhamnoides	-	+	-	-	-	+	-	-	-
26	P.rhamnicola Scott	Rhamnus spp.	-	+	-	-	-	+	-	-	-
27	P.hartigi Flor	Betula spp.	+	+	-	-	-	-	+	-	-
28	P.albipes Flor	Sorbus spp.	+	+	-	-	-	-	+	-	-
29	P.ambigua Frst	Salix spp.	-	+	-	-	-	+	+	-	-
	Family Homotomidae										
30	Homotoma ficus (L.)	Ficus carica	+	-	-	-	-	-	-	-	+
	Family Calophidae										
31	Calophya rhois Löw	Cotinus coggygria	-	+	-	-	-	+	-	-	-
	Family Triozidae										
32	Heterotrioza obliqua (Thoms.)	Atriplex sp.	-	-	-	+	-	+	-	-	+
33	Trioza galii Frst	Galium spp.	-	-	-	+	-	-	+	+	-
34	T.femoralis Frst.	Alchimilla spp.	-	-	-	+	-	-	+	+	-
35	T.nigricornis Frst.	Rumex, Daucus	-	-	-	+	-	-	+	+	+
36	T.rumicis Löw	Rumex spp.	-	-	-	+	-	-	+	+	-
37	T.apicalis Frst.	Anthriscus, Daucus	-	-	-	+	-	-	+	-	+
38	T.viridula (Zett.)	Cirsium spp.	-	-	-	+	-	-	+	-	-
39	T.valerianae Gegechk.	Valeriana spp.	-	-	-	+	-	-	+	-	-
40	T.urticae (L.)	Urtica spp.	-	-	-	+	-	+	+	+	+
41	T.magnisetosa Log.	Elaegnus spp.	-	+	-	-	-	+	-	-	-
42	T.rhamni (Schrnk.)	Rhamnus spp.	-	+	-	-	-	+	-	-	-
43	T.scotti Löw	Berberis spp.	-	+	-	-	-	+	-	-	-
44	T.proxima Flor	Taraxacum spp.	-	-	-	+	-	-	+	+	-
45	T.brassicae Vasil.	Allium cepa	-	-	-	+	-	-	-	-	+
46	T.remota Frst.	Quercus spp.	+	-	-	-	-	+	-	-	-
47	T.salicivora Reut.	Salix spp.	+	+	-	-	-	-	+	-	-
48	T.albiventris Frst.	"---"	+	+	-	-	-	+	+	-	-
49	T.striola flor	"---"	+	+	-	-	-	-	+	+	-

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ფსილიდების (Psylloidea) შესწავლის შედეგები შიდა ქართლის პირობებში (მდინარე მეჯუდას ხეობა)

გეგეჭკორი არნ., გინტური ზ., ჭუნაშვილი თ.

ზოოლოგიის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.10.2005)

რეზიუმე

შესწავლილია პირველად მდ. მეჯუდას ხეობის ფსილიდების ფაუნა. შეგროვილია ფსილიდების 49 სახეობა. მათგან 31 სახეობა განეკუთვნება დენდროფილურს, 18 კი - ჰორტოფილურს. შესწავლილი რაიონის ფსილიდების ფაუნა გავრცელებულია ხეობის სამი სიმაღლებრივი სარტყლის მიხედვით: ტყის სარტყელში სპეციალიზირებულია 31 სახეობა, სუბალპურში - 27, ალპურში - 12. 4 სახეობა პოლიზონალურია. განსაზღვრულია ყველა სახეობის ტროფიკული კავშირები. აგროცენოზებში გამოვლენილია 14 სახეობა, რომელთაგან 12 სასოფლო-სამეურნეო კულტურებზე იკვებება და სხვადასხვა სახის ზიანი მოაქვს, ხოლო 2 სახეობა იკვებება სარეველა მცენარეებზე.

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ეროვნული ბიბლიოთეკა

Zoology

BIOFORMULATIONS OF ENTOMOPATHOGENIC NEMATODES

LORTKIPANIDZE M.

Institute of Zoology of the Georgian Academy of Sciences

(Received September 21, 2005)

Abstract

The work deals with the description and evaluation of most of the well-known techniques of entomopathogenic nematodes formulations applied for insect-pest control. Privileges are given to the application of the silkworm pupa.

Key words: cultivation, invasion start, nematode suspension, harmful insects, transportation of the start of nematode suspension, feeding media.

Introduction:

The majority of nematodes (roundworms) are parasites of plants and animals. They include such families of entomopathogenic nematodes, as Steinernematidae and Heterorhabditidae, which being effective biological control agents for insect-pests, are of great potential.

In natural conditions a host-insect infected by entomopathogenic nematodes dies in 24-48 hrs. After the intrusion into the host-insect body, entomopathogenic nematodes release the bacteria *Xenorhabdus, Photorhabdus*, the toxin of which is responsible for the death of the host-insect. Nematodes together with their symbiotically related bacterium form a nematodobacterial complex [Poinar, Thomas, 1965].

Application of modern technologies opened great possibilities to study numerous entomopathogenic nematode species and apply them for insect-pest control [Grewal, 1998].

The cost of nematode formulations is much lower compared to that of chemicals, which promotes their successful distribution in many countries as biological means of pest control. Additionally, application of nematodes against pests has proved to be more effective compared with pest insecticides.

Entomopathogenic nematodes of the genus Steinernema and Heterorhabditis are the best control agents for insect-pests, as they possess many positive characteristic traits: they are safe for non-target organisms, they are easily reproduced en masse in favorable conditions, they apply the foraging strategy for target insects, are responsible for rapid death of host-insects and, as biological means, are capable to compete with chemicals utilized in agriculture. Moreover, they are amenable to genetic selection. These characteristic properties have promoted their rapid commercialization.

In the last 15 years the technology of entomopathogenic nematodes formulations has been progressively developed and broadened beginning with techniques of soaking by artificial sponges and ending with their advanced granular formulations. However, storage of formulations for long periods, still remains to be the major problem limiting the large-scale commercialization of nematodes.

This article gives the description of a number of nematode storage techniques worked out and used in recent years in various advanced laboratories of the world.

Entomopathogenic nematodes can be stored in refrigerators. However, there are some difficulties dealing with storage of concentrated nematode suspensions, which is due to their high oxygen demand, that lessens the chance of nematode survival. Therefore the invasive juveniles are formulated immediately after harvesting from the fermentation medium in inert carriers, granules and powders.

Materials and Methods.

Inert carriers are used to store small quantities of nematodes under refrigerated conditions. The nematode suspension is soaked by the polyether sponge. Then the sponge is placed in a plastic bag. This method allows us to store nematodes for 1-2 months at 5-10°C. Removal of nematodes from the sponge is carried out by soaking and squeezing the sponge in water. This method of nematode storage and application is convenient only for small-scale lawns, home and vegetable gardens but not for large-scale areas.

Among active materials - gels are worth mentioning. Encapsulation of entomopathogenic nematodes in calcium algenate gel beads was first described by Kaya and Nelson [Kaya M.A. & Helsen C.A.,1985] developed a new gel formulation in which the nematode solution was mixed with anhydrous polyacrylamide. The obtained mixture possesses properties similar to water. But this formulation is characterized by a limited shelf-life (dates of storage, maintenance of vitality).

Later activated charcoal was applied by Yukawa and Pitt [Grewal, 1998]. They described the system of nematode storage and transportation, wherein nematodes were mixed with such absorbent material as activated charcoal powder. But this formulation did not offer any advantages either (including high cost and storage instability).

Among other active materials clay sandwiches were applied. The method implied mixture of nematodes with clay. The authors described this formulation as a sandwich consisting of a layer of nematodes between two layers of clay. This formulation was commercialized by Biotechnology Australian Ltd, but it had certain disadvantages: 1. lack of stability at room temperature; 2. difficulty to be dissolved in water; 3. frequently clogged spay particles and 4. a very low nematode to clay ration. Hence the project failed [Grewal, 1998].

Granules proved to be more advantageous. Gapinera and Hibbard [Gapinera & Hibbard, 1987] gave the description of a pellet nematode formulation, where pallets contained alfalfa ground meal and wheat flour. Nematodes were uniformly distributed in the granular product, which was called "Pesta". But this product had certain disadvantages: granules dried hard very easily and it was difficult to dissolve them in water. Hence, low nematode survival.

Significant success was achieved by application of water dissolvable granules [Grewal, Georgis,1998], in which infective juveniles were encased in 10-12 mm diameter granules - a mixture of silica, clay, cellulose, lignin and starch. These granules are prepared very easily: droplets of a nematode suspension are sprayed on a tilted rotating pan on which there is dry powder. As soon as droplets of the nematode suspension come in contact with the powder, granules start to form. Droplets roll over dry powder and absorb more powder. Then granules are sieved out of the powder and packaged for transportation. The granular matrix allows access of oxygen to nematodes during their storage and transportation.

Water dissolvable formulations offer a number of advantages: 1. prolonged nematode storage stability at room temperature and under refrigeration; 2. nematode tolerance promoting low cost and ease of transportation; 3. ability to dissolve rapidly in water; 4. capability of S.carpocapsae nematodes to be stored for 5-6 months at 25°C. The characteristic property of this formulation is

also moisture content (temperature and rate of water loss are the most significant factors affecting nematode survival in granules).

A liquid concentrate has also been obtained. It is used for those formulations in which a proprietary metabolic inhibitor and an antimicrobial agent are added to the liquid nematode suspension. The metabolic inhibitor allows reduction of nematode demand on O_2 and thus increases nematode survival for extended periods under anoxic conditions.

Results and discussion

A new method, which implies transportation of nematodes via infected pupae in silkworm cocoons and preparation of a suspension in places, has been developed by us [Kakulia, Lortkipanidze, 1992]. We consider this method to be of great potential.

The silkworm cocoon is much more advantageous compared to all the above mentioned formulations. Invasion (inoculation) of the silkworm pupae by nematodes is carried out by means of injection of the invasive material into the cocoon. 2 ml of nematode suspension with 500 nematodes is injected either into the top or the central part of the silkworm cocoon. Dissection of cocoons begins from the 7th day. Initially only a few nematodes appear, but 11 days later their number considerably increased.

While testing the insect organism it was found out that after injecting the central part of the cocoon, the degree of infestation was higher than after injecting the top-part of the cocoon, which indicated that during inoculation there appears more chance to get with a needle exactly into the body of the silkworm pupa, which is much more effective. In this case nematodes are both found within the whole pupa and on the silkworm cocoon wall. The number of nematodes in each cocoon reaches 450 000-500 000.

The advantages of the silkworm cocoon formulation are demonstrated in the product - with more nematode concentration (approximately 450 000 nematodes per pupa), prolonged storage and much more convenient transportation. In such formulations nematodes can be stored for 2-3 months at 5-6°C. Before application of the pupae in natural conditions they are to be cut open, dissolved in certain amount of water and filtered. Then the obtained suspension is applied for spraying and for soaking. The only drawback of this product is a low indicator of storage, as it can be kept at room temperature for only 2 months.

As mentioned above, the most important factors having a decisive role in formulation advancement are complete satisfaction of nematode O_2 demand and extended shelf life stability (temperature and moisture).

Thus, as it has been shown from our data, application of silkworm cocoons for cultivation and transportation of entomopathogenic nematodes is of great potential. Our work dealing with the further development of this method is being continued

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ენტომოპათოგენური ნემატოდების ბიოფორმულირებები

ლორთქიფანიძე მ.

საქართველოს მეცნიერებათა აკადემიის ზოოლოგიის ინსტიტუტი

(მიღებულია 21.09.2005)

რეზიუმე

შემუშავებულია ახალი მეთოდი, რომელიც ითვალისწინებს აბრეშუმის პარკმხვევიას ჭუპრის გამოყენებას Steinernematidae-სა და Heterorhabditida-ს ენტომოპათოგენური ნემატოდების კულტივირებისა და ტრანსპორტირებისათვის. მოცემულია მსოფლიოს მოწინავე ლაბორატორიებში ნემატოდების შენახვის ახალი ტექნოლოგიები და შედარებულია ჩვენ მეთოდთან.

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ეროვნული ბიბლიოთეკა

Zoology

THE ECOLOGICAL AND ZOOGEOGRAPHICAL REVIEW OF THE SPIDERS (FAMILY PHILODROMIDAE) DISTRIBUTED IN EAST GEORGIA

MKHEIDZE T., GEGECHKORI ARN., PKHAKADZE V.

Department of Zoology, Iv. Javakhishvili Tbilisi State University

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Abstract

The ecological investigation of Family Philodromidae spiders from East Georgia has shown that 6 species and 1 subspecies are mesophillous, 2 species belong to xerophillous ecological unit and 2 - to hygrophillous. It was established that in studied fauna from autochthonous element 1 genus and 1 subspecies are characterized with South Caucasian distribution. From allochronous element of fauna with Holarctic distribution are characterized 2 genera, 4 species, with Palaearctic - 2 genera, 3 species; one by one genus and as much species belong to the following zoogeographical units: European, Mediterranean, Europe-Siberian.

Key words: taxonomy, mesophillous, xerophillous, hygrophillous.

Introduction

3 genera and 10 species and 1 subspecies of the family Philodromidae were registered in East Georgia. The family Philodromidae comprises the following genera: Philodromus Walck., - 4 species, Tibellus Sim. - 3 species and 1 subspecies, Thanatus Koch. - 3 species [Mkheidze, 1992].

Studies of spiders fauna of the family Philodromidae in different landscape zones and altitudinal mountain belts in Georgia were carried out from the beginning of XX century, but in ecological and zoogeographical viewpoint it was not discussed till now.

Materials and Methods

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

Results and Discussion

Mesophillous group of spiders of the family Philidromidae, according to abiotic factors, includes 6 species and 1 subspecies (Phillodromus dilutus, Philodromus rufus, Philodromus aureolus, Thanatus liniatipes, Thanatus imbecilus, Tibellus macellus, Tibellus macellus Sim., sub species - georgicus), xerophillous group - 2 species (Philodromus histrio, Thanatus arenarius), and

hygrophillous one - 2 species (Tibellus maritimus, Tibellus oblongus) (Table 1.) [Azheganova, 1968; Mkheidze, 1992].

Studied spiders as a predators hunt on their preys on all living forms of vegetation - grasses, bushes and trees (Philodromus dilutus, Philodromus histrio, Philodromus rufus, Philodromus aureolus, Thanatus liniatipes, Thanatus arenarius, Thanatus imbecilus, Tibillus oblongus, Tibellus maritimus, Tibellus macellus, Tibellus macellus georgicus sub spn.).

From the feeding point of view spiders are typical predators (zoophagus), which hunt for insects: Coleoptera, Myriapoda, Aphidodea (Hemiptera), Diptera.

As regards to the zoogeographical studies of the spiders of the family Philodromidae from autochtonous element of fauna 1 genus and 1 subspecies are characterized with South Caucasian distribution (*Tibellus macellus Sim., georgicus sub spn.*).

From allochtonous element of fauna with Holarctic distribution characterized 2 genera, 4 species (*Philodromus rufus, Philodromus aureolus, Tibellus maritimus, Tibellus oblongus*) [Azheganova, 1968; Mikhailov, 1997; Mkheidze, 1992; Tyschenko, 1971], with Palaeractic - 2 genera, 3 species (*Philodromus histrio, Thanatus arenarius, Thanatus imbecilus*), one by one genus and as much species belong to the following zoogeographical units: European (*Philodromus dilutus*), Mediterranean (*Thanatus liniatipes*), Europe-Siberian (*Tibellus macellus*) [Mikhailov, 1997; Mkheidze, 1992].

Table 1. Data of Ecological and Zoogeographical Studies of Spider's (Family Philodromidae) Fauna of East Georgia.

N				Family, Genera, Species	Distribution	Areal types	The relation to humidity			landscape genetic-type	The relation to plant's life forms			Food (prey)
							Hygrophillous	Mesophillous	Xerophillous		Tree	Brush	Grass	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1				Philodromidae										
	1			Philodromus (Walck., 1826)										
		1		*Philodromus dilutus* (Thor., 1875)	North Caucasus (Russia), European countries of the former Soviet Union, South Caucasus (Georgia)	E	-	+	-	Fr.f	-	-	+	Insecta (Diptera, Coleoptera)

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		2		Philodromus histrio (Latr., 1819)	Europe (wide), Carpathians, Russia, Estonia, Latvia, Lithuania, Ukraine, South Caucasus (Georgia) Middle Asia (Turkmenistan, Uzbekistan, Tajikistan), Kazakhstan, the Urals, Amur-Maritime area.	P	-	-	+	f	-	+	+	Insecta (Diptera, Aphidodea Hemi-ptera)
		3		[1]Philodromus rufus (Walck., 1826)		H	-	+	-	Fr.f	+	+	-	Insecta
		4		[2]Philodromus aureolus (Clerck., 1757)		H	-	+	-	f	-	-	+	Insecta (Aphido-dea Hemiptera, Pieris
	2			Thanatus (Koch., 1837)										
		5		Thanatus liniatipes (Simon, 1870)	Syria, Tunisia, Spain, Portugal, South Caucasus (Georgia)	M	-	+	-	Fr.f	-	-	+	Insecta (Hemiptera)
		6		Thanatus arenarius (Thor., 1872)	Europe (wide), Turkey, Carpathians, Russia, Latvia, Ukraine, Byelorussia, Moldavia, South Cau-casus (Azer-baijan, Ge-orgia), Ka-zakhstan, the Urals, Siberia, Middle Asia	P	-	-	+	Fr.f	-	+	+	Insecta (Coleiptera)
		7		Thanatus imbeciles (Koch., 1878)	Europe (wide), Turkey, Russia, South Caucasus (Georgia), Middle Asia (Uzbekistan, Turkmenistan, Tajikistan)	P	-	+	-	Fr.f	-	-	+	Insecta (Diptera, Hemiptera)

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	3			Tibellus (Simon, 1878) (=Metastenus Bert., 1878)										
		8		Tibellus macellus (Simon, 1878)	Russia, Ukraine, South Caucasus (Georgia), the Urals	ES	-	+	-	Fr.f	-	+	+	Insecta (Diptera)
			8.1	Tibellus macellus (Sim., georgicus Sub.spn.)	South Caucasus (Georgia)	SC	-	+	-	Fr.f	-	-	+	Insecta (Coleoptera)
		9		Tibellus maritimus (Menge., 1875)	Carpathians, Russia, Estonia, Latvia, Lithuania, South Caucasus (Azerbaijan, Georgia) Kazakhstan, Middle Asia (Uzbekistan, Turkmenistan), the Urals, Siberia, Kamchatka, Amurmaritime area, North America	H	+	-	-	Fr.f	-	+	+	Insecta (Coleoptera, Hemiptera)
		10		Tibellus oblongus (Walck., 1802)	Europe (wide), Carpathians, Russia, Estonia, Latvia, Lithuania, Ukraine, Moldavia, South Caucasus (Azerbaijan, Armenia, Georgia), Middle Asia (Uzbekistan, Kyrgyzstan, Turkmenistan, Tajikistan), Sakhalin, Kamchatka, Japan, Amurmaritime area, North America	H	+	-	-	ff	-	+	+	Insecta (Aphidodea)

Abbreviations: E – European, P – Palaearctic, H – Holarctic, M – Mediterranean, ES – Europe-Siberian, SC – South Caucasian, Frf – Forest-field, f – field.

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აღმოსავლეთ საქართველოში გავრცელებული ობობების ოჯახ Philodromidae -ს ეკოლოგიური და ზოოგეოგრაფიული მიმოხილვა

მხეიძე თ., გეგჭკორი არნ., ფხაკაძე ვ.

ზოოლოგიის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 26.09.2005)

რეზიუმე

ეკოლოგიური ანალიზით დადგენილია, რომ აღმოსავლეთ საქართველოში გავრცელებული ობობების 6 სახეობა და 1 ქვესახეობა მეზოფილური ბუნებისაა, ქსეროფილური და ჰიგროფილურებია - 2-2 სახეობა. შესწავლილი ზოოფაგები მცენარეთა სასიცოცხლო ფორმების მიხედვით მსხვერპლზე ნადირობენ როგორც ხეებზე, ისე ბალახებსა და ბუჩქებში. Philodromidae-ის ოჯახის ზოოგეოგრაფიული ანალიზით დადგინდა, რომ ავტოქტონური ჯგუფიდან სამხრეთ კავკასიური გარცელებით ხასიათდება 1 გვარი და 1 ქვესახეობა; ალოქტონური ჯგუფიდან ჰოლარქტიკული გავრცელებით - 2 გვარი და 4 სახეობა, პალეარქტიკულით - 2 გვარი და 3 სახეობა; თითო-თითო გვარსა და ამდენივე სახეობას ახასიათებს ევროპული, ევროპულ-ციმბირული და ხმელთაშუაზღვიური არეალები.

ინსტრუქცია ავტორთათვის

ეროვნული ბიბლიოთეკა

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:

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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.

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