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Biochemistry

EFFECT OF DIFFERENT CONCENTRATIONS OF ALKANES ON MAIZE, RYEGRASS AND KIDNEY BEAN SEEDLINGS

BETSIASHVILI M., SADUNISHVILI T., KUPRAVA N., AMASHUKELI N., TSULUKIDZE N., NUTSUBIDZE N.

Durmishidze Institute of Biochemistry and Biotechnology, Georgian Academy of Sciences

(Received January 25, 2004)

Abstract

The influence of natural gas and pentane different concentrations on main metabolic and energetic enzymes activities in plant leaves have been studied. Glutamate dehydrogenase, malate dehydrogenase and glutamine synthetase activities, as well as protein content changes in maize, ryegrass and kidney bean seedling leaves have been revealed. It has been stated that with the increase of alkanes concentration simultaneous decrease of studied enzyme activities and the increase of protein content in leaves were observed. Among the tested alkanes pentane is the strongest inhibitor of energy and nitrogen metabolism enzymes in all studied plants. At the same time, the most sensitive to the effect of the toxicants is ryegrass and the most tolerant kidney bean.

Key Words: Natural gas, pentane, glutamate dehydrogenase, malate dehydrogenase and glutamine synthetase.

Introduction

Plant protein is the main source of food. Plant productivity and their protein content in particular are determined by the plants ability of nitrogen uptake. The latter is mainly determined by ammonium assimilation that is the only product of transformation of main nitrogen sources, nitrate and urea, as well as molecular nitrogen fixation [Sadunishvili, 1996]. Incorporation of ammonium into amino acids and thus in proteins is mainly realized via glutamate syntheses cycle, in which enzymes - glutamine synthetase and glutamate synthetase have an important role [Lea. et al. 1992]. Although it is known, that plants are capable to absorb and detoxicate toxicants in definite dozes, by means of incorporation of their transformation products into a standard metabolic cycle [Korte, Kvesitadze et al. 2000]. Besides oxidative enzymes, the enzymes of plants main metabolic pathways, namely enzymes of energy and nitrogen metabolism are involved in this process [Kvesitadze et al. 2001].

The goal of the present work was to study the changes in enzyme activities – glutamate dehydrogenase, malate dehydrogenase and glutamine synthetase, that occupy the key position in plant cell energy and nitrogen metabolism.

Materials and Methods

Maize *(Zea mayse L.)* and ryegrass *(Lolium perenne)* from cereals family and kidney bean *(Phaseolus vulgaris L.)* from legumes were selected for study. Experiments were carried out on young seedlings of above mentioned plants grown on tap water at ordinary illumination, at 25°C. From 6-th till 12-th days of growing the seedlings were kept in hermetic chambers in the atmosphere, containing vapor of natural gas (mixture of C_1-C_4 alkanes) and pentane (as vapors). The toxicants effect was assessed by the following parameters: changes in the protein content and in the activities of glutamate and malate dehydrogenases and glutamine synthetase.

The Glutamate dehydrogenase and malate dehydrogenase (E.C.1.1.1.37) activities were determined by a spectophotometric method according to the rate of oxidation of NADH at 340 nm [Sadunishvili et al. 1996, Kvesitadze et al. 1993]. As a unit of activity, we took the amount of enzyme that induced oxidation of 1 μmol of NADH in 1 min at 20°C. The reaction mixture for determining the aminating activity of glutamate dehydrogenase contained 0.1 ml of the solution to be investigated (40-60 μg of protein), 10 mM 2-oxoglutarate, 0.13 mM NADH, and 100mM NH_4Cl in a 0,05 M Tris-HCl buffer in a final volume of 3 ml (pH 7.8). Variants without ammonia served as the control.

The reaction mixture for determining the activity of malate dehydrogenase in reaction contained 0.02 ml of the solution to be investigated (10 μg of protein), 1.9 mM oxaloacetate, and 0.13 mM NADH in a 0,05 M Tris-HCl buffer in a final volume of 3 ml, pH of the mixture 7.8. Variants without oxaloacetate served as the control.

Glutamine synthetase activity (EC 6.3.1.2) was determined by a colorimetric method, according to the amount of γ-glutamylhydroxamic acid (γ-GHA) formed in the transferase reaction [Sadunishvili et al. 1996]. The amount of the enzyme catalyzing the formation of 1 μmol γ-GHA in 1 min at 37°C was taken as the Glutamine synthetase activity unit.

Protein was determined according to Bradford [Bradford, 1974]. The specific activity of the enzymes was calculated as the number of units per 1 mg of protein in the solution under investigation.

Results and Discussions

The results of changes in activities of the principle metabolic and energetic enzymes: glutamate dehydrogenase, malate dehydrogenase and glutamine synthetase in maize, ryegrass and kidney bean seedlings leaves at the effect of different concentrations of natural gas and pentane are presented in the Tables 1-3.

Similar to maize, pentane is an inhibitor for ryegrass and kidney bean seedlings too. The strongest inhibition of enzymes activities in ryegrass seedlings leaves is observed in the case of glutamate dehydrogenase at pentane concentration – 10% by volume. The mixture of alkanes (C_1-C_4) at concentration – 10% by volume cause the inhibition of glutamate dehydrogenase activity in leaves of ryegrass seedlings and the stimulation of malate dehydrogenase (for 44%) and glutamine sythetase (for 63%) activities.

As is seen from experimental results, in most cases with the increase of alkanes concentration simultaneous decrease of studied enzymes activities was observed.

Table 1. Effect of different concentrations of natural gas and pentane vapor on glutamate dehydrogenase, malate dehydrogenase and glutamine synthetase activities and on protein content in maize leaves.

Hydrocarbon	Quantity of toxicant vapor in a chamber, % of volume	Protein, mg/ml	Specific activity		
			Glutamate dehydrogenase, μmol NADH/min ×mg protein	Malate dehydrogenase, μmol NADH/min ×mg protein	Glutamine synthetase, μmol γ-GHA*/min× protein
Pentane	0	1.38	0.035	0.31	1.88
	1	1.82	0.027	0.22	1.02
	5	3.11	0.005	0.12	0.7
	10	1.88	0.034	0.27	1.42
Natural gas	0	0.90	0.106	0.44	3.13
	1	1.27	0.038	0.34	2.50
	5	1.21	0.026	0.31	2.51
	10	1.03	0.015	0.38	2.75

* - γ - glutamilhydroxamic acid

Table 2. Effect of different concentrations of natural gas and pentane vapor on glutamate dehydrogenase, malate dehydrogenase and glutamine synthetase activities and on protein content in ryegrass leaves.

Hydrocarbon	Quantity of toxicant vapor in a chamber, % of volume	Protein, mg/ml	Specific activity		
			Glutamate dehydrogenase, μmol NADH/min ×mg protein	Malate dehydrogenase, μmol NADH/min ×mg protein	Glutamine synthetase μmol γ-GHA*/min× protein
Pentane	0	0,27	0,42	0,36	2,5
	1	0,26	0,03	0,25	2,3
	5	0,25	0,02	0,23	2,5
	10	0,24	0,01	0,30	2,5
Natural gas	0	0,28	0,56	0,34	2,64
	1	0,35	0,02	0,23	1,70
	5	0,36	0,01	0,27	1,72
	10	0,13	0	0,49	4,31

* - γ - glutamilhydroxamic acid

Table 3. Effect of different concentrations of natural gas and pentane vapor on glutamate dehydrogenase, malate dehydrogenase and glutamine synthetase activities and on protein content in kidney bean leaves

Hydrocarbon	Quantity of toxicant vapor in a chamber, % of volume	Protein, mg/ml	Specific activity		
			Glutamate dehydrogenase, μmol NADH/min ×mg protein	Malate dehydrogenase, μmol NADH/min ×mg protein	Glutamine synthetase μmol γ-GHA*/min× protein
Pentane	0	1,25	0,064	0,19	0,26
	1	1,26	0,063	0,23	0,19
	5	2,40	0,020	0,12	0,12
	10	1,70	0,038	0,19	0,17
Natural gas	0 (Control)	0,035	0,14	0,21	2,3
	0,1	0,028	0,14	0,18	2,5
	0,5	0,018	0,12	0,29	2,2
	1	0,020	0,12	0,38	1,8

* - γ - glutamilhydroxamic acid

Mainly, the most effective for maize seedlings is pentane vapor in concentration – 5% by volume that causes a strong activity inhibition of almost all metabolic enzymes. Contrast to natural gas concentration – 10% by volume, the increase of malate dehydrogenase and glutamine synthetase activities was observed in maize seedlings.

In kidney bean seedlings a strong inhibition of glutamate dehydrogenase, malate dehydrogenase and glutamine synthetase activities is observed under the effect of natural gas (mixture of C_1-C_4 alkanes), at concentration 5% by volume.

As a result of toxicant penetration into plant cell the increase of protein content in plant leaves have also been demonstrated [Kvesitadze et al. 1993], which is supposed to be determined by the stimulation of biosynthesis processes of those proteins, peptides and enzymes, that participate in detoxication processes – oxidation and conjugation with protein/peptides.

Thus, pentane is the strongest inhibitor of enzymes of energy and nitrogen metabolism for all the studied types of plants. The most sensitive to the effect of the toxicants, namely alkanes, is ryegrass and the most tolerant - kidney bean.

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სხვადასხვა კონცენტრაციის ალკანების ზეგავლენა სიმინდის, კოინდარისა და ლობიოს ნაზარდებზე

ბეციაშვილი მ., სადუნიშვილი თ., ამაშუკელი ნ., კუპრავა ნ., წულუკიძე ნ.

საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 25.01.2004)

რეზიუმე

შესწავლილია სხვადასხვა კონცენტრაციის ბუნებრივი აირისა და პენტანის ზეგავლენა მცენარის ფოთლებში ძირითადი მეტაბოლური და ენერგეტიკული ცვლის ფერმენტების აქტივობაზე. გლუტამატდჰიდროგენაზის, მალატდჰიდროგენაზისა და გლუტამინსინთეტაზის აქტივობის, ისევე როგორც ცილის რაოდენობის ცვლილება გამოვლენილია სიმინდის, კოინდარისა და ლობიოს ნაზარდებში. დადგენილია, რომ ალკანების კონცენტრაციის ზრდის პარალელურად ადგილი აქვს შესწავლილი ფერმენტების აქტივობის თანდათანობით შემცირებას. თითქმის ყველა მცენარის შემთხვევაში შეინიშნება ცილის რაოდენობის მატება. შესწავლილი ნახშირწყალბადებიდან პენტანი წარმოადგენს თითქმის ყველა შესწავლილი მცენარისათვის ენერგიისა და აზოტის ცვლის ფერმენტების ძლიერ ინჰიბიტორს. ამასთან ტოქსიკანტების ზეგავლენის მიმართ ყველაზე მგრძნობიარე აღმოჩნდა კოინდარი, ხოლო ყველაზე ტოლერანტული – ლობიო.

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Biochemistry

STUDY OF THE EFFECT OF LIGHT ON THE ACCUMULATION OF STEROLS AND STEROID GLYCOSIDES *IN VITRO* HETEROTROPHIC AND PHOTOMIXOTROPHIC CULTURES OF *YUCCA GLORIOSA*

GOGAVA M., GOGOBERIDZE M., ZAMBAKHIDZE N., MIMINOSHVILI T.

Durmishidze Institute of Plant Biochemistry and Biotechnology, Georgian Academy of Sciences

(Received January 20, 2004)

Abstract

The interrelation between free and bound sterols and steroid glycosides biosynthetic processes in *Yucca gloriosa* heterotrophic and photomixotrophic callus tissues has been studied. In heterotrophic culture compared to the intact plant, transformation of sterols into steroid glycosides is blocked. In photomixotrophic culture compared to the heterotrophic culture, the total amount of sterols is reduced, the interrelation of their free and bound forms is changed, individual sterol spectrum becomes poorer, the quantity of steroid glycosides is enhanced. The light complicates cytodifferentiation of isolated cells of *Yucca gloriosa* and at the same time it stimulates the transformation of sterols into steroid glycosides.

Key words: *Yucca gloriosa,* sterols, steroid glycosides, heterotrophic culture, photomixotrophic culture

Introduction

Steroid glycosides are a diverse family of secondary metabolites. They commonly have potent antifungal activity and their natural role in plants is likely to be in protection against attack by pathogenic microbes. Steroid glycosides are widely used in medicine and agriculture as biologically active compounds [Arthan et al., 2002, Osbourn et al., 2003, Plock et al., 2002, Yokosuka. et al., 2002].

In vitro dedifferentiated cultures represent the best model system for the study of many physiological processes, metabolism, regulation of biosynthesis and biological functions of different compounds. Considering the totypotency of plant cells, it is important to find ways to realize the plant genome information.

It has been stated that *Yucca gloriosa* heterotrophic callus culture, like most proliferous cells, has a lower biosynthetic potential compared to the intact plant. In cultivated cells the spectrum of formed metabolites is also changed [Gogoberidze et al., 1989]. The changes in spectrum of secondary metabolites in isolated cultures and the decrease of their concentration can be explained by the physiological characteristics of plant cells, which in *in vitro* cultures can not

reach the differentiation level characteristic of intact plant specialized cells [Lucner et al., 1977]. This supposition is confirmed in our experiments by showing the correlation of steroids and sapogenins biosynthesis at different stages of *Yucca gloriosa* callus tissue morphogenesis [Gogoberidze et al., 2003].

Ensuingly, we have chosen the greening process as one of the best documented types of cell specialization exhibited by cultivated plant cells.

Materials and Methods

The composition of nutrient medium, the regime of cultivation and the methods of growth analysis of the *Yucca gloriosa* callus culture were described earlier [Gogoberidze et al., 1988]. In order to obtain photomixotrophic culture, the callus tissue culture has been cultivated at the following light regime: 16 h light + 8 h dark, at 3.5 thousand lux intensity of light, using luminescence bulbs, at $26^0 \pm 1^0$ temperature.

Determination of chlorophyll quantity was carried out by Arnon [Arnon, 1949].

Determination furostanol glycosides performed by Vasilieva et al. methods [Vasilieva et al., 1947].

Sterol analysis was carried out in the following way: 25 g. of dry, crushed callus tissue lipid extraction was carried out by Folch method [Folch et al., 1959]. Lipid fraction was detached from pigments and other nonpolar components [Kuiper, 1968] and evaporated to dry residue. Presence of sterols free and bound forms (sterylglycosides and esterified sterols) was stated by thin-layer chromatographic analysis of lipid fraction. After acid and alkaline hydrolysis of bound forms, the precipitation of free sterols occurred by Digitonin method [Grundwald, 1970]. Quantitative analysis of sterols was carried out by spectrophotometric method [Cowley et al., 1971], while the qualitative analysis of total sterols was made by gas-liquid chromatography. SERVA-sterols were used as markers.

Results and Discussion

In plant cells sterols are known to be the necessary intermediate products, from which biosynthesis of other steroid compounds, including steroid glycosides biosynthesis occur [Heftman, 1971]. Firstly the interrelation of the two compounds of accumulation process in the *Yucca gloriosa* heterotrophic tissue has been studied. Callus tissue obtained from buds were compared to intact plant buds. By the method of thin-layer chromatography it has been stated that free and bound are present in the total preparations of lipids extracted from *Yucca gloriosa* intact plant buds and tissue culture. It has also been observed, that the total quantity of sterols in the callus tissue was 1.5 times higher than in the intact plant. As for the furostanol glicosides, their accumulation in heterotrophic cells was decreased by 0.17% (Table 1).

The quantitative analysis of total preparations of sterols revealed that the esterified form of sterols in heterotrophic culture predominates over free and glycosidized forms (0.028%, 0.019% and 0.013% respectively). According to gas-liquid analysis of free and bound sterols it has been stated that the content of heterotrophic culture total preparations was not homogenous (Table 3). The free form contained 5, sterileglycosides – 7, esterified forms – 11 different sterols. Sitosterole dominates in free and glycosidized sterols (35.5% and 32% respectively), and 4α-methylsterol representative - in esterified form (28%).

Table 1. Quantitative analysis of total preparations of sterols and furostanols extracted from *Yucca gloriosa* various differentiation tissues. 1. Free sterols. 2. Esterified sterols. 3. Sterylglycosides

Tissues	Sterols, %			Total sterols, %	Furostanols, %
	1	2	3		
Intact plant buds	+	+	+	0.040	1.50
Heterotrophic culture	0.019	0.028	0.013	0.060	0.17
Photomixotrophic culture (Sample I)	0.020	0.005	0.008	0.033	0.31
Photomixotrophic culture (Sample II)	0.008	0.005	0.004	0.017	0.55

As in the process of dedifferentiation quantitative changes of sterols and steroid glycosides occur, the total quantity of sterols is increased and that of steroid glycosides is decreased, further research was focused on finding the factors which would prevent the blocking of transformation of sterols into steroid glycosides. Light has been suggested to be the factor that stimulates cytodifferentiation in cells of a number of tissue cultures and plays an important role in the induction and repression of secondary metabolites biosynthesis [Dalton et al., 1983].

Having been exposed to light for 25 days the callus tissue started going black and was necrosed Therefore the tests lasted for 25 days (including the control sample). The obtained photomixotrophic tissue (Sample I) differed morphologically from the heterotrophic tissue. Under the effect of light dense light green callus was formed. Chlorophyll formation became visually noticeable on the 15th day; later its content gradually increased. On the 25th day of the growth cycle its quantity equal to 0.022 mg per fresh biomass. The light caused strong inhibition of the growth of *Yucca gloriosa* tissue culture. According to the accumulation of fresh biomass the growth was inhibited almost twice, while according to the dry biomass accumulation - 1.5 times. Growth reduction was followed by an increase of the amount of furostanol glycosides (0.31%) (Table 2).

In order to study the effect of light duration the cultivation of photomixotrophic tissue on light during 11 passage (II sample) was carried out. For transplantation green cells were mainly selected, and as a result, we have obtained stable, homogenously pigmented cultures of highly chlorophyllous cells which contain partially developed chloroplasts. At long exposition to light compared to the tissue growing under the light during one passage the figures of growth changed insignificantly, the chlorophyll and furostanol glycosides content increased. As it seems, the biosynthetic activity of *in vitro* tissues depends on the duration of exposition to light.

Like intact plant buds and heterotrophic culture, total preparations extracted from both samples of photomixotrophic cultures contained free, as well as bound sterols. Under the effect of light the total quantity of sterols in both samples decreased. In sample I their content equal 0,033% (per dry biomass), in sample II – 0,017% (per dry biomass). Obviously the quantitative decrease of sterols is resulted from their fast transformation into glycosides (Table 1).

Table 2. The effect of light on accumulation of callus tissue biomass and chlorophyll of *Yucca gloriosa* cultivated in the different conditions

Tissues	Fresh biomass, g	Dry biomass, g	Clorophyll, mg/g fresh biomass
Heterotrophic culture	21.95	0.61	-
Photomixotrophic culture(Sample I)	13.18	0.44	0.022
Photomixotrophic culture(Sample II)	11.28	0.37	0.034

Quantitative changes caused by the experimental conditions were expressed mostly in decrease of the quantity of esterified sterols. Their number at the end of 1st passage was decreased by 5.4 and did not change at long exposition to light. As for free sterols, their quantity in sample 1 was almost unchanged, and later it was decreased by 2.5. The reduction of the number of sterylglycosides was gradual.

Table 3. Gas-liquid analysis of total preparations of sterols extracted from *Yucca gloriosa* differentiated tissues. 1. Free sterols, 2. Esterified sterols, 3. Sterylglycosides (% per dry biomass)

Individual sterols	Heterotrophic culture			Photomixotrophic culture(Sample I)			Photomixotrophic culture(Sample II)		
	1	2	3	1	2	3	1	2	3
Unidentified sterol	10.5	5.2	8.0	-	-	-	-	-	-
Cholesterol	16.6	3.5	3.2	30.2	32.4	23.3	12.8	26.0	23.6
Campesterol	16.6	6.4	15.0	15.5	16.2	21.0	20.2	15.0	18.2
Stigmasterol	21.0	10.0	25.0	20.6	14.2	17.0	25.5	14.0	12.7
24-ethylen-cholesterol	-	6.4	-	-	-	-	-	-	-
Sitosterol	35.2	14.5	32.0	33.7	37.2	38.6	41.5	45.0	45.5
24-ethyl-$\Delta^{5,25}$-cholestadienol	-	2.0	-	-	-	-	-	-	-
Unidentified sterol	-	28.0	5.0	-	-	-	-	-	-
24–ethyldienolphenol	-	13.5	12.0	-	-	-	-	-	-
Unidentified sterol	-	5.0	-	-	-	-	-	-	-
Unidentified sterol	-	5.0	-	-	-	-	-	-	-

Gas-liquid analysis showed that the contents of all total preparations in both samples cultivated under light were identical and contained cholesterol, campesterol, stigmasterol and sitosterol (Table 3). Apparently, under the effect of light the spectrum of individual sterols becomes poorer; this induces the growth of their share in photomixotrophic cultures total preparations compared to heterotrophic culture. The influence of cytodifferentiation on steroid compounds biogenesis is confirmed by the changes of quantitative content of individual sterols. In both samples, as well as in total preparations of free sterols and sterylglycosides from the tissue cultivated in the dark, sitosterol is the dominant sterol. It is important to mention the change of the share of sitosterol and cholesterol in the preparations of photomixotrophic cultures. The quantity of sitosterol in all total preparations of tissues cultivated in light during 11 passages is higher than in sample I. In contrast the share of cholesterol is much higher in the total preparation of free sterols of tissues grown in the light during one cycle of cultivation. Two minor sterols - campesterol and stigmasterol undergo insignificant changes.

The results lead us to suppose that during cytodifferentiation stimulated by the light in cells of *Yucca gloriosa* tissue culture the factor blocking the transformation of sterols into steroid glycosides is partially removed. The mechanisms and conditions which block the cell proliferation and activate growth, at the same time appear to be the activators of the synthesis of secondary metabolite enzymes.

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განათების გავლენის შესწავლა სტეროლებისა და სტეროიდული გლიკოზიდების დაბროვებაზე იუკა დიდებულის *IN VITRO* ჰეტეროტროფულ და ფოტომიქსოტროფულ კულტურებში

გოგავა მ., ღოღობერიძე მ., ზამბახიძე ნ., მიმინოშვილი თ.

საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 20.01.2004)

რეზიუმე

შესწავლილია ურთიერთდამოკიდებულება თავისუფალი და ბმული სტეროლებისა და სტეროიდული გლიკოზიდების ბიოსინთეზის პროცესებს შორის იუკა დიდებულის იზოლირებულ ქსოვილებში. ჰეტეროტროფულ უჯრედებში, ინტაქტურ მცენარესთან შედარებით, ბლოკირებულია სტეროლების სტეროიდულ გლიკოზიდებად გარდაქმნა; ხოლო ფოტომიქსოტროფულ კულტურაში, ჰეტეროტროფულთან შედარებით მცირდება სტეროლების ჯამური რაოდენობა, იცვლება მათი თავისუფალი და ბმული ფორმების ურთიერთშეფარდება, ღარიბდება ინდივიდუალური სტეროლების სპექტრი, იზრდება სტეროიდული გლიკოზიდების რაოდენობა. განათება ართულებს იუკა დიდებულის იზოლირებული უჯრედების ციტოდიფერენცირებას და ამასთანავე, ხელს უწყობს სტეროლების სტეროიდულ გლიკოზიდებად გარდაქმნის სტიმულირებას.

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Biochemistry

THE CHARACTERIZATION OF GLUCOAMYLAZE FROM THE MICROFUNGUS *ASPERGILLUS AWAMORI L – 56*

KUTATELADZE L., IASHVILI T., ZAKARIASHVILI N., DAUSHVILI L., JOBAVA M.

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

(Received January 28, 2004)

Abstract

The enzyme preparation of glucoamylase has been received from the cultural filtrate of micro fungus *Aspergillus awamori.* The maximal activity of the enzyme was obtained by the precipitation of the enzyme with ethanol from the culture medium, in ratio 3.5:1. The effect of pH and temperature of the incubating medium on glucoamylase synthesis was investigated. The thermal and acid resistance of the enzyme was established. In particular, at 70°C the time of semi inactivation of the enzyme was equal to 140min., and at pH 2.0 its activity was fully retained. The preparation of the enzyme might be considered to be responsible for the hydrolysis of the starch to glucose at high temperature, which would protect it from the bacterial contamination and it enhance the yield of glucose.

Key words: glucoamylase, enzyme preparation, incubation, buffer, thermo resistance, acid resistance

Introduction

Glucoamylase is one of the central hydrolytic enzymes, widely used for producing the crystal glucose, in bakery, confectionery, alcohol industry and also in medicine. Quite often glucoamylase is produced from the liver, kidneys, placenta and intestines [Kelly I.I., et al., 1973; Tsujno K., et al., 1974]. But its application in medicine, in enzymotherapy, in curing the glycogenezic diseases of liver and other organs demands high purified preparations of the enzyme with thermal and pH resistance. For this purpose only the glucoamylases of microorganisms are industrially important. Especially high quantity of this enzyme is produced by some representatives of microfungi from the genus *Aspergillus*, actually *A. niger, A. awamori, A. batatae, A. foetidus* [Kvesitadze G., 1984; Linicka O., et al., 1989; Manjunath T., et al., 1983.].

Materials and Methods

The activity of glucoamylase is determined by means of the glucose amount, released at the process of hydrolysis of soluble starch by this enzyme. The amount of glucose formed during the fermentation was measured according to the State Standards [The state standards of the USSR, 1975]. The amount of proteins was determined according to Lowry [Lowry O.H., et al., 1951]. To make the calibrating curve the bull serum albumin ("Sigma" USA) served as a standard. To obtain the enzyme preparation the filtrate of the cultivating medium was removed from the biomass, the

medium was cooled up to 4°C and mixed with cold ethanol in ratio 3,5:1. The mixture was held at 4°C for 20min. The formed sediment was removed by centrifugation (5000 – 6000 rot/min) during 10min. The sediment was dried in vacuum desiccator on the dehydrolyzed $CaCl_2$ at 4°C.

Results and Discussion

Data confirming the difference by some features of glucoamylases, obtained from particular representatives of *Aspergillus*, [Manjunath T., et al., 1983; Svensson B., et al., 1982] stipulate the importance of characterization of glucoamylase of the given strains.

The enzyme preparation of the mutant strain *Aspergillus awamori L – 56* was obtained by means of the following precipitators: ethanol, acetone, ammonium sulfate (Table1).

Table 1. Precipitation of Glucoamylase obtained from the filtrate of cultivating medium of *Aspergillus awamori L – 56* by different volumes of organic solvents and ammonium sulfate

Precipitants	Ratio of volumes of the precipitants	activity of glucoamylase U/g
Ethanol	1:3	31500
	1:3.5	84000
	1:4	73500
Acetone	1:3	10500
	1:3.5	21000
	1:4	14000
Ammonium sulfate	1:3,3	38500
	1:2,2	59500
	1:1,4	4200

Activity of filtrate of the cultivating medium was - 130 U/ml.

From the table it is clear that the maximal activity of glucoamylase was reached by precipitation of the protein with ethanol, when its ratio to cultivating medium was 3.5: 1.According to this result, in further experiments the enzyme was precipitated by ethanol cooled to 4°C, adding 3.5 vol. of the solvent, per one vol. of filtrate of the cultivating medium (evaporated 5 – 7 times). Sediment was removed by means of centrifugation (3500 rot/min, for 10 min), and dried.

The activity of glucoamylase of the obtained preparation was 84000U/g. The maximal productivity of the preparation following the glucoamylase was 75 – 80%. Amount of protein per gram of preparation was 100 mg, output of the protein – 25%.

It has been established the pH and optimal temperature of enzyme activity. The optimal activity for glucoamylase is pH 1.5 – 5.0. Its maximal activity is revealed at 70°C during 10 min. Higher than 80°C fast degradation of the enzyme begins and the enzymatic reaction sharply decreases.

The most important characteristics of the preparation are thermal and acid resistance. To study the influence of temperature on the stability of glucoamylase preparation obtained from *A. awamori L – 56*, the solution of the enzyme with the initial activity 25 U/ml was incubated at 70°C without the substrate. Concentration of the enzyme preparation was 3mg/100ml, duration of the incubation from 5min till 4h. After incubation the glucoamylase activity was studied in 0.05M acetate buffer, with pH4.6, at 30°C. The results of the experiment are given in Fig.1. The residual activity (A%) was expressed in percents of the initial activity. The period of half inactivation of the enzyme at 70°C was 140min.

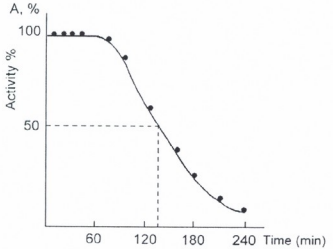

Fig.1. Thermal inactivation of the enzyme preparation of glucoamylase from *Aspergillus awamori L – 56.* Conditions of the experiment: temperature of the incubating medium – 70°C, temperature of the reaction medium – 30°C. pH of the medium – 4.6, 0.05M acetate buffer. The substrate – 1% soluble starch. The samples were taken in every 5, 15, 40, 60, 90, 150, 180, 210, 240 min.

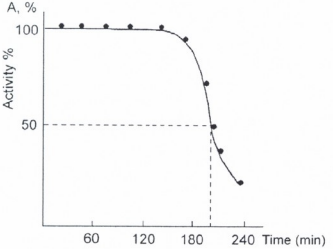

Fig.2. pH inactivation of the enzyme preparation of glucoamylase from *Aspergillus awamori L – 56.* Conditions of the experiment: pH of the incubating medium – 2.0. pH of the reaction medium – 4.6, 0.05M acetate buffer, temperature of the reaction medium – 30°C, substrate - 1% soluble starch. The samples were taken in every 5, 15, 40, 60, 90, 150, 180, 210, 240 min.

To study the effect of pH on the stability of glucoamylase, the enzyme was incubated in buffer solution with pH2.0, at 30°C. Duration of the incubation was changed from 5min till 4h. After incubation the glucoamylase activity in 0.05M acetate buffer was measured, with pH4.6, at 30°C. 1% solution of soluble starch was used as substrate. The residual activity was expressed in percents of the initial (Fig. 2). The obtained results reveal that holding the enzyme at pH2 during 3h fully retained the enzymatic activity.

Summarizing the results, it must be mentioned that the preparation of glucoamylase, obtained from the strain *A. awamori L – 56* is highly thermal and acid resistant. It is supposed that the practical application of glucoamylase, obtained from *A. awamori L – 56* will be possible to

perform starch hydrolysis at high temperatures avoiding the bacterial infection and correspondingly increasing the production of glucose.

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მიკროსკოპული სოკო *ASPERGILLUS AWAMORI L–56*-დან მიღებული გლუკოამილაზის ფერმენტული პრეპარატის დახასიათება

ქუთათელაძე ლ., იაშვილი თ., ზაქარიაშვილი ნ., დაუშვილი ლ., ჯობავა მ.

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

(მიღებულია 28.01.2004)

რეზიუმე

მიკროსკოპული სოკო *Aspergillus awamori l-56*-დან მიღებულია გლუკოამილაზის ფერმენტული პრეპარატი. გლუკოამილაზის აქტივობის მაქსიმალური გამოსავალი მიღებულია კულტურალური სითხიდან ცილის ეთანოლით დალექვისას შეფარდებით 3.5:1.

შესწავლილია გლუკოამილაზის პრეპარატზე ინკუბაციური არის pH-ისა და ტემპერატურის გავლენა. დადგენილია გლუკოამილაზის პრეპარატის თერმო და მჟავამედეგობა, კერძოდ 70°C-ზე ფერმენტის ნახევრადინაქტივაციის პერიოდია 140 წთ, ხოლო pH 2.0 პირობებში მისი ფერმენტული აქტივობა თითქმის მთლიანადაა შენარჩუნებული. ამდენად მოცემული პრეპარატი საშუალებას იძლევა სახამებლის პიდროლიზი ჩავატაროთ მაღალ ტემპერატურაზე, რაც დაიცავს მას ბაქტერიული დაბინძურებისაგან და ამით გაზრდის გლუკოზის გამოსავალს.

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EFFECT OF DIFFERENT INHIBITORS ON PHENOLOXIDASE ACTIVITY FROM GREEN HUSK OF WALNUT (*JUGLANS REGIA L.*)

MCHEDLISHVILI N., ZAMTARADZE R., SADUNISHVILI T., OMIADZE N., GULUA L.

Durmishidze Institute of Biochemistry and Biotechnology, Academy of Sciences of Georgia

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Abstract

The effect of various concentrations of different inhibitors (DEDTC, sodium azide, cysteine, ascorbic acid and *p*-nitrophenol) on the activity of walnut green husk phenoloxidase has been studied. The order of inhibitory efficiency of the tested inhibitors was found as follows: DEDTC > ascorbic acid > cycteine > *p*-nitrophenole > sodium azide. DEDTC affected on both V_{max} and K_m values. This fact indicated to the complex (mixed) mode of inhibition by DEDTC.

Key words: walnut green husk, phenoloxidase, inhibitor concentration

Introduction

Phenoloxidase (EC 1.14.18.1) is a copper containing enzyme which catalyzes two basic reactions: hydroxylation of the *o*-position adjacent to an existing hydroxyl group of the phenolic substrate (monophenol oxidase activity), and oxidation of diphenol to *o*-benzoquinones (dipehnol oxidase activity). Both reactions utilize molecular oxygen as a co-substrate [Marshall et al, 2000].

Phenoloxidases are widely distributed in the plant kingdom and they have been isolated and purified from different plants. Substrate specificity, effect of inhibitors and various other properties of phenoloxidases have been studied [Fenoll et al., 2002, Griffith, 1994, Kim et al., 2001, Marshall et al., 2000, Mayer A. M., 1987, Mayer and Harel, 1979]. There are two main types of catechol oxidase inhibitors –reagents which interact with the copper in the enzyme (metal-chelating agents) such as sodium diethyldithiocarbamate (DEDTC), sodium azide, cyanide, thiourea, etc. and compounds which affect the site of the phenolic substrate (or analogue of the phenolic substrates) [Mayer and Harel, 1979], besides reducig agents serve as phenoloxidase inhibitors [Marshall et al., 2000]. Some inhibitors affect in different ways e.g. cysteine has been shown to reduce *o*-quinones to their phenol precursors [Walker, 1977]. It has particularly high affinity for Cu^{2+} since, apart from having NH_2 and COOH groups, cysteine has a thiol group, which has metal binding capacity [Bell, 1977]. Ascorbic acid, inhibitor of a reductant nature, also acts as an oxygen scavenger to remove molecular oxygen in phenol oxidase reactions. [Walker, 1977].

In our previous paper we reported about the isolation of phenoloxidase from green husks of walnut [Zamtaradze et al., 2003].

The aim of the presented work is to investigate the effect of some inhibitors on the activity of phenoloxidase from green husks of walnut.

MATERIALS AND METHODS

Fresh green husks of walnut (*Juglans regia L*) were used in all experiments.

Isolation of phenoloxidase from walnut husks was carried out as follows: fresh husks of walnut were crushed in the presence of dry ice and added the polyamide powder at a ratio 1:1 (w/w) as well as 0.05M citrate-phosphate buffer containing 1% of Twin. The ratio of crushed material and buffer was 1:15 (v/v). The mixture was homogenized for 5 min, filtrated through two layers of cheesecloth and centrifuged at 3000g for 40 min. The supernatant was concentrated in a dialysis bag surrounded by dry sucrose for 18-20 h and dialyzed against running water for 24 h. The obtained solution was used as phenoloxidase crude preparation. All steps were performed at 4°C.

Phenoloxidase activity was determined spectrophotometrically according to the method of Lanzarini using 32 mM pyrogallol as substrate [Lanzarini et al., 1972] from the increase in the optical density at 430 nm and expressed in arbitrary units. The duration of incubation was 1 min. One unit was defined as the amount of the enzyme sufficient to change the absorption spectrum by 0.05 in 1 min. Specific activity was expressed in units per mg protein.

Protein content of enzyme preparation was determined by the method using Amido Black reagent [Plum et al., 1955].

RESULTS AND DISCUSSION

The effect of different concentration (from 0.02 to 2. 5mM) of various known inhibitors of phenoloxidase such as *p*-nitrophenol (analogue of the phenolic substrates), sodium diethyldithiocarbamate, sodium azide (metal-chelating agents) [Anosike and Ayaebene, 1982], cysteine and ascorbic acid on the activity of walnut husk phenoloxidase has been investigated at pH 4.9 (pH optimum of phenoloxidase of walnut green husk) without preincubation of the enzyme with inhibitor. All these compounds significantly decreased the activity of phenoloxidase of walnut green husk (Fig.1). Among them DEDTC was shown to be the most potent inhibitor for phenoloxidse of walnut green husk. 50% inactivation of phenoloxidase was observed at 0.06 mM concentration of DEDTC and the enzyme was completely inhibited by the addition of 0.08 mM of this inhibitor.

As shown in Fig.1 inhibitory efficiency of the tested inhibitors decreased in the following order: DEDTC > ascorbic acid > cycteine > *p*-nitrophenol > sodium azide. Though it should be noticed that at low concentrations (up to 0.8mM) *p*-nitrophenol exhibited higher inhibitory effect on walnut green husk phenoloxidase than cysteine (62.7 % and 40.0% by *p*-nitrophenol and cysteine respectively), but at concentrations higher than 0.8mM cycteine was much more effective. At concentration of about 1.8 mM cycteine caused complete inhibition of phenoloxidase, while *p*-nitrophenol inhibited the enzyme only by 72.0 %.

In order to determine the mode of inhibition by DEDTC (most potent inhibitor) the effect of different concentrations of pyrogallol (substrate) with and without the inhibitor (0.06mM) has been studied. The Lineweaver - Burk plots with and without DEDTC (Fig.2) were linear. This inhibitor had effect on both V_{max} and K_m values. For phenoloxidase reaction without DEDTC V_{max}= 243.9 ΔE/mg protein/min and K_m=2.49 mM, but for phenoloxidase with this inhibitor $V_{max(i)}$=92.6 ΔE/mg protein/min and $K_{m(i)}$= 12.54 mM. This fact indicated the complex (mixed) mode of inhibition by DEDTC.

Thus according to the experimental data it can be suggested that the activity of phenoloxidase of green husk from walnut may be regulated both by metal-chelating agents and substrate analogues as well as by reducing agents.

This work has partially been supported by INTAS-Food grant, N 00-0727

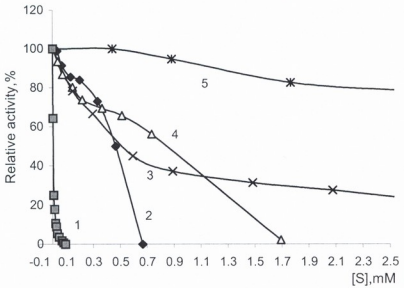

Fig.1. Effect of different inhibitors on the activity of phenoloxidase from walnut green husk. 1-DEDTC, 2- ascorbic acid, 3-*p*-nitrophenol, 4-cysteine, 5-sodium azide

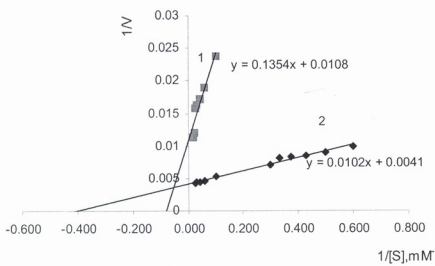

Fig.2. Effect of substrate (pyrogallol) concentration on apple phenoloxidase activity: double reciprocal plots with (1) and without (2) DEDTC (V = specific activity ΔE/mg protein/min).

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სხვადასხვა ინჰიბიტორის გავლენა კაკლის (*JUGLANS REGIA L.*) ფენგოს ფენოლოქსიდაზას აქტივობაზე

მჭედლიშვილი ნ., ზამთარაძე რ., სადუნიშვილი თ., ომიაძე ნ., გულუა ლ.,

(მიღებულია 09.02.2004)

რეზიუმე

გამოკვლეულია სხვადასხვა ინჰიბიტორის (ნატრიუმის დიეთილდითიოკარბამატი, ნატრიუმის აზიდი, ასკორბინის მჟავა, ცისტეინი და p-ნიტროფენოლი) კონცენტრაციების გავლენა კაკლის წენგოს ფენოლოქსიდაზას აქტივობაზე. საკვლევი ინჰიბიტორების მაინჰიბირებელი მოქმედება მცირდება შემდეგი თანმიმდევრობით: ნატრიუმის დიეთილდითიოკარბამატი > ასკორბინის მჟავა > ცისტეინი > p-ნიტროფენოლი > ნატრიუმის აზიდი. დიეთილდითიოკარბამატი გავლენას ახდენს ფენოლოქსიდაზას რეაქციის როგორც V_{max} ისე K_m მნიშვნელობებზე. ეს ფაქტი მიუთითებს დიეთილდითიოკარბამატით ინჰიბირების რთულ (შერეულ) ხასიათზე.

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Biochemistry

STUDY OF ADSORPTIVE PROPERTIES OF THERMOPHILIC MICROMYCETES ENDO-1,4-β–GLUCANASES

URUSHADZE T., KHVEDELIDZE R., BERULAVA A., ALEKSIDZE T., ZAKARIASHVILI N., IASHVILI T., METREVELI E.

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

(Received January 30, 2004)

Abstract

Thermophilic strains *Aspergillus versicolor, Aspergillus wentii, Sporotrichum pulverulentum* produce exogenous endo-1,4-β–glucanases. Endoglucanases according to the ability of the enzyme adsorption on dissolved substrate have been comparatively investigated. The presence of weakly and firmly absorbed forms of the enzyme is stated. Relative content and coefficients of equilibrum distribution of weakly and firmly absorbed forms are calculated.

Key words: endoglucanase, cellulose, adsorption, MCC-microcrystalline cellulose, coefficient of distribution

Introduction

In spite of successful demonstration of some processes of cellulose enzymatic hydrolysis using experimental-industrial technologies, it hasn't been achieved a wide industrial application yet. This is connected with a fact, that enzymes of cellulase complex known today, have not those qualities which might ensure the high rate and conversion degree in industrial conditions. For the realization of this process, enzymes of cellulase complex should have: high thermal resistance, high adsorption ability on cellulose, high catalytic activity, and low inhibition by the final products. It has been stated that on the first stage of degradation the adsorption of enzyme takes place on the substrate surface. Bioconversion efficiency is highly determined by degree of adsorption and desorption, and index of enzyme distribution on substrate surface [Klesov A., et al., 1987].

The main goal of our investigations was to study the absorbing potential of cellulase producer thermophilic micromycetes – *Sporotrichum pulverulentum, Aspergillus versicolor, Aspergillus wentii* endo-1,4-β–glucanases from the microorganisms collection created as a result of several-years studies at Durmishidze Institute of Biochemistry and Biotechnology.

Materials and methods

Endoglucanase preparations obtained from proper culture solutions, precipitated by ethyl alcohol, have been used in the experiment [Gogilashvili L. et al., 1991]. Endoglucanase activity has been measured by viscosimetric method [Rabinovich M. et al., 1983], microcrystalline cellulose (MCC) served as a sorbent. The procedure of adsorption has been evaluated by the coefficient of equilibrate distribution (K_p-constant of Henry),which indicates the correlation between the enzyme quantity, absorbed on substrate surface, and enzyme concentration in solution .

$$[K]_p = \frac{[E]_p - [E]_p}{[MCC][E]_p} \quad (1)$$

Here $[E]_o$- is initial enzyme activity, $[E]_p$ - enzyme activity in a supernatant at the end of adsorption.

K_p has been estimated in thermostate test tubes with magnetic stirrers at 25°C. Each tube was supplemented with 10 g sorbent, 2 ml 0.05 M acetate buffer pH 4.5. 2 ml enzyme solution with determined activity was added after 1 hour stirring.

The sorbent was removed from suspension between the centrifuging intervals and the residual activity in supernatant was determined. According to – (E_p - E_o) we calculated the amount of absorbed enzyme. K_p numerical values were calculated according to the experimentally obtained data diagram with $[E]_o/[E]_p$ and [MCC] coordinates [Dong W. et al., 1995]. This correlation is expressed by hyperbole, where the tangent of inclination angle gives the numeric expression of K_p. In case when the enzyme contains two or more multiple forms with different absorbing capacity, the curve has slightly changed hyperbole shape [Lu Y., et al., 2002]. At the end of adsorption process we removed the supernatant and added adsorbent into the initial buffer. In accordance with the increase of endoglucanase activity of this solution we estimated the kinetics of desorption.

Results and discussion

According to the experimental data we have determined K_p for all three preparations. The results obtained in the experiments on the adsorption of endoglucanase of *A. wentii*, are demonstrated as an example. The initial activity of the solution of *A. wentii* $[E]_o$=740U/l. At the end of the adsorption activity of the supernatant was $[E]_p$=240U/l. As the supernatant was removed, it was replaced by the initial acetate buffer. The kinetics of the desorption was estimated by increasing the endoglucanase activity of the solution. The final activity of the desorbed endoglucanase was $[E]_p$=60U/l.

According to the given data the constant of distribution of Henry K_p is calculated:

$$[K]_p = 0.21 g/l \quad (1)$$

Meaning of K_p makes possible to calculate the theoretical activity of the volume phase after the process of desorption finished, using the ratio:

$$[E]_{p\,theor} = [E]_p \left(1 - \frac{[E]_p}{[E]_0}\right) \quad (2)$$

Following (2) $E_{p\ theor}$=162U/l which is much more than experimental result [E]p=60U/l. It may be supposed that endoglucanase of *A. wentii* is an integration of several molecular forms, which differ by the absorbing ability. For their identification the correlation between $[E]_o/[E]_p$ and the concentration of the absorbent (MCC) must be studied. In the case when the system consists of several molecular forms with the coefficient of distribution K_i and α_i – the i - firm of the enzyme, following correlation is:

$$\frac{[E]_o}{[E]_p} = \frac{1}{\sum \alpha_i / (1 + K_i [MCC])} \qquad (3)$$

On Fig.1 (a) experimentally obtained dependence between $[E]_o/[E]_p$ and [MCC] is shown. From the figure it is clear that the linear dependence is not observed through the whole length of the diagram.

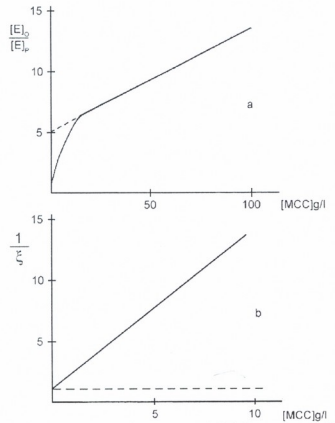

Fig. 1 Adsorbtion of endoglucanase of *A. wemtii* on MCC. Dependence of $[E]_o/[E]_p$ on [MCC]
a-for weakly adsorbing form
b-for strongly adsorbing form

$$\frac{1}{\xi} = \left(\frac{[E]p}{[E]o} - \frac{\alpha_1}{1 + K_1 [MCC]} \right)^{-1}$$

It may be concluded that the investigated preparation consists of several molecular forms of endoglucanase. The line corresponding to high meanings of MCC includes the parameters characteristic of weakly absorbed molecular form α_1 and K_1. According to the calculations $1/\alpha_1$ = 3.0 i.e. α_1=0.33 or 33%. tg $\alpha_1=K_1/\alpha_1$=0.96 or K_1=0.31 l/g. At the same time, following the equation (3) for two molecular forms

$$\left(\frac{[E]_p}{[E]_o} - \frac{a_1}{1+K_1[MCC]}\right)^{-1} = \frac{1}{a_2} + \frac{K_2[MCC]}{a_2} \qquad (4)$$

The diagram of the equation (4) makes possible to calculate the parameters of the second - firmly absorbed form, i. e. α_2 and K_2.

Fig.1 (b) demonstrates the corresponding diagram. Following the calculations 1/ α_2=1.5, i.e. α_2= 0.67 or 67%. tgα_2=1.27 i.e. K_2=0.83 l/g. The same results were obtained in the case of the endoglucanase of *Sp. purverulentum*.

Analyzing the obtained results it may be concluded that the weakly and firmly absorbed forms of endoglucanase of *A. wentii* and *Sp. purverulentum* were revealed.

Experiments on the adsorption of endoglucanase of the strain *A. versicolor* cleared the presence of 97% of firmly absorbing forms with the constant of Henry 1.6 l/g. (Fig. 2). The results of calculations are given in Table 1.

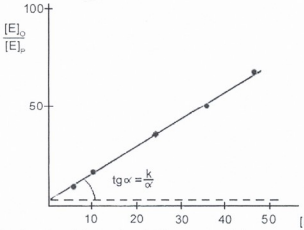

Fig. 2. Absorbtion of endoglucanase of *A. versicolor* on MCC
Dependence of $[E]_o/[E]_p$ on [MCC]

Table 1. Characteristics of the studied endoglucanases

Endoglucanase	MCC (g/l)	$[E]_o$ (u/l)	$[E]_e$ (u/l)	Enzyme (mol.)	Content (%)	K_p (l/g)
Sp.pulverulentum	10	620	100	1 2	20 80	0,08 0,96
A.wentii	10	740	240	1 2	33 67	0,31 0,83
A.versi-color	10	1425	1000	1	98	1,55

Thus, studied endoglucanases are characterized by high percentage of molecular forms, with strong absorbing ability on substrate, that makes them technologically useful.

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თერმოფილური მიკრომიცეტების ენდო-1,4-β-გლუკანაზას ადსორბციული თვისებების შესწავლა

ურუშაძე თ., ხვედელიძე რ., ბერულავა ა., ალექსიძე თ., ზაქარიაშვილი ნ., იაშვილი თ., მეტრეველი ე.

ს. დურმიშიძის სახ. ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, საქართველოს მეცნიერებათა აკადემია

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რეზიუმე

შესწავლილია თერმოფილური მიკრომიცეტების ენდოგლუკანაზების ადსორბციული თვისებები. ადსორბციის უნარის მიხედვით დადგენილია ფერმენტის განსხვავებული მოლეკულური ფორმების არსებობა. გამოთვლილია ძლიერ და სუსტად სორბირებადი მოლეკულური ფორმების შემცველობა და სუბსტრატის ზედაპირზე მათი განაწილების კოეფიციენტები.

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ALLESCHERIA TERRESTRIS AND *ASPERGILLUS WENTII* – PRODUCERS OF XYLANASE

JANELIDZE T., GOMARTELI M., BUTSKRIKIDZE N., MANVELIDZE N.

Department of Biotechnology, Technical University of Georgia

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Abstract

Among the thermophyle fungies of high xylanase activity two active producers *Allescheria terrestris* and *Aspergillus wentii* have been selected. In order to obtain the maximum quantity of xylane an inducing nature of its synthesis have been studied and the cultural medium optimized. The maximum effect was reached in case of xylane (52-55%), using waists of agriculture as a source of carbon, and using sodium nitrate (50-60%) as a source of nitrogen.

Key words: Microscope fungi, Cultural medium, Substrate, Xylane, Xylanase;

Introduction

The synthesis of xylanase is an inducible process and depends on the contents of the cultural medium. For the optimization of the cultural medium the effect of carbon, nitrogen and phosphor sources on the capability of *Allescheria terrestris* and *Aspergilius wentii* to synthesize xylanase were studied.

The cultures were grown on synthesized liquid area, where as a source of carbon di- and monosacharides and the agricultural waists were used.

Materials and Methods

Microorganisms - microscopic fungi *Allescheria terrestris* and *Aspergillus wentii*.

Substrate - cultural medium:

Allescheria terrestris – (g/l) Microcrystal cellulose – 20; Hop sprouts – 10; KH_2PO_4 – 6.8; $(KH_4)_2SO_4$ – 1.3; $MgSO_4 \cdot 7H_2O$ – 0.5; $CaCl_2$ – 0.2; Peptone – 1.5; Corn extract – 15; pH- 5.5;

Aspergillus wentii – (g/l) Microcrystal cellulose – 20; Sprouts of hops – 10; $NaNO_3$ – 3; KH_2PO_4 – 2; $MgSO_4 \cdot 7H_2O$ – 0.5; Reagent of Myxlin – 4.5; Corn extract – 15; pH – 4.5;

Assay Procedure. The strains were cultivated for 96 hours, at 40°C, on the shaker (200r/m), in 250ml Erlen-Mayer flasks with 70 ml of cultural medium.

To study xylanase activity 1g of substrate (1% of birch xylane) was diluted in 80ml of 0.05 M Na - citrate buffer (pH 5.3), at 60°C and brought to boiling temperature on the magnetic shaker.

The mixture was cooled for 24 hours, with constant shaking and filled up to 100ml with buffer.

The substrate was stored at +4°C for 1 week. The reaction medium consisted of 1.8 ml substrate and 0.2ml enzyme. It was incubated for 5 min. at 50°C. 3 ml of DNS reagent was added to reaction medium to assay the reducing sugars, then it was boiled in water bath for 5 min. and cooled rapidly under the cold water flow. The sample was measured spectrophotometrically at 540 against the control.

Control was prepared in the same way, with only one difference, instead of 0.2ml of enzyme solution we added 0.2ml of buffer solution in the reaction medium. The standard solution was prepared using pure xylose.

Results and Discussion

The obtained data showed that xylane had the best inducing effect. In order to avoid low molecular admixture it was not pretreated with alcaline and acid. Using an above mentioned substrate the synthesis of enzyme xylanaze was increased by 52% in case of *Allescheria terrestris* and by 55% in case of *Aspergillus wentii* (Table 1).

We found that the plant waists were characterized by specific inducing effect: in case of corn an increase reaches 45%, wheat straw or bran - 49-51%. Addition of hop sprouts, extract of corn, beet extract to the medium caused enhance of xylanase synthesis. As for manose, fructose and galaktose, they significantly suppress synthesis of the xylanase.

Further the effect of different sources of nitrogen on the ability of xylanase synthesis by these cultures were studied at deep cultivation. From the mineral sources of nitrogen we used: K, Na, Ca and ammonium nitrates. The concentration of above mentioned salts, in the medium, against nitrogen was equal to 0.04 g/l.

The organic nitrogen was used as peptone, yeast extract, urea and growth factor obtained by methane boiling in anaerobic conditions. It turned out that among the mineral sources the most effective was sodium nitrate. When 0.24% of sodium nitrate was introduced in to the medium the quantity of the synthesized enzyme increased by 50-60%. We have also obtained important results using the growth factor and peptone (see Table 2).

In order to find an effective source of phosphor, we tested the following salts: K_2HPO_4 and KH_2PO_4, NaH_2PO_4, $(NH_4)_2HPO_4$. The tests showed that K_2HPO_4 gave the best results.

The final optimization of the culture medium allowed us to choose the cultural mediums with the below mentioned contents for *Allescheria terrestris* and *Aspergillus wentii:*

For *Allescheria terrestris* – (g/l) impure xylane -10.0; sprouts of hops -10.0; extract of corn - 25ml; peptone - 1.0; $NaNO_3$ - 9,0; KH_2PO_4 – 1,5; KCl - 0.5; $MgSO_4 \cdot 7H_2O$ - 0.5; growth factor 5ml

For *Aspergillus wentii* – (g/l) impure xylane -10.0; sprouts of hops -10.0; product of thermophyl methane boiling - 4.5; extract of corn - 25ml; $NaNO_3$ - 3.0; KH_2PO_4 - 2.0; $MgSO_4 \cdot 7H_2O$ - 0.5; growth factor 5ml.

Table 1. Effect of the different carbon sources on xylanas production by *Allescheria terrestris* and *Aspergillus wentii* in conditions of deep cultivation.

Additives	*Allescheria terrestris*		*Aspergillus wentii*	
	Xylanase activity (unit/ml)	increase (+) decrease (-) %	Xylanase activity (unit/ml)	increase (+) decrease (-) %
Xylane (purified)	66.4	+14.5	77.8	+18
Xylane (unpurified)	88.1	+52	102.3	+55
Hay Hydrolyzate	67.8	+17	78.5	+19
Sawdust Hydrolyzate	64.9	+12	73.2	+11
Corn Wastes	84	+45	93.7	+42
Sprouts of hops	77.7	+34	89.7	+36
Wine wastes	67.8	+17	79.2	+20
Beet extract	72.5	+25	85	+29
Wheat	86.4	+49	100.3	+52
Wheat	87	+50	100.3	+52
Corn extract 1.5%	69.6	+20	80.5	+22
Arabynose	14.5	-75	10.5	-84
D-xylose	59.4	+3	68.9	+4.5
Fructose	7.5	-87	17.1	-74
Galactose	15.08	-74	21.1	-8
Control	58		66	

Table 2. Effect of the different nitrogen sources on xylanase production by Allescheria terrestris and Aspergillus wentii in conditions of deep cultivation.

Additives	*Allescheria terrestris*		*Aspergillus wentii*	
	Xylanase activity (unit/ml)	increase (+) decrease (-) %	Xylanase activity (unit/ml)	increase (+) decrease (-) %
NaNO3	69.6	+20	80.5	+22
KNO3	59.1	+2	62.7	-5
Ca(NO3)2	56.2	-3	60.7	-8
NH4NO3	49.3	-15	58	-12
Peptone 0.25%	80	+38	76.4	+15
Urea	20.3	-65	26.4	-60
Growth factor 0.1%	65	+12	75.2	+14
Growth factor 0.5%	72.5	+25	84.5	+28
Control	58		66	

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ALLESCHERIA TERRESTRIS და *ASPERGILLUS WENTII* – ფერმენტ ჭსილანაზას პროდუცენტი სოკოები

ჯანელიძე თ., გომართელი მ., ბუცხრიკიძე ნ., მანველიძე ნ.

ბიოტექნოლოგიის კათედრა, საქართველოს ტექნიკური უნივერსიტეტი

(მიღებულია 3.12.2003)

რეზიუმე

საკვები არის ოპტიმიზაციის მიზნით შესწავლილია ნახშირბადის, აზოტის და ფოსფორის სხვადასხვა წყაროების გავლენა *Allescheria terrestris* და *Aspergillus wentii*-ის მიერ ქსილანაზას სინთეზის უნარზე. მკვეთრად გამოხატული ინდუცირებადი ეფექტით გამოირჩეოდა ქსილანი, რომელიც წინასწარ არ იყო დამუშავებული ტუტით ან მჟავით. ფერმენტ ქსილანაზას სინთეზი აღნიშნული სუბსტრატის გამოყენებისას *Allescheria terrestris*-ის შემთხვევაში გაიზარდა 52%-ით, ხოლო *Aspergillus wentii*-ის შემთხვევაში – 55%-ით. მინერალური წყაროებიდან ყველაზე ეფექტურია ნატრიუმის ნიტრატი (50 და 60%).

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Botany

EMBRYOLOGY OF *GENTIANA ANGULOSA* AND *G. PONTICA* (GENTIANACEAE)

AKHALKATSI M., GVALADZE G., GACHECHILADZE M., TARALASHVILI N.

Institute of Botany, Georgian Academy of Sciences

(Received February 20, 2004)

Abstract

Embryology of two gentians - *Gentiana angulosa* and *G. pontica*, has been studied for the first time. They show similar morphology and are considered to be relative species. Embryological study has revealed considerable similarity in structure of reproductive organs of *G. angulosa* and *G. pontica*. The common characters are: four-loculate anther; 2-celled mature pollen; superior, cenocarpous and paracarpous ovary; unitegmic, tenuinucellate and anatropous ovule; hypodermal unicellular archesporium; linear tetrad of megaspores; *Polygonum* type embryo sac; pre-mitotic type of fertilization; nuclear endosperm and *Solanad* type of embryogenesis. The investigated species differ mainly by two quantitative features – numbers of cell layers in the integument (5-8 in *G. angulosa*, 9-10 in *G. pontica*) and the antipodals (3-6 in *G. angulosa*, 3 in *G. pontica*). The structural difference is only slightly expressed in seed coat structure, which has taxonomic significance.

Key Words: Embryology, ovule, seed, Gentiana.

Introduction

Gentiana angulosa and *G. pontica* often are identified as subspecies or varieties of one species [Gagnidze, 1985; Halbmayr, 1990]. In "Flora of Europe" [Tutin et al., 1972] one of them is identified as variety (*G. verna* var. *angulosa* Kusn.) and the other as subspecies (*G. verna* ssp. *pontica* (Soltok.) Hayek) of *G. verna*. All three species are perennial herbs. Vegetative parts and flowers of *G. verna* are smaller than those of *G. angulosa* and *G. pontica*. The main difference, however, is a form of calyx. Teeth and tube of calyx in *G. angulosa* and *G. pontica* are with clearly visible wings, which are absent in *G. verna*. *G. pontica*, in addition *G. pontica* has wider, ovate and obtuse leaves. These data show that morphological characteristics are not sufficient criteria for their identification. It is necessary to conduct further study of these species using embryological and molecular systematic approaches.

Embryological investigations [Stolt, 1921; Öhler, 1927; Bouman, Schrier, 1979; Shamrov, 1987, 1988, 1991, 1996; Akhalkatsi, Wagner, 1997] of the family Gentianaceae have shown that reproductive organs reveal number of variable features on species and genus level which have a taxonomic value. For example, ovule in most species is anatropous -*Gentiana* spp., *Gentianopsis* spp., but sometimes might be orthotropous - *Halenia elliptica* [Stolt, 1921], *Cotylanthera tenuis*, *Voyriella parviflora*, *Voyria* spp. [Öhler, 1927], hemitropous - *Comastoma tenellum*, *Gentianella*

spp.[Stolt, 1921], or ana-campilotropous - *Swertia* spp. [Shamrov, 1991, 1996]. Ovules in all species are tenuinucellate and unitegmic. The number of layers of the cells in a single integument varies among species from 2 to 20. Megagametophyte develops according to *Polygonum* type in all Gentianaceae. However, mature embryo sacs differ among species in number of antipodals. There are three groups of species in the family Gentianaceae differing in antipodal structure [Shamrov, 1987, 1988]: 1. antipodals uninucleate, ephemeral, degenerate before fertilization. They might be 3 (*Gentiana prostrata*), or 3-9 (*Gentiana asclepiadea, G. lutea, G. tibetica*); 2. antipodals always 3, degenerate after fertilization (*Enicostema littorale, Exacum affine, Hoppea dichotoma*); 3. antipodals multinucleate, or with polyploid nuclei, hypertrophic, 3 (*Halenia elliptica*), 6 (*Comastoma tenellum*), or more than 6 (*Gentianella amarella, G. caucasea, G. germanica, G. uliginosa*) .

On the basis of these suggestions we have decided to conduct embryological investigation of two gentians, which have not been studied before. The determination of embryological features will contribute to the further taxonomic evaluation of these species.

Materials and Methods

Two species of the family Gentianaceae have been studied – *G. angulosa* M. Bieb. and *G. pontica* Solotk. [Czerepanov, 1995]. Both are perennial mountain plants (2n=26) distributed in the Caucasus, Asia Minor and Iran. *G. angulosa* is widely distributed on the Great Caucasus. *G. pontica* is more common in the Minor Caucasus. They grow between 2000-3600 m. above see level. Depending on altitude they are flowering in May-June. Fruit matures in July-August.

Plant material was collected during 1985-1989 in Kazbegi and Bakuriani. Buds, flowers and fruits at different stages of development were 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 during 2001-2003 using light microscope (Polivar, Reichert, Austria). Photographs were taken with a digital camera (NikonCoolpix5000).

Results

Comparative embryological investigation has revealed number of features common for *G. angulosa* and *G. pontica*. Anther is four-loculate, opens longitudinally. Anther wall consists of epidermis, endothecium and 1-3 layers of glandular tapetum. Tetrad of microspores is tetrahedral. Mature pollen contains two cells.

The gynoecia of the investigated species are superior, unilocular, bicarpellate (very rare tricarpellate) and paracarpous. They are terminated by a short style and a 2-lobed stigma. The gynophore is 4-5 mm long. Numerous ovules develop on the parietal placentae along the fused margins of the carpels. Vascular bundles reach chalaza, but do not enter ovule. The Ovule is anatropous, tenuinucellar and unitegmic (Fig. 1). Nucellus degenerates during the extension of the embryo sac. The archesporium is hypodermal, unicellular and functions directly as megaspore mother cell. Tetrad of megaspores is linear. *Polygonum* type of embryo sac develops from chalazal megaspore. The polar nuclei (Fig. 2) fuse before fertilization and secondary nucleus is located near the egg cell (Fig. 3). The mature embryo sac consists of the three-celled egg apparatus, the central cell and different number of ephemeral antipodals. The synergids have a well developed filiform apparatus.

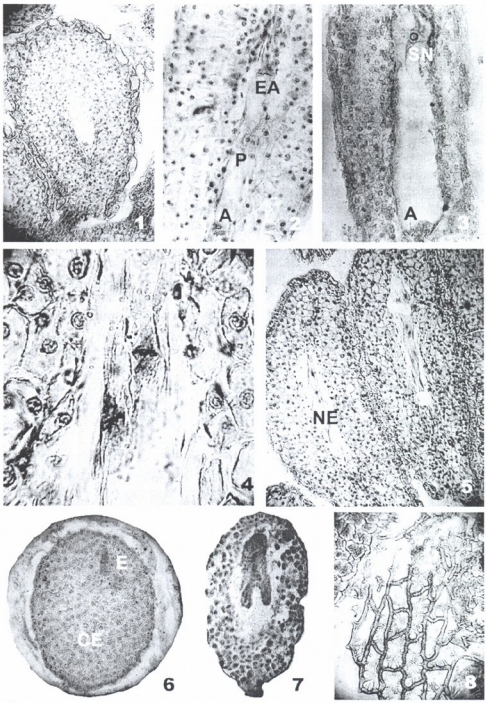

Figure legends:
Fig.1 – Ovule of *Gentiana pontica*, x 110;
Fig.2 – Ovule and embryo sac of *G. angulosa* with egg apparatus (EA), polar nuclei (P) in the center and three antipodals (A). x 200;
Fig.3 - Embryo sac of *G. angulosa* with secondary nucleus (SN) and six antipodals (A). x 180;
Fig.4 – Metaphase of second division of the endosperm nuclei in *G. pontica*, x 400;
Fig.5 – Nuclear endosperm (NE) in developing seeds of *G. pontica*, x 85;
Fig.6 – Seed of *G. pontica* with Solanad type of embryo (E) and cellular endosperm (CE), x 80;
Fig.7 – Mature seed of *G. angulosa* with embryo at torpedo stage and cellular endosperm, x 80;
Fig.8 – Seed coat texture in *G. angulosa*, x 320.

Fertilization is porogamous. The pollen tube enters the embryo sac through the micropyle and discharges its contents into one of the synergid. Triple fusion precedes syngamy. Gametic fusion is of the pre-mitotic type. The primary endosperm nucleus moves towards the middle of the embryo sac and undergoes successive synchronous divisions (Fig. 4). Endosperm is of a nuclear type (Fig. 5). Cell wall formation proceeds centripetally after 128 endosperm nuclei have developed. At the same time, first division of the zygote takes place. Embryogenesis follows the *Solanad* type (Fig. 6). Mature seed contains embryo at early torpedo stage, cellular endosperm and seed coat (Fig. 7). The seed coat consists of a sculptured outer layer, derived from the epidermis of the ovule, and a transparent membranous envelope originated from the crushed cells of the inner layers of the integument. The sculpturing is of the reticulate type (Fig. 8) in both species. However the pit like structure of periclinal walls is more prominent in *G. pontica.*

Investigated species differ by some quantitative characters, which usually are variable within the family Gentianaceae. These features are the number of antipodals and cell layers in the integument. Integument of *G. angulosa* consists of 5 layers of cells in the lateral, adjacent to the embryo sac region, and 8 layers in the micropylar part (Fig. 2). The micropyle is 100 μm long. *G. pontica* possesses 9-10 layers of cells of the integument (Fig. 1). The micropyle is 160 μm long. Antipodals are mostly 3 (Fig. 2), rarely 6 (Fig. 3) in *G. angulosa.* They are ephemeral and degenerate during fertilization. In *G. pontica* there are always 3 ephemeral reduced antipodals degenerating during or before fertilization.

Number of anomalous ovules has been found in both investigated species. Most of the anomalies cease the developmental process in the megagametophyte and make impossible the fertilization. The following anomalies have been observed: 1. gynoecium consists of 3 instead of 2 carpels. Consequently the stigma is 3- lobed, ovules form 6 marginal rows in the ovary; 2. archesporium is absent in the ovule; 3. megaspore mother cell degenerates; 4. tetrad of megaspores is formed but degenerates completely; 5. two or more megaspores begin to develop but degenerate at later stages; 6. polarization 0-2 is observed in two-nucleate embryo sac; 7. polarization 1-3 is found in four-nucleate embryo sac; 8. twin embryo sacs containing 16 or 12 nuclei randomly located into one cenocyte are observed; and, 9. polar nuclei do not fuse before fertilization and are located in the center of the embryo sac.

Discussion

The structure of reproductive organs of *G. angulosa* and *G. pontica* revealed considerable similarity. Most of these common characters usually occur in all species of the family Gentianaceae. These are: 4-loculate anther; 2-celled mature pollen; superior, cenocarpous and paracarpous ovary; unitegmic and tenuinucellate ovule; hypodermal unicellular archesporium; linear tetrad of megaspores; *Polygonum* type embryo sac; pre-mitotic type of fertilization; nuclear endosperm and *Solanad* type of embryogenesis.

There are several features, which are variable within the Gentian family, such as ovule type, number of cell layers of the integument, number and structure of antipodals and synchronization of embryogenesis and endosperm development. Only two of them are different in the investigated species – thickness of the integument and number of the antipodals. The ovule is anatropous in both species. First division of the zygote takes place similarly in the investigated species after 128 endosperm nuclei are formed.

Integument thickness varies within the family Gentianaceae from 2-3 (*Gentianopsis ciliata*) to 20 cells [*Gentiana lutea*, Shamrov, 1987, 1988]. The investigated species differ by this character. *G. pontica* has 9-10 layers of cells, *G. angulosa* – 5-8 layers. The number of the layers of integumentary cells and length of micropyle determine seed coat texture. It is known [Miège, Wüest, 1984; Yuan, 1993] that shape of seeds and seed coat structure are variable within the genus

Gentiana and have taxonomic significance. The studied species possess oblong seeds with reticulate structure of the seed coat. The texture differs insignificantly among these species.

Ovule with a single integument is common feature within the family Gentianaceae. Unitegmic ovules occur in other sympetalous taxons, such as *Asteranae* and *Lamianae* [Netolitzky, 1926]. Single integument is usually formed by both epidermal and subepidermal cells of a placenta. [Bouman, Schrier, 1979]. Unitegmy is originated from bitegmy. The studies of ovules showing transitional stages between bi- and unitegmy make it apparent that there are three possible ways in which the change-over from bitegmy to unitegmy took place: 1. the reduction of one of the two integuments; 2. the fusion of integument primordia; and, 3. the process of integument shafting, when subepidermal cells are dividing more intensively and overlappe the epidermal layer resulting in their fusion [Bouman, 1977].

The investigated species differ only slightly by number and structure of antipodals, which is a variable feature within the family Gentianaceae. Both species might have 3 ephemeral antipodals, or 6 antipodal cells can be observed in some ovules of *G. angulosa*. It was shown [Akhalkatsi, Wagner, 1996, 1997] that there is definite relations between number and structure of antipodals and the life history of a species. The short-lived monocarpic *Gentianella* species with proliferated antipodal tissue show a high plasticity in habitat colonization and flowering time. In *G. caucasea* the time span for seed development amounts to 16-20 days [Akhalkatsi, Wagner, 1996], in *G. germanica* to 20-25 days [Wagner, et al., 1995]. It was supposed that such rapid development of a seed might be promoted by hypertrophous antipodals, an apparently nutritive tissue, which substitute the endosperm in the early stages and accelerate embryogenesis. The first division of a zygote in these species takes place at 8-nuclate stage of endosperm development. For comparison, in the perennial species *G. pyrenaica,* with ephemeral antipodals, the first division of a zygote occurs only after formation of 128 nuclei in the endosperm. The total duration of seed formation in this species exceed 30 days [Wagner, et al., 1995]. The investigated perennial polycarpic *G. angulosa* and *G. pontica* show similar synchronization of embryogenesis and endosperm development as it is described for *G. pyrenaica*. These data confirm earlier suggestion concerning the role of proliferated antipodals in acceleration of seed development [Akhalkatsi, Wagner, 1996, 1997].

Thus, the investigated species differ mainly by two quantitative features – the number of cell layers in the integument and that of antipodals. The structural difference is only slightly expressed in seed coat structure, which has taxonomic significance.

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GENTIANA ANGULOSA AND *G. PONTICA* (GENTIANACEAE)-Ს ემბრიოლოგია

ახალკაცი მ., ღვალაძე გ., გაჩეჩილაძე მ., თარალაშვილი ნ.

საქართველოს მეცნიერებათა აკადემია, ნ.კეცხოველის სახ. ბოტანიკის ინსტიტუტი

(მიღებულია 20.02.2004)

რეზიუმე

ემბრიოლოგიურად პირველადაა შესწავლილი სისტემატიკურად ახლომდგომი სახეობები: *Gentiana angulosa* და *G. pontica*. დადგენილია, რომ მათ ახასიათებთ რიგი საერთო ნიშნები: ოთხბუდიანი მტვრიანა; ორუჯრედიანი მწიფე მტვრის მარცვალი; ზედა, ცენოკარპული, პარაკარპული ნასკვი; ერთსაფარველიანი, ტენუინუცელარული და ანატროპული თესლკვირტი; ჰიპოდერმიული, ერთუჯრედიანი არქესპორიუმი; მეგასპორების ხაზური ტეტრადა; Polygonum-ტიპის ჩანასახის პარკი; პრემიტოზური ტიპის განაყოფიერება; ბირთვული ენდოსპერმი; Solanad-ტიპის ჩანასახი. შესწავლილი სახეობები ერთმანეთისაგან განსხვავდებიან ძირითადად 2 რაოდენობრივი ნიშნით: ინტეგუმენტის შრეების რაოდენობით (5-8, *G. angulosa*-ში, 8-10, *G. pontica*-ში) და ანტიპოდების რიცხვით (3-6, *G. angulosa*-ში, 3, *G. pontica*-ში). მცირედენი სტრუქტურული სხვაობაა თესლის გარსის ზედაპირის აგებულებაში, რასაც ტაქსონომიური მნიშვნელობა აქვს.

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LICHEN FLORA OF THE TBILISI BOTANICAL GARDEN

BATSATSASHVILI K.

Department of Botany, Iv. Javakhishvili Tbilisi State University

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Abstract

Presented paper is the first most complete systematic, morphological, ecological and geographic survey of the lichen flora of the Central (Tbilisi) Botanical Garden of Georgian Academy of Sciences. 116 species from 46 genera and 23 families have been registered in the garden and its vicinity. The following ratios of morphological and ecological groups of lichens have been determined: crustose (63%) > foliose (28%) > fruticose (9%) and saxicolous (59%) > epiphitic (22%) > terricolous (19%), respectively. The geographic analysis have shown that the arid (18%) and nemoral (11%) elements define the character of the lichen flora in the garden on the background of multizonal species (51%).

Key words: Tbilisi Botanical Garden, lichen flora, floristic analysis.

Introduction

The Central (Tbilisi) Botanical Garden of Georgian Academy of Sciences is situated in the southern part of the historical centre of Tbilisi, in the gorge of the river Tsavkisistskali (Leghvtakhevi). The garden is located in the arid vegetation belt (semi-desert, steppe, arid light forest, xerophilous and hemixerophilous shrubbery, rock phryganoid communities). Natural vegetation occupies approximately 45% of the total area of the garden. The number of vascular plant species presented in the garden is about 700 [Kereselidze J., Loria M., 2000].

This research has been the first attempt to list and analyze the lichen diversity of the Tbilisi Botanical Garden, although there is quite large background information obtained from lichen collections of G. Woronow, J. Steiner, V. Pakhunova, Ts. Inashvili, N. Chelidze.

Materials and Methods

The lichen flora of the garden was surveyed several times. During each survey samples were taken from different substrates: rocks, stones, ground, bark of woody plants, mosses, dead plant material, etc. Environmental conditions of a habitat were recorded for each sample. The collected species were identified and listed. The list was completed with species recorded previously by other researchers. The latter data were obtained from the Lichen Herbaria of the Georgian State Museum and Institute of Botany of the Georgian Academy of Sciences and appropriate literature [Inashvili Ts., Chelidze N., 2000; Kanchaveli K. et al., 1986].

Results and Discussion

116 species from 46 genera and 23 families have been registered in the garden and its vicinity since the end of the XIX century. 115 species belong to ***Ascolichenes*** and one species, namely, *Lepraria aeruginosa* represents ***Deuterolichenes*** (***Lichenes imperfecti***). We have recorded the presence of *Psorotichia moravica* (*Pyrenopsidaceae*) – a multizonal saxicolous species – for the first time in the garden. The species list is given in the Table and the numbers of species of each family are shown on Diagram 1.

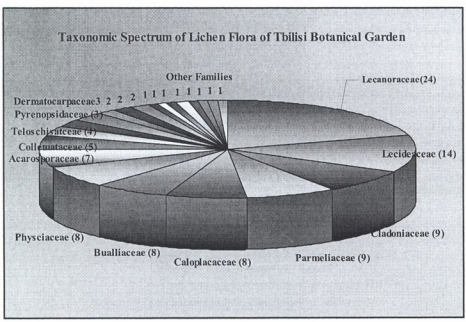
Table 1. Lichens of the Tbilisi Botanical Garden.

Family	Species
Verrucariaceae	*Verrucaria lecideoides Trevis.*
	Verrucaria nigrescens Pers.
Dermatocarpaceae	*Endopyrenium rufescens (Ach.) Koerb.*
	Dermatocarpon miniatum (L.) Mann.
	Dermatocarpon vellereum Zsch.
Polyblastiaceae	*Staurothele clopima (Walnbg.) Th. Fr.*
Endocarpaceae	*Endocarpon adscendens (Anzi.) Mull. Arg.*
Arthopyreniaceae	*Pseudosagedia cerasi (Schrad.) Arn.*
Diploschistaceae	*Diploschistes bryophylus (Ehrh.) Zahlbr.*
	Diploschistes scruposus (Schreb.) Norm.
Pyrenopsidaceae	*Thyrea pulvinata (Schaer.) Mass.*
	Gonohymenia mesopotamica Stnr.
	Psorotychia moravica Zahlbr.
Collemataceae	*Collema crispum (Hads.) Web.*
	Collema cristatum (L.) Web.
	Collema minor (Pachunoff) Tomiu
	Collema polycarpon Hoffm.
	Collema tenax (Sw.) Ach.
Pannariaceae	*Placyntium tremniacum (Mass.) Fetta*
Peltigeraceae	*Peltigera canina (L.) Willd.*
	Peltigera rufescens (Weis.) Humb.
Lecideaceae	*Lecidea atroocarpa (Ach.) Ach.*
	Lecidea crustulata (Ach.) Sprgl.
	Lecidea glomerulosa (DC.) Stend.
	Lecidea goniophila Floerk.
	Lecidea mosigii (Hepp.) Anzi.
	Psora lurida (Dill.in With.) DC.
	Catillaria athallina (Hepp.) Hellb.
	Bilimbia sphaeroides (Dicks.) Koerb.
	Bacidia muscorum (Sw.) Mudd.
	Toninia coeruleonigricans (Lightfl.) Th. Fr.
	Rhizocarpon concentricum (Dav.) Vain.
	Rhizocarpon geographicum (L.) DC.
	Rhizocarpon lindsayanum Ras.
	Rhizocarpon montagnei (Flot. ax Koerb.) Koerb.
Cladoniaceae	*Cladonia chlorophaea (Floerk.) Spreng.*
	Cladonia coniocraea (Floerk.) Spreng.
	Cladonia convoluta (Lam.) P.Cout.
	Cladonia fimbriata (L.) Fr. em. Vain.
	Cladonia furcata (Huds.) Schrad.

	Cladonia pityrea (Floerk.) Fr.
	Cladonia pocillum (Ach.) Rich.
	Cladonia pyxidata (L.) Fr.
	Cladonia rangiformis Hoffm.
Stereocaulaceae	*Stereocaulon quisquiliare (Leers) Hoffm.*
Acarosporaceae	*Sarcogyne regularis Koerb.*
	Acarospora cervina Mass.
	Acarospora fuscata (Rohl.) Arn.
	Acarospora glaucocarpa (Wahlnb.) Koerb.
	Acarospora heufleriana Koerb.
	Acarospora oligospora (Nyl.)Arn.
	Acarospora oxytona (Ach.)Mass.
Pertusariaseae	*Pertusaria lactea (L.) Arn.*
Lecanoraceae	*Aspicilia caesiocinerea (Nyl.)Arn.*
	Aspicilia calcarea (L.) Mudd.
	Aspicilia cinerea (L.) Korb.
	Aspicilia contorta (Hoffm.) Kphbr.
	Aspicilia desertorum Mer.
	Aspicilia hoffmannii (Ach.) Flag.
	Aspicilia reticulata Kphbr.
	Lecanora atra (Huds.)Ach.
	Lecanora atrynea (Ach.) Rohl.
	Lecanora badia (Hoffm.)Ach.
	Lecanora carpinea (L.) Vain.
	Lecanora cenisea Ach.
	Lecanora crenulata (Dicks.) Hook.
	Lecanora dispersa (Pers.) Rohl.
	Lecanora frustulosa (Dicks.) Ach.
	Lecanora hageni (Ach.) Ach.
	Lecanora rugosella Zahlbr.
	Lecanora subrugosa Nyl.
	Placolecanora alphoplaca (Wnbg.) Ras.
	Placolecanora garovaglii (Korb.) Ras.
	Placolecanora muralis (Schreb.) Ras.
	Placolecanora radiosa (Hoffm.) Ras.
	Candelariella aurella (Hoffm.) Zahlbr.
	Candelariella vitellina (Ehrh.) Mull. Arg.
Parmeliaceae	*Candelaria concolor (Dicks.) Stein.*
	Hypogymnia physodes (L.) Nyl.
	Parmelia caperata (L.) Ach.
	Parmelia conspersa (Ach.) Ach.
	Parmelia isidiotyla Nyl.
	Parmelia pulla Ach.
	Parmelia ryssolea Nyl.
	Parmelia stenophylla (Ach.) Heugel
	Parmelia vagans (Nyl.) Nyl.
Usneaceae	*Cornicularia steppae Sav.*
Caloplacaceae	*Protoblastenia rupestris (Scop.) Stnr.*
	Blastenia teicholyta Bausch.
	Caloplaca aurantiaca (Lightf.) Th. Fr.
	Caloplaca cerina (Ehrh.) Th. Fr.
	Caloplaca flavovirescens (Wulf.) D. Torre et Sarnth.
	Caloplaca pyracea (Ach.) Th. Fr.

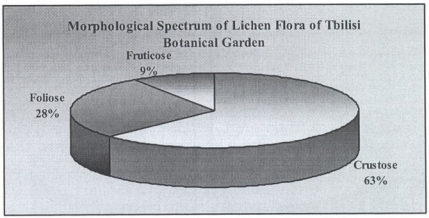
	Caloplaca stilicidiorum (Vahl.) Lynge
	Fulgensia bracteata (Hoffm.) Ras.
Teloschistaceae	*Xanthoria candelaria Kirckh.*
	Xanthoria parietina (L.) Beltr.
	Xanthoria substellaris (Ach.) Vain.
	Xanthoria ulophylodes Ras.
Buelliaceae	*Buellia dispersa Mass.*
	Diplotomma alboatrum (Hoffm.) Fw.
	Diplotomma epipolium Arn.
	Diplotomma porphiricum Arn.
	Rinodina bischoffii (Hepp.) Mass.
	Rinodina demissa (Flk.) Arn.
	Rinodina milvina (Wahlbg.) Th. Fr.
	Rinodina pyrina (Ach.) Arn.
Physciaceae	*Phaeophyscia orbicularis (Neck.) Moberg.*
	Physcia adscendens Oliv.
	Physcia aipolia Hampe
	Physcia biziana (Mass.)Zahlbr.
	Physcia stellaris (L.) Nyl.
	Physcia tribacia (Ach.)Nyl.
	Physciopsis adglutinata (Flk.) Choisy
	Physconia pulverulenta (Hoffm.) Poelt
Lichenes imperfecti	*Lepraria aeruginosa Sm.*

Diagram 1.



The lichen species were divided into 3 morphological groups: crustose, foliose and fruticose. The ratio of the noted groups is shown on Diagram 2.

Diagram 2.



The abundance of crustose species (73 species of *Lecanoraceae, Lecideaceae, Caloplacaceae, Buelliaceae, Acarosporaceae,* etc.) is explained by the location of the garden in the arid vegetation belt. However, owing to the relatively mild climate formed under the influence of the topographic peculiarities and green plantations of the garden, the number of foliose species is also relatively high (32 species of *Parmeliaceae, Physciaceae, Peltigeraceae*, etc.). The group of fruticose lichens (11 species) consists almost completely of terricolous species of *Cladonia.*

The distribution of the lichens among 3 ecological groups distinguished according to the substrate type and general systematic structure of each group is shown on Diagram 3.

8 geographic elements have been distinguished in the lichen flora of the garden according to the species general distribution among the latitudinal and vertical vegetation belts. These are multizonal, arid, nemoral, boreal, mountain, alpine, arcto-alpine and hypoarcto-mountain elements [Golubkova N. S., 1983; Guide-book of Lichens of the USSR. 1971-77; van Herk C. M., Aptroot A., van Dobben H. F., 2002]. The ratio of the noted elements is shown on Diagram 4 (2 species of 116 - Xanthoria ulophylodes Ras. and Buellia dispersa Mass. have been excluded because of the insufficient data on their distribution).

Diagram 3.

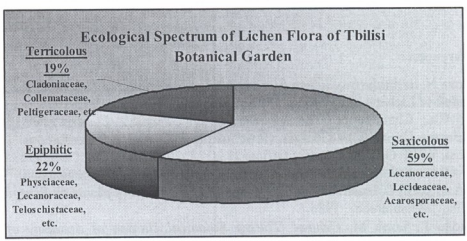
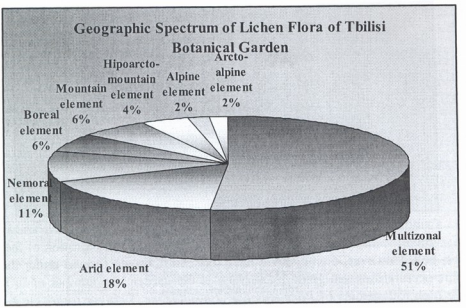


Diagram 4.



The arid (18%) and nemoral (11%) elements define the character of the lichen flora of the garden on the background of multizonal species (51%). The relatively high percentage of arid species is due to the location of the garden in the arid vegetation belt. The relatively great number of nemoral species typical of plain forests is explained as follows: the major part of the arid vegetation of Tbilisi and its vicinity has been formed in consequence of deforestation of plains and foothills. The regrowth of forests was precluded by further human intrusions. However, a number of lichen species characteristic to woodlands survived in remnants of those forests; and moreover, green plantations of the garden encouraged the subsistence of such species.

Thus, the lichen flora of the Tbilisi Botanical Garden is quite diverse and its structure reflects the environmental conditions of the arid vegetation belt as well as peculiarities of the garden.

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თბილისის ბოტანიკური ბაღის ლიქენები

ბაცაცაშვილი ქ.

ბოტანიკის კათედრა, თბილისის ივ. ჯავახიშვილის სახელობის უნივერსიტეტი

(მიღებულია 20.01.2004)

რეზიუმე

წარმოდგენილია საქართველოს მეცნიერებათა აკადემიის ცენტრალური (თბილისის) ბოტანიკური ბაღის ლიქენოფლორის პირველი ყველაზე სრული ანალიზის (სიტემატიკური, მორფოლოგიური, ეკოლოგიური, გეოგრაფიული) შედეგები. თბილისის ბოტანიკური ბაღსა და მის შემოგარენში აღრიცხულია ლიქენების 116 სახეობა 23 ოჯახის 46 გვარიდან. მორფოლოგიურ და ეკოლოგიურ ჯგუფებში სახეობები შემდეგნაირადაა განაწილებული: ქაფისებრი (63%) > ფირფიტისებრი (28%) > ბუჩქისებრი (9%) და ეპილითური (59%) > ეპიფიტური (22%) > ეპიგეური (19%). გეოგრაფიულმა ანალიზმა აჩვენა, რომ თბილისის ბოტანიკური ბაღის ლიქენოფლორის ხასიათს მულტიზონური ელემენტის (51%) სახეობათა ფონზე არიდული (18%) და ნემორალური (11%) ელემენტები განსაზღვრავს.

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ANALYSIS OF THE FLORA OF THE SUBALPINE BELT IN THE CHIRUKHISTSKALI CANYON (COLKHIS, GEORGIA)

KHARAZISHVILI D., MEMIADZE N.

Batumi Botanical Garden of the Georgian Academy of Sciences

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Abstract

The flora of the subalpine belt in a gorge of Colkhis, Chirukhistskali was studied. The data of the high-mountain meadows of this region is poor. The present investigation has revealed 40 endemic species, 34 of which are essentially endemic for the Caucasus, 2 for Georgia and 4 for Colkhis.

Key words: flora, subalpine belt, endemism, Colkhis, Georgia.

Introduction

Colchis and, particularly, its southern part is the richest refugium of relicts of the Tertiary's (Neogenic period) thermo-mesophilous dendroflora in the western Eurasia. Summer-green broad-leaved forests with spots of mountain dark coniferous forests occurring at the treeline represent the prevailing landscape of the region. However, the Colkhic forest vegetation differs from the woody landscapes of the western Eurasia in the participation of vegetative motile prostrate trees and shrubs, many of which are evergreen. These plants mainly occur in the subalpine belt. The following well-known relicts dominate over the forest vegetation: *Betula medwedewii, Quercus pontica, Rhamnus imeretina, Daphne alboviana*, etc. And the following evergreen shrubs are distributed in these forests: *Rhododendron ponticum, R. ungernii, R. smirnovii, Epigaea gaulterioides, Laurocerasus officinalis* [Dolukhanov, 1980].

The forest flora and vegetation of the southern Colkhis is studied quite well [Dolukhanov A. G., et. al., 1942; Dolukhanov, 1980]; however, the same cannot be said of the vegetation of the subnival belt.

The aim of our research was to study the subnival vegetation of a canyon of the southern Colkhis, Chirukhistskali (Fig.1). The paper considers floristic peculiarities of the subalpine belt of the noted canyon between 1900 and 2400 a.s.l.

The Chirukhistskali canyon is located in the southeastern part of Adjara (Western Georgia). It is bordered by the Shertuli range to the north and is connected with the canyon of the river Adjaristskali to the northwest. The area of the canyon is 314sq. km. The river Adjaristskali flows at elevations from 2700m to 550m a.s.l. The canyon is constituted by huge volcanogenic rocks of the Middle Eocene. Its most part is deeply penetrated into the mountain massifs. Owing to river erosion the Chirukhistskali canyon is divided into hardly accessible parts.

Precipitations (1690mm per year) are almost evenly distributed throughout a year. Spring is relatively dry and autumn and winter are the most humid. The average annual temperature is 3.9°C.

Fig. 1

Materials and methods

The present report is the result of the field investigations carried out in 2000-2003. The research was done using conventional fieldwork floristic method.

The names of the taxa are given according to "Key of Plants of Adjara" [Dmitrieva, 1990], "Flora of Georgia" [1971-2003]. Plant endemism has been determined according to literature data [Kolakovsky, 1980-1986; Gagnidze, Davitadze, 2000; Gagnidze, et al., 2003].

Herbarium specimens are kept in the Herbarium of the Batumi Botanical Garden (BAT) of the Georgian Academy of Sciences.

Results and Discussion

The following altitudinal belts are presented in the Chirukhistskal canyon: the middle mountain forest (500-1000/1100m), the upper mountain forest (1200-2000m), subalpine (2000-2400m) and alpine (2400-2992m) belts [Zazanashvili, et al., 2000].

The vegetation of the subalpine belt and ecotone of the treeline of the canyon is represented by elfin forests (*Betula litwinowii, Fagus orientalis*), shrubberies, swampy meadows (*Alopecurus aequalis, Deschampsia caespitosa,* etc.), subalpine meadows, rock and scree communities (*Arenaria rotundifolia, Psephellus schavscheticus*).

According to the classification system of the vegetation in relation to the degree of human impact, the vegetation cover of the canyon is referred to the second degree of hemeroby, i.e. the semi-natural plant communities, which are used as pasture [Pott, 1996].

Ecotopological distribution of species. Plants of various phytocoenoses and ecological groups constitute the subalpine vegetation. The floristic core of the meadow communities comprises species of the genuine subalpine meadows (57% of the total number of the species). Such endemic and rare species dominate over these communities as *Ranunculus cappadocicus, R. makaschwili, Geum latilobum, Grossheimia polyphylla, Paeonia macrophylla,* etc.

Tall herbaceous vegetation does not form independent communities in the canyon. Its separate constituents are scattered over subalpine meadows and forest edges. Rare endemic species typical of the tall herbaceous vegetation such as *Grossheimia polyphylla, Lilium kesselringianum* are worth mentioning.

The presence of such components of the forest vegetation as *Brachypodium silvaticum, Lathyrus pratense, Anemone caucasica, Geranium robertianum, Digitalis schischkinii,* etc. is due to the occurrence of the subalpine meadows at the treeline. The subalpine meadows are also enriched with species of swamps (*Juncus effusus, Primula auriculata, Caltha polypatala*), which in this canyon occur mainly in the forest belt. There are no endemic species among these plants. Plants of rocks and scree occur in comparatively rarefied and short-grass communities and their proportion is low (5%). A rare endemic species, *Psephellus schavscheticus* is among these plants.

Table1. Plants of the subalpine belt in the Chirukhistskali canyon.

Family	Species	Biotope	Frequency	Endemism
Aspleniaceae	*Asplenium pseudolanceolatum Fomin.*	rock	freq.	
Athyriaceae	*Athyrium filix-femina (L.)Roth.*	forest	freq.	
	A.distentifolium Tausch.	meadow	freq.	
	Cystopteris fragilis (L.) Bernh.	forest	freq.	
	Woodsia alpina (Bolton)S.F.Gray	rock	rare	
Cryptogrammaceae	*Cryptograma crispa (L.) R.Br.*	rock	rare	
Hypolepidaceae	*Pteridium aquilinum Kuhn.*	forest	freq.	
Polypodiaceae	*Polypodium vulgare L.*	forest	freq.	
Aspidiaceae	*Polystichum braunii (Spenn.)Fee*	forest	rare	
	P.lonchitis (L.) Roth.	rock	rare	
	Dryopteris filix-mas (L.)Schott.	forest	freq.	
	D. oreades Fom.	forest	rare	
Ophioglossaceae	*Botrychium lunaria (L.)Sw.*	meadow	rare	
Lycopodiaceae	*Lycopodium annotinum L.*	forest	rare	
	L. alpinum (L.)Rothm.	meadow	rare	
Equisetaceae	*Equisetum hiemale L.*	forest	freq.	
	E. palustre L.	Marsh	freq.	
Pinaceae	*Pinus kochiana Klotzsch.*	forest	freq.	
	Picea orientalis (L.) Link.	forest	freq.	
	Abies nordmanniana (Steven) Spach	forest	freq.	
Cupressaceae	*Juniperus sabina L.*	rock	freq.	
	J. pigmaea C.Koch.	rock	freq.	
Paeoniaceae	*Paeonia macrophylla (Albov) Lomak.*	tall erb.veget.	rare	Georgian
Helleboraceae	*Caltha polypetala Hochst.*	marsh	freq.	
	Trollius ranunculinus (Smith.) Stearn.	meadow	freq.	
	Aquilegia caucasica Bieb.	meadow	sporad.	
	Delphinium dzavakhischwili Kem.-Nath.	tall erb.veget.	sporad.	
	Aconitum orientale Mill.	tall erb.veget.	freq.	
	A. nasutum Fisch.	tall erb.veget.	freq.	Caucasian
Ranunculaceae	*Ranunculus repens L.*	marsh	freq.	
	R. cappadocicus Willd.	forest	freq.	
	R. makaschwilii Kem.-Nat.	meadow	rare	Georgian
	Pulsatilla albana Stev.	meadow	rare	
	P. violacea Rupr.	meadow	rare	
	Anemone fasciculata L.	meadow	freq.	

	A. ranunculoides L.	meadow	freq.	
	Thalictrum foetidum L.	forest	rare	
	T. triternatum Rupr.	forest	rare	
Crassulaceae	*Sempervivum armenum Boiss.et Huet.*	rock	freq.	
	Sedum caucasicum (Grossh.) Bor.	forest	freq.	
	S. spurium Bieb.	rock	freq.	
	S. tenellum Bieb.	rock	freq.	
	S. gracile C. A. Mey	rock	freq.	
	S. annuum L.	rock	freq.	
	S. pallidum Bieb.	rock	freq.	
	Rosularia pilosa Bor.	rock	rare	
Rosaceae	*Aruncus vulgaris Rafin.*	forest	freq.	
	Cotoneaster integerrimus Medik.	rock	rare	
	Pyrus caucasica Fed.	forest	rare	
	Sorbus boissieri Schneid	forest	freq.	
	S. subfusca (Ledeb.) Boiss.	forest	rare	Caucasian
	S. colchica Zinserl.	forest	rare	Caucasian
	Rubus saxatilis L.	forest	freq.	
	R. buschii (Rozanova) Grossh.	forest	freq.	
	R. caucasicus Focke.	forest	freq.	Caucasian
	Fragaria vesca L.	forest	freq.	
	Potentilla brachypetala Fisch.et Mey	rock	rare	Caucasian
	P. elatior Willd. ex Schlecht.	meadow	freq.	
	P. ruprechtii Boiss.	meadow	freq.	Caucasian
	P. recta L.	meadow	rare	
	P. adscharica Somm.et Lev.	meadow	sporad.	
	P. crantzii (Crantz .)Beck.	meadow	freq.	
	Sibbaldia parviflora Willd.	meadow	freq.	
	Alchemilla oxysepala Juz.	meadow	freq.	
	A. tredecimloba Bus.	tall erb.veget.	freq.	Caucasian
	A. retinervis Bus.	meadow	freq.	
	A. dura Bus.	forest	freq.	
	A. languida Bus.	meadow	freq.	
	Rosa boissieri Crep.	rock	rare	
	R. woronowii Lonacs.	forest	rare	
	Geum latilobum Somm.et Lev.	forest	rare	
Saxifragaceae	*Saxifraga mollis Smith.*	rock	freq.	
	S. cymbalaria L.	forest	freq.	
	S. cartilaginea Willd.	rock	freq.	
	S. exerata Vill.	rock	rare	
Grossulariaceae	*Ribes alpinum L.*	forest	rare	
Leguminosae	*Trifolium ambiguum Bieb.*	meadow	freq.	
	T.repens L.	meadow	freq.	
	T. trichocephalum Bieb.	meadow	freq.	
	T. canescens Willd.	meadow	freq.	
	T. pratense L.	meadow	freq.	
	Anthyllis caucasica (Grossh.)Juz.	meadow	rare	
	Lotus caucasicus Kupr.	tall erb.veget.	freq.	
	Asragalus incertus Ledeb.	rock	rare	
	A. bachmarensis Grossh.	rock	freq.	Colkhic
	A. polygala Pall.	rock	rare	

	Oxytropis lazica Boiss.	meadow	freq.	
	Coronilla balansae (Boiss.) Grossh.	meadow	freq.	
	Lathyrus aureus (Stev.) Brandza	forest	freq.	
	L. roseus Stev.	forest	sporad.	
	Vicia cassubica L.	forest	freq.	
	V. balansae Boiss.	meadow	freq.	
	V. sepium L.	forest	freq.	
Linaceae	*Linum hypericifolium Salisb.*	meadow	rare	
Oxalidaceae	*Oxalis acetosella L.*	forest	freq.	
Geraniaceae	*Geranium pallens Bieb.*	meadow	freq.	
	G. gracile Ledeb.	forest	freq.	
	G. platypetalum Fisch.et Mey	meadow	freq.	
	G. psilostemon Ledeb.	meadow	freq.	
	G. gymnocaulon DC.	meadow	freq.	
Euphorbiaceae	*Euphorbia macroceras Fisch.et Mey.*	forest	freq.	Caucasian
	E. oblongifolia (C.Koch.)C.koch.	meadow	freq.	
Thymelaeaceae	*Daphne mezereum L.*	forest	freq.	
	D. glomerata Lam.	meadow	rare	
	D. pontica L.	forest	freq.	
	D. alboviana Woron. ex Pobed.	meadow	rare	
Onagraceae	*Ludwigia palustris (L.) Elliott.*	Marsh	rare	
	Epilobium montanum L.	forest	freq.	
	E. prionophyllum Haussknech	forest	freq.	
	E. gemmascens C. A. Mey	Marsh	freq.	
	E. algidum Bieb.	Marsh	freq.	
	E. palustre L.	Marsh	freq.	
	Chamerion dodonaei (Vill.) Holub.	forest	freq.	
Polygalaceae	*Polygala caucasica Rupr.*	meadow	freq.	
Aceraceae	*Acer trautvetteri Medw.*	forest	freq.	
Rhamnaceae	*Rhamnus imeretina Booth.*	forest	rare	
	Rh. microcarpa Boiss.	forest	rare	
Aquifoliaceae	*Ilex colchica Pojark.*	forest	freq.	
Umbelliferae	*Chaerophyllum astrantiae Boiss. et Bal.*	meadow	freq.	
	Anthriscus kotschyi Fenzl. ex Boiss.	rock	rare	
	A. nemorosa (Bieb.) Spreng.	forest	freq.	
	Astrantia maxima Pall.	meadow	freq.	
	Bupleurum nordmannianum Ledeb.	rock	rare	
	B. polyphyllum Ledeb.	meadow	rare	
	Carum carvi L.	meadow	freq.	
	C. meifolium (Bieb.) Boiss.	meadow	freq.	
	Pimpinella saxifraga L.	forest	rare	
	Heracleum sosnovskyi Manden.	forest	freq.	Caucasian
	H. apiifolium Boiss.	meadow	freq.	
	Laserpitium affine Ledeb.	meadow	rare	
Caprifoliaceae	*Viburnum lantana L.*	forest	freq.	
	Lonicera caucasica Pall.	forest	freq.	
Rubiaceae	*Asperula prostrata (Adam.)C.Koch.*	rock	freq.	
	A. odorata L.	forest	freq.	
	Galium cruciata (L.) Scop.	meadow	freq.	
	G. rotundifolium L.	forest	freq.	
	G. palustre L.	Marsh	freq.	

	G. album Mill.	meadow	freq.	
Valerianaceae	*Valeriana alliariifolia Adam.*	forest	freq.	
	V. eriophylla (Ledeb) Utk.	forest	freq.	
Dipsacaceae	*Knautia montana (Bieb.) DC.*	tall herb.veget.	freq.	
	K. involucrata Somm. Et Lev.	meadow	freq.	
	Cephalaria gigantea (Ledeb) Bobr.	tall erb.veget.	freq.	Caucasian
	Scabiosa caucasica Bieb.	meadow	freq.	
	S. adzharica Schchian	tall erb.veget.	rare	
Betulaceae	*Batula litwinowii Doluch.*	forest	freq.	
	B. medwedewii Regel.	forest	rare	
Coryllaceae	*Coryllus avellana L.*	forest	freq.	
Fagaceae	*Quercus pontica, C. Koch.*	forest	rare	
	Fagus orientalis Lipsky	forest	freq.	
Oleaceae	*Fraxinus excelsior L.*	forest	freq.	
Gentianaceae	*Gentiana cruciata L.*	forest	freq.	
	G. septemfida Pall.	meadow	freq.	
	G. schistocalyx C.Koch. (C.koch.)	meadow	freq.	
	Gentianella caucasea (Lodd. ex Sims) Holub	meadow	rare	Caucasian
	Swertia iberica Fisch et C.A. Mey	forest	freq.	
Menyantaceae	*Menyantes trifoliata L.*	Marsh	rare	
Boraginaceae	*Macrotomia echinoides (L.) Boiss.*	meadow	freq.	
	Cerinte glabra Mill.	meadow	freq.	
	Symphytum asperum Lepech.	tall erb.veget.	freq.	Caucasian
	Nonea intermedia ledeb.	meadow	freq.	
	Myosotis caespitosa K.F.Schultz.	Marsh	freq.	
	M. sylvatica Ehrh. ex Hoffm.	forest	freq.	
	M. alpestris F. W. Schmidt	meadow	freq.	
Scrophulariaceae	*Scrophularia chrysantha Jaub. et Spach.*	meadow	rare	
	S. chlorantha Kotschy et Boiss.	meadow	rare	
	S. olympica Boiss.	rock	rare	
	S. variegata Bieb.	meadow	freq.	
	Veronica filiformis Smith.	meadow	freq.	
	V. beccabunga L.	Marsh	freq.	
	V. officinalis L.	forest	freq.	
	V. peduncularis Bieb.	meadow	freq.	
	V. gentianoides Vahl.	meadow	freq.	
	V. chamaedrys L.	meadow	freq.	
	V. monticola Trautv.	meadow	freq.	Caucasian
	Digitalis schischkinii Ivanina	forest	freq.	Caucasian
	Euphrasia hirtella Jord.	meadow	freq.	
	E. pectinata Ten.	meadow	freq.	
	Paederotella pontica (Rp.exBoiss.)Kem.-Nath.	forest	rare	Caucasian
	Pedicularis crassirostris Bunge	meadow	freq.	
	P. nordmanniana Bunge.	meadow	freq.	
	P. acmodonta Boiss.	meadow	freq.	
	P. atropurpurea Nordm.	meadow	freq.	
	Rhynchocorys elephas (L.) Griseb.	meadow	freq.	
Labiatae	*A juga orientalis L.*	meadow	freq.	

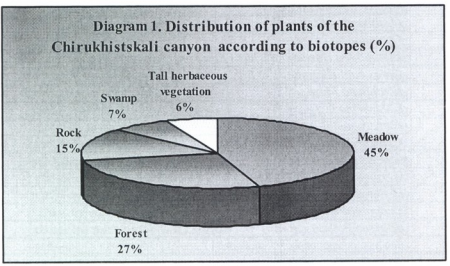
	Teucrium orientale L.	meadow	freq.	
	Prunella vulgaris L.	meadow	freq.	
	Lamium tomentosum Willd.	rock	rare	
	Betonica grandiflora Steph.ex Willd.	meadow	freq.	
	Stachys iberica Bieb.	meadow	freq.	
	S. pubescens Ten.	rock	freq.	
	Salvia glutinosa L.	forest	freq.	
	S. verticillata L.	forest	freq.	
	Calamintha grandiflora (L.) Moench.	forest	freq.	
	Mentha aquatica L.	Marsh	freq.	
	Thymus grossheimii Ronn.	rock	sporad.	
Orobanchaceae	*Orobanche caryophyllaceae Smith.*	forest	rare	
Plantaginaceae	*Plantago major L.*	meadow	freq.	
	P. lanceolata L.	meadow	freq.	
Papaveraceae	*Papaver monanthum Trautv.*	meadow	rare	
	P. fugax Poir.	rock	rare	
Cruciferae	*Dentaria quinquefolia Bieb.*	forest	freq.	
	Cardamine impatiens L.	forest	freq.	
	C. uliginosa Bieb.	forest	freq.	
	Hesperis matronalis L.	forest	freq.	
	Draba hispida Willd.	meadow	freq.	
	D. siliquosa Bieb.	rock	freq.	
	Bunias orientalis L.	meadow	rare	
	Sobolewskia clavata (Boiss.) Fenzl.	rock	rare	
	Alyssum trichostachyum Rupr.	rock	sporad.	
Cistaceae	*Helianthemum tomentosum (Scop) S.F.Gray.*	meadow	rare	
Violaceae	*Viola orthoceras Ledeb.*	meadow	rare	Colkhic
	V. reichenbachiana Jord.ex Boreau	forest	rare	
	V. vespertina Klok.	meadow	rare	Caucasian
	V. arvensis Murr.	meadow	freq.	
Campanulaceae	*Campanula grossheimii Charadze*	forest	rare	Caucasian
	C. collina Bieb.	meadow	freq.	Caucasian
	C. hemschinica C.Koch.	meadow	rare	
	C. latifolia L.	tall erb.veget.	freq.	
	Gadellia lactiflora (Bieb.)Schulkina	tall erb.veget.	freq.	Caucasian
	Asyneuma campanuloides (Bieb.)Bornm.	meadow	rare	
Compositae	*Solidago caucasica Kem.-Nath.*	meadow	freq.	Caucasian
	Erigeron caucasicus Stev.	rock	freq.	Caucasian
	E.orientalis Boiss.	meadow	freq.	
	Inula magnifica Lipsky	tall erb.veget.	freq.	Caucasian
	I.orientalis Lam.	meadow	freq.	
	Bellis perennis L.	meadow	freq.	
	Telekia speciosa (Schreb.) Baumg.	tall erb.veget.	rare	
	Aster alpinus L.	meadow	freq.	
	Achillea latiloba Ledeb.ex nordm.	meadow	freq.	
	Pyrethrum macrophyllum (Waldst.et Kit.) Willd.	tall erb.veget.	freq.	
	P. roseum (Adam.)Bieb.	meadow	freq.	
	P. punctatum (Desr.) Bordz.	Marsh	rare	
	Petasites albus (L.) Gaertn	forest	freq.	

	Anthemis marschalliana Willd.	rock	freq.	Caucasian
	A. schischkiniana Fed.	rock	rare	
	A. iberica Bieb.	meadow	rare	
	Taraxacum stevenii (Spreng.) DC.	meadow	freq.	
	Doronicum macrophyllum Fisch.	tall erb.veget.	freq.	
	Senecio caucasigenus Schischk.	meadow	freq.	Caucasian
	S. cladobotrys Ledeb.	tall erb.veget.	freq.	Caucasian
	S. othonnae Bieb.	tall erb.veget.	freq.	
	S. platyphylloides Somm.et Lev.	tall herb.veget.	freq.	
	S. propinquus Schischk.	forest	freq.	Caucasian
	S. rhombifolius (Willd.) Sch. Bip.	tall herb.veget.	freq.	Caucasian
	S. taraxacifolius (Bieb.) DC.	rock	freq.	
	Centaurea nigrofimbria (C.Koch.) Sosn.	meadow	freq.	
	Cirsium echinus (Bieb.) Hand.-Mazz.	forest	freq.	
	C. obvallatum (Bieb.) Bieb.	meadow	freq.	
	C. simplex C.A.Mey	meadow	freq.	
	C. kosmelii (Adam.) Fisch.ex Hoh.	meadow	freq.	
	C. adjaricum Somm.et Lev.	meadow	freq.	
	C. hypoleucum DC	meadow	freq.	
	C. cephalotes Boiss.	meadow	freq.	
	C. rhizocephalum C. A. Mey	Marsh	freq.	
	Grossheimia polyphylla (Ledeb.) Holub.	tall herb.veget.	rare	Caucasian
	Prenanthes abietina (Boiss.et Bal.) Kirp.	tall herb.veget.	freq.	
	Psephellus schavscheticus Khokhr.	rock	sporad.	Colkhic
	Mycelis muralis (L.) Dumort.	forest	freq.	
	Kemulariella caucasica (Willd.) Tamamsch.	meadow	freq.	
	Antennaria caucasica Boriss.	rock	freq.	
	Gnaphalium supinum L.	rock	freq.	
	G. caucasicum Somm. et Lev.	rock	freq.	
	Helichrysum graveolens (Bieb.) Sweet.	rock	freq.	
	H. polyphyllum Ledeb.	meadow	freq.	
	Jurinea subacaulis (Fisch.et Mey) Iljin	rock	sporad.	
	Aethopappus pulcherrimus (Willd.) Cass.	rock	sporad.	
	Scorzonera seidlitzii Boiss.	meadow	rare	
	Leontodon hispidus L.	meadow	freq.	
	Cicerbita racemosa (Willd.) Beauv.	rock	freq.	
Hypericaceae	*Hypericum caucasicum (Woron.) Gorschk.*	meadow	freq.	
	H. orientale L.	meadow	freq.	
	H. bupleuroides Griseb.	meadow	sporad.	
Ericaceae	*Rhododendron caucasicum Pall.*	forest	freq.	
	R. luteum Sweet.	forest	freq.	
	R. ponticum L.	forest	freq.	
Vacciniaceae	*Vaccinium myrtillus L.*	meadow	rare	
	V. arctostaphyllos L.	forest	freq.	
Drozeraceae	*Drozera intermedia Hayne*	Marsh	rare	

Santalaceae	*Thesium alpinum L.*	meadow	rare	
Caryophyllaceae	*Stellaria persica Boiss.*	meadow	freq.	
	S. media (L.)Vill.	forest	freq.	
	Arenaria rotundifolia Bieb.	rock	freq.	
	Cerastium fontanum Baumg.	forest	freq.	
	C. purpurascens Adam.	meadow	freq.	
	C. cerastoides (L.) Britt.	rock	freq.	
	C. sosnovskyi Schischk.	forest	rare	
	Sagina procumbens L.	meadow	freq.	
	Moechringia trinervia (L.)Clairv.	forest	freq.	
	Silene multifida (Adam.) Rohrbach.	forest	freq.	
	S. wallichiana Klotzsh.	tall herb.veget.	freq.	
	S. saxatilis Sims.	meadow	rare	
	S. italica (L.) Pers.	meadow	rare	
	S. physocalyx Ledeb.	meadow	rare	
	Gypsophylla tenuifolia Bieb.	rock	rare	Caucasian
	Dianthus multicaulis Boiss.et Huet.	meadow	rare	
	Minuartia circassica (Albow.) Woronow.	rock	rare	
	M.colchica Charadze	rock	rare	
	M.biebersteinii (Rupr.) Schischk.	rock	rare	
	Scleranthus polycarpos L.	rock	freq.	
	S. uncinatus Schur.	rock	freq.	
Polygonaceae	*Polygonum cognatum Meissn.*	meadow	freq.	
	Rumex acetosella L.	meadow	freq.	
	R. alpinus L.	meadow	freq.	
	R. scutatus L.	rock	freq.	
Primulaceae	*Androsace intermedia Ledeb.*	rock	freq.	Caucasian
	A. armeniaca Duby	rock	rare	
	A. villosa L.	rock	freq.	
	Primula auriculata Lam.	Marsh	freq.	
	P. pallasii Lehm.	meadow	freq.	
Liliaceae	*Colkhicum speciosum Stev.*	meadow	freq.	
	C. umbrosum Stev.	forest	freq.	
	Fritillaria latifolia Willd.	meadow	freq.	Caucasian
	Gagea anisanthos C.koch.	meadow	rare	
	Lilium kesselringianum Miscz.	forest	rare	Colkhic
	L. szowitsianum Fisch.	forest	freq.	
	Muscari sosnovskyi Schchian.	meadow	rare	Caucasian
	Ornithogalum balansae Boiss.	meadow	freq.	
	Polygonatum verticillatum (L.) All.	forest	sporad.	
	Scilla winogradowii Sosn.	meadow	freq.	Georgian
	Veratrum lobelianum Bernh.	meadow	freq.	
	Allium kunthianum Vved.	meadow	rare	
	Smilax excelsa L.	forest	freq.	
Iridaceae	*Crocus vallicola Herbert.*	meadow	freq.	
Juncaceae	*Juncus alpigenus C. Koch.*	Marsh	freq.	
	J. effusus L.	Marsh	freq.	
	Luzula forsteri (Smith.) DC.	forest	freq.	
	L. campestris (L.) DC.	forest	freq.	
Orchidaceae	*Coeloglossum viride (L.) C.Hartman.*	meadow	rare	

	Dactylorhiza lancibracteata (C.koch.) Renz.	meadow	rare	
	D. flavescens (C.koch.)Holub.	meadow	rare	
	D. euxina (Nevski)Czer.	meadow	freq.	
	Orchis mascula (L.) L.	meadow	freq.	
	O. sphaerica Bieb.	meadow	rare	
	Gymnadenia conopsea (L.) R.Br.	meadow	rare	
	Platanthera chlorantha (Cust.) Reichenb.	forest	rare	
Cyperaceae	*Carex capitellata Boiss.et Bal.*	meadow	freq.	
	C.echinata Murr.	Marsh	freq.	
	C. leporina L.	Marsh	freq.	
	C.micropodioides V.Krecz.	meadow	freq.	
	C. pallescens L.	meadow	freq.	
	C. sylvatica Huds.	forest	freq.	
	C. szovitsii V.Krecz.	meadow	freq.	
	C. diandra Schrank.	Marsh	freq.	
	C. appropinquata Schum.	Marsh	freq.	
	C. tristis Bieb.	rock	freq.	
	Scirpus sylvaticus L.	Marsh	freq.	
Graminae	*Agrostis tenuis Sibth.*	meadow	freq.	
	Alopecurus aequalis Sobol.	meadow	freq.	
	Agropiron canium L.	meadow	freq.	
	Anthoxanthum odoratum L.	meadow	rare	
	Brachypodium sylvaticum (Huds.) Beauv.	forest	freq.	
	Briza elatior Sibth. et Smith.	meadow	freq.	
	Bromopsis benekenii (Lange) Holub.	forest	freq.	
	B. variegata (Bieb.) Holub.	meadow	freq.	
	Calamagrostis arundinacea (L.)Roth.	meadow	freq.	
	Colpodium colchicum (Albov.)Woronow.	meadow	freq.	Caucasian
	Catabroza aquatica (L.) Beauv.	Marsh	freq.	
	Dactylis glomerata L.	meadow	freq.	
	Deschampsia caespitosa (L.) Beauv.	Marsh	freq.	
	Festuca drymeja Mert.et Koch.	forest	freq.	
	F. pratensis Huds.	meadow	freq.	
	F. rubra L.	meadow	freq.	
	F. varia Haenke	rock	freq.	
	Helictotrichon pubescens (Huds.) Pilg.	meadow	rare	
	Koeleria albovii Domin.	meadow	freq.	
	Milium schmidtianum C.Koch.	tall herb.veget.	rare	
	Nardus stricta L.	meadow	freq.	
	Phleum pratense L.	meadow	freq.	
	Poa alpina L.	meadow	freq.	
	P. iberica Fisch.et Mey	meadow	freq.	
	P. palustris L.	Marsh	freq.	
	P. pratensis L.	meadow	freq.	
	Trisetum turcicum Chrtek.	meadow	freq.	
Lemnaceae	*Lemna trisuloa L.*	Marsh	rare	
Salicaceae	*Populus tremula L.*	forest	freq.	
	Salix caucasica Anderss.	forest	freq.	
	S. kikodzeae Goerz.	forest	rare	

It is worth mentioning that plants of the meadow biotope are the most numerous in the subalpine belt of the studied region (Diagram 1); they are followed by plants of forests, rocks, swamps and tall herbaceous vegetation.



Statistic analysis of the flora of the Chirukhistskali canyon. We have recorded 381 species of vascular plants in the subalpine belt of this canyon. They refer to 214 genera and 67 families (Table 1). The great number of the genera is especially worth attention. The proportion of genera to species is 1:1.8. The species ratio in the group of seed plants is as follows (Table 2):

Table 2. Number of the taxa in the flora of the subalpine belt in the Chirukhistskali canyon.

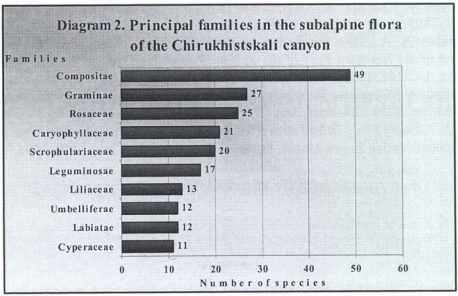
Divisions	Families		Genera		Species	
	Number	%	Number	%	Number	%
Pteridophyta	9	13	12	5.6	17	4.5
Gymnospermae	2	3	44	1.8	5	1.3
Angiospermae	56	83.5	196	92.5	359	94.2
Dicotyledoneae	50	74.6	157	73.3	295	77.4
Monocotyledoneae	6	9	41	19.1	64	16.7

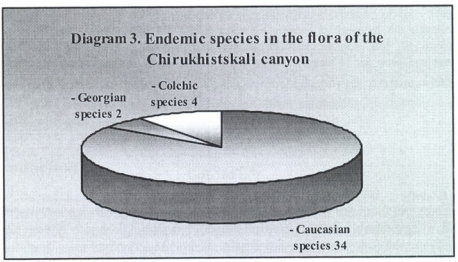
Here and below proportions (%) are estimated on the basis of the total number of the taxa (families, genera, species) recorded in the subalpine belt of the Chirukhistskali canyon.

Species ratio within the group of the seed plants is as follows: dicots - 77.4%: monocots - 16.7%, ptredophytes are far behind the noted groups and conifers are represented by the insignificant number of species.

The following families predominate in the number of species (Diagram 2): *Compositae, Graminae, Rosaceae, Caryophyllaceae, Scrophulariaceae, Leguminosae, Liliaceae, Umbelliferae, Labiatae, Cyperaceae.* These 10 families together are represented by 207 species, which is more than 50% of the total number of the species recorded in the canyon. The other 57 families comprise 174 species. The following families are distinguished by the fewest number of species: *Iridaceae* (1 species), *Valerianaceae* (2 species), *Ericaceae* (3 species), *Gentianacae, Primulaceae* (5 species in each).

The number of Caucasian endemic species is 40, i.e. 11% of the total number of the species recorded in this canyon; 2 of them are endemic to Georgia and 4 to Colkhis (Diagram 3).





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ჭირუხისწყლის ხეობის სუბალპური სარტყლის ფლორის ანალიზი

ხარაზიშვილი დ., მემიაძე ნ.

საქართველოს მეცნიერებათა აკადემიის ბათუმის ბოტანიკური ბაღი

(მიღებულია 29.01.2004)

რეზიუმე

შესწავლილია ჭირუხისწყლის ხეობის (კოლხეთი) სუბალპური სარტყლის ფლორა. ამ რეგიონის მაღალმთის მდელოებს შესახებ მონაცემები ძალიან ღარიბია. გამოვლინებულია 40 ენდემური სახეობა, რომელთა შორის კავკასიის ენდემია 34, მათგან 2 საქართველოსი და 4 საკუთრივ კოლხეთის.

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TYPOLOGY OF THE JUNIPER COMMUNITIES OF THE IORI PLATEAU

LACHASHVILI N., KHACHIDZE M., IASHAGHASHVILI K.

N. Ketskhoveli Institute of Botany of the Georgian academy of Sciences

Abstract

The typology of the Juniper forests (*Junipereta; Juniperus foetidissima, J. polycarpos*) spread on the Iori plateau (East Georgia) has been studied. 9 associations have been distinguished. 5 of them have been described in Georgia for the first time. The paper covers natural distribution ranges and short diagnostic characterization of each association. The juniper forests spread on the Iori plateau are similar to those of the adjacent Sheki plateau and Bozdaghi foothills in their typological composition and phytocenological structure; however, they are different from the juniper forests of the southern part of the South Caucasus (Armenia).

Key words: juniper forest, association, plant community, characteristic species, dominant-edificator.

Introduction

The Caucasian range of the juniper community (*Junipereta*) – one of the principal formations of the relict arid forests of the Tertiary – comprises in the main the central, eastern and southern parts of the South Caucasus [Grossheim A.A, 1948, Ivanova A.V.,1946, Prilipko L.I.,1970]. Juniper forests also occur in the Northern Caucasus and the Crimea [Grossheim A.A, 1948, Gulisashvili V.Z. et al., 1975, Rubtsov N.I., 1956]. These communities are widespread in the Near and Middle East [Rubtsov N.I., 1956].

Caucasian juniper communities, including Georgian ones are mainly made up of *Juniperus foetidissima* and *J. polycarpos*. Large tracts of these forests occur on the Iori plateau and fragments are found in Shida Kartli – on the Sarkineti and Armazi ranges (Mtskheta-Shiomghvime and Mtskheta-Dzegvi vicinity). In Georgia the juniper forests grow between 200-800m a.sl.

The typological composition of Georgia's juniper forests is quite diverse, which is due to the diversity of the topographic and edaphic conditions within their range as well as the contact of these forests with various types of vegetation (semi-deserts, phriganoid vegetation, steppe, arid deciduous forests, hemi-xerophilous broad-leaved forests, etc.), human impact, etc. Data concerning the typological composition of Georgia's juniper communities are reported [Khachidze M.N., 1984, Gulisashvili V.Z., et al., 1975, Svanidze M.A., 2001, Kvachakidze R.K., 2001]. However, the information presented in these works does not reflect the entire typological sintaxonomical diversity of Georgia's juniper communities. The data require further research and revision.

The purpose of our research was to determine the typological composition of the juniper communities in their principal range in Georgia – Iori plateau, to find out the phytocoenological structure of associations of the distinguished sintaxons and compare the typological composition and phytocoenological structure of the juniper forests of Iori plateau with those of other regions of the Caucasus.

Materials and methods

Geobotanical and floristic data were obtained by means of field trips undertaken during 1980-1991. Geobotanical descriptions were made on different areas determined according to topographic and edaphic conditions. Separate geobotanical descriptions were summarized in a form of tables that were used to find out the phytocoenological structure and distinguish sintaxons. Associations were distinguished in compliance with standard geobotanical methods [Rabotnov T.A., 1983, Shennikov A.P. 1964, Vasilevich V. Ch., 1985].

Results and discussion

The typological review of the juniper forests of Iori plateau determined on the basis of new data obtained during our geobotanical research are given below.

1. ***Juniperus foetidissima, J. polycarpos* + *Pistacia mutica* + *Jasminum fruticans*.** This is one of the most characteristic and widespread associations of Iori plateau communities of the association grown on north- and north-east-facing slopes of 15-35° inclination covered with brown forest soils of the middle depth. Stands are 6-9m tall. Density of the canopy is 0.5-0.7; however, there can also be found communities with the density of the canopy equal to 0.3-0.5. Two species of juniper: *Juniperus foetidissima* and *J. polycarpos* are the dominant-edificators and *Pistacia mutica* is a co-dominant. Projectional coverage of the understory is about 30-40%. It is 1-3m tall. The understory is constituted by approximately 20 species. The dominants are *Jasminum fruticans* and *Paliurus spina-christi.* Characteristic species are: *Ephedra procera* (frequency of occurrence about 80%), *Juniperus oxycedrus*, *Lonicera iberica* (65%), *Cotoneaster suavis* (55%). The grass cover is quite rich floristically and comprises about 60 species. Characteristic species are: *Achnatherum bromoides* (frequency of occurrence 100%), *Dictamnus caucasicus* (about 90%), *Teucrium nuchense* (80%), *Cleistogenes bulgarica* (65%), *Thalictrum colinum*, *Carex bordzilowskii* (55%). Ephemeral communities do not occur. These communities have been described by us for the first time.

2. ***Juniperus foetidissima, J. polycarpos* + *Pistacia mutica* + *Botriochloa ischaemum*.** Communities of this association occur sporadically. They are spread in the extreme eastern part of Iori plateau in gorges directed towards the river Alazani (Arfadara, Chaibulaki) on slopes of various aspects and about 30° inclination. Density of the canopy is 0.4-0.6. Stands are 5-7m tall. *J. foetidissima* and *J. polycarpos* are the dominant-edificators and *Pistacia mutica* is a co-dominant. Understory is not well formed. Characteristic species are: *Jasminun fruticans* (under crowns of trees), *Paliurus spina-christi*, *Ephedra procera*, *Juniperus oxycedrus. Bothriochloa ischaemum* and *Cleistogenes bulgarica* are typical of the grass cover open places and *Stipa capillata* and *Achnatherum bromoides* are characteristic of the sward under crowns of trees. Communities of this association existing in Georgia have been for the first time described by us.

3. ***Juniperus foetidissima* + *Pistacia mutica* + *Quercus iberica*.** Communities of this association are relatively rare. They are spread in Arfadara and Chaibulaki gorges and grow on slopes of various aspects (north-, north-east- and south-east-facing ones) and about 20-30° inclination, constituted by conglomerates and covered with brown soils of the middle depth.

Density of the canopy is 0.5-0.6 (0.7). *J. foetidissima* is the dominant-edificator and *Pistacia mutica* is a co-dominant. *Quercus iberica* is a characteristic species. The latter usually occurs in a form of wilted trees. Understory is well formed. Projectional coverage of the understory is 25-35%. Characteristic species are: *Paliurus spina-christi*, *Juniperus oxycedrus* (frequency of occurrence 100%), *Lonicera iberica*, *Carpinus orientalis*, *Cerasus microcarpa* (70%). Participation of species characteristic of broad-leafed forests is noteworthy; these species are: *Swida australis, Ligustrum vulgare, Euonymus verrucosa.* Species characteristic of the grass cover are the following: *Achnatherum bromoides, Botriochloa ischaemum, Cleistogenes bulgarica* (frequency of occurrence 100%), *Aegonychon purpureo-caeruleum* (70%). On open places the sward is made up of various perennial herbs. Communities of this association existing in Georgia have been for the first time found and described by us.

4. ***Juniperus foetidissima, J. polycarpos* + *Pistacia mutica* + *Caragana grandiflora***. Communities of the association are spread in the Lekistskali gorge, on massifs of Usakhelo Mta and Mijniskure and partially in the Pantishara gorge on north-facing slopes of 20-30° inclination covered with gypseous soils. The topography is more or less partitioned. The habitat is more xerophilous compared to the previously described associations. *Juniperus foetidissima* and *J. polycarpos* are the dominant-edificators and *Pistacia mutica* is a co-dominant. Density of the canopy is 0.3-0.5. *Caragana grandiflora* dominates over the understory. Projectional coverage is about 30%. The understory comprises: *Ephedra procera*, *Cerasus microcarpa*, *Atraphaxis spinosa*, *Paliurus spina-christi*, *Lonicera iberica*, *Acantholimon fominii.* The third tier is made up of semi-shrubs, dwarf semishrubs and herbs and is floristically rich. Characteristic species are: *Crinitaria villosa, Agropyron pectinatum, Reaumuria alternifolia, Astragalus stevenianus, Stachys fruticulosa, Tulipa eichleri, Iris iberica.* Moss and lichen communities are formed under juniper crowns. Communities of this association existing in Georgia have been for the first time described by us.

5. ***Juniperus foetidissima, J. polycarpos* + *Caragana grandiflora***. Communities of the association are spread in the Lekistskali gorge, on massifs of Usakhelo Mta and Mijniskure and partially in the Pantishara gorge on north-facing slopes of 20-30° inclination and plateaus covered with gypseous soils. *J. foetidissima* and *J. polycarpos* are the dominant-edificators. Density of the canopy is 0.3-0.5. The structure of the understory and sward is similar to the previously described associations.

6. ***Juniperus foetidissima, J. polycarpos* + *Jasminum fruticans* + *musci***. Communities of the association are spread on the ranges of Kumros Mta and Kaladara, particularly, on the upper parts of north-facing slopes of 20-40° inclination covered with thin brown soils. Trees of different ages form the canopy. Density of the canopy is 0.6-0.7. Stands are 6-9m tall. *Jasminum fruticans* dominates over the understory. The shrub makes up 1-1.2m tall impassable clumps. The following species occur as individual shrub in the undersory: *Lonicera iberica*, *Cerasus incana, Paliurus spina-christi*, *Ephedra procera.* The third tier is made up of mosses (*Tortula ruralis, Pleurochaeta squarrosa, Thuidium abietinum, Hipnum cupressiforme*). Lichen cushions (*Cladonia pocillum, C. foliacea, C. rangiformis*) are formed among mosses. Degree of coverage of mosses and lichens amounts to 95%. There is no grass cover, only individual plants of *Dictamnus caucasicus* and *Astragalus verticillatus* occur in this community.

7. ***Juniperus foetidissima, J. polycarpos* + *mixtofruticosa***. Communities of the association are spread in Chachuna-Chatma, Katari, Pantishara, Mamachai and other gorges. They occur on eroded slopes of 30-40° inclination, on solid limestone rock, where rarely can be found only remnants of soil cover. Communities are open. *Juniperus foetidissima* and *J. polycarpos* are the dominants. Presence of individual wilted trees of *Pistacia mutica* is a feature of these communities (frequency of occurrence 100%). Density of the canopy is 0.2-0.4. Stands are 2-4m tall. Understory is open; however, it is rich floristically and comprises about 15 species. These

species are: *Paliurus spina-christi*, *Cotynus coggygria, Berberis iberica, Cotoneaster multiflorus, Ephedra procera, Juniperus oxycedrus* (frequency of occurrence 100%), *Rhamnus pallassii* (about 80%), *Atraphaxis spinosa, Astragalus caucasicus, A. microcephalus* (50%). The third tier is rich floristically, although rather open. Characteristic species are: *Bothriochloa ischaemum, Stipa caspia, Thymus tiflisiensis, Teucrium polium* (frequency of occurrence 100%), *Teucrium nuchense, Pimpinella aromatica* (about 80%), *Euphorbia sequierana, Rubia iberica, Lappula barbata, Astragalus stevenianus, Ziziphora serpillacea, Fumana procumbens* (50%). We suppose that the association is of secondary origin and it is formed in consequence of the human impact (from the juniper community with *Pistacia mutica* and understory made up of *Jasminum fruticans* and *Paliurus spina-christi*); however, at present its phytocenological structure is already well formed and stable.

8. ***Juniperus foetidissima* + *Carpinus orientalis*.** Communities of the association are rare and occur in relatively humid places. They are spread in the Datviskhevi, Mamachai and Chaibulaki gorges and grow along the thalwegh a gorge forming a line of 20-40m wide on north-facing slopes of 20-30° (rarely 50°) of inclination constituted by conglomerates and sandstone and covered with brown forest soil. They can be found on weathered limestone too. Density of the canopy is 0.4-0.6. *Juniperus foetidissima* is the dominant-edificator. Individual plants of *Juniperus polycarpos* and *Pistacia mutica* admix the community. *Carpinus orientalis* dominates over the understory. Characteristic species are: *Paliurus spina-christi, Cotoneaster integerrimus, Juniperus oxycedrus* (frequency of occurrence 100%), *Lonicera iberica, Ligustrum vulgare, Euonymus verrucosa, Cerasus microcarpa, Ephedra procera* (65%). The following species are characteristic of the grass cover: *Dictamnus caucasicus* (100%), *Achnatherum bromoides, Aegonychon purpureo-caeruleum, Tragopogon tuberosus, Thalictrum colinum, Thymus tiflisiensis* (65%). The communities growing on weathered sandstone are open and floristically poor. The communities of the described association existing in Georgia have been for the first time described by us.

9. ***Juniperus polycarpos* + *Stipa lessingiana, Festuca sulcata, Bothriochloa ishaemum***. Communities of the association are relatively rare. They are spread around the lower part of the river Iori on the south-facing side of the Kotsakhura range, namely, on slopes of 30-45° inclination and flat ridges with skeleton sandy substrate. Density of the canopy is 0.3-0.5. *Juniperus foetidissima* is the dominant-edificator. Occasionally *Juniperus polycarpos* and *Pistacia mutica* are present as co-dominants. The understory is constituted by *Paliurus spina-christi* (which sometimes dominates over the understory), *Ephedra procera, Jasminum fruticans, Juniperus oxycedrus, Astragalus microcephalus, Atraphaxis spinosa,* etc. Projectional coverage of the sward is 30-40%. The dominants are: *Festuca sulcata, Stipa lessingiana, Bothriochloa ishaemum.* Characteristic species are: *Teucrium polium, Agropyron pectinatum* (The description of the association communities is given on the basis of unpublished materials rendered by G. Arabuli).

Thus, we distinguish nine associations with clear phytocoenological structure and distribution ranges among Iori plateau juniper communities.

The typological structure of the arid juniper forests spread on Iori plateau is similar to that of the juniper forests of the Sheki plateau and Bozdaghi foothills [Gulisashvili V.Z. et al., 1975, Prilipko L.I., 1970]. This mainly due to the similarity of the physico-geographical and geomorphological parameters of the noted regions of Azerbaijan and Iori plateau. The juniper forests of Iori plateau are geobotanically different from those of the southern part of the South Caucasus – Armenia [Ivanova A.V., 1946].

Vascular plants constituting the juniper forests of Iori plateau are given in the table below. All the species recorded at least once in a geobotanical description enter into the list. The associations are arranged according to their order in the text.

Species	Association								
	1	2	3	4	5	6	7	8	9
Trees									
1. *Acer ibericum*	–	–	+	–	–	–	–	+	–
2. *Celtis caucasica*	–	–	–	–	–	–	–	+	–
3. *Fraxinus excelsior*	–	–	–	–	–	–	–	+	–
4. *Juniperus foetidissima*	+	+	+	+	+	+	+	+	+
5. *J. polycarpos*	+	+	+	+	+	+	+	+	–
6. *Pistacia mutica*	+	+	+	+	+	+	+	+	+
7. *Quercus iberica*	–	–	+	–	–	–	–	+	–
Shrubs									
1. *Acantholimon fominii*	–	–	–	+	+	–	–	–	–
2. *Astragalus caucasicus*	+	–	–	–	–	–	–	+	–
3. *A. microcephalus*	+	–	–	–	–	–	–	+	+
4. *Atraphaxis caucasica*	+	–	–	–	–	–	–	–	–
5. *A. spinosa*	–	+	–	+	+	–	+	–	+
6. *Berberis iberica*	+	+	–	–	–	–	+	+	+
7. *Caragana grandiflora*	+	+	+	+	+	–	+	–	+
8. *Carpinus orientalis*	–	–	+	–	–	–	–	+	–
9. *Cerasus incana*	+	–	–	–	–	–	–	+	–
10. *C. microcarpa*	+	+	+	+	+	–	+	+	–
11. *Colutea cilicica*	–	–	+	–	–	–	–	–	–
12. *C. orientalis*	+	+	–	–	–	–	+	–	–
13. *Cotynus coggygria*	+	–	+	–	–	–	+	+	+
14. *Cotoneaster integerrimus*	–	–	+	–	–	–	–	+	–
15. *C. meyeri*	+	–	–	–	–	–	+	–	–
16. *Ephedra procera*	+	+	–	+	+	+	+	+	+
17. *Evonimus verrucosa*	+	–	–	–	–	–	–	+	–
18. *Ligustrum vulgare*	+	–	+	–	–	–	+	+	–
19. *Lonicera iberica*	+	–	+	–	–	+	+	+	–
20. *Jasminum fruticans*	+	–	+	–	–	+	+	+	+
21. *Juniperus oxycedrus*	+	+	+	+	+	–	+	+	+
22. *Paliurus spina-christi*	+	+	+	–	–	+	+	+	+
23. *Punica granatum*	–	+	–	–	–	–	–	–	–
24. *Rosa canina*	–	–	–	–	–	–	–	+	–
25. *Swida australis*	–	–	+	–	–	–	–	+	–

Semishrubs and dwarf semishrubs

1.	*Artemisia fragrans*	–	–	–	–	–	–	+	–	+
2.	*Capparis herbacea*	–	+	–	–	–	–	–	–	–
3.	*Kochia prostrata*	+	–	–	+	+	–	–	–	+
4.	*Noaea mucronatha*	–	–	–	–	–	–	+	+	+
5.	*Reaumuria alternifolia*	–	+	–	+	+	–	–	–	–
6.	*Salsola dendroides*	–	–	–	+	+	–	–	–	–
7.	*S. ericoides*	–	–	–	+	+	–	–	–	–
8.	*S. glauca*	–	+	–	–	–	–	–	–	–
9.	*S. nodulosa*	–	–	–	+	+	–	–	–	–
10.	*Stachys fruticulosa*	+	+	+	+	+	–	+	+	+
11.	*Teucrium nuchense*	+	–	–	–	–	–	+	+	–
12.	*T. polium*	+	+	–	–	–	–	+	–	+
13.	*Thymus tiflisiensis*	+	–	+	–	–	–	+	+	–
14.	*Ziziphora serpillacea*	–	–	–	–	–	–	+	–	–
15.	*Fumana procumbens*	–	–	–	–	–	–	+	–	–

Herbs

1.	*Achillea nobilis*	+	–	–	–	–	–	–	–	–
2.	*Achnatherum bromoides*	+	+	+	–	–	–	–	+	–
3.	*Aegilops cylindrica*	–	+	–	–	–	–	+	–	–
4.	*Aegonychon purpureo-caeruleum*	+	–	+	–	–	–	–	+	–
5.	*Aethionema carneum*	–	–	–	–	–	–	–	+	–
6.	*Agropyron pectinatum*	–	–	–	+	+	–	–	–	+
7.	*Allium rubelum*	–	+	–	+	–	–	+	–	–
8.	*Alyssum calycinum*	–	–	–	–	–	–	–	–	+
9.	*Alyssum turkestanicum*	–	–	–	–	–	–	+	–	–
10.	*Amberboa glauca*	–	–	–	+	+	–	–	–	–
11.	*Arabidopsis thaliana*	+	–	–	+	+	–	+	–	–
12.	*Arenaria serpyllifolia*	+	–	–	–	–	–	+	–	–
13.	*Asparagus caspicus*	+	–	–	+	–	+	–	–	–
14.	*A. verticillatus*	–	–	–	–	–	–	–	+	–
15.	*Asperula arvensis*	–	–	–	–	–	–	–	+	–
16.	*A. humifusa*	+	–	–	–	–	–	–	–	–
17.	*Astragalus brachycarpus*	+	–	–	–	–	–	–	–	–
18.	*A. sphaerocephalus*	–	–	–	–	–	–	+	–	–
19.	*A. stevenianus*	–	+	–	+	–	–	+	–	–
20.	*Botriochloa ischaemum*	+	+	+	+	+	–	+	–	+

21. *Bromus japonicus*	–	+	–	–	–	–	+	–	–
22. *Campanula hohenackeri*	+	–	–	–	–	–	–	–	–
23. *Carex bordzilowskii*	+	–	–	–	–	–	+	–	–
24. *Cephalaria media*	–	–	–	–	–	–	+	–	–
25. *Cerastium glutinosum*	+	–	–	–	–	–	–	–	–
26. *Cleistogenes bulgarica*	+	+	+	+	–	–	+	+	–
27. *Clypeola jonthlaspi*	–	–	–	–	–	–	+	–	–
28. *Crinitaria villosa*	+	–	–	+	+	–	–	–	–
29. *Dactylis glomerata*	+	–	–	–	–	–	–	–	–
30. *Daucus carota*	–	–	–	–	–	–	+	–	–
31. *Dictamnus caucasicus*	+	–	–	–	–	+	+	+	–
32. *Erodium cicutarium*	–	–	–	–	–	–	+	–	–
33. *Erysimum repandum*	+	–	–	–	–	–	+	–	–
34. *Euphorbia seguierana*	–	–	–	–	–	–	+	–	–
35. *Falcaria vulgaris*	+	–	–	+	+	–	–	–	–
36. *Festuca sulcata*	+	–	–	–	–	–	–	–	+
37. *Filipendula vulgaris*	+	–	–	–	–	–	–	+	–
38. *Fragaria viridis*	–	–	–	–	–	–	–	+	–
39. *Fylago pyramidata*	–	–	–	–	–	–	+	–	–
40. *Gagea chlorantha*	–	+	–	–	–	–	+	–	–
41. *Galium tenuissimum*	+	–	–	–	–	–	–	–	–
42. *G. verum*	+	–	–	+	+	–	+	+	–
43. *Geranium tuberosus*	+	–	–	–	–	–	–	–	–
44. *Gladiolus italicus*	–	+	–	+	–	–	–	+	–
45. *Helianthemum lasiocarpum*	–	–	–	–	–	–	+	–	–
46. *H. salicifolium*	–	–	–	–	–	–	+	–	–
47. *Holosteum glutinosum*	+	–	–	–	–	–	–	–	–
48. *Hypericum perforatum*	+	–	–	–	–	–	–	–	–
49. *Inula britanica*	+	–	–	–	–	–	–	+	–
50. *I. germanica*	+	–	–	–	–	–	+	–	–
51. *Iris iberica*	–	–	–	+	+	–	–	–	–
52. *Iuno caucasica*	–	+	–	–	–	–	–	–	–
53. *Iurinea arachnoidea*	–	–	–	–	–	–	–	+	–
54. *Koeleria cristata*	+	–	–	–	–	–	+	–	–
55. *Lappula barbata*	+	–	–	–	–	–	+	–	–
56. *Limonium meyeri*	–	–	–	–	–	–	–	–	+

57. *Linum austriacum*	+	–	–	–	–	–	–	–	–
58. *L. tauricum*	–	+	–	–	–	–	–	–	–
59. *Lolium rigidum*	–	+	–	–	–	–	–	–	–
60. *Melica transsilvanica*	+	–	–	–	–	–	–	–	–
61. *Melilotus albus*	–	+	–	–	–	–	–	–	–
62. *M. officinalis*	–	+	–	–	–	–	–	–	–
63. *Nonea setosa*	–	–	–	–	–	–	–	–	+
64. *Onobrychis cyri*	+	+	–	–	–	–	+	+	–
65. *O. kachetica*	–	–	–	–	–	–	+	–	–
66. *O. komarovii*	–	+	–	–	–	–	+	–	–
67. *Papaver arenarium*	–	–	–	–	–	–	+	–	–
68. *Phleum phleoides*	+	–	–	–	–	–	–	+	–
69. *Phlomis pungens*	+	–	–	–	–	–	–	–	–
70. *Ph. tuberosus*	+	–	–	–	–	–	–	–	–
71. *Pimpinella aromatica*	+	–	–	–	–	–	+	–	–
72. *Poa bulbosa var. vivipara*	–	–	–	–	–	–	+	–	–
73. *Polygala transcaucasica*	+	–	–	–	–	–	–	–	–
74. *Potentilla recta*	+	–	–	–	–	–	–	–	–
75. *Prangos ferulacea*	–	–	–	+	+	–	–	–	–
76. *Psephellus carthalinicus*	+	–	–	–	–	–	–	–	–
77. *Pyrethrum corimbulosum*	+	–	–	–	–	–	–	+	–
78. *Rostraria glabriflora*	–	–	–	–	–	–	+	–	–
79. *Rubia tinctorum*	+	–	+	–	–	–	+	–	–
80. *R. transcaucasica*	–	–	–	–	–	–	+	–	–
81. *Rumex tuberosus*	+	–	–	–	–	–	–	+	–
82. *Salvia nemorosa*	+	–	–	–	–	–	–	–	–
83. *Schismus arabicus*	–	–	–	–	–	–	+	–	–
84. *Scleranthus annuus*	–	–	–	–	–	–	+	–	–
85. *Sedum hispanicum*	–	–	–	–	–	–	+	–	–
86. *Serratula biebersteiniana*	+	–	–	–	–	–	–	–	–
87. *Sideritis montana*	–	–	–	–	–	–	+	–	+
88. *Silene cyri*	–	–	–	–	–	–	+	–	–
89. *S. italica*	+	–	–	–	–	–	–	–	–
90. *Stachys atherocalyx*	+	–	–	–	–	–	–	+	–
91. *Stipa capillata*	+	+	–	–	–	–	–	–	+
92. *S. caspia*	–	+	–	+	+	–	+	+	–

93. *S. lessingiana*	+	–	–	+	+	–	–	–	+
94. *Stipa pulcherrima*	+	–	–	–	–	–	–	–	–
95. *S. tirsa*	–	–	–	+	+	–	–	–	–
96. *Taraxacum praticola*	–	–	–	–	–	–	–	+	–
97. *Thalictrum colinum*	+	–	–	–	–	–	–	+	–
98. *Thlaspi perfoliatum*	+	–	–	+	+	–	–	–	–
99. *Torularia torulosa*	+	–	–	–	–	–	+	+	–
100. *Tragopogon graminifolius*	–	+	–	–	–	–	–	–	–
101. *T. tuberosus*	+	–	–	–	–	–	+	+	–
102. *Trisetum rigidum*	–	–	–	–	–	–	+	–	–
103. *Tulipa eichleri*	–	–	–	+	+	–	–	–	–
104. *Veronica multiflora*	+	–	–	–	–	–	–	–	–
105. *Vinca herbacea*	+	–	–	–	–	–	–	–	–
106. *Viola alba*	–	–	–	–	–	–	–	+	–
107. *V. kitaibeliana*	–	–	–	–	–	–	–	+	–
108. *Ziziphora capitata*	–	–	–	–	–	–	+	–	–
109. *Zygophyllum fabago*	–	+	–	–	–	–	–	–	–

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ივრის ზეგნის ღვიიანების ტიპოლოგია

ლაჩაშვილი ნ., ხაჩიძე მ., იაშაღაშვილი კ.

საქართველოს მეცნიერებათა აკადემიის ნ. კეცხოველის სახელობის ბოტანიკის ინსტიტუტი

(მიღებულია 12.01.2004)

რეზიუმე

შესწავლილია ივრის ზეგნის (აღმოსავლეთ საქართველო) ღვიიანი ტყეების (*Junipereta; Juniperus foetidissima, J. polycarpos*) ტიპოლოგიური შემადგენლობა. გამოყოფილია 9 ასოციაცია. მათგან 5 ასოციაცია საქართველოსთვის პირველად არის აღწერილი. მოცემულია თითოეული ასოციაციის გავრცელების კანონზომიერებანი და მოკლე დიაგნოსტიკური დახასიათება. ივრის ზეგანზე გავრცელებული ღვიიანები თავისი ტიპოლოგიური შემადგენლობითა და ფიტოცენოლოგიური სტრუქტურით ახლოს დგას მუზობელი შექის ზეგნისა და ბოზდაღის მთისწინების ღვიიან ტყეებთან, ამავე დროს განსხვავებულია სამხრეთ კავკასიის სამხრეთი ნაწილის (სომხეთი) ღვიიანებისაგან.

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Cytology

THE INFLUENCE OF WHITE RAT PROTEIN FACTOR ON TRANSCRIPTIONAL ACTIVITY OF NORMAL AND TRANSFORMED CELLS

[1]DZIDZIGURI D., [1]ASLAMAZISHVILI T., [1]CHKHOBADZE M., [3]KHORAVA P., [2]CHIGOGIDZE T., [2]MANAGADZE L.

[1]Department of Cytology, Histology and Development Biology, Laboratory of Development Biology, Iv. Javakhishvili Tbilisi State University,
[2] A. Tsulukidze National Center of Urology,
[3]National Center of Oncology, Tbilisi, Georgia

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Abstract

The comparative study of influence of protein factor obtained from kidney cells of white rat on the genes expression in tumor cells nuclei of different tissue has been performed. It was established, that the tissue specificity of protein factor is preserved in case of neoplastic cells. In particular, the transcriptional activity of nuclei isolated from the cells of kidney-cellular cancer of human and Erlich ascitic carcinoma cells (epithelial cell) of white mice, is suppressed by 40% under the influence of kidney protein factor. At the same time the activity of RNA synthesis of cells nuclei of mice connective tissue sarcoma (M1) remains constant. It was also found, that the accessibility of target genes for protein factor is limited on the level of nuclear envelope in nuclei of epitheliocytes of both distant from cancer area (human post operational materials) and of intact kidney of mice with Erlich ascitic carcinoma.

Key words: protein factor, tumor cells, transcription, tissue specificity.

Introduction

The kidney cells of intact adult rat contain thermostable protein, which inhibits growth of homologous cells. Its effect is realized on the level of genes primary activation. The investigations carried out on the different species have shown that this factor is tissue-specific but does not reveal species-specificity [Dzidziguri D., et al., 2002; Giorgobiani N., et al, 1999]. It is also estimated that protein factor suppresses RNA-synthesis in nuclei of human tumor cells and does not influence the transcriptional activity of cells distant from tumor cells. This may be caused by restriction of accessibility of the target genes tumor cells [Mepharishvili A., et al., 2002]. Therefore, the goal of the proposed work is to study the influence of protein factor of kidney cells of white rat on gene expression in tumor cells nuclei of different tissues.

Materials and methods

The cells of white rat Erlich ascitic carcinoma and cells of M1 sarcoma (supplied from National Center of Oncology), also the post operation material of human origin (derived from A. Tsulukidze National Center of Urology) were used in experiments. The synthesis of RNA was evaluated by using ^{14}C-UTP [Dzidziguri D., et al., 1994]. The method of alcoholic extraction was used for the isolation of proteins from adult rat kidney tissue. The thermal processing of protein fraction was realized during 15-20 minutes at 100^{0}C and then the lyophilization was carried out [Tumanishvili G., et al., 1994].

Results and discussions

The comparative study of influence of white rat kidney protein factor on the genes expression in tumor cells of the different tissues was performed. It was established, that the transcriptional activity of nuclei isolated from Erlich ascitic carcinoma cells (epithelial cells) is suppressed approximately by 40% due to influence of rat kidney protein factor (Fig. 1).

Table 1. The influence of white adult rat kidney protein factor on transcriptional activity of various tumor cells nuclei

Type of tumor	Type of cell	Effect
The renal-cell tumor of human kidney	epithelial cells	+
Erlich ascitic carcinoma of white rat	epithelial cells	+
M1 connective tissue sarcoma of rat	spindle-cells	-

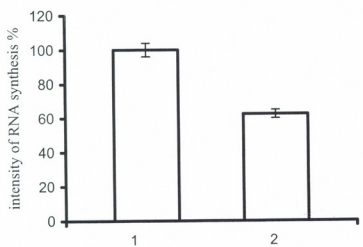

Fig. 1. The influence of rat kidney protein factor on transcriptional activity of Erlich ascitic carcinoma cells nuclei. 1. Control group (Erlich ascitic carcinoma cells nuclei); 2. Erlich ascitic carcinoma cells nuclei + rat kidney protein factor.

The inhibition of transcriptional activity by mentioned factor also takes place in kidney carcinoma cell nuclei. The RNA-synthesizing activity of nuclei isolated from tumor area (post operation material) *in vitro* is decreased by 60% in comparison with control group (Fig. 2).

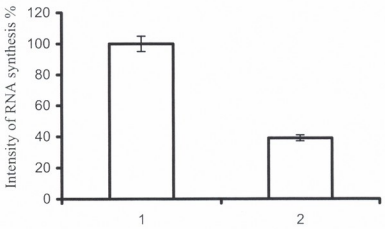

Fig. 2. The influence of rat kidney endogenic factor on the transcriptional activity of human renal tumor cells nuclei. 1. Control group (human renal tumor cells nuclei); 2. Human renal tumor cells nuclei + rat kidney protein factor.

The RNA-synthesizing ability of nuclei in case of M1 sarcoma cells (connective tissue cells) remains unchanged. Hence, it may be concluded, that the specificity of white rat protein factor is revealed even on nuclear level of tumor cells (Fig. 3).

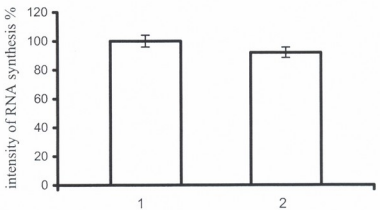

Fig. 3. The influence of rat kidney protein fraction on transcriptional activity of M1 tumor cells nuclei. 1. Control group (M1 tumor cells nuclei); 2. M1 tumor cells nuclei + rat kidney protein factor.

As it was already mentioned the influence of protein factor on transcriptional activity of nuclei in areas distant from tumor is not revealed [Mepharishvili A., et al., 2002]. The analogous results were received in experiments on nuclei of the renal cells of mice with Erlich ascitic carcinoma (Fig. 4). It may be supposed on the basis of literary data that the accessibility of target genes for protein factor is changed in both cases [Gedevanishvili M., et al., 1999]. In addition, the tissue-specificity of white rat protein factor was substantiated by data obtained on the tumor cells. The data presented in the table indicates, that the inhibitory effect of rat kidney protein factor is revealed only on nuclei of epithelial origin.

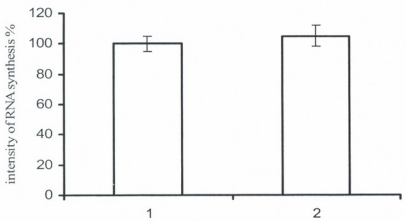

Fig. 4. The change of transcriptional activity of kidney nuclei of mice with Erlich ascitic carcinoma after the influence of rat protein factor. 1. Control group (kidney nuclei of mice with Erlich ascitic carcinoma); 2. Kidney nuclei of mice with Erlich ascitic carcinoma + rat kidney protein factor.

On the basis of received results it may be concluded, that the tissue-specificity of rat kidney protein factor is realized on the terminal stage of cell differentiation.

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ვირთაგვის თირკმლის ცილოვანი ფაქტორის ზემოქმედება ნორმალური და ტრანსფორმირებული უჯრედების ბირთვების ტრანსკრიპციულ აქტიურობაზე

ძიძიგური დ.,[1] ასლამაზიშვილი თ.,[1] ჩხობაძე მ.,[1] ხორავა პ.,[3] ჩიგოგიძე თ.,[2] მანაგაძე ლ.[2]

[1] ციტოლოგიის, ჰისტოლოგიის და განვითარების ბიოლოგიის კათედრა, თვ. ჯავახიშვილის სახელობის თბილისის სახ. უნივერსიტეტი,
[2]უროლოგიის ეროვნული ცენტრი, საქართველო
[3]ონკოლოგიის ნაციონალური ცენტრი, საქართველო

(მიღებულია 10.02.2004)

რეზიუმე

შესწავლილია სხვადასხვა ქსოვილის სიმსივნური უჯრედების ბირთვებში გენების ექსპრესიაზე თეთრი ვირთაგვის თირკმლის ცილოვანი ფაქტორის ზემოქმედება. დადგინდა, რომ ცილოვანი ფაქტორისთვის დამახასიათებელი ქსოვილოვანი სპეციფიკურობა შენარჩუნებულია სიმსივნური უჯრედების შემთხვევაშიც. კერძოდ, ვირთაგვას ცილოვანი ფაქტორის ზემოქმედებით დაახლოებით 40%-ით ითრგუნება ადამიანის თირკმელუჯრედოვანი კიბოს და თეთრი თაგვის ერლიხის ასციტური კარცინომის უჯრედებიდან იზოლირებული ბირთვების ტრანსკრიპციული აქტიურობა, მაშინ როდესაც თაგვის შემაერთებელქსოვილოვანი სარკომის (M1) უჯრედების ბირთვების რნმ-მასინთეზირებელი აქტიურობა უცვლელი რჩება. მიღებული შედეგებიდან გამომდინარე, შეიძლება ვიფიქროთ, რომ თირკმლის ცილოვანი ფაქტორისთვის ბირთვის გარსის დონეზე შეზღუდულია სამი'ხნე გენების მიღწევადობა როგორც სიმსივნური კერიდან დაშორებული (ადამიანის პოსტოპერაციული მასალა), ასევე ერლიხის ასციტური კარცინომით დაავადებული თაგვის ინტაქტური თირკმლის ეპითელიოციტების ბირთვებში.

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Ecology

FAUNISTIC COMPLEXES OF VINEYARD PESTS IN VINE-GROWING REGIONS OF GEORGIA

ABASHIDZE E.

Department of Ecology, Iv. Javakhishvili Tbilisi State University

(Received February 27, 2004)

Abstract

The distribution of noxious faunistic complex of vineyards has been studied in different vine-growing regions of Georgia. Based on the natural-climatic peculiarities and administrative division of Georgia the vine-growing regions were united in 5 zones and 8 subzones. Faunistic complex of vineyard pests of these zones, their distribution and harmfulness were determined. It is established the most harmful and dominant pests for East Georgia are *Lobesia botrana Schiff, Schizotetranychus pruni (Oudem.)* and *Neopulvinaria innumerabilis (Rathvon)*. In the West Georgia the most dangerous species are *Theresia ampelophaga, Schysotetranychus pruni, Pseudococcus viburni* (*Signoret*). Last years the vineyards of Kakheti were injured very intensively by *Calliptamus italicus*, *Dociostaurus maroccanus* (Dedoplistskaro) and bush crickets.

Key words: faunistic complex, vineyard, vine-growing zones, pests, dominant species.

Introduction

Historically Georgia is divided on vineyard regions depending on different climatic peculiarities and vine sorts [Vakhushti Bagrationi, 1973].

Ecosystem ability to resist negative influence of environment is determined by its structure and biological peculiarities of organisms forming these ecosystems. Vineyard ecosystem as artificial ecosystems created by anthropogenic activity consists of complex of interrelating organisms.

Study and assessment of natural – economic peculiarities of different regions, climatic zones and reaction of plants and associated pests promotes to work out differentiated approach to plant protection from noxious phytophages.

Material and methods

Field survey and collecting of the material were carried out in different zones of vine-growing regions of Georgia. Phenology of leading sorts of grapevine, dominant and economically important pests of different zones were established.

The density and harmfulness of the most dangerous species was studied by using periodical calculation method. About 38 species of pests were registered in vineyards (5-7 age) of Georgia, but we indicate only economically important pests which need control measures.

Results and discussion

Based on the administrative and natural-economic division vine-growing regions of Georgia were united in 5 zones and 8 subzones, characterizing of different climatic, ecological conditions, sorts and different faunistic complexes. Phenological-climatic characteristic of different vine-growing zones is given in Table 1.

Table 1. Phenoclimatic assessment of vine-growing zones

N	Zones, Subzones	Leading sort	Dates of grapevine phenophases					t^0C (average)	average year sum of precipitations (mm)
			I phase of III leaf	II blossoming	III ripening	IV technical ripeness	V fall of the leaves		
I	Kakheti								
	1. Inner Kakheti	Rkatsiteli	1.05-10.05	1.04-15.04	15.08-25.08	10.09-25.09	10.11-15.11	10-13	580-1000
	2. Outer Kakheti	Rkatsiteli	5.05-8.05	5.04-20.04	15.08-25.08	10.09-20.09	15.11-20.11	10-12	380-800
II	Kartli								
	1. Middle and lower Kartli	Rkatsiteli (Chinuri et al.)	8.05-10.05	12.04-21.04	15.08-25.08	20.09-30.09	10.11-15.11	10-13	350-600
	2. Upper Kartli	Chinuri	18.05-25.05	20.04-30.04	15.09-20.09	10.10-20.10	20.10-30.10	9-11.5	450-700
III	Imereti								
	1. Upper Imereti	Tsolikauri	1.05-6.05	8.04-15.04	10.09-16.09	15.10-20.10	10.11-15.11	10-14	800-1300
	2. Middle and lower Imereti	Tsolikauri	25.04-30.04	18.05-30.05	10.09-20.09	10.10-25.10	15.11-20.11	13.5-14.5	1200-1500
IV	Racha-Lechkhumi	Tsolikauri	4.05-10.05	5.06-15.06	25.08-30.08	25.10-28.10	5.11-15.11	0-1.0	900-1300
V	Guria-Adjara Samegrelo-Abkhazeti								
	1. Guria-Adjara	Tsolikauri	16.04-24.04	8.06-10.06	15.09-20.09	20.09-3.10	20.11-30.11	10-14	1500-2700
	2. Samegrelo-Abkhazeti	Tsolikauri	20.04-25.04	8.06-20.06	25.08-5.09	25.10-30.10	15.11-25.11	9,5-14,5	1300-2100

Many of grapevine phytophages is connected with the plant and accompanied it in the different zones. There are principal difference in rate of dominating. As a result of carried out researches the complex of economically important and dominant pest for each zone was established.

The first zone – Kakheti is main vine-growing zone of Georgia. The climatic conditions are favorable for grapevine cultivation. The following pests injure vineyards of this zone: *Lobesia botrana Schiff., Neopulvinaria innumerabilis (Rathvon), Schizotetranychus pruni Oudem, Eriophyes vitis Nal., Planococcus ficus (Signoret).* Last years *Calliptamus italicus L., Dociostaurus*

maroccanus Thunb., and bush crickets – *Tettigonia caudate* and *T. viridissima* seriously injured vineyard in Gurjaani, Sagarejo and Dedopliskaro. Leading sort of this zone is Rkatsiteli.

The second zone – Kartli; because of low temperatures grapevine is not always able to ripen. The following pests are recorded: *Schyzotetranychus pruini, Eriophyes vitis, Brevipalpus levisi,* sometimes *Theresia ampelophaga.* Principal sorts are Rkatsiteli, Chinuri.

The third zone – Imereti is the second leading zone of vine-growing of Georgia. There are good conditions of grapevine growing. Leading sorts are Tsolikauri, Tsitska, associated pests are: *Theresia ampelophaga, Pseudociccus viburni, Schyzotetranychus pruni* and *Eriophyes vitis.*

In the forth zone – Racha-Lechkhumi grapevine is cultivated mostly in Ambrolauri and Tsageri where produce high quality wine. The leading sorts are Tsolikauri, Alexandrouli. In this zone the following complex of pests is recorded: *Schizotetranychus pruni, Eriophyes vitis, Theresia ampelophaga. Pseudococeus viburni, Neopulvinaria innumerabulis.*

In the fifth zone – Guria-Ajara-Samegrelo-Abhazeti the principal cultures are subtropical cultures and tea. Vine is cultivated in the limited territory. The leading sorts are Tsolikauri, Isabella and Aladasturi (Chokhatauri). In this zone grapevine is seriously injured by different deceases. Complex of pests are *Shyzotetranychus pruni, Brevipalpus lewisi* and *Pseudococcus viburini.*

Study of the noxious fauna of grapevine showed the diversity of faunistic complex and harmfulness of pests depends on climatic factors and sort peculiarities.

The most dangerous pest of vineyards of Georgia is *Lobesia botrana*. Its harmfulness changes from year to year and by zones.

Lobesia botrana is recorded in all vine-growing zones, but it has economic importance in Kakheti zone where it is dominant species of fauna [Abashidze E., 1990].

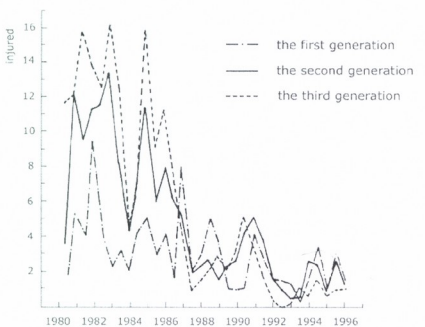

Fig. 1. Dynamic of vine injury by *Lobesia botrana* (Kakheti region)

Specialized pest *Theresia ampelophaga* injured only grapevine. It is prevalent in vineyards of Imereti and occasionally in Kartli, Racha and in Samegrelo-Abkhazeti.

Schizotetranychus pruni is the most dangerous species from Acarina complex and the principal pest of vineyards of Kakheti, Kartli and Imereti [Malchenkova N., et al., 1980].

Increasing of number and harmfulness of Schizotetranychus pruni last years is stipulated by intensive use of chemical pesticides against *Lobesia botrana* (Fig. 1). As a result more sensitive acariphagues killed and *Schyzotetranychus pruni* quickly developed resistant population.

Several species of coccids are recorded in vineyards of Georgia. The cottony scale *Neopulvinaria innumerabilis (Rathvon)* and the mealybug *Planococcus ficus (Signoret)* are the most serious pests of vineyards in dry regions of East Georgia.

In the west Georgia *Pseudococcus viburni (Signoret)* is the noxious species. Other coccids are rare and have no economic importance [Yasnosh V., et al., 2002]. In 1997-2000 there are serious damages of vineyards of Kakheti caused by *Calliptanus italicus, Dociostaurus maroccanus* and bush crickets. Study of distribution of the complexes of noxious pests in different zones of vinegrowing promotes working out the differentiated system of vineyard protection.

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ვაზის მავნე ფაუნის კომპლექსები საქართველოს მევენახეობის რაიონებში

აბაშიძე ე.

კოლეგიის კათედრა, ილ. ჭავჭავაძის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 27.02.2004)

რეზიუმე

შესწავლილია ვაზის მავნე ფაუნის კომპლექსები საქართველოს სხვადასხვა კლიმატურ ზონაში. დადგენილია ვაზის ფენოკლიმატური კანონზომიერებანი და მავნე ფიტოფაგების დომინანტი სახეობები, მათი მავნეობის ხარისხი ზონების მიხედვით, რაც საშუალებას იძლევა დამუშავდეს ვაზის დაცვის დიფერენცირებული სისტემა.

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Genetics

THE REGULARITIES OF HEAVY METALS DISTRIBUTION IN POTABLE AND IRRIGATION WATERS AND IN SOILS OF KVEMO BOLNISI AND PROTEIN ANALYSIS OF SECOND CYCLE LINES SEEDS OF MAIZE

MAMULASHVILI L., ZVIADADZE U., NASKIDASHVILI P.

Department of Genetics and Selection, Georgian State Agriculture University

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Abstract

Using the Atomic-Absorption Spectroscopy method the quantitative values of heavy metals – Cu, Cd, Pb, Co, Zn, Mn, Sr, Ni, Fe in potable and irrigation waters, as well as in the soils of village Khatissopeli of Bolnisi district were determined. Exceeding their actual contents above the Maximum Admissible Concentrations (MAC) was established. The reasons of pollution of mentioned ecosystems by inorganic toxicants were revealed. Analysis of water-soluble proteins received from the second cycle line seeds of maize grown on polluted soils was carried out by isoelectric focuses method. The changes in protein spectrum in comparison with the control was revealed.

Key words: Heavy metals, potable and irrigation water, soil, heterosis hybrids of maize, water soluble proteins.

Introduction

The industrial and consumption waste represent the direct danger for the normal development of natural cycle of biosphere, since they cause the negative change of natural processes [Ilin V.,1991].

The longtime accumulation of heavy metals in human organism by pollution of vital ecosystems, such as potable and irrigation waters, soils, as well as foodstuffs, causes a number of grave diseases – Anemia, cardiovascular and nervous diseases, cancer, etc. [Bochkov N., 1989].

Hence the grave ecological situation within the Bolnisi region (namely village Khatissopeli), where the experimental site is disposed, the study of heavy toxic metals is an acute problem at present to reveal ecological conformities, which stipulate the pollution of soils and waters by heavy metals.

Analysis of water soluble proteins received from the food products grown on heavy metals polluted soils is an urgent problem, hence the proteins as a product of genes activity can induce

biochemical, morphological-anatomical and physiological changes of genotypes of plant, as well as of humans feeded with polluted products [Konarev V.G., 1998]

Materials and Methods

The investigation objects are the samples, picked out from the water pipe and irrigation canals of village Khatissopeli, as well as the soil samples from the experimental site. The preparation of water samples and further determination of heavy metals contents have been carried out using the Atomic-Absorption Spectroscopy method [Arinushkina A.V., 1982; Slavin V., 1971]. Analogically the extracts from soil samples have been prepared and microcomponental analysis was carried out to establish the quantitative values of active forms of metals in the soil solutions. The analysis was carried out on the spectral photometer C-302, ensuring the high precision of determination in the regime of absorption and emission depending on the kind of metals. In particular, Sr was determined in emission regime; Cu, Cd, Pb, Co, Zn, Mn, Ni and Fe – in absorption regime. Cathodic lamp LSP –1 was used. Obtained data were treated by statistical method [Shcklette H.T. et al., 1984].

In the second series of experiments the second cycle line seeds of maize received from the high heterosis hybrids (Enguri, Kartli 9) grown on heavy metals polluted soils (experimental plot of village Khatissopeli of Bolnisi district) were compared with control seed lines received from unpolluted plots (experimental plot of the Institute of Agriculture, Tserovani, Mtskheta district). Comparative analysis of water soluble proteins was carried out on Phast System (Pharmacia, Swiss). To the extraction area (Tris–HCl 20mM, pH 8.0, 0.1M NaCl) 20 mg powdered seeds of maize was added. Extraction was lasted 2-3 hours at 4^0C. Mixture was centrifuged at 10 000g during 5min. For isoelectric focuses 1-3 µl supernatant was used and carried out on 0.2 mm thickness 5%-polyacrilamide gel in 3-10 pH range.

Results and Discussions

The results of microcomponental analysis of water and soil samples are given in the Table 1.

According to the obtained data, the actual contents of toxic metals in studied objects - soils and natural waters, very often significantly exceed international norms of Maximum Admissible Concentrations (MAC). Namely, in Potable water: Cd – 29, Mn – 1.6 times; in irrigation water: Cd – 41, Mn – 8.3, Pb – 14 times; in soils: Cd – 2.8, Mn – 1.1, Cu – 5.8, Co – 3.4, Zn – 1.5, Ni – 3 times.

The above anomalies are caused mainly by Madneuli Mining Complex of large Copper-Pyrite deposit [Gvakharia V., et al.,1997] and village Khatissopeli and it's surroundings are under this influence. Especially, it concerns the surface and underground waters of the region and it's soils.

The abnormally high contents of especially toxic Cadmium in potable water is alarming. To reveal the real reason of this phenomenon it is necessary to carry out the detailed investigations in the territory. Increased concentration of heavy metals in irrigation waters and in the soils is connected directly with the fact of pollution of the river Mashavera [Gvakharia V., et al., 1997], since the irrigation system of the region functions on the basis of the Mashavera.

The pikes of heavy metals concentration exactly coincide with the periods of the Mashavera pollution from Madneuli dressing plant and from the quarry.

Table 1. The contents of heavy metals in potable and irrigation waters and soils village Khatissopeli

№	Elements	The contents of microelements													
		Water mg/l							soils, mg/kg						
		Potable water				Irrigation water									
		M.A.C.	1	2	3	1	2	3	M.A.C.	1	2	3	4	5	6	7
1.	Cu	1,0	0,096	0,15	0,1	0,33	1,2	0,66	10,0	47,97	62,07	70,02	68,84	59,01	49,11	53,14
2.	Cd	0,001	0,0225	0,037	0,03	0,0275	0,057	0,041	1,0	2,5	2,906	3,1	3,05	2,84	2,6	2,714
3.	Pb	0,03	Trace	0,0014	0,001	0,15	0,94	0,18	20(11)	2,5	5,61	6,4	5,93	4,404	2,7	3,4
4.	Co	0,1	0,06	0,077	0,091	0,08	0,12	0,093	4,5	5,2	19,07	25,5	22,01	15,01	8,3	12,02
5.	Zn	1,0	0,062	0,11	0,084	0,28	0,73	0,43	50(36)	68,0	80,4	88,0	85,0	78,1	73,1	75,4
6.	Mn	0,1	0,14	0,171	0,16	0,75	0,91	0,84	100	100	114,7	120	119,5	109,2	104,1	106,2
7.	Sr	2,0	0,083	0,104	0,098	0,05	0,14	0,073	150	4,83	14,09	20,24	18,15	11,5	5,303	8,17
8.	Ni	0,1	0,04	0,09	0,06	0,076	0,11	0,083	2,0	5,3	6,0	6,9	6,6	5,909	5,404	5,67
9.	Fe	0,3	0,1	0,21	0,18	0,21	0,3	0,27	150	25,0	27,13	28,0	27,8	26,88	25,4	26,3

The effect of heavy metals on the maize lines is shown on the figures which represent the spectrums of isoelectric focuses of water soluble proteins of maize seeds lines: "Enguri 11/28" (Fig. 1) and "Kartuli 9 15/36" (Fig. 2), grown in Bolnisi and Mtskheta plots. The differences between these samples were revealed: In Bolnisi samples alkali proteins (expressed as A) disappeared, while neutral proteins (expressed as B) appeared.

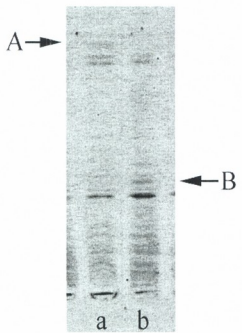

Fig. 1. Spectrum of isoelectric focuses of water soluble proteins of "Enguri I_4 11/28" grown on a) Mtskheta plot and b) Bolnisi plot

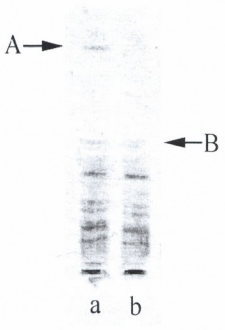

Fig. 2 Spectrum of isoelectric focuses of water soluble proteins of "Kartuli 9 I_4 15/36" grown on a) Mtskheta plot and b) Bolnisi plot

Observed differences of proteins spectrum indicate to the changes of genetical constitution of proteins. Hence the protein is the product of activity of gene or genes group the changes of protein point to the changes of gene or genes group. High concentration of heavy metals of the investigated plot is reflected on the changes of the genotype of maize.

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ძვიმო ბოლნისის სასმელ-საირიგაციო წყლებსა და ნიადაგებში მძიმე მეტალების განაწილების კანონზომიერებები და ამ ნიადაგზე მიღებული სიმინდის მეორე ციკლის ხაზების თესლის წყალში ხსნადი ცილების ანალიზი

მამულაშვილი ლ., ზვიადაძე უ., ნასყიდაშვილი პ.

გენეტიკისა და სელექცია-მეთესლეობის კათედრა, საქართველოს აგრარული უნივერსიტეტი

(მიღებულია 29.08.2003)

რეზიუმე

ატომურ-აბსორბციული სპექტროსკოპიის მეთოდით განსაზღვრულია ბოლნისის რაიონის სოფ. ხატისოფლის სასმელ და ხარისგაციო წყლებში და ნიადაგებში მძიმე მეტალების – Cu, Cd, Pb, Co, Zn, Mn, Sr, Ni, Fe შემცველობის რაოდენობრივი მაჩვენებლები. დადგენილია მათი ფაქტობრივი კონცენტრაციების აღმატების ხარისხი ზღვრულად დასაშვებ კონცენტრაციებთან (ზ.დ.კ.) შედარებით, გამოვლენილია აღნიშნული ეკოსისტემების არაორგანული ტოქსიკანტებით გაჭუჭყიანების კონკრეტული მიზეზები. იზოელექტრული ფოკუსირების მეთოდით ჩატარებულია ამ ნიადაგებზე მიღებული სიმინდის მეორე ციკლის ხაზების წყალში ხსნადი ცილების ანალიზი. ნაჩვენებია ცვლილებები ცილების სპექტრში კონტროლთან შედარებით.

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Genetics

THE MUTAGENIC EFFECT OF HEAVY METAL SALTS ($PB(NO_3)_2$, $CdCl_2$, $NiCl_2$) IN DIFFERENT COMBINATIONS ON DROSOPHILA

NIBLADZE N., JMUKHADZEN., TADUMADZE N., DADUNASHVILI E.

Department of Genetics, Iv. Javakhishvili Tbilisi State University

(Received October 20, 2003)

Abstract

Combinative activities of low- and non- mutagenic doses of heavy metal salts ($Pb(NO_3)_2$, $CdCl_2$, $NiCl_2$) have been investigated in Drosophila by the method of "Meller-5" assessing sex-linked recessive lethal and sublethal mutations. All studied combinations showed more or less expressed synergistic effect.

Key words: drosophila, heavy metals, mutations.

Introduction

In recent years increased amount of pollutants in environment caused by various exogenous factors causes growing interest to metals as to potential mutagens, carcinogens or teratogens.

As it is apparent from published data different metals express different mutagenic activities when acting in combinations [Sharma A. et al., 1987]. The effect may be antagonistic, synergistic, or simply additive, depended on different variables.

Material and methods

The goal of the presented work was to study mutagenic effect of three heavy metal salts in combinations on Drosophila. The standard method of "Meller-5" for sex-linked mutations was applied to register the frequencies of recessive lethal and sublethal mutations.

We have defined the lowest mutagenic doses and non-mutagenic doses for studied salts, that for each compounds were found to be 10^{-3} M and 10^{-4} M – respectively. The combinative actions of low- and non-mutagenic doses of compounds were studied. The exposition time lasted for 24 h. The standard nutrient medium served as a solvent.

Results and discussion

As shown in Table 1 (Fig. 1), a well -expressed synergistic effect was observed in the flies treated by 10^{-3} M $Pb(NO_3)_2$ and 10^{-4} M $CdCl_2$. The total number of sex-linked recessive mutations equal to 12,71± 2,17% (4,66± 1,37% - lethal and 8,05± 1,77% - sublethal mutations).

That significantly exceeds the mutagenic effect caused by a single action of 10^{-3} M $Pb(NO_3)_2$ (the total number of mutations – 8,60± 1,86%).

10^{-3} M $Pb(NO_3)_2$ and 10^{-4} M $NiCl_2$ in combinations also revealed synergistic effect but it was less expressed (see Table 1).

Table 1. The frequency of sex-linked recessive lethal and sublethal mutations after treatment with combination of 10^{-3} M $Pb(NO_3)_2$, 10^{-4} M $CdCl_2$ and 10^{-4} M $NiCl_2$.

compounds and combinations	invest crom. numb.	mutation frequency					
		lethal		sublethal		total	
		n	%±m	n	%±m	n	%±m
$Pb(NO_3)_2$ 10^{-3} M+$CdCl_2$ 10^{-4} M	236	11	4,66 ±1,37	19	8,05± 1,77	30	12,71± 2,17
$Pb(NO_3)_2$ 10^{-3} M+$NiCl_2$$10^{-4}$ M	232	9	3,88± 1,27	16	6,90± 1,66	25	10,78± 2,04
$Pb(NO_3)_2$ 10^{-3} M	230	7	3.04±1,13	13	5,65± 2,38	20	8,69 ±1,86
control	276	0	0	0	0	0	0

Table 2. The frequency of sex-linked recessive lethal and sublethal mutations after treatment with combination of 10^{-3} M $CdCl_2$, 10^{-4} M $Pb(NO_3)_2$ and 10^{-4} M $NiCl_2$.

compounds and combinations	invest crom. numb.	mutation frequency					
		lethal		sublethal		total	
		n	%±m	n	%±m	n	%±m
$CdCl_2$ 10^{-3} M+ $Pb(NO_3)_2$$10^{-4}$ M	221	12	5,43 ±1,52	21	9,50± 1,97	33	14,93± 2,39
$CdCl_2$ 10^{-3} M+$NiCl_2$$10^{-4}$ M	228	9	3,95± 1,29	18	7,89± 1,79	27	11,84± 2,14
$CdCl_2$$10^{-3}$ M	215	6	2,79±1,12	8	3,72±1,29	14	6,51±1,68
control	276	0	0	0	0	0	0

Table 3. The frequency of sex-linked recessive lethal and sublethal mutations after treatment with combination of 10^{-3} M $NiCl_2$, 10^{-4} M $Pb(NO_3)_2$ and 10^{-4} M $CdCl_2$

compounds and combinations	invest crom. numb.	mutation frequency					
		lethal		sublethal		total	
		n	%±m	n	%±m	n	%±m
$NiCl_2$$10^{-3}$ M+ $Pb(NO_3)_2$ 10^{-4} M	238	8	3,36 ±1,17	12	5,04± 1,42	20	8,40 ± 1,79
$NiCl_2$ 10^{-3} M+ $CdCl_2$ 10^{-4} M	241	7	2,91± 1,08	11	4,56± 1,81	18	7,47±1,69
$NiCl_2$$10^{-3}$ M	231	4	1,73±0,86	7	3,03±1,13	11	4,76±1,40
control	276	0	0	0	0	0	0

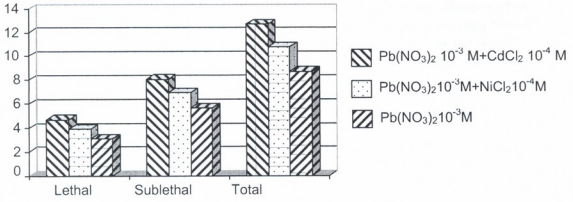

Fig. 1.

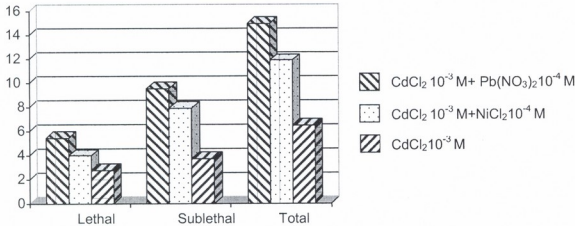

Fig. 2.

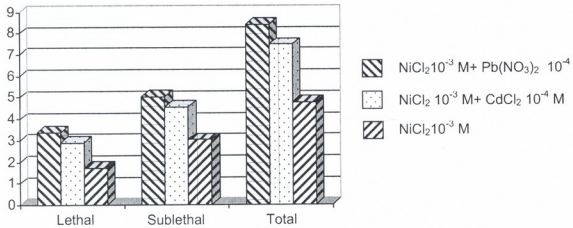

Fig. 3.

As a result of obtained data, Cd and Pb salts showed drastic changes in mutagenicity when the two metals are used in combination with other metals [Dhir H., Sharma A., et al., 1985].

The mutagenic effect of $Pb(CH_3COO)_2$ was reduced by action of $CaCl_2$ in the somatic and germ cells of mice while $Pb(NO_3)_2$ reduced the mutagenic activity of $AgNO_3$ [Ezzat l., 2002]. As to the mechanism of Cd and Pb action, it was found that neither single, nor combined action of noncytotoxic doses of $Pb(CH_3COO)_2$ and $CdCl_2$ induced direct damages in DNA. It was supposed, that the mutagenic effect of these compounds was caused by an oxidative stress, revealed in lipid peroxidation and an increase of free radical levels in the different organs of CD – 1 male mice [Valverde M., Trejo C., Rojas E., 2001].

The analogic data is presented in Table 2 (Fig.2) in the case of 10^{-3} M $CdCl_2$ combination with 10^{-4} M $Pb(NO_3)_2$ and 10^{-4} M $NiCl_2$. These combinations had the most power synergistic effect than other combination of the same salts. The total number of sex-linked recessive mutations (14,93± 2,19% and 11,84± 2,14% - respectively) increased two-fold and more in comparison to the treatment of single 10^{-3} M $CdCl_2$ (6,51± 1,68%).

The total number of sex-linked mutations were redubled in the case of combinatoins - 10^{-3} M $NiCl_2$+10^{-4} M $Pb(NO_3)_2$ and 10^{-3} M $NiCl_2$+10^{-4} M $CdCl_2$ (Table 3, Fig.3). It is known, that Ni has the lower mutagenic properties then Cd and Pb [Fracasso M., et al., 2002; Seoana A., Dulous F., 2001; Wang T., Lee M., 2001]. However, the weak mutagenic activity of Ni was strengthened by the presence of nonmutagenic doses of Cd and Pb.

In conclusion, the combinations of weak- and nonmutagenic doses of heavy metal salts ($Pb(NO_3)_2$, $CdCl_2$ and $NiCl_2$) revealed different levels of synergistic effect in Drosophila.

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მძიმე მეტალთა მარილების ($Pb(NO_3)_2$, $CdCl_2$, $NiCl_2$) კომბინირებული მოქმედების ბენეტიკური ეფექტი დროზოფილაზე

ნიბლაძე ნ., ჯ̌მუხაძე ნ., თადუმაძე ნ., დადუნაშვილი ე.

გენეტიკის კათედრა, ი.ჯ̌ავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 20.10.2003)

რეზიუმე

შესწავლილია მძიმე მეტალთა მარილების - $Pb(NO_3)_2$, $CdCl_2$, $NiCl_2$ დაბალ-მუტაგენური და არამუტაგენური დოზების კომბინირებული მოქმედების მუტაგენური ეფექტი დროზოფილაზე. გამოყენებულ იქნა "მელერ-5"-ს სტანდარტული მეთოდი. ყველა გამოკვლეულმა კომბინაციამ გამოავლინა სხვადასხვა ხარისხით გამოხატული სინერგისტული ეფექტი.

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Plant Physiology

IODINE CONTENT IN PLANT SPECIES *URTICA URENS, CHENOPODIUM ALBUM* AND *RUMEX CRISPUS*

ALEXIDZE G.[1], MANGALADZE N.[2], BAKRADZE M.[2]

[1] *Department of Plant Physiology, Iv. Javakhishvili Tbilisi State University,*
[2] *Department of Botany and Ecology, Kutaisi State University*

(Received December 10, 2003)

Abstract

Present study was aimed to reveal plant species enriched with iodine as well as to learn which vegetative organs (leaves or roots) are distinguished by iodine content. Iodine salts were shown to have positive influence on the development of leaves of *Urtica urens* and *Rumex crispus* grown in hydrophonic solution, while roots were not significantly extended compared to control specimen, grown in solution, free from iodine. Luminescent spectrography was used for analysis of iodine content in the roots and leaves of experimental specimen. Higher concentration of iodine was registered in leaves of *Urtica urens* and *Rumex crispus* compared with roots of the same specimen. Obtained data provide us to suggest that plant organs, enriched with iodine can be revealed by the observation of the effects of iodine on plant vegetation. Obviously, plant organs, (leaves in a given case), capable of accumulating iodine in relatively high concentrations, display enhanced vegetation compared with other organs, poor in iodine.

Key words: Plant, iodine content, vegetation

Introduction

Iodine deficient diet remains still problematic all round the world [Van der Haar, 1997] and also in Georgia [Metreveli D. et al., 1996, Sekhniashvili Z., et al., 2000]. Marine plant and animal species provide the richest food in this respect [Sekhniashvili Z., et al., 2000, Torres-Duran et al., 1999]. At the same time, buckwheat and spinach, as well as some citrus plants (among them lemon) contain iodine in relatively higher concentrations compared with other land dwelling plants, studied earlier in connection with iodine content [Sekhniashvili Z., et al., 2000]. The global elimination of iodine deficiency disorders greatly depends on providing people with an iodine enriched food.

Present study is aimed at revealing plant species rich in iodine. Plant species - *Urtica urens*, *Chenopodium album* and *Rumex crispus* were tested on iodine content in regard to their extension over a wide area in Georgia. At the same time, these plants are widely used as a vegetable food by general community, especially by rural dwellers.

In our view, the first step towards the quantitative analysis of iodine content in plants should be aimed at revealing particular organs (roots, leaves, stem or fruits), which could be suspected as containing iodine in the highest concentrations and thus, serve as a target for further spectrographic analysis on iodine content.

Material and Methods

In a first series of experiments, *Urtica urens, Chenopodium album* and *Rumex crispus* (50 control and 50 experimental specimen of each species) were grown in hydrophonic nutritious solution (Table 1). Experimental solution was enriched with iodine salt, while the control one was free of iodine. The length of leaves and roots was registered in April, May, June and July 2000. Hastened growth of leaves and roots in experimental specimen as compared with control objects was considered an indication of the effect of iodine on plant vegetation.

Table 1. Solution content for experimental and control specimen

Salts	Control solution g/l	Experimental solution g/l
$Ca(NO_3)_2$	5.0	5.0
NH_4NO_3	0.2	0.2
$MgSO_4$	0.5	0.5
KCl	0.36	0.36
KNO_3	0.51	0.51
$Fe_2(SO_4)_3$	0.32	0.32
KI		0.028

In the second series of experiment, spectrophotometer Specord M-40 (Carl Zeis, Yena) was used for luminescent spectrographic computed analysis of iodine content in roots and leaves of experimental and control specimen (40 plants of each species). Material for spectrographic analysis was prepared according to well-known methods, widely used in practice [Akkerman Iu., 1964, Chernavina Iu., et al., 1978].

Results and Discussion

As it is shown in Table 2 the length of leaves in experimental plants *Rumex crispus* and *Urtica urens* exceeded that of control specimen. Difference in the length of leaves between experimental and control specimen of *Chenopodium album* was not statistically authentic. At the same time, the intensification of vegetation was evident in May, June and July in *Urtica urens*, while *Rumex crispus* displayed enhanced development of leaves earlier in April. Significant difference in the length of leaves between experimental and control specimen of *Urtica urens* and *Rumex crispus* was registered in April and May. No significant difference in root length between experimental and control specimen was found in plant species under examination.

Evidently, growth of plants in nutritious solution, enriched with iodine salts, leads to the increase in the length of leaves in *Urtica urens* and *Rumex crispus*, while iodine does not influence the development of roots in the same plant species. Presumably, leaves represent the target organs for iodine and thus, should be mostly enriched with iodine as compared with roots.

Table 2. The length of leaves in control and experimental specimen (parameters represent control and experimental specimen respectively)

Plant species	The length of leaves cm (Average of 50 specimen)			
	April	May	June	July
Urtica urens	2.1/3.4	5.7/8.6	8.9/9.5	10.0/10.5
Rumex crispus	2.0/3.9	7.0/9.3	7.1/7.8	7.9/8.3
Chenopodium album	2.7/2.8	7.0/7.9	8.0/8.1	13.1/12.8

Table 3. Iodine content in experimental specimen

Plant species	Iodine content mcg/kg (Average of 40 specimen)			
	April	May	June	July
Urtica urens	190.8	70.0	30.0	40.0
Rumex crispus	140.0	90.0	50.0	50.0
Chenopodium album	80.0	65.0	40.0	40.0

To check this assumption, vegetative organs of experimental specimen of *Urtica urens*, *Chenopodium album* and *Rumex crispus* were analysed on iodine content (Table 3). The higher content of iodine was registered in *Urtica urens* and *Rumex crispus* compared to *Chenopodium album*. At the same time, iodine content in roots was significantly lower compared to leaves in *Urtica urens* and *Rumex crispus*.

Data obtained provide argument for suggestion, that plant organs, enriched with iodine can be revealed by the observation of the effects of iodine on plant vegetation. Obviously, plant organs, (leaves in a given case), capable of accumulating iodine in relatively high concentrations in spring, display enhanced vegetation compared to other organs, poor in iodine. At the same time, iodine content in the leaves of *Urtica urens* and *Rumex crispus* gradually decreased in parallel to plant growth in spring and summer. Presumably, iodine was efficiently used by the leaves for determined processes of growth and metabolism.

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იოდის შედგენილობა მცენარეთა სახეობებში *URTICA URENS, CHENOPODIUM ALBUM* და *RUMEX CRISPUS*

ალექსიძე გ.[1] , მანგალაძე ნ.[2], ბაქრაძე მ.[2]

[1] მცენარეთა ანატომიისა და ფიზიოლოგიის კათედრა, ივ.ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი
[2] ჟუთაისის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.12.2003)

რეზიუმე

წყლის კულტურებში შესწავლილია იოდის ზეგავლენა მცენარეთა ფოთლების და ფესვების ზრდაზე. ნაჩვენებია, რომ საკვებ ხსნარში იოდის დამატება აჩქარებს ჭინჭრის და ღილოს ფოთლების სიგრძეში ზრდას, მაგრამ არ მოქმედებს ფესვების ზრდაზე. ლუმინესცენტური სპექტროგრაფიის მეთოდის გამოყენებით დადგინდა, რომ ამ მცენარეების ფოთლებში იოდის კონცენტრაცია საკმაოდ მაღალია და იმავდროულად, ბევრად აღემატება ფესვებში არსებულ იოდის რაოდენობას. მცენარის ვეგეტაციაზე იოდის ხემოქმედების მიხედვით შეიძლება ვიმსჯელოთ, თუ რომელ ორგანოში არის განსაკუთრებით დიდი რაოდენობით აკუმულირებული იოდი. მცენარეები, რომლებიც გარემოდან აქტიურად ითვისებენ იოდს, სწრაფად უვითარდებათ ფოთლები.

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Plant physiology

ISOPRENE EMITTING ARBOREAL AND GRASSY PLANTS OF GEORGIAN FLORA

TARKHNISHVILI G., LOLADZE T., JAIANI G., PKHACHIASHVILI S., MGALOBLISHVILI M., KHETSURIANI N., NACHKEBIA K., SANADZE G.

Laboratory of Photosynthesis, Iv. Javakhishvili Tbilisi State University

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Abstract

71 species of 24 families (22% of species investigated), with Isoprene photobioemission ability were revealed. Among them, 61 species are arboreal plants, which are combined in 35 botanical genus and 19 families, and 10 species – grassy plants combined in 8 genus and 19 families. All species studied belong to plants with C_3 type metabolism. Rapidly growing plants with high photosynthetic activity are distinguished by strong ability of photobioemission. Isoprene emitting species occupy about 24% ($657x10^3$ha) of total area covered with forest in Georgia. Total annual production of photobioemission of Isoprene emitting tree-plants spread in forests of Georgia was estimated approximately $700x10^3$ ton/year.

Key words: Photosynthesis, Isoprene Effect, Isoprene emitting pants, Species diversity, Greenhouse Effect, Photochemistry of Atmosphere, Leaf Area Index.

Introduction

Grass cover has a crucial role in establishment of current chemical composition of the earth's atmosphere and maintenance of its balance, which totally distinguishes from other components of biota by its exclusive ability of photosynthesis and transpiration, as well as strong excretory function of organic matters.

Besides the main components (N_2, O_2, CO_2, H_2O) the atmosphere contains small admixtures of organic and non-organic gases of biogenetic and anthropogenic origin. They are mainly accompanying products of ongoing photosynthetic, respiratory and other biochemical processes in biota and anthropogenic effecting on environment. Those are: Methane, Isoprene, Carbonic Acid, NH_3, NO_X, Hydrogen sulphide, Carbon disulfide and other hydrocarbons. Though, concentrations of the above mentioned gases are several orders lower than the main components of atmosphere, main part of them have high efficacy of absorbing rays in infrared area of light spectrum and actively participate in photochemical transformations going in atmosphere [Smith W., 1981; Brauseur G., Chatfield R., 1991; Baldochi D., 1991].

Isoprene takes an important place among biogenic hydrocarbons whose photobioemission intensity from grass cover considerably exceeds emission rates of other biogenic gases (except Methane) [Monson R., et al., 1991].

Free Isoprene photobiosynthesis and its emission from leaves under CO_2 deficiency was discovered by G. Sanadze in 50s. This phenomenon was named Isoprene Effect (IE) [Sanadze G., 1957, 1964]. Localization of the main reaction of Isoprene photobiosynthesis in chloroplasts was defined in the further researches [Sanadze G., et al., 1972, 1976, 1982, 1986, Baazov D., Sanadze G., 1987; Tarkhnishvili G., et al., 1985; Khananashvili V., Sanadze G., 1980; Mgaloblishvili V., et al., 1978; Rasmussen R., 1973; Tingey D., et al., 1979; Monson R., 1989; Sharky T., et al., 1991] held in Laboratory of Photosynthesis of Tbilisi State University and other scientific centers of the World. IE intensity kinetics and its functional relationship to the major limiting factors of photobiosynthesis (Co_2, O_2, light, temperature), was studied. G. Sanadze's hypothetic scheme on Isoprene photosynthesis [Sanadze G., 1991] theoretically proved Dimethylial Pyrophosphate as metabolic predecessor of Isoprene instead of isomer isopentenil pyrophosphate, which had been considered a priori. In the end of the last century American and Georgian scientists isolated synthesizing enzyme of Isoprene – Isoprensynthase from different species of Isoprene emitting plants [Silver G., 1995; Kuzma J., 1995; Schnitzer J., et al., 1996; Datukishvili N., et al., 2001].

Isoprene effect function in green cells metabolism is successfully studied in leading scientific centers of the USA, Germany, Japan, Israel, China and Russia. Importance of photogenic Isoprene molecules in atmosphere chemistry is being intensely studied along with physiological and biochemical mechanisms of Isoprene photobiosynthesis in plants.

Isoprene emitting plants in forest community and their natural habitat expand, as well as Isoprene and other gases emission intensities and concentrations in different layers of troposphere were defined due to researches carried out by USA atmosphere scientific research centers in tropical forests of Amazon basin [Zimmerman P., et al., 1988; Rasmussen R., 1988].

Isoprene bioemission map of USA plant cover was created on the basis of the data obtained [Olson R., 1980; Lemb B., et al., 1987], where Isoprene emission intensity rates and dynamics of their changes for different regions are given.

Nowadays it is known that about 800 million tones of non-methane hydrocarbons of biogenic origin are being globally emitted as gas in the Earth's atmosphere per year, from which approximately 300-450 million tones comes to photogenic Isoprene and equals to annual emission intensity of Methane. These data are enrolled in General Circulation Model of Gases (GCM) [Monson R., et al., 1991] as one of the meteorological parameters.

Observations carried out on troposphere photochemical transformations showed that interaction of photogenic Isoprene and OH radicals are closely related to generation of components such as O_3, CO, CO_2 and other organic peroxides. In addition, competition between Methane and Isoprene for OH was found in favor of the latter, which increases possibility of Methane accumulation in the atmosphere; hence, it may become one of the main reasons for growing greenhouse effect [Monson et al., 1991].

Consequently, it may be concluded that vast amounts of Isoprene emitted from phytocenosis and its high photochemical activity should determine regulation level of OH radicals and especially O_3 concentration in the atmosphere, which indefinably increases the role of these gaseous matters in ecology of atmosphere and issues regarding climate changes.

Today a number of species emitting Isoprene are known. Mostly they are trees, as well as grassy plants, ferns, mosses and etc.

Forests represent main source for Isoprene and other biogenic gases emission [Smith W., 1981; Baldochi D., 1991; Rasmussen R., 1988]. About 5000 species of wild and running wild, as well as *Angiospermae* and *Gymnospermae* and about 8300 species of spore plants grow in Georgia. Forest community takes particular place in plant cover, which occupy 43% of territory of the Republic ($6950x10^3$ hectare). Forests occupy 2,75 million hectare of the territory (39,6% of the territory of Georgia). Around 395 species of arboreal plants widely grow in forests. They are joined in 123 genus and 56 families, more than 182 forms and 91 variations of the mentioned species are

also described. Beside that, more than 3600 introduced species grow in botanical gardens and built-up areas [Gigauri G., 2000].

Due to biodiversity of Georgian flora and wide natural habitat of its expansion a number of new Isoprene emitting species typical to our region may be revealed, as well as gas exchange between atmosphere and phytocenosis and contribution of Isoprene photobioemission processes in regulation of chemical balance of air basin of Georgia is to be assessed, which may be upset by growth of anthropogenic factors.

The aim of the present work is: 1. to reveal Isoprene emitting arboreal and grassy plants of Georgian flora, and create a taxonomy list; 2. to determine area occupied by dominant and subdominant Isoprene emitting tree-plants which create forests of Georgia and estimate average annual production of Isoprene photobioemission of these plants.

Material and Methods

During the years Isoprene emitting plants were discovered in Laboratory of Photosynthesis with the members of Plant Physiology and Anatomy department of Tbilisi State University [Kvariani L., et al., 1985]

Plants growing in Tbilisi Botanical Garden and Tbilisi suburb represent subject of the research. We collected investigational leaves and coniferous branches in June-July and measured intensity of photosynthesis and Isoprene bioemission in microclimate chamber constructed by us [Tarkhnishvili G., et al., 1985]. Experiment exposition time was 15-30 minutes, temperature-28-30^{0}C, light-saturation of photosynthesis - 800mc.mol/m^{2}min; oxygen concentration in chamber was 21%. We defined photosynthesis intensity through infrared gas analyzer (INRED USA). CO_2 concentration in chamber changed from 500 ppm (mcl/l) to compensation point of Carbonic Acid (50-70 ppm). The error was $\pm$ 1-2 ppm.

We determined amount of Isoprene emitted by leaves on flame ionized chromatograph (Fractovap-420, "Karlo Erba", Italy). Sensitivity of the device to Isoprene molecules was $6x10^{-9}$ mg/ml. Measurement error did not exceed 1-3%.

Results and Discussion

We have studied 321 species of 103 families, from which 71 species of 26 families were Isoprene emitting, i e. 22% of the species investigated. Among them 61 species of 19 families are arboreal and 10 species of 6 families - grassy plants. All species belong to plants with C_3 metabolism. Most of them are endemic species.

List of Isoprene emitting arboreal and grassy plants according to their taxonomy are given in table 1 [Makashvili A., 1991]. The table shows, that *Gymnospermae* (Coniferous) plants are presented by seven species of two genus and three families. *Angiospermae* combines 55 species belonging to 17 different families and 31 genus of *Dicotyledonae* class. *Monocotiledonae* (grassy) class is presented by 10 species belonging to 6 families and 8 genus of *Gramineae*. Representative of *Dicotyledonae* class prevail over investigated Isoprene emitting plants.

Table 1. Arboreal Isoprene Emitting Plants of Georgian Flora

#	Species	Genus	Family	Class	Type
1	*A.nordmannia Spach*	*Abies*	*Pinaceae*	*Coniferopsida*	*Gymnospermae*
2	*A.pinsapos Boiss*				
3	*A.cilicica Carr*				
4	*P.pungens Engelm*	*Picea*			
5	*P.orientalis Link*				
6	*Ephedra procera Fisch*	*Ephedra*	*Ephedraceae*		
7	*Q.imeretina Stev*	*Quarcus*	*Fagaceae*	*Dicotyledoneae*	*Angiospermae*
8	*Q.longipes Stev*				
9	*Q.hartwissiana Stev*				
10	*Q.pontica C. Koech*				
11	*Q.iberiana Stev*				
12	*Q.macranthera F.et M*				
13	*Q.dschorochensis C.Koch*				
14	*Cl.Vitalba L*	*Clematis*	*Ranunculaceae*	" "	" "
15	*A.fructicosa L*	*Amorfa*	*Lecuminosae juss*	" "	" "
16	*R.pseudoacacia L*	*Robinia*	" "	" "	" "
17	*A.julibrissin Duras*	*Albizzia*	" "	" "	" "
18	*C.arboroscens Len*	*Caragana*	" "	" "	" "
19	*C.lutea (mich) C.koch*	*Cladrastis*			
20	*Gdioicus (L) C.Koch*	*Gimnocladus*	" "	" "	" "
21	*W.chinensis (sime) Sweet*	*Wisteria*	" "	" "	" "
22	*C.cucasus Cirosh*	*Citisus*	" "	" "	" "
23	*P.hirsute Thnb*	*Pueraria*	" "	" "	" "
24	*P.orientalis*	*Platanus*	*Platanacea Lind*	" "	" "
25	*P.acerifolia Wild*				
26	*P.tremula*	*Populus*	*Salicaceae Lind*	" "	" "
27	*P.nigra*				
28	*P.hibrida*				
29	*P.piramidalis*				
30	*P.deltodies*				
31	*S.discolor*	*Salix*	" "	" "	" "
32	*S.alba*				
33	*S.dophoides Vill*				
34	*S.arbuscula L*				
35	*S.babilonica L*				
36	*S.caprea L*				
37	*S.fragilis L*				
38	*S.pentadral*				
39	*S.vinimalis L*				
40	*S.wilhelmsiana M.B.*				

#	Species	Genus	Family	Class	Type
41	*E.glofulus*	*Eucaliptus*	*Myrtaceae*	" _____ "	" _____ "
42 43 44	*B.colchica* *B.hircana* *B.jempervirens*	*Buxus*	*Buxacee*	" _____ "	" _____ "
45	*B.vulgaris*	*Berbaris*	*Berbaridaceae*	" _____ "	" _____ "
46	*P.chinenais Bge*	*Pistacia*	*Anacardiaceae*	" _____ "	" _____ "
47 48	*C.preacot L* *Calycanthus floridus*	*Chimonantus*	*Calycanthaceae*	" _____ "	" _____ "
49	*R.ponticum Schreb*		*Ericaceae*	" _____ "	" _____ "
50 51 52	*H.virginiana* *L.stiraciflua L* *P.persica C.A. Mey*	*Hamamelis* *Liguidambar* *Parotia*	*Hamamelideae*	" _____ "	" _____ "
53	*J.nigra L*	*Juglans*	*Jugalandaceae*	" _____ "	" _____ "
54	*B.papyrifera L'Herit*	*Braussonetia*	*Moraceae*	" _____ "	" _____ "
55	*F.pennsylvanica Marsch*	*Fraxinus*	*Oleaceae*	" _____ "	" _____ "
56	*H.duleis Thnb*	*Hovenia*	*Rhamnaceae*	" _____ "	" _____ "
57	*P.spinachristi Mill*	*Paliurus*			
58	*R.frangula L,* *R.cathartica L*	*Rhamnus*			
59	*C.salicifolia Franch*	*Cotonoaster*	*Rosaceae*	" _____ "	" _____ "
60	*E.grandiflora Schneid*	*Exochorda*	" _____ "	" _____ "	" _____ "
61	*P.coccinea Roem*	*Phiracanta*	" _____ "	" _____ "	" _____ "

Grassy Plants

#	Species	Genus	Family	Class	Type
1 2	*A.capillaries L* *A.planifolia C.koch*	*Agrostics*	*Gramineae*	*Monocotiledonae*	*Angiospermae*
3	*Adonax L*	*Arundo*	" "	" "	" "
4 5	*B.vulgaris* *B.arundinaceae*	*Bambusa*	" "	" "	" "
6	*J.Ep*	*Juncus*	*Juncaceae*	" "	" "
7	*B.nigra L*	*Balltoa*	*Lamiaceae Lindley*	*Dicotyledoneae*	" "
8	*M.polychoa Grossh*	*Medicago*	*Leguminosae Juss*	" "	" "
9	*H.caucasicus ABc*	*Heleborus*	*Ranunculaceae*	" "	" "
10	*Ch.majus L*	*Chelidonium*	*Papaveraceae*	" "	" "

Dominant and subdominant tree-plants, creating forests of Georgia, are widely presented in the list of Isoprene emitting plants (table 1), particularly, species of *Quarcus, Populus, Robinia, Salix, Buxus, Abies* and *Picea*. On the basis of species composition and territorial distribution we have calculated total area occupied by this species which amounted to $658{,}2 \times 10^3$ hectare, which makes about 24% of total area of Georgia covered with forest.

Monitoring of gas change processes of different species of tree-plants showed that rapidly growing species of *Populus, Salix* and *Robinia* with high photosynthesis activity have high ability of Isoprene photobioemission, IE intensity of which from $1m^2$ of leaf area on Carbonic Acid compensation point, at $28\text{-}30^0$C temperature and light-saturation of photosynthesis, was 18-20mgr/hr on average; then comes species of *Quarcus* and *Buxus* - 8-10mgr/m^2hr; comparatively low intensity of Isoprene emission is characteristic of species of *Abies* and *Picea* - 3-5 mgr/m^2hr.

On the basis of obtained data we approximately estimated annual (vegetation period) amount of Isoprene production of dominant and subdominant Isoprene emitting tree-plants of the Georgian cenosis from occupied land area. The data are given in table 2. For calculation we took into consideration leaf area index (LAI) which showed plant leaves area grown per soil unit: $L = \frac{S}{P}$; where S is leaves area, P- soil area located under tree-plants (tree branching) [Zelitch I. 1971]. According to the existing data [Kramer P.J., 1979], LAI for deciduous plants varies within 4-6, for coniferous this index is comparatively high - 8-12.

Isoprene photobioemission intensity i.e. the product of LAI (L) and Isoprene amount (A) emitted from unit area of leaf surface within time unit results in Isoprene production within time

unit from land area unit occupied by plants. $E = A \times L = \frac{\Delta C}{S \times \Delta t} = \frac{1}{P} \times \frac{\Delta C}{\Delta t}$; $\Delta C -$ amount of Isoprene emitted in Δt period.

For Isoprene emission period (Δt) sun light duration within vegetation period was taken. For temperate climate zone it approximately totals to: Δt =200 days x14 hrs =2800 hrs, 200- is annual quantity of vegetation days, 14 hours- average duration of daily sun light [Rasmussen R., et al.,1988].

Calculations showed (Table 2), that annual Isoprene production of dominant and subdominant Isoprene emitting tree-plants, which create forests of Georgia, from total area of land occupied (I=ExP) is rather high and probably amounts to 700 thousands tones.

Table 2. Annual Production of Isoprene Photobioemission of Dominant and Subdominant Arboreal Plants Creating Forests of Georgia

Genus	Number of species naturally spread in Georgia	P Area of land occupied $10m^3$	% From total area covered with forest	LAI L=S/P	Isoprene photobioemission intensity from area unit A mgr/m^2hr	Isoprene bioemission from land area unit E=AxL t/ha year	Annual Isoprene production from total area of land occupied by forest I=ExP $x10^3$t/year
Quarcus	7	291	10,6	4	10	1,12	326
Populus	8	19,1	0,7	6	20	3,36	64,6
Robinia	1	10	0,36	4	18	2,02	20,2
Salix	17	1,3	0,047	4	17	1,9	2,5
Buxus	6	8,4	0,03	4	8	0,9	7,6
Abies	1	189,8	6,9	8	3	0,7	133
Picea	1	138,6	5,04	8	5	1,12	155
Total	41	658,2	23,7	-	-	-	708

Thus, we can conclude:

1. Isoprene emitting plants of the Georgina flora are distinguished by diversity of species. At present 71 species of 24 genus with Isoprene photobioemission ability are revealed (22% of species investigated). Among them, 61 species are arboreal plants which are combined in 35 genus and 19 families, and 10 species of grassy plants combined in 8 genus and 6 families. Majority of Isoprene emitting plants are representatives of *Dicotyledonae* class. All species studied belong to plants with C_3 type metabolism.

2. Species of Isoprene emitting plants occupy about 24% ($657{,}5x10^3$ ha) of forest area, which indicates high potential of Isoprene photobioemission of Georgian forests and wide expand of tree-plants species emitting Isoprene.
3. Isoprene photobioemission intensity rate of different species at identical environmental conditions considerably differ from each other. Rapidly growing species with high photosynthesis activity are distinguished by high capacity of Isoprene emission.
4. Total annual production of photobioemission of Isoprene emitting tree-plants of Georgian forests was estimated approximately $700x10^3$ t/year. Thus we consider that considerable amount of Isoprene coming from the forests to the atmosphere should actively affect photochemical transformations going in different layers of the air basin, including creation of ozone molecules.

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საქართველოს ფლორის იზოპრენგამომყოფი მერქნიანი და ბალახოვანი მცენარეები

თარხნიშვილი გ., ლოლაძე ტ., ჯაიანი გ., ფხაჭიაშვილი ს., მგალობლიშვილი მ., ხეცურიანი ნ., ნაჭყებია ქ., სანაძე გ.

ფოტოსინთეზის პრობლემური სამეცნიერო-კვლევითი ლაბორატორია, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 23.02.2004)

რეზიუმე

გამოვლენილია იზოპრენის ფოტობიოემისიის უნარის მქონე 24 ოჯახის 71 სახეობა (გამოკვლეული სახეობათა 22%). მათ შორის 61 სახეობა მერქნიანი მცენარეა, რომლებიც გაერთიანებულნი არიან 35 გვარსა და 19 ოჯახში, ხოლო ბალახოვანი მცენარეების 10 სახეობა – 8 გვარსა და 19 ოჯახში. ყველა შესწავლილი სახეობა მიეკუთვნება C_3 ტიპის მეტაბოლიზმის მქონე მცენარეებს.

მაღალი ფოტოსინთეზური აქტივობის მქონე სწრაფმზარდი სახეობები გამოირჩევიან მძლავრი ფოტობიოემისიის უნარით.

იზოპრენ გამომყოფ მცენარეთა სახეობებს საქართველოში ტყით დაფარული ფართის დაახლოებით 24% (657×10^3 ჰა) უკავიათ.

პირველი მიახლოებით შეფასებულია საქართველოში გავრცელებული იზოპრენგამომყოფი ხე-მცენარეების ფოტობიოემისიის საერთო წლიური პროდუქცია, რომელიც 700×10^3 ტ/წ უნდა შეადგენდეს.

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Zoology

SOME BIOLOGICAL AND MORPHOMETRIC CHARACTERISTICS OF A VENDACE (*COREGONUS ALBULA* L.) OF THE LAKE PARAVANI

JAPOSHVILI B.

Institute of Zoology, Georgian Academy of Sciences

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Abstract

Some morphometric characteristics, condition factor, age groups of vendace (*Coregonus albula* L.) of Paravani Lake (South Georgia) have been given for the first time since 1970. Growth rates in accordance with the age were compared using Fulton's condition factor. The study of annual growth was based on back-calculations of length. It was shown that nowadays in Paravani Lake quantity of vendace, as well as length and weight rate were diminished. The reasons of such changes are discussed.

Key words: Vendace (*Coregonus albula*), Paravani, Georgia.

Introduction

Paravani Lake is the largest lake in Georgia, its surface in average is 37km². The maximum length is 10 km, the maximum width - 5.75km; an average depth is 1.87m, maximum depth - 2.80m. The lake is located at 2080m above sea level in the north-east of Akhalkalaki and belongs to Ninotsminda region. The distance from the regional centre is 25km [Baratch, 1964].

Vendace was introduced into the Paravani Lake during the twentieth century, from Volkhov fish hatchery. In a new condition (location) it adapted easily and since the early 1940 vendace has been the dominant species in the commercial catches. Vendace was the main species in the total catch from Paravani in 1947 (203.5 tones) and 1952 (119.25 tones), from 1952 the annual catch of vendace declined, but in 1957 it again restored, from 1989 the annual catches finally decreased, from 1994 till 2001 whole catch achieves 100 tones [Japoshvili, 2002. Japoshvili et al. 1999]. Initially this decline was caused by intensive fishery, unfavorable combinations of water condition and by other ecological factors.

Vendace in Lake Paravani grow rapidly, young fish usually attain length of 10-14 cm during the first year and 17-23 cm. during the second year. Females reach maturity in the fall of their second year.

Material and methods

Sampling was started from 1999 and continued in 2002. In winter when the lake is ice-covered, seines are operated below the ice through a series of hole, using ropes driven under the ice between holes with long, floating poles, with gillnets during the summer, and with trap nets in autumn. All fishes were sorted by species, age or size groups. Total length, weight and sex of each

individual were recorded [Pravdin, 1966]. Age determinations were based on scales. The study of annual growth was based on back calculations of length [Lea, 1910]:

$$\frac{L}{C} = \frac{L_x}{C_x}$$

where L - length of fish, C- distance from the scale to the edge, C_x - distance from the scale centre to each of the rings, L_x- length of X age fish. Back-calculations are used to estimate vendace length at previous ages. For this purpose using the scales removed from each individual fish, the distance from the scale centre to each of the rings and to the edge was measured. To estimate the condition of fish Fulton's formula was used:

$$K = \left(\frac{W}{TL^3}\right) x100$$

where K is condition factor, W-weight of fish, TL- total length of fish.

Results

Our research has shown that 0+, 1+, 2+, 3+ age groups are found mainly in Paravani Lake at present and three years old fishes prevail among them. Our study of vendace (*Coregonus albula* Linnaeus) was aimed primarily at determining the biology, diet variation with season and age, as well as growth rate, condition factor, morphometric characters of vendace.

Table 1. Some characteristics of vendace in the Lake Paravani.

Age	TL	length of fish without C	weight	Condition factor	By Demetrashvili (1960)data		
					TL	length without C	weight
0+	11.2	10.6	52	_	14	13.5	30
1+	23.2	19.7	84	0.72	24.8	23.9	173
2+	26	22.8	138	0.78	29	27.2	277
3+	28.3	25.2	161	0.83	32.9	31.7	487

From Table 1 the condition factor, length and weight of fish reach maximum in the fish of 3+ years age. By Demetrashvili's data length and weight of same age fish are higher then our results. Namely, if earlier an average weight of the fish was 250-350g, at present it decreased to 120-150g.

We measured morphometric characteristics of 2+ years age vendace and results are given in Table 2.

Table 2. Morphometric characteristics of 2+ age *Coregonus albula* L.

Character	Female	Male
TL	25.86	24.66
SL	23.6	22.55
Snout length	1	0.9
Eye diameter	0.9	0.88
Postorbital distance	1.96	1.86
Head length	4	3.8
Head depth	2.9	2.77
Maximum body depth	4.26	4.1
Minimum body depth	1.39	1.34
Predorsal distance	9.2	8.72
Postdorsal distance	8.7	8.3
Caudal peduncle length	2.7	2.5
D length	2.12	2.1
D depth	3.55	3.5
P length	0.7	0.67
P depth	2.94	2.9
P-V distance	6.4	5.86
V-A distance	5.46	5.1
Number of rays in D	II-III 9-12	II-III 8-11
Number of rays in A	II-III 8-13	II-III 8-13

Abbreviations used are: TL- total length, SL- standard length, D- dorsal fin, P- pectoral fin, A- anal fin, V- ventral fin.

Conclusions

We compared vendace growth rate in Paravani Lake to those of Russian and Finish lakes [Potopova, 1972; Sarvala et al. 1988; Sarvala et al. 1999; Helminen et al. 1992; Helminen et al. 1997], it was revealed that in Paravani Lake vendace growth rate is high and on the third year of life becomes the object of fishery.

The results have shown that nowadays in Paravani Lake quantity of vendace as well as length and weight rate are diminished. In our opinion it depends on ecological factors as much as on the government's lack of attention. There is much illegal fishing in Lake Paravi, everyday all age group vendace is caught in the lake. This all hinders the fish to have active feeding, and it causes decrease in the size of vendace.

Thus, vendace as ichthyofaunistic rare and economically important species must be regarded as greatly endangered species that requires special attention and protection.

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ფარავნის ტბაში გავრცელებული ევროპული ჭაფალას (***COREGONUS ALBULA* L.**) ბიოლოგიური და მორფომეტრული თავისებურებები

ჯაფოშვილი ბ.

საქართველოს მეცნიერებათა აკადემიის ზოოლოგიის ინსტიტუტი

(მიღებულია 22.12.2003)

რეზიუმე

პირველად 1970 წლის შემდეგ მოცემულია ევროპული ჭაფალას (*Coregonus albula* L.) მორფომეტრული მაჩვენებლები, ნაკეებობის კოეფიციენტი, ასაკობრივი ჯგუფები. ფულტონის ნაკეებობის კოეფიციენტის გამოყენებით შედარებულია ზრდის ტემპი ასაკის მიხედვით. წლიური ზრდა შესწავლილია სიგრძის უკუგამოთვლის მეთოდის გამოყენებით. ჩვენებია, რომ ამჟამად შემცირებულია ევროპული ჭაფალას, როგორც რიცხოვნობა, ასევე სიგრძე და წონა ასაკის მიხედვით ფარავნის ტბაში. განხილულია ამ ცვლილებების გამომწვევი მიზეზები.

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Letter to Editor

GLOBAL WARMING AND TREELINE

NAKHUTSRISHVILI G., ABDALADZE O., AKHALKATSI M.

Institute of Botany of the Georgian Academy of Sciences

The problem of the global warming of the earth's surface and atmosphere has become the focus of attention of the modern science. Naturally, biologists cannot treat these problems with indifference. Ecologists are rather concerned about the fact, that an alteration of temperature might cause a drastic transformation of living organisms yet in the nearest 30-50 years and destroy biological interconnections developed during millions of years.

It is worth mentioning that climate warming was observed in the last century too. The temperature of the earth's surface increased by approximately 0.6°C; however, temperature was rising unevenly during the whole period. The first remarkable warming was observed in the first half of the last century, between 1900 and 1945. The second and stronger warming has been recorded during the last 25 years. Besides, between these two events temperature stabilized to a certain degree and in some cases even decreased. It is worth paying attention that temperature changes have mainly taken place in the northern hemisphere. In the southern hemisphere temperature rose insignificantly supposedly owing to heavy precipitation.

Apparently, first of all factors underlying the impending ecological catastrophe must be found out, available data must be compared and the reasons for the alteration of temperature must be studied. According to the most convincing explanation, factors of the industrialization, increasing level of CO_2 in the atmosphere and the so-called Greenhouse effect, which is gradually establishing, are most responsible for this alteration. Existing data have proved the noted statements: the CO_2 level did not exceed 280 ppm before the industrial revolution and 300 ppm in 1900. At present this index has reached 350 ppm. It is supposed that the CO_2 level will be doubled in the middle of this century leading to a sudden rise in temperature (Ozenda, Borel, 1990).

Scientists have suggested 3 scenarios concerning the problem of the rise in the CO_2 concentration. Scenario 1 – in consequence of the doubling of the CO_2 concentration, by 2030-2050 temperature will have become 3.5°C-4°C greater than it is now. Scenario 2 – it is supposed that temperature will rise only by 1°C. Scenario 3 – in case of the threefold decrease in the industrialization, the CO_2 level will stabilize.

According to the first hypothesis it is supposed that the events will take place in the following order: the rise in temperature will lead to the increase in precipitation and exert influence on their distribution over a year; profound alterations of the intensity of evaporation, atmospheric circulation and condensation are inevitable.

Taking into account the present state of the climate in warm countries, it should be supposed that the amount of precipitation will rise. However, it might occur in case the ocean warms and, consequently, the intensity of evaporation rises. There is danger that events will move in the opposite direction: the ocean will maintain its current temperature for a long time by inertia, the intensity of evaporation will alter insignificantly. Therefore, the warmed atmosphere will not

receive an additional dose of water vapour; the probability of condensation, i.e. precipitation will drastically decrease, thereby disrupting the pattern of the atmospheric circulation. The so-called Young drought will set in. All these changes are very much connected with the snowfall conditions, floods, moisture supply in the soil, etc. For instance, at 1800m the duration of the snow cover will shorten from 6 to 3 months. It is possible that the results of such alterations will prove to be harder than those caused by the warming of the atmosphere.

Rising of the sea level is another serious problem. The successive events suggested in the first noted hypothesis can be traced considering an example of Europe. Within 30-50 years temperature will rise by 4.0°C±1.0° on average and precipitation by 0.22mm daily, i.e. by less than 77mm annually. Such situation is rather favourable to the development and distribution of fungus diseases, pests, etc. High temperature and early-spring droughts will bring about a danger of forest fires. A logical conclusion should be drawn that events moving in such a way will lead to the disturbance of the correlation between photoperiodism, pollination, maturation of seeds. Temperature rising by 4.0°C will result in alterations in the vegetation zones. These zones will ascend and every zone located higher will be replaced by the lower one. Such a phenomenon has already been observed in the department Izera (France), where the lower zone of the hill vegetation is being replaced by the Mediterranean vegetation, in particular, evergreen oak forests. Furthermore, hornbeam and chestnut will replace the vegetation of the middle zone (silver fir), the latter will ascend to the present-day treeline and the subalpine coniferous forests will occupy the alpine meadows (Ozenda, Borel, 1990).

In the course of time phytoecological changes will manifest themselves in the high mountains of Georgia in shifts of the altitudinal belts by 700m leading to the narrowing of the alpine and, especially, subnival belts.

The alpine-nival belt in Europe will remain only in the region of Mt. Mont Blanc, in western Switzerland and partially in the Tirollian Alps (Austria) and the Caucasus. In the Pyrenees and Carpathians the nival belt will completely disappear.

According to the preliminary data 120 species of plants will most likely become extinct in the Alps. In Georgia the most sensitive ecosystems and, in particular, the treeline vegetation, i.e. elfin forests, naturally rare forests, subalpine tall herbaceous vegetation, broad-leaved meadows, alpine carpets (occurring at the snow line), subnival and nival plants will be under stress.

In the Kazbegi region (the Central Greater Caucasus) remarkable growth of seedlings and young birch trees (*Betula litwinowii*) of 6-8 years of age have been observed beyond the treeline (2200-2450m) for the last several years. This is due to dry winters with no snowfall having become more frequent. Cases of wilting of an evergreen shrub, *Rhododendron caucasicum* in the high mountains of Georgia, probably related to dry winters, have also been observed in the same belt. Plants apt to be found in snowy and moist habitats such as *Botrychium lunaria,* species of *Selaginella, Lilium georgocum* have also disappeared there.

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გლობალური დათბობა და ტყის საფარი

ნახუცრიშვილი გ., აბდალაძე ო., ახალკაცი მ.

საქართველოს მეცნიერებათა აკადემიის ნ. კეცხოველის სახ. ბოტანიკის ინსტიტუტი

რეზიუმე

გასული საუკუნის მე-2 ნახევარში, ინდუსტრიალიზაციის მძლავრი განვითარების შედეგად, ატმოსფეროში CO_2-ის კონცენტრაცია მკვეთრად გაიზარდა. ამ მოვლენამ გამოიწვია ე. წ. სათბურის ეფექტი და ტემპერატურის აწევა. მეცნიერთა ვარაუდით, 30-50 წლის შემდეგ მოსალოდნელია CO_2-ის რაოდენობის გაორმაგება და იმ უარყოფითი პროცესების გაღრმავება, რაც გლობალური დათბობის ფონზე დედამიწის ამა თუ იმ ნაწილში უკვე აშკარად შეინიშნება. აქედან გამომდინარე, რეალური საფრთხე ემუქრება ეკოსისტემურ თუ სახეობრივ მრავალფეროვნებას.

ამჟამად, განიხილება კლიმატის ცვლილების სამი ჰიპოთეზა და მისი შესაძლო შედეგები: 1) 2030-2050 წლებისთვის CO_2-ის კონცენტრაციის გაორმაგება გამოიწვევს ტემპერატურის გაზრდას 3,8^{0}C-ით; 2) ტემპერატურა გაიზრდება მხოლოდ 1^{0}C-ით და 3) ინდუსტრიალიზაციის თანამედროვე დონის სამჯერ შემცირების შედეგად, ტემპერატურა მხოლოდ უმნიშვნელოდ შეიცვლება. პირველი ჰიპოთეზის მიხედვით, საქართველოს მაღალმთის მცენარეულ საფარში მოსალოდნელია ძლიერი ტრანსფორმაცია – მცენარეული სარტყლები ვერტიკალურად დაახლოებით 700 მ-ით აიწევს. შეიცვლება ალპური სარტყელი. სუბნივალური და ნივალური სარტყლები ზოგან საერთოდ გაქრება. სტრესულ სიტუაციაში აღმოჩნდებიან სენსიტური ეკოსისტემები: ტყის ზედა საზღვარი, მაღალბალახეულობა, ფართოფოთლოვანი მდელოები, "ალპური ხალები", სუბნივალური და ნივალური ნანოცენოზები. ცენტრალურ და მცირე კავკასიონზე დღეს უკვე შეინიშნება ტყის ზედა საზღვრის ეკოტონში მცენარეული საფარის ცვლილების ტენდენცია (ტანბრეცილი არნარი ტყის ქქსანსია, დეკიანების ხმობა და სხვ.). მეორე ჰიპოთეზის თანახმად, საქართველოს მაღალმთაში მცენარეული სარტყლების ვერტიკალური გადანაცვლება მხოლოდ 150-180 მ-ით არის მოსალოდნელი. ამ შემთხვევაშიც მოსალოდნელია სენსიტური ეკოსისტემების, უპირველეს ყოვლისა, ტყის ზედა საზღვრის გარკვეული ტრანსფორმაცია.

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