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CONTENTS

Biochemistry

Biotechnology

Botany

Microbiology

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 3-4, 2008.

Biochemistry

INFLUENCE OF AMMONIUM NITRATE INTRODUCTION ON GLUTAMATE DEHYDROGENASE AND GLUTAMINE SYNTHETASE ACTIVITIES AND PROTEIN CONTENT IN ROOTS AND LEAVES OF MAIZE

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(Received July 15, 2008)

Abstract

Activities of the enzymes participating in ammonia assimilation - glutamine synthetase and glutamate dehydrogenase were studied in roots and leaves of maize against the background of fertilizing soil with rising doses of ammonium nitrate. The activity of enzymes as well as protein content changed in roots and leaves according to the doses of introduced ammonium nitrate.

Key words: glutamine synthetase, glutamate dehydrogenase, maize, ammonia assimilation, spectrophotometry, activity.

Introduction

Productivity of plants is mostly considered by presence of usable nitrogen and its recycling efficiency. In order to increase plants productivity it is necessary to provide them with additional forms of bound nitrogen.

Plants receive nitrogen by means of assimilation of ammonium [Kretovich, 1972], which is produced as a result of binding of nitrates - the main source of nitrogenous compounds. That's why it is important to study the enzymes participating it the process of ammonium assimilation.

Glutamine synthetase (GS) is the main enzyme taking part in the assimilation of fixed nitrogen and ammonium, which is supplied by the form of ammonium nitrate [Sadunishvili, 1996]. In addition to this one, other enzymes participate as well in the processes aiming at the maintaining of required balance of nitrogen in the plant, one of which is glutamate dehydrogenase (GDH). It secures the recurrence of carbon to amino acids by means of metabolism and Krebs cycle. Besides, the aforesaid enzymes are important for supply of energy and nitrogen to the plant [Kvesitadze et al., 2001].

Synthesis of glutamine is one of the most important parts of ammonium assimilation by the plant. This reaction is catalyzed by GS. Enzymatic synthesis of glutamine runs in the presence of glutamic acid, ammonium, ATP and Me^{+} [Evstigneeva, 1993; Kretovich, 1972; Miflin, Lea, 1973; Lea et al., 1992; Lea, Miflin, 1974]. According to the reaction:

$$\text{L-glutamate} + NH_3 + \text{ATP} \rightarrow \text{L-glutamine} + \text{ADP} + H_3PO_4$$

L-glutamine + 2-oxoglutarate + NAD(P)H + H^+ or Fd-red.→ 2L-glutamate+ NAD(P) or Fd-oxid.

GDH catalyzes the interconversion reaction of 2-oxoglutarate and glutamic acid, as a result of which the simultaneous interconversion of non-organic nitrogen of ammonium and alpha-amine nitrogen takes place:

$$\text{L-glutamate} + H_2O + NAD(P)^+ \rightarrow \text{2-oxoglutarate} + NH_3 + NAD(P)H$$

The increase of GDH activity *in vivo* by means of ammonia has been revealed in many higher plants: cucumber leaves [Matsumoto, Wakiucht, 1972], rice roots [Katamori, Matsumoto, 1972], oat leaves [Barash et al., 1973; Barash et al., 1975], etc.

The increase of nitrogen assimilation, especially in cereal crops, is one of the most important tasks of agriculture. Such an increase can be reached by means of permanent fertilization with nitrogenous compounds, which in its turn guarantees the increase of crop capacity and the higher content of proteins.

Our goal was to study the changes in glutamine synthetase and glutamate dehydrogenase activities in maize according to the various doses of nitrogen in the applied fertilizers (N60, N90, N120, N150, N180, N210).

Materials and Methods

The field test was conducted at podzol soils of Adjameti Experimental Station on the local maize species "Adjametis Tetri". The following doses of ammonium nitrate were applied: 1. none; 2. P120K60 (background); 3. P120K60N60; 4. P120K60N90; 5. P120K60N120; 6. P120K60N150; 7. P120K60N180; 8. P120K60N210.

The influence exerted by nitrogenous fertilizers was rated according to those changes in the protein contents and activity of main energetic and metabolic enzymes, such as glutamate dehydrogenase and glutamine synthetase.

The tests were conducted during the following growth phases of the plant: after 20 days since fertilizers had been applied, at starting of head formation, and at the beginning of milk ripeness.

Reductive amination activity of GDH was determined spectrophotometrically according to the rate of oxidation of NADH at 340 nm [Kvesitadze et al., 1993; Betsiashvili et al., 2004]. As the unit of enzyme activity we counted the amount of enzyme, which caused the oxidation of 1 micromole of NADH in 1 min at 20^0C.

GS activity was determined by means of colorimetric analysis, according to amount of gamma-glutamilhydroxamic acid (gamma-GHA) produced in a transferase reaction [Sadunishvili et al., 1996]. As the unit of GS we considered the amount of enzyme, which catalyzed the synthesis of 1 micromole of gamma-GHA in 1 min at 37^0C.

The protein content was determined by means of Bradford method [Bradford, 1976]. The specific activities in the enzymes were calculated as the number of enzyme units per 1 mg of protein.

Results and Discussion

In Table 1 the changes in the activities of enzymes and protein content in roots and leaves of maize, caused by introduction of different doses of nitrogenous fertilizers are presented.

Together with the increase of the amount of nitrogenous fertilizers in roots of the plant (especially in case of introduction of N150 and N180 doses of nitrogenous fertilizers) the considerable (71-172%) rise of GDH specific activity against its inhibition in leaves was observed.

It should be mentioned that depending on the concentration of nitrogen in the introduced nitrogenous fertilizers the protein content in leaves during the milk ripeness phase increased 1.5 times.

During the II phase in case of introducing higher doses of nitrogenous fertilizers (N120, N150 and N180) the significant increase (45%) in the specific activity of GDH was still observed, whereas the activity of GS in leaves increases at introduction of smaller doses of nitrogen and on the contrary considerably decreases in case of introduction of higher doses of nitrogen, which is evidently related to the increase of protein amount in leaves.

As a result of introduction of nitrogenous fertilizers the plant undergoes qualitative changes, which are reflected by means of changes of activity of studied enzymes both in roots and leaves.

Table 1. Influence of nitrogenous fertilizers on glutamate dehydrogenase and glutamine synthetase activities and protein content in maize leaves and roots

	No	Variation	I stage					II stage					III stage				
				GDH		GS			GDH		GS			GDH		GS	
			Protein, mg/ml	micromole NADH/min	micromole NADH per 1mg of protein	micromole γ-GHA/min	micromole γ-GHA per 1 mg of protein	Protein, mg/ml	micromole NADH/min	micromole NADH per 1mg of protein	micromole γ-GHA/min	micromole γ-GHA per 1 mg of protein	Protein, mg/ml	micromole NADH/min	micromole NADH per 1mg of protein	micromole γ-GHA/min	micromole γ-GHA on 1 mg of protein
Leaves	1	None	0.48	0.06	0.12	1.8	3.7	0.08	0.02	0.24	0.29	3.6	0.04	0.05	1.2	1.2	30
	2	P120K60 (backgr)	0.69	0.09	0.13	1.9	2.7	0.22	0.04	0.18	0.92	4.2	0.03	0.02	0.8	1.4	48
	3	P120K60N60	0.42	0.11	0.27	1.5	3.5	0.08	0.03	0.36	0.47	5.9	0.06	0.02	0.4	1.2	20
	4	P120K60N90	0.45	0.1	0.22	1.6	3.6	0.09	0.03	0.36	0.59	6.6	0.04	0.04	1	1.3	33.25
	5	P120K60N120	0.49	0.1	0.2	1.3	2.7	0.07	0.03	0.46	0.32	4.6	0.05	0.06	1.3	2.6	51.2
	6	P120K60N150	0.31	0.1	0.31	1.3	4.1	0.08	0.03	0.4	0.06	7.5	0.03	0.05	1.6	1.6	54
	7	P120K60N180	0.43	0.11	0.26	1.5	3.4	0.08	0.03	0.4	0.33	4.1	0.02	0.06	3.2	1.5	77
	8	P120K60N210	0.58	0.12	0.21	1.3	2.3	0.07	0.02	0.34	0.4	5.7	0.06	0.07	1.2	1.4	24
Roots	1	None	2.1	0.1	0.05	2.2	1	1.8	0.1	0.06	3.1	1.7	0.67	0.06	0.1	3.8	5.6
	2	P120K60 (backgr)	3.12	0.1	0.03	2.3	0.73	0.92	0.04	0.05	4.4	4.8	2	0.11	0.06	3.8	2
	3	P120K60N60	2.6	01	0.04	1.9	0.75	2.6	0.05	0.02	3.6	1.4	1.4	0.08	0.06	3.9	2.8
	4	P120K60N90	4.5	0.1	0.02	2.9	0.64	1.1	0.06	0.06	3.3	3.1	1.8	0.08	0.04	3.8	2.1
	5	P120K60N120	4.2	0.06	0.02	2.2	0.52	2.3	0.07	0.03	3.8	1.7	1.3	0.11	0.09	4	3.1
	6	P120K60N150	3.5	0.06	0.02	1.9	0.54	3	0.08	0.03	3.4	1.1	1.6	0.1	0.06	4.8	3
	7	P120K60N180	3.6	0.11	0.03	2.7	0.75	2	0.08	0.04	3.2	1.6	1.8	0.08	0.05	4.3	2.5
	8	P120K60N210	4.2	0.08	0.02	2.2	0.52	2.1	0.06	0.03	1.5	0.7	3	0.11	0.04	2.7	0.9

The quantitative parameters of GDH activity in roots increase twice in the II phase, and even more significantly in the III phase of growth; however, the same does not take place in leaves.

In contrast to GDH, the increase in activity of GS according to the phases of growth is observed in leaves but not in roots.

Comparison of the results of the first phase showed, that the quantitative parameters of studied enzymes are almost the same.

Thus, taking into consideration the test results it can be said that changes in activities of main metabolic and energetic enzymes - glutamate dehydrogenase and glutamine synthetase, depend on the doses of introduced nitrogenous fertilizers. In case of introduction of smaller doses of nitrogen the activity of glutamine synthetase increases in maize leaves, whereas at higher doses of nitrogen the activity of glutamate dehydrogenase is stimulated.

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(მიღებულია 15.07.2008)

რეზიუმე

შესწავლილია ეწერ ნიადაგში ამონიუმის ნიტრატის მზარდი დოზების შეტანის გავლენა სიმინდის მცენარის (ჯიში "აჯამეთის თეთრი") ფესვებსა და ფოთლებში ცილის რაოდენობაზე, აგრეთვე აზოტის ასიმილაციაში მონაწილე ფერმენტების გლუტამინსინთეტაზასა (გს) და გლუტამატდეჰიდროგენაზას (გდჰ) აქტივობებზე მცენარის განვითარების სამ ფაზაში. მცენარის ასაკის მატებასთან ერთად აზოტის ზეგავლენით ფერმენტების აქტივობა იცვლება როგორც ფესვებში, ისე ფოთლებში. გდჰ-ს აქტივობა მცენარის ასაკთან ერთად მატულობს ფესვებში, მაგრამ არა ფოთლებში, ხოლო გს-ს აქტივობა მატულობს ფოთლებში, მაგრამ არა ფესვებში, რაც უშუალო კავშირშია ფოთლებში ცილის რაოდენობის ზრდასთან. გს-ს გააქტივება შეიმჩნევა აზოტის დაბალი დოზების გამოყენების დროს, გდჰ-ს კი აზოტის მაღალი დოზების გამოყენებისას.

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Biotechnology

DNA MICROSATELLITE ANALYSIS OF CULTIVATED AND WILD GEORGIAN GRAPES

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(Received May 5)

Abstract

DNA microsatellite analysis of cultivated and wild Georgian grapes were studied. Seven Georgian cultivars: Rkatsiteli, Saperavi, Tavkveri, Goruli mtsvane, Aleksandrouli, Chkhaveri and Ojaleshi were genotyped at six (VVS2, VVMD5, VVMD7, VVMD27, ssrVrZAG62, and ssrVrZAG79) polymorphic microsatellite loci and seven specimens of wild grape from different geographic zones of Georgia and one from Turkey were analyzed at four polymorphic microsatellite loci (VVMD7, VVMD27, VVS2, ZAG62). Plylogenetic tree of Georgian cultivars was constructed. Microsatellite analysis revealed high level of polymorphism among investigated cultivated and wild grapes detecting at the same time their individual, unique allelic profile. Extent of genetic polymorphism in wild accessions is slightly higher from that observed in cultural varieties.

Key words: microsatellite, DNA microsatellite markers, *Vitis vinifera, V. vinifera spp. sylvestris,* simple sequence repeats (SSR)

Introduction

From ancient times grapes have been one of the most important agricultural crops in the world. The predominant cultivated variety of grapevine is *Vitis vinifera* L., the only species, which produces high quality wines. Within *V. vinifera* two subspecies exist: The cultivated subpsecies, *Vitis vinifera* subsp. *vinifera* that includes thousands of cultivars (5000 to 7000) [Alleweldt and Detweiller, 1994] and the wild subspecies, *Vitis vinifera* subsp. *silvestris*, which is widespread Eurasian species occurring as a climbing vine in forests from Spain to Turkmenistan. [Alleweldt G., Dettweiler E. 1994; Levadoux, L, 1956; Zohary D., Hopf M., 2000]. They are dioecious plants, with female and male flowers, having small, acidic fruits and occurring in roughly the same proportion in populations.

In Georgia, several hundreds cultivated varieties of *Vitis vinifera* are described [Ketskhoveli et al., 1960]. Botanical investigation of grape in Georgia until now was done by ampelographic methods. By using this method the morphological differences between cultivars are detected. In spite of activity of many well-known Georgian scientists the classification of Georgian grape cultivars is not definitely established. Georgian varieties express the features of two ecological-geographical groups of grape, from one side very felty and from other side naked plant area and Georgian grapes express the features of both groups [Tsertsvadze, 1989]. At the same time *Vitis vinifera ssp. sylvestris* is also very abundant and morphologically variable in Georgia. Many intermediate wild forms were observed, including those with characters associated with cultivated forms such as white fruits, hermaphroditic flowers, and larger sized seeds [Negrul A.M., 1946].

Our paper reports on the use of DNA microsatellite analysis for the comparative study of Georgian cultivated and wild grapes. We also intend to investigate systematics of grape cultivars based on DNA analysis.

Materials and Methods

The grape cultivars were taken from the ampelographic collections of Georgian Institute for Horticulture, Viticulture, and Wine-making and Georgian Agrarian University. As a control, samples of two French cultivars, Cabernet-Sauvignon and Pinot noire were used. The grape grafts were grown in water at room temperature, until fully-grown leaves were formed. The wild grape samples were collected from different geographic zones of Georgia and one sample was taken from Turkey. Trueness to wild accessions was based on ampelographical analysis of collected grape leaves, which were confirmed by comparing with the morphological descriptions of typical *Vitis vinifera ssp.sylvestris*. In most cases the flowers were also investigated. DNA isolation from grape leaf samples and PCR conditions are described in our earlier paper [Tabidze V. et al., 2006, Gamkrelidze M. et al., 2008]. Amplification was carried out by using Techne PCR amplifier TC412. For the electrophoresis 2.5 μl of each PCR product was loaded on a 8% polyacrylamide/urea sequencing gel, electrophoresed by 2117 Multiphor II LKB Electrophoretic system and visualized by silver staining technique according to Promega Manual. Allele sizes were determined using defined size markers: DNA of two French cultivars Cabernet Sauvignon and Pinot Noire [This P., et al., 2004]. Phylogenetic tree was constructed by comparison of data obtained by DNA microsatellite analysis using UPGMA clustering method [Fitch W.M., Margoliash E. 1967].

Results and Discussion

Table 1, which was given in our previous paper, represents genetic profiles of seven highly valued Georgian grapevine cultivars at 6 microsatellite loci [Tabidze V. et al., 2006]. For investigation of systematics of grape cultivars, based on these DNA microsatellite analysis data, UPGMA clustering method was used [Fitch W.M., Margoliash E. 1967]. Dendrogram in Fig.1 represents genetic relationships of seven most valued Georgian cultivars with one wild sample from Gardabani district of Georgia and two French cultivars: Cabernet Sauvignon and Pinot Noire. According this dendrogram three different groups of cultivars were detected. Group 1 appeared the largest, consisting five varieties: Goruli mtvane, Ojaleshi, Rkatsiteli, Ckhaveri and Aleksandrouli. Group 2 consists of wild grape samples from Gardabani and two cultivars: Tavkveri and Saperavi. Group 3, which revealed certain similarity with cultivars from Group 2, comprised almost entirely of French cultivars: Cabernet Sauvignon and Pinot Noire.

Comparative study of Georgian cultivated and wild grapes by DNA microsatellite analysis was performed. Earlier we presented genotypes of eight wild populations from different geographic zones of Georgia and Turkey at four polymorphic microsatellite loci (Table2) [Gamkrelidze M. et al., 2008]. Comparative study of Georgian cultivated and wild grapes detected a high level of polymorphism. The number of alleles among cultivars varied from 6 at locus VVMD 7 to 10 at locus ssrVrZAG79 and among wild samples between 9-12, with mean value 9.75. Calculation of standard genetic parameters also revealed high extent of diversity. The expected heterozygosity among grape cultivars is ranged from 0.847 to 0.879, with mean value 0.861 and the observed heterozygosity from 0.714 to 1.000, with mean value 0.833. The extent of genetic polymorphism in wild accessions is only slightly higher, from that observed in cultural varieties, representing 0.897

and 0.879 for observed and expected heterozygosity respectively. Genetic variability measures of Georgian cultivated and wild grapes are presented in Table 3.

The results obtained by these comparative studies revealed higher level of variability than that have been reported by analysis of cultivated and wild accessions obtained from different grape growing regions of the world [Aradhya M.K. et al., 2003]. Vouillamoz J.F. et al. (2006) representing genetic characterization of traditional grape cultivars from Transcaucasia and Anatolia, which included 41 Georgian accessions, also reported high extent of variability among Georgian cultivars. As usual, such high rate of heterozygisity is always observed among clonally propagated, outbreeding perennial species [Aradhya M.K. et al., 2003].

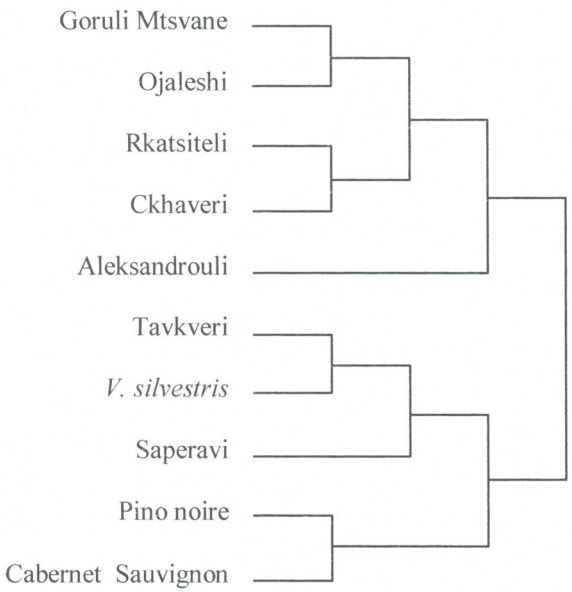

Fig. 1. Plylogenetic tree of seven cultivars from Georgia, one wild sample from Gardabani district and two French cultivars: Cabernet Sauvignon and Pinot Noire, constructed from six microsatellite markers.

Table 1. Genetic profiles of Georgian grapevine cultivars at 6 microsatellite loci. Allele sizes are given in base pairs (bp).

Cultivars	*VVMD5*	*VVMD7*	*VVMD27*	*VVS2*	*VrZAG62*	*VrZAG79*
Rkatsiteli	234 : 242	248 : 254	181 : 183	133 : 141	192 : 202	252 : 260
Saperavi	224 : 238	240 : 240	189 : 193	133 : 145	190 : 202	246 : 264
Tavkveri	228 : 238	238 : 244	191 : 195	135 : 147	192 : 202	254 : 254
Goruli Mtsvane	232 : 232	248 : 254	175 : 181	135 : 143	198 : 200	254 : 262
Alexandrouli	234 : 238	246 : 246	179 : 185	143 : 153	194 : 204	244 : 256
Chkhaveri	230 : 240	248 : 256	179 : 185	133 : 133	204 : 204	244 : 258
Ojaleshi	234 : 238	250 : 254	175 : 179	135 : 141	199 : 199	250 : 260

Table 2. Genetic profiles of Georgian wild grapevine populations at 4 microsatellite loci. Allele sizes are given in base pairs (bp).

Vitis vinifera spp. sylvestris specimens	*ZAG 62*	*VVMD 27*	*VVS2*	*VVMD 7*
Gardabani	196 –192	189 – 177	139-131	254 - 235
Ksovrisi	190 –190	227 – 201	145 - 129	259 - 247
Borjomi-1	206 – 202	191 – 191	139 - 135	249 - 243
Borjomi-3	198 –198	227 – 217	141 - 135	252 - 240
Sagarejo	204 –198	187 – 181	143 - 133	245 - 235
Vashlovani	200 – 188	195 –175	139 - 137	246 - 240
Ruispiri	202 –196	185 –179	143 - 141	-
Artvin (Turkey)	-	189 – 185	135 - 133	246 - 246

Table 3. Genetic variability measures of Georgian cultivated and wild grapes.

		VVMD5	*VVMD7*	*VVMD27*	*VVS2*	*VrZAG62*	*VrZAG79*	mean
Number of alleles	Cultivars	9	8	9	6	8	10	8.33
	Wild varieties	–	9	12	9	9	_	9,75
Observed heterozygosity (Ho)	Cultivars	0.857	0.714	1.0	0.857	0.714	0.857	0.833
	Wild varieties	–	0.857	0.875	1.0	0.857	_	0,897
Expected heterozygosity (He)	Cultivars	0.847	0.847	0.868	0.879	0.847	0.878	0.861
	Wild varieties	–	0.856	0.906	0.867	0.867	_	0.874

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ქართული კულტურული და ველური ვაზის მიკროსატელიტური ანალიზი

გამყრელიძე მ.

დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 05.05.2008)

რეზიუმე

ჩატარებულია ქართული კულტურული და ველური ვაზის მიკროსატელიტური ანალიზი. ქართული ვაზის შვიდი სხვადასხვა ჯიშის: რქაწითელის, საფერავის, თავკვერის, გორული მწვანეს, ალექსანდროულის, ჩხავერის და ოჯალეშის მიკროსატელიტური პოლიმორფიზმი შესწავლილი იქნა ექვსი (VVS2, VVMD5, VVMD7, VVMD27, ssrVrZAG62, ssrVrZAG79) პრაიმერის, ხოლო საქართველოს სხვადასხვა გეოგრაფიული ზონებიდან და თურქეთიდან აღებული ველური ვაზის შვიდი ნიმუშის მიკროსატელიტური პოლიმორფიზმი ოთხი (VVMD7, VVMD27, VVS2, და ZAG62) სხვადასხვა პრაიმერის გამოყენებით. მიღებულ მონაცემებზე დაყრდნობით კონსტრუირებულ იქნა შესწავლილ კულტურულ ჯიშთა ფილოგენეტიკური ხე. საქართველოს კულტურული და ველური ვაზის ნიმუშების შედარებითმა შესწავლამ მათში გენეტიკური პოლიმორფიზმის მაღალი ხარისხი გამოავლინა, რაც ამავე დროს მათი ალელების ინდივიდუალურ და უნიკალურ განაწილებაში გამოიხატება. ველური ვაზის პოპულაციის გენეტიკური პოლიმორფიზმის ხარისხი უმნიშვნელოდ აჭარბებს შესწავლილ კულტურულ ჯიშთა ვარიაბელობის ხარისხს.

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Biotechnology

NOVEL PLANT COMPOSITES AS ANTIOXIDANT FOOD ADDITIVES

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Abstract

Content of polyphenols and soluble pectin in different fruits and vegetables as well as in green tea extract was determined. Two novel antioxidant composites from plant extracts were proposed as antioxidant food additives. One composite consisted of green tea extract and red wine lees, another composite included lemon peels extract in addition. The composite from green tea extract and red wine lees showed by 30% more antioxidant activity than the other composite.

Key words: antioxidant, polyphenols, tea, wine, citrus, composite, food additive

Introduction

Since many people are concerned about the presence of "chemicals" in the food and worry about possible ill-effects of eating them, search for novel natural sources of food additives are still very actual problem.

The antioxidant activity of polyphenolic compounds has long been of primary interest [Halliwell, 1994]. Green tea catechins and theaflavins in black tea are effective antioxidants [Leung et al, 2001]. Other particular rich sources of the polyphenols are grapes [Kader and Barret, 2005]. There are also many patents for helpful products to be made from citrus substances: bioflavonoids from citrus also have antimicrobial properties and help milk preserve freshness if added before pasteurization [Braddock, 1999].

The objective of this work was to determine content of polyphenolic compounds in some commercial sources of plant raw materials in order to create novel composites of plant extracts and test their antioxidant power.

Materials and Methods

Plant materials: green tea extract with 23%, 60% and 80% total polyphenols content made from leaves of *Camellia sinensis L.* was obtained from the local plant extracts producing JS company "Kolkheti 93", red wine lees produced from Georgian variety of *Vitis vinifera* "Saperavi" was obtained from the local wine producing company "Tbilgvino Ltd", lemons of Georgian variety of "Kartuli", apple (*Malus domestica* Borkh), pear (*Pyrus* sp. div. cult.), plum (*Prunus divaricata* Ldb. and *Prunus Domestica* L.), medlar (*Mespilus germanica* L.), cherry (*Cerasus vulgaris* Mill),

mulberry (*Morus* alba L.), strawberries (*Fragaria vesca* L.), were commercially available at local market. All plant materials were analysed directly or stored at 4^0C for few days.

Aqueous extract samples preparation: 1 kg of fresh plant material was carefully cut into small pieces. 10 g of chopped material was immediately homogenized with 40 ml of distilled hot water in commercial food processor; the homogenate was poured into 100 ml Erlenmeyer flask and placed on the water bath for 30 min, centrifuged at 10 000 g for 20 min and the sediment re-extracted with the same amount of water and centrifuged. The combined supernatant was diluted with distilled water to 100 ml into volumetric flask. The obtained solution was transferred into 250 ml Erlenmeyer flask and shaken vigorously for 10 min and filtered through paper filter under vacuum.

Antioxidant power was estimated according to Benzie, Strain [1996].

An improved colorimetric assay using Folin-Ciocalteu reagent was applied to determine the **total polyphenol** content in plant materials [Bond et al., 2003].

Pectin substances were quantitatively analyzed by the method, which is based on the reaction of galacturonic acid with carbazole in sulphuric acid medium. Purple colour is developed when uronic acid reacts with carbazole [Demchenko et al., 1981].

Amino acids were determined by method based on color reaction of free amino acids with nynhydrin reagent [Burkina et al., 2000] with some modifications.

Mineralization of samples was done at 525°C in a combustion oven.

Macroassay for reducing sugars. The standard reducing-sugar assay was that of Nelson and Somogyi [Somogyi, 1952; Nelson, 1944].

Preparation of composites:

Composite from green tea extract and red wine lees. The composite was prepared by mixing liquid red wine lees and dry powder of green tea (instant tea) with the subsequent spray-drying up to the powder form. Wine lees was preliminarily left over night in cylindrical glass vessel at the 4 ^{0}C temperature in order to separate insoluble sediment. After sedimentation the clear supernatant was pumped over by decantation and filtered through a dense filter under vacuum. The filtered wine lees was heated up to 60 ^{0}C and green tea dry extract was added gradually by small portions and with constant stirring. The ratio of dry substances of wine lees and green tea extract was 1:2 respectively. The obtained solution was spray-dried up to powder form.

Composite from green tea extract, red wine lees and lemon peels. The filtered supernatant of the red wine lees was poured into vessel with pressed peels of lemon (ratio was 6:1 m/m), the mixture was heated up to 80 ^{0}C, kept for 15 min and then filtered through a dense filter under vacuum. The filtered extract was added by green tea extract (1:2 respectively, based on dry matter), and spray-dried up to powder form.

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

Results and Discussion

Content of polyphenols and soluble pectin in fruits and vegetables as well as in green tea extract was determined; results are given in Table 1.

Table 1. Content of polyphenols and soluble pectin in some plants (%, dry weight basis)

Vegetable	**Polyphenols**	**Soluble pectin**
Peach	1.21 ± 0.05	15.1 ± 0.3
Cherry	1.52 ± 0.05	13.2 ± 0.3
Apple	1.51 ± 0.05	14.0 ± 0.3
Mulberry	1.01 ± 0.05	18.2 ± 0.5
Pear	0.61 ± 0.04	42.3 ± 0.3
Medlar	1.11 ± 0.05	38.4 ± 0.5
Strawberry	0.42 ± 0.03	45.6 ± 0.6
Plum	1.11 ± 0.05	25.1 ± 0.5
Cherry plum	0.43 ± 0.02	31.4 ± 0.3
23% Green tea extract	23.52 ± 0.90	9.5 ± 0.6

From Table 1 it can be seen that green tea extract contained the highest amount of polyphenols (23.5 %). Content of pectin was significant in the fruits and vegetables. Strawberries and plums were especially rich in pectin substances. Abundant polyphenols and pectin were found in red wine lees as well (22 and 12.8% respectively) (Table 2).

Table 2. Proximate chemical composition of red wine lees from red wine "Saperavi", (% dry matter)

Constituents / Product name	Polyphenols	Soluble pectin	Reducing sugars	Amino acids	Organic acids
Red wine lees	16.4 ± 0.6	12.8 ± 0.5	14.0 ± 0.5	19.0 ± 0.6	27.7 ± 0.7

Red wine lees was rich in other bioactive compounds, such as sugars, amino acids and organic acids. Therefore, it was proposed to create a bioactive composite from green tea extract and red wine lees.

Tea is a good source of antioxidants and addition of lemon to tea increases its antioxidant power [Tewari et al., 2000]. Lemon peels were found to be a very rich source of polyphenols, pectin, sugars and organic acids in comparison with pulps (Table 3). Hence, it is the one more commercial source for production of bioactive composites.

Table 3. Proximate chemical composition of lemons (sort "Kartuli") peels and pulps (%, dry weight basis)

Polyphenols		Reducing sugars		Organic acids		Soluble pectin	
pulp	peel	pulp	peel	pulp	peel	pulp	peel
68.0± 1.0 mg per 100g	300.0±5.5mg per 100g	2.1±0.1%	6.5±0.3 %	5.6±0.3%	0.30±0.05%	12.1±0.6 %	33.2±0.9%

Based on these results the second composite was composed of green tea and lemon peels extracts and red wine lees. Both composites consisted of large amount of polyphenols (17-21%), soluble pectin (11-30 %), amino acids (7-15 %), organic acids (5-14 %), sugars (22-30 %) and minerals (8-9%) (Table 4). Accordingly, the plant composites might have a wide spectrum of high bioactivity. In particular their antioxidant power was estimated.

As it can be seen from Table 5 both composites showed high antioxidant power, though the composite from green tea extract and red wine lees revealed by 30% more antioxidant power than the other composite. The composite from green tea extract and red wine lees and the

composite from green tea and lemon peels extracts and red wine lees showed only about 4 and 5 times less antioxidant power (respectively) than ascorbic acid.

Table 4. Proximate chemical composition of plant extract composites (% dry matter)

Composite	Polyphenols	Soluble pectin	Organic acids	Amino acids	Reducing sugars	Minerals
Green tea + red wine lees + lemon peels	21.3 ± 0.5	14 ± 0.4	13.9 ± 0.6	7.0± 0.4	30± 0.6	8± 0.3
Green tea + red wine lees	17.3 ± 0.5	11.5 ± 0.3	16.0 ± 0.6	15.3 ± 0.5	22.5 ± 0.7	8.5 ± 0.5

Table 5. Ferric reducing antioxidant power of different plant products

Plant product	Antioxidant power [Fe2+] mM *
23% Green tea extract	2.1 ± 0.1
Lemon peels extract	0.25 ± 0.05
Ascorbic acid	9.4 ± 0.7
Composite of green tea extract and red wine lees	2.3 ± 0.3
Composite of green tea and lemon peels extracts and red wine lees	1.8 ± 0.2

*- Figures are calculated on the base of 1 g/l concentration of the extracts

Thus, both plant composites with high antioxidant activity may be successfully used in the food industry as antioxidant natural food additives.

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ახალი მცენარეული კომპოზიტები, როგორც ანტიოქსიდანტური საკვები დანამატები

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(მიღებულია 10.07.2008)

რეზიუმე

დადგენილია პოლიფენოლებისა და ხსნადი პექტინის შემცველობა სხვადასხვა ხილსა და ბოსტნეულში, აგრეთვე მწვანე ჩაის ექსტრაქტში. შემოთავაზებულია მაღალი ანტიოქსიდანტური აქტივობის მქონე ორი ახალი მცენარეული კომპოზიტი, როგორც ანტიოქსიდანტური საკვები დანამატები. ერთი კომპოზიტი მიღებულია მწვანე ჩაის ექსტრაქტისა და წითელი ღვინის ლექის საფუძველზე, ხოლო მეორე კომპოზიტი დამატებით შეიცავს ლიმონის კანის ექსტრაქტს. მწვანე ჩაისა და წითელი ღვინის ლექიდან მიღებულ კომპოზიტს მეორე კომპოზიტთან შედარებით გააჩნია 30%-ით უფრო მეტი ანტიოქსიდანტური აქტივობა.

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Botany

INVASION OF *CENTAUREA SOLSTITIALIS* L. IN STEPPE VEGETATION OF TBILISI SURROUNDINGS

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Abstract

The paper presents the results of a research on invasion specificities of a weed *Centaurea solstitialis* L. in steppe vegetation (Bothriochloeta; *B. ischaemum* and Festuceta; *F. valesiaca*) of Tbilisi surroundings. In steppe vegetation coenoses with sustainable (or slightly disturbed structure) the coenotic role of *C. solstitialis* is insignificant; its vitality is low: populations are represented by faded and weak individuals with poor renewal. In structure disturbed semi-ruderal coenoses the coenotic role of *C. solstitialis* is high (high projective coverage and frequency of occurrence). Its populations are represented by middle-sized and big (strongly branched) individuals with the ability to form a great number of heads. Their vitality is high.In steppe vegetation coenoses invasion of *C. solstitialis* is directly proportional to disturbance of coenosis structure and soil erosion. The most important factor promoting the process is the human impact.

Key words: projective coverage, frequency of occurrence, species load, plant height, number of heads.

Introduction

Centaurea solstitialis L. [Fam. Compositae (Asteraceae)] is a widespread annual weed. Its natural distribution range covers the Mediterranean region, southeastern part of Middle Europe (the Black Sea shore), the Caucasus, S.W. Asia (Front Asia) and southwestern part of Middle Asia (mountainous Turkmenistan, Pamir and Altay). Its secondary distribution range is much broader and includes Western and Central Europe, Eastern Asia (Far East), North and South America, etc. [Czerepanov, 1963; Wagenitz, 1975; Dostal, 1976; Jinjolia, 2007; etc.].

C. solstitialis has invaded vast areas within its secondary range and it often even dominates on these areas. A great number of researches have been done to find out reasons for invasion of *C. solstitialis* [D'Antonio et al., 1999; Callaway and Aschehoug, 2000; Dukes, 2002; Callaway et al., 2005; Hierro et al., 2005; etc.]. Various scientific projects are being implemented to solve the problem. Investigations are carried out both in countries of the species secondary distribution and wild vegetation within the natural distribution range. Georgia is involved in one (№ GRBI.2833-TB-06) of the projects. The project activities are carried out in the USA (California), Argentina, Armenia and Georgia.

The aim of our work was to study invasion of *C. solstitialis* into the steppe vegetation of Tbilisi surroundings; find out its coenotic role in connection with principal indices of the vegetation

structure and some components of vitality in different coenoses of the steppe vegetation as well as in structure disturbed semi-ruderal coenoses strongly weeded with *C. solstitialis*; and to determine *C. solstitialis* invasion extent as well as some reasons of this invasion in the steppe vegetation in parallel to a field experiment envisaged by the above project.

Materials and Methods

Geobotanical and ecological data were collected using itinerary methods during 2007-2008. Geobotanical surveys were conducted and coenoses were distinguished according to conventional geobotanical methods [Shennikov, 1964; Vasilevich, 1985]. Species load per area unit and frequency of occurrence were studied using methods by A. A. Korchagin [Korchagin, 1964], J. Braun-Blanquet [Braun-Blanquet, 1964] and V. M. Ponyatovskaya [Ponyatovskaya, 1964].

In order to study height, the number of heads, extent of damage of roots and heads (presence of caterpillars in inflorescences and burrows and galls in roots) of *C. solstitialis* with the range of each coenosis 100 individuals (less in some cases, when the populations were small) were randomly collected during flowering peak.

Various coenoses of the formations Bothriochloeta (*B. ischaemum*) and Festuceta (*F. valesiaca*) that are common in Tbilisi surroundings were selected as the study objects. For comparison investigations were conducted in structure disturbed semi-ruderal coenoses strongly weeded with *C. solstitialis*, which were conditionally nam,ed as Centauretum, and in similarly strongly weeded Cynodonetum (*C. dactylon*) occurring along roads.

Plant nomenclature follows R.Gagnidze [Gagnidze, 2005].

Results and Discussion

Brief geobotanical description of the studied coenoses, coenotic role of *C. solstitialis* in these coenoses and some principal indices of vitality are given below.

1. *Bothriochloa ischaemum+Festuca valesiaca*+mixtoherbosa (surroundings of the village Tsavkisi). The coenosis is studied in the gorge of the river Tsavkisistskali between the villages Tsavkisi, Shindisi and Okrokana at 810-835 m a.s.l. The coenosis is formed on an east-facing slope. The slopes are terraced. Inclination of separate parts of the terraces is from 2-3° to 15-16°, while that of short segments between terraces is 22-27°. General projective coverage is from 77-78% to 97-98%, sodding ranges from 15-17% to 32-35 %. No litter is present. Projective coverage of *Bothriochloa ischaemum*, the edificator, ranges from 15-17% to 70-72% on different parts of the range. *Festuca valesiaca* (projective coverage from 9-10% to 25-27%) is the sub-edificator. Species load per 1 m^2 is 27.6. 70 vascular plant species were recorded in 15 geobotanical relevés.

Frequency of *C. solstitialis* is 13.3%. It can be found in a form of separate individuals – the projective coverage is indicated by "+" mark. Average height of individuals is 16 cm, minimum – 5 cm and maximum – 42 cm. 1.4 heads on average are formed on each plant. Proportion of individuals according to the number of heads is as follows:

1-headed – 70%	3-headed – 9%
2-headed – 20%	4-headed – 1%

2. *Festuca valesiaca+Bothriochloa ischaemum*+mixtoherbosa (surroundings of the village Tsavkisi). The coenosis is studied in the gorge of the river Tsavkisistskali between the

villages Tsavkisi, Shindisi and Okrokana at 810-835 m a.s.l. The coenosis is formed on an east-facing slope. General inclination of the slopes is between 18-22°, while in separate places it ranges from 5-7° to 20-22°. General projective coverage is from 75-78% to 94-95%. Sodding ranges from 23-25% to 40%. No litter is present. Projectrive coverage of *Festuca valesiaca*, the edificator, ranges from 25-26% to 60-62% on different parts of the range. *Bothriochloa ischaemum* (projective coverage from 14-15% to 30-35%) is the sub-edificator. Species load per 1 m^2 is 31.5. 73 vascular plant species were recorded in 15 geobotanical relevés.

Frequency of *C. solstitialis* is 2.7%. It can be found in a form of separate individuals – the projective coverage is indicated by "+" mark. Average height of individuals is 11.7 cm, minimum – 4 cm and maximum – 27 cm. 1.4 heads on average are formed on each plant. Proportion of individuals according to the number of heads is as follows:

1-headed – 72%
2-headed – 20%
3-headed – 4%
4-headed – 4%

3. ***Festuca valesiaca*+mixtoherbosa (surroundings of the village Shindisi).** The coenosis is studied on the western end of Tabori range in surroundings of the village Shindisi at 820-850 m a.s.l. The coenosis is formed on a north- and partially northwest-facing slope. Mean slope inclination is 20-25° and the range of inclination variability is from 10-12° to 30-32°. General projective coverage of the coenoses ranges from 70% to 93-95%. Sodding ranges from 20% to 53-55%. No litter is present. Projective coverage of *Festuca valesiaca*, the edificator, on separate parts of the area ranges from 35-36% to 70-72%. Species load per 1 m^2 is 28.7. 79 vascular plant species were recorded in 16 geobotanical relevés.

Frequency of *C. solstitialis* is 43.7%. It can mostly be found in a form of separate individuals – the projective coverage is indicated by "+" mark; rarely the projective coverage is 1-3%. Average height of individuals is 15.7 cm, minimum – 4 cm and maximum – 40 cm. 2.6 heads on average are formed on each plant. Proportion of individuals according to the number of heads is as follows:

1-headed – 23%
2-headed – 36%
3-headed – 25%
4-headed – 5%
5-headed – 4%
6-headed – 4%
7-headed – 2%
8-headed – 1%

4. ***Bothriochloa ischaemum*+*Festuca valesiaca*+mixtoherbosa (surroundings of the village Dighomi).** The coenosis is studied in the gorge of the river Dighmistskali between the villages Dighomi and Didgori at 775-800 m a.s.l. The coenosis id formed of a south-facing slope, while separate micro-slopes are southeast-facing. General slope inclination is 15-20° and ranges from 5-7° to 20-22° on various micro-plots. General projective coverage is from 74-75% to 91-92%. Sodding ranges from 15-17% to 33-35%. No litter is present. Projective coverage of *Bothriochloa ischaemum*, the edificator, varies from 38-40% to 65-67%. *Festuca valesiaca* is the sub-edificator. Its projective coverage ranges from 1-3% to 18-19%. Species load per 1 m^2 is 24.6. 61 vascular plant species were recorded in 11 geobotanical relevés.

Frequency of *C. solstitialis* is 63.6%. It can mostly be found in a form of separate individuals – the projective coverage is indicated by "+" mark; rarely the projective coverage is from 0.5% to 4%. Average height of individuals is 9.9 cm, minimum – 3 cm and maximum – 25 cm. 1.2 heads on average are formed on each plant. Proportion of individuals according to the number of heads is as follows:

1-headed – 85%
2-headed – 11%
3-headed –3%
4-headed – 1%

5. **Centauretum; *C. solstitialis* (surroundings of the village Dighomi)**. The coenosis is formed adjacent to the previous coenosis (№ 4) on the same slope. General projective coverage is from 55-60% to 90-92%. No sodding is present. Projective coverage of litter ranges from 6-7% to 20% (mean 15-17%). *Centaurea solstitialis* is the edificator. Its projective coverage varies from 55-60% to 82-85%. Average height of the 1st layer mainly made up of *Centaurea solstitialis*, is 20-30 cm. Species load per 1 m^2 is 9.6. 39 vascular plant species were recorded in 10 geobotanical relevés. Proportion of *Botriochloa ischaemum* and *Festuca valesiaca*, the principal species of the adjacent *B. ischaemum* formation, is high on some plots (projective coverage of *B. ischaemum* is 10-20% and that of *F. valesiaca* is 1-2%). Also, one or two of the following species are abundant in places, which indicates structure degradation and instability of the coenosis: *Elytrigia repens*, *Achillea biebersteinii*, *Salvia nemorosa*, *Euphorbia seguieriana*, *Carthamus lanatus*, *Scabiosa micrantha*, *Daucus carota*, etc.; while high proportion of the species characteristic to the adjacent coenosis of *B. ischaemum* formation proves that the latter coenosis is formed as a result of degradation of the former one.

Average height of individuals is 24 cm, minimum – 8.5 cm and maximum – 48 cm. 2.9 heads on average are formed on each plant. Proportion of individuals according to the number of heads is as follows:

1-headed – 31%
2-headed – 23%
3-headed – 20%
4-headed – 9%
5-headed – 4%
6-headed – 3%
7-headed – 3%
8-headed – 4%
9-headed – 1%
10-headed – 1%
12-headed–1%

6. **Centauretum; *C. solstitialis* (surroundings of the village Okrokana)**. The coenosis is studied at the lower part of the south-facing macro-slope of Mama-Daviti range in surroundings of the village Okrokana at 850-870 m a.s.l. In places micro-slopes are southeast-facing. Slope inclination is 5-12°. General projective coverage varies from 60-62% to 88-90%. No sodding is present. Litter projective coverage ranges from 5-6% to 30-35% (mean 14-15%). *Centaurea solstitialis* has the role of the edificator. Its projective coverage varies from 32-33% to 56-58%. Average height of the 1st layer mainly made up of *C. solstitialis*, is 25-30 cm. Species load per 1 m^2 is 12.5. 48 vascular plant species were recorded in 10 geobotanical relevés. Frequently (in 30-50% of the surveys) coenotic role of *Eryngium campestre* and *Daucus carota* is very high (projective coverage varies from 5% to 40-42%). Along with these species one or two of the following are abundant on separate parts of the area: *Trachynia distachya*, *Chondrilla juncea*, *Aegilops triuncialis*, *Filago arvensis*, etc. All these indicate structure degradation and instability of the coenosis.

In contrast to the above coenosis of Centauretum (surroundongs of the village Dighomi) participation of steppe edificators (*Bothriochloa ischaemum* and *Festuca valesiaca*) as well as typical perennials is insignificant, which indicates that formation of Centauretum within this range has started longer ago.

Average height of individuals is 23.6 cm, minimum – 4 cm and maximum – 54 cm. 4.2 heads on average are formed on each plant. Proportion of individuals according to the number of heads is as follows:

1-headed – 9%
2-headed – 20%
3-headed – 22%
8-headed – 4%
9-headed – 2%
10-headed – 1%

4-headed – 15%
5-headed – 13%
6-headed – 3%
7-headed – 7%
11-headed – 1%
12-headed – 1%
13-headed – 1%
14-headed – 1%

7. Cynodonetum; *C. dactylon* (roadsides; surroundings of the villages Tsavkisi, Shindisi, Okrokana). The coenosis is formed on roadside east- and northeast-facing micro-slopes. Slope inclination is 15-35°. General projective coverage varies from 68-70% to 98-99%. Sodding is not present. Litter projective coverage is insignificant ("+" – 5-6%). *Cynodon dactylon* is the edificator. Its projective coverage on different parts of the range varies from 42-45% to 90-92%. Average height of the 1st layer mainly made up *C. solstitialis* starting from the second half of summer, is 45-55 cm. Species load per 1 m^2 is 16.1. 49 vascular plant species were recorded in 10 geobotanical relevés.

Participation of steppe edificators (*Bothriochloa ischaemum* and *Festuca valesiaca*) as well as typical perennials is insignificant, which is quite normal to semi-ruderal coenoses formed on roadsides.

Frequency of *C. solstitialis* is 100%. Its projective coverage ranges from 35-36% to 70-72%. Average height of individuals is 47.1 cm, minimum – 15 cm and maximum – 99 cm. 16.8 heads on average are formed on each plant. It is worth mentioning that individuals with 468 and 600 heads were found. Proportion of individuals according to the number of heads is as follows:

1-headed – 1%
2-headed – 3%
3-headed – 9%
4-headed – 3%
5-headed – 8%
6-headed – 12%
7-headed – 6
8-headed – 7%
9-headed – 7%
10-headed – 4%
10-20-headed – 28%
21- and more-headed – 12%

Table 1. Frequency of occurrence and coenotic role of *Centaurea solstitialis* in different coenoses

Coenosis	Average sodding of a coenosis (%)	Species load (per 1 m^2)	Average projective coverage of a coenosis (%)	Centaurea solstitialis	
				Frequency of occurrence (%)	Average projective coverage (%)
1. *Bothriochloa ischaemum+Festuca valesiaca*+mixtoherbosa (Tsavkisi)	21-23	27.6	92-95	13.3	+
2. *Festuca valesiaca+Bothriochloa ischaemum*+mixtoherbosa (Tsavkisi)	32-34	31.5	88-90	2.7	+
3. *Festuca valesiaca*+mixtoherbosa (Shindisi)	35-37	28.7	85-87	43.7	+, 1-3
4. *Bothriochloa ischaemum+Festuca valesiaca*+mixtoherbosa (Dighomi)	26-27	28.0	83-85	63.6	0.5-1
5. Centauretum (Dighomi)	-	9.8	75-77	100	67-70
6. Centauretum (Okrokana)	-	12.5	72-75	100	42-45
7. Cynodonetum (roadsides)	-	16.1	80-83	100	48-50

Comparison of some indices of the structure of the studied coenoses shows that in structure disturbed semi-ruderal coenoses sodding is not present in contrast to coenoses of steppe vegetation. It is also important that species load (per 1 m^2) is markedly decreased, while frequency of occurrence of *C. solstitialis* and its coenotic role are increased (Table 1).

The study has shown that in coenoses of steppe vegetation populations of *C. solstitialis* are mainly represented by small, faded individuals (Fig. 1), while in structure disturbed semi-ruderal coenoses *C. solstitialis* individuals are much taller (especially in roadside coenoses). Such a difference clearly indicates that in coenoses of steppe vegetation there are less favourable conditions for growth of *C. solstitialis.*

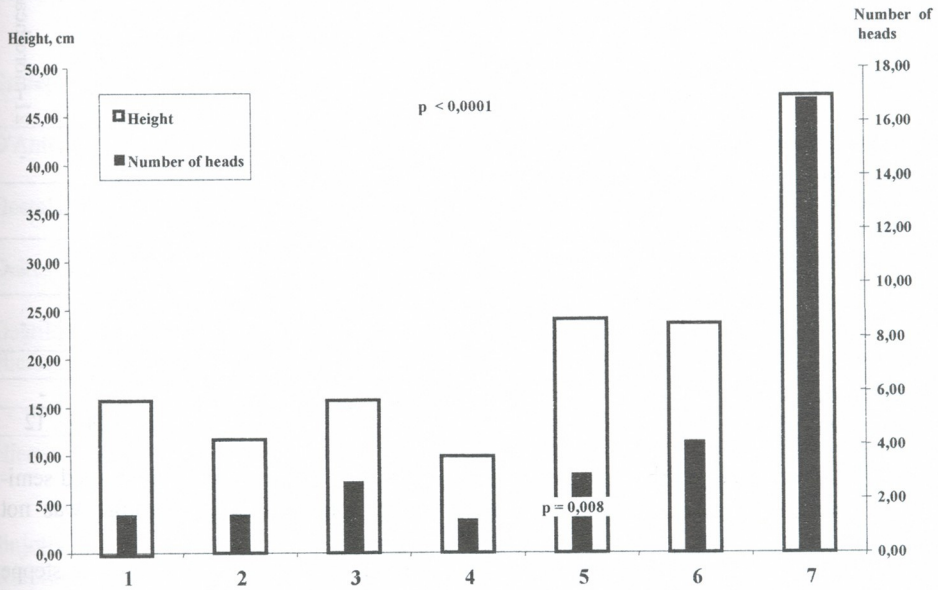

Fig. 1. Height (cm) and number of heads of *Centaurea solstitialis* in different coenoses

1. *Bothriochloa ischaemum+Festuca valesiaca*+mixtoherbosa (Tsavkisi)
2. *Festuca valesiaca+Bothriochloa ischaemum*+mixtoherbosa (Tsavkisi)
3. *Festuca valesiaca*+mixtoherbosa (Shindisi)
4. *Bothriochloa ischaemum+Festuca valesiaca*+mixtoherbosa (Dighomi)
5. Centauretum (Dighomi)
6. Centauretum (Okrokana)
7. Cynodonetum (roadsides, surroundings of the village Tsavkisi, Shindisi, Okrokana)

Degree of head formation is also quite different. In studied steppe vegetation coenoses of *C. solstitialis* each individual forms 1.4-2.6 heads on average, while in structure disturbed semi-ruderal coenoses the index varies from 2.9 to 16.8 (Fig. 1). Proportion of individuals of *C. solstitialis* according to the number of heads is another important index (Table 2). In populations of *C. solstitialis* presented in the coenoses of steppe vegetation 1-headed individuals are most numerous – from 70% to 85% (except *Festuca valesiaca*+mixtoherbosa), while in the structure disturbed semi-ruderal coenoses the number of 1-headed individuals is small.

In contrast the number of 6-10- and more-headed individuals is high: in roadside Cynodonetum their summarized proportion is 76% and in the coenosis at Okrokana it is 21%. In the coenoses of steppe vegetation 6-10-headed individuals were recorded only in a single coenosis, while 10- and more-headed were not recorded in any of the coenoses. Development of heads on *C. solstitialis* plants as well as height of individuals indicated that in the coenoses of steppe vegetation vitality of the species is low and conditions for growth and renewal are unfavourable; while in structure disturbed semi-ruderal coenoses conditions are much more suitable for

development of the plant. It is worth mentioning that also in non-native regions disturbance of the coenosis structure is a factor promoting invasion of *C. solstitialis* [Elton, 1958; D'Antonio et al., 1999; Callaway et al., 2005; Hierro at al., 2006].

Table 2. Proportion (%) of individuals of *Centaurea solstitialis* according to the number of heads in different coenoses

Coenosis	1-headed	2-headed	3-headed	4-headed	5-headed	6-10-headed	11-20-headed	21-more-headed
1. *Bothriochloa ischaemum* +*Festuca valesiaca*+mixtoherbosa	70	20	9	1	-	-	-	-
2. *Festuca valesiaca* +*Bothriochloa ischaemum*+mixtoherbosa (Tsavkisi)	72	20	4	4	-	-	-	-
3. *Festuca valesiaca* +mixtoherbosa (Shindisi)	23	36	25	5	4	7	-	-
4. *Bothriochloa ischaemum* +*Festuca valesiaca*+mixtoherbosa (Dighomi)	85	11	3	1	-	-	-	-
5. Centauretum (Dighomi)	31	23	20	9	4	12	1	-
6. Centauretum (Okrokana)	9	20	22	15	14	17	3	-
7. Cynodonetum (roadsides)	1	3	9	3	8	36	28	12

Study of *C. solstitialis* head damage by insects in the steppe and structure disturbed semi-ruderal coenoses has not revealed any clear correlation. In some coenoses damage was not recorded, while in some of them just single heads were damaged.

Degree of *C. solstitialis* root damage caused by insects is relatively lower in the steppe vegetation coenoses (0-15%). The damage is more pronounced (15-60%) in structure disturbed semi-ruderal coenoses, especially roadside ones.

On the basis of statistical analysis of structure the above described coenoses and some indices of plant vitality in different populations of *C. solstitialis* the following conclusions were drawn:

In steppe vegetation coenoses with sustainable (or slightly disturbed structure) the coenotic role of *C. solstitialis* is insignificant; its vitality is low: populations are represented by faded and weak individuals with poor renewal.

In structure disturbed semi-ruderal coenoses the coenotic role of *C. solstitialis* is high (high projective coverage and frequency of occurrence). Its populations are represented by middle-sized and big (strongly branched) individuals with the ability to form a great number of heads. Their vitality is high.

In steppe vegetation coenoses invasion of *C. solstitialis* is directly proportional to disturbance of coenosis structure and soil erosion. The most important factor promoting the process is the human impact (mainly, overgrazing and mechanical disturbance of coenoses).

Results of the field experiment (study of effects of anthropogenic factor, competition with other ruderal species, natural enemies and soil biota on development of *C. solstitialis*) will show the role of other factors acting together with human impact in the process of *C. solstitialis* invasion.

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Centaurea solstitialis L.-ის ინვაზია თბილისის მიდამოების სტეპის მცენარეულობაში

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თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

(მიღებულია 08.09.2008)

რეზიუმე

დადგენილია თბილისის მიდამოების სტეპის მცენარეულობაში (Bothriochloeta; *B. ischaemum* და Festuceta; *F. valesiaca*) სარეველა მცენარის *Centaurea solstitialis* L.-ის ინვაზიის თავისებურებები. სტრუქტურაშენარჩუნებულ სტეპის ცენოზებში *C. solstitialis*-ის ცენოზური როლი უმნიშვნელოა; მისი სიცოცხლიანობა დაბალია – პოპულაციებს ქმნის დაჩაგრული სუსტი ინდივიდები, რომელთა გენერაციული განახლება მცირეა. სტრუქტურადარღვეულ ნახევრად რუდერალურ ცენოზებში *C. solstitialis*-ის ცენოზური როლი მაღალია – მისი პოპულაციები წარმოდგენილია საშუალო და დიდი ზომის ძლიერ დატოტვილი ინდივიდებით, რომლებიც მრავალ კალათას ინვითარებენ. სტეპის მცენარეულობაში *C. solstitialis*-ის ინვაზია ცენოზების სტრუქტურისა და ნიადაგური საფრის რღვევის პირდაპირ პროპორციულია. ამ პროცესის ერთ-ერთი ყველაზე ხელშემწყობი ფაქტორია ანთროპოგენური ზეგავლენა.

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MORPHOGENESIS AND REPRODUCTION BIOLOGY OF *MUSCARI ALPANICUM* SCHCHIAN

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Abstract

Structural aspects of reproduction biology, seed production processes and germination capacity have been studied in *Muscari alpanicum* Schchian - plant endemic to Georgia. High potential for sexual reproduction and high rate of full value seed formation have been established in conditions of outcrossing. Formation of both male and female generative spheres and reproductive activity are within the limits of norm in conditions of outcrossing and correspondingly great number of seeds having high germination capacity is produced. Autofertility of the studied plant was revealed, which is of facultative character and can be regarded as an auxillary mechanism of seed formation in unfavorable environmental conditions.

Key words: *Muscari alpanicum*, microsporogenesis, embryogenesis, seed germination.

Introduction

Muscari alpanicum Schchian, plant endemic to Georgia, is one of 12 species of the genus *Muscari* (family Hyacinthaceae) distributed on the territory of Georgia [Gagnidze, 2005]. The species was first described by A. Schchian in 1938 from its native distribution range, the surroundings of the settlement of Alpana, situated in the West Georgia [Schchian, 1938].

The name "muscari" derives from Arabian word "muscarimi", which in its turn originates from the Greek word "moschos" referring to the musk, due to specific fragrance emitted by certain species of the genus, though some species of Muscari are lacking such specific smell. Georgian name of *Muscari alpanicum* is "alpanuri kazakha". Plants of *Muscari alpanicum* are distinguished with bigger size as compared with other species of the genus Muscari. Plant is highly ornamental, with beautiful inflorescence of light blue coloration and delicate aroma. *Muscari alpanicum* is distributed in the regions of West Georgia - Racha-Lechckhumi and Imereti. The species grows on calcareous soils from the lower mountain belt up to the upper mountain boundary. At present the species is under the threat of extinction. This makes especially urgent the study of reproduction peculiarities of the species.

It should be outlined also that embryology of *Muscari alpanicum* is completely unstudied.

The aim of our investigation was to study the processes of formation of male and female generative spheres and the processes of fertilization and seed formation in *Muscari alpanicum*, determination of productivity of the formed seeds, establishment of their germination capacity and creation of *ex-situ* collections of this rare plant.

Materials and Methods

Material for investigations was taken during the period of 2006-2007 from the ranges of natural distribution of *Muscari alpanicum* - the settlement Alpana (Tsageri district) and village of Barakoni (Ambrolauri district) (N42°32. 345; E43°13.069') at 582 m altitude ASL.

Investigations were made both in field and laboratory (sowing on Petri dishes, in pots and open ground). Seedling biology was observed in pots, placed in glasshouse at the temperature approaching the ambient conditions.

Embryology was studied using cytoembryological methods [Baskin F., Baskin C., 2002].

Material was fixed using FAA, Carnua, FPA. Chromosome number was determined in mother cell of pollen grain in Prophase, M1. Microscopic study was performed using the light microscope of Carl Zeiss (Germany) firm and microphotographs made on the film МФН-12.

Seed production capacity was determined according to the method by Rabotnov [Rabotnov, 1960]. 50 capsules with mature seeds were taken from each population and number of formed seeds and undeveloped ovules was counted.

Germination capacity was tested at room temperature 18-20°C.

Results and Discussion

Morphology. *Muscari alpanicum* is a perennial bulbous plant. Bulb is oval-spherical, containing numerous bulblets. In addition to the main root it develops a great number of adventitious roots as well. Stem is 20-35 cm high, leaves linear. Flowers are gathered in 6-10 cm long and 2-3 cm wide apical raceme. The inflorescence is oblong-ovate first and it becomes cylindrical later. Flower is zygomorphic. Two different zones can be distinguished in the raceme. The upper apical flowers are infertile and differ in appearance from fertile flowers by smaller size. Androecium and gynoecium are not developed in these flowers. The upper zone is nearly 2-3 cm long.

The main zone is 4-6 cm long and mainly fertile flowers are gathered in it. Coloration of flowers is nearly the same in both zones. Perigone of fertile flower is cylindrical, 5-8 mm long and 4-5 mm wide, light violet, with whitish denticles at the border. Stamens are cylidrical at the base. The fruit is roundish capsule, 7-11 mm long and 7-9 mm wide (Fig.1, Flora of Georgia, Vol. II, 1941). Seed is of black color with lustrous surface.

Flowering takes place in the 3rd decade of March or the first half on April and lasts for a month in moist soil. Seed becomes mature in June. Leaves die away after the seed maturation and the bulbs pass to the stage of dormancy.

After the period of summer dormancy at the beginning of September plant starts the second vegetation.

Propagation. Nearly all species of *Muscari* are known to propagate mainly vegetatively, by bulblets, though propagation by seeds also takes place. Plants of the genus *Muscari* are cross-pollinating and pollinated by insects.

Structural studies of processes of microsporogenesis and embryogenesis have been carried out with the aim to clarify the sexual reproductive cycle of *Muscari alpanicum*.

Flower of *Muscari alpanicum* is characterized with slightly expressed protogyny. The 6 stamens are arranged within the perigone tube in 2 rows like the other species of the genus Muscari [Speta F., 1998]. At an early stage of flowering the receptive stigma is located behind the closed anthers, but on the next step of development filiform style elongates placing stigma between the anthers. This process is followed by the dehiscence of anthers.

At an early stage of development the anther is four-locular but upon maturation it becomes two-locular. The wall of pollen grain consists of epidermis, single layer endothecium, intermediate

layer and the tapetum of secretory type. Cells of tapetum are binucleate. Meiosis proceeds normally. The 18 bivalents are found in the prophase of the first meiotic splitting, in particular the diplonema and diakinesis. Bivalents are mainly of closed type, though sometimes 1-3 open bivalents are formed. Chromosomes differ in size. The arms of the most chromosomes are of equal length. In some cases the wall of pollen forming mother cell is not fully decomposed and the tetrad doesn't disassemble. In some cases it is split into two dyads or assembled in groups. Both simple and complex pollen is found. Mononucleate pollen grain splits to form generative and vegetative cells of different sizes. Vegetative cell is spindle-shaped and is attached to the wall of pollen grain. Small size is horseshoe-shaped generative cell is located opposite to the pore of pollen grain. Mature pollen grain is monopore, double layered. The outer layer is smooth. Mature pollen is bicellular. Generative cell division takes place in the pollen tube. Nearly all mature pollen grains are fertile.

Fig. 1. 1) perigone; 2) perianth; 3) gynoecium; 4) raceme; 5) fruit(capsule); 6. seed (Scheme is given according to Flora of Georgia, Vol. II, 1941)

The superior ovary is three-locular, with two ovules in each locule. Ovule is anatropous, with two integuments, crassinucellate. In the progamic phase of fertilization active growth of pollen tube into the depth of embryo sac was observed. Polar nuclei merge with each other prior to fertilization. Embryo develops in the way characteristic to the Asterad type. Embryo is linear. Endosperm is of helobial type, with haustorium. Endosperm is preserved in mature seed.

The studied material has shown that in conditions of outcrossing the processes of double fertilization, embryo- and endosporogenesis proceed normally.

While studying the processes of fertilization and embryogenesis in conditions of isolation, when self-pollination takes place, far slow rate of pollen tube growth is observed. Sometimes

penetration of pollen tube into embryo sac is hampered. The process of embryo differentiation is extended in time.

In conditions of outcrossing *Muscari alpanicum* produces great number of seeds. Weight of 100 seeds from the single population is equal to ≈450±0.3 mg. Number of fertile flowers in inflorescence varies from 10 to 34 (n=17; 18.2±3.81). Seed number per inflorescence (individual plant) in the same population varies from 20 to 95 (n=7; 39.6±7.2). Each capsule contains from 2 to 3 seeds (Fig. 2). In conditions of isolation number of seeds developed per inflorescence varies from 8 to 20, 1 or 2 seeds develop in each capsule. Some of the ovules aborted at the time of fertilization.

Our investigation allowed to establish optimum terms of seed germination for *Muscari alpanicum*. Our observations have revealed that the certain period, namely 4-5 months are needed for seeds to germinate after seed set, though for another species of the genus *Muscari*, in particular *Muscari tenuiflorum*, this process takes 7-8 months [Herman et al., 2006].

Percent of seed germination on Petri dishes was rather high – it approached ≈100% (Fig. 3). From Petri dishes the seedlings were transferred to the pots, open ground and the closed space of glasshouse. After two years from planting in the open ground nearly all plants started to blossom. In conditions of plant isolation germination capacity of seeds obtained by means of self-fertilization as well as the rate of natural propagation of seedlings were significantly reduced and the cycle of development from seed to flower was delayed by two years.

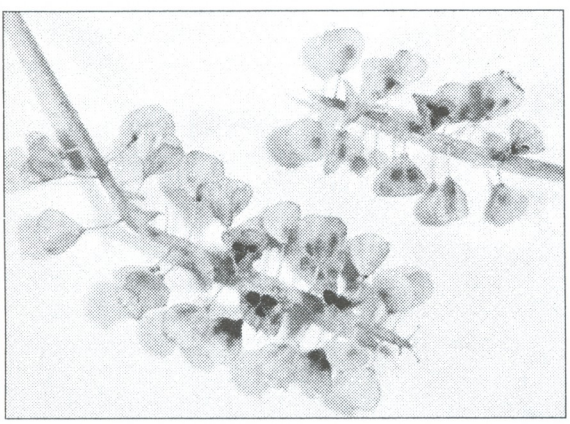

Fig 2. Raceme of *Muscari alpanicum* with mature seeds (2or 3 seeds in each capsule)

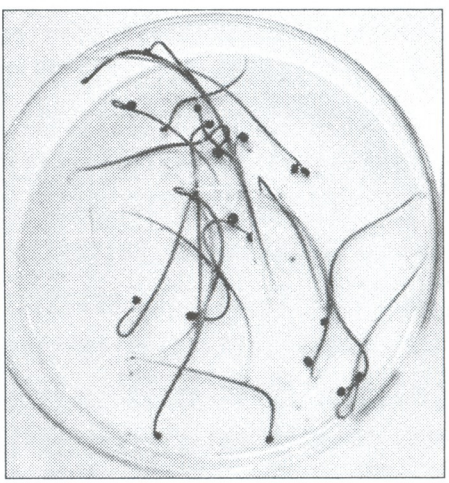

Fig. 3. Seeds of Muscari alpanicum germinated on Perti dish

Conclusions

Our investigations have shown that environmental conditions are responsible for seed production capacity of a species *Muscari alpanicum*. In conditions of outsrossing formation of both male and female generative sphere of *Muscari alpanicum* as well as reproduction activity are within the limits of norm. Consequently significant amount of germinable seeds is produced. In favorable conditions the species remains cross-pollinating, though in conditions of isolation, in the absence of pollinator insects, the species reveals the ability for self-pollination in order to maintain fertility. In such case, as it was proved by embryological investigations, fertilization process is somehow hampered and the percent of seed set is comparatively low, ≈50-60%. Despite this the

seedlings emerged from such seeds had normal cycle of ontogenesis, though the process of development was delayed.

Proceeding from this we consider autofertility of *Muscari alpanicum* to be of facultative character. It can be regarded as an auxillary mechanism of seed formation. The mentioned fact indicates generative plasticity of a species and can be regarded as a strategy of survival in unfavorable environment.

As to the reduction of populations of *Muscari alpanicum* in nature, it seems that a number of secondary factors operate in the range of natural distribution of a species and anthropogenic factor is one of the most significant ones among them.

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ალპანური ყაზახას *Muscari alpanicum* Schchian მორფოგენეზი და რეპროდუქციული ბიოლოგია

მელია ნ., გაბედავა ლ., ბარბლიშვილი თ., ჟღენტი ლ.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

(მიღებულია 25.07.2008)

რეზიუმე

შესწავლილია საქართველოს ენდემის *Muscari alpanicum* Schchian სქესობრივი გამრავლების ზოგიერთი სტრუქტურული ასპექტი და თესლწარმოქმნის პროცესები. დადგენილია თესლის გაღივებისა და აღმონაცენების უნარი. თავისუფალი ყვავილობის რეჟიმში მცენარის როგორც მამრობითი, ასევე მდედრობითი გენერაციული სფეროს ფორმირება ნორმის ფარგლებშია და შესაბამისად, მიიღება აღმოცენებისუნარიანი თესლის მნიშვნელოვანი რაოდენობა. *Muscari alpanicum*-ის ავტოფერტილობის უნარი ფაკულტატური ხასიათისაა და მისი ამოქმედება არახელსაყრელი გარემო პირობებისას თესლწარმოქმნის პროცესებში დამხმარე მექანიზმის როლს ასრულებს.

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Botany

EX-SITU CONSERVATION OF SOME ECONOMICALLY IMPORTANT RELICT PLANT SPECIES DISTRIBUTED IN GEORGIA

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Abstract

The paper presents distribution ranges of economically important relict plant species: *Gymnospermium smirnowii* (Trautv.) Takht. (Berberidaceae); *Hibiscus ponticus* Rupr. (Malvaceae); *Kosteletzkya pentacarpa* (L.) Ledeb (Malvaceae); *Paeonia steveniana* Kem.-Nath. (Paeoniaceae); *Pancratium maritimum* L. (Amaryllidaceae) and *Trapa colchica* Albov (Trapaceae) within the territory of Georgia. As a result of investigations GIS-based distribution maps are produced, the present state of populations is estimated and the capacity for propagation by seeds is evaluated for each of these relict plant species. Our investigations have revealed high vitality and fertility of the studied species and quite good potential for self renewal, expressed in high rates of seed germination and seedling growth. In our opinion environmental conditions (anthropogenic pressure, zoo factor, habitat degradation, etc.) are considered to be the main factors responsible for the reduction of population number of these species. Works are undertaken for *ex-situ* conservation of species under investigation: seed bank and collection of seedlings are created.

Key words: conservation, seed reproduction, germination, embryology.

Introduction

The territory of Georgia is very rich in biological diversity due to complex geological, hydrological, climatic and other environmental factors. The botanical diversity of Georgia is particularly remarkable when compared with similar countries of the temperate zone. According to current evaluations of experts nearly 60-65% of Georgia's territory is covered with natural plant cover which embraces more than 4100 species of vascular plants.

It is noteworthy that many species occurring in the country originated from various floristic regions of the world. The territory of West Georgia, referred to as Colchis in ancient Greek sources, supports many relict plant species of the Tertiary period.

More or less stable climatic conditions of Colchis refugium (the highest concentration of relict species are found in SW Georgia) and almost Tertiary appearance of local phytolandscapes (dense polydominant forests, evergreen arborescent understory, abundance of typical sylvan species of ground vegetation, Black Sea littoral sandy beach, ancient Mediterranean plant groupings, etc.) contributed to the presence of a number of relict species that are more or less well represented in the flora of Colchis but are extinct elsewhere. In addition there are a number of refugia of smaller areas throughout Georgia that are marked by the presence of relict species. Examples include the area of Black Sea sandy littoral with plants associated with peat bogs, where

the presence of relict plants is marked. The territory of Lagodekhi Reserve in the North-Eastern Georgia, the Borjomi gorge in Southern Georgia and some other isolated territories in both eastern and western parts of the country also belong to the marked refugia.

Relict plants are of conservation concern due to their limited geographical distribution, isolated taxonomic position, habitat destruction, and threat of extinction.

The goal of the present research was identification of precise distribution of target plants within the territory of Georgia; creation of GIS-based maps showing the geographical distribution of target plant species; evaluation of the current state of populations, establishing the capacity for propagation by seeds, collection of seeds and *ex-situ* conservation via creation of seed banks and living collections at the Department of Plant Conservation of Tbilisi Botanic Garden and Institute of Botany. Six of the most vulnerable and economically important relict plant species have been selected as objects of investigation based on a survey of available literature and through personal communications with colleagues from other Georgian botanical institutions. These species are gymnospermium - *Gymnospermium smirnowii* (Trautv.) Takht. (Berberidaceae); hibiscus -*Hibiscus ponticus* Rupr. (Malvaceae); kosteletzkya - *Kosteletzkya pentacarpa* (L.) Ledeb (Malvaceae); peony - *Paeonia steveniana* Kem.-Nath. (Paeoniaceae); sea daffodil - *Pancratium maritimum* L. (Amaryllidaceae) and water chestnut - *Trapa colchica* Albov (Trapaceae).

Materials and Methods

Below we give brief descriptions of the economic properties and habitats of relict species selected for investigation.

***Gymnospermium smirnowii* (Trautv.) Takht. Bot. Journ. 55, 8 (1970) 1119. – Leontice smirnowii Trautv. Acta Hort. Petrop. 7 (1880) 405; Kem.-Nath. in Fl. Georgiae, 2, 2 (1973)172 (Georg.)** - is a perennial herbaceous plant, 20-25 cm tall, with fleshy underground tuber, bottom leaves and yellow petals, known from only limited localities within Georgia. Populations of gymnospermium are found in Kakheti region, near Lagodekhi. This Tertiary relict is associated with dense deciduous broad-leaved forests as a component of ground vegetation. Due to its medicinal properties gymnospermium has been intensively used in folk medicine for the treatment of tuberculosis, anemia, and epilepsy and at the lack of appetite. Flowering period of the plant falls on February-March and fruit-bearing takes place in May. The species is included in the Red Book of Georgia.

***Hibiscus ponticus* Rupr. Mem. Acad. Sci. Petersb. (Phys.-Math.) 7, 15, 2 (1869) 25; Grossh. Flora Cauc., III (1932) 64; Davlianidze in Fl. Georgiae, 2, 8 (1983)** - is a perennial herbaceous plant. Stem is simple, 5-160cm tall. The plant is described from the river mouth of riv. Supsa, near the town of Poti. Plant belongs to a group of Tertiary relicts that are associated with the wetlands of Western Georgia. As referenced above, human induced transformation of wetland habitats has caused drastic reduction of populations of hibiscus. This plant has enormous economic potential as a highly productive source of bast fiber. It can be used as a material for making ropes and fishing nets. Seeds are rich in fat. Fruits of hibiscus can be used in cookery. The species is also prospective for ornamental purposes.

Period of flowering falls on July-August, fruit bearing takes place in September. Seeds are of reddish color, protruded, 2mm in diameter, with scabrous surface. The species is not included in the Red Book of Georgia.

***Kosteletzkya pentacarpa* (L.) Ledeb. Fl. Ross. 1 (1812) 437; Kolak. Rast. mir Kolkhidy (1961) 356 (Russ.); Davlianidze in Fl. Georgiae, 2, 8 (1983) 310 (Georg.). – *Hibiscus pentacarpos* L., Sp.pl. (1753) 697** - is a perennial herbaceous plant, up to 2m tall. Stem is erect, cylindrical, branched in the upper part and lignified in the lower parts. Leaves have long stalks,

petals are of reddish-rose color. Presumably kosteletzkya is the rarest plant of all the Tertiary relicts known in the Caucasus. It grows is Lenchoran. In Georgia it is described from a single location (the shores of Lake Paliastomi). The plant is a component of peat bog vegetation. Plant is on the verge of extinction due to habitat destruction, as peat extraction activities are still in progress on the shores of Lake Paliastomi. Kosteletzkya is a fibrous plant known to produce high quality and durable bast fiber. In addition, it also may be used in beekeeping as a melliferous plant. Period of flowering falls on July-August and fruit bearing takes place in September-October. The species is not included in the Red Book of Georgia

***Paeonia steveniana* Kem.-Nath. Tr. Tiphl. Bot. Inst. XXI (1961) 20; edem, in Fl. Gruzii, 2, II (1973) 12 (Georg.)** - is a perennial herbaceous plant, up to 100 cm tall, with yellow scyphoid corolla of big size, relict of the Tertiary forest flora. Local populations of peony are associated with high-mountain forests of the Minor Caucasus. At present *Paeonia steveniana* is know to be distributed in 3-4 localities, where the plant individuals are being intensively collected by local population. *Paeonia steveniana* is highly ornamental plant to be used in ornamental gardening. It also possesses curative features and is used in folk medicine for the treatment of various gastric disorders, high blood pressure and epilepsy. Flowering takes place in June and fruit-bearing in August. Fruit is a capsule, with numerous seeds of black color. The species is included in the Red Book of Georgia.

***Pancratium maritimum* L. Sp. pl. (1753) 291; Grossh. Flora Cauc. 2, 2 (1940) 198 (Russ.); Kem.-Nath. in Fl. Georgiae 2 (1941) 522 (Georg.)** - is a perennial herbaceous plant with big bulb. Stem is 40-70 cm tall, perianth of white color. *Pancratium maritimum* is distributed in several regions of Georgia – Abkhazia, Ajara and Guria. This is a showy relict plant of sandy ancient littoral areas. The species belongs to the relicts of the oldest Mediterranean flora. Because the natural flora of sandy beaches is almost totally eliminated from its natural habitat, the sea daffodil is under the threat of extinction. The sea daffodil is a highly decorative wild plant with enormous potential as a garden plant in seaside areas. The plant contains volatile oils. Flowering takes place in August, fruits mature in September. Seeds are of black color, flat. The species is included in the Red Book of Georgia.

***Trapa colchica* Albov. Bull. Herb. Boiss. III, 5 (1895) 237; Khintibidze, in Fl. Georgiae 2, IX (1984) 119 (Georg.)** - is a perennial plant, 60-80 cm tall, relict of the Tertiary thermophilic flora. The aquatic plant was widely distributed in freshwater ponds of Colchis, but as a result of heavy anthropogenic pressure (drainage of wetlands, pollution with liquid and solid domestic wastes, sewage waters, eutrophication due to excess discharge of organic matter, etc.) its distribution range has drastically reduced. Mature fruits of water chestnut are rich in fat and edible raw and cooked. Fruits are widely used in cookery. Especially delicious are water chestnut fruits boiled with sugar syrup or honey. The seeds are also a source of food. Green mass is valuable material as forage. Flowering period of the plant falls on June-July and fruit-bearing - on August-September.

Different methods have been applied to achieve the goals of the research. Herbarium specimens of target species, deposited at the National Herbarium of Georgia (TBI), herbaria of the Tbilisi State Museum (TGM), Iv. Javakhishvili Tbilisi State University, Institute of Pharmacological Chemistry, Batumi Botanical Garden (BATU) and Lagodekhi State Reserve have been examined. Relevant information was extracted from unpublished manuscripts and phytosociological archives kept at the Institute of Botany, Batumi Central Botanic Garden, and the administrative building of Lagodekhi State Reserve. Based upon the information gathered in the initial stage, data on geographical distribution/places of occurrences of target plants were collected.

Both field studies and laboratory investigations have been carried out. Field work was planned to all populations growing in the wild. Expeditions embraced the localities of distribution of target species. In particular, field works connected with *Gymnospermium smirnowii* were carried

out in Lagodekhi State Reserve, Telavi District of Kakheti region (East Georgia), right bank of river Iori, and environs of villages Gulgula and Kurdghelauri. Works aimed at studying *Paeonia steveniana* were located in environs of Bakuriani (mount Sakochavi, Kuziani khidi (humpbacked bridge), Nato valley) and environs of Tskhratskaro pass. In Ajara and Guria region (Batumi, Green Cape, Gonio, Kobuleti, Supsa and Ureki environs) data on *Trapa colchica* and *Pancratium maritimum* were collected and studied, though field works connected with *Hibiscus ponticus* and *Kosteletzkya pentacarpa* were concentrated in environs of Poti and Lake Palisatomi.

Data on the occurrences of the populations, their vitality, sociability and fertility were collected in the places of geographical distribution of target plant populations. Meetings with locals were arranged in order to collect data on plant uses existed in local population.

More exact data on geographical positioning - GPS readings - were obtained in the process of field works for each target taxon for compiling GIS-based distribution maps. Method by Rabotnov [Rabotnov T. A., 1960] was applied to assess quantitatively seed production capacity, germination rates and development of viable seedlings for each target plant species. Seed germination capacity was tested on Petri dishes in the laboratory at room temperature in the range of 18-20°C. Seed collection, processing and deposition in seed bank was performed using the techiniques described in [Bowers B.G., Bowers B.G. 1998; Baskin, F. and Baskin, C. 2002].

Results and Discussion

To fulfill the goals and obejctives of the present research we specified herbarium samples and vouchers kept in herbaria of different scientific institutions of Georgia. Total of 105 herbarium samples relevant to six species under investigation have been checked and thoroughly examined. Selected species are quite poorly presented in the studied herbaria and it is necessary to fill the depositories with new material.

Among plant species under investigation *Kosteletzkya pentacarpa* - one of the rarest plants of Georgia's flora with very local distribution range - turned out to be the most noteworthy. The first specimens of this plant were collected by Sh. Tsertsvadze in 1930 near the town of Poti, in sedge swamps of Lake Paliastomi. These samples are preserved until now at the Herbarium of the State Museum of Georgia (TGM, N5899-900). Later, in 1953 A. Kolakowskii and N.N. Kaden have revealed the population of this species in the same location (the shore of Lake Paliastomi) and the specimen was deposited in the herbarium of Sokhumi Botanic Garden under the specified scientific name. Due to known events of 1990s and as a result of fire in the mentioned garden this specimen was lost along with many others. Recovery of the herbarium with a new specimen of kosteletzkya obtained by us can be regarded as an important acquisition.

Total of 30 herbarium specimens of the target plants collected and prepared by us have been delivered to different scientific institutions to deposit in their herbaria. The duplicates of herbarium specimens of four species - Gymnospermium, Hibiscus, Peony, Kosteletzkya have been deposited in the herbarium of the Royal Botanic Gardens, Kew, UK.

Based on the material obtained during the field works in different parts of Georgia, the distribution ranges of the target plants have been established. GPS readings have been recorded in the localities of species occurrences, GIS-based maps were produced. Data collected will be used in the future for assigning the IUCN categories to the studied plant species.

On plots, outlined prior to experiments the population viability analysis was undertaken through the counting of numbers of individuals of the target populations during the flowering phase. Fertility of each species was investigated, which implies the seed production capacity. Also the capacity for vegetative propagation was assessed. Al these data are necessary for the assessment of vitality degree of a species.

Our investigations have shown that all flowering plants of the studied species have developed seeds. The level of vegetative propagation was also good for all studied species.

Paeonia steveniana, Pancratium maritimum, Gymnospermium smirnowii, Hibiscus ponticus, Kosteletzkya pentacarpa and *Trapa colchica* reveal adaptation ability to the given ecological conditions (of distribution ranges). Life cycle of plants proceeds well and is regularly completed. Other factors seem to be responsible for drastic reduction of their population numbers.

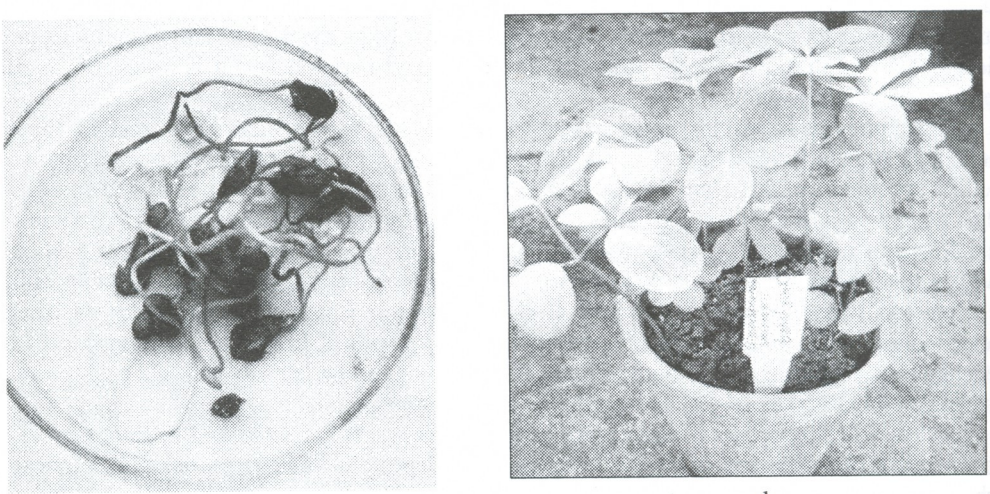
To survive in certain environmental conditions (ecosystem) the given plant species needs to be fertile – to produce great number of full value seeds and further successful survival of the obtained seedlings (new generation) in suitable ecological conditions.

For the first time the capacity for propagation by seeds has been checked by us for all plant species under investigation: potential and real capacities for seed formation were assessed. Observations were made on the processes of fruit and seed development, maturation and dispersal.

Seed germination rates and development of viable seedlings was quantitatively assessed in laboratory conditions.

Seeds were sown in pots, in the open ground on the experimental plot of the Department of Plant Conservation, on the territory of Tbilisi Botanic Garden in order to establish *ex-situ* living collections and on Petri dishes, with the aim to test germination rates of seeds and assess quantitatively establishment capacity of seedling.

The studied species *Gymnospermium smirnowii, Hibiscus ponticus, Kosteletzkya pentacarpa* and *Pancratium maritimum* have revealed high rate of germination and capacity of seedling development both in pots and on Petri dishes (Fig. 1, 2, 3).



a b

Fig 1. *Gymnospermium smirnowii* : a) seeds germinated on Petri dish: b) seedlings, developed from seeds germinated on Petri dish, transferred to the pot

The following data were obtined on germination capacity and seedling development for the studied species: sea daffodil – 84-85% on Petri dish and 82% in the pot; for kozteletzkya – 90-92% on Petri dish and 87% in the pot; for hibiscus – 88-90% on Petri dish and 85% in the pot; for gymnospermium – 72% on Petri dish and 75% in the pot (low temperature -3-4°C is required for germination); peony is propagated by seeds, but germination and growth takes a certain time - 8-9 months, germination percentage on Petri dish is 60%, and 67% in the pot. Seeds of water chestnut also need a long time to germinate. Due to this its capacity for propagation by seeds was not

established so far, but in the future it is envisaged to continue investigation of this problem together with British colleagues within the frame of the Millennium Seed Bank Project.

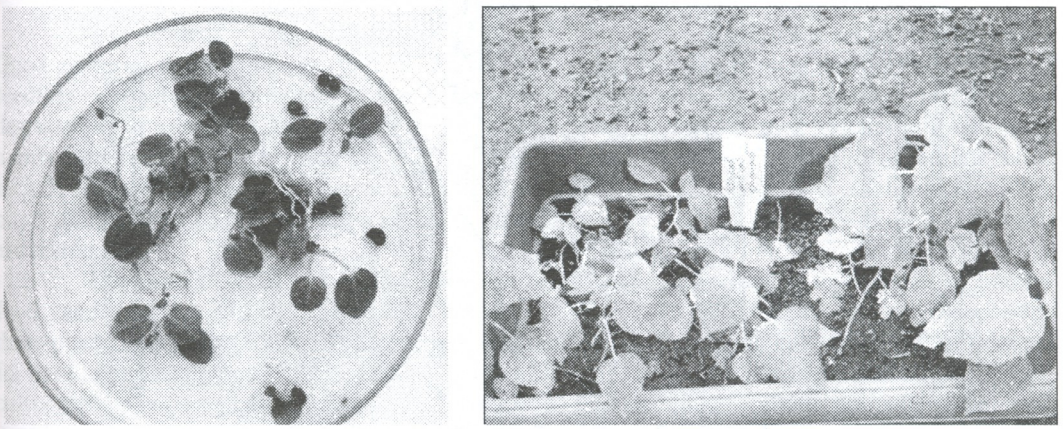


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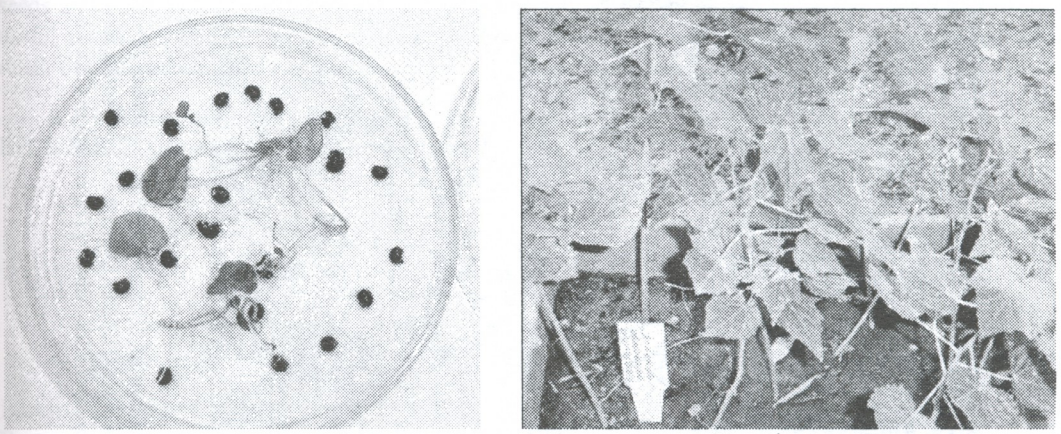
Fig. 2. *Hibiscus ponticus*: a) seeds germinated on petri dish; b) seedlings emerged from seeds sown in the pot



a b

Fig. 3. *Kosteletzkya pentacarpa*: a) seeds germinated on petri dish; b) seedlings growing in the pot

New localities of distribution of sea daffodil (sand lily) and peony have been revealed as a result or our surveys. Transformation and trends of changes proceeding in populations under the anthropogenic pressure have been evaluated in the process of studies. New facts of extinction of sea daffodil populations have been revealed in already known places of occurrence of a species. Namely, populations of the species have drastically reduced or even eliminated in the environs of Ureki settlement due to construction of summer residences in this part of Black Sea coast.

During the process of investigation *ex-situ* conservation activities were performed: seeds of the target species were deposited at the Regional Caucasus Seed Bank, located in the building of the former Institute of Botany. The duplicates have been sent to the Millennium Seed Bank of the Kew Royal Botanic Garden. Collection plots of seedlings were established on the collection plot of the NGO Wild Plants Conservation Association situated on the territory of Tbilisi Botanic Garden.

During field works the issues of importance and potential use of target species, technical aspects and prospects of their propagation have been discussed on the meetings with locals.

Fig. 4. Map showing distribution of *Gymnospermium smirnowii* (Trautv.) Takht. (Berberidaceae); *Hibiscus ponticus* Rupr. (Malvaceae); *Kosteletzkya pentacarpa* (Ledeb.) (Malvaceae); *Paeonia steveniana* Kem.-Nath. (Paeoniaceae); *Pancratium maritimum* L. (Amaryllidaceae) and *Trapa colchica* Albov[1] (Trapaceae) in Georgia

Conclusions

Our research has revealed high vitality and fertility of six species under investigation. Good potential for self renewal was stated being expressed in high rates of seed germination and seedling growth. Environmental conditions (anthropogenic pressure, zoo factor, habitat degradation, etc.) are considered to be the main factors responsible for the reduction of population number of those species. Based on references and our data we consider that it is reasonable to include *Kosteletzkya pentacarpa* and *Hibiscus ponticus* into the Red Book of Georgia as the populations of these species are extremely reduced in number and the localities of their occurence are scarce.

The studied relict species are of enormous economic potential as the sources of natural food, medicines, wood, oils and other important herbal products. Sustainable use of resources of these plants may greatly contribute to the development of local economy considering great demand for natural and ecologically pure products existed both in Georgia and throughout the world. Thus, cultivation of ecomonically valuable plants may became a profitable commercial activity for local population and small- and medium-scale businesses. This will facilitate *in-situ* conservation of decreasing natural resources and will significantly improve livelihoods of local population.

As a result of fulfilled work a great bulk of information is accumulated on reproduction by seeds and establishment of seedlings for 6 species of economically important relict plants. These useful recommendations on propagation of ecenomically important plant species, elaborated as a result of undertaken research can be disseminated to all interested parties.

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საქართველოში გავრცელებული ეკონომიკური მნიშვნელობის ზოგიერთი რელიქტური სახეობის ex-situ კონსერვაცია

მიქატაძე-ფანცულაია ც., დავლიანიძე მ., კობახიძე ლ., ერისთავი მ.

(მიღებულია 22.07.2008)

რეზიუმე

ნაშრომში წარმოდგენილია ეკონომიკური მნიშვნელობის რელიქტური სახეობების: მრგვალი წამლის *Gymnospermium smirnowii* (Trautv.) Takht. (Berberidaceae); ჰიბისკუსის *Hibiscus ponticus* Rupr. (Malvaceae); კოსტელეცკიას *Kosteletzkya pentacarpa* (L.) Ledeb (Malvaceae); სტევენის იორდასალამის *Paeonia steveniana* Kem.-Nath. (Paeoniaceae); ზღვის შროშანის *Pancratium maritimum* L. (Amaryllidaceae) და წყლის კაკლის *Trapa colchica* Albov (Trapaceae) საქართველოს ტერიტორიაზე გავრცელების არეალები. შექმნილია გეოგრაფიულ საინფორმაციო სისტემებზე დაფუძნებული რუქები, შეფასებულია მათი პოპულაციების თანამედროვე მდგომარეობა და თესლით გამრავლების შესაძლებლობა. დადგენილია, რომ შესწავლილი სახეობები სიცოცხლისუნარიანი და ფერტილურია და გააჩნიათ თვითგანახლების პოტენციური შესაძლებლობები, რაც გამოიხატება თესლწარმოქმნის, თესლის გაღივების და აღმოცენების კარგი უნარით. ჩვენი აზრით, ზემოთ აღნიშნული სახეობების რიცხობრივ შემცირებას ძირითადად განაპირობებს გარემო ფაქტორები (ანთროპოგენული, ზოოფაქტორი და საარსებო ადგილსამყოფელის დეგრადაცია). შესრულებულია საკვლევი სახეობების ex-situ კონსერვაციის სამუშაოები: შექმნილია თესლის ბანკი და აღმონაცენთა კოლექცია.

სამუშაო შესრულებულია **BP**-სა და მისი პარტნიორების ინიციატივითა და დაფინანსებით გამართულ კონკურსში გამარჯვებული პროექტის **S-05-BPCS-66887** ფარგლებში.

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Microbiology

MICROFLORA OF SOME SOIL TYPES IN SAMEGRELO REGION

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Abstract

General number and peculiarities of distribution of separate groups of microorganisms in alluvial calcareous, alluvial-saturated, alluvial-acid and subtropical gley soils of Samegrelo region have been studied. It has been established that according to the number of microorganisms alluvial calcareous soil type has the highest index ($822.3x10^5$), while their amounts in alluvial-saturated, alluvial-acid and subtropical gley soils are comparatively small - $606.0x10^5$, $218.0x10^5$, $480.0x10^5$ cells, respectively (per 1 g of dry soil). The distribution of actinomycete-antagonists was also investigated in those soils.

Key words: fungi, actinomycetes, cellulose-destroying microorganisms, oligonitrophyles, saprophytes, actinomycete-antagonist.

Introduction

The soil is inhabited by numerous and diverse organisms, participating in different processes proceeded in the soil. Among them are: bacteria, cellulose-destroying microorganisms, microscopic fungi, oligonitrophyles, actinomycetes [Gusev, Mineeva, 2003; Lengeler et al., 2005].

At present the study of quantitative and qualitative content of the microorganisms in the soil has a special importance. Their specific diversity and quantitative ratio determine definitely the soil fertility and the activity of biochemical processes taking place in the soil. Study of the effect of soil types on specific and quantitative content appears to be no less important.

The goal of our investigation was to study the microorganisms in some soil types in Samegrelo region (Western Georgia).

Materials and Methods

The following four soil types appeared to be the object of our investigation in Samegrelo region: alluvial calcareous - village Shuakalaki; alluvial-saturated - vil. Zanati; alluvial acid - vil. Marani and subtropical gley podzols - vil. Tskemi.

In order to reveal the antagonistic peculiarities of actinomycetes phytopathogenic bacteria - *Agrobacterium Tumefaciens, Xanthomonas Campestris* and *Pectobacterium aroideae* have been studied. The pure cultures of the bacteria were taken from the collection of microorganisms of microbiological department of Institute of Botany.

The isolation of microorganisms from the soil was performed using Tepper method [Selibor, 1992]. Krasilnikov synthetic medium was used for the actinomycetes, for oligonitrophyles - Ashbi nutrient medium, for fungi - Chapek medium, for cellulose-destroying bacteria -

Hutchinson medium and for saprophytic microorganisms - Burkholter's nutrient medium [Tepper, 2003].

The antagonistic characteristics of investigated cultures were studied using Egorov block methods [Tepper, 2003].

Results and Discussion

At the initial stages of the research the amount of moisture was determined in the investigated soils. It has been established that among above mentioned soil samples the alluvial-saturated and subtropical-gley podzol soils are characterized by high moisture (0.2708% and 0.16395%, respectively), while alluvial-calcareous and alluvial-acid types are characterized by relatively low indices of moisture (0.099% and 0.0602%, respectively) (Fig.1).

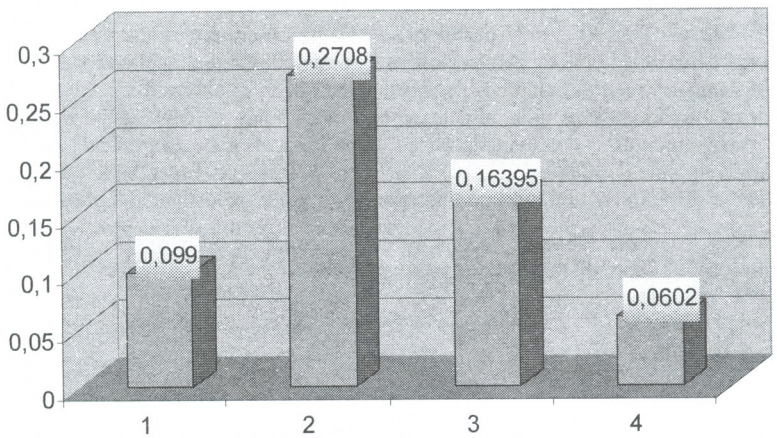

Fig.1. The amount of moisture in the samples of soil in Samegrelo region. 1 - Alluvial-calcareous soil; 2 - Alluvial-saturated soil; 3 - Subtropical gley podzols; 4 - Alluvial acid soil.

The results of determination of microorganism numbers, and namely of fungi, actinomycetes, cellulose-destroying microorganisms, oligonitrophyles and saprophytes, in studied soil samples are given in Table 1.

Table 1. Microflora of alluvial-calcareous, alluvial-saturated, alluvial acid and subtropical gley podzol soil types in Samegrelo region

Soil types	Groups of the microorganisms	The amount in 1 g of dry soil
Alluvial-Calcareous	Fungi	$18.1x10^5$
	Actinomycetes	$6.46x10^5$
	Cellulose-destroying	$1.74x10^5$
	Oligonitrophyles	$334.5x10^5$
	Saprophytes	$462.2x10^5$
Alluvial-Saturated	Fungi	$30.06x10^5$
	Actinomycetes	$37.67x10^5$

	Cellulose-destroying	$5.91x10^5$
	Oligonitrophyles	$247.6x10^5$
	Saprophytes	$284.5x10^5$
Subtropical-gley podzols	Fungi	$19.12x10^5$
	Actinomycetes	$21.691x10^5$
	Cellulose-destroying	$1.02x10^5$
	Oligonitrophyles	$174.8x10^5$
	Saprophytes	$2.83x10^5$
Alluvial-acid	Fungi	$3.49x10^5$
	Actinomycetes	$185.1x10^5$
	Cellulose-destroying	$10.64x10^5$
	Oligonitrophyles	$851.1x10^5$
	Saprophytes	$34.0x10^5$

The antagonistic properties of actinomycetes isolated from different soil types of Samegrelo region have been studied. As the analysis of the results has shown the investigated actinomycetes are characterized by a high antagonistic activity (Table 2).

Table 2. The effect of actinomycetes isolated from some soil types of Samegrelo region on the growth and development of phytopathogenic bacteria

NN	Antagonist-strain / Test strain	1^1	2^1	2^2	3^2	3^3	4^2
		The zone of suppression in mm					
1	*Agrobacterium Tumefaciens*	4.0	0.0	0.0	0.0	1.0	0.0
2	*Xanthomonas Campestris*	4.5	0.0	0.8	1.3	1.3	0.0
3	*Pectobacterium aroideae*	5.0	0.0	0.0	6.0	0.0	0.0

As is shown from Table 2 *Agrobacterium Tumefaciens* is resistant to the following strains: 2^1, 2^2, 3^2 and 4^2. The strains 1^1 and 3^2 suppress the growth of phytopathogenic bacterium by different intensity.

Xanthomonas campestris appeared to be stable to 2^1 and 4^2 strains and was sensitive to 1^1, 2^2, 3^2 and 3^3 strains.

Pectobacterium aroideae as well as *Agrobacterium tumefaciens* are resistant to 2^1, 2^2, 3^3 and 4^2 strains, but 1^1 and 3^2 strains have influence on the growth and development of the given pathogene.

Thus, different soil types of Samegrelo region are distinguished by the qualitative and quantitative content of the microorganisms. The actinomycetes are characterized by antagonistic peculiarities in these soils.

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სამეგრელოს რეგიონის ზოგიერთი ტიპის ნიადაგის მიკროფლორა

ბულისკერია ი., ლომთათიძე ზ.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი, მიკრობიოლოგიის განყოფილება

(მიღებულია 15.09.2008)

რეზიუმე

შესწავლილია სამეგრელოს რეგიონის სხვადასხვა ტიპის, კერძოდ: ალუვიურ-კარბონატულ, ალუვიურ-მაძღარ, ალუვიურ-მჟავე და სუბტროპიკულ-ლებიან ნიადაგებში მიკროორგანიზმების საერთო რაოდენობა და ცალკეული ფიზიოლოგიური ჯგუფების გავრცელების თავისებურებანი. დადგენილ იქნა, რომ მიკროორგანიზმთა რაოდენობის მიხედვით ყველაზე მაღალი მაჩვენებლით ხასიათდება ალუვიურ-კარბონატული ტიპის ნიადაგი $82.23 \cdot 10^6$, ხოლო ალუვიურ-მაძღარ, ალუვიურ-მჟავე და სუბტროპიკულ-ლებიან ნიადაგებში მიკროორგანიზმების რაოდენობა შედარებით მცირეა $60.6 \cdot 10^6$, $21.8 \cdot 10^6$, $48.0 \cdot 10^6$ მიკროორგანიზმი, შესაბამისად (1 გ მშრალ ნიადაგში). შესწავლილ ნიადაგებში გვხვდება დიდი რაოდენობით აქტინომიცეტ-ანტაგონისტები.

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THE POTENTIAL OF 2,4,6-TRINITROTOLUENE APPLICATION BY BACTERIA AS THE SOLE SOURCE OF CARBON AND NITROGEN

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Abstract

To reveal the potential of 2,4,6-trinitrotoluene (TNT) application as the sole source of carbon and nitrogen 40 bacteria isolated from contaminated soils of Georgia and 12 strains of genera *Rhodococcus* and *Mycobacterium* kept in the collection of microorganisms at Durmishidze Institute of Biochemistry and Biotechnology have been investigated. All tested strains grew better on TNT-containing media in the presence of rapidly assimilated sources of carbon and nitrogen. Some of them grew better on TNT-containing nutrient media in the presence of carbon sources and others – in the presence of nitrogen sources. The growth intensities of all strains were suppressed when TNT was used as the sole source of both elements. At deep cultivation utilization of TNT in case of *Pseudomonas* sp.44 and *Rhodococcus* sp. VCM Ac 1170 str.44 was most intensive on complete nutrient media (89% and 67%, respectively); however, the process also occurred in the media, in which TNT was the sole source of carbon and nitrogen. In these variants the amount of residual TNT was 33-42%.

Key words: *Rhodococcus, Pseudomonas,* residual TNT, biomass accumulation

Introduction

At the end of the last century the massive mobilization of natural resources, the industrial synthesis of chemical compounds, and the involvement of the compounds into the biogenic migration of atoms made ecological problems for biosphere. 2,4,6-trinitrotolouene is the most frequent applicable nitroaromatic compound among anthropogenic contaminants [Elovitz, Weber, 1999].

There are many publications on the toxicity and mutagenicity of TNT and its metabolites for mammalians, fishes, plants and bacteria [Dilley et al., 1982; Drzizga et al., 1995; Hankenson et al., 1991; Palazzo et al., 1986]. The high concentrations of TNT are hazardous for human health and cause various diseases. Consequently, Environmental Protection Agency of USA (EPA, 1991) and Integrated Risk Information System (IRIS) (1991) rank TNT among C-group carcinogenic toxicants.

Only few aerobes are able to apply TNT as the sole source of carbon and nitrogen. Some microorganisms are capable of using TNT as the sole source of nitrogen in mineral medium enriched with carbon sources [Duque et al., 1993; Jones et al., 1995].

The goal of our investigation was to study the potential of TNT application as the sole source of carbon and nitrogen by some microorganisms with high capability of TNT degradation.

Materials and Methods

Objects of investigation were bacteria isolated from soils of Georgia [Gagelidze et al., 2002]. During investigation typical strains of genera *Rhodococcus* and *Mycobacterium* kept in the collection of microorganisms at Durmishidze Institute of Biochemistry and Biotechnology were also used.

Czapek's medium (g/l: glucose, 20; $NaNO_3$, 2; K_2HPO_4, 1; $MgSO_4 \times 7H_20$, 0.5; KCl, 0.5; $FeSO_4 \times 7H_20$, 0.01; pH 7.0) and synthetic medium modified for rhodococci (g/l: urea, 1.5; Na_2HPO_4, 4; KH_2PO_4, 3; $MgSO_4 \times 7H_2O$, 1; glucose, 30; sucrose, 10; $FeCl_3$, 8 mg/l; B_1, 1mg/l; pH 7.0) [Daraselia et al.1989] were used to cultivate bacteria.

For revealing biodegradation ability of bacteria they were grown on agar media at the concentration of TNT 200 mg/l in thermostat and also in liquid media with the same concentration of TNT in 750-ml flasks, with 50 ml of nutritional media, at rotational shaker (180 resolutions per minute) at 28-30°C during 8 days. TNT serves as the sole source of carbon or nitrogen or the both element together. Nutrient media with other sources of carbon and nitrogen were taken as control. Inoculate was obtained on suitable media without TNT. Liquid media were inoculated by 10% (by vol.) of bacterial suspension at the exponential phase of growth.

The intensity of culture growth on solid nutritional media has been evaluated visually by 5-point system (– - no growth, + - a trace, 2+ - poor growth, 3+ - average growth, 4+ - intensive growth).

Amounts of biomass and residual TNT [Oh et al., 2000] in liquid media were determined by spectrophotometer at 660 nm and under high alkaline (pH>11) conditions at 447 nm, respectively.

Suitable media in the presence of TNT, without inoculation of microorganisms and media in the absence of TNT with microorganisms served as controls for quantitative analysis.

Results and Discussion

Growth ability of bacteria was studied on solid nutrient media containing 200mg/l TNT. The following variants were tested:

- TNT + carbon source + nitrogen source,
- TNT + carbon source,
- TNT + nitrogen source,
- TNT only

12 collection strains and 40 bacterial strains isolated from military grounds of Georgia were investigated. The bacteria were selected previously by growth intensity on solid nutrient media, containing 300 mg/l of TNT, together with glucose and mineral sources of nitrogen [Gagelidze at al., 2004].

Based on obtained data it might be concluded that all tested strains grew better on TNT-containing media in the presence of easily assimilated sources of carbon and nitrogen. Some strains

under conditional numbers 44, 74, 88*, 107, 139, 228, 236 grew better on TNT-containing nutrient media in the presence of carbon sources; some strains, under conditional numbers 81, 88*, 209, 124, 140 - on TNT-containing nutrient media in the presence of nitrogen sources; the growth intensities of all strains were suppressed when TNT was applied as the sole source of both elements.

Presumably, different sources of nitrogen and carbon serve as a certain biochemical cofactor, increasing the amount of transformed TNT. The intensive accumulation of biomass is one of the demonstrations of the fact.

The experiment was continued on *Rhodococcus* sp. VCM Ac 1170 str. 44 and *Pseudomonas* sp. TNT-44 in condition of deep cultivation.

The liquid medium for each strain was different: synthetic medium modified for *Rhodococcus* sp. VCM Ac 1170 str. 44 and Czapek's medium for *Pseudomonas* sp. TNT-44. Growth of cultures was investigated in variants mentioned above. The biomass and residual TNT were determined in dynamics during 8 days. The results are given in Fig. 1.

Growth characters of the bacteria *Rhodococcus* sp. VCM Ac 1170 str.44 and *Pseudomonas* sp. TNT-44 do not differ essentially from each other in perfect media without TNT (Fig.1, variants a and d). The difference is observed only in growth intensity: *Rhodococcus* sp. VCM Ac 1170 str.44 accumulates more biomass than *Pseudomonas* sp. TNT-44; it could be explained by growing of *Rhodococcus* sp. VCM Ac 1170 str.44 on the medium optimized for the culture [Daraselia, Daushvili, 1982].

In all other variants without TNT biomass increase was not observed in case of *Pseudomonas* sp.TNT-44 (Fig.1, variant a); and in case of *Rhodococcus* sp. VCM Ac 1170 str.44 insignificant growth was found, especially in the absence of nitrogen source in the medium (Fig.1, variant d). It should be explained by physiological specificity of the genus *Rhodococcus*. However, even in this variant the amount of biomass was half in comparison with the biomass accumulated on the perfect medium.

The characters of bacterial growth curves on perfect media with TNT and without TNT confirms that *Rhodococcus* sp. VCM Ac 1170 str.44 is more sensitive to toxicant than *Pseudomonas* sp. TNT-44. As regards to other media containing TNT, growth of *Pseudomonas* sp. TNT-44, in the absence of additional sources of nitrogen doesn't occur practically. In variants, where TNT is applied as a source of carbon or both of carbon and nitrogen, the cultures growth intensity is very weak, but still better than in corresponding variant without TNT. In case of *Rhodococcus* sp. VCM Ac 1170 str.44, in all TNT-containing variants growth take place, but in all cases are equally weak.

The utilization of TNT in case of *Pseudomonas* sp.44 was the most intensive on complete nutrient media (89%), however the process also occurred on the media, in which culture applies TNT as the sole source of carbon and nitrogen and on which better growth was revealed in comparison with corresponding control. The amount of residual TNT is 35% and 42%, respectively.

In case of *Rhodococcus* sp. VCM Ac 1170 str.44, the utilization of TNT was occurred most intensively on the medium, which besides TNT contained additional sources both carbon and nitrogen (the amount of residual TNT – 33%), however the process of TNT utilization took place nearly with the same intensity as in the variants, in which the additional source of nitrogen was not introduced (the amount of residual TNT – 35%).

It has been established [Price et al., 2000] that glucose and sucrose, readily convertible carbon containing substrates promote TNT transformation. Application of molasses or yeast extract as additional substrates caused intensification of TNT conversion [Amerkhanova, Naumova, 1978; Yin et al., 1998; Frische et al., 1999].

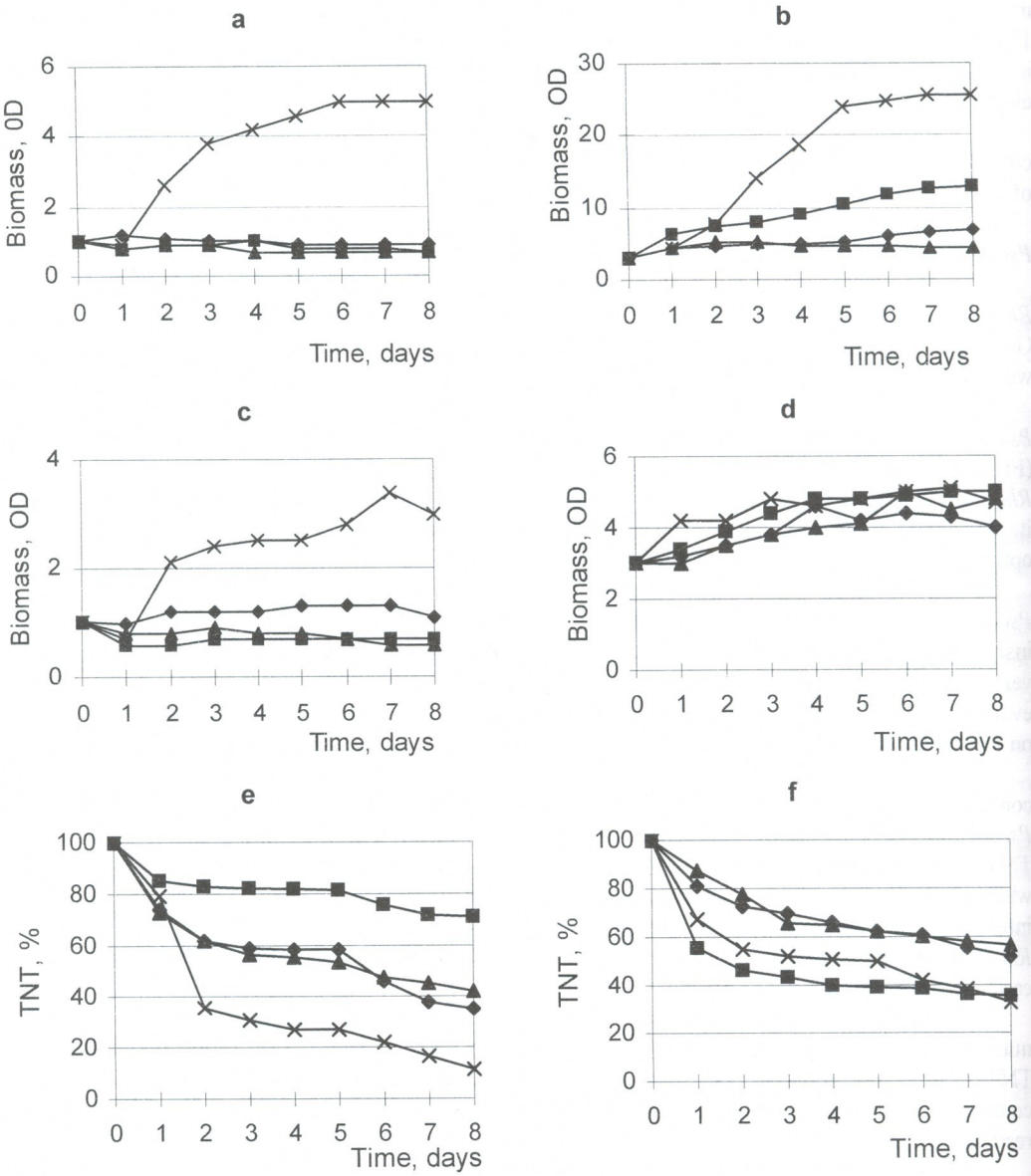

Fig. 1. Accumulation of biomass by *Rhodococcus* sp. VCM Ac 1170 str.44 and *Pseudomonas* sp. TNT-44 and utilization of TNT in dynamics during cultivation on different nutrient media

--■-- –N+C; --◆-- +N–C; -- ▲ -- –N–C; -- × -- +N+C

a – Biomass (g/l) of *Pseudomonas* sp. TNT-44 on the media without TNT;

b – Biomass (g/l) of *Pseudomonas* sp. TNT-44 on TNT-containing media;

c – Residual TNT (%) after cultivation of *Pseudomonas* sp. TNT-44;

d – Biomass (g/l) *Rhodococcus* sp. VCM Ac 1170 str.44 on the media without TNT;

e – Biomass (g/l) *Rhodococcus* sp. VCM Ac 1170 str.44 on TNT-containing media;

f – Residual TNT (%) after cultivation of *Rhodococcus* sp. VCM Ac 1170 str.44.

The highest level of TNT degradation was also achieved in case of *Sac. oviformis* in the presence of glucose and yeast extract in nutrient medium [Gagelidze et al., 2005].

It should be mentioned that the rate of TNT degradation during the first three days was high. Then the amount of TNT changed slightly and at that, unidentified coloring compounds were excreted in cultivation medium. Particularly, during cultivation of *Pseudomonas* sp.TNT-44 on complete nutrient medium yellow metabolites were accumulated. This process was observed more distinctly during cultivation of *Rhodococcus* sp.124 on Czapek's medium. As concerns to *Rhodococcus* sp. VCM Ac 1170 str.44 cultivation on complete medium or in the absence of carbon source, red metabolites were accumulated in cultural liquid. The effect was displayed more distinctly when the latter was cultivated on complete medium that might be caused by diffusion of carotenoid pigments into the medium. Some authors [Kaplan, Siedle, 1971; Suen, et al., 1996 Fant et al., 2001] suppose that electron deficiency in the aromatic nucleus of TNT promotes nucleophilic impact on it. As a result, Meizenhymer's complex, an aromatic structure with red-orange color and negative charge, is formed. Presumably, during cultivation of *Rhodococcus* sp. VCM Ac 1170 str.44 on complete medium or in case of the absence of carbon source, the red coloring of cultivation medium is caused by synthesizing of compounds of the same type.

Thus, according to the adduced data, the tested bacteria, *Pseudomonas* sp.TNT-44 and *Rhodococcus* sp. VCM Ac 1170 str.44, utilize TNT more effectively in the presence of readily utilizable sources of carbon and nitrogen that points to degradation of TNT by co-metabolism fate. However, the cultures utilize TNT even in the absence of additional sources of nitrogen and carbon.

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ბაქტერიების მიერ 2,4,6-ტრინიტროტოლუოლის ნახშირბადისა და აზოტის წყაროდ გამოყენების შესაძლებლობა

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რეზიუმე

შესწავლილია საქართველოს ნიადაგებიდან გამოყოფილი 40 ბაქტერიისა და დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტის მიკროორგანიზმების კოლექციაში დაცული *Rhodococcus*-ისა და *Mycobacterium*-ის გვარის 12 შტამის 2,4,6-ტრინიტროტოლუოლის (ტნტ) აზოტისა და ნახშირბადის ერთადერთ წყაროდ გამოიყენების უნარი. ყველა გამოცდილი შტამი უკეთ იზრდება ტნტ-ს შემცველ არეზე ადვილად ასიმილირებად ნახშირბადისა და აზოტის წყაროების არსებობისას. საკვებ არეში ტნტ-ს შემცველობისას, ზოგი მათგანი უკეთ იზრდება ნახშირბადის დამატებითი წყაროს, ხოლო ზოგი – აზოტის დამატებითი წყაროს არსებობის შემთხვევაში. ყველა შტამის ზრდის ინტენსივობა ფერხდება როდესაც ტნტ გამოყენებულია ორივე ელემენტის ერთადერთ წყაროდ. სიღრმული კულტივირებისას *Pseudomonas* sp.44-ისა და *Rhodococcus* sp. VCM Ac 1170 str.44-ის მიერ ტნტ-ს უტილიზაცია ყველაზე ინტენსიურია სრულ საკვებ არეებზე (89% და 67%, შესაბამისად), თუმცა, ეს პროცესი მიმდინარეობს აგრეთვე ნახშირბადისა და აზოტის ერთადერთ წყაროდ ტნტ-ს გამოყენებისას. ამ შემთხვევაში ნარჩენი ტნტ-ს რაოდენობა შეადგენს 33-42%.

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THE MICROORGANISMS PARTICIPATING IN OLIGOCARBOPHYLIC AND HUMUS MINERALIZATION OF SHIDA KARTLI SOILS

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Abstract

The quantitative and qualitative content of the microorganisms participating in oligocarbophylic and humus mineralization of different soil types in Shida Kartli have been determined. It has been established that the samples of the given soils are distinguished by the amount of investigated microorganisms. The cannimonic calcareous soil type is rich in oligocarbophyle microorganisms (the amount of microorganisms – $574x10^3 \pm 1\%$), relatively poor are cinnamonic leached, alluvial-calcareous and meadow cinnamonic soil types (the amount of microorganisms - $52x10^3 \pm 1\%$, $69x10^3 \pm 1\%$ and $67x10^3 \pm 1\%$, respectively). It has been also established that the microorganisms of *Nocardia* family dominate.

Key words: soil, oligocarbophyles, microorganism, mineralization of humus

Introduction

The amount of microbial biomass in the soil directly correlates with the amount of organic substances in the soil. Accordingly a microbial activity is high on the surface of the soil and it gradually decreases in the depth. By participation in soil microfauna, the fungi and bacteria cooperatively perform the mineralization and transformation of plant and animal remains. Thus, the microorganisms including oligocarbophyles play an important role in humus formation that appears to be a factor stabilizing and participating in ion metabolism and holds significant nutritive matters in the soil [Gusev, Mineeva, 2003; Lengeler et al., 2005].

For the future it will be much perspective that these microorganisms would be used for the purification of the environment polluted by the oil, as the final products of microbiological decay of oil hydrocarbons appear to be nontoxic oxygen-containing compounds.

The goal of our research was to study the content of microorganisms participating in oligocarbophyle and humus mineralization of some soils in Shida Kartli.

Materials and Methods

Four types of soil in Shida Kartli appear to be the object of our investigation:

- Cinnamonic Leached – village Ateni
- Alluvial Calcareous – vil. Karaleti
- Meadow Cinnamonic – vil. Kheltubani

- Cinnamonic Calcareous – vil. Nadarbazevi

The isolation of oligocarbophylic microorganisms from the soil is performed using the method offered by Tepper; Vinogradski's medium was used as nutrient medium [Tepper, 2003].

The amount of microorganisms was determined by the method of statistical variation [Asatiani, 1995].

Results and Discussion

The content of microorganisms taking part in the mineralization of oligocarbophyles and the humus has been studied in cinnamonic leached, alluvial calcareous, meadow cinnamonic and cinnamonic calcareous soil types.

The amount of moisture was determined in the investigated soils. Sample were taken at the following meteorological conditions: temperature - $+25^0$C, atmospheric pressure – 728 mm, the amount of precipitations – 0, direction of the wind – north-western.

It has been established that under those conditions the investigated soils differ in moisture amount (Fig. 1); in particular, medow cinnamonic and cinnamonic calcareous soil types are distinguished by a high amount of moisture (15,90% and 15,60%, accordingly), in cinnamonic leached and alluvial calcareous soil types relatively low index of moisture was noted (9,20% and 12%, respectively).

The content of microorganisms, participating in oligocarbophyle and humus mineralization in the investigated soils is given in Table 1.

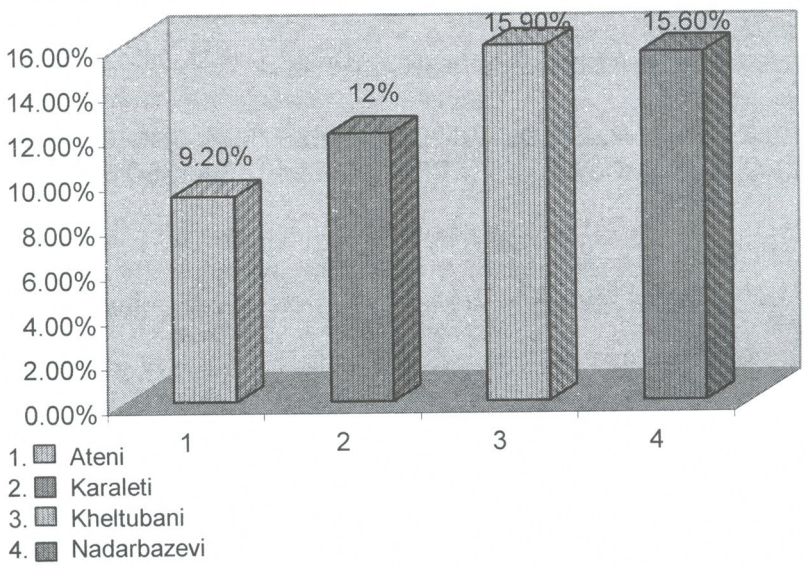

Fig. 1. The amount of moisture in different types of soils in Shida Kartli

Table 1. The content of microorganisms, participating in oligocarbophyle and humus mineralization in 1 g of dry soil

The type of soil	Moisture in %	Amount in 1 g of dry soil
Cinnamonic Leached	9.2	$52x10^3$±1%
Alluvial Calcareous	12	$69x10^3$±1%
Meadow Cinnamonic	15.9	$67x10^3$±1%
Cinnamonic Calcareous	15.6	$574x10^3$±1%

As seen in Table 1, the samples of the given soils differ in the content of oligocarbophyle microorganisms. A maximal index was noted in cinnamonic calcareous type soil (Nadarbazevi: the amount of microorganisms - $574x10^3$±1%), while a minimal index – in cinnamonic leached soil (Ateni - $52x10^3$±1%). A low index of oligiocarbophyles was also noted in alluvial calcareous type soil (Karaleti - $69x10^3$±1%) and meadow cinnamonic soil (Kheltubani - $67x10^3$±1%).

The results of our investigation have shown that in the investigated soils among the autoctonic microorganisms participating in oligocarbophyle and humus mineralization the representatives of *Nocardia* family dominate.

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გოროზია ი., ლომთათიძე ზ., კოტია ნ., ბულისკერია ი., ლომთათიძე ნ.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

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რეზიუმე

განსაზღვრულია შიდა ქართლის სხვადასხვა ტიპის ნიადაგში ოლიგოკარბოფილური და ჰუმუსის მინერალიზაციაში მონაწილე მიკროორგანიზმების რაოდენობრივი და თვისობრივი შედგენილობა. დადგენილია, რომ მოცემული ნიადაგის ნიმუშები ერთმანეთისაგან განსხვავდება საკვლევი მიკროორგანიზმების რაოდენობით. ოლიგოკარბოფილური მიკროორგანიზმებით მდიდარია ყავისფერი-კარბონატული ტიპის ნიადაგი (მიკროორგანიზმების რაოდენობა - $574•10^3$±1%), შედარებით ღარიბია ყავისფერი გამოტუტული ნიადაგი ($52•10^3$±1%), ალუვიურ-კარბონატული ($69•10^3$±1%) და მდელოს ყავისფერი ტიპის ნიადაგი ($67•10^3$±1%). აგრეთვე დადგენილ იქნა, რომ გამოკვლეულ ნიადაგებში დომინირებს *Nocardia*–ს გვარის მიკროორგანიზმები.

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SCREENING OF EXTREMOPHILIC ACTINOMYCETES – DESTRUCTORS OF HYDROCARBONS AND PESTICIDE 2,4-DICHLOROPHENOXYACETIC ACID

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Abstract

Detoxification capability of 403 strains of extremophilic actinomycetes isolated from various soil types of Georgia to hydrocarbon (hexane, benzene, benzpyrene, naphthalene, crude oil) and pesticide 2,4-diclorophenoxyacetic acid has been studied. The following actinomycetes have been selected: 26 – hexane, 28 – benzene, 3- crude oil, 19 – benzpyrene and 37 - naphthalene destructor strains among halophilic actinomycetes and 25 – benzpyrene, 31 – dichlorobenzene, 15 – naphthalene, 9 – hexane and 7 benzene destructing strains among alkaliphilic actinomycetes. Almost all 97 investigated strains of thermophilic actinomycetes exhibit hydrocarbons transformation ability. From 2,4-D-destructor strains 64 – halophilic, 10 ackaliphilic active strains have been selected; no active strain has been established among thermophiles.

Key words: actinomycetes, extremofiles, hydrocarbone, pesticide, detoxication.

Introduction

Petroleum hydrocarbons and pesticides are principle pollutants of environment. Today interest is focused on elaboration of ecologically safe technologies which are aimed at the restoration of contaminated soils. Bioremediation of toxicant-contaminated soils is generally based on activation of endemic microflora and introduction of environmentally adapted microorganisms (bioaugmentation) into soil [Daane, 2001, Margesin, 2001].

In Georgia, pesticides and among them 2,4–dichlorophenoxyacetic acid were used in farms and at present left as residues are still in soil and they do a great ecological danger for every living organism. It has been stated, that pesticides in soil are characterized by high tolerance for a long time. The use of extremophilic actinomycetes makes possible bioremediation of various types of contaminated soils.

There are known bacterial strains capable to 2,4-D transformation, and among them haloalkalophilic bacteria. Their growth optimum in 2,4-D contaminated regions was pH 8.4-9.4 and 0.6-1.0 M Na-ion concentration. According to salt concentration and pH-optimum these strains belong to moderate halophilic, alkalophilic bacteria [Maltseva, 1996; Haggblom, 2003].

Perspective method for detoxification of soils contaminated with crude oil is the use of preparations in which microorganisms – active destructors of hydrocarbons are incorporated. Introduction of pure cultures capable to perform oxidation of aliphatic, aromatic and other

hydrocarbons into contaminated soils enhances soil detoxification and stabilization of the processes of biological degradation with comparatively lower economical expenses and it is no danger for environment. Halophilic cultures were isolated from saline soils of Oklahoma by Nicolson K. These cultures use benzene as a sole source of carbon. The process of complete transformation of benzene lasts 1-2 weeks [Nicholson, 2004].

The carried out investigation had following objectives:

1. Selection of extremophilic actinomecets – destructors of hydrocarbons (benzene, hexane, benzpyrene, naphthalene, crude oil).
2. Selection of extremophilic actinomecets– destructors of 2,4–dichlorophenoxyacetic acid.

Materials and Methods

Growth of extremophilic actinomycetes on hydrocarbons (hexane, benzene, benzpyrene, naphthalene, crude oil) and pesticide - potassium salt of 2,4–dichlorophenoxyacetic acid containing nutrient media has been investigated. Toxicant's uptake ability was established on the bases of growth intensity. At selection of hydrocarbons destructing strains hexane, benzene, benzpyrene, naphthalene, crude oil concentration was 0.5% in the nutrient medium and those of benzpyrene – 0.005 and 0.01%. Selection of alkaliphilic actinomycetes on 0.01mM dichlorobenzene containing nutrient medium has been performed.

Actinomycetes growth ability was studied on 0.2, 0.5, 1, 2, 3% 2.4-D containing nutrient media.

As a nutrient medium both in case of hydrocarbons and 2.4-D a synthetic medium Gause 1 [Krassilnikov, 1966] was used for the selection of halophilic and alkaliphilic actinomycetes, but at selection of destructive thermophiles – TSA [Uridil, 1959].

Results and Discussion

197 halophilic, 109 alkaphilic, and 97 thermophilic actinomycetes strains from various types of soils of Georgia have been investigated.

From 197 strains of halophilic actinomycetes 37 strains, growing well on 0.5% naphthalene containing nutrient medium have been selected. Most of studied halophiles develop well on nutrient medium with naphthalene in concentration 0.02 and 0.1% as a sole source of carbon. From halophilic actinomycetes 26 – hexane, 28 – benzene, 3- crude oil, 19 – benzpyrene destructing strains have been revealed [Pataraya, 2008].

While study of alkaliphilic actinomycetes in one case hydrocarbons were used in nutrient media as a sole of C, in other cases 0.5% starch was introduced as a co-substrate.

Among the studied alkaliphilic actinomycetes a good growth on nutrient media containing benzpyrene and dichlorobenzene was observed in strains isolated from calcareous soil of Kaspi region. Among them 25 – benzpyrene, 31 – dichlorobenzene, 15 – naphthalene, 9 – hexane and 7 benzene destructing strains have been established [Pataraya, 2006]. The strains grow on crude oil containing medium only at the presence of starch. Among them are distinguished 3 strains - *Streptomyces pruniviolaceus* 214A, *Streptomyces sporocaneris* 215A, *Streptomyces globisporus* 216A, isolated from cinnamonic calcareous soils of Sagarejo Region growing on crude oil as a sole source of C containing nutrient medium. Among naphthalene destructing alkaliphilyc actinomycetes are distinguished: *Streptomyces coeruleus* 200A, *Streptomyces levoris* 201A, *Streptomyces streptomycini* 202A, *Streptomyces streptomycini* 204A, isolated from mountain-meadow soddy and mountain-forest-meadow types of soils of Stepantsminda Region, as well as strains isolated from cinnamonic calcareous soils of Sagarejo Region.

While studying thermophiles, isolated from various regions of Georgia it has been stated that all the strains intensively uptake hexane, benzene, as well as crude oil. As the strains develop at 0.5% concentration of the indicated hydrocarbons, their concentration was increased up to 1% for the next selection. On crude oil containing nutrient medium most of strains develop well, but form no aerial mycelium. Only 8 strains form aerial mycelium (Tab.1).

Table 1. Detoxication ability of hydrocarbon by extremophilic actinomycetes

#	Strains	Hydrocarbons				
		Hexane	Benzene	Benzpyrene	Naphthalene	Crude oil
	Halophyles					
1	*Streptomyces albocrustosus* 28H	3	4	5	5	3
2	*Streptosporangium album* 101H	2	3	0	5	1
3	*Streptomyces ravus* 107H	3	3	4	5	1
4	*Streptomyces alborobeus* 115H	4	4	5	5	2
5	*Streptomyces sindenensis* 169H	4	4	5	3	2
6	*Streptomyces rectivolaceus* 173H	4	3	3	5	1
7	*Streptomyces* spp. 270H	4	4	2	4	0
8	*Streptomyces* spp.276H	4	4	4	3	3
9	*Streptomyces sporostellatus* 326H	4	3	-	3	1
10	*Streptomyces griseofavillus* 327H	4	3	4	3	1
11	*Nocardia septisporus* 138H	3	3	5	5	3
12	*Nocardia alborectus* 168H	2	2	3	3	1
13	*Saccharopolyspora* spp.181H	2	3	3	0	2
	Alkaliphiles					
14	*Streptomyces naphteutilis* 78A	2	2	3	3	0
15	*Streptomyces setosus* 127A	3	3	5	1	0
16	*Streptomyces coeruleus* 200A	4	3	-	4	1
17	*Streptomyces levoris* 201A	4	3	-	4	1
18	*Streptomyces streptomycini* 202A	3	3	-	3	1
19	*Streptomyces streptomycini* 204A	3	3	-	4	1
20	*Streptomyces pruniviolaceus* 214A	4	4	-	3	3
21	*Streptomyces sporocaneris* 215A	4	4	-	4	4
22	*Streptomyces globisporus* 216A	3	3	-	4	3
23	*Streptomyces* spp. 218A	4	4	-	4	0

24	*Streptosporangium* sp. 39A	3	3	5	5	2
25	*Streptosporangium* sp. 112A	4	4	5	3	2
	Thermophiles					
26	*Thermoactinomyces* spp. 32Th	5	5	-	-	5
27	*Thermoactinomyces* spp. 59Th	5	5	-	-	4
28	*Thermoactinomyces* spp. 82Th	5	5	-	-	5
29	*Thermoactinomyces* spp. 91Th	5	5	-	-	5
30	*Thermoactinomyces* spp. 94Th	5	5	-	-	5

[a] 0 – no uptake; 1 and 2 – weak uptake; 3 – moderate uptake; 4 and 5 – strong uptake; -, not defined.

It is worth mentioning that among oil hydrocarbone destructing extremophilic actinomycetes thermophiles are distinguished most of which belong to *Theromoactinomyces* genera.

Selection of pure cultures of extremophilic actinomycetes on nutrient medium containing different concentration (0.2, 0.5, 1.0%) of pesticide - potassium salt of 2.4-D was carried out. The concentrations were not suppressive for development of all investigated strains, optimum concentration was 0.5%. At a given concentration 64 active strains have been selected, but in case of 1% - 2 strains: *Streptomyces* spp.89H, *Streptomyces ravus* 107H. Among alkaliphilic actinomycetes 10 strains have been selected which develop well at 1%. For 97 studied thermophilic actinimycetes 2,4-D was suppressive. Only one strain *Thermoactinomyces* spp. 62Th isolated from raw humus calcareous soils of Tetritskaro Region, village Asureti has been selected.

Thus, as a result of carried out investigation it has been established, that among extremophiles alkaliphilic and halophilic actinomycetes reveal detoxification ability of 2,4-D, and thermophilic actinomycetes - of hydrocarbons.

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ნახშირწყალბადების და პესტიციდ – 2,4-დიქლორფენოქსიძმარმჟავას დესტრუქტორი ექსტრემოფილური აქტინომიცეტების სპრინინგი

გურიელიძე მ., ბერიშვილი თ., ჭოლოკავა ნ., პატარაია დ., ნუცუბიძე ნ.

ს.დურმიშიძის ბიოქიმიის და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 08.09.2008)

რეზიუმე

შესწავლილია საქართველოს სხვადასხვა ნიადაგებიდან გამოყოფილი ექსტრემოფილური აქტინომიცეტების 403 შტამის დეტოქსიკაციის უნარი ნახშირწყალბადების (ჰექსანის, ბენზოლის, ბენზ-პირენის, ნაფტალინის, ნედლი ნავთობის) და პესტიციდ - 2,4-დიქლორფენოქსიძმარმჟავას კალიუმის მარილის მიმართ. ჰალოფილური აქტინომიცეტებიდან შერჩეულ იქნა ჰექსანის დესტრუქტორი - 26, ბენზოლის - 28, ბენზ-პირენის – 19, ნაფტალინის – 37, ნავთობის – 3 შტამი. ალკალიფილებიდან შერჩეულ იქნა ბენზპირენის – 25, დიქლორბენზოლის – 31, ნაფტალინის – 15, ჰექსანის 9 და ბენზოლის 7 დესტრუქტორი შტამი. გამოკვლეული თერმოფილური აქტინომიცეტის 99 შტამიდან თითქმის ყველა ავლენს ნახშირწყალბადების გარდაქმნის უნარს. 2,4-დ-ს დესტრუქტორი შტამებიდან შერჩეულ იქნა ჰალოფილების 64, ალკალიფილების 10 აქტიური შტამი. თერმოფილებიდან არ გამოვლინდა აღნიშნული აქივობის მქონე შტამები.

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Molecular Biology

INFLUENCE OF IRON IONS ON THE POLY(ADP-RIBOSE)POLYMERASE AND DNA TOPOISOMERASE II ACTIVITIES OF EUKARYOTIC CELLS

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Abstract

The influence of iron salts on the rat brain and liver cell nuclear poly(ADP-ribose)polymerase and nuclear matrix DNA topoisomerase II activities has been studied. The inhibition of both poly(ADP-ribose) polymerase and topoisomerase II activities by Fe^{2+} and Fe^{3+} ions has been shown. It is supposed that iron ions can inhibit nuclear poly(ADP-ribose)polymerase activity not only by affecting nucleoprotein system but also by participating in the formation of such complexes with the substrate of poly(ADP-ribose)polymerase (NAD), which are not convertible by the enzyme..

Key words: DNA, NAD, iron, nuclei, nuclear matrix, poly(ADP-ribose)polymerase, DNA topoisomerase

Introduction

Iron is a chemical element that plays an essential role in many biologically important processes including oxygen transport, electron transfer and enzymatic reactions. Living organisms have developed systems to store the iron in a nontoxic form. These systems avoid the cells from toxic effects of iron excess. In many diseases iron accumulation is observed. The cause of tissue iron accumulation may be the disruption of the expression of iron metabolism proteins induced by genetic or non-genetic factors [Quinata et al., 2006, Thompson et al., 2001].

Based on the above mentioned the study of iron influence on the enzymatic systems participating in genetic processes is important. Poly(ADP-ribose) polymerase (PARP) catalyses conversion of NAD into $(ADP\text{-}ribose)_n$ and provides covalent or noncovalent post-translational modification of proteins by ADP-ribosylation [Burkle, 2005]. Both PARP and DNA topoisomerase II, which use double strand cleavage and rejoining of DNA to modulate DNA topology, are involved in various genetic processes [Burkle, 2005, Champoux, 2001].

The aim of the present work was to study the influence of iron ions on rat liver and brain nuclear PARP and nuclear matrix DNA-topoisomerase II activities.

Materials and Methods

The nuclei from liver and whole brain were isolated from rats weighing ~150 g, according to Chauveau et al., (1956) with some modifications, while nuclear matrices - by extraction method

with high concentration of saline [Zaalishvili et al., 2000]. Solubilization and purification of PARP from isolated nuclei were performed according to [Zaalishvili, Sabelashvili, 1989].

PARP activity was measured by inclusion the adenine ring labeled ^{14}C-NAD into the acid insoluble product [Zaalishvili et al., 2000, Zaalishvili, Sabelashvili 1989], using buffer solution containing 20 mM Tris-HCl, pH 8.0, 20 mM $MgCl_2$ and 2,4% ethanol.

For the determination of DNA topoisomerase II activity of matrices the reaction medium with the volume of 20 μl containing 20 mM Tris-HCl, pH 8.0, 0,2 M NaCl, 10 mM $MgCl_2$, 1 mM ATP, 30 μg/ml bovine serum albumin, 1 μg plasmid DNA-pTZ and nuclear matrices (3 μg protein) was used (plasmid DNA was prepared by standard alkaline lysis method). The samples were incubated at 30°C for 30 min. DNA topoisomerase activity was determined by relaxation of plasmid DNA. Electrophoresis in 1% agarose gel was used [Philauri et al., 2000].

Protein concentration was determined according to Bradford [Bradford, 1976].

Results and Discussion

Table 1 clearly shows that iron salts decrease incorporation of ^{14}C-NAD into acid insoluble product of rat brain as well as in the case of liver nuclei. $FeS0_4$ at a concentration of 0.5 mM causes significant, approximately 2-fold decrease of ^{14}C-NAD incorporation into acid insoluble product – (ADP-ribose)$_n$, while $FeCl_3$ only slightly reduces ^{14}C-NAD incorporation.

Table 1. Influence of iron ions on the rat liver and brain nuclear PARP activity

Treatment	PARP activity Incorporation of ^{14}C-NAD into acid insoluble product (%)	
	Rat liver Nuclei	Rat Brain Nuclei
None	100	100
50 μM 1,5-isoquinolinediol	1.1	1.2
0.5 mM $FeSO_4$	48.0	46.1
50 μM 1,5-isoquinolinediol + 0.5 mM $FeSO_4$	1.2	1.3
1 mM $FeSO_4$	61.1	55.0
50 μM 1,5-isoquinolinediol + 1 mM $FeSO_4$	16.2	14.0
2 mM $FeSO_4$	92.5	176.9
50 μM 1,5-isoquinolinediol + 2 mM $FeSO_4$	90.1	174.8
5 mM $FeSO_4$	174.5	330.6
50 μM 1,5-isoquinolinediol + 5 mM $FeSO_4$	167.2	325.7
0.5 mM $FeCl_3$	87.0	78.1
50 μM 1,5-isoquinolinediol + 0.5 mM $FeCl_3$	1.3	1.2
1 mM $FeCl_3$	107.3	104.4
50 μM 1,5-isoquinolinediol + 1 mM $FeCl_3$	27.1	26.7
2 mM $FeCl_3$	207.6	388.6
50 μM 1,5-isoquinolinediol + 2 mM $FeCl_3$	199.5	386.3

Addition of 50 μM 1,5-isoquinolinediol, PARP inhibitor in a reaction mixture for the determination of PARP activity almost completely inhibits incorporation of ^{14}C-NAD into acid insoluble product both in the absence and presence of iron salts at a concentration of 0.5 mM, while at a higher concentrations of iron salts incorporation of ^{14}C-NAD into acid insoluble product increases and is not inhibited by 1,5-isoquinolinediol. These data indicate that at low concentrations (0.5 mM) iron salts inhibit PARP activity by influence the nucleoprotein system, while at higher concentrations iron ions are involved in formation of such complexes with NAD, which are not convertible by PARP into $(ADP\text{-}ribose)_n$ molecules and incorporate into acid insoluble product.

Based on the above mentioned we can conclude that iron ions can inhibit nuclear PARP activity not only by affecting nucleoprotein system but also by participating in the formation of such complexes with NAD, which are not convertible by PARP and remain on a glass filter (GF/C, Whatman) during washing radioactivity by trichloroacetic acid and ethanol for removing noncorverted ^{14}C-NAD radioactivity. Analogical effects of iron salts have been revealed during the study of nuclear matrices and purified PARP from liver and brain which excluded participation of free radicals in these processes.

Negative effects of iron salts in case of DNA topoisomerase activity of nuclear matrices has been also observed. As can be seen from Fig.1, the activity of DNA topoisomerase II of the isolated rat brain nuclear matrix is inhibited by iron salts. Analogical picture was observed in case of rat liver nuclear matrix.

Thus, the results of this work show that iron ions can directly inhibit the nuclear PARP and DNA topoisomerase II activities and in this way damage the cell.

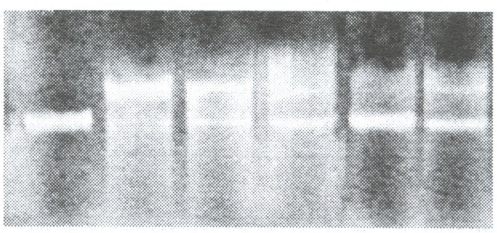

Fig. 1. Rat brain nuclear matrix DNA topoisomerase II activity. Plasmid DNA (1), activity in the absence (2) and presence of 0.05 mM (3), 0.5 mM (4), 1 mM $FeSO_4$ (5) and 1 mM $FeCl_3$ (6).

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რკინის იონების ზეგავლენის შესწავლა ეკარიოტული უჯრედების ბირთვული პოლი(ADP–რიბოზა) პოლიმერაზის და დნმ ტოპოიზომერაზა II-ის აქტივობებზე

ზაალიშვილი გ., მარგიანი დ., გაბრიაძე ი., კუტალია ქ., კოლხიდაშვილი ქ., ზაალიშვილი თ.

მოლეკულური ბიოლოგიის და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 05.09.2008)

რეზიუმე

შესწავლილია რკინის მარილების ზეგავლენა ვირთაგვას თავის ტვინის და ღვიძლის უჯრედების ბირთვული პოლი(ADP-რიბოზა)პოლიმერაზას და დნმ ტოპოიზომერაზა II-ის აქტივობებზე. აჩვენებია, რომ Fe^{2+} –ის და Fe^{3+}-ის იონები აინჰიბირებენ როგორც პოლი(ADP-რიბოზა)პოლიმერაზას, ასევე დნმ ტოპოიზომერაზა II-ის აქტივობებს. ნავარაუდევია, რომ პოლი(ADP-რიბოზა)პოლიმერაზის ინჰიბირების მიზეზი შესაძლოა იყოს არა მარტო რკინის იონების ზემოქმედება ნუკლეოპროტეინულ სისტემაზე, არამედ რკინის იონებსა და პოლი(ADP-რიბოზა)პოლიმერაზის სუბსტრატს (NAD) შორის ისეთი კომპლექსების წარმოქმნა, რომლებიც ვერ გარდაიქმნება ფერმენტის მიერ.

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Mycology

CONTRIBUTION TO THE MYCOBIOTIC DIVERSITY OF GEORGIA: FUNGI ASSOCIATED WITH MYRICARIA GERMANICA (L.) DESV.

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Abstract

16 species of microfungi associated with Myricaria germanica in Georgia are briefly reviewed. Among them *Diplodia tamaricina* and *Phomopsis myricariae* are new records to Georgia and other species listed are new fungus-host combination.

Key words: Myricaria germanica, fungi, Georgia.

Introduction

The paper continues representing the results of studies of systematic composition of mycobiotic complexes of woody plants in Georgia. It must be noted that until now on M. germanica growing on proluvial riverside stony soils in middle mountain zones, only 2 fungal species, viz. *Cytospora myricariae* Petrak (Voronikhin, 1927) and *Cytospora schulzeri* Sacc et Syd. (Gvritishvili, 1982) have been known.

Materials and Methods

The materials investigated have been collected near Mtskheta in the gorge of left tributary of riv. Mtkvari (Kura), Dzegvi village environs (March 1998, April and November 2000). Routine light microscopic method has been used for identification of collected specimens on the base of macro and micromorphological features.

Results and Discussion

Botryosphaeria dothidea (Moug ex Fr.) Ces. & de Not.(Sivanesan,1984)
This plurivorous fungus causing or associated with many diseases of wild and cultivated woody plants in Georgia and other countries is recorded on M. germanica for the first time.

Camarosporium sp.
Pycnidia up to 450μm in diameter. Conidia light brown, elliptic, with 3 transversal and 1 incomplete septa, 12-18x7-10 μm.

Cytospora leucosperma (Pers.) Fr.

This fungus with very wide range of host plants (Gvritishvili,1982) was first recorded in Georgia by Voronov in 1923 (Voronikhin, 1927 as *C. myricariae* Petrak).

Cytospora sacculus (Schwein.) Gvrit.

This fungus having also very wide and heterogeneous range of host plants (Gvritishvili, 1982) is recorded on Myricaria for the first time.

Diaporthe eres Nitschke (Wehmeyer, 1933)

The fungus is distinguished with wide range of hosts belonging to woody plants.

D. eres - M. germanica is new fungus-host combination.

Diplodia tamaricina Sacc. (Diedicke, 1915)

Conidia 18-25x 8-10 μm. (12 x 8 μm. in Saccardo, 1884).

Macrophoma spp.

Our list of fungi on M. germanica includes two representatives of the genus *Macrophoma* with conidia 13-23 x 6-7.5 μm. and 20-30 x 7-8 μm.

Microsphaeropsis olivacea (Bonord.) Höhn. (*Coniothyrium olivaceum* Bonord.) (Sutton, 1980)

Plurivorous species associated with stems and branches of woody and herbaceous plants. M. germanica - *M. olivacea* is new host-fungus combination.

Phomopsis myriocariae H. Syd. (Uecker,1988)

Conidiophores 10-18 x 2.5-3.5 μm. Alpha conidia 5-10(12.5) x 2-3.5 μm. Beta conidia not observed.

Pleospora sp.

Asci cylindrical 8 spored, 65-85 x 32-87 μm. Ascospores elliptical yellow to pale brown with 5-7 transversal and 1 incomplete vertical septa, constricted at the septa, 20-30 x 6-9 μm.

Stagonospora sp.

Conidia hyaline, fusiform, guttulatae, 1-3 septate, 25-33 x 4-5μm.

From other plurivorous fungi first recorded on M. germanica, can be noted: *Alternaria alternata* (Fr.) Keissl., *Botrytis cinerea* Pers., *Pleospora herbarum* (Pers.) Rabenh.

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მასალები საქართველოს სოკოების მრავალფეროვნების შესწავლისათვის: მთის იალღუნთან (Myricaria germanica Royle) ასოცირებული სოკოები

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(მიღებულია 03.09.2008)

რეზიუმე

სტატიაში მოცემულია ახალი ცნობები საქართველოს მიკობიოტის სისტემატიკური შემადგენლობის შესახებ. მთის იალღუნზე რეგისტრირებული 16 სახეობიდან ორი სახეობა, სახელდობრ, *Diplodia tamaricina* და *Phomopsis myricariae* საქართველოში პირველად აღინიშნება. დანარჩენი მონაცემები ეხება სოკო-მცენარის დღემდე უცნობ ახალ კომბინაციებს.

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Paleobiology

FORAMINIFERS AND PALYNOMORPHS IN THE SARMATIAN DEPOSITS OF KARTLI (EASTERN GEORGIA): STRATIGRAPHICAL AND PALAEOCLIMATOLOGICAL IMPLICATIONS

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Abstract

The foraminifers and palynomorphs from Sarmatian deposits of two sections, Nadarbazevi and Uplistsikhe, were studied. The foraminiferal assemblages allow establishing Late Volkhynian (beds with *Elphidium aculeatum)* and Early-Middle Bessarabian (beds with *Affinetrina voloshinovae* and *Porosononion aragviensis*) ages. The palynological analysis reflects the changes of ecological-systematical composition of the flora and allows interpreting the evolution of vegetation on the territory of Kartli depending on climatic fluctuations.

Key words: Eastern Georgia, Sarmatian, foraminifers, pollen and spores.

Introduction

Sarmatian deposits are widely distributed on the territory of Eastern Georgia (Fig.1). The mollusks of these deposits have been studied in detail [Buleishvili, 1960; Gruzinskaya, 1967], but investigation of the microfauna began only recently [Koiava, 2006].

Concerning pollen, there is only one work of P. Mchedlishvili and N. Mchedlishvili (1953), who studied the materials from a bore-hole in the central part of Kartli. The authors analyzed samples from Early, Middle and Late Sarmatian deposits and determined 28 forms. The Late Sarmatian palynological assemblages reflect the domination of the pollen of grasses.

First results of the micropalaeontological investigation of Sarmatian deposits of Eastern Georgia were published in 2008. In this work [Maissuradze et al., 2008], the lists of foraminifers and flora from the Nadarbazevi section are given and the changes in composition of micropalaeontological and palynological associations during the Middle Sarmatian are traced.

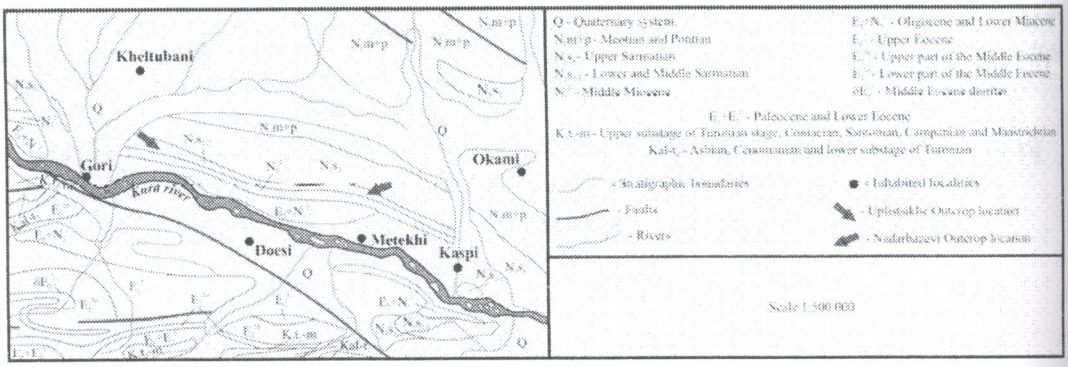

Fig.1. Geological map of the Gori area and location of the Nadarbazevi and Uplistsikhe sections (according to G. Gudjabidze, 2003)

Materials and Methods

In Figure 2, the stratigraphical schemes of the Sarmatian deposits in the Nadarbazevi and Uplistsikhe sections are given based on detailed sedimentological logging. The biostratigraphic zonation (N_1S_1, N_1S_2) is based on Koiava (2006).

The list of foraminifers from the Nadarbazevi section is already published [Maissuradze et al., 2008]. Here only the list of microfauna from the Uplistsikhe section is given:

Elphidium macellum (Fichtel et Moll.), *E.aculeatum* (Orbigny), *E.rugosum* (Orbigny), *E.crispum* (Linne), *E.flexuosum* (Orbigny), *E.fichtelianum* (Orbigny), *E.hauerinum* (Orbigny), *E.reginum* (Orbigny), *E.grilli* Papp., *Porosononion subgranosum* (Egger), *P.granosum* (Orbigny), *P.martkobi* (Bogdanowicz), *P.subgranosum umboelatum* (Bogdanowicz), *Cycloforina complanata* (Gerke et Issaeva), *C.*aff.*predcarpatica* (Serova), *Sinuloculina consobrina sarmatica* (Gerke), *Varidentella reussi* (Bogdanowicz), *V.sarmatica* (Karrer), *Affinetrina guriana* (O.Djanelidze), *A.*aff.*ucrainica* (Serova), *Nonion bogdanowiczi* (Voloshinova), *N.*aff.*tumidulus* Pishvanova. In addition, *Ostracoda* are very common in this section.

In previous work [Maissuradze et al., 2008], the list of Sarmatian flora was published. It includes the results of palynological analysis of the Nadarbazevi section and of large plant remains from Sarmatian deposits of Kartli [Chelidze, 1979; 1987]. Later, new palynological material from Uplistsikhe as well as from the Nadarbazevi section was investigated that enriched our knowledge about the Sarmatian flora of Eastern Georgia. The full list of palynoflora from both sections is given below:

Sphagnum sp., *Lycopodium serratum* Thumb., *Lycopodium* sp., *Selaginella fusca* N.Mtchedl., *Selaginella* sp., *Bothrychium sp., Osmunda* sp., *Schizaea* sp., *Schizaeaceae* gen.indet., *Anemia* sp., *Mohria* sp., *Lygodium digitatum* Presl., *L.japonicum* Sw., *Lygodium* sp., *Cryptogramma* sp., *Pteridacidites boerzoenyensis* St. et Sh.(Nagy), *P.dentatiformis* Sh. et St., *P.grandifoliiformis* St. et Sh., *P.guriensis* Sh. et St., *P.longifoliiformis* Sh., et St., *P.remotifolioides* Sh. et St., *P.venustaeformis* St. et Sh., *P.verus* (N.Mtchedl.) Sh. et St., *Pteridacidites* sp., *Marsilea* sp., *Anogramma* sp., *Onychium* sp., *Pityrogramma* sp., *Clavifera* sp., *Gleichenia* sp., *Gleicheniaceae* gen.indet., *Polypodium aureum* L., *P.pliocenicum* Ram., *P.verrucatum* Ram., *Polypodium* sp., *Verrucatosporites histiopteroides* W.Kr., *Pyrossia* sp., *Polypodiaceae* gen.indet.,

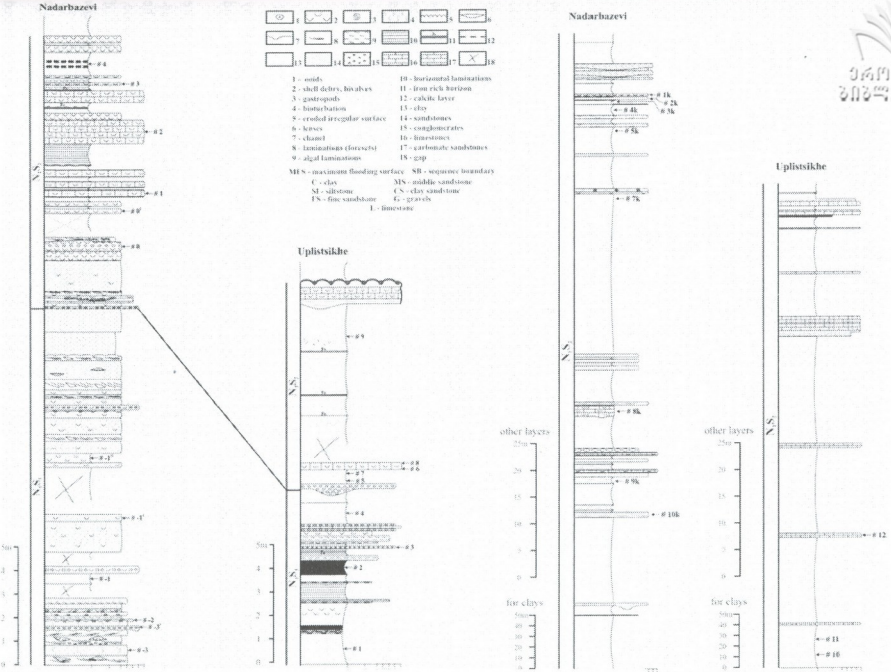

Fig.2. The stratigraphical schemes of the Nadarbazevi and Uplistsikhe sections.

Hymenophyllum sp., *Cibotium* sp. *Dicksonia reticulata* Purc., *Dicksonia spanditocincta* Purc., *Dicksonia unitotuberata* Purc., *Dicksonia* sp., *Alsophylla* sp., *Cyathea* sp., *Hemitelia* sp., *Asplenium* sp., *Cystopteris* sp., *Microlepia* sp., *Dryopteris* sp., *Thelypteris* sp., *Polystichum* sp., *Ginkgo* sp., *Dacrydium* sp., *Podocarpus* sp., *Phyllocladus* sp., *Araucaria* sp., *Abies ciliticaeformis* N.Mtchedl., *A.nordmanniana* (Stev.) Spach., *Abies* sp., *Cathaya* sp., *Cedrus sauerae* N.Mtchedl., *Cedrus* sp., *Keteleeria caucasica* Ram., *Picea minor* N.Mtcheld., *Picea* sp., *Pinus* sp., *Pseudolarix* sp., *Pseudotsuga* sp., *Tsuga* aff.*canadensis* (L.) Carr., *T.*aff.*pattoniana* Engelm., *Tsuga* sp., *Sciadopitys* sp., *Cryptomeria* sp., *Cunninghamia* sp., *Metasequoia* sp., *Sequoia* sp., *Taxodium* sp., *Taxodiaceae* gen.indet., *Libocedrus* sp., *Juniperus* sp., *Cupressaceae* gen.indet., *Ephedra* sp., *Comptonia* sp., *Myrica* aff.*notabilis* Gladk., *Myrica* sp., *Carya aquatica* (Michx.) Nutt., *C.*aff.*cordiformis* (Wandh.) C.Koch, *Carya* sp., *Engelhardia* sp., *Platycarya* sp., *Pterocarya* sp., *Juglans cinerea* L., *J.regia* L., *Juglans* sp., *Juglandaceae* gen.indet., *Salix* sp., *Alnus* sp., *Betula* sp., *Carpinus betulus* L., *Carpinus caucasica* Grossh., *Carpinus* sp., *Corylus* sp., *Castanea* sp., *Castanopsis* sp., *Fagus* sp., *Lithocarpus* sp., *Quercus* sp., *Celtis* sp., *Ulmus foliacea* Gilib., *Ulmus* sp., *Zelkova* aff.*serrata* (Thunb.) Macino, *Zelkova carpinifolia* (Pall.) Dipp., *Eucommia ulmoides* Oliv., *Ficus* sp., *Polygonum* sp., *Caryophillaceae* gen.indet., *Chenopodiaceae* gen.indet., *Liriodendron* sp., *Magnolia grandiflora* L., *Magnolia megafigurata* (W.Kr.) Ram., *Magnolia* sp., *Annona* sp., *Cinnamomum* sp., *Laurus* sp., *Lauraceae* gen.indet., *Ranunculus* sp., *Menispermum* sp., *Platanus* sp., *Liquidambar styraciflua* L., *Liquidambar* sp., *Corylopsis* sp., *Disanthus* sp., *Distyliopsis* sp., *Fothergilla* sp., *Hamamelis* sp., *Parrotia* sp., *Sycopsis colchica* Ram., *Hamamelidaceae* gen.indet., *Cercidiphyllum* sp., *Sorbus* sp., *Rosaceae* gen.indet., *Fabaceae* gen.inet., *Geranium* sp., *Rhus* sp., *Acer* sp., *Aesculus* sp., *Ilex* sp., *Euonymus* sp., *Staphylea* sp., *Parthenocissus* sp., *Vitis* sp., *Tilia* sp., *Sterculia* sp., *Viola* sp., *Myrtaceae* gen.indet., *Alangium* sp., *Nyssa* sp., *Cornaceae* gen.indet., *Aralia* sp., *Dendropanax* sp., *Araliaceae* gen.indet., *Apiaceae* gen.indet., *Rhododendron* sp., *Sapotaceae* gen.indet., *Symplocos* sp., *Fraxinus* sp., *Oleaceae* gen.indet., *Lonicera* sp., *Lamiaceae* gen.indet., *Plantago* sp., *Boraginaceae* gen.indet., *Artemisia* sp., *Cirsium* sp., *Asteraceae* gen.indet., *Poaceae* gen.indet., *Nipa* sp., *Arecaceae* gen.indet., *Sparganium* sp., *Typha* sp., *Tricolporopollenites edmundi* (R.Pot.) Th. et Pf., *Tricolporopollenites wackersdorfensis* Thiele-Pfeiffer.

Results and Discussion

The foraminifers in the Nadarbazevi and Uplistsikhe sections are characterized by identical complexes. However, the Nadarbazevi section is richer in number of species and specimens (Figs. 3, 4).

According to the foraminifers, the lower beds (samples 1-4) in the Uplistsikhe section are dated as Early Sarmatian (N_1S_1) [Koiava, 2006], which corresponds to the Volkhynian substage. Only the Late Volkhynian layers with *Elphidium aculeatum* are exposed here [Koiava, 2006]. The sediments are represented by sandy-argillaceous facies with intercalated beds of sandstone, conglomerate, and limestone. They are characterized by more or less monotonous assemblages of foraminifers, in which typically *Porosononion subgranosum* (sample 1) or *Elphidium macellum* (sample 2) are dominant. Together with these predominant species also *Porosononion martkobi, P.granosum, Elphidium aculeatum, E. hauerinum, E. flexuosum, E. grilli, Nonion bogdanowiczi* are present. *Varidentella reussi, Cycloforina complanata,* and small specimens of *Ostracoda* are rare.

The deposits of the upper part of the Uplistsikhe section (samples 5-12) are characterized by foraminifer assemblages indicating a Middle Sarmatian age (N_1S_2). The sediments correspond to the Early Bessarabian substage or to layers with *Affinetrina voloshinovae*, which are widely distributed in the Sarmatian of Eastern Georgia [Koiava, 2006]. In Uplistsikhe this interval is represented by shallow water facies (marls including sandstone and limestone beds) and

characterized by numerous many-chambered (10-14) specimens of *A. voloshinovae*, not typical for this species. Such are *Porosononion subgranosum* and *P.subgranosum umboelatum*, or big thick-walled many-chambered representatives of the genus *Elphidium (macellum, crispum, fichtelianum, flexuosum*). More rarely (samples 3, 10), single species of *Affinetrina, Cycloforina, Varidentella* are present, which display large, coarse, and thick tests. Such morphological features are characteristic also for *Ostracoda*, which are enriched in this complex.

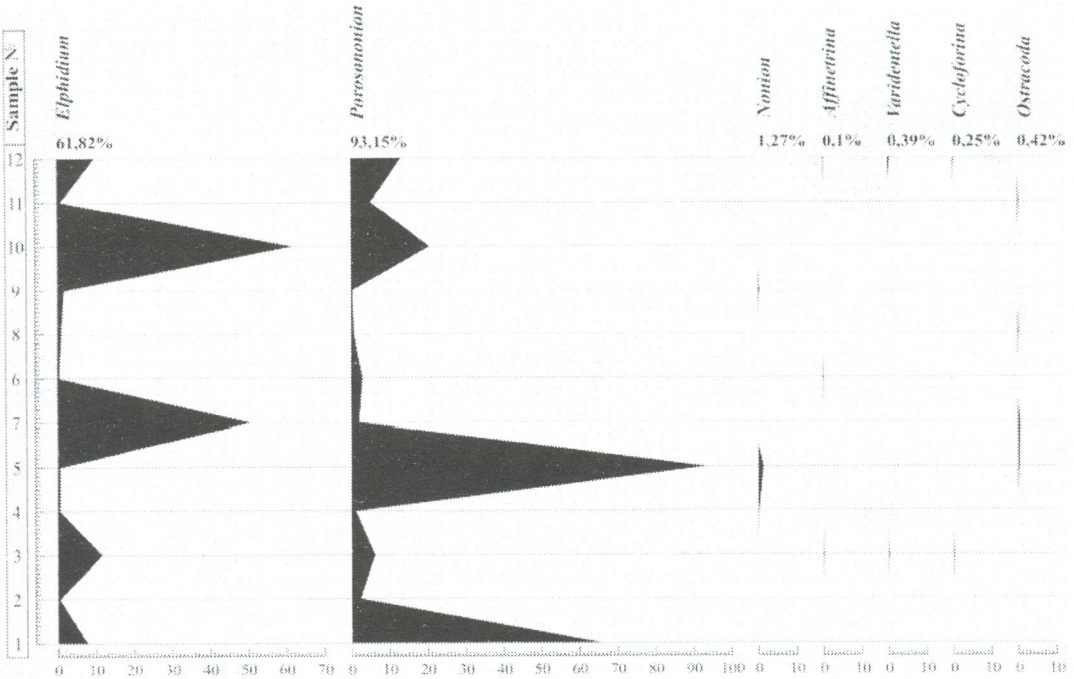

Fig.3. Relative abundance of foraminifer genera in the Uplistsikhe section (for sample position see Fig. 2).

Consequently, in theUplistsikhe section, the Late Volkhynian (beds with *Elphidium aculeatum)* and the Early Bessarabian (beds with *Affinetrina voloshinovae*) are well established.

The first results of micropalaeontological investigations of the Nadarbazevi section have already been published [(Maissuradze et al., 2008]. The study of additional material now allows establishing new stratigraphical levels with characteristic assemblages of foraminifers (Fig. 4.). The section is composed of Early Sarmatian (N_1S_1, Late Volkhinian) and Middle Sarmatian (N_1S_2, Early-Middle Bessarabian) deposits. The Late Volkhinian assemblage (samples -3; -1") is characterized by very small, typically small-chambered (6-8) *Porosononion subgranosum* and *P. martkobi* (10-15 specimens) and single (not more than 3-4 specimens) *Elphidium hauerinum, E. macellum, Nonion bogdanowiczi,* and *Varidentella reussi.* Numerous otoliths of fishes, fragments and embryos of mollusks and - seldom - small ostracodes are also present.

The lower part of the Middle Sarmatian (Early Bessarabian) is defined by beds containing *Affinetrina voloshinovae* (samples 0-8). This part is characterized by a rich and diverse assemblage of foraminifers, in which bigger than usual *Porosononion* and *Elphidium* are dominant, similar to synchronous forms from theUplistsikhe section. Only the assemblage of sample 3 is different. This is a typical miliolidian complex, which is characteristic for argillaceous deposits: *Varidentella*

reussi, V. sarmatica, V. latelaculata, Cycloforina complanata, C. aff. *predcarpatica, C.* aff. *hauerina, Sinuloculina consobrina sarmatica, S. angustioris, Affinetrina* sp., *Spiroloculina kolesnikovi, Articulina sarmatica, A. problema,* and numerous *Ostracoda.*

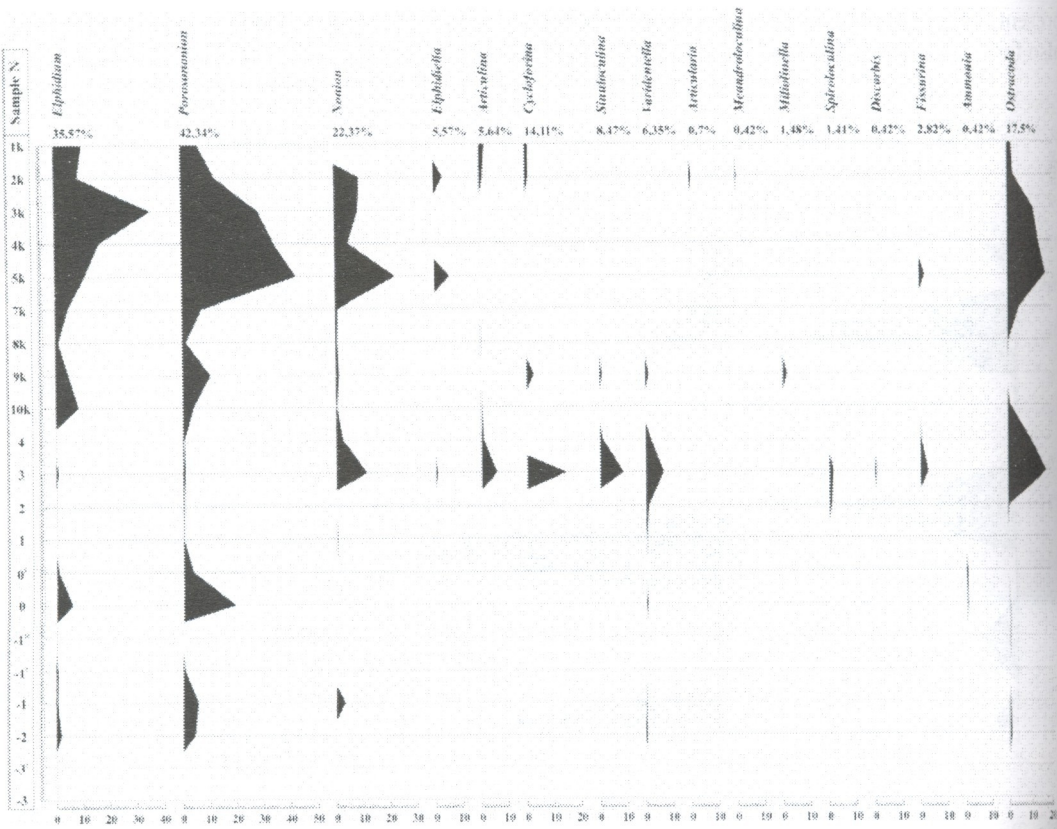

Fig. 4. Relative abundance of foraminifer genera in the Nadarbazevi section (for sample position see Fig. 2).

The deposits of the upper part of the Nadarbazevi section (samples 7-1k) have already been described in detail [Maissuradze et al., 2008]. Additional material allows only specifying their age as Middle Bessarabian (beds with *Porosononion aragviensis)* [Koiava, 2006].

In the deposits of the Nadarbazevi and Uplistsikhe sections, pollen and spores were seen mainly in the Middle Sarmatian and in the upper part of the Early Sarmatian. However, not in all samples the number of grains was large enough for calculating the percentages.

The diagrams in Figs. 5 and 6 reflect the ecological-systematical composition of the flora. In the beginning of these diagrams the composition of palyno-assemblages is given: pollen of woody plants, herbaceous plants, and cryptogams. The assemblages are dominated by woody plants, followed by spores of ferns. Grasses are presented by a small number of grains, except in the upper beds of the Nadarbazevi section.

The woody plants are divided into two groups: conifers and angiosperms. In turn, the conifers are also divided into two groups: the forms of temperate climate (*Abies, Picea, Tsuga*), with only minor participation in the composition of the palynocomplex; and the forms of warm-temperate and subtropical climate (*Podocarpus, Dacrydium*, some *Cedrus, Cathaya, Phyllocladus,*

etc.). By percentage these forms predominate over conifers of temperate climate. The curve of pine is given separately, as a form with wide ecological range. Among angiosperm woody plants, we distinguish two groups: the plants of warm-temperate climate (*Fagaceae, Ulmaceae, Betulaceae, Juglandaceae*) and forms of subtropical climate (*Myricaceae, Hamamelidaceae, Magnoliaceae, Alangium, Sapotaceae*, etc.).

The distribution of spores is given separately in the diagram. They are presented mainly by subtropical forms: *Schizaeaceae, Anemiaceae, Lygodiaceae, Pteridaceae, Gleicheniaceae, Polypodiaceae, Dicksoniaceae,* etc.

Finally, the distribution of pollen of subxerophilous forms (*Chenopodiaceae, Asteraceae, Ephedra*) is given.

The second type of diagram (Fig. 7) is built on the basis of the landscape-phytocenological method. The aim of this diagram is to show the character of vegetation cover and its evolution through time, and to distinguish the stages of development of vegetation and climate.

The assemblages were divided into three groups: temperate-dark-conifer forest of the upper mountain belt, and subtropical-warm-temperate polydominant forests of the lower and middle mountain belts. We also include subtropical conifers and also ferns, which formed the lower layer of the forests. The curve of pine – intrazonal plants as indicators of humidity is given separately in the diagram.

The analysis of the palynological distribution through time (Figs. 5, 6, 7) allows distinguishing 9 stages in the development of vegetation and climate in Kartli during the Early and Middle Sarmatian.

Stage I (upper part of the Early Sarmatian, Uplistsikhe section, sample 4) is characterized by a small area of dark conifers and the domination of pine forests over the polydominant forests. The climate was subtropical with low humidity.

Stage II was established in the Uplistsikhe section (sample 5). The area of polydominant forests became smaller. The area of pine increased. The territory of dark conifers was the same as in stage I. Stages I and II differed by the regime of humidity, which in stage II was lower.

Stage III is distinguished by the material from the Uplistsiche section (sample 7) and the Nadarbazevi section (sample 0). The characteristic feature of this stage is the high percentage of plants of polydominant forests and the low proportion of pine and dark conifers. The climate was subtropical with high humidity and probably corresponded to the climatic optimum of the Middle Sarmatian. According to the foraminifers, the upper boundary of this stage is likely the boundary between lower and middle parts of Middle Sarmatian (see Fig. 2).

Stage IV is also established in both sections (sample 9 in Uplistsicke and sample 3 in Nadarbazevi). The characteristic feature of this stage is the increase in number of plants of temperate climate, the reduction of the area of polydominant forests, and some increase of pine. In comparison with the previous stage, temperature and humidity were decreasing.

Stage V is distinguished by the palynological data of the Uplistsiche section (samples 10, 11, 12). To this stage we attribute also the samples 4 and 8k of the Nadarbazevi section. The assemblages of both sections reflect a similar picture of development of vegetation: the predominance of polydominant forests and reduction of area of dark conifer and pine forests. Probably it also was the climatic optimum, with subtropical and humid climate.

About the other stages of development of vegetation and climate of Kartli during the Middle Sarmatian we can judge only by material from the Nadarbazevi section.

Stage VI (samples 7k, 6k) reflects the increase of area of dark conifers and pine, and reduction of area of polydominant forests. The climate was warm-temperate with somewhat reduced humidity.

Stage VII (samples 5k, 4k) is characterized by reduction of the role of pine and by increase of the area of polydominant forests. The climate was close to subtropical with high humidity.

During stage VIII (sample 3k), the area of polydominant forest was reduced and the territory of pine increased. The dark conifers continued to occupy stable areas during the stages VI, VII, and VIII. The climate was warm-temperate with low humidity.

Stage IX (samples 2k, 5, 6) is distinguished by reduction of area of dark conifers and pine. The area of polydominant forests increased but their composition changed. The number of subtropical ferns reduced. Predominant components were warm-temperate plants colonizing flood-plain forests. Judging from the diagram of the Nadarbazevi section (Fig. 5), characteristic features of this stage were reduction of forests at whole and increase of open spaces.

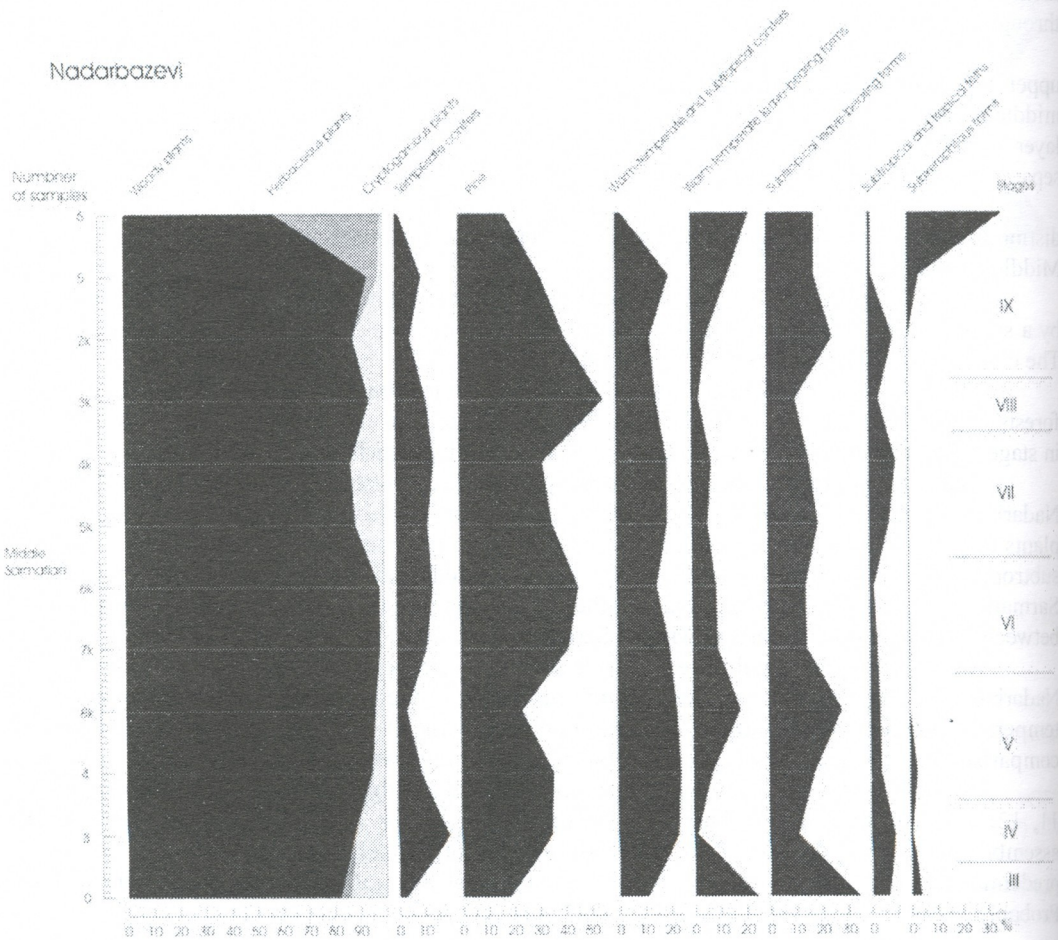

Fig. 5. Pollen diagram of Sarmatian deposits of the Nadarbazevi section (for sample position see Fig. 2). Temperate conifers: *Abies, Picea, Tsuga*, some species of *Cedrus*; warm-temperate and subtropical conifers: *Dacrydium, Podocarpus, Phyllocladus, Cathaya*, some species of *Cedrus, Ginkgo*, etc.; warm-temperate leave-bearing forms: *Fagaceae, Ulmaceae, Betulaceae, Juglandaceae*; subtropical leave-bearing forms: *Myricaceae, Magnoliaceae, Hamamelidaceae, Araliaceae, Arecaceae*, etc.; subtropical and tropical ferns: *Pteridaceae, Polypodiaceae, Gleicheniaceae, Schizaeaceae,* etc.; subxerophilous forms: *Ephedra, Chenopodiaceae, Asteraceae,* etc.

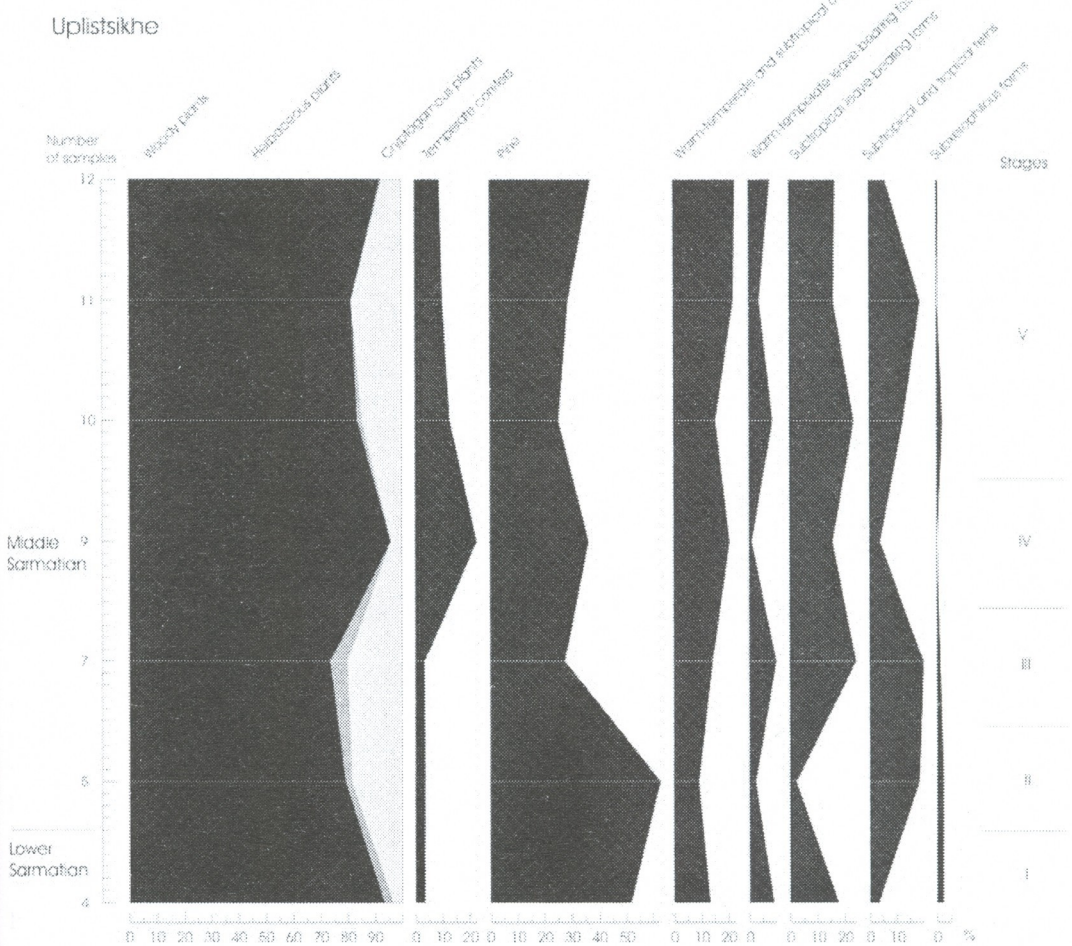

Fig.6. Pollen diagram of Sarmatian deposits of the Uplistsikhe section (for sample position see Fig. 2). Temperate conifers: *Abies, Picea, Tsuga*, some species of *Cedrus*; warm-temperate and subtropical conifers: *Dacrydium, Podocarpus, Phyllocladus, Cathaya*, some species of *Cedrus, Ginkgo*, etc.; warm-temperate leave-bearing forms: *Fagaceae, Ulmaceae, Betulaceae, Juglandaceae;* subtropical leave-bearing forms*: Myricaceae, Magnoliaceae, Hamamelidaceae, Araliaceae, Arecaceae*, etc.; subtropical and tropical ferns*: Pteridaceae, Polypodiaceae, Gleicheniaceae, Schizaeaceae,* etc.; subxerophilous forms*: Ephedra, Chenopodiaceae, Asteraceae,* etc.

Conclusions

The Sarmatian deposits of Kartli (Eastern Georgia) in the Nadarbazevi and Uplistsikhe sections were analyzed by microfaunistical and palynological methods.

In these sections, Late Volkhinian, Early Bessarabian and Middle Bessarabian deposits are present. They are characterized by homotypic complexes of foraminifers, in which shallow water euryhaline forms are dominated. The similarity of the assemblages, their ecological characteristics, the morphological peculiarity of the dominant forms (sizes, sculpture of the tests) and the

composition of accompanying microorganisms (otoliths, mollusks, bryozoans, algae, ostracodes) testify that in this region, during Early-Middle Sarmatian, the sea was brackish (absence of stenohaline forms). The water was shallow, warm, saturated by oxygen (big sizes of tests) and by calcium carbonate (thickness, ornamentation or sculpture of walls of tests).

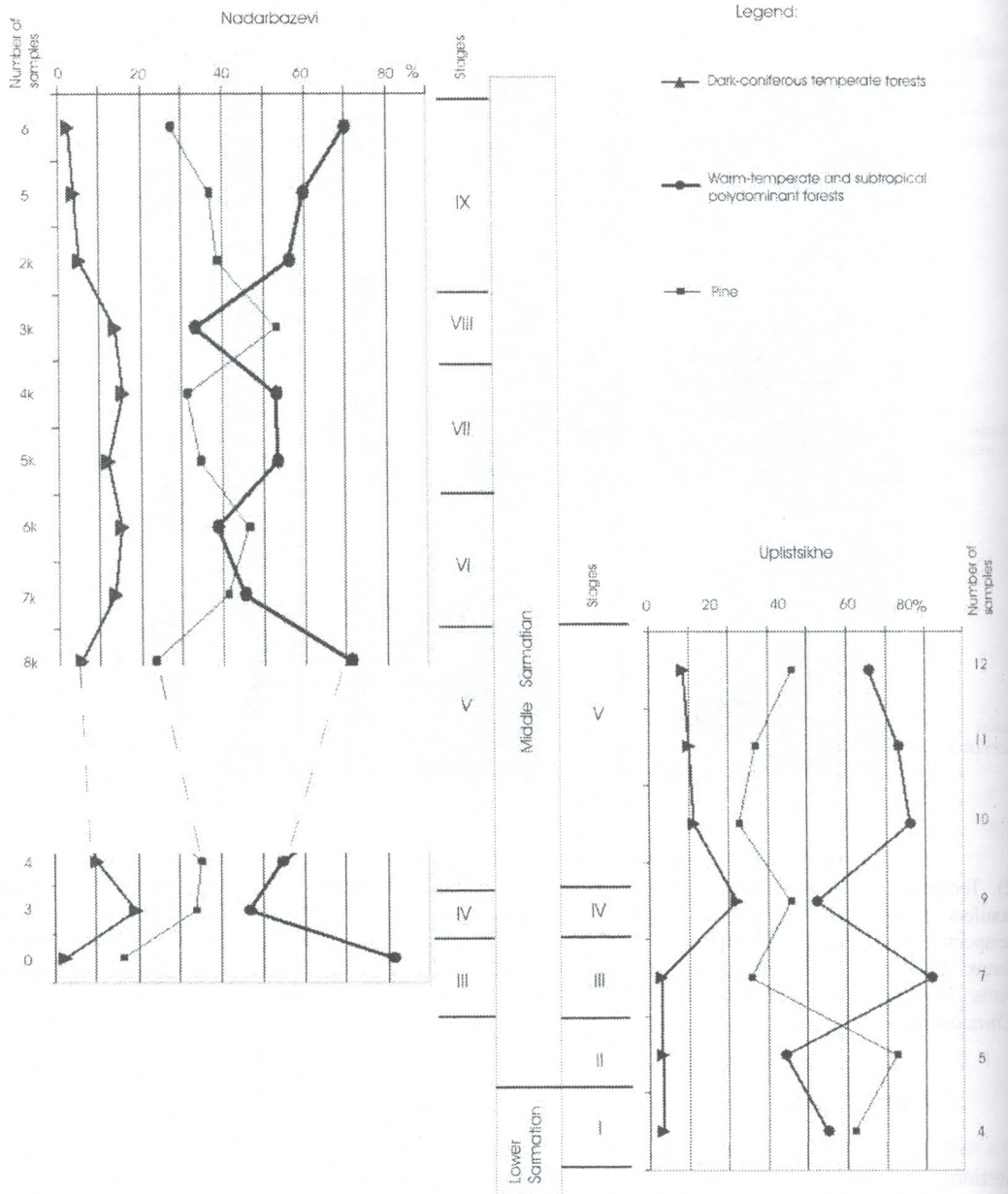

Fig.7. The main stages of climate and vegetation development in the territory of Kartli (Eastern Georgia) during the Middle Sarmatian according to palynological analysis of deposits of the Nadarbazevi and Uplistsikhe sections.

The Late Volkhinian and Early Bessarabian deposits in the Nadarbazevi and Uplistsicke sections are well correlated due to the similarity of foraminifer assemblages.

The palynological analysis of these two sections allows distinguishing 9 stages in the history of development of vegetation and climate in Kartli.

In stages I and II the temperatures were rather high close to those of subtropical zones, but the humidity was low, especially in stage II, when subtropical polydominant forests occupied a comparably small area. In stage III, the humidity was higher and the subtropical forests increased their area, which was the largest for the whole Middle Sarmatian. Therefore, stage III can be considered as climatic optimum. Nearly the same situation occurred in stage V, which also can be considered as climatic optimum. These two optima were separated by an interval with lower temperatures (stage IV). In this time temperate dark-conifer forests increased their area. In stage V their territory decreased again. In the following stages (VI, VII, VIII) they preserved nearly equal areas till the middle part of the Middle Sarmatian (stage IX). During the whole time of deposition of the Nadarbazevi and Uplistsicke sections the area of dark-conifers never intersected with the area of pine and polydominant forests. The evolution of vegetation of Eastern Georgia during the Middle Sarmatian is expressed mainly in periodical domination of either polydominant forests or pine forests. This was accentuated in the middle part of the Middle Sarmatian (stages VI, VII, VIII). Towards the end of the middle part of the Middle Sarmatian (stage IX) the forests began to decline as a whole as did the number of subtropical ferns, and the role of plants characteristic for vegetation of open spaces increased. Warm-temperate and subtropical plants composing riparian forests continued to exist.

We can conclude that the main climatic factor, which influenced the development of vegetation of Eastern Georgia was the regime of humidity. This is in accordance with the paleogeographical changes that took place at the end of the Middle Sarmatian, one of the turning points in the Neogene history of the Caucasus [Tsagareli, Astakhov, 1971]. As a result of orogenic movements the surface area of the sea decreased and on the territory of Eastern Georgia dry land with continental climate originated. In the Late Sarmatian and following time, according to palaeontological data [Mchedlishvili P.A., Mchedlishvili N.D. 1953; Meladze, 1967], steppes and semi-deserts dominated on the territory of Kartli .

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ქართლის სარმატული ნალექების კვლევა მიკროფაუნისტური და პალინოლოგიური მეთოდების საშუალებით

შატილოვა ი.[1], მაისურაძე ლ.[1], ქოიავა კ.[2], მჭედლიშვილი ნ.[1], რუხაძე ლ.[1], სპეზაფერი ს.[3], სტრასერ ა.[3]

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(მიღებულია 22.07.08)

რეზიუმე

შესწავლილია ფორამინიფერები და პალინომორფები ნადარბაზევისა და უფლისციხის ჭრილების სარმატული ნალექებიდან. მოცემულია რეგიონის რუკა, სტრატიგრაფიული ჭრილები, ნამარხი ფორმების სიები, დიაგრამები. მიკროფაუნისტური დიაგრამები ასახავს ფორამინიფერების ცალკეული გვარების პროცენტულ ცვლილებას ჭრილებში. პალინოლოგიური დიაგრამები ორი ტიპისაა. პირველი გვიჩვენებს ფლორის ეკოლოგიურ-სისტემატიკური შემადგენლობის ცვლილებებს, მეორე კი - ქართლის ტერიტორიაზე კლიმატური ფლუქტუაციების ზეგავლენით მცენარეული საფარის ევოლუციას.

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Plant Physiology

ADAPTIVE POTENTIALS OF *ARTEMISIA* L. SPECIES TO HEAVY METAL CONTAMINATION DEPENDING ON THEIR HABITATS

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Abstract

Adaptive possibilities and heavy metal (HM) accumulating capacity of two indigenous dominant *Artemisia* L. species (*A.fragrans* and *A.szovitsiana*) widespread on different contaminated locations of Azerbaijan with various soil pollution levels has been studied. The contents of Cd, Cu, Ni, Pb and Zn in plant parts and surrounding soil samples were determined. Tested *Artemisia* species displayed the distinctive strategies of toleration to HM levels in their environment. They proved as tolerant, accumulator and indicator plants depending on their habitats. Due to high resistance to various soil contamination they could be recommended for use in natural long-term restoration of stressed soils.

Keywords: heavy metals, *Artemisia* species, accumulation capacity, growing location

Introduction

Elucidation of the relations between an adaptive potential of plants, their ability of survival and reproduction under man-caused stress conditions and ecological situations in their habitats draws a proper attention in connection with a possibility to use green plants for the restoration and cleaning up of environment from various pollutants.

In particular, some plant species developed a tolerance and an accumulative capacity of heavy metals (HM) which are considered as one of the most dangerous pollutants of all biosphere components (air, water and soil) due to their negative effects on the plant growth and productivity and risk for human and animal health [Marschner, 1983]. The toxic effect of HM on a plant metabolism is mainly nonspecific and similar the effect of other stress factors on plants [Marschner, 1983; Ernst, 2006; Seregin and Kojevnikova, 2008].

Plants are known to differ on their response reactions to HM excess in an environment [Baker, 1981] and display a tolerance or sensitivity to this stress factor due to their individual morphological-physiological peculiarities. Tolerant plants are able to survive and vigorously grow under heavily contaminated conditions without any symptoms of damage [Ernst, 2006]. Tolerant species accumulating high amounts of HM and storing them in their above ground parts are considered to be accumulator plants [Lasat, 2000].

Revelation of indigenous plant species which are not only tolerant to high soil contamination, but also able to grow vigorously, reproduce under these adverse conditions and accumulate HM in their organs is of great importance for the use of these species in long-term natural restoration of local soils [Willey, 2007].

Our previous study on selection of HM-tolerant and HM-hyperaccumulator *Artemisia* L. species from local flora growing in two contaminated regions of Azerbaijan (Binegedi and Ali-Bayramli) revealed a marked difference between ecotypes in their HM accumulation capacity depending on the growing locations [Alirzayeva et al., 2006].

An aim of the present work is a continuation of study of differences in adaptive possibilities and HM accumulating capacities of two indigenous *Artemisia* L. species (*A.fragrans* and *A.szovitsiana*), which are typical representatives of local flora and widespread on other contaminated regions of Azerbaijan, depending on species/ecotypes and the soil contamination levels in their habitats.

Materials and methods

Two *Artemisia* L. species (*A.fragrans* and *A.szovitsiana*) growing in different regions of Azerbaijan (Sumgayit, Lokbatan, Garadag, Silk route) contaminated by various pollutants (oil products, chemical, metallurgical wastes, cement dust and automobiles emissions) were collected for testing of their HM accumulation capacity. Soil samples (0-10 cm of surface soil) from all locations tested were also taken for analysis.

The plants collected were washed thoroughly with de-ionized water and separated into shoots and roots. Plant samples were analyzed by ICP-AES (Varian-Vista PRO, Australia) to determine Cd, Cu, Ni, Pb and Zn concentrations. The total content of Cd, Cu, Ni, Pb and Zn in soil samples were analyzed in Aqua Regia ($1HNO_3$:3HCL by volume).

All analyses were run in three replications. Data were evaluated by analysis of variance using MS Excel.

Results and Discussion

To clarify the differences in HM accumulation capacity of ecotypes of *Artemisia* L. plants depending on their habitat and soil pollution levels, *A.fragrans* and *A.szovitsiana* were collected from grey-brown soils of some Azerbaijan locations with varied pollution types: Lokbatan location contaminated by various chemical wastes and oil products, Garadag location contaminated by cement dust, Sumgayit location polluted by chemical emissions and near Silk route location contaminated by metallic wastes (Table 1).

In previous investigations, while among the tested species *A.scoparia* collected from Binegedi location was revealed as plant with the highest HM accumulative ability (Zn concentration ranged from 600 to 900 mg kg^{-1} DW in the shoots), *A.fragrans* also distinguished by high concentrations of HM in its shoots. At the same time, in latter species collected from more contaminated sites in Ali-bayramli location, insignificant concentrations of HM were found [Alirzayeva et al., 2006]. A number of species/ecotypes of *Artemisia* L. from different world regions were also found to accumulate different HM in the large amounts in their organs depending on contamination substrates, soil types and season [Morishita and Boratynski, 1992; Bashmakov and Lukatkin, 2002; Li et al., 2003; Alirzayeva et al., 2006].

The present comparative study also showed that ecotypes of *A.fragrans* widespread in investigated locations have different accumulation capacity for all HM tested than the ecotypes of the same species growing in other locations. In particular, concentrations of HM in their parts were significantly lower than in Binegedi ecotype shoot/roots and at the same time higher than in Ali-Bayramli ones. While Zn concentration was found to be 380 mg kg^{-1} in the shoots of *A.fragrans* from the Binegedi and 20 mg kg^{-1} in Ali-Bayramli ones [Alirzayeva et al., 2006], amounts of Zn in

the shoots of *A.fragrans* ecotypes growing at present tested regions were about 60 mg kg^{-1} (Table1).

Table 1. Heavy metal concentrations in Artemisia L. species collected from different contaminated locations of Azerbaijan

Location	Plants		Elements, mg kg-1 DW					Source of contamination
			Cd	Cu	Ni	Pb	Zn	
Sumgayit	*Artemisia szovitsiana*	shoot	0.29±0.13	14.01±1.20	2.64±0.57	2.90±0.20	66.49±16.68	Aluminium and other chemical plants
		root	0.90±0.20	26.74±11.50	4.69±2.64	2.24±1.39	48.55±16.71	
Lokbatan	*Artemisia szovitsiana*	shoot	0.10±0.02	17.04±4.49	2.74±0.92	1.80±0.55	72.30±10.31	Oil production, chemical plants and intensive road
		root	0.22±0.08	16.92±1.94	1.84±0.24	1.91±0.26	34.33±2.04	
	Artemisia fragrans	shoot	0.25±0.05	14.46±4.03	3.30±1.75	2.10±0.73	58.70±12.87	
		root	0.54±0.24	18.58±3.74	3.22±0.73	3.45±1.43	39.46±6.25	
Silk route	*Artemisia szovitsiana*	shoot	0.11±0.03	17.48±1.45	2.58±0.36	1.79±0.30	106.24±13.06	Metallic wastes and intensive road
		root	0.07±0.03	12.39±1.23	3.21±1.82	1.05±0.18	31.00±4.47	
	Artemisia fragrans	shoot	0.17±0.17	12.29±2.26	2.85±0.90	1.01±0.39	60.99±6.60	
		root	0.28±0.41	14.45±0.94	3.64±0.69	0.67±0.12	29.47±3.19	
Garadag	*Artemisia fragrans*	shoot	0.12±0.02	15.37±1.49	4.33±0.57	1.59±0.11	59.46±14.04	Cement plant and intensive road
		root	0.10±0.08	14.52±0.00	3.47±0.63	1.76±0.36	32.94±2.76	

Other tested species *A.szovitsiana* displays somewhat different accumulative properties depending on its growing locations. When slight differences in HM concentrations were found in the ecotypes of *A.fragrans* collected from the all four locations tested (Sumgayit, Lokbatan, Garadag and Silk route), there was significant difference in the Zn accumulation capacity of *A.szovitsiana* ecotypes from these locations. The highest Zn concentration - 106 mg kg^{-1}, was found in the shoots of *A.szovitsiana* growing near the Silk route (Table 1). The bio-accumulation factor (shoot/soil HM concentration ratio), which is known to be more important parameter for phytoextraction than the shoot concentration *per se* [Zhao et al., 2003], in this ecotype for Cu was 2.8 and for Zn - 5.5. This factor for Cu and Zn in *A.szovitsiana* ecotype from Lokbatan was also 1.1 and 1.5, respectively. And plants are considered to be metal accumulators when their bio-accumulation factor is higher than 1. The lowest Cu and Zn concentrations were revealed in *A.szovitsiana* ecotype collected around the chemical plants in Sumgayit (Table 1) and bio-accumulation factor for all HM was also lower than 1. However, surrounding soils of this species were more contaminated by all HM tested than soils in other studied locations: total Zn concentration was 101 mg kg^{-1} and Cu – 60 mg kg^{-1} (Fig. 1). It is significant that soil samples collected near the Silk route location distinguished by lowest concentrations of all HM tested, whereas plants growing in this location differed by their highest accumulating capacity (Table 1, Fig. 1).

It should be noted that all studied *Artemisia* plant species accumulated mainly zinc both in their shoots and roots regardless the contamination source and their growing habitat. However, as a rule, majority of Zn accumulated by their roots further was translocated to the shoots. Therefore, the Zn store in the shoots in all *Artemisia* plants was found to be significantly higher than Zn contents in their roots (Table 1). Copper in *A.szovitsiana* from the Lokbatan and near the Silk route locations was also presented in amounts close to the generally accepted critical toxicity levels [Lobnik, 2004].

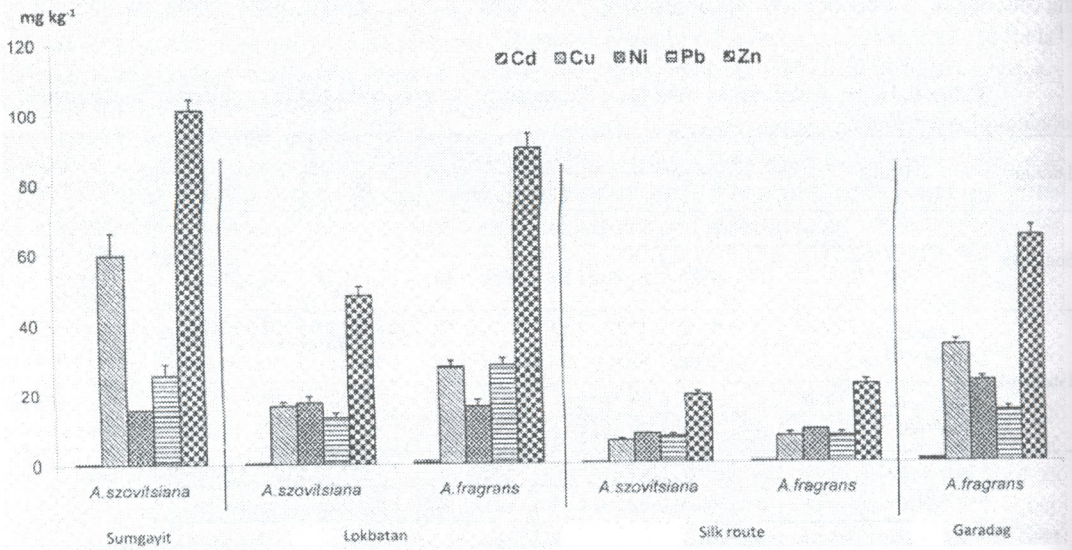

Fig. 1. Total heavy metal concentrations in surrounding soils of Artemisia L. species from different locations of Azerbaijan

The adaptive strategies of plants to HM toxicity are known to vary between species/ecotypes and depend on some soil factors [Harter, 1983)] local habitat and plant accumulation capacity [Lasat, 2000; Bashmakov and Lukatkin, 2002; Seregin and Kojevnikova, 2008]. Results obtained in the present study being in agreement with these comments also indicated that two *Artemisia* species growing in the different locations with various source and levels of pollution displayed the distinctive strategies of tolerating to a presence of HM in their environment. In particular, *A.szovitsiana* ecotype growing near Silk route and Lokbatan at very low concentrations of HM in their surrounding soils, nevertheless accumulating the high amounts of metals in their shoots displays itself as accumulator plants for Zn and Cu, while ecotype of this species from Sumgayit was found to be tolerant plant. But *A.fragrans* ecotype from Garadag was identified as indicator plant, since Zn concentration in their shoots is reflected by this in the soil [Baker, 1981].

Thus, one can conclude that all ecotypes of both *Artemisia* L. species tested demonstrating a high resistance to soil contaminations irrespective of their accumulative abilities could be recommended for the use in restoration of stressed soils.

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მძიმე მეტალებით დაბინძურების მიმართ *ARTEMISIA* L. სახეობების ადაპტაციური პოტენციალი განსხვავებულ გარემო პირობებში

ალირზაევა ე., შირვანი ტ., ბაბაევა გ., ალი-ზადე ვ.

აზერბაიჯანის მეცნიერებათა ეროვნული აკადემიის ბოტანიკის ინსტიტუტი

(მიღებულია 28.08.2008)

რეზიუმე

შესწავლილია ნიადაგის დაბინძურების განსხვავებული ხარისხის მქონე აზერბაიჯანის სხვადასხვა რეგიონში ფართოდ გავრცელებული ორი აბორიგენი დომინანტი სახეობის (*A.fragrans* და *A.szovitsiana*) ადაპტაციური პოტენციალი და მძიმე მეტალების აკუმულაციის უნარი. განსაზღვრულია Cd, Cu, Ni, Pb და Zn-ს შემცველობები მცენარის სხვადასხვა ნაწილში და ახლომდებარე ნიადაგის ნიმუშებში. *Artemisia*-ს შესწავლილი სახეობები ავლენენ განსხვავებულ შეგუების უნარს მათ საარსებო გარემოში მძიმე მეტალების სხვადასხვა შემცველობის მიმართ. თავიანთ ჰაბიტატზე დამოკიდებულებით ისინი გვევლინება როგორც ტოლერანტული, აკუმულაციის უნარის მქონე და ინდიკატორი მცენარეები. ნიადაგის სხვადასხვაგვარი დაბინძურებისადმი მაღალი მდგრადობის გამო ისინი შეიძლება რეკომენდირებულ იქნეს სტრესით გამოწვეული დეგრადირებული ნიადაგების გრძელვადიანი ბუნებრივი აღდგენისათვის.

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INSECTICIDAL PROPERTIES OF MANNOSE-BINDING LECTIN FROM *DIOSCOREA BATATAS* TUBERS

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Abstract

Mannose-binding lectin DB1 from *Dioscorea batatas* tubers was examined for insecticidal activity against *Helicoverpa armigera* and *Helicoverpa assulta* (Lepidoptera: Noctuidae) larvae at different stages of development. The rate of adults successfully emerging from pupae fed on DB1 was 33% when incorporated into artificial diet at a level of 0.01% (w/w). DB1 had no, or revealed marginal inhibitory effects on gut proteolytic and hydrolytic enzymes measured by FITC assay. The results show that mannose-binding lectin DB1 from *Dioscorea batatas* may fulfill defense role against insect pests.

Key words: *Dioscorea batatas*, insecticidal activity, mannose-binding lectin.

Introduction

Lectins are among wide range of natural defense proteins found in plants [Rudiger, H and Gabius, H.J. 2001]. While the physiological functions of plant lectins have not yet been fully elucidated, one possible function that of serving as a chemical defense against large array of insect pests is well documented [Carlini, C.R. and Grossi-de-Sá, M.F. 2002, Vasconcelos, I.M. and Oliveira, J.T.A. 2004]. With the exception of some enzymes, e.g. some types of chitinases, glucanases, glycosidases, lectins are only plant proteins that are capable of recognizing and binding glycoconjugates on exoskeleton of invertebrates or specific sites exposed along the intestinal tract. Such interaction is considered to be prerequisite for insecticidal action.

We isolated and characterized tuber proteins from the tubers of a typical Japanese yam, *Dioscorea batatas*, and demonstrated that mannose- and maltose-binding lectins (DB1 and DB3, respectively) were present as major proteins in the yam [Gaidamashvili, M., et al., 2004]. Dioscorin-like storage protein itself proved to be a maltose-binding lectin, which could not be classified into any of known plant lectin families. DB1 was homologous to snowdrop lectin, GNA (*Galanthus nivalis* agglutinin) which was shown to be insecticidal to a range of economically important pests [Fitches, E., et al., 2001].

In this paper we analyzed monocot mannose-binding lectin (DB1) from Japanese yam *Dioscorea batatas* for insecticidal activity toward *Helicoverpa* pests of the Lepidoptera family, which is a serious pest of many important crops and claims a major share in crop losses every year throughout Europe, Africa, Asia and Australia [Sharma, H.C. 2001]. We demonstrated obvious anti-nutritive effects of DB1 on *Helicoverpa armigera* and *Helicoverpa assulta* larvae. Supposedly,

DB1 plays defense role in yam tubers and might be promising tool to develop resistance in crop species via genetic engineering.

Materials and Methods

The tubers of *Dioscorea batatas* were harvested in Aomori prefecture, Japan in May, and stored at 4°C until use. For isolation of proteins whole tubers were used. Larval cultures of *Helicoverpa armigera* and *Helicoverpa assulta*, originally obtained from Zennoh (Hiratsuka, Japan) and JA (Oyama. Japan), respectively, were reared continuously at Laboratory of Insect Science and Bioregulation (Tohoku University, Japan) on an artificial diet at 24 ± 1°C under a L16:D8 light regime. In all assays larvae were placed on Insecta LFS (Nosan. Co, Yokohama, Japan) with or without lectin.

Third instar larvae of *H. armigera* and *H. assulta* were reared on control diet or artificial diets containing lectins (DB1, DB2+DB3+DB4 (50:20:10) or SBA) at concentrations of 0.01%. Fifteen larvae were used per treatment. Control diet was supplemented with an equivalent weight of casein to the test protein (i.e., DB1 or DB3) added to experimental diets. Insect survival was estimated daily, and the weights of pupae were measured.

Midguts isolated by dissecting the fifth instar larvae were stored at –80°C until use. The gut tissue was mixed with 3 volumes of 0.1 M Gly-NaOH buffer (pH 10.0) and allowed to stand for 15 min on ice to extract protease. The gut luminal contents were recovered by centrifugation at 10,000 g for 10 min at 4°C [Harsulkar, A.M., et al. 1999]. The resulting supernatant was analyzed for protease assays. Total gut protease activity was measured by FITC-casein assay. DB1 was pre-incubated with gut extract at 37°C for 15 min, prior to addition of the substrate. The enzyme solution (15 µl) was added to 40 µl of FITC-casein (1 µg/ml, in 0.1 M Gly-NaOH buffer (pH 10.0)) and incubated at 37°C for 1 h. The reaction was stopped by adding 5 µl of 60% trichloroacetic acid (TCA). The solution was mixed with 200 µl of 0.2 M Tris-HCl buffer (pH 9.0) containing 0.5% SDS and 0.02% NaN_3. The fluorescence polarization of samples was measured by BEACON 2000 (TaKaRa) with Ex: 490 nm and Em: 520 nm.

Fifth instar larvae were cold immobilized and dissected to remove the whole midguts. The midguts were homogenized in cold distilled water (1 ml/gut), and centrifuged at 10,000 g for 5 min at 4°C. The supernatant was collected and used as enzyme sources for enzymatic assays. Total gut amylase activity was measured using glucose B test Wako kit (Wako, Osaka, Japan) by using soluble starch as substrate in 100 mM sodium phosphate buffer (pH 5.8) containing 20 mM NaCl and 0.1 mM $CaCl_2$. The midgut extracts (10 µl) were pre-incubated with DB1 or DB3 at 37°C for 15 min prior to the addition of 10 µl of substrate solution (1% soluble potato starch (Wako). After 1 h incubation the reaction was stopped by the addition of 300 µl of coloring reagent followed by developing color by placing the reaction tubes at 37°C for 20 min. The absorbance was read at 510 nm.

Results and Discussion

In bioassay of the *H. armigera* from third instar to adult, the rates of adults successfully emerging from pupae fed on DB1 or DB2+DB3+DB4 were 33% and 47%, respectively (Fig. 1A). These rates were much lower than that of control insects (67%). Final instar larvae and pupae, fed on DB1 or DB2+DB3+DB4, also showed lower surviving rates than control insects. On the other hand, SBA had no effect on the rates.

For *H. assulta* from third instar to adult, SBA had the strongest insecticidal activity. The rates of pupae emerging from larvae and adult were 40% was 33%, respectively (Fig. 1B). The

control insects showed higher emerging rates (80-87%). The rates of pupation and hatch of the insects fed on DB1 were 73% and 40%, respectively. The number of adults significantly decreased. *H. assulta* usually advances from the egg to pupation in 30 days, with 5 larval stages. The insects fed on SBA emerged seventh instar larvae (13%). Days to reach pupation, pupae period, and pupae weight were not significantly deferent among each treatment on both insects (Table 1). Antinutritive effects of DB1 were observed when incorporating in artificial diet of insects at concentration 0.01% resulting in survival of larva and pupae 33% compare that of control insects (67%). This is an advantage of DB1 toxicity over other plant lectins produced 50% mortality and mass loss when incorporated into an artificial diet at levels of 1.5% and 1.0% (w/w) [Coelho, M. B., et al. 2007]. Changes in days to reach pupation and pupal period were not significant. However, DB1 has influenced *H. armigera* more effectively at all developmental stages in compare to *H. assulta*. DB1 showed dramatic decrease in survival of *H. assulta* between pupal and adult stages. Similar result was observed with SBA. Gal/GalNAc-binding SBA remarkably affected *H. assulta* from fifth instar to adult stages. Supposedly, *H. assulta* larvae are less susceptible to deleterious effects of lectins at their early developmental stages. Insecticidal action is more evident at the later stages of insect development when gut glycosilated structures are apparently more defined.

Proteolytic activity of the midgut extracts from fifth instar *H. armigera* was measured by fluorescence polarization spectroscopy using FITC-casein as substrate. DB1 had no effect on midgut proteases of *H. armigera* (Fig. 2). Effects of yam lectins on *H. armigera* amylase activity were examined using soluble starch as a substrate (Fig. 3). DB3, which was maltose-binding lectin, had no inhibitory activity. Mannose-binding lectin, DB1, showed a dose-dependent inhibition on insect amylase activity, but the inhibitory activity was marginal. The highest inhibition was 28% at 150 μM DB1.

The precise mechanism how plant lectins exert their toxic effects is not fully understood. One presumed mode is binding and alteration of glycosylated enzymes of digestive tract of insects. That is concerning serine and cysteine proteinases found in midguts of both Coleoptera and Lepidoptera [Terra, W.R. and Ferreira, C. 1994]. The same is true also for amylase, which is the main hydrolytic enzyme found in insect [Silva, C.P., et al. 2001]. Macedo et al. [2007] reported *in vitro* influence of plant lectins on gut α-amylase activity. In controversy, sufficiently purified PHA was found actually nontoxic to cowpea weevil [Murdock, L.L., et al. 1990], and the active compound of *Phaseolus vulgaris* beans that is toxic to insect is the α–amylase inhibitor [Huesing, J.E., et al. 1991]. We found DB1 had no effect on proteolytic activity of gut protease measured by FITC-casein assay (Fig. 2). Influence of DB1 on gut amylase activity was not significant as well (Fig. 3). We monitored final 28% decrease in amylase activity when exposed to high concentration (150 μm) of lectin. The results indicate that insecticidal activity may not always involve direct interaction of lectins with gut enzymes and these interactions might be dose-depended to some extent.

Helicoverpa species of Lepidoptera family are serious pests on various agricultural crops including Solanaceae, Poaceae, Fabaceae, Cucurbitaceae. Dioscoreaceae species are also undergoing to attack, but losses are rather moderate in compare to other crops. Yam tubers of *D. batata* are stored for a year after harvesting and are particularly vulnerable, since they are more attractive to potential parasites. In addition, as the resting storage organs yam tubers may lack an active defense system to resist various pests. DB1 existed in yam tubers at significant amounts (20% of total protein content), where it may fulfill storage protein function. Preferential association of GNA-like protein with those parts of plant that is most susceptible to attack by foreign organisms might be an additional argument for proposed protective role of DB1.

Table 1. Effect of lectins on larval development of *H. armigera* and *H. assulta*

		Days to reach pupation days	Pupae period days	Pupae weight mg
H. armigera	DB1	21.86 ± 1.08	13.40 ± 0.81	286.84 ± 5.42
	DB2/3/4	20.70 ± 1.45	13.86 ± 0.70	283.07 ± 6.41
	SBA	19.50 ± 0.45	13.50 ± 0.45	314.37 ± 25.38
	control	20.00 ± 0.56	13.70 ± 0.33	314.37 ±25.38
H. assulta	DB1	21.27 ± 1.95	13.86 ± 0.34	220.70 ± 9.12
	DB2/3/4	20.40 ± 2.15	14.78 ± 0.46	239.27 ± 11.56
	SBA	18.17 ± 1.45	13.80 ± 0.73	224.37 ± 18.06
	control	16.69 ± 0.50	12.92 ± 0.48	217.23 ± 15.65

Values are means ± standard deviation, n = 15.

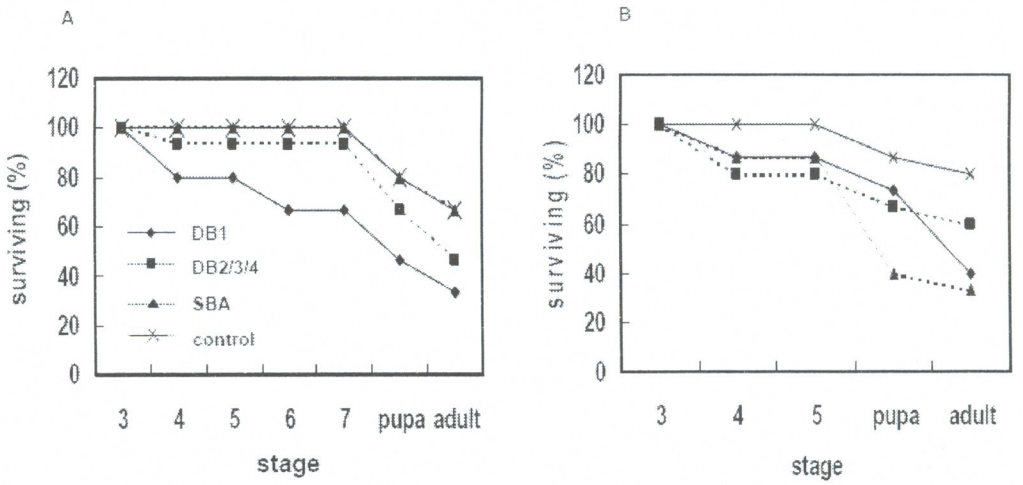

Fig. 1. Effect of lectins on survival and development of *H. armigera* and *H. assulta* when incorporated into an artificial diet at 0.01%. Insects were newly emerged third instar larvae at the start of assay. (A) *H. armigera* (B) *H. assulta.*

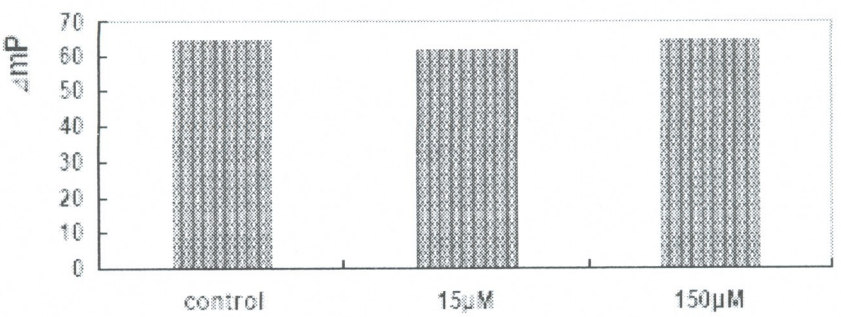

Fig. 2. Effect of DB1 on proteolytic activity of midgut extracts from *H. armigera.* Total midgut proteolytic activity was measured by FITC-casein assay in 0.1 M Gly-NaOH buffer (pH 10.0) in the presence of 15 μM or 150 μM DB1.

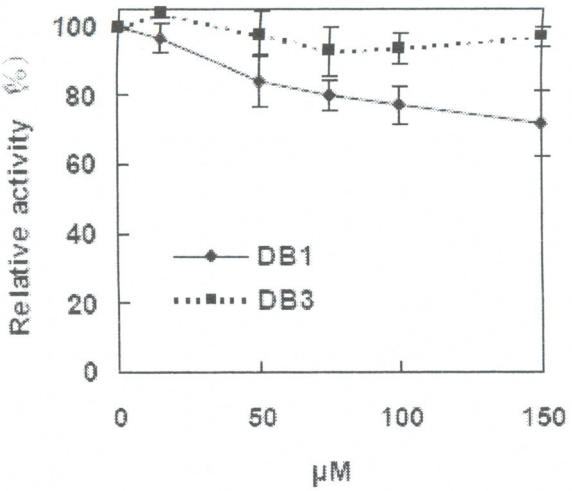

Fig. 3. Effect of DB1 on amylase activity of midgut extracts from *H. armigera.* Midgut extract was assayed against soluble starch in 0.1 M phosphate buffer (pH 5.8) containing 20 mM NaCl and 0.1 mM $CaCl_2$ in the presence of 0~150 μM DB1 or DB3.

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DIOSCOREA BATATAS ტუბერის მანოზა-დამკავშირებელი ლექტინის ინსექტიციდური თვისებები

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(მიღებულია 21.07.2008)

რეზიუმე

შესწავლილია *Dioscorea batatas* ტუბერების მანოზა-დამკავშირებელი ლექტინის ინსექტიციდური აქტივობა *Helicoverpa armigera* და *Helicoverpa assulta* (Lepidoptera: Noctuidae) ლარვების მიმართ განვითარების სხვადასხვა საფეხურზე. 0.01% DB1-ის შემცველი ხელოვნური საკვებით მკვებავი ჭუპრის სტადიიდან გამოსული ზრდასრული მწერების რაოდენობა შეადგენდა 33%. FITC ანალიზის საფუძველზე DB1 არ ახდენდა, ან ავლენდა მარგინალურ ინჰიბიტორულ ეფექტს ნაწლავის პროტეოლიზურ და ჰიდროლიზურ ფერმენტებზე. შედეგები მიუთითებს, რომ *Dioscorea batatas* ტუბერის მანოზა-დამკავშირებელი DB1 ლექტინი შეიძლება ასრულებდეს დამცველობით როლს მავნებელი მწერების მიმართ.

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Letter to Editor
Genetics

THE GENETIC EFFECT OF LEAD NITRATE ON *ALLIUM CEPA* AT THE EARLY STAGE OF ONTOGENESIS

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Abstract

Short-term (24 hours) and prolonged (240 hours) genetic effects of lead nitrate have been studied in *Allium cepa* at the early stage of ontogenesis. Examination of different doses (0.001M, 0.01M and 0.1M) of the compound revealed mutagenic activity of all tested doses. In addition, the highest dose was cytotoxic and induced extremely high level of chromosome aberrations leading to the death of the plant within 2-3 weeks after sprouting.

Key words: Heavy metal; Allium cepa; Mitotic activity; Chromosome aberration.

The adverse effect of heavy metal compounds on living organisms is well established. However, intensity of harmful influence mostly depends on exposure time, concentration of the agent and the age of affected organisms [Bochkov, Chebotarev, 1989; Dobrovolsky,1980].

Different doses of lead nitrate [$Pb(NO_3)_2$] have been tested for their cytogenetic activity at short-term (24 hrs) and prolonged (240 hrs) exposure on developing onions at the early stage of ontogenesis. Onion seeds were air-dried and subjected to 0.1M, 0.01M and 0.001M lead nitrate solutions for the two mentioned periods. All the tested and control samples were then washed in flowing water for another 2 hours, rinsed with distill water and placed in the thermostat for sprouting. 10 days later all the sprouts were planted in boxes with soil and placed at room temperature.

The samples were harvested on the 5^{th} (I), 15^{th} (II) and 25^{th} (III) days after treatment. Temporary microscope slides of onions were made by pressing the cells with cover slips to flatten the specimen.

The analysis confirmed cytogenetic and cytotoxic activities of lead nitrate in meristem tissue cells of tested plants. With the increase of exposure time and concentrations of lead nitrate decrease in percentage of sprouted seeds and the tendency to slow down the growth process were observed in affected seeds.

Cytogenetic analysis of all the three variants of samples harvested at different time revealed elevated rates of chromosome aberrations (such as: chromosome fusion, anaphase bridges and fragments, heteroploidy) with a dose-dependant relationship.

It should be noticed, that the late cytogenetic effect of short-term treatment stayed unchanged in all the cases. On the 5^{th} day after treatment the frequency of aberrations was within the range of 3.7-11.4%(control-1.5±1.0); on the 15^{th} day – 4.8-9.5%(control-1.6±1.2) and on the 25^{th} day it varied from 4.5 to 10.2%(control-2.0±1.2). Such results should be attributed to the specificity of chemical mutagens to cause significant changes while prolonged acting. In case of

short-term exposure chromosome fusions, chromosome bridges and fragments were most common disordered, however, at the lately harvested specimens high incidence of anaphases with 3-4 poles as well as heteroploid cells were observed. We suppose that cells with severe chromosome disorders can neither survive nor give new cell generations. Polyploid, hypo- or hyperploid cells have more chances to stay alive and multiply. Consequently, they are better accounted.

When treated with higher doses (0.01M and 0.1M) of lead nitrate other intracellular changes were also revealed. They were: unequal chromatin distribution, vacuolization of nuclei and emergence of cells with micronuclei. Such cells are stopped in interphase, since they cannot pass prophase. As a rule, nuclei in such cells undergo degeneration resulting in cells elimination. Proceeding from that, mitotic index of meristem tissue in roots falls down by 30-50%.

When plants were exposed to lead nitrate for prolonged time (240 hrs) the cytogenetic and cytotoxic effects got sharply increased. The rates of chromosome aberrations positively correlated with the doses of the agent: on the 5^{th} day after exposure cytogenetic alterations in the fixated material ranged from 20.3 ± 3.7% to 41.2 ± 4.7% (control – 1.5 ± 1.1) and from 47.9 ± 5.6% to 68.9 ± 8.5% (control – 1.6 ± 1.2) on the 15^{th} day. Only the specimens treated with 0.001M $Pb(NO_3)_2$ were available to be harvested after 25 days of treatment (78.9 ± 7.4%, control – 2.0 ± 1.2). Only the nuclei degenerated or containing fused chromatin were accessible to harvest. Mitotic activity was completely blocked in the other cells.

As it has been stated, meristem tissue cells of roots undergo differentiation during the process of plant growth and development. Having been transformed, some of them make up vascular bundles. Under the effect of lead nitrate this process was partly altered. In particular, tissue architectonics was changed and abnormally shaped vascular tissue was formed.

Proceeding from the above we can conclude that biotesting of lead nitrate revealed all the three examined doses to be mutagenic. In addition, relatively high doses of the agent exhibited cytotoxic activity and even late effect of early exposure to lead nitrate is very harmful to the plant. As for prolonged treatment with high doses of the agent, it is considered to be crucial for the plants, as it causes significant increase in the rate of chromosome aberrations that in combination with other cytological changes in meristem tissue and cells causes death of the plants at the age of 2-3 weeks.

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ტყვიის ნიტრატის ზემოქმედების გენეტიკური ეფექტი ონტოგენეზის ადრეულ ეტაპზე *Allium cepa*-ში

შევარდნაძე გ., გოგინაშვილი ქ., შარია შ., შალიკაშვილი ლ.

ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი, ზუსტ და საბუნებისმეტყველო მეცნიერებათა ფაკულტეტი

(მიღებულია 15.07.2008)

რეზიუმე

შესწავლილია ტყვიის ნიტრატის ხანმოკლე (24 სთ) და ხანგრძლივი (240 სთ) ზემოქმედების გენეტიკური ეფექტი ონტოგენეზის ადრეულ ეტაპზე *Allium cepa*-ში. აღნიშნული ნაერთის სხვადასხვა დოზების (0.001M, 0.01M და 0.1M) ტესტირებამ გვიჩვენა, რომ სამივე დოზა მუტაგენურია, მათ შორის მაღალი დოზა ციტოტოქსიკურ მოქმედებასაც ავლენს. ამასთანავე, მაღალი (0.1M) დოზის ზემოქმედება კატასტროფულად ზრდის ქრომოსომული აბერაციების სიხშირეს და იწვევს მცენარის სიკვდილს 2-3 კვირის ასაკში.

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Letter to Editor
Zoology

ENCHODELUS MUCHURIENSIS N. SP. (NEMATODA, DORYLAIMIDA) FROM WESTERN GEORGIA

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(Received May 19, 2008)

Abstract

The paper deals with the description of the new species *Enchodelus muchuriensis* n. sp. (Nematoda, Dorylaimida) from Western Georgia. Its measurements, differential diagnosis and original pictures are given.

Key words: odontostile, odontophore, spicule, supplement.

***Enchodelus muchuriensis* n. sp.**

Measurements:

7♀: L = 0.78-0.95 mm; a=14-20; b=3.5-4.9; c=22-29; v=52-59%, odontostile - 6.6-7.7 μm; odontophore - 16.5 μm.

5♂: L =0.78-0.82 mm; a=14-19.5; b=3.5-4.9; c=19.6-23.0; spicules: 36-36.5 μm; supplements: 6-9; odontostile - 7-7.7 μm; odontophore - 16.5 μm.

Holotype -♀**:** L=0.78 mm; a=15; b=4.1; c=22.5; v=56%; odontostile - 7.7 μm; odontophore - 6.5 μm.

Allotype - ♂**:** L=0.79 mm; a=16; b=4.8; c=20; spicules: 37.4 μm; supplements: 6. odontostile - 7.7 μm; odontophore - 16.5 μm.

The body ventrally curved. Cuticle smooth and thin. Labial region distinctly set off from body contour. Bowl-shaped amphids with wide opening occupy about 1/2 of the body-width. Odontostile narrow, short, awl-shaped, about 6,6-7,7 μm, shorter than the labial diameter (8,8 μm). Odontophore with evident flanges two times longer than the odontostile (16.5 μm). The odontostile opening small, about 1/7 of its length. The guiding ring located in the middle of odontostile.

Oesophagus widen after the centre. Cardium hemispherical. Vulva transversal, postequatorial; vagina sclerotized, about ½ of the body width. Gonads amphidelphic, curved on the 1/2-1/3 for till vulva location. Uterus is full with sperms. Egg size 58.5-33 x 60.5-36.3μm. Length of robust spicules of males 36-35.5 μm, supplements 6-9. Prerectum of females and males 1.3-1.6 times longer than the anal body width and 2 times longer than the rectum; rectum is smaller than the anal body diameter.

Tail short, conical, curved ventrally, slightly longer than body diameter.

Differential diagnosis: the new species ***Enchodelus muchuriensis* n. sp.** belongs to the group of species of genus Enchodelus with conical, ventrally curved tail; it resembles *Enchodelus brevidentatus* Thorne, 1939 [Thorne, Capita, 1939; Eliava, Eliashvili, 1990], but differs by some distinguished features: body shorter (1.2-2 mm for *E. brevidentatus*); odontostile shorter (10-12 μm for *E. brevidentatus*); odontophore with flange (flange absent in *E.brevidentatus*); the spicules shorter (39-42 μm for *E. brevidentatus*) and only 6-7 supplements (9-10 in *E. brevidentatus*).

Habitat: near village Muchura (Western Georgia, Imereti), Racha Range, sluggy, moss.

Materials: glycerin slides with *Enchodelus muchuriensis* n.sp. (slides N1-4) are kept in the laboratory of Nematology of Institute of Zoology (Georgia).

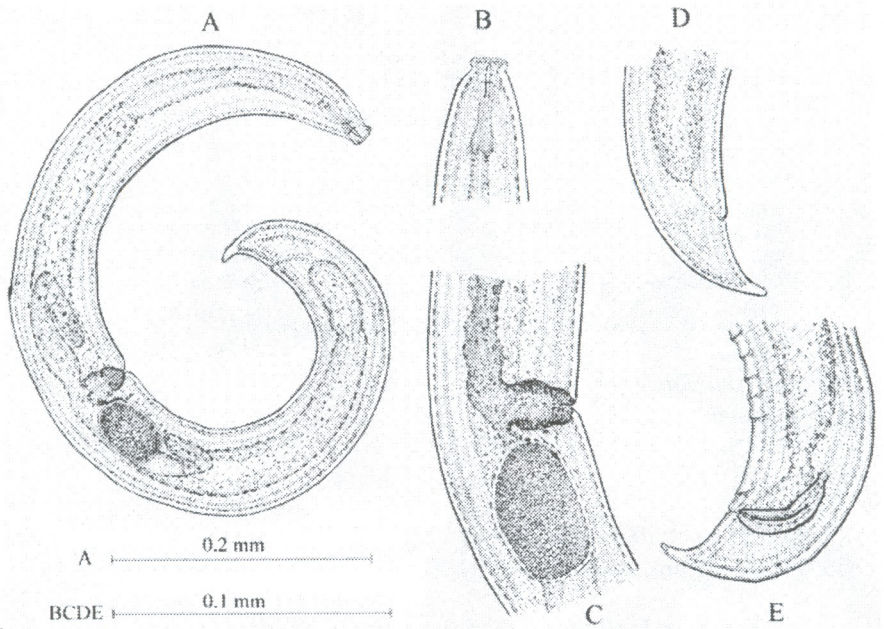

Fig. 1. *Enchodelus muchuriensis* n.sp. (Nematoda Dorylaimida) from Western Georgia. **A** - Overall view of *E.muchuriensis;*. **B** - Female had; **C** - Vulva region; **D** - Female tail; **E** - Male tail, spicules and supplements

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Enchodelus muchuriensis n. sp. (Nematoda: Dorylaimida) დასავლეთ საქართველოდან

ბადათურია ნ., კუჭავა მ., ჭუჭულაშვილი ნ.

ზოოლოგიის ინსტიტუტი

(მიღებულია 19.05.2008)

რეზიუმე

აღწერილია მეცნიერებისათვის ახალი სახეობა ***Enchodelus muchuriensis n.sp.*** დასავლეთ საქართველოდან. მოცემულია მისი განაზომები, დიფერენციალური დიაგნოზი და სურათები.

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Letter to Editor
Zoology

SPREADING OF NEMATODES IN THE PREDATORY MAMMALS OF IMERETI REGION (WESTERN GEORGIA)

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(Received march 12, 2008)

Abstract

Helminthofauna of predatory mammals of Imereti region was studied. It was found that 11 species of predatory mammals in Imereti region are characterized by their own, specific helminthofauna from Nematode class. 63.18% of investigated animals turned out to be infested. 25 species of parasitic nematodes were found in studied predatory mammals.

The area of Imereti region (Western Georgia) is 6 500 km^2 and is characterized with expressed natural borders. There is a mixed climate. Air temperature plays great role on the diversity of living organisms. Mixed natural conditions support the development of various species of flora and fauna. Inside the above-mentioned area there is a great number of mammals from Carnivora family. The purpose of our research was to study the helminths from Nematode class of predatory mammals in Imereti region. The material was collected during 1991-2005 and studied by the method of total helminthological cut. The material was taken from 255 specimens of predatory mammals. Among those were: Canidee - 110; Felidae - 66; Ursidae - 3; Mustelidae - 76; 163 species of these animals turned out to be infested by helminths, that is 63.18% of the whole investigated predatory mammals.

The list of helminths found among predatory mammals spread in Imereti region, their distribution areas, host animals, locations, extensiveness of invasion are presented in the Table. Indicated literature is cited according to Rodonaia, 1972.

species	distribution	host	location	Extensiveness of invasion (%)	literature
**Ancylostoma caninum* (Ercolani, 1859)	all over the world; widespread in Georgia; in Imereti - Kutaisi and Tskaltubo districts	dog, cat, wild cat, wolf, fox	small gut	5.7-11.6	Blazhin, 1928; Djikhia, 1934; Gamdsemlidze, 1941; Chubabria, 1958; Rodonaia, 1979
**Ancylostoma tubaeforme* (Zeder, 1800)	all over the world; widespread in Georgia; in Imereti - Tkibuli district	wild cat	small gut	7.1	Rodonaia, 1972
**Uncinaria stenocephala* (Railliet, 1884)	all over the world; in Imereti - Tskaltubo, Tkibuli, Zestafoni, Sachxere districts	dog, cat, wolf, badger, marten	small gut	5.26-12.8	Rodonaia, 1972
*Crenosoma vulpis	all over the world;	fox	bronchia	17.6	Rodonaia, 1972

(Dujardin, 1845)	widespread in Georgia; in Imereti - Bagdati district.				
Crenosoma petrovi (Morosow, 1939)	Russia; in Imereti - Bagdati	marten	lungs	6.8-11.76	Petrov, 1941; Rodonaia, 1972; Kurashvili, et al., 1991
**Aelurostrongylus falciformis* (Schlegel, 1933)	Crimea, Georgia; in Imereti - Samtredia district	marten	lungs.	15.78	Petrov, 1941; Rodonaia, 1972
**Molineus patens* (Dujardin, 1845)	Europe, Asia and North America; Georgia; Imereti - Samtredia district	marten	small gut	15.78	Rodonaia, 1972
**Ascaris columnaris* (Leidy, 1856)	Europe and America; Caucasus - Azerbaijan, Georgia	marten	small gut	11.76	Sadikhov, 1981; Rodonaia, 1972
**Toxascaris leonina* (Linstow, 1902)	all over the world; in Imereti - Kutaisi, Tskaltubo, Tkibuli, Zestapponi, Samtredia districts	dog, jackal, fox	small gut	14.5-26.8	Blazhin, 1928; Djikhia, 1934; Rodonaia, 1951, 1956, 1972
**Rictularia affinis* (Sägerskiold, 1904)	North Africa, Europe and Asia; in Imereti - Tskaltubo district	wolf	small gut	3.7	Rodonaia, 1972
**Rictularia lupi* (Panin et Lavrov, 1962)	Kazakhstan; in Imereti - Tkibuli	wolf	small gut	3.8	Panin, Lavrov, 1962
**Rictularia petrowi* (Sadichov, 1954)	Azerbaijan; in Imereti - Bagdati	marten	small gut	5.26	Sadichov, 1954, 1981
**Spirocerca lupi* (Rudolphy, 1809)	all over the world; widespread in Georgia; in Imereti - Kutaisi and Tkibuli districts	dog, wolf	pharynx	1.7-14.3	Blazhin, 1928; Djikhia 1934; Kamalov, 1935; Burdjanadze 1937; Rodonaia, 1965, 1972
**Spirocerca melesi* (Tazieva, 1971)	Kazakhstan; in Imereti - Samtredia district	marten	stomach	5.26	Tazieva, 1971
**Onchocerca lupi* (T.Rodonaia, 1967)	Imereti - Tkibuli district	wolf	eyes, connective tissue	1.7	Rodonaia, 1967; Demiashkevich, Madsaberidze, 1991
**Dirofilaria repens* (Railliet et Henry,1911)	all over the world; in Imereti - Kutaisi district	dog	connective tissue	3.0	Blazhin, 1928; Rodonaia, 1966.
Trichocephalus vulpes (Froelich, 1789)	all over the world; in Imereti – Kutaisi, Tskal- tubo districts	dog	large gut	11.76	Blazhin, 1928: Burdjanadze, 1937; Rodonaia, 1972
**Trichocephal-us asadovi* (Sadychov, 1970)	Azerbaijan; in Imereti - Vani district	weasel	small gut	4.5	Sadykhov 1970, 1981
**Capillaria feliscati* (Bellingham, 1815)	all over the world; in Imereti - Kutaisi, Tskaltubo districts	cat	urinary bladder	7.89	Skriabin, Zakharov, 1927; Rodonaia, 1951, 1965, 1972

*Capillaria plica (Rudolphi, 1819)	Europe and North America. in Imereti - Kutaisi, Zestaponi districts	dog, jackal	urinary bladder	7.6-9.12	Blazhin, 1928; Burdjanadze, 1937; Gamtsemlidze, 1941; Rodonaia, 1956, 1972; Petrov, Borovkova, 1942
*Capillaria putori (Rudophy, 1849)	Europe and North America; in Imereti - Zestaponi, Sachkhere districts	weasel	small gut	12	Rodonaia, 1965, 1972; Arbilovich, 1945; Kozlov, 1977
*Thominx aerophilus (Creplin, 1839)	Europe and North America; in Imereti - Zestaponi and Bagdati	cat, wild cat	trachea, bronchia	5.76-10.71	Kamalov, 1936; Gamtsemlidze, 1941; Rodonaia, 1951, 1956, 1972
*Trichinella spiralis (Owen, 1835)	all over the world; in Imereti - Zestaponi, Tskaltubo, Tkibuli, Kharagauli districts	cat, fox, jackal, bear, badger, wolf, dog	muscles of diaphragm and thigh	0.8-11.7	Gamtsemlidze, 1941; Rodonaia, Matsaberidze, 1967; Rodonaia, 1972; Kurashvili, et al., 1971
*Trichinella nativa (Britov et Boev, 1972	Euro-Asia and North America; in Imereti - Tkibuli	fox	muscles of diaphragm	3.7	Kurashvili, et al., 1989

* - indicates that the species was found for the first time in predatory mammals on the researched territory.

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ნემატოდების კლასის წარმომადგენელთა გავრცელება იმერეთის რეგიონის მტაცებელ ძუძუმწოვრებში

გორდაძე ე., ჟორჟოლიანი ც.

ქუთაისის ა. წერეთლის სახელმწიფო უნივერსიტეტი

(მიღებულია 12.03.2008)

რეზიუმე

შესწავლილია იმერეთის რეგიონში გავრცელებული მტაცებელი ძუძუმწოვრების ჰელმინთოფაუნა. გამოკვლეულია იმერეთის 77 სხვადასხვა რაიონში გავრცელებულ მტაცებელ ძუძუმწოვართა 255 ეგზემპლარი. აღმოჩნდა, რომ მათგან 163 ეგზემპლარი (63.18%) დაინვაზირებულია ჰელმინთებით. რეგიონში გავრცელებულ მტაცებელ ძუძუმწოვრებში აღმოჩენილ იქნა 25 სახეობის პარაზიტული ნემატოდა.

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Letter to Editor
Zoology

CASE OF CESTODE INVASION OF AFRICAN ROCK PYTHON (*PYTHON SEBAE* GMELIN, 1788) IN TBILISI ZOOLOGICAL PARK

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Abstract

The paper deals with morphological description of *Cestoda* found in small intestine of African rock python (*Python sebae*) delivered from Tanzania and a month after deceased. According to the scolex structure the helminth was belonged to the inferior cestodes of order *Pseudophyllidea,* family *Bothriocephalidae.*

In December 2007 in Tbilisi Zoological Park two individuals of African rock python (*Python sebae*) were delivered from Tanzania. One of them died after the month. In the anterior part of small intestine of python one specimen of *Cestoda* was found. Because of original form of scolex and several morphological characters, we decided to publish the description of this helminth.

Information about cestode invasions in pythons is represented in the work of N.G. Kamalov [1948], who studied 5 dead specimens of reticulated python (*Python reticulatus*). One species of cestod found in small intestines was identified as *Salenophorus megalocephalus* (Creplin, 1839), syn.: *Bothriocephalus pythonis* (Retzias, 1829). Unfortunately description of the helminth is not given in this work. It is only mentioned that the length of the longest specimen was about 50 cm. At different times *Bothridium pythonis* (Grohmann, 1906) [Shults, Gvozdev, 1972] *and B. pitonis* (Blainville) [Dubinina, 1980] were also found in pythons. In spite of existed available information on pythons and their parasites, we couldn't find recent data on python cestods.

Morphological characters of cestode found in the small intestine of the rock python are as follows (Fig. 1): colour of the parasite is milk white, flattened dorso-ventrally. The length of the body is 96 cm and consists of the original scolex divided evidently from the body, neck and strobila.

The scolex (Fig. 2, 3, 4, 5) is quite thick, much wider than the neck, consists of two spherical parts. The scolex with medially depression, the surface is smooth, without any hooks. On two elevated upper parts of the scolex, there are two deep adhesions. The edges of the adhesions are surrounded with well developed, comparably darker semicircular muscle ridges, openings of which are directed to the outside. The maximal width of the scolex is – 6mm, height – 3 mm, distance between the depression and the neck – 2.5 mm, diameter of each spherical part – 3 mm.

The width of **the neck** (Fig. 1,2) at the narrowest side is 1.5 mm and widens gradually.

The strobila (Fig. 1, 6, 7) is tape-shaped. It is important to mention, that strobila is not entirely divided, but with evident longitudinal striation (false division). In one cm of the strobila

13-14 false divisions are counted, which make characteristic for cestodes serrated (dentate) surface at the edges. The strobila reaches the maximal width (15mm) at the first third of the body and then narrows to the 12-13 mm. The minimum width is 9-10 mm. Along the central part of the body depressions are found. The reproductive system is undivided, it is located in one row on the full length and is filled with eggs in different developmental stages.

The eggs (Fig. 8) are classic oval, with two layered, transparent, reddish envelope, which has well developed valve at one pole and small, dark, subterminal thickening at the opposite one. The main part of the eggs is large in size: length – 0.16 mm, width – 0.11-0.13 mm. Another part is small: length – 0.066 mm, width – 0.044 mm.

According to the scolex structure, we belong the above described cestode to the inferior cestodes of order *Pseudophyllidea,* family *Bothriocephalidae.* The morphological characters of the strobila are like those of representatives of family *Ligulidae.* We do not discuss the further systematic belonging of the specimen. It may be very possible, that the given helminth is identical to those, identified by N.G.Kamalov (1948) in reticulated python.

The material is preserved in the laboratory of parasitology of the Institute of Zoology.

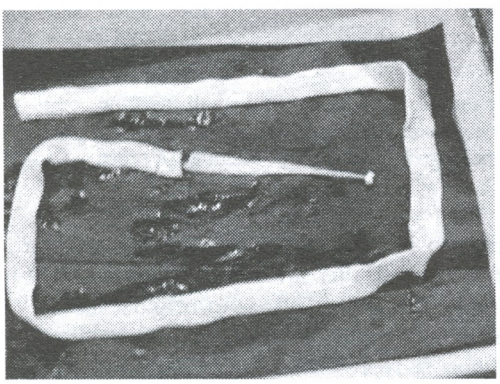


Fig. 1. The cestode body

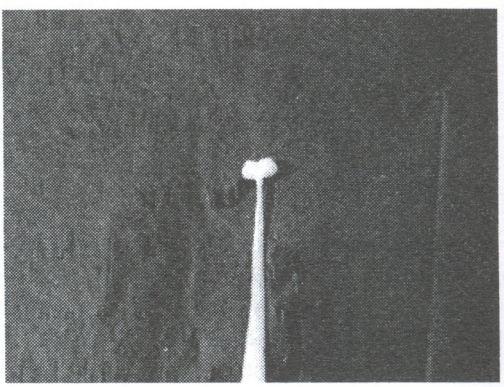
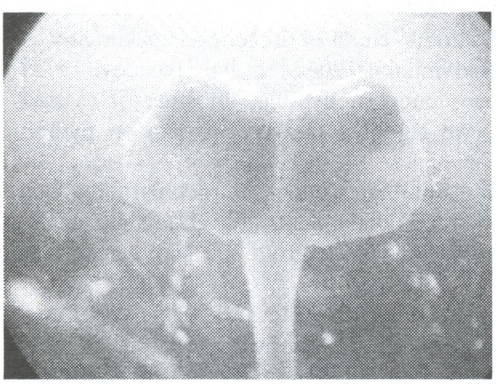


Fig. 2. The scolex and the neck



Fog. 3. The scolex; dorsal view

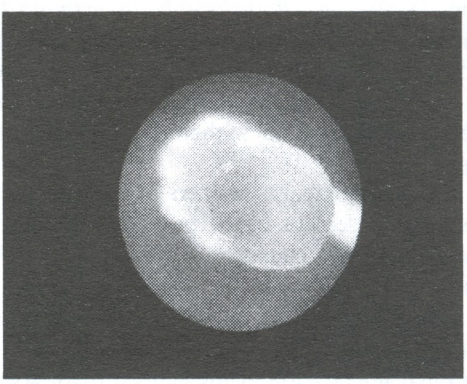


Fig. 4. The scolex; lateral view

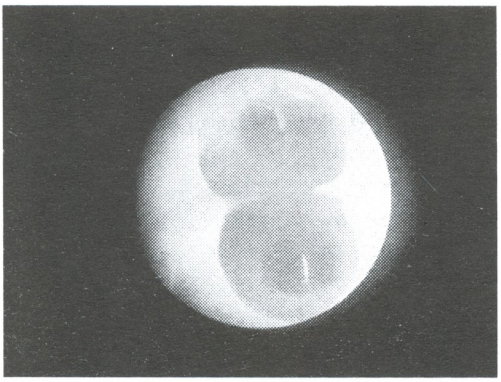


Fig. 5. The scolex; view from above

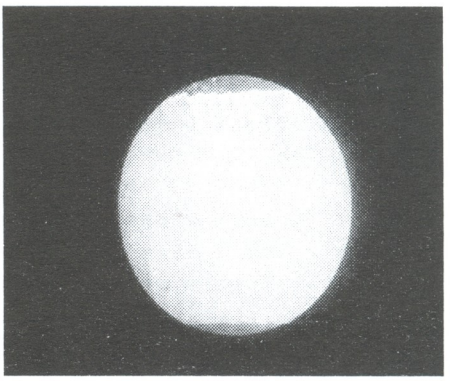


Fig. 6. The part of strobilae

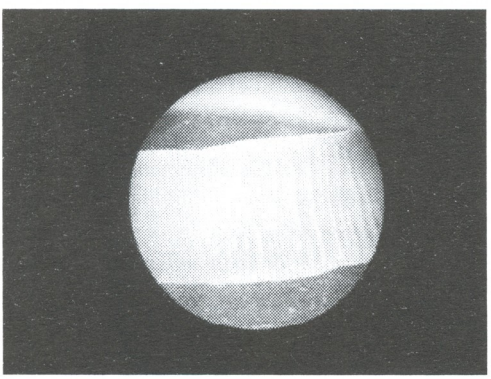


Fig. 7. The strobila; dorsal view

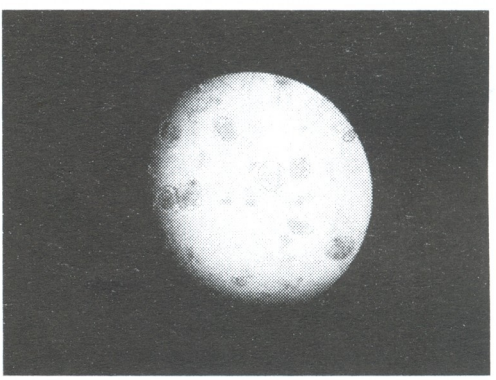


Fig. 8. The eggs of the cestoda

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აფრიკული იეროგლიფური პითონის (*Python sebae*) ცესტოდით ინვაზიის შემთხვევა თბილისის ზოოპარკში

მურვანიძე ლ.[1], ლომიძე ც.[1], ნიკოლაიშვილი ქ.[1], ვაჩნაძე ნ.[2]

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[2] თბილისის ზოოლოგიური პარკი

(მიღებულია 16.06.2008)

რეზიუმე

ნაშრომში მოცემულია ზოოპარკში ტანზანიიდან ჩამოყვანილი და დაღუპული აფრიკული იეროგლიფური პითონის (*Python sebae*) წვრილ ნაწლავში აღნიშნული *Cestoda*-ს მორფოლიგიური აღწერა. სკოლექსის აგებულების მიხედვით პარაზიტი მიეკუთვნება უმდაბლესი ცესტოდების Pseudophyllidea-ს რიგს, ოჯახი Bothriocephalidae.

ინსტრუქცია ავტორთათვის

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

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

მითითებული ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

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

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

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

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

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