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SOME KINETIC PROPERTIES OF Cu-ATPase

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(Received September 8, 2010)

Abstract

Cu²⁺-induced change of Mg-ATPase activity has been found in rat brain synaptic fraction. Study of molecular mechanism of Cu-ATPase revealed that it is a multi-sited enzyme system whose functional unit is a dimer at the minimum and substrate of this system is MgATP complex. The numbers of sites for Cu²⁺ as for essential activators and that of full-effect inhibitors are equal (n=m=1). From the point of peculiarities of transporting P-type ATPase, it is presumable that Cu-ATPase is responsible for Cu²⁺ transport.

Key words: Cu-ATPase, Mg²⁺, MgATP, multi-sited enzyme system.

Introduction

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Copper is an essential trace element present in all tissues and is required for cellular respiration, peptide amidation, neurotransmitter biosynthesis, pigment formation, and connective tissue strength. Copper is a cofactor for numerous enzymes and plays an important role in central nervous system development [Mercer, 1999; Desai, Kaler, 2008]. In spite of a crucial importance of this microelement for the living organisms, low concentrations of copper may result in incomplete development, whereas excess of copper maybe injurious. Copper is implicated directly or indirectly in the pathogenesis of numerous neurological diseases [Madsen & Gitlin, 2007; Zucconi, 2007]. Thus, for a living organism to function normally, it is of vital importance to maintain homeostasis of the mentioned element. This is possible through the transport systems which mediate Cu²⁺ transport across the membrane. As to P-type ATPase system, it has been found the Cu²⁺ activated ATPase in rat brain synaptosomal and microsomal fractions [Nozadze et al., 2005]. This enzyme system is characterised with a complex geometric shape of kinetic curves that cannot be analyzed by the classical Michaelis-Menten kinetics. The only vehicle for this problem to be solved is the method for kinetic study of multi-sited enzyme systems [Kometiani, 2007].

In order to identify the function and regulation ways of Cu²⁺-activated ATPase, it is necessary to study the molecular mechanism of its activity. The some of these questions are the purpose of the given work.

Material and Methods

As a preparation the synaptic membrane fraction (1.2-0.9 M between sucrose layers) obtained from a rat brain by differentiated centrifugation was used [De Robertis et al., 1969]. Protein concentration was measured by Lowry method [Lowry, Rosenbrogh, 1951]. For

measurement of inorganic phosphorus a modified Fiske-Subbarow [Fiske, Subbarow, 1925] and Kazanov-Maslova methods [Kazanov, Maslova, 1980] were applied.

ATPase activity was judged by amount of inorganic phosphorus per mg protein in an hour released during ATP hydrolysis by the enzyme. Cu-ATPase activity was determined with change of Mg-ATPase activity induced by addition of Cu^{2+} directly. Concentration measurement of free ATP_f , Mg_f^{2+} , Cu_f^{2+} , CuATP and MgATP complexes was made by application of the following equations:

$$\begin{aligned} [\text{ATP}_f] \cdot [\text{Mg}_f^{2+}] &= [\text{MgATP}] \cdot K_{\text{Mg}} \\ [\text{ATP}_f] \cdot [\text{Cu}_f^{2+}] &= [\text{CuATP}] \cdot K_{\text{Cu}} \\ \Sigma \text{Mg}^{2+} &= [\text{Mg}_f^{2+}] + [\text{MgATP}] \\ \Sigma \text{Cu}^{2+} &= [\text{Cu}_f^{2+}] + [\text{CuATP}] \\ \Sigma \text{ATP} &= [\text{ATP}_f] + [\text{MgATP}] + [\text{CuATP}] \end{aligned}$$

where, K_{Mg} and K_{Cu} are dissociation constants of the CuATP and MgATP complexes correspondingly and $K_{\text{Mg}} = 0,0603\text{mM}$; $K_{\text{Cu}} = 0,00074\text{mM}$ [Iacimirski et al., 1972].

To analyse the experimental curves and to estimate numerical values for n and m parameters the method of kinetic analysis of multi-sited enzyme systems and a special computer program was used [Kometiani, 2007]. Experiments were subjected to strict statistical treatment.

Results and Discussion

At the first stage of investigation of molecular mechanism of Cu-ATPase, it has been studied the enzyme activity (V) dependence upon Cu^{2+} concentration (Fig.1). Composition of reaction medium was: $[\text{Mg}_f^{2+}] = [\text{ATP}_f] = 0.3\text{mM}$, $[\text{MgATP}] = 1.5\text{mM}$, $0.0000004\text{mM} < [\text{CuATP}] < 0.02\text{mM}$. Cu^{2+} concentration varied within rather large concentration domain $0.001\text{nM} < [\text{Cu}_f^{2+}] < 50\text{nM}$ (that is why decimal logarithm was taken on the abscissa). As it seen from Fig.1, the $V=f(\lg X)$ (where $X=\text{Cu}^{2+}$) function has bell-like shape, where activation of enzyme system is revealed in the low concentrations of Cu^{2+} , while the inhibition is reflected in the high concentration areas. Proceeding from transport nature, the transport system affinity for a transportable ion is more on one side and less on the other. So, the transportable ion represents an activator on one side of the membrane and an inhibitor, on the other side, what is kinetically expressed just by the bell-like shape curve. Existence of kinetic curve of such shape is the necessary, but insufficient condition for saying that Cu^{2+} -activated ATPase is responsible for Cu^{2+} transport.

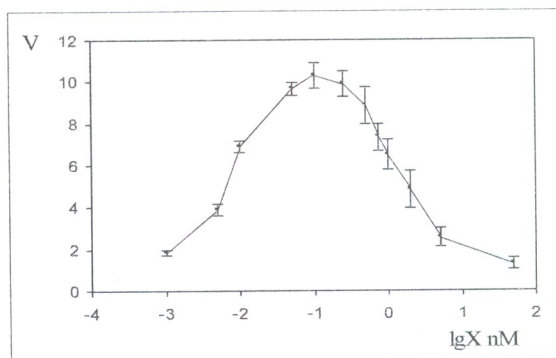
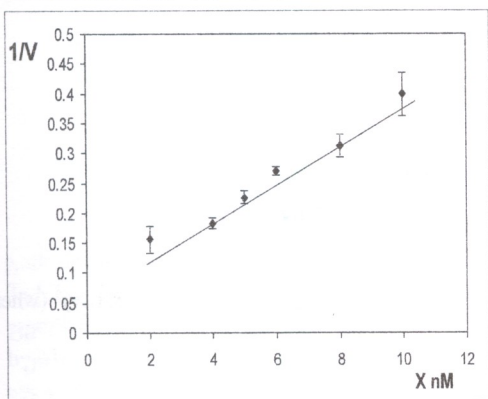
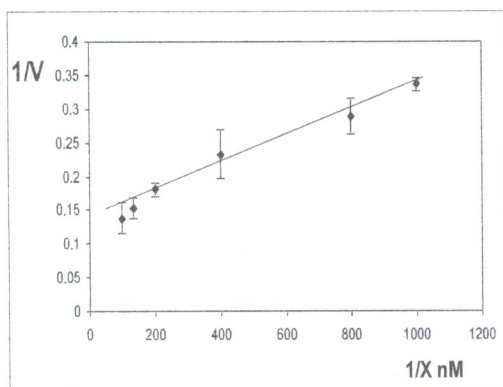


Fig. 1. Dependence of enzyme activity upon Cu^{2+} concentration in $V=f(\lg X)$ (where $X=\text{Cu}^{2+}$) coordinate system.

For a transportable ion a necessary condition is also equality of the number of sites for essential activators and that of full inhibitors. For determining the sites of essential activators (n) and full inhibitors (m) for Cu^{2+} , it was inevitable to study ${}^n\sqrt{u} = f(t)$ and ${}^m\sqrt{u} = f(x)$ functions (where $U=1/V$, $t=1/X$, X - Cu^{2+} -concentration) in the extremely low (for n) and high (for m) concentrations of Cu^{2+} correspondingly. Linearization of mentioned functions gives possibility to establish the number of essential activators and full inhibitors. The ${}^n\sqrt{u} = f(t)$ function in extremely low concentrations of Cu^{2+} ($0,001\text{nM} < [\text{Cu}_f^{2+}] < 0,01\text{nM}$) is presented on Fig.2A. The reaction medium was as follow: $[\text{MgATP}] = 1.5\text{mM}$, $[\text{Mg}_d] = [\text{ATP}_d] = 0.3\text{mM}$, $0,000004\text{mM} < [\text{CuATP}] < 0,000004\text{mM}$. As it obvious from Fig.2A, that function has rectilinear dependence, i.e. $n=1$. This means that the number of sites for essential activators for Cu^{2+} is equal to 1. The analysis of ${}^m\sqrt{u} = f(x)$ function in the high concentration area of Cu^{2+} (composition of reaction medium was: $2\text{nM} < [\text{Cu}_f^{2+}] < 10\text{nM}$, $[\text{Mg}_d] = [\text{ATP}_d] = 0.3\text{mM}$, $[\text{MgATP}] = 1.5\text{mM}$, $0,0008\text{mM} < [\text{CuATP}] < 0,004\text{mM}$) has shown that rectilinearity dependence is achieved, when $m=1$ (Fig.2B). This represents the necessary and sufficient condition to say that the number of sites for full inhibitors for Cu^{2+} is 1.



A



B

Fig. 2. Dependence of enzyme activity upon extremely low concentrations of Cu^{2+} in $1/V=f(1/X)$ (where $X=\text{Cu}^{2+}$) coordinate system (A) and high concentrations of Cu^{2+} in $1/V=f(X)$ (where $X=\text{Cu}^{2+}$) coordinate system.

At the next stage of investigation, it has been studied enzyme activity dependence upon MgATP concentration in $1/V=f(1/X)$ (where $X=\text{MgATP}$) coordinate system (Fig.3). Reaction medium contained: $0.16\text{mM} < [\text{Mg}_f^{2+}] < 0.35\text{mM}$, $0.16\text{mM} < [\text{ATP}_d] < 0.35\text{mM}$, $[\text{Cu}_f^{2+}] = 0.1\text{nM}$, $0.4\text{mM} < [\text{MgATP}] < 2\text{mM}$, $0,000022\text{mM} < [\text{CuATP}] < 0,000047\text{mM}$. As seen from Fig.3, in low concentrations of MgATP ($[\text{MgATP}] < 1\text{mM}$) enzyme system undergoes activation, while in high concentrations of MgATP ($[\text{MgATP}] > 1.4\text{mM}$) enzyme system inhibition occurs. At the same time, in high quantity of argument ($1/\text{MgATP} > 1\text{mM}$), function yields a rectilinear dependency, that in consistent with kinetic analysis of multi-sited enzyme system is the necessary condition for saying that MgATP represents a substrate for this enzyme system. In low concentration domain of argument ($1/\text{MgATP} < 0,43\text{mM}$), function asymptotically approximates the ordinate axis, i.e. in this range MgATP is a full inhibitor for the enzyme system. On the experimental curve simultaneous existence of one turning and one inflexion point is the necessary and sufficient condition to say that

MgATP has also the site allotted for the partial-effect modifiers. Thus, Cu-ATPase possesses the substrate binding activatory, inhibitory and partial-effect modifier's sites, i.e. the number of MgATP binding sites exceeds 1. If it be assumed that as in the case of Na,K-ATPase, one subunit has one MgATP binding site [Robinson, Plashner, 1979], it can thus be presumed that Cu-ATPase is a multi-sited enzyme system whose functional unit is a dimer at the minimum.

So, Cu-ATPase represents a multi-sited system whose functional unit is at the minimum a dimer and its substrate is the MgATP complex. In addition, Cu-ATPase has the substrate binding activatory, inhibitory and partial-effect modifiers' sites. A bell-like shape of $V=f(\lg X)$ (where $X=Cu^{2+}$) function and equal number of sites for Cu^{2+} of essential activators and full inhibitors constitute the necessary, but insufficient kinetic evidence for saying that Cu-ATPase is responsible for Cu^{2+} transport.

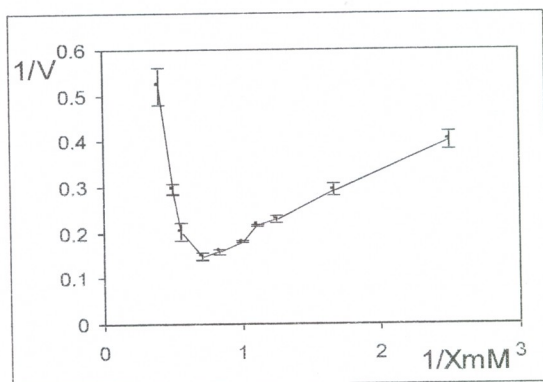


Fig. 3. Dependence of enzyme activity upon MgATP concentration in $1/V=f(1/X)$ (where $X=MgATP$) coordinate system.

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Cu-ATP-ასას ზოგიერთი კინეტიკური თავისებურება

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(მიღებულია 08.09.2010)

რეზიუმე

ვირთავას თავის ტვინის სინაფსურ ფრაქციებში ნანახი იქნა Cu^{2+} -ით გამოწვეული Mg-ATP-ის აქტიობის ცვლილება. Cu-ATP-ასას მოლეკულური მექანიზმის შესწავლამ აჩვენა, რომ Cu-ATP-ასა წარმოადგენს მრავალუბნიან ფერმენტულ სისტემას, რომლის ფუნქციური ერთეული მინიმუმ დიმერია. მისი სუბსტრატი არის MgATP-ის კომპლექსი. დადგინდა, რომ Cu^{2+} -ის, როგორც აუცილებელი აქტივატორის, ისე სრული ინჰიბიტორის უბნების რიცხვი ტოლია ($n=m=1$). გამოითქვა მოსაზრება, რომ Cu-ATP-ასა ახორციელებს Cu^{2+} -ის ტრანსპორტს.

THE EFFECT OF NEUROTRANSMITTERS AND SYNAPTIC FACTORS ON THE ANION-ACTIVATED ATPase ACTIVITY

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Abstract

The effects of neurotransmitters (NT): 5-hydroxytryptamine (5-HT), noradrenaline (NA) and endogenous synaptosomal factors (SF) on anion ATPases (Cl-activated ATPase, E.C.3.6.3.11, Mg²⁺-dependent, E.C. 3.6.1.3. and Mg²⁺-independent HCO₃⁻-activated ATPase) were studied in the rat brain fraction greatly enriched in synaptic junctional complexes (SJC).

NT and SF was shown to inhibit Cl-ATPase each separately, whereas their combined action (SF/NT) resulted in the withdrawal of the inhibitory effect and passed into activation. The effect of Mg²⁺-dependent and Mg²⁺-independent compounds on HCO₃⁻-ATPases is insignificant. The neurotransmitter's regulation by a synaptic factor is universal for those transport enzyme systems whose involvement in synaptic transmission has been corroborated and does not extend to other membrane enzymes, both transporting and "Ecto" ATPases.

Key words: synaptic junctional complex (SJC), Cl-ATPase, Mg²⁺-dependent and Mg²⁺-independent ATPases, neurotransmitters (NT), synaptosomal factors (SF).

Introduction

To the anionic ATPases belong the enzyme systems which dependence on anions accomplish the ATP hydrolysis.

The anion-dependent Mg-stimulated ATP hydrolysis is reported in the literature, in particular, HCO₃⁻- and Cl-ion activation [Ivaschenko, 1977]. Their action has been recorded mainly in bacteria and eucaryotes. They play a principal role in the regulation of intracellular pH, cell volume, nerve excitability and determination of rest potential [Gerenser, 1996; 2003].

Cl-ion activated ATPase and HCO₃⁻-ion activated Mg²⁺-dependent ATPases satisfy the necessary kinetic specificities of transport ATPases [Dzneladze et al., 2009].

It has been assumed [Gerenser, 2003] that of ATPases Cl-ATPase, like Na,K-ATPase, presumably takes part in the regulation of synaptic transmission mechanisms. The neurotransmitters represent one of the groups of Na,K-ATPase modifiers and their effect on Na,K-ATPase is due to SF. In the cytosol of nerve ending endogenous factor SF (inhibitory and activatory) with MW=60kD was found, which regulates the synaptic Na,K-ATPase system. It is remarkable that this factor alongside with neurotransmitters activates the synaptic Na,K-ATPase [Tsakadze, Kometiani, 1989].

The Na,K-ATPase regulation with the involvement of NT and SF determines synaptic transmission process that is essential for the integrative activity of brain.

We aimed at studying the influence of neurotransmitters and SF factor on anionic ATPases, in particular, on synaptic membrane Cl-ATPase and HCO₃⁻-ATPase (Mg²⁺-dependent and Mg²⁺-independent).

Materials and Methods

The fractions greatly enriched in synaptic junctional complexes (SJC), microsomes (0.32M), mitochondria (1.4M), vesicles (0.4M) obtained from the albino rat brain with the method of differential centrifugation in sucrose gradient [1.2-0.8M] were used as the object of exploration [Kometiani et al., 1984].

The SF was obtained from the fraction isolated from the crude mitochondrial fractions by means of osmotic shock with further thermotreatment at 80°C for 5 min and anew centrifugation at 15000g for 40 min [Tsakadze et al., 1989]. The concentration of the factor in the preparation was directly proportionate to the protein amount available in it. The preparation was stored at -25°C.

The ATPase activity (V) was assessed by the amount of isolated inorganic phosphorus at ATP dissociation per mg obtained protein per hour. The protein concentration was measured by the Lowry method [Lowry et al., 1951].

The measurement of inorganic phosphorus was made with the modified method of Kazanova-Maslova [Kazanova, Maslova, 1984].

Cl-ATPase was determined by the difference in the medium with and without Cl-ion. The incubation medium contained Tris-malate buffer 30mM, pH7.7, ATP - 2mM, MgCl₂ - 2mM, Oubain - 0.2mM, EDTA - 0.4mM.

HCO₃⁻-ATPase was determined by the difference in the medium with and without HCO₃⁻-ion.

The incubation medium for Mg²⁺-dependent HCO₃⁻-ATPase was: Tris-HCl (or Tris-malate) buffer - 30mM, ATP - 2mM, MgCl₂ - 2mM, EGTA - 0.4mM, Oubain - 0.2mM.

The incubation medium for Mg²⁺-independent HCO₃⁻-ATPase was: Tris-HCl (or Tris-malate) buffer - 30mM, ATP - 2mM, EGTA - 0.4mM, Oubain - 0.2mM.

Experimental data were treated statistically.

Results and Discussion

Table 1 represents distribution of Cl-ATPase within some subcellular fractions of brain (SJC, microsomes, mitochondria and vesicles). Calculation of specific and fraction activities shows that there is no principal difference in Cl-ATPase distribution between fractions, though relatively higher activity is noted in the microsomes. Qualitatively similar result was revealed in Mg²⁺-dependent and Mg²⁺-independent ATPases [Tsakadze et al., 2007, 2009]. Further investigation was continued on anionic ATPase of SJC fraction, since NT and SF effect is specific only for the membranes [Kometiani et al., 1988].

Table 1. Distribution of Cl-ATPase activity in subcellular fractions

activity	SJC	microsomes	mitochondria	vesicles
specific μmolP/hour.mg protein	145.14±8	174.92±12	143.79±14	154.12±26
of fraction μmolP/ hour	9.68±0.52	14.58±1.05	9.59±1.64	19.265±4.58

The effect of neurotransmitter (5-HT) and SF on the synaptic membrane anion dependent ATPases is given in Table 2. From the results it is seen that by addition of 5-HT (0.1mM) the

inhibitory effect is evidenced only in relation with Cl-ATPases, whereas HCO₃-ATPases do not show sensitivity.

Table 2. Effect of 5-HT and SF on anion sensitive ATPase activity of SJC

Addition into the incubated medium	μmolP _i /hour.mgP		
	*Cl ⁻ -ATPase	**HCO ₃ ⁻ -ATPase (Mg≠0)	**HCO ₃ ⁻ -ATPase (Mg=0)
Control	5.96±0.67	8.75±0.3	4.75±0.72
5-HT (0.1mM)	3.73±0.11	8.94±0.46	5.0±0.80
SF (0.15 mg/ml)	3.88±0.30	9.63±1.13	4.5±0.61
SF/5-HT	10.41±0.29	9.55±0.61	4.2±0.73

*P < 0.05 ** P ≥ 0.05

Similar result is seen at the addition of only the factor into the incubation solution: Cl-ATPase activity gets reduced, HCO₃-ATPase activity does not alter reliably. While under combined influence of SF/NT it is seen that NT- and SF-induced inhibitory effect on Cl-ATPase withdraws and passes into activation. Under the impact of 5-HT/SF HCO₃-ATPases are authentically unchanged. 5-HT manifests similar effect on the three types of anionic ATPases under the influence of noradrenaline and the factor. Thus, NT and SF affect Cl-induced ATPase activation, whereas HCO₃-ATPases are insensitive to these substances. It seems that the mechanisms that underlie the synaptic membrane Cl-ATPase, do not apply to HCO₃-ATPases.

NT represents the principal component of the synaptic transmission regulating system across the chemically excitable membranes. NT was shown to be responsible for the regulation of the synaptic membrane Na,K-ATPase system [Kometiani et al., 1990]. Elucidation of the mechanism subserving this process has much contributed to the study of the brain integrative activity. Study of the regulatory mechanism of Na,K-ATPase system by neurotransmitters has become possible since the discovery of endogenous synaptic factor (SF). NT and SF cause the system's inhibition, while NT/SF unitary action results in a substantial elevation of the activity. This effect is not detected on other membrane enzymes such as Ca,Mg-ATPase, acetylcholine esterase, Mg-ATPase and parantitrophenylphosphatase indicating specificity of the effect.

There raises a question as to how much universal is the involvement of SF/NT regulating electrogenic Na,K-ATPase system in the synaptic transmission process. Like an electrogenic component, Cl-ions participate in the generation of membrane potential. It is known that Cl-ion concentration in the membrane is measured by a membrane potential, i.e. ratio [Cl_{out}/Cl_{in}]. Such a distribution of Cl in the membrane coincides with that of Na [Yaot et al., 1981]. It is worth mentioning that one of the major pathways of Cl-anion transport is symport with Na and antiport with HCO₃ [Gerenser, 1996].

Proceeding from the facts cited above we have studied how much sensitive is the anion activated ATPase to neurotransmitters and NT/SF. SF/NT effect appeared qualitatively similar to the result obtained for Na,K-ATPase. The enzyme is inhibited by neurotransmitters (5-HT, NT and dopamine) and SF, while SF/NT is activated additionally markedly [Kometiani et al., 1988]. Cl-ATPase (Table 2) is inhibited separately by 5-HT and SF action, while SF/5-HT activates the enzyme. Similar effect is seen while introducing noradrenaline into the incubation medium. There is no effect on HCO₃-ATPases, both Mg-dependent and Mg-independent, of which the first belongs to the transport ATPases group and the other to the so called "Ecto" ATPases.

From the foregoing it may be said that SF regulation of neurotransmitters is universal for those transport enzyme systems whose participation in synaptic transmission has been testified and does not extend over other membrane enzymes, either transporting or "Ecto" ATPases.

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ანიონებით აქტივირებულ ATP-ზე რეაქციაში ნეიროტრანსმიტერისა და სინაფსური ფაქტორის ეფექტის შესწავლა

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(მიღებულია 06.09.2010)

რეზიუმე

ვირთავს თავის ტვინის სინაფსური მემბრანების ფრაქციაში შესწავლილია ნეიროტრანსმიტერების (NT): 5-ჰიდროქსიტრიფტამინის (5-HT), ნორადრენალინის (NA) და ენდოგენური სინაფტოსომური ფაქტორის (SF) ეფექტი ანიონდამოკიდებულ ATP-ზე (Cl-ით აქტივირებული ATP-ზე, E.C.3.6.3.11, Mg^{2+} -დამოკიდებული, E.C.3.6.1.3. და Mg^{2+} -არადამოკიდებული HCO_3 -იონებით აქტივირებული ATP-ზე).

აღმოჩნდა, რომ საკუთრივ NT და SF აინჰიბირებს Cl-ATP-ზე, ხოლო მათი ერთობლივი მოქმედებით (SF/NT) ინჰიბიციური ეფექტი იხსნება და გადადის აქტივაციაში. HCO_3 -ATP-ზე (Mg^{2+} -დამოკიდებული და Mg^{2+} -არადამოკიდებული) აღნიშნული ნაერთები არ ხასიათდებიან სარწმუნო ეფექტით.

ამდენად, სინაფსური ფაქტორით ნეიროტრანსმიტერების რეგულაცია უნივერსალურია იმ ტრანსპორტული ფერმენტული სისტემებისათვის, რომელთა მონაწილეობა დამტკიცებულია სინაფსური გადაცემის პროცესში და არ ვრცელდება სხვა მემბრანულ ფერმენტებზე, როგორც სატრანსპორტო, ისე „Ecto“ ATP-ზე.

FLAVONOID COMPOUNDS OF GEORGIAN (KAKHETIAN) AND EUROPEAN WINE TYPES

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Abstract

Flavonoid compounds of Georgian (Kakhetian) and European types of wines are studied. It is shown that white and red wines of Kakhetian type considerably exceed white and red wines of European type by the total phenolics, catechins, proanthocyanidins, anthocyanins and antiradical efficiency.

Kew words: wine, catechins, proanthocyanidins, anthocyanins, antiradical efficiency, 2,2-diphenyl-1-picrylhydrazyl.

Introduction

Georgia is the most ancient country of viticulture and winemaking. According to some authors "Viticulture, or grape-growing, began in Georgia (which lies on the eastern shore of Black Sea, near the Caucasus Mountains) some 9000 years ago [Norrie, 2003]. On the basis of the archeological data, the winemaking in Georgia originated 8000 years ago [The Independent, 2003].

Adverse ecological conditions observable in the most part of the modern world, unbalanced nutrition and the various illnesses break the counterbalanced free-radical processes proceeding in living cells. The reasons causing this problem are pollution of an environment, the stressful influences, radiation, chronic intoxications, smoking and other conditions, as a result of which the uncontrollable free-radical reactions develop. Under influence of these reactions the toxic effects of xenobiotics are amplified, and carcinogenesis, mutagenesis, atherosclerosis and autoimmune diseases are stimulated. In this respect, to reveal such foodstuff which contains compounds having antioxidant activity in large quantities is important. In this point the Kakhetian technology of wine-making which is since old days used in Georgia is especially interesting [Beridze, 1970]. The wines, exclusively rich in flavonoids, are prepared by means of this technology which are characterized by high antioxidant activity and considerably reduce injury of tissues and cells [Middleton et al., 2000; Robles-Sardin et al., 2010; Williams et al., 1997].

According to the ancient Kakhetian traditional technology of wine-making, the crushed grape is placed in a clay vessel ("kvevri") dug in the ground, and alcoholic fermentation is carried out together with components of a cluster. During fermentation plenty of phenolic compounds are extracted from a stem, skin and seeds of a grape, which define the composition and essence of Kakhetian wine. Interaction of these compounds with the oxidizing enzymes contained in a skin, pulp and stem of a grape, defines taste and aroma, characteristic for Kakhetian wine. Grape seeds

play a main role during formation of Kakhethian wine as seeds increase basically the content of phenolic compounds in a wine, and give to it characteristic aroma. Stems promote clarification of a wine and enrich it with flavonoids and extractive substances. The grape skin gives to a wine gentle, specific, varietal aroma. Flavonoid compounds define character of a wine, at ageing these compounds are oxidized, therefore the wine becomes soft, velvety and of pleasant taste. Quality of the wine made in a clay vessel is much better than that of made in a wooden vessel. The wine made in “kvevri” has specific fruit taste and is characterized by integrity. Therefore, “kvevri” is the best vessel for making of Kakhethian wine. Besides, in the “kvevri” dug in the ground fermentation occurs in more normal conditions, because of smaller fluctuation of temperature.

The aim of this study is to estimate total amount of phenols, catechins, proanthocyanidins and anthocyanins in European and Kakhethian wines, and to compare their anti-radical efficiency in the system forming a radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH*).

Materials and Methods

White and red wines of the Kakhethian and European type, made with grape varieties (*Vitis vinifera* L.) cultivated in Georgia, Italy and Slovenia, were given to us by wineries and physical persons whom we express gratitude. The technology of making of white (W) and red (R) wines, variety of a grape, the country of cultivation, wine factory and year of making are given in Tab. 1.

In samples of analyzed wines the total content of phenols is determined with Folin-Chiocalteu reagent [Singleton & Rossi, 1965], catechins and proanthocyanidins - by method of Swain & Hillis (1959), anthocyanins - by Durmishidze & Sophromadze (1983). Standard curves are constructed: for phenols - on a basis of gallic acid ("Sigma", maximum absorption 765 nm), for catechins - on a basis of (+) catechin ("Theodor Schuchard", maximum absorption 500 nm), for proanthocyanidins - on a basis of cyanidin isolated from a grape skin (maximum absorption 548 nm) and for anthocyanins - on a basis of malvidin-3-monoglucoside (maximum absorption 536 nm).

Table 1. Wine Samples*

Wine	Technology of preparation	Grape Variety	Country and Winery	Vintage
1W	Kakhethian	<i>Rqatsitheli</i>	Georgia, Kakhethi, „Okros Kvanchkara”	2005
2W	Kakhethian	<i>Khikhvi</i>	Georgia, Kakhethi, Physical Person	2005
3W	Kakhethian	<i>Ribolla</i>	Italia, Azenda Agricola, Osalavia Francesco Joško Grauner	2003
4W	European	<i>Rqatsitheli</i>	Georgia, Kakhethi, „Badagoni”	2005
5W	European	<i>Kakhuri Mtsvane</i>	Georgia, Kakhethi, Physical person	2004
6W	European	<i>Tsulukidzis tetra</i>	Georgia, Racha, „Okros Khvanchkara”	2004
7W	European	<i>Rebula</i>	Slovenia, Vipavska dolina, Vinorodni okolis, deadami Azelen	2006
8R	Kakhethian	<i>Sapheravi</i>	Georgia, Kakhethi, „Vazi+Ltd ”	2005
9R	Kakhethian	<i>Cabernet Sauvignon</i>	Georgia, Kakhethi, „Vazi+Ltd “	2005
10R	Kakhethian	<i>Ojaleshi</i>	Georgia, Samegrelo, „Vazi+Ltd ”	2005
11R	European	<i>Sapheravi</i>	Georgia, Kakhethi, Physical person	2007
12R	European	<i>Aleksandreuli</i>	Georgia, Racha, „Okros Khvanchkara ”	2003
13R	European	<i>Merlot</i>	Georgia, Kakhethi, „Besini ”	2008

* W – White wine; R – Red wine

For determination of antiradical efficiency of wine the stable radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH*) with maximum absorption 520 nm was used [Sanchez-Moreno et al., 1998]. Alcohol was removed from samples of analyzed wine (50 ml) by evaporation on the vacuum-rotational evaporator at 40°C and after that the volume of samples supplemented again up to initial volume with distilled water. Spectrophotometric measurements were carried out on spectrophotometer CΦ-26 (Russia). Each experimental variant was repeated five times. Experimental data were processed statistically by computer program, MS Excel”.

Results and Discussion

According to the data obtained (Tab. 2), it is obvious that white and red wines of the Kakhethian type (1W, 2W, 3W, 8R, 9R, 10R) considerably surpass the appropriate wines made with European technology (4W, 5W, 6W, 7W, 11R, 12R, 13R) in the content of phenolic compounds.

In white wines made by the Kakhethian technology, the total content of phenols varies from 1296 mg (3W) up to 2290 mg (1W), and in red wines of the Kakhethian type - from 2848 mg (10R) up to 4416 mg (8R) per liter, while in white wines made by the European technology, these parameters varies from 210 mg (7W) up to 456 mg (6W), and in red wines of the European type - from 1630 mg (12R) up to 3130 mg (11R) per liter. Thus, the wine made by the Kakhethian technology is considerably enriched by phenolic compounds.

Table 2. The Content of Total Phenolics, Catechins, Proanthocyanidins, Antocyanins in White and Red Wines and Antiradical Efficiency

Wine	Total phenolics, mg/l	Catechins, mg/l	Proanthocyanidins, mg/l	Anthocyanins, mg/l	EC ₅₀ (g antioxidant/ Kg ⁻¹ DPPH*)	T _{EC50} (min)	AE (x10 ⁻³)
1w	2290±38	640±007	690±7.1		510±11.7	4.5	0.44
2w	2000±13	453±01	1097±2.4		515±16	4.5	0.43
3w	1296±46	509±4	392±16		847±35	5	0.23
4w	346±11	39±1	47.8±2		1191±14.1	5	0.16
5w	278±7	27±2	43.2±1.1		1447±22.4	5	0.12
6w	456±26	77±2	165±8.7		1219±18.4	4.5	0.18
7w	210±4	8±2	-		893±13.3	5	0.038
8R	4416±100	1010±23	1203±15	1270±45	516±16.5	3.2	0.62
9R	2848±72	798±2	728±13	317±24	382±14.9	5	0.52
10R	3700±85	862±11	872±18	414±20	342±11.5	5	0.58
11R	3130±76	582±5	610±55	1456±36	519±5.1	4.4	0.43
12R	1630±50	378±15	980±69	53.2±5	595±14.5	5	0.34
13R	2318±73	636±33	826±4	322±18	880±4.3	4.5	0.25
α-Tocopherol					625±22.7	5	0.32

Study of phenolic compounds of the wines made with the Kakhethian and European technologies, from the same variety of a grape is of special interest. We have compared the wines made by the Kakhethian (1W, 3W, 8R) and European (4W, 7W, 11R) technologies, from the autochthonous Georgian varieties of grape (*Rqatsitheli*, *Sapheravi*) and from a grape variety cultivated in some countries of Europe (*Ribolla*) (Tab. 2).

In the Kakhethian wine (1W) of grape variety *Rqatsitheli* total amount of phenolic compounds, catechins and proanthocyanidins, is 6.6, 16, and 15 times higher, respectively, than in 4W, made from the same variety by the European technology. Approximately the same patterns of

relationship are observed in case of the wines made in Italy by the Kakhethian technology from a grape variety *Ribolla*, and in Slovenia from the same variety by the European technology. In the wine made by the Kakhethian technology (3W), the total phenols are 1296 mg/l, and in the wine made by the European technology (7W) from the same variety of a grape, this parameter is 210 mg/l. Kakhethian wine (3W) contains catechins and proanthocyanidins in amount of 509 mg/l and 392 mg/l, respectively, and the wine made from the same variety by the European technology (7W) contains insignificant amount of catechins, and practically does not consist of proanthocyanidins.

Among the white wines made by Kakhethian technology, the high content of proanthocyanidins characterizes a wine (2W), made with autochthonous Georgian grape variety *Khikhvi*, in which the content of those compounds makes 1097 mg/l, and the content of catechins - 453 mg/l (the total of phenols in this wine makes 2 g/l). The wine made by Kakhethian technology from a grape variety *Khikhvi* has straw color, contains high amount of extractive substances, is perfect and harmonious [Tabidze, 1954].

Among the white wines made from the autochthonous Georgian grape varieties by the European technology, the wine (6W) made by variety *Tsulukidzis Tetra* is distinguished, in which the total content of phenols makes 456 mg/l, catechins - 77 mg/l, and proanthocyanidins - 165 mg/l. It should be also noted a wine (5W) made with grape variety *Kakhuri Mtsvane* by the European technology, in which the total content of phenols makes 278 mg/l, catechins - 27 mg/l, and proanthocyanidins - 43.2 mg/l. This wine tastes very gentle and aromatic.

In red wine made from grape variety *Sapheravi* by Kakhethian (8R) and European (11R) technologies, the total content of phenols makes 4416 mg/l and 3130 mg/l, respectively. The total content of catechins and proanthocyanidins in wine made by Kakhethian technology is 1.7 and 2 times higher, than in wine made by European technology.

Partially other interrelation is observed in case of anthocyanins. In red wine made by European technology (11R) the content of anthocyanins is 1456 mg/l, but in red wine made by Kakhethian technology (8R) their content makes 1270 mg/l. It is apparently the result of long process of maceration in a "kvevri" causing sedimentation of anthocyanins.

Among Georgian autochthonous red varieties of grape is distinguished *Ojaleshi*. This wine made by Kakhethian technology is characterized by good color, typical viscosity for red wines, extract content, and harmonicity [Ramishvili, 1948]. In this wine (10R), the total content of phenols makes 3700 mg/l, catechins - 862 mg/l, proanthocyanidins - 872 mg/l, and anthocyanins - 414 mg/l.

The characteristics of the wine made by Kakhethian technology from the French variety *Cabernet Sauvignon* cultivated in Georgia are also important. In this wine (9R), the total content of phenols makes 2848 mg/l, catechins - 798 mg/l, proanthocyanidins - 728 mg/l, and anthocyanins - 317 mg/l. Comparison of the wine made by European technology from the French variety *Merlot* cultivated in Georgia with the wine made in Argentina from the same variety and by the same technology is rather interesting. In wine made in Kakhethi (13R) the total content of phenols, catechins, proanthocyanidins and anthocyanins makes 2318 mg/l, 636 mg/l, 826 mg/l and 322 mg/l, respectively (Tab. 2), while in red wine made in Argentina the total content of phenols is 1637 mg/l, the total content of catechins and proanthocyanidins is 13.30 mg/l, and content of anthocyanins is 52.61 mg/l [Sanches-Moreno et al., 2003]. It is also worth mentioning the wine (12R) made from the autochthonous Georgian grape variety *Aleksandreuli*, which is characterized by pleasant taste and delicate aroma. In this wine the total content of phenols makes 1630 mg/l, catechins - 378 mg/l, proanthocyanidins - 980 mg/l, and anthocyanins - 53.2 mg/l.

Thus, study of total amount of phenolic compounds, catechins, proanthocyanidins, and anthocyanins of white and red wines prepared by Kakhethian and European technologies has demonstrated that by content of those compounds Kakhethian wines considerably exceed European wines specifying medical and prophylactic properties of Kakhethian type wines. This conclusion

has been confirmed by investigation of antiradical efficiency (AE) of wines of Kakhethian and European types (Tab. 2). According to these findings average value of AE of white and red wines of Kakhethian type 2.3 and 1.7 times exceeds average value of AE of white and red wines of European type, respectively. Among wines, the white (1W, 2W) and red (8R, 9R, 10R) wines of Kakhethian type are especially distinguished by their antiradical efficiency.

The data of some authors on the enrichment of white wines of European type by polyphenols should be noted. Williams & Elliot (1997) have carried out researches aiming the development of a seed-enhanced (polyphenol-enriched) white wine with higher positive health potential. The potential effect of such seed-enhanced white wines on the cardiovascular system and inhibition of platelet aggregation has been shown. White wine prepared from grape variety *Chardonnay*, enriched by polyphenols possesses a protective effect against early forms of atherosclerosis in hamsters [Auger et al., 2005], and the white wine enriched by polyphenols possesses similar to red wine antiradical properties [Fuhrman et al., 2001].

Conclusions

As a result of comparison of white and red wines made by Kakhethian and European technologies it is shown that by content of total amount of phenolic compounds, catechines, proanthocyanidins, and anthocyanins, and by antiradical efficiency, the Kakhethian white and red wines considerably exceed European white and red wines specifying medical and prophylactic properties of Kakhethian type wines.

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ქართული (კახური) და ევროპული ტიპის ღვინოების ფლავონოიდური ნაერთები

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ს. დურმიშვილის ბიოქიმიის და ბიოტექნოლოგიის ინსტიტუტი

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რეზიუმე

შეწავლილია ქართული (კახური) და ევროპული ღვინოების ფლავონოიდური ნაერთები. ფენოლების ჯამის, კატეხინების, პროანტოციანდინების და ანტოციანების შემცველობის და ანტირადიკალური ეფექტურობის მიხედვით კახური ტიპის თეთრი და წითელი ღვინოები მნიშვნელოვნად სჭარბობს ევროპული ტიპის თეთრ და წითელ ღვინოებს.

INFLUENCE OF NEUROTRANSMITTER AND SYNAPTOSOMAL FACTOR ON DIVALENT CATION ACTIVATED ATPase ACTIVITY

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Abstract

Study of divalent cation activated ATPases (Cu-ATPase, Zn-ATPase and Ni-ATPase) from rat brain synaptic membrane fraction has shown that neither neurotransmitter or synaptosomal factor alone, nor together have effect on given ATPases activity. This ATPases do not take part in the synaptic transmission, which is likely the reason of insensitivity of those ATPases to the neurotransmitter and synaptosomal factor.

Key words: Cu-ATPase, Zn-ATPase, Ni-ATPase, noradrenalin, synaptosomal factor.

Introduction

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Divalent cation activated ATPases, Cu-ATPase, Zn-ATPase and Ni-ATPases, obtained from rat brain fractions are multi-sited enzyme systems regulating cellular level of these microelements [Chkadua et al., 2009]. As Na,K-ATPase, which is perfectly studied, these ATPases also belong to the transport ATPases. Investigation of an impact of various neurotransmitters (NT) on Na,K-ATPase has shown that one group of NT, such as acetylcholine, noradrenalin, dopamine and serotonin change its activity in a concentration dependent manner. Effect of NT on Na,K-ATPase is characterized with tissue-specificity, i.e. the effect is specific towards the chemically excitable membranes and is not found in erythrocyte shadows, in microsomal fraction of Na,K-ATPase of liver and kidney [Tsakadze et al., 1990]. The NT effect on Na,K-ATPase have shown in different ways in subcellular fractions of the brain as well. There were no effects in mitochondrial, myelin and vesicular fractions. Relatively weak effect was found in microsomal fraction and the most strong effect was revealed in the synaptosomal fraction [Jariashvili, Kometiani, 1975]. Later it was found that the NT effect, in its turn, is regulated by the synaptosomal factor (SF) from a supernatant obtained after osmotic shock of the rough mitochondrial fraction. Its edition into reaction medium inhibits the Na,K-ATPase activity. In a case of its introduction into the reaction medium together with NT, inhibition elicited either by SF as such or NT, is abolished and enzyme is sharply activated [Jariashvili et al., 1995]. So, to study the mechanisms which have influence on transport ATPase is very important. The subject of our interest was identification of NT and SF effects on divalent cation activated ATPases (Cu-ATPase, Zn-ATPase and Ni-ATPase).

Materials and Methods

The object of investigation was synaptic membrane fraction (1.2 – 0.9M between sucrose layers) [De Robertis et al., 1969; Whittaker, 1962] obtained from rat brain by centrifugation in sucrose density gradient. Protein concentration was determined by Lowry method [Lowry, Rosenbrogh, 1951], whereas inorganic phosphorus was measured using Fiske-Subbarow [Fiske, Subbarow, 1925] and Kazanov-Maslova methods [Kazanov, Maslova, 1980].

ATPase activity was judged by the amount of isolated inorganic phosphorus at ATP hydrolysis per mg protein in an hour.

The synaptosomal factor was isolated from the regions rich in nerve-endings by means of osmotic shock of the rough mitochondrial fraction, with farther centrifugation of the fraction obtained.

The experimentally obtained data were subjected to strict statistical treatment. Was used Student distribution and Fisher criteria. For reliable estimation of significant difference between experimental data $p < 0.02$, and of nonsignificant difference $p > 0.05$.

Results and Discussion

On the one hand it has been studied the influence of noradrenalin (NA) and SF alone and on the other hand their joint effect on the divalent cation activated ATP-ases (Cu-ATPase, Zn-ATPase and Ni-ATPase). The composition of the reaction medium was: $[MgATP]=1.5$ mM, $[Mg_i]=[ATP_i]=0.3$ mM, $[NA]=0.1$ mM, $[SF]=0.03$ mg/ml. Those concentrations of the divalent cations $[Cu^{2+}]=0.1$ nM, $[Ni^{2+}]=100$ nM, $[Zn^{2+}]=1$ nM were chosen, at which maximal enzyme activity was expected. The results have shown, that neither NA or SF alone, nor together give any effect on Cu-ATPase, Zn-ATPase and Ni-ATPase. Results are summarized end given in tables 1, 2, 3.

So results have shown, that NA and SF have no effect on bivalent cation activated ATPases. Supposedly effect of NA and SF on Na,K-ATPase activity is stipulated by its participating in synaptic transmission process. Bivalent cation activated ATPases don't take part in synaptic transmission and presumable this is the reason of their insensitivity to NA and SF.

Table 1. Influence of NA and SF on the Cu-ATPase, localized in the synaptic membrane fraction. The reaction medium composition was $[MgATP]=1.5$ mM, $[Mg_i]=[ATP_i]=0.3$ mM, $[NA]=0.1$ mM, $[SF]=0.03$ mg/ml, $[Cu^{2+}]=0.1$ nM.

active substance	Cu-ATPase activity
$[NA]=0$ $[SF]=0$	10.65±0.765
$[NA]=0.1$ mM	9.68±0.484
$[SF]=0.03$ mg/ml	10.374±1.2
$[NA]=0.1$ mM $[SF]=0.03$ mg/ml	11.26±1.01

Table 2. Influence of NA and SF on the Zn-ATPase, localized in the synaptic membrane fraction. The reaction medium composition was $[MgATP]=1.5$ mM, $[Mg_i]=[ATP_i]=0.3$ mM, $[NA]=0.1$ mM, $[SF]=0.03$ mg/ml, $[Zn^{2+}]=1$ nM.

active substance	Zn-ATPase activity
$[NA]=0$ $[SF]=0$	7.26±0.76
$[NA]=0.1$ mM	6.62±0.63
$[SF]=0.03$ mg/ml	7.018±0.726
$[NA]=0.1$ mM $[SF]=0.03$ mg/ml	7±0.998

Table 3. Influence of NA and SF on the Zn-ATPase, localized in the synaptic membrane fraction. The reaction medium composition was [MgATP]=1.5 mM, [Mg_i]=[ATP_i]=0.3 mM, [NA]=0.1 mM, [SF]=0.03 mg/ml, [Ni²⁺]=100nM.

active substance	Ni-ATPase activity
[NA]=0 [SF]=0	6.05±0.65
[NA]=0.1mM	6.77±0.92
[NA]=0.1mM [SF]=0.03mg/ml	6.65±0.807

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ნეიროტრანსმიტერებისა და სინაფტოსომური ფაქტორის გავლენა ორვალენტიანი კათიონებით აქტივირებულ ATP-ზე აქტივობაზე

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ივანე ბერიტაშვილის ფიზიოლოგიის ინსტიტუტი

(მიღებულია 06.09.2010)

რეზიუმე

ვირთავს თავის ტვინის სინაფტურ ფრაქციაში ლოკალიზებული ორვალენტიანი კათიონებით აქტივირებული ATP-ების (Cu²⁺-ATP-ისა, Zn²⁺-ATP-ისა და Ni²⁺-ATP-ისა) შესწავლამ აჩვენა, რომ როგორც ცალ-ცალკე ნორადრენალინი და სინაფტოსომური ფაქტორი, ისე მათი ერთდროული მოქმედება გავლენას არ ახდენს აღნიშნული ფერმენტული სისტემების მუშაობაზე. ვინაიდან ზემოთ ჩამოთვლილი ATP-ები არ მონაწილეობენ სინაფტურ გადაცემაში, სავარაუდოდ, ამით აიხსნება მათი არამგრძობელობა ნორადრენალინისა და სინაფტოსომური ფაქტორის მიმართ.

WOODY PLANTS OF IMERETI

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Abstract

The total number of the higher plants in Imereti is about 900 species. We have revealed 143 woody plant species, of which 11 are Caucasian and 6 Georgian endemics. This species of the woody plants are included in 82 genera and 48 families. Imereti dendroflora consists of the Mediterranean, Caucasus, Front Asia and Asia Minor, Palaearctic and Holarctic species with different share in the flora composition.

Key words: woody plants, systematics, phytogeography,

Introduction

Imereti is one of the oldest provinces in Georgia. It is prominent for ancient historical wealth: t. Vani dating back to 6th – 3rd c.c. B.C. should be noted; Gelati monastery is conspicuous among churches and monasteries, while Bagrati monastery is included in the UNESCO cultural heritage list. Ajameti and Sataplia reserves occur in Imereti. Dinosaur traces have been preserved in Sataplia reserve. Borjomi-Kharagauli National Park is located to the east of the territory. Beech, oak, chestnut, Zelkova and other forest formations are present in the Park. Almost all vegetation belts typical for Georgia and Caucasus occur in the area.

Natural Conditions

Location, Area, Borders. Imereti is located in the east of the Western Georgia. The floristic district is bordered by Askhi massif to the north, Likhi and Gethsemane ridges to the east, Adjara-Imereti ridge (Meskheta ridge section) and Supsa-Tsablariškhalı watershed to the south, r. Tskhenistskhalı to the west. The area is 6552 km², which is 9.7 % of Georgia.

Terrain. The relief is plain-hilly, low and moderately mountainous. The absolute heights of the lowlands (the eastern part of Colchis lowland) vary from 20 m to 1000 m a.s.l. Imereti plateau is noteworthy with absolute height of 1200-1500 m at Dzirula-Chkherimela watershed. Mepistskharo (2850 m) and Sametskhvario (2642 m) are prominent from peaks.

Geology. In geological terms the floristic district is composed of Quaternary, Neogenic, Cretaceous, Jurassic and Paleozoic strata, namely: Quaternary - shingles, sands, clays, Neogenic - clays, sandstones, limestones, volcanic formations; Cretaceous - limestones, dolomites, marls, sandstones, volcanic formations, Jurassic - slates, sandstones, conglomerates, limestones, porphyrites, Paleozoic granitoids [<http://www.moe.gov.ge>].

Soil. Soils of the district are represented by turf-peat, Chernozem, turf-carbonate, yellow, sub-tropical podzol and Chernozem soils [Urushadze, 1977].

Climate. The climate of the district is of Colchic type and characterized by a number of sub-types: excessively humid climate with warm winter and hot summer occurs in the western part, humid climate with moderately cold winter and long hot summer – in the central part, while climate with cool summer – in the eastern part. Humid climate with long cold winter and cool summer is observed in the mountainous part. The precipitations fluctuate within 700-2000 mm. The average January temperature is 0 - +5, average July temperature - +20 - +27 [Maruashvili, 1969].

Hydrological Regime. The district is rich in rivers and reservoirs, while lakes are less abundant and smaller in size. The hydrographic system is formed by the waters of Tskhenistskhali, Rioni, Kvirila, Dzirula, Jrutchula, Cholaburi, Tsablaristskhali, Sakrauli, Rikotuli, Chkherimela and Khanistskhali, of which Rioni and Tskhenistskhali are especially plentiful [Maruashvili, 1969].

The purpose of the research: identification of the diversity of Imereti woody plants, their taxonomic and phytogeographical analysis.

Materials and Methods

Field studies were conducted using traditional field methods [Grossheim, 1936; Tolmachev, 1947, 1986; Gagnidze, Ivanishvili, 1975].

To determine the geographical element the recent geographical distribution of taxa was accepted [Gagnidze, 1974, 2005].

In addition to our field research, the botanical-geographic analysis was based on the first (1941-1952) and second (1-15 volumes 1971-2007) editions of the “Flora of Georgia”.

Species nomenclature was adopted according to S. Cherepanov [Cherepanov, 1995] and R. Gagnidze [Gagnidze, 2005] lists.

Results and Discussion

The History of Botanical Research of Imereti

The detailed study of Caucasian and Georgian flora has started in the beginning of the 19th c. Imereti flora was described during the general research of the vegetation of the Western Georgia by: Kolakovski, Dolukhanov, Grossheim, Medvedev, Lomakin, Ruprecht, Iuzepchuk, Eikhvald (Ledeburg Herbarium), Shishkin, Kemularia-Natadze, Mandenova, Sosnovski, Kutateladze, Gagnidze, Sokhadze, Makashvili, Khintibidze, Lachashvili, Tchelidze, Mikeladze, etc.

Imereti flora had been researched for half a century since 1933 by A. Kutateladze, L. Kemularia-Natadze (A. Tsereteli State University) who studied the limestone flora of Imereti, described several new plant species. In the recent period T. Tcheishvili under Gagnidze’s supervision has worked on the endemic species of Imereti flora

Flora Peculiarity

The number of the higher plants of Imereti comprises about 900 species [Gagnidze, 2005] (however, this issue should be confirmed, which is the goal of our future research), while the number of the arborescent species is 143, of which 11 are Caucasian and 6 Georgian endemics.

143 species of the woody plants are included in 82 genera and 48 families. Genera are distributed within the families in the following way (Table 1):

Rosaceae – 12 genera; Caprifoliaceae, Corylaceae, Fagaceae, Leguminosae, Oleaceae, Pinaceae and Rhamnaceae – 3 by 3 genera; Anacardiaceae, Betulaceae, Ericaceae, Juglandaceae, Loranthaceae, Moraceae, Salicaceae, Tamaricaceae and Ulmaceae – 2 by 2 genera; other families

of Aceraceae, Aquifoliaceae, Araliaceae, Asclepiadaceae, Berberidaceae, Buxaceae, Celastraceae, Cistaceae, Cornaceae, Crossulariaceae, Cupressaceae, Ebenaceae, Elaeagnaceae, Empetraceae, Euforbiaceae, Hydrangeaceae, Lauraceae, Platanaceae, Punicaceae, Ranunculaceae, Simaroubaceae, Solanaceae, Staphyleaceae, Taxaceae, Thymelaeaceae, Tiliaceae, Vacciniaceae, Verbenaceae and Vitaceae – 1 by 1 genera.

The first seven genera by species abundance are as follow: 1. *Salix* – 7 species; 2. *Populus* – 6 species; 3. *Rosa* – 6 species; 4. *Acer* – 5 species; 5. *Daphne* – 4 species; 6. *Rhamnus* – 4 species; 7. *Rubus* – 4 species.

36 species are included in the most abundant 7 genera, which is 25% of the total number of species.

The first nine families by species abundance are as follow: 1. Rosaceae – 24 species; 2. Salicaceae – 13 species; 3. Rhamnaceae – 6 species; 4. Aceraceae – 5 species; 5. Caprifoliaceae – 5 species; 6. Corylaceae – 5 species; 7. Fagaceae – 5 species; 8. Leguminosae – 5 species; 9. Thymelaeaceae – 5 species.

The number of species in the most abundant 9 families reaches 73, which is 51% of the total number of species.

Brief Review of Vegetation

Vertical belting of Imereti vegetation is represented as follows: mixed leaved forest belt (20-800 m), broad leaved forest belt (800-1200 m), dark coniferous forest belt (1200-2000 m), sub-alpine forest-meadow belt (2000-2600 m) and alpine belt (2600-3000 m).

Mixed leaved forest belt: the belt comprises the areas of the lower reaches of r. Rioni tributary water catchment basins (Imereti lowland, Simoneti plain). It is a belt with natural climbers, the vegetation of which is formed by *Quercus imeretina* Stev., *Quercus hartwissiana* Stev., *Alnus barbata* C., *Pterocarya pterocarpa* (Michx.) Kunth, *Castanea sativa* Mill; liana plants, namely: *Hedera colchica* (C. Koch), *Smilax excelsa* L. Evergreen undergrowth comprises *Rhododendron luteum* Sweet, *Ilex colchica* Pojark. K. Ajameti Reserve is located within the belt. Relict species of *Quercus imeretina* Stev., *Zelkova carpinifolia* (Pall.) Dipp., as well as *Quercus hartwissiana* Stev. have been preserved in the Reserve.

Broad leaved forest belt: the territory comprises the major part of the district including Imereti highland. Beech, oak, hornbeam, chestnut, lime with Colchic understory occur in the area.

Dark coniferous forest belt: the belt is mainly distributed in the southern part of the district and the vegetation is formed by fir, spruce and evergreen undergrowth.

Sub-alpine forest-meadow belt: it is located even more to the south and the vegetation comprises sycamore maple, ash, birch, high mountainous tall grass along with Colchic understory.

Alpine belt: it comprises fairly small part of the territory at Adjara-Imereti border. The vegetation is formed by meadows, rhododendron, mountain crowberry, whortleberry, paradise plant. Sedge meadows, stains and shrubby belt are also present.

The geographic structure of the endemic woody plants of Imereti, conspectus of woody plants and total number of families and genera are given below (Table 1).

Phytogeography

Imereti belongs to the ancient Mediterranean world, Sub-Mediterranean province, Colchis or East Euxeinus province, Racha-Lechkhumi and Imereti limestones, Colchis lowland and foothills and Guria-Southern Imereti provinces.

Imereti flora is formed by Mediterranean, Caucasian, Front Asia and Asia Minor, Palaearctic and Holarctic species with different shares in flora composition.

Table 1. Woody plants of Imereti

Family	Number of genera	Genera	Number of species	Number of endemic species
1. Cupressaceae	1	Juniperus	2	
2. Pinaceae	3	Abies Picea Pinus	3	
3. Taxaceae	1	Taxus	1	
4. Aceraceae	1	Acer	5	
5. Anacardiaceae	2	Cotinus Rhus	2	
6. Aquifoliaceae	1	Ilex	1	
7. Araliaceae	1	Hedera	2	
8. Asclepiadaceae	1	Periploca	1	
9. Berberidaceae	1	Berberis	1	
10. Betulaceae	2	Alnus Betula	4	
11. Buxaceae	1	Buxus	1	
12. Caprifoliaceae	3	Lonicera Sambucus Viburnum	5	
13. Celastraceae	1	Euonymus	3	1
14. Cistaceae	1	Cistus	1	1
15. Cornaceae	1	Swida	1	
16. Corylaceae	3	Carpinus Corylus Ostrya	5	1
17. Ebenaceae	1	Diospyros	1	
18. Elaeagnaceae	1	Hippophae	1	
19. Empetraceae	1	Empetrum	1	
20. Ericaceae	2	Arctostaphylos Rhododendron	4	1
21. Euforbiaceae	1	Leptopus	1	1
22. Fagaceae	3	Castanea Fagus Quercus	5	1
23. Grossulariaceae	1	Ribes	2	
24. Hydrangaceae	1	Philadelphus	1	1
25. Juglandaceae	2	Juglans Pterocarya	2	
26. Lauraceae	1	Laurus	1	
27. Leguminosae	3	Cytisus Genista Robinia	5	2
28. Loranthaceae	2	Arceuthobium Viscum	2	
29. Moraceae	2	Ficus Morus	2	
30. Oleaceae	3	Fraxinus Jasminum Ligustrum	3	
31. Platanaceae	1	Platanus	1	
32. Punicaceae	1	Punica	1	
33. Ranunculaceae	1	Clematis	1	
34 Rhamnaceae	3	Frangula Rhamnus Zizyphus	6	1
35. Rosaceae	12	Cerasus Cotoneaster Crataegus Laurocerasus Malus Mespilus Prunus Pyracantha Pyrus Rosa Rubus Sorbus	24	3
36 Ruscaceae	1	Ruscus	2	1
37 Salicaceae	2	Populus Salix	13	3
38 Scrophulariaceae	1	Pacderotella	1	1
39 Simarubaceae	1	Ailanthus	1	
40 Smilacaceae	1	Smilax	1	
41 Solanaceae	1	Lycium	1	
42 Staphylaeaceae	1	Staphylea	2	
43 Tamaricaceae	2	Myricaria Tamarix	2	
44 Thymelaeaceae	1	Daphne	5	1
45 Tiliaceae	1	Tilia	1	
46 Ulmaceae	2	Ulmus Zelkova	4	
47 Vacciniaceae	1	Vaccinium	3	
48 Verbenaceae	1	Vitex	1	
49 Vitaceae	1	Vitis	3	

We discuss the composition of each geographical element below:

Caucasian Species: 1) *Cytisus caucasicus* Grossh. 2) *Euonymus leiophloea* Stev. 3) *Genista patula* Bieb. 4) *Pyrus caucasica* Fed. 5) *Paederotella pontica* (Rupr. ex Kem.-Nath. Boiss.) 6) *Philadelphus caucasicus* Koehne 7) *Populus hyrcana* L. 8) *Rubus ponticus* (Focke) Juz. 9) *Ruscus colchicus* P. F Yeo. 10) *Salix kazbekensis* A. 11) *Salix micans* Anderss.

Western Caucasian: 1) *Arctostaphylos caucasica* (Kvaratzchelia) Lipsch.

Trans-Caucasian: 1) *Daphne axilliflora* (Keissl.) Pobed.

Georgian: 1) *Corylus imeretica* Kem.-Nath. 2) *Rubus mochus* Juz.

Western Georgian: 1) *Genista sachokiana* A. 2) *Leptopus colchicus* (Fisch. Et Mey.) 3) *Quercus imeretina* Stev. 4) *Rhamnus cordata* Medw.

Caucasian-Asian Minor: 1) *Abia nordmanniana* Stev. 2) *Acer trautvetteri* Medw. 3) *Betula Litwinowii* Doluch. 4) *Buxus colchica* Pojark. 5) *Cytisus hirsutissimus* C. 6) *Empetrum caucasicum* (V. Vassil.) Juz. 7) *Hedera colchica* (C. Koch) 8) *Quercus hartwissiana* Stev. 9) *Rhamnus imeretina* Booth. 10) *Rhamnus depressa* Grub. 11) *Salix elbursensis* Boiss. 12) *Salix pseudomedemii* E. 13) *Sorbus Boissierii* C. 14) *Viburnum orientale* Pall.

Caucasian, Mediterranean, Asian Minor: 1) *Daphne alboviana* Woronow 2) *Daphne pontica* L. 3) *Ilex colchica* Pojark. K. 4) *Laurus nobilis* L. 5) *Ostrya carpinifolia* Scop. U. 6) *Pinus sosnowskyi* Nakai, 7) *Pyracantha coccinea* Roem. 8) *Rhododendron caucasicum* Pall. D. 9) *Rhododendron ponticum* L. 10) *Staphylea colchica* Stev.

Caucasian, Asian Minor and Front Asian: 1) *Acer laetum* C. 2) *Alnus barbata* C. 3) *Crataegus pseudoheterophylla* Pojark. 4) *Juniperus oblonga* M. 5) *Malus orientalis* Uglitzk. 6) *Pterocarya pterocarpa* (Michx.) Kunth 7) *Quercus iberica* Stev. 8) *Ribes Biebersteinii* Berl. K. 9) *Zelkova carpinifolia* (Pall.) Dipp.

Mediterranean, Asian Minor and Front Asian: 1) *Carpinus caucasica* Grossh. 2) *Crataegus microphylla* C. 3) *Euonymus latifolia* (L.) Mill. 4) *Fagus orientalis* Lepsky 5) *Juniperus depressa* Stev. 6) *Laurocerasus officinalis* M. 7) *Lonicera caucasica* Pall. 8) *Tilia begoniifolia* Stev. 9) *Vaccinium arctostaphylos* L.

Mediterranean and Front Asian: 1) *Juglans regia* L.

Caucasian, Front and Central Asian: 1) *Salix excelsa* Gmelin.

Mediterranean, Asian Minor, Central Asian: 1) *Platanus digitifolia* Palib.

Mediterranean, Asian Minor, Front and Central Asian: 1) *Prunus divaricata* Ledeb. 2) *Punica granatum* L. 3) *Rhus coriaria* L. 4) *Rubus anatolicus* (Focke).

Asian Minor, Front and Central Asian: 1) *Rosa foetida* J.

Western-Palaeartic-Mediterranean: 1) *Acer campestre* L. 2) *Acer pseudoplatanus* L. 3) *Arceuthobium oxycedri* (DC.) Bieb. 4) *Berberis vulgaris* L. 5) *Carpinus orientalis* Mill. 6) *Castanea sativa* Mill. 7) *Cerasus silvestris* (Kirschl.) Gars. 8) *Cistus salviifolius* L. 9) *Clematis vitalba* L. 10) *Corylus avellana* L. 11) *Cotoneaster integerrimus* Medik. 12) *Crataegus pentagyna* Waldst. 13) *Daphne mezereum* L. 14) *Euonymus europaea* L. 15) *Ficus carica* L. 16) *Fraxinus excelsior* L. 17) *Hedera helix* L. 18) *Ligustrum vulgare* L. 19) *Lonicera caprifolium* L. 20) *Mespilus germanica* L. 21) *Myricaria germanica* (L.) 22) *Periploca graeca* L. 23) *Populus alba* L. 24) *Populus canescens* (Ait.) Smith 25) *Populus pseudonivea* Grossh. 26) *Rhododendron luteum* Sweet, 27) *Ribes alpinum* L. 28) *Rosa canina* L. 29) *Ruscus ponticus* Woronow ex Grossh. 30) *Rosa corymbifera* Borkh. 31) *Rosa gallica* L. 32) *Rosa micrantha* Borrer 33) *Rosa mollis* Smith 34) *Sambucus nigra* L. 35) *Smilax excelsa* L. 36) *Sorbus graeca* (Spach) Lodd. 37) *Sorbus torminalis* (L.) Crantz, 38) *Staphylea pinnata* L. 39) *Swida australis* (C. A. Mey.) Pojark. 40) *Tamarix smyrnensis* Bunge 41) *Taxus baccata* L. 42) *Ulmus elliptica* C. Koch 43) *Ulmus minor* Mill 44) *Ulmus suberosa* Moench 45) *Vitex agnus-castus* L.

Palaeartic-Mediterranean: 1) *Acer platanoides* L. 2) *Cotinus coggygria* Scop. 3) *Daphne glomerata* Lam. 4) *Frangula alnus* Mill. 5) *Hippophae rhamnoides* L. 6) *Morus alba* L. 7) *Populus*

nigra L. 8) *Populus tremula* L. 9) *Ramnus cathartica* L. 10) *Rubus idaeus* L. 11) *Salix caprea* L. 12) *Salix triandra* L. 13) *Viscum album* L. 14) *Viburnum opulus* L.

Holarctic-Central-American-Mediterranean: 1) *Alnus incana* (L.) Moench, Meth. 2) *Vaccinium myrtillus* L. 3) *Vaccinium Vitis-idaea* L.

Mediterranean-South-East-Palaeartic: 1) *Zizyphus jujuba* Mill.

Holarctic-Neotropical: 1) *Vitis vinifera* L.

Geographic structure of Imereti endemic woody plants

In total there are 13 Caucasian and 6 Georgian endemics occur among 143 woody plants occurring in Imereti; these are as follow:

- | | |
|---------------------------------------|--------------------|
| 1. <i>Arctostaphylos caucasica</i> | (Ericaceae) |
| 2. <i>Corylus imeretica</i> | (Corylaceae) |
| 3. <i>Cytisus caucasicus</i> | (Leguminosae) |
| 4. <i>Daphne axilliflora</i> | (Thymelaeaceae) |
| 5. <i>Euonymus leiophloea</i> | (Celastraceae) |
| 6. <i>Genista patula</i> | (Leguminosae) |
| 7. <i>Genista sachokiana</i> | (Leguminosae) |
| 8. <i>Leptopus colchicus</i> | (Euforbiaceae) |
| 9. <i>Paederotella pontica</i> | (Scrophulariaceae) |
| 10. <i>Philadelphus caucasicus</i> | (Hydrangeaceae) |
| 11. <i>Populus hyrcana</i> | (Salicaceae) |
| 12. <i>Pyrus caucasica</i> | (Rosaceae) |
| 13. <i>Quercus imeretina</i> | (Fagaceae) |
| 14. <i>Rhamnus cordata</i> | (Rhamnaceae) |
| 15. <i>Rubus mochus</i> | (Rosaceae) |
| 16. <i>Rubus ponticus</i> | (Rosaceae) |
| 17. <i>Ruscus colchicus</i> P. F. Yeo | (Ruscaceae) |
| 18. <i>Salix kazbekensis</i> | (Salicaceae) |
| 19. <i>Salix micans</i> | (Salicaceae) |

Imereti endemic woody plants could be divided into 5 groups according to their distribution:

1. Trans-Caucasus: *Daphne axilliflora* (Keissl.) Pobed.
2. Western Caucasus: *Arctostaphylos caucasica* (Kvaratzchelia) Lipsch.
3. Caucasus: *Cytisus caucasicus* Grossh., *Euonymus leiophloea* Stev., *Genista patula* Bieb., *Pyrus caucasica* Fed., *Paederotella pontica* (Rupr. ex Kem.-Nath. Boiss.), *Philadelphus caucasicus* Koehne, *Populus hyrcana* L., *Rubus ponticus* (Focke) Juz., *Ruscus colchicus* P. F. Yeo, *Salix kazbekensis* A., *Salix micans* Anders.
4. Western Georgia: *Genista sachokiana* A., *Leptopus colchicus* (Fisch. et Mey.), *Quercus imeretina* Stev., *Rhamnus cordata* Medw.
5. Georgia: *Corylus imeretica* Kem.-Nath., *Rubus mochus* Juz.

Landscape Changes throughout the Recent 50 Years

Like the entire Georgia, Imereti has been populated since ancient times (t. Vani remnants). Hence, the forests have been cut for centuries. Grass and shrubbery has been intensively grazed. As a result, oriental hornbeam and steppes have developed causing the reduction and even extinction of populations of many plant species.

Imereti Protected Areas

As we have already mentioned above, protected areas are represented by Kharagauli district part of Borjomi-Kharagauli National Park and Sataplia and Ajameti Reserves in Imereti. Borjomi-Kharagauli National Park is also tourist centre, various tourist routes have been arranged in the Park. The main function of Ajamaneti Reserve is the conservation of the oldest Zelkova stands and that of Sataplia Reserve is the protection and preservation of the dinosaur traces.

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იმერეთის დენდროფლორის მრავალფეროვნების შესწავლისათვის

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რეზიუმე

განხილულია იმერეთის ფლორისტული რაიონის მერქნიანი მცენარეების მრავალფეროვნება. გამოვლენილია 143 სახეობა, რომელთაგან 11 არის კავკასიის და 6 საქართველოს ენდემი. მოცემულია სისტემატიკური სტრუქტურა და გეოგრაფია. მერქნიანი მცენარეების ეს სახეობები გაერთიანებულია 82 გვარსა და 48 ოჯახში. იმერეთის დენდროფლორა მოიცავს ხმელთაშუაზღვიურ, კავკასიურ, წინა აზიურ, მცირე აზიურ, პალეარქტიკულ და ჰოლარქტიკულ სახეობებს.

GENETIC STUDY OF ALCOHOLIC FERMENTING WINE YEASTS OF KAKHETI (EASTERN GEORGIA) AND ADJARA (WESTERN GEORGIA) SMALL FARM WINERIES

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Abstract

The antagonistic activity of the strains isolated from wine yeast natural populations of ecologically distinct regions (Kakheti and Adjara) has been studied. The populations were isolated from the small farm wineries, where fermentation of grape juice is occurred spontaneously. The strains of K, N and S phenotypes were revealed in natural populations of the wine yeast. The populations differ with composition structure. The frequency of killer and neutral phenotype strains was high in Kakheti populations, whilst prevalence of sensitive strains was observed in Adjara populations. The ecological niche in Kakheti and Adjara wine yeast populations was found to belong to the K2 system.

Key words: wine yeast, killer strains, neutral strains, sensitive strains, natural populations

Introduction

Viniculture is the basic and traditional branch of Georgian economics. Yeast fungi take key place in determination of wine quality. Yeast fungi – saccharomycetes are classical objects of genetics and molecular biology. In this viewpoint, unlike other organisms, they are fully researched and are used successfully in genetic engineering and microbiological industries. Study of gene pool of wine yeast is an actual problem of population genetics. Gene pool structure of wine yeast population is determined by ecological conditions and mainly by vine culture. Georgia is considered as the homeland of cultural vine [Jacobson, 1994; Vouillamoz et al., 2006], up to 500 unique endemic cultivars occur here. Existence of viniculture in Georgia from 3rd millennium B.C. is confirmed by archeological data [Constantini et al., 2006]. It is consider that formation of wine yeast and its distribution happened in the central part of Near East [Jackson, 2000].

Small farm wineries are those nidi where during the thousands of years both, formation and conservation of gene pool of wine yeast fungi occurred. Diversity of vine cultivars, varieties of soil-climatic conditions in Georgia caused formation of unique gene pool of wine yeast [Shatirishvili & Chuchulashvili, 2000].

In the near future small farm wineries should become noncompetitive and disappear due to the development of wine industry, using monocultural strains in management of wine technological processes. So, genetic study of wine yeast gene pool in Georgia is significant.

Molecular-genetic study of wine yeast endemic gene pool, revealing the perspective strains for selection, conservation in zymological museums, obtaining industrially perspective and competitive strains via genetic selection of endemic strains, their manufacturing application are the urgent problems. Such strains preserved in zymological museums will give the great profit to the country.

Study of interspecific and intraspecific interdependence of wine yeast is very important. One of the ways of exposure of intraspecific competition in wine yeasts is via toxic protein mycocine isolated by “killer” cells and another way is elimination of sensitive cells [Marquin et al., 2002]. In natural habitats “killer” strains compete with sensitive strains in nutriment earning forcing them from habitats or limiting their reproduction and inhabitation area [Schmitt & Breining, 2002].

Yeast “killer” strains are infested permanently with virus-like particles consisting of two-stranded RNA. They are surrounded with protein covering – capsid. Two forms of virus-like particle are found in yeast cells. One of them has key, i.e. L genome and the other one – minor M genome. L genome encodes enzyme RNA-polymerase and capsid composing proteins and M genome – exotoxin and determines resistance against toxin. L genome controls M genome action. “Killer” strains have particles of both forms, neutral strains – particles with M genome and as a result they can not synthesize toxin. Neutral cells sometimes have particles of both forms, but due to the mutation synthesized toxin is inactivated [Wincler, 1985; Magliani et al., 1997; Yap et al., 2000]. Up to 40 nucleus genes participated in this process are revealed. “Killer’s” activity is controlled by nucleus-cytoplasmic system [Sommer & Wickner, 1984; Magliani et al., 1997].

Three phenotypic classes – K, N and S are found in yeast natural populations [Magliani et al., 1997. Gulbinienė et al., 2004]. “Killer” (K) strain produces exotoxin (protein or glycoprotein) affecting lethally on sensitive (S) strains of the same species. Producer cells are immune against own toxin, but against other toxins it should appear sensitive. Neutral (N) strains do not synthesize toxin, but they are resistant against them [Magliani et al., 1997; Marquin et al., 2002; Gulbinienė et al., 2004]. “Killer’s” activity may be detected by corresponding sensitive strains. The temperature, pH and salinity of environment have effect on activity [Yasuyuki & Wickner, 1995].

At first killer strains were recorded in strains of *Saccharomyces cerevisiae* [Bevan & Makower, 1963; Somers & Bevan, 1969; Naumov & Naumova, 1973], and later in other yeast genera [Phillbiskirk & Young, 1975; Mitchel & Bevan, 1983; Schmitt & Breining, 2002]. In Georgian populations “killer” systems for the first time were studied by Chuchulashvili [Chuchulashvili, 1980]. Action spectrum of definite killer toxin is usually limited - it affects on species united in the genus [Bevan & Makower, 1963; Somers & Bevan, 1969], but later killer reaction was registered on species belonging to various genera [Bussey, 1981; Tipper & Bostian, 1984]. In saccharomycetes “killer” system of three types was revealed: K1, K2 and K28. They differ from each other by genome structure and action spectrum [Magliani et al. 1997].

Materials and Methods

Isolation of strains from natural populations. In yeast fungi, and particular, in saccharomycetes it is not worked out yet features of population characterizing systematic category. *Drosophila* determines the area of reproductive activity of wine yeast which involves small territories (400 m). Respectively, village and surrounding vineyard territory we consider as wine yeast population area and distributed there species group – as population. Material (wine crust) was

taken from 10 small farm wine-cellars of the village according to the method described previously [Menabde et al., 2004; Shatirishvili et al., 2007]. Yeast strains isolated from the wine crust of separate wine-cellars (isolates) represent a micropopulation. Wine-cellars and vineyards from where the farmer has been harvesting were far from each other (at more than 500 m). Between those places gene streams (transmission of wine yeast cells via drosophila) are limited. Wine crust was taken after fermentation is over. In studied farms fermentation proceeds spontaneously and inlet of industrial strains does not occur. Then wine crust was put in sterile vessels.

From the vessels wine crust was transferred by microbiological loop into the sterile flask of 4 ml filled with grape juice. Dilute material was sown on the agar overlaid grape juice medium according to the streak method. Incubation of culture lasts for 4 days at 25°C. Isolation of typical colonies and their cloning was conducted on the YEPD medium. Determination of species was carried out by existed criteria [Kreger-van, 1984; Krasilnikov & Shchokolova, 1991].

Test-strains. Antagonistic activity was determined by the following test-strains: K7 (MAT α arg9 (KIL-1)); S14 (MAT α) strain sensitive to K1 killer; M437 (wild type HM/HM (KIL-2)); 7A-p192 (MAT α ade2) strain sensitive to K2 killer; MS 300 (MAT α leu2 ura3 (KIL-28)); S6 (wild type HM/HN) strain sensitive to K28 killer.

Nutrient media. Cultivation was carried out on YEPD medium (glucose - 2%, bactopectone - 2%, yeast autolysate - 1%, agar - 2%). Induction of sporulation was conducted on acetated medium (Na acetate - 1%, KCl - 0.5%, agar - 2%). Killer activity was observed on YEPD-MB medium (YEPD with methylene blue - 0.003%, acetate-phosphate buffer). Pure culture was isolated on the agar overlaid grape juice medium (grape juice - 50%, agar - 2%) [Somers & Bevan, 1969; Gulbinienė et al., 2004].

Determination of killer and resistant phenotypes. Identification of K1 and K2 killer systems was carried out at pH 4.6 and of K28 system at pH 5.2 [Bevan & Makower, 1963]. Test-strains were sown on Petri dishes like lawns; strains isolated from the natural population were brought in over them as streaks. Killer strains lysed both sensitive and unrelated killer test-strains. Around the streaks sterile zones of various sizes are formed. Neutral strains reveal resistance against the killer strains and do not lyse sensitive test-strains. Sensitive strains are killed on the killer test-strain lawn, and the culture is dyed blue [Gulbinienė et al., 2004].

Results and Discussion

Antagonistic activity of wine yeast populations of two regions of Adjara (Khetsubani – Kobuleti district and Kapreshumi – Batumi district) was studied (Tables 1 and 2). Strains are isolated from material taken from 20 small farm wine-cellars. Yeast populations are isolated from wine crust extracted from vine cultivars “Nova” (hybrid cultivar of American origin) and “Izabela” (half-wild form of American origin). Strains composing the both populations were divided into three phenotypic classes: killer (K) producing exotoxins, sensitive (S) which are affected lethally by toxins and neutral (N) resistant to toxins.

Phenotypes of studied strains are determined according to the relations to the test-strains. Strains consisted of K2 plasmid lyse both, strains with K2 plasmid and sensitive strains. Strains consisted of K1 plasmid lyse sensitive strains, as well as strains with K2 plasmid. In Khetsubani and Kapreshumi populations strains with K2 plasmid occur only.

We researched 10 micropopulations of each population (Tables 1 and 2). Composite micropopulations of one population appeared distinct according to killer-sensitivity. Only 5 micropopulations of Khetsubani population (IV, V, VI, VII, IX) have strains of all three phenotypic classes. In 2 micropopulations (III, X) only sensitive strains are met, and in 4 micropopulations (II, III, VIII, X) “killer” strains do not occur at all. In spite of the fact that some micropopulations are monomorphic, the structure of the whole population of Khetsubani wine yeast appeared

polymorphic. It consists of 7.2% of “killer”, 37.6% of neutral and 55.6% of sensitive strains. Sensitive strains are significant in fermentation process.

Kapreshumi wine yeast population does not differ from Khetsubani population by its structure. In all wine yeast micropopulations of Kapreshumi S phenotype strains are distributed, but their frequencies are distinct. In some micropopulations “killer” and neutral strains do not occur at all. I micropopulation consists of only sensitive strains. In VII, VIII, X micropopulations only N and S phenotype strains are distributed. K phenotype strains occur only in 6 micropopulations. In 6 micropopulations basically sensitive strains took part in fermentation.

Two wine yeast populations are studied in Kakheti region (Kardenakhi, Mirzaani). The material was taken from 20 small farm wine-cellar. K, N and S phenotype strains are distributed with various frequencies in both populations. “Killer” phenotype strains occur in all micropopulations of Kardenakhi, but with distinct frequencies. IV, VII and VIII micropopulations are distinguished with high frequencies of K phenotype strains: 32%, 36% and 52% respectively. Neutral phenotype strains are spread with the highest frequencies in micropopulations. Their total percentage in Kardenakhi wine yeast population equals to 57.6%. In III and VIII micropopulations sensitive strains do not occur at all. In VI micropopulation their frequency was rather high (44%). Alcoholic fermentation is mainly depended on K and N phenotype strains.

Wine yeast population of Mirzaani by its structure appeared distinct from Kardenakhi population. The total amount of “killer” phenotype strains in the population is 21.6% and of neutral strains – 75%. Frequency of neutral phenotype strains in micropopulations varies from 42% to 92%. Sensitive phenotype strains are distributed with low frequencies in micropopulations with exception of X micropopulation where their percentage is 40%. In Adjara (Khetsubani, Kapreshumi) high vine is cultivated, grapes are rather high from the ground (3-5 m). Due to high precipitations characterizing this region yeast fungi occur not much on grapes. Spontaneous fermentation is caused by yeast fungi got into the grape juice occasionally.

Unlike Adjara, the common characteristic of wine yeast populations and their composite micropopulations of traditional viticulture region – Kakheti is that distribution frequencies of K and N phenotype strains are high and they dominate in fermentation process [Menabde et al., 2004; Shatirishvili et al., 2007; 2009]. It should be taken into consideration that in traditional viticulture regions low vine is grown, which phyllosphere is diverse and differ from high vine phyllosphere. Species composition of the last one is very poor.

Ability of toxin synthesis in killer strains always correlates positively with resistance against it. Immunity against toxin is not universal. Immunity of producer cell against its own toxin does not cause their resistance against toxin of different type isolated from other strains. Each strain should be characterized by resistance-sensitivity against toxin, and killer strains by the spectrum of action on other killers and strains [Bevan & Makower, 1963; Magliani et al., 1997; Gulbiniene et al., 2004]. Exotoxin affects on young cells of yeast being in the phase of fission [Baeza et al., 2008].

Detection of “killer” phenotype depends on ecological conditions (temperature, pH, salinity) [Magliani, 1997; Marquin et al., 2002]. In Georgian wine yeast populations high frequency of neutral strains is caused by inactivation of cytoplasmic determinant, as well as by action of nucleus genes. Intrapopulation polymorphism in wine and beer yeast is revealed and confirmed [Naumov & Naumova, 1973; Magliani et al., 1997; Gulbiniene et al., 2004; Shatirishvili et al., 2007]. As a result of studies of strains isolated from materials obtained from wine factories and museums, it was established that some “killer” systems occupy definite ecological niche. Among wine yeasts ecological niche takes up K2 system [Naumov & Naumova, 1973; Gulbiniene et al., 2004; Shatirishvili et al., 2007].

Wine fermentation is complicated and multistage process, in which bacteria, yeast fungi, viruses (like killer systems) take part. Complex biochemical, genetic, and ecological interactions

are formed between microorganisms [Fleet & Heard, 1993; Zagorc et al, 2001; Sangorrín et al., 2002]. Specific form of interspecific competition - amensalism acts [Rib'ereu-Gayon et al., 2006]. Representatives of wine yeast population are subjected to the intra- and interspecific competition. In intraspecific competition elimination of forms sensitive to toxin isolated by "killer" strains is significant [Yap et al., 2000; Lowes et al., 2002]. During the process of natural selection viability of organisms and their adaptation towards vital conditions are examined.

To find out and study "killer" phenotype strains having wide spectrum of action has great practical importance. Control of alcohol fermentation should be carried out by "killer" strains. "Killer" strains expel weed species from grape juice got in occasionally from environment and alcohol fermentation should be conducted with only "killer" strains obtained by selection and having the best characteristics.

Table 1. Frequencies of K, N and S phenotype strains in micropopulations of Khetsubani (Kobuleti) wine yeast population

micropopulation	number of strains	Killer (K)		Neutral (N)		Sensitive (S)	
		number	%	number	%	number	%
I	25	1	4	1	4	23	92
II	25	-	-	23	92	2	8
III	25	-	-	-	-	25	100
IV	25	1	4	4	20	20	80
V	25	4	16	7	28	14	56
VI	25	1	4	20	80	4	16
VII	25	6	24	11	44	8	32
VIII	25	-	-	19	76	6	24
IX	25	5	20	6	24	14	56
X	25	-	-	-	-	25	100
total	250	18	7.2	93	37.2	139	55.6

Table2. Frequencies of K, N and S phenotype strains in micropopulations of Kapreshumi (Batumi) wine yeast population

micropopulation	number of strains	Killer (K)		Neutral (N)		Sensitive (S)	
		number	%	number	%	number	%
I	25	-	-	-	-	25	100
II	25	2	8	-	-	23	92
III	25	2	8	-	-	23	92
IV	25	18	72	2	8	5	20
V	25	4	16	7	28	14	56
VI	25	1	4	20	80	4	16
VII	25	-	-	13	52	12	48
VIII	25	-	-	19	76	6	24
IX	25	2	8	3	12	20	80
X	25	-	-	9	36	16	64
total	250	29	11.6	73	29.2	148	59.2

Table 3. Frequencies of K, N and S phenotype strains in micropopulations of Kardenakhi wine yeast population

micropopulation	number of strains	Killer (K)		Neutral (N)		Sensitive (S)	
		number	%	number	%	number	%
I	25	2	8	19	76	4	16
II	25	2	8	17	68	6	24
III	25	7	28	18	72	-	0
IV	25	8	32	12	48	5	20
V	25	1	8	15	60	9	36
VI	25	5	20	9	36	11	44
VII	25	9	36	8	32	8	32
VIII	25	13	52	12	48	-	0
IX	25	4	8	18	72	3	12
X	25	3	12	16	64	6	24
total	250	54.0	21.6	144	57.6	51	20.4

Table 4. Frequencies of K, N and S phenotype strains in micropopulations of Mirzaani wine yeast population

micropopulation	number of strains	Killer (K)		Neutral (N)		Sensitive (S)	
		number	%	number	%	number	%
I	50	9	18.0	41	82.0	-	-
II	50	1	2.0	46	92.0	3	6.0
III	50	8	16.0	37	74.0	5	10.0
IV	50	8	16.0	35	70.0	7	14.0
V	50	17	34.0	27	54.0	6	14.0
VI	50	5	10.0	44	88.0	1	2.0
VII	50	5	10.0	43	86.0	2	4.0
VIII	50	7	14.0	41	82.0	2	4.0
IX	50	10	20.0	40	80.0	-	-
X	50	9	18.0	21	42.0	20	40.0
total	500	79	15.8	375	75.0	46	9.2

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კახეთისა და აჭარის მცირე ფერმერულ მეურნეობებში ალკოჰოლურ ფერმენტაციაში მონაწილე ღვინის საფუარის ბენეტიკური უმსწავლა

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რეზიუმე

ეკოლოგიურად განსხვავებული ორი რეგიონის: კახეთისა (კარდენახი, მირზაანი) და აჭარის (კაპრეშუმი, ხუცუბანი) ღვინის საფუარის ბუნებრივი პოპულაციიდან გამოყოფილ შტამებში შესწავლილია ანტაგონისტური აქტივობა. პოპულაციები გამოყოფილია მცირე ფერმერულ მეურნეთა მარნიდან, სადაც ყურძნის წვენი ფერმენტაციის პროცესი სპონტანურად მიმდინარეობდა. ღვინის საფუარის ბუნებრივ პოპულაციებში გამოვლენილია K, N და S ფენოტიპის შტამები. პოპულაციები შემადგენელი სტრუქტურით განსხვავებული აღმოჩნდა. კახეთის ღვინის საფუარის პოპულაციაში მაღალი სიხშირითაა კილეური და ნეიტრალური ფენოტიპის შტამები, ხოლო აჭარის პოპულაციებში მგრძობიარე შტამები. კახეთისა და აჭარის ღვინის საფუარის პოპულაციებში ეკოლოგიური ნიში K2 სისტემას უჭირავს.

COMPARISON OF THE IMMUNOGENICITY OF A MUTANT RECOMBINANT hCG β -PROTEIN CONJUGATED TO DIFFERENT CARRIER MOLECULES

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Abstract

Human chorionic gonadotropin (hCG) is a heterodimeric hormone consisting of an α -chain, shared with other glycoprotein hormones, and an hCG-specific β -chain. Since hCG is instrumental in the early maintenance of pregnancy, and is an important tumor growth factor, the hormones and its components have been a target for a development of immunocontraceptive and anti-cancer vaccines.

We have constructed three prototypes of anti-hCG vaccines based upon the recombinant hCG β -chain (hCG β) with a single point mutation of arginine⁶⁸ to glutamic acid [hCG β (R68E)]. In two of them hCG β (R68E) has been conjugated to heat shock protein 70 (hCG β (R68E)-HSP70) or to keyhole limpet hemocyanin (hCG β (R68E)-KLH) as carrier molecules, and the third contained hCG β (R68E) alone. Mice were immunized with these preparations, and specificity and immunogenicity of the sera against native hCG $\alpha\beta$, hCG β -chain and C-terminal peptide of hCG β (CTP) was studied using a direct binding ELISA. All three preparations induced appreciable antibody immune responses to the proteins studied. However whilst hCG β (R68E)-KLH and hCG β (R68E) alone elicited high titers of anti-CTP antibodies, the sera reacted weaker with the native hCG. In contrast, the anti-hCG β (R68E)-HSP70 sera contained high titers of anti-CTP, anti-hCG $\alpha\beta$ and anti-hCG β antibodies. Our data argues in favor of the effectiveness of hCG β (R68E)-HSP70 for a vaccine formulation.

Key words: Human chorionic gonadotropin (hCG), mutant recombinant hCG β protein, anti-hCG vaccines

Introduction

Human chorionic gonadotropin (hCG) is a placental member of the glycoprotein hormone (GPH) family which also includes the pituitary hormones: follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH) [Stenman et al., 2006]. They are heterodimers consisting of a common alpha and a hormone-specific beta subunit. The β -subunits are partly homologous to each other. The closest homology is between hCG β and hLH β [Lapthorn et al., 1994].

hCG is a pregnancy hormone secreted by the pre-implantation blastocyst and, subsequently, by the trophoblast [Fox & Kharkongor, 1970]. Since it is responsible for the

maintenance of the corpus luteum in early pregnancy, blocking the action of this hormone with antibodies prevents pregnancy. Thus, vaccines based on hCG can be used for immunocontraception [Stevens, 1999; Talwar, 1999; Delves et al., 2002].

Later it has been shown that the levels of serum hCG strongly correlate with trophoblastic germ cell tumors, and it was used for diagnosis and monitoring tumor burden and for evaluating the effectiveness of therapeutic intervention [Bidart et al., 1999; Braunstein, 1990]. Elevated serum hCG β levels and/or tissue expression were since used as an independent predictor of an unfavorable disease outcome and were associated with a more aggressive disease course in renal, colorectal, bladder, and pancreatic cancers [Hotakainen et al., 2002; Shah et al., 2008]. It was proposed that hCG may act at different levels to facilitate cancer progression: as a transforming growth factor, an immunosuppressive agent, an inducer of metastasis, and/or an angiogenic factor [Triozzi & Stevens 1999]. Therefore, the hCG-based vaccines are been tested in anti-tumor therapy [Triozzi & Stevens 1999; Delves et al., 2007].

Vaccines, which incorporate the full-length hCG β -chain, stimulate the production of antibodies that cross-react with hLH due to the 85% amino acid sequence homology of the first 110 amino acids of the β -chains of the two hormones. As a strategy aimed at reducing hLH cross-reactivity with hCG several hCG β -chain mutants were engineered by using oligonucleotide-directed site-specific mutagenesis [Delves et al., 2007; Porakishvili et al., 2002]. One of the mutant molecules hCG β (R68E) containing an arginine to glutamic acid replacement at a position 68 in the protein sequence has grossly diminished ability to provoke hLH cross-reactive antibodies whether administered intranasally to mice or intramuscularly to rabbits [Delves et al., 2007; Porakishvili et al., 2002]. Much of anti-hCG β (R68E) antibodies were re-focused towards normally weakly immunogenic C-terminal peptide (CTP) unique for hCG β and not shared by hLH. In the mutant hCG molecule CTP acquires the properties of an immunodominant epitope [Porakishvili et al., 2002; Charrel-Dennis et al., 2005].

However a vaccine preparation based on the mutant hCG β (R68E) has not been finalised regarding an optimal carrier molecule and an adjuvant. The aim of this study was to assess immunogenicity of the sera elicited in response to hCG β (R68E) conjugated to heat shock protein 70 (HSP 70) or to keyhole limpet hemocyanin (KLH) compared to the hCG β (R68E) alone.

Materials and Methods

Antigens

Native hCG $\alpha\beta$: hCG $\alpha\beta$ -heterodimer purified from human pregnancy urine (Sigma, USA).

Native hCG β : hCG β -chain purified from human pregnancy urine (Sigma, USA).

CTP: the C-terminal peptide sequence synthesized as a peptide representing amino acids 110-145 of the hCG β -chain (Sigma, USA).

Mutant hCG β (R68E): recombinant hCG β containing a single amino acid substitution expressed in High FiveTM cell cultures (Invitrogen, USA) using baculovirus expression system. Recombinant protein was purified from supernatants of High Five cell cultures using Immobilized metal affinity chromatography (IMAC). Supernatants were loaded on HiTrap Chelating HP 1ml columns (Pharmacia, USA), charged with Ni ions and His-tagged proteins eluted with Imidazole step gradient (0.04-0.4M). Purity was examined by SDS PAGE on Phast Gel system (Pharmacia, USA) followed by Silver staining or Western Blotting procedure (Fig.1).

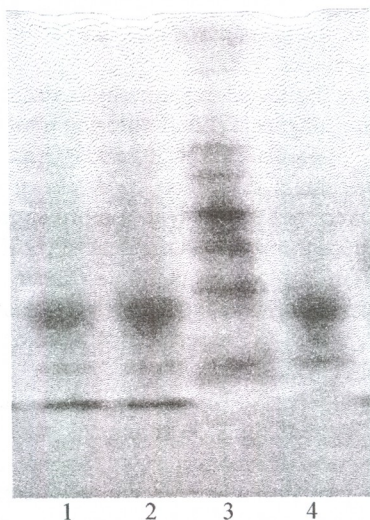


Fig. 1. SDS PAGE analysis of purified R68E samples

The samples were analyzed on Phast System Using PhastGel 12.5% homogeneous, followed by Western Blotting. Lanes 1, 2, 4 R68E purified from High Five cell supernatants. Lane 3- molecular weight markers- 150, 100, 75, 50, 35, 15 kDa.

Carrier protein-conjugation

2 mg of HSP70 or KLH was dissolved in 200 μ l conjugation buffer (0.1 M MES (2-[*N*-morpholino] ethane sulfonic acid), pH 4.5-5). 2mg hCG β (R68E) was dissolved separately in 500 μ l of conjugation buffer. 500 μ l protein solution was added to the 200 μ l carrier protein solution and 50 μ l of a zero-length crosslinking agent EDC (1-Ethyl-3-(3-Dimethylaminopropyl) carbodiimide Hydrochloride) solution at a concentration of 10mg/ml was immediately added to the carrier-peptide solution. After incubation for 2 hrs at RT, the conjugated protein was isolated through Sephadex G-25M column.

Immunization of mice

6 week old female Balb/c mice (seven mice per each of the antigen preparation) were immunized at the University College London Animal house. Montanide ISA720 was used as adjuvant in all cases prepared as follows: 70 μ g of immunogen was diluted in 450 μ l of sterile saline and added to 1,050 μ l of ISA720 to achieve a ratio of antigen/ISA720 30:70 according to the manufacturer's instructions (SEPPIC, Paris, France). The mixture was vortexed for 30 minutes and further mixed in syringe to form emulsion. The mice were immunized 2x 100 μ l s.c. with 10 μ g total immunogen/mouse, boosted after 5 weeks as above, and sera collected a week after the boost.

Determination of serum titres:

The titres of the sera were determined using a direct binding ELISA. Nunc MaxisorpC 96-well flat-bottomed microtiter plates were coated overnight at 4 $^{\circ}$ C with 50 μ l of native hCG $\alpha\beta$, native hCG β or ovalbumin (control), all at 2 μ g/ml and hCG β CTP at 1 μ g/ml in 0.05M carbonate-bicarbonate buffer (CBB, pH=9.6.). The plates were washed three times with PBS (Sigma)

containing 0.05% Tween 20 (PBS-T), followed by blocking with 200 μ l/well of 2% dried skimmed milk powder (marvel, UK) in CBB overnight at 4°C. Serum from the immunized mice was diluted in PBS-T supplemented with 2% bovine serum albumin (PBS-T-BSA) in serial dilutions from 1:50 to 1:25,600. After washing the plates three times with PBS-T, 50 μ l of each dilution of the sera was added to the plates in duplicates and incubated for 2hrs at 37°C. The plates were washed three times with PBS-T before 50 μ l of rabbit anti-mouse IgG peroxidase conjugated antibody (Sigma) was added for 2 hrs at 37°C. Following a further four washes with PBS-T, 100 μ l of the substrate 3,3',5,5'-Tetramethylbenzidine (TMB) was added, the plates left for 5 min at RT, and then the reaction was stopped with 100 μ l of 0.05M H₂SO₄. The plates were read at A450nm in a spectrophotometer (Humanreader, Human GmbH). The 50% titer was calculated as the dilution of serum corresponding to 50% of the plateau and the end-point titer was chosen as the highest but one dilution giving an o.d. above the control.

Results and Discussion

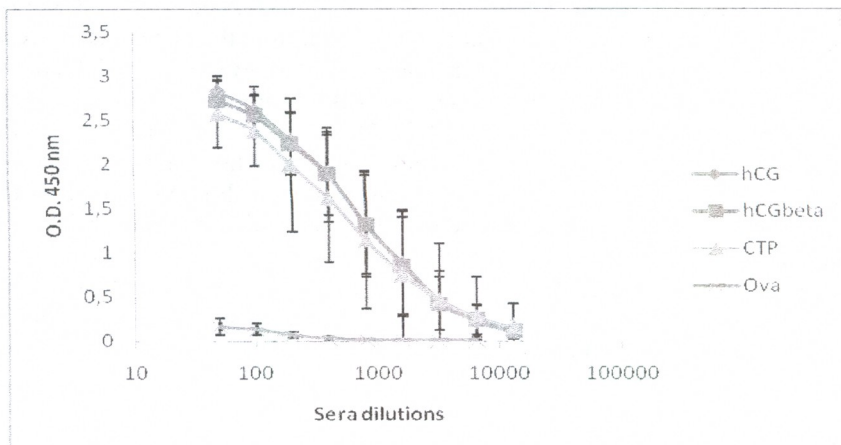
In order to study specificity and immunogenicity of the immune sera the titers of anti-hCG, anti-hCG β and anti-CTP were determined in mice immunized with recombinant protein hCG β (R68E) conjugated with HSP70 and KLH as carrier molecules, compared to the immunogen hCG β (R68E) alone. All sera showed appreciable levels of antibodies to the protein studied compared to the control ovalbumine protein.

Immunization with hCG β (R68E)-HSP70 raised equally high titers of antibodies to the native hCG $\alpha\beta$ heterodimer, native hCG β -chain and CTP. The 50% titer of these antibodies was defined as 1:800, whilst the end point titer was detected as 1:12800, indicating a high concentration of the respective antibodies (Fig. 2a). However antibodies raised in response to hCG β (R68E)-KLH and to hCG β (R68E) alone (Fig. 2, b and c) reacted strongly with CTP (50% titer 1:400 and end-point titer 1:6400), but not with the native hCG $\alpha\beta$ or hCG β (50% of titer 1:200, end point titer-1:3200).

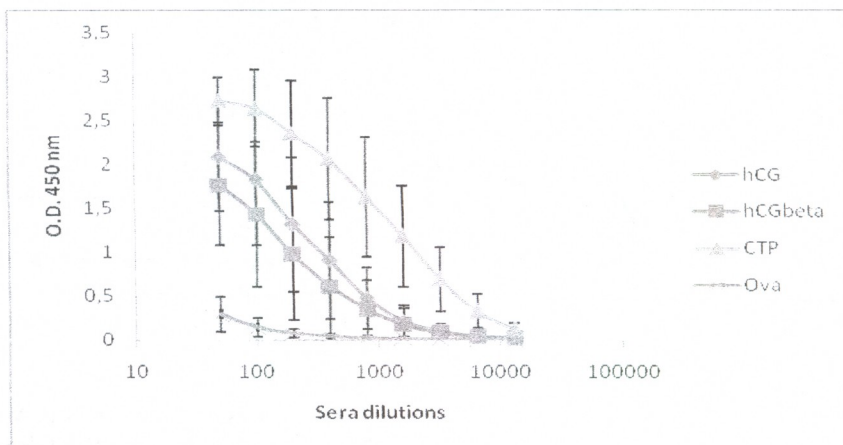
Sera from mice immunized with a) hCG β (R68E)-HSP70 (n=7), b) hCG β (R68E)-KLH (n=7) and c) hCG β (R68E) (n=7) were analyzed using direct ELISA as described in Materials and Methods, plates coated with proteins as indicated. The sera was diluted in PBS-T-BSA at 1:50 - 1:25,600 and the results were expressed as optical density, M \pm SD.

Detection of the high titers of the anti-CTP antibodies in experimental sera was expected (Fig. 2a,b,c). We have previously shown that a single arginin⁶⁸ to glutamic acid substitution dramatically alters the antigenicity and immunogenicity of the mutant molecule by re-focusing it to the CTP region of the β -chain [Porakishvili et al., 2002; Charrel-Dennis et al., 2005]. It was notable in this study that sera of the mice immunized with hCG β (R68E)-HSP70 reacted with CTP with the same strength as with the native hCG $\alpha\beta$ and hCG β . It indicates that these sera could be directed to a CTP epitope which is equally attainable in a free CTP subunit as well as in native hCG $\alpha\beta$ and hCG β . It is supported by our previous observation that in rabbits immunization with hCG β (R68E) lead to re-focussing of antibody response to CTP [Porakishvili et al., 2002]. Alternatively, the sera main contain high affinity antibodies to CTP as well as to another unidentified co-dominant epitope expressed on hCG β . Indeed, sera immunized with hCG β (R68E)-KLH or hCG β (R68E) alone contained higher titres of anti-CTP antibodies than of those directed to hCG $\alpha\beta$ and hCG β .

a)



b)



c)

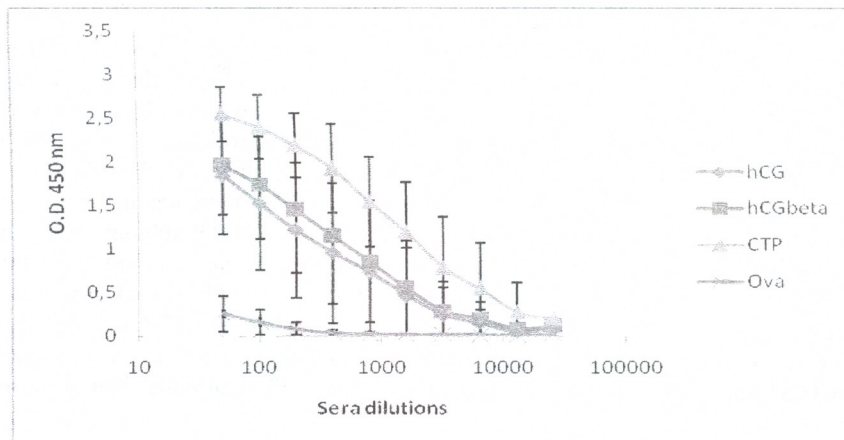


Fig. 2. Titration of anti-hCG, anti-hCGβ and anti-CTP antibodies after immunization with a) hCGβ(R68E)-HSP70, b) hCGβ(R68E)-KLH and c) hCGβ(R68E).

This makes HSP70 the most efficient carrier for mutant hCG β (R68E) molecule for a proposed vaccine formulation. The fact that the immunization with this conjugate induced equally strong anti-CTP and anti-native hCG $\alpha\beta$ /hCG β immune responses means that such sera *in vivo* would bind with high affinity to the endogenous hormone and its beta subunit. This makes hCG β (R68E)-HSP70 conjugate a good potential candidate for the development of hCG-based vaccine, particularly that both hCG holohormone and its beta subunit have been identified as tumor growth factors [Braunstein, 1990; Triozzi & Stevens, 1999]. Recently we have demonstrated that the immune complexes formed by anti-hCG β (R68E) rabbit sera and native hCG are successfully phagocytosed *in vitro* by monocytes and neutrophils, indicating a good clearance capacity *in vivo*.

Furthermore, the C-terminal peptide of hCG does not take part in binding to or in activation of the hCG/hLH receptor expressed by variety of cancer cells [Matzuk et al., 1990]. Therefore anti-CTP antibodies would be able to bind to hCG, already bound to the receptor and trigger a cytotoxic attack against the cancer cells. Indeed, peptides chaperoned by the HSP70 can be taken up quite efficiently in a receptor-mediated manner (CD91, LOX-1) by antigen-presenting cells such as dendritic cells (DCs) [Basu et al., 2001; Srivastava, 2002a], resulting in the peptide cross-presentation via HLA class I molecules [Castellino et al., 2000]. Heat shock proteins, especially HSP70, have gained much attention as enhancers of antigen-specific CD8⁺ cytotoxic as well as CD4⁺ T-helper cell (Th1) responses by re-presentation of antigens [Srivastava, 2002b]. We are currently studying cellular immune responses elicited upon immunization with hCG β (R68E) preparations.

In conclusion, this study demonstrated that the HSP70 increases immunogenicity of the mutant molecule hCG β (R68E) since antibodies generated against hCG β (R68E)-HSP70 conjugate bind with high affinity to the CTP region of hCG, as well as to the native holohormone hCG $\alpha\beta$ and its hCG β subunit. Thus, hCG β (R68E)-HSP70 is an effective prototype of hCG-based vaccine characterized by a high degree of immunogenicity.

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**ადამიანის ქრონიული გონადოტროპინის β -ჯაჭვის ანალოგი
მუტანტური, რეკომბინანტული ცილის და სხვადასხვა
მოლეკულა-მატარებლების კონუგაციით მიღებული კონუგატების
იმუნოგენურობის შეფარებითი დასასიათება**

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(მიღებულია 09.09.2010)

რეზიუმე

ადამიანის ქრონიული გონადოტროპინი (hCG) ა და β ჯაჭვების შემცველი პეტროლიმერული ჰორმონია, რომლის ა ჯაჭვიც იგივეა რაც დანარჩენი გლიკოპროტეინული ჰორმონების ა ჯაჭვი, ხოლო β ჯაჭვი კი hCG სპეციფიურობისაა. გამოძინარე იქიდან, რომ hCG ხელს უწყობს ფეხმძიმობის შენარჩუნებას ადრეული პერიოდიდან და ამასთან ერთად წარმოადგენს სიმსივნური ზრდის ფაქტორს, ეს ჰორმონი და მისი კომპონენტები წარმოადგენს სამიზნეს იმუნოკონტრაცეპტული და სიმსივნესაწინააღმდეგო ვაქცინის შექმნისათვის. ჩვენ შევქმენით hCG-ს საწინააღმდეგო ვაქცინის სამი პროტოტიპი, რომელთა ძირითადი კომპონენტია ერთი წერტილოვანი მუტაციის შედეგად 68-ე ადგილზე მდგომი არგინინის გლუტამინის მუტაციით ჩანაცვლების შედეგად მიღებული რეკომბინანტული hCG β ჯაჭვი (hCG β (R68E)). ვაქცინის ერთ პროტოტიპში აღიშნული მოლეკულა კონუგირებულია სითბური შოკის ცილა 70-თან (hCG β (R68E)-HSP70), მეორე პროტოტიპში მოლუსკის ცილა keyhole limpet hemocyanin-თან (hCG β (R68E)-KLH), ხოლო მესამე პროტოტიპი წარმოადგენს მხოლოდ რეკომბინანტულ ცილას მოლეკულა-მატარებლის გარეშე (hCG β (R68E)). აღნიშნული კონუგატები გამოყენებულ იქნა თავგების იმუნიზაციისათვის. ჩვენ შევისწავლეთ მიღებული შრატების სპეციფიურობა და იმუნოგენურობა hCG $\alpha\beta$, hCG β ცილის და hCG β -ჯაჭვის C-ტერმინალური პეპტიდის – CTP მიმართ არაპირდაპირი იმუნოფერმენტული ანალიზით (ELISA). სამივე კონუგატით იმუნიზაციის შედეგად მიღებულ შრატებში აღმოჩნდა ექვირემენტული ცილების სპეციფიურობის ანტისხეულების მაღალი დონე. თუმცა, მიუხედავად იმისა, რომ hCG β (R68E)-ის და არაკონუგირებული მუტანტური ცილის საპასუხოდ სინთეზდება მაღალი ტიტრის ანტი-CTP ანტისხეულები, ეს შრატები სუსტად რეაგირებენ ნატიურ hCG-სთან. საპირისპიროდ, ანტი- hCG β (R68E)-HSP70 შრატი შეიცავს ანტი-CTP, ანტი-hCG $\alpha\beta$ და ანტი-hCG β ანტისხეულების თანაბრად მაღალ ტიტრებს. ჩვენი შედეგები გამოხატავს კონუგატი hCG β (R68E)-HSP70-ის ეფექტურობას ვაქცინის შექმნისათვის.

CONTRIBUTION TO THE FUNGAL DIVERSITY OF GEORGIA: FUNGI ASSOCIATED WITH MAHALEB CHERRY, *PRUNUS* *MAHALEB* L.

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Abstract

New records of fungi to Georgia and new host-fungus combinations recorded in Tbilisi environs are given for *Prunus mahaleb* (*Cerasus mahaleb*).

Key words: Fungi, Mahaleb cherry, Tbilisi Botanical Garden (TBG), Tbilisi environs (TE), Georgia.

Introduction

Prunus mahaleb is deciduous tree or shrub up to 5-6 (10) m tall. Flowers April-May, fruits ripen (June) July-August. Grows in semi-arid forests and shrublands on slopes mainly stony and skeleton substrates and forest edges. Used as ornamental, dye, very suitable rootstock for cultivated cherries. In some countries (Armenia, Greece, Syria, Lebanon, Cyprus) mahaleb cherry is known as a spice and also used for sweet breads and confectionery, special type of Easter cheese pie cake, liquor, etc.

It should be noted that in environs of Tbilisi variability in the shape of fruits from globose to ovoid with a pointed kernels are being observed. However as it is known to us these features are not properly assessed and nomenclaturally formalized. In Georgia according to the mycological and phytopathological sources [Voronikhin, 1927; Bedoidze, 1975; Gvritishvili, 1982; Ulianishchev et al., 1985; Gvritishvili et al., 2003] on *Prunus (Cerasus) mahaleb* the following 7 species of microfungi have been recorded: *Coccomyces hiemalis*, *Cytospora leucosperma* (*Valsa ambiens*), *C. leucostoma* (*V. leucostoma*), *Taphrina wiesneri*, *Tranzschelia discolor*, *T. pruni-spinosae*.

Materials and Methods

The materials include specimens collected during the field observations. They contain biotrophic and necrotrophic parasitic micromycetes associated with living and dead stems and leaves of Mahaleb cherry including fire injured plants. Routine light microscopic method has been used for identification of collected specimens on the base of macro- and micromorphological features.

Results and Discussion

The list of all parasitic and other fungi occurring on mahaleb cherry in TBG and TE is given below.

List of fungi associated with *Prunus mahaleb* in TBG and TE

- Alternaria tenuis* Nees (*A alternata* (Fr.: Fr.) Keissl.)
Blumeriella jaapii (Rehm) v.ArX (*Coccomyces hiemalis* Higg.)
 (Anamorph: *Phloeosporella padi* (Lib.) v. ArX (*Cylindrosporium padi* (Lib.) P. Karst.: Sacc.)
Botryosphaeria dothidea (Schwein.) Shoemaker
Botrytis cinerea Per.: Fr.
Cladosporium herbarum (Pers.) Link
Cryptovalsa pruni Fuck.
Cyclothyrium juglandis (Schum.: Rabenh.) B. Sutton
Cytospora leucosperma (Pers.: Fr.) Fr.
Cytospora leucostoma (Pers.: Fr.) Fr.
Dermea cerasi (Pers.; Fr.) Fr. (Anamorph: *Micropera drupacearum* Lév.)
Diatrypella quercina (Pers.) Cooke
Diplodia cerasorum Fuck.
Diplodia pruni Fuck.
Eutypella microsperma Karst.& Malbr.
Eutypella prunastri (Pers.; Fr.) Sacc.
Fusicoccum sp.
Hypoxylon coccineum Bull. (*H. fragiforme* (Pers.: Fr.) Kickx
Microsphaeropsis olivacea (Bonord.) Höhn.
Monilia cinerea Bonord.
Nectria cinnabarina (Tode: Fr.) Fr. (Anamorph *Tubercularia vulgaris* Tode.: Fr.)
Phoma pruni-japonicae Syd.
Phomopsis perniciososa Grove
Polyporus varius (Pers.: Fr.) Fr
Schizophyllum commune Fr.
Sclerophoma pruni Died.
Septobasidium sp.
Septoria magnispora Peck.
Sphaerellopsis filum (Biv., Berm.) B.Sutton (on *Tranzschelia discolor* and *T. pruni-spinosae*)
Stigmia carpophila (Lev.) M.B.Ellis.
Taphrina wiesnerii (Ráthay) Mix
Trametes versicolor (L.) Pilát
Tranzschelia discolor (Fuck.) Tranz & Litv.
Tranzschelia pruni-spinosae (Pers.) Diet.
Tubercularia vulgaris Tode: Fr.
Valsa ambiens (Pers.: Fr.) Fr. (Anamorph: *Cytospora leucosperma* (Pers.: Fr.) Fr.)
Valsa leucostoma (Pers.: Fr.) Fr. (*Leucostoma persoonii* (Nitschke) Höhn.; Anamorph: *Cytospora leucostoma* (Pers.: Fr.) Fr.).

This list contains mainly biotrophic and necrotrophic parasites (micromycetes) such as two rust fungi *Tranzschelia discolor*, *T. pruni-spinosae*, causative agents of leaf disease appearing from second half of June. From other fungi the biotrophic parasites *Blumeriella jaapii* causing leaf spot of *Prunus* spp. can be mentioned.

More interesting finding is *Septobasidium* sp. with brown resupinate basidiocarps, brightly colored in the peripheral part, felty in texture. As it is known “Species of *Septobasidium* appear to have a mutualistic relationship with their insect associates and to cause them little harm. A colony of scale insects can be covered entirely by a fungal colony and thus derive partial protection from predators. In return a few individuals of the colony are invaded by helical haustoria of the fungus. Infected individuals do not die, in fact they may outlive uninfected neighbours. The only apparent disadvantage is that they cannot reproduce.” [Benjamin et al., 2004].

It is interesting that up to now the only one species of the genus *Septobasidium*, *S. pedicellatum* associated with *Mespilus germanica*, *Malus domestica* and *Pyrus communis* [Voronikhin, 1927, Shoshiashvili, 1940] is known to occur in West Georgia.

Unfortunately, the insect associates in these old collections as well as in new samples of a great number of different woody plants collected by M. Gvritishvili in East Georgia (mainly in TE) still remain to be unidentified.

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მასალები საქართველოს სოკოების მრავალფეროვნების უმსწავლისათვის: ბაღლოჯთან (*Prunus mahaleb* L.) ასოცირებული სოკოები თბილისის მიდამოებში

გვრიტიშვილი მ., ყაჭეიშვილი-თავართქილაძე ქ., გოცაძე ნ.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

(მიღებულია 04.10.2010)

რეზიუმე

ნაშრომში წამოდგენილია ბაღლოჯთან ასოცირებული ლიტერატურაში ცნობილი და ავტორთა მიერ გამოვლენილი საქართველოში აქამდე უცნობი სოკოებისა და მცენარე-სოკოს ახალი კომბინაციების სია და მათთან დაკავშირებული ზოგიერთი შენიშვნა.

NATURAL OCCURRENCE AND DISTRIBUTION OF *BEAUVERIA BASSIANA* IN PINE FOREST ECOSYSTEM OF EAST GEORGIA

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Abstract

The study on 10 different sites of different altitudes (600-1700 m a.s.l) of pine forest in East Georgia was conducted during 2009-2010. A total 30 soil samples were investigated and 6 entomopathogenic fungal species: *Beauveria bassiana*, *Beauveria brongniatii*, *Metarhizium anisopliae*, *Metarhizium flavoviride*, *Lecanicillium* sp. and *Aspergillus flavus* were isolated. Entomopathogenic fungi were isolated from 22 of the 30 (73.3 %) soil samples. In these samples *B. bassiana* was the most common. Of the 30 soil samples, 17 yielded *B. bassiana* (56.6 %), 2 - *M. anisopliae* (6.7 %), 2 - *M. flavoviride* (6.7 %), 3 - *Lecanicillium* sp. (10%) and 4 - *Aspergillus flavus* (13.3%). *Beauveria brongniatii* (3.3%) was isolated from only 1 sample, 2 soil samples (6.7%) harboured with *B. bassiana* and *Lecanicillium* sp. and *B. bassiana* and *Aspergillus flavus*, no entomogenous fungi were isolated from 8 of the soil samples (26.7%). Only 1 species was recorded in any individual infected larva, except the case when the larvae was infected with two fungal species - *B. bassiana* and *Lecanicillium* sp.

Key words: Pine forest, soil, entomopathogenic fungi, *Beauveria bassiana*, *B. brongniatii*, *Metarhizium anisopliae*, *M. flavoviride*, *Lecanicillium* sp., *Aspergillus flavus*

Introduction

Pine forests play a key ecological and social-economical role for the Georgia which is represented by *Pinus eldarica*, *Pinus nigra*, *Pinus sosnovskyi*. Pines as well as other species are damaged by numerous pest insects. Most of them are in close contact with soil during their life cycle. However, we don't have the complete representation of biological factors which are responsible for the collapse, their role in regulations of population dynamics.

Soil-inhabiting entomopathogenic fungi are an important and widespread component of most terrestrial ecosystems and play a key role in regulating insect populations, particularly soil-dwelling insect pests [Keller & Zimmerman 1989; Jackson et al., 2000]. Many species belonging to the Hypocreales (Ascomycota) inhabit the soil for a significant part of their life cycle when they are outside of their insect host. Among them, *Beauveria* spp., *Metarhizium anisopliae*, and *Paecilomyces* spp. are especially common [Keller & Zimmerman 1989].

Currently, fungal biological control agents frequently perform inconsistently in the soil due to a lack of environmental competence [Jackson et al., 2000]. An understanding of the

parameters that determine the diversity and distribution of entomopathogenic fungal species in the soil would help to identify those species best suited to a particular environment and improve biological control efficacy. The effects of factors such as geographical location, climatic conditions, habitat type, cropping system, and soil properties on the occurrence and distribution of insect pathogenic fungi have been studied by several authors [Tkaczuk & Mietkiewski 1996; Tarasco et al., 1997; Keller et al., 2003; Bruck, 2004; Meyling & Eilenberg 2006].

Entomopathogenic fungi (EPF) naturally present in the soil constitute a reservoir of inoculums of antagonists for insect pest. The use of local EPF will be appropriate to control insects since these strains may be better adapted to local environmental conditions.

The cosmopolitan anamorphic fungus *Beauveria bassiana* (Ascomycota: Hypocreales) is a well recognized entomopathogen known to infect hundreds of host species belonging to most insect orders. This ability has prompted extensive research on the potential of *B. bassiana* for biological control of pests and several commercial products have been developed [Inglis et al., 2001]. *B. bassiana* is a facultative pathogen and the fungus can survive saprotrophically in the soil environment for extended time periods [Keller & Zimmermann, 1989].

The aim of our work was study of the occurrence and spatial distribution of EPF *Beauveria bassiana* in the soil from different habitats in pine forest. The use of local EPF will be appropriate to control insects since these strains may be better adapted to local environmental conditions.

Materials and Methods

The study was conducted in 2009-2010, on 10 different sites at different altitudes of pine forests in East Georgia: 3 locations in Lower Kartli (600-700 m a.s.l.); 3 locations in Middle Kartli (850-900 m a.s.l.), 3 locations in Samtskhe-Javakheti (900-1700 m a.s.l.) and 1 location in Borjomi-Kharagauli National Park.

EPF were isolated from soil samples by the 'Galleria bait method' [Zimmermann, 1986]. The wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) and *Tenebrio molitor* larvae were used for baiting the soil samples. Each soil sample was baited with 10 larvae and the cups were incubated in the dark in closed cardboard boxes at room temperature (20–25°C). Incubated larvae were inspected for presence of external fungal growth. The soil samples were suspended in a distilled water with solution of 2.5 g per 50 ml in a 100 ml flask, and then strongly shaken. For the isolation and presence of EPF different artificial media (SDAY, PDA) were used.

The fungi were identified morphologically both by low magnifying stereomicroscopy (40x magnification) of cadavers and by preparing slides for light microscopy (300-1500 x magnification). The material was studied using generally accepted methods in insect fungal pathology [Evlakhova et al., 1961; Humber, 1997].

Results and Discussion

A total 30 soil samples were investigated. In the soils from Pine forest ecosystem of East Georgia six entomopathogenic fungal species: *Beauveria bassiana*, *Beauveria brongniatii*, *Metarhizium anisopliae*, *Metarhizium flavoviride*, *Lecanicillium* sp. and *Aspergillus flavus* were isolated. The dominance of particular species depended on soil type and habitat (Fig.1).

EPF were isolated from 22 of the 30 (73.3 %) soil samples. In these samples *B. bassiana* was the most common. Of the 30 soil samples, 17 yielded *B. bassiana* (56.6 %), 2 - *M. anisopliae*, 2 - *M. flavoviride* (6.7 %), 3 - *Lecanicillium* sp. (10%) and 4 - *Aspergillus flavus* (13.3%). *Beauveria brongniatii* was isolated from only 1 sample (3.3%), 2 soil samples harboured with *B.*

bassiana and *Lecanicillium* sp. and *B. bassiana* and *Aspergillus flavus* (6.7%), no entomogenous fungi were isolated from 8 of the soil samples (26.7%). Only one species was recorded in any individual infected larva, except the case when the larvae was infected with two fungal species - *B. bassiana* and *Lecanicillium* sp.

Names and parameters of studied areas of pine forests are presented in Table 1.

Table 1. Sites of investigation area

N	Place of samples collection	GPS		altitude a.s.l. (m)
		Longitude (L)	Latitude (N)	
1	Teleti	41° 38' 29"	43° 48' 75"	600
2	Shavnabada	41° 46' 12"	43° 22' 27"	700
3	Sagarejo	41° 46' 12"	43° 22' 27"	1300
4	Surami	42° 00' 55"	43° 35' 21"	780
5	Likani	41° 52' 40"	43° 22' 35"	900
6	Patara Tsemi	41° 44' 12"	43° 32' 14"	1600
7	Bakuriani	41° 45' 45"	43° 30' 58"	1750
8	Rabati	41° 34' 29"	42° 51' 47"	1400
9	Ackuri	41° 44' 51"	43° 10' 22"	1200
10	Nunisi	41° 43' 37"	43° 52' 26"	980

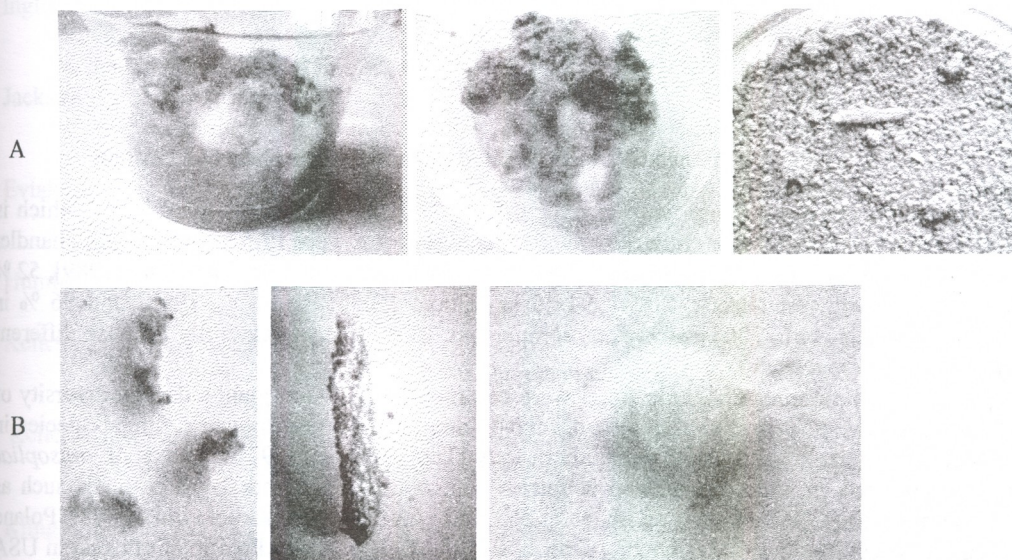


Fig.1. Isolation of EPF from soil using "Bait" method: A – Soil sample with *Tenebrio molitor*, B - infected larvae by entomopathogenic fungi.

Comparison of the number of entomopathogenic fungi isolated from pine forest soils is given in Fig. 2.

This is the first investigation of insect pathogenic fungi in the soil environment in pine forest of East Georgia. The insect pathogenic fungal species detected in Georgia were similar to those from other parts of the world [Vänninen, 1995; Bidochka et al., 1998; Meyling & Eilenberg, 2006]. The wide distributions and high occurrences of *Beauveria bassiana* in natural soil habitats of Georgia have been showed. *B. bassiana* was the most frequently found and most dominant, then come *Metarhizium* and *Lecanicillium* species.

In approximately 22 % of soil samples the occurrence of *Beauveria* on artificial media was recorded, whereas *Metarhizium* spp. and *Lecanicillium* spp. were found at lower density in only one region (Samtskhe–Javakheti region). Identification of isolated fungi showed that *Beauveria bassiana* is most dominant.

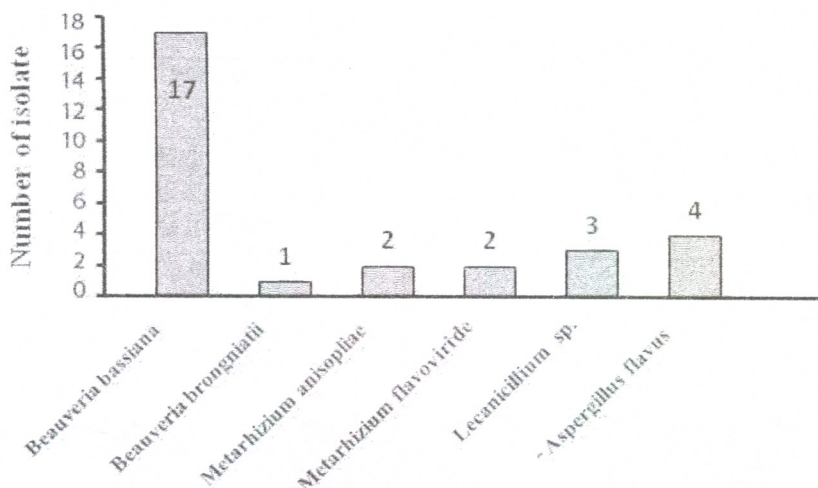


Fig.2. Comparison of the number of EPF isolated from pine forest soils in East Georgia

Entomopathogenic fungi were recovered from 73.3 % of the 30 sampled fields, which is similar to the reports from other countries. Typical recovery rates were 17.5 % in the UK [Chandler et al., 1998], 32 % in Tasmania [Rath et al., 1992], 44.6 % in Finland [Vänninen et al., 1989], 52 % in the Pacific Northwest [Bruck, 2004], 91 % in Ontario [Bidochka et al., 1998] and 96 % in Switzerland [Keller et al., 2003]. However, comparisons must be made carefully because different assay protocols were used.

Although entomopathogenic fungi were common in East Georgian soils, the diversity of the fungi was low. *B. bassiana* was also the most common entomopathogenic fungal species in other Mediterranean countries, such as Southern Italy [Tarasco et al., 1997], whereas *M. anisopliae* was more common in soils of northern countries with more humid and frigid climates such as Finland [Vänninen, 1996], Norway [Klingen et al., 2002], Switzerland [Keller et al., 2003], Poland [Tkaczuk & Mietkiewski, 1996], Canada [Bidochka et al., 1998] and the Pacific Northwest in USA [Bruck, 2004]. Exceptions occur in UK and Denmark where *B. bassiana* was more common than *M. anisopliae* [Chandler et al., 1997; 1998; Meyling & Eilenberg, 2006]. A cosmopolitan species *Lecanicillium* sp. recorded in our study was also found in the soils of other localities [Chandler et al., 1997; Bidochka et al., 1998; Keller et al., 2003]. *Paecilomyces fumosoroseus* has been recorded commonly in natural habitats elsewhere, particularly in hedges and forest soils [Vänninen 1996; Chandler et al. 1997; Klingen & Haukeland, 2006]. The absence of *P. fumosoroseus* from forest

soils in our study could be due to differences in the physicochemical properties of East Georgia forests.

This is the first record for the establishment of the EPF resource in Georgia. We are performing further study to isolate effective entomopathogenic fungi against various hard control insect pests.

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აღმოსავლეთ საქართველოს ფიტონარი ტყეების ეკოსისტემაში *Beauveria bassiana* –ს გავრცელება და გამოვლენა

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(მიღებულია 04.10.2010)

რეზიუმე

2009-2010 წწ ენტომოპათოგენური სოკოების გამოვლენის მიზნით შესწავლილი იყო აღმოსავლეთ საქართველოს ფიტონარ ტყეებში, 10 სხვადასხვა ადგილიდან (600-1700 მ ზ.დ.) აღებული ნიადაგის ნიმუშები, სადაც გამოვლინდა ექვსი სახეობის ენტომოპათოგენური სოკო: *Beauveria bassiana*, *Beauveria brongniatii*, *Metarhizium anisopliae*, *Metarhizium flavoviride*, *Lecanicillium* sp. და *Aspergillus flavus*.

30 ნიადაგის ნიმუშიდან ენტომოპათოგენური სოკოები გამოვლინდა 22 ნიმუშში (73.3 %) და *B. bassiana* წარმოადგენს დომინანტურ სახეობას. 30 ნიმუშიდან 17-ში გამოვლინდა *B. bassiana* (56.6 %), 2-ში - *M. anisopliae*, და 2-ში *M. flavoviride* (6.7 %), 3-ში *Lecanicillium* sp. (10%) და 4-ში *Aspergillus flavus* (13.3%). მხოლოდ ერთი ნიმუშში გამოვლინდა *Beauveria brongniatii* (3.3%). ასევე 2 შემთხვევაში (6.7%) აღიღი ქონდა ერთდროულად ორი პათოგენური სოკოს გამოვლენას: *B. bassiana* და *Lecanicillium* sp. და *B. bassiana* და *Aspergillus flavus*. ენტომოპათოგენური სოკოები არ გამოვლინდა 8 ნიმუშიდან (26.7%). “Bait” მეთოდით ძირითადად ერთი სამიზნე მწერიდან ხდებოდა ერთი პათოგენური სოკოს იზოლირება, გარდა ერთი გამონაკლისისა, სადაც გამოვლინდა ორი სხვადასხვა სოკოთი *B. bassiana* და *Lecanicillium* sp. ინფიცირებული მწერი.

FAUNA OF AFRICAN SAVANNAS IN KAKHETI (EAST GEORGIA) TERRITORY

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Abstract

Some decades ago, the scientists of the Institute of Paleobiology of the Georgian Academy of Sciences found a big "burial" of fossilized bones of animals in the surroundings of a place called "Kvabebi" (bank of the river Iori, Signaghi district). Nowadays in special literature this "burial" having great scientific significance is known as a site of representatives of vertebrate fauna from Kvabebi. Fauna of Kvabebi by its composition is diverse, but mammals occupy main place in it. The most surprising finding of Kvabebi is the skull and lower jaw of gigantic daman, which differ from all known fossil and modern damans by the peculiarities of skull and teeth. According to ecological analysis of Kvabebi fauna 2.5-3 million years ago in the territory of East Georgia, during the period of distribution of Kvabebi fauna, savana-like forest-steppe dominated with marshlands on the banks of basin. Climate was probably warm and moderately humid.

Key words: Mammalian fauna, Akchagyl, Hyracoidea, Kvabebi

Introduction

Georgian nature and organic world are diverse, but its present-day state does not give even approximate idea on the richness and diversity of plants and animals characteristic to recent geological past of our country. It is known that organic world is constantly changeable and its present-day state is the result of very complicated and prolonged evolution. Geologists and paleontologists come across evolutionary evidences nearly every day in nature, collect them and study. It can be said without exaggeration that in this regard remains of fossil animals provide us with interesting and effective material. Many generations of paleontologists and geologists have been studying fossil, or as it is often called, petrified animal remains (bones). So, there is no wonder that today it is quite possible to determine the history of development of organic world of geological past rather completely.

Certainly, everything has not been discovered and defined up to date. Paleontologists still have been keeping on intensive searching and their every new achievement enriches our knowledge about organic world of geological past.

Recent years' discovery of hominid relics on Dmanisi site is the best illustration of abovementioned, which totally changed widespread idea in science on the origin of man, time and routes of its settling [Gabunia et al., 1997; 2000].

Strata constituting the earth crust are often called annals of evolution, where major stages of development of organic world are printed. Study and analysis of every new paleontological discovery is one more read, deciphered line of the history of living nature [Gabunia, Vekua, 1978].

The aim of our article is to get readers acquainted with contents of one of such discovered and studied line, to tell about the world of those animals and natural environment, which was spread about 2.5-3 million years ago in the territory of East Georgia, namely Kakheti, in the valley of the river Iori.

Some decades ago, the scientists of the Institute of Paleobiology of Georgian Academy of sciences found a big "burial" of fossilized bones of animals in Signaghi district, in the valley of the river Iori, in the surroundings of place called "Kvabebi". Nowadays in special literature this "burial" is known as a site of representatives of vertebrate fauna from Kvabebi. Its scientific significance is the acknowledged fact [Vekua, 1972].

Result and Discussion

Fossil bones of Kvabebi are located in strata, dated by sea mollusca, where anatomically jointed skulls and parts of skeletons of animals are often found, that means that bones are brought to the basin by water-flows from nearby.

Fauna of Kvabebi by its composition is diverse, but mammals occupy main place in it. Among reptiles land tortoise is found, from birds – gigantic ostrich (*Struthio transcausicus* Burt.et Vekua) the height of which is probably about three meters, from rodents porcupine is presented (*Hystrix primigenia* Wagner). Plenty of predators also occur there, represented by raccoon-like dog (*Nictereutes megamastoides* Pomel), spotted jackal, small size bear (*Ursus arvernensis* Cr.et Job), lynx (*Lynx issiodorensis* Cr.et Job.) and sabre-toothed tiger (*Homotherium davitashvili* Vekua). The last one especially attracts researchers' attention by its uncommon (long, flat and notched) fangs. Sizes and form of mentioned fangs are so peculiar that researchers have not yet decided what purpose had long and bent like sabre fang. In some researcher's opinion, this strange fang was not quite useful and even hampered it in acquiring food [Kurten, 1952; Romer, 1966; Schaub, 1934; Flerov, 1957]. But most researchers justly think that this fang was a weapon in the struggle for existence, and, at the same time, the secondary mark of sex, attracting the representatives of opposite sex (Fig.1) [Davitashvili, 1961].

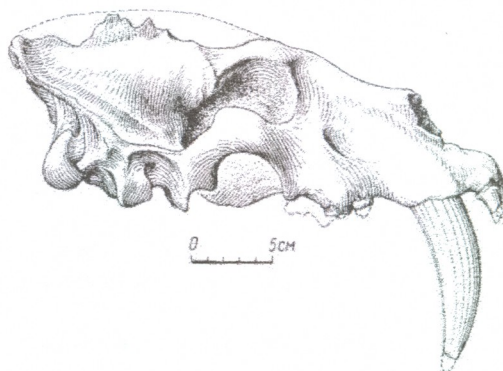


Fig. 1. Skull of Kvabebi *Homotherium davitashvili*

From proboscideans hill-toothed mastodont (*Anancus arvernensis*) is represented in Kvabebi fauna, which resembles real elephant very much by its body and fangs. Representatives of Perissodactyla are three-toed horse-hypparion (*Hipparion crusafonti* Villalta) and rhinoceros (*Diccerorhinus megarhinus* Chr.). Group of artiodactyls is especially diverse, represented by Asian pig (*Propotamochoerus provincaialis* Gerv.), deer with branched antlers, elk, roe and antelopes with twisted and sabred antlers, characteristic to African animals. Here is also found syncerus (*Eosyncerus ivericus* Vekua), related form of caffer buffalo, hornless cow and others. But the most strange and unexpected form is gigantic daman (*Procavia*), the place of which in mammals we could not determine for a long time (Fig.2).

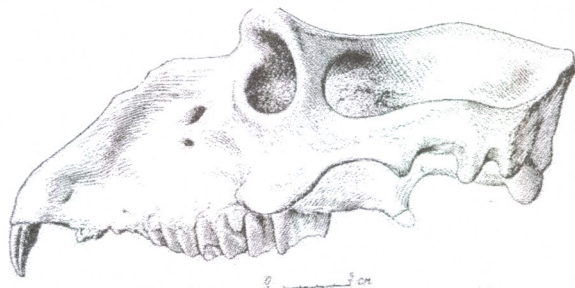


Fig.2. Skull of *Kvabebihyrax kakheticus* Gabunia et Vekua

It appeared that zoologists found an animal of size of hare in African mountains, which did not resemble any group of mammals. Some researchers considered this animal to be close with proboscideans, others – to rodents, but most of them – to solidungulates, because of their teeth structure. Finally, scientists have chosen the most optimal method and isolated from mammals quite a new order Hyracoidea. In modern fauna only three families of this group of animals are known. All of them are grass-feeding. One family inhabits on the rocks, two – in the trees (Fig.3).

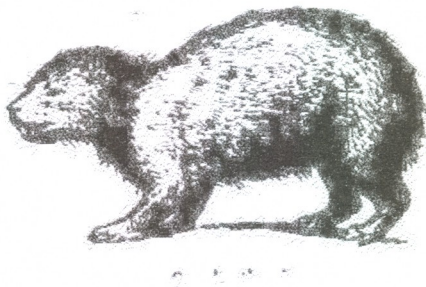


Fig.3. Recent *Dendrohyrax Dorsalis*

We have come across the remains of this very animal on the site of Kvabebi with very well preserved material: skull jointed with mandibula, separate mandibula, some isolated teeth and lower end of the shoulder bone. As we noted, it was difficult to us to determine this material; at last, Academician L. Gabunia found the description of similar animal in special literature. It appeared to be the representative of daman group, which sharply differed from modern damans. For comparison, it must be noted that modern damans are of size of hare, but damans from Kvabebi are not inferior to modern horses in height.

Damans from Kvabebi essentially differ from all known fossil and modern damans by the peculiarities of skull and teeth, because of which we picked them out as a new family – *Kvabebihyrax kachethicus* [Gabunia & Vekua, 1966]. The name of the family is given according to the site (Kvabebi) and geographical designation of territory (Kakhethi).

Peculiarities of skull and structure of teeth of Kvabebi damans enable us think that they inhabited in the marshlands of basin and were mainly connected with water surroundings. It had eyelids like a hippopotamus on the eminence, bony prominence covered eyelids, short bones and deep hole of nose indicate that animal had a short trunk (Fig.4).

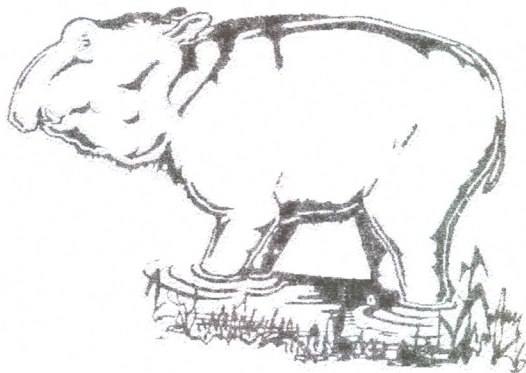


Fig.4. *Kvabebihyrax kachethicus* Gabunia et Vekua (L. Gabunia drawing)

It is difficult to discuss in detail on every representative of Kvabebi fauna in our short letter. We stress only that fauna like Kvabebi inhabits today in South African savannas and is extinct for a long time on the continents of Asia and Europe. Before discovery of Kvabebi fauna, it was believed that fauna contemporary to African one is created owing to migration of Eurasian Hyparionian fauna to Africa. But today it becomes clear that this migration happened comparatively later, namely, after the migration of Kvabebi-like faunas to Africa, which probably is connected to the second part of the Pliocene.

Ecological analysis of Kvabebi fauna makes us think that 2.5-3 million years ago in the territory of East Georgia, in the period of distribution of Kvabebi fauna, savana-like forest-steppe dominated, with marshlands on the banks of basin. Climate was probably warm and moderately humid. Nearby the site of vertebrates' fauna of Kvabebi the site of fossil plants is found too. According to J. Dolidze [Dolidze, 1968] in East Georgia, namely, in the territory of Kvabebi willow, asp, alder, forest hazel-nut, oak, elm, zelkova, mountain ash, guelder rose, etc. were spread.

Finally, it must be said that discovery and study of Kvabebi fauna is of great scientific importance and considered as one of the most significant paleontological discoveries of XX century. Excavations of Kvabebi site have not been finished and we hope, it will offer us more unexpected and interesting surprises.

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აფრიკის სავანების ფაუნა კახეთის ტერიტორიაზე

გეგუა ა., ბენდუქიძე ო., ჩაგელიშვილი რ., კილაძე ს., გოცირიძე თ.

საქართველოს ეროვნული მუზეუმი

(მიღებულია 14.09.2010)

რეზიუმე

სიღნაღის რაიონში მდ. ივრის პირას, ადგილ ქვაბების მიდამოებში პალეობიოლოგიის ინსტიტუტის თანამშრომლებმა პლიოცენური (3 მილონი წლის წინანდელი) ცხოველების ნამარხი სასაფლაო აღმოაჩინეს. ქვაბების გათხრების შედეგად სპეციალისტებმა დაადგინეს, რომ სამი მილიონი წლის წინ აღმოსავლეთ საქართველოში ბინადრობდა აფრიკის სავანის დღევანდელი ფაუნის მსგავსი ცხოველები. ქვაბებში აღმოჩენილი თავის ქალების, ქვედა ყბებისა და ჩონჩხის ნაწილების მიხედვით დადგენილია აფრიკის სავანისათვის დამახასიათებელი ფორმები: გიგანტური სირაქლემა, ჟირაფი, მარტორქა, ხვეულრქიანი ანტილოპა, კაფრის კამეჩის მსგავსი სინცერინა და სხვა. აქვეა ნაპოვნი ჰიპარიონის, ხმალებილა ვუფხვის ენოტისებური მგლის, ოვერანის დათვის, მანჯღლარბას და სხვა ცხოველთა ნაშთები.

ყველაზე საინტერესო და მოულოდნელია ქვაბებში გიგანტური დამანის თავის ქალის და ქვედა ყბის პოვნა. თანამედროვე აფრიკაში ბინადარი დამანი კურდღლის ზომისაა, ხოლო ქვაბების დამანი თითქმის ძროხის ზომის ყოფილა, რომელსაც პატარა ხორთუმიც ჰქონია. აფრიკის თანამედროვე დამანი კლდეებსა და ტყეში ბინადრობს, ქვაბების დამანი კი ჰიპოპოტამის მსგავსად ძირიდან დროს წყალში ატარებდა, ალბათ უფრო ჭაობიან ადგილებს ირჩევდა. როგორც ფაუნა და ფლორა გვიჩვენებს, პლიოცენის ბოლოს აღმოსავლეთ საქართველოში გაბატონებული უნდა ყოფილიყო თბილი და ზომიერად ტენიანი ჰავა.

PALYNOLOGICAL INVESTIGATION OF MARINE, LAGOON AND OTHER SEDIMENTS FROM THE BLACK SEA AREA IN GEORGIA

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Abstract

Analysis and synthesis of 26 pollen diagrams of Black Sea area allowed us to make a stratigraphic subdivision of Holocene sediments and reveal climatic fluctuations for the last 10 000 years. The most informative pollen spectra were those of marine formations with no gaps in sediment accumulation. Three main stages of climate warming have been revealed, reaching a maximum in the periods 6000-5500 BP, 3800-2400 BP and 1350-600 BP. Rather significant warming is indicated for the Middle Ages (7th-11th cent. B. C.). In all these periods the Black Sea level on the Georgian coast was some meters higher than previously. In the mountains of Georgia the tree line may be 300-500-600 m higher. During climatic optima new cultures appeared in the Georgian archaeological record. Early agriculture penetrates not only into the middle mountain belt, but also into higher areas. The influence of human activity on development of the landscapes had been observed since the Subboreal time when deforestation took place not only on the Colchis lowland, but also in the mountains of Western and Southern Georgia.

Key words: palynology, Black Sea, marine sediments, palaeoecology.

Introduction

The palynological study of numerous profiles of Holocene sediments at different altitudes of Georgian mountains as well as the study of cultural layers of archaeological monuments showed the presence of several main climatic fluctuations given in the regional standard diagrams. In Georgia, palynological studies of Holocene marine sediments began in the early 1970s [Shatilova, 1974]. Extensive drilling of the Black Sea shelf and estuarine sediments was performed as part of engineering-geological surveys near coastal resorts and sanatoriums (Fig.1). Drilling was performed on the scientific ship "Geochimik" and during 1978-1984 a substantial body of core material was collected and studied using many methods of the natural sciences, including palaeogeographical, paleontological, geomorphological, geochronological, and climatostratigraphical methods [Ostrovskii et al., 1977; Balabanov et al., 1981; Balabanov & Gei, 1981; Kvavadze & Jeiranashvili 1985; Balabanov & Kvavadze, 1985; Fiodorov, 1988].

Palynological and sedimentological results showed that the Black Sea reached its lowest level 17-18 thousand years ago. During this regression, corresponding to the last phase of the Würm Ice Age, the sea level was 120m lower than today. Cooling was followed by intensive

warming, glaciers began to melt and the new Black Sea transgression began. By the onset of the Holocene (10 thousand years ago) the sea-level rose by nearly 70 m [Tvalchrelidze et al., 2004]. How sea-level changes developed later, the climate in different Holocene transgression phases and the human activity at the very beginning of the Holocene and in all the subsequent periods in the mountains of Georgia – these are the points we will try to address in the present paper. The Caucasus and Georgia in particular, is the region where the manufacturing economy that forms the basis of civilization originated during the first stages of the Holocene [Trifonov & Karakhanyan, 2004]. According to radiocarbon dates, early agricultural settlements in the southern part of Georgia are dated to the 60-54 centuries B.C. [Hansen et al., 2007].



Fig.1. Map of Georgia and the location of the profiles studied.

Material and Methods

Study area

Physical-geographic conditions adjacent to the easternmost coast of the Black Sea are characterized as follows. The length of the coastline is 355 km. Mountain ranges of the Caucasus hug the coast, especially in the northern part (Gagra range). The Bzyb, Kodori and Meskheta ranges ring the Black Sea shore. The maximum altitudes of these mountains are 2756 m (Bakhmaro) and 3309 m (Khojali).

The climate is very mild. Average annual air temperature in the coastal lowlands is +14°C, +15°C. At an altitude of 900-1000 m, it decreases to +9°C and, at an altitude of 2000 m, +3,5°C. Above 2600 m the annual temperature is below zero. Precipitation is more plentiful in the southern part than in the north, and reaches 2600-2700 mm in the Kobuleti region. In Gagra and Sokhumi, average annual precipitation totals 1500-1400 mm [Lominadze, Chirakadze 1971]. This index is even lower in the Gudauta area (~1000 mm).

The humid climate has led to the development of an extensive hydrographic network in the region. There are many large rivers, including the Kodori, Bzyb, Kelasuri, Choloki and Kintrishi.

Vegetation in the coastal lowlands is of secondary character; however, around the estuaries of the larger rivers, forests of *Alnus barbata* are preserved. Relict *Pinus pithyusa* grows on limestone slopes at Pitsunda Cape. At an altitude of 300-700 m low mountain Colchis forests with predominance of oak (*Quercus iberica*, *Q. imeretina*, *Q. hartwissiana*) and chestnut (*Castanea sativa*) occur. At higher altitudes *Carpinus caucasica* becomes a prevalent species in broad-leaved forests, while above 1000 m *Fagus orientalis* is dominant. Beech-dominated forests occupy nearly half of the forest area, as they do throughout Georgia. *Abies nordmanniana*, which ascends to the upper tree limit (1800 – 2200 m), is widespread in the mountain forest belt. In the higher

mountains, dark coniferous forests of *Picea orientalis* and *Pinus sosnowsky* are recorded [Kolakovsky, 1961].

Subalpine vegetation is formed at the timberline of beech and birch elfin woodland at an altitude of 2200 – 2400 m. Above this altitude subalpine and alpine meadows appear. The subnival and nival vegetation belt is located above 2700-2800 m.

More than 2000 plant species are distinguished in the regional flora, including 450 Colchic endemic species. There are also many Tertiary relicts, which is peculiar for this unique Colchis refugium.

The investigation covered five areas of the eastern part of the Black Sea (Fig.1), where many boreholes were drilled during voyages of the “Geokhimik”. Drilling was performed with a Russian-made “UGB” drill, with a core diameter of 10.8 cm.

The Black Sea shelf sediments studied comprise mostly fine grained sands, silt and clay. The depth of Holocene sediments in the region varies from 17 m (borehole 120) to 27.8 (borehole 511). Material from 26 boreholes was studied palynologically; 7 of these were located in the Gagra area, i.e. boreholes 603, 607, 609, 613, 424 and Gagra-1. Borehole 471 was drilled nearby in the continental zone (Table 1).

Table 1. Database of the profiles studied in Georgia

Permanent station Name	Date and sampling frequency of profiles	Lower depth of profiles	Number of samples	Sediment type
Gagra area:				
Gagra-603	1981, every 50 cm	25.5m	5	Marine
Gagra-1	1971, every 10 cm	70cm	10	Marine + peat
Gagra-607	1981, every 50 cm	37m	12	Marine
Gagra-609	1981, every 50 cm	15m	12	Marine
Gagra-613	1981, every 50 cm	32m	9	Marine
Gagra-424	1978, every 50 cm	12m	5	Marine
Gagra-471	1978, every 10 cm	28m	66	Peat
Gudauta area:				
Gudauta-120	1983, every 50 cm	9.5m	17	Marine
Gudauta-521	1983, every 50 cm	31.8m	20	Marine
Akhali Atoni area:				
AkhalAt-511	1984, every 10 cm	21.1m	35	Marine
AkhalAt-55	1982, every 50 cm	24m	12	Alluvial
AkhalAt-128	1983, every 50 cm	9m	6	Alluvial
AkhalAt-182	1983, every 50 cm	16m	7	Alluvial
AkhalAt-149	1983, every 50 cm	17m	4	Alluvial
AkhalAt-239	1983, every 50 cm	12.5m	8	Alluvial
Sokhumi area:				
Sokhumi-721	1980, every 10 cm	21m	60	Marine
Sokhumi-723	1980, every 10 cm	26m	50	Marine
Sokhumi-36	1980, every 10 cm	45m	120	Alluvial + peat
Kobuleti area:				
Kobuleti-22	1984, every 10 cm	22.6m	99	Marine
Kobuleti-35	1984, every 10 cm	120m	120	Alluvial + peat
Kobuleti-39	1984, every 10 cm	27m	25	Alluvial + peat
Ispani II	2003, every 10 cm	9.5m	47	Peat
Supsa-1	1972, every 20 cm	6.5m	33	Alluvial + lake
Supsa-2	1972, every 20 cm	7.8m	42	Alluvial + lake
Supsa-3	1972, every 20 cm	3m	17	Alluvial
Supsa-4	1972, every 20 cm	2.1m	17	Alluvial

To the south-west of Gagra the Gudauta shelf zone lies, where boreholes 120 and 521 were drilled to a depth of 9.5 and 31.8 m respectively.

In the Akhali Atoni area, borehole 511 (at a depth of 21.1 m) was collected. Boreholes 55, 39, 182, 128 and 149 were drilled nearby on the alluvial-marine terrace.

Boreholes 732 and 721 come from the Sokhumi coastal area, at a depth of 9.8 and 14.9 m on the shelf, where Holocene sediments are represented most completely. Borehole 36 was drilled here in the continental part near the shelf (Table 1).

The Kobuleti area lies on the southernmost sector of the Georgian coastline. Here borehole 22 was drilled between the Choloki and Kintrishi river mouths at a depth of 7.3 m on the shelf. Boreholes 35 and 39 come from the continental part. Profiles Ispani II, Supsa 1, 2, 3, 4 were also studied in this area.

Table 2 gives the results of radiocarbon dating of organic remains found in these marine and continental sediments (Uncalibrated age, ^{14}C yr. BP).

Table 2. Radiocarbon dating of borehole sediments on the shelf of the Black Sea (eastern part)

Borehole	Core depth (m)	Material analyzed	Age (^{14}C yr BP)
Gagra-416	4.5	Peat	2450±80
Sokhumi-723	6.4	Shell	3335±50
Sokhumi-723	7.5	Archaeolog.	3500±50
Gagra-I	6.5	Peat	3690±120
Gagra-609	24.4	Shell	4000±140
Gagra-607	21.1	Shell	4140±160
Akhali Atoni-55	15	Shell	5200±80
Gagra-607	34.5	Shell	5410±320
Sokhumi-723	11.2	Shell	5540±60
Akhali Atoni-55	23	Shell	6780±120
Sokhumi-723	14	Shell	7630±250
Sokhumi-723	20.1	Shell	8690±300
Sokhumi-722	26	Peat	9310±80
Gudauta-120	17	Shell	11000±150
Supsa-I	2.20	Wood	1260±120
Ispani-II	5.20	Wood	1940±40
Ispani-II	6.95	Wood	4060±40
Ispani-II	9.45	Wood	4900±40

Results and Discussion

Palynological analysis of Holocene sediments in 5 study areas showed that in the more complete pollen diagrams 5 regional palynozones can be distinguished (Fig. 2, 3, 4, 5). Palynozone I is assigned to the Pre-Boreal period, palynozone II to the Boreal period, palynozone III to the Atlantic period, palynozone IV to the Sub-Boreal period and palynozone V covers the Subatlantic period.

Palynozone I differs from the underlying Late Dryas sediments by its greater representation of thermophilous arboreal taxa such as chestnut and oak (Fig.2). However, this zone is indicated by a *Fagus-Carpinus-Abies* association. Note for comparison that in the Late-Dryas only high mountain vegetation elements prevailed. Palynozone I is divided into two subzones, the first reflecting more arid climatic conditions compared to the second. Redeposited pollen is found in significantly less quantities (up to 25 – 27% of the total pollen) in Pre-Boreal strata compared to Late Dryas (up to 46%).

Gudauta, core No 521, AP

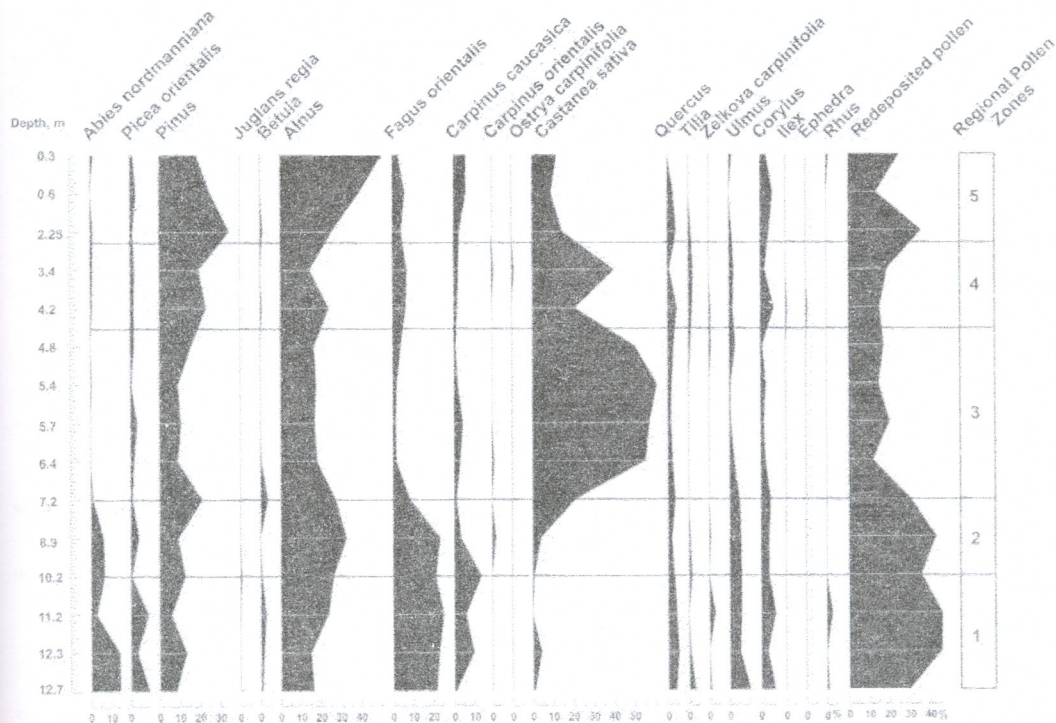


Fig.3. Palynological diagram of marine sediments on the Gudauta shelf exposed by borehole 521.

Palynozone V corresponds to the Subatlantic period. It is indicated by a *Alnus-Pinus-Castanea* association in the northern part and *Pinus-Alnus-Castanea* in the southern part (Kobuleti area). High mountain vegetation indicators decrease to minimum values in palynozone V. Fir, spruce and birch pollen is found only as single grains. In the Gagra area, which has been more thoroughly investigated, palynozone V is divided into three subzones. The second subzone reflects warmer and more humid climatic conditions. *Pterocarya pterocarpa* pollen content increases rather significantly and redeposited pollen are few throughout the whole palynozone (up to 20%).

The occurrence of distinct palynozones is due to vegetation and climate dynamics in the eastern part of the Black Sea coast during the Holocene. The close proximity of mountain ranges facilitated a detailed and clear reflection of change in all the altitudinal vegetation belts of the region. This is explained by the specific character of pollen spectra of marine sediments where regional vegetation is more clearly reflected compared to the spectra of continental deposits.

The regional palaeozones derived from analysis of marine, lagoon and alluvial sediments represent the major events in the Holocene development of vegetation in the Caucasus region. Marine sediments, in particular, show major shifts in species dominance that reflect changes in vegetation in all altitudinal belts, thanks to the proximity of mountain ranges to the Black Sea. In this case the Black Sea can be regarded as a natural pollen trap in which pollen accumulated for the last ten thousand years. Importantly, there are no gaps in the record and therefore we have a complete and detailed picture of palaeoecological events. Statistical processing of the palynological material using the software "Paleoclimate 1" [Bukreeva 1990] allowed us to reconstruct quantitative indices of the climate and to reconstruct oscillations of the upper tree limit

in response to climatic fluctuations [Kvavadze et al., 1992; 1994]. In addition to marine palynospectra, palaeoecological reconstruction also incorporates pollen data from alluvial and lagoon sediments along the coastline and from the high mountains of Abkhasia [Kvavadze et al., 1992].

Akhali Athoni, core No 511, AP

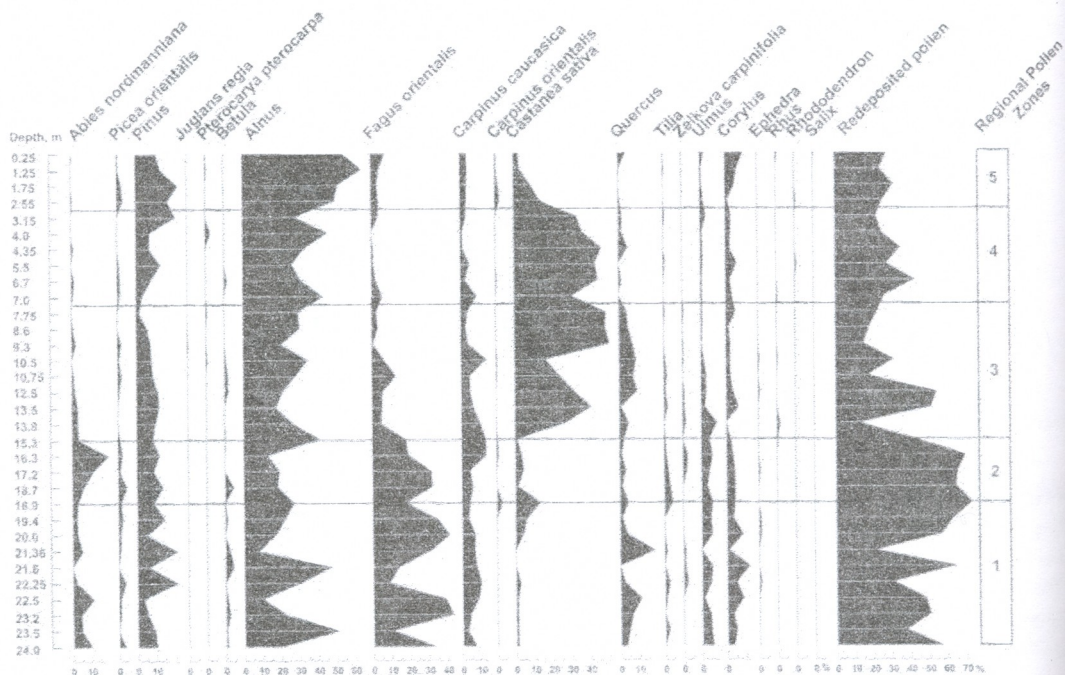


Fig.4. Palynological diagram of marine sediments on the Akhali-Athoni shelf exposed by borehole

511.

At the first stage, very early in the Holocene, sedimentological data [Tvalchrelidze et al., 2004] indicates that the Black Sea level was lower than nowadays by 50-60 m. The timberline was 800 – 850 m lower than now [Kvavadze & Connor, 2005]. However, comparing pollen spectra dated 10 000 BP with those of the Younger Dryas, it is clear that the areas of broad-leaved forests with hornbeam, oak, chestnut and wing-nut expanded. This process was due to warmer, wetter climatic conditions. During the Younger Dryas, July mean temperature on the coast was reconstructed at 18.3°C, while in the Preboreal it rose to 23.3°C.

The second stage of landscape development corresponds to the Boreal period when the process of warming was interrupted by short-term cooling. The sea-level lowered by approximately 1-1.5 m. The timberline also descended significantly. Forests with beech and fir broadened. Reconstructed temperatures on the coastline were 19.4°C in July and 3.3°C in January (mean annual temperature 10.7°C). Precipitation was about 1827 mm per year.

The third stage occurred during the warming of the Atlantic period. This stage was quite long and resulted in rather substantial changes in altitudinal vegetation zones. Rapid ascent of all vegetation belts initiated during this stage. Upper mountain belts of dark coniferous forests migrated upslope and narrowed in their altitudinal range. At the same time, the area of broad-leaved chestnut, oak, wing-nut and zelkova forests expanded substantially [Kvavadze&Connor,

2005]. Paludification of the coastline led to the expansion of boggy areas. Temperatures and humidity increased, reaching a maximum 5500-6000 years ago. Compared with the Boreal, winter temperatures on the coastline nearly doubled and reached 6 – 6.5°C. In the mountains remote from the sea climate warming was very intensive. In Abkhasia and Adjara the timberline ascended 300 m during the peak of the Holocene climatic optimum, while in the mountains of the South Georgian Upland it ascended no less than 400-500 m.

Sokhumi, core No 723, AP

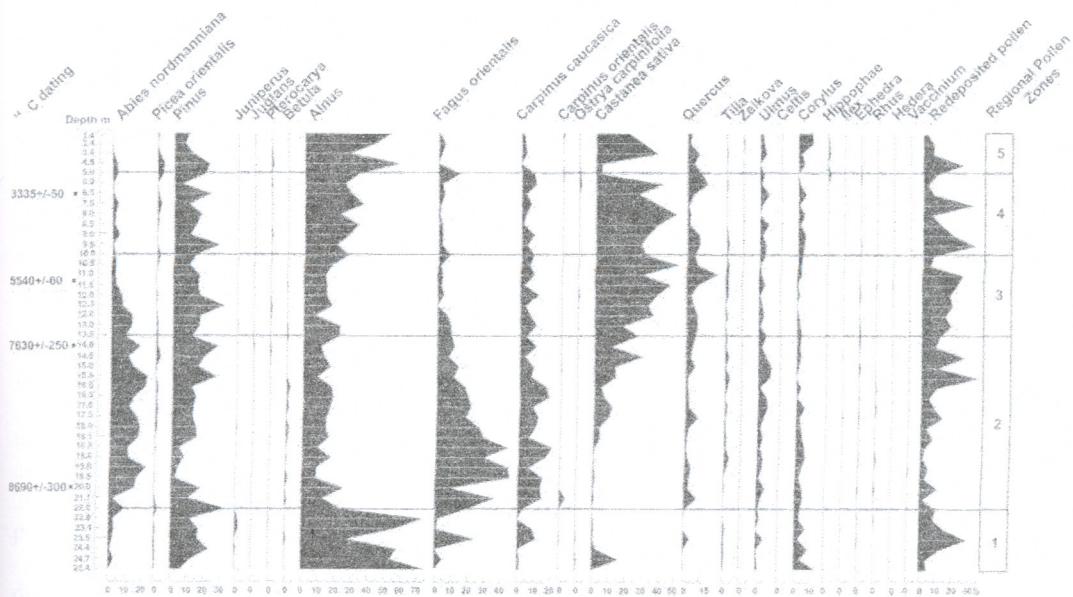


Fig.5. Palynological diagram of marine sediments on the Sokhumi shelf exposed by borehole 723.

The sea level during the Atlantic period rose rapidly and, between 6000-5500 years, exceeded its present-day level by some meters [Tvalchrelidze, 2004].

It was under the warm and humid conditions of the Atlantic period that the first early agricultural settlements appeared in the south-east of Georgia. Palynological studies of cultural layers in the settlement mounds of Gadachrili Gora and Arukhlo, dated to the 6th-5th millennia B. C., indicated a warmer, more humid climate. In place of the present-day steppes there grew forests of alder, wing-nut, hornbeam and oak. Humans, besides grain-growing, were engaged in viticulture. In the lower layers of Gadachrili Gora, a piece of loom-woven flax fabric was found and imprints of hand-knitted flax fabric were discovered in ceramic vessels. These finds, as well as macroremains of toadflax seeds [Rusishvili, 1990], suggest the existence of local weaving. Toadflax, *Linum usitatissimum*, grows only under humid climatic conditions [Zohary & Hopf, 1993]. Microremains of numerous flax fibres and woolen fabric, including coloured fibres, were also revealed during the palynological study of material (cultural layers and pot contents) from Neolithic layers at these sites. Pollen analysis of organic material found in ceramic vessels provides evidence of beekeeping.

Data from the investigation of archaeological monuments in the South Georgian Uplands are also very interesting. Material from the Early Kurgan epoch of Javakheti (beginning of the third millennium B. C.) shows the existence of agriculture at an altitude of 2000-2800 m. In this period forest vegetation with oak and lime occurred at these altitudes [Kvavadze et al., 2007], whereas

today these forests occur at lower altitudes: 1700-1800 m [Dolukhanov, 1989; Nakhutsrishvili, 1999].

Kobuleti, core No 39, AP

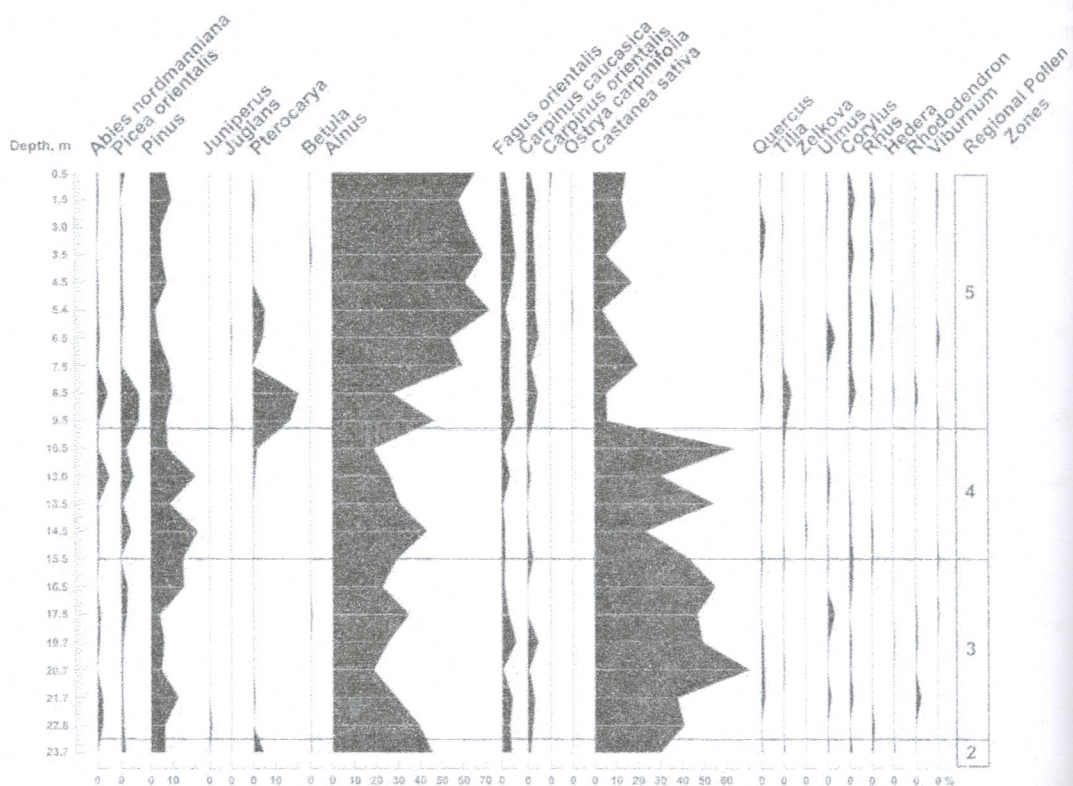


Fig.6. Palynological diagram of marine sediments on the Kobuleti shelf exposed by borehole 39.

During the Atlantic period, the process of warming was interrupted twice by mild, short-cooling. This process is seen in the curves of both timberline oscillations and Black Sea fluctuations [Kvavadze et al., 1992; Tvalchrelidze et al., 2004].

The fourth stage in landscape development is characterized by significant climate cooling, causing the Phanagorian regression in the Black Sea. In the Subboreal period, erosion processes accelerated, indicated by the increase in redeposited pollen in marine sediments. This increased erosion is explained by base-level lowering in Colchic river systems.

This period witnessed a lowering of zonal vegetation belts. The timberline was 600-550 m lower than nowadays. At the same time, the area of chestnut and other thermophilous species decreased, as indicated in all pollen diagrams. Cooling was accompanied by a moderate decrease in humidity.

In the second half of the Subboreal period, between 3800-2400, a change in climatic conditions is observed. The Black Sea rose to levels somewhat higher than today. This warming manifested itself clearly, not only in the lowland territories adjacent to the sea [Kvavadze&Connor, 2005; Connor et al., 2007a,b; Arabuli et al., 2007], but also on the mountain plateau of Southern Georgia. Palynological studies of archaeological sites (Safar-Kharaba and Imera burials) indicate that, during the 15th-14th centuries B.C., agriculture, horticulture and viticulture were practised at an

altitude of 1700-1800 m. Forests comprised oak, lime, zelkova and other species [Kvavadze et al., 2007]. Here, for the first time, cotton fabric and fibres were discovered, indicating a well-developed trade between India and the Caucasus [Kvavadze&Narimanishvili, 2006a,b]. In Colchis and in the regions more remote from the Black Sea, signs of forest destruction can be observed in this stage [Connor et al., 2007a].

The fifth stage coincides with the Subatlantic period. Around 2500 years ago, a short-term, but pronounced cooling took place, resulting in sea regression. The sea level lowered nearly 20 m compared to the end of the Subboreal (Fig.2). In high mountain areas, agriculture was replaced by stock-breeding. Viticulture went into decline at high altitudes and was not even developed in the middle mountains [Bieniek & Licheli, 2007]. The timberline descended nearly 350-400 m compared to the present-day level. Then, five centuries later (2000 years ago), significant climate warming occurred, resulting in the Nymphaean transgression in the Black Sea. The sea level again rose some metres higher than nowadays. Climate warming was accompanied by an increase in humidity on the Black Sea coast. In antiquity, according to palynological and palaeoethnobotanical studies of cultural layers in the Eshera and Nokalakevi settlements, flax-growing was very intensively developed [Rukhadze et al., 1988; Bokeria et al., 2009]. Moreover, the population of Colchis of this time was engaged in grain-growing, gardening and viticulture. Cultivation of olives imported from Greece began.

With developing agriculture on the coastal lowlands and in the piedmonts of Colchis, intensive deforestation took place. This process is indicated in palynological spectra by the increasing role of pollen from secondary vegetation.

The 3rd-4th centuries A.D. were rather cool, followed by a period of warming from the 7th to 11th centuries. At that time, the population density in the high mountains increased and agriculture, including viticulture, occupied a prominent place in the economy [Kvavadze et al., 2007a, b; Arabuli et al., 2008]. The areas under olive plantations increased, as indicated by the pollen record [Connor & Kvavadze, 2005].

During the 12th-14th centuries, climatic conditions again deteriorated, but switched back during the 15th-16th centuries, according to the palynological data. Viticulture and wine-making developed intensively in the mountains of Southern Georgia. Palynological study of material from cultural layers and vessels from the Atskuri settlement at an altitude of 1200 m showed that here, besides the vine, olives were also cultivated [Kvavadze&Licheli, 2009]. According to historical documents, olive plantations were also found in the gorge of the River Khrami, in its headwaters, and in many places of the lowland part of Colchis [Ketskhoveli, 1959].

This rather significant and long-term warming lasted nearly 200 years and was the last warming. In the second half of the 17th century, the short-term, but very strong, global cooling of the Little Ice Age took place. Though it lasted perhaps only 40 years (1675 – 1715) [Grove, 1997], it exerted a strong influence on landscapes. Since then, in Atskuri and in other mountains settlements, viticulture was no longer practised [Kvavadze & Licheli, 2009]. Olive plantations were completely destroyed by frost everywhere in Georgia [Ketskhoveli 1959].

The comparison of our scheme of Holocene climate changes with similar schemes from mountain territories in southern Europe and the Near East shows a very good agreement, especially for the second half of the Holocene [Le Roy Ladurie, 1971; Grove, 1997; Ramezani et al., 2008]. It perfectly demonstrates the global character of climatic fluctuations that had feedback effects on sea-levels in Southern Europe, including the Black Sea.

Conclusions

The created database for palynological studies of marine, lagoon, alluvial and bog sediments of the Black Sea coastline on the territory of Georgia includes 26 profiles of Holocene sediments. The analysis and synthesis of the plotted pollen diagrams allowed us to make a

stratigraphic subdivision of Holocene sediments and to reveal climatic fluctuations for the last 10 000 years.

According to palynological data, Black Sea transgressive phases can be clearly identified by the combination of a vast number of pollen of thermophilous arboreal species and low values of redeposited pollen in shelf sediments. Conversely, during Black Sea regressions, the role of heat-loving elements decreases and there is a sharp increase in the quantity of redeposited pollen due to enhanced erosion caused by base-level lowering.

During the Holocene, transgressive phases with warm climatic conditions lasted longer than regressive phases. The most significant warming and sea transgression took place in the Atlantic period, lasting nearly three millennia (8000-5500 BP). The climatic trend was increased temperatures and precipitation. This process reached its peak 5500-6000 years ago and the sea-level in Colchis exceeded its present-day level by several metres for the first time for the whole post-glacial period.

At the beginning of the Atlantic period, with the establishment of humid, warm conditions, the first Neolithic agricultural settlements appeared on the alluvial plains of Southern Kartli, where, besides grain-growing, gardening, viticulture, beekeeping and weaving were developed. During the Eneolithic period, this warming process continued and mild climatic conditions facilitated the rise of new cultures and penetration of agriculture into mountainous areas.

The second significant ingressions of the Black Sea took place at the end of the Subboreal period (3800 – 2400 years ago), which was also due to climate warming. The sea level was again higher than nowadays. Broad-leaved forests with chestnut, lime, wing-nut and zelkova expanded. In the high mountains, traditional stock-breeding was replaced by agriculture, viticulture and horticulture. Trade may have developed at this time, judging by the presence of traded goods, such as cotton, in archaeological material from the 15-14th centuries B.C.

The last 2000 geological years are characterized by more frequent transgressions and climatic fluctuations, among which the rather long climatic optimum of the Middle Ages (7th-11th centuries) is distinguished. The last warming and significant transgression of the Black Sea lasted for 200 years and took place in the 15th-16th centuries.

The influence of human activity on landscape development can be observed since the Subboreal period, when deforestation took place not only on the Colchis lowland, but also in the mountains of Western and Southern Georgia.

The comparison of our scheme of climatic changes with similar schemes of mountain territories of the southern part of Europe and the Near East shows their nearly full identity which is explained by their global character.

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საქართველოს შავი ზღვის რეგიონის ზღვიური, ლაბუნური და სხვა სხვის ნალექების პალინოლოგიური კვლევა

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რეზიუმე

შავი ზღვის სანაპირო ზოლის 26 პალინოლოგიურ დიაგრამის ანალიზი და სინთეზი საშუალებას გვაძლევს მოვახდინოთ ჰოლოცენური ნალექების სტრატეგრაფიული დანაწევრება და კლიმატის ფლუქტუაციების გამოვლენა ბოლო 10 000 წლის განმავლობაში. ყველაზე ინფორმატიული აღმოჩნდა ის ზღვიური ნალექები, სადაც მათი აკუმულაციის დროს ხარვეზები არ უნდა ყოფილიყო. გამოვლენილია კლიმატის დათბობის სამი მთავარი ეტაპი, რომელთა მაქსიმუმი დროის შემდეგ მონაკვეთებს ემთხვევა: 6000-5500 წლის წინ, 3800-2400 წლის წინ და 1350-600 წლის წინ. საკმაოდ მნიშვნელოვანი დათბობა დადგენილია ასევე შუასაუკუნეებისთვისაც (ჩვ.წ.ად. 7-11 სს). დათბობის ყველა პერიოდში შავი ზღვის დონე ზევით იწევდა და წინა დონეს აჭარბებდა. კლიმატური ოპტიმუმების დროს ახალი კულტურები აღმოცენდებოდა, რაზეც საქართველოში არქეოლოგიური მონაცემები მეტყველებს. ადრეული მიწათმოქმედების პერიოდის კულტურა ჩნდებოდა და ვლინდებოდა როგორც მთის შუა სარტყელში, ისე მადაღმთიანეთში. ადამიანის სამეურნეო მოღვაწეობის შედეგად გამოვლენილი ზეწოლა ბუნებრივ ლანდშაფტებზე მკვეთრად აისახა სუბბორეალური პერიოდში, როდესაც დაიწყო ტყის განადგურება კოლხეთში და დასავლეთ და აღმოსავლეთ საქართველოს სხვა მთიან რეგიონებშიც.

ANTINUTRITIVE EFFECTS OF *VISCUM ALBUM* LECTIN (MChbL) ON *APAMEA SORDENS* HUFN. AND *AGROTIS SEGETUM* SCHIFF. (LEPIDOPTERA: NOCTUIDAE)

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Abstract

The insecticidal activity of *Viscum album* chitin-binding lectin (MChbL) against *Apamea sordens* Hufn. and *Agrotis segetum* Schiff. larvae have been investigated. MChbL exhibited proteinase inhibitory activity and affected larval development at different growth stages. MChbL produced 40% mortality of larvae when incorporated into an artificial diet at a level of 0.001% (w/w). Possible implication of chitin-binding mistletoe lectin in entomotoxic effects on herbivore pests is discussed.

Key words: Chitin-binding lectin, Insecticidal activity, Lepidoptera, *Viscum album*.

Introduction

Apamea sordens Hufn. and *Agrotis segetum* Schiff. are serious herbivore Lepidoptera pests causing substantial crop losses throughout the world. They are responsible also for significant damage of stored seeds and post harvest loss of agricultural production. Due to the environmental concerns of pesticide use and limited list of effective alternatives it is therefore urgent to develop biopesticides from natural sources that have low mammalian and environmental toxicity. Lectins are among wide range of natural defense proteins found in plants [Rudiger & Gabius, 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 et al., 2002; Vasconcelos et al., 2004]. Lectins are capable of recognizing and binding to exoskeleton specific sites or glycoconjugates exposed along intestinal tract of insects. Such interaction is considered to be prerequisite for insecticidal action.

In the present paper the insecticidal activity of chitin-binding lectin from *Viscum album* L. was investigated against *Apamea sordens* Hufn. and *Agrotis segetum* Schiff. larvae and antinutritive effects of MChbL on larval development in long-term bioassays was studied.

Materials and Methods

The fruits of European Mistletoe were harvested in mountainous region of East-Georgia, in winter and stored at -15°C until use. Mistletoe chitin-binding lectin (MChbL) was prepared as described with some modifications [Keburia, Alexidze, 2001].

The larvae of *Apamea sordens* Hufn. (*Hadena basilinea* Schiff. (F.) and *Agrotis segetum* Schiff. were obtained from Khashuri region (East Georgia). Larval cultures were reared continuously at $25\pm 1^\circ\text{C}$ and relative humidity of 65-75%, under a L16/D8 light regime. The lectins were incorporated into natural diet daily at a level of 0.001% (w/w). 10-15 larvae were used per treatment. Insect survival was estimated daily, the weights of larvae and pupae were measured and the duration of developmental stages was determined. The effect of MChbL on the development was assessed by determining the number and mass of surviving larvae.

Total gut protease activity was measured by FITC-casein assay. Midguts were isolated by dissecting the fifth instar larvae. The gut tissue was mixed with 3 volumes of 0.1M Gly-NaOH buffer (pH 10.0) and allowed to stand for 15 min on ice to extract proteases. The gut luminal contents were recovered by centrifugation at 10000g for 10 min at 4°C . The resulting supernatant was analyzed for protease assays. MChbL was preincubated with gut extract at 37°C for 15 min, prior to addition of the substrate. The enzyme solution (20 μl) was added to 40 μl of FITC-casein (1 $\mu\text{g/ml}$, in 0.1M 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 0.2M Tris-HCl buffer (pH 9.0) containing 0,5% SDS and 0.02% NaN_3 . The fluorescence polarization of samples was measured with Ex: 490 nm and Em: 520 nm.

Results and Discussion

In long-term bioassays the anti-nutritive effects of dietary MChbL on the growth and surviving of *A. sordens* and *A. segetum* from 2nd instar to adult were examined. The results showed that MChbL affected the larval development as well as their survival and showed increased pupation time for both insects. The results obtained are shown in Table 1. The rates of pupae formation as well as the rate of adults successfully emerging from pupae fed on MChbL in all experimental groups were lower than those of control insects (41.4% and 41.7%, respectively). The effects of MChbL on surviving of the larvae at different developmental stages is shown in Fig. 1. Generally, in all experimental groups the larval mortality observed were higher than that of control group. The mortality of third and fourth instar larvae fed on MChbL were 36% and 50%, respectively, compare to that of control insects (83%). The results suggest that the influence of lectins were much evident at early stages of larval development. Apparently, this might be related to extended sensitivity of glycosylated gut structures of young insects toward carbohydrate-binding proteins. At the following stages of development lectin did not show significant influence on *A. sordens* larvae survival. Supposedly, larvae are less susceptible to deleterious effects of lectins at their late developmental stages. Pupae period and pupae weight were not significantly different among each treatment of both insects.

Table 1. The effects of MChbL on the development of *A. sordens* and *A. segetum* larvae.

groups	Days to reach pupation	Rate of pupation (%)	Rate of adults emerging from pupa (%)
<i>A. sordens</i>	30 ± 1	28,6%	33%
<i>A. segetum</i>	25 ± 1	10%	5%
control	23 ± 1	41,4%	41,7%

In the following series of experiments larvae midgut enzyme extracts were prepared and inhibitory effects of MChbL on midgut proteases and amylases activity were determined. Proteolytic activity of the midgut extracts from fifth instar larvae was measured by fluorescence polarization spectroscopy using FITC-labeled casein as substrate. The results showed that MChbL

influenced larval gut proteolytic enzyme activity *in vitro* (decrease of total protease activity of the midgut extracts was monitored). The highest inhibition rate was 60% at a concentration of 0.25 μ g/ μ l MChbL (Fig.2). When incubated with the insect enzymes MChbL showed resistance to digestion and no inhibition of sugar-binding activity of lectin was observed. Resistance to degradation by pest metabolic systems is clearly beneficial for plant defensive proteins, production of which represents an effective strategy developed by some plants [Brunelle et al., 2004].

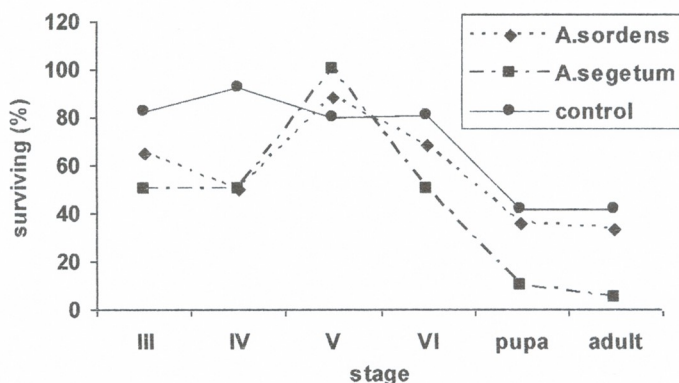


Fig. 1. Effect of MChbL on the survival of experimental larvae when incorporated into an artificial diet at 0.001% (w/w).

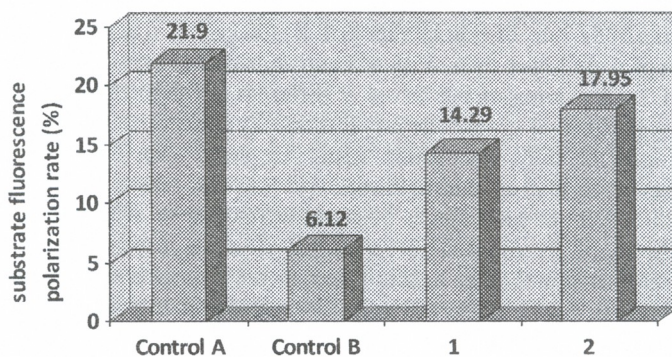


Fig. 2. Effect of MChbL on proteolytic activity of midgut extract from *A. sordens* and *A. segetum* measured by FITC-casein assay. control A, FITC-casein substrate solution; control B, substrate+midgut extract; 1-2, substrate+midgut extracts preincubated with ChbL.

The results obtained demonstrate that mistletoe chitin-binding lectin have obvious anti-nutritive effects on Lepidoptera pests. Apparently, lectin exerts its antinutritive effect on larvae at the early stages of development by interaction with midgut structures. The precise mechanism how the lectin exerts the insecticidal activity has not been fully elucidated, however, lectins with specificity for GlcNAc residues appear to reveal toxic effects to many insects [Macedo et al., 2007]. Since glycoproteins are major constituents of insect gut structures, it is possible that specific interaction take place between the glycosylated gut structures and plant lectins. It appears that surviving the hostile proteolytic environment of the insect midgut, specific binding to insect gut chitin components and alteration of glycosylated enzymes of digestive tract are basic prerequisites

for MChbL lectin to exert its deleterious effects on insects. The insecticidal activity of MChbL may be attributed to the lectin-induced reduction in diet ingestion resulting starvation of larvae. Possible implication of mistletoe chitin-binding lectin as potential entomotoxic biopesticide for the control of Lepidoptera pests is under consideration.

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Viscum album ლექტინის (MChbL) ანტი-კვეპიანი ბავშვანა *A. sordens* Hufn. და *A. segetum* Schiff. (Lepidoptera: Noctuidae) ბანგობარეგაზე

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ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი,
ბიოლოგიის დეპარტამენტი, მცენარეთა ფიზიოლოგიის ლაბორატორია

(მიღებულია 26.07.2010)

რეზიუმე

შესწავლილია *Viscum album* ქიტინ-დამკავშირებელი ლექტინის (MChbL) ინსექტიციდური თვისებები *Apamea sordens* Hufn. და *Agrotis segetum* Schiff. ღარვების მიმართ. MChbL ავლენდა პროტეაზას მაინჰიბირებელ აქტივობას და მოქმედებდა ღარვების ჩამოყალიბებაზე მათი განვითარების სხვადასხვა სტადიაზე. MChbL იწვევდა ღარვების 40% სიკვდილიანობას საკვებში მისი 0.001% (w/w) დანამატის სახით ჩართვისას. განიხილება ქიტინ-დამკავშირებელი ფითრის ლექტინის ენტომოტოქსიკური გავლენა აგრომაგნებლების მიმართ.

BIOLOGY AND ECOLOGY OF *PSEVDORASBORA PARVA* IN THE BASALETI LAKE

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Abstract

Pseudorasbora parva is characterized by strongly pronounced sexual dimorphism according to the size and weight indices. The species of the both sexes reach the pubescence simultaneously at the same age in the second spring season of the life. It has a simple age, size-weight and sexual structure presented by 4 age groups ($0^+ - 3^+$). During the whole year the sexual ratio approaches to 1:1. By the selection of spawn substrate it is lithophilous and expresses pretensions only to the "clearness" of spawn substrate. The spawning takes place in portions; the interval between the hard-roed portions makes up 10-11 days. *Pseudorasbora parva* belongs to the species which produce spawns in summer (Bazaleti Lake). The individual productiveness varies between 839-2816 samples. The function of defender guarantees a maximal receipt of new generation from fertilized spawns. By the character of nutrition it is characterized by the selectivity. *Pseudorasbora parva* has been formed as a resistant form of the ichthyocomplex in the reservoirs and belongs to the species of balanced populations.

Key words: Sexual dimorphism, hard-roed portion, ichthyological complex, *Pseudorasbora parva*

Introduction

In the ichthyofauna of the East Georgia, in spite of an impressive amount of "dwarf" or so-called "weed" fishes, the problems of their biology and ecology are not basically studied what is explained by the fact that they have no trade significance. For economic development and domestic use of the reservoirs, a certain practical importance has their faunistic and bioecological study (supply of biological resources, degree of production). It is much to do in respect of study of "dwarf" fishes in the Georgian reservoirs what prompts our interest to these problems. *Pseudorasbora parva* has been selected as the subject for the investigation which was accidentally introduced in the Bazaleti Lake. The main goal of our investigation was to establish the ways of *Pseudorasbora parva* introduction into the Bazaleti Lake and other reservoirs, to study the possibilities of the acclimatization to the new ecological environment, to discover the levels of adaptive properties and to elaborate preventive measures for their use in case of getting in unknown environment.

Pseudorasbora parva appears to be a widely spread Eastern-Asiatic species, a natural habitat includes the rivers of west seaside of the Japanese sea, as well as the reservoirs of south part of Japan as far as to the rivers of Vietnam. It is also distributed in the basin of the river Amur

and Lake Khanka. It was found in the lower part of the river Kume, etc. Taking into account a capacity for its adaptation, the broadening of its natural habitat is not yet completed.

Materials and Methods

During 1999-2003 we have obtained the actual material of *Pseudorasbora parva* in 5 selected stations (points) according to the seasons (Fig. 1).

The I and the IV points appear to be fractional small-sized pebble sandstone biotopes which are close to biocenoses rich in vegetation; the II and III points mainly are fractional small-sized pebble sandstone biotopes and the V point is uliginous, here and there stony biotopes are observed between vegetation zones (Fig. 1, 2).

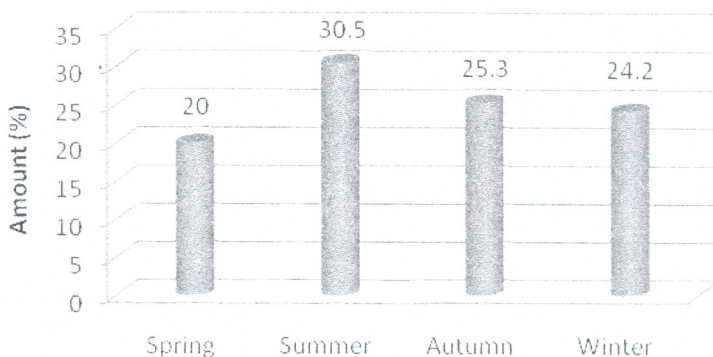


Fig.1. Samples of *P.parva* obtained in Bazaleti lake according to the seasons.

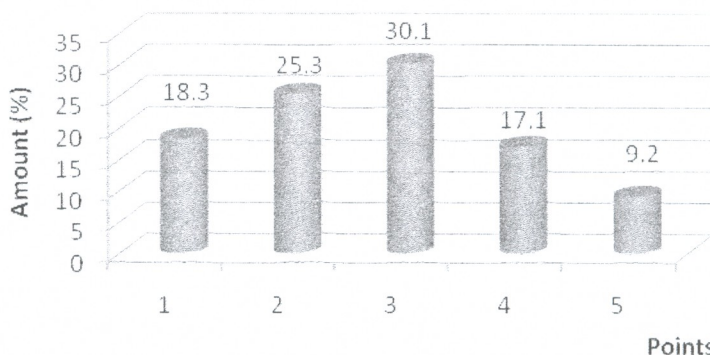


Fig.2. Samples of *P.parva* obtained in Bazaleti lake according to the selected points.

The fishing of *Pseudorasbora parva* was mainly accomplished on the shore area of the lake by means of small-celled net (length – 6 m, width – 1 m, the cell size – 3 mm with fishing floats on each 30 cm of the cell top, on the bottom – leaden weights on each 10 cm) and trap net, consisting of combined weaving cloth: length – 6 m, lower part – 1 m with 3 mm cells and upper part – 5 m with 6 mm cells. The diameter of the area of its entrance “mirror” was 25 cm³. The

effectiveness of the fishing was depended on the weather, hydrologic and hydrothermal conditions of the lake, etc. Out of the material, size and weight were determined in 770 (100%) samples, sex and stages of maturing - in 641 (83.2%) samples; prolificacy - of 309 (40.1%) samples, morphological niche - of 134(17.4%) samples, age – of 550 (71.4%) samples and feeding - in 320 (41.5%) samples. Those parameters were assessed by means of biological analysis accepted in ichthyology [Ivanov 1988; Monastyrski 1952; Pravdin 1966]. The material was fixed in 4% formalin solution. The laboratory study of the obtained material was carried out at the Institute of Zoology. The map - “Distribution of *Pseudorasbora parva* in the Bazaleti Lake” is elaborated by the GIS program [Bogutskaja & Naseka 1998; Rass 1983].

Results and Discussion

In 1987 the following 12 fish species were recorded in Bazaleti Lake: 1. *Capoeta capoeta* (Gueldenstaedtii), 2. *Cyprinus carpio* (Linnaeus), 3. *Barbus lacerta* (Heckel), 4. *Ctenopharyngodon idella* (Valenciennes), 5. *Hypophthalmichthys molitrix* (Valenciennes), 6. *Leuciscus cephalus orientalis* (Nordman), 7. *Vimba vimba* (Linnaeus), 8. *Carassius carassius* (Linnaeus), 9. *Alburnus bipunctatus eichwald* (Filippi), 10. *Alburnus filippi* (Kessler), 11. *Romanogobio persus* (Günther), 12. *Pseudorasbora parva* (Temminck et Shleg.). The percentage of only 10 species of tadpoles was established (Table 1).

Table 1. Nutrient composition and distribution frequency of *P. parva* in the Bazaleti Lake

№	Species	Percentage of samples in different biotypes (%)	
		Plant biotypes	Stony biotypes
1	<i>Capoeta capoeta</i>	4.8	95.5
2	<i>Barbus lacerta</i>	-	100
3	<i>Cyprinus carpio</i>	95.2	4.8
4	<i>Vimba vimba</i>	-	100
5	<i>Leuciscus cephalus orientalis</i>	2.7	97.3
6	<i>Carassius Carassius</i>	99.1	0.9
7	<i>Alburnoides bipunctatus eichwald</i>	100	-
8	<i>Alburnus filippi</i>	6.1	93.3
9	<i>Romanogobio persus</i>	-	100
10	<i>Pseudorasbora parva</i>	93.8	6.2

Ctenopharyngodon idella, *Hypophthalmichthys molitrix*, *Capoeta capoeta*, *Lacerta cyri* and *Cyprinus carpio* are found in single amounts. In general, faunistic complex of the hydroecosystems are resistant to natural invasion of foreign species existing in the fauna. In spite of it, from time to time the introduction of new species takes place which is connected to the procedures of fish industry. The existence of fishes of Chinese complex fully depends on artificial reproduction. They show a complete biological stability and are considered to be defective-anthropogenic elements, while they are considered to be valuable in case, if there are all necessary conditions for the species introduced in the given ecosystem which are the following: 1) transfer an accent from local, native species being under a high risk of extinction and dying out to the introduced species; 2) increasing fish production in the reservoirs by means of reconstruction of ichthyocomplex, in particular, by substitution of low value fishes by fast-growing expensive species; 3) to infill trophic connections in the reservoirs; 4) fulfillment of genetic-selective works followed by revelation of the complex of “concealed” adaptive properties of introduced foreign species by full-value or inferior forms. The latter completely depends on anthropogenic factors: artificial nutrition, reproduction or the both together taken, while the existence of all necessary

vitaly important conditions is considered to be full valued. During complete acclimatization of introduced species, an artificial migration appears to be an altered “forced” form and results in artificial widening of natural habitat of the species.

Presumably *P. parva* was brought to Georgia in 1987 together with introduced *Ctenopharyngodon idella*. *P. parva* together with *C. idella* was found in different reservoirs. At first it was noted in the Bazaleti Lake (1987), and later on – in Jandari, Kumisi, Algeti Shaori Lakes, and Tkibuli reservoir. An absolute length of *P. parva* does not exceed 7 cm; it has an elongated form, oblique-directed upturned muzzle with small lengthwise mouth. *P. parva* has longitudinally situated large eyes in upper part of the head. Between the mouth and eyes the nasal fossa are located. The upper lip is thicker than the lower one. There are 5 teeth along the both sides of the head and they are located in one line (Table 2).

Table 2. Morphological characteristics of *P. parva* in the Bazaleti Lake.

Morphological features of <i>P. parva</i>	Females, n=67			Males, n=67		
	M±m	δ	Fluctuation	M±m	δ	Fluctuation
Length of whole body	49.99±0.57	2.89	34.06 – 65.93	61.60±0.71	3.85	60.00 – 62.50
in % from the whole length of the body						
Body length	58.09±0.51	2.00	37.66-62.93	63.09±0.60	3.00	57.50 – 63.5
Head length	21.42±0.17	0.79	9.31 – 13.11	24.30±0.22	1.05	9.33 – 13.33
Length of tail stalk	17.82±0.21	1.67	16.11 - 24.00	19.80±0.23	1.07	16.50 -24,45
Antedorsal distance	41.66±0.27	1.43	48.68 – 53.69	43.55±0.28	1.44	48.60 – 54.39
Postdorsal distance	32.14±0.23	1.24	32.77 – 36.26	37.70±0.25	1.28	33.21 – 38.95
Maximal height	19.00±0.20	1.03	15.50 – 18.06	23.11±0.40	1.10	15.29 – 19.30
Minimal height	9.50± 0.09	0.37	6.24 – 7.50	10.78±0.11	0.40	-
Length of D foundation	10.71±0.39	1.29	-	-	-	-
D Height	22.71±0.29	1.42	14.28 – 21.01	13.67±0.31	1.43	15.26 – 20.10
Length of A foundation	8.33±0.28	1.44	-	-	-	-
A Height	11.90±0.27	1.46	12.22 – 17.16	10.29±0.32	1.44	13.15 – 16.11
P Height	13.09±0.23	1.21	17.53 – 22.19	15.77±0.25	1.21	19.35 – 24.45
V Length	10.70±0.30	1.56	12,18 – 18.10	16.40±0.34	1.58	15.00 – 18.75
P-V Distance	17.85±0.42	2.20	28.60 – 40.87	20.01±0.44	1.88	29.09 – 40.05
V-A Distance	17.85±0.42	2.20	28.60 – 40.87	20.01±0.44	1.88	29.09 – 40.05
Diameter of eye	4.76±0.10	0.41	2.76 – 3.86	6.60±0.17	0.56	2.80 – 3.88
Length of muzzle	7.10±0.15	0.84	8.58 – 11.52	9.15±0.18	0.68	10.40 – 12.49
Distance behind the eye	7.10±0.18	0.70	2.37 – 4.00	8.03±0.21	0.71	2.44 – 4.20
Length of the forehead	29.34±0.11	0.60	23.00 – 35.73	27.43±0.13	0.25	23.01 – 35.80
Height of the head	11.9±0.23	0.80	41.61 – 64.52	15.3±0.26	1.02	42.59 – 63.58
D fin	III- 7					
A fin	III- 6					
The amount of vertebrae	31 - 33					
The amount of gullet teeth	5 – 5					
Lateral line	L.L. 36 5/3 37					

Total of 37-38 scales are located in side line which as distinct from other scales are of dark colour and have no moon-like edging. The body has a yellowish-silver tint. The fins are rounded.

In the Bazaleti Lake the population of *P. parva* is characterized by a simple age and size structure. The young age groups (0+ 1+) make up 72% of the obtained material. Age structure is similar in the samples of both sex, i.e. their utmost age corresponds to each other and does not exceed 3+. In the Bazaleti Lake the structure of *P. parva* population is presented according to the following scheme: 1) impuberal, 2) primary maturing and 3) repeated maturing samples. The spawning part is presented by three age groups. The natural loss between separate age groups is: 0+ 1+ – 10.15%; 1+ 2+ – 24.9%; 2+ 3+ – 39.0%. The structure and character of the dynamics show their specific connection to the environment factors. As well as age structure, the size-weight structure is also subjected to the changes of environmental conditions and it is specific for the species but does not overstep the limits of genetically coded growth fins. In the lake its maximum size is 65 mm and weight – 3070 g (male species) (Fig. 3).

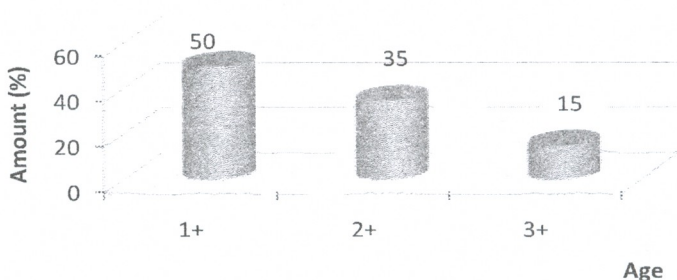


Fig.3. Age structure of mature *P.parva*.

The start time for maturing of *Pseudorasbora parva* is connected to definite size but not to chronological age. The individual productiveness of *P. parva* varies between 839-2816 samples. The pubescence of male and female species takes place at the same time at the age of 1+, when the length of samples of the both sexes reaches 30 mm. The spawning season is connected to temperature mode of the water and begins at 16-17°C. The process of development and maturing of the spawn in gonads is extended in time which is corroborated by the existence of spawns of three generations (in average 1.04 mm, 0.76 mm, 0.47 mm). The production of spawn is fulfilled in portions. During all the period of reproduction fish produce spawn of 978-2017 samples. The mature spawn has yellowish colour and elliptic form. As usual the spawning season for *P. parva* is the summer. *P. parva* appears to be lithophilous and produces spawn in the shore line of the lake on 60-70 cm depth (in dried up roots of reeds and *typha latifolia*, etc). The male species cleans the territory of 130-310 mm (sediments, lower seaweeds) and attracts female species which like “a clean” territory and produce spawn in this zone after which male species fertilize the spawn. 2-15 minutes later the fertilized spawn produces a viscidulous (gluten) matter and tightly sticks to the substrate. The physiological process taking place in male species results in morphological changes as well as in severe aggression making possible maintenance of a complete quantity of a future generation. Till complete formation of the embryo under optimal conditions the spawn undergoes 7 stages during 7-10 days. Then a free embryo gets off from the spawn tunic and it takes 2-3 days for a complete formation. After this from time to time it begins to float to the upper layers of the water.

Reproductive products are not developed synchronously. In the Bazaleti Lake the stages of development and maturing of *P. parva* are presented by the following scheme: I stage – juvenile; it is impossible to determine the sex by unaided eye. Before the I hard-roed portion, the following stages of spawn development should be noted: II, III, IV, V, VI-III; before the II portion: (VI)-III; (VI)-IV; (VI)-V; (VI)-VI-III; before the III portion: (VI-VI)-III; (VI-IV)-IV; (VI-VI)-V which is directly followed by the II stage without the I stage. The interval of maturing between portions is 10-11 days.

The main food for *P. parva* is algae, oligochets and detritus composing 84% of the Bazaleti Lake. It should be also noted caddis flies, nematodes and ostracoda. The season spectrum of their nutrition points to the fact that the specimens which are in abundance in all seasons appear to be the main food. By the occurrence frequency the chironomides from the animal organisms and detritus dominate during the whole year. As to small bristling worms, seasonal dayflies and caddis flies dominate. *P. parva* is characterized by the selectivity, fast adaptation to the unknown environment and competes with local species; it shows a high resistance to different diseases. *P. parva* belongs to the balanced populations, with **K**-type of selection, but by some properties it belongs to **R**-type. It is sufficiently tolerant to temperature variation and decrease of water level in the lake. The possibilities of its spreading are also well expressed in Georgia (Fig. 1).

Pseudorasbora parva may have some practical values: 1) it is a dwarf, exotic fish, has a small size and weight. According to morphological and biological peculiarities it may be considered as a perspective aquarian species; 2) dried *P. parva* may be used for birds nutrition in poultry firming (ducks, geese), as well as for fish nutrition in fish industry. *P. parva* contains a considerable quantity of vitamin A which makes it possible to produce high quality flour; 3) in the literature *P. parva* may be determined as an alternative of *Gambusia holbrooki* and it may be used against the malaria mosquito spread on humid territory of West Georgia.

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ბაზალეთის ტბის მცირე ფეკდორასბორას ბიოლოგია და ეკოლოგია

დ. შონია, თ. ქოქოსაძე, ბ. ჯაფოშვილი.

ივანე ჯავახიშვილის სახელმწიფო უნივერსიტეტი, თბილისი

(მიღებულია 02.09.2010)

რეზიუმე

მცირე ფეკდორასბორას ახასიათებს მკვეთრად გამოხატული სქესობრივი დიმორფიზმი ზომა-წონით მაჩვენებლებში. სქესობრივ სიმწიფეს ორივე სქესის ეგზემპლარები აღწევენ ერთდროულად, ერთსა და იმავე ასაკში, სიცოცხლის მე-2 გაზაფხულს. გააჩნია მარტივი ასაკობრივი, ზომა-წონითი და სქესობრივი სტრუქტურა 4 ასაკობრივი ჯგუფით (0^+ - 3^+). მთელი წლის განმავლობაში სქესობრივი თანაფარდობა მიახლოებულია 1:1-თან. სატოფე სუბსტრატის არჩევითობის მიხედვით იგი ლითოფილია, პრეტენზიულია სატოფე სუბსტრატის მხოლად “სისუფთავის” მიმართ. ქვირითობა პორციულია, ხოლო ინტერვალი პორციებს შორის კი 10-11 დღეს მოიცავს, იგი ზაფხულში მოქვირითე თევზია (ბაზალეთის ტბა); ინდივიდუალური ნაყოფიერება მერყეობს 839-2816 ცალ ქვირითამდე. მცველის ფუნქცია უზრუნველყოფს განაყოფიერებული ქვირითებიდან ახალი თაობის მაქსიმალურ მიღებას. კვების მხრივ იგი ხასიათდება სელექტიურობით. იგი ჩამოყალიბდა ჩვენი წყალსატევების იქთიოკომპლექსის მდგრად ფორმად და მიეკუთვნება გაწონასწორებულ პოპულაციათა რიცხვს.

ON THE NEW LOCALITIES OF *IRIS IBERICA* IN GEORGIA

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During the research of Georgian flora and vegetation (2008-2010) in the Eastern Georgia we discovered *Iris iberica* Hoffm. (Iridaceae) in the south-eastern part of Trialeti ridge. This species was not recorded for this area either in herbarium material or literature sources (TBI, TGM).

I. iberica was described from Tbilisi vicinities by Hoffman in the beginning of XIX c. It occurs in the eastern part of the southern Caucasus [Kakheladze, Gavrilenko 1955]. According to D.Sosnovski [Sosnovski, 1941], the distribution area of this species is identified as Kartli-Borchalo and outside Georgia - Azerbaijan. Georgian iris also occurs on Teleti ridge, Delisi, Ponitchala-Krtsanisi plain and in Kiziki region [Lachashvili et al., 2007].

Iris iberica is a rare and endangered plant. It is included in the Red Book (1982) and Red List of Georgia and Red Book of the USSR (1984). As a result of large-scale and uncontrolled extraction, grazing and impact of other factors, its populations have significantly decreased.

We found *Iris iberica* in the vicinities of vill. Marabda (N 410 32. 264' Eo 440 44. 771', 574 m a. s. l.) and Durnuki (N 410 32. 136' Eo 440 41. 353', 743 m a. s. l.).

Iris iberica Hoffm. occurs in steppes and semi-deserts. It is presented in the phytocenoses of phryganoid vegetation and foothill deserts associated with: *Paliurus spina-christi*, *Poa bulbosa*, *Stipa lessingiana*, *Botriochloa ischaemum*, *Allium rubellum*, etc.

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Iris iberica -ს ბავრცელების ახალი აღბილსამყოფელის შესახებ

თედორაძე გ.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

(მიღებულია 11.10.2010)

რეზიუმე

Iris iberica იშვიათი და გადაშენების საფრთხის ქვეშ მყოფი სახეობაა. იგი შეტანილია საქართველოს წითელ წიგნში. მასობრივი და უკონტროლო მოპოვების, ძოვების და სხვა ფაქტორების ზემოქმედების შედეგად მისი პოპულაციები საგრძნობლად შემცირებულია. *Iris iberica* ჩვენ მიერ ნაპოვნია თეთრიწყაროს რაიონის სოფ. მარაბდის (N 41° 32. 264' E₀ 44° 44. 771'. 574 მ ზ. დ.) და დურნუკის (N 41° 32. 136' E₀ 44° 41. 353'. 743 მ. ზ. დ.) მიდამოებში.



Iris iberica Hoffm.

STUDY OF KARYOLOGY OF CHERRY PLUM (*PRUNUS CERASIFERA* EHRH.) DISTRIBUTED IN SAMTSKHE-JAVAKHETI REGION OF GEORGIA

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(Received October 4, 2010)

Abstract

In the process of karyological study of *Prunus cerasifera* in Samtskhe-Javakheti Region of Georgia along with diploid forms ($2n=16$) the polyploid form ($2n=48$) was revealed as well. Polyploid form of cherry plum ("tkemali") should have been emerged as a result of allo-polyploidization of diploid forms.

Key words: diploidy, polyploidy, autopolyploidy.

Cherry plum ("tkemali") (*Prunus cerasifera* Ehrh.) is a diploid species ($2n=16$). On the basis of investigation of cherry plum specimens in the Caucasus it was concluded that all samples distributed in this region are diploid and the wild relative of the type of *Prunus domestica* ($2n=48$) was not found there [Rubin, 1936]. In the North Caucasus, in particular the locality of Shuntuk, only forms with $2n=24$, $2n=32$ chromosomes have been detected, which were attributed to the natural hybrids of myrobalan-blackthorn [Rubin, 1936].

Wild form of *Prunus* with $2n=48$ chromosomes was not known up to present.

Karyological study of rich material of plum specimens collected from Kartli and as a result of expeditions in other regions of Georgia, has shown that along with diploid - $2n=16$ forms of cherry plum and tetraploid - $2n=32$ forms of blackthorn, the polypliod forms (cherry plum $2n=48$, $2n=96$ and blackthorn $2n=48$, $2n=64$) were found as well [Baiashvili, 1973; 1980; 1983].

The present study deals with karyological study of cherry plum specimens collected as a result of expeditions carried out in Samtskhe-Javakheti region [Baiashvili, 2010].

For karyological studies the root meristem of plant was used. Roots were fixed according to the technique developed by Navashina. Material was cut to 12-16 μ thick slices and stained by Iron Hematoxylin, according to Heiden-Hain's method.

Karyological analysis of material has revealed that along with diploid forms ($2n=16$) polyploid forms ($2n=48$) present also (Fig. 1).

Karyological study of cherry plum made it clear that chromosome number of somatic cells of this form is $2n=48$.

It is worth to note the mosaicism of chromosome number in root somatic cells, which is one of characteristic features of ploidy.

Polyploid form of cherry plum ($2n=48$) (specimen N3), which was found in the village Khizabavra of Aspindza district, differs from the diploid form ($2n=16$) not only by chromosome number, but by morphology as well. Its fruit is big (weighing 10.5g), of conic shape, dark red. Fruit is fleshy, juicy, with thick skin, very sour around the stone. Flesh can be hardly removed from the

stone, stone is big (0.5g), flattened, with well manifested wing. According to narrative descriptions of local inhabitants the plant has emerged from the stone.

Fruits of forms of cherry plum distributed in Meskheta are small in size. They are rarely planted on farm-yards attached to the house. These trees usually grow near the fences, out of fences or at the roadsides.

Specimen of cherry plum N20 was found in Aspindza. Fruit is big (10.3 g), red, of round shape. On some fruits line is visible. Flesh is easily separated from the stone. Stone is of medium size. Similar form with big size fruit was found in Akhaltsikhe as well. Trunk of the tree is big; fruit is of medium size, very sour at the stone. Stone is small (0.3g).

When collecting the material we were focused mainly on forms with big fruits, as ploidy is also manifested in morphological changes.

It is worth to note that fruits of all polyploid forms of cherry plum and blackthorn are of big size, but not all forms with big fruits are polyploid.

The polyploid form of cherry plum ($2n=48$) (N3) revealed in Samtskhe-Javakheti is characterized mainly by the features of cherry plum and none of characters of any other species of the genus *Prunus* are manifested in it.

We suppose that this polyploid form ($2n=48$) of cherry plum should have been emerged as a result of allo-autopolyploidization of diploid forms ($2n=16$).

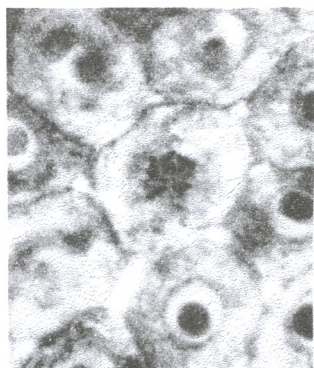


Fig. 1. Microphoto and drawing. Somatic mitosis $2n=48$ of the cherry plum (*Prunus cerasifera* Ehrh.)

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**სამცხე-ჯავახეთში გავრცელებული ტყემლის (*Prunus cerasifera*
Ehrh.) კარიოლოგიური შესწავლისათვის**

ბაიაშვილი ე.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

(მიღებულია 04.10.2010)

რეზიუმე

სამცხე-ჯავახეთში გავრცელებული ტყემლის კარიოლოგიური შესწავლისას ტყემლის დიპლოიდურ ($2n=16$) ფორმებს შორის, რომელსაც ეს სახეობა მიეკუთვნება, პოლიპლოიდური ($2n=48$) ფორმაც აღმოჩნდა. პოლიპლოიდური ფორმის სომატურ უჯრედებში შეინიშნება ქრომოსომა რიცხვის მოზაიკურობა, სიჭრელე, რაც პლოიდურობის დამახასიათებელი ნიშანია. ვვარაუდობთ, რომ ტყემლის ეს პოლიპლოიდური ფორმა დიპლოიდურ ფორმათა ალლო-ავტოპოლიპლოიდიზაციის შედეგს უნდა წარმოადგენდეს.

TWO DORYLAIMID NEMATODE SPECIES NEW FOR GEORGIAN FAUNA FROM SAMCKHE-JAVAKHETI (EASTERN GEORGIA)

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(Received October 5, 2010)

Abstract

As a result of study of soil and plant nematodes of Samtske-Javakheti region (Eastern Georgia) two species of nematodes new for the fauna of Georgia were found. Description and measurements of these species are given.

Key word: Nematode, spear, cuticle, specula

Aporcelaimellus medius, Andrassy, 2002

Measurement:

Females (2): L=1.71-1.79mm; a=19.5-21.3; b=3.7-4.1; c=92-98; c'=0.4-0.5; v=52%-54%;

Males (1): L=1.75mm; a=27; b=3.8; c=82; c'=0.5; spic=73µm; suppl.=11

Body dense enough, 80-87 µm wide at middle. Head strongly set off from the body contour. Lip region 18-19 µm wide, hardly separated. Cuticle thin, 2-3 µm. Length of spear 22-24 µm. Orifice equals to 1/2 of stylet length or slightly more.

Vagina strongly sclerotized. Ovaries paired, outstretched. In uterus of one of the two females three eggs (86.8-58.8 µm; 89.6-64.4 µm; 98-56 µm) are elongated. Rectum 1.2-1.6 of anal body widths. Tail hemispherical, about half as long as anal body diameter.

Male genital organs occupy about half of the body length. Spicula slender, 71 µm long. Ventromedial supplements 10, mostly separated [Andrassy, 2002]

Labronemella ruttneri, Schneider, 1937

Measurement:

Female: L=1.23mm; a=30.2; b=3.8; c=64; v=52.7%;

Small species. Body stout, lip region is set off from the body contour. Spear 27 µm long, 1.2 times longer than the head diameter. The aperture occupy one-third of its length. Guiding ring double. Oesophagus strongly muscular, enlarged slightly anterior to middle. Total length of oesofagus is 133mm. Body at posterior and of esophagus 2.3 times as wide as the head. Outer lips discolaimoid. Female gonads paired. Prerectum about one and one-half times as long, as anal body

diameter. Tail blunt, rounded, about two-thirds as long as the anal body diameter [Thorne, 1939]. Both species were found in soil samples.

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საქართველოს ფაუნისათვის ორი ახალი სახეობა *Aporcelaimellus medius* და *Labronemella ruttneri*

ცქიტიშვილი ე., ცქიტიშვილი თ., კუჭავა მ., გიგოლაშვილი მ.

(მიღებულია 05.10.2010)

რეზიუმე

აღწერილია საქართველოს ფაუნისათვის ნემატოდების ორი ახალი სახეობა *Aporcelaimellus medius*, Andrassy, 2002 და *Labronemella ruttneri*, Schneider, 1937. მოცემულია მათი ახალი აღწერა და განაზომები.

ინტერუქცია 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 გვართა და წლით [Lernmark, Hagglof 1981].

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

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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.

