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CONTENTS

Microbiology

Phytopathology

Plant Physiology

Zoology

Letter to Editor

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 3, No. 3, 2005.

Biochemistry

STUDY OF CHEMICAL COMPOSITION OF GRAIN PROTEIN OF *VICIA FABA*

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(Received September 14, 2004)

Abstract

The chemical composition of seeds of Vicia faba has been studied. The quantitative content of proteins, lipids, polysaccharides, mineral substances and some vitamines has been calculated. Separate protein fractions of albumines, globulines and glutelines have been obtained. Alcohol soluble prolamines fraction has been found in trace amount. Amino acid composition of separate protein fractions of Vicia faba seeds are determined.

Introduction

The elaboration of efficient methods of nontraditional application of plants rich in delicious proteins is of practical importance for bread baking industry. Vicia faba besides improving the degree and nutritional value of a ready product, provides the economy of basic and additive raw materials and intensification of paste preparation processes [Dug, et al., 1999; Crofton, et al. 2000; Dug, et al., 2001]. So, it is important to state the chemical composition of Vicia faba seeds.

Materials and Methods

Vicia faba seeds from the agricultural scientific station of Tskaltubo were used. The separate protein fractions were obtained by Osborn method. For quantitative estimation of total proteins and separate protein fractions Bredford method was used [Bredford, 1976]. Amino acid composition was estimated by "Hitachi" – 835 amino analyzer. The glucose content was calculated according to Somogyi [Somogyi, 1952].

Results and Discussion

To use the plants for bread baking plant characterized by high protein content should be chosen. Besides, the proteins themselves must be characterized by abundance of unreplaceable amino acids and easy digestion as well.

To establish the possibility of using Vicia faba in bread baking chemical composition of meal was studied. For analyses the sort "imereti" of Vicia faba was selected. The data on main organic compounds, namely proteins, lipids and carbohydrates content are presented in table 1 and diagram.

Table 1. The chemical composition of meal of Vicia faba

The list of substances	Quantity % (as dry mass)
Polysaccharides	
monosaccharides	2,47
(glucose)	1,02
disaccharides	3,40
dextranes	2,60
starch	42,20
pectine	2,60
hemicellulose	3,40
cellulose	2,50
pentosine	4,80
(soluble fraction)	3,35
Proteins	
soluble in water	21,30
unsoluble in water	3,40
Lipids	1,94
Ashes	3,85
Vitamins	
B_1 - thiamine	0,350
B_2 - riboflavin	0,239
PP - nicotine acid	3,144

As it is seen from the table carbohydrate content reaches 63,97 % calculated per unit of dry weight material. Content of starch (42 %) is about a half of total saccharides. Among other polysaccharides prevail hemicellulose (3,4%) and pentosine (4,8%).

Saccharide composition of Vicia faba will not disbalance the digestion system and hence it may be used in bread baking.

It is known that plants with high lipid content can't be used in bread baking. The lipid content (1,94%) is comparatively low in Vicia faba. So, this plant has to be recommended for this purpose.

To investigate the mineral content of Vicia faba seeds some macro and micro substances were determined. Among the microelements the highest are aluminum and the iron contents, less copper; among macro substances potassium prevails, which composes about 70 % of the total content.

Some vitamin content is determined in Vicia faba meal. In particular, B_1 and B_2 vitamins compose of 0,350 mg/g and 0,239 mg/g respectively; but the content of nicotinic acid reaches 3, 144 mg/g; this quantity is enough for these vitamin's daily supply.

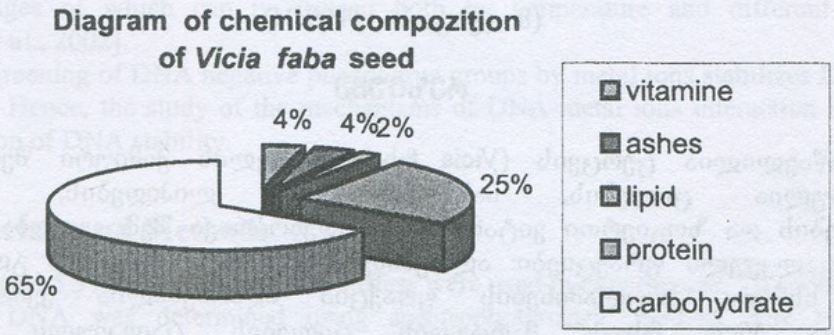
Vicia faba seeds contain a high level of proteins (24,70 %). Total proteins were fractionated by different solvents and as a result fractions of water soluble - albumins, salt soluble - globulins and alkaline soluble - glutelins were received. It must be indicated that alcohol soluble fraction - prolamines, which is characteristic of cereals are represented in Vicia faba only in trace amounts.

It is known that for plants selection in bread baking the quality of soluble fractions and its balancing by unreplaceable amino acids must be considered. Therefore, separate protein fractions were tested on amino acid content. The data of amino acid composition of the separate protein fractions are represented in table 2.

Table 2. The amino acid composition of separate protein fractions of Vicia faba

List of the amino acids	albumin %	globulin %	glutelin %
Lysine	7,10	1,40	4,20
Hystidine	2,30	4,27	28,00
Arginine	7,10	8,00	4,00
Aspartic acid	8,50	11,00	9,15
Treonine	3,90	5,05	4,90
Serine	5,00	6,30	4,80
Glutamine acid	18,10	27,80	11,00
Proline	4,40	4,60	-
Glicine	4,80	6,58	9,20
Alanine	4,20	5,00	-
Cistine	2,45	2,39	-
Valinine	2,38	1,90	-
Methionine	2,20	2,92	-
Isolysine	9,39	3,10	-
Leuicine	7,40	9,25	22,10
Thyrosine	2,40	3,70	-
Phenilalanine	1,60	3,00	-
Triptofine	0,84	0,90	-

Separate protein fractions - albumines, globulines and glutelines have been received and their amino acid compositions have been determined. The content of unreplaceable amino acid in albumins reaches more then one third (35%), and in globulins - 25,5%. In addition both fractions contain all unreplaceable amino acids. Between unreplaceable amino acids of albumine fraction lysin (7,1%), isoleuicine (9,39%) and leucine (7,40%) prevail, but in globulin fraction - leucine (8,25%) and treonine (5,05%). It is interesting to analyze gluteline fraction. The whole content of unreplaceble amino acids reaches 31,2%, which is represented by only 3 amino acids: leucine (22,1%), triptophane - 4,90% and lysine - 4,20%.



Thus, according to the analysis of saccharides, proteins and some vitamins we can say that Vicia faba is the sufficient object to be used as additional material. Chemical composition of Vicia faba points to its nutritional value and high protein content and quality stipulates its application as additive in bread baking.

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ცერცვის (*Vicia faba*) მარცვლის ქიმიური შემადგენლობის შესწავლა

შენგელია ნ.

ს. დურმიშიძის სახელობის ბიოქიმიის და ბიოტექნოლოგიის ინსტიტუტი, საქართველოს მეცნიერებათა აკადემია

(მიღებულია 14.09.04)

რეზიუმე

შესწავლილია ცერცვის (Vicia faba) მარცვლის ქიმიური შემადგენლობა. განსაზღვრულია ცილების, ნახშირწყლების, ლიპიდების, მინერალური ნივთიერებების და ზოგიერთი ვიტამინის რაოდენობრივი შემცველობა. მიღებულია ცილების ცალკეული ფრაქციები: ალბუმინები, გლობულინები და გლუტელინები. სპირტში ხსნადი პროლამინების ფრაქცია აღმოჩენილია კვალის სახით. დადგენილია Vicia faba-ს მარცვლის ცილების ცალკეული ფრაქციების ამინომჟავური შემადგენლობა.

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Biophisics

EFFECT OF IONS (Ni^{2+}, F^- AND Ce^{3+}) ON DNA MELTING. CALORIMETRIC STUDY

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(Received February 22, 2005)

Abstract

The interaction between DNA and metal ions (Ni^{2+} and Ce^{3+}) has been studied. The increase of Ni^{2+} concentration leads to the decrease of DNA transition temperature and enthalpy. Trivalent Ce^{3+} has different effect on DNA: Two thermostable regions exposed at 53.4±0.1° C and 91.9±0.1° C (when $[Ce^{3+}]/[P]=0.2$). The change Cl^- by F^- is reflected in DNA heat capacity increment.

Keywords: DNA melting, heat capacity increment, denaturation, DNA hydration.

Introduction

The DNA hydration degree and DNA-ions interaction have a significant influence on DNA conformation. The DNA duplex exists in B form in conditions of high relative humidity, but in the case of humidity decrease DNA acquires A or C form. The experimental results show that there are some hydration water layers on DNA double helix surface [Bloomfield et al., 2000], the structural changes of which can be caused both by temperature and different metal ions [Mrevlishvili et al., 2002].

The screening of DNA negative phosphorus groups by metal ions stabilizes DNA double-helix structure. Hence, the study of the mechanisms of DNA-metal ions interaction is significant for determination of DNA stability.

Materials and Methods

Citric (pH 5.9) and acetic (pH 5.2) buffers were used in experiments. The concentration of Calf Thymus DNA was determined using spectrophotometric method. The heat capacity measurements were performed by means of differential scanning calorimeter (DACM-4A), with operational cell volume of 0.46 ml and the operating temperature range of –10 to 150 ° C; the heating rate was 2 K min^{-1}. The specific heat capacity (Cp) values were calculated using a value of 0.54 ml/g for partial volume of native DNA.

Results and Discussion

It is well known that Calf Thymus DNA melting curve (0.15 mM NaCl; pH 5.9) has distinguishable peaks. DNA investigation by means of centrifuge in CsCl density gradient and

microcalorimetry [Klump et al., 1991] showed that the peaks of so called satellite DNA melting correspond to the number of G-C pair.

One of the most important feature of DNA denaturation is the existence of heat capacity increment, in our case ΔC_p=0.27±0.03 J/gK (when solution contains NaCl). As it was shown in [Mrevlishvili et al., 2001] the value of ΔC_p is mainly attributed to destruction of hydration water structure and hydrophobic effect — exposition of bases into water environment.

Both cations and anions have effect on the helix-coil transition in DNA. We have changed NaCl by NaF and tried to emphasize the role of anions on DNA conformational stability. The thermal denaturation curves of Calf Thymus DNA are given in Fig. 1 (when solution contains NaCl (curve a) and NaF (curve b)). Comparison of the curves (Fig. 1) did not reveal significant changes in the thermodynamic parameters of DNA melting (T_{mNaCl}=87.6±0.1° C, T_{mNaF}=88.4±0.1° C; ΔH_{mNaCl}=52±5 J/g, ΔH_{mNaF}=56±6 J/g). Positions of satellite DNA melting peaks also have not changed. Effect of Cl^- changing by F^- was exhibited in denaturation increment of heat capacity. ΔC_p increased up to 0.58±0.06 J/gK for NaF containing solution. This fact can be considered as reason of two phenomena: 1. increase of hydration water ordering at DNA surface; 2. the hydrophobic effect. The first reason should not be taken into consideration, because enthalpy of DNA melting does not change in the case of Cl^- change by F^-. This means that energetic contribution of hydrated water destruction in enthalpy does not change in the case of F^-. Thus we should pay attention to hydrophobic effect on double helix unwinding in environment which contains F^-. One can assume that F^- destroys H-bonds in bulk water lattice increasing number of monomeric water molecules interacting with base pairs at DNA melting. This circumstance leads to the increase of DNA denaturation heat capacity increment.

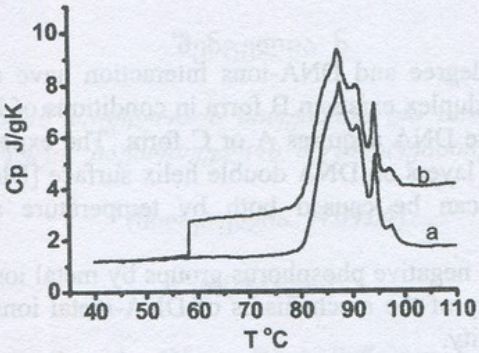

Fig. 1. The dependence of specific heat capacity of DNA on temperature at pH5.9.
a - 150 mM NaCl; b - 150 mM NaF (curve b is manually rised for clearity).

Heat capacity temperature dependence for Calf Thymus DNA (0.15M NaCl; pH5.9 C_{DNA}=0.77 mg/ml) is given on Fig. 1 (curve a). Thermodynamic parameters in this case are T_{mNaCl}=87.6±0,1 °C; ΔH_{mNaCl}=52±5 J/g. DNA melting curves in the presence of 10mM Ni^{2+} are represented on Fig. 2 (curve a). The thermodynamic parameters have changed (T_m=82.0±0.1 °C; ΔH_m=54±5 J/g). This result confirms that Ni^{2+} interacts with DNA (particularly with G-C rich fraction of DNA).

The results obtained by us correlate with the results described in other papers [Kankia 2000]. In mentioned paper Ni^{2+} effects on regularly alternative DNA purine-pyrimidine sequence have been studied. It has been shown that Ni^{2+} preferably binds to G-C pairs, causing dehydration of DNA double-strand. The release of water molecules from hydrated shells results, in its turn, in decrease of DNA thermostability.

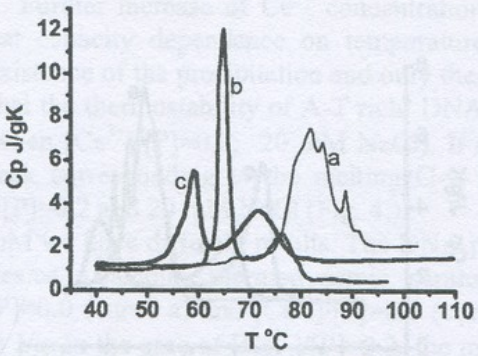

Fig. 2. The dependence of specific heat capacity of DNA on temperature at pH 5.9.
a - 10 mM Ni^{2+}; b - 20 mM Ni^{2+}; c - 40 mM Ni^{2+}.

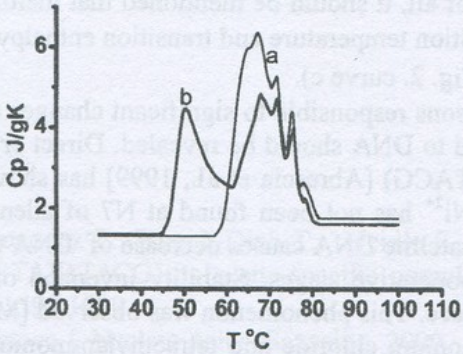

Fig. 3. The dependence of specific heat capacity of DNA on temperature at pH 5.2, 20 mM NaCl. a - $[Ce^{3+}]/[P] = 0.0$; b - $[Ce^{3+}]/[P] = 0.1$.

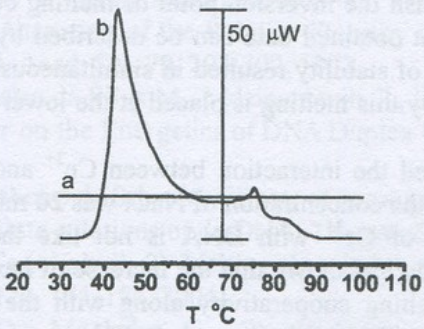

Fig. 4. DSC scan for DNA at pH 5.2, 20 mM NaCl. $[Ce^{3+}]/[P] = 0.2$.
a - baseline; b - DNA melting curve. Scanning rate 2 K/min.

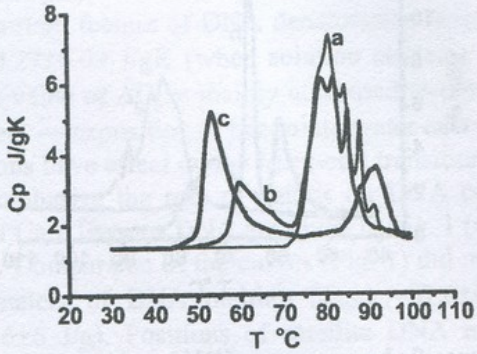

Fig. 5. The dependence of specific heat capacity of DNA on temperature at pH 5.2, 100 mM NaCl. a - [Ce3+]/[P] = 0.0; b - [Ce3+]/[P] = 0.1; c - [Ce3+]/[P] = 0.2.

The DNA melting curves at significant increase of Ni^{2+} ion concentration (from 10mM to 40mM) is presented on Fig. 2. Increase of Ni^{2+} ion concentration results in sensible difference of DNA melting process. First of all, it should be mentioned that melting process is divided into two heat absorption stages. Transition temperature and transition enthalpy have decreased to 66.0±0.1°C and 40±4 J/g, respectively (Fig. 2. curve c).

To elucidate the reasons responsible to significant changes in thermodynamic parameters, the sites where Ni^{2+} ions bind to DNA should be revealed. Direct crystallographic investigation of oligonucleotide d(CGTATATACG) [Abrescia et al., 1999] has shown that Ni^{2+} makes complexes with N7 of guanine, while Ni^{2+} has not been found at N7 of adenine. Thus it makes clear why binding of Ni^{2+} to G-C rich satellite DNA causes decrease of DNA thermostability and division of melting process into two cooperative stages. Stability inversion of A-T and G-C pairs at high concentration of Ni^{2+} takes place. This phenomenon was observed [Melchior W and Von Hippel P., 1973] using tetramethylammonium chloride and tetraethylammonium chloride salts. Inversion of stability of A-T and G-C pares at 2-3M salt concentration was observed in mentioned paper, hence A-T rich region of DNA gets more stabile than G-C ones and at the inversion point melting curve narrows. This phenomenon was also studied using $(C_2H_5)_4NBr$ [Voskoboinik et al., 1975].

We could not establish the inversion point of melting curves observed in the presence of $(C_2H_5)_4NBr$. We suppose that obtained data can be described by existence of A-T and G-C pairs stability inversion. Inversion of stability resulted in simultaneous melting of satellite DNA and the heat absorption represented by this melting is placed at the lower temperature region than the main heat absorption peak.

We have also studied the interaction between Ce^{3+} and Calf Thymus DNA at different concentrations of Na^+ (when the concentration of NaCl was 20 mM and 100mM). As it is shown on Fig. 3, 4, 5 the interaction of Ce^{3+} with DNA is not like the interaction between DNA and $Co(NH_3)_6^{3+}$. Namely, the study has shown that the increase of hexamminecobalt (III) concentration causes increase of DNA melting cooperativity along with the increase of melting temperature maintaining transition enthalpy [Mrevlishvili et al., 2004].

Taking into account that positions of peaks corresponding to G-C rich sites melting have not changed, it can be supposed that Ce^{3+} interacts with A-T rich sites of DNA causing decrease of thermostability of these parts of DNA. This fact is represented by the appearance of thermostable region of DNA which melts at 50.3±0.1 °C (when $[Ce^{3+}]/[P]$=0.1; 20 mM NaCl), but enthalpy of transition maintains its initial value 57±6 J/g. This allows us to suggest that Ce^{3+} does not destruct

the hydration shell of DNA. Further increase of Ce^{3+} concentration precipitates the DNA. It is impossible to determine heat capacity dependence on temperature and transition enthalpy of melting process because of existence of the precipitation and only thermogram is represented (Fig. 4). The thermogram shows that the thermostability of A-T rich DNA region decreased even more and melts at 44.1±0.1 °C (when $[Ce^{3+}]/[P]=0,2$; 20 mM NaCl). It should be mentioned that the height of heat absorption peak corresponding to the melting G-C rich sites of DNA has been reduced in the case of $[Ce^{3+}]/[P]=0.2$ and 20 mM NaCl (Fig. 4.).

In the case of 100 mM we have different results. The DNA precipitation did not appear at $[Ce^{3+}]/[P]=0.2$ and this enables us to calculate thermodynamic parameters of melting process (Fig. 5). In conditions of $[Ce^{3+}]/[P]=0.0$ (curve a) and $[Ce^{3+}]/[P]=0.1$ (curve b) enthalpy value has not changed and is equal to 58±6 J/g. In the case of $[Ce^{3+}]/[P]=0.2$, the melting process is divided into two cooperative stages (curve c). It is supposed that the peaks with maximum at 53.4±0.1°C and 91.9±0.1°C correspond to the melting of A-T and G-C rich sites of DNA accordingly. Transition enthalpy has dropped from 58±6 to 43±4 J/g and it may be assumed that Ce^{3+} binds to DNA at grooves and destructs the hydration water structure.

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იონების (Ni^{2+}, F^- და Ce^{3+}) გავლენა დნმ-ის ლღობაზე. კალორიმეტრული შესწავლა

გოგოლაძე გ., მრევლიშვილი გ.

მაკრომოლეკულების ფიზიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 22.02.2005)

რეზიუმე

შესწავლილია ურთიერთქმედება დნმ-სა და ლითონის იონებს (Ni^{2+} და Ce^{3+}) შორის. Ni^{2+} იონების კონცენტრაციის გაზრდამ დნმ-ის ლღობის ტემპერატურის და ენტალპიის შემცირება გამოიწვია. Ce^{3+} განსხვავებულად მოქმედებს დნმ-ზე: ორი თერმოსტაბილური უბანი გამოვლინდა 53.4±0.1 °C და 91.9±0.1°C-ზე (როცა [Ce]/[P]=0.2). Cl^- -ის შეცვლა F^- -ით აისახა დნმ-ის სითბოტევადობის დენატურაციულ ინკრემენტზე.

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Vol. 3, No. 3, 2005.

BIOCONVERSION OF LIGNOCELLULOLYTIC PLANT SUBSTRATE-*SILPHIUM PERFOLIATUM L.* BY MEANS OF BASIDIAL FUNGUS *PLEUROTUS OSTERATUS 41* UNDER THE SUBMERGED CULTIVATION CONDITIONS

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

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(Received March 14, 2005)

Abstract

The chemical composition of perennial herbaceous plant – Silphium perfoliatum L. (cup-plant) has been studied. The dynamics of cup-plant bioconversion under the conditions of submerged cultivation of white rot fungus-Pleurotus ostreatus 41 was investigated. The maximal accumulation of crude protein (23%) in the fungus biomass was achieved on the 7th day of fermentation at the expense of degradation of 50% of hemicellulose of the main substrate.

Key words: bioconversion, Basidiomycetes, hemicellulose, lignin

Introduction

In 1980-ies perennial grass Silphia- Silphium perfoliatum L. was introduced for moisture limitation and soil erosion prevention in west Georgia where the unused semi-swamped area came to 40 000 ha. Preliminary estimations show that the above perennial grass could be used as a potential energy crop for biofuels and/or protein-reach feed or food by-products production. It should be underlined that nowadays low cost energy crops are recognized as one of the effective feedstock for decentralized energy and protein-reach products production in high developed and developing countries as well [Reith, den Uil, 2002]. The choice of Silphium perfoliatum L. as the suitable energy crop could be justified in the way that it grows on semi-swamped soils that usually are used neither for crops cultivation nor as the pastures, it has a high dry matter yields: 20 -25 t/ha per year even when using low quantities of nitrogen fertilizers, essentially eliminates moisture content and soil erosion and improves air quality. Referring to investigations, performed at the Ajameti selection station, cup-plant's (Silphium perfoliatum L.) productivity of semi swampy soils in West Georgia makes about 80-100 ton/ha in a year. The highest productivity of plant is reached on the 3 rd year of cultivation (120t/ha) and is retained for 10 years. After 15 years of cup-plant cultivating the soil's structure significantly improves because of extra humidity and erosion abating. Since Silphia's production can be concentrated, enough material should be available within a reasonable transportation radius to supply the biofuel and/or protein-reach products producing facilities.

This study reports the feasibility for microbial bioconversion of Silphium perfoliatum L. in protein-reach product.

Materials and Methods

White rot fungus - Pleurotus ostreatus 41, obtained from the collection of basidial fungi of the Durmishidze Institute of Biochemistry and Biotechnology, served as an experimental object. The perennial plant - Silphium perfoliatum L. (cup-plant) was used as a substrate for biotransformation.

At the first stage of experimental works cup-plant's chemical composition was determined, using the following methods: for measuring dry weight, samples were dried till the constant weight, at 105°C in the thermostat. Content of total nitrogen was calculated in percents [Termkhitarova, 1974], amount of cellulose was studied by Apdegraph method [Apdegraph, 1969], lignin - by Zadrazhil method [Zadrazhil, 1977], hemicellulose - after [Katkevich et al., 1982], tannins - by Levental method described in [Practical Works in Tea Chemistry, 1976], amount of soluble sugars- after the modified Shomodi-Nelson method [Klesev et al., 1980].

For preliminary treating of the mentioned lignocellulosic substrate, procedures of mechanical grinding and freezing at -20°C were used. For this purpose leaves and stems of plant were dried till the constant weight at 70°C, after they were grounded and weighed at equal amounts (5g), added 70ml of distilled water and stored at room temperature for swelling, for 24h.The amount of soluble sugars was measured in supernatant. Swelled substrate was placed in the refrigerator and stored at -20°C for 24h. After repeat of this procedure determination of soluble sugars was done in the supernatant. The substrate was again dried at 70°C in thermostat. For extracting the soluble sugars from cup plant, to the equal amounts of leaves and stems (5-5g) was added 70ml of distilled water and sterilized at 1 atm, for 45 min, then the substrate was washed, using 140ml of water, and dried at 60°C in the thermostat.

For preparing the sowing material 250ml conic flasks were filled with nutrient medium of the following composition: Na_2HPO_4-0,4; KH_2PO_4- 0,8; $MgSO_4$ – 0,5; NH_4NO_3 – 3,0; yeast extract – 3,0. 1 ml of 1% solution of microelements: $CaCl_2$, $ZnSO_4$, $CuSO_4 \cdot 7H_2O$ and 2 ml of 5% solution of $FeSO_4$ which was sterilized at 0.7 atm, for 45 min. After inoculation with basidial fungus Pleurotus ostreatus 41, flasks were shacked (180rot/min) at 28°C for 10days. After these procedures the sowing material was homogenized.

Dried up to the constant weight, preliminary treated and not treated substrate was placed in 250ml volume conic flasks in equal amounts, so that the final concentration made 2%. Substrate was added to 100ml of above mentioned nutrient medium, without glucose and was sterilized at 0.7 atm for 45 min. After this procedure flasks were sowed with 5 ml of homogenized sowing material. Cultivation of basidial fungus Pleurotus ostreatus 41 on cup-plant substrate was done identically to the conditions of preparing sowing material. On the 3rd, 4th, 6 and 7th days of fermentation the content of flasks was centrifuged (5000rot/min) and the concentration of soluble sugars and pH in the supernatant were measured. The sediment was dried at 70°C, and the percentage of protein, cellulose, lignin and hemicellulose was studied in it.

Results and discussion

The purpose of the performed study was to determine the feasibility of using perennial herbaceous plant - cup-plant as a substrate in the process of biotransformation. As there were no data about the chemical composition of the plant, at the first stage of experiment the chemical composition of cup plant was investigated.

It was revealed that by content of protein cup-plant surpassed the plant substrates like citrus flour (7.05%), tomato press cake (3.86%), maize straw (4.62%), vine cuttings (5.20%) etc. It contains also enough amounts of easy-assimilative biopolymers –cellulose and hemicellulose. For this reason it represents a potential source for cultivating the cellulase- producing mycelial fungi. Besides these biopolymers cup-plant contains high amount of easy-metabolizing sugars (2184 γ/ml) and small amount of tannins (Table 1). Such composition makes possible to use this yet unknown plant substrate as a unique source of carbon in the process of biotransformation.

At the same time, cup-plant contains high concentrations of hardly-assimilative biopolymer – lignin (22.39%). High content of lignin, together with other purposes, was the reason for selecting the white rot fungus Pleurotus ostreatus 41 as an object for investigation.

Table 1. Chemical composition of Silphium perfoliatum L. (cup-plant)

Water content (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Soluble sugars mg/g	Total nitrogen (%)	Tannins (%)
80	15, 34	38,15	22,39	15,8	9,22	1,30

It is known that for increasing the processing quality of lignocellulolytic substrate preliminary methods of chemical or physical treating has been used. Beforehand treating of material, used in our experiments (grinding and freezing at -20°C, with further sterilizing and extracting the substrate) could not significantly influence the processing quality of cup-plant (Table 2).

Table 2. Influence of preliminary treating of plant substrate by physical method on chemical composition of cup-plant.

Pretreatment method	total nitrogen %	cellulose %	lignin %	hemicellulose %	tannins %
non-pretreated substrate	9,22	15,34	22,39	38,15	1,3
grinding and chilling at (-20°C)	8,42	15,20	21,19	38	1,2
extraction and sterilization	7,49	15,34	21,08	38	1,1

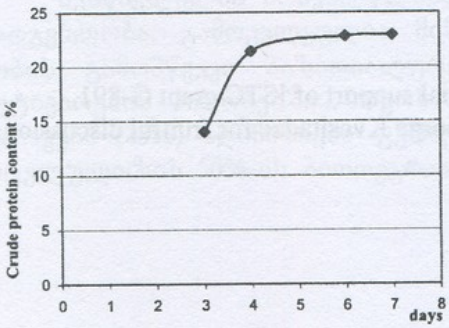

Fig.1. Biodegradation dynamics by Pleurotus ostreatus 41 on treated plant substrates.

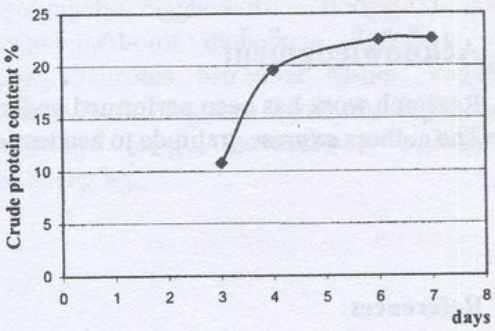

Fig.2. Biodegradation dynamics by Pleurotus ostreatus 41 on untreated plant substrates.

In our experiments biodegradation of both - untreated and treated substrates of cup plant has been studied (Table 3).

Table 3. Biodegradation dynamics of cup plant by Pleurotus ostreatus 41-under submerged cultivation conditions

substrate	cultivation time (days)	pH	Losses (%)		
			cellulose	lignin	hemicellulose
pretreated substrate	3	6,2	0,43	0,67	2
	4	6,8	1,09	0,75	8
	6	7,6	2,10	1,46	14
	7	7,6	2,70	2,74	19
non-pretreated substrate	3	6,6	2,84	0,78	6,15
	4	6,4	3,34	1,09	12,18
	6	6,6	4,05	1,95	14,15
	7	6,6	5,12	3,07	20,15

Pleurotus ostreatus 41 revealed the ability of biotransformation of the experimental plant. The fungus developed successfully on both- treated and untreated plant substrates (Fig. 1, Fig. 2).

On the third day of fermentation the culture reached the exponential phase of growth. On the 7th day of experiment the content of crude protein in the biomass came to 22.6-22.93%. By this period the basidial fungus was at the stationary growth phase (Fig. 1, Fig. 2). As cup-plant represented the only source of carbon in the nutrient medium, the successful growth of Pleurotus ostreatus 41 (accumulation about 23% of protein) would be performed at the expense of lignocellulose uptake. In Table 3 there are presented the data on degradation of main biopolymers of the plant substrate. From the table it is clear that Pleurotus ostreatus 41 received the necessary energy for growing from easy metabolizing biopolymer - lignocellulose of the plant substrate. At the end of the experiment about 10-20.15% of hemicellulose had been degraded by Pleurotus ostreatus 41, which makes 48-51% of the initial amount of polymer. Presumably because of intensive uptake of hemicellulose, slight degradation of hard-assimilative biopolymers - cellulose and lignin, had performed (Table 3). At the 7th day of cultivation on the untreated plant substrate the basidial fungus had metabolized only 5.12% of cellulose and 3.07% of lignin.

Acknowledgment

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ლიგნოცელულოზური მცენარეული სუბსტრატის - სილფიას *SILPHIUM PERFOLIATUM L.* ბიოკონვერსია მერქნის თეთრი ლპობის გამომწვევი ბაზიდიალური სოკოთი (*PLEUROTUS OSTERATUS 41)* სიღრმული კულტივირების პირობებში

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

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

(მიღებულია 14.03.2005)

რეზიუმე

შესწავლილია მრავალწლიანი ბალახოვანი მცენარის – სილფიას ქიმიური შემადგენლობა. გამოკვლეულია მისი ბიოკონვერსიის დინამიკა მერქნის თეთრი ლპობის გამომწვევი ბაზიდიალური სოკო Pleurotus ostreatus 41-ით სიღრმული კულტივირების პირობებში. სოკოს მიერ ბიომასაში ნედლი ცილის მაქსიმალური დაგროვება (23%) აღინიშნება ფერმენტაციის მე-7 დღეს, ძირითადად სუბსტრატის, ჰემიცელულოზის 50%-ის ბიოდეგრადაციის ხარჯზე.

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Botany

SYSTEM OF REPRESENTATIVES OF GENUS CENTAUREA L. (COMPOSITAE) OCCURRING IN GEORGIA

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Abstract

The paper deals with critical study of species of the genus Centaurea (Compositae) occurring in Georgia. Synonyms, ecology and distribution in Georgia, the Caucasus and general distribution are indicated for each taxon. Available data on species chromosome numbers are indicated.

Key words: Centaurea, Compositae, diversity, Georgia.

Introduction

In Georgia the Genus Centaurea is represented by 25 species belonging too 11 subgenera and 10 sections. Two species: C. bella and C. transcaucasica are endemic to the Caucasus, and one – C. bagadensis – to Georgia.

Genera: Hyalinella, Lopholoma, Acrolophus, Phalolepis are diverse and include endemic subspecies, rare and relict species.

Meadow and lithophilous species predominate. C. carduiformis, C. bella C. simplicicaulis, C. bagadensis are bound to calcareous ecotopes.

Materials and Methods

A system of the genus Centaurea is presented at the level of subgenera and sections. Geographical distribution is indicated according to "Flora of Georgia" (1971-2003). Chromosome numbers are given according to "Chromosome numbers of flowering plants of the flora of the USSR" (1990).

Results and Discussion

S u b g e n. 1. **Cyanus** (Mill.) Spach 1841, Nat. Veg. (Phan.) 10:11,68 Cyanus Mill. 1745, Gard. Dict. ed 4:422

S e c t. 1. **Cyanus** Mill. Spach

1. ***C. depressa*** Bieb. 1808, Fl. Taur.-Cauc. 2:346. Distributed in the lower mountain belt, on croplands, in ruderal places, along roads. 2n = 16.

Georgia: 5 – Imereti, 7 – Adjara, 9 – Kartli, 12 – Kakheti, 15 – Gardabani, 16 – Trialeti, 19 – Meskheti.

Caucasus: North Caucasus (western and central parts); Transcaucasus: Azerbaijan, Armenia.

General distribution: East Europe (Crimea), South East Europe, Minor, Middle and Central Asia, Iran, Afghanistan.

2. ***C. cyanus*** L. 1753, Sp. Pl. 2:911.

Grows on croplands and in ruderal places, rare. 2n = 24.

Georgia: 1 – Abkhazeti, 5 – Imereti, 8 – Shida Kartli, 9 – Kartli, 15 – Gardabani.

Caucasus: North Caucasus; Transcaucasus: Black Sea shore, Azerbaijan, Armenia.

General distribution: North and Middle Europe, Mediterranean, Minor Asia, Iran, Pakistan, Indo-Malaysia, Siberia, Far East.

S e c t. 2. **Protocyanus** Dobrocz.

3. ***C. cheiranthifolia*** Willd. 1794, Phitogr. 1:12 – C. fischei Willd. 1813, Enum. Hort. Berol. (Suppl.):61.

3.1 subsp. **cheiranthifolia**

Distributed in the subalpine and alpine belts, on meadows, in places with skeleton substrate. 2n = 18.

Georgia: throughout the country. The species is represented by a great number of varieties.

Caucasus: North Caucasus; Transcaucasus: Azerbaijan, Armenia.

General distribution: Minor Asia (northeastern and eastern parts); Iran (northern and western parts).

3.2 subsp. ***willdenovii*** (Czer.) Mikheev, 2000, Bot. Journ., 84, 9:104. – C. willdenovii Czer. 1963, Fl. SSSR, 28:449; Galushko, 1980, Fl. North Cauc. 3:237.

Distributed in the subalpine and alpine belts, on meadows.

Georgia: 1 – Abkhazeti.

Caucasus: North Caucasus; Transcaucasus: Black Sea shore. Endemic to the Caucasus.

4. ***C. nigrofimbria*** (C. Koch) Sosn. 1926, Bull. Tifl. Bot. Garden, new ser. 2:77. – C. montana var. nigrofimbria C. Koch, 1851, Linnaea, 24:426.

Distributed in the subalpine and alpine belts, on meadows, in places with skeleton substrate.

Georgia: 1 – Abkhazeti, 2 – Svaneti, 3 – Racha-Lechkhumi, 4 – Samegrelo, 5 – Imereti, 6 – Guria, 7 – Adjara, 8 – Shida Kartli, 10 – Mtiuleti, 19 – Meskheti.

Caucasus: North Caucasus (western and central parts); Transcaucasus: Azerbaijan, Armenia.

General distribution: Minor Asia (northeastern part).

5. ***C. triumfettii*** All. 1773, Auct. Syn. Stirp. Horti Ttaur.: 16. – C. atrata Willd. 1803, Sp. Pl. 3:2290 – C. huetii Boiss. 1856, Diagn. Pl. Or. Nov. ser.2,3:69 - C. fischeri subsp. cyanea Sosn. 1926, Bull. Tifl. Bot. Garden, new ser. 2:84, p. p.

Distributed in the middle and upper mountain belts, on meadows, rocks, at forest edges.

Georgia: 9 – Kartli, 16 – Trialeti, 17 – Kvemo Kartli, 18 – Javakheti, 19 – Meskheti.

Caucasus: Transcaucasus: Azerbaijan, Armenia.

General distribution: Minor Asia (northeastern and eastern parts).

6. ***C. woronowii*** Bornm. ex Sosn. 1926, Bull. Tifl. Bot. Garden, new ser. 2:91.

Grows on dry, stony slopes.

Georgia: 19 – Meskheti (Abastumani).

General distribution: Minor Asia (northeastern part).

S u b g e n. 2. **Lopholoma** (cass.) Spach 1841, Hist. Nat. Veg. (Phan.) 10:11. Acrocentron Cass. 1826, Dict Sci. Nat. 44:37.

S e c t. 1. **Acrocentron** (Cass.) DC

7. ***C. reflexa*** Lam. 1785, Encycl. Meth. Bot. 1:657.

Distributed in the lower and middle mountain belts, on dry stony ecotopes. 2n = 20.

Georgia: 5 – Imereti, 8 – Shida Kartli, 14 – Gare Kakheti, 15 – Gardabani, 16 – Trialeti, 17 – Kvemo Kartli, 18 – Javakheti, 19 – Meskheti.

Caucasus: North Caucasus; Transcaucasus: Azerbaijan, Armenia (very rare).

General distribution: Minor Asia (northeastern part).

S e c t. 2. **Orientales** (Hayek) Tzvel.

8. ***C. orientalis*** L. 1753, Sp. Pl.: 913.

Distributed in the middle mountain belt, on stony ecotopes, steppe and dry slopes. 2n = 20.

Georgia: 8 – Shida Kartli, 9 – Kartli (Mikheev, 2000).

Caucasus: North Caucasus.

General distribution: Europe (central, southeastern, eastern parts).

S e c t. 3. **Lopholoma**

9. ***C. carduiformis*** DC*. 1838, Prodr. 6:590.

Distributed in the lower and middle mountain belts, on stony, calcareous and argillaceous slopes. 2n = 20.

Georgia: 9 – Kartli, 19 – Meskheti (Mikheev, 2000).

Caucasus: Transcaucasus: eastern part – Karabakh, southern part – Yerevan, Nakhichevan.

General distribution: Minor Asia (central and eastern parts), Iran (northwestern part).

10. ***C. pseudoscabiosa*** Boiss. et Buhse 1860, Nouv. Met. Soc. Nat Mos. 12:131. – C. scabiosa Subsp. pseuodpscabiosa (Boiss. et Buhse) Mikheev; var. glehnii (Trautv.) Mikheev, 2000, Bot .Journ., 85,3:117.

10.1. subsp. ***glehnii*** (Trautv.) Wagenitz 1972, Willdenowia, 6:500 – C. glehnii Trautv. 1876, Proc. Peterb. Bot. Garden, 4, 1:382.

Distributed in the middle mountain belt, on dry slopes, lakeshores, meadows, roadsides. 2n = 18.

Georgia: 9 – Kartli, 10 – Mtiuleti, 16 – Trialeti, 17 – Kvemo Kartli, 18 – Javakheti, 19 – Meskheti.

Caucasus: Northern Caucasus (Dagestan); Transcaucasus: Azerbaijan, Armenia.

General Distribution: Minor Asia (northeastern part).

10. 2. subsp. ***ossethica*** (Sosn.) Gabr. 1999 Fl. Arm. 9:416 – C. ossethica Sosn. 1963, Fl. SSSR 28:614 – C.scabiosa subsp. pseudoscabiosa (Bioss. et Buhse) Mikheev; var. ossetica (Sosn.) Mikheev, 2000, Bot. Journ., 85,3:117.

Distributed in the middle and upper mountain belts, on stony and dry meadows.

Georgia: 8 – Shida Kartli, 11 – Tush-Pshav-Khevsureti.

Caucasus: Transcaucasus: Armenia.

General Distribution: Crimea (Dubovik, 1990).

S u b g e n. 3. **Jacea** (Mill.) Spach 1841, Nat. Veg. (Phan.) 10:67 – Jacea Mill. 1754, Gard. Dict. ed 4.

S e c t. 1. **Jacea**

11. ***C. jacea*** L. 1753, Sp. Pl.:914.

11.1 subsp. **substituta** (Czer.) Mikheev, 1999, Bot. Journ., 84,9:105 – C. substituta Czer. 1963 Fl. SSSR, 28:612.

Distributed in the forest belt, at forest edges, in shrubberies.

Georgia: 1 – Abkhazeti.

Caucasus: Northern Caucasus (western part).

General distribution: Crimea, East Europe (southern part).

S e c t. 2. **Lepteranthus** (DC.) Dumort

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* Recorded in Georgia for the first time.

12. ***C. salicifolia*** Bieb. ex Willd. 1803, Sp. Pl. 3,3:2283. – C. salicifolia Bieb. ex Willd. subsp. salicifolia Wagenitz in Davis, 1975, Fl. Turkey. 5:519. – C. phrygia L.subsp. salicifolia (Bieb. ex Willd.), Mikheev, 1999, Bot. Journ., 84,9:105.

12.1 subsp. ***salicifolia***

Distributed in the lower, middle, rarely upper mountain belts. 2n = 22.

Georgia: throughout the country.

Caucasus: Northern Caucasus; Transcaucasus: Azerbaijan, Armenia.

General Distribution: Minor Asia (northern part).

12.2 subsp. ***abbreviata*** (C. Koch) Hand. – Mazz. 1909 Ann. Mus. (Wien) 23; 198. - C. abbreviata C. Koch Linnaea 1843, 17:39. – C. salicifolia subsp. abbreviata Wagenitz in Davis, 1975, Fl. Turkey 5:506. – C. phrygia subsp. abbreviata C. Mikheev, 1999, Bot. Journ., 84,9:105.

Distributed in the middle and upper mountain belts, on forest and subalpine meadows, at forest edges.

Georgia: 7 – Adjara, 8 – Shida Kartli, 9 – Kartli, 11 – Tush-Pshav-Khevsureti, 16 – Trialeti, 18 – Javakheti, 19 – Meskheti.

Caucasus: Northern Caucasus; Transcaucasus: Armenia.

General distribution: Crimea, Minor Asia (northeastern part), Iran.

13. ***C. alutacea*** Dobrocz. 1949, Bot. Journ. Acad. Sci. USSR, 6, 2:74; Dubovik,1990, Bot. Journ. 75, 11:1579. C. pseudophrygia C. A. Mey. subsp. alutacea (Dobrocz.) Mikheev, 1999, Bot. Journ. 84, 9:106.

Distributed in the forest and subalpine belts, on meadows, at forst edges, in shrubberies.

Georgia: 1 – Abkhazeti, 5 – Imereti, 7 – Adjara, 8 – Shida Kartli, 9 – Kartli.

Caucasus: North Caucasus.

General distribution: Europe (southeastern part).

S u b g e n. 4. **Hyalinella** (Tzvel.) Tzvel. 1963, Fl. SSSR, 28:488. Centaurea sect. Hyalinella Tzvel. 1959, Bot. math. Inst. of Acad. Sci. of the USSR, 19:426.

14. ***C. bella*** Trautv. 1866, Bull. Acad. Sci. Peterb. 10:394.

14.1. subsp. ***C. bella***

Distributed in the lower and middle mountain belts, on dry, stony slopes, in cracks of rocks. 2n = 60.

Georgia: 9 – Kartli, 16 – Trialeti, 18 – Javakheti.

Caucasus: Transcaucasus: Armenia (Tonyan, 1980). Endemic to Transcaucasus.

a) ***C. bella*** var. ***svanetica*** (Mardalejshvili) Jinjolia, 1990, Proc. III conference of young botanists (Leningrad) 1:35. – C. svanetica Mardalejschvili, 1985, Bot. Journ. 70, 2:265.

Distributed in the forest belt, at forest edges, on marl clays.

14.2 subsp. ***nathadzeae*** (Sosn.) Djindjolia comb. nov. – C. nathadzeae Sosn. 1959, Notes syst. geogr. pl. (Tbilisi), 21:58. – C. bella Trautv. var. nathadzeae Djindjolia, 1990, Proc. III conference of young botanists (Leningrad) 1:34.

Distributed in the forest belt, in cracks of calcareous rocks.

Georgia: 5 – Imereti. Endemic to Imereti.

15. ***C. simplicicaulis*** Boiss. et Huet, 1856, in Boiss, Diagn, Pl. Or. 2, 3:67, Wagenitz in Davis, 1975, Fl. Turkey 5:519; Mikheev, 1999 Bot. Journ. 84, 9:109.

15.1 subsp. ***simplicicaulis***

Georgia: 19 – Meskheti.

General Distribution: Minor Asia (northeastern part)

15.2 subsp. ***adjarica*** (Albov) Djinjolia comb. nov. – C. adjarica Albov, 1894, Bull. Herb. Boiss. 2:639. – C. dimitrieiewiae Sosn. 1959, Notes syst. geogr. pl. (Tbilisi) 21:59.

Georgia: 7 – Adjara.

General Distribution: Minor Asia (northeastern part).

16. ***C. bagadensis*** Woronow; 1905, Proc. Peterb. Soc. of naturalists 34:31

Distributed in the forest belt, in cracks of calcareous rocks. 2n = 30.

Georgia: 1 – Abkhazeti, 4 – Samegrelo (Mikheev, 1999). Endemic to calcareous habitats of West Transcaucasus.

S u b g e n. 5. **Acrolophus** (Cass.) Spach 1841, Hist. Nat. Veg. (Phan.) 10:11. Acrolophus Cass. 1827, Dict. Sci. Nat. Sci. Nat, 50:253.

S e c t. 1. **Arenariae** (Hayek) Dostal

17. ***C. ovina*** Pall. ex Willd. 1803, Sp. Pl. 3,3:2292.

Distributed in the middle mountain belt, steppe, stony places. 2n = 18.

Georgia: 9 – Kartli, 12 – Kakheti, 14 – Gare Kakheti, 15 – Gardabani, 16 – Trialeti, 17 – Kvemo Kartli.

Caucasus: Northern Caucasus; Transcaucasus: Azerbaijan (Karabakh, Len Koran), Armenia (Zangezur).

General Distribution: Minor Asia, Iran (northeastern part).

18. ***C. gulisaschvilii*** Dumbadze, 1946, Proc. Acad. Sci. of Arm. SSR, 5,2:49.

Distributed in the steppe belt, on dry slopes. 2n = 18.

Georgia: 19 – Meskheti.

Caucasus: Armenia (Lori, Sevan, Zangezur).

General Distribution: Minor Asia (northeastern part).

S e c t. 2. **Cylindracea** (Hayek) Dostal

19. **C. diffusa** Lam. 1785, Encycl. Meth. Bot. 1:675.

Georgia: 1 – Abkhazeti, 5 – Imereti, 8 – Shida Kartli, 9 – Kartli, 10 – Mtiuleti, 12 – Kakheti, 13 – Kiziki, 14 – Gare Kakheti, 15 – Gardabani, 16 – Trialeti, 17 – Kvemo Kartli, 18 – Javakheti, 19 – Meskheti.

Caucasus: Northern Caucasus; Transcaucasus: Azerbaijan, Armenia.

General Distribution: South and East Europe; Minor Asia.

S u b g e n. 6. **Phalopelis** (Cass.) Spach 1841, Hist. Nat. Veg. (Phan.) 10:11. Phalolepis Cass. 1827, Dict. Sci. Nat. 50:248.

20. ***C. transcaucasica*** Sosn. ex Grossh. 1934, Fl. Cauc. 4:212

20.1. subsp. ***transcaucasica***

Distributed on dry slopes, rocks.

Georgia: 8 – Shida Kartli, 9 – Kartli, 12 – Kakheti, 15 – Gardabani, 16 – Trialeti.

Caucasus: Northern Caucasus (eastern part); Transcaucasus: Armenia. Endemic to Caucasus.

20.2. subsp. ***georgica*** (Klok.) Mikheev, 2000, Bot. Journ. 85 3:121 – C. georgica Klok. 1963, Fl. SSSR, 28:619.

Distributed in the steppe belt on dry slopes, rocks.

Georgia: 8 – Shida Kartli, 16 – Trialeti. Endemic to East Georgia.

20.3 subsp. ***alexandri*** (Bordz.) Mikheev*, 2000; Bot. Journ. 85, 3:121 – C. alexandri Bordz. 1934, Feddes Repert. 36:306.

Distributed in the steppe zone on dry, stony slopes. 2n = 18.

Georgia: 19 – Meskheti.

Caucasus: Armenia (Megri). Endemic to South Transcaucasus.

S u b g e n. 7. **Pseudoseridia** (Wagenitz) Gabr. 1995, Fl. Arm. 9:421.

21. ***C. stevenii*** Bieb. 1808, Fl. Taur. – Cauc. 2:356.

21.1. subsp. ***stevenii***. – Phaeopappus stevenii (Bieb.) C. Koch. 1851. var. pinnatifolius E. Bordz. 1932, Feddes Repert. 30:396.

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* Recorded in Georgia for the first time.

Distributed in the middle mountain belt on slopes with herbaceous cover.

Georgia: 8 – Shida Kartli, 9 – Kartli, 14 – Gare Kakheti, 18 – Javakheti.

Caucasus: Transcaucasus: Armenia (Mikheev, 2000).

S u b g e n. 8. **Rhizocalathium** (Tzvel.) Tzvel. 1963, Fl. SSSR 28:565. – Centaurea sect. Rhisocalathum Tzvel. 1959, Bot. math. (Leningrad) 19:438.

22. ***C. rhizantha*** C.A. Mey. 1831, Verzeichn.: 64 – C. grossheimii Sosn. 1949, Bot. Journ. 34, 3:288 – C. sessilis auct. non Willd.: Boiss, 1875, Fl. Or. 3:676.

Distributed in the upper mountain and subalpine belts, on dry, skeleton slopes, meadows, lakeshores.

Georgia: 8 – Shida Kartli, 9 – Kartli, 18 – Javakheti.

Caucasus: Transcaucasus: Azerbaijan, Armenia.

General distribution: Minor Asia (southwestern part), Iraq, Iran, Turkmenistan.

S u b g e n. 9. **Solstitiaria** (Hill.) Dobrocz. 1949, Bot. Journ. Acad. Sci. USSR 6,2:64,69 – sect. Solstitiaria Hill. 1762, Veg. Syst. 4:21.

23. ***C. soltitialis*** L. 1753, Sp. Pl.: 917. – C. adamii Willd. 1803, Sp. Pl. 3,3:2310.

Distributed in the steppe belt on dry slopes, in ruderal places, croplands, roadsides. 2n =16.

Georgia: 8 – Shida Kartli, 9 – Kartli, 10 – Mtiuleti, 12 – Kakheti, 13 – Kiziki, 14 – Gare Kakheti, 15 – Gardabani, 16 – Trialeti, 17 – Kvemo Kartli, 18 – Javakheti, 19 – Meskheti.

Caucasus: Caucasus: Northern Caucasus; Transcaucasus: Azerbaijan, Armenia.

General distribution: Europe (central, southeastern parts), Minor Asia, Middle and Central Asia. Invaded from East Europe.

S u b g e n. 10. **Calcitrapa** (Hill.) Spach 1841, Hist. Veg. Nat. (Phan.) 10:11, 72. – sect. Calcitrapa Hill 1762, Veg. Syst. 4:20.

24. ***C. iberica*** Trev. ex Spreng. 1826, Syst. Veg., 3:406.

S u b g e n. 11. **Tetramorphaea** (DC.) Czer. 1963, Fl. SSSR 28:576. – sect. Tetramorphaea DC. 1833, In Guill, Arch. Bot. 2:331.

25. ***C. bruguieriana*** (DC.) Hand. – Mazz. 1913, Ann. Naturh. Muz. (Wien), 27:451. – Tetramorphaea bruguieriana DC. 1838, Prodr. 6:609.

25.1. subsp. **belanguieriana** (DC.) Bornm. 1939, Beih. Bot. Centralbl. 60 B:214. – Tetramorphaea belangeriana DC. 1838, Prodr. 6:609.

Georgia: 1 – Abkhazeti (Mikheev, 2000) (According to material collected by E. Shengelia).

Caucasus: Transcaucasus: Azerbaijan (Nakhichevan), Armenia (Yerevan) (Mikheev, 2000; Gabryelyan, 1995).

General distribution: Iran, Middle, South Asia (Indo-Hymalaya).

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საქართველოს ფლორის გვარ Centaurea L. (Compositae)-ს წარმომადგენელთა სისტემა

ჯინჯოლია ლ.

საქართველოს მეცნიერებათა აკადემიის ნ. კეცხოველის სახ. ბოტანიკის ინსტიტუტი

(მიღებულია 15.02.2005)

რეზიუმე

წარმოდგენილი სისტემა საქართველოს ფლორის გვარ Centaurea L. (Compositae)-ს სახეობათა კრიტიკული შესწავლის შედეგად შეიქმნა. თითოეული ტაქსონისთვის მოცემულია სინონიმები, ეკოლოგია და გეოგრაფიული გავრცელება საქართველოში, კავკასიში და საერთო გავრცელება. მოყვანილია არსებული მონაცემები სახეობათა ქრომოსომთა რიცხვის შესახებ.

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Botany

MORPHOLOGICAL PECULIARITIES OF THE ROOT SYSTEM OF SEA-BUCKTHORN, *HIPPOPHAË RHAMONIDES L.* AND ULTRASTRUCTURE OF MYCORRHIZAL NODULES

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Abstract

Morphology of sea-buckthorn root system is studied. Fluctuation of roots summary length in 2 year-old male and female plants, viz. from (♀)95,8+3,24m to (♂)119,7+4,55 m, and in 5-6 year-old plants - from (♀)1110,8+6,11 to (♂)1186,3+25,20 has been established. Growing small roots and germ tubes account for 80-85% of roots total length. Diameter of root system is almost 3 times more than that of crone and its adsorption surface significantly oversteps the limits of crone projection. The nitrogen-fixation activity of mycorrhizal nodules is not invariable. It changes during all vegetative period. The maximum of nitrogen fixation is registered in July, 96 μ mole C_2H_4/g raw mass in 1 hour, and minimum, 26 μ mole in December. In summer period in the cells of mycorrhizal nodules large amounts of cytoplasm, hyphae, vesicles and other cell structures have been observed. But, in the cells the wintering nodules strengthening of destructive processes and beginning of noticeable nitrogen fixation reduction took place.

Key words: root, morphology, mycorrhizal nodules, nitrogen-fixation, ultrastructure.

Introduction

At present there is more and more increasing interest in cultivation of sea-buckthorn. For this purpose the wild forms and varieties are being introduced in new conditions. In this respect the detailed study of biological peculiarities of sea-buckthorn, as well as its reaction to the new environmental conditions, etc. are considered to be of great significance. It is also important to study formation of nitrogen-fixing nodules on the sea-buckthorn roots and determine their participation in growth and development processes. There are restricted data about the root system of sea-buckthorn in Georgia and they mainly concern the activity of nitrogen fixation in wintering mycorrhizal nodules [Todua,1985].

The purpose of the study was to investigate wild sea-bukthorn root system, determine the intensity of nitrogen fixation by means of mycorrhizal nodules, and also define some details of ultra-structural changes taking place in the cells of mycorrhizal nodules during all vegetative period.

Materials and methods

We tested fruit-bearing forms of wild sea-buckthorn. The studies on root systems have been conducted in the beginning of June. In experiments the sea-buckthorn seedlings and root-sprouts from Ksani river valley, consisting of both male and female 2- and 5-6 year-old plants were used. The study of root system was carried out by "monolithic" method [Kolesnikov, 1973]. Taking roots from soil was conducted by soil strata, in each 10-12 cm. The roots were washed and sorted according to their size (width): a) with diameter up to 1mm, b) from 1 to 3mm and c) over 3 mm. After this the length of a roots was measured using millimetre ruler.

Also in Abkhazian populations of sea-buckthorn was investigated the nitrogen fixation activity and morphology as well as some ultra-structural changes characteristic of mycorrhizal cells in the process of ontogenesis of endophyte. The studies were conducted on different aged plants (2, 5 and 6 year-old), growing in the Kodori river delta natural conditions, in poor soils (Black Sea, village Adziujba).The samples were collected from various plots in May-July and October-November-December. Sowing of mycorrhizal nodules under analyze and conservation were made in glass jars with sand. The defining of nitrogen-fixing ability of nodules was carried out in the Timirijazev Institute of Plant Physiology, Russian Academy of Sciences. The nitrogen-fixing activity of the nodules has been determined by means of acetylene method. Quantitative definition of ethylene was conducted by gas chromatograph "Chrom-4" (Czechia). The nitrogen-fixing activity has been expressed in micromole of the produced ethylene per 1 g raw mass of nodules for 1 hour incubation. The methodology is completely described in [Andreeva et al., 1982].

Results and discussion

Morphological study of sea-buckthorn root system was carried out in East Georgia on the left bank of the river Ksani, within the territory of artificial borehole in front to village Agaiani and Borjomi Region (village Kviratskhoveli) [Todua, 2001]. There is the meadow-brown soil with clay-sandy mechanical composition, law thickness alluvial cover, the average amount of humus 1.4%, and nitrogen amount 0.17%. Reaction of soil solution is neutral (in water extract pH=6,5-7,6 and doesn't depend on depth of soil).

The excavations of the root systems revealed, that sea-buckthorn has a well-developed root system and reproduces successfully by root sprouts. On the territory under consideration sea-buckthorn has horizontally disposed root system with largely spread radial surface. It has neither the roots spread into depth longer then 1 m, nor the fibrous roots. Due to periodical stratification of sand cover sea-buckthorn produces underground trunk on which very soon new layers of adventitious roots develop. This process repeatedly takes place during each flood time.

The data of biometric measurements of sea-buckthorn root system are given in table 1. As shown from the table the summarized length of roots fluctuates in 2 year-old male and female plants, from 95,8+3,24 m to 119,7+4,55 m, and in 5-6 year-old plants these indices are from ♀1110,8+6,11 to ♂1186,3+25,20. The negligible differences between 5-6 year-old male and female individuals can be explained by individual peculiarities of studied plants within the limits of given population.

From the table 2 is evident, that in 2 year-old individuals of sea-buckthorn growing small roots and germ tubes account for 80-85% of roots total length. Main mass in 2 year-old individuals is disposed within 0-30 cm deep strata of the soil, but in 5-6 years old ones within 0,50 cm depth. When the soil is moist, in the most cases the root system develops radially (horizontally) and it is similar to fibrous root system.

Table 1. The length of sea-buckthorn roots by fractions

Location of investigated plant	Age (years)	Sex	Total length of roots (cm)	The length of a root by fractions		
				up to 1 mm	up to 1-3 mm	over 3 mm
The left bank of the riv. Ksani	2*	female	958±3,24	47,8	34	14
	2*	male	119,7±4,55	56,7	40	23
	5-6 **	female	1110,8±6,11	600,8	401	109
	5-6 **	male	1186,3±25,2	618,3	366	202

*The main mass of roots is included within strata of soil of 30 cm depth.
**- in 40-50 cm depth.

This fact is also pointed out by others in the soils of various type [Veshchenko,1986]. This characteristic feature of sea-buckthorn is certainly response reaction on deficit of moisture in soil. The similar fact is also described in other plant species of high-mountain zone [Nakhutsrishvili, 1986; Larcher, 1994]. In dry soils the root reaches the deeper layers of soil to uptake larger amount of water and minerals. In such cases the top root system with adventitious roots develops (Fig.1).

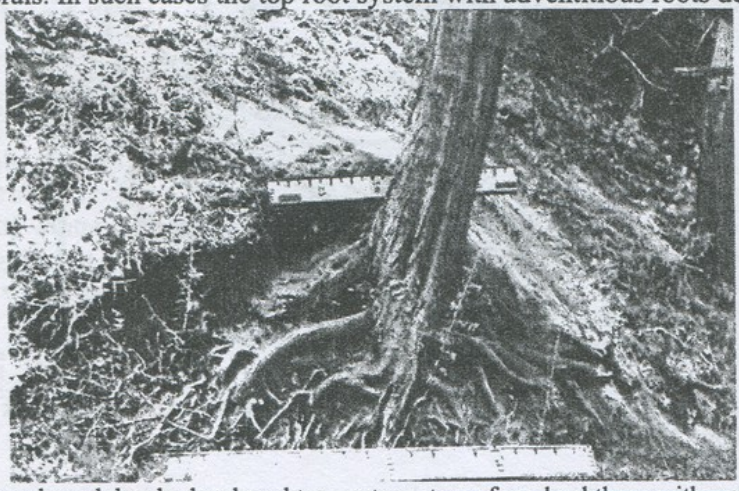

Fig.1. Strongly and deeply developed top roots system of sea-buckthorn with well developed lateral roots (Borjomi, Kviratskhoveli).

The observations showed (Fig.2) that on the first stage of growth of plants from seeds development of root system significantly precedes development of aboveground parts. Also later roots strongly develop both vertically and horizontally. According to the data shown in Tab. 2 the diameter of root system is 3-times more than the crone diameter and absorption surface of root system is much wider than projection of crone.

Table 2. Morphology of roots of sea-buckthorn (river Ksani)

sex	height of a plant, cm	mean diameter of a crone, cm	total length of roots, m
female	224,7±24,49	294,5±5,09	1143,9±12,49
male	290±5,57	383,5±2,62	1221,8±16,82

- Each variant includes data from 10 individuals concerning measurements of height, crone diameter and average length of root systems

The notable peculiarity of sea-buckthorn is the presence of nitrogen-fixing nodules on the roots (mycorrhiza) that allow the plant to use the atmospheric nitrogen and feel well on the soils with low content of minerals. It is a pioneer plant on the poor soils with low content of humus.

As mentioned above, we have been studying nitrogen fixation by mycorrhizal nodules and their ultra-structure. It was established that in different stages of development nodule bodies morphologically differ from each others. Oval, yellowish, white coral-like growths and swellings of different forms of 1-3 mm have been observed (Fig. 2). The nodules mainly are formed on adventitious roots, in which the strong development of fungal hyphae takes place. The nitrogen-fixing activity of the nodules has been determined by means of acetylene method. Quantitative definition of ethylene was conducted by gas chromatograph "Chrom-4".

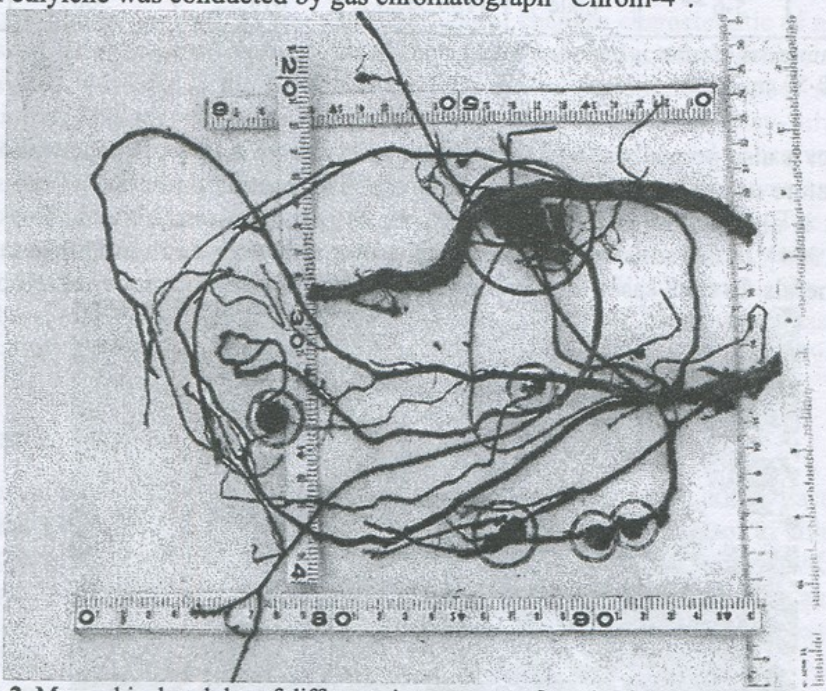

Fig. 2. Mycorrhizal nodules of different size on roots of sea-buckthorn (left bank of the river Ksani, water bore-hole territory, Mtskheta district)

It was revealed, that the nitrogen-fixation activity of mycorrhizal nodules is not invariable and constant. It changes during all vegetative period. The maximum of nitrogen fixation is registered in July, 96 μ mole C_2H_4/g raw mass in 1 hour, and minimum, 26 μ mole, in December. Although in early spring time, in May, nitrogen-fixation is low - 44 μ mole, however it has an increasing tendency. The results are reflected in diagram (Fig.3).

As a result of investigation of wintering nodules sections it was determined, that in November- December majority of cells are poor in cytoplasm and in some cells cytoplasm is not observed at all. Unfortunately our data in this concern obtained during our work in Abkhazeti are lost. Only the part of results of studies are given here [Todua, 1985, 2000]. Certain changes are also observed in other cell structures. For example, in transversal sections of nodules took in October little number of hyphae and vesicles has been observed (this time activity of nitrogen fixation was accounted for 48 μ mole C_2H_4/g raw mass per 1 hour); in November in cells strengthening of destructive process and the beginning of notable decreasing of nitrogen-fixing activity (up to 32μ mole) have been observed. In December nitrogen-fixing activity was accounted for 26μ mole. Similar phenomena are described for the plants of genus Taxaceae [Miroslavov, 2002].

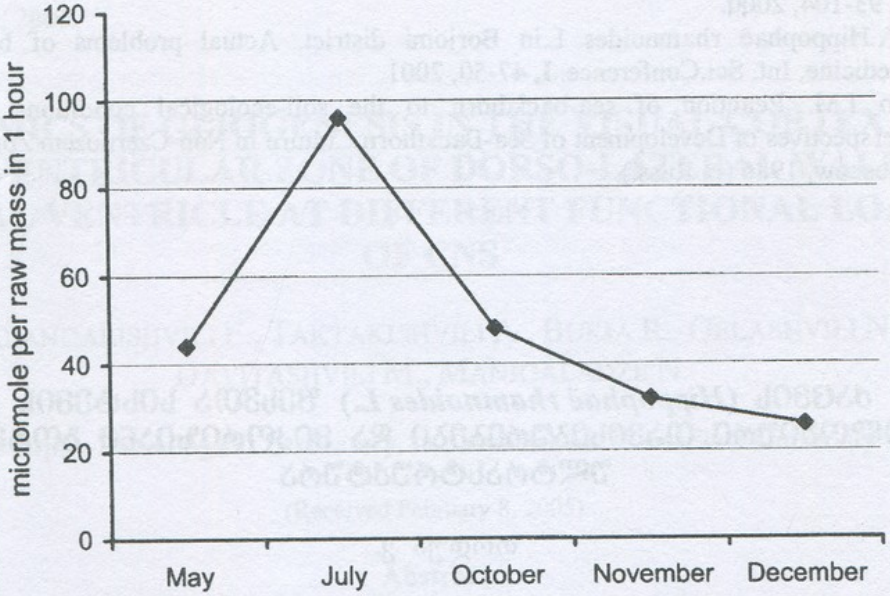

Fig. 3. Intensity of nitrogen fixation in mycorrhizal nodules

Taking into account above mentioned data, it can be concluded that the nitrogen-fixation activity of mycorrhizal nodules is not invariable. It changes during whole vegetative period. Majority of cells of wintering nodules is poor in cytoplasm. And in some cells cytoplasm is not observed at all. Significant changes take place also in other structures of the cells.

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ქაცვის (*Hippophaë rhamnoides L.*) ფესვთა სისტემის მორფოლოგიური თავისებურებები და მიკორიზიანი გორგლების ულტრასტრუქტურა

თოდუა ვ.

საქართველოს მეცნიერებათა აკადემიის ნ. კეცხოველის სახ. ბოტანიკის ინსტიტუტი

(მიღებულია 14.02.2005)

რეზიუმე

შესწავლილია ქაცვის ფესვთა სისტემის მორფოლოგია. ორწლიან მდედრობით და მამრობით მცენარეებში დადგენილია ფესვის ჯამური სიგრძის მერყეობა – ♀ 95,8±3,24 მ-დან ♂ 119,7± 4,55 მ-ის ფარგლებში, ხოლო 5-6-წლიან ინდივიდებში – ♀ 1110,8±6,11 მ-დან ♂ 1186,3±25,20 მ-მდე. მცირე ზომის მოზარდი ფესვებისა და ღივების წილად (3 მმ-მდე) მოდის ფესვის საერთო სიგრძის 80-85%. ფესვთა სისტემის დიამეტრი თითქმის 3-ჯერ აღემატება ვარჯის დიამეტრს და მათი შემწოვი ზედაპირი ბევრად სცილდება ვარჯის საზღვრებს.

ქაცვის მიკორიზიანი გორგლების აზოტფიქსაციური აქტიურობა სავეგეტაციო პერიოდის მანძილზე მუდმივად იცვლება. აზოტფიქსაციის მაქსიმუმი აღრიცხულია ივლისში – 96 მკ მოლი, ხოლო მინიმუმი დეკემბერში – 26 მკ მოლი C_2H_4/გ ნედლი მასა 1 საათში. ზაფხულის პერიოდში მიკორიზიანი გორგლების უჯრედებში დიდი რაოდენობით აღინიშნა ციტოპლაზმა, ჰიფები, ვეზიკულები და უჯრედის სხვა სტრუქტურები, რასაც ვერ ვიტყვით მოზამთრე გორგლების უჯრედებზე - ადგილი ჰქონდა დესტრუქციული მოვლენების გაძლიერებას და იწყებოდა აზოტფიქსაციის აქტივობის შესამჩნევი კლება.

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Cytology

DYNAMICS OF GLIOGENESIS IN THE VISUAL CORTEX AND PERIVENTRICULAR ZONE OF DORSO-LATERAL WALL OF LATERAL VENTRICLE AT DIFFERENT FUNCTIONAL LOADING OF CNS

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Abstract

Proliferated activity of glial cells of visual cortex and dorso-lateral wall of lateral ventricle was studied in visual deprived and light stimulated rats during 30 days from the birth. By quantitative indices of mitotic and DNA-synthesizing cells it was established that in visual cortex of deprived rats proliferated activity compared to the norm is low, in light stimulated animals – high. With differentiation of cortex neurons amount of perineuronal satellite glia is increased. Neurons with three or more satellites in light stimulated rats are more than in the norm, in deprived animals they occur rarely. The amount of proliferated glial populations in periventricular zone during the first 10 days is lower compared to the norm, and further is higher. Intensification of proliferated processes in light stimulated rats during the first 14 days is noticed. By method of autoradiography the main mass of labeled glial cells in cortex is presented by their postmitotic derivates migrated from lateral ventricle. The amount of the last one is varied at different functional loading. This fact indicates that postnatal realization of indifferent stem cells can be conducted by outer factors.

Key words: visual cortex, proliferated activity, satellite glia, postmitotic derivates

Introduction

Shifts in the amount of perineuronal satellites and connected with this process postnatal dynamics of postnatal gliogenesis are important parameters of functional state of neurons at different effects on CNS. Postnatal proliferation of neuroglia takes place due to mitotic activity of glial elements locally in the brain cortex, as well as due to migration of postmitotically differentiated into glia derivates of matrix cells from periventricular zone to neocortex.

It is known that during the process of brain formation and especially in the extreme conditions considerable shifts in the amount of neuroglia in the neocortex are noticed [Pevzner, 1974; Krivitskaia, 1974; Luskin, 2001; Gage, 2000; Svanidze, Museridze, 1974; Mepisashvili et al., 1986; Kalandarishvili et al., 2004].

The goal of our work is to study dynamics of gliogenesis in the visual cortex and periventricular zone of dorso-lateral wall of lateral ventricle of rats.

Materials and Methods

We studied 1-30 days old white rats. By keeping in dark camera from the birth till the age of 30-days, the first group of rats was experienced to visual deprivation. During the same period the second group was stimulated by glimpsed light of frequency 3 hertz for 1 hour daily. The third – control group was in the normal light regime. By the indices of mitotic activity and the number of labeled cells intensity of cell proliferation was revealed. The amount of mitotically divided and DNA-synthesizing glial cells in the visual cortex was counted on 1 section of hemisphere. Mitotic activity index (MAI) and labeling index (LI) were determined (in ppm %) in periventricular zone. By the method of autoradiography using ^{3}H thymidine premitotic synthesis of DNA was determined. Part of serial sections of the width of 7μm were dyed according to Bemer & Heidengain, and other part was covered with photoemulsion. After development autographs were dyed with cresyl-violet.

Results and Discussion

Proliferated activity of glial cells in the visual cortex of normal rats is gradually reduced with the aging. Process of gliogenesis in deprived rats is inferior to the control animals of the corresponding age. Proliferated activity in light-stimulated rats is characterized by higher indices (Table 1).

On the early stages of postnatal development of rats main mass of glial population is presented by free neuroglia. Further, as far as cortex neurons are differentiated, the amount of perineuronal satellite glia is considerably increased. Dynamics of redistribution of free and satellite glia in visual deprived rats shows that this process is slower compared with the control group, but in stimulated animals – is faster. At the same time, in every age group number of neurons with three and more satellites in stimulated rats happens frequently, compared to control, but in deprived – rarely (Table 2).

Table 1. Amount of mitosis and labeled glial cells in visual cortex of rats at norm, visual deprivation and light stimulation on one section.

Age of	rats (days)	3	7	10	14	16	21	30
norm	mitosis	25,1	22,6	18,3	10,9	9,9	5,8	3,3
	Labeled cells	59,8	48,6	38,4	27,2	25,2	12,3	8.0
deprivation	mitosis	23,5	20,0	15,0	12,4	11,1	8,9	5,8
	Labeled cells	57,1	40,2	35,5	34,3	28,7	13,8	9,6
stimulation	Mitosis	28,2	26,1	24,1	16,9	10,9	2,1	1,0
	Labeled cells	63,9	62,8	58,7	50,6	29,5	8,3	3,1

Study of dynamics of LI of glial population produced by periventricular zone has shown that in deprived rats during the first 10 days amount of labeled cells is lower than in control group. Later these parameters prevailed on control ones and the gliogenesis is stretched out in longer periods. The amount of DNA-synthesizing cells of light stimulated rats in periventricular zone during the first two weeks is higher compared to control ones. Then the amount of labeled cell is considerably decreased, i.e. intensification of proliferated processes is significant (Table 3).

Analogous regularity is observed in parameters of mitotic activity (Table 3).

Compare the amount of mitotically divided and DNA-synthesizing cells of visual cortex with those of periventricular zone, revealed considerable prevailing of the last ones.

Table 2. Distribution of perineuronal satellites in visual cortex of rats in norm, visual deprivation and light stimulation.

Age of rats (days)		Amount of neurons with satellites (per 100 neurons)	Total amount of satellites	Number of neurons with one satellite	Number of neurons with two satellites	Number of neurons with three and more satellites
3	norm	11	13	9	2	-
	deprivation	10	12	8	2	-
	stimulation	13	19	10	3	1
7	norm	18	27	11	5	2
	deprivation	14	19	10	3	1
	stimulation	24	37	14	7	3
14	norm	34	56	18	12	4
	deprivation	26	44	16	8	3
	stimulation	43	69	23	14	6
21	norm	44	77	21	16	8
	deprivation	33	51	18	12	3
	stimulation	52	90	25	19	9
30	norm	48	82	22	18	8
	deprivation	38	60	20	14	4
	stimulation	64	110	29	24	11

Table 3. LI and MAI of cells of periventricular zone of dorso-lateral wall of lateral ventricle of rats in norm, visual deprivation and light stimulation (%)

Age of	rats (days)	3	7	10	14	16	21	30
norm	MAI	9,8	7,2	6,2	5,6	3,0	1,8	0,7
	LI	107,1	71,1	50,0	34,2	20,1	10,2	7,1
deprivation	MAI	8,7	5,1	4,2	4,0	3,8	2,5	1,8
	LI	95,0	62,2	41,0	38,3	34,2	22,1	14,7
stimulation	MAI	10,5	8,8	6,4	5,8	3,0	0,7	0,2
	LI	120,1	80,1	70,0	42,0	17,2	6,4	0,5

Results of long-term autoradiography have shown that the main mass of labeled glial cells in the visual cortex was presented by previously labeled postmitotic derivates of matrix cells migrated from periventricular zone, the amount of which is notably varied in accordance with functional loading of brain.

Thus, at different levels of functional loading of CNS, shifts of cells proliferative activity of periventricular zone give rise to changes in intensity of replenishment of cortex with neuroglia, which is necessary for glioneuronal complex formation providing structural and functional maturing of neocortex, i.e. this process can be conducted by medium factors. So, the plastic reorganizations in hemisphere cortex at different steps of postnatal development should be realized by mobilization of residual reserves of frigid (stem) cells, migrated from periventricular zone to neocortex.

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გლიოგენეზის დინამიკა მხედველობის ქერქში და გვერდითი პარკუჭების დორსოლატერალური კედლის სუბვენტრიკულურ ზონაში ცენტრალური ნერვული სისტემის სხვადასხვა ხარისხის ფუნქციონალური დატვირთვისას

კალანდარიშვილი ე., თაქთაქიშვილი ა., ბუკია რ., გელაშვილი ნ., დავითაშვილი მ., მანჯგალაძე ნ.

მორფო-ფიზიოლოგიის ლაბორატორია, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 8.02.2005)

რეზიუმე

მხედველობით დეპრივირებულ და სინათლის სხივით სტიმულირებულ ვირთაგვებში დაბადებიდან 30 დღის მანძილზე შესწავლილია მხედველობის ქერქის და გვერდითი პარკუჭების დორსო-ლატერალური კედლის პერივენტრიკულური ზონის გლიური უჯრედების პროლიფერაციული აქტიობა. მიტოზური და დნმ-მასინთეზირებელი უჯრედების რაოდენობრივი მაჩვენებლების მიხედვით დადგენილია, რომ დეპრივირებულ ცხოველების მხედველობის ქერქში პროლიფერაციული აქტიობა ნორმასთან შედარებით დაბალია. სინათლით

სტიმულირებულ ცხოველებში კი მაღალი. ქერქის ნეირონების დიფერენცირებასთან ერთად პერინეირონალური სატელიტური გლიის რაოდენობა მატულობს. ნეირონები სამი და მეტი სატელიტებით სინათლით სტიმულირებულ ცხოველებში მეტია, ვიდრე ნორმაში. დეპრივირებულში კი ასეთები იშვიათად გვხვდება. პირველი 10 დღის განმავლობაში პერივენტრიკულურ ზონაში პროლიფერირებადი გლიური პოპულაციების რაოდენობა დეპრივირებულ ცხოველებში ნარმასთან შედარებით დაბალია, შემდგომ კი აღემატება ნორნის მონაცემებს. სინათლით სტიმულირებულ ცხოველებში პირველ 14 დღეში აღინიშნება პროლიფერაციული პროცესების ინტენსიფიკაცია. ავტორადიოგრაფიის მონაცემებით ქერქში მონიშნული გლიური უჯრედების ძირითადი მასა წარმოდგენილია გვერდითი პარკუჭებიდან მიგრირებული მათი პოსტმიტოზური დერივატებით. ამ უკანასკნელის რაოდენობა განსხვავებულია სხვადასხვა ფუნქციონალური დატვირთვისას, რაც იმის მაჩვენებელია, რომ სუბვენტრიკულარული ზონის ინდიფერენტული ღეროვანი უჯრედების პოსტნატალური რეალიზაცია შესაძლებელია იმართებოდეს გარემო ფაქტორებით.

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Genetics

EARLY DEFINITION OF SEX IN ACTYNIDIA BY THE LEAF PROTEIN ELECTROPHORESIS

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Abstract

The protein spectrum of Actinidia spp. has been studied by the method of electrophoresis in polyacrilamide gel in the presence of sodium dodecylsulphate. Specific polypeptides were revealed in protein markers of male and female plants of A. chineusis and A. kolomikta. The possibility of using protein markers to reveal plant sex at the early stage of development is considered.

Key words: Actinidia, electrophoresis, polyacrilamide gel

Introduction

Actinidia belongs to the group of unisexual, dioecious plants and, hence, its productivity as well as other dioecious plants depends on correlation of male and female individuals in population. It was established that one male plant is enough for 5-6 female actinidia plants to receive optimum yield. At the same time, the splitting of seedlings according to the sex is 4:1. At the yearly stages of development the seedlings practically don't differ by morphological peculiarities and only reaching the period of puberty, i.e. approximately, 4-6 years when the flowers with reduced pistil or with sterile anther appear, it is possible to identify their sex.

Consequently, it is important to find such markers of actinidia's sex that will allow to differentiate male and female plants at the early stages of their development and to choose the necessary forms for selective and economic aims. According to literature data it is well known that individuals of different sex of some dioecious plants differ by enzymes, phytohormones, tannin substances, etc. The experiments were carried out to determine actinidia sex using peroxidase test and other biochemical markers [Hirsch et al., 1997; Harvey et al., 1997]. The goal of the presented paper is identification of actinidia sex by selection of protein markers.

Materials and Methods

Leaves of two species of actinidia: A. chineusis (Chinese actinidia) and A. kolomikta were studied.

Total protein extract was isolated from plant leaves of different sex, at different stages of vegetative cycle. For protein extraction several buffer solutions were used. The most complete extraction was achieved using 0,5 M tric-glycine buffer (pH-7,0). The leaves were collected in the day time, they were cut into small pieces and put into distilled water for 1-1,5 hours. At the same

time, partially water soluble albumin fraction of proteins is moved off, which, as it was found out, doesn't reveal sex specificity and only blocks up total protein spectrum, impeding identification of polypeptide lines.

Leaves cut into small pieces were taken out of water and dried on filter paper, put into preliminary cooled porcelain mortar, adding a little amount of liquid nitrogen, polyvinilpyrolidon (Poliklar AT) in correlation 1:4 and quickly grinded. The obtained powder was put into centrifuge test-tubes, for protein extraction buffer in ratio 1:3 was added, mixed and left in a refrigerator for 1 hr. Then it was centrifuged at 3000 g, for 20 min. The supernatant was carefully separated from sediment. The sediment was put away, the experiment was performed by the method of electrophoresis [Laemmli, 1970] in polyacrilamide gel (PAAG), using PAAG in different concentrations. Comparatively clear separation of protein components was reached using 12,5% PAAG. Molecular masses of these components were determined according to Osterman [Osterman, 1981], using standard protein markers: cytochrom C (12,3 kDa), myoglobin (17,8 kDa), egg albumin (45,0 kDa) and bull serum albumin (67,0 kDa).

Electrophoresis was carried out for 3,5-4 hr at 10 mA/cm. Protein dissociation up to subunits was carried out at the presence of 1% sodium dodecylsulphate (DS-NA) [Eggi, 1984]. To study polypeptide spectra β-mercaptoethanol was added into this mixture, destructing subunits up to polypeptides. Protein components were fixed in the mixture: isopropanol – acetic acid – water (25:7:68), gel was dyed by 0,2% solution of cumassi R-250. The redundant dye was washed off and the preparations were dried out.

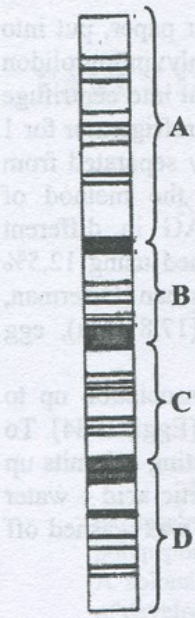
Results and discussion

No distinctions were observed between male and female individuals in any of the studied species on electrophoregramms of actinidia proteins, separated at the presence of DS-Na. Addition of destroying disulphide bridges β-mercaptoethanol promotes formation of peptides of various intensity distributed along the electric field.

Analysis of the obtained electrophoregramms of polypeptide spectra showed that leaves proteins of the studied species of actinidia form almost 30 components. The standard spectrum is given on the Fig. 1. Four zones of components were marked out on the standard spectrum: A-polypeptides with molecular mass 89-65 kDa, B - 65-45 kDa, C - 45-20 kDa and D - lower than 20 kDa. Each zone is distinguished by specific set of components. Their bulk is in the C and D zones with molecular masses, approximately 12-45 kDa.

On the electrophoregramms of male plants an intensive component in zone D with mol. mass approximately 18 kDa (Fig.2, a) is marked out, it is absent in spectra of female plants. On electrophoregramms of female plants a clear line of polypeptide with mol. mass approximately 45 kDa and two less intensive components with mol. masses ~67 kDa were observed (Fig.2,b). These two components probably are polypeptides of one polymorphic protein which is coded with specific multiallelic gene for female plants. In protein spectrum of male plants these components are either absent, or form minor lines of polypeptides. The experiments were carried out during the whole period of vegetation of mature plants as well as on plants obtained in leaves tissue culture in vitro (Fig.3).

However, differences in polypeptide spectra of mature plant were found only during the period of flowering evocation, at the end of May and beginning of June, and during the period of reproduction, at the beginning of November. No differences were observed during the rest periods of vegetation in polypeptide spectra of both male and female plants. On electrophoregramms of the same plants, received in tissue culture from auxiliary buds, polypeptide component, characteristics only for male plant was fixed.



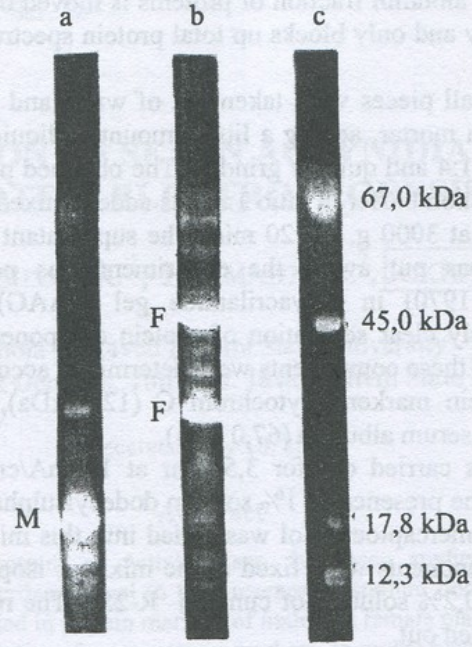


Fig. 1. **Fig. 2.** **Fig. 3.**

Fig. 1. Standard spectra of protein polypeptides from Actinidia L. leaves A-zone - polypeptides with mol. mass 89-65 kDa. B-zone - 65-45 kDa, C-zone - 45-20 kDa, D-zone - 20 kDa.

Fig. 2. Electrophoregrams of Actinidia L. leaves proteins. a - Polypeptide spectrum of male plants; b - Polypeptide spectrum of female plants; c - Standard protein markers; M-Protein markers of male plants; F - rotein markers of female plants.

Fig.3. Electrophoregram of actinidia L. leaves proteins received in tissue culture in vitro. M - protein markers of male plants.

According to references, at the period of flower evocation in some dioecious plants increase of nucleic acids and proteins synthesis, as well as formation of specific phytohormones in plants of both sex takes place. Distinguished protein antigens were found in female and male plants leaves [Chailakhyan et al., 1982].

Thus, in leaves of actinidia at the very beginning of sex morphogenesis a process of gene differential expression takes place. So, polypeptides of total protein extract from actinidia leaves may be used as biochemical markers for early identification of this plant sex.

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აქტინიდიის სქესის ადრეული იდენტიფიკაცია ცილების ელექტროფორეზული სპექტრის მიხედვით

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(მიღებულია 10.05.04)

რეზიუმე

შესწავლილია აქტინიდიის ფოთლის ცილები ელექტროფორეზის მეთოდით პოლიაკრილამიდის გელში. გამოვლენილია სპეციფიური პოლიპეპტიდები ორი სახეობის ***A. kolomikta*** **and** ***A. Chineusis*** მდედრობითი და მამრობითი მცენარეების მაგალითზე. განხილულია პოლიპეპტიდური სპექტრის მიხედვით ამ მცენარეთა სქესის იდენტიფიკაციის შესაძლებლობა განვითარების ადრეულ სტადიებზე.

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ACTIVITY OF NUCLEOLAR ORGANIZING REGIONS OF ACROCENTRIC CHROMOSOMES AND ASSOCIATIVE INDEX OF THE INDIVIDUALS CONTACTED WITH FERROMANGANESE AND SILICOMANGANESE

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Abstract

Ag^+ positive nucleolar organizing region's (NOR) number is varied under the influence of different substances. Studies of acrocentric chromosomes transcriptional activity show that in the individuals contacted with Si and Mn compounds – ferromanganese and silicomanganese, indices of NOR's frequency, size and participation in associations is sharply decreased. To avoid the negative results of the effect of external mutagens, it is necessary to carry out genetical monitoring on the individuals belonging to the risk group.

Key Words: nucleolar organizing region, transcriptional activity, ribosomal cistrons, satellite associations.

Introduction

It is known that Ag^+ positive NOR's number, sizes and their dyeing intensity depend on the degree of functional activity of ribosomal cistrons in the premitotic interphase which provides intensity of protein synthesis [Lezhava, 1999].

The number of Ag^+ positive NORs is variable value and changes under the influence of different substances [De Capoa, et al., 1996].

The goal of the work is to study transcriptional activity of acrocentric chromosomes of ribosomal genes.

Materials and Methods

To determine activation degree of synthesizing processes in the cells, transcriptional activity of ribosomal cistrons of chromosomes in the peripheral blood lymphocytes cultures of Zestaponi non-ferrous factory 10 workers (age 26-42) were studied.

Activity of ribosomal genes of acrocentric chromosomes and satellite associations frequency were determined.

To reveal NORs Bloom and Goodpasture's modified method was used [Bloom&Goodpasture, 1976; Khavinson et al., 2003]. Activity of ribosomal cistrons was estimated by several criteria: by calculation of silver dyeing regions frequency; by the size of silver-covered

segments (the size was estimated by 3-mark system); by determination of satellite associations [Lezhava, 1999]. The results statistically were worked out by Student's method.

Results and Discussion

To study transcriptional activity of acrocentric chromosomes NORs, 300 metaphases of 10 workers of non-ferrous factory were researched.

In tested individuals average number of Ag^+ positive chromosomes per one cell (4,06±0,12) is reduced compared to control one (6,35±0,10). Mentioned parameter varied from 3,13 to 5,53 (Fig.1).

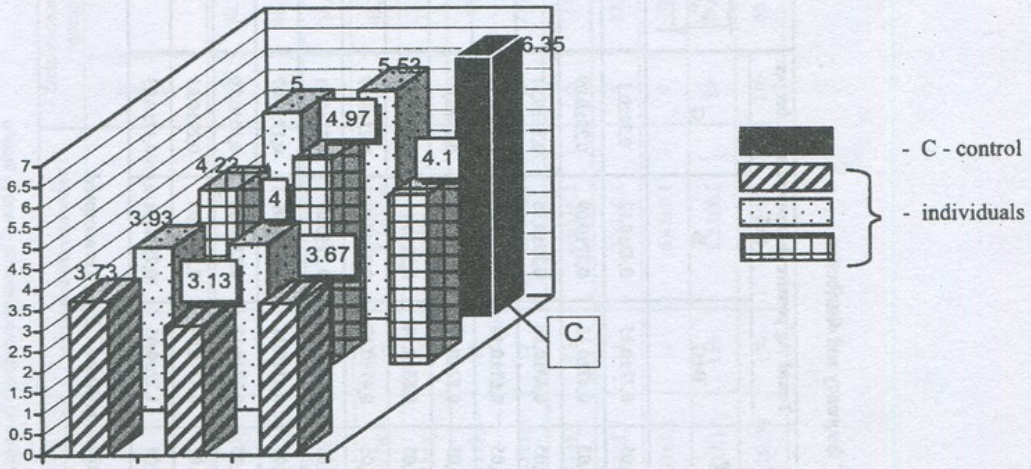

Fig.1 The variability of the amount of Ag^+ positive chromosomes

The frequency of Ag^+ positive chromosomes was also studied in the case of other metals effect. The ions of nickel and cobalt don't cause the change of this parameter, but ions of mercury and zink, like Si and Mn decrease it [Jokhadze, 2001].

In all tested individuals, as well as in control group, silver dyeing capacity of D chromosomes was increased compared to G chromosomes. At calculation of the big size (2-mark) NORs, their reliable reduction was noticed in all cases (Table 1). The same results were received in the case of the effect of Ni, Co, Zn, Hg [Jokhadze, 2001].

We studied parameters of associations of acrocentric chromosomes. It was revealed that this parameter is also decreased compared to control. Average percentage parameter (25,67±2,5) reliably differed from the control parameter (52,0±3,2). Analyzing the types of associations it was shown that equal decrease of the frequency of all three types of associations (D/D, D/G and G/G) occur (Table 2).

Studies of functional characteristics of acrocentric chromosomes NORs showed that in peripheral blood cells of individuals working with Si and Mn, in particular with ferromanganese and silicomanganese, the parameters of NORs' frequency, size and participation in associations are sharply lowered compared to control group parameters, which indicates homeostasis disorder and should be the cause of the rise of pathologies.

So, to avoid the negative effect of external mutagens, it is necessary to carry out genetical monitoring among the individuals of high professional risk group.

Table 1. Ag^+ positive NORs' frequency and distribution

Individual№	Ag^+ positive chromosomes number per 1 cell			$\frac{P_D - P_G}{\sqrt{\frac{P_D(1-P_D)}{6n} - \frac{P_G(1-P_G)}{4n}}}$	P	2-mark Ag^+positive chromosomes number per 1 cell			$\frac{P_D{}^{2+} - P_G{}^{2+}}{\sqrt{\frac{P_D{}^{2+}(1-P_D{}^{2+})}{6n} - \frac{P_G{}^{2+}(1-P_G{}^{2+})}{4n}}}$	P
	D+G	D	G			D+G	D	G		
1.	3,73±0,35	2,17±0,27	1,57±0,23	0,54	>0,05	0,73±0,15	0,43±0,12	0,30±0,1	0,11	>0,05
2.	3,13±0,32	1,70±0,24	1,43±0,22	1,36	>0,05	0,47±0,12	0,27±0,09	0,23±0,09	* 0,54	<0,05
3.	3,67±0,35	2,20±0,27	1,63±0,23	* 0,72	>0,05	0,63±0,14	0,30±0,10	0,33±0,11	1,33	>0,05
4.	5,0±0,41	2,87±0,31	2,13±0,27	* 0,93	>0,05	0,63±0,14	0,27±0,09	0,37±0,11	* 1,66	<0,05
5.	5,53±0,43	2,20±0,27	1,17±0,24	0,88	>0,05	0,57±0,14	0,30±0,10	0,27±0,09	* 0,61	<0,05
6.	4,23±0,38	2,10±0,27	2,13±0,27	* 3,10	<0,05	0,63±0,14	0,33±0,11	0,30±0,10	* 1,10	<0,05
7.	4,10±0,37	2,30±0,28	1,80±0,25	* 0,29	<0,05	0,47±0,12	0,23±0,09	0,23±0,09	0,73	<0,05
8.	4,97±0,41	2,23±0,27	1,73±0,24	1,06	<0,05	0,60±0,14	0,27±0,09	0,33±0,11	* 2,29	<0,05
9.	3,93±0,36	2,20±0,27	1,73±0,24	1,14	>0,05	0,43±0,12	0,27±0,09	0,17±0,07	* 0,08	>0,05
10.	4,0±0,37	2,4±0,28	1,60±0,22	* 0,14	<0,05	0,53±0,13	0,23±0,09	0,30±0,10	1,29	<0,05
Average	4,06±0,12	2,24±0,09	1,74±0,08	* 3,5	>0,05	0,57±0,04	0,29±0,03	0,29±0,03	* 0,29	<0,05
control	6,35±0,11	3,17±0,23	2,32±0,20	5,131	>0,05	1,22±0,05	0,75±0,04	0,47±0,03	0,71	>0,05

* - For these individuals the alternative admission - $P_G > P_D$ is accepted

Table 2. The frequency and types of uniting in associations of the chromosomes of D and G groups

individual #	metaphases with associations (%±m)	The types of associations						associated chromosomes		Dchromosomes of group		Gchromosomes of group	
		D/D		D/D		D/D							
		number	%	number	%	number	%	total	per 1 association	number	%	number	%
1.	23,3±7,7	3	10,0±5,5	3	10,0±5,5	1	3,33±3,2	15	0,50±0,1	10	33,33±8,6	5	16,67±6,8
2.	36,7±8,8	3	10,0±5,5	6	20,0±7,3	2	6,67±4,5	23	0,77±0,2	12	40,0±18,9	11	36,67±8,8
3.	20,0±7,3	2	6,67±4,6	3	10,5±5,5	1	3,33±3,2	13	0,43±0,1	8	26,67±8,1	5	16,67±6,8
4.	36,7±8,8	3	10,0±5,5	6	20,0±7,3	2	6,67±4,8	22	0,73±0,2	13	43,33±9,1	9	30,0±8,4
5.	20,0±7,3	2	6,67±4,6	3	10,0±5,5	1	3,33±3,2	12	0,40±0,1	7	23,33±7,7	5	16,67±6,8
6.	33,3±8,6	3	10,0±5,5	6	20,0±7,3	1	3,33±3,2	15	0,50±0,1	12	40,0±8,9	9	30,0±8,4
7.	23,3±7,7	2	6,67±4,6	4	13,3±6,2	1	3,33±3,2	15	0,50±0,1	8	26,67±8,1	7	23,33±7,7
8.	20,0±7,3	1	3,33±3,2	4	13,3±6,2	1	3,33±3,2	13	0,43±0,1	6	20,0±7,3	7	23,33±7,7
9.	16,7±6,8	0	0	4	13,3±6,2	1	3,33±3,2	11	0,37±0,1	4	13,33±6,2	7	23,33±7,7
10.	23,3±7,7	3	10,0±5,5	3	10,0±5,5	1	3,33±3,2	14	0,47±0,1	9	30,0±8,4	5	16,67±6,8
Average	25,67±2.5	22	7,3±1,5	42	14,0±2,5	12	4,0±1,13	159	0,53±0,1	89	29,67±2,64	70	23,33±2,44
control	52,0±3,2	32	42,10±5,6	34	44,73±5,7	10	13,16±38	190	2,5±0,1	130	68,42±3,3	60	31,57±3,4

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აკროცენტრული ქრომოსომათა ბირთვაკ-მაორგანიზებელი უბნების აქტივობა და ასოციაციური მაჩვენებელი ფერომანგანუმისა და სილიკომანგანუმთან საწარმოო კონტაქტის მქონე პირებში

ლოლაძე თ.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 15.02.2005)

რეზიუმე

Ag^+ პოზიტიური ბირთვაკ-მაორგანიზებელი უბნების რიცხვი ვარიაბელური სიდიდეა და იცვლება უჯრედებზე სხვადასხვა ნივთიერებების ზემოქმედებით. Si-ისა და Mn-ის შენაერთებთან, კერძოდ სილიკომანგანუმსა და ფეროსილიციუმთან საწარმოო კონტაქტის მქონე პირებში, აკროცენტრული ქრომოსომათა ტრანსკრიპციული აქტივობის შესწავლამ გვიჩვენა, რომ ბირთვაკმაორგანიზებელი უბნების სიხშირის, ზომის და მათი ასოციაციებში მონაწილეობის მაჩვენებლები, მკვეთრად არის დაქვეითებული. გარემო მუტაგენთა მოქმედების უარყოფითი შედეგების თავიდან ასაცილებლად საჭიროა განხორციელდეს გენეტიკური მონიტორინგი ე.წ. მაღალი პროფესიული რისკის მქონე ჯგუფებში.

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LYMPHOCYTE SUBPOPULATIONS IN THE BLOOD OF WOMEN WITH MALIGNANT AND BENIGN TUMORS OF REPRODUCTIVE SYSTEM

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Abstract

Peripheral blood lymphocytes from patients with malignant and benign tumors of reproductive system have been analyzed for the expression of phenotypic and activation markers by flow cytometry. The results were compared with those of a control group of healthy women. No important differences have been found in the expression of CD3+, CD19+ or CD8+ in patients with uterus benign tumor (UBT), although the percentages of CD4+ cells and therefore CD4/CD8 ratio was found to be decreased. Low numbers of CD4+ cells were also characteristic for the blood of patients with uterus body cancer (UBC) and cervical cancer (CC), however the percentages of CD19+ and CD8+ cells were found to be increased almost twofold, which further affected the CD4/CD8 ratio. Both these diseases were also characterized by extremely low number of circulating CD25+T lymphocytes. In patients with ovarian cancer (OVC) very similar changes have been detected, apart from the normal number of CD19+ B cells. Although a sharp increase of cytotoxic CD8+ T cells in blood of the patients with UBC, CC and OVC suggests a potential for anti-tumor responses, these cytotoxic cells seem to be unable to react by activation due to the lack of the expression of CD25 (IL-2Rα) receptor. In addition, elevated number of CD19+ B cells may reflect the production of antibodies to tumour-associated antigens (TAA) in patients with UBC and CC which would prevent CD8+ T cells from the recognition of the tumours. These results suggest that gynecological cancer is associated with specific alterations in the T cell population and up-regulation of the IL-2Rα receptor may be important for potential therapeutic strategies.

Key Words: Uterus benign tumor, cervical cancer, uterus body cancer, ovarian cancer.

Introduction

UBC is the most common cancer of the female reproductive system. It is the fourth most common cancer in women, following lung, breast, and colon cancer. CC is the second most common cancer in women with lethal outcome. OVC is the fifth most common cancer in women, and it is responsible for the majority of gynecological fatalities. Because patients with cancer of reproductive organs have high recurrence rates and poor long-term survival, there is interest in developing adjunctive therapies, including immunotherapy [Edward, et al.,2004]. The prerequisite

for an immunotherapeutic approach to cancer is the expression by the malignant neoplasm of tumor–associated antigens [Urban, Schreiber, 1992]. On the other hand, successful immunotherapy requires a functional immune system and defect in the immune responses may contribute to tumor growth. Such defects, especially T-cell dysfunction, can be partially due to the active suppression by the tumor [Wojtowicz-Praga, 1997; Levey, Srivastava, 1996].

The aim of the present study was quantitation of the main circulating blood cell population in patients with malignant and benign tumors of reproductive system by measuring percentages of CD3+, CD4+, CD8+, CD19+ and CD25+ lymphocyte in peripheral blood mononuclear cells (PBMC). CD3 delineates all circulating T cells, CD4 is expressed by T helper and T suppressor cells, CD8 is expressed by cytotoxic T lymphocytes, CD19+ cells belong to B cell subset and CD25 (IL-2α receptor) is acquired by predominantly T cells upon their activation [Boon et al.,199; Gupta et al.,1997; Westermann, Pabst, 1990].

Materials and Methods

10 ml of peripheral blood has been obtained from 4 women with UBT, 5 women with UBC, 5 women with CC, 4 women with OVC and 5 healthy donors, aged 42-65. All patients were newly diagnosed and untreated.

Peripheral blood mononuclear cells (PBMC) from heparinized blood were separated by centrifugation on Ficoll-Hypaque (Sigma, 1.077g/l) gradient. After the separation cells were washed twice with phosphate-buffered saline (Sigma, PBS). The cells were stained with appropriate mAbs (Pharmingen) for 30min on ice and washed three times in PBS supplemented with 2% bovine serum albumin (Sigma) and 0.01% sodium azide (Sigma). Cells were incubated for 30min on ice with secondary mAbs: FITC – conjugated rabbit anti-mouse $F(ab)_2$ fragments (Dako), washed twice in PBS and fixed with 2% solution of paraformaldehyde (Sigma) in PBS before flow cytometric analysis. All samples were analyzed using FACScan flow cytometer (Becton & Dickinson). Data were expressed as histograms of the fluorescence intensity versus cell number. In each case the dot-plot was gated on the lymphocytes in side scatter-forward scatter (SSC-FSC) plot. Within this gate the makers were set on the isotype control to define the negative population. For each sample percentages of positive cells have been calculated.

The results were statistically analyzed according to the Student's t-test. The values on the charts represent an average and a standard deviation.

Results and Discussion

Percentages of CD19+ and CD3+ cells in patients with UBT did not differ from the control values. This means that there are no substantial changes in the total number of B and T lymphocytes respectively in the blood of patients with UBT. Although the number of CD8+ T cells also was found to be normal, the percentages of CD4+ cells were significantly decreased in UBT (23.04 ± 5.2 vs. 34.17 ± 3.9, $P<0.05$). This resulted in the reduction of CD4+/CD8+ ratio (1.4 ± 0.5 vs. 2.06 ± 0.9, $P<0,001$. Fig.1).

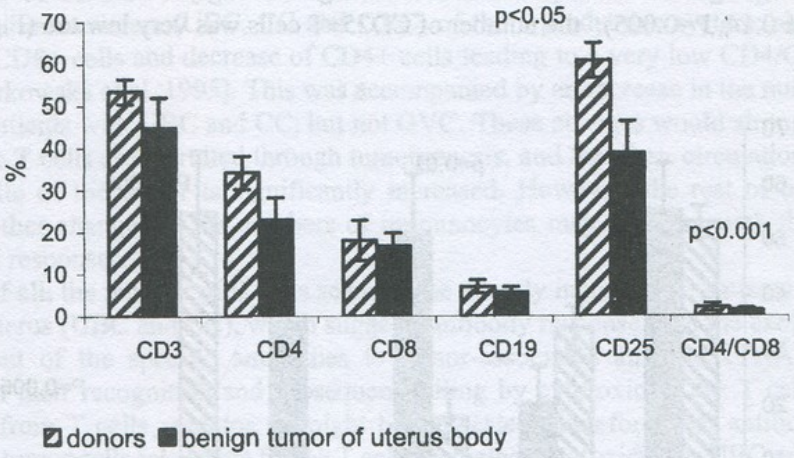

Fig.1. Percentages of various lymphocyte populations in peripheral blood of patients with BTU and controls.

In contrast to UBT, in the blood of the UBC patients the percentages of CD8+ cells and CD19+ cells were twice as high as the control values (35.52 ± 10.7 vs. 17.9 ± 4.8, P<0.005 and 15.8±1.7 vs 7.06 ± 1.3, P<0.01), although the percentages of CD4+ cells and therefore CD4/CD8 ratio were decreased: CD4+ T cells 22.35 ± 7.2, CD4/CD8=0.7±0.3 vs CD4+ 34.17±3.9 and CD4/CD8 =2.06 ± 0.9 in controls, P< 0.001. Only a very small number of CD3+ T cells expressed CD25: 0.27±0.06 compared to 60.8±4.1 in normal controls, P=0.005 (Fig.2).

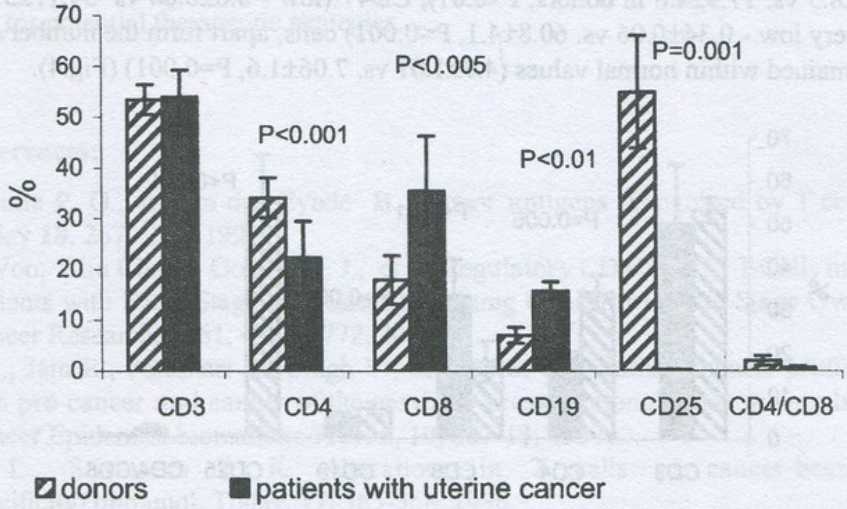

Fig.2. Percentages of various lymphocyte populations in peripheral blood of patients with UBC and controls.

Very similar changes were found in patients with CC: dramatic increase in the relative number of CD19+ and CD8+ lymphocytes, compared to control values. A slight decrease was

noted in the percentages of CD4+ cells. These changes strongly affected the CD4/CD8 ratio (CD4/CD8=0.5 ± 0.14, P=0.005); the number of CD25+T cells was very low too (Fig.3).

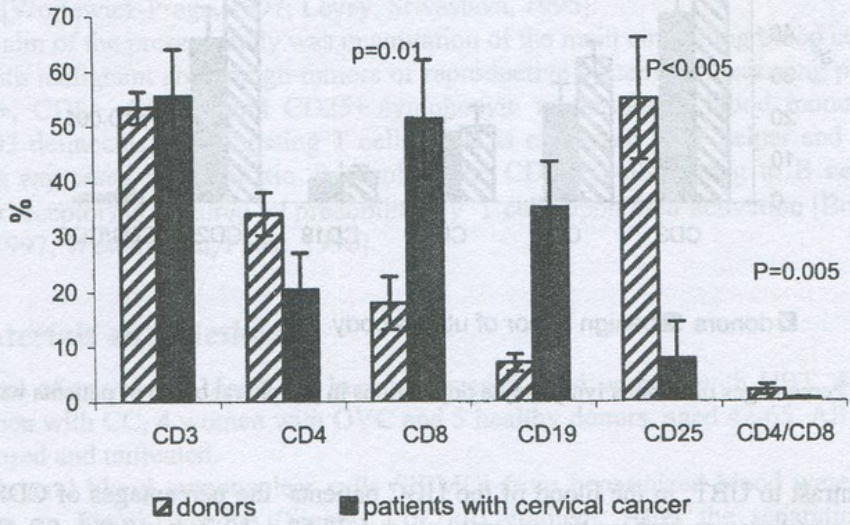

Fig.3. Percentages of various lymphocyte populations in peripheral blood of patients with CC and controls.

Patients with OVC were characterized by similar changes in the percentages of CD8+ (high - 33.02±8.5 vs. 17.9±4.8 in donors, P<0.01), CD4+ (low - 0.6±0.04 vs 34.17±3.9, P=0.005) and CD25+ (very low - 0.34±0.06 vs. 60.8±4.1, P<0.001) cells, apart form the number of CD19+ B cells which remained within normal values (4.9±1.01 vs. 7.06±1.6, P=0.001) (Fig.4).

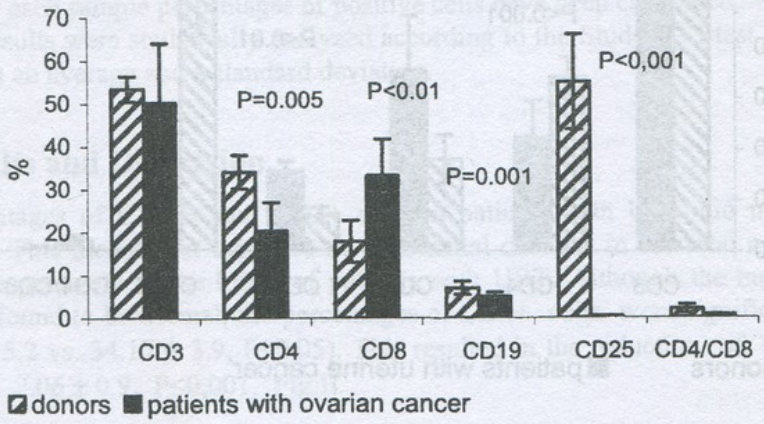

Fig.4. Percentages of various lymphocyte populations in peripheral blood of patients with OVC and controls.

Our study shows that the only quantitative changes in the main populations of T cells and B cells in blood of the patients with UBT are slight decrease in the percentages of CD4+T cells

and, as a result, of CD4/CD8 ratio. However, dramatic and quite shared changes were found in patients with various cancers (UBC, CC and OVC) of the reproductive system: sharp increase in the number of CD8+ cells and decrease of CD4+ cells leading to a very low CD4/CD8 ratio [Sheu et al.,1999; Markowska et al.,1995]. This was accompanied by an increase in the number of CD19+ B cells in the patients with UBC and CC, but not OVC. These changes would strongly suggest that CD8+ cytotoxic T cells are recruited through tumorgenesis, and that their circulation and, possibly, traffic to the site of the tumor is significantly increased. However, the rest of our current data indicates that other changes in the numbers of immunocytes may interfere with the efficiency of cytotoxic T cell responses.

First of all, the number of B cells seems to be sharply increased in patients with cancerous tumors of the uterus (UBC and CC), which suggests antibody response with the exclusion of OVC. The involvement of the specific antibodies to tumor-associated antigens (TAA) could play a negative role in their recognition and subsequent killing by cytotoxic CD8+ T cells by shielding these antigens from T cells receptor. It might be suggested, therefore, that antibody response to TAA may help tumor cells to survive CD8+ T cell–mediated cytotoxicity in UBC and CC.

Secondly, the number of activated CD25+ T cells in all three cancerous tumors (less in case of CC) has been dramatically decreased. This may indicate that CD8+ cytotoxic cells are unable to express CD25 (IL-2Rα) receptor which will render them unresponsive to IL-2, as it was shown before [Edward et al.,2004; Sheu et al.,1997]. T cells lacking expression of CD25 are functionally inhibited. There is a possibility that a detected down-regulation of the expression of CD25 is caused by the soluble factors produced by the tumor. Recently it was demonstrated, that OVC cells may suppress T cell proliferation through inhibition of IL-2 dependent signaling pathways, which may be a mechanism of immunosuppression induced by OVC [Wang et al.,2004]. Similar mechanisms might be employed by the tumors in UBC, CC and OVC.

In summary, our results suggest that gynecological cancer is associated with specific alterations in the T cell population and that up-regulation of the expression of IL-2α receptor may be important for potential therapeutic strategies.

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რეპროდუქციული სისტემების სიმსივნეებით დაავადებულ ქალთა სისხლის ლიმფოციტების სუბპოპულაციები

ახვლედიანი ლ.[1], ჭიკაძე ნ.[2], ჯანიკაშვილი ნ.[2], ქართველიშვილი მ.[2], ბალოიანი დ.[2], ფორაქიშვილი ნ.[2]

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რეზიუმე

დადგენილია, რომ საშვილოსნოს ტანის კეთილთვისებიანი სიმსივნით დაავადებულთა ლიფოციტების სუბპოპულაციებში საკონტროლო ჯგუფთან შედარებით არ გვხვდება მნიშვნელოვანი ცვლილებები. საშვილოსნოს ტანის, ყელის და საკვერცხეების კიბოს შემთხვევაში ცვლილებები თითქმის ერთნაირია. T ლიმფოციტების (CD3+) საერთო რაოდენობა უცვლელია კონტროლთან შედარებით, მომატებულია ლიმფოციტების (CD19+) და T ციტოტოქსიურ/სუპრე–სორულ (CD8+) უჯრედთა პროცენტული რაოდენობა, ხოლო CD4+ T უჯრედთა დონე მცირედ დაქვეითებულია. ამის შედეგია CD4/CD8 შემცირებაც, რომელიც გადახრილია იმუნოსუპრესიის მიმართულებით. CD25+ T ლიმფოციტების შემცველობა გინეკოლოგიური კიბოთი დაავადებულთა პერიფერიული სისხლის უჯრედებში უმნიშვნელოა, ვიდრე საშვილოსნოს ტანის კეთილთვისებიანი სიმსივნის დროს. B ლიმფოციტების მატების შედეგად შესაძლებელია მოიმატოს სპეციფიკური ანტისხეულების რაოდენობამაც, რომლებიც ბლოკავენ რა სიმსივნურ ანტიგენებს, იცავენ სიმსივნეს განადგურებისაგან. ციტოტოქსიური T უჯრედების მატება მიუთითებს, რომ ისინი ჩართული არიან ანტისიმსივნურ პროცესში, მაგრამ CD25 (IL-2Rα) დაქვეითებული ექსპრესიის გამო, არ შესწევს უნარი დაიკავშიროს IL-2. აღნიშნული სახის სიმსივნეების იმუნოთერაპიისათვის მნიშვნელოვანი იქნებოდა სწორედ T უჯრედების ზედაპირზე ამ რეცეპტორის ექსპრესიის გაზრდა.

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ANTI GAD65 ANTIBODIES AND GLUCOSE TOLERANCE TEST IN THE DIAGNOSIS OF DIABETES MELLITUS

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Abstract

The aim of the present study was to evaluate the importance of the phases of insulin secretion, measured during an intravenous glucose tolerance test (IVGTT) and anti GAD 65 antibodies as a beta cell autoimmune marker in the proper early diagnosis of the type of diabetes. 42 newly diagnosed diabetic patients were enrolled in the study, 19 subjects with normal glucose tolerance served as a control group. Using the data from IVGTT and anti GAD 65 antibodies, the diabetic patients were divided in to 2 groups – type 1 diabetic patients (n=19), showing loss of first (FPIS) and second (SPIS) phases of insulin secretion and type 2 diabetic patients (n=23), who demonstrated declined FPIS and relatively preserved, but retarded SPIS, none of them being anti GAD 65 positive. Our results demonstrate that IVGTT allows precise assessment of the phases of insulin secretion and helps to set proper early diagnosis of the type of diabetes. The presence of anti GAD 65 antibodies alone can set the diagnosis type 1 diabetes, while anti GAD 65 negativity requires the performance of IVGTT, which can then distinguish between anti GAD 65 negative type 1 diabetic patients and type 2 diabetic patients. The combinations of IVGTT and anti GAD 65 antibodies appear to be of clinical importance in distinguish between type 1 and type 2 diabetes mellitus in newly diagnosed patients.

Key words: IVGTT, insulin secretion, anti GAD 65 antibodies, type 1 and type 2 diabetes mellitus.

Introduction

Diabetes Mellitus is a markedly heterogeneous disease. The distinct disorders under the term "diabetes mellitus" differ in pathogenesis, natural history, responses to therapy and preventive measures. Each type of diabetes mellitus has characteristics that distinguish it from other. The assignment of a patient to a particular type of diabetes is important, as it goes hand in hand with determining the most appropriate therapy.

Type 1 diabetes, also termed insulin-depended diabetes mellitus (IDDM), is generally caused by insulinopenia, due to a diminution of insulin secretory capacity associated with loss of pancreatic beta cells. [Lernmark, Hagglof 1981; Leslie, Atkinson 1999]. Type 2 diabetes, or non-insulin-depended diabetes mellitus (NIDDM), is a result of concomitant defects in both insulin

secretion and insulin action. The pancreatic beta cell presents functional abnormalities in its very early stages of development [DeFronzo, Bonadonna 1992; Porte, Banting 1991].

Type 1 diabetes is the most prevalent type among children and young adults and it was formerly termed as "juvenile diabetes". However classification based on age at onset has been discontinued because clinical onset can occur at any age. Onset of IDDM in adult subjects is not uncommon [Lernmark, Hagglof 1981; Leslie, Atkinson 1999; Porte, Banting 1991; Zimmet, Tuomi 1994]. There are sometimes individuals, who present clinically as having NIDDM, but within a year or so they become insulin-depended.

The different patterns of insulin secretion and presence of certain markers of beta-cell autoimmune disorder have been proposed as tools in distinguishing between type 1 and type 2 diabetes mellitus [Zimmet, Tuomi 1994; Turner, Stratton 1997; Welborn, Garsia-Webb 1981; Zimmet, Turner 1999]. It is well established, that insulin is secreted in a biphasic pattern with an early burst of insulin release within first 10 minutes followed by a gradually increasing second phase of insulin secretion [Grodsky 1997]. This biphasic response is readily demonstrable after intravenous glucose tolerance test [DeFronzo, Bonadonna 1992]. IDDM present the usual pattern of beta-cell function shows lack of first and second phase insulin response to glucose. Basal insulin secretion in patients with NIDDM has been reported as elevated, reduced or normal. The loss of first phase insulin secretion is always present and it occurs at the very early stage of the disease [DeFronzo, Banadonna 1992; Porte, Banting 1991]. It has been suggested that loss of the first phase of insulin secretion is the earliest detectable abnormality in patients, who are destined to develop NIDDM [DeFronzo, Banadonna 1992; Porte, Banting 1991].

The aim of present study was to evaluate the impact of the phases of insulin secretion measured during an intravenous glucose tolerance test (IVGTT) and the presence of anti GAD65 antibodies as a beta-cell autoimmune marker in the proper early diagnosis of type of diabetes mellitus in normal weight newly diagnosed patients.

Materials and Methods

Forty-two newly diagnosed diabetic patients with normal body weight were referred to Tbilisi Central I Hospital, Department of Endocrinology for diagnostic and therapeutic reasons. Nineteen subjects with normal glucose tolerance test (NGT), according to WHO criteria during 75g oral glucose tolerance test (OGTT) [Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997], served as a control group. The diabetic patients had not received any antidiabetic therapy before the study and none of the studied subjects was taking any drugs that could affect glucose tolerance.

An IVGTT was performed in all subjects. We used 0.5 kg body weight glucose with no upper limit of dose [Gianani, Pugliese 1992]. Time zero was taken as the end of the infusion [Bingley, Colman 1992]. Blood samples for glucose and insulin levels were taken at 0, 1, 3, 10, 60 minutes. Blood glucose was measured by glucose analyzer (Beckman) and plasma insulin concentration by MEIA, using a commercial kit (Abbott, USA) (normal range; 2-2,5mU/L). First phase insulin secretion (FPIS) was defined as the sum of first and third minute insulin levels [Gianani, Pugliese 1992] and the second phase insulin secretion (SPIS) by the values at 30 and 60 minutes. Stimulated insulin secretion (SIS) was defined as first min + third min insulin –2x basal insulin [Bleich, Jackson 1990]. The area under the curve (AUC) was calculated for FPIS, SPIS and total insulin secretion, as well as for plasma glucose concentration during IVGTT, using the trapezoidal method [Tallaride, Murray 1981].

Anti GAD65 antibodies we measured using a commercial kit (BRAHMS, Germany), at the cut-off value being 0,9 u/mL to distinguish between antibody positivity and negativity.

Statistical analysis was performed by repeated measures analysis of variance (ANOVA). The results are presented as mean ±SEM.

Results and discussion

Plasma insulin concentrations of the studied groups of subjects during INGTT are presented in Figure 1. According to the values of the FPIS and SPIS, the diabetic patients were divided into 2 groups. I group consisted of 19 patients, who showed very low first and second phases of insulin secretion ($p<0.001$for both compared with the control group) and they were classified as having type 1 diabetes (table 1). The diabetic patients (n=23), who demonstrated declined FPIS: $p<0.001$ compared with the healthy controls and relatively preserved but retarded SPIS ($p>0.1$compared with the healthy controls) were classified as having type 2 diabetes mellitus. There is a significant difference in the FPIS between the healthy controls and all diabetic patients, the difference between the two types of diabetes also being significant ($p<0.01$, when comparing type 1 and type 2 diabetes patients). As far as SPIS is concerned, there is a significant difference between the healthy controls and type 1diabetic patients ($p<0.001$), while there is no difference between subjects with NGT and type 2 diabetic patients ($p>0.1$).

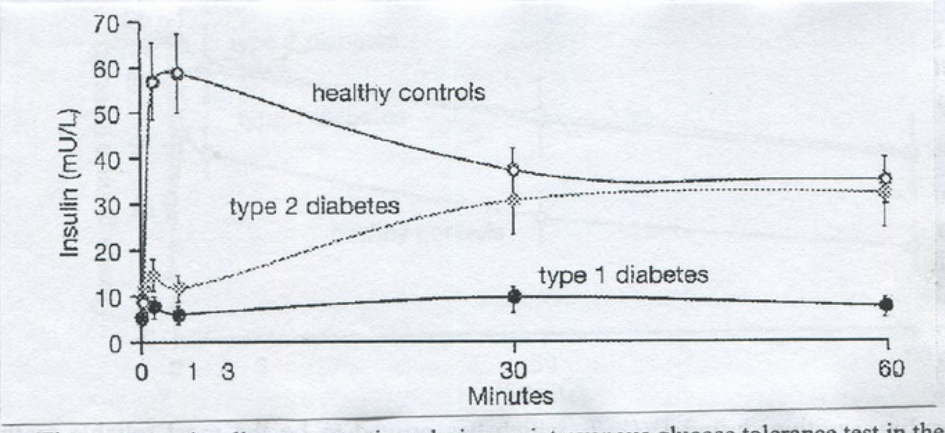

Fig. 1. Serum insulin concentrations during an intravenous glucose tolerance test in the different groups

Using trapezoidal method we have calculated the area under the curve (AUC) for FPIS, SPIS and total insulin secretion and the data are presented in Table 1. It can be clearly seen, that as far as AUC for total insulin secretion is concerned type 1 diabetic patients are markedly insulin deficient as compared with both the healthy controls ($p<0.001$) and type 2 ($p<0.001$) diabetic patients. There is a relative insulin deficiency in type 2 diabetic patients as well ($p<0.01$ compared with the control group), may be due to the low FPIS and the retarded SPIS. There is a significant difference in stimulated insulin secretion (SIS) between type 1 diabetic patients and the healthy controls ($p<0.001$) as well as between type 2 diabetic patients and the subjects with NGT ($p<0.001$). Plasma glucose concentrations during IVGTT in the tree groups (NGT, type 1 and type 2 diabetic patients) are presented in Figure 2. There is a significant difference in the AUC for plasma glucose between the healthy controls and both type 1 (999 ±196 versus 574.1 ± 94.7mmol/L x 60min, $p<0.001$) and type 2 diabetic patients (1010.2 ± 203.7 versus 574.1 ± 94.7 mmol/L x 60min, $p<0.001$), the difference between the two groups of diabetics being not

significant. Therefore we consider that the change in plasma glucose level during IVGTT cannot be used to assign a patient to either type of diabetes.

Table 1. Insulin secretion (FPIS, SPIS, SIS and AUC for FPIS, SPIS and total secretion) during an intravenous glucose tolerance test in patients with type 1 and type 2 diabetes and in the control group with normal glucose tolerance. Values are mean±SEM

Parameter	Control group (n=19)	Type 1 diabetic patients (n=19)	Type 2 diabetic patients (n=23)
FPIS (mU/L)	134.4±43.7	12.9±5.1**	25.9±11.5**#
SPIS (mUlL)	64.4±23.6	15.4±6.4**	61.1±17.7##
SIS (mU/L)	116.2±50.2	2.3±2.0**	2.9±2.7**
AUC for FPIS (mU/L x 60mm)	147 ± 28.7	19.7 ± 7.8**	38.7± 34**#
AUC for SPIS (mUlL x 60mm)	2176±603	420.9±165**	1481±384*#
AUC for total insulin secretion (mU/L x 60mm)	2323±804	440.6±1 71**	1520±598*##

FPIS, first-phase insulin secretion; SPIS, second-phase insulin secretion; 515, stimulated insulin secretion; AUC, area under the curve; *<0Q1 compared with the healthy controls; p<0.001 compared with the healthy controls; #p<0.01 compared with type 1 diabetic patients; ##p<0 001 compared with type 1 diabetic patients

Antibodies to GAD65 were present in one out of 19 subjects with NGT (5,2%) and in 14 out of 19 patients with type 1 diabetes (73,7%), while none of type 2 diabetic patients had anti GAD65 antibodies.

Diabetes mellitus is a heterogenous disease and sometimes it is rather difficult to distinguish between its different types, mainly the most common ones – type 1 and type 2. The define diagnosis of the type of diabetes is often complicated. Bearing in mind the differences in the patterns of insulin secretion in the two main types of diabetes, we measured the first and second phases of insulin secretion during IVGTT, which has proved to be the most reliable method to assess the biphasic pattern of insulin secretion [DeFronzo, Bonadonna 1992; Grodsky 1997].

Those patients, who lacked both phases, were considered insulin depended, while those with reduced FPIS but relatively preserved SPIS were classified as having non-insulin depended, or type 2diabtes mellitus. This division of the patients was confirmed by the presence of anti GAD65 antibodies. It was found, that 73.7% of type 1 diabetic patients were anti GAD65 positive, which is similar to the observations of other authors [Lernmark, Hagglof 1981], while non of type 2 diabetic patients was anti GAD65 positive.

In fact, IVGTT helped to distinguish between the two types of diabetes mellitus. The pattern of insulin secretion – the two phases- appeared to be quite indicative of the different types of diabetes, i.e. insulin-depended and non-insulin–depended ones. As far as measurement of anti GAD65 antibodies is concerned, it can be assumed, that this parameter alone cannot distinguish between two types of diabetes. If one starts with this measurement first, it can be of great help in those newly diagnosed diabetic patients who appear anti GAD65 positive, as they are certainly type 1 diabetic patients. As far as the antiGAD65 negative patients are concerned they might be type 2 diabetic patients appear also to be anti GAD 65 negative (26.3%).

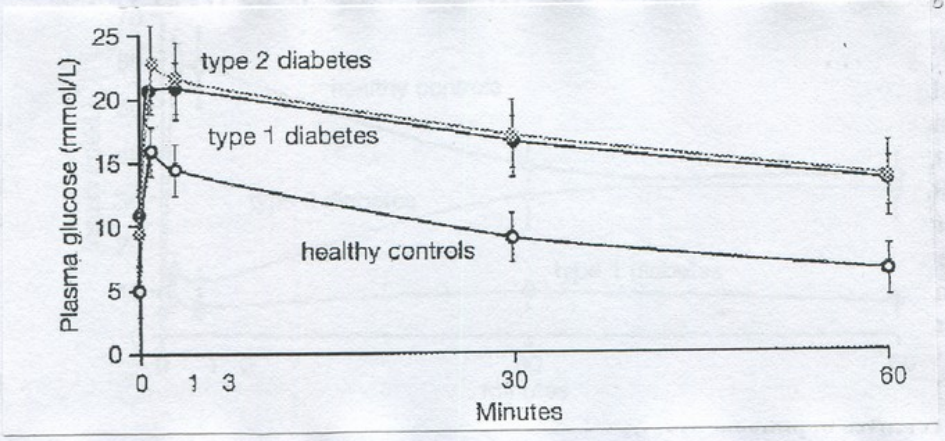

Fig. 2. Plasma glucose levels during an intravenous glucose tolerance test in the different groups

Therefore, bearing in mind the good accordance between the two tests, IVGTT and anti GAD65 antibodies, we can propose two diagnostic approaches in distinguishing between the two types of diabetes mellitus in newly diagnosed patients. IVGTT can distinguish between type 1 and type 2 diabetes mellitus in the majority of cases on the basis of the different pattern in insulin secretion in the two types.

Another approach is to start with measuring anti GAD 65 antibodies. It is clear, that anti GAD 65 antibodies are present in only type 1diabetic patients, therefore anti GAD 65 positive patients can be assigned to type 1 diabetes mellitus and they don't require the performance of IVGTT. However, in anti GAD65 negative patients it is worth performing IVGTT and then assigning them to either type 1 or type 2 diabetes mellitus.

Conclusion:

- The patients who showed loss of both FPIS and SPIS during IVGTT were considered type 1 diabetic patients.
- The patients, who demonstrated declined FPIS and relatively preserved but retarded SPIS during IVGTT were classified as having type 2 diabetes.
- IVGTT allows precise assessment of the phases of insulin secretion and helps to set proper early diagnosis of the type of diabetes in newly diagnosed patients.
- The measurement of anti GAD 65 antibodies in such patients just proves the already set diagnosis of the type of diabetes. The presence of anti GAD65 antibodies alone can set the diagnosis type 1 diabetes, while anti GAD65 negativity requires the performance of IVGTT, which can be distinguish between anti GAD 65 negative type 1 diabetic patients and type 2 diabetic patients.
- These two alternative approaches – starting with either IVGTT or anti GAD 65 antibodies – appear to be of clinical importance in distinguishing between type 1 and type 2 diabetes mellitus and in prescribing adequate treatment in newly diagnosed patients.

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ანტი GAD 65 ანტისხეულები და გლუკოზის მიმართ ტოლერანტულობის ტესტი, როგორც შაქრიანი დიაბეტის სადიაგნოსტიკო მაჩვენებლები

გაჩეჩილაძე ნ.[1], ბურჯანაძე ლ.[1], ამაღლობელი ნ.[1], მიცკევიჩი ნ.[1], შელეგია ქ.[1], ლომიშვილი ნ.[1], ჩიქობავა მ.[2], ბობოხიძე ც.[2]

[1]ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტის იმუნოლოგიის კათერდა,

[2]ქ. თბილისის ცენტრალური საავადმყოფოს ენდოკრინოლოგიური განყოფილება.

(მიღებულია 7.03.2005)

რეზიუმე

შესწავლილია ინსულინის სეკრეციის ფაზებისა და ანტი GAD 65 ანტისხეულების როლი შაქრიანი დიაბეტის სწორ დიაგნოსტირებაში. ანტი GAD 65 ანტისხეულები გამოყენებული იყო, როგორც β უჯრედების აუტოიმუნური მარკერი, ხოლო ინსულინის სეკრეციის შესასწავლად გამოყენებული იქნა გლუკოზის მიმართ ტოლერანტულობის ტესტი (გტტ). შესწავლილ იქნა 42 შაქრიანი დიაბეტით დაავადებული პირი, რომლების გაიყო ორ ჯგუფად: I ტიპის დიაბეტის მქონე პირები, რომლებიც ავლენდნენ ინსულინის სეკრეციის როგორც პირველი, ასევე მეორე ფაზის სრულ დათრგუნვას და II ტიპის დიაბეტით დაავდებული პირები, რომლებსაც დათრგუნული ქონდათ ინსულინის სეკრეციის პირველი და შენელებული - ინსულინის სეკრეციის მეორე ფაზა. პირველი ჯგუფის პაციენტების უმრავლესობა იყო ანტი GAD 65 დადებითი, მაშინ, როდესაც არც ერთ პაციენტს მეორე ჯგუფიდან არ აღმოაჩნდა ანტი GAD 65 ანტისხეულები. დადგენილი იქნა, რომ გტტ შეიძლება გამოყენებული იქნას ადრეულ ეტაპზე დიაბეტის ტიპის გამოსავლენად. ანტი GAD 65 ანტისხეულები აბსოლუტურად ადასტურებს I ტიპის დიაბეტის (ინსულინდამოკიდებული შაქრიანი დიაბეტის - იდშდ) არსებობას, მაშინ, როდესაც ანტი GAD 65 ანტისხეულების არმქონე იდშდ და II ტიპის დიაბეტის განსამიჯნავად და ზუსტი დიაგნოზისათვის საჭირო ხდება გტტ ტესტის ჩატარება.

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Microbiology

MICROSCOPIC FUNGI STRAINS – THE PRODUCERS OF PROTEOLITIC ENZYMES

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Abstract

To reveal acid and alkali proof active producers of proteinases screening of microscopic fungi: Mucor plumbeus, Mucor-T, Aspergillus flaus, A. versicolor, A. terreus, A. niger, A. awamori 44, Penicillium has been done. Optimal conditions for the cultivation of studied strains – the duration of cultivation, growth temperature, initial pH of the nutrient medium, its composition and aeration - have been established. The best sources of carbon, nitrogen, sulfur and phosphorus were revealed. The strains were tested on toxicity and pathogenicity. Studied strains were not toxic and pathogenic.

Key words: microscopic fungi, proteolitic activity, alkalic and acid proteinases.

Introduction

Particular representatives of proteolitic enzymes are widely used in different fields of medicine and industry. They are used in cheese producing, in leather industry, for softening and cleaning the leather, in stomatology and other fields of medicine – skin burn, surgery. For a long period only the proteinases of the plant origin were known, but later it was found out that some microscopic fungi also possess the ability to produce the proteolitic enzymes [Beshay, 2003; Lowe, 2002]. According to high demand for proteolitic enzymes, the purpose of our study was to select producers of the acid and alkali proteinases among the microscopic fungi and determine the optimal conditions for their cultivation.

Materials and Methods

For experimental investigations were used strains of: Mucor plumbeus, Mucor-T, Aapergillus flaus, A. versicolor, A. terreus, A. niger, A. awamori 44, Penicillium, obtained from different soil and climatic conditions of Georgia.

Microorganisms were grown by means of submerged cultivation on different nutrient media in 750ml conic flasks at 37°C, rotation-180rot/min, during 72h. Both alkalic and acid proteinases activities were measured by means of Anson's modified method [Shakhov, 1978]. The amount of enzyme forming 1 microequivalent of nonsedimentogene tyrosine in trichloracetic acid during 1h was considered as an unit of activity. The amount of released tyrosine was determined spectrophotometrically, following the calibration curve for tyrosine. To select the active producer of proteinase among the microscopical fungi obtained from different soil-climatic zones of

Georgia, the screening has been done. About 120 strains of micro-fungi were tested. Cultivation was done on Chapek's modified medium at 37°C, 180 rot/min.

Results and Discussion

Studying of the enzymatic activity has revealed that 9 strains possessed the proteolitic activity (Table1). From the Table1 it is clear that the strain Mucor plumbeus revealed the highest acid proteinase activity, while the strain M. 44 possessed alkalic proteinase activity. These two strains were used in further investigations.

It is known that enzyme's activity is highly influenced by the cultivating conditions. To establish the favorable growth conditions for the optimal sources of main nutritional elements, like carbon, nitrogen, phosphorus and sulfur were selected [Alekseeva, 1986].

Table 1. Proteolitic activity of the micromycetes, obtained from Georgian soils

Species	Activity U/ml	
	Acid	Alkalic
Rhizopus	0.6	0.8
Mucor K-44	1.2	0.9
Aspergillus tamar	1.1	0.7
A. awamorii	1.4	1.2
Trichoderma	0.7	1.0
Sporotrichum	0.7	0.9
Mucor plumbeus	1.8	1.1
Mucor t-44	1.0	2.0
A. niger	1.6	1.1

To study the dynamics of proteinases accumulation the strains were grown on Chapek's modified medium for 7 days. Enzyme's activity was determined every 24h. The maximal activity of proteinases was recorded on the 3-rd day of cultivation, whereupon decreasing of the activity was mentioned (Fig.1). These results made us to investigate the proteolitic activity on the third day of cultivation in further experiments.

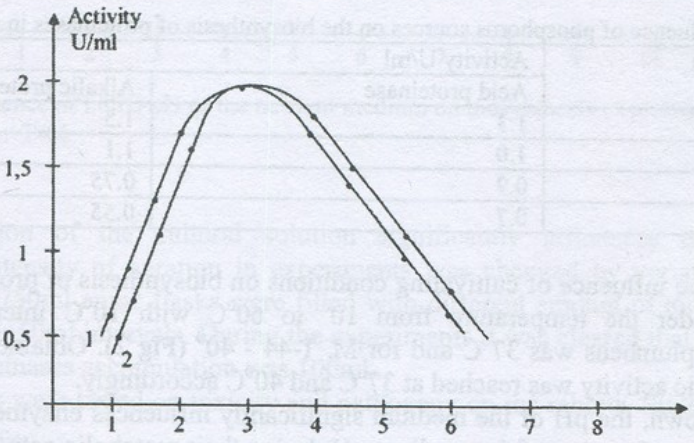

Fig.1. Dynamics of proteinase accumulation of 1)Mucor plunbeus and 2)Mucor T-44

To study the influence of carbon sources on proteinase biosynthesis micromycetes were grown on media containing sucrose, glucose, fructose, maltose, yeast extract, rye flour and starch

as sources of carbohydrates. From the Table 2 it is clear that for biosynthesis of the alkalic proteinases the most effective was yeast extract, while for acid proteinases the rye flour did.

Table 2. Influence of carbon sources on proteolitic activity of microscopic fungi

Sources of carbon	Activity U/ml	
	Acid	Alkalic
Sucrose	0	0
Glucose	0	0
Fructose	0	0
Maltose	0	0
Yeast extract	1.0	1.5
Rye flour	1.7	0.4
Starch	0.8	0.5

Peptone, yeast extract and casein were tested to select the optimal source of nitrogen. It was revealed that the most effective was KNO_3 (Table 3). Adding of this substance to the medium increased the enzyme's activity, while testing the phosphorus sources the best results were obtained using KH_2PO_4 (Table 4).

Table 3. Influence of nitrogen sources on the biosynthesis of proteinases in microscopic fungi

Sources of nitrogen	Activity U/ml	
	Acid proteinase	Alkalic proteinase
peptone 0,5%	0.3	0.2
peptone 1,0%	0.5	0.25
peptone 1,5%	0.54	0.3
KNO_3	0.9	1.2
$NaNO_3$	1.0	1.1
$(NH_4)_2HSO_4$	0.7	0.9
$(NH_4)H_2PO_4$	0.8	0.7
Yeast Extract	1.0	1.5
peptone 1,5% + $(NH_4)_2SO_4$	0.6	0.5

Table 4. Influence of phosphorus sources on the biosynthesis of proteinases in microscopic fungi

Sources of phosphorus	Activity U/ml	
	Acid proteinase	Alkalic proteinase
KH_2PO_4	1.3	1.5
K_2HPO4	1.0	1.1
NaH_2PO_4	0.9	0.75
$(NH_4)_2HPO_4$	0.7	0.55

Studying the influence of cultivating conditions on biosynthesis of proteinases the strains were cultivated under the temperature from 10° to 60°C with 10°C intervals. The optimal temperature for M. plumbeus was 37°C and for M. T-44 - 40^0 (Fig 2). Obtained results show that the highest proteolitic activity was reached at 37°C and 40^0C accordingly.

As it is known, the pH of the medium significantly influences enzymes biosynthesis. As microorganisms cause changing of the medium pH during their metabolic activity, maintaining the pH of the area on the same level during the whole experiment was impossible.

To establish the optimal pH of the given nutrient medium, pH of the medium was changed from 2.0 to 8.0, with 0.5 intervals. The optimal pH of the medium for acid proteinases was 3.0,

while for alkalic proteinases it reached 8.0. Changing of the pH negatively affected the accumulation of the enzyme (Fig.3.).

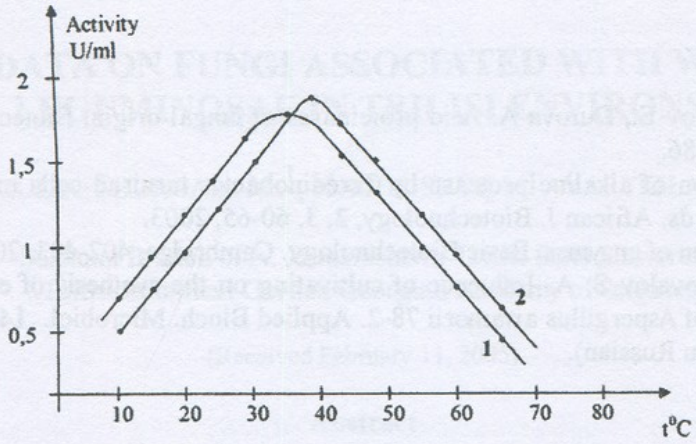

Fig. 2. Influence of temperature on biosynthesis of proteinases in 1)Mucor plunbeus and 2)Mucor T-44

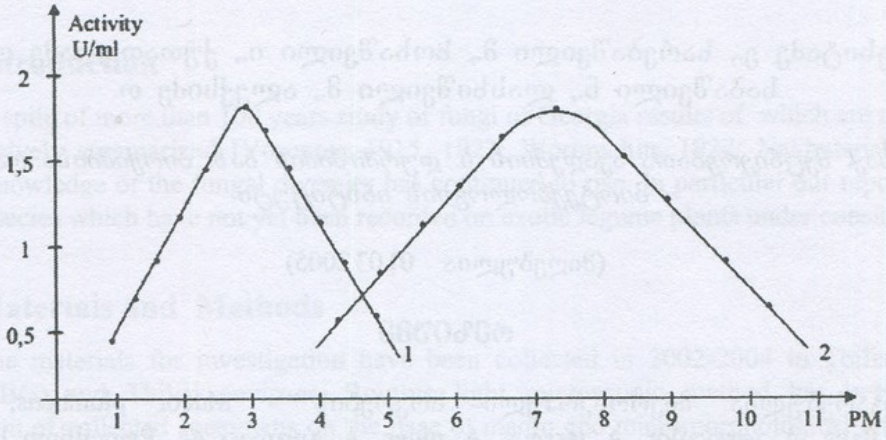

Fig. 3. Influence of initial pH of the nutrient medium on biosynthesis of proteinases in 1)Mucor plunbeus and 2) Mucor T-44

The aeration of the cultural solution significantly influences the biosynthesis of proteinases. The intensity of aeration in experiments was changed by variation of the nutrient solution's volume. 750ml conic flasks were filled with different amount of nutrient medium from 50ml to 300ml, with 50ml intervals. During the experiments it was cleared that the optimal volume of medium for proteinases accumulation was 100ml.

The strains were tasted on toxicity and pathogeny on the rabbits. Studied strains were not toxic and pathogenic.

Summarizing the experimental data, it may be said that the optimal conditions of submerged cultivation for proteinases established in our experiments are following: 1. For acid proteinases – rye flour of 10g/l, duration of cultivation 72h, KH_2PO_4-5g/l, $MgSO_4$-5g/l, $FeSO_4$-0.02g/l, KNO_3-1g/l, initial pH-3.0, and optimal growth temperature - 37°C. 2. For alkalic

proteinases – yeast extract-5g/l, duration 72h, KNO_3-1g/l, KH_2PO_4-1g/l, $FeSO_4$-0.02g/l, $FeSO_4$-0.07g/l, initial pH-8.0, optimal growth temperature - 40°C.

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მიკროსკოპული სოკოები – პროტეოლიზური ფერმენტების პროდუცენტები

კვესიტაძე ე., ხარებაშვილი მ., ხოხაშვილი ი., ქუთათელაძე ლ., საბაშვილი ნ., ლასხიშვილი მ., ალექსიძე თ.

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

(მიღებულია 01.03.2005)

რეზიუმე

ჩატარებულია მიკროსკოპული სოკოების - Mucor plumbeus, Mucor-T, Aspergillus flaus, A. versicolor, A. terreus, A. niger, A. awamori 44, Penicillium სკრინინგი მჟავა და ტუტე-გამძლე პროტეინაზების აქტიური პროდუცენტების გამოსავლენად. დადგენილია აღნიშნული შტამების კულტივირების ოპტიმალური პირობები: კულტივირების ხანგრძლივობა, ზრდის ოპტიმალური ტემპერატურა, კულტურალური სითხის საწყისი pH და ოპტიმალური აერაცია, საკვები არის ოპტიმალური შემადგენლობა. გამოვლენილია ნახშირბადის, აზოტის, გოგირდის და ფოსფორის საუკეთესო წყაროები. შტამები შემოწმებულია ტოქსიკურობაზე და პათოგენურობაზე. აღნიშნული შტამები არატოქსიკური და არაპათოგენურია.

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Phytopathology

NEW DATA ON FUNGI ASSOCIATED WITH WOODY LEGUMINOSAE IN TBILISI ENVIRONS

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Abstract

The paper deals with 34 species of fungi from Georgia first recorded on Albizia, Caesalpinia, Cercis, Gleditsia, Robinia, Sophora

Key words: Georgia, fungi, woody legumes.

Introduction

In spite of more than 100 years study of fungi of Georgia results of which are more or less comprehensively summarized [Voronov, 1915, 1923; Woronichin, 1927; Nakhutsrishvili, 1986; etc.], our knowledge of the fungal diversity has continued to rise. In particular our report concerns the fungi species which have not yet been recorded on exotic legume plants under consideration.

Materials and Methods

The materials for investigation have been collected in 2002-2004 in Tbilisi Botanical Garden (TBG) and Tbilisi environs. Routine light microscopic method has been used for identification of collected specimens on the base of macro and micromorphological features using classic and modern guide books for identification of fungi [Saccardo, 1882-1931; Grove, 1935, 1937; Sutton, 1980; Ellis,Ellis, 1985; Sivanesan, 1985; Melnik, 1992, etc.].

Results and Discussion

This contribution to the mycobiotic diversity of Georgia includs list of legume species and their fungal inhabitants with location, and data of collection. It must be noted that Gleditsia sinensis is not listed among the host plants of fungi of Georgia till now.

Albizia julibrissin

Cladosporium tenuissimum Cooke, on dead fruits in association with Cladosporium sp. and Phoma leguminum Westend. Tbilisi,Saburtalo, 25. 01. 2003.

Microsphaeropsis olivacea (Bonord.)Höhn.,on dead branches. Tbilisi, Didube, 23. 09. 2004.

Caesalpinia gilliesii

Botryosphaeria dothidea (Moug.) Ces. & De Not., on dead branches. TBG, 05. 12. 2003.
Camarosporium aequivocum Sacc., on dead branches in association with Dothiorella berengeriana Sacc. and Diplodia cavanillesiana Frag. TBG, 05. 12 .2003 .
Colletotrichum sp., on dead branches. TBG, 29 .01. 2004.
Dothiorella berengeriana Sacc., on dead branches. TBG, 05. 12. 2003.
Microsphaeropsis olivacea (Bonord.) Höhn., on dead branches in association with Alternaria alternata (Fr.) Keissler and Fusarium lateritium Nees. TBG, 29.01.2004.
Shizophyllum commune Fr., on dead branches. TBG, 03.12.2003; 18.07.2003.
Trimmatostroma salicis Corda, on dead branches. TBG, 07.05.2004.

Cercis siliquastrum

Botryosphaeria dothidea (Moug.) Ces. & De Not.. Tbilisi, Saburtalo, University street, 22.06.2004.
Camarosporium siliquastri P. Henn., on dead branches in association with Diplodia siliquastri Westend. TBG, 01.12. 2003.
Dinemasporium decipiens Sacc., on dead branches. Tbilisi, Saburtalo, University street, 28.02.2004.
Hendersonia sarmentorum Westend., on branches in association with Microdiplodia cercidis Died. Tbilisi, Saburtalo, University street, 28.02.2004.
Phellinus sp., on dead stems. TBG, 23.10.2000.
Seimatosporium caudatum (Preuss.) Shoemaker, on dead branches. TBG, 05.01.2004, 29.01.2004.
Trichothecium roseum Link, on dead fruits and leaves. TBG, 13.10.2003.

Gleditsia sinensis

Camarosporium spartii Trail, on dead branches in association with Dothiorella berengeriana Sacc. TBG, 05.01.2004.
Cladosporium herbarum (Pers.) Link, on thorns and fruits. TBG,24.12.2003; 05.01.2004.
Cyclothyrium juglandis(Schum. ex Rabenh.) B. Sutton, on dead branches in association with Camarosporium sp. and Dothiorella berengeriana Sacc.TBG, 05.01.2004.
Eutypella stellulata (Fr.) Sacc., on dead branches. TBG, 03.11.2003.
Hendersonia gleditsiae Kickx, on dead branches. TBG, 05.01.2004.
Kaskaskia gleditsiae Born & Crane, on dead branches. Tbilisi, Digomi massif, 21.05.2004.
Leptosphaeria sp.(asci 60-73x8μm; ascospores 15-22x4-5μm), on dead branches. TBG, 07.11.2003.
Microdiplodia gleditsiae Died., on dead branches. TBG, 24.12.2003; 29.01.2004.
Pseudovalsa profusa (Fr.) Wint., on dead branches. TBG,03.11.2003.

Robinia pseudoacacia

Alternaria alternata (Fr.) Keissler, on dead branches in association with Camarosporium pseudoacaciae Brun. Tbilisi, Saburtalo, 15.02.2004.
Camarosporium laburni (Westend.) Sacc., on dead branches. Tbilisi, Saburtalo, University street, 13.08.2004.
Chaetomella atra Fuckel,on dead branches in association with Alternaria alternata (Fr.) Keissler and Fusarium lateritium Nees. Tbilisi, Saburtalo, 13.08.2004.
Chaethomella sp., on dead branches. Tbilisi, Saburtalo, 13.08.2004.
Cyclothyrium juglandis (Schum. ex Rabenh.) B. Sutton, on dead branches. Tbilisi, Saburtalo, University street, 28.02.2003.

Cytospora leucosperma (Pers.) Fr., on dead branches. Tbilisi, Saburtalo, University street, 17.10.2002; 07.08.2004, Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 13.06.2004.

Cytospora leucostoma Fr., on dead branches. Tbilisi, Saburtalo, University street, 22.06.2004.

Cucurbitaria laburni (Pers.:Fr.) De Not., on branches in association with Camarosporium laburni (Westend.) Sacc. Tbilisi, Saburtalo, University street, 25.01.2003.

Dothiorella sp., on fire injured branches. Mtskheta distr., left bank of the river Ksani near village Dzveli Kanda, 13.06.2004.

Microdiplodia microsporella (Sacc.) Allesh., on dead branches in association with Alternaria alternata (Fr.) Keissler and Fusarium lateritium Nees. Tbilisi, Saburtalo, University street, 13.08.2004.

Microsphaeropsis olivacea (Bonord.) Höhn., on dead branches. Tbilisi, Saburtalo, University street, 25.12.2002.

Sophora japonica

Cladosporium herbarum (Pers.) Link,on dead branches in assotiation with Diplodia sophorae Speg. & Sacc. and Macrophoma sophorae Kantschaveli. Tbilisi, lake Kus Tba, 02.07.2003.

Dothiorella berengeriana Sacc., on dead branches in assotiation with Diplodia sophorae Speg. & Sacc. TBG,26.01.2004.

Hendersonia sophorae (Peil) Sacc. & Traverso, on dead branches in association with Botryosphaeria dothidea (Moug.) Ces. & De Not. TBG, 16.02.2004.

Microdiplodia gleditsiae Died., on dead fruits. TBG, 11.02.2003.

Schizophyllum commune Fr., on branches. TBG, 26.01.2004.

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ახალი მონაცემები თბილისის შემოგარენში პარკოსან მერქნიან მცენარეებთან ასოცირებული სოკოების შესახებ

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(მიღებულია 11.02.2005)

რეზიუმე

წარმოდგენილია მოკლე ცნობები იმ სოკოების შესახებ, რომლებიც ავტორების მიერ საქართველოში პირველად არის გამოვლენილი თბილისის ბოტანიკურ ბაღსა და თბილისის შემოგარენში გავრცელებულ ხეებისა და ბუჩქების ამა თუ იმ სახეობაზე. მათ შორის Gleditsia sinensis აქამდე საერთოდ არ ფიგურირებდა საქართველოს სოკოების მკვებავ მცენარეთა სიაში.

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NEW DATA ON MICROFUNGI ASSOCIATED WITH TREES AND SHRUBS OF TBILISI ENVIRONS

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Abstract

The papers deals with parts of new records of fungi and new fungus-host combinations for Georgia. The genus Cryptosphaerella (with C.parca) together with Cucurbitaria amorphae, Didymella physocarpi, Gnomoniella rubicola, Lopadostoma turgidum, Lopadostoma sp., Melanospora sp., Pleospora sp. (Ascomycota), Phomopsis cuginiana, Septoria laburni, S.myrobalanae, Phoma sp., Phomatospora sp., Phomopsis sp. (Deuteromycota) are recorded for the first time in Georgia. The new fungus-host combinations for Georgia are: Camarosporium cytisi - Laburnum anagyroides; Cryptodiaporthe salicina-Salix alba; Cytospora rubescens - Rubus sp.; Diplodia cerasorum - Cerasus incana; Exosporium tiliae - Paliurus spina-christi.

Key words: Georgia, Tbilisi environs, microfungi, Ascomycota, Deuteromycota, trees, shrubs.

Introduction

Among the biotic factors influencing vitality of plants, fungi play more or less significant role. In particular, it is evident the significance of the researches aimed at determination of the species composition (diversity) of the parasitic and non-parasitic fungal inhabitants of bark, leaves, fruits and other parts of trees and shrubs growing on the territory under consideration. It must be noted that mycological studies in this area with expressed purpose have not been carried out before. Only incomplete data are available [Gvritishvili,1998; Gvritishvili, Kacheishvili-Tavartkiladze, 2000; Gvritishvili, Kacheishvili-Tavartkiladze, 2002].

Materials and Methods

Samples have been collected in Vere river valley during 2000-2004. Moreover, the materials studied include specimens collected by M.Gvritishvili in 1987-1989. In the cases of samples with unripen fungal fruit bodies different moist chambers were used for their ripening (Petri dishes, Ziplok packets, etc.). Identification of fungi species were carried out on the base of light microscopic examination of the macro and micromorphological characters to determine the form, structure and size of the fruiting bodies, asci, ascospores, conidia, etc.

Results and Discussion

This contribution to the mycobiotic diversity of Georgia is presented by the list of necrotrophic micromycetes species and their host plants with location, data of collection and some notes concerning new fungus-host combinations.

Camarosporium cytisi Berl. & Bres. [Diedicke,1915]

Laburnum anagyroides, on dead twigs and branches, in assotiation with Tubercularia vulgaris Tode. Tbilisi environs, Vere river valley, Kus tba, 26.06.2004.

This is the first record of L.anagyroides as a host plant for C.cytisi in Georgia.

Cryptodiaporthe salicina (Curr.) Wehmeyer [Wehmeyer,1933]

Salix alba, on dead branches and twigs. Tbilisi environs, Vere river valley, Delisi, 05.1989 (M.Gvritishvili).

This is the first record of S.alba as a host plant for C.salicina in Georgia.

Cryptosphaerella parca Sacc. [Traverso,1906]

Mespilus germanica, on dead branches. Tbilisi environs, Vere river valley, Kus tba, 15.11.2003

The genus Cryptosphaerella is first recorded in Georgia.

Cucurbitaria amorphae (Wallr.) Fuckel [Sivanesan,1984]

Celtis caucasica, on dead branches. Tbilisi environs, Vere river valley, Delisi, near Nutsubidze street 221, 15.02.2004 (M.Gvritishvili).

This is the first record of C.caucasica as a host plant for C.amorphae in Georgia.

Cytospora rubescens Fr. [Gvritishvili,1984]

Rubus sp., on dead branches. Tbilisi environs, Vere river valley, Kus tba, 15.11.2003.

This is the first record of Rubus sp. as a host plant for C.rubescens.

Didymella physocarpi Ell. & Ev. [Sivanesan,1984]

Rubus idaeus, on dead branches. Tbilisi environs, Vere river valley, Gvevi village, 05.07.2003.

Salix alba, on dead branches. Tbilisi environs, Vere river valley, Delisi, 15.07.1987 (M.Gvritishvili).

Swida australis (≡Cornus australis), on dead branches. Tbilisi environs, Vere river valley, Delisi, 12.09.1987 (M.Gvritishvili).

Vitis vinifera var. sylvestris, on dead stems. Tbilisi environs, Vere river valley, Delisi, 18.07.1987 (M.Gvritishvili).

These are the first records of R.idaeus, S.alba, S.australis, V.vinifera, as host plants for D.physocarpi in Georgia.

Diplodia cerasorum Fuckel [Merezhko,1980]

Cerasus incana, on fire injured stems and branches, in assotiation with Cytospora leucostoma Fr., C.rubescens Fr. and Valsa leucostoma (Pers.) Fr. Tbilisi environs, Vere river valley, 23.01.2000 (M.Gvritishvili).

This is the first record of C.incana as a host plant for D.cerasorum in Georgia.

Exosporium tiliae Link. [Ellis,1971]

Paliurus spina-christi, on the base of dead stem. Tbilisi environs, Vere river valley, 05.03.2000 (M.Gvritishvili)

This is the first record of P.spina-christi as a host plant for E.tiliae in Georgia.

Gnomoniella rubicola Pass. [Ellis,Ellis,1985]

Rubus sp., on dead branches. Tbilisi environs, Vere river valley, Kus tba, 15.11.2003.

This is the first record of Rubus sp. as a host plant for G.rubicola in Georgia.

Lopadostoma sp.

Asci 137 X 17,5 μm; ascospores dark-brown, subglobosus, 16-18 X 13-16μm.

Sorbus graeca, on dead stems and branches. Tbilisi environs, Vere river valley, Delisi, 13.05.1989 (M.Gvritishvili).

Lopadostoma turgidum (Pers.) Traverso [Traverso,1906]

Cotoneaster sp., on dead branches. Tbilisi environs, Vere river valley, Delisi, near Nutsubidze street 221, 04.01.2004.

This is the first record of Cotoneaster sp. as a host plant for L.turgidum in Georgia.

Melanospora sp.

Spores dark-brown, 22,5-27,5 X 11,2-12,5 μm.

Spiraea hypericifolia, on dead branches. Tbilisi environs, Vere river valley, Delisi, 29.08.1987 (M.Gvritishvili).

Phomatospora sp.

Asci 32,5-37,5 X 6,2 μm., ascospores hyaline, fusoid, 5-8 X 2,5-3,7 μm.

Rubus sp., on dead branches. Tbilisi environs, Vere river valley, 07.03.1987 (M.Gvritishvili).

Phomopsis cuginiana (Traverso) Traverso (≡***Phoma cuginiana*** Traverso) [Traverso,1906]

Paliurus spina-christi, on dead branches, in assotiation with Diaporthe meridionalis Sacc. and Microsphaeropsis olivacea (Bonord.) Höhn. Tbilisi environs, Vere river valley, Delisi, 05.03.2000 (M.Gvritishvili).

Phomopsis sp.

Spores hyaline, 7-10 X 3-3,3 μm.

Melia azederach, on dead branches, in association with Alternaria alternata (Fr.) Keissler and Phoma sp. (spores 6-7 X 3,3 4,5μm). Tbilisi environs, Vere river valley, Park Mziuri, 26.11.2003.

Phomopsis sp. and other fungi listed, are recorded for the first time on Melia azederach in Georgia.

Pleospora sp.

Asci 85 X 15 μm., ascospores 17-22 X 7,5-10 μm.

Lonicera iberica, on dead stems. Tbilisi environs, Vere river valley, 19.09.1987 (M.Gvritishvili).

Solanum persicum, on dead stems. Tbilisi environs, Vere river valley, Delisi, 05.03.1987 (M.Gvritishvili).

Septoria laburni Pass. [Teterevnikova-Babajan,1987]

Laburnum anagyroides, on leaves. Tbilisi environs, Vere river valley, Kus tba, 15.10.1989 (M.Gvitishvili).

This is the first record of L.anagyroides as a host plant for S.laburni in Georgia.

Septoria myrobalanae Brun. [Teterevnikova-Babajan,1987]

Prunus domestica, on leaves. Tbilisi environs, Vere river valley, Delisi, near Nutsubidze street 221, 05.10.2003 (M.Gvritishvili).

This is the first record of P.domestica as a host plant for S.myrobalanae in Georgia.

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ახალი მონაცემები თბილისის მიდამოების (ვერეს ხეობა) მერქნიან მცენარეებთან ასოცირებული მიკრომიცეტების შესახებ

გოცაძე ნ.

ბოტანიკის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 11.02.2005)

რეზიუმე

მოცემულია მოკლე ცნობები საკვლევ ტერიტორიაზე შეგროვილი მიკოლოგიური მასალის იდენტიფიკაციის შედეგად გამოვლენილი სიახლეების ნაწილის შესახებ. მოყვანილია საქართველოს მიკობიოტისათვის აქამდე უცნობი სოკოების სახეობები, აგრეთვე ზოგიერთი სახეობისათვის აქამდე უცნობი მკვებავი მცენარე. სახელდობრ, საქართველოში პირველად აღინიშნება გვარი Cryptosphaerella, აგრთვე სახეობები: Cucurbitaria amorphae, Didymella physocarpi, Gnomoniella rubicola, Lopadostoma turgidum (ჩანთიანი სოკოები), Phomopsis cuginiana, Septoria laburni, S.myrobalanae (უსრული სოკოები). ექვსი სახეობის სოკო იდენტიფიცირებულია გვარის დონეზე (Lopadostoma sp., Melanospora sp., Pleospora sp. (ჩანთიანი), Phoma sp., Phomatospora sp., Phomopsis sp. (უსრული)). დანარჩენი სახეობები პირველად არის რეგისტრირებული ამა თუ იმ მკვებავ მცენარეზე.

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ON THE EFFECTS OF BORON DEFICIENCY ON THE STRUCTURE OF CELLS OF LEAVES IN *SOLANUM LICOPERSICUM*

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(Received December 8, 2004)

Abstract

Widened cell wall and stretching of sells as well as clusters of brown-coloured pigment inclusions and extension of intercellular substance have been revealed in microscopic sections of parenchimatous tissue of leaves in Solanum licopersicum grown under the conditions of boron deficiency. Abnormalities of osmotic relationships and disorders of cell metabolism, as a result of deficiency in boron, are suggested to cause structural alterations of the tissue in plant specimen under examination.

Key words: Plant cell, structure, boron deficiency.

Introduction

The term essential mineral element was proposed by Arnon and Stout in 1939 [Mengel, Kirby, 1987]. They concluded three criteria must be met for an element to be considered essential. These criteria are: 1) a plant must be unable to complete its life cycle in the absence of the mineral element, 2) the function of the element must not be replaceable by another mineral element and 3) the element must be directly involved in plant metabolism.

These criteria are important guidelines for plant nutrition but exclude beneficial mineral elements. Beneficial elements are those that can compensate for toxic effects of other elements or may replace mineral nutrients in some other less specific function such as the maintenance of osmotic pressure [Rea, 2003; Salt, 2004].

There are actually 20 mineral elements necessary or beneficial for plant development. Boron belongs to essential trace elements. Boron is necessary for plant cell wall formation, membrane integrity, calcium uptake and may aid in the translocation of sugars. Boron affects vital functions in plants. These functions include flowering, pollen germination, fruiting, cell division, water relationships and the movement of hormones.

Boron must be available throughout the life of the plant. It is not translocated and is easily leached from soils. Deficiencies in boron kill terminal buds leaving a rosette effect on the plant. Leaves are thick, curled and brittle. Fruits, tubers and roots are discolored, cracked and flecked with brown spots [Gallardo-Williams, et al., 2004; Lukaski, 2004; Marshner, 1995; Yermiyahu et al., 2001].

Copper is a synergist of boron. These elements may replace each other at some extent, however, as true essential minerals, neither copper, nor boron are fully replaceable by another

mineral element [Snowball et al., 1980; Williams, 2001]. Boron antagonists are manganese and calcium [Sanders et al., 2002].

The study was aimed at revealing possible alteration of cell structure in plant specimen treated under conditions of boron deficiency. Solanum licopersicum has been chosen as experimental subject as long as there is a lack of modern information on the effects of boron on the formation of cell and tissue structure in agricultural plant specimen.

Material and Methods

Total of 132 Solanum licopersicum specimen have been treated in nutrient solution, enriched with all non-organic components, necessary for plant development and reproduction. Experimental plants were grown under the conditions of boron deficiency (Group A). Normal content of boron was retained in nutrient solution for control plants (Group B).

Anatomical structure of columnar parenchymatous tissue of leaves was studied in microscopic sections under the apparatus MBY-3.

Results and Discussion

Microscopic examination of the leaves revealed several structural alterations in experimental plant species as compared to control specimen. In particular, parenchimatous cells of leaves were found stretched at some extent and fulfilled with brown-coloured pigment inclusions in experimental subjects.

As it is shown in the Fig. 1, cell wall was widened in experimental plant specimen as compared to control subjects. According to authors [Mengel, Kirby, 1987; Rea, 2003; Williams, 2001; Yermiyahu, 2001] boron plays important role in plant metabolism and in sugar translocation in particular. Evidently, increase in the size of cell wall in experimental plants is caused by the disorders of sugar distribution among cell structural elements.

Presumably, cell stretching is related to the weakening of water intake, which in turn, is caused by the increase in the width of cell wall in experimental plants. At the same time, excess of water in interstitial tissue leads to the increase of the volume of intercellular space in experimental plant species.

Concentration of the excess of pigment in cells is suggested due to the abnormalities of metabolic processes.

In sum, plant specimen treated under conditions of deficiency in boron underwent several structural alterations presumably due to disorders of cell metabolism and water intake. Data obtained provide one another argument for crucial role of boron in plant development.

However, experimental data on the necessity of boron for plant development is not an argument for excessive, overdosed use of these element in plant nutrition. Plant development may be affected by the deficit of mineral elements. The excess of these chemical compounds in plant tissues may have negative influence on plant growth and reproduction as well [Vinogradova, Martinov, 1998; Marshner, 1995].

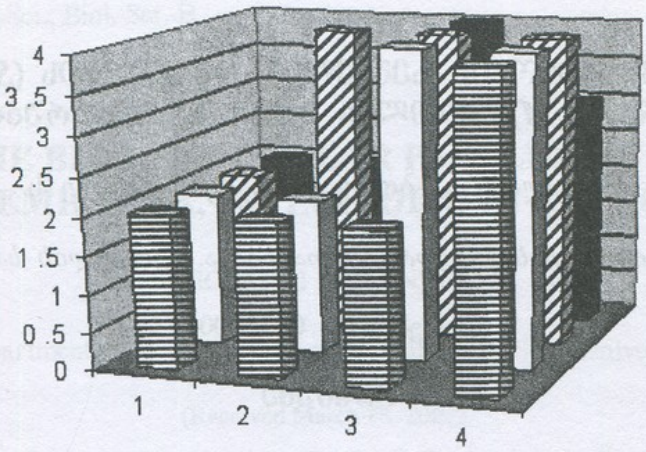

Fig. 1. The width (in μm) of cell wall in control (1) and experimental (2,3,4) specimen. Each column in the row (1,2,3,4) represents data, collected from 34 plant specimen.

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ბორის უკმარისობის ზეგავლენა პომიდვრის (*SOLANUM LICOPERSICUM*) ფოთლის უჯრედების სტრუქტურაზე

მანგალაძე ნ., ბოჯგუა ა., მერკვილაძე ს.

ბოტანიკისა და ეკოლოგიის კათედრა, ქუთაისის ა. წერეთლის სახ. უნივერსიტეტი.

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რეზიუმე

შესწავლილია ბორის ზეგავლენა პომიდვრის ფოთლის უჯრედების ჩამოყალიბებაზე საკვებ არეში ბორის სიმცირის პირობებში. წყლის კულტურის ფოთლის ანათლებში განსაზღვრულია პარენქიმული უჯრედების სტრუქტურული თავისებურებები. ნაჩვენებია, რომ საკვებ არეში ბორის მცირე რაოდენობით შეტანის შემთხვევაში, პომიდვრის პარენქიმული უჯრედების კედლის სისქე საგრძნობლად მეტია, ვიდრე საკონტროლო ქსოვილში. საცდელ მცენარეთა უჯრედებში აღინიშნება ყავისფერი პიგმენტის გროვები. ამ მცენარეთა უჯრედები წაგრძელებულია, ხოლო უჯრედშორისი სივრცის მოცულობა მომატებულია საკონტროლო მცენარეებთან შედარებით. სავარაუდოა, რომ ბორის ნაკლებობა იწვევს ოსმოსის და უჯრედის მეტაბოლიზმის დარღვევას, რაც, თავის მხრივ, იწვევს პომიდვრის ფოთლის უჯრედების ზემოთ აღწერილ სტრუქტურულ ცვლილებებს.

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Zoology

STUDY OF THE BIOLOGY OF PEAR PSYLLID - *PSYLLA BIDENS (ŠULC)* (HEMIPTERA, PSYLLOIDEA) IN EAST GEORGIA

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Abstract

Chorological and biological analysis of 5 pest psyllids of cultivated pears in Georgia is presented. Among them P.pyricola is orophilous one. P.pyrisuga Frst., P.Pyri (L.), P.permixta Burck.et Hodk. are widely distributed in Georgia in cultivated landscapes, as well as in wild environment (different decidious forests of the country). During the last 15 years new pest psylla, P.bidens (Šulc) of cultivated pear penetrated in east Georgia from the arid landscapes of South Caucasus - river Arax gorge – Nakhichevan and part of Armenia. This species is the most important pest of cultivated pears in Central Asia countries of the Former Soviet Union.

Key words: Psyllinea, chorology, pest psyllids of horticulture

Introduction

Psyllids are trophically linked with flowering plants. They are of small size (1-5 mm) insects with limited mobility. Psyllids occur throughout the globe. Up to 2500 species have been recorded in the world [White, Hodkinson, 1995], more than 200 species are represented in the Caucasus [Gegechkori, 1984].

In Palaearctic zoogeographical realm there are known approximately 20 pest psyllids of cultivated and wild pears (Pyrus spp.). Most of them are distributed in China [Fields, Zwick, 1981; Yang Chi-Kun, Li Fasheng, 1985].

In 1990-1992 in East Georgia - Kakheti, Kvemo Kartli, from 2000 in Zemo Kartli new pest psyllid for Georgia – P.bidens was discovered. Nowadays cultivated pears in Georgia are the most infested by P.pyri (in the border of all agrocoenosis) and P.bidens (within the arid regions' horticulture plantations of East Georgia).

From ecological viewpoint P.bidens is thermophilous species. Therefore it is distributed only in dry climate (200-400 mm average annual precipitation) regions and landscapes: deserts, semi-deserts, steppe and arid forest habitats. It occurs in plains and low-mountain zones (400-600 m.a.s.l.)

In recent time the area of this species distribution includes following countries of Eurasia: European part of the former USSR, Kazakhstan, Middle Asia, Greece, Italy, France, Israel, Iran, Mongolia [Gegechkori, Loginova, 1990].

As it was mentioned above, among the pear pest psyllids in Central Asia and southern part of South Caucasus, P.bidens is the most dangerous one. Harmfulness is caused by its biological peculiarities. It is the most polyvoltine species (has 3-7 generation per year). So, applied

entomologists should pay more attention to the biology of this species and control its further distribution in Georgia.

Materials and Methods

To research the distribution of P.bidens, in 2000-2003 we carried out series of special field studies in Zemo Kartli (Gori-Khashuri horticultural fields). We determined further expansion of above mentioned pest on the territory of East Georgia.

Investigation of the biology of P.bidens of Gori district – the midrivers region of Didi Liakhvi and Mejuda gorges agrocoenosis was carried out in 2001-2003, during all vegetation period of the pears plantation.

To study the phenology of P.bidens we distinguished pure populations of this species from P.pyri.

150 specimen of adult overwintered P.bidens were gathered from the host plants in Mejuda river gorge horticulture plantations and placed in the nursery on young pears plants. The seedlings of 50 cm length were planted in woody pots and isolated from the environment by gauze cloth.

Results and Discussion

P.pyri and P.bidens are narrow oligophagous psyllids, closely connected by feeding habits with cultivated and wild pears (Pyrus spp.). In East Georgia both ones occur on the same host plant [Gegechkori,1996 a, 1996 b, 2000].

The observations took place during the whole vegetation period of food plant in 2003.

Both, male and female adult P.bidens hibernate on its host plant, under fallen leaves, in the cracks of trunk, etc. Overwintering adults exhibit at the end of February and in very begining of March. After mating female lay the eggs superficially on the leaf or bud of trees. Hatching of eggs in the spring (the second decade of March) occurs at or about bud burst and newly emerged larva (of 1st generation) move into the flush of new foliage.

The first instars of larvae are hatched after 5-7 days. They began to suck the sap of buds and leaves and soon turn into nymphs. The winged adults of the first generation come out in the middle of April.

According to the table 1, development of the 1st generation takes place 24-30 days, 2nd – 20-26 days, 3rd – 16-22 days and 4th – 18-23 days (Table 1).

Consequently, in the ecological (abiotic) conditions of the midrivers region of Didi Liakhvi and Medjura gorges P.bidens gives at least 4 generations during the pear cultures vegetation period. These data coincide with the data of the development of P.pyri in East Georgia [Gegechkori, 1996].

The time for the development of the eggs and nymphs of different generations are not similar. The development of eggs of P.bidens ranges between 4-8 days, larvae – 4-6 days, nymphs – 12-18 days.

Individual capacity of eggs production of different generations isn't the same. The less productivity shows the overwintered specimen (45-152 eggs), the autumn populations are also characterized by rather low productivity (52-265 eggs).

In early spring and late autumn the insects get poor food and the climate is far from being favourable. The second and the third generations are characterized by the highest fertility, laying accordingly ±400 and ±600 eggs. The productivity of one female varies from 45 to 515 eggs, the

average amount is 190. The highest quantity of adult P.bidens occurring in nursery conditions is coming on July and August.

According to our observation nymphs are sucking the sap of leaves, buds, flowers and young shoots that cause physiological and morphological changes in the plant. As a result damaged pear trees fruit becomes dry and tasteless. The harvest of pears considerably decreases. Feeding damage can be traced through systematic toxaemia caused by salivary injection. Visible damage of cultivated pears ranges from localized necrosis of plant leaf, bud, and stem tissue to the deformed leaves.

Our studies revealed that in stationary conditions and also in former horticulture farms of the midriver region of Didi Liakhvi and Mejuda gorges P.bidens and P.pyri are serious damaging pests of the pear-trees.

During 2002 in neglected gardens of above-mentioned regions 70-80% of trees were infested by P.bidens and the harvest was very low.

Therefore, besides P.pyri, recently expanded in Georgia P.bidens is one of the most dangerous pest causing important economic disadvantage in the horticulture of the eastern part of this country.

Table 1. Life-cycle features of Psylla bidens (Šulc) observed in the village Mejvriskhevi (midriver region of Didi Liakhvi and Mejuda gorges)

Generation	The duration of development of different phases (days)			The duration of development of different generations (days)	The period of development of different generations (months)	Daily average temperature (C^0) for different generations
	egg	larva	nymph			
I	4-8	4-6	16-18	24-32	III-IV	5,5-15,3
II	3-6	3-4	14-16	20-26	IV-V	14,1-22,4
III	2-5	2-3	12-14	16-22	VI-VII	18,2-28,1
IV	2-3	3-5	13-15	18-23	VII-VIII	22,2-30,2

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მსხლის ფსილას - *Psylla bidens (Šulc)* (Hemiptera, Psylloidea) – ბიოლოგიური კვლევის შედეგები აღმოსავლეთ საქართველოში (გორის რაიონი)

გეგეჭკორი არნ., გინტური ზ.

ზოოლოგიის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 14.03.2005)

რეზიუმე

ამჟამად საქართველოს მეხილეობაში მსხლის კულტურაზე რეგისტრირებულია ფსილიდების 5 სახეობა. მათ შორის, უკანასკნელი 15 წლის განმავლობაში, აღმოსავლეთ საქართველოში, როგორც ჩანს, სომხეთიდან შემოიჭრა და გავრცელდა ფსილიდების მანამდე უცნობი სახეობა - Psylla bidens (Šulc). ეს სახეობა პოლივოლტურია (ცენტრალურ აზიაში ახასიათებს 6-7 თაობა წელიწადში). მსხლის კულტურაზე მისი ნიმფები იკვებებიან ახალგაზრდა ფოთლებზე, კვირტებზე, რაც უარყოფით გავლენას ახდენს მოსავალზე. ფსილიდებიდან მეხილეობის ერთერთი ყველაზე საშიში მავნებელია. შესწავლილია P.bidens-ის არეალი აღმოსავლეთ საქართველოს კულტურულ სავარგულებსა და ბიოცენოზებში. პირველად დადგინდა, რომ საკვლევ ტერიტორიაზე P.bidens ვითარდება 4 თაობის სახით.

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ON TAXONOMY OF SOME WEEVIL BEETLE SPECIES (COLEOPTERA, CURCULIONIDAE) OF SHIDA KARTLI (EAST GEORGIA)

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Abstract

Based on literature sources and collections from different museums correct identification of some weevil beetle species (Coleoptera, Curculionidae) distributed both in Shida Kartli and throughout Georgia is given. 250 species of the weevil beetles recorded in Shida Kartli have been checked. Results of identification showed 20 doubtful species. In addition 15 species due to incorrect identification should be excluded from the lists of weevil beetles recorded in Shida Kartli. For 5 species their real taxonomic position has been established.

Key words: Weevil beetle, dynamics, taxons, Coleoptera, phytophagous, phytosaprophagous, endemic.

Introduction

When studying the problems of fauna, biology, ecology, etiology, zoogeography, evolution of species of any animal groups, exact identification of taxons plays the decisive role. The fauna of the weevil beetle of Georgia (Coleoptera, Attelabidae, Apionidae, Dryophthoridae, Curculionidae) has been fundamentally studied [Cholokava,1968] and over 900 species have been revealed, more than half of which are mentioned for the first time, and 12 species are entirely newly described. While making an annotated list of the weevil beetle species, we used all the existing literature sources and materials of collections kept in museums of various countries dealing with Georgian fauna. It has been discovered that from known weevil beetle species about 100 species have never been encountered in Georgia and names of those species, as a result of their incorrect identification, were used improper in publications for many years, which was the cause of numerous misunderstandings. Therefore it became necessary to delete those species from the annotated list of weevil beetles of Georgia, based on scientifically confirmed arguments. At present all above mentioned can be filled up by material obtained from Shida Kartli region during the study of the weevil beetle fauna in dynamics in 2001 – 2003.

Materials and Methods

Shida Kartli covers the territory of central Georgia and is bordered by: the Northern part of Trialeti range from the South, foothills of the Southern part of the Caucasian range from the North, Likhi range from the West, and Aragvi river from the East.

Doubtful species from Georgian and foreign literature sources and entomological collection funds of museums registered as Shida Kartli specimens were studied. We searched required material in collections of Zoology Institute of Russian Academy of Sciences (St. Petersburg), Zoology Department of Georgian State Museum, Zoology museum of Lomonosov Moscow State University, collection of Natural-Historical Museum of Hungary (Budapest) and personal entomological collection of Acad. Iablokov-Khnzoryan (Yerevan). We revised the range and bioecology of each studied species.

Results and Discussion

Weevil beetles represent one of the main groups of the largest order - Coleoptera, which unites more than 45000 species in certain families. Their majority is phytophagous, only a small group is phytosaprophagous. The ancient age of the fauna of the above mentioned group, a wide range of distribution of most of those species, and the high level of endemism in a number of cases play the most important role in studying the ways of zoogeographical and faunal formations in some regions. The species of the weevil beetle of Shida Kartli, known from literature sources and the systematic belonging of which requires exact definition, are given below:

Phyllobius sinuatus Fabricius, 1801 – was incorrectly identified for years and introduced in literature under this name. It was mentioned by different authors [Schneider, Leder, 1878; Radde, 1899; Reck, Kobakhidze, 1957; Supatashvili,1949; Tulashvili,1953; Vashadze,1962; etc.] as distributed in Shida Kartli, Tbilisi, Borjomi, Batumi, East Georgia and South Osetia (without exact location), Kolkheti, Lagodekhi State Reserve, Kojori, Mtsvane Kontskhi, Chakvi, Ajameti and Sokhumi. This species, introduced under this name is, in fact, Phyllobius schneideri Schilsky, 1811, the name of which was applied once in the work of Batiashvili and Baghdavelidze, 1941. But in other works of the same authors this species was still named as Ph. sinuatus. Moreover, in the monograph of Lozovoy [Lozovoy, 1965] both of these names are mentioned as independent species of Georgia, although Ph. sinuatus has never been found in Georgia, neither in the whole Caucasus. As for Ph. schneideri, it is commonly encountered in Georgia, included Shida Kartli. Studying the dynamics of weevil beetle in 2001 – 2003, representatives of this species were revealed in great numbers in Shida Kartli, viz.: the Ateni gorge; villages: kvakhvreli, Zemo Khvedureti, Okami; gorges of the rivers Liakhvi and Prone.

According to literature sources [Radde, 1899; Eichler, 1930; Cholokava,1968; Batiashvili, Bagdavelidze, 1941; etc.] the following species of genus Polydrosus from different regions of Georgia, including Shida Kartli have been known: P.cocciferae Kiesenwetter, 1864; P. pilosus Gleder, 1866 and P. caucasicus Desbrochers, 1871. But none of the above mentioned species are encountered in Georgia. For example, P. cocciferae is spread in Greece and Crete Is., P. pilosus – in Central and Northern Europe. Moreover, P. caucasicus was referred to the Caucasus in Winkler's catalogue (1924 – 1932) and later it was applied by many authors for Georgia [Batiashvili, Bagdavelidze, 1941; Eichler, 1930; Cholokava,1968; etc.] which is a result of wrong systematic categorization of species name from one literary source to another.

Over the years many authors [Kalandadze, Lozovoy,1937; Batiashvili, Bagdavelidze, 1941; Kobakhidze, 1957; Cholokava,1968; etc.] mentioned one of the species of the genus Chlorophnus as Ch. voluptificus Gyllenhal, 1834 distributed in Shida Kartli and even in whole Georgia. Now it is stated that in fact it is Ch. vittatus Menetries, 1832. Moreover, it has been found out that among the materials obtained from Georgia and displayed in the Georgian State Museum the three forms, viz.: 1.an insect identified by Suvorov as Ch.micans Stewen, 1829; 2. Ch. gibbosus Paykull., 1792, found by Eichler (1930) in the surroundings of Tbilisi, and 3. Minicollis smycronyx Gyllenal, 1834, mentioned by Radde (1899) in Borjomi, were incorrectly identified and all of them proved to be Ch. vittatus, a species which is widely distributed throughout Georgia, included Shida

Kartli. In 2001 – 2003 large numbers of this species were found in the villages of Shida Kartli: Uplistsikhe, Kvemo Chala, Ergneti, Mereti, Dvani, Kvakhvreli, Bobnevi, and in the gorges of Liakhvi and Mejuda rivers. Thus, instead of four species only one species Ch. vittatus, of the genus Chlorophnus is distributed in Georgia.

Schneider, Leder (1878) and Radde (1899) referred to Bagous frit (Herbst) non Beder, 1884 – a species from the village Surami, Shida Kartli, which was not discovered by us among the studied material as it has never been encountered in the Caucasus; it is known from the central and Northern regions of Europe. Another species B.tataricus Faust, 1786, mentioned for Mtskheta [Eichler,1930], is not distributed on the territory of Georgia. It is spread in Kazakhstan. Anthonomus celtidis T.M.,1952, described in Georgia according Ter-Minasyan (1952) and then applied in other literature sources [Forest Pests, 1955; Lozovoy, 1965; Cholokava, 1968] proved to be the synonym of A. Koenigi Pic (1912), which is distributed in Eastern and Southern regions of Georgia, including Shida kartli. We found this species in the Ksani gorge. Baris pilicornis Marsham,1802, registered by Eichner (1930) in Mtskheta, and another species registered by us on the region of Bolnisi [Cholokava, 1968] and mistakenly defined as Baris chlorsans Germar,1824, in fact is Baris concina Boheman, 1844, first registered by us in studied regions of East Georgia.

Two specimens of the weevil beetle obtained from the gorge of the river Tana (Ateni gorge) and identified by Schultze as Ceutorhynchus latierryi Brisout,1866 are deposited in the Georgian State Museum. This species was registered in the Caucasus (Georgia incl.) by a number of authors [Schneider,Leder, 1878; Radde, 1899]. The above mentioned species, together with Ceutorhyns Schultze incisuschu,1899 registered by Korotyaev (1980) in Georgia, proved to be Glocianus (=Ceutorhynchus) brevicollis Schultze, 1996, which is commonly spread in Georgia and has been incorrectly identified as Ceutorhynchus incisus up to now.

Finally, among not existing species in Shida Kartli, Miarus scutelaris Brisout, 1865, which is preserved in the entomological collection of the Georgian State Museum and is registered in Mtskheta, has been recognized by us [Cholokava,1968] as the one distributed in the surroundings of Tbilisi. As a result of thorough studies it has been stated that its identification was wrong and it proved to be M.graminis (Gyllenal),1813.

Hence, among studied 20 species 15 species have not been discovered in Georgia at all, and 5 species that over the years have been defined as belonging to definitely other species, have regained their real position in systematics.

Acknowledgment

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შიდა ქართლის ცხვირბრძელა ხოჭოების (COLEOPTERA, CURCULIONIDAE) ზოგიერთი სახეობის ტაქსონომიური მდგომარეობის დაზუსტებისთვის

ღოღობერიძე ლ.[1], ჭოლოკავა ა.[2]

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(მიღებულია 14.03.05)

რეზიუმე

შესწავლილია შიდა ქართლიდან ლიტერატურულ წყაროებში გამოქვეყნებული ცხვირგრძელა ხოჭოების **(Coleoptera, Curculionidae)** 20 საეჭვო სახეობის სისტემატიკური კუთვნილების სიზუსტე. დადგენილია, რომ 15 სახეობა არასწორი იდენტიფიკაციიდან გამომდინარე საერთოდ არ არსებობს საქართველოში და ამდენად ისინი ამოღებული უნდა იქნას საქართველოს ცხვირგრძელა ხოჭოების ანოტირებული სიიდან. 5 სახეობას, რომლებიც გავრცელებულია, როგორც შიდა ქართლში, ისე საერთოდ საქართველოში და ლიტერატურულ წყაროებში ასევე არასწორი იდენტიფიკაციის გამო მოიხსენებიან სრულიად სხვა სახეობებად, დაუბრუნდათ რეალური ტაქსონომიური კუთვნილება.

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Letter to Editor

PHYTONCIDE ACTIVITY OF PEACH (*PERSICA VULGARIS MILL.*) LEAVES

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Abstract

Seasonal dynamics of the intensity of volatile substances in the leaves of some peach cultivars and its dependence with leaves seasonal phytoncide activity was studied. It was established that intensity of volatile organic substances and their phytoncide activity is high in summer, but in autumn and spring both these processes are weakened.

Key words: volatile substances, phytoncide activity, dynamics, peach leaves.

Introduction

In the viability processes the separate organs of plants evolve organic substances, which take important place among the atmospheric biogenic substances. The plants by volatile organic substances interact not only with each other, but they affect the animal organisms too. To study the substances of this group evolved by the different cultures and cultivars, as well as their influence on the environment, is an urgent problem [Kvesitadze G. et al., 1999]. The organic substances evolved by plants during their vital activities might be used by other organisms as nutrition, on the other hand, these volatile substances may have poisonous effect on the environment. Especially it is worth to mention such components of volatile substances as phytoncide substances that have killing effect on pathogenic microorganisms [Kretovich V.L., 1980; Georgievski V.P., 1990].

Hence, the chemical analyses of plant volatile substances are of great interest.

The aim of our research was to determine the seasonal dynamics of phytoncide activity in the leaves of some sorts of peach culture, the intensity of volatile substances and dependence between phytoncide activity and intensity of plant volatile substances.

Materials and Methods

Investigations have been carried out on the peach (Persica vulgaris Mill.) leaves obtained from the experimental station of the Scientific-Research Institute of Gardening, Viticulture and Winemaking, Academy of Agriculture of Georgia, in Skra.

The quantity of volatile organic substances and their seasonal dynamics was determined by different methods: permanganate method [Gammerman, 1959], sulphuric acid metod and by gas

analyzer [Sanadze, 1961]. Leaves phytoncide activity determined by hung drop method [Ershakov, 1987].

Table 1. Seasonal dynamics of volatile substances and phytoncide activity by permanganate method (a), sulphuric acid method (b), gas analyzer method (c).

Peach cultivars	Spring						Summer						AAutumn					
	volatile substances (mg)			phytoncide activity (min)			volatile substances (mg)			phytoncide activity (min)			volatile substances (mg)			phytoncide activity (min)		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
krimchaki	0,52	2,9	2,9	4	4	4	5,6	5,4	2,82	1	1	1	12,0	2,4	0,34	9	9	9
elberta	0,62	5,6	5,6	3	3	3	18,8	5,0	1,57	1	1	1	11,7	4,6	0,70	52	53	53
bestavashvili	0,56	0,85	0,85	5	5	5	18,0	1,8	0,90	1	1	1	20,0	1,62	0,69	9	9	9
bel	0,55	3,2	3,2	6	6	6	4,8	5,2	2,03	1	1	1	12,3	4,65	0,90	9	9	9
geokchai	3.0	2,8	2,8	4	4	4	10,2	2,07	3,14	3	3	3	11,5	6,10	0,67	41	41	41
vazhuri	2,7	2,6	2,6	27	27	27	17,0	6,08	1,51	5	5	5	21,0	1,84	0,17	40	40	40
Eristavis vardisperi	0,11	0,25	0,25	4	4	4	1,0	1,7	0,70	1	1	1	1,12	trace	0,05	20	20	20

Volatile substances were collected in special apparatus. Special treatment and quantitative titration was carried out by previously determined titre.

To determine phytoncide activity as a test object infusorium Stilonichia was used. In the glass trap 1g of pound leaf was put which was closed by glass plate. On the inner side of this plate there was water drop containing infusorium. Microscopic observations were carried out. Phytoncide activity was determined as a time necessary for killing infusorium.

Results and Discussion

Table 1 presents data of seasonal dynamics of volatile substances and its connections with seasonal changes of leaves phytoncide activity by permanganate method (a), sulphuric acid method (b), gas analyzer method (c). The amount of volatile substances and phytoncide activity reaches maximum in summer (less time for killing infusorium). In spring and autumn their amount decreases (phytoncide activity expressed in minutes is higher).

As it is seen from the tables the intensity of volatile substances of plant leaves and their phytoncide activity are changed during the vegetation period. In summer period (June, July) the regularity between the intensity of volatile substances and phytoncide activity was revealed. But in spring and autumn both these processes are decreased. Although, in some cases excretion of substances is high and phytoncide activity is low that may be caused by different chemical nature of volatile substances.

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ატმის ფოთლის *(Persica vulgaris Mill.)* ფიტონციდური აქტივობა

ბლიაძე გ.[1], ჩუბინიძე ვ.[2]

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

(მიღებულია 07.02.05)

რეზიუმე

შესწავლილია ატმის ფოთლის აქროლადი ნივთიერებების გამოყოფის სეზონური დინამიკა და მისი კავშირი ფოთლის სეზონურ ფიტონციდურ აქტიობასთან. დადგენილია, რომ ნივთიერებების გამოყოფის ინტენსიობა და მათი ფიტონციდური აქტიობა მაღალია ზაფხულის პერიოდში; ხოლო გახაფხულსა და შემოდგომაზე ორივე ეს პროცესი შესუსტებულია.

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Letter to Editor

PECULIARITIES OF HEAVY METALS ACCUMULATION IN THE BLACK SEA MUSSELS *MYTILUS GALLOPROVINEIALIS LAM.*

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The mussels of the South-West nearshore of the Black Sea are more or less well studied [Dragoli, 1966; Diasamidze, 1983]. Although their ecological peculiarities need more precise specification.

The goal of our studies was to research peculiarities of heavy metals (Zn, Cr, Cd) accumulation in the mussels distributed in the sea shore of Georgia (Gonio, Green Cape) by biotopes. The studies were carried out in March and June, 2002.

We studied mussels species – Mytilus galloprovineialis Lam., which is characterized by wide distribution on above mentioned territory. It is known from scientific literature that this species is effective bio-filterer and at the same time it should be used as reliable indicator of the accumulation of heavy metals in sea ecosystems [Sadikhova, 1964; Pavlova, 1986].

We carried out experiments by known methods [Skurikhina, 1998]. 2 g weighted mussels we put in wide-mouthed jars of 100ml which were placed in drying box at 100-105°C temperature for 2 hours. To the dried material 10 ml concentrated HNO_3 was added and this mixture was leaved for about 12 hours. Then NHO_3 was evaporated so that the specimen remained in the damp state. To the material 10 ml distilled water was added and then it was evaporated again. Remains in the jar was filtered and added 25 ml distilled water. The amount of heavy metals was determined by atomic-absorption spectrophotometrical method.

It was revealed that on the studied territory Mytilus galloprovineialis Lam. reside in two different biotopes – rock and mud. Here they are presented by two specimen - black and brown mussels. The amount of heavy metals was determined by the coloration and biotopes. The results are presented in Table 1.

Table 1. Average amount of heavy metals (μg/g) in mussels by the coloration and biotopes

Biotopes	Mussels coloration	Amount of specimen	Mussels size (mm)	Zn	Cd
rock	black	2	23-25	4,27	1,87
	brown	2	24-25	8,09	4,73
rock	black	6	38-52	1,22	1,23
	brown	7	41-60	1,17	1,07
rock	black	3	84-86	0,98	0,97
	brown	4	93-95	0,96	1,03
mud	black	2	43-46	1,86	1,26
	brown	4	50-60	1,74	1,30

As it is seen from the table in brown mussels of rock with mussel size less than 25mm the amount of Zn and Cd considerably exceeded these parameters in black mussels. The amount of these microelements in black mussels of rock with mussel size of 38-52 mm and 41-60 mm is higher than in brown mussels. Mussels of 84-86 mm and 93-95 mm sizes consist of equal amount of Zn and Cd. Analogous situation is the case of black and brown mud mussels.

While comparing the heavy metals concentrations according to mussels sizes, the regularity is evident. With the increase of size of mussels of both coloration concentration of Zn and Cr obviously decreased, except the mud mussels – the regularity in the case of cadmium is slightly violated.

We have also shown that concentration of chrome in black and brown coloration of both, rock and mud mussels is stable and it consists of 0,01 μg/g.

Besides, in rock and mud biotopes we studied the concentration of heavy metals in different organs of mussels – muscles and liver (but only in black mussels). The size of rock mussels was 41-57 mm and mud mussels – 51-63 mm (Table 2).

Table 2. Average amount of heavy metals (μg/g) in mussels in muscles and liver

biotopes	Amount of specimen	Size of mussel (mm)	muscles			liver		
			Zn	Cr	Cd	Zn	Cr	Cd
Rock	4	41-57	18,35	0,23	0,37	31,45	0,37	1,57
mud	4	51-63	25,73	0,29	0,38	24,76	0,39	1,19

As it is seen from the table concentration of Zn is considerably more than Cr and Cd both, in muscles and liver. Concentration of Cd and Cr in liver is higher than in muscles in both biotopes. In the muscles of mud mussels the amount of all studied microelements is higher than of rock mussels, in the liver the picture is nearly reversed but to find out clear regularity is difficult.

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მძიმე ლითონების დაბროვების თავისებურებები შავი ზღვის მიდიებში *(Mytilus galloprovineialis Lam.)*

ლურსმანაშვილი ლ., მაზმანიდი ნ.

ზღვის ეკოლოგიის და თევზის მეურნეობის სამეცნიერო-კვლევითი ინსტიტუტი

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რეზიუმე

შესწავლილია საქართველოს შავი ზღვის სანაპირო ზოლში (გონიო, მწვანე კონცხი) გავრცელებულ მიდიებში ზოგიერთი მძიმე ლითონების (Zn, Cr, Cd) დაგროვების თავისებურებები ბიოტოპების მიხედვით.

ინსტრუქცია ავტორთათვის

სამეცნიერო ნაშრომს ჟურნალი ბეჭდავს 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.

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

ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

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

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