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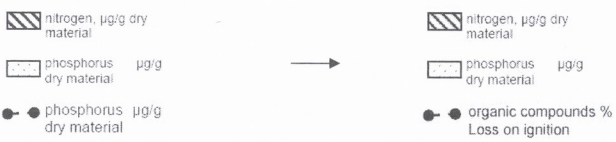

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HUMAN SPERMATOZOA GLYCOCONJUGATES: ISOLATION, PURIFICATION, CHARACTERIZATION AND POSSIBLE ROLE IN SPERM-EGG RECOGNITION

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(Received December 12, 2005)

Abstract

Lectin-binding glycoconjugates from the human spermatozoa were isolated and characterized. Sperm proteins inhibit hemagglutination activity of various lectins, according to the relative L/LBP ratio decreases in the following order: ConA, PSA, WGA, SNA, PNA, SBA, LAL. An inhibiting effect of ConA (100 μg/ml) on movement of sperm cells, which is expressed in decreased movement ("Local Movement" phenomenon) and in the mixed type, heavy agglutination was established. The presence of ConA-bs with specific (α-D-Man, α-D-Glc, α-D-GlcNAC) carbohydrate moieties has been revealed on sperm cell plasma membrane, using cyto-chemical methods. Lectin-binding glycoconjugates have been purified on ConA 4B-Sepharose afinal sorbent, with high pressure liquid chromatography on Gel-filtrated TSK G3000SW Column. It was suggested the possibility that Con A-binding sites of sperm glycoconjugates play structural and functional role during fertilization via recognition of egg *zona pellucida* glycoproteins followed by gamete recognition and adhesion.

Key words: human sperm, normozoospermy, ConA binding glycoconjugates, lectin recognition systems.

Abbreviations: ZP-*zona pellucida*; L/LBP lectin-lectin binding protein; ConA-bs - ConA -binding sites

Introduction

Optimal performance of biological processes on different levels of cell organization requires complementarity that is often implemented by the carbohydrate-containing informative molecules. Fertilization is the joint process of adhesion-recognition including sperm capacitating, binding to the egg and final intrusion. The complementary molecules are distributed over the surfaces of both gametes and regulate different stages of fertilization [Wasseraman et al., 2001]. Capacitated sperm cell is recognized and binds specifically to the glycoproteins of egg *zona pellucida* (ZP1, ZP2, ZP3) based on protein-carbohydrate interaction. Binding is selective, species-specific process and varies according to glycosilation [Gabriele et al., 1998; Yoshitani et al., 2001]..

Maturation of sperm cells is accompanied by molecular changes on plasma membrane. Accordingly, it is reflected on changes in lectin-recognition systems as well as on lectin-binding sites [Yoshida-Komiya et al., 1999; Silvestroni et al., 2004]. Lectins have specificity towards the oligosaccharide determinants of glycoconjugates thus providing the possibility of their use for evaluation of differentiation and functional mechanisms in the tissue and cell structures [Khunsook et al., 2003]. The aim of present study was to make probing of carbohydrate sites of human sperm glycoconjugates by lectins, isolate and study their allocation, quantitative and characteristic distribution.

Materials and Methods

Human sperm was used as an object of investigation. The normal ejaculate with normative characteristics (concentration, morphology, viability and moving ability) in particular, normozoospermy according Elliason's classification [Eliasson R. 1981] was used in experiments. The evaluation of spermogram and morphological parameters of sperm cells were carried out by computer analysis and phase-contrast microscopy.

Sperm cells were obtained by centrifuging of an ejaculate at 3 000g/15 min. Pellet was washed in PBS containing 150 mM NaCI, 40 mM KH_2PO_4 (pH 7.4) and centrifuged at 8 000g/20 min. The pellet obtained contained pure sperm cells. The purity of sperm cells was examined with light microscope under 400 fold magnification (objective x10, ocular x10).

Sperm cells were homogenized in 40 mM phosphate buffer (pH 7.4) containing 150 mM NaCI, 1mM PMSF, 0.1 mM DDT. The homogenate was centrifuged at 8 000g for 15 min. After removing of soluble protein fraction pellet was treated with 0.1 M sodium acetate buffer (pH 4.0) containing nonionic detergent 0.5% Triton X-100, 150 mM NaCI, 1mM PMSF and frozen for 1 h. After defrosting the material was homogenized again and centrifuged at 8 000g for 15 min. Supernatant was subjected to dialysis against PBS at 4°C and centrifuged at 12 000g at 15 min.

Lectin activity was determined using 2% (v/v) trypsin-treated rabbit erythrocytes on 96 well immunological plates in agglutination buffer containing 0.15 M NaCl, 40 mM KH_2PO_4 (pH 7.4). Hemagglutination was evaluated visually after 1-1,5 h at the room temperature [Nowak. et al., 1977].

Lectin-binding activity was determined by the inhibition of 2% (v/v) trypsin-treated rabbit erythrocytes (ligand-receptor inhibition method) as follows: to 100 μl 2 fold gradually diluted protein solutin 50 μl lectin solution (titer 1:8) was added and incubated for 30-45 min at the room temperature. Then 50 μl 2% rabbit erythrocytes suspension was added. The inhibitory activity was determined by the minimal concentration of protein causing inhibition of agglutination via reactivity with the lectin glycoconjugates and expressed in conventional units of lectin/lectin-binding protein [Lutsik et al., 1981]. Commercial plant lectin preparations were used in experiments (Sigma, USA).

Proteins were purified on ConA 4B-Sepharose column (1.5x3 cm, Sigma, USA) equilibrated in 0.1 M sodium acetate buffer (pH 6.0) containing 1 M NaCI, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$. Bound proteins were eluted by 0.1 M borate buffer (pH 8.0) containing 1 M NaCI. Elution rate 0.2 ml/min, detection at 280 nm.

Gel filtration of purified proteins was performed on HPLC (Waters USA; Gilson France) using TSK G3000SW column (7.5x300 mm, LKB-prdukter AB, Sween) equilibrated with 0.1M phosphate buffer (pH 6.6) containing 150 mM NaCI, 0.02% Na_3N. Elution rate 0.6 ml/min, detection at 280 nm.

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

Results and Discussion

In primary series of the experiments, for studies of human spermatozoa carbohydrate moieties of glycoconjugates plant lectins (Sigma, USA) were used. Plant lectins have the ability to inhibit glycanes and respective haptenes in specific manner, which is employed in histochemical studies for characterization of wide spectrum of glycoconjugate structures in animal cells.

Using the ligand-inhibition method we studied influence of the total membrane protein fraction of sperm cells on hemagglutination activity of lectins and partly characterized their monosaccharide determinants. However, some plant lectins bind complex determinants with high affinity and recognition takes place via specific anomer configuration and side sugar residue. We found that proteins revealed affinity to plant lectins and inhibited their hemagglutination activity. Lectin-binding activity was determined by ligand-receptor inhibition method and expressed in conventional units of lectin/lectin-binding protein (L/LBP). As it was found, proteins extracted from spermatozoids contain various carbohydrate moieties and are characterized by different abilities of hemagglutination inhibition of lectins (Table 1). It should be mentioned, that determination of lectin-binding activity in immunological plates is highly sensitive rapid method and convenient for quantitative characterization.

Table 1. Lectin-binding activiti of human spermatozoa membrane glycoconjugates and inhibition of hemaglutination activity of plant lectins

Lectin Abbreviation	Source of lectin	Sugar specificity	Inhibitor lectin activity Lectin/Lectinbinding protein (L / LBP) mkg/150mμ
Con A	Canavalia ensifirmis	Terminal and internal α–D-Man >α-D-Glc >GlcNAC	8.3×10^{-2}
WGA	Triticum vulgaris	Terminal and internal β-D-GlcNAc>NeuNAc	3.3×10^{-2}
PSA	Pisum sativum	Terminal and internal α–D-Man >α-D-Glc >GlcNAC	5.2×10^{-2}
SNA	Sambucus nigra	NeuNAca2,6 Gal/GalNAc	6.4×10^{-3}
SBA	Glycine max	Terminal α/β-D-GalNAc	3.2×10^{-3}
PNA	Arachis hypogea	Terminal β-D-Gal(1-3)-GalNAc	3.9×10^{-3}
LAA	Laburnum alpinum	Terminal α-L-Fuc	1.6×10^{-3}

$P < 0.05$

As is shown in the Table 1, according to the L/LBP ratio highest binding ability was attributed to ConA, which reacts with α-anomer containing mannose and glucose glycoconjugates. ConA binds firmly to mannose-type N-glycanes and weakly to hybrid and biantennal N-glycanes, while not binds to the branched complex N- and O-type glycanes at all [Chamutovski et al., 1986]. PSA has the similar specificity, however, reveals less affinity to the abovementioned proteins and occupies next position. The active site of WGA shows more complementarity to the di- and trisaccharides of GlcNAc in compare to monosaccharides and reveals less affinity. Among galactose-specific lectins the binding ability attributed to SNA, less to PNA and SBA. The ability of PNA to bind O-type glycoproteins is of interest. Hence, binding with PNA is often indicates the presence of O-type links in the molecule. Fucose-specific lectin LAL takes the last position in binding row.

Thus, an intensity of inhibition of hemagglutination activity of plant lectins by the total membrane protein fraction decreases according to L/LBP by the following order: ConA, PSA, WGA, SNA, PNA, SBA, LAL.

Due to high glycoconjunate binding ability of ConA, on the following stage of the research of cyto-chemical studies the influence of lectins on the movement of sperm cells was studied by using of ConA. At the same, the ability of ConA to agglutinate some cells (lymphocytes and erythrocytes) is known.

Before conducting the experiment an ejaculate was incubated at 37°C for 15-60 min. Since temperature bear the great influence on movement abilities and speed of sperm cells, the test was conducted at 20-24°C or 37°C. The sperm cells with concentration of 60 x 10^6/ml and with moving ability more then 65 % were used in the experiments (Fig 1.1).

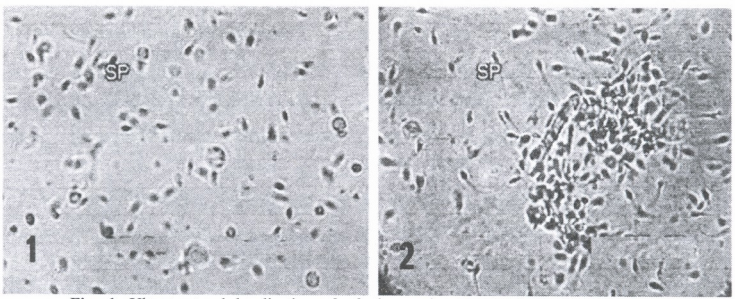

Fig. 1. Ultrastructural localization of of glycoconjugate with lectin binding sites in human spermatozoa. Localization of ConA-bs was visualized at x400 with microscope and photographed on 35 mm/400 ASA black and white film, before (1.1) and after (1.2) further incubation with a ConA.

It has been determined an inhibiting effect of ConA (100 μg/ml) on movement of sperm cells, which is expressed in decreased movement and agglutination. That agglutination of sperm cells implies the adhesion of moving sperm cells via their heads, tails or heads to tails. Adhesion of non-movable sperm cells or adhesion of movable sperm cells to slime threads, with other cells, or cell derivatives is not considered as agglutination and qualified as non-specific aggregation. Agglutination was evaluated dynamically monitoring the movement of sperm cells.

ConA (100 μg/ml) was added to the sperm cell suspension and agglutination pattern (by heads, tails or mixed type) was monitored. To evaluate agglutination rate the half-quantitative method was used rating from the mark "-" (no agglutination) to "+++" (high level). We found that agglutination corresponded to the mixed type and corresponded to "+++" level, indicating that all sperm cells are unviable due to agglutination (Fig. 1.2).

The presence of Con A-bs with specific (α-D-Man, α-D-Glc, α-D-GlcNAC) carbohydrate moieties has been revealed on sperm cell plasma membrane. The presence of ConA-bs on plasmatic membranes of sperm cells has led to the necessity of isolation and further purification of glycoconjugates followed by studies of their some biochemical characteristics. For this purpose we have used ConA immobilized on Sepharose-4B guided by the general application of immobilized plant lectins for affinity purification of glycoproteins, glycopeptides and glycolipids.

The total membrane protein fraction of sperm cells was loaded on ConA Sepharose-4B column (1.5 x 3 cm, Sigma, USA) equilibrated by 0.1 M Na-acetate buffer. Bivalent cations Mn^{+2} and Ca^{+2} (1mM) were included in buffer for the binding of glycoproteins to ConA active site. The column was maintained for 1-1.5 h at room temperature. Bound proteins were eluted with 0.1 M

Na-borate buffer (pH 8.0). Elution was performed at 0.2 ml/min and detection was monitored at 280 nm ("Gilson", France).

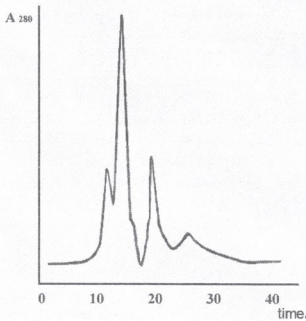

Fig. 2. Purifiфcation of human spermatozoa ConA binding proteins obtained by gel filtration chromatography, fractionated on a TSK G3000SW column (7.5 x 300mm, LKB-producter AB, Sween) equibrilated with 0.1M phosphate buffer (pH 6.6), containing 150 mM NaCl, 0.02% Na_3N. Elution rate was 0.6 ml/min, detectioned at 280nm.

Gel filtration of proteins was performed on HPLC (Waters USA; Gilson France) using TSK G3000SW column (7.5x300 mm, LKB-prdukter AB, Sween) equilibrated with 0.1M phosphate buffer (pH 6.6). Elution rate was 0.6 ml/min, detection was monitored at 280 nm. Fractionating of the proteins by HPLC purified on ConA 4B-Sepharose afinal column revealed heterogeneity of glycoconjugates with the presence of four peaks (Fig.2).

Thus, human sperm cells lectin-binding glycoconjugates were isolated and characterized. ConA influences spermatozoon, which is expressed in their decreased movement and in the mixed type, heavy agglutination. The research of lectin-recognition systems and ConA-bs are of importance in pathology as well for evaluation of physiological and biochemical conditions of cells to reveal their possible participation *in vitro* fertilization both in norm and pathology.

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ადამიანის სპერმატოზოიდების გლიკოკონიუგატები: გამოყოფა, გასუფთავება, დახასიათება და შესაძლო როლი სპერმატოზოიდ-კვერცხუჯრედის შეცნობაში

ბოლოთაშვილი თ.[1], მდებრიშვილი ნ.[1], გაგნიძე თ.[2], კობეშავიძე მ.[1], ალექსიძე ნ.[1]

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(მიღებილია 12.12.2005)

რეზიუმე

ადამიანის სპერმატოზოიდებიდან გამოყოფილი და დახასიათებულია ლექტინდამაკავშირებელი გლიკოკონიუგატები. მათი მოქმედებით სხვადასხვა ლექტინების ჰემაგლუტინაციური აქტივობა ინჰიბირება და ლ/ლდც შეფარდების რაოდენობრივი მაჩვენებლები მცირდება შესაბამისად: ConA, PSA, WGA, SNA, PNA, SBA, LAL. დადგენილია სპერმატოზოიდების მოძრაობაზე ConA-ს (100გ/მლ) შემაკავებელი გაელენა, რაც გამოიხატება მათი მოძრაობის შემცირებასა ("ადგილზე მოძრაობის" ფენომენი) და შერეული ტიპის, მძიმე ხარისხის აგლუტინაციაში. სპერმატოზოიდების პლაზმურ მემბრანაზე ციტოქიმიური მეთოდებით გამოვლინდა ConA-bs სპეციფიკური ნახშირწყლოვანი (α-D-Man, α-D-Glc, α-D-GlcNAC) ნაშთებით. ლექტინდამაკავშირებელი გლიკოკონიუგატები გასუფთავებულია ConA 4B-სეფაროზას აფინურ სორბენტზე და შემდგომი ფრაქციონირებით მაღალი წნევის ქრომატოგრაფიული სისტემით გელ-ფილტრაციულ TSK G3000SW სვეტზე.

გამოთქმულია მოსაზრება სპერმის გლიკოკონიუგატების ConA-bs-ის სტრუქტურულ და ფუნქციურ როლზე განაყოფიერებისას, შეიცნობენ რა კვერცხუჯრედის *zona pellucida*-ს გლიკოპროტეინებს, ჩაერთვებიან გამეტების შეცნობისა და ადჰეზიის მექანიზმებში.

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GREEN TEA EXTRACT AS A STIMULATOR OF HUMAN WOUNDS RECOVERY PROCESS

KHUTSIDZE T.[1], GULUA L.[1], MCHEDLISHVILI N.[1], KVESITADZE E.[1], OMIADZE, N.[1], AKVLEDIANI K.[1], ABUTIDZE M.[1], LOSABERIDZE P.[2]

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(Received February 6, 2006)

Abstract

Green tea extract was tested clinically to stimulate recovery process of wounds. More than twenty cases of treatment of wounds of different conditions with green tea extract topical application showed definitely positive results. On the base of these results we can suggest that green tea extract stimulates a process of regeneration of tissue of wounds and it can successfully be used in treatment of the wounds along with other common used medication. Use of green tea extract in clinic may help to accelerate wound healing and regeneration of new skin tissue, and subsequently prevent scar tissue formation.

Keywords: green tea extract, wound, regeneration, polyphenols, catechins

Introduction

Green tea polyphenols (referred to as GTPPs), including the four major polyphenols: epicatechin, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate (EGCG)) have been identified to possess chemopreventive and apoptotic activity against certain cancers, whereas normal epidermal keratinocytes follow a survival pathway that has not been fully elucidated [Ahmad et al. 2001; Hsu et al 2002]. The most abundant green tea polyphenol, EGCG, was reported to induce differentiation and decreased cell proliferation in epidermal keratinocytes rather than apoptosis as it does in tumor cells [Balasubramanian et al., 2002].

Results from the study [Hsu et al., 2003] demonstrated that 1) by promoting biological energy production and new DNA synthesis, both EGCG and GTPPs "reenergized" the aged keratinocytes; thus, these compounds can presumably stimulate the regeneration of keratinocytes in aging skin; and 2) by induction of p57, keratin 1 and filaggrin expression, and activation of transglutaminase, EGCG also stimulated the differentiation of the keratinocytes found in the basal layer of the epidermis. The combination of these two effects may help to accelerate wound healing and regeneration of new skin tissue, and subsequently prevent scar tissue formation. In addition, certain epithelial conditions may be amenable to treatment by topical applications of green tea polyphenols.

These results suggest that tea polyphenols may be used for treatment of wounds or certain skin conditions characterized by altered cellular activities or metabolism.

The objective of this work was to examine a possibility of the using of green tea extract in healing of different human wounds in clinical practice.

Materials and Methods

Obtaining of green tea extract. Green tea leaves were subjected to the high temperature (100°C) treatment for 5 min, as a result, enzymes present in the leaves were inactivated and the chemical composition was fixed. Next, the leaves were undergone extraction process with 70°C water, the extract was filtered, concentrated and spray dried.

Chemical analysis of the green tea extract. Content of total polyphenols was determined by Levental method based on titration of the extract with $KMnO_4$ solution; content of sugars was determined by Berthran method; pectin compounds were quantitatively analyzed by method based on precipitation of soluble pectic acids with calcium salts; amino acids were determined by method based on color reaction of the amino acids with ninhydrine reagent and then measuring absorption of the colored products at 584 nm spectrophotometrically; caffeine was determined from the calibration curve of the caffeine content in chloroform extract vs. optical density at 275 nm; catechins were separated by paper chromatography method and quantitatively determined by detection in UV spectra; water (moisture) content was determined as loss in mass at the 103°C, mineralization of the extract was done at the 525°C in combustion oven – all these procedures were done as described in [Jinjolia et al, 1983], content of organic acids was determined by titration with 0.1 N NaOH and calculated on the base of tartaric acid [Begunova, 1972].

Preparation and application of tampons of the green tea extract. 2 g of green tea extract was dissolved in distilled water at room temperature and then sterilized at 180°C. Sterile tampons were dipped into the sterilized extract of green tea extract and then wounds were plugged with the tampons. The GTPPs concentrations used are within the physiological range in humans, given the fact that daily topical application of 30 mg/ml EGCG for 30 days failed to induce dermal toxicity [Stratton et al., 2000].

Determination of wounds area, volume and regeneration velocity. A transparent sterile plate was applied on the top of the wound and a contour of the wound was marked by marker. The plate with marked contour was placed on the millimeter paper and number of square millimeters was counted. The area of the wound was expressed in sq. cms. The wound area was determined periodically with 3-4 days' intervals until full recovery. A relative velocity of regeneration of the surface of the wound was calculated according to [Fenchin, 1979] with some modification:

$$V = 100\ (S_i - S_j)\ /S_iN$$

S_i and S_j is an area of the wound at the i-th and j-th days respectively;

$N=j-i$

V was expressed in % per days.

An absolute velocity of recovery was calculated as difference in wound area ΔS divided by the number of days in which this difference was observed.

The volume of the wound divided by its area gives a velocity of granulation process towards normal direction of the wounds surface area:

$$V_g = (V_i - V_j)/S_iN$$

here V_g is a granulation velocity expressed in cm per days;

Vi and V_j are the volumes of the wounds at the i-th and j-th days expressed in cm^3. To calculate the volumes the shape of the wound was assumed to be a geometric figures such as cone, prism, cylinder, hemisphere and the like, and the volume was calculated according to respective formula.

Results and Discussions

Chemical composition of the green tea extract was as follows: 23.5 % polyphenols (including 9 % catechins), 4.0 % caffeine, 14.3% amino acids, 27.2 % sugars, 9.5 pectin, 5.3% organic acids, 10 % minerals, 6.0% water (Table 1). The extract was of yellowish color, fully dissolvable in hot/cold water; when dissolved in distilled water pH was equal to 5.6.

Table 1. Chemical composition of the green tea extract in %

Polyphenols	(among them) Catechins	Caffeine	Amino acids	Sugars	Pectin	Organic acids	Minerals	water	Total
23.5 ±0.9	(9.0) ± 0.5	4.0±0.1	14.3±0.5	27.2±0.9	9.5±0.6	5.3±0.4	10.0±0.5	6.0±0.4	99.8±0.9

Case 1. A case of patient with a cylindrical wound on the thigh; the wound of initial area 50 cm^2 and initial volume of 250 cm^3. The wound was treated with common surgery methods and drainage was put into the wound. Additionally to the condition, peritonitis was developed and as a result disorder in metabolism, namely critical shortage of albumin was observed. Because of this, regeneration process was intensively suppressed and though, the rotting process was not developed, the wound was not recovering and the brims of the wound were opening spontaneously after removing the stitches. Along with common treatment (antibiotics, physiological solution, glucose etc.) well known preparations stimulating tissue regeneration processes locally such as a *Solcoseryl gel*, 1% *ungvetum Methyluracili* were applied. Nevertheless, there was no regeneration process during first 12 days. From the 10-th day a green tea extract tampons were plugged into the wound three times a day. On the second, third and fourth day of the green tea extract treatment a hemorrhagic excretion was observed from the wound. On the sixth day the excretion was reduced and the next day it ceased at all. On the ninth day a granulation tissue appeared at the brims of the wound. On the 14-th day granulation tissue increased and respectively wound area and depth decreased; so it did until full recovery on the 30th day of treatment. Dynamics of regeneration process is represented in Fig.1. As we can see from Fig. 2 a relative velocity of recovery of the wound increases gradually over days as it could be expected. As for a velocity of granulation process towards normal direction of the wounds surface area its curve over time is not of monotonic characteristics (Fig.3).

Case 2. Patient with rotting wound on the right foot was treated with antibacterial and antiseptic preparations (solutions of 3% hydrogen peroxide and 1% *Dioxydini*, *ungvetum Laevosini*). Inflammation process was reduced though regeneration was still suppressed. Only weak granulation tissue was formed and no further improvement was achieved regardless stimulating medicines such as pills of *Solcoseryl* and *Vobenzyme*, capsules of *Imuno-ritz* and ampoules of *Tactivini* used. Patient was such treated for 23 days but with negative result. From the 24-th day green tea extract tampons were used to stimulate a regeneration process. On 7th day of green tea extract treatment it was observed an intensive growing of granulation tissues and on 27-th day the wound was fully recovered without scar tissue formation. Dynamics of recovery of the wound area is shown in Fig.4.

We have accounted more than twenty cases of treatment of wounds of different conditions with green tea extract topical application and all of them showed definitely positive results (unpublished data). On the base of these results we can suggest that green tea extract stimulates a

process of regeneration of tissue of wounds and it can successfully be used in treatment of the wounds along with other common used medication.

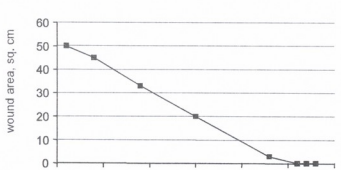

Fig. 1. Dynamics of wound area reduction over days (S - sq. cm), case 1

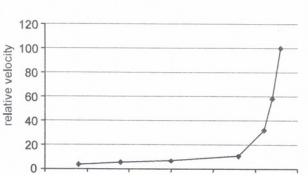

Fig. 2. Dynamics of changes of relative velocity of wound recovery over days (V - % per day), case 1

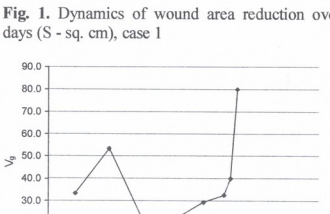

Fig. 3. Dynamics of changes of velocity of granulation process over days (Vg–cm per day), case 1

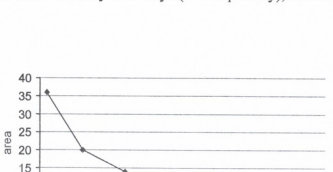

Fig. 4. Dynamics of wound area reduction over days (S - sq. cm), case 2

Acknowledgement: This work was carried out with the financial assistance of STCU (Science and Technology Center in Ukraine) within the framework of the project No: GG-122 "Creating plant bioconcentrates with antiradiation and anticancerogenic action on the base of polyphenols and working out industrial technology for their production".

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მწვანე ჩაის ექსტრაქტი როგორც ადამიანებში ჭრილობების შეხორცების სტიმულატორი

ხუციძე თ.[1], გულუა ლ.[1], მჭედლიშვილი.[1], კვესიტაძე ე.[1], ომიაძე ნ.[1], ახვლედიანი ქ.[1], აბუთიძე მ.[1], ლოსაბერიძე ა.[2]

[1] *ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი*

[2] *საქართველოს რკინიგზის ქუთაისის საავადმყოფო*

(მიღებულია 06.02.2006)

რეზიუმე

მწვანე ჩაის ექსტრაქტი გამოცდილ იქნა კლინიკაში როგორც ჭრილობის შეხორცების სტიმულატორი. ოცზე მეტ შემთხვევაში მწვანე ჩაის ექსტრაქტის ზედაპირულად გამოყენებისას სხვადასხვა სახის ჭრილობების მკურნალობის დროს მიღებულ იქნა დადებითი შედეგი. აღნიშნული შედეგების საფუძველზე ვიგარაუდებთ, რომ მწვანე ჩაის ექსტრაქტი შესაძლებელია ეფექტურად იქნას გამოყენებული ჭრილობების მკურნალობისას სხვა ტრადიციულ სამკურნალო მეთოდებთან ერთად. მისი გამოყენება აჩქარებს ქსოვილის რეგენერაციის პროცესს და შესაბამისად ხელს უწყობს ჭრილობის უნაწიბუროდ შეხორცებას.

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Biochemistry

MECHANISMS OF NA,K-ATPASE SYSTEM REGULATION BY NORADRENALINE

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Abstract

Recently it has been shown that noradrenaline (NA) inhibits Na,K-ATPase system and transfers it from OPS regime to OPM regime inducing the increase of electrogenic coefficient of Na,K-ATPase. As a result of simultaneous action of protein synaptic factor (SF) and NA, the inhibiting effect of NA is removed and Na,K-ATPase is activated. SF appears to be a catalytic activator for Na,K-ATPase.

Key words: neurotransmitters, synaptic factor, OPS regime, OPM regime,

Introduction

In the synaptic membranes of rat brain a new system is found which regulates Na,K-ATPase by neurotransmitters (NT). It is fulfilled by: noradrenaline (NA), dopamine, serotonine and acetylcholine. In its turn, their action is regulated by protein factor (SF) found in synaptosomal cytozole. After its addition, an inhibition induced by NT action on Na,K-ATPase is removed and the enzyme is sharply activated [Kometiani & Jariashvili, 2000].

Regulation of Na,K-ATPase by NT (particularly, by NA) and SF has a functional importance. Their action is specific for synaptic transfer [Kometiani & Jariashvili, 2000]. At present molecular mechanism of NA and SF acting on Na,K-ATPase is not studied, the elucidation of which gives an information about functional importance of this regulation. The presented work appears to be an attempt to clear up this problem.

Materials and Methods

Synaptic fraction from the rat brain served as Na,K-ATPase preparation. Na,K-ATPase activity (V) was determined as an ouabain sensitive part of total ATPase in $\mu molP_i$/hour/mg protein units [Kometiani & Jariashvili, 2000; Kometiani et al., 2001].

In the standard conditions the reaction medium contained: 30 mM Tris-HCl, pH 7.73 and [MgATP], [Mg^{++}], [ATP_f], [NaCl] and [KCl] of corresponding concentrations, while the reaction medium of Mg-ATPase contained: 1 mM ouabain, 145 mM KCl, 30 mM Tris-HCl, pH 7.7 and [MgATP], [Mg^{++}], [ATP_f] of corresponding to the regimes concentrations. As a dissociation constant for MgATP was taken 0.085 mM^{-1}. During OPS regime reaction medium contains: 2.08 mM MgATP, 0.42 mM free Mg^{++} and ATP_f, while during OPM regime – 1 mM MgATP, 3 mM Mg^{++} and 0.028 mM ATP_f [3, 4, 5]. Synaptic factor (SF) was obtained using the method developed

recently [Kometiani & Jariashvili, 2000]. Experimental data were subjected to strict statistical processing.

The number (n) of sites for essential activators was determined using the method of qualitative transformation of the function [Kometiani, 2005]. The essence of this method appears to be in the analysis of geometrical form of functions obtained as a result of V=f(x) function transformation-induced type $y(r,t) = \sqrt[r]{u} = f(t)$ (t=1/x, u=1/V) having different r, what means the ascertainment of asymptote existence, as such function has asymptote only in case when r=n; if a working interval of V=f(x) function is chosen properly, then it is concave (r<n) or convex (r>n) curve or maximally approaches a straight line (r=n). In this case weighed mean square error and linear measure are minimal, while choosing working interval properly ln(u))=f(t) is a monotone, convex function and ln(u)=f(lnt) function have no bending and turning sites. Calculation of the number (n) of sites for essential activators and evaluation of reliability of the result was carried on by means of computer program developed in our laboratory. The results of calculations are present as experimentally obtained number (R), its error (σ_R), and as the difference (R-n) in first approximation.

Results and Discussion

In case of relatively small free Mg^{++} and high substrate (S) concentrations ($[ATP_f] \approx 0$), the Na,K-ATPase system works in so-called OPS regime, while in case of low concentrations of S and free Mg^{++} it works in so-called OPM regime [Kometiani & Leladze, 2001; Chkadua et al., 2002; Kometiani, 1987]. Na^+ and K^+ transport simultaneously takes place in OPS regime at constant stoichiometry ($Na^+/K^+=3/2$), while in OPM regime the transport has a consecutive character and changeable stoichiometry. In OPM regime in case of high concentrations of $[K^+] > 100$ mM, the number (n) of sites for essential activators changes for Na^+ and becomes four, while transfer of three Na^+ ions may be followed by the transfer of 0 or 1 K^+ ion. Therefore, a goal of our investigation was to study the effect of NA and SF on stoichiometry of Na^+/K^+ transport.

As a result of 0.1 mM NA influence in OPS regime, the Na,K-ATPase activity is inhibited by 62.9% (Fig. 1). While adding SF into the reaction medium, NA inhibiting effect is removed. Addition of 0.001 mg/ml SF increases activity by 53%, 0.008 mg/ml SF totally removes NA inhibiting effect, while addition of 0.016 mg/ml SF activates Na,K-ATPase by 18.5%.

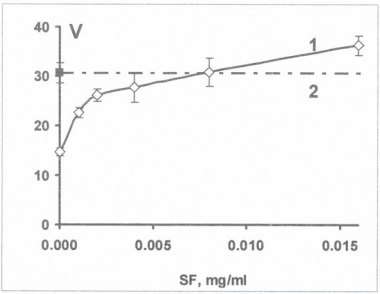

Fig. 1. Dependence of Na,K-ATPase activity on NA and SF concentrations in OPS regime. OPS reaction medium: [MgATP]=2.08 mM, $[Mg^{++}]=[ATP_f]=0.42$ mM, [NaCl]=145 mM, [KCl]=5 mM. 1. [NA]=0.1 mM, 2. [NA]=0.

In case of Na,K-ATPase in OPM regime we have an analogous situation. The data obtained come to an agreement with data given in the literature [Kometiani & Jariashvili, 2000]. Proceeding from above-mentioned the changes in the reaction medium after addition of 0.001 and 0.016 mg/ml SF have been studied, in particular, amount of essential activatory sites for Na^+ and K^+ and correspondingly transport stoichiometry as a result of NA and SF influence on Na,K-ATPase.

In OPS regime the amount of essential activatory Na-sites is three, while the amount of K-sites is two. In case of addition of 0.1 mM Na into the reaction medium their amount changes and correspondingly becomes 4 and 1. But if 0.001 NA and 0.016 mg/ml SF are simultaneously added, the situation does not change and we have $n(K^+)=2$ and $n(Na^+)=3$ (Table 1). As seen from the Fig. 2, SF does not change Na^+ affinity to its binding site, the straight lines are intersected in one point of abscissa (t_0=-0.399±0.011). On the other hand, K^+ and Na^+ ions were simultaneously and randomly bound to the enzyme [Kometiani & Leladze, 2001; Chkadua et al., 2002; Kometiani, 1987]. So it may be concluded that for Na,K-ATPase SF appears to be catalytic activator, $V=\varphi(SF)\cdot f(Na, K)$.

Table 1. Dependence of the amount of essential activatory sites on [SF] in OPS regime ([MgATP]=2.08 mM, $[Mg^{++}]=[ATP_f]$=0.42 mM). 0.1 mM NA presents in the reaction medium

Conditions of experiments	[SF], mg/ml	Number of sites of essential activators for Na^+; $n(Na^+)$ and K^+, $n(K^+)$
[NaCl]=144 mM=const $\sqrt[z]{1/V} = f(1/[K^+])$	0	R=2.0004±0.043; (R-n)=-0.0874; ⇒ $n(K^+)$=2
	0.001	R=2.0038±0.163; (R-n)=-0.2704; ⇒ $n(K^+)$=2
	0.016	R=2.0014±0.073; (R-n)=-0.1714; ⇒ $n(K^+)$=2
[KCl]=125 mM=const $\sqrt[z]{1/V} = f(1/[Na^+])$	0	R=3.0001±0.094; (R-n)=-0.2438; ⇒ $n(Na^+)$=3
	0.001	R=3.0371±0.182; (R-n)=p0.0974; ⇒ $n(Na^+)$=3
	0.016	R=3.0015±0.083; (R-n)=p0.0631; ⇒ $n(Na^+)$=3

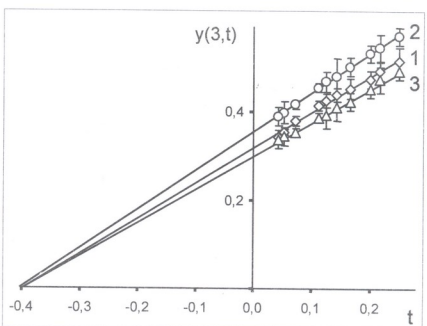

Fig. 2. Amount of essential activatory sites for Na^+ during simultaneous action of NA and SF on Na,K-ATPase in OPS regime: ([MgATP]=2.08 mM, $[Mg^{++}]=[ATP_f]$=0.42 mM).

The reaction medium: [NA]=0.1 mM; [KCl]=125 mM; 4 mM ≤ $[Na^+]$ ≤ 24 mM

(1) [SF]=0 y(3,t)=0.8000t+0.3180 -a/b= -0.3975±0.0022

(2) [SF]=0.001 mg/ml, y(3,t)=0.8784t+0.3542 -a/b= -0.4033±0.0030

(3) [SF]=0.016 mg/ml, y(3,t)=0.7509t+0.2983 -a/b= -0.3972±0.0056.

As it was noted for Na,K-ATPase in OPM regime the existence of four essential activatory sites is characteristic for Na ions and one essential activatory site – for K^+ ions. At simultaneous addition of 0.1 mM NA and 0.016 mg/ml SF, in OPM regime ([MgATP]=1 mM, $[Mg^{++}]$=3 mM and $[ATP_f]$=0.028 mM), n(Na) and n(K) (amount of essential activatory sites) do not change (Figs 3 and 4), while in case of addition of only 0.1 mM NA, n(Na)=3 and n(K)=2. It should be mentioned that at different concentrations of SF, asymptotes of $1/V = f(1/[K^+])$ and $\sqrt[3]{1/V} = f(1/[Na^+])$ functions are intersected in one point of the abscissa. So, SF appear to be a catalytic activator for Na,K-ATPase as well as in case of OPS regime.

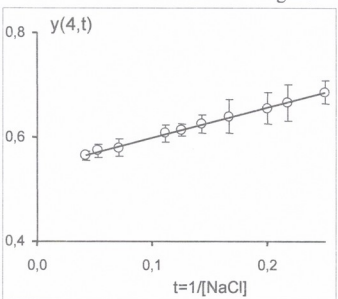

Fig. 3. Amount of essential activatory sites for Na^+ during simultaneous action of NA and SF on Na,K-ATPase in OPM regime: ([MgATP]=1 mM, $[Mg^{++}]$=3 mM, $[ATP_f]$=0.028 mM). The reaction medium: [NA]=0.1 mM; [SF]=0.016 mg/ml; [KCl]=125 mM; 4 mM ≤ $[Na^+]$ ≤ 24 mM
R=4.022±0.034; (R-n)= -0.133; ⇒ $n(Na^+)$=4

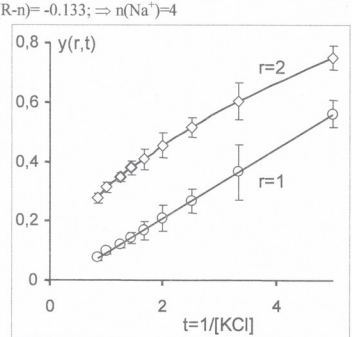

Fig. 4. Amount of essential activatory sites for K^+ during simultaneous action of NA and SF on Na,K-ATPase in OPM regime: ([MgATP]=1 mM, $[Mg^{++}]$=3 mM, $[ATP_f]$=0.028 mM). The reaction medium: [NA]=0.1 mM; [SF]=0.016 mg/ml; [NaCl]=144 mM; 0.2 mM ≤ $[K^+]$ ≤ 1.2 mM
R=1.002±0.005; (R-n)= -0.013; ⇒ $n(K^+)$=1.

Na,K-ATPase, localised in the fraction of synaptic membranes is regulated by two special systems: NT-dependent inhibiting and NT/SF-dependent activatory mechanisms. In our case, NA-induced inhibitory mechanism consists in the transfer of Na,K-ATPase from OPS to OPM regime what is followed by the changes in transport stoiciometry of cathions: $3Na^+//2K^+ \Rightarrow 3Na^+//0\text{-}1K^+$ (the amount of essential activatory sites also changes: $n(Na)=3 \Rightarrow n(Na)=4$ and $n(K)=2 \Rightarrow n(K)=1$). So electrogeneity of Na,K-ATPase system sharply increased. As a result of simultaneous action of NT and SF the process of inhibition is blocked and SF has an influences on Na,K-ATPase, as a catalytic activator.

Apparently, these two Na-dependent, intercompetition processes have an important role in the functioning of chemical synaptic transfer, especially when taking into account the fact that in these processes a sharp change in electrogeneity of Na,K-ATPase system takes place.

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Na,K-ATPაზას სისტემის რეგულაციისესისტემის რეგულაციის მექანიზმი ნორადრენალინის ზემოქმედებით

შიოშვილი ლ.

ივ. ბერიტაშვილის სახელობის ფიზიოლოგიის ინსტიტუტი

(მიღებულია 10.01.2006)

რეზიუმე

ადრე ნაჩვენები იყო, რომ ნორადრენალინი (NA) აინჰიბირებს Na,K-ATPაზას და გადაჰყავს ის OPS რეჟიმიდან OPM რეჟიმში, რაც იწვევს Na,K-ATPაზის ელექტროგენური კოეფიციენტის ზრდას. ცილოვანი ბუნების სინაფსური ფაქტორისა (SF) და NA ერთდროული მოქმედების შედეგად NA-ის ინჰიბიტორული ეფექტი იხსნება და Na,K-ATPაზა აქტივდება. Na,K-ATPაზისათვის SF წარმოადგენს კატალიზურ აქტივატორს.

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THE INOSIT-SPECIFIC LECTIN (BVL-I) FROM SYNAPTIC VESICLE MEMBRANES AND ITS INFLUENCE ON THE CA^{2+}-ATPASE ACTIVITY OF THE SYNAPTOSOMAL FRACTION

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Abstract

Inosit-specific neurolectin has been isolated from bovine head brain (BVL-I). It was established that BVL-I is a Ca^{2+}-dependent lectin, which means that its carbohydrate-binding activity reveals only in the presence of Ca^{2+} in the active center of molecule. Moreover, in presence of free Ca^{2+} ions in the incubation medium, specific activity of the BVL-I sharply increases. The lectin reveals high affinity to phosphatidilinositol and phosphatidilcholine, especially at the presence of the free Ca^{2+} ions. Hence, the participation of BVL-I in the process of neuroexocytoses was supposed. BVL-I is a sufficiently effective modulator for the Ca^{2+}-ATPase of synaptosome fraction. In a case of its specific quantity, activation of enzyme occurs. Judging by the changes of kinetic parameters (V_{max}, $K_{m,}$), it could be suggested that interaction of the BVL-I with the synaptosomal Ca^{2+}-ATPase must have a non-competitive character. It was shown that activating effect of the lectin best manifests at 100μM Ca^{2+}. This allows suggesting that calcium not only increases the BVL-I lectin activity, but in parallel amplifies its modulator influence on the enzyme. It should be considered that BVL-I participates not in the membranes fusion only, but also in the fusion-induced modulation of the Ca^{2+}-ATPase activity.

Key words: neurolectin, specific activity, modulator, hemagglutination activity

Introduction

Discovery of the neurolectins, the proteins with agglutination capacity, in the nervous tissue should be considered as one of the major achievements of the modern neurochemistry. Lectins are the proteins of non-immune origin and glycoprotein nature, which selectively and irreversibly bind with carbohydrates, without their chemical alterations. Specificity of a lectin interaction with the target molecules is determined by the characteristic carbohydrate group of the latter. Specific binding of the lectin and respective carbohydrate plays an important role in such processes as are cell-cell interaction [Nelson et al., 1995], cell recognition and adhesion [Ashraf & Khan, 2003; Dodd & Drickamer, 2001] regulation of the immune processes [Lutomski et al., 1995], cyclation of the plasma membrane, synaptogenesis [Aleksidze, 1992], regulation of cell division [Lew et al., 1994], modulation of nucleus membrane activity [Hubert & Seve, 1994], migration of neurons [Zanetta, 1998], etc. At the same time lectins are modulators of many enzyme activities [Gabius, 1997]. These interactions add more dynamic aspects to the function of lectins.

It is known that after binding of the lectins to the carbohydrate residues of cellular membrane, activity of the membrane transporter enzymes alters [Adamkiewiez et al., 1994]. This effect of the lectins may be due to structural changes in the membranes or altering of an enzyme microenvironment, which is followed with alteration of the ions' permeability. Out of these enzyme systems the best investigated is the influence of mitogenic and non-mitogenic lectins on the Na^+, K^+-ATPase system [Drickamer & Trylor, 1993]. Notwithstanding the vast number of the animal lectins' investigations, they still are less studied as compared to the plant lectins. This is particularly true in regard of the nervous system, specifically – of the brain lectins.

Considered the above mentioned, the present work was aimed to study the influence of inosit-specific lectin BVL-I isolated from synaptic vesicle membranes, on the Ca^{2+}-ATPase activity of the synaptosomal fraction.

Material and Methods

Bovine brain was used as unit for test in all experiments. Fraction of the synaptic vesicles was isolated via differential centrifuging according to De-Robertis [De-Robertis, 1969].

Assay of hemagglutination activity - Hemagglutination activity of the lectin was assessed on special titration plane-table with U-shaped wells, in 2% suspension of the rabbits trypsinized erythrocytes with an aid of 50μl titration strips, the solution under study was tittered on the plane-table, suspension was added and during two hours an agglutinations of erythrocytes was observed. Lectin activity was expressed in the lectin-specific activity (titer$^{-1}\times$ mg/ml protein $^{-1}$) [Lutsik et al., 1981]

Assay of lectin specificity - Specificty of the lectins against ligands was evaluated with hapten-inhibitory method [Lutsik et al., 1983], on the titration plane-table with U-shaped wells. After prior titration of lectin, different ligands of similar concentration were introduced into each well. Following 30 min preincubation, 2% suspension of the rabbits' trypsinized erythrocytes (50μl) was added into each well and agglutination of erythrocytes was observed for two hours. Inhibition of agglutination in presence of ligands pointed at binding of the ligand to active center of lectin, as a hapten and inactivation of a lectin did occur.

Protein Determination – was made by the method of Lowry at al. using bovine serum albumin as a standard. To 0.2ml samples of protein 2.0ml reactive C was added, which contains reactive A (20g Na_2CO_3/ 1*l* 0.1NaOH) and reactive B (10g $Na_2C_4H_4O_6$ + 5g $CuSO_4$ / 1*l* distilated water). Mixture was incubated for 10 min. Then folin was added and incubated for 30 min at room temperature. The dying intensity was estimated photocolorimetrically at λ = 750 nm [Lowry, et al., 1951].

Measurement of ATPase activity - The ATPase activity was determined according to volume of inorganic phosphorus liberated after hydrolysis of ATP (μg/ml P/mg/ml protein/ 1min). Activity of the Ca^{2+}-ATPase was determined in synaptosomal fraction of the bovine brain, according to difference between activities of the Ca^{2+}, Mg^{2+}- and Mg^{2+}-ATPases, in conditions of different concentrations of Ca^{2+} and ATP, other conditions being permanent (Tris-HCl 20mM, pH 7.4, EGTA 1mM), incubation medium contained lectin solutions of different concentrations.

The results obtained throughout the study were evaluated statistically with the students t-test. Significance was accepted at $p<0.05$.

Results

In order to extract lectins from the synaptic vesicles, several extraction buffers were tested. Maximal lectinic activity was found in the protein fraction extracted with 0.5% solution of

Triton X-100. Conditions of extraction indicate that the proteins with lectinic activity, obtained from the synaptic vesicles, are of membrane origin.

Following determination of the carbohydrate specificity of isolated lectins, the inosit-specific lectin, designed BVL-I (Bovine Brain Vesicular Inosit-Specific Lectin), has been isolated from the sum protein fraction from synaptic vesicles as described previously [Surguladze, et al., 2002]. We have also shown [Surguladze et al., 2004] that BVL-I, is a member of Ca^{2+}-dependent lectins, the so called C-type lectins. It means that lectin carbohydrate-binding activity reveals only in the presence of Ca^{2+} in the active center of lectin molecule. Furthermore, in the presence of free Ca^{2+} ions in the reaction medium, increase of the lectin activity does occur and minimal concentration of the ion which still exerts an effect, is 0.047mM. In presence of calcium, specific activity of the BVL-I increases 16-fold, which points an activator property of Ca^{2+} for the BVL-I lectin. In other words, Ca^{2+} ions participate in the regulatory mechanisms of the BVL-I activity.

BVL-I lectin manifests a sensitivity to the phospholipids. Especially BVL-I shows high affinity to the phosphstidilinositol and phosphatidilcholine, which are located primarily in the inner layer of lipid bilayer. Insofar the Ca^{2+} ions increase activity of the lectin, interaction of the BVL-I with the phospholipids was studied in a presence of the Ca^{2+} ions in the incubation medium and without these ions. The results obtained are presented in Table 1. The table shows that in the absence of the Ca^{2+} in the incubation medium, the BVL-I exerts an affinity to phosphatidilinositol and phosphatidilcholine only in presence of 200μM of Ca^{2+} in the medium, sensitivity of the lectin to the phospholipids increases somewhat - an elevated affinity is observed not to separate phospholipids only, but the total spectrum of interaction with the phospholipids increases as well.

These data verify that the BVL-I is a phospholipid-binding protein. Moreover, its affinity to the phospholipids significantly increases in presence of the calcium ions and because phospholipids are involved in the membrane fusion [Vanhesebroeck et al., 2001], it could be suggested that the BVL-I lectin must play a serious role in interactions of the synaptic vesicles and presynaptic membranes and in exocytosis of the neuromediators.

In order to clarify the inosit-specific lectin contribution into the process of synaptic vesicles activity, we studied a problem of intramembranal orientation of active center of BVL-I. It was found that the native synaptic vesicles are characterized with capacity to agglutinate the rabbit thrypsinized erythrocytes, which may be inhibited by inositol only. None of the other haptens influenced a degree of the lectin agglutination property. Thus, it could be suggested that the BVL-I in the synaptic vesicle is oriented with its active center to the outer surface of the synaptic vesicular membrane.

Since we have proposed an idea about participation of the BVL-I in synaptic transmission, it seems interesting to investigate an influence of this lectin on the Ca^{2+}-ATPase of the synaptosomal fraction. As it is known, the basic function of Ca^{2+} pumps is to maintain the Ca^{2+} gradient across the plasma membrane via the highly regulated active extrusion of Ca^{2+} from the cell. Ca^{2+} concentration up to 200μM triggers neuroexocytoses – synaptic vesicles move to the presynaptic membrane and fuse there. After neurotransmitters release Ca^{2+} pump restore Ca^{2+} homeostasis in the synapse. In that way Ca^{2+} ATPase plays a critical role for the normal function of the synapse [Khukho, 1990]. In this report we try to clarify the mechanism by which BVL-I modulates the hydrolytic activity of the Ca^{2+}-ATPase and Mg^{2+}-ATPase of the synaptosomal fraction. It was shown that in conditions of gradually increasing concentration of the lectin (within 2 μg/ml – 60 μg/ml), sharp increase of activity was noted in both Ca^{2+}- and Mg^{2+}-ATPase, as compared to the control (Fig.1). Increase of activity was directly proportional to the lectin concentration. Such effect was best revealed in a case of Mg^{2+}-ATPase. For instance, at the lectin concentration - 60 μg/ml, Ca^{2+}-ATPase activity increased by 300% against the control, and in a case of Mg^{2+}-ATPase by 400%.

The data obtained showed that above effect should be attributed to different affinity of the enzyme to the lectin as an activator. Specifically, K_m for Mg^{2+}-ATPase amounts 2.34±0.27, while for Ca^{2+}-ATPase – 17.5±0.61 (Fig.2).

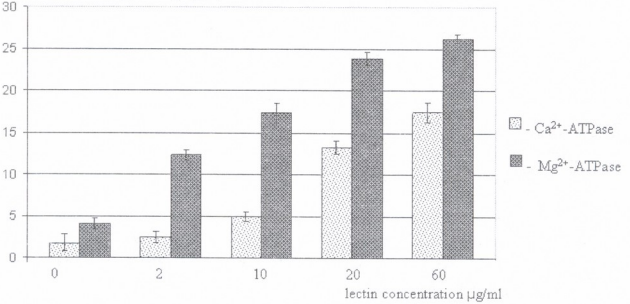

Fig. 1. Abscissa – concentration of the lectin (µg/ml); Ordinate – enzyme activity (PµM/ml protein/1min).

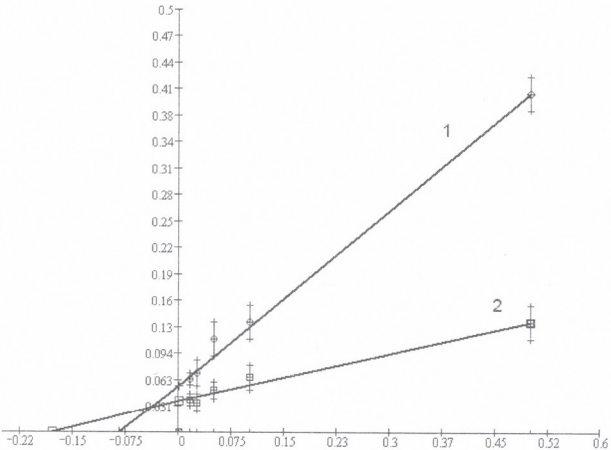

Fig. 2. The kinetic parameters of the Ca^{2+}- and Mg^{2+}-ATPases of the synaptosomal fraction in the presence of BVL-I.. Abscissa – inverse value of the lectin concentration; ordinate – inverse value of the enzymatic reaction velocity. 1 – Ca^{2+}-ATPase; 2 – Mg^{2+}-ATPase.

In the next series of experiments the above procedures were repeated in conditions of different concentration of calcium. Experiments were aimed at assessment of the Ca^{2+}-ATPase activity only, because this enzyme was an object of our particular interest.

The Fig. 3 shows that activating effect of the lectin best manifests at 100 μM Ca^{2+}. This allows to suggest that calcium not only increases the BVL-I lectinic activity, but in parallel amplifies its modulator influence on the enzymes. Ca^{2+} concentration found in our experiments, is close to that, which exists in synaptic terminal during excitation [Matthews, 2000]. Modulator influences of BVL-I on the enzymes, must be amplified because of increased lectin activity following interaction with the ion. In support for this suggestion those experiments could be invoked, in which Ca^{2+}-ATPase activity was studied during gradually increasing concentration of the Ca^{2+} ions at presence of EGTA in the reaction medium (Table 2). It is evident from the Table that in presence of 1 mM EGTA, V_{max} of the enzyme significantly decreases against the control experiments (EGTA=0). Although presence of the lectin increases the enzyme activity, it however does not return to initial values (when EGTA=0). From Table 2 it is clear also that the lectin significantly increases enzyme affinity to the Ca^{2+} ions both in presence of EGTA and in its absence (as compared to the respective control). Therefore, the data obtained show that the BVL-I lectin somewhat eliminates inhibitory influence of EGTA on the enzyme activity, although in presence of EGTA its modulator effect is attenuated.

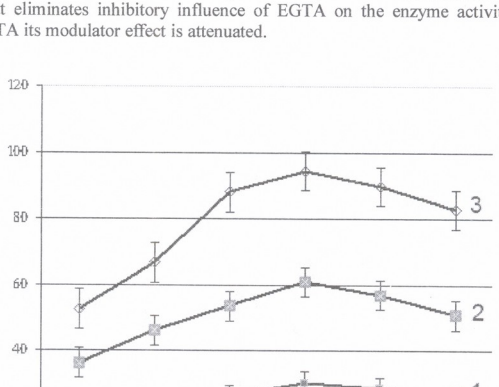

Fig. 3. Alteration of the synaptosomal Ca^{2+}-ATPase activity in the presence of the lectin in the incubation medium, at different concentrations of Ca^{2+}. Abscissa – concentration of the lectin (μg/ml); ordinate – enzyme activity (P μM/ml protein/ 1min).
1 - 5μM Ca^{2+}; 2 - 50μM Ca^{2+}; 3 - 100μM Ca^{2+}

In order to more precisely assess a character of interaction between the BVL-I lectin and synaptosomal Ca^{2+}-ATPase, we studied character of relation to the substrate, in presence of the lectin and in its absence for which different concentration were used (0.1 mM, 0.5 mM, 1.0 mM, 1.5 mM, 2.0 mM, 5.0 mM, and 10.0 mM). It was found that incubation medium containing 2 mM Mg^{2+}-ATP is the most optimal for revealing the enzymatic activity of the Ca^{2+}-ATPase. Analogous results were obtained when the BVL-I was presented in the incubation medium. From the data

presented in Table 3 is clear that the lectin increases both V_{max} and K_m (statistically not reliable. $p>0.1$).

Judging by the changes of kinetic parameters, it could be suggested that interaction of the BVL-I lectin with the synaptosomal Ca^{2+}-ATPase must have uncompetitive activation character.

Table 1. Influence of the phospholipids on hemagglutination activity of the BVL-I lectin

Phospholipids	Minimal amount of a phospholipid (mg/ml), which inhibits hemagglutination activity of the lectin	
	Without Ca^{2+}	In presence of Ca^{2+}
Phosphatydilinositol	0.00625	0.000195
Sphyngomyelin	>100	0.0179
Phosphatic acid	>100	0.0025
Phosphatydilcholine	0.00078	0.00019

Table 2. Alterations of kinetic parameters in presence of EGTA

Kinetic parameters	EGTA=0			EGTA=1 mM		
	L=0	L=20 μg/ml	p	L=0	L=20 μg/ml	p
Vmax	12.78±0.008	24.7±0.039	<0.02	10.35±0.0025	16.68±0.016	<0.02
Km	0.49±0.006	0.34±0.013	<0.05	0.19±0.018	0.056±0.007	<0.05

Table 3. Alterations of kinetic parameters, in the presence of the lectin, at various concentrations of Mg^{2+}/ATP

Kinetic parameters	L=0	L=20 μg/ml	p
V_{max}	13.06±0.6	18.7±1.6	<0.05
K_m	0.25±0.078	0.11±0.076	>0.1

Discussion

In the present work, we demonstrated that BVL-I is a Ca^{2+}-dependent lectin. Moreover, at the presence of free Ca^{2+} ions in the incubation medium its hemagglutination activity sharply increases. In other words, BVL-I may play a role of some kind of Ca^{2+}-sensor. Our results show that BVL-I have a high affinity to the negativly-charged phospholipids such as phosphatidilinositol and phosphatidilcholine. We demonstrate that its affinity to the phospholipids significantly increases in presence of Ca^{2+} ions and because phospholipids are involved in the membrane fusion, it could be suggested that the BVL-I must play a serious role in interactions of the synaptic vesicles and presynaptic membranes and in exocytosis of the neuromediators. Moreover, BVL-I in the synaptic vesicle membrane is oriented with its active center to the outer surface of vesicle membrane to the synaptic terminal. According to above mentioned, it could be suggested that at final stage of fusion of synaptic vesicle with the presynaptic membrane, along with the other vesicular proteins, the BVL-I should participate too. We suggest that the main function of the BVL-I is attachment to the lipid components of target membrane, in response to increased concentration of the Ca^{2+}-ions. It is fairly feasible just this interaction is a prerequisite of the two membranes fusion. It was shown that BVL-I induces activation of the synaptosomal Ca^{2+}-ATPase in a dose-

dependent menner. Activation of the enzymes, including ATPases was demonstrated previously for different vegetable lectins [Conrad & Rudiger, 1994], but little is known about role of animal lectins in such interactions. We have shown that in a case of BVL-I specific quantity, activation of the synaptosomal Ca^{2+}-ATPase does occur, which manifests in pumping of excess of Ca^{2+} ions outside a synapse. It should be considered, therefore, that during neuroexocytosis, because of binding of certain number of the vesicles to the presynaptic membrane, in which must participate BVL-I, the synaptic membrane Ca^{2+}-ATPase is activated automatically, or, in our opinion, the BVL-I participates not in the membranes' fusion only, but also in the fusion-induced modulation of the Ca^{2+}-ATPase activity. Notably, the lectin alters the enzyme activity in such a way that transfers it into more "economic" regime and increases enzyme affinity to the substrate. As a result, even in conditions of the lower concentration of Mg^{2+}/ATPase, a V_{max} of the enzyme is tangibly higher against the control one.

Thus, BVL-I should be one of those regulatory components of such complex processes, as the synaptic transmission is.

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ინოზიტ-სპეციფიკური ლექტინი **(BVL-I)** სინაფსური ვეზიკულების მემბრანებიდან და მისი გავლენა სინაფტოსომური ფრაქციის Ca^{2+}-ატფ-აზას აქტივობაზე.

სურგულაძე ნ., კოშორიძე ნ., ვარდიაშვილი თ.

ბიოქიმიისა და ბიოტექნოლოგიის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 25.01.2006)

რეზიუმე

ხარის თავის ტვინის სინაფსური ვეზიკულებიდან გამოყოფილია ინოზიტ-სპეციფიკური ლექტინი BVL-I. დადგენილია, რომ BVL-I არის Ca^{2+}-დამოკიდებული ლექტინი, რაც იმას ნიშნავს, რომ მისი ნახშირწყალ-დამაკავშირებელი აქტივობა მუდავნდება მხოლოდ ლექტინის მოლეკულის აქტიურ ცენტრში კალციუმის იონების თანაობისას. საინკუბაციო არეში თავისუფალი Ca^{2+}-ის იონების თანაობისას BVL-I ლექტინის სპეციფიკური აქტივობა მკვეთრად იმატებს. ლექტინი ამცდავნებს მაღალ თვისობას ფოსფატიდილინოზიტოლისა და ფოსფატიდილქოლინის მიმართ, განსაკუთრებით კალციუმის თანაობისას. შემოთავაზებულია BVL-I-ის ნეიროეგზოციტოზში მონაწილეობის მოდელი. BVL-I არის ეფექტური მოდულატორი სინაპტოსომური ფრაქციის Ca^{2+}-ატფ-აზასათვის. მისი კონკრეტული რაოდენობის პირობებში ადგილი აქვს ფერმენტის აქტივაციას. კინეტიკური პარამეტრების ცვლილების მიხედვით სავარაუდოა, რომ ლექტინ BVL-I-ის ურთიერთქმედებას სინაფტოსომურ Ca^{2+}-ატფ-აზაზე ჰქონდეს არაკონკურენტული აქტივაციის ხასიათი. ლექტინის აქტივატორული ეფექტი მაქსიმალურად მუდავნდება 100μM Ca^{2+}-იონის თანაობისას. ეს გვაფიქრებინებს, რომ კალციუმი არამარტო ზრდის ლექტინ BVL-I-ის აქტივობას, არამედ პარალელურად აძლიერებს ფერმენტებზე მის მოდულატორულ გავლენასაც. სავარაუდოა, რომ BVL-I მონაწილეობს არამარტო მემბრანათა შერწყმაში, არამედ შერწყმის შემდეგ, სამიზნე მემბრანის Ca^{2+}-ატფ-აზას აქტივობის რეგულაციაშიც.

denaturation transition of troponin T has not been detected [Morosova et al., 1988] neither by calorimetric, nor by fluorescence methods. It was concluded that troponin T molecule in solution does not have stable, highly ordered structure [Morosova L.A., et al., 2001]. That is why we carried out the experiments to investigate the thermostability of troponin T.

Materials and Methods

Troponin was extracted from rabbit skeletal muscle according to Staprans [Staprans, et al., 1972]. Chromatography of troponin was carried out on DEAE-celluloze [Perry, et al., 1974] to separate its components. The polyacrylamide gel electrophoresis in the presence of sodium dodecile sulfate was used for examination of homogenity of troponin T preparations. The thermal denaturation of troponin T was studied by means of the differential adiabatic scanning microcalorimetry DASM–4 ("Biopribor" Puschino, Russia). The heating rate was 1 K/min, concentration of protein – 3 mg/ml. The intrinsic fluorescence of proteins was carried out by Shimadzu RF-5000 spectrofluorimeter (Japan) in thermostatic cell at excitation wavelength λ_{exc}=296 nm. The heating mean-rate was 0.5 K/min and the protein concentration of 0.2 –0.3 mg/ml.

As troponin T is badly dissolved at physiological ionic strength and the study of the intrinsic fluorescence spectrum of trpopnin T showed no essential changes in the fluorescence parameters at high concentration of KCl, in our experiments we increased KCl concentration in protein solution up to 1 M.

Results and Discussion

We were interested if troponin T melecule restores its conformation after the heating, i.e. if renaturation occurs. Therefore we studied the dependence of intrinsic fluorescence parameters on the temperature and investigated the melting curves of troponin T placed in the cell of the differential adiabatic scanning microcalorimetry. After the primary thermal denaturation the protein was situated in refrigerator for 18 hours and the measuring was carried out again. While the primary heating of troponin T at λ_{exc}=296 nm shift of the spectrum to the long wavelength (3.5 nm) was observed (Fig 1). This proves the change of the conformational state of troponin T molecule and rearrangement of tryptophan residues towards water environment. At the second heating of troponin T the curves of fluorescence spectrum don't almost differ from the primary curves. They are slightly smoother and are situated above the primary heating curves on 0,5nm. At the same time fluorescence intensities of troponin T decrease with the heating temperature growth. The conformational changes of troponin T can be observed as a small derivation of the smooth temperature dependence of protein fluorescence intensity. The intensity curve of the second heating of troponin T is identical with the curve of primary heating of the protein. It allows us to conclude that the structure of troponin T restores after the first thermal denaturation. Hence, one can conclude that troponin T molecule is thermostable in investigating conditions (20 mM Tris –HCl ; pH 7,3; 1 M KCl).

Skeletal muscle troponin T contains the following fluorescence amino acid residues: tyrosine in 156, 159, 210, 227 positions and tryptophan in 206 and 259 positions [Pearlstone, et al, 1976; Stefancsik, et al, 1998]. At λ_{exc}=296 nm only triptophans are got exited [Chernitsky et al, 1972]. They are situated in C-terminal domain of troponin T. It is known that the chymotripsin effect on the bond between Y-158 and L-159 amino acid residues results in the formation of two fragments of troponin T: THT_1 (residues 1-158) and THT_2 (residues 159-256) [Pearlstone, et al,1976]. Chymotripsin acts like this on the whole troponin complex. In the soft proteolysis

conditions the same N-tail fragment of troponin T (THT_1) and complex consisting of troponin I, troponin C and C-tail fragment of troponinT (THT_2) are received [Sehaertl et al, 1995]. So tryptophans are placed in THT_2–fragment of troponinT. During the fluorescence process the amino acid residues that are located at up to 10 nm distance from fluorophore, influence the intrinsic fluorescence parameters of protein [Lacovich, 1986]. Therefore tryptophans in 206 and 259 positions located in C-terminal domain of troponin (THT_2) stay insensible to the process that occur in N-tail fragment (THT_1) of troponin T. That's why the conclusion about thermostability of troponin T molecule based on the study of dependence of intrinsic fluorescence parameters on temperature may concern only THT_2 –fragment of troponin T, but not the whole troponin T molecule in case of stretched one.

This conclusion is confirmed by our microcalorimetric investigations. The microcalorimetric curves are shown in Fig.2. The primary heating curve is represented by two separated peaks with additional transition among them. The dotted line in Fig.2 shows the melting after the cooling of protein (the second melting). As is seen, after thermal denaturation only a part of a structure of troponin T molecule renaturates. The denaturation of troponin T is not reversible: only a part of troponin T molecule (~60%) that corresponds to the peak at high transition temperature renaturates. The denaturation of the region of molecule which is represented by the melting peak at low temperature is not practically reversible. In previous study [Getashvili et al., 2005] we demonstrated that the melting profiles of microcalorimetric curves of troponin T are greatly depended on solvent pH. Considering the process of deprotonization of ionized groups in troponin T molecules we can assumed that the C-terminal globular fragment of troponin T participates in the formation of high-temperature peak of thermal denaturation, while the low-temperature peak is represented by the melting of N-terminal tail of the protein. Proceeding from the data of protein secondary heating described above we can assume that C-terminal region of troponin T molecule renaturates, while the N-tail part of molecule doesn't restore its primary structure. So while investigating the thermostability of proteins it is not enough to use only the method of intrinsic fluoroscence as it can lead to wrong conclusions.

Thus, the C-terminal fragment of troponin T molecule is supposed to have more stable structure, which is able to restore at least partially after the thermal influence. It is just the part of molecule where troponin T binding sites with tropomyosin, troponin I and troponin C are placed.

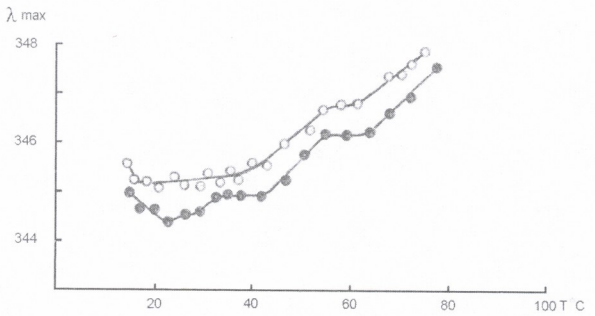

Fig.1. The dependence of troponin T intrinsic fluorescence spectrum position on the temperature. λ_{exc}=296 nm, buffer: 20mM Tris-HCl, 1M KCl, pH 7.3. • – primary heating. o – secondary heating.

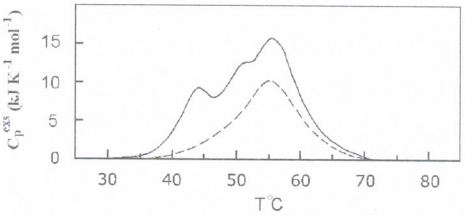

Fig.2. Temperature dependence of apparent excess heat capacity of troponin T Buffer: 20mM Tris-HCl , 1M KCl, pH 7.3. Solid line - primary heating, dotted line - secondary heating.

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ტროპონინ T-ს თერმოსტაბილურობა

ბუთხუზი ნ., ერისთავი თ., გვრიტიშვილი ა., ბუტი ე., ზაალიშვილი მ.

მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 17.01.2006)

რეზიუმე

ნაჩვენებია, რომ ტროპონინ-T მოლეკულის N-ტერმინალური ნაწილი არ რენატურირებს თერმული ზემოქმედების შემდეგ, მაშინ როცა C-ტერმინალური ფრაგმენტი აღდგება 60%-ით. ვვარაუდობთ, რომ ტროპონინ-T მოლეკულის C-ტერმინალური ნაწილს გააჩნია უფრო სტაბილური სტრუქტურა, რომელსაც შეუძლია აღდგეს. სწორედ მასშია განლაგებული ის საიტები, რომლებიც აკავშირებს ტროპონინ-T მოლეკულას ტროპომიოზინთან, ტროპონინ I- და ტროპონინ C-სთან.

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Biophysics

INFLUENCE OF BIOREGULATOR VILON ON VITAL FUNCTIONS OF BIOPOLIMERS AND CHROMATINS *IN SITU*

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Abstract

Influence of the synthetic bioregulator dipeptide Vilon (Lis-Glu) on vital functions of human lymphocyte cell cultures and thermodynamic stability of membranes, cytoplasmic protein and chromatin was investigated *in situ*. It was shown that Vilon does not affect on the structural changes of cytoplasmic proteins and membranes *in situ*, and DNA *in vitro* at physiological NaCl concentration and pH 7.0. However, it has an influence on the metabolic heat (-Q) and causes partial decondensation of "silent"-heterochromatin.

Key words: bioregulator, microcalorimetry, metabolic heat, denaturation heat

Introduction

New type of synthesized preparations – peptide bioregulators have been investigated and successfully applied in gerontological and geriatric practice [Khavinson, 2002]. One of such synthetic bioregulators, dipeptide Vilon (Lis-Glu) belongs to those compounds, which are not directly bound to DNA at selected sites, but interfere with transcription of DNA or the replication process. It was shown that this synthetic preparation activates some metabolic changes regulated through the genes in chromatin domains [Lezhava, 2004; Meskhi, 2004]. In the given work we tried to evaluate an influence of Vilon on metabolic heat (-Q), denaturation heat (Q_d), and thermodynamic stability of cytoplasmic proteins, chromatin complex in cell culture of human lymphocytes and on the melting parameters of calf thymus DNA in dilute solutions at physiological concentration of sodium salt at pH7.0. A goal of this work was to establish what influence have low concentrations of Vilon on biochemical processes proceeding in lymphocytes, if Vilon is bound to DNA *in vitro* at physiological conditions and if it influences structural organization of protein complexes and hetero- "silent" and "active' chromatin.

Materials and Methods

Modified Differential scanning microcalorimetric (DSC) investigation was carried out at sensitivity of 0.1 μW, temperature range - 5–150°C, and the measuring capsule volume - 0.3 cm^3. The accuracy of absolute temperature measurement is better than 0.05°C. The calculation of metabolic heat (−Q), heat of denaturation (Q_d), denaturation temperature (T_d) and width of the peak

at half height (ΔT_d) was carried out by a program developed by us. Deconvolution of curves was conducted with Origin 6.0 (MicrocalTM Software Inc.). The error in determination –Q and Qd of lymphocytes was less than 12%. T_d was determined up to ±1°C and ΔT_d to ~ 0.5°C [Monaselidze, 2006]. Vilon – dipeptide (Lys-Glu) – was prepared by a directed chemical synthesis on the base of amino acid analysis of the complex preparation of thymus – thymalin [Khavinson, 2002].

Results and Discussions

Fig. 1 shows the microcalorimetric record of thermal effect observed in the process of heating of human lymphocytes in the culture medium. The intensive asymmetric exothermic peak with maximum at about 37°C is observed in the temperature range from 8 to 40°C. The value of heat evolution (-Q) calculated from area under the peak is equal to 13.4±2.0 J/g of dry biomass. According to numerous data, the value of metabolic heat (-Q) is generally associated with respiration of cells with biochemical reactions connected with modification of nucleic acids and macromolecular synthesis, and reflects degree of cell survival and apoptosis [Monaselidze, 2006]. 4 peaks and 3 shoulders are observed in the temperature range from 40 to 120°C. The integral heat calculated from areas under the endotherms is equal to 26.5±3.0 J/g of dry biomass. Systematic calorimetric measurements show that in the temperature range 40-85°C cytoplasmic structures, nuclear matrix, and chromatin in 80-120°C, are denaturated [Monaselidze, 2006]. We carried out the deconvolution of this curve on Gaussian constituents and obtained eight thermal transitions [Meskhi, 2004]. Q_d was estimated on the basis of endotherms VII and VIII, the value equal to 90.5±9.0 J/g DNA was obtained. This value coincides with the value of Q_d of chromatin in solution, which is equal to 75.5±7.5 J/g DNA [Monaselidze, 2006].

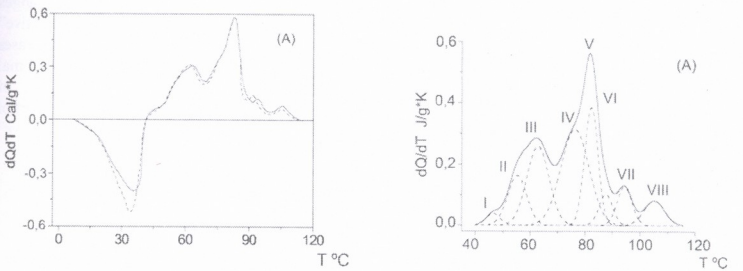

Fig. 1 (A) Thermal effects observed during heating of lymphocytes from donors in culture medium (pH7.1), heating rate 0.1°C/min. Curve 1 (solid line): lymphocytes non-treated with Vilon ~ $2x10^{-7}$ cells (dry biomass is 4.8mg; DNA is 0.095mg). Curve 2 (dash line): lymphocytes treated with 0.05 mM/ml Vilon for 48 hours, $1.5x10^{-7}$ cells (dry biomass is 4.4mg; DNA is 0.092mg). (B) Deconvolution of curve 1.

So we come to the conclusion that chromatin in the composition of human lymphocytes has two thermal transitions at 100 and 105°C and denaturation heat of chromatin is equal to 90.5±9.0 J/g of DNA. The comparison of the curves presented in Fig.1 shows that Vilon does not cause any significant effect on the curve profile of the dependence Q_d=f(T) and value Q_d in the temperature range 40-80°C, that confirms that Vilon does not have influence on stability of proteins and protein-membrane complexes, but 10% decrease of –Q is observed in this range. Vilon

causes the redistribution of heat between of the endotherms VII and VIII, corresponding to denaturation of active heterochromatin [Meskhi, 2004; Monaselidze, 2006]; particularly, Vilon increases Q_d of the endotherm VII at 95°C. We also showed that T_m and ΔH_m of DNA at molar ratio Vilon/$DNA_{b.p.}$ (in the range R=0.02-0.06) at physiological conditions did not change. So we come to the conclusion that a law concentration of Vilon in physiological conditions does not influence directly on stability of DNA *in situ* and nucleosomal DNA in the composition of active chromatin, but it influences on biochemical process proceeding in a living cell and causes decondensation of "silent" chromatin.

The authors of work [Studitsky, 2004] proposed a two-step model of chromatin structure change connected with gene indication on the basis of last achievements in the field of chromatin remodulation. Each chromosome occupies a certain area in a nucleus called a chromosome territory. Inactive genes are located in the inner part of this territory – "silent" chromatin, while active genes are concentrated on the surface of the territory – "active" chromatin. Induction of gene has two stages. Untranscriptional "silent" heterochromatin, existing in high compacted state, not very accessible state for regulator protein complexes, is partially decondensed and transits into a state ready for transcription. In this unfolded part of heterochromatin, nucleosome DNA is, mainly, in composition of 30nm fibers projecting from heterochromatin as loops. The structure of loop of 30 nm fibers is decondensed (unfolded) on the second stage and a degree of decondensation depends on activity of regulator enzymes. It was shown that at high effectiveness of transcription, the 30nm fiber turns into 10nm fiber with tightly packed nucleosomes "side-to-side", nucleosomes separated from each other by linear areas of DNA and 5nm fiber consisting of unfolded nucleosomes.

Our data confirm the proposed model. According to them, Vilon has a specific influence on chromatin *in situ*, decondensing only the heterochromatin structure (T_d=105°C). Basing on the fact that Vilon causes decrease of metabolic heat by about 10%, and it does not cause any changes in thermodynamic stability of membranes, proteins, and DNA, and weakly influences on "active" chromatin (T_d=90°C), we suppose that Vilon initiates demethylation of specific methyltransferases [Bannister, 2001], which label Lis-9 at the end of histone H3 in composition of the nucleosomal core of the "silent" chromatin [Bannister, 2001]. As a result, chromatin domains Hp1 of heterochromatin protein 1 does not recognize the binding sites on heterochromatin and this part of chromatin remains unprotected. Consequently, this part of chromatin exposes properties of "active" chromatin and denaturates at 90°C, which is observed experimentally.

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ბიორეგულატორ ვილონის ზემოქმედება ბიოპოლიმერების სასიცოცხლო ფუნქციასა და ქრომატინზე *IN SITU*

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(მიღებულია 13.02.2006)

რეზიუმე

შესწავლილი იქნა სინთეზური ბიორეგულატორის, დიპეპტიდ ვილონის (Lis-Glu) ზემოქმედება ადამიანის ლიმფოციტების უჯრედების კულტურის სიცოცხლისუნარიანობაზე და მემბრანების, ციტოპლაზმატური ცილებისა და ქრომატინის თერმოდინამიკურ სტაბილურობაზე *in situ*. ნაჩვენები იქნა, რომ NaCl-ის ფიზიოლოგიური კონცენტრაციისა და pH7.0 პირობებში ვილონი გავლენას არ ახდენს ციტოპლაზმური ცილებისა და მემბრანების სტრუქტურულ ცვალებადობაზე *in situ*, და დნმ-ის სტრუქტურაზე *in vitro*. ამავე დროს იგი ზეგავლენას ახდენს მეტაბოლურ სითბოზე (-Q) და იწვევს ჰეტეროქრომატინის ნაწილობრივ გაშლას (დეკონდენსაციას).

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ALTERATION OF TOBACCO RAW MATERIAL QUALITY INDICES IN THE PROCESS OF "AGING"

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Abstract

Possibility of processing of tobacco raw material under the conditions of partial fermentation has been studies. Organoleptic and other qualitative indices of tobacco, like the dynamics of oxygen index variation during aging under the full and partial fermentation conditions have been investigated. It was established that prolonging of the process of aging may be responsible for shortening of the expensive process of fermentation, while the qualitative indices of the production remain the same.

Key words: aging process, partial fermentation, oxygen index, nicotine

Introduction

Tobacco is one of the important technical crops among the cultivated plants in Georgia. For today tobacco industry is mainly destroyed there and users' needs for tobacco are fully satisfied on the expense of imported production.

It must be motioned that native raw material of tobacco is distinguished with its qualitative and sorts diversity (Djaparidze, 1975). It is known that 90% of the cost of smoking production depends on tobacco raw material. This material possesses a complex of qualitative indices, which are responsible for the quality of the integrated products.

In processing of tobacco raw material "aging" or long-term storing after fermentation is one of the leading technological processes [Chouteau et al, 1955; Kintsurashvili, 2006; Tasheva, 1979]. While determining the qualitative changes of the raw material, the main obstacle is necessity of simultaneous testing of different age tobacco samples [Mokhnachev & Babenko, 1986; Mokhnachev et al, 1984; Mokhnachev, 1984; 1985]. The question of improving the quality of tobacco raw material, especially perfection of technologies of its processing, became very popular.

The purpose of our study was intensification of the process of tobacco fermentation, also modification and development of fermentation industry; in particular, improving the qualitative characteristics of tobacco under the conditions of partial fermentation.

Materials and Methods

Tobacco raw material of the sort "Trapezondi" of the 1st, 2nd, and 3rd brand, cultivated in a private section of Imereti (West Georgia), has been used for investigations.

For studying the processes taking place during "aging", tobacco raw material was stored for a long period under usual conditions in the form of standard packs. From time to time samples for analyzing were taken from different packs and qualitative characteristics were determined there. Samples of one and the same quality tobacco were thoroughly mixed, dried, chopped and analyzed. The taste and flavor of tobacco, indices of cremating, humidity and content of nicotine were investigated in experimental material. For studying different indices of the testing material the method of spectrophotometrical analysis has been used [Mokhnachev, 1984].

Content of nicotine in tobacco was determined using our modified method [Kintsurashvili, Melkadze, 2005].

Results and Discussion

From the literature it is known that perfection of the fermentation process is evaluated according to the limiting value of "oxygen index" (OI<0.1). We decided to study the variation of oxygen index in the process of tobacco aging in the cases of full and partial fermentation.

Analyzing samples were divided in two groups. One was fully fermented, the other – only partially. The samples were stored for "aging". The qualitative indices determined before "aging" were taken as control. Once in every 3 month, during 15 months both type of experimental samples were tested. The results of investigations are given in the table.

Table 1. Qualitative indices of tobacco integrated products during the process of aging

Quality	I brand		II brand		III brand	
	Full fermentation	Partial fermentation	Full fermentation	Partial fermentation	Full fermentation	Partial fermentation
Oxygen index (OI)	0.05	0.06	0.07	0.07	0.10	0.12
Nicotine content (mg/g)	16.7	16.0	15.39	15.25	15.24	15.0
Humidity (%)	15.3	15.2	16.0	16.2	16.7	16.4
Organoleptic properties Flavor(mark)	18.0	7.5	17.4	17.2	17.5	17.2
Taste(mark)	18.8	19.0	17.6	17.65	17.6	17.6
Burning capacity (puff per piece of cigarette)	16.6	16.5	18.2	18.0	20.1	20.0

First of all attention must be paid on smoking properties, which is reflected on flavour and taste of tobacco smoke. Usually "aging" for one or more year is responsible for improving of smoking properties, but later (especially at the end of "aging") significant worsening of the test qualities of tobacco smoke takes place.

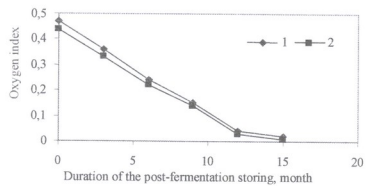
The results obtained on cremating properties of tobacco raw material are interesting from the practical point of view. The experimental results have revealed that post-fermenting storing of tobacco is due to basic improving of burning quality. In this regard "aging" is undoubtedly favourable for obtaining the raw material with low burning quality.

During aging regularly decreases the humidity of tobacco raw material. This takes place in the process of fermentation and prolongs in post-fermentation period of storing. According to this fact, it has been concluded that the raw material with low humidity (less than 12%) can not be hidden for aging.

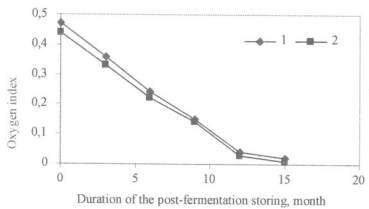
As it was expected, decreasing of nicotine content in tobacco (on the expense of oxidation) was mentioned. Therefore, storing of tobacco, containing much nicotine (more than 2%) may cause improving of its quality. But if tobacco material with low nicotine content (about 1%) is kept, the expectable result is that it will become "empty", i.e. it will lose smoking qualities.

According to the obtained results, it may be concluded that aging of tobacco for a year period finally is responsible for improving the quality properties of the raw material, which may be used in manufacturing technology.

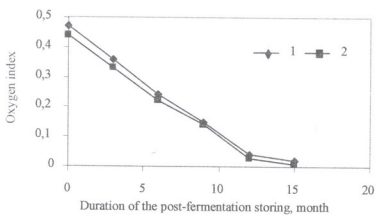
Also the dynamics of variation of the oxygen index has been studied in fully and partially fermented samples. Results of measuring are given on Figure 1 (a, b, c).



a – I brand



b – II brand



c – III brand

Fig.1. Dynamics of oxygen index variation during long-term storing of tobacco raw material
1 – full fermentation, 2 – partial fermentation

From the Figure it is clear that the oxygen index are almost the same in both cases (1-full fermentation, 2-partial fermentation), while the duration of fermentation was significantly reduced in the case of partial fermentation.

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COMPARATIVE STUDY OF THE QUANTITATIVE PARAMETERS OF BERRIES AND SEEDS IN THE AUTOCHTHONOUS RED GRAPE VARIETIES OF THE KOLKHIS (WESTERN GEORGIA)

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Abstract

Seed number per fruit, berry length, breadth, index=breadth/length, seed length, breadth and seed stalk length have been determined and biometric analysis of obtained data has been carried out to identify similarity distances and intercultivar variation of old Kolkhic red grapevine varieties. Total ten varieties of red grapevine have been studied. Nine of them – Aladasturi, Chodi, Kachichi, Koloshi, Machkvaturi, Makhvateli, Odjaleshi, Paneshi and Shonuri, are old aboriginal varieties of the Kolkhis. One variety – Saperavi is currently widespread in Kakheti. Intercultivar variability of quantitative parameters has been revealed and similarity distances among varieties have been determined by Tree Clustering method.

Key words: Berry, cluster analysis, grapevine varieties, seed.

Introduction

Worldwide, grapes (*Vitis* spp.) are among the most important plant species cultivated with an area of about 9 million ha. The leading cultivated species by far is *Vitis vinifera* L. (Vitaceae). Domestication of the grapevine (*V. vinifera* ssp. *sativa* DC.) and development of the technology of viticulture and winemaking initially occurred in the southern Caucasus about 6000 to 4000 BC [Mullins et al., 1992; Jackson, 1994; Damania et al., 1997; Sefc et al., 2003], which determines high importance to study the autochthonous grape varieties in the Caucasus and namely in Georgia.

From this ancestral center of the grape origin, viticulture spread around the Mediterranean Basin to the Far East and was later introduced to the New World, particularly by settlers from the winegrowing countries of the Mediterranean area [Sefc et al., 2003]. The rapid domestication of the grapevine was supported by the manifold uses of grapes for producing table fruit, wine, juice, and raisins. The modern grapevine varieties show great genetic diversity and broad adaptability to different soils and climates. Genetic differentiation among extant human populations was used to show that the spread of agriculture through Europe was accompanied by demic diffusion of Neolithic farmers [Sokal et al., 1991]. It is assumed that spread of viticulture mainly involved the dissemination of domesticated varieties of grapevine, while the use of indigenous wild vines is discussed as alternative origin of grapevine cultivars [Sefc et al, 2003[. It seems that variation in *V. vinifera* was insufficient to permit universal cultivation and led to the domestication of other wild species, such as *V. vinifera* ssp. *silvestris* Gmel. in the Caucasus, Europe and Near East; *V.*

labrusca L. and *V. rotundifolia* Michaux in America; and, *V. amurensis* Ruprecht in China. This caused some taxonomic confusion in the taxonomic status of the modern grapevine varieties.

Only *V. vinifera* has some 10 000 varieties worldwide. Among them, about 500 names of Georgian autochthonous grapevine varieties are known [Javakhishvili, 1986; Ketskhoveli et al., 1960]. In 1860, the *V. vinifera* was virtually wiped out in the places of its origin, when an aphid, *Phylloxera vastatrix* had accidentally introduced into France, and within a few years had ravaged all vineyards in Europe and as well in the Caucasus, including Georgia. It normally lives in the roots of some American vines without doing any great harm, but once they get into the root system of *V. vinifera*, it soon kills the plant. It was soon noticed that the few American vines found growing in Europe as exotics were not affected, and grafting quickly began. Currently, almost all Georgian grape varieties are grafted on rootstocks of American vines.

This disaster made it necessary to undertake urgent steps for *ex situ* conservation of old, endangered and autochthonous grapevine varieties by set up of living collections in Georgia, which was started in 30es of the XX century. Nowadays, 929 varieties are protected in the living collections of the State Agrarian University in Dighomi and Mukhrani; and, in the collections of the Georgian Scientific-Research Institute of Horticulture, Viticulture and Winemaking in Telavi and Skra. Among them, 701 are "original" and only 248 are autochthonous Georgian varieties remained from the known 524 varieties [Chkhartishvili, Tsertsvadze, 2004]. Some aboriginal Georgian grape varieties is still possible to find in the private grounds of peasants and in several small living collections, such as grapevine collection of the G. Eliava National Museum in Martvili district founded in 1972 [Eliava, Tsotsoria, 2002]. This collection containing 24 old Kolkhic grapevine varieties was used in the present study as source for material collection.

Currently, big attention is given to conservation, characterisation, collection and utilization of genetic resources of grapevine varieties, especially of old, endangered and autochthonous ones worldwide. The European Gene Banks (EURISCO) and EU program Genres081 attempt to develop database for all grape varieties including that from Georgia. Main approach used today for identification of genetic diversity of grapes is based on DNA technology for detection of grape intracultivar variation [This et al., 2004]. However, several micromorphometric and QTL methods for evaluation of berry and seed quantitative parameters of taxonomic importance are widely used in the studies of genetic differentiation of grapevine varieties [Negrul, 1960; Mangafa, Kotsakis, 1996; Jacquat, Martinoli, 1999, Doligez et al., 2002]. Ampelometric and economic-technological parameters are studied for most Georgian grapevine varieties by different authors [Ramishvili, 1948, Ketskhoveli et al., 1960, Frolov-Bagreev et al., 1946-1970, Tsertsvadze, 1989]. However, computer derived new statistical programs offers an opportunity to conduct biometric analysis of quantitative parameters of berry and seed sizes and obtain new results.

The aim of the present study was to determine berry and seed sizes and carry out biometric analysis of obtained data to identify similarity distances and intercultivar variation of old Kolkhic red grapevine varieties.

Material and Methods

Total ten varieties of red grapevine (*V. vinifera* L.) have been studied. Nine of them - Aladasturi, Chodi, Kachichi, Koloshi, Machkvaturi (=Machkadina), Makhvateli, Odjaleshi, Paneshi and Shonuri, are old aboriginal varieties of the Kolkhis. One variety – Saperavi is widespread in Kakheti, currently, and was included in the list as out-group species for comparative statistical analysis.

All studied varieties belong to the eco-geographic group: convar. *pontica* subconvar. *georgica* Negr. provar. *tomentosae* Tserts. [Tsertsvadze, 1989]. Odjaleshi, Makhvateli and Shonuri

are unified into classification group 2 according to Tsertsvadze (1989). Machkvaturi and Chodi are included in the group 14. In separate groups are distributed Paneshi, Koloshi, Kachichi, Saperavi and Aladasturi.

Typical representatives of the aboriginal grape varieties endemic for Samegrelo are Odjaleshi, Machkvaturi and Paneshi. Chodi occurs both in Samegrelo and Adjara. Makhvateli and Koloshi are cultivated in Samegrelo, Guria and Adjara. Aladasturi is typical grape variety of Guria. Shonuri (Svanuri) is spread in Lechkhumi, Samegrelo and Guria. Kachchi is recognized as Abchasian variety, although the origin is not clearly known. Saperavi now occurs mainly in the Eastern Georgia. Although, I. Javakhishvili (1986) suggests that it might be originated in Kolkhis. Therefore, we have chosen it as out-group species in the statistical analysis.

Plant material has been collected in the grapevine living collection of the G. Eliava National Museum in v. Martvili, except Saperavi, which was obtained from private ground in Kakheti, v. Shilda, Kvareli distr. 30 berries have been collected from different plants of each variety for statistical analysis. Photographs have been taken for each collected samples (plant, leaf, branch, cluster, berry and seed) using digital photo cameras Nikon Coolpix5000 and Canon 3,2 megapixel CCP. Berry and seed morphology was studied using stereomicroscope Stemi DV4, Karl Zeiss, Germany. Number of seeds per fruit was estimated. Morphometric parameters have been measured according to known methods [Mangafa, Kotsakis, 1996; Jacquat, Martinoli, 1999] used in studies of grape berries and seeds. The measurements have been done using micrometer and millimeter paper. The following parameters have been measured: berry length, berry breadth, seed length, seed breadth, stalk length.

Statistical analysis of the data has been performed by computer programs MS Exel and Statistica 6. Mean, standard deviation and variance have been determined for every data set. Index representing breadth/length ratio has been determined for berries. Linear regression analysis has been done to compare berry and seed lengths. Multivariate cluster analysis was performed to determine similarity distances among grape varieties. Different horizontal hierarchical tree plots have been developed. The distances between objects used when forming the clusters were: 1) Euclidean distance, which is simple geometric distance in the multidimensional space calculated for raw data; 2) Chebychev distance, which allows to define two objects as different if they are different on one of the dimensions. Both single and complete linkages have been used in the analysis. In the single linkage method, the distances between clusters are determined by the distance of the two closest objects in the different clusters. In the complete linkage method, the distances between clusters are determined by the greatest distance between any two objects in the different clusters. Different parameters of berry and seed sizes of studied grape varieties have been tested by the tree clustering method.

Results

Table 1 represents mean, standard deviation and variance of berry sizes of the ten red grape varieties - Aladasturi, Chodi, Kachichi, Koloshi, Machkvaturi, Makhvateli, Odjaleshi, Paneshi, Saperavi and Shonuri. The length and breadth of the berries show that the size of seeds is smallest in Kachichi (length =10,20±0,51 mm), medium size berries (length < 15 mm) have Chodi, Koloshi, Makhvateli and Odjaleshi. Large berries (length >15 mm) are characteristics for Aladasturi, Machkvaturi, Paneshi, Saperavi and Shonuri. The index representing ratio of berry breadth and length is indicative of berry shape. Almost round seeds (with index > 0.9) possess Aladasturi, Chodi, Kachichi, Koloshi, Makhvateli, Odjaleshi and Shonuri. Nearly similar indexes have Machkvaturi (0.86) and Saperavi (0.88). More ellipsoid form has berry of Paneshi (0.81).

Table 2 shows mean, standard deviation and variance of seed length, breadth and stalk length. Stalk represents modified funiculus of the ovule and is considered as feature of taxonomic importance for the varieties. As it was the case with berries, Kachichi has smallest seeds (length = 4.92±0.18). The length of the seeds in other varieties varies from 6 to 8 mm. The shortest stalk has Kachichi (0.85±0.19) and longest Saperavi (2.07±0.14).

Table 1. Mean, standard deviation (Sd) and variance (V) of berry length, breadth and index (breadth/length ratio) for ten red grape varieties. n=30.

N	Variety	Length			Breadth			Index - breadth/length		
		mean	Sd	V	mean	Sd	V	mean	Sd	V
1.	Aladasturi	17,20	0,64	0,41	15,87	0,63	0,40	0,92	0,036	0.001
2.	Chodi	14,51	1,09	1,18	13,97	1,14	1,29	0,96	0,040	0,002
3.	Kachichi	10,20	0,51	0,26	9,99	0,64	0,40	0,98	0,033	0,001
4.	Koloshi	13,63	1,36	1,85	13,38	1,32	1,74	0,98	0,026	0,001
5.	Machkvaturi	16,50	0,80	0,62	14,24	0,71	0,51	0,86	0,047	0,001
6.	Makhvateli	13,86	0,64	0,41	13,41	0,61	0,37	0,96	0,029	0,004
7.	Odjaleshi	13,79	0,97	0,94	12,75	0,75	0,57	0,92	0,040	0,001
8.	Paneshi	18,44	0,79	0,60	15,00	0,59	0,35	0,81	0,030	0,001
9.	Saperavi	15,87	0,63	1,87	13,89	1,26	1,60	0,88	0,070	0,005
10.	Shonuri	15,36	0,85	0,79	14,95	0,79	0,63	0,97	0,036	0,001

Table 2. Mean, standard deviation (Sd) and variance (V) of seed length, breadth, and stalk length for ten red grape varieties. n=30.

N	Variety	Length			Breadth			Stalk length		
		mean	Sd	V	mean	Sd	V	mean	Sd	V
1.	Aladasturi	7,69	0,35	0,127	4,05	0,18	0,032	1,66	0,20	0,041
2.	Chodi	6,76	0,27	0,071	4,34	0,27	0,075	1,73	0,20	0,040
3.	Kachichi	4,92	0,18	0,033	3,34	0,33	0,060	0,85	0,19	0,039
4.	Koloshi	7,48	0,37	0,067	4,88	0,21	0,039	1,82	0,12	0,015
5.	Machkvaturi	7,09	0,47	0,085	4,14	0,32	0,058	1,87	0,19	0,038
6.	Makhvateli	5,93	0,30	0,055	3,92	0,25	0,046	1,70	0,22	0,048
7.	Odjaleshi	6,86	0,16	0,028	4,27	0,32	0,058	1,71	0,15	0,023
8.	Paneshi	7,49	0,29	0,054	4,55	0,36	0,066	1,82	0,20	0,041
9.	Saperavi	6,41	0,42	0,026	3,83	0,16	0,022	2,07	0,14	0,074
10.	Shonuri	6,30	0,41	0,170	4,01	0,20	0,041	1,53	0,19	0,039

The linear regression analysis between berry and seed lengths shows slight correlation (R^2=0,58; Fig.1). The number of seeds per fruit was variable feature among samples. It varies from one to four in different berries. However, some tendency to be one-, two-, three- or four seeded was

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MECHANISMS OF NA,K-ATPASE SYSTEM REGULATION BY NORADRENALINE

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Abstract

Recently it has been shown that noradrenaline (NA) inhibits Na,K-ATPase system and transfers it from OPS regime to OPM regime inducing the increase of electrogenic coefficient of Na,K-ATPase. As a result of simultaneous action of protein synaptic factor (SF) and NA, the inhibiting effect of NA is removed and Na,K-ATPase is activated. SF appears to be a catalytic activator for Na,K-ATPase.

Key words: neurotransmitters, synaptic factor, OPS regime, OPM regime,

Introduction

In the synaptic membranes of rat brain a new system is found which regulates Na,K-ATPase by neurotransmitters (NT). It is fulfilled by: noradrenaline (NA), dopamine, serotonine and acetylcholine. In its turn, their action is regulated by protein factor (SF) found in synaptosomal cytozole. After its addition, an inhibition induced by NT action on Na,K-ATPase is removed and the enzyme is sharply activated [Kometiani & Jariashvili, 2000].

Regulation of Na,K-ATPase by NT (particularly, by NA) and SF has a functional importance. Their action is specific for synaptic transfer [Kometiani & Jariashvili, 2000]. At present molecular mechanism of NA and SF acting on Na,K-ATPase is not studied, the elucidation of which gives an information about functional importance of this regulation. The presented work appears to be an attempt to clear up this problem.

Materials and Methods

Synaptic fraction from the rat brain served as Na,K-ATPase preparation. Na,K-ATPase activity (V) was determined as an ouabain sensitive part of total ATPase in $\mu molP_i$/hour/mg protein units [Kometiani & Jariashvili, 2000; Kometiani et al., 2001].

In the standard conditions the reaction medium contained: 30 mM Tris-HCl, pH 7.73 and [MgATP], $[Mg^{++}]$, $[ATP_f]$, [NaCl] and [KCl] of corresponding concentrations, while the reaction medium of Mg-ATPase contained: 1 mM ouabain, 145 mM KCl, 30 mM Tris-HCl, pH 7.7 and [MgATP], $[Mg^{++}]$, $[ATP_f]$ of corresponding to the regimes concentrations. As a dissociation constant for MgATP was taken 0.085 mM^{-1}. During OPS regime reaction medium contains: 2.08 mM MgATP, 0.42 mM free Mg^{++} and ATP_f, while during OPM regime – 1 mM MgATP, 3 mM Mg^{++} and 0.028 mM ATP_f [3, 4, 5]. Synaptic factor (SF) was obtained using the method developed

recently [Kometiani & Jariashvili, 2000]. Experimental data were subjected to strict statistical processing.

The number (n) of sites for essential activators was determined using the method of qualitative transformation of the function [Kometiani, 2005]. The essence of this method appears to be in the analysis of geometrical form of functions obtained as a result of V=f(x) function transformation-induced type $y(r,t) = \sqrt[r]{u} = f(t)$ (t=1/x, u=1/V) having different r, what means the ascertainment of asymptote existence, as such function has asymptote only in case when r=n; if a working interval of V=f(x) function is chosen properly, then it is concave (r<n) or convex (r>n) curve or maximally approaches a straight line (r=n). In this case weighed mean square error and linear measure are minimal, while choosing working interval properly ln(u))=f(t) is a monotone, convex function and ln(u)=f(lnt) function have no bending and turning sites. Calculation of the number (n) of sites for essential activators and evaluation of reliability of the result was carried on by means of computer program developed in our laboratory. The results of calculations are present as experimentally obtained number (R), its error (σ_R), and as the difference (R-n) in first approximation.

Results and Discussion

In case of relatively small free Mg^{++} and high substrate (S) concentrations ($[ATP_f] \approx 0$), the Na,K-ATPase system works in so-called OPS regime, while in case of low concentrations of S and free Mg^{++} it works in so-called OPM regime [Kometiani & Leladze, 2001; Chkadua et al., 2002; Kometiani, 1987]. Na^+ and K^+ transport simultaneously takes place in OPS regime at constant stoichiometry ($Na^+/K^+=3/2$), while in OPM regime the transport has a consecutive character and changeable stoichiometry. In OPM regime in case of high concentrations of $[K^+] > 100$ mM, the number (n) of sites for essential activators changes for Na^+ and becomes four, while transfer of three Na^+ ions may be followed by the transfer of 0 or 1 K^+ ion. Therefore, a goal of our investigation was to study the effect of NA and SF on stoichiometry of Na^+/K^+ transport.

As a result of 0.1 mM NA influence in OPS regime, the Na,K-ATPase activity is inhibited by 62.9% (Fig. 1). While adding SF into the reaction medium, NA inhibiting effect is removed. Addition of 0.001 mg/ml SF increases activity by 53%, 0.008 mg/ml SF totally removes NA inhibiting effect, while addition of 0.016 mg/ml SF activates Na,K-ATPase by 18.5%.

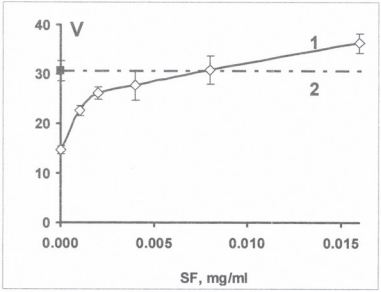

Fig. 1. Dependence of Na,K-ATPase activity on NA and SF concentrations in OPS regime. OPS reaction medium: [MgATP]=2.08 mM, $[Mg^{++}]=[ATP_f]=0.42$ mM, [NaCl]=145 mM, [KCl]=5 mM. 1. [NA]=0.1 mM, 2. [NA]=0.

In case of Na,K-ATPase in OPM regime we have an analogous situation. The data obtained come to an agreement with data given in the literature [Kometiani & Jariashvili, 2000]. Proceeding from above-mentioned the changes in the reaction medium after addition of 0.001 and 0.016 mg/ml SF have been studied, in particular, amount of essential activatory sites for Na^+ and K^+ and correspondingly transport stoichiometry as a result of NA and SF influence on Na,K-ATPase.

In OPS regime the amount of essential activatory Na-sites is three, while the amount of K-sites is two. In case of addition of 0.1 mM Na into the reaction medium their amount changes and correspondingly becomes 4 and 1. But if 0.001 NA and 0.016 mg/ml SF are simultaneously added, the situation does not change and we have $n(K^+)=2$ and $n(Na^+)=3$ (Table 1). As seen from the Fig. 2, SF does not change Na^+ affinity to its binding site, the straight lines are intersected in one point of abscissa (t_0=-0.399±0.011). On the other hand, K^+ and Na^+ ions were simultaneously and randomly bound to the enzyme [Kometiani & Leladze, 2001; Chkadua et al., 2002; Kometiani, 1987]. So it may be concluded that for Na,K-ATPase SF appears to be catalytic activator, $V=\varphi(SF)\cdot f(Na, K)$.

Table 1. Dependence of the amount of essential activatory sites on [SF] in OPS regime ([MgATP]=2.08 mM, $[Mg^{++}]=[ATP_f]$=0.42 mM). 0.1 mM NA presents in the reaction medium

Conditions of experiments	[SF], mg/ml	Number of sites of essential activators for Na^+; $n(Na^+)$ and K^+, $n(K^+)$
[NaCl]=144 mM=const $\sqrt{1/V} = f(1/[K^+])$	0	R=2.0004±0.043; (R-n)=-0.0874; ⇒ $n(K^+)=2$
	0.001	R=2.0038±0.163; (R-n)=-0.2704; ⇒ $n(K^+)=2$
	0.016	R=2.0014±0.073; (R-n)=-0.1714; ⇒ $n(K^+)=2$
[KCl]=125 mM=const $\sqrt{1/V} = f(1/[Na^+])$	0	R=3.0001±0.094; (R-n)=-0.2438; ⇒ $n(Na^+)=3$
	0.001	R=3.0371±0.182; (R-n)=p0.0974; ⇒ $n(Na^+)=3$
	0.016	R=3.0015±0.083; (R-n)=p0.0631; ⇒ $n(Na^+)=3$

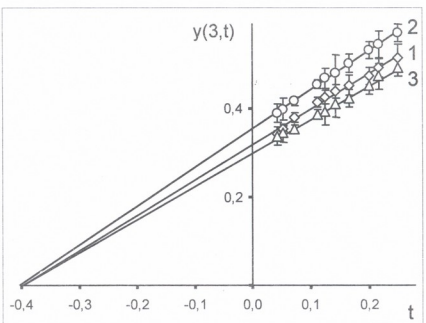

Fig. 2. Amount of essential activatory sites for Na^+ during simultaneous action of NA and SF on Na,K-ATPase in OPS regime: ([MgATP]=2.08 mM, $[Mg^{++}]=[ATP_f]$=0.42 mM).

The reaction medium: [NA]=0.1 mM; [KCl]=125 mM; 4 mM ≤ $[Na^+]$ ≤ 24 mM

(1) [SF]=0 y(3,t)=0.8000t+0.3180 -a/b= -0.3975±0.0022

(2) [SF]=0.001 mg/ml, y(3,t)=0.8784t+0.3542 -a/b= -0.4033±0.0030

(3) [SF]=0.016 mg/ml, y(3,t)=0.7509t+0.2983 -a/b= -0.3972±0.0056.

As it was noted for Na,K-ATPase in OPM regime the existence of four essential activatory sites is characteristic for Na ions and one essential activatory site – for K^+ ions. At simultaneous addition of 0.1 mM NA and 0.016 mg/ml SF, in OPM regime ([MgATP]=1 mM, $[Mg^{++}]$=3 mM and $[ATP_f]$=0.028 mM), n(Na) and n(K) (amount of essential activatory sites) do not change (Figs 3 and 4), while in case of addition of only 0.1 mM NA, n(Na)=3 and n(K)=2. It should be mentioned that at different concentrations of SF, asymptotes of $1/V = f(1/[K^+])$ and $\sqrt[3]{1/V} = f(1/[Na^+])$ functions are intersected in one point of the abscissa. So, SF appear to be a catalytic activator for Na,K-ATPase as well as in case of OPS regime.

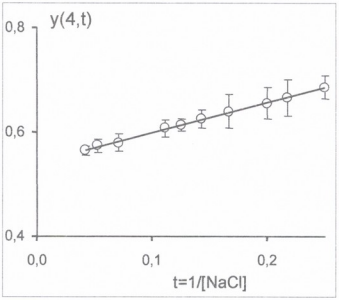

Fig. 3. Amount of essential activatory sites for Na^+ during simultaneous action of NA and SF on Na,K-ATPase in OPM regime: ([MgATP]=1 mM, $[Mg^{++}]$=3 mM, $[ATP_f]$=0.028 mM). The reaction medium: [NA]=0.1 mM; [SF]=0.016 mg/ml; [KCl]=125 mM; 4 mM ≤ $[Na^+]$ ≤ 24 mM
R=4.022±0.034; (R-n)= -0.133; ⇒ $n(Na^+)$=4

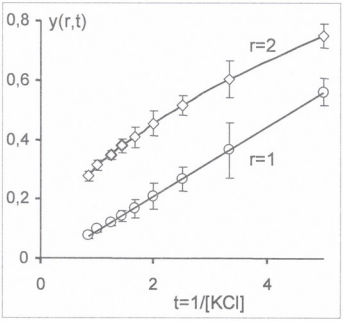

Fig. 4. Amount of essential activatory sites for K^+ during simultaneous action of NA and SF on Na,K-ATPase in OPM regime: ([MgATP]=1 mM, $[Mg^{++}]$=3 mM, $[ATP_f]$=0.028 mM). The reaction medium: [NA]=0.1 mM; [SF]=0.016 mg/ml; [NaCl]=144 mM; 0.2 mM ≤ $[K^+]$ ≤ 1.2 mM
R=1.002±0.005; (R-n)= -0.013; ⇒ $n(K^+)$=1.

Na,K-ATPase, localised in the fraction of synaptic membranes is regulated by two special systems: NT-dependent inhibiting and NT/SF-dependent activatory mechanisms. In our case NA-induced inhibitory mechanism consists in the transfer of Na,K-ATPase from OPS to OPM regime what is followed by the changes in transport stoiciometry of cathions: $3Na^+//2K^+ \Rightarrow 3Na^+//0\text{-}1K^+$ (the amount of essential activatory sites also changes: $n(Na)=3 \Rightarrow n(Na)=4$ and $n(K)=2 \Rightarrow n(K)=1$). So electrogeneity of Na,K-ATPase system sharply increased. As a result of simultaneous action of NT and SF the process of inhibition is blocked and SF has an influences on Na,K-ATPase, as a catalytic activator.

Apparently, these two Na-dependent, intercompetition processes have an important role in the functioning of chemical synaptic transfer, especially when taking into account the fact that in these processes a sharp change in electrogeneity of Na,K-ATPase system takes place.

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Na,K-ATPაზას სისტემის რეგულაციისესისტემის რეგულაციის მექანიზმი ნორადრენალინის ზემოქმედებით

შიოშვილი ლ.

ივ. ბერიტაშვილის სახელობის ფიზიოლოგიის ინსტიტუტი

(მიღებულია 10.01.2006)

რეზიუმე

ადრე ნაჩვენები იყო, რომ ნორადრენალინი (NA) აინჰიბირებს Na,K-ATPაზას და გადაჰყავს ის OPS რეჟიმიდან OPM რეჟიმში, რაც იწვევს Na,K-ATPაზის ელექტროგენური კოეფიციენტის ზრდას. ცილოვანი ბუნების სინაფსური ფაქტორისა (SF) და NA ერთდროული მოქმედების შედეგად NA-ის ინჰიბიტორული ეფექტი იხსნება და Na,K-ATPაზა აქტივდება. Na,K-ATPაზისათვის SF წარმოადგენს კატალიზურ აქტივატორს.

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THE INOSIT-SPECIFIC LECTIN (BVL-I) FROM SYNAPTIC VESICLE MEMBRANES AND ITS INFLUENCE ON THE CA^{2+}-ATPASE ACTIVITY OF THE SYNAPTOSOMAL FRACTION

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Abstract

Inosit-specific neurolectin has been isolated from bovine head brain (BVL-I). It was established that BVL-I is a Ca^{2+}-dependent lectin, which means that its carbohydrate-binding activity reveals only in the presence of Ca^{2+} in the active center of molecule. Moreover, in presence of free Ca^{2+} ions in the incubation medium, specific activity of the BVL-I sharply increases. The lectin reveals high affinity to phosphatidilinositol and phosphatidilcholine, especially at the presence of the free Ca^{2+} ions. Hence, the participation of BVL-I in the process of neuroexocytoses was supposed. BVL-I is a sufficiently effective modulator for the Ca^{2+}-ATPase of synaptosome fraction. In a case of its specific quantity, activation of enzyme occurs. Judging by the changes of kinetic parameters (V_{max}, K_m), it could be suggested that interaction of the BVL-I with the synaptosomal Ca^{2+}-ATPase must have a non-competitive character. It was shown that activating effect of the lectin best manifests at 100μM Ca^{2+}. This allows suggesting that calcium not only increases the BVL-I lectin activity, but in parallel amplifies its modulator influence on the enzyme. It should be considered that BVL-I participates not in the membranes fusion only, but also in the fusion-induced modulation of the Ca^{2+}-ATPase activity.

Key words: neurolectin, specific activity, modulator, hemagglutination activity

Introduction

Discovery of the neurolectins, the proteins with agglutination capacity, in the nervous tissue should be considered as one of the major achievements of the modern neurochemistry. Lectins are the proteins of non-immune origin and glycoprotein nature, which selectively and irreversibly bind with carbohydrates, without their chemical alterations. Specificity of a lectin interaction with the target molecules is determined by the characteristic carbohydrate group of the latter. Specific binding of the lectin and respective carbohydrate plays an important role in such processes as are cell-cell interaction [Nelson et al., 1995], cell recognition and adhesion [Ashraf & Khan, 2003; Dodd & Drickamer, 2001] regulation of the immune processes [Lutomski et al., 1995], cyclation of the plasma membrane, synaptogenesis [Aleksidze, 1992], regulation of cell division [Lew et al., 1994], modulation of nucleus membrane activity [Hubert & Seve, 1994], migration of neurons [Zanetta, 1998], etc. At the same time lectins are modulators of many enzyme activities [Gabius, 1997]. These interactions add more dynamic aspects to the function of lectins.

It is known that after binding of the lectins to the carbohydrate residues of cellular membrane, activity of the membrane transporter enzymes alters [Adamkiewiez et al., 1994]. This effect of the lectins may be due to structural changes in the membranes or altering of an enzyme microenvironment, which is followed with alteration of the ions' permeability. Out of these enzyme systems the best investigated is the influence of mitogenic and non-mitogenic lectins on the Na^+, K^+-ATPase system [Drickamer & Trylor, 1993]. Notwithstanding the vast number of the animal lectins' investigations, they still are less studied as compared to the plant lectins. This is particularly true in regard of the nervous system, specifically – of the brain lectins.

Considered the above mentioned, the present work was aimed to study the influence of inosit-specific lectin BVL-I isolated from synaptic vesicle membranes, on the Ca^{2+}-ATPase activity of the synaptosomal fraction.

Material and Methods

Bovine brain was used as unit for test in all experiments. Fraction of the synaptic vesicles was isolated via differential centrifuging according to De-Robertis [De-Robertis, 1969].

Assay of hemagglutination activity - Hemagglutination activity of the lectin was assessed on special titration plane-table with U-shaped wells, in 2% suspension of the rabbits trypsinized erythrocytes with an aid of 50µl titration strips, the solution under study was tittered on the plane-table, suspension was added and during two hours an agglutinations of erythrocytes was observed. Lectin activity was expressed in the lectin-specific activity ($titer^{-1}\times$ mg/ml protein $^{-1}$) [Lutsik et al., 1981]

Assay of lectin specificity - Specificty of the lectins against ligands was evaluated with hapten-inhibitory method [Lutsik et al., 1983], on the titration plane-table with U-shaped wells. After prior titration of lectin, different ligands of similar concentration were introduced into each well. Following 30 min preincubation, 2% suspension of the rabbits' trypsinized erythrocytes (50µl) was added into each well and agglutination of erythrocytes was observed for two hours. Inhibition of agglutination in presence of ligands pointed at binding of the ligand to active center of lectin, as a hapten and inactivation of a lectin did occur.

Protein Determination – was made by the method of Lowry at al. using bovine serum albumin as a standard. To 0.2ml samples of protein 2.0ml reactive C was added, which contains reactive A (20g Na_2CO_3/ 1*l* 0.1NaOH) and reactive B (10g $Na_2C_4H_4O_6$ + 5g $CuSO_4$ / 1*l* distilated water). Mixture was incubated for 10 min. Then folin was added and incubated for 30 min at room temperature. The dying intensity was estimated photocolorimetrically at λ = 750 nm [Lowry, et al., 1951].

Measurement of ATPase activity - The ATPase activity was determined according to volume of inorganic phosphorus liberated after hydrolysis of ATP (µg/ml P/mg/ml protein/ 1min). Activity of the Ca^{2+}-ATPase was determined in synaptosomal fraction of the bovine brain, according to difference between activities of the Ca^{2+}, Mg^{2+}- and Mg^{2+}-ATPases, in conditions of different concentrations of Ca^{2+} and ATP, other conditions being permanent (Tris-HCl 20mM, pH 7.4, EGTA 1mM), incubation medium contained lectin solutions of different concentrations.

The results obtained throughout the study were evaluated statistically with the students t-test. Significance was accepted at $p<0.05$.

Results

In order to extract lectins from the synaptic vesicles, several extraction buffers were tested. Maximal lectinic activity was found in the protein fraction extracted with 0.5% solution of

Triton X-100. Conditions of extraction indicate that the proteins with lectinic activity, obtained from the synaptic vesicles, are of membrane origin.

Following determination of the carbohydrate specificity of isolated lectins, the inosit-specific lectin, designed BVL-I (Bovine Brain Vesicular Inosit-Specific Lectin), has been isolated from the sum protein fraction from synaptic vesicles as described previously [Surguladze, et al., 2002]. We have also shown [Surguladze et al., 2004] that BVL-I, is a member of Ca^{2+}-dependent lectins, the so called C-type lectins. It means that lectin carbohydrate-binding activity reveals only in the presence of Ca^{2+} in the active center of lectin molecule. Furthermore, in the presence of free Ca^{2+} ions in the reaction medium, increase of the lectin activity does occur and minimal concentration of the ion which still exerts an effect, is 0.047mM. In presence of calcium, specific activity of the BVL-I increases 16-fold, which points an activator property of Ca^{2+} for the BVL-I lectin. In other words, Ca^{2+} ions participate in the regulatory mechanisms of the BVL-I activity.

BVL-I lectin manifests a sensitivity to the phospholipids. Especially BVL-I shows high affinity to the phosphstidilinositol and phosphatidilcholine, which are located primarily in the inner layer of lipid bilayer. Insofar the Ca^{2+} ions increase activity of the lectin, interaction of the BVL-I with the phospholipids was studied in a presence of the Ca^{2+} ions in the incubation medium and without these ions. The results obtained are presented in Table 1. The table shows that in the absence of the Ca^{2+} in the incubation medium, the BVL-I exerts an affinity to phosphatidilinositol and phosphatidilcholine only in presence of 200μM of Ca^{2+} in the medium, sensitivity of the lectin to the phospholipids increases somewhat - an elevated affinity is observed not to separate phospholipids only, but the total spectrum of interaction with the phospholipids increases as well.

These data verify that the BVL-I is a phospholipid-binding protein. Moreover, its affinity to the phospholipids significantly increases in presence of the calcium ions and because phospholipids are involved in the membrane fusion [Vanhesebroeck et al., 2001], it could be suggested that the BVL-I lectin must play a serious role in interactions of the synaptic vesicles and presynaptic membranes and in exocytosis of the neuromediators.

In order to clarify the inosit-specific lectin contribution into the process of synaptic vesicles activity, we studied a problem of intramembranal orientation of active center of BVL-I. It was found that the native synaptic vesicles are characterized with capacity to agglutinate the rabbit thrypsinized erythrocytes, which may be inhibited by inositol only. None of the other haptens influenced a degree of the lectin agglutination property. Thus, it could be suggested that the BVL-I in the synaptic vesicle is oriented with its active center to the outer surface of the synaptic vesicular membrane.

Since we have proposed an idea about participation of the BVL-I in synaptic transmission, it seems interesting to investigate an influence of this lectin on the Ca^{2+}-ATPase of the synaptosomal fraction. As it is known, the basic function of Ca^{2+} pumps is to maintain the Ca^{2+} gradient across the plasma membrane via the highly regulated active extrusion of Ca^{2+} from the cell. Ca^{2+} concentration up to 200μM triggers neuroexocytoses – synaptic vesicles move to the presynaptic membrane and fuse there. After neurotransmitters release Ca^{2+} pump restore Ca^{2+} homeostasis in the synapse. In that way Ca^{2+} ATPase plays a critical role for the normal function of the synapse [Khukho, 1990]. In this report we try to clarify the mechanism by which BVL-I modulates the hydrolytic activity of the Ca^{2+}-ATPase and Mg^{2+}-ATPase of the synaptosomal fraction. It was shown that in conditions of gradually increasing concentration of the lectin (within 2 μg/ml – 60 μg/ml), sharp increase of activity was noted in both Ca^{2+}- and Mg^{2+}-ATPase, as compared to the control (Fig.1). Increase of activity was directly proportional to the lectin concentration. Such effect was best revealed in a case of Mg^{2+}-ATPase. For instance, at the lectin concentration - 60 μg/ml, Ca^{2+}-ATPase activity increased by 300% against the control, and in a case of Mg^{2+}-ATPase by 400%.

The data obtained showed that above effect should be attributed to different affinity of the enzyme to the lectin as an activator. Specifically, K_m for Mg^{2+}-ATPase amounts 2.34±0.27, while for Ca^{2+}-ATPase – 17.5±0.61 (Fig.2).

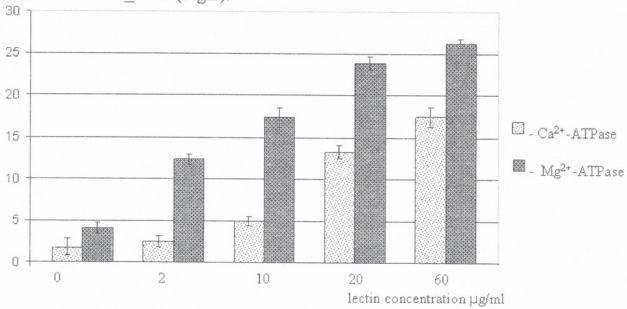

Fig. 1. Abscissa – concentration of the lectin (µg/ml); Ordinate – enzyme activity (PµM/ml protein/1min).

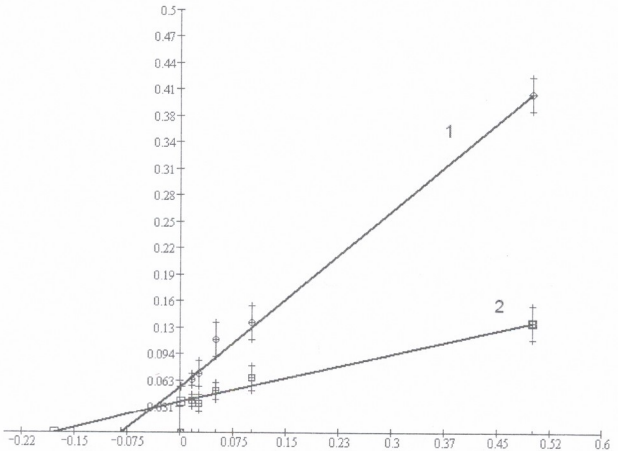

Fig. 2. The kinetic parameters of the Ca^{2+}- and Mg^{2+}-ATPases of the synaptosomal fraction in the presence of BVL-I.. Abscissa – inverse value of the lectin concentration; ordinate – inverse value of the enzymatic reaction velocity. 1 – Ca^{2+}-ATPase; 2 – Mg^{2+}-ATPase.

In the next series of experiments the above procedures were repeated in conditions of different concentration of calcium. Experiments were aimed at assessment of the Ca^{2+}-ATPase activity only, because this enzyme was an object of our particular interest.

The Fig. 3 shows that activating effect of the lectin best manifests at 100 μM Ca^{2+}. This allows to suggest that calcium not only increases the BVL-I lectinic activity, but in parallel amplifies its modulator influence on the enzymes. Ca^{2+} concentration found in our experiments, is close to that, which exists in synaptic terminal during excitation [Matthews, 2000]. Modulator influences of BVL-I on the enzymes, must be amplified because of increased lectin activity following interaction with the ion. In support for this suggestion those experiments could be invoked, in which Ca^{2+}-ATPase activity was studied during gradually increasing concentration of the Ca^{2+} ions at presence of EGTA in the reaction medium (Table 2). It is evident from the Table that in presence of 1 mM EGTA, V_{max} of the enzyme significantly decreases against the control experiments (EGTA=0). Although presence of the lectin increases the enzyme activity, it however does not return to initial values (when EGTA=0). From Table 2 it is clear also that the lectin significantly increases enzyme affinity to the Ca^{2+} ions both in presence of EGTA and in its absence (as compared to the respective control). Therefore, the data obtained show that the BVL-I lectin somewhat eliminates inhibitory influence of EGTA on the enzyme activity, although in presence of EGTA its modulator effect is attenuated.

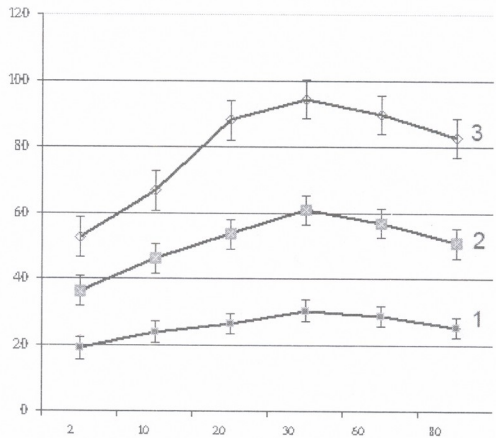

Fig. 3. Alteration of the synaptosomal Ca^{2+}-ATPase activity in the presence of the lectin in the incubation medium, at different concentrations of Ca^{2+}. Abscissa – concentration of the lectin (μg/ml); ordinate – enzyme activity (P μM/ml protein/ 1min).
1 - 5μM Ca^{2+}; 2 - 50μM Ca^{2+}; 3 - 100μM Ca^{2+}

In order to more precisely assess a character of interaction between the BVL-I lectin and synaptosomal Ca^{2+}-ATPase, we studied character of relation to the substrate, in presence of the lectin and in its absence for which different concentration were used (0.1 mM, 0.5 mM, 1.0 mM, 1.5 mM, 2.0 mM, 5.0 mM, and 10.0 mM). It was found that incubation medium containing 2 mM Mg^{2+}-ATP is the most optimal for revealing the enzymatic activity of the Ca^{2+}-ATPase. Analogous results were obtained when the BVL-I was presented in the incubation medium. From the data

presented in Table 3 is clear that the lectin increases both V_{max} and K_m (statistically not reliable, $p>0.1$).

Judging by the changes of kinetic parameters, it could be suggested that interaction of the BVL-I lectin with the synaptosomal Ca^{2+}-ATPase must have uncompetitive activation character.

Table 1. Influence of the phospholipids on hemagglutination activity of the BVL-I lectin

Phospholipids	Minimal amount of a phospholipid (mg/ml), which inhibits hemagglutination activity of the lectin	
	Without Ca^{2+}	In presence of Ca^{2+}
Phosphatydilinositol	0.00625	0.000195
Sphyngomyelin	>100	0.0179
Phosphatic acid	>100	0.0025
Phosphatydilcholine	0.00078	0.00019

Table 2. Alterations of kinetic parameters in presence of EGTA

Kinetic parameters	EGTA=0			EGTA=1 mM		
	L=0	L=20 μg/ml	p	L=0	L=20 μg/ml	p
Vmax	12.78±0.008	24.7±0.039	<0.02	10.35±0.0025	16.68±0.016	<0.02
Km	0.49±0.006	0.34±0.013	<0.05	0.19±0.018	0.056±0.007	<0.05

Table 3. Alterations of kinetic parameters, in the presence of the lectin, at various concentrations of Mg^{2+}/ATP

Kinetic parameters	L=0	L=20 μg/ml	p
V_{max}	13.06±0.6	18.7±1.6	<0.05
K_m	0.25±0.078	0.11±0.076	>0.1

Discussion

In the present work, we demonstrated that BVL-I is a Ca^{2+}-dependent lectin. Moreover, at the presence of free Ca^{2+} ions in the incubation medium its hemagglutination activity sharply increases. In other words, BVL-I may play a role of some kind of Ca^{2+}-sensor. Our results show that BVL-I have a high affinity to the negativly-charged phospholipids such as phosphatidilinositol and phosphatidilcholine. We demonstrate that its affinity to the phospholipids significantly increases in presence of Ca^{2+} ions and because phospholipids are involved in the membrane fusion, it could be suggested that the BVL-I must play a serious role in interactions of the synaptic vesicles and presynaptic membranes and in exocytosis of the neuromediators. Moreover, BVL-I in the synaptic vesicle membrane is oriented with its active center to the outer surface of vesicle membrane to the synaptic terminal. According to above mentioned, it could be suggested that at final stage of fusion of synaptic vesicle with the presynaptic membrane, along with the other vesicular proteins, the BVL-I should participate too. We suggest that the main function of the BVL-I is attachment to the lipid components of target membrane, in response to increased concentration of the Ca^{2+}-ions. It is fairly feasible just this interaction is a prerequisite of the two membranes fusion. It was shown that BVL-I induces activation of the synaptosomal Ca^{2+}-ATPase in a dose-

dependent menner. Activation of the enzymes, including ATPases was demonstrated previously for different vegetable lectins [Conrad & Rudiger, 1994], but little is known about role of animal lectins in such interactions. We have shown that in a case of BVL-I specific quantity, activation of the synaptosomal Ca^{2+}-ATPase does occur, which manifests in pumping of excess of Ca^{2+} ions outside a synapse. It should be considered, therefore, that during neuroexocytosis, because of binding of certain number of the vesicles to the presynaptic membrane, in which must participate BVL-I, the synaptic membrane Ca^{2+}-ATPase is activated automatically, or, in our opinion, the BVL-I participates not in the membranes' fusion only, but also in the fusion-induced modulation of the Ca^{2+}-ATPase activity. Notably, the lectin alters the enzyme activity in such a way that transfers it into more "economic" regime and increases enzyme affinity to the substrate. As a result, even in conditions of the lower concentration of Mg^{2+}/ATPase, a V_{max} of the enzyme is tangibly higher against the control one.

Thus, BVL-I should be one of those regulatory components of such complex processes, as the synaptic transmission is.

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ინოზიტ-სპეციფიკური ლექტინი **(BVL-I)** სინაფსური ვეზიკულების მემბრანებიდან და მისი გავლენა სინაფტოსომური ფრაქციის Ca^{2+}-ატფ-აზას აქტივობაზე.

სურგულაძე ნ., კოშორიძე ნ., ვარდიაშვილი თ.

ბიოქიმიისა და ბიოტექნოლოგიის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 25.01.2006)

რეზიუმე

ხარის თავის ტვინის სინაფსური ვეზიკულებიდან გამოყოფილია ინოზიტ-სპეციფიკური ლექტინი BVL-I. დადგენილია, რომ BVL-I არის Ca^{2+}-დამოკიდებული ლექტინი, რაც იმას ნიშნავს, რომ მისი ნახშირწყალ-დამაკავშირებელი აქტივობა მჟღავნდება მხოლოდ ლექტინის მოლეკულის აქტიურ ცენტრში კალციუმის იონების თანაობისას. საინკუბაციო არეში თავისუფალი Ca^{2+}-ის იონების თანაობისას BVL-I ლექტინის სპეციფიკური აქტივობა მკვეთრად იმატებს. ლექტინი ამჟღავნებს მაღალ თვისობას ფოსფატიდილინოზიტოლისა და ფოსფატიდილქოლინის მიმართ, განსაკუთრებით კალციუმის თანაობისას. შემოთავაზებულია BVL-I-ის ნეიროეგზოციტოზში მონაწილეობის მოდელი. BVL-I არის ეფექტური მოდულატორი სინაპტოსომური ფრაქციის Ca^{2+}-ატფ-აზასათვის. მისი კონკრეტული რაოდენობის პირობებში ადგილი აქვს ფერმენტის აქტივაციას. კინეტიკური პარამეტრების ცვლილების მიხედვით სავარაუდოა, რომ ლექტინ BVL-I-ის ურთიერთქმედებას სინაფტოსომურ Ca^{2+}-ატფ-აზაზე ჰქონდეს არაკონკურენტული აქტივაციის ხასიათი. ლექტინის აქტივატორული ეფექტი მაქსიმალურად მჟღავნდება 100μM Ca^{2+}-იონის თანაობისას. ეს გვაფიქრებინებს, რომ კალციუმი არამარტო ზრდის ლექტინ BVL-I-ის აქტივობას, არამედ პარალელურად აძლიერებს ფერმენტებზე მის მოდულატორულ გავლენასაც. სავარაუდოა, რომ BVL-I მონაწილეობს არამარტო მემბრანათა შერწყმაში, არამედ შერწყმის შემდეგ, სამიზნე მემბრანის Ca^{2+}-ატფ-აზას აქტივობის რეგულაციაშიც.

denaturation transition of troponin T has not been detected [Morosova et al., 1988] neither by calorimetric, nor by fluorescence methods. It was concluded that troponin T molecule in solution does not have stable, highly ordered structure [Morosova L.A., et al., 2001]. That is why we carried out the experiments to investigate the thermostability of troponin T.

Materials and Methods

Troponin was extracted from rabbit skeletal muscle according to Staprans [Staprans, et al., 1972]. Chromatography of troponin was carried out on DEAE-celluloze [Perry, et al., 1974] to separate its components. The polyacrylamide gel electrophoresis in the presence of sodium dodecile sulfate was used for examination of homogenity of troponin T preparations. The thermal denaturation of troponin T was studied by means of the differential adiabatic scanning microcalorimetry DASM–4 ("Biopribor" Puschino, Russia). The heating rate was 1 K/min, concentration of protein – 3 mg/ml. The intrinsic fluorescence of proteins was carried out by Shimadzu RF-5000 spectrofluorimeter (Japan) in thermostatic cell at excitation wavelength λ_{exc}=296 nm. The heating mean-rate was 0.5 K/min and the protein concentration of 0.2 –0.3 mg/ml.

As troponin T is badly dissolved at physiological ionic strength and the study of the intrinsic fluorescence spectrum of trpopnin T showed no essential changes in the fluorescence parameters at high concentration of KCl, in our experiments we increased KCl concentration in protein solution up to 1 M.

Results and Discussion

We were interested if troponin T melecule restores its conformation after the heating, i.e. if renaturation occurs. Therefore we studied the dependence of intrinsic fluorescence parameters on the temperature and investigated the melting curves of troponin T placed in the cell of the differential adiabatic scanning microcalorimetry. After the primary thermal denaturation the protein was situated in refrigerator for 18 hours and the measuring was carried out again. While the primary heating of troponin T at λ_{exc}=296 nm shift of the spectrum to the long wavelength (3.5 nm) was observed (Fig 1). This proves the change of the conformational state of troponin T molecule and rearrangement of tryptophan residues towards water environment. At the second heating of troponin T the curves of fluorescence spectrum don't almost differ from the primary curves. They are slightly smoother and are situated above the primary heating curves on 0,5nm. At the same time fluorescence intensities of troponin T decrease with the heating temperature growth. The conformational changes of troponin T can be observed as a small derivation of the smooth temperature dependence of protein fluorescence intensity. The intensity curve of the second heating of troponin T is identical with the curve of primary heating of the protein. It allows us to conclude that the structure of troponin T restores after the first thermal denaturation. Hence, one can conclude that troponin T molecule is thermostable in investigating conditions (20 mM Tris –HCl ; pH 7,3; 1 M KCl).

Skeletal muscle troponin T contains the following fluorescence amino acid residues: tyrosine in 156, 159, 210, 227 positions and tryptophan in 206 and 259 positions [Pearlstone, et al, 1976; Stefancsik, et al, 1998]. At λ_{exc}=296 nm only triptophans are got exited [Chernitsky et al, 1972]. They are situated in C-terminal domain of troponin T. It is known that the chymotripsin effect on the bond between Y-158 and L-159 amino acid residues results in the formation of two fragments of troponin T: THT_1 (residues 1-158) and THT_2 (residues 159-256) [Pearlstone, et al,1976]. Chymotripsin acts like this on the whole troponin complex. In the soft proteolysis

conditions the same N-tail fragment of troponin T (THT_1) and complex consisting of troponin I, troponin C and C-tail fragment of troponinT (THT_2) are received [Sehaertl et al, 1995]. So tryptophans are placed in THT_2 –fragment of troponinT. During the fluorescence process the amino acid residues that are located at up to 10 nm distance from fluorophore, influence the intrinsic fluorescence parameters of protein [Lacovich, 1986]. Therefore tryptophans in 206 and 259 positions located in C-terminal domain of troponin (THT_2) stay insensible to the process that occur in N-tail fragment (THT_1) of troponin T. That's why the conclusion about thermostability of troponin T molecule based on the study of dependence of intrinsic fluorescence parameters on temperature may concern only THT_2 –fragment of troponin T, but not the whole troponin T molecule in case of stretched one.

This conclusion is confirmed by our microcalorimetric investigations. The microcalorimetric curves are shown in Fig.2. The primary heating curve is represented by two separated peaks with additional transition among them. The dotted line in Fig.2 shows the melting after the cooling of protein (the second melting). As is seen, after thermal denaturation only a part of a structure of troponin T molecule renaturates. The denaturation of troponin T is not reversible: only a part of troponin T molecule (~60%) that corresponds to the peak at high transition temperature renaturates. The denaturation of the region of molecule which is represented by the melting peak at low temperature is not practically reversible. In previous study [Getashvili et al., 2005] we demonstrated that the melting profiles of microcalorimetric curves of troponin T are greatly depended on solvent pH. Considering the process of deprotonization of ionized groups in troponin T molecules we can assumed that the C-terminal globular fragment of troponin T participates in the formation of high-temperature peak of thermal denaturation, while the low-temperature peak is represented by the melting of N-terminal tail of the protein. Proceeding from the data of protein secondary heating described above we can assume that C-terminal region of troponin T molecule renaturates, while the N-tail part of molecule doesn't restore its primary structure. So while investigating the thermostability of proteins it is not enough to use only the method of intrinsic fluoroscence as it can lead to wrong conclusions.

Thus, the C-terminal fragment of troponin T molecule is supposed to have more stable structure, which is able to restore at least partially after the thermal influence. It is just the part of molecule where troponin T binding sites with tropomyosin, troponin I and troponin C are placed.

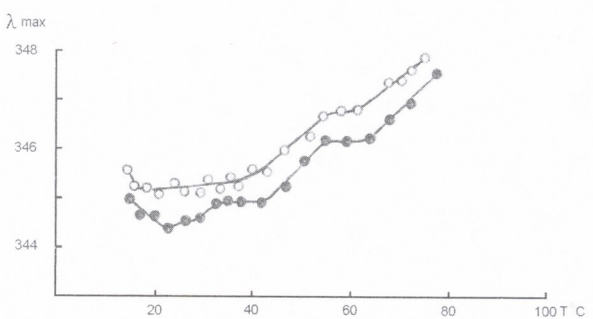

Fig.1. The dependence of troponin T intrinsic fluorescence spectrum position on the temperature. λ_{exc}=296 nm, buffer: 20mM Tris-HCl, 1M KCl, pH 7.3. ● – primary heating. o – secondary heating.

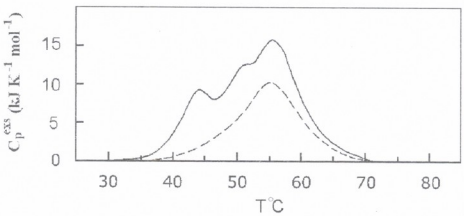

Fig.2. Temperature dependence of apparent excess heat capacity of troponin T Buffer: 20mM Tris-HCl , 1M KCl, pH 7.3. Solid line - primary heating, dotted line - secondary heating.

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ტროპონინ T-ს თერმოსტაბილურობა

ბუთხუზი ნ., ერისთავი თ., გვრიტიშვილი ა., ბუტი ე., ზაალიშვილი მ.

მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 17.01.2006)

რეზიუმე

ნაჩვენებია, რომ ტროპონინ-T მოლეკულის N-ტერმინალური ნაწილი არ რენატურირებს თერმული ზემოქმედების შემდეგ, მაშინ როცა C-ტერმინალური ფრაგმენტი აღდგება 60%-ით. ვვარაუდობთ, რომ ტროპონინ-T მოლეკულის C-ტერმინალური ნაწილს გააჩნია უფრო სტაბილური სტრუქტურა, რომელსაც შეუძლია აღდგეს. სწორედ მასშია განლაგებული ის საიტები, რომლებიც აკავშირებს ტროპონინ-T მოლეკულას ტროპომიოზინთან, ტროპონინ I- და ტროპონინ C-სთან.

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INFLUENCE OF BIOREGULATOR VILON ON VITAL FUNCTIONS OF BIOPOLIMERS AND CHROMATINS *IN SITU*

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Abstract

Influence of the synthetic bioregulator dipeptide Vilon (Lis-Glu) on vital functions of human lymphocyte cell cultures and thermodynamic stability of membranes, cytoplasmic protein and chromatin was investigated *in situ.* It was shown that Vilon does not affect on the structural changes of cytoplasmic proteins and membranes *in situ,* and DNA *in vitro* at physiological NaCl concentration and pH 7.0. However, it has an influence on the metabolic heat (-Q) and causes partial decondensation of "silent"-heterochromatin.

Key words: bioregulator, microcalorimetry, metabolic heat, denaturation heat

Introduction

New type of synthesized preparations – peptide bioregulators have been investigated and successfully applied in gerontological and geriatric practice [Khavinson, 2002]. One of such synthetic bioregulators, dipeptide Vilon (Lis-Glu) belongs to those compounds, which are not directly bound to DNA at selected sites, but interfere with transcription of DNA or the replication process. It was shown that this synthetic preparation activates some metabolic changes regulated through the genes in chromatin domains [Lezhava, 2004; Meskhi, 2004]. In the given work we tried to evaluate an influence of Vilon on metabolic heat (-Q), denaturation heat (Q_d), and thermodynamic stability of cytoplasmic proteins, chromatin complex in cell culture of human lymphocytes and on the melting parameters of calf thymus DNA in dilute solutions at physiological concentration of sodium salt at pH7.0. A goal of this work was to establish what influence have low concentrations of Vilon on biochemical processes proceeding in lymphocytes, if Vilon is bound to DNA *in vitro* at physiological conditions and if it influences structural organization of protein complexes and hetero- "silent" and "active' chromatin.

Materials and Methods

Modified Differential scanning microcalorimetric (DSC) investigation was carried out at sensitivity of 0.1 μW, temperature range - 5–150°C, and the measuring capsule volume - 0.3 cm^3. The accuracy of absolute temperature measurement is better than 0.05°C. The calculation of metabolic heat (−Q), heat of denaturation (Q_d), denaturation temperature (T_d) and width of the peak

at half height (ΔT_d) was carried out by a program developed by us. Deconvolution of curves was conducted with Origin 6.0 (MicrocalTM Software Inc.). The error in determination –Q and Q_d of lymphocytes was less than 12%. T_d was determined up to ±1°C and ΔT_d to ~ 0.5°C [Monaselidze, 2006]. Vilon – dipeptide (Lys-Glu) – was prepared by a directed chemical synthesis on the base of amino acid analysis of the complex preparation of thymus – thymalin [Khavinson, 2002].

Results and Discussions

Fig. 1 shows the microcalorimetric record of thermal effect observed in the process of heating of human lymphocytes in the culture medium. The intensive asymmetric exothermic peak with maximum at about 37°C is observed in the temperature range from 8 to 40°C. The value of heat evolution (-Q) calculated from area under the peak is equal to 13.4±2.0 J/g of dry biomass. According to numerous data, the value of metabolic heat (-Q) is generally associated with respiration of cells with biochemical reactions connected with modification of nucleic acids and macromolecular synthesis, and reflects degree of cell survival and apoptosis [Monaselidze, 2006]. 4 peaks and 3 shoulders are observed in the temperature range from 40 to 120°C. The integral heat calculated from areas under the endotherms is equal to 26.5±3.0 J/g of dry biomass. Systematic calorimetric measurements show that in the temperature range 40-85°C cytoplasmic structures, nuclear matrix, and chromatin in 80-120°C, are denaturated [Monaselidze, 2006]. We carried out the deconvolution of this curve on Gaussian constituents and obtained eight thermal transitions [Meskhi, 2004]. Q_d was estimated on the basis of endotherms VII and VIII, the value equal to 90.5±9.0 J/g DNA was obtained. This value coincides with the value of Q_d of chromatin in solution, which is equal to 75.5±7.5 J/g DNA [Monaselidze, 2006].

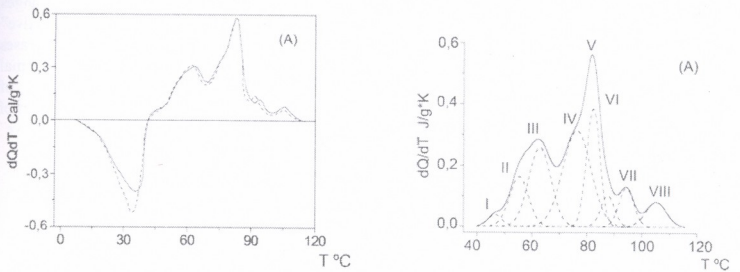

Fig. 1 (A) Thermal effects observed during heating of lymphocytes from donors in culture medium (pH7.1), heating rate 0.1°C/min. Curve 1 (solid line): lymphocytes non-treated with Vilon ~ $2x10^{-7}$ cells (dry biomass is 4.8mg; DNA is 0.095mg). Curve 2 (dash line): lymphocytes treated with 0.05 mM/ml Vilon for 48 hours, $1.5x10^{-7}$ cells (dry biomass is 4.4mg; DNA is 0.092mg). (B) Deconvolution of curve 1.

So we come to the conclusion that chromatin in the composition of human lymphocytes has two thermal transitions at 100 and 105°C and denaturation heat of chromatin is equal to 90.5±9.0 J/g of DNA. The comparison of the curves presented in Fig.1 shows that Vilon does not cause any significant effect on the curve profile of the dependence Q_d=f(T) and value Q_d in the temperature range 40-80°C, that confirms that Vilon does not have influence on stability of proteins and protein-membrane complexes, but 10% decrease of –Q is observed in this range. Vilon

causes the redistribution of heat between of the endotherms VII and VIII, corresponding to denaturation of active heterochromatin [Meskhi, 2004; Monaselidze, 2006]; particularly, Vilon increases Q_d of the endotherm VII at 95°C. We also showed that T_m and ΔH_m of DNA at molar ratio Vilon/$DNA_{b.p.}$ (in the range R=0.02-0.06) at physiological conditions did not change. So we come to the conclusion that a law concentration of Vilon in physiological conditions does not influence directly on stability of DNA *in situ* and nucleosomal DNA in the composition of active chromatin, but it influences on biochemical process proceeding in a living cell and causes decondensation of "silent" chromatin.

The authors of work [Studitsky, 2004] proposed a two-step model of chromatin structure change connected with gene indication on the basis of last achievements in the field of chromatin remodulation. Each chromosome occupies a certain area in a nucleus called a chromosome territory. Inactive genes are located in the inner part of this territory – "silent" chromatin, while active genes are concentrated on the surface of the territory – "active" chromatin. Induction of gene has two stages. Untranscriptional "silent" heterochromatin, existing in high compacted state, not very accessible state for regulator protein complexes, is partially decondensed and transits into a state ready for transcription. In this unfolded part of heterochromatin, nucleosome DNA is, mainly, in composition of 30nm fibers projecting from heterochromatin as loops. The structure of loop of 30 nm fibers is decondensed (unfolded) on the second stage and a degree of decondensation depends on activity of regulator enzymes. It was shown that at high effectiveness of transcription, the 30nm fiber turns into 10nm fiber with tightly packed nucleosomes "side-to-side", nucleosomes separated from each other by linear areas of DNA and 5nm fiber consisting of unfolded nucleosomes.

Our data confirm the proposed model. According to them, Vilon has a specific influence on chromatin *in situ*, decondensing only the heterochromatin structure (T_d=105°C). Basing on the fact that Vilon causes decrease of metabolic heat by about 10%, and it does not cause any changes in thermodynamic stability of membranes, proteins, and DNA, and weakly influences on "active" chromatin (T_d=90°C), we suppose that Vilon initiates demethylation of specific methyltransferases [Bannister, 2001], which label Lis-9 at the end of histone H3 in composition of the nucleosomal core of the "silent" chromatin [Bannister, 2001]. As a result, chromatin domains Hp1 of heterochromatin protein 1 does not recognize the binding sites on heterochromatin and this part of chromatin remains unprotected. Consequently, this part of chromatin exposes properties of "active" chromatin and denaturates at 90°C, which is observed experimentally.

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ბიორეგულატორ ვილონის ზემოქმედება ბიოპოლიმერების სასიცოცხლო ფუნქციასა და ქრომატინზე *IN SITU*

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(მიღებულია 13.02.2006)

რეზიუმე

შესწავლილი იქნა სინთეზური ბიორეგულატორის, დიპეპტიდ ვილონის (Lis-Glu) ზემოქმედება ადამიანის ლიმფოციტების უჯრედების კულტურის სიცოცხლისუნარიანობაზე და მემბრანების, ციტოპლაზმატური ცილებისა და ქრომატინის თერმოდინამიკურ სტაბილურობაზე *in situ*. ნაჩვენები იქნა, რომ NaCl-ის ფიზიოლოგიური კონცენტრაციისა და pH7.0 პირობებში ვილონი გავლენას არ ახდენს ციტოპლაზმური ცილებისა და მემბრანების სტრუქტურულ ცვალებადობაზე *in situ*, და დნმ-ის სტრუქტურაზე *in vitro*. ამავე დროს იგი ზეგავლენას ახდენს მეტაბოლურ სითბოზე (-Q) და იწვევს ჰეტეროქრომატინის ნაწილობრივ გაშლას (დეკონდენსაციას).

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ALTERATION OF TOBACCO RAW MATERIAL QUALITY INDICES IN THE PROCESS OF "AGING"

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Abstract

Possibility of processing of tobacco raw material under the conditions of partial fermentation has been studies. Organoleptic and other qualitative indices of tobacco, like the dynamics of oxygen index variation during aging under the full and partial fermentation conditions have been investigated. It was established that prolonging of the process of aging may be responsible for shortening of the expensive process of fermentation, while the qualitative indices of the production remain the same.

Key words: aging process, partial fermentation, oxygen index, nicotine

Introduction

Tobacco is one of the important technical crops among the cultivated plants in Georgia. For today tobacco industry is mainly destroyed there and users' needs for tobacco are fully satisfied on the expense of imported production.

It must be motioned that native raw material of tobacco is distinguished with its qualitative and sorts diversity (Djaparidze, 1975). It is known that 90% of the cost of smoking production depends on tobacco raw material. This material possesses a complex of qualitative indices, which are responsible for the quality of the integrated products.

In processing of tobacco raw material "aging" or long-term storing after fermentation is one of the leading technological processes [Chouteau et al, 1955; Kintsurashvili, 2006; Tasheva, 1979]. While determining the qualitative changes of the raw material, the main obstacle is necessity of simultaneous testing of different age tobacco samples [Mokhnachev & Babenko, 1986; Mokhnachev et al, 1984; Mokhnachev, 1984; 1985]. The question of improving the quality of tobacco raw material, especially perfection of technologies of its processing, became very popular.

The purpose of our study was intensification of the process of tobacco fermentation, also modification and development of fermentation industry; in particular, improving the qualitative characteristics of tobacco under the conditions of partial fermentation.

Materials and Methods

Tobacco raw material of the sort "Trapezondi" of the 1st, 2nd, and 3rd brand, cultivated in a private section of Imereti (West Georgia), has been used for investigations.

For studying the processes taking place during "aging", tobacco raw material was stored for a long period under usual conditions in the form of standard packs. From time to time samples for analyzing were taken from different packs and qualitative characteristics were determined there. Samples of one and the same quality tobacco were thoroughly mixed, dried, chopped and analyzed. The taste and flavor of tobacco, indices of cremating, humidity and content of nicotine were investigated in experimental material. For studying different indices of the testing material the method of spectrophotometrical analysis has been used [Mokhnachev, 1984].

Content of nicotine in tobacco was determined using our modified method [Kintsurashvili, Melkadze, 2005].

Results and Discussion

From the literature it is known that perfection of the fermentation process is evaluated according to the limiting value of "oxygen index" (OI<0.1). We decided to study the variation of oxygen index in the process of tobacco aging in the cases of full and partial fermentation.

Analyzing samples were divided in two groups. One was fully fermented, the other – only partially. The samples were stored for "aging". The qualitative indices determined before "aging" were taken as control. Once in every 3 month, during 15 months both type of experimental samples were tested. The results of investigations are given in the table.

Table 1. Qualitative indices of tobacco integrated products during the process of aging

Quality	I brand		II brand		III brand	
	Full fermentation	Partial fermentation	Full fermentation	Partial fermentation	Full fermentation	Partial fermentation
Oxygen index (OI)	0.05	0.06	0.07	0.07	0.10	0.12
Nicotine content (mg/g)	16.7	16.0	15.39	15.25	15.24	15.0
Humidity (%)	15.3	15.2	16.0	16.2	16.7	16.4
Organoleptic properties Flavor(mark)	18.0	7.5	17.4	17.2	17.5	17.2
Taste(mark)	18.8	19.0	17.6	17.65	17.6	17.6
Burning capacity (puff per piece of cigarette)	16.6	16.5	18.2	18.0	20.1	20.0

First of all attention must be paid on smoking properties, which is reflected on flavour and taste of tobacco smoke. Usually "aging" for one or more year is responsible for improving of smoking properties, but later (especially at the end of "aging") significant worsening of the test qualities of tobacco smoke takes place.

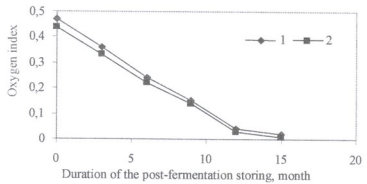
The results obtained on cremating properties of tobacco raw material are interesting from the practical point of view. The experimental results have revealed that post-fermenting storing of tobacco is due to basic improving of burning quality. In this regard "aging" is undoubtedly favourable for obtaining the raw material with low burning quality.

During aging regularly decreases the humidity of tobacco raw material. This takes place in the process of fermentation and prolongs in post-fermentation period of storing. According to this fact, it has been concluded that the raw material with low humidity (less than 12%) can not be hidden for aging.

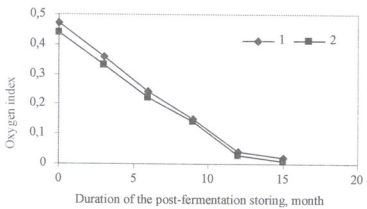
As it was expected, decreasing of nicotine content in tobacco (on the expense of oxidation) was mentioned. Therefore, storing of tobacco, containing much nicotine (more than 2%) may cause improving of its quality. But if tobacco material with low nicotine content (about 1%) is kept, the expectable result is that it will become "empty", i.e. it will lose smoking qualities.

According to the obtained results, it may be concluded that aging of tobacco for a year period finally is responsible for improving the quality properties of the raw material, which may be used in manufacturing technology.

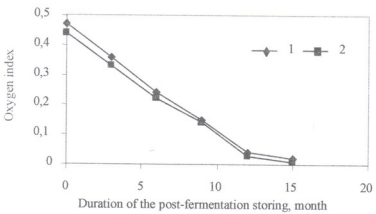
Also the dynamics of variation of the oxygen index has been studied in fully and partially fermented samples. Results of measuring are given on Figure 1 (a, b, c).



a – I brand



b – II brand



c – III brand

Fig.1. Dynamics of oxygen index variation during long-term storing of tobacco raw material
1 – full fermentation, 2 – partial fermentation

From the Figure it is clear that the oxygen index are almost the same in both cases (1-full fermentation, 2-partial fermentation), while the duration of fermentation was significantly reduced in the case of partial fermentation.

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COMPARATIVE STUDY OF THE QUANTITATIVE PARAMETERS OF BERRIES AND SEEDS IN THE AUTOCHTHONOUS RED GRAPE VARIETIES OF THE KOLKHIS (WESTERN GEORGIA)

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Abstract

Seed number per fruit, berry length, breadth, index=breadth/length, seed length, breadth and seed stalk length have been determined and biometric analysis of obtained data has been carried out to identify similarity distances and intercultivar variation of old Kolkhic red grapevine varieties. Total ten varieties of red grapevine have been studied. Nine of them – Aladasturi, Chodi, Kachichi, Koloshi, Machkvaturi, Makhvateli, Odjaleshi, Paneshi and Shonuri, are old aboriginal varieties of the Kolkhis. One variety – Saperavi is currently widespread in Kakheti. Intercultivar variability of quantitative parameters has been revealed and similarity distances among varieties have been determined by Tree Clustering method.

Key words: Berry, cluster analysis, grapevine varieties, seed.

Introduction

Worldwide, grapes (*Vitis* spp.) are among the most important plant species cultivated with an area of about 9 million ha. The leading cultivated species by far is *Vitis vinifera* L. (Vitaceae). Domestication of the grapevine (*V. vinifera* ssp. *sativa* DC.) and development of the technology of viticulture and winemaking initially occurred in the southern Caucasus about 6000 to 4000 BC [Mullins et al., 1992; Jackson, 1994; Damania et al., 1997; Sefc et al., 2003], which determines high importance to study the autochthonous grape varieties in the Caucasus and namely in Georgia.

From this ancestral center of the grape origin, viticulture spread around the Mediterranean Basin to the Far East and was later introduced to the New World, particularly by settlers from the winegrowing countries of the Mediterranean area [Sefc et al., 2003]. The rapid domestication of the grapevine was supported by the manifold uses of grapes for producing table fruit, wine, juice, and raisins. The modern grapevine varieties show great genetic diversity and broad adaptability to different soils and climates. Genetic differentiation among extant human populations was used to show that the spread of agriculture through Europe was accompanied by demic diffusion of Neolithic farmers [Sokal et al., 1991]. It is assumed that spread of viticulture mainly involved the dissemination of domesticated varieties of grapevine, while the use of indigenous wild vines is discussed as alternative origin of grapevine cultivars [Sefc et al, 2003[. It seems that variation in *V. vinifera* was insufficient to permit universal cultivation and led to the domestication of other wild species, such as *V. vinifera* ssp. *silvestris* Gmel. in the Caucasus, Europe and Near East; *V.*

labrusca L. and *V. rotundifolia* Michaux in America; and, *V. amurensis* Ruprecht in China. This caused some taxonomic confusion in the taxonomic status of the modern grapevine varieties.

Only *V. vinifera* has some 10 000 varieties worldwide. Among them, about 500 names of Georgian autochthonous grapevine varieties are known [Javakhishvili, 1986; Ketskhoveli et al., 1960]. In 1860, the *V. vinifera* was virtually wiped out in the places of its origin, when an aphid, *Phylloxera vastatrix* had accidentally introduced into France, and within a few years had ravaged all vineyards in Europe and as well in the Caucasus, including Georgia. It normally lives in the roots of some American vines without doing any great harm, but once they get into the root system of *V. vinifera*, it soon kills the plant. It was soon noticed that the few American vines found growing in Europe as exotics were not affected, and grafting quickly began. Currently, almost all Georgian grape varieties are grafted on rootstocks of American vines.

This disaster made it necessary to undertake urgent steps for *ex situ* conservation of old, endangered and autochthonous grapevine varieties by set up of living collections in Georgia, which was started in 30es of the XX century. Nowadays, 929 varieties are protected in the living collections of the State Agrarian University in Dighomi and Mukhrani; and, in the collections of the Georgian Scientific-Research Institute of Horticulture, Viticulture and Winemaking in Telavi and Skra. Among them, 701 are "original" and only 248 are autochthonous Georgian varieties remained from the known 524 varieties [Chkhartishvili, Tsertsvadze, 2004]. Some aboriginal Georgian grape varieties is still possible to find in the private grounds of peasants and in several small living collections, such as grapevine collection of the G. Eliava National Museum in Martvili district founded in 1972 [Eliava, Tsotsoria, 2002]. This collection containing 24 old Kolkhic grapevine varieties was used in the present study as source for material collection.

Currently, big attention is given to conservation, characterisation, collection and utilization of genetic resources of grapevine varieties, especially of old, endangered and autochthonous ones worldwide. The European Gene Banks (EURISCO) and EU program Genres081 attempt to develop database for all grape varieties including that from Georgia. Main approach used today for identification of genetic diversity of grapes is based on DNA technology for detection of grape intracultivar variation [This et al., 2004]. However, several micromorphometric and QTL methods for evaluation of berry and seed quantitative parameters of taxonomic importance are widely used in the studies of genetic differentiation of grapevine varieties [Negrul, 1960; Mangafa, Kotsakis, 1996; Jacquat, Martinoli, 1999, Doligez et al., 2002]. Ampelometric and economic-technological parameters are studied for most Georgian grapevine varieties by different authors [Ramishvili, 1948, Ketskhoveli et al., 1960, Frolov-Bagreev et al., 1946-1970, Tsertsvadze, 1989]. However, computer derived new statistical programs offers an opportunity to conduct biometric analysis of quantitative parameters of berry and seed sizes and obtain new results.

The aim of the present study was to determine berry and seed sizes and carry out biometric analysis of obtained data to identify similarity distances and intercultivar variation of old Kolkhic red grapevine varieties.

Material and Methods

Total ten varieties of red grapevine (*V. vinifera* L.) have been studied. Nine of them - Aladasturi, Chodi, Kachichi, Koloshi, Machkvaturi (=Machkadina), Makhvateli, Odjaleshi, Paneshi and Shonuri, are old aboriginal varieties of the Kolkhis. One variety – Saperavi is widespread in Kakheti, currently, and was included in the list as out-group species for comparative statistical analysis.

All studied varieties belong to the eco-geographic group: convar. *pontica* subconvar. *georgica* Negr. provar. *tomentosae* Tserts. [Tsertsvadze, 1989]. Odjaleshi, Makhvateli and Shonuri

are unified into classification group 2 according to Tsertsvadze (1989). Machkvaturi and Chodi are included in the group 14. In separate groups are distributed Paneshi, Koloshi, Kachichi, Saperavi and Aladasturi.

Typical representatives of the aboriginal grape varieties endemic for Samegrelo are Odjaleshi, Machkvaturi and Paneshi. Chodi occurs both in Samegrelo and Adjara. Makhvateli and Koloshi are cultivated in Samegrelo, Guria and Adjara. Aladasturi is typical grape variety of Guria. Shonuri (Svanuri) is spread in Lechkhumi, Samegrelo and Guria. Kachchi is recognized as Abchasian variety, although the origin is not clearly known. Saperavi now occurs mainly in the Eastern Georgia. Although, I. Javakhishvili (1986) suggests that it might be originated in Kolkhis. Therefore, we have chosen it as out-group species in the statistical analysis.

Plant material has been collected in the grapevine living collection of the G. Eliava National Museum in v. Martvili, except Saperavi, which was obtained from private ground in Kakheti, v. Shilda, Kvareli distr. 30 berries have been collected from different plants of each variety for statistical analysis. Photographs have been taken for each collected samples (plant, leaf, branch, cluster, berry and seed) using digital photo cameras Nikon Coolpix5000 and Canon 3,2 megapixel CCP. Berry and seed morphology was studied using stereomicroscope Stemi DV4, Karl Zeiss, Germany. Number of seeds per fruit was estimated. Morphometric parameters have been measured according to known methods [Mangafa, Kotsakis, 1996; Jacquat, Martinoli, 1999] used in studies of grape berries and seeds. The measurements have been done using micrometer and millimeter paper. The following parameters have been measured: berry length, berry breadth, seed length, seed breadth, stalk length.

Statistical analysis of the data has been performed by computer programs MS Exel and Statistica 6. Mean, standard deviation and variance have been determined for every data set. Index representing breadth/length ratio has been determined for berries. Linear regression analysis has been done to compare berry and seed lengths. Multivariate cluster analysis was performed to determine similarity distances among grape varieties. Different horizontal hierarchical tree plots have been developed. The distances between objects used when forming the clusters were: 1) Euclidean distance, which is simple geometric distance in the multidimensional space calculated for raw data; 2) Chebychev distance, which allows to define two objects as different if they are different on one of the dimensions. Both single and complete linkages have been used in the analysis. In the single linkage method, the distances between clusters are determined by the distance of the two closest objects in the different clusters. In the complete linkage method, the distances between clusters are determined by the greatest distance between any two objects in the different clusters. Different parameters of berry and seed sizes of studied grape varieties have been tested by the tree clustering method.

Results

Table 1 represents mean, standard deviation and variance of berry sizes of the ten red grape varieties - Aladasturi, Chodi, Kachichi, Koloshi, Machkvaturi, Makhvateli, Odjaleshi, Paneshi, Saperavi and Shonuri. The length and breadth of the berries show that the size of seeds is smallest in Kachichi (length =10,20±0,51 mm), medium size berries (length < 15 mm) have Chodi, Koloshi, Makhvateli and Odjaleshi. Large berries (length >15 mm) are characteristics for Aladasturi, Machkvaturi, Paneshi, Saperavi and Shonuri. The index representing ratio of berry breadth and length is indicative of berry shape. Almost round seeds (with index > 0.9) possess Aladasturi, Chodi, Kachichi, Koloshi, Makhvateli, Odjaleshi and Shonuri. Nearly similar indexes have Machkvaturi (0.86) and Saperavi (0.88). More ellipsoid form has berry of Paneshi (0.81).

Table 2 shows mean, standard deviation and variance of seed length, breadth and stalk length. Stalk represents modified funiculus of the ovule and is considered as feature of taxonomic importance for the varieties. As it was the case with berries, Kachichi has smallest seeds (length = 4.92±0.18). The length of the seeds in other varieties varies from 6 to 8 mm. The shortest stalk has Kachichi (0.85±0.19) and longest Saperavi (2.07±0.14).

Table 1. Mean, standard deviation (Sd) and variance (V) of berry length, breadth and index (breadth/length ratio) for ten red grape varieties. n=30.

N	Variety	Length			Breadth			Index - breadth/length		
		mean	Sd	V	mean	Sd	V	mean	Sd	V
1.	Aladasturi	17,20	0,64	0,41	15,87	0,63	0,40	0,92	0,036	0.001
2.	Chodi	14,51	1,09	1,18	13,97	1,14	1,29	0,96	0,040	0,002
3.	Kachichi	10,20	0,51	0,26	9,99	0,64	0,40	0,98	0,033	0,001
4.	Koloshi	13,63	1,36	1,85	13,38	1,32	1,74	0,98	0,026	0,001
5.	Machkvaturi	16,50	0,80	0,62	14,24	0,71	0,51	0,86	0,047	0,001
6.	Makhvateli	13,86	0,64	0,41	13,41	0,61	0,37	0,96	0,029	0,004
7.	Odjaleshi	13,79	0,97	0,94	12,75	0,75	0,57	0,92	0,040	0,001
8.	Paneshi	18,44	0,79	0,60	15,00	0,59	0,35	0,81	0,030	0,001
9.	Saperavi	15,87	0,63	1,87	13,89	1,26	1,60	0,88	0,070	0,005
10.	Shonuri	15,36	0,85	0,79	14,95	0,79	0,63	0,97	0,036	0,001

Table 2. Mean, standard deviation (Sd) and variance (V) of seed length, breadth, and stalk length for ten red grape varieties. n=30.

N	Variety	Length			Breadth			Stalk length		
		mean	Sd	V	mean	Sd	V	mean	Sd	V
1.	Aladasturi	7,69	0,35	0,127	4,05	0,18	0,032	1,66	0,20	0,041
2.	Chodi	6,76	0,27	0,071	4,34	0,27	0,075	1,73	0,20	0,040
3.	Kachichi	4,92	0,18	0,033	3,34	0,33	0,060	0,85	0,19	0,039
4.	Koloshi	7,48	0,37	0,067	4,88	0,21	0,039	1,82	0,12	0,015
5.	Machkvaturi	7,09	0,47	0,085	4,14	0,32	0,058	1,87	0,19	0,038
6.	Makhvateli	5,93	0,30	0,055	3,92	0,25	0,046	1,70	0,22	0,048
7.	Odjaleshi	6,86	0,16	0,028	4,27	0,32	0,058	1,71	0,15	0,023
8.	Paneshi	7,49	0,29	0,054	4,55	0,36	0,066	1,82	0,20	0,041
9.	Saperavi	6,41	0,42	0,026	3,83	0,16	0,022	2,07	0,14	0,074
10.	Shonuri	6,30	0,41	0,170	4,01	0,20	0,041	1,53	0,19	0,039

The linear regression analysis between berry and seed lengths shows slight correlation (R^2=0,58; Fig.1). The number of seeds per fruit was variable feature among samples. It varies from one to four in different berries. However, some tendency to be one-, two-, three- or four seeded was

revealed for varieties (Fig. 2). Chodi, Koloshi and Odjaleshi have less than 2 seeds. 2-3 seeds were found in Machkvaturi, Makhvateli, Kachichi and Saperavi. More than 3 seeds are characteristic for Aladasturi, Paneshi and Shonuri.

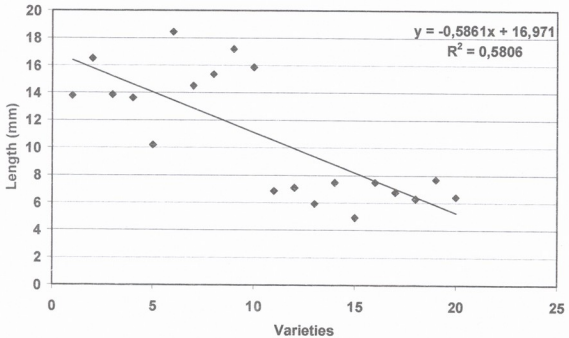

Fig. 1. The linear regression analysis between berry and seed lengths in ten red grape varieties. n=10.

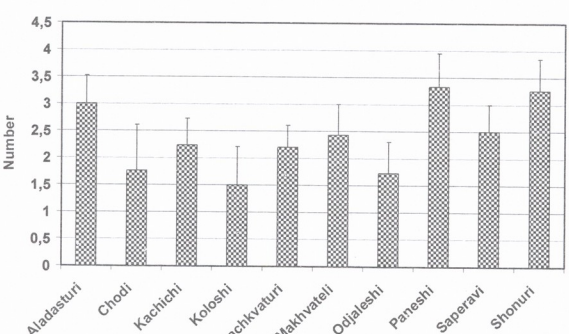

Fig. 2. The number of seeds per fruit in ten red grape varieties. n=30.

Raw data of length of the seed stalk have been statistically analysed with Tree Clustering method. Different horizontal hierarchical tree plots have been developed to determine similarity distances among studied varieties. Single and complete linkage and Euclidean and Chebychev distance metrics have proved as most suitable for this analysis. The Euclidean distance analysis (Fig. 3) has revealed that almost all West Georgian varieties were very similar with linkage

distance less than 1.5. More distant positions showed Shonuri and Saperavi. Very distant position has Kachichi. Chebychev distance metric analysis (Fig. 4) revealed five cluster groups, which are less different within group when compare with other linkages. This linkage groups are: 1) Odjaleshi and Makhvateli; 2) Paneshi and Koloshi; 3) Shonuri, Chodi and Aladasturi; 4) Machkvaturi and Saperavi; and, 5) Kachichi.

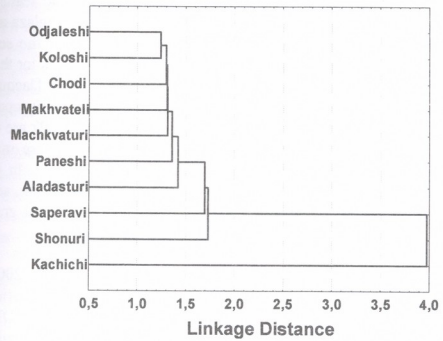

Fig. 3. Horizontal hierarchical tree plot of Kolkhic grape varieties developed by tree clustering method with single linkage and Euclidean distance analysis. Raw data of length of the seed stalk have been statistically analysed.

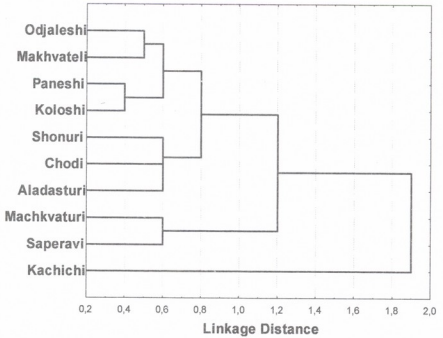

Fig. 4. Horizontal hierarchical tree plot of Kolkhic grape varieties developed by tree clustering method with complete linkage and Chebychev distance analysis. Raw data of length of the seed stalk have been statistically analysed.

Discussion

Morphometric study of grape organs is widely used in ampelographic characterization of grapevine species or cultivars and by far represents main diagnostic methods for grapevine classification (Tsrtsvadze, 1989). Among different quantitative and qualitative parameters the sizes and morphology of berries and seeds is considered to be most informative diagnostic features [Jacquat, Martinoli, 1999]. The length, breadth, length of stalk, length and breadth of chalaza and the sculpture of the fossete were considered as the most important characteristics of the seed [Negrul 1960]. Almost all these dimensions and ratios have been used by various scholars for their taxonomic works, especially in connection with the origin of *V. vinifera* ssp. *vinifera* [Jacquat, Martinoli, 1999]. However, although such data are available in both West and East European literature, the dimensions and ratios of the whole berry and pip as well as the length of stalk are the only criteria used in the most works [Mangafa, Kotsakis, 1996]. These parameters are main passport characteristics, as well, for a variety included in the European *Vitis* Database. In the present study, the statistically sufficient data on berry length, breadth, index =breadth/length, seed length, breadth and stalk length are done for the first time for West Georgian aboriginal grape varieties, which will be used further for passport data.

Morphological parameters are used together with molecular genetic studies for determination of genetic similarity and origin history of grape varieties worldwide [Doligez, 2002]. Recently, genetic analysis has been included in the investigations of crop domestication [Maletic et al., 1999, Sefc et al., 2003]. The morphometric data, however, are used parallel to PCR-based DNA analysis; hence, molecular markers oft describe only one phenotypic feature while morphological description allows overall characterization of a variety. For determination of genetic relations among varieties most informative parameter is seed stalk length, which by many authors is considered to be a feature of taxonomic importance [Tsertsvadze, 1989; Mangafa, Kotsakis, 1996]. In our study we have used this parameter for cluster analysis and determination of similarity indexes among varieties.

Caucasus is considered to be a primary centre for the domestication of grapevine, with high relevance for the further distribution of the crop towards the Mediterranean basin and for the development of the European modern cultivars [Mullins et al., 1992; Jackson, 1994; Damania et al., 1997; Sefc et al., 2003, Constantini, 2004]. Therefore, to study aboriginal grape varieties in the place of its domestication is of high importance to determine genetic relations among modern grape cultivars.

In the present study, berry and seed quantitative parameters of taxonomic importance have been analyzed for ten autochthonous red grape varieties of Kolkhis. Three of them -Odjaleshi, Machkvaturi and Paneshi, are endemic varieties for Samegrelo. Makhvateli and Koloshi are cultivated in Samegrelo, Guria and Adjara. Chodi is spread in Samegrelo and Adjara. Shonuri (Svanuri) occurs in Lechkhumi, Samegrelo and Guria. One, Saperavi is highly commercial variety widespread in Kakheti. However, suggestion about its Kolkhic origin is known [Javakhishvili, 1986].

According to classification of Tsertsvadze (1989) all studied varieties belong to the eco-geographic group: convar. *pontica* subconvar. *georgica* Negr. provar. *tomentosae* Tserts. Odjaleshi, Makhvateli and Shonuri are unified into classification group 2nd [Tsertsvadze, 1989]. According to our data Odjaleshi and Makhvateli show high similarity when analyzed by Chebychev distance. The main difference between these two varieties is thicker berry skin occurred in Makhvateli [Eliava, Shonuri, 2002], which determines name of this variety – "crisp" in Megruli language [Javakhishvili, 1986]. According to some authors Shonuri is considered to be a synonym for Odjaleshi [Tsertsvadze, 1989]. However, in the Ampelography [Ketskhoveli et al., 1960], it is said that it differs from Odjaleshi by number of morphological features. According to our data

Shonuri is quit different form Odjaleshi and is situated in separate cluster after Euclidean distance analysis. By Chebychev analysis it is linked with Chodi and Aladasturi.

Very interesting data to our opinion is grouping of Saperavi and Machkvaturi in one cluster after Chebychev distance analysis. As well, both varieties have similar size of berries and breadth/length ratio, 0.88 an 0.86 respectively, while all other studied varieties have more rounded seeds with index more than 0.9. Another similarity is that berry of the both varieties are connected with stalk very weekly and falls down when ripe. Machkvaturi, therefore is called as "Mtsvivani" in Megruli language which means "falling down". From ancient times, Machkvaturi was used in religious rituals for medicinal purpose against reproductive sterility in men. Healthy properties of Saperavi are well known. We suggest that maybe these two varieties have common ancestor and Saperavi was first originated in Kolkhis.

In general, all studied varieties are characterized by short similarity distances except one - Kachichi. The Euclidean linkage distance in most varieties is less than 1.5, while in case of Kachichi it is 4. The same is typical for Chebychev linkage distance. We assume that Kachichi has different origin than other studied varieties. The origin of Kachichi is not well determined [Ketskhoveli et al., 1960]. It is unified with Abkhasian varieties according to Tsertsvadze (1989). Our data shows that it differs strongly from autochthonous Kolkhic grapes.

In conclusion we have to mention that morphometric parameters represent good opportunity to study similarity distances and genetic relations among different grapevine varieties, which will have big importance to study origin and domestication history of the varieties of grapevine.

Acknowledgement

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მარცვლის და თესლის რაოდენობრივი მაჩვენებლების შედარებითი შესწავლა კოლხეთის ველური ყურძნის აუტოქტონურ ჯიშებში

ეხვაია ჟ., ახალკაცი მ.

ნ. კეცხოველის სახ. ბოტანიკის ინსტიტუტი

(მიღებულია 02.12.2005)

რეზიუმე

განსაზღვრული იქნა თესლის რიცხვი, მარცვლის სიგრძე, სიგანე, ინდექსი = სიგანე/სიგრძე, თესლის სიგრძე, სიგანე და თესლის ნისკარტის სიგრძე და განხორციელდა მონაცემთა ბიომეტრული ანალიზი წითელი ყურძნის ძველ კოლხურ ჯიშებს შორის მსგავსების მანძილის და შედარებითი ვარიაბელობის გამოვლენის მიზნით. შესწავლილ იქნა წითელი ყურძნის კოლხეთის 9 აუტოქტონური ჯიში – ალადასტური, ჭოდი, კაჭიჭი, კოლოში, მაჭყვატური, მახვატელი, ოჯალეში, პანეში, შონური და ერთი კახეთში გავრცელებული საფერავი, როგორც ჯგუფის გარეთ მდგომი ჯიში. გამოვლინდა რაოდენობრივი მაჩვენებლების ჯიშთაშორისი ვარიაბელობა და დადგინდა ჯიშთა შორის მსგავსების მანძილი კლასტერული ანალიზის მეთოდის გამოყენებით.

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EFFECT OF INDOLE ACETIC ACID AND SACCHAROSE ON VEGETATIVE PROPAGATION OF SOME DECORATIVE EXOTIC PLANTS GROWN IN BATUMI BOTANICAL GARDEN

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Abstract

Effect of 0.01% aqueous solution of indole acetic acid and 2% aqueous solution of saccharose on the process of vegetative propagation of 16 highly ornamental exotic plant species belonging to 6 families (Hamamalidaceae, Illiciaceae, Proteaceae, Rosaceae, Bignoniaceae, Myrtaceae) introduced in Batumi Botanical Garden has been studied.

Key words: introduction, rooting, cutting

Introduction

Great number of introduced exotic plant species grown in Batumi Botanical Garden are distinguished by highly ornamental, valuable technical, nutritive, curative and other features. Many trees, lianas or bushes introduced from different floristic regions have been tested here for decades. Some of them were successfully implemented in agriculture, green construction, decorative gardening and forestry. Some of species exist only in the collection of Botanical Garden and are not found anywhere else. These are highly decorative evergreen plants of various geographical origin belonging to Hamamalidaceae, Illiciaceae, Proteaceae, Rosaceae, Bignoniaceae and Myrtaceae families, characterized with effective blossoming during winter and spring (Table 1). Vegetative development of these species proceeds normally, but in spite of adaptation to local conditions some of them are characterized by weak fruitage. Besides, stratification of different duration or in some cases scarification is needed for seeds to germinate [Trees and shrubs of BBG, 1987; Trees and shrubs of USSR, 1950-1962]. Certain time is needed for these processes. Considering this we decided to study capacity of these plants for vegetative propagation by grafts using 0.01% solution of indole acetic acid (IAA) and 2% solution of saccharose.

Materials and Methods

In case of deciduous plants grafts were taken at the end of February, before bud breaking and starting of sap movement. Grafts of 15-25 cm length were taken from annual lignified shoots. Grafts of 15-20 cm length of evergreen species were prepared at the end of August. In both cases grafts have been taken from shoots of comparatively young samples [Kapianidze, N. V., Krialashvili L.G., 1980; Klimovich V. I., Klimovich I.V., 1987].

Green shoots contain less amount of nutrients in contrast to lignified ones. Considering this fact grafts with several leaves were taken from lateral well developed shoots. In case of young green cuttings, grafting was performed by half-lignified ones. With this aim cuttings were taken in July-August and soft shoot tip was removed. Cuttings of 15-20 cm length with 2-3 nodes were taken from comparatively maturated middle and lower parts of a shoot. Leaves were removed from lower node and the area of leaf blade situated on the upper first or second nodes was restricted by 1/3 or 1/2, in order to reduce transpiration and avoid intershading. Aqueous solutions of IAA (0.01%) and saccharose (2%) were prepared on the same day when the cuttings were taken. Green cuttings were immersed into solutions by 1/3 of the whole length and lignified cuttings up to the half of the whole length for 24 hours.

Cuttings treated by the above mentioned solutions were transferred to greenhouse and planted out into substrate composed of soil and sand mixture. The cuttings were planted on the substrate to 1.5-2 cm depth in inclined position; distance between cuttings and beds was 3-4 cm. Constant air and substrate humidity, illumination and temperature regime were kept in the greenhouse. Temperature interval - 18-30°C and air humidity - 80% were the most favourable for rooting. One of the environmental factors - light is very essential for rooting of treated cuttings, as very soon after planting root-forming process is started and correspondingly the demand for nutrients and the necessity of synthesis of organic substances increase.

Results and Discussion

The experiment has shown that the process of rooting was more active in cuttings taken in August. Rooting was started after 30-35 days from planting. In cuttings taken in February the process of rooting started 50-55 days after planting. Root system was better developed on cuttings taken in August. Treatment with growth stimulators (IAA, saccharose) yielded positive results (Table 2).

Table 1. Life forms and geographical origin of some exotic ornamental plants grown in Batumi Botanical Garden

N	Plant species	Family	Life form	Place of origin
1.	*Bignonia unguis-kati* L.	Bignoniacea	evergreen liana	Argentina
2.	*Raphiolepis delacouri* Andre	Rosaceae	hybrid, evergreen bush	China
3.	*Raphiolepis umbellata* (Thunb.)	"-"	evergreen bush	Japan, Korea
4.	*Lomatia longifolia* R. Br	Proteaceae	evergreen bush	Australia
5.	*Tristania laurina* R. Br.	Myrtaceae	evergreen bush	Australia
6.	*Illicium parviflorum* Michx.	Illiaceae	evergreen bush	North America
7.	*Illicium religiosum (anisatum)* Sieb. et Zucc.	"-"	evergreen bush	China, Japan
8.	*Distylum racemosum* Sieb. et Zucc	Hamamelidaceae	evergreen tree	Japan
9.	*Corylopsis sinensis* Hemsl.	"-"	deciduous bush	China
10.	*Corylopsis spicata* Sieb et Zucc.	"-"	"-"	Japan
11.	*Corylopsis veitchiana* Beam.	"-"	"-"	China
12.	*Hamamelis mollis* Oliv.	"-"	"-"	China
13.	*Hamamelis vernalis* Sarg.	"-"	"-"	North America
14.	*Hamamelis virginiana* L.	"-"	"-"	North America
15.	*Loropetalum chinense* Oliv.	"-"	evergreen bush	China
16.	*Sycopsis sinensis* Oliv.	"-"	evergreen bush	China

Table 2. Results of rooting of experimental cuttings

N	Plant species	Percentage of survival			
		Term of taking cuttings	Control	IAA, 0.01%	Saccharose, 2%
1.	*Bignonia unguis-kati* L.	29.08	65	90	85
2.	*Raphiolepis delacouri* Andre	29.08	40	75	75
3.	*Raphiolepis umbellata* (Thunb.)	29.08	30	65	85
4.	*Lomatia longifolia* R. Br	29.08	35	70	75
5.	*Tristania laurina* R. Br.	29.08	45	60	75
6.	*Illicium parviflorum* Michx.	30.08	25	65	60
7.	*Illicium religiosum (anisatum)* Sieb. et Zucc.	30.08	20	75	80
8.	*Distylum racemosum* Sieb. et Zucc	30.08	35	100	100
9.	*Corylopsis sinensis* Hemsl.	27.02	15	80	65
10.	*Corylopsis spicata* Sieb et Zucc.	27.02	35	75	90
11.	*Corylopsis veitchiana* Beam.	27.02	35	70	90
12.	*Hamamelis mollis* Oliv.	27.02	25	80	75
13.	*Hamamelis vernalis* Sarg.	27.02	20	75	75
14.	*Hamamelis virginiana* L.	27.02	20	70	65
15.	*Loropetalum chinense* Oliv.	30.08	-	25	20
16.	*Sycopsis sinensis* Oliv.	30.08	15	60	55

In this case the percentage of survival of rooted cuttings made at average 65-75%, while in case of rooting without treatment, percentage of survival made 20-30%.

Vegetative propagation was especially successful in highly ornamental species belonged to the genera Corylopsis, Hamamelis, Raphiolepis, Bignonia, blossoming in winter and early spring. The percentage of survival approached 100% in case of evergreen plant, Distylium of good habitus. At the end of vegetation period rooted cuttings have been transferred to pots, left in greenhouse during winter and transferred to open ground at the beginning of spring.

Thus it can be concluded, that 2% aqueous solution of saccharose and 0.01% solution of IAA can be successfully applied for vegetative propagation of exotic plants.

It seems to be advisable wide implementation of the studied original highly ornamental exotic plants in landscaping of Black Sea Coast of Adjara.

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ბათუმის ბოტანიკური ბაღის ზოგიერთი მაღალდეკორატიული ეგზოტური მცენარის ვეგეტაციური გამრავლება ინდოლილმარმჟავას და საქაროზას ხსნარების მოქმედებით

მეტრეველი მ., ბრეგვაძე მ., ბოლქვაძე გ., ლომთათიძე ნ.

ბათუმის ბოტანიკური ბაღი

(მიღებულია 20.02.2006)

რეზიუმე

შესწავლილია ბათუმის ბოტანიკურ ბაღში მოზარდი მსოფლიოს სხვადასხვა ფლორისტული ოლქიდან ინტროდუცირებული 6 ოჯახის (Hamamalidaceae, Illiciaceae, Proteaceae, Rosaceae, Bignoniaceae, Myrtaceae) 16 მაღალდეკორატიული სახეობის ვეგეტაციური გამრავლების, კერძოდ, ღეროს კალმებით გამრავლების შესაძლებლობანი ინდოლილმარმჟავას 0.01% და საქაროზას 2% ხსნარების მოქმედებით. აჭარის შავიზღვისპირეთის გამწვანებაში ეს ორიგინალური ჰაბიტუსის, ზამთარსა და გაზაფხულზე ეფექტურად მოყვავილე ეგზოტები დღემდე უმნიშვნელოდ ან საერთოდ არ არის გამოყენებული. მათი გამრავლების სირთულის დასაძლევად წარმატებით შეიძლება იქნეს გამოყენებული საქაროზას 2%-იანი და ინდოლილმარმჟავას 0.01%-იანი ხსნარები.

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Botany

FUNGI AND CRYPTOGAMS OF HIGH MOUNTAIN ECOSYSTEMS OF LAGODEKHI STATE RESERVE

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Abstract

Vegetation and ecological state of the treeline, a rich and original habitat, is particularly important to high mountainous regions of Georgia. Fungi and cryptogamic plants are its principal components and, therefore, taxonomic and ecological investigation of mycobiota and lichen and moss complexes of separate plant species as well as plant communities is essential with respect to biodiversity assessment. Taxonomic structure of fungi (micro-fungi), lichens and mosses of the high mountain zone of the Lagodekhi reserve, distribution of these groups of organisms according to vertical belts and plant formations and their relation to particular phorophyte species are presented for the first time.

Key words: subalpine, alpine, subnival belts, micro-fungi, lichens, mosses.

Introduction

The main purpose of the research was to find out species of micro-fungi, lichens and mosses principal and characteristic to the vegetation of subalpine, alpine and partly subnival belts of the Lagodekhi reserve.

Altitudinal limit of the high-mountainous zone passes between 1800-3500 m a. s. l. in the eastern part of the Greater Caucasus and, particularly, in the Lagodekhi reserve [Dolukhanov et al., 1941]. Subalpine, alpine and partly subnival belts are distinguished within the mentioned zone.

Forest, meadow and transitional forest-meadow ecosystems are distinguished in the subalpine belt (1800-2500 m). Tall herbaceous vegetation, grass-forb densely sodded meadows and alpine carpets are characteristic to alpine belt (2500-3000 m). Rocky ridges and scree between them above 3000 m represent subnival belt (3000-3500 m). These are communities and micro-communities of the alpine carpets – sedge-grass and grass-forb carpets [Dolukhanov, 1941; Kvachakidze, 1999].

Materials and Methods

Similar to the research on cryptogams and fungi of the forest belt of the Lagodekhi reserve [Chikovani et al., 2005] the study was carried out using detailed itinerary and semi-stationary techniques with further laboratory treatment of the material. The material was identified via microscopic analysis of fruitages.

In order to find out maximum possible number of complexes of saprotrophic and biotrophic/necrotrophic fungi on separate host plants, latent forms of fungi were extracted in

laboratory conditions along with direct investigation of the material. The following mycological manuals and keys were used [Saccardo, 1882-1972; Dennis, 1968; Sutton, 1980; Ellis, 1983; Hawksworth et al., 1996. Lichens and mosses were identified according to the following sources: Oxner, 1993; Poelt, 1969; Melnichuk, 1970].

Results and Discussion

Table 1 presents distribution of parasitic and saprotrophic micro-fungi on principal and characteristic species of subalpine beech, oak, maple and birch forests, shrubberies, tall herbaceous vegetation and meadows. Micro-fungi of alpine shrubberies, high mountain meadows and meadow-carpets of subnival belt are also given.

In consequence of the investigation of the micro-fungi of high mountain ecosystems in the Lagodekhi Reserve, principal patterns of their distribution were revealed; particularly, it was determined that there are numerous representatives of *Coelomycetes* with minute spores on the studies area, which indicates their adaptation to severe environmental conditions related to increasing altitude. Mostly forms of the rust fungi with shortened life cycle occur on the study area. Small number of the powdery mildew species is due to negative influence of low temperature. Only genera and forms with short growing period and those adapted to minimum amount of heat occur there.

In contrast with the forest belt of the Lagodekhi Reserve, phylophilous complexes of micro-fungi obviously predominate in the high mountain ecosystems of the studied area.

Distribution of lichens and mosses in the designated belts corresponds to specificity of their geographic distribution and ecology, i.e. their proportion in the plant cover is increased. Cushions and mats are the predominant life forms of lichens and mosses there.

Prevalence of epiphytic lichens is noticeable in the subalpine belt, while representatives of epilithic and epigeal synusiae of lichens and mosses are in abundance in the alpine and subnival belts.

Table 1. Micro-fungi of high mountain ecosystems of the Lagodekhi reserve.

Subalpine belt (1800-2500 m a. s. l.)	
Host plants	**Micro-fungi**
Acer trautvetteri	*Ascochyta asclepiadearum* var. *macrospora, Camarosporium acerinum, Cercospora acericola, Coryneum foliicolum, Cucurbitaria acerina, Cylindrosporium pseudoplatani, Diplodia acerina, Discosia artocreas, Gloeosporium acericola, Gnomonia inclinata, Hendersonia sarmentorum, Hymenoscypha epiphyllum, Leptothyrium platanoides, Phoma platanoides, Phyllosticta aceris, P. pseudoplatani, Tubercularia vulgaris*
Achillea biserrata	*Erysiphe cichoracearum* f. *achilleae, Phomopsis achilleae*
Aconitum nasutum	*Cercospora aconiti, Erysiphe communis* f. *aconiti, Leptothyrium vulgare*
Althaea rugosa	*Ascochyta parasitica, Phoma nebulosa, Puccinia malvacearum*
Anemone fasciculata	*Septoria anemones, Sphaerotheca fuliginea* f. *anemones*
Anthemis sosnovskyana	*Microdiplodia chrysanthemi, Phoma chrysanthemicola*
Anthoxanthum odoratum	*Helminthosporium dematioideum, Puccinia poae-sudeticae*
Betula litwinowii	*Camarosporium betulinum, Colletotrichum betulae, Didymosphaeria massarioides, Diplodia betulae, Hendersonia punctoidea, Hypoxylon udum, Melamsporidium betulae, Microsphaera alni, Septoria betulina, Steganosporium muricatum*
Brachypodium silvaticum	*Epichloë typhina*
Bupleurum polyphyllum	*Coniothyrium olivaceum, Phyllosticta bupleuri, Puccinia bupleuri, Septoria buplearicola*

Calamagrostis arundinacea	*Coniothyrium tenue, Leptosphaeria fuskelii, Septoria calamagrostidis*
Campanula lactiflora	*Ascochyta campanulae, Coleosporium campanulae, Mycosphaerella campanulae, Puccinia campanulae, Ramularia macrospora, R. coleospori, Septoria obscura*
Cirsium obvallatum	*Phyllosticta cirsii, Puccinia cnici, Ramularia cirsii, Septoria cirsii*
Convolvulus arvensis	*Erysiphe communis* f. *convolvuli, Septoria convolvuli, S. longispora*
Dactylis glomerata	*Erysiphe communis* f. *dactylidis, Diplodia herbarum, Leptosphaeria eustoma*
Daphne mezereum	*Diaporthe eres, Didymosphaeria analeptoides, Dothidea tetraspora, Melampsora daphnicola*
Doronicum macrophyllum	*Cercosporella aronicicola, Phyllosticta aronici, Ramularia doronici*
Epilobium montanum	*Didymella fenestrans, Mycospaerella leptoasca, Puccinia vagans, Ramularia montana, Sphaerotheca macularis* f. *epilobii*
Euphorbia macroceras	*Leptosphaeria euphorbiae, Melampsora euphorbiae* f. *gerardianae, Phyllosticta euphorbiaecola, Sphaerotheca tomentosa*
Fagus orientalis	*Ascochyta fagi, Coccomyces coronatus, Diatrype stigma, Discosia artoceras, Gloeosporium fagicolum, G. fuskelii, Hymenoscypha epiphyllum, Hypoxylon serpens, Melogramma bulliardii, Mycosphaerella fagi, M. punctiformis, M. virgultorum, Phoma antarctica, Quaternaria quaternata, Septoria fagi, Tubercularia, vulgaris, Xylaria carpophila, X. polymorpha.*
Gentiana schistocalyx	*Mycosphaerella gentianae, Phyllosticta gentianella, Puccinia gentianae, Ramularia evanida*
Geranium ibericum	*Phyllosticta geraniicola, Ramularia geranii, Sphaerotheca macularis* f. *geranii, Uromyces geranii*
Heracleum sosnowskyi	*Cylindrosporium heraclei, Erysiphe umbelliferarum* f. *heraclei, Phoma complanata, Phomopsis eryngiicola, Septoria heraclei*
Impatiens noli-tangere	*Cercospora campi-silii, Puccinia argentata, Ramularia impatiensis, Sphaerotheca fuliginea* f. *impatientis*
Inula helenium	*Coleosporium inulae, Erysiphe cichoracearum* f. *inulae, Leveilluta taurica* f. *inulae, Ramularia virgaureae*
Lamium album	*Erysiphe labiatarum* f. *lamii, Ramularia lamiicola, Septoria lamii*
Paulownia tomentosa	*Phomopsis imperalis, Phyllosticta paulowniae*
Petasites albus	*Phyllosticta petasites, Ramularia variegata*
Poa longifolia	*Didymaria graminella, Erysiphe graminis* f. *poae, Stagonospora graminella*
Polygonum carneum	*Cercospora avicularis, Erysiphe communis* f. *polygonorum, Ovularia bistorta, Ramularia rufomaculans, Septoria polygonorum, Uromyces polygoni*
Primula macrocalyx	*Cercosporella primulae, Phyllosticta primulicola, Ramularia primulae, Septoria primulae*
Pyrethrum roseum	*Diplodina pyrethri, Erysiphe cichoraciarum* f. *pyrethri, Ramularia bellunensis*
Quercus macranthera	*Anthostoma atro-punctatum, Coccomyces coronatus, Coryneum depressum, Diatrypella quercina, Didymella quercina, Diplodia quercina, Discosia artocreas, Gloeosporium quercinum, Gnomonia quercina, Hymenoscypha virgultorum, Macrophoma fusispora, Melogramma bulliardii, Microsphaera alphitoides, Monochaetia pachyspora, Phyllosticta quercus, P. quercus-ilicis, Septoria dubia, Stigmella dryina, Stilbospora angustata*
Ranunculus caucasicus	*Cylindrosporium ranunculi, Ovularia decipiens, Phoma exigua, Septoria ranunculi, Urocystis anemones, Vermicularia ranunculi*
Rhododendron caucasicum	*Didymosphaeria rhododendri, Exobasidium discoideum, E. magnusii, Monochaetia rhododendri, Mycosphaerella rhododendri, Pestalotia guepini, Phoma rhododendri, Torula rhododendri*
Rhododendron luteum	*Cladosporium oxycocci, Coniothyrium rhododendri, Diplodina azaleae, Exobasidium discoideum, E. magnusii, Monochaetia monochaeta, Phoma rhododendri, Phyllosticta rhododendricola*

Rubus idaeus	*Coniothyrium fuckelii, Coryneum microstictum, Didymosphaeria brunneola, Hendersonia rubi, Leptosphaeria dumetorum, Pestalotia suffocata, Phoma rubicola, Phragmidium rubi, Ramularia rubi, Rhabdospora ramicola, R. uniseptata, Septoria rubi* var. *rubi, Sphaerotheca macularis* f. *rubi, Tubercularia rubi*
Rumex alpinus	*Cercospora acetosellae, Erysiphe communis* f. *rumicus, Phyllosticta acetosellae, Ramularia decipiens, Uromyces rumicis*
Salvia glutinosa	*Erysiphe labiatarum* f. *salviae, Leveillula taurica* f. *salviae, Phoma salviae, Ramularia salviae, Septoria salviae*
Senecio rhombifolius	*Coleosporium senecionis, Diplodina jacobaeae, Phoma senecionis, Puccinia expansa, P. schoeleriana, Ramularia senecionis-platyphylli, Spherotheca fuliginea* f. senecionis
Sorbus caucasigena	*Cytospora rubescens, Diplodia sorbi, Hendersonia torminalis, Nummularia rependa, Phoma aucuparia, Ramularia sorbi, Rhabdospora inaequalis, Tubercularia vulgaris*
Stachys sylvatica	*Erysiphe labiatarum* f. *stachydis, Ramularia stachydis, Rhabdospora betonica, Septoria stachydis*
Symphytum asperum	*Erysiphe horridula* f. *symphyti, Cylindrosporium myosotidis, Ovularia asperifolii, Ramularia farinosa*
Teucrium chamaedrys	*Leveillula taurica* f. *teucrii, Phyllosticta obliqua, Puccinia annularis*
Thymus transcaucasicus	*Erysiphe labiatarum* f. *thymi, Stagonospora thymi, Trematosphaeria thymi*
Trifolium ambiguum	*Ascochyta trifolii, Cercospora zebrina, Erysiphe communis* f. *trifolii, Phyllosticta trifolii, Polytrincium trifolii, Uromyces fallens, U. trifolii-repentis*
Trollius patulus	*Cercosporella trolliicola, Mycosphaerella pulsatillae, Phyllosticta trollii, Septoria trollii*
Vaccinium arctostaphylos	*Phyllosticta leptidae, P. vaccinii, Thekopsora myrtilli*
Valeriana alliariaefolia	*Cercosporella valerianae, Oidium eryngii* f. *valerianae, Ramularia basarabica, R. valerianae, Rhabdospora valerianae, Septoria valerianae, Uromyces valerianae*
Veratrum lobelianum	*Cercosporella veratri, Diplodia veratri, Phyllosticta albina, P. melanoplaca, Puccinia veratri, Uromyces veratri*
Alpine belt (2500-3000 m a. s. l.)	
Host plants	**Micro-fungi**
Agrostis planifolia	*Ascochyta graminicola, Cercospora agrostis, Erysiphe graminis* f. *agrostidis, Hadrotrichum microsporum* var. *macrosporum*
Alchemilla caucasica	*Ramularia alchimillae, Sphaerotheca macularis* f. *alchimillae, Trachyspora alchimillae*
Anthemis sosnowskyana	*Microdiplodia chrysanthemi, Ramularia anthemidis*
Astrantia maxima	*Cercosporella astrantiae, Ramularia oreophila*
Betonica grandiflora	*Erysiphe labiatarum, Ovularia betonicae, Phyllosticta stachydis*
Bromus variegatus	*Erysiphe graminis* f. *bromi, Phyllosticta bromi, Septoria bromi*
Calamagrostis arundinacea	*Coniothyrium tenue, Diplodina calamagrostidis, Septoria calamagrostidis*
Centaurea salicifolia	*Cercospora centaureae, Erysiphe cichoracearum* f. *centaureae-scabiosae, Ramularia centaureae*
Cephalaria gigantea	*Leptosphaeria taurica, Ramularia silvestris, Septoria amicabilis, S. scabiosicola, Sphaerotheca fuliginea* f. *cephalariae*
Cerastium multiflorum	*Helminthosporium exasperatum, Isariopsis alborosella*
Chaerophyllum maculatum	*Cercospora depressa, Puccinia chaerophylli, Septoria weissii*
Cirsium obvallatum	*Phyllosticta cirsii, Puccinia suaveolens, Ramularia cirsii, Septoria cirsii*
Dactylis glomerata	*Erisiphe graminis* f. *dactylidis, Leptosphaeria eustoma*
Dianthus raddeanus	*Phoma caryophylli*

Festuca varia	*Erysiphe graminis* f. *festucae, Ophiobolus graminis, Vermicularia lineola*
Gentiana schistocalyx	*Darluca filum, Mycosphaerella gentianae, Puccinia gentianae, Ramularia evanida, Septoria rhaphidospora*
Geranium ibericum	*Cercosporella magnusiana, Erysiphe communis* f. *geraniacearum, Ramularia geranii*
Inula grandiflora	*Coleosporium inulae, Erysiphe cichoracearum* f. *inulae, Ramularia inulae, R. virgaureae, Septoria inulae*
Lapsana grandiflora	*Phyllosticta lampsanae, Puccinia grandiflora, P. lapsanae, Ramularia lampsanae, Sphaerotheca fuliginea* f. *lampsanae*
Leontodon hispidus	*Rhabdospora leontodontis*
Minuartia oreina	*Phoma schischkiniana, Septoria schischkiniana*
Myosotis alpestris	*Cercosporella myosotis, Cylindrosporium myosotidis*
Pedicularis condensata	*Ramularia obducens*
Phleum alpinum	*Cercospora graminicola, Leptosphaeria rousseliana, Septoria culmifida, S. phyllachoroides*
Plantago saxatilis	*Cercospora plantaginis, Phyllosticta plantaginis, Ramularia plantaginis, Septoria plantaginis-majoris, Sphaerotheca fuliginea* f. *plantaginis*
Poa alpina	*Didymaria graminella, Erysiphe graminis* f. *poae, Phyllachora poae, Septoria poae-annuae, Stagonospora graminella*
Potentilla crantzii	*Phragmidium potentillae, Phyllosticta potentillica, Ramularia arvensis, Septoria tormentillae*
Primula algida	*Cercosporella primulae, Phyllosticta primulicola, Ramularia primulae, Septoria primulae*
Ranunculus caucasicus	*Cercosporella ranunculi, Cylindrosporium ranunculi, Didymaria didyma, Vermicularia ranunculi*
Rhododendron caucasicum	*Coniothyrium rhododendri, Pestalotia guepini, Torula rhododendri*
Rumex alpinus	*Erysiphe communis* f. *rumicis, Phoma durandiana, Ramularia pratensis, Septoria acetosae, Uromyces rumicis*
Senecio rhombifolius	*Cercospora senecionis, Phoma senecionis, Phyllosticta albo-brunea, Septoria senecionis, Sphaerotheca fuliginea* f. *senecionis*
Taraxacum crepidiforme	*Erysiphe cichoracearum* f. *taraxaci, Puccinia taraxaci, Ramularia taraxaci, Sphaeotheca fuliginea* f. *taraxaci*
Thymus transcaucasicus	*Erysiphe labiatarum* f. *thymi, Pleospora thymi, Stagonospora thymi, Trematosphaeria thymi*
Trifolium canescens	*Cercospora zebrina, Erysiphe communis* f. *trifolii, Polythrincium trifolii, Stemphylium sarciniforme, Uromyces fallens*
Trollius patulus	*Cercosporella trollii, Erysiphe communis* f. *trollii, Mycosphaerella pulsatillae, Phyllosticta trollii, Septoria trollii*
Vaccinium myrtillus	*Phyllosticta leptidae*
Veratrum lobelianum	*Cercosporella veratri, Fusoma veratri, Phyllosticta albina, P. melanoplaca, Uromyces veratri*
Veronica gentianoides	*Erysiphe communis* f. *veronicae, Gloeosporium veronicae, Phoma veronicae, Sphaerotheca fuliginea* f. *veronicae*
Viola minuta	*Ascochyta violae, Phyllosticta tricoloris, Ramularia lactea*
Subnival belt (3000-3500 m a. s. l.)	
Host plants	**Micro-fungi**
Campanula biebersteiniana	*Ascochyta campanulae, Phyllosticta alliariaefolia, Ramularia campanularum*
Carex oreophila	*Cercospora microstigma, Mycosphaerella pusilla, Stagonospora macropycnida*
Festuca supina	*Erysiphe graminis* f. *festucae, Septoria festucae*
Gentiana schistocalyx	*Puccinia gentianae*
Phleum alpinum	*Cercospora graminicola*

Poa alpina	*Didymaria graminella, Erysiphe graminis* f. *poae*
Potentilla crantzii	*Cercosporella potentillae, Ramularia arvensis*
Primula algida	*Ramularia arvensis*
Scrophularia minima	*Ramularia scrophulariae, Sphaerotheca fuliginea* f. *scrophulariae*
Senecio sosnowskyi	*Erysiphe cichoracearum* f. *senecionis, Ramularia jacobaea*
Taraxacum crepidiforme	*Erysiphe cichoracearum* f. *taraxaci, Puccinia taraxaci*
Veratrum lobelianum	*Phyllosticta albina, P. melanoplaca, Puccinia veratri, Uromyces veratri*
Veronica minuta	*Erysiphe communis* f. *veronicae, Ramularia coccinea*
Viola minuta	*Cercospora lilacina, Erysiphe cichoracearum* f. *violarum*

Table 2. Distribution of lichens and mosses according to high mountain vertical belts, principal ecosystems and substrate.

Species	High mountain vertical belts and principal ecosystems				
	Subalpine belt (1800-2500 m a.s.l.)			Alpine belt meadows (2500-3000 m a.s.l.)	Subnival belt rocks and scree (> 3000 m a.s.l.)
	Forest	Shrubbery	Meadow		
1	2	3	4	5	6
Lichenes					
Anaptychia solenaria	⊙				
Aspicilia cinerea		○	○	○	○
Bellemeria cupreoatra		○	○	○	
Bryoria chalibeiformis	⊙				
B. implexa	⊙				
Buellia disciformis	⊙				
Caloplaca cerina	⊙				
C. holocarpa	⊙				
Cetraria ericetorum			●	●	
C. islandica		●	●	●	●
C. nivalis		●	●	●	●
Cetrelia cetrarioides	⊙				
Cladonia arbuscula		●		●	
C. bacillaris	⊙				
C. cariosa			●	●	
C. chlorophaea	●	●	●		
C. coccifera	⊙●				
C. coniocraea	⊙●				
C. deformis	●	●			
C. fimbriata	⊙●				
C. furcata	●	●	●		
C. gracilis		●	●		
C. ramulosa	●				
C. pyxidata	●	●	●	●	
C. rangiferina		●	●	●	●
Collema cristatum	○				
C. flaccidum	⊙○				
C. tenax	●	●			
Cornicularia normoerica		○		○	○
Dactilina madreporiformis					●
Dermatocarpon miniatum	○	○	○		
Diploschistes bryophylloides	⊙○				

D. scruposus	○	○			
Flavocetraria cucullata		●	●	●	●
Heterodermia speciosa	⊙○				
Hypogimnia encausta		○	○	○	○
H. physodes	⊙				
H. villata	⊙	●			
Lacanora allophana	⊙				
L. carpinea	⊙				
L. glabrata	⊙				
L. frustulosa	○	○	○	○	
Lecidea cinereoatra	○				
L. glomerulosa	⊙				
Leptogium cyanescens	⊙○	○			
L. lichenoides	●				
L. saturninum	⊙				
L. sinuatum	●				
Lobaria amplissima	⊙				
Melanelia glabra	⊙				
Nephroma laevigatum	⊙				
N. parile	⊙○	○			
N. resupinatum	⊙○	○			
Ochrolechia parella	⊙				
Opagrapha atra	⊙				
O. rufescens	⊙				
Pannaria pezizoides		●	●	●	
Parmelia saxatilis	⊙○	○			
P. sulcata	⊙				
Parmelina carporrhizans	⊙				
P. tiliacea	⊙				
Parmeliopsis ambigua	⊙				
Parmotrema chinense	⊙				
Peltigera aphthosa		●	●	●	
P. canina	●	●	●	●	
P. horizontalis	●				
P. leucophlebia		●	●		
P. polydactyla	●				
P. venosa	●	●			
Pertusaria globulifera	⊙				
Physcia aipolia	⊙				
Ph. caesia		○	○		
Physconia muscigena			●	●	●
Ph. pulverulenta	⊙				
Placolecanora alphoplaca			○	○	○
P. melanophthalma			○	○	○
P. rubina				○	○
Pseudoevernia furfuracea	⊙				
Pseudocyphellaria scrobiculata	⊙				
Ramalina farinacea	⊙				
R. fastigiata	⊙				
R. fraxinea	⊙				
R. pollinaria	⊙	⊙			

R. strepsilis		○	○	○	
Rhizocarpon geographicum			○	○	○
Rh. petraeum			○	○	
Solorina bispora		●	●	●	
S. crocea				●	●
S. saccata	●	●			
Stereocaulon alpinum				●	●
S. paschale				●	●
Thamnolia vermicularis				●	●
Umbilicaria cylindrica	○	○	○		
U. deusta			○	○	
U. vellea		○	○		
U. virginis				○	○
Usnea glabrescens	⊙				
Vulpicida pinastri	⊙				
Xanthoparmelia conspersa	○	○	○		
X. solmoensis			○	○	
Xanthoria elegans				○	○
Musci					
Bartramia halleriana	○	○	○		
B. ithyphylla			○	○	○
Brachythecium populeum	○	○	○		
B. reflexum			○	○	
Campylium stellatum		●	●		
Cirriphyllum cirrosum			●	●	
Climacium dendroides	●	●	●	●	
Cynodontium polycarpum	○	○	○		
Desmatodon latifolius			●	●	
Dicranoweisia crispula			○	○	○
Dicranum scoparium	●	●			
D. spadiceum			○	○	○
Distichium capillaceum	●		○		
Encalypta vulgaris			○	○	
Grimmia funalis			○	○	○
G. elongata			○	○	○
Hedwigia ciliata	○	○	○	○	
Hylocomium splendens	●	●	●		
Leskea incurvata		○	○	○	
Leskeela nervosa	⊙	⊙			
Leucodon sciuroides	⊙				
Mnium rugicum	●	●	●	●	
M. spinolosum	●	●	●		
Paraleucobryum longifolium		○	○	○	
Plilonothis seriata		●	●	●	
Plogiothecium roseanum		○	○	○	○
Pogonatum urginerum	○	○	○		
Pohlia nutans	●	●	●		
P. cruda			○	○	
P. longicollis			○	○	○
Polytrichum piliferum	○	○	○	○	○
P. alpestre	○	○	○	○	

P. juniperinum	○	○	○		
Rhytidiadelphus triquetrus	●	●	●		
Rhytidium rugosum	○	○	○		
Saelania glaucescens		○	○	○	○
Schistidium gracile		○	○	○	○
Sch. brunescens			○	○	○
Tortella tortuosa	●	●	●		
Thuidium abietinum	●	●	●		
Racomitrium canescens			○	○	

○ – epilithic ● – epigeal ⊙ – epiphytic

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SOIL POPULATION OF NEMATODES OF GOMBORI RIDGE (EAST GEORGIA)

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Abstract

Nematode population of different slopes of Tsiv-Gombori Ridge was studied during 22 months. Some regularities of ecological character, species composition and population density were established. 203 nematode forms have been recorded on studied plot, among them 148 forms are identified up to species. Representatives of Dorylaimida class dominate. Species *Enchodelus georgiensis* is described for the first time in the scientific literature.

Key words: species diversity, ecosystem, trophic specialization, Dorylaimida.

Introduction

Study of faunistic population of various soil types enables us to understand some features of those soils, and at the same time, has a diagnostic significance.

Gombori Ridge by its geological structure represents intermontane area of Georgia. It is closely connected with Central Caucasus; divided into two parts. Bigger one is typical intermontane territory with cinnamonic soil and montane black earth. Up to 1 200 m a.s.l. platyphyllous forests (oak, hornbeam, beech) occur. Pine-tree happens in Mariamjvari reserve. The top of Ridge is forest-uncovered, which is caused by antropogenic factors: development of cattle-breeding and forest cutting. Actually, subalpine belt is a pasture.

While studying of species composition of soil nematodes, their number and distribution in separate ecosystems throughout different height levels, all abovementioned was taken into consideration.

Materials and Methods

Studies for soil nematodes of Gombori Ridge were carried out from January 2002 to November 2003. 6 biotopes different by characteristics (vegetation cover, height a.s.l.) were chosen. Soil samples were taken from each biotope at 3 points separated by about 15-20 m, up to a depth of 25 cm; soil samples capacity – 100 cm^3. Separation of nematodes was carried out from small capacity of soil samples (20 cm^3) in laboratory environment via the method considering sinking of sieves with cotton filter in water, at 36 h exposition and 18-20°C. After thermal treatment up to 50°C, fixation by 5% formalin was realized. Then, via generally accepted method of phytohelminthology, temporary glycerin preparations were prepared.

Results and Discussion

Along with identification of nematodes, calculation of their number in the samples, number of eugamic and larval forms, sex ratio, was carried out.

While investigation 202 forms of nematodes corresponded to 7 classes, 32 families and 62 genera were revealed. 149 forms were identified up to species [Kiknadze, Eliava, 1985].

Distribution of nematodes throughout the plots is presented in Table 1.

93 forms (45.5%) from registered nematodes belong to Dorylaimida class, by 13-13 forms (6.4%) – to Tylenchida and Enoplida classes, 12 forms (5.9%) – to Mononchida class, and the rest 17 forms (8.4%) to the classes: Monchysteria, Areolaimida and Rabditida.

Above noted distribution of nematode species and genera by classes is common for natural ecosystems of Georgia studied earlier. Distribution of families, genera and species by classes is presented in Table 2.

Table 1. Distribution of nematodes throughout the plots

#	Species	I plot	II plot	III plot	IV plot	V plot	VI plot
1	*Alaimus meyli*						+
2	*A. minor*				+		
3	*A. parvus*	+			+		+
4	*A. primitivus*						+
5	*Alaimus sp.*			+			
6	*Trypilina arenicola*	+	+				
7	*Trypilina sp.*		+				
8	*Tryshistoma monohistera*			+			
9	*Tryshistoma sp.*			+			
10	*Paratrypila intermedia*	+	+				
11	*Trypila afinis*	+	+	+			
12	*Trypila filicaudata*		+		+		
13	*Trypila glomerans*	+	+	+	+	+	+
14	*Trypila longicaudata**	+					
15	*Trypila sp.*	+	+		+	+	
16	*Trypila gen. nova*					+	
17	*Tobrylus abberans*		+				
18	*Tobrylus sp*	+	+	+	+	+	
19	*Epytobrilus setosus**		+				
20	*Epytobrilus sp.*						+
21	*Monhystera agilis*	+					
22	*Monhystra sp.*	+		+		+	
23	*Geomonhystera villosa*	+	+	+			
24	*Cylindrolaimus communis*		+	+			+
25	*Plectus acuminatus*					+	
26	*P. annulatus*		+	+	+	+	
27	*P. elongatus*			+			

28	*P. longicaudatus*					+	
29	*P. parietinus*	+	+	+	+	+	
30	*P. parvus*	+	+	+			
31	*P. ryzophilus*				+		
32	*Plectus sp.*	+	+	+	+		
33	*Anaplectus granulosus*	+	+			+	+
34	*Anaplectus submersus*	+		+		+	
35	*Anaplectus sp.*	+					
36	*Ceratoplectus assimilis*				+		
37	*Ceratoplectus sp.*				+		
38	*Aquatydes aquaticus*		+				
39	*Nygolaimus brachiuris*			+		+	+
40	*Paravulvus paraamphigonicus*	+					
41	*Paravulvus hartingi*			+			
42	*Nygolaimus sp.*	+		+	+		
43	*Mesodorylaimus abberans*			+	+	+	
44	*M. Arvensis**	+					
45	*M. bastiani*	+	+	+	+	+	+
46	*M. centrocercus*		+			+	
47	*M. filicaudata*	+	+	+	+		+
48	*M. mesonictius*	+	+	+	+	+	
49	*M. Recurvus**	+					
50	*M. Signatus**	+	+				
51	*Mesodorylaimus sp.*	+		+	+	+	
52	*Thornenema mauritianum*	+					
53	*Thornenema silphoides*			+			
54	*Thornenema sp.*						
55	*Prodorylaimus longicaudatus***					+	
56	*Prodorylaimus paralongicaudatus**					+	
57	*Dorylaimus montanus*			+			
58	*Dorylaimus sp.*			+			
59	*Dorylaimellus sp.*			+			
60	*Eudorylaimus acuticauda*	+	+	+	+	+	
61	*E. acutus*	+	+			+	+
62	*E. alltheri*				+		
63	*E. brachicephalus*		+				
64	*E. carteri*	+	+	+	+	+	+
65	*E. centrocercus*	+	+		+	+	
66	*E. confusus*					+	
67	*E. curvatus*	+					
68	*E. curvicaudatus*	+					

69	*E. Incisus**	+		+		+	
70	*E. franzi*						+
71	*E. jurrasicus*		+				
72	*E. leucarti*	+	+		+		+
73	*E. leptus*					+	
74	*E. maritus*			+	+		+
75	*E. obesus*						
76	*E. opistohistera*			+			
77	*E. productus*	+					
78	*E. pseudocarteri*				+		+
79	*E. perspicuus*			+			
80	*E. paramonovi*			+			
81	*E. paracirculifer*				+		
82	*E. paracentrocercus*		+				
83	*Eudorylaimus sp.*	+	+	+	+	+	+
84	*Allodorylaimus diadematus*	+					+
85	*Allodorylaimus irritans*	+			+		
86	*Allodorylaimus granulifer*	+	+	+			
87	*Allodorylaimus holdemani*	+					
88	*Allodorylaimus husmanni*		+				
89	*Microdorylaimus dubius*	+	+				
90	*Epidorylaimus lugdunensis*	+	+		+	+	+
91	*Tacamangai lautus*	+					
92	*Discolaimus laevinae*	+					
93	*Discolaimus major*						+
94	*Discolaimus sp.*	+					
95	*Discomictus sp.*					+	
96	*Aporcelaimellus adriani**	+	+				
97	*A. amilovorus*					+	+
98	*A. capitatus*	+			+	+	
99	*A. krigeri*	+	+	+	+	+	
100	*A. micropunctatus*			+			
101	*A. obscurus*	+		+		+	+
102	*A. obscuroides*	+	+	+	+		
103	*A. obtusicaudatus*		+	+	+	+	+
104	*A. paraobtusicaudatus*	+	+	+	+	+	+
105	*A. Silvanus**		+				
106	*A. tailory*	+			+		
107	*A. williamsi*			+	+		
108	*Aporcelaimellus sp.*	+		+	+	+	+
109	*Aporcellaimus sp*		+				

110	*Paraxonchium striatum*	+	+	+	+	+	
111	*Paraxonchium sp.*					+	
112	*Dorydorella pratensis*	+	+	+			
113	*Longidorella cuspidata*	+					
114	*Longidorella sp.*	+					
115	*Pungentus angulosus*		+				
116	*Pungentus obscurus*			+			
117	*Pungentus sp.*	+		+	+	+	+
118	*Enchodelus brevidentatus**				+	+	+
119	*E. georgiensis***					+	
120	*E. macrodorus*	+				+	+
121	*E. hopedorus*	+		+	+	+	
122	*E. hopedoroides*	+			+		
123	*E. teres*	+		+			
124	*Enchodelus sp.*			+	+	+	
125	*Longidorus caespiticola*	+					
126	*L. levicapitatus*				+		
127	*L. macramphis**						+
128	*L. tardicauda*				+		
129	*Longidorus sp.*	+			+	+	+
130	*Xyphinema brevicole*	+			+		
131	*Xyphinema sp.*	+	+		+	+	
132	*Paralongidorus sp.*					+	
133	*Tylencholaimus aerolensis*	+					
134	*T. minimus*		+		+	+	
135	*T. mirabilis*		+		+		
136	*T. mongolicus*		+				
137	*T. pusilus*				+		
138	*T. stekki*	+	+	+	+	+	
139	*T. vigil*		+				
140	*Tylencholaimus sp.*	+	+	+	+	+	
141	*Belondira apitica*	+				+	+
142	*B. cylindrica*				+		
143	*B. ortha*			+			
144	*Belondira sp.*				+	+	+
145	*Axonchium dolichodorum*		+				
146	*Axonchium siddiqi*	+	+				
147	*Axonchium sp.*	+					
148	*Trachactinolaimus sp*			+	+	+	
149	*Paractinolaimus sp.*		+	+	+	+	
150	*Oxydirus oxycephalus*						+

151	*Oxydirus sp.*						+
152	*Tylencholaimellus eskei*	+				+	
153	*Tylencholaimellus sp*	+					
154	*Dyphterophora perplexans*						+
155	*Dyphterophora sp*						+
156	*Trychodorus sp.*				+		
157	*Clarcus papilatus*	+		+	+	+	+
158	*Clarcus sp.*			+	+	+	+
159	*Comansus parvus*	+	+	+		+	
160	*Comansus sp.*	+	+	+		+	+
161	*Prionchulus muscorum*	+	+	+	+	+	
162	*Prionchulus vescus**	+					
163	*Prionchulus sp.*	+			+		
164	*Mylonchulus branchiuris*					+	+
165	*Mylonchulus index*	+	+	+			
166	*Mylonchulus rotundicaudatus*						+
167	*Mylonchulus sp.*		+	+			
168	*Jotonchus geminus**			+			
169	*Jotonchus sp.*	+		+	+		+
170	*Anatonchus alleni*	+					
171	*Anatonchus ginglimodontus**	+		+			
172	*Anatonchus tridentatus*	+	+			+	+
173	*Anatonchus sp.*	+			+		+
174	*Myconchus sp.*	+				+	
175	*Panagrolaimus rigidus*	+			+		
176	*Panagrolaimus sp.*						+
177	*Cephalobus parvus*					+	
178	*Cephalobus persegnis*						+
179	*Eucephalobus mucronatus*	+		+	+		
180	*Eucephalobus oxyuroides*	+					
181	*Teratocephalus terrestris*	+	+			+	
182	*Aglenchus agricola*		+				
183	*Tylenchus davainei*	+	+				
184	*Tylenchus majus*		+				
185	*Tylenchus sensulato*						+
186	*Tylenchus sp.*		+				
187	*Fylenchus filiformis*				+		
188	*Fylenchus orbus*				+		
189	*Fylenchus sp.*					+	+
190	*Psylenchus hilarulus*			+			
191	*Psylenchus sp.*				+		

192	*Neotylenchidae gen. sp.*				+		
193	*Tylenchorynchus capitatus*				+		
194	*H. crenicauda*	+					
195	*H. exallus*	+					
196	*H. digonicus*		+		+		
197	*H. dihistera*				+		
198	*H. vulgaris*						+
199	*Helicotylenchus sp.*		+				+
200	*Criconema sp.*	+	+		+		
201	*Nothocriconema sp.*						+
202	*Lobocriconema sp.*		+				

Table 2. Distribution of nematode species and genera by classes

N	Class	Number of family	Number of genus	Number of species
1.	*Enoplida*	3	7	13
2.	*Monchysterida*	1	2	2
3.	*Areolaimida*	2	4	10
4.	*Dorylaimida*	15	29	93
5.	*Mononchida*	4	6	12
6.	*Rabditida*	3	4	6
7.	*Tylenchida*	4	10	13
		32	66	149

Class Dorylaimida is most of all distinguished by species diversity. Species *Enchodelus georgiensis sp. nov.,* described for the first time in the scientific literature belongs just to this class [in press], and those species also, which were registered for the first time in Georgia (in the list of Tab.1 they are marked by *). Such distribution of nematodes could be accounted for wide spectrum of adaptation of Dorylaimidas and also for the fact that studied territory is occupied with larch forest characterized by great diversity of soil nematodes, and especially of Dorylaimidas. Observed differences in nematode species composition is probably caused by variety of main characteristics of studied ecosystems. But, it should be mentioned that for all six biotopes common species are revealed which could be ranked among euconstants. Wide spread nematodes, found in all ecosystems, are the following ones: *Eydorylaimus carteri, Mesodorylaimus bastiani, Mesodorylaimus mszonictius, Clarcus papilatus, Trypila glomerans.*

By ecological classification in the viewpoint of trophic specialization the following groups should be singled out: bacteriotrophs, euryphagous, predators, typical phytoparasites (phytohelminths) and nematodes of unestablished trophic nature. According to our data overwhelming majority of nematodes belong to euryphagous group, then come predators and phytohelminths [Eliava I., 1985; Shesteperov, Savotikov, 1995; Twinn, 1973].

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ბომბორის ჭედის ნიადაგის ნემატოდები

ცქიტიშვილი ე.

ზოოლოგიის ინსტიტუტი

(მიღებულია 20.01.2006)

შესწავლილია ცივ-ბომბორის სხვადასხვა ექსპოზიციის ნიადაგის ნემატოდოფაუნა 22 თვის განმავლობაში. დადგენილია ეკოლოგიური ხასიათის ზოგიერთი თავისებურებანი, ნემატოდების სახეობითი შედგენილობა და პოპულაციური სიმჭიდროვე. საკვლევ ტერიტორიაზე გამოვლენილია ნემატოდების 202 ფორმა, რომელთაგან 148 იდენტიფიცირებულია სახეობამდე. დომინირებს მაღალი ადაპტაციის მქონე რიგი ***Dorylaimida***. აღწერილია მეცნიერებისთვის ახალი სახეობა ***Enchodelus georgiensis.***

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EFFECT OF GEROPROTECTOR LIVAGEN ON CHROMOSOME CHANGES INDUCED BY COBALT IONS AND γ-RAYS AT AGEING

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Abstract

Changes of functional characteristics of chromosomes (level of chromosomal aberrations and activity of nucleolus organizers) induced by heavy metal – cobalt, and by single-shot and fractionated γ-irradiation, have been studied in 70-86 years old individuals. Possibility of their correction by geroprotector livagen was also shown. It was registered that livagen sharply reduced chromosomal instability induced by cobalt ions in both age groups: it was decreased from 8.5-1.9% to 2.0-0.9% in middle age group and from 13.17-2.3% to 3.25-1.2% in old age group. Livagen also reduced considerably mutagenic effect of γ-rays in cells of old individuals at single-shot, as well as at fractionated irradiation. Antimutagenic influence of livagen is explained by its decondensing effect on chromatin, which increases effectiveness of reparative processes.

Key words: nucleolus organizers, chromosomal aberrations, synthetic peptide, antimutagenic effect

Introduction

It is common knowledge that changes of chromatin state at earlier stage of ontogenesis provide normal development, and at later stage it is connected with disorder of homeostasis, which causes gradual loss of organism functions. Thus, it is of interest to study functional characteristics of chromosomal apparatus at later stage of ontogenesis.

Level of structural-numerical disorders, activity of nucleolus organizers are those functional characteristics which are subjected to changes at earlier, as well as at later stages of ontogenesis [Lezhava, 2001; Warburton, 2005]. Study of functioning of chromosomal apparatus during ontogenesis in the conditions of certain influence on it, enables us to reveal variability of sensitivity of chromosomes and specific function of separate segments, and to prevent undesirable results.

The goal of the given work was to determine character of changes of structural variations and parameters of activity of nucleolus organizers at the effect of cobalt chloride and γ-irradiation during the later period of ontogenesis (72-86 years old individuals), and to study the remedial action of bioregulator, synthetic peptide – livagen, affecting as geroprotector on these processes.

Materials and Methods

Short term lymphocyte cultures of 20-37 years old (control) and 72-86 years old clinically healthy individuals, stimulated by mitogen, were researched. The following test-systems were used: 1) counting of structural-numerical disorders of chromosomes; 2) assessment of activities of ribosomal genes. Cultivation of peripheral blood lymphocytes was carried out by standard method. RPMI-1640 (Sigma) was used as a nutrient medium, phytohemagglutinin (Sigma-P and ПанЭко) – as mitogen. 10^{-4}M of $CoCl_2$ solution and peptide bioregulator livagen with a final concentration of 0.0005 μg/ml was added to the cultures after 24 hours and remained there till fixation.

γ-irradiation of Cs^{137} was used as a source of ionizing radiation. Irradiation of peripheral blood was carried out on γ-instrument of the Institute of Radiology (irradiation capacity of 1 grey/min). Irradiation was conducted by the following schedule: in one case of experiments the dose of 2 grey was given entirely, in another case – the dose was given fractionated, first 0.2 grey, and 2.5-3 h later – the main testing dose, up to 2 grey.

Assessment of structural-numerical disorders of chromosomes was realized according to International System for Cytogenetic Nomenclature (ISCN).

To determine activity of ribosomal genes, frequency of active nucleolus organizers of acrocentric chromosomes was studied according to Bloom and Goodpasrure method [Bloom, Goodpasrure, 1976]. Size of Ag^+-nucleolus organizing regions (Ag^+-NORs) was estimated by 3-point system: 0 – nonexistence of segment; 1 – small size segment, less than chromatid width, and 2- large size segment, of chromatid width or more [Lezhava, 2001b].

Results and Discussions

Results of analysis of chromosomal disorders induced by cobalt ions are presented in Table 1. Analysis of chromosomal aberrations showed that frequency of cells with chromosomal disorders in the intact cultures of old age individuals is equal to 4.2±1.2%. Authentic low spontaneous level of this parameter – 1.7±0.5% was recorded in the control group.

Scientific literature data [Lezhava, 2001; Warburton, 2005] indicate the increase of cells comprising aberrations during the process of ageing. Our data are in accordance with above mentioned data. Phenomenon of increasing of chromosomal disorders along with ageing could be explained by the fact that structural disorders of chromosomes induced spontaneously and by the effect of various physical-chemical agents occur mainly in the heterochromatic regions of the chromosomes [Lezhava, 2001 a, b]. It was established that at ageing progressive hetero-chromatinization of chromatin takes place, due to which primary disorders of DNA are unavailable for reparation enzymes, and consequently, the frequency of chromosome structural disorders increases [Lezhava, 1991].

While testing in both systems cobalt revealed clastogenic effect, in the middle-age group its effect made up 0.09 aberrations per cell and in cultures of the old-age group – 0.14 aberrations per cell. Single and pair fragments and chromatid terminal deletions were recorded mainly.

Obtained results, in respect of sensitivity of chromosomal apparatus against destructive influence at the later stage of ontogenesis, are in accordance with scientific literature data [Jokhadze, Lezhava, 1994; Vorobtsova et al., 2001].

It is of interest, at what extend variations of chromosomal apparatus could be subjected to correction at the later stage of ontogenesis. In this viewpoint we choose synthetic peptide bioregulator - livagen, having geroprotective effect. It was shown that livagen reduces the rate of ageing process and the risk of arousing of pathologies related with age.

Livagen doesn't change spontaneous level of chromosomal disorders in the middle age individuals. While testing the cultures of the old age individuals it causes decrease of high spontaneous level of aberrations (Table 1).

Along with cobalt ions livagen revealed drastic antimutagenic effect. The high level of chromosomal aberrations induced by metal ions in the cultures of both, middle age and old age individuals at the effect of bioregulator was reduced practically up to spontaneous.

Changes of transcriptional activity of ribosomal genes by effect of cobalt ions and livagen in different age groups.

Assessment of transcriptional activity of ribosomal genes in the cells of middle- and old age individuals was conducted via the frequency of Ag^+-positive acrocentric chromosomes (Table 2). It is known that the number and sizes of Ag^+-positive NORs show the degree of functional activity of ribosomal cistrons in previous interphase [Lezhava, 1999].

Analysis of the results shows that cobalt inhibited the frequency of 2-point Ag^+-chromosomes in both, middle- and old age group cultures. Livagen doesn't cause correction of total parameter of Ag^+-chromosomes recorded at the effect of metals in the middle age group. But in old age group livagen increases parameter of 2-point Ag^+-chromosomes recorded at the effect of cobalt.

Distinct data obtained by age groups indicate to the fact that chromosomal apparatus of various age groups presents two different modeling systems, which at certain specific influence should function differently.

Changes of adaptive response at a single-shot and fractionated irradiation by γ-rays and its correction by livagen in various age groups.

It is known that adaptive response is caused by low dose of genotoxic stress, which revealed protective potency against a following higher dose [Upton, 2001; Johansen, 2003; Miura, 2004]. Potential of adaptive response is shown for several chemical compounds and ionized irradiation. In the last case previous irradiation with low doses causes radioprotective effect against the following testing irradiation.

Results of chromosomal disorders induced by γ-irradiation in different age groups are presented in the Table 3. As is seen, one and the same dose of γ-rays in different age groups causes dissimilar effect. Destructive influence is more significant in older age group. In this viewpoint our results are in good accordance with scientific data. Vorobtsova et al. [Vorobtsova et al., 2001] have shown that along with the growing, from 3 up to 70 years, 15 times rise of translocations induced by radiation occurs. Frequency of unstable chromosomal exchanges was also rose along with ageing.

Adaptive response with respect to chromosomal aberrations at fractionated irradiation was registered in both age groups. Frequency of aberrant cells at irradiation of dose of 2 grey of the middle age individuals comprises 24.5%, and at fractionating of the dose – 16.0%. Frequencies of cells comprising aberration in the old age group under the effect of the same doses of single-shot and fractionated irradiation, equal to 31.0% and 21.1%, respectively.

Adaptive response induced by γ-rays in the old age group cells, in the conditions of geroprotector – livagen action, was also studied. Reduction of the effect of a single-shot (up to 25.5%), as well as fractionated irradiation (up to 17.5%) was induced at the adding of livagen. Hence, abovementioned parameter was reduced up to the value of parameter of middle age individuals by adding of livagen.

Thus, protective potential of synthetic geroprotector – livagen, at the effect of testing metal – cobalt ions, as well as at the effect of a single-shot and fractionated γ-irradiation, was recorded. Protective potential of livagen could be explained by the fact that it has ability of chromatin decondensation [Khavinsos et al., 2002], which is especially well observed on the cell systems of later stage of ontogenesis. Abovementioned condensing effect makes chromatin available for reparation enzymes, and as a result, the level of chromosomal aberrations reduces.

Table 1. Effect of livagen on chromosomal instability induced by cobalt ions in two age groups

Experimental conditions	Cells of middle age group individuals			Cells of old age group individuals		
	Amount of analyzed cells	Metaphases with aberrations		Amount of analyzed cells	Metaphases with aberrations	
		total	%±m		total	%±m
Intact	640	11	1.7±0.3	260	11	4.2±1.2
livagen	210	4	1.9±0.5	200	5	2.5±1.1
$CoCl_2$	200	17	8.5±1.9	205	27	13.17±2.90
Livagen+$CoCl_2$	200	4	2.0±0.9	215	7	3.25±1.20

Table 2. Effect of livagen on the frequency of Ag^--positive acrocentric chromosomes at the influence of cobalt ions

N	Experimental conditions	Cells of middle age individuals Ag -chromosomes per 1 cell			$\frac{P_D - P_G}{\sqrt{\frac{P_D(1-P_D)}{6n} + \frac{P_G(1-P_G)}{4n}}}$	P	Cells of old age individuals Ag -chromosomes per 1 cell			$\frac{P_D - P_G}{\sqrt{\frac{P_D(1-P_D)}{6n} + \frac{P_G(1-P_G)}{4n}}}$	P
		total	D	G			total	D	G		
1	Control	6.5	3.90	2.53	0.6	>0.05	5.86	3.53	2.33	0.48	>0.05
2	Livagen	6.36	3.80	2.56	0.28	>0.05	7.36	4.50	2.86	2.1	<0.01
3	$CoCl_2$	6.7	3.90	2.80	-0.28	>0.05	5.83	3.33	2.50	-0.3	>0.05
4	Livagen+$CoCl_2$	5.8	3.42	2.38	-0.32	>0.05	6.2	3.65	2.55	-1	>0.05
	For 2-point Ag -chromosomes per 1 cell										
1	Control	1.56	1.0	0.56	-0.25	>0.05	1.83	1.13	0.7	3.13	<0.01
2	Livagen	1.32	0.82	0.4	1.47	>0.05	2.16	1.39	0.77	2.22	<0.01
3	$CoCl_2$	1.1	0.8	0.3	1.5	>0.05	1.1	0.76	0.33	2.6	>0.05
4	Livagen+$CoCl_2$	0.86	0.6	0.23	1.7	>0.05	1	0.67	0.33	1.2	>0.05

Table 3. Frequency of structural disorders of chromosomes and spectrum at fractionated γ-irradiation in the lymphocytes of different age group individuals

Experimental conditions. Irradiation dose	Cells of old age group individuals					Cells of middle age individuals				
	Amount of analyzed cells	Cells with aberrations (% ± m)	Dicentric chromosomes per 1 cell	Chromosomal fragments per 1 cell	Closed chromosome per 1 cell	Amount of analyzed cells	Cells with aberrations (% ± m)	Dicentric chromosomes per 1 cell	Chromosomal fragments per 1 cell	Closed chromosome per 1 cell
control	400	2.5±0.7	0.01	0.01	-	400	2.5±0.7	0.01	0.01	-
2 Gy	380	31.0±2.4	0.01	0.33	0.07	380	31.0±2.4	0.01	0.33	0.07
0.2 Gy	370	6.2±1.6	-	0.06	-	370	6.2±1.6	-	0.06	-
0.2 Gy+2Gy	360	21.1±2.15	0.02	0.27	0.05	360	21.1±2.15	0.02	0.27	0.05
2 Gy+livagen	360	25.5±2.29	0.005	0.43	0.07	360	25.5±2.29	0.005	0.43	0.07
0.2 Gy+livagen	320	3.7±1.05	-	0.04	0.006	320	3.7±1.05	-	0.04	0.006
0.2 Gy + 2 Gy + livagen	320	17.5±2.12	-	0.27	0.02	320	17.5±2.12	-	0.27	0.02

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გერონტოპროტექტორ ლივაგენის გავლენა კობალტის იონებითა და γ-სხივებით ინდუცირებულ ქრომოსომათა ცვალებადობაზე დაბერებისას

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ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტის გენეტიკის კათედრა

(მიღებულია 15.03.2006)

რეზიუმე

შესწავლილ იქნა მძიმე მეტალით, კობალტით და γ-რადიაციით ერთჯერადი და ფრაქციონირებული დასხივებით ინდუცირებული ქრომოსომათა ფუნქციური მაჩვენებლების (ქრომოსომული აბერაციებისა და ბირთვაკის ორგანიზატორთა აქტივობის დონე) ცვალებადობა 70-86 წლის ინდივიდებში, და ამ მაჩვენებლის კორექციის შესაძლებლობა გერონტოპროტექტორ ლივაგენით. აღმოჩნდა, რომ ლივაგენი მკვეთრად აქვეითებს კობალტის იონებით ინდუცირებულ ქრომოსომულ არასტაბილურობას ორივე ასაკობრივ ჯგუფში (8,5-1.9% საშუალო ასაკის და 13.17-2.3% ხანდაზმულ ინდივიდებში, ლივაგენის გარეშე; და შესაბამისად 2.0-0.9% და 3.25-1.2% ლივაგენის ზემოქმედებისას). ლივაგენი ასევე შესამჩნევად აქვეითებს γ-სხივების მუტაგენურ ეფექტს ხანდაზმულ ინდივიდთა უჯრედებში როგორც ერთჯერადი, ასევე ფრაქციონირებული ზემოქმედების პირობებში. ლივაგენის ანტიმუტაგენური მოქმედება ახსნილია ქრომატინზე მისი მადეკონდენსირებელი ზეგავლენით, რაც ზრდის რეპარაციული პროცესების ეფექტურობას.

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EFFECT OF POLLEN GRAIN ON POLYEMBRYONY DEGREE IN THE CITRUS PLANTS

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Abstract

Results of study of pollen effect on the degree of polyembryony in the citrus are analyzed. The pollen of *C.wilsoni* and *C.ichangesis* were used as a pollenizer, lemons *L.gruzinxki, L.Mayer* and indigenous variety of orange (*citrus sinensis var.*) as a mother-plant. It was established that potential of development of additional embryo and stimulating effect of pollen on the degree of polyembyony are caused by specific biological characteristics of plants and according to genotype features are often distinctive.

Key words: apomixic, nucellus, polyembryonic plant, biembryonic plant.

Introduction

Some families of metasperm plants are characterized by apomictic reproduction potential. Development of new organisms via somatic cells of seed-buds enables them to maintain unchanged genotype of mother plant in the further generations. Such generations, by cloning of mother genotypes, remain balanced proportions of adaptively available gene combinations within species, and consequently are not submitted to segregation. Evolutionary priority of apomictic reproduction should be explained by such mechanisms [Bengtsson et al., 2000].

The citrus are characterized by apomixic of facultative type. Along with zygotic embryo apomictic (nucellar) embryo are developed in the seed. Most species of this genus are characterized by polyembryony of induced type [Mamporia, 1951; Maisuradze, 1956; Koltunow et al., 1996; Schranz et al., 2005; Mender-Rodrigues et al., 2005]. Additional embryos in citruses are not zygotic and are developed from structurally changed nucellus somatic cells [Mamporia, 1951] or from subepidermal tapetal cells [Kapanadze, 1967]. Genetical differences of zygotic and nucellar embryo were also registered using protein markers [Tutberidze, 1964; Khukhunaishvili, 1988].

In scientific literature there are various opinions in regard to the effect of pollenizer plant on the degree of polyembryony in the citruses. Some authors consider that development of additional embryo depends on mother plant, as well as on pollenizer [Maisuradze, 1956], in the opinion of others – only on mother plant [Kapanadze, 1967], and others consider that it mainly caused by biological features of pollenizer [Diasamidze, 1994; 1995].

The aim of our work was to study polyembryony degree in citrus cultivars (*L. Mayer, L. gruzinski* and local variety of orange - *citrus sinensis var.*) at their pollination by wild forms of citruses (*C.ichangensis and C.Wilsoni*), and to analyze the results of the effect of pollenizer plant on the polyembryony.

Materials and Methods

As a material for our experiments we used lemons *L. Mayer, L. gruzinski,* local variety of orange, *C.ichangensis and C.Wilsoni.*

For conducting of hybridization unblown flowers were opened by tweezers on the mother plant branches, pollen tubes were removed and gauze bags were put on them. After some days, when the liquid appeared on the stigma, pollination was carried out by brush.

For pollen preparation pollenizer plant flowers were opened by tweezers, corolla was removed and unblown yet pollen sacks were collected on Petri dish. Material was stored at room conditions. When pollen sacks were blown, they were put in desiccator and stored up to maturing of mother plan flowers for pollination.

Pollination was carried out in the morning hours. After pollination gauze bags with corresponding marks were put on the branches with flowers. After fruit-set gauze bags were removed.

After fruitage seeds were taken, and their stratification was carried out. In early spring they were sowed in the wood boxes at intervals of 10 cm on prepared specially soil. Every sprout was analyzed.

Data obtained by free pollination were used as a control in every case.

Results and Discussion

C.ichangensis and *C.wilsoni* belong to the wild forms of citruses. They are characterized by sharp features and dominate at hybridization with cultivars. As a result, it is possible to distinguish accurately by outward signs hybrid (zygotic) embryos from nucellar ones (clones of mother plant) grown from young sprouts of one seed.

According to experimental results citrus *sinensis var.* revealed especially high potential of development of additional embryo (43.3%), this parameter for lemon species was lower; for *L. Mayer* it equals 5.3%, and for *L. gruzinski* – 2.8%.

At the usage of various species as a mother plant effect of pollenizer plant on the polyembryony degree is different. While using *C.wilsoni* as pollenizer certain stimulation of polyembryony was observed in lemons, compared with free pollination. Namely, for *L.Mayer* it equals 4.8%, but in the case of control – 3.65%; for *L.gruzinski* it is 3.2%, and for control – 2.8%. Analogous results were recorded at pollination of lemons by *C.ichangensis*. But stimulating effect of pollenizer plant on polyembryony is more aloud: *L.Mayer* – 7.8% and control – 3.9%; *L.gruzinski* – 3.9% and control – 2.85%.

In the case of local variety of orange opposite picture is observed: at free pollination polyembryony degree is 44.3%, whereas at the usage of *C.wilsoni* (9.3%) and *C.ichangesis* as pollenizer this parameter is lower considerably. Probably, in oranges in the case of self-pollination (and more probably at free pollination) inducing effect of pollen tube entrails on additional embryo production is higher than at pollination by different genotypes. Above mentioned regularity considers fruit-set of two-embryonic seeds, as well as poly-embryonic seeds (Tables 1, 2).

It should be mentioned that while using *C.ichangesis* as pollenizer its inducing effect in additional embryo production is higher in every variant of hybridization than in the case of using *C.wilsoni.*

There are various opinions in scientific literature about genetical nature of apomictic embryo. According to our results in nucellar sowings hereditary features of pollenizer at the level of organism were not recorded. We can suppose that pollenizer has only inducing influence on additional embryo production on corresponding somatic cells and doesn't take part in

determination of their genetic characteristics. Hence, consideration about hybrid nature of nucellar sowings should be groundless.

Consequently, according to above mentioned, potential of additional embryo production and stimulating effect of pollen on the degree of polyembryony could be reasoned by specific characteristics of citrus plants.

Table 1. *C.ichangesis* pollen effect on polyembryony degree at its using in hybridization

Mother plant	Amount of seedlings	polyembryony		Among them			
				biembryonic		polyembryonic	
		amount	%	amount	%	amount	%
L.Mayer	195	15	7.7±2.7	14	93.4	1	6.4
control	167	6	3.6±1.9	6	100	-	-
L.gruzinski	128	5	3.9±1.9	5	100	-	-
control	142	4	2.8±1.6	4	100	-	-
Citrus sinensis var.	345	45	10.0±3.4	36	80.0	9	20.0
control	330	147	44.5±4.9	118	80.3	29	19.7

Table 2. *C.wilsoni* pollen effect on polyembryony degree at its using in hybridization

Mother plant	Amount of seedlings	polyembryony		Among them			
				biembryonic		polyembryonic	
		amount	%	amount	%	amount	%
L.Mayer	108	5	4.6±2.1	5	100	-	-
control	167	6	3.6±1.8	6	100	-	-
L.gruzinski	187	6	3.2±1.8	6	100	-	-
control	142	4	2.8±1.6	4	100	-	-
Citrus sinensis var.	118	11	9.3±2.9	8	72.7	3	27.3
control	330	147	44.3±4.9	118	81.2	29	19.2

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მტვრის მარცვლის გავლენა პოლიემბრიონიის ხარისხზე ციტრუსებში

დოლიძე ქ., დიასამიძე ა.

შ. რუსთაველის სახელობის ბათუმის სახელმწიფო უნივერსიტეტი

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რეზიუმე

შესწავლილია მტვრის მარცვლის გავლენა პოლიემბრიონიის ხარისხზე ციტრუსებში. დამამტვრიანებლად გამოყენებულ იქნა *C.wilsoni* და *C.ichangesis*-ის მტვერი, დედა მცენარედ კი ლიმონები - *L.gruzinxki, L.Mayer* და ადგილობრივი ფორთოხალი (*citrus sinensis var.*). დადგენილია, რომ დამატებითი ჩანასახების წარმოქმნის უნარი და მტვრის მასტიმულირებელი გავლენა პოლიემბრიონიის ხარისხზე ციტრუსოვან მცენარეთა სპეციფიკური ბიოლოგიური თავისებურებებით არის განპირობებული და ხშირად გენოტიპური თავისებურებების მიხედვით ინდივიდუალურ ხასიათს ატარებს.

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STUDY OF GENETIC ALTERATIONS INDUCED BY THE PESTICIDE KURZAT IN ARTIFICIAL AND NATURAL POPULATIONS OF YEAST

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Abstract

Influence of the pesticide Kurzat on artificial and natural populations of wine and baker's yeasts has been investigated. The preparation induced with high frequency intragene segregants. The pesticide manifested genetic activity in test-systems where the process of reparation was disturbed. The strains resistant to the pesticide Kurzat were rarely found in natural populations of wine yeast.

Key words: xenobiotic, fungicide, mitotic segregation, gene toxic effect

Introduction

Pollution of the environment by xenobiotics of different types is a phenomenon accompanying technogenic progress. Pesticides represent one of most harmful groups of environmental pollutants. Some of them being chemically steady circulate invariably in the environment for years. Many of species turned out to be capable for xenobiotics' accumulation. Due to this, every higher step of the trophic web contains pesticide in much more amount [Kovaltsova, Korolev, 1996, Dubinin, 2000].

The majority of xenobiotics along with toxicity are characterized by gene toxicity as well. These compounds affect genetic apparatus established in the process of evolution thus making threat to all living system [Dubinin, 2000; Kadagishvili et al. 2006].

Materials and Methods

Intergene recombinations have been analyzed using the lines T5 (the genotype MAT a/α ADE2 his 8::ade2::HIS8) and T423 (the genotype MAT a/α ADE2 his8 spl2::ade2::HIS8 spl2) obtained form the strain GU-51 of wine yeast (*Saccharomyces cerevisiae var vini*). The ade2 and his8 loci are localized in XV chromosome and are presented in the genotype in *trans* state. Spl2 mutation is pleiotropic and at high temperature (37°C) it blocks such important processes of life cycle as mitosis, meiosis and reparation [Shatirishvili et al., 2000].

Intragene mitotic segregation was analyzied by the strains constructed from Petergof genetic lines of the yeast (*S. cerevisiae*): T1 (the genoptype MAT a/α ade2-192::ade2-Δ45) and T2 (the genotype MAT a/α rad2ade2-192:: α rad2ade2-Δ45) [Zakharov et al., 1979].

In our experiments $1 \cdot 10^6$ cell titer suspension of the yeast has been exposed to the 1% aqueous solution of the fungicide for 30, 60, 90 and 120 minutes. The standard nutrition media and methods were used for cultivation of yeasts [Zakharov et al., 1984].

Results and Discussion

Kurzat is a combined fungicide composed of copper oxychloride and cymoxaline. To study the alterations evoked by the fungicide the artificial populations composed of cells of four strains with different genotypes were used. The strains T1 and T2 belong to the race of baker's yeast. Using these strains the intragene segregation induced by the pesticide was studied. Mutations ade2-192 and ade2- Δ45 partially suppress adenine biosynthesis (Leaky-mutation) and are characterized by differing phenotypic effect. The first develops the colonies of rose color and the second one develops yellowish colonies [Zakharov et al., 1979].

In T51 and T423 forms of wine yeast (*Saccaromyces cerevisiae var. vini*) constructed from the genetic line of the strain GU-51 it was possible to study intergene mitotic segregation induced by the pesticide. With this aim the system ade2-his8 was used. These genes are localized in Xv chromosome and distance between them makes 43.7 cM.

The main criterion for evaluation of lethal effect of the pesticide under study is dependence of survival rate of diploid vegetative cells on the time of population exposure to the compound. Kurzat evoked different lethal effects on populations of various races and genotypes. T1 and T2 lines turned out to be more susceptible to the fungicide, than T51 and T423 lines of wine yeast. Different susceptibility to the pesticide was observed even in different populations of the same race. In particular, the rad2 and spl2 genes containing strains where the process of reparation was impaired, were distinguished by higher susceptibility. 50% lethal effect for the strain T1 turned out to be 33 min; for T2 -21 min; T51 – 66 min; T423 – 39 min.

In parallel to toxic effect gene toxic action of Kurzat has been studied (Table 1). The fungicide induces both mitotic segregation and crossing-over in T1 and T2 strains. This process was much more frequent in rad2 gene-containing populations.

Using the test-system developed for the strains T51 and T423 mitotic segregation caused by Kurzat has been investigated. Frequency of segregation increased with time of exposition (the phenomenon of dose-effect). Induction of segregants by the pesticide was comparatively frequent in spl2 gene-possessing cells. It should be marked also, that the frequency of intergene recombinations induced by Kurzat exceeded that of intragene recombinations.

This is no chance to study in natural conditions the recombinations and mutations evoked by xenobiotics. In the strains isolated from the natural populations only the quantity of fungicide-susceptible and fungicide-resistant forms can be determined.

Resistance to the fungicide Kurzat of the yeast strains isolated from wine yeast populations of two most important wine-growing regions of East Georgia (Telavi and Kvareli regions) has been evaluated (Table 2).

The strains resistant to Kurzat are rarely found in both populations. In the same population high incidence of forms resistant to copper ions was stated [Nikabadze, Shatirishvili, 1997]. In contrast to Kurzat copper salts have been used for centuries as fungicides. This has contributed to the establishing and spreading the forms resistant to this metal in wine yeast populations.

Using the test-system developed for T1 and T2 strains genetic activity of 8 pesticides has been evaluated. Only two of them Butiphos and 2, 4-D have revealed the ability for recombinants'

induction. Recombinants were induced only in T2 strain. As it was mentioned, this line contains rad2 gene, which suppresses the process of reparation [Davronov, 1984].

Table 1. Mitotic segregation induced by the pesticide Kurzat in genetic strains of Saccharomycetes

Test system	Time of exposure	Survival rate, %	Number of analyzed colonies	Mitotic segregation			
				Number of segregants	%	Number of cross-overs	%
T1	0	100	4200	1	0.02±0.001	0	0
	30	67.2	7430	6	0.08±0.01	1	0.06±0.01
	60	56.2	11802	15	0.13±0.02	5	0.04±0.01
	90	39.3	16506	23	0.14±0.03	7	0.04±0.01
	120	16.2	34131	51	0.17±0.02	11	0.03±0.01
T2	0	100	5463	1	0.02±0.01	0	0
	30	41.1	5617	9	0.09±0.01	1	0.01±0.01
	60	22.1	6053	14	0.12±0.09	2	0.02±0.01
	90	20.1	13240	28	0.16±0.01	7	0.04±0.01
	120	9.9	27100	73	0.28±0.03	34	0.13±0.02
T51	0	100	4183	1	0.02±0.01	-	-
	30	73.1	3057	5	0.14±0.02	-	-
	60	60.9	2531	13	0.51±0.03	-	-
	90	47.2	1974	17	0.86±0.03	-	-
	120	29.3	1225	12	0.98±0.04	-	-
T423	0	100	3779	1	0.03±0.01	-	-
	30	76.2	3511	8	0.23±0.01	-	-
	60	51.3	4029	14	0.35±0.02	-	-
	90	18.5	3302	25	0.75±0.04	-	-
	120	13.8	5903	75	1.27±0.07	-	-

Table 2. Study of sensitivity to the pesticide Kurzat in natural populations of wine yeast

Population	Total number of analyzed strains	Number of resistan strains	Number of sensitive strains
Kvareli "Rkatsiteli"	157	19 (12,1%)	138 (87,9%)
Telavi "Rkatsiteli"	139	24 (17,3%)	115 (82,7%)

By the same test-system as developed in *S. cerevisiae* genetic activity of Hexathiuram, Heptathiuram and Sumilex of wine and baker races was evaluated. Preparations have manifested specific effect. High incidence of induced intragene recombinants was stated in case of Hexathiuram application [Shatirishvili et al., 2000].

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პესტიციდ კურზატით ინდუცირებული გენეტიკური ცვლილებების შესწავლა საფუვრის ხელოვნურ და ბუნებრივ პოპულაციებში

ქადაგიშვილი ე., სადაგიშვილი თ., მენაბდე მ., ზარნაძე თ., შათირიშვილი ა.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.02.2006)

რეზიუმე

შესწავლილია საფუვრის (*Saccharomysces cerevisiae*) ღვინისა და პურის რასების ხელოვნურ და ბუნებრივ პოპულაციებზე ფუნგიციდ კურზატის გავლენა. პრეპარატმა მაღალი სიხშირით გამოიწვია გენთაშორისი სეგრეგანტების ინდუქცია. გენეტიკური აქტივობა პრეპარატმა გამოავლინა იმ ტესტ-სისტემებში, სადაც რეპარაციის პროცესი იყო დარღვეული. ღვინის საფუვრის ბუნებრივ პოპულაციებში დაბალი სიხშირით გვხვდება კურზატისადმი რეზისტენტული შტამები.

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THE SIGNIFICANCE OF INDUCED CHROMOSOME REARRANGEMENTS IN SOMATIC TISSUES ON THE AGEING PROCESS OF DROSOPHILA

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Abstract

The aim of this investigation was to establish the significance of γ-induced chromosome rearrangements in somatic tissues on the ageing process of Drosophila. The emergence rate and lifespan parameters of imago of radiosensitive strain bearing rad(2)201 ^{G1} mutation (rad) and of wild-type strain (rad^+) after exposure to γ-irradiation of larvae were studied. After irradiation of 96-hour-old larvae of rad strain with doses of 2.5-10.5 Gy, the emergence rate equals 84.7-31.8%. After irradiation of rad^+ strain at the same age with doses of 10.0-40.0 Gy, the emergence rate equals 84.7- 54.6%. The lifespan is decreased in irradiated populations. The tendencies of the adult Drosophila lifespan changes depending on their genotypes, sexes and the doses treatment are described in the study. The influence of the induced chromosomal rearrangements in somatic tissues on the normal vital process which occur during ageing of adult Drosophila was shown.

Key words: DNA repair, Drosophila, irradiated larvae, radiosensitive mutant, lifespan

Introduction

Drosophila is widely used in ageing research, a massive amount of data have been collected that bear directly on the problems of longevity and ageing in this one organism (Lints F. et al., 1988).Clearly, the progress we make is not directly proportional to the number of facts we collect [Martin et al., 1988; Arking, Dudas, 1989]. As proposed, the inconsistency of the sex differences and the presence of DNA repair systems presents an entirely new level of possible control of lifespan that remains to be fully evaluated [Mayer P. Backer G., 1985]. Similar gene expression patterns characterize ageing and oxidative stress in *Drosophila melanogaster* [Landis et al., 2004].

The study of radiosensitive mutations in yeast and Neurospora play a significant role in resolving of many problems of radiation genetics. Also, it is important to know the effect of radiation and UVA on the biological activities of organisms, such as induction of somatic-cell mutation in Drosophila larvae [Nigeshi T. et al., 2001]. The relative contribution of DNA repair and other events to survive DNA damage in Drosophila larvae was shown recently [Jaklevik R., Su T., 2004].

Isolation and characterization of radiosensitive strains of *Drosophila melanogaster* from natural population has the aim to study the radiation-induced injury manifestations which are

typical for multicellular eukaryotic organisms. The use of Drosophila radiosensitive mutant rad(2)201^{Gl}, which is characterized by increased rate of chromosomal aberrations in somatic cells as a result of irradiation [Levina V.,et al., 1980; Kromikh Yu., Levina V., 1990] gives us a chance to estimate the role of induced damages in genetical material in the lifeshortening of irradiated individuals.

It has previously been reported that rad(2)201^{Gl} mutation causes significant changes in lifespan of females [Tsikoridze N., 2002]. In this study we have the aim to show the significance of chromosome aberrations in somatic tissues that influence the ageing process of the insect *Drosophila melanogaster* after irradiation of larvae by γ -rays.

Materials and Methods

Radiosensitive rad(2)201^{Gl} mutant strain and wild-type radioresistant Drosophila strain (Canton-S) were used in this study. Material for carrying out the tests was obtained from the 2-hour of the egg-position cultures. The conditions of culture maintaining were as same as described earlier [Tsikoridze N., 2002].

The larvae irradiated were 96-hour-old, as at that time the highest level of chromosome aberrations (the autosomal chromatid deletions) in somatic cells of irradiated rad(2)201^{Gl} mutant larvae were registered [Khromikh Yu., Levina V.1990].

γ irradiations were made in a ^{137}Cs irradiator –"MIDI" (E=662 KeV, dose rate equals to 5,41 Gy · min^{-1}). The doses tested were: 1.5; 2.5; 3.0; 5.0; 7.5; 10.0 Gy for radiosensitive strain (rad), and the doses tested were: 10.0; 12.5; 15.0; 20.0; 25.0; 30.0; 40.0 Gy for radioresistant (rad$^+$) strain. In the experiments each of doses were repeated from 2 to 5 times, with its own uniradiated (controls) variants.

The number of pupae and adults were counted. The number of emerged imagoes was registered during 10 days from the beginning of eclosion. The criterion of surviving of pupae was the emergence rate of imago from puparіums. The adult flies were reared in glass "Drosophila bottles" at 25±1°C, males and females of both strains separately. Insects were transferred on fresh medium every 2 days. 19 664 pupae of rad$^+$ and 15 279 pupae of rad were tested alltogether, among them survived adults in rad$^+$ were 16 051, or 80.4%, and in rad were 6 891, or 45,1%. The number of imagoes lifespan studied for rad$^+$ was 9 085 (45.5% of pupae) and the number of rad imagoes lifespan studied was 6 235 (40.8% of pupae). The effects of each dose on the lifespan were studied in the following four groups of flies: rad$^+$ females, rad$^+$ males, rad females and rad males. For each groups the values of average lifespan and standard root-mean-square deviation, LT50, the maximal LT, and average LT(ALT) relatively to control groups' ALT were calculated. The survival curves were constructed in each case. The data obtained were analyzed separately, as it is needed for the specificity of the study.

Results and Discussions

The emergence rates of flies irradiated as late larvae, before they are going to begin pupariation, are given in Fig.1. The first curve shows that in radioresistant strain up to dose 20.0 Gy the rates of emergence remained normal, since they were similar in control and treated flies. The difference between control and irradiated populations composed 7%. However, when the dose reached 30.0 Gy, the emergence rate in radioresistant strain populations fell by 31%, when the dose reached 40.0 Gy, the emergence rate in population fell by 38%. The data are in agreement with the results obtained by J. Seugé et al., 1985, who studied the aging of the insect *Drosophila melanogaster* (Oregon) strain by γ-irradiation of pupae.

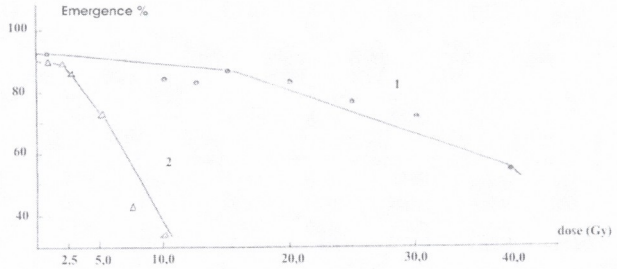

Fig.1. Emergence rates of flies in relation to the dose of irradiation during the third larval stage.

• = rad$^+$; △ = rad.

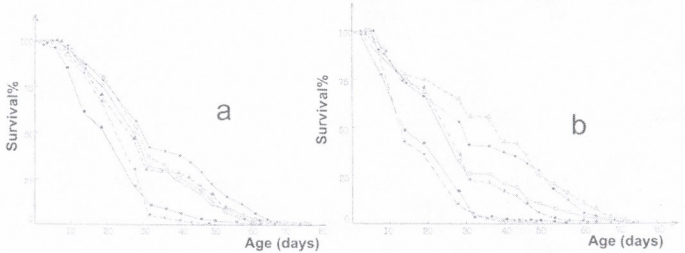

Fig.2. The survival of Drosophila melanogaster radioresistant strain, females (a) and males (b).
•, control group, Δ -10,0 Gy irradiated group; □- 15,0 Gy irradiated group;
▲- 20,0 Gy irradiated group; * - 30,0 Gy irradiated group; ■ -40,0 irradiated group.

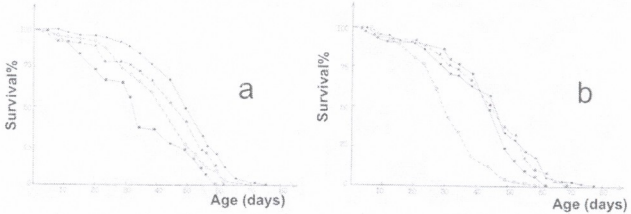

Fig.3. The survival of Drosophila melanogaster radiosensitive strain, females (a) and males (b).
•, control group,○- 5,0 Gy irradiated group; ▲ - 7,5 Gy irradiated group;
■ - 10,0 Gy irradiated group.

The second curve in Fig.1 shows that in radiosensitive strain up to dose 2.5 Gy the rates of emergence remained normal, when the dose reached 10.0 Gy the emergence rate of radiosensitive strain population fell by 60%. It must be noted that the presented material includes the results obtained in one of the subpopulations of radiosensitive strain. It must be noted too, in some subpopulations of radiosensitive strains the lethality of flies after exposure of late larvae to 10.0 Gy reached to 100%. The data are in a good agreement with the results, which concerned the realization of the effects of rad(2)201^{Gl} mutation on the cell and organism level [Khromykh Yu., Levina V. 1990].

The duration of life of Drosophila imagoes, which were treated at late larval stage, was studied. For radioresistant populations (males' and females' were analyzed separately) six survival curves from many obtained are presented in Fig. 2. They indicate that the treated populations had reduced lifespan. Also, the difference between females and males lifespan was shown. A statistically significant diminution of the average lifespan was observed in females (10.0 Gy: 30.4 ±1.1 days; 15.0 Gy: 29.6 ±1.1 d; 20.0 Gy: 29.7 ±1.3 d; 30.0 Gy: 20.5 ±0.9 d; 40.0 Gy: 22.4± 0.7 d; controls: 33.5± 1.3 d; that is, a ratio of treated/control LT 50 reduction is in the range of 8.3% to 26.7%). Four of the presented curves show rectangular shape while the fifth and sixth (30.0 Gy and 40.0 Gy) are more linear. A statistically significant diminution of the average lifespan was observed in males too (10.0 Gy: 35.7± 1.9 days; 15.0 Gy: 26.6± 1.2 d; 20.0 Gy: 25.2 ±1.0d; 30.0 Gy: 17.5 ± 0.8 d; 40.0 Gy: 16.7 ±0.7 d; controls: 32.1± 1.3d; that is a ratio of treated/control LT50 reduction is in the range of 17.0% to 51.9%). Two curves show the commonly observed rectangular shape while the other four are linear.

For radiosensitive populations (males and females were analyzed separately) four survival curves from the many obtained are shown in Fig.3. They indicate that the treated populations had reduced lifespan. Also, the difference between lifespan changes of females and males depending on doses was shown. A statistically significant changes of the average lifespan was observed in females (5.0 Gy: 39.4 ±1.7 days; 7.5 Gy: 40.8 ±2.4 d; 10.0 Gy: 32.5 ±2.9 d; control: 47.2 ±1.5d; a ratio of treated/control LT50 reduction is in the range of 7.2% to 34.0%). Three of the presented curves show rectangular shape while the fourth (10.0 G y) is more linear. A statistically significant diminution of the average lifespan was not observed in all cases of treated males (5.0 Gy: 29.5 ±1.3d; 7.5 Gy: 41.2±2.1 d; 10.0 Gy: 40.3± 2.5 d; control: 42.0 ±1.8 d; a ratio of treated/control LT50 reduction is in the range 1.1% to 36.3%). One curve shows linear shape while others are rectangular.

Ageing in eukaryotic organisms refers to the sequential changes of endogenous processes (biochemical, physiological, etc.), that regularly occur over time in the phenotype and that influence the duration of life. In a population of organisms, ageing ultimately reflected in differential (adult) survival and, along with the effects of the environment, determines the shape of the survivorship curve [Mayer P., Baker G. 1985].

Mutation rad (2)201^{Gl} becomes effective essentially in divisional cells. This suggestion is correct in both cases: when the difference of an average lifespan of these strains after irradiation of 1-day-old imagoes were compared [Tsikoridze et al., 2005] and, when the role of DNA repair deficiency in survive of irradiated larvae was studied [Tsikoridze, 1988; Dalakishvili, Tsikoridze 1996; 1998]. Thus, the effect of irradiation which was registered at imago stage represents the result of postmitotic cells' damages connected with a high resistance of adults to radiation treatment.

After irradiation of late larvae of rad strain different abnormalities of flies' development were registered. So, DNA damages that provoke double strand breaks or alter the transcription, i.e. are realized on the tissue level in different ways and expressed in disfunctioning of these tissues.

As far as we know, the literature includes no reports concerning the effect of the irradiation of late larvae on the ageing of adult *Drosophila melanogaster*. In the adults emerged from those pupae which were irradiated as late larvae, we observed the following:

1. An average lifespan of the adults which developed from irradiated larvae was significantly less than an average lifespan of the adults developed from unirradiated larvae in males and females of radioresistant (Canton-S) and radiosensitive – rad(2)201^{GI} flies as well. Herewith, the changes of the absolute values are possible.

2. An average lifespan of the adult females of radiosensitive strain developed from irradiated larvae was significantly less than an average lifespan of the adult males developed from irradiated larvae, in the same conditions; that has been reflected on survival curves too. But, with the low doses of γ-irradiation treatment (5.0 Gy; 7.0 Gy) an average lifespan turned out to be the shortest in the radiosensitive males which were developed from irradiated larvae. For this case, the difference to control composed in males -35.2%, and in females -16.5%.

3. An average lifespan of the adult radioresistant strain females which developed from irradiated larvae does not differ from an average lifespan of the radioresistant adult males, in the same conditions. However, the females' average lifespan was prevailed in some cases, though with the low doses of treatment (10.0 Gy; 15.0Gy) the developed males lived through.

4. It must be noted that the registered differences and tendencies of lifespan changes between radioresistant and radiosensitive strains are less expressed when the one-day old imagoes radiation treatment takes place.

As known, components of the DNA damage checkpoint are essential for surviving exposure to DNA damaging agents. Checkpoint activation leads to cell cycle arrest, DNA repair and apoptosis in eukaryotes [Jaklevic, Su, 2004). After exposure to ionizing radiation, wild-type Drosophila larvae regulate the cell cycle and repair DNA. We supposed rad(2)201^{GI}, as like as okra (DmRAD54) mutants regulate the cell cycle but are deficient in repair of DSB; mei-41(DmATR) mutants cannot regulate the cell cycle and are deficient in DSB repair show progressive degeneration of imaginal discs and die as pupae.

Double mutants mei-41^{D5}; rad(2)201 ^{GI} and mei-9^{a}; rad(2)201^{GI} were constructed to study the interaction of these mutations in Drossophila exposed to γ -rays. The results obtained showed that the interaction of mei9 and rad201 mutations is additive and the interaction of mei-41 and rad201 mutations is epistatic [Varentsova, Khromikh,1997]. The emerged flies had shorter lifespan. The main effects induced by the irradiation of late larvae are the same as those observation in adults, irradiated with doses 20 fold higher, that were pointed by J. Seugé et al. (1985).

The obtained results of the lifespan studying display the significance of the genetic rearrangements in somatic tissues which have some influence on the normal vital processes which occur during ageing of adult Drosophila.

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სომატურ ქსოვილებში ინდუცირებულ ქრომოსომულ ცვლილებათა მნიშვნელობა დროზოფილას დაბერების პროცესში

წიქორიძე ნ.

ალ. ნათიშვილის სახ. ექსპერიმენტული მორფოლოგიის ინსტიტუტი

(მიღებულია 07.02.2006)

რეზიუმე

გამოკვლევის მიზანს შეადგენდა დროზოფილას სომატურ ქსოვილებში γ-ინდუცირებულ ქრომოსომულ დაზიანებათა მნიშვნელობის დადგენა დაბერების პროცესში. შევისწავლეთ ლარვის სტადიაზე დასხივებული რადიომგრძნობიარე rad(2)201) ხაზის(rad) და საკონტროლო Canton-S ხაზის (rad^+) გადარჩენადობა და სიცოცხლის ხანგრძლივობის პარამეტრები. ლარვის სტადიის 96-სთ-ზე rad ხაზის 2.5 – 10.0 გრეი დოზით დასხივების დროს გადარჩენადობა საშუალოდ 84.7-31.8%-ს შეადგენდა. ამავე ასაკის rad^+ ხაზის 10.0-40.0 გრეი დოზით დასხივების დროს გადარჩენადობა 84.7-54.6%-ს შეადგენდა. დასხივებულ პოპულაციებში აღინიშნება სიცოცხლის ხანგრძლივობის (სხ) კლება. განხილულია ზრდასრული დროზოფილას სხ–ის ცვლილებათა ტენდენციები სქესის, გენოტიპისა და დასხივების დოზებთან მიმართებაში. ნაჩვენებია სომატურ ქსოვილებში ინდუცირებულ ცვლილებათა გავლენა ნორმალურ საციცოცხლო პროცესებზე, რომლებიც ზრდასრული დროზოფილების დაბერების დროს აღმოცენდება.

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ULTRASTRUCTURAL CHANGES OF MICE RETINA GANGLIONIC CELLS BY THE INFLUENCE OF ACTINOMYCIN-D

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Abstract

The ultrastructural changes of adult mouse eye retina ganglionic cells by the influence of inhibitor of transcription, actinomycin-D, was investigated. After 1 and 4 hours of actinomycin-D injection in BA/B line adult mice the ultrastructure of ganglionic cells changes drastically: the space of rough endoplasmic reticulum, the Golgi complex and the quantity of polyribosomes reduce; the cristae of mitochondria are destroyed. These changes indicate mainly to the inhibition of function of protein-synthesis apparatus and mitochondria. After 24 hours of inhibitor injection, the ultrastructure of ganglionic cells resembles the norm. These data show that the reaction of ultrastructure of ganglionic cell organoids on the inhibition of transcription in adult animals has reparative character.

Key words: ganglionic cells, actinomycin-D, mice.

Introduction

It is known, that the regulation of synthesis processes in any cells takes place according to the information, which exists in its genotype. But correction of these syntheses always happens by means of different exogenous factors of the environment. Thus, it is of a great interest to study the ultrastructural changes of vertebrate eye retina cells by the influence of the exogenous factor - actinomycin-D, which inhibits transcription. Actinomycin-D forms chemical bond with DNA nucleotides - guanine and cytosine and inhibits RNA transcription. Accordingly, mRNA adequate protein-synthesis process is inhibited [Gale et al., 1972].

According to above-mentioned it was interesting to find out whether actinomycin-D influences the ultrastructure of adult mouse retina ganglionic cells.

Material and Methods

To solve this problem, BA/B line adult mice (5 specimens) were injected with actinomicin D in a ratio: 1γ actinomicin-D / 1g animal weight.

The material was treated according to the routine electron microscopy methods. After 1, 4, and 24 hours of actinomycin –D injection material was fixed in 2% OsO_4 with prefixation in 2.5% glutaraldehyde. Than material was embedded in 812 Epon epoxide. Ultrathin slides were studied by electronic microscope- JEM-100B.

Results and Discussion

BA/B line adult mice' retina ganglionic cells are represented by one layer of rather thick, rounded cells. The nucleus is large and takes almost the whole cell. It is surrounded by well-expressed dual membrane with numerous holes. The nucleolus is large, with clearly expressed fibrillar and granular components. Near the nucleus the Golgi complex often exists, which is represented by parallel oriented saccules and rounded vesicles to the sides. In the cytoplasm we can see many polysomes, which contain 5-6 ribosomes. The mitochondria are large, the cristae are well expressed. (Fig.1).

After 1 hour of actinomycin-D injection the changes in nucleus are quite inconsiderable: nucleolus becomes a bit steady, distance between the nuclear membranes reduces. The quantity of polysomes in cytoplasm quite diminishes. Endoplasmic channels get wider, but their granulars diminishes. The vesicles of Golgi complex are broad and the vacuoles are seen in cytoplasm. Mitochondria reduce in size. Some of them get steady and dark, the others – have light matrix, but their cristae are reduced and degraded (Fig 2).

After 4 hours of actinomycin–D injection the nucleolus becomes compact. The vesicles of Golgi complex become narrow. There are many polysomes in cytoplasm, endoplasmic reticulum channels are narrower and they almost have no ribosomes. The mitochondria are replaced near the nuclear membrane. Some of them have quite decomposed cristae, but some of them have well defended ones (Fig.3).

After 24 hours of actinomycin–D injection the whole cytoplasm is covered with polysomes. Separated ribosomes are seldom seen. Near the nuclear membrane rough elements of endoplasmic reticulum are seen. There are noticed ribosomes on the interior nuclear membrane. Cytoplasm contains many large mitochondria with well expressed cristae, though some of them have very light matrix and destroyed cristae. The nucleus has dual membrane. The nucleolus is characterized with expressed fibrillar and granular components (Fig.4).

As our investigation show, after 1 and 4 hours of actinomycin–D injection in mouse ganglionic cells the quantity of polysomes is reduced, the channels of endoplasmic reticulum and Golgi complex become wider; the ultrastructure of nucleolus and karyoplasm is considerably changed. The mitochondria are represented with light matrix and destroyed cristae.

It is interesting that after 24 hours the ultrastructure of ganglionic cells reestablishes and approximates the norm.

These dates show that in adult animals the inhibition of transcription doesn't cause so many molecular and ultrastructural changes, as in embryonic period. For example: after actinomycin–D injection in hen by inhibition of transcription the ultrastructure of membrane discs of photoreceptors outer segments was destroyed. It was considered that the inhibition of mRNA transcription, which is necessary for protein-opsin, the component of photoreceptors membrane discs. As a result, drastic ultrastructural changes were noticed in the process of differentiation in outer discs of 12-day embryos photoreceptors [Kvinikhidze, Djanelidze, 1986; Kvinikhidze et al., 2004].

The same ultrastructural changes, caused by the inhibition of transcription, and which we have observed in adult mouse ganglionic cells, occur in adult hen and lizard retina ganglionic cells [Akhalkatsi, 1982].

As is seen, in adult animals the inhibition of transcription doesn't have decisive role for synthesis of the specific structural proteins, as their syntheses happen on the long lifespan mRNA.

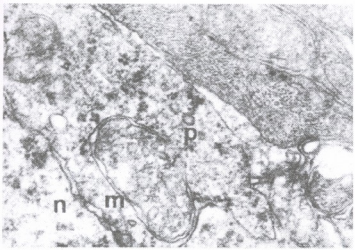

Fig.1. Ganglionic cell of adult mouse retina. (the norm), x35000; n nucleus, m-mitochondria, p- polysomes.

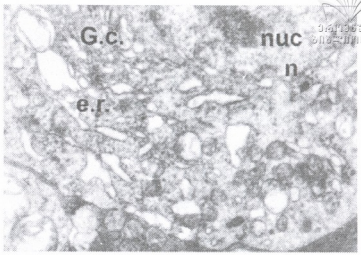

Fig.2. Ganglionic cell of adult mouse retina after 1 h of actinomycin-D injection, x 35000; n- nucleus, nuc.- nucleolus, G.c.-Golgy complex, e.r.- endoplasmic reticulum.

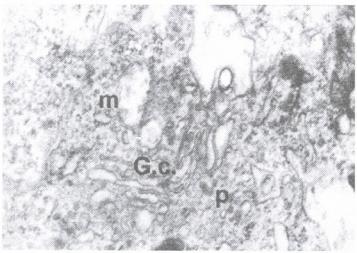

Fig.3. Ganglionic cell of adult mouse retina after 4 h of actinomycin-D injection, x 35000. G.c.-Golgy complex, m- motochondria, p-polysomes.

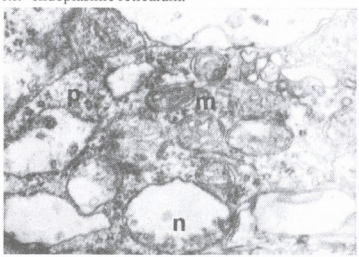

Fig.4. Ganglionic cell of adult mouse retina after 24 h of actinomycin-D injection, x 35000. n-nucleus, m-mitochondria, p-polisomes.

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თაბვების თვალის ბადურის განგლიოზური უჯრედების ულტრასტრუქტურული ცვლილებების შესწავლა აქტინომიცინ- D-ს მოქმედების შედეგად

გრატიაშვილი ნ.

ზოოლოგიის ინსტიტუტი

(მიღებულია 17.03.2006)

რეზიუმე

შესწავლილია ზრდასრული თაგვების თვალის ბადურის განგლიოზური უჯრედების ულტრასტრუქტურა ტრანსკრიპციის ინჰიბიტორის, აქტინომიცინ-D-ს მოქმედების შედეგად. BA/B ხაზის ზრდასრულ თაგვებში აქტინომიცინ-D-ს შეყვანიდან 1 და 4 სთ-ის შემდეგ ბადურის განგლიოზურ უჯრედებში მკვეთრად იცვლება ულტრასტრუქტურა: მცირდება გრანულარული ენდოპლაზმური ბადის ფართი, პოლირიბოსომული კომპლექსების რიცხვი, გოლჯის კომპლექსის არხების ფართი, შეიმჩნევა მიტოქონდრიების კრისტების დაშლა. აღნიშნული ცვლილებები მეტყველებენ ძირითადად ცილა-მასინთეზირებელი აპარატის და მიტოქონდრიების ფუნქციის დაქვეითებაზე. ინჰიბიტორის შეყვანიდან 24 საათისთვის კი განგლიოზური უჯრედების ულტრასტრუქტურა ემსგავსება ნორმას. მიღებული მონაცემები მეტყველებენ იმაზე, რომ ტრანსკრიპციის ინჰიბიციაზე ზრდასრული თაგვების ბადურის განგლიოზური უჯრედების ორგანოიდების ულტრასტრუქტურის რეაქცია რეპარაციულ ხასიათს ატარებს.

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THE STUDY OF T AND B LYMPOCYTES AND IL-6 AT HELICOBACTER PYLORI INFECTION

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Abstract

We aimed to investigate peripheral blood T-, B-lymphocytes and IL-6 during gastroduodenal pathological process. We have observed patients with ulcer disease (20), chronic gastroduodenal disease (32 patients) and mixed form of ulcer and chronic gastroduodenal diseases (37 patients). There was no difference found between groups while comparing quantities of T- and B-lymphocytes, T-suppressors. The active fraction of T-lymphocytes was statistically reliably decreased in the mixed group in comparison with the chronic group. A tendency of T-active lymphocytes increase in the group of patients with chronic course of disease in comparison with all other groups was recorded. IL-6 concentration in peripheral blood was the highest in the chronic group. Accordingly, there is increase in T-active and T-helper lymphocyte quantity, as well as IL-6 concentration in peripheral blood during chronic gastroduodenal diseases. Thus, quantitative determination of T-lymphocytes subpopulations and IL-6 is significant factor for the study of chronic forms of gastoduodenal pathology.

Key words: Helicobacter pylori, T- and B-lymphocytes, IL-6.

Introduction

Immune reactions induced by Helicobacter pylori (H.P.) infections play leading role in the damage of mucous membrane of gastroduodenal zone [Calam et al., 1994]. The peptic ulcer may be the result of CD4 immune reaction on the H.P. antigen and imunopathological processes caused by it [Di Tommaso et al., 1995]. At the same time it is known that cytokines production by peripheral blood cells has some influence on the stomach epiteliocytes and development of inflammatory process in stomach mucous membranes [Pasechnikov & Chukov, 2000].

Proceeding from the above-mentioned we aimed to investigate T- and B-lymphocytes population and IL-6 production in the peripheral blood during gastroduodenal disorders.

Methods and Materials

The estimation of the T-lymphocytes' general population and active T-lymphocytes in the peripheral blood of the patients was performed by method of rosettes [Lyapov et al., 1980]. The investigation of T-helpers and T-suppressors was made according to the quantity of sensitive and resistant to teophyllin populations [Shohat, 1982]. IL-6 was investigated by ELISA method. B-lymphocytes were measured by the method of direct immunofluorescention.

We have studied the following groups of patients: I – ulcer disease (20 patients), II – chronic gastroduodenal disease (32 patients), III – mixed form of ulcer and chronic gastroduodenal disease (37 patients); IV – control group (13 healthy persons).

Results and Discussion

The quantitative parameters of T-lymphocytes general population, their active fraction and B-lymphocytes are presented in the Fig.1. As it is shown in the diagram there was no difference in the quantity of general population of T lymphocytes between control and research groups, but in the mixed group there was tendency of decline in T lymphocytes general population. The quantitative parameters of T-lymphocytes active fractions were low in comparison with chronic group ($p<0,05$). It should be mentioned that in spite of statistically unreliable difference between all other groups there is tendency of increase in T-lymphocytes number. There is no statistically reliable difference in any group for B-lymphocytes.

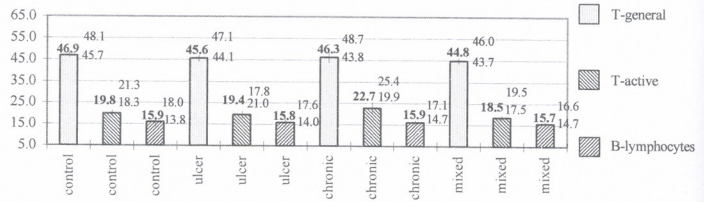

Fig.1. Average and confidential intervals for T-general, T-active and B-lympocytes parameters in different groups.

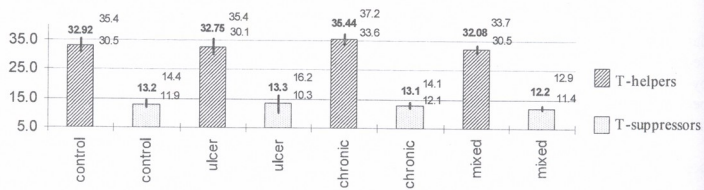

Fig.2. Average and confidential intervals for T-helpers, and T-suppressors parameters in different groups.

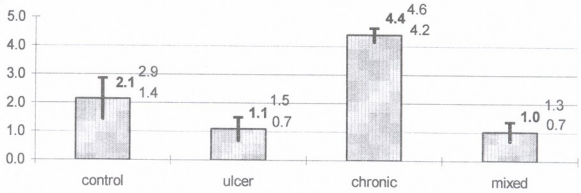

Fig.3. Average and confidential intervals for IL-6 general parameters in different groups.

T-helpers and T-suppressors (consequently resistant and sensitive to teophyllin) quantitative parameters are shown in the Fig.2. As it could be seen from the diagram the difference in T-helpers quantitative parameters between chronic and mixed groups approaches statistically reliable scope. There is the tendency of T-lymphocytes parameters to increase in chronic patients' group in comparison with all other groups.

The immunoregulation index didn't show any statistically reliable difference between the groups. Except T- and B-lymphocytes investigation of IL-6 was performed in peripheral blood. The received data is presented in the Fig.3. It shows that IL-6 quantity is the highest in patients with chronic gastroduodenitis. This difference is statistically reliable.

The tendency of some correlation between the quantitative increase of T-active lymphocytes, T-helpers and concentration of IL-6 could be seen from all three diagrams. This correlation logically proceeds from the fact that IL-6 is product of T-helpers (3). According to the received data we could conclude that quantitative analysis of IL-6, T-lymphocytes helper subpopulation and T-lymphocytes active fraction in peripheral blood provides important information for study of chronic gastroduodenic process.

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T და B ლიმფოციტებისა და IL-6-ის შესწავლა Helicobacter pylori ინფექციის დროს.

გაბადაძე ნ., ბუკია თ., მაცაბერიძე გ., ხუციშვილი მ.

ივ. ჯავახიშვილის სახელობის სახელმწიფო უნივერსიტეტის სამკურნალო დიაგნოსტიკური ცენტრი
სახელმწიფო სამედიცინო აკადემიის კლინიკურ-ლაბორატორიული დიაგნოსტიკის კათედრა

(მიღებულია 14.03.2006)

რეზიუმე

შესწავლილია პერიფერიული სისხლის T და B ლიმფოციტები და IL-6 გასტროდუოდენური პათოლოგიის დროს. ჩვენ გამოვიკვლიეთ გასტროდუოდენური წყლულით დაავადებული, ქრონიკული გასტროდუოდენიტით და შერეული ფორმით დაავადებულები. T და B ლიმფოციტებისა და აგრეთვე T სუპრესორების რაოდენობის განსაზღვრისას ჯგუფებს შორის სხვაობა არ აღმოჩნდა. T ლიმფოციტების აქტიური ფრაქცია იძლევა სტატისტიკურად სარწმუნო დაქვეითებას პაციენტების შერეულ ჯგუფში ქრონიკულ ფორმასთან შედარებით. T აქტიური ლიმფოციტების რაოდენობა მატულობს ქრონიკული ფორმის დროს, ასევე აღინიშნება IL-6-ის კონცენტრაციის მკვეთრი მატება ქრონიკული ფორმის დროს. ამგვარად, T ლიმფოციტების სუბპოპულაციებისა და IL-6-ის შესწავლა მეტად მნიშვნელოვანია HP-ით ინდუცირებული ქრონიკული ანთებითი პროცესების შესასწავლად.

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STUDY OF DISTRIBUTION OF HLA ANTIGENS IN CASE OF DIFFUSE-ENDEMIC GOITER (OF EUTHYREODICAL FORM) IN GEORGIAN POPULATION

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Abstract

The distribution of HLA antigens revealed a comparatively high frequency of HLA B17, B22 antigens among the patients with euthyreoid goiter disease. The character of the distribution of HLA antigens among the Georgian population with euthyreoid goiter disease differs from the features typical to other population that, evidently, can be explained by the peculiarities of the distributional character of HLA antigens among the healthy Georgian population.

Key words: HLA B17, HLA B22, euthyreoid goiter.

Introduction

The HLA System coding genes are localized at the shorter shoulder of the 6th autosomic chromosome of a human. It contains up to 4 millions nucleotides and is spread on two centimorgans, i.e. covers 1/1000 part of a human's genetic fund [Karseladze et al. 2003]. This genetic structure is a compact one that explains an uneven cohesion and a low frequency of a recombination – 0.8 – 1.0 of the genes of this system. Expression of two haploids in a generation, one from mother and the other one - from father, takes place with an equal degree. An uneven cohesion contradicts to an incidental sorting of genes in a generation, while in rare cases of Crossingover, a new haplotype is generated. At the same end of the 6th chromosome, there are placed the genes, which participate in coding the Chido and Rogers erythrocyte systems, ME-1, PGM3, Pg5, GLO, complement structural genes C2, C4a C4b and the Bf factor [Zaretskaya & Abramov, 1986; Sochnev et al., 1987]. Until 1980s, the associations had been revealed mainly with the I class antigens. Later on, when typing of the II class antigens has become widely possible, some stronger associations just with these antigens were reported [Mayr et al., 1981; Gyodi et el., 1982; Wee et al., 1992a; Wee et al., 1992b]. However a positive contact of HLA-B27 antigen with the ankylosing spondylitis is a classic example. Up to 800 patients with this disease in Europe, Asia, and America were examined. The results have shown that a frequency of HLA-B27 in these patients achieved to 81-97%, while in healthy individuals this figure varied within 4-12% [Zaretskaya et al., 1986].

By help of the HLA markers it is possible to define the risk groups of populations in relation with various diseases. In order to determine the relationship between the HLA system and the diseases, two methods are applied: the Population and the family studies: In case of the population study, together with determining the immune-genetic profile, a comparison of distribution of different frequencies in sick and healthy individuals is conducted, while in case of family studies, it is determined not an association, but a cohesion of a disease with the HLA Haploid type. In this case, a relation of a disease with the HLA system is expressed in a certain regress of the HLA Haploid Type in line with the diseases of a family members [Robinson et al., 1993].

The goal of our research is to reveal the immune-genetic markers for patients with the diffuse-endemic Goiter (of euthyreodical form) of the Georgian population.

Materials and Methods

For the purpose of determining the features of distribution of the HLA antigens in cases of the diffuse-endemic goiter (of euthyreodical form), the typing of HLA antigens has been conducted through the microlymphocytotoxic method introduced by Terasaki and the Mc Cleand (1964) [Gyodi et al., 1982], enabling us to define the I and II classes antigens of the HLA system. The genetic analysis implied a determination of a percentage frequency of distribution of the HLA antigens, genic frequency, standard deviations, hametic association and correlation rates, a comparative risk of all HLA markers, etiological and preventive fractions.

Results an Discussion

Distribution of the I and II classes HLA Antigens in Georgian Population in case of Diffuse-endemic Goiter (of euthyreodical form) was studied (Table 1). The data obtained as a result of this study were compared with the data of a control group. As a result of the comparison a high relative risk and the antigen frequency of HLA B17 (Fa% = 18.18, RR = 5.35. Control Group Fa% = 3.97), and HLA B22 (Fa% = 27.27, RR = 9.41, Control Group Fa%=3.97) in the individuals with Diffuse-endemic goiter disease, was revealed, that is statistically proved. A high relative risk and the antigen frequency was reported in case of HLA B73 (Fa% = 12.90 RR = 38.51, Control Group Fa% = 0.38) too, but, according to other statistical data (The P immune-genetic correction) this cannot be considered statistically proved.

As to the HLA II class DR locus antigens, the HLA DR3 antigen (Fa5=40.90, RR = 1.87, Control Group Fa%=26.94) was distinguished with a higher frequency, that, if taking into account the P immune-genetic correction, cannot be considered statistically proved.

Thus, HLA-B17, B22 are the immune-genetic markers for patients with the diffuse-endemic Goiter (of euthyreodical form) of the Georgian population.

Table 1. Distribution of the HLA Antigens in Georgian Population in case of Diffuse-endemic Goiter

Antigens	Fa% Healthy	Fa% Sick	RR Relative Risk	Pg Gene Frequency	+SD Standard Deviation
A1	10,19	18,18	1,95	0,0955	0,04
A2	58,03	31,82	0,33	0,1743	0,06
A3	23,14	27,27	1,26	0,1472	0,05
A9	26.25	40,90	1,94	0,2313	0,07
A10	15,37	27,27	2,09	0,1472	0,05
A11	8,64	13,64	1,67	0,0707	0,04
A19	5,87	13,64	2,53	0,0707	0,04
A28	8,81	-	0,22	-	-
Blank				0,0369	
B5	34,20	27,28	0,72	0,0473	0,05
B7	13,99	18,18	1,36	0,0955	0,04
B8	7,94	13,63	1,82	0,0707	0,04
B12	12,95	18,18	1,49	0,0955	0,04
B13	8,29	22,72	3,25	0,121	0,052
B14	4,84	9,09	1,96	0,0468	0,03
B15	9,33	18,18	2,16	0,0955	0,04
B16	5,33	-	0,37	-	-
B17	3,97	18,18	5,37	0,0955	0,04
B18	2,76	-	0,75	-	-
B21	4,15	9,09	2,31	0,0468	0,03
B22	3,97	27,27	9,41	0,1473	0,05
B27	3,80	9,09	2,53	0,0468	0,03
B35	26,25	-	0,06	-	-
B37	1,15	-	1,64	-	-
B40	3,28	-	0,63	-	-
B73	0,38	4,54	12,38	0,023	0,02
Blank				1,0017	
DR1	19,17	18,18	0,94	0,0955	0,04
DR2	30,57	40,90	1,57	0,2313	0,07
DR3	26,94	40,90	1,87	0,2313	0,07
DR4	24,35	27,27	1,16	0,1473	0,05
DR5	26,94	18,18	0,60	0,0955	0,04
DR7	20,21	13,63	0,62	0,0707	0,04
Blank				0,8311	

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HLA სისტემის ანტიგენების განაწილების შესწავლა დიფუზურ – ენდემური (ეუთირეოიდული ფორმის) ჩიყვის დროს ქართულ პოპულაციაში

ქარსელაძე ც., მეუნარგია ვ., ჩაგიაშვილი ც., შავლაყაძე ნ., ვარდოსანიძე თ.

გ. მუხაძის სახელობის პემატოლოგიისა და ტრანსფუზიოლოგიის სამეცნიერო კვლევითი ინსტიტუტი

(მიღებულია 06.02.2006)

რეზიუმე

შესწავლილ იქნა HLA სისტემის ანტიგენების განაწილება ქართულ პოპულაციაში დიფუზურ – ენდემური (ეუთირეოიდული ფორმის) ჩიყვით დაავადების დროს. ჯანმრთელებთან შედარებით მაღალი სიხშირით გვხვდება HLA-B17, B22 ანტიგენები, მიღებული მაჩვენებლები საშუალებას გვაძლევს ვივარაუდოთ, რომ ქართულ პოპულაციაში დიფუზურ–ენდემური (ეუთირეოიდული ფორმის) ჩიყვით დაავადების იმუნოგენეტიკური მარკერებია HLA-B17, B22.

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APPLICATION OF SUGARED ENZYMATIC HYDROLYZATES IN BAKING AND CONFECTIONARY INDUSTRY

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Abstract

The thermophilic strain of *Aspergillus versicolor* has been distinguished as an active producer of cellulase. The optimal conditions for its cultivation were determined and the commercial preparation of high-active cellulase has been obtained. The enzymatic hydrolysis of cellulose, containing residuals of flour was performed. Influence of obtained hydrolyzate on the quality of bakery was studied. It was established that application of sugared hydrolyzates significantly improved both, the appearance and organoleptic indices of integrated products.

Key words: cellulase, Aspergillus, dampness, acidity, shape stability, volatile acids, reducing sugars, organoleptic properties

Introduction

Filling up the food and energetic deficiency on the expense of nontraditional recycling raw materials is one of the urgent problems of the recent world. From this point of view the cellulose containing waste appears to be perspective. A number of industrially valuable products, like sugared hydrolyzates, may be obtained by means of its biotransformation [Klesov, 1985]. Application of these hydrolyzates in different branches of food industry and especially in bakery significantly increases quality and biological value of the integrated products [Bikovskaya, 2000; Petrash et al., 1989]. High content of reducing sugars and amino acids, together with other products of fermentation, create a favorable background for biochemical processes, improving the organoleptic indices of integrated products, especially taste and flavor [Kislukhina, 2000].

The biotransformation of cellulose containing raw material is realized by means of enzyme preparations, obtained from microorganisms. According to this fact, producing of high-active cellulase preparations is of great importance [Liungaki, 1985].

The purpose of our study was to select the strains of microscopic fungi, actively synthesizing cellulase, also, obtaining the sugared hydrolyzates from cellulose containing residuals of floury industry and their application as sweeteners in bread making and cake preparation.

Materials and Methods

The preparation of cellulase was used to receive the hydrolyzate. This preparation was obtained from thermophilic culture of microscopic fungi, taken from the collection of

microorganisms of S. Durmishidze Institute of Biochemistry and Biotechnology. In particular, the extremophilic culture of the microscopic fungi Aspergillus versicolor has been used. The submerged cultivation of the mentioned strain of fungi was done, for the purpose to receive a commercial preparation of cellulase. Composition of the nutrient medium was as follows: (%) - microcrystal cellulose – 1.0, extract of maize – 1.5, $NaNO_3$ – 0.3, $KHPO_4$ – 0.2, $MgSO_4 \cdot 7H_2O$– 0.05, KCl – 0.05, $FeSO_4 \cdot H_2O$ – 0.02. Cultivation took place for 96h, at 40°C. After cultivation was finished, the enzyme's sedimentation was performed by means of ethanol. Later the enzyme was lyophilized and its activity toward the filter paper was determined.

Wheat bran - the waste of floury industry, served as a substrate; its enzymatic hydrolysis was performed at 50°C, for 5h. The quality of the process was evaluated by the amount of reducing sugars, synthesized during the process. While determining the bread quality following indices were taken into account: dampness, acidity, porosity, shape stability, content of volatile acids, reducing sugars and bisulphite binding substances, also, the structural and mechanical properties of the crumb (flexible and plastic D-formula).

Dampness of dough, extreme tension of moving, swelling ability in a ready product, alkalinity, and content of: total sugars, reducing and aromatic substances, and organoleptic properties (surface, shape) were studied, while evaluating the quality of the cake.

Results and Discussion

For the purpose to select the active producers of cellulase, different genera of microscopic fungi were tested on the first step of investigation; in particular: Aspergillus, Chaetomium, Penicillium, Trichoderma, Fusarium, Cladosporium and Sporotrichum.

According to the experimental data it was concluded that the cellulase activity was characteristic only for four genera of the studied fungi: Aspergillus, Chaetomium, Penicillium and Trichoderma (Table 1). From the obtained results is clear that Aspergillus versicolor revealed the highest cellulase activity.

For receiving a commercially convenient preparation with high cellulase activity, the optimal conditions for cultivation and effective composition of the cultivating medium were established.

Table 1. Cellulase activity of microscopic fungi

Strain	Activity, U/ml(to a filter paper)	Cellulase activity, U/ml
Aspergillus terreus	0.35	0.3
Aspergillus versicolor	0.85	0.65
Aspergillus wentii	0.50	0.40
Chaetomium thermophile	0.55	0.40
Aspergillus flaus	0.25	0.15
Aspergillus fumigatus	0.26	0.15
Penicillium can.	0.38	0.25
Trichoderma lignorium	0.55	0.36
Sporotrichum puverulentum	0.25	0
Fusarium mon. f.	0.31	0.04
Sporotrichum thermophile	0.35	0.15

On the next step of investigation the commercial preparation of cellulase has been obtained, and the enzymatic hydrolysis of wheat flour residuals (bran) was performed. As a result, solution containing 55% of glucose was obtained. This hydrolyzate was used as a sweetener in

bread baking, and the influence of sugared hydrolyzate on dough properties and quality of integrated products in bread baking and cake preparing processes was studied.

To optimize the process of baking, influence of hydrolyzate amount on dough and integrated products quality was investigated. The sugared hydrolyzates were added to the dough during the preparing process, with amount of 1% to 15%, compared with the total quantity of flour. The results of these experiments are given in tables 2 and 3.

The results demonstrate that adding of hydrolyzate significantly improved all indices of bread and cake quality. The best results were obtained in the case of 12% (relatively to flour mass) hydrolyzate adding. Bread, containing this amount of hydrolyzate possessed the beast marketable appearance, with dark crust, flexible crumb, and retained freshness for a long period. Especially must be mentioned the taste and flavor of the bread. This may be explained by the fact, that the hydrolyzate increases the amount of volatile carbonyl substances.

Table 2. Influence of sugared hydrolyzate on dough properties and bread quality

Index	Control (bread without hydrolizate)	Indices of dough properties and bread quality obtained using the hydrolizate				
		Amount of hydrolyzate in % (compared with flour mass)				
		1	3	6	9	12
Dough Moisture, %	43.5	43.5	43.8	44.6	44.8	45.2
Acidity, %	2.6	2.6	2.6	2.8	3.4	3.6
Rising force, min	17	16	13	9	11	15
Density, g/cm^3	0.600	0.596	0.582	0.554	0.576	0.620
Duration of rising, min	180	180	120	90	90	90
Bread Moisture, %	42.2	42.2	43.0	43.8	44.0	44.2
Porosity, %	76	78	84	86	76	69
Shape stability (H:D)	0.46	0.48	0.52	0.54	0.45	0.39
Acidity, %	2.0	2.0	2.2	2.4	3.0	3.4
Volatile acids, % (compared with total acidity)	15.8	19.2	28.5	34.0	39.4	54.2
Reducing sugars, % (on dry weight)	0.68	1.19	2.67	3.48	3.80	4.02
The crumb quality	Normal		Elastic		Slightly raw	Raw

Table 3. Influence of sugared hydrolyzate on cake properties and integrated product quality

Index	Control	With hydrolyzate
Dough moisture, %	25.6	25.8
Temperature, °C	34-40	38-40
Duration of kneading, min	46	34
Extreme tension of moving, KPa	87	68
Cake moisture, %	6.7	6.5
Duration of baking, sec.	240	210
Swelling, %	129	152
Alkalinity, °H	0.55	0.40
Surface	Plane, bright brown	Polished, brown, plane
Shape	equal	equal
Total sugars, calculated by sucrose, %(on dry weight)	18.9	20.16
Reducing substances, calculated by inverted sugars, %(on dry weight)	1.64	5.80

Finally it may be concluded that in baking industry substitution of sugar with hydrolyzate of wheat bran improves the integrated products quality, and makes possible to abate sugar and flour costs.

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დაშაქრებული ფერმენტული ჰიდროლიზატების გამოყენება პურისა და საკონდიტრო მრეწველობაში

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

ს. დურმიშიძის სახ. ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

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რეზიუმე

შერჩეულია ცელულაზის აქტიური პროდუცენტი თერმოფილური კულტურა *Aspergillus versicolor*, დადგენილია კულტივირების ოპტიმალური პირობები და მიღებულია მაღალაქტიური ცელულაზის ტექნიკური პრეპარატი. ჩატარებულია ფქვილის ცელულოზაშემცველი ნარჩენის ფერმენტული ჰიდროლიზი. შესწავლილია ჰიდროლიზატის გავლენა პურ-ფუნთუშეულის ხარისხზე. დადგენილია, რომ დაშაქრებული ჰიდროლიზატების გამოყენება მნიშვნელოვნად აუმჯობესებს ნაწარმის როგორც გარეგნულ, ისე მის ორგანოლეპტიკურ მაჩვენებლებს.

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BRUCELLOSIS IN GEORGIA AND RELATED PROBLEMS

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Abstract

In 2003-2005, 32567 horned cattle were investigated on brucellosis in Mtskheta, Gardabani districts and near Tbilisi. Permanent persistence of brucellosis pathogen and percentage growth of disease in cows with calf in following years was established also.

Key words: *B.aburtus*, *B.melitensis*, Rose-Bengal Reaction, agglutination reaction.

Introduction

Animal brucellosis is widespread in many countries and among them in Georgia. Horned cattle brucellosis is more frequent, then comes goat and sheep brucellosis, and in hog this infection occur with restricted propagation [Sigafoose, 2006].

It is known that brucellosis is chronic disease of animal and human induced by Brucella group bacteria. The relation between animal and human brucellosis was discovered in 1920 by American bacteriologist Alice Evans. *B.aburtus* and *B.melitensis* play the decisive role in human infection. Human can be infected via direct contact with animal, respiratory and also by meet, milk and milk products [Callahan, 2006 a]. The probability of infection from human to human is very low, but some cases of infection at blood transfusion, spinal cord implantation and breast-feeding were recorded.

According to scientific literature data, the occurrence of human infection with brucellosis is increased in Latin America, East Europe, Near East, Asia, and some places of Africa [Payeur, 2006]. 78 incidents per 100 000 humans was recorded in the Near East [Callahan, 2006 b]. Especially great attention is paid to *B.aburtus*, and some countries could reduce this infection.

Due to hard social and economic conditions of Georgia, to carry out the systematic and radical measures against brucellosis, was impracticable, which causes wide propagation of this disease among animal. It should be mentioned that more than 1.2 millions of horned cattle are registered today in Georgia, among them 70% inhabit those areas where diseased animals are revealed [Lominaishvili, Tavmamaishvili, 1999].

It is worth mentioning that existence of brucellosis disease is established in animals of personal property of Tbilisi population. According to the data of 1999, in Isani region 3 infected animals were recorded, in Samgori region – 4, in Nadzaladevi – 7, in Gldani 5 [Lominaishvili, Tavmamaishvili, 1999].

Thus, the goal of our research is to reveal the distribution of brucellosis in horn cattle of Mtskheta and Gardabani districts and near Tbilisi for assessment of the actual state of affairs.

Materials and Methods

To establish brucellosis infection special antigens were fixed in blood serum. Accumulation of agglutinins in the organism is coincided with the exacerbation of infection, and hence, with the intensive release of microbes in environment. Blood samples were taken from axillary artery, kept for 25-30 min at the room temperature and then stored in fridge.

Hemolysis blood serum was not researched.

The following standard serological methods were used:

1. Rose-Bengal Reaction (RBR) - agglutination reaction with pink Bengal antigen, which is carried out in acid medium. Today this reaction is used in many countries (France, Germany, USS, Great Britain, etc.)

2. Agglutination (Raite) reaction (AR) – when agglutination reaction is researched in different dilutions of serum.

3. Complement long-term fixation reaction (CLFR). Unlike to complement fixation reaction (CFR) when the first phase of reaction is carried out in warm, at CLFR the first stage of reaction or binding-fixation of complement in the case of antigen-antibody binding phenomenon is realized in fridge at +4°C for 18-20 hours, and the second phase, adding of hemolytic system – at 37°C for 45 min.

Results and Discussion

The characteristic clinical feature of brucellosis is abortion, but until disease detection process of latent phase is possible, when no symptoms are revealed. Consequently, blood serum samples taken from the disease suspected animals, and with no clinical signs ones were used as researched material. The cows with calf were studied.

During 2003 in Mtskheta district 6096 blood serum samples were investigated, among them in 166 samples brucellosis was revealed. In 98 cases abortion at the third month of pregnancy was noted, in 10 cases - asomus, and in 8 cases – mortinatus. In 2004 6692 samples were studied, among them in 122 ones brucellosis was established, in 122 cases abortions up to 5th month of pregnancy was registered, in 4 cases – asomus, and in 9 cases – mortinatus. In 2005 6683 samples were studied, among them in 186 samples brucellosis was revealed, in 153 cases abortion was recorded, in 10 cases – asomus, and in 23 cases – mortinatus.

In 2003, while studying the blood serum samples taken in Gardabani district, from 1392 studied samples in 36 ones brucellosis was found, among them in 30 cases abortion was recorded, and only in 6 cases – mortinatus. In 2004 2020 samples were investigated, in 164 ones brucellosis was found, among them in 132 cases abortion was noted, and in the rest cases – mortinatus. In 2005 1558 samples were researched, in 98 ones brucellosis was found, among them in 91 cases abortion was registered, in 4 cases – asomus, and in the rest cases – mortinatus.

As for Tbilisi outskirts, in 2003 2838 samples were studied, among them 40 samples appear positive to brucellosis and in the rest cases the clinical course with abortions occur. In 2004 2657 samples were studied. 42 samples appear positive to brucellosis, among them in 36 cases abortions were noted, in 2 cases – asomus, and in the rest ones – mortinatus. In 2005 we studied 1841 samples, among them 50 samples appear positive, and the rest ones happen with abortions.

As is seen from above mentioned data, in more than 80% of researched material abortions happen. Brucellosis pathogens are released abundantly with fetus liquid during abortion. It is known that after abortion short sterile period is possible, which should be remained only in 5% of cases. In these animals further pregnancy occur in norm, and they present just a risk-group for spreading of brucellosis in environment.

Table 1. Results of serological investigations

District, years	Number of studied samples	Number of positive reactions	%
2003			
Mtskheta district	6096	116	1.9
Gardabani district	1382	36	2.6
Near Tbilisi	2838	40	1.4
2004			
Mtskheta district	6692	135	1.01
Gardabani district	2820	164	5.80
Near Tbilisi	2657	42	1.58
2005			
Mtskheta district	6683	186	2.73
Gardabani district	1558	99	6.35
Near Tbilisi	1841	50	2.71

The results of serological study show that brucellosis infection of horned cattle is progressive for the last 3 years. For example, in 2003 diseased animals amount in Mtskheta district was 1.9%, while in 2005 it equaled to 2,73%. In Gardabani district this index in 2003 was 2.6%, and in 2005 it composed 6.35%. As for animals of personal property of Tbilisi population increase of the index was the following: 2003 – 1.4%, 2005 – 2.71%.

If we take into consideration the fact that the number of studied animals is about 35% of the total number of animals inhabited in these areas, the percentage mentioned above will increase correspondingly.

Obtained data indicate to brucellosis spreading in studied regions. It should be mentioned that the disease is invaded in Tbilisi too, that makes the population to be upon the high risk of brucellosis infection. So, it is necessary to find out new resources for diagnostics and further preventive measures.

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ბრუცელოზი საქართველოში და მასთან დაკავშირებული პრობლემები

ღვინჯილია გ., ღვინჯილია მ.

საქართველოს ვეტერინარული დიაგნოსტიკისა და ექსპერტიზის ცენტრალური ლაბორატორია

(მიღებულია 27.02.2006)

რეზიუმე

ბრუცელოზზე 2003-2005 წლებში გამოკვლეულ იქნა 32567 სული მსხვილი რქოსანი პირუტყვი მცხეთის, გარდაბნის და ქ. თბილისის გარეუბნებში. დადგენილია ბრუცელოზის გამომწვევის მუდმივი პერსისტენტობა, ასევე დაავადების პროცენტული ზრდადობა მაკე ძროხებში წლების მიხედვით.

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INFLUENCE OF SOME PHYSICAL AND CHEMICAL FACTORS ON DEVELOPMENT OF MICROSCOPIC FUNGI DEGRADING 2,4,6-TRINITROTOLUENE

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Abstract

The extent of extremophilicy of strains of microscopic fungi: *Aspergillus niger* K1-2, *Mucor sp.* T8-2, *Fusarium sp.* K15 and *Trichoderma sp.* H6-1, able to degrade 2,4,6-trinitrotoluene (TNT) has been investigated. The strain *Mucor sp.* T8-2, which appeared to be extremophilic, is interesting object because of its alkaliphilicy and halophilicy too. The favourable conditions for submerged cultivation of the tested cultures were established, in particular, the optimal meanings of temperature and pH, when the degradation of TNT was maximal.

Key words: extremophils, biodegradation, submerged cultivation.

Introduction

Environmental pollution is one of the critical and global problems of the recent world. Intensive utilization of fossil fuel and huge production of chemical materials became the reason for many problems in the nature, because of limited merging of natural and synthesized molecules in biological cycles [Esteve et al., 2001].

Great attention has been paid to clearing of the places, polluted at the process of military activity with heavy metals and organic substances, which stay unchanged in the soil for many years and keeping toxicity because of their chemical inertness [Rugh et al., 1996]. 2,4,6-trinitrotoluene (TNT) must be especially mentioned among them. This substance is more stable than mono- and dinitrotoluenes, partially because of symmetric situation of nitro groups in the aromatic ring [Reiger et al., 1999]. Contacting with this chemical becomes reason for many professional diseases and intoxication.

Microorganisms have a significant role in detoxification of soils, polluted with TNT. Recently many countries try to reveal active strains of microorganisms, able to detoxify and mineralize stable chemicals. The cultures, destructing 2,4,6-trinitrotoluene and belonging mainly to bacteria and basidial or white-rot fungi, have been studied by many scientists [Hodgson et al.,2000; Bumpus & Taratko, 1994; Boopathy, 2000].

Detoxifying activity of microscopic fungi is less studied. According to the latest data, representatives of Zigo- and Deiteromycetes classes revealed the ability of 2,4,6-trinitrotoluene biotransformation [Weber et al., 2002; Bennet et al., 1995; Bayman & Radkar, 1997].

Materials and Methods

Four cultures of microscopic fungi, relatively active as TNT biodegrading, from the collection of S. Durmishidze Institute of Biochemistry and Biotechnology: *Aspergillus niger* K1-2, *Mucor sp.* T8-2, *Fusarium sp.* K15 and *Trichoderma sp.* H6-1, able to converse TNT served as an objects for testing.

Experimental cultures were grown on Chapek's modified, solid nutrient medium, with following composition (%): agar – 2.0, $NaNO_3$ – 0.91, KH_2PO_4 – 0.1, KCl – 0.05, $MgSO_4 \cdot 7H_2O$ – 0.05, $FeSO_4 \cdot 7H_2O$ - 0.0002. TNT added in different concentrations (100, 200 and 300mg·l^{-1}) was the only source of carbon.

For determining the amount of assimilated and degraded TNT, the submerged cultivation of the selected cultures of microscopic fungi has been done on liquid nutrient medium with following composition (%): glucose – 6.0, $NaNO_3$ – 0.91, KH_2PO_4 – 0.1, KCl – 0.05, $MgSO_4 \cdot 7H_2O$ – 0.5, $FeSO_4 \cdot 7H_2O$ - 0.0002 and malt – 0.3. The suspension of conidia of experimental cultures, grown at 30°C for 10 days, served as a sowing material.

The influence of temperature, pH and salt concentration on selected strains has been studied for establishing the extent of their extremophilicy. In particular, for the purpose to establish the range of the growth temperature, the experimental fungi were grown at 5° up to 50°C temperatures, with 5°C intervals.

The selected cultures of the microscopic fungi were grown at pH2.0 to pH9.0, with 0.5 intervals, for determining their alkali or acidophilicy.

Different concentrations of sodium chloride (1, 1.5, and 2M) was added to the nutrient medium to reveal the halophilic nature of cultures.

750ml conic flasks were used for submerged cultivation, for the purpose to establish the TNT degrading ability of cultures, grown at different temperature and pH. Incubation was done on thermostated shaker, with 200rot/min, at 30°C, for 72h. Later the cultivating medium was centrifuged (4000rot/min) for 10min. The amount of residual TNT was determined spectrophotometrically.

Results and Discussion

For establishing the extent of extremophilicy influence of temperature, pH and salt concentration on development of the tested cultures was investigated.

These observations made possible to establish the extent of extremophilicy of the investigated cultures of microscopic fungi. Exactly, the strain of microscopic fungi *Mucor sp.* T8-2 is thermotolerant. *Fusarium sp.* K15 is psychrotolerant, the strains *Aspergillus niger* K1-2 and *Trichoderma sp.* H6-1 are mesophylls. Particularly noteworthy is the strain *Mucor sp.* T8-2, because of its alkaliphilicy and halophilicy too.

The purpose of our investigations was also to determine the optimal conditions for submerged cultivation of the tested cultures, in particular - the optimal meanings of temperature and pH for maximal degrading of TNT.

During the submerged cultivation the temperature ranges of growth for each studied strain was taken into account. Temperature was changed from 20° to 50°C, with 5°C intervals in the nutrient medium, where the only source of carbon was TNT. Results for all tested cultures are given on figures 1-4.

As it clear, the maximal assimilation of TNT was reached at 40°-45°C for the strains *Aspergillus niger* K1-2 and *Mucor sp.* T 8-2, while for the strain *Fusarium sp.* K15 the optimal was 25°-30°C, and for *Trichoderma sp.* H6-1 – 30°C did.

The pH of the nutrient medium has an important role in submerged cultivation. It is impossible to maintain the constant level of pH of the medium during the experiment, because of metabolic activity of microorganisms.

To investigate the optimal pH of the medium for developing, the TNT-destructor strains preliminary studied for alkali- or acidophilicy, were grown on a liquid nutrient medium, where the pH was changed from 2.0 up to 9.0. Cultivation was performed at an optimal for each microscopic fungus, temperature. The results of the experiments are done in the Table 1.

Table 1. TNT degrading by selected microscopic fungi at different pH

Strains	Amount of residuary TNT at different pH in %		
	pH_2	pH-6	pH_9
Aspergillus niger K1-2	55	20	40
Mucor sp. T 8-2	35	12	0
Fusarium sp. K 15	78	15	75
Trichoderma sp. H 6-1	33	15	70

From the obtained data it is clear that the strains *Aspergillus niger* N 2-2, *Fusarium sp.* K 15 and *Trichoderma sp.* N 2-6 entirely metabolized TNT at pH 6.0, while *Mucor sp.* T 8-2 was the most effective when the reaction of the medium was alkali – pH 9.0.

Summarizing our experimental data we can say that the optimal conditions for cultivation of microscopic fungi, able to assimilate and destruct TNT have been elaborated.

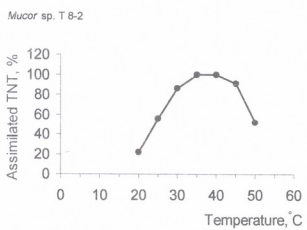


Fig.1. Degradation of TNT by *Mucor sp.* T 8-2 at different temperatures

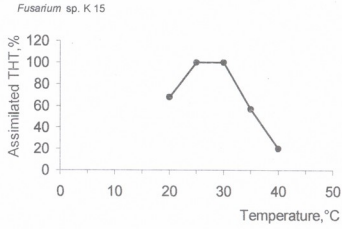
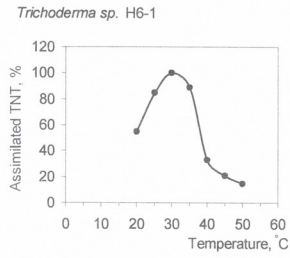


Fig.2. Degradation of TNT by *Fusarium sp.* K 15 at different temperatures



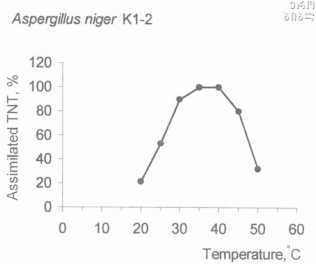


Fig. 3. Degradation of TNT by *Trichoderma sp.* H6-1 at different temperatures

Fig. 4. Degradation of TNT by *Aspergillus niger* K1-2 at different temperatures

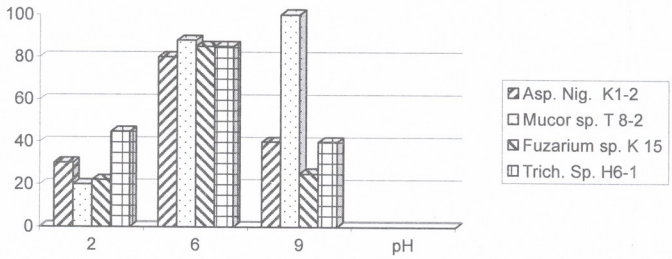


Fig. 5. Changing of TNT degrading abilities of *Aspergillus niger* K1-2, *Mucor sp.* T8-2, *Fusarium sp.* K15, and *Trichoderma sp.* H6-1 at different pH

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ზოგიერთი ფიზიკო-ქიმიური ფაქტორის გავლენა ტნტ-ს მაღებრადირებელი მაღალაქტიური მიკროსკოპული სოკოების ზრდა-განვითარებაზე

წულუკიძე ტ., ქუთათელაძე ლ., ხოხაშვილი ი., ჯობავა მ., კვესიტაძე ე.

ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 01.03.2006)

რეზიუმე

შევისწავლეთ ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტის მიკროსკოპული სოკოების კოლექციაში არსებული ტნტ-ს დეგრადაციის უნარის მქონე შტამების: *Aspergillus niger* K1-2, *Mucor sp.* T8-2, *Fusarium sp.* K 15, და *Trichoderma sp.* H6-1 ექსტრემოფილობის ხარისხი. შესწავლილ შტამებს შორის ექსტრემოფილი აღმოჩნდა შტამი *Mucor sp.* T8-2, რომელიც საინტერესოა იმითაც, რომ არის ალკალიფილი და ჰალოფილი. დადგენილია შერჩეული შტამების სიღრმული კულტივირების პირობები, კერძოდ, ოპტიმალური ტემპერატურა და pH, რომლის დროსაც აღნიშნული შტამები გარდაქმნიან ტნტ-ს მაქსიმალურ რაოდენობას.

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EVALUATION OF DNA EXTRACTION METHODS FOR PCR ANALYSIS OF FRUIT SEEDS

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Abstract

Genomic DNAs were isolated and purified from seeds of several fruit species, in particular: grape (*Vitis* spp), apple (*Malus* spp), and three *Citrus* spp., such as lemon, orange, and grapefruit using two DNA extraction protocols of commercially available kits: Qiagen DNeasy plant mini kit and Promega Wizard genomic DNA purification kit. The quality and quantity of the obtained DNA samples were evaluated by spectrophotometric technique and agarose gel electrophoresis after staining with ethidium bromide. The amplifiability of DNA templates was assessed by polymerase chain reaction (PCR) using 18S ribosomal RNA gene conserved primer sequences. The results obtained indicate that DNA samples extracted by Wizard method are not useful as templates for PCR because they are contaminated with polysaccharide and polyphenolic compounds. However all of the DNAs extracted by Qiagen kit were successfully used in the same PCR system. Therefore it is concluded that Qiagen method is suitable for preparation of amplifiable genomic DNA from studied fruit crops.

Key words: Genomic DNA extraction, PCR amplification, fruit crops, seeds.

Introduction

Polymerase chain reaction (PCR) is a DNA amplification technique recognized as a reference method for variety identification, genetically modified organisms (GMO) detection and seed genetic testing. The analytical procedure of PCR consists of several steps, in particular: sample preparation, genomic DNA extraction and purification, DNA quality and quantity assessment, PCR primer design, PCR conditions development and optimization, PCR product evaluation, and result interpretation. The efficiency of amplification reaction is largely defined by suitability of template DNA. The amplifiable quality of DNA is determined by quantity, purity and integrity of template and it is dependent on analyte matrix and DNA extraction method. Therefore, proper choice of suitable DNA isolation procedure for each analyte is most significant. To date numerous extraction methods and commercially available kits have been developed, several of them were successfully applied to plant [Lenstra, 2002] including fruit leaf material [Lodhi et al., 1994, Kim et al., 1997, Cheng et al., 2003] or cereal seeds [Kutateladze et al., 2005], however much less effort were spent so far on the genomic DNA extraction from fruit seeds.

The objective of this study was to obtain amplifiable genomic DNAs from seeds of several fruit species, in particular: grape (*Vitis* spp), apple (*Malus* spp), and three *Citrus* species: lemon, orange, and grapefruit. These fruits are subjects of extensive molecular studies, such as DNA

fingerprinting and genetic transformation [Cervera et al., 2005, Guo et al., 2005, Szankowski et al., 2003, Vigne et al., 2004] because they are cultivated worldwide and are in significant use in food production. Investigation of these fruits is most important in Georgia because all of them are cultivated in different parts of the country, in addition there are even local endemic cultivars of grape and apple. There is a lack of molecular genetic studies of Georgian cultivars. Investigation of these species by DNA technologies is essential for there certification and breeding application.

Materials and Methods

Sample preparation

The fruits of apple, orange, lemon, grapefruit and grape were purchased from a local market. The sample preparation procedure was optimized for each analyte especially. The seeds were cleaned and air-dried at room temperature (grape and apple) or at 30°C (lemon, orange and grapefruit) during 2-3 weeks. In addition, seeds of citrus were cleaned from a skin. The grape seeds were ground by mixer, however seeds of apple, lemon, orange and grapefruit were ground to a fine powder using mortar and pestle.

Genomic DNA extraction and analysis

The total DNAs were isolated and purified using two DNA extraction protocols of commercially available kits, in particular DNeasy Plant Mini Kit (Qiagen) and Wizard genomic DNA purification kit (Promega). The purity, degradation and quantity of the extracted DNA samples were evaluated by agarose gel electrophoresis in TBE buffer (50 mM Tris base, 50 mM Boric acid, 1mM Na_2EDTA) after ethidium bromide staining and visualization under ultra violet (UV) light. DNA concentration was estimated by comparison of band intensities of the analyte with known concentration of lambda DNA on the agarose gel. The quantity and purity of the DNAs were estimated also using spectrophotometric technique.

PCR analysis

The eukaryote-specific PCR system with primers on 18S ribosomal RNA gene conserved sequences developed in our previous study [Kutateladze et al., 2005] was used to assess amplifiability of DNA templates. Polymerase chain reactions were performed using GoTaq™ DNA polymerase with 5X green GoTaq™ Reaction Buffer (Promega). PCR contents and conditions were optimized to amplify PCR product of expectable size. DNA amplification was performed in 25 μl reaction mixture containing 1X Green Go Taq™ Reaction Buffer (pH 8.5) with 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each primer, 0.625 unit of GoTaq™ DNA polymerase, and 1-10 ng of template DNA. PCR was run on a thermocycler Techne TC-412 using the following cycle profile. Initial denaturation 1 cycle at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 2 min. The amplified fragments were electrophoresed in 1.5% agarose gels and the bands were visualized and photographed under UV after staining with ethidium bromide.

Results and Discussion

The seeds of several fruit species, in particular grape, apple, lemon, orange and grapefruit were analyzed to obtain amplifiable genomic DNA. These crops are perennial woody plants known with high level of polysaccharide and polyphenolic compounds, therefore many DNA extraction protocols were unable to isolate pure amplifiable DNA from these crops, however a few methods were successful for the leaves or wood tissue of grape and apple [Kim et al., 1997, Lodhi et al., 1994] and for citrus [Cheng et al., 2003]. In this study we have tested two DNA extraction

protocols of commercially available kits from Qiagen and Promega to isolate total DNA from seeds of investigated crops.

DNA solutions with unsolvable precipitations were obtained after the Wizard genomic DNA extraction procedure. Therefore, the centrifugation at 8000xg was used as the last step for removing of these sediments. The obtained DNAs were assessed by agarose gel electrophoresis.

No significant degradation is observed on the Fig.1B representing genomic DNAs isolated by Wizard method, however PCR analysis exhibited no amplifiable quality of these DNA samples. The results obtained indicate that DNAs extracted by Wizard method are not useful as templates for PCR.

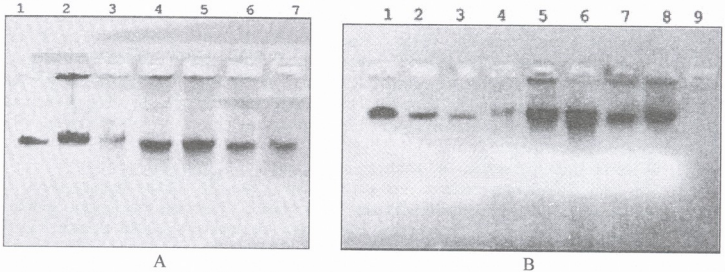

Fig.1. Agarose gels (1.5%) with genomic DNAs from different plant species extracted by **A**. Qiagen method from 100 mg grounded seeds. Lane 1. λ DNA (50 ng), lane 2. λ DNA (100 ng), lane 3. grape, lane 4. apple, lane 5. lemon, lane 6. orange, lane 7.grapefruit. **B**. Wizard method from 50 mg seed powder. Lane 1. λ DNA (50 ng), lane 2. λ DNA (20 ng), lane 3. λ DNA (10 ng), lane 4. grape, lane 5. apple, lane 6. lemon, lane 7. orange, lane 8. grapefruit, 9. negative water control.

PCR inhibition might be caused by either insufficient amount of template, or/and template degradation or/and contamination. However, a lack of template molecules or template degradation are excluded in this case based on the results of gel electrophoresis (Fig. 1B) and spectrophotometric analysis. DNA quantity and purity was estimated by spectrophotometer. The analysis of spectrophotometric measurements revealed that investigated fruit species gave DNAs with A260/A280 less than 0.9, indicating presence of protein contaminations. The A260/A230 ratio was between 0.2 and 0.6 corresponding to the high level of polysaccharides and polyphenols. Therefore, we suggested that contamination of template DNA samples with polysaccharide and polyphenolic compounds might be the reason of PCR inhibition. Our results corresponds to the previously published data on DNA extraction problems for fruit leaf materials [Kim et al., 1997, Cheng et al., 2003].

The clean DNA solutions were obtained using Qiagen DNeasy Plant Mini Kit. The agarose gel electrophoresis did not reveal any visible degradation (Fig.1A). Analysis of results from spectrophotometric and electrophoretic measuremets exhibits different amount of DNA yields ranging from 1.3 to 9.3 μg DNA/g seed powder. PCR testing identified that DNAs extracted by Qiagen method served as suitable templates for PCR amplification using above mentioned primers. As shown in Fig.2 amplification by PCR has generated PCR products of expected size. Therefore, we can conclude that Qiagen DNeasy plant mini kit might be used successfully for isolation of amplifiable genomic DNA from higher plant species.

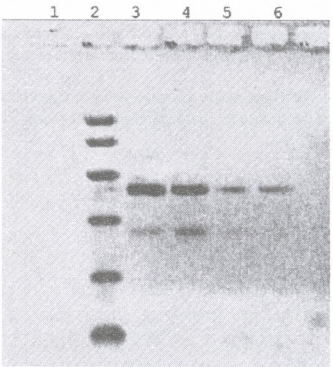

Fig. 2. Agarose gel (2%) with PCR products obtained after amplification of genomic DNAs from different fruit crops extracted by Qiagen protocol. Lane 1. negative water control, lane 2. Molecular weight PCR markers: 1 kb, 750 bp, 500 bp, 300 bp, 150 bp, 50 bp (Promega), lane 3. grape, lane 4. apple, lane 5. lemon, lane 6. orange.

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დნმ-ის ექსტრაქციის მეთოდების შეფასება ხილის თესლების პჯრ-ით ანალიზისთვის

დათუკიშვილი ნ., ქუთათელაძე თ., ქარსელაძე მ., ზაალიშვილი გ., გაბრიაძე ი., ზაალიშვილი თ.

მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 09.01.2006)

რეზიუმე

ხილის რამდენიმე სახეობის, კერძოდ, ყურძნის (*Vitis spp.*), ვაშლის (*Malus spp.*), ციტრუსების (*Citrus spp.*): ლიმონის, ფორთოხლის და გრეიფრუტის თესლებიდან გამოყოფილ და გაწმენდილ იქნა გენომური დნმ-ები ორი კომერციული კრებულის (Qiagen-ის მცენარის დნმ-ის გამოყოფის მინი კრებული და Promega Wizard-is გენომური დნმ-ის გაწმენდის კრებული) დნმ-ის ექსტრაქციის მეთოდებით. მიღებული დნმ-ის ნიმუშების თვისება და რაოდენობა შეფასებულ იქნა სპექტროფოტომეტრული ტექნიკითა და აგაროზის გელზე ელექტროფორეზით ეთიდიუმის ბრომიდით შეღებვის შემდეგ. დნმ-ის თარგების ამპლიფიკაციის უნარი შემოწმდა პოლიმერაზული ჯაჭვური რეაქციით (პჯრ) 18S რიბოსომული რნმ-ის გენის კონსერვატიული პრაიმერული თანმიმდევრობების გამოყენებით. მიღებული შედეგები მიუთითებს, რომ Wizard-ის მეთოდით ექსტრაგირებული დნმ-ის ნიმუშები არ გამოდგება თარგებად პჯრ-თვის რადგანაც ისინი დაბინძურებულია პოლისაქარიდული და პოლიფენოლური ნაერთებით მაშინ, როდესაც Qiagen-ის კრებულით ექსტრაგირებული ყველა დნმ წარმატებით გამოიყენება იგივე პჯრ-ის სისტემაში. ამგვარად, შეიძლება დავასკვნათ, რომ Qiagen-ის მეთოდი ვარგისია შესწავლილი ხილის კულტურებიდან ამპლიფიკაციის უნარის მქონე გენომური დნმ-ის მოსამზადებლად.

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NEMATODA *STEINERNEMA GURGISTANA* SP.N. (RHABDITIDA:STEINERNEMATIDAE) FROM *AGRIOTES GURGISTANA* F.

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Abstract

The paper deals with the description of nematode *Steinernema gurgistana* sp.n. (Sg. sp.n.) representative of genus steinernema revealed in worms of the pest (*Agriotes gurgistana* F.) near Tbilisi in 2004 is given. New species is more similar to *Steinernema disparica*. The infested larvae have four cuticular rings on the lateral side of the body, which is characteristic to *S.gurgistana* sp.n., relating it closely to *S.disparica*. Besides, *S.gurgistana sp.n.* differs from *S.disparica* by spiculla, gubernaculum shape and excretory pore location.

Key words: entomopathogenic nematodes (EPN), biological struggle, generation, control agent, saprophage

Introduction

The representatives of genus Steinernema have a great potential as pest control agents [Ke L.P., 2001; Stock.P.,2005]. The nematodes are spread widely in insect populations, more than 300 species of insect hosts are known [Klein; 1990]. The ability of EPN for searching the insect fast, making in it bacterial septicaemy, causing the host's death, stipulate the necessity of mass cultivation on the infectious basis of which biological preparations are developed.

S.georgica [Kakulia, Veremchuk, 1965], S.thesami [Gorgadze, 1988] and S.disparica [Gorgadze, 2001] species of local EPN studied different years in Georgia, are discussed as effective and safe facilities for pests' biocontrol.

Morphological and morphometrical analyses of the nematode revealed in pest *Agriotes gurgistanus* worms in 2004 showed, that *S.gurgistana* represents new entomopathogenic species for Georgia.

Materials and Methods

EPN were studied in the kitchen-garden and melonfield plots of forest border private sectors in Tskneti. Pests were collected on both, small plots of potato and haricot beans growings, and on vegetable culture (beets, carrots, etc.) root crops. The root crops of carrots and beets are damaged basically with (*A.gurgistanus*) worms. A new species of EPN from genus Steinernema is revealed in one of the pests' worm cadaver. The new species of nematodes were cultivated on *Galleria mellonella* worms according to the method and stored in fridge at +7°C [White, 1927].

Morphological and morphometrical measurements of the new species were made according to the international index formulae of nematodology [De Man, 1884; Micoletzky, 1922; Stanuszek, 1974].

Results and Discussion

Discription of the species *Steinernema gurgistana* sp. n. (Fig.1 and Fig.2. Table 1)

Holotype (Male, first generation); L=1641 mm; body width=104 mm; distance from anterior part to excretory pore =115 mm; oesophagus length =187 mm; tail length =50 mm; spicula length = 72 mm; gubernaculum length =46 mm; a=15,7; b=8,7; c=32,5; P%=62; E% =2,3; SW=1,3.

Paratype measurements are given in Table 1.

Table 1. Morphometrics (inum) of *Steinernema gurgistana* sp.n

Character	Male paratypes		Female paratypes		Infective juvenile (n=15)
	1st generation (n=7)	2nd generation (n=10)	1st generation (n=10)	2nd generation (n=10)	
Body length	1695(1494-1828)	1200(810-1591)	3612(2876-4348)	2007(1674-2340)	864(684-900)
Body width	121(100-144)	108(82-133)	267(241-306)	172(165-180)	38(36-43)
Stoma lengt	16(14-18)	7	20(18-21)	10	-
Stoma width	8(7-10)	5(4-6)	17(14-18)	7	-
EP	115(108-126)	99(82-115)	135(122-144)	93(90-100)	71(66-75)
NR	127(126-129)	126(115-136)	166(162-180)	134(126-144)	102(97-108)
Total oesophagus	180(176-187)	165(147-183)	223(212-241)	194(180-216)	126(115-144)
Anal body width (ABW)	50(48-46)	42(38-43)	-	-	-
Tail length	49(46-54)	57(54-61)	58(54-62)	99(93-104)	86(75-90)
Spicule lengt (SP)	72(68-75)	55(50-61)	-	-	-
Gubernaculum length	45(43-50)	32(28-36)	-	-	-
Gubernaculum width	13(10-14)	8(6-10)	-	-	-
Vulva (%)	-	-	55,3(53,3-59)	50(48,3-52)	-
a	14,1(11,8(17,8)	10,8(7,5-13,1)	14,1(11-14,4)	13(10,4-17,8)	21,0(18-25)
b	9,3(8,3-10,4)	6,5(4,5-9)	16(12,8-18)	11,8(8-16,4)	6,5(5,6-7,6)
c	34,3(32-39)	19,2(14-26)	62,1(47-75,1)	23,2(18,4-32)	9,8(8-12)
D%=EP:ES	63(61-67)	60(55-62)	60(57-59)	47(47-46)	0,56(0,57-0,52)
E%=EP:tail	2,3(2,3-2,5)	1,7(1,5-1,8)	2,3(2,2-2,3)	0,9	0,82(0,88-0,83)
SW=SP:ABW	1,4(1,5-1,6)	1,2(1,1-1,3)	-	-	-
GS=GU:SP	0,62(0,63-0,66)	0,5(0,5-0,6)	-	-	-
Hyaline lengt	-	-	-	-	33(31-35)
H=H:tail	-	-	-	-	0,3(0,3-0,4)

Male (Adult, first saprophage generation). Body nearly of equal width (Fig.1A) curved towards ventral side. At 36°C nematodes die, at that time the tail tapers to ventral side hardly. Body - smooth surfaced. Round head is stick out bluntly from the body, on the posterior part of which 6 labial and 4 cephalic papillae are located (Fig.1B). Amphids - indistinct; mouth opening - funnel-

shaped usually; 3 micro onchs are noticed on the metastome; oesophagus muscular; procorpus cylindrical; metacorpus convex and is not valved; isthmus distinct; cardial bulbus is great and pyriform without bar. Nerve ring surrounds isthmus above basal bulb. Excretory pore located on the border of metacorpus. Excretory pore is hardly noticed in cadavers. Excretory duct less cuticulized, than excretory pore. A single ventral-precloacal and 12 pair genital papillae present in genital part. 7 pairs are located preanalsubventrall on two rows, and one pair of lateral amphids are on the level of second preanal papilla. Spicula is pair and curved (Fig.1C) spicula membrane is hardly noticeable. Tail compressed and ends with blind edge. Tail length is 2 mm longer than of spiculla length. Bluntly rounded tail terminate with mucron.

Female (Adult first saprophage generation). Female is twice bigger then male. Body thick. Cadaver curved, c - shaped (Fig.2A). Frontal end rounded. 6 labial and 4 cephalic papillae are located in the epical part of the head. The nematode stoma shape changes from cup-shape to funnel-shape. Cuticle smooth-surfaced. Excretory pore is located in the middle of oesophagus, anterior to nerve ring. Oesophagus muscular, procorpus - cylindar-shaped. Metacorpus is not swollen. Distinct isthmus, but cardial bulbus is big and rounded. Its bar is indistinct. Nerve ring surrounds isthmus, located dorsal to bulb (Fig. 2B). Vulva located almost in the mid-body with perpendicular vagina. Vulva lips swollen from the body (Fig. 2D). Vagina short, with straight muscular walls. Tail conoid (Fig.2C). It is longer than the width of the body at anal hole. Most of the ovums round, oval forms are also met.

Infective juvenile. Larvae are characterized with rhabditidoid motion. Frontal body is more active than back one. Larva is characterized with both ambush and vagrant motion behaviour. At 36°C nematodes die in 20 minutes, and its body curves ventrally (Fig.2E). Head is not always round. 4 prominent cephalic papillae are located in the apical part of the head. Pore amphids are anterior to cephalic papillae. Anus closed. Oesophagus tapering with thin isthmus, which is surrounded with nerve ring. Cardial bulb - wide and elongated. Excretory pore located anterior to mid-oesophagus. Posterior lateral field with 4 cuticular rings. Tail elongated conoid and blunt. Hyaline part is bright. Tail length occupies about 36% of the body. Phasmids noticeable in anterior part of the tail.

3% of collected *Agriotes gurgistanus F.* were infected by new nematode species. The number of nematodes varied from 650 to 1000 species in one host.

New species is pathogenic and causes infection and mortality of such pests as: Galleria mellonella, Tenebrio molitor, Pieriss brassicae, Leptiotarsa deremlineata, Operophtore brumata, Agriotes gurgistanus, etc.

Age differentiation of new species during development in T.molitor and G.mellonella worms in parasitic and saprophage generations was studied.

Differential diagnosis

It is established, that the described new species *S.gurgistanus sp.n.* is most similar to *S.disparica* [Gorgadze, 2001] revealed in Georgia than to other nematodes. The infective juveniles of this nematode have 4 cuticular rings on lateral part of the body. By those characteristic data the new species is related closely to *steinernema disparica*, but by some morphological and anatomic features is differed.

1. *S.gurgistana* sp.n. infective juvenile length, width and distance of excretory pore from the apical end of the head, the length of oesophagus and tail is greater (accordingly 864,38,73,126,86) than of *S.disparica* infective juvenile (accordingly 789, 33,69,120,82).

2. *S.gurgistana* sp.n. spiculla is 2 mm shorter than that of *S.disparica.*

3. There is difference in spiculla head shape, the head of the new species is rounded, but that of *S.disparica* is squared.

4. Tail of the new species male is 6 mm longer, with 2 mm longer mucron at the end than that of *S.disparica.*

5. The length of the new species oesophagus is 18 mm shorter than that of *S.disparica*, but excretory pore is 21 mm far from the anterior head.

6. *S.disparica* has 6 mm shorter tail than the new species.

7. The new species female vulva is located almost in mid-body, but vulva of *S.disparica* 54% is on tail part.

8. Compared nematodes differ by De Man (1884) index:

Female *S.gurgistana* sp.n.	Female *S.disparica*
a = 13(10,4-17,8)	a = 15,4(13,2-17)
b = 11,8(8-16,4)	b = 10,4(8,4-12,2)
c = 23,2(18,4-32)	c = 29,7(25,8-33,7)
V% = 50(48,3-52)	V% = 55,4(53-59,4)

Male of the new nematode has no sexual relations with S.carpocapsae "agriotos" and vice versa.

The anatomic-morphological data of the described nematode indicate that this nematode is related to *Steinernema gurgistana* sp.n new species for Georgia. It is named by pest in which it was revealed.

The described species both in vivo and in vitro cultures are deposited in the Entomonemathodology Laboratory of the Institute of Zoology.

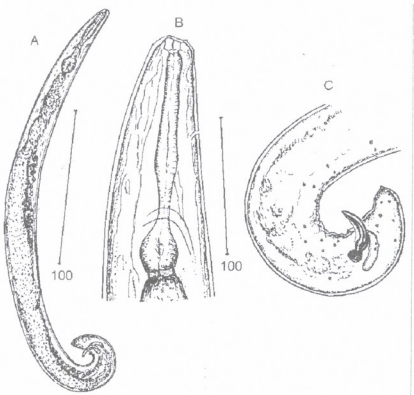

Fig.1. *Steinernema gurgistana* sp.n. Male
A: Entire body; B: Anterior region; C: Posterior region

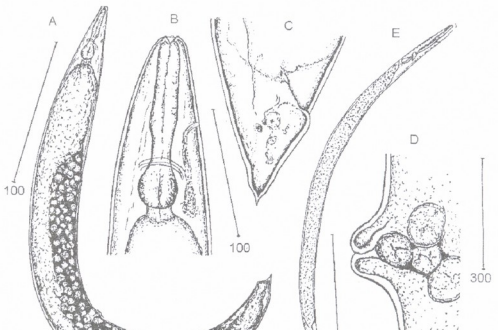

Fig.2. *Steinernema gurgistana* sp.n. Female
A - Entire body; B - Anterior region; C - Posterior region;
D - Vulva region; E - Infective juvenile

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ნემატოდა *Steinernema gurgistanus* sp.n. (Rhabditida: Steinernematidae) ქართული ტკაცუნადან (*Agriotos gurgistanus* F.)

გორგაძე ო., ლორთქიფანიძე მ.

ზოოლოგიის ინსტიტუტი

(მიღებულია, 07. 03. 2006)

რეზიუმე

მოცემულია გვარი Steinernema-ს წარმოდგენილი ნემატოდის *Steinernema gurgistana* sp.n. (S.g.sp.n.) აღწერა, რომელიც გამოვლენილია მავნე მწერის ქართული ტკაცუნას (*Agriotes gurgistana* F.) მატლში 2004 წელს თბილისთან ახლოს, დაბა წყნეთში. აღწერილ ახალ სახეობას ყველაზე მეტი მსგავსება აქვს საქართველოში გამოვლენილ *Steinernema disparica*-სთან. ინვაზიურ ლარვებს სხეულის ლატერალურ მხარეზე გააჩნიათ 4 კუტიკულარული ნაწიბური. ეს დამახასიათებელი თვისებაა *S.gurgistana* sp.n.-თვის, რომელიც აახლოებს მას *S.disparica*-სთან. გარდა ამისა *S.gurgistana* sp.n განსხვავდება *S.disparica*-სგან სპიკულის და გუბერნაკულუმის ფორმით და ექსკრეტორული არხის მდებარეობით.

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Phytopathology

EFFECT OF THE *FUSARIUM OXYSPORUM* F.SP.LYCOPERSICI ON PATHOANATOMICAL CHANGES OF THE PLANTLET STEMS OF VARIOUS CULTIVARS OF TOMATO

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Abstract

Pathological changes of plantlet stem anatomical structure of the resistant ("Eureka") and susceptible ("Utro") cultivars of tomato infested with fungus *Furasium oxiporum* were studied. Pathological changes in anatomical structure of the plantlet stem of resistant cultivar were established. In particular, active development of plasmodesms, thickening and lignification of the cell membrane round infection, development of additional bundles in phloem cells, accumulation of volatile oils and starch in the peripheral cells of pith occur. Such changes could be explained by the plant response defense reactions against infection which are aimed at it isolation and determine plant resistance to pathogen.

Key words: resistant cultivar, susceptible cultivar, pathogen, plasmodesm

Introduction

The principal factor of plant resistance is its anatomical structure [Goiman, 1954]. Structure of cover tissues and stomata, size and number of fibres and fringes determine invasion of parasite in a plant. It is known that infection of tomato fruits by macrosporiosis (*Macrosporium solani*) and fungus *Botrytis cinerea* depends on the thickness of cuticle. While ripening the thickness of cuticle increases and resistance of tomato fruit against infection increases accordingly.

Data about propagation of *Fusarium oxysporum* f.sp.radicis-lycopersici, its biology and histological changes in host plant caused by this fungus, occur in scientific literature [Benhamou et al., 1989; Adejuwon & Olutiola et al., 2005; Hassni et al., 2005].

Epidemic of fungus *Fusarium oxysporum* f.sp.radicis-lycopersici in potato plant depends on its competitiveness against soil mycobiota and for its biological control the usage of allelopathic control is recommended [Jarvis 1989a; 1989b].

Fungus *F. oxysporum* f.sp.lycopersici was revealed on the plants of 12 various genera. Effect of micro- and macroelements and fungicides on the physiology of the fungus was studied [Engelhard et al., 1989].

The goal of our work was to establish pathological changes of anatomical structure of plant stem of resistant and susceptible tomato cultivars infested with the fungus *F. oxysporum* f.sp.lycopersici.

Materials and Methods

Anatomical structure of tomato stem tissues of resistant "Eureka" and susceptible "Utro" cultivars infested artificially with fungus *F. oxysporum* f.sp.lycopersici were compared with intact plant stem tissues, after 5, 10, 20, 30 days of invasion by means of microscopic studies.

Invasion was carried out in similar ecological conditions, in laboratory. Experiments were realized in the following ways:

1. Plantlets of both cultivars in the phase of 3-4 leaves were infested via bringing of mycelium of fungus *F. oxysporum* f.sp.lycopersici in the wounds near root collar.

2. Intact plantlets of both cultivars were sown in the soil infested with fungus *F. oxysporum* f.sp.lycopersici previously.

Results and Discussion

As a result of our experiments pathological changes in the tissues of non-infested tomato stem, with control wounds (at 5 cm above wound) of both, "Eureka" and "Utro" cultivars, are not observed and their anatomical structure does not differ from the structure of intact plant stem, even 20 days after wounding. Whereas the fungus mycelium hyphae in stem cells and intercellular structures, and deplasmolysis - in epidermis and pericycle cells were noted 5 days after infection of susceptible cultivar "Utro" (Fig.1).

10 days after infection, when characteristic features of disease are visible on plant, fungus hyphae occur in fibrovascular bundle cells. Development of gumma-like substance, sharp darkening of tissues is observed in vessels. 20 days after darkening of rind tissue, maceration of pith and timber tissues is already observed (Fig. 2). After 30 days fibrovascular bundles are corked up, darkened, pith and timber tissues are destructed, and the plant die.

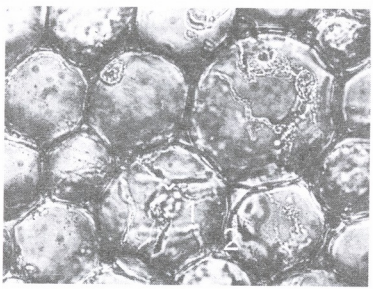

Fig. 1. Fungus Hyphae in the stem cells (1) and intercellular structures (2) of tomato susceptible cultivar "Utro", 5 days after infection.

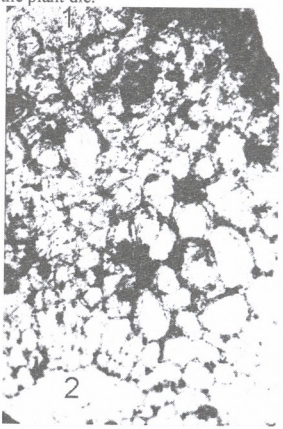

Fig. 2. Maceration of timber (1) and pith (2) tissues of tomato susceptible cultivar "Utro", 20 days after infection.

After 5 days pathological changes in infested plant stem wounds of resistant cultivar "Eureka" were not noted. Slight changes of coloration of tissues should be caused by activation of metabolism under the effect of pathogen. 10 days after infection plasmodesms are observed in stem endoderm and pericycle cells (Fig. 3); 20 days after thickening and lignification of membrane in pericycle cells are noted. Inside the pericycle, in phloem tissues additional bundles were developed (Fig. 4). Starch is accumulated in peripheral cells of pith. After 30 days additional bundles, which consist only phloem elements, were differentiated in pith. Cells have thick membrane, starch and volatile oils happen in pith and rind cells.

According to analysis of obtained results it was also found that pathological changes of stem tissues induced by fungus *Fusarium oxysporum* f.sp.lycopersici, regardless of the way of fungus invasion in the plant, are similar, that should be explained by the fact that pathological changes in plant tissues are mainly caused by fungus invasion and spreading.

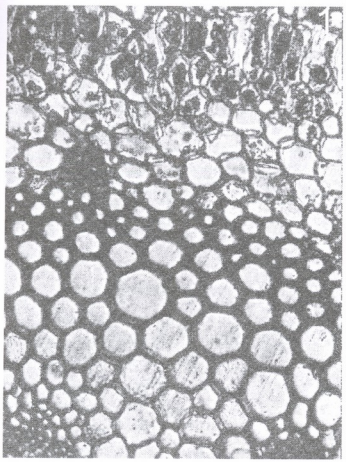

Fig. 3. Development of plasmodesms (1) in stem pericycle cells of tomato resistant cultivar "Eureka", 10 days after infection.

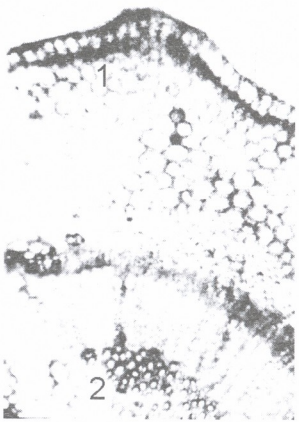

Fig. 4. Development of starch and volatile oils in stem pith (1) and rind (2) cells of tomato resistant cultivar "Eureka", 20 days after infection.

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Jarvis W.R. *Allelopatic control of Fusarium oxysporum f. sp. radicis-lycopersici.* Nato ASI Series, H **28,** Vascular Wilt Diseases of Plants Ed. E.C. Tyamos & C. Beckman, Sprisger-Verlag, Berlin Heidenbergs, 397-411, 1989b.

სოკო ***Fusarium oxysporum*** **F. sp lycopersici-**ით გამოწვეული პათოანატომიური ცვლილებები პამიდვრის სხვადასხვა ჯიშის ჩითილების ღეროში

კუპრაშვილი თ.[1], ზარქუა დ.[2], რაზმაძე ლ.[2], ღაღანიძე დ.[1]

[1]ლ. ყანჩაველის სახელობის მცენარეთა დაცვის ინსტიტუტი.
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(მიღებულია 12.12.2005)

რეზიუმე

დადგენილია სოკო *Fusarium oxysporum* f.sp lycopersici-ით დასენიანებულ პამიდვრის გამძლე ჯიშის („ევრიკა") ჩითილების ღეროს ანატომიურ აგებულებაში მიმდინარე პათოლოგიური ცვლილებები: პლაზმოდესმების აქტიური წარმოქმნა, ინფექციის ირგვლივ უჯრედების გარსის გასქელება, გახევება, ლაფნის უჯრედებში დამატებითი კონების წარმოქმნა, გულგულის პერიფერიულ უჯრედებში სახამებლისა და ეთეროვანი ზეთების დაგროვება, რაც უნდა აიხსნას მცენარის საპასუხო თავდაცვითი რეაქციებით ინფექციის შეჭრაზე, რომელიც მიმართულია მისი იზოლირებისაკენ და განსაზღვრავს მცენარის გამძლეობას პათოგენის მიმართ.

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SURVEY OF MAIZE DISEASES IN GEORGIA

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Abstract

As a result of phytosanitary monitoring of maize fields, conducted in different geographic zones of Georgia, the following diseases were recorded: Northern and Southern corn leaf blights, Northern corn leaf spot, Anthracnose leaf blight, Bacterial leaf spot, Brown spot (Physoderma), Common smut, Common leaf rust and Fusarium kernel. All the mentioned diseases except bacterial leaf spot were indicated in Western Georgia. Northern leaf blight was dominant disease. But in Eastern Georgia only bacterial leaf spot and common smut were occurred widely. Causal agents were isolated into pure culture from the disease samples collected during the maize observation, and kept in collection of phytopathogens of Plant Immunity Institute.

Key words: maize, diseases, phytosanitary monitoring.

Introduction

Total value of maize yield losses in world under the influence of biotic factors according to FAO is 36%. As American Phytopathological Society informs, over 70 maize diseases caused by fungi, bacteria and virus have been already recorded [APS, 2004].

Among cereals, maize by its importance is on the second place in Georgia after wheat, and according to the occupied area (215 000-220 000 ha) - on the first place. Its products are used in food production and in livestock breeding, as well. However, because of uncontrolled business, barter changes and import of plant products between the former republics of USSR, these is a significant threat for the worsening of phytosanitary situation of agriculture of Georgia. Besides, nonobservance of crop rotation, particularly of the monoculture, makes favorable conditions for development of pests and diseases. During the last several years, regular observations of maize fields, caused by some objective reasons, were not realized in Georgia. But, taking into account all the above mentioned, the annual phytosanitary monitoring can be treated as the most important national problem. As a contemporary monitoring provides with wide information characterizing qualitative, and also quantitative potential of pathogen populations, particularly the infected areas, intensity of development of diseases, species and intraspecies structure of pathogenic complexes, sort resistance, etc.

Materials and Methods

All the observed diseases of maize were to be registrant and scored. The disease scoring is made during the maize field observations according to beforehand planned route, at one and the

same areas of 2-3 most typical farms of the region. The main scoring unit is a sample. Quantity of samples is fixed depending on the size of the observed field. The samples are distributed on plot diagonal, at the same distances from each other. The scoring comprises: evaluation of plant conditions, collecting the samples and their studying. The isolation of pathogens into pure culture was conducted according to generally accepted methodology [Grigoryev, 1986].

Results and Discussion

Among the cereals sorts, maize occupies the most part of sown area (200-220 000 ha), but its productivity is very low 2.0-2.2 tone per 1 ha [Jinjikhadse et al., 2004]. One of the reasons of low yield of this crop is its low resistance to diseases, as well as common practiced for the last 15 years in Georgia monoculture, contributing to low productivity. It should be also mentioned the fact that the pesticides are not used on plants sowings that causes accumulation of the infections in the soil, which in its turn weakens the level of extreme environmental influence on the photogene vitality. No monitoring of maize diseases took place during the last 10 years in Georgia caused by some objective reasons. Observations of maize fields in different agro-climate zones of Georgia realized in 2004-2005 during the all vegetation periods. As a result of phytosanitary monitoring of maize sowing, the following diseases were recorded: Southern and Northern corn leaf blights, Northern leaf spot, Antracnoze leaf spot, Physoderma, Common smut, Common leaf rust and Fusarium kernel (Fig.1).

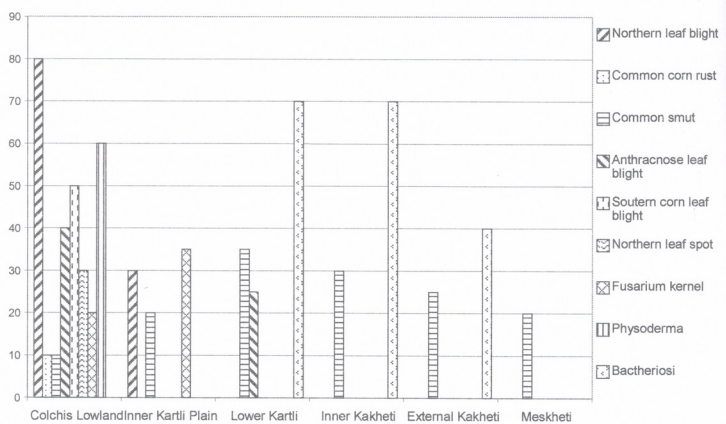

Fig. 1. Maize diseases distribution in different agro climate zones of Georgia (2004-2005).

Northern corn leaf blight caused by fungus *Setosphaeria turcica* (Luttrell) K.I. Leonard E.G. Suggs (anamorph: *Exserohilum turcicum* (Pass.) K.I. Leonard E.G. Suggs= *Helmintosporium turcicum* (Pass.) occupies the definite ecological niche in the local biocenose. This disease was observed in Georgia in 1914 in Colkhis Lowland by Voronikhin. When the main part of the leaf is

damaged, the development of starch is detained. Thus, the leaves can not by used for animals feeding. Depending on intensity level, the loses of productivity can reach 50%. Moderate temperature and high air humidity is favorable for fungi development. The infection is distributed during the vegetation period by conidiums. Fungus mycelium spends the whole winter in the infected debris, where conidia develop during the next vegetation under the favorable conditions.

The evaluation of 65 lines, hybrids and synthetics of maize for resistance to the Northern corn leaf blight conducted against a provocation background of the Plant Immunity Institute trial plot, showed that all the tested samples were susceptible.

Northern corn leaf blight was observed nearly in every region of the Western Georgia (40-90%) and in Khashuri region - 30% (in the Eastern Georgia) on both, the white and yellow maize. The severity of disease in 2004 was 80-100%, but in 2005 it was only 20-45%, as weather conditions in 2005 (high temperature and very low moderate air humidity) were not favorable for disease development.

Southern corn leaf blight (*Cochliobolus heterostrophus (Drechs.)* Drechs anamorph: *Bipolaris maydis* (Nisikado & Miyake) Shoemaker = *Helminthosporium maydis* (Nisikado & Miyake)) - was indicated in Kobuleti, Ozurgeti, Abasha and Samtredia regions; disease is not widely distributed in the Western Georgia, and severity does not exceed 0.5 (<10%). It is necessary to notice, that 0-race which has been found, has no economical importance different from T-race, which is a quarantine object. The characteristics of 0-race are spindle-shaped greenish–fired spots (0.2-2.0 cm long; 0.8 cm wide) with narrow dark border, bloom is very weak.

Common smut (*Ustilago zeae* (Beckm.) Unger = *U. maydis* (DC.) Corda) is fixed every year and absolutely in all regions of Western and Eastern Georgia, but its distribution was about 10%. The disease infects stems, cobs and leaves. The harmfulness of common smut depends on infected organ: it is high when cobs and stems above cobs are infected; weak - when stems lower than cobs and leaves are diseased. Leaves infection was registered in Kobuleti, Zestaphoni, and Telavi regions, but damaged cingulum – in Keda district. This disease has no economical importance for Georgia.

Fusarium kernel is widely distributed disease, especially in the regions of Western Georgia (Kolkhis Lowland and Southern-Eastern part of the Black See coast), characterized with high humidity. Abundant rain falls during the mature period and harvesting increase the number of infected cobs. Mainly, Fusarium appears on the upper part of maize cobs. The disease continues its development when cobs are kept under the conditions of bad airing [Ivashenko et al., 2000]. Mechanic injures and injures caused by pests make favorable conditions for development of Fusarium of cobs. Infection can be kept in the seeds during 2-3 years [Kirimelashvili, 1975; 1978] causing different pathologies of growth. Shortage of biomass of vegetative and reproductive organs is one of the characteristic features of Fusarium fungi damage. One of the main features of harmfulness of these fungi is that they may produce mycotoxins which are cancerogenic for human and animals [WHO, 1993; Rheedar et al., 2002].

According to mycological analyses of diseased cobs the following pathogens were determined: *Fusarium avenaceum* (Fr.: Fr.) Sacc; *Fusarium moniliforme* J. Sheld; *Fusarium culmorum* (Wm. G. Sm.) Sacc., *Alternaria tenuis* Nees, *Cladosporium herbarum* (Pers.) Link and *Penicillium sp. Fusarium moniliforme* – is dominant.

Northern helmintosporium blotch (*Cochliobolus carbonum* (anamorph: *Bipolaris carbonum* Ulls) was indicated on maize sowings in Keda, Khobi, Ozurgeti regions. But the severity of disease was very low (< 10%). This disease is also indicated in maize sowings, but is not widely distributed (30%). Has no trade importance.

Physoderma or Brown spot, caused by the fungus of *Physoderma maydis* Miyabe, infect leaf sheaths and the leaves. Diseased tissues are covered with small round spots of dark-brown and red-brown color. The size of dots is 1-5 mm in diameter. The disease begins from sheaths of leaves,

where water gathered after the rain, enough for infection. Humid and warm climate of Western Georgia is favorable for the disease development. Physoderma has been recorded in: Ozurgeti (50%), Senaki (70%), Abasha (40%) and Lantchkhuti (40%). This disease has no importance in economic trade.

Anthracnose leaf blotch caused by fungus *Colletotrichum graminicola* (Ces.) G. W. Wils. is considered as minor disease. It is fixed in fields in the end of June, mainly on 2 lower leaves, when the plants are at the stage of the 4-7 leaves. Though the spots incidence was on 70% of plants, the severity doesn't exceed 10%. The disease was found in Kobuleti (70%), Khelvachauri (50%) and Keda regions (70%) every year.

Common rust caused by the fungus of *Puccinia sorgi* Schwein, was indicated in Keda, Chokhatauri, Ozurgeti, Lantchkhuti and Khobi regions of Western Georgia. In the sowings they are found mainly by locuses, but pustules are usually placed only on 2-3 lower leaves and the severity was between 10-15%. According to our observations, rust can be put into the maize minor disease groups, as it appears very late, when the crops has already been formed.

Bacterial blotch of leaves and leave sheaths, is also called as red bacterios, mainly infects leaves sheaths and then spreads to the leaves themselves. At first, the disease is noticed on the inner side of sheath as a spot of irregular shape and outside they are nearly invisible. But after they coalesce, they become visible as spots with indistinct merges of red-brownish color.

Bacterios has been found only in Eastern Georgia in maize fields in Lagodekhi, Kareli, Signakhi, Dedoplistskaro, and Tetritskaro regions. Though it is widely distributed disease, its very weak intensity has been fixed in Georgia (spots on 2-3 lower sheaths), having no economic importance.

Thus, phytosanitary monitoring of maize, which took place in 2004-2005 in Georgia, enables us to conclude:

- Common smut is found absolutely in all regions where maize is grown, but it has no importance of economic trade.
- All the above described diseases are found in all regions of Western Georgia, except Bacterial blotch. In revealed pathogenic complex, the Northern corn leaf blight is dominant.
- In the Eastern parts of Georgia only Common smut and Bacterial blotch have been fixed.

Acknowledgment: This study was supported by ISTC grant G-1093

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საქართველოში გავრცელებული სიმინდის დაავადებათა მიმოხილვა

მეფარიშვილი გ., მეფარიშვილი ს.

ქობულეთის მცენარეთა იმუნიტეტის ინსტიტუტი

(მიღებულია 20.02.2006)

რეზიუმე

საქართველოს სხვადასხვა გეოგრაფიულ ზონაში არსებული სიმინდის ნათესების ფიტოსანიტარული მდგომარეობის მონიტორინგის შედეგად გამოვლენილი იქნა შემდეგი დაავადებები: ჩრდილოეთის ჰელმინტოსპორიოზი, სამხრეთის ჰელმინტოსპორიოზი, ჩრდილოეთის ჰელმინტისპორიოზული სილაქავე, ანთრაქნოზი, სიმინდის ფოთლებისა და ხალთის ბაქტერიული სილაქავე, ფიზოდერმა, ბუშტოვანი გუდაფშუტა, ჟანგა და ტაროს ფუზარიოზი. დასავლეთ საქართველოში აღრიცხული იქნა ყველა ზემოთაღნიშნული დაავადება ბაქტერიული სილაქავის გარდა. მთავარი დაავადება იყო ჩრდილოეთის ჰელმინტოსპორიოზი. მაშინ, როცა აღმოსავლეთ საქართველოში ფართო გავრცელებით გამოირჩეოდა მხოლოდ ბაქტერიოზი და ბუშტოვანი გუდაფშუტა. ნათესების გამოკვლევის დროს შეგროვებული დაავადებების ნიმუშებიდან სუფთა კულტურაში გამოყოფილი იქნა დაავადებათა გამომწვევები, რომლებიც ინახება ინსტიტუტის ფიტოპათოგენთა კოლექციაში.

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THE BIOGEOGRAPHICAL ANALYSIS OF RODENT NATTFLIES (LEPIDOPTERA: NOCTUIDAE, NOCTUINAE) DISTRIBUTED ON THE TRIALETI RANGE

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(Received February 20, 2006)

Abstract

The biogeographical analysis of rodent Nattflies distributed on the Trialeti Range is discussed. 57 species of subfamily Noctuinae are established. The landscape-genetic belonging, types of the area and relationship with life forms of plants is studied. The basis of the fauna is made with elements of steppe and light forest – 54,4%, where the main part have Old Mediterranean and more wide distributed species; the forest elements includes 33,4%, with predominance of boreal-nemoral forms; the quantity of orobiom species is equal to 10,5%; the desert element of fauna is presented only with one species with Tethis area; endemism is poor and is expressed with only one species. In the composition of faunal spectrum the particular parts have pluriregional groups (3,5%). The general analysis shows that the fauna of rodent Nattflies on the Trialeti Range is united basically in the former Laurasian mainlands – Arctogea (94,7%), but some species area includes Palaeogea and Notogea as well. This fact indicates complicated history of formation of fauna of this region.

Key words: *Lepidoptera, Noctuidae, Noctuinae*, zoogegoraphical areas, trophical links, landscape-genetic belonging, Arctogea, Palaeogea, Notogea.

Materials and Methods

The Nattflies are night butterflies and for collected the need materials were used different sources of light. On the stationers – 500-1000 watt tungsten lamps with so colled Sakharov sweeping net and in field conditions – quartz apparatus with PRK-2 lamp. 272 species of Nattflies were collected [Didmanidze, 1971; 1978; Emelianov, 1976], 57 species (20,9%) from them belong to the subfamily of rodent Nattflies. The presented work is an analysis of these materials.

Results and Discussion

Trialeti Range represents the eastern part of the Caucasus Minor. It begins from Mtatsminda plateau (Tbilisi), goes to southwest and finishes in Borjomi gorge within the limits of Atskur-Tashiskari. The whole length of the range is 153 km, width – 25-40 km. Relief is volcanic, rich by gorges, rivers, cawes and plateaus. Climate is subtropical-continental. As medium mountainous region (400-2000 m. s. l), Trialeti Range lays in forest zone, but by the reason of anthropogenic influence, the great part of the territory is covered with wood grove of secondary origin and typical mountain steppe vegetation. The peculiarity of Trialeti Range vegetation is

wedge-shape entered elements of colchic flora in its western part. On the other hand volcanic relief provides its xerophytization.

The bio- and zoogegraphical data of rodent Nattflies distributed on the Trialeti Range is characterized by special peculiarities. According to the schemes of bio- and zoogeographical division of Palaearctis [Gegechkori, 1974; 1984; 1985; Gegechkori et al., 1996; Krijanovski, 1965; 2002] we united the fauna of *Noctuinae* in 20 zoogeographical units. (See the table 1).

Table 1. The Biogeographical data of Rodent Nattflies Distributed on the Trialeti Range

	Species	**General distribution**	The types of area	The landscape-genetic belonging	The trophycal links with the host plants
1	2	3	4	5	6
	Euxoa Hb.				
1.	E. agricola Bsd	Palaearctic	P	SL	PT
2.	E. obelisca Schiff	Caucasus, Transcaucasus, Middle and South Europe, Mediterranean, Central Asia	T	SL	PT
3.	E. tritici L	Caucasus, Transcaucasus, Middle and East Europe, Crimea, Siberia, Central Asia, Mongolia	WP	SL	HT
4.	E. nigrikan L	Caucasus, Transcaucasus, Europe, Crimea, Siberia, Central Asia, Mongolia, North China, Korea	P	F	PT
5.	E. temera Hb.	Caucasus, Transcaucasus, Western Europe, Asia Minor, North Africa, Palestine	WT	SL	PT
6.	E. hastifera Dzl.	Caucasus, Transcaucasus, South Europe, North Iran, Central Asia	MT	SL	HT
7.	E. distigueuda Led.	Caucasus, Transcaucasus, Middle and South Europe, Asia Minor, Central Asia	MT	SL	PT
8.	E. aquiline Schiff.	Caucasus, Transcaucasus, Asia Minor, Central Asia, Siberia, Manchuria, Korea, Japan	P	SL	PT
9.	E. cos Hb	Caucasus, Transcaucasus, Asia Minor, Central Asia, Siberia, Manchuria, Korea, Japan	P	SL	HT
10.	E. birivia Schiff.	Caucasus, Transcaucasus, East Europe, Crimea, Balkan	EM	M	HT
11.	E. recussa Hb.	Peninsula, Asia Minor, Syria, Palestine, North Iran	P	M	DN
	Scotia Hb.				
12.	S. cinerea Schiff.	Caucasus, Transcaucasus, Middle Europe(Alps), Kazakhstan, Central Asia, Siberia, Amur Riverside	EM	SL	HT

13.	S. segetum Schiff	Caucasus,Transcaucasus, Middle and South Europe, Crimea, Asia Minor	OP	F	PT
14.	S. clavis Hfn.	Caucasus, Transcaucasus, Europe, Asia Minor, Central Asia, Siberia, Japan, India	H	F	PT
15.	S.exclamationis L.	Caucasus, Transcaucasus, Europe, Asia Minor, Central Asia, Siberia, Far East, North America	P	F	PT
16.	S. ypsilon Hfn.	Caucasus, Transcaucasus, Europe, Siberia, Asia Minor, Central Asia, Far East, Amur Riverside	HOA	F	PT
17.	S. crassa Hb.	Caucasus, Transcaucasus, Europe, Siberia, Asia Minor, Central Asia, China, Korea, America, Australia, New Zealand	P	SL	PT
18.	S. obesa Hb	Caucasus, Transcaucasus, Middle and South Europe, Central Asia, Siberia, Mediterranean	T	SL	HT
	Ochropleura Hb				
19.	O. renigera Hb.	Caucasus, Tran Caucasus, South Europe, Asia Minor, Central Asia, Palestine, Iran, Mediterranean	ME	M	HT
20.	O. crimea Kozh.	East Caucasus, Transcaucasus, Western and Middle Europe	WM	SL	HT
21.	O. forcipula Schiff.	Transcaucasus, Mediterranean	WM	SL	HT
22.	O. signifera Schiff.	Caucasus, Transcaucasus. South Europe, Asia Minor	T	D	HT
23.	O. flavina Hs.	Caucasus, Transcaucasus, Kazakhstan, Afghanistan, North Iran, Mediterranean	EM	SL	HT
24.	O. praecox L.	Transcaucasus, East Europe, Asia Minor, Iran	PP	SL	HT
25.	O. candelisequa Schiff.	Caucasus, Tran Caucasus, Middle Europe, Siberia, Sakhalin, Mongolia, Manchuria, Korea, Japan	MT	SL	HT
26.	O flammatra Schiff.	North Caucasus, Tran Caucasus, Crimea, Asia Minor, Iraq, North Iran, Central Asia	MT	SL	PT
27.	O. plecta L.	Caucasus, Transcaucasus, Middle Europe, Asia Minor, Iran, Iraq, Palestine, Central AsiAPalaearctic, Tibet, South Africa, America	EPON	SL	PT
	Eugnorisma Brsn.				
28.	E. depuncta L.	Caucasus, Transcaucasus, Middle Europe, Crimea	E	F	PT
	Rhyacia Hb.				
29.	R. grisecens F.	Caucasus, Transcaucasus, Europe (Alps), Siberia	ESC	M	HT

30.	R. simulans Hufn.	Caucasus, Transcaucasus, Middle and Western Europe, Kazakhstan, Siberia	Sk	F	HT
31.	R. lucipeta Schiff.	Caucasus, Transcaucasus, Middle and South Europe, Crimea, Mediterranean	WM	SL	HT
	Chersotis B.				
32.	C. rectangular Schiff.	Caucasus, Transcaucasus, Middle Europe, Crimea	P	SL	HT
33.	C. alpestris B.	Caucasus, Transcaucasus, East and Middle Europe, Asia Minor, Iran, Central Asia	MT	SL	HT
34.	C. multangula Hb.	Caucasus, Transcaucasus, Middle Europe, Asia Minor, Central Asia, Iran	MT	SL	HT
35.	C. margaritacea Vill.	Caucasus, Transcaucasus, Middle and South Europe, Asia Minor, Kazakhstan, Central Asia	NT	M	HT
36.	C. anachoreta Hs.	Caucasus, Transcaucasus, Western Europe, Siberia, Far East, North China	PP	F	DN
37.	C. lupezinoides	Caucasus, Transcaucaus	C	M	HT
	Noctua L.				
38.	N. degeniata Chr.	Transcaucasus, Central Asia, Iran	ITMA	SL	HT
39.	N. pronuba L.	Caucasus, Transcaucasus, Western Europe, North Iran, Mediterranean	WM	SL	PT
40.	N. orbona Hfn.	Caucasus, Tran Caucasus, South and Middle Europe, Asia Minor, Iran, Iraq, Pakistan, Afghanistan, Central Asia, Mediterranean	T	SL	PT
41.	N. fimbriata Schiff.	Caucasus, Transcaucasus, Middle and South Europe, Mediterranean, North Africa	EM	SL	HT
42.	N. ianthina Schiff.	Caucasus, Transcaucasus, Middle and South-East Europe, Asia Minor, North Iran, North Africa	WP	SL	HT
	Epilecta				
43.	E. linogrisea Schiff.	Caucasus, Transcaucasus, Europe-Black See side, Asia Minor, Syria, Palestine	EM	SL	HT
	Spaelotis B.				
44.	S. ravida Schiff.	Caucasus, Transcaucasus, Europe, Asia Minor, North Iran, Kazakhstan, Central Asia, Siberia, Korea, China, Japan	PP	F	HT
	Peridroma Hb.				
45.	P. saucia Hb.	Caucasus, Transcaucasus, North - East. middle and South Europe, Asia Minor, Syria, North America	P	F	PT
	Diarsia Hb.				
46.	D. mendica F.	North Caucasus, Transcaucasus, Western and Middle Europe, Siberia, Sakhalin, Mongolia	ESC	F	PT

47.	D. rubi View.	Caucasus, Transcaucasus, Middle Europe, Siberia, China, Tibet	OP	F	HT
	Amathes Hb.				
48.	A. c-nigrum L.	Caucasus, Transcaucasus, Siberia, Kazakhstan, Central Asia, Sakhalin, North America	H	F	PT
49.	A. ditrapezium Schiff.	Caucasus, Transcaucasus, Middle Europe, Central Asia, North Mongolia, China, Manchuria, Sakhalin, North America	P	SL	PT
50.	A. triangulum Hfn.	Caucasus, Transcaucasus, Western Europe, Central Asia, Siberia,	P	F	HT
51.	A. boja Schiff.	Caucasus, Transcaucasus, North and middle Europe, Siberia, Central Asia, Mongolia, Manchuria, Korea, Japan, North America	H	F	PT
52.	A. rhomboidae Esp.	Caucasus, Transcaucasus, Middle Europe, Crimea, Kazakhstan	WSk	SL	HT
53.	A. xanthographa Schiff.	Caucasus, Transcaucasus, Middle and South Europe, Asia Minor, Syria, Palestine, Central Asia	MT	SL	HT
	Naenia Stph.				
54.	N. tipica L.	Caucasus, Transcaucasus, North-East Europe, Siberia	ESC	F	PT
	Anaplectiodes Md.				
55.	A. Prazina Schiff.	Caucasus, Transcaucasus, North and South Europe, Siberia, Sakhalin, Far East, North Africa	P	F	PT
	Cerastis O.				
56.	C. rubricosa Schiff.	Caucasus, Transcaucasus, Middle and South-East Europe, Siberia, Far East	ESC	F	PT
	Mesogona B.				
57.	M. acetosellae Schiff.	Caucasus, Transcaucasus, Middle Europe, Asia Minor, West And South Siberia	WP	F	DN

The types of area: P – **Palaearctic.** WP – **Western Palaearctic**, MT – **Middle Tethis (Old Mediterranean area),** PP – **Panpalaearctic,** EM – **Eastern Mediterranean,** WT – **Western Tethis,** T – **Tethis,** WSk – **Western Skiff,** ESC – **Euro-syberian-Caucasian,** E –**European,** WM – **Wide Mediterranean,** ME – **Mountainous European (Euxinean),** C – **Caucasian,** S – **Skiff (Steppe),** H – **Holarctic,** NT – **Northen Tethis,** OP – **Oriental–palaearctic,** HOA – **Holarctic–Oriental-Australian,** EPON – **Ethiopian-Palaearctic-Orienta-Neotropical.**

The landscape – genetic belonging: M – **Mountainous,** F – **Forest,** D – **Desert,** SL – **Steppe and light forest.**

The trophic links with the host plants: HT – **Hortophylous,** PT – **Polytrophous,** DN – **Dendrophylous.**

These zoogeographical units are (through decreasing of the number of species): Palaearctic, Middle Tethis (Old Mediterranean area), Eastern Mediterranean, Tethis, Euro-

Syberian-Caucasian, wide Mediterranean, Caucasian, Holarctic, Western Palaearctic, Panpalaearctic, Western Tethis, Western Skiff, European, Mountainous European (Euxinean), Skiff (steppe), Northern Tethis, Oriental-Palaearctic, Holarctic-Oriental-Australian and Ethiopian-Palaearctic-Oriental-Neotropical.

The arhaeological analysis of the species shows the following: the fauna of moderate cold and humid loving boreal-nemoral group of rodent Nattflies unites 28 species from 9 zoogeographical units. This fauna from the genetic closeness and origin point of view corresponds with fauna of Mediterranean proper and Old Mediterranean one (humid, subarid and arid) groups – 29 species (together with multiregional groups – 35%). This peculiarity adequately indicates on progressive process of xerophytisation on Trialeti Range (especially on its eastern part). The last tendency is strenghten by volcanic relief of this part of Trialeti Range and anthropogenic factor as well.

Nattflies as the phytophagous insects trophically are bound basically with vascular plants. They feed on the as annual so on perennial forms of vagetation. Analysis of the trophycal links with the host plants show that the main part of rodent Nattflies are hortophylous – 50,9%; they inhabit mainly in the following habitats: mountain steppes, arid light forests and xerophitic bushes (shrubs); 43,8% are the polytrophous one, which are characterized by wide biotopical distribution; dendrophylous species are presented only by 5,3%, what indicates on preference by rodent Nattflies of the open landscapes.

According to the above mentioned issue, rodent Nattflies distributed on the Trialeti Range may be divided the following landscape-genetic groups:

The kernel of the fauna is made with steppe and light forest elements – 31 species (54,4%). It includes 11 zoogegoraphical regions, where the great parts have wide Mediterranean and Ancient Mediterranean distributed complexes – 20 species.

By richness of species is distinguished the forest element of the fauna as well – 19 species (33,4%), which are united in 9 zoogeographical types. Here predominate the boreal-nemoral forms.

The element of the mountain fauna is represented only by 6 species (10,5%), but this small group is very specific for orobiom. It contains 6 zoogegoraphical unites.

From desert elements of the fauna only one species with Tethis area is presented. The endemism is expressed with one species.

The pluriregional groups must be divided into 3 units: biregional – (Oriental-Palaearctic), three regional (Holarctic-Oriental-Australian) and four regional (Ethiopian-Palaearctic-Oriental-Neotropical) species. These species have insignificant part (3,5%) in the fauna, but their participation in the faunal spectrum is a face of the faunal genesis of this territory. However, from the zoogeographical point of view their participant in the region's fauna has an important value.

So, we can conclude that the biogeographical peculiarity of the discussed fauna is that the fauna of the rodent Nattflies distributed on the Trialeti Range, as well as the fauna of Caucasus, belongs to the Arctogea realm (94,7%) and only some species centre of origination is presented by the other realms, as are Palaeogaea, Neogaea and Notogaea.

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თრიალეთის ქედზე გავრცელებული ძვეოჯახ მღრღნელი ხვატარების **(LEPIDOPTERA: NOCTUIDAE, NOCTUINAE)** ბიოგეოგრაფიული ანალიზი

გეგეჭკორი არნ., დიდმანიძე ე., მანდარია ნ.

საქართველოს ეროვნული მუზეუმი
სიმონ ჯანაშიას სახელობის მუზეუმი, თბილისი

(მიღებულია 20.02.2006)

რეზიუმე

განხილულია თრიალეთის ქედზე გავრცელებული მღრღნელი ხვატარების ბიოგეოგრაფიული ანალიზი. გამოვლენილია მღრღნელი ხვატარების 57 სახეობა. თითოეულისათვის ლანდშაფტურ-გენეტიკური კუთვნილება, არეალის ტიპები და დამოკიდებულება მცენარეთა სასიცოცხლო ფორმებთან. დადგენილია, რომ ფაუნის ბირთვს ქმნის სტეპისა და ნათელი ტყის ელემენტები–54,4%, რომელთა შორის განმსაზღვრელია ფართო და ძველხმელთაშუაზღვიური გავრცელების სახეობები; ტყის ელემენტებს უკავიათ 33,4%, რომელთა შორის ჭარბობს ბორეალურ–ნემორალური ფორმები; მთის ფაუნის ელემენტს ქმნის ორობიომისათვის სპეციფიკური სახეობები–10,5%; უდაბნოს ელემენტებიდან წარმოდგენილია მხოლოდ I–ტეთისური არეალით; ენდემიზმი გამოხატულია 1 სახეობით. ფაუნის სპექტრის შექმნაში განსაკუთრებული წვლილი შეაქვთ პლიურირეგიონალურ ჯგუფებს (3,5%). საერთო ანალიზიდან გამომდინარე თრიალეთის ქედზე გავრცელებული მღრღნელი ხვატარების ფაუნა, ისე როგორც მთელი საქართველოსი, მართალია ძირითადად არქტოგეაშია გაერთიანებული (94,7%), მაგრამ მოიცავს პალეოგეასა და ნოტოგეას, რაც ამ რეგიონის ფაუნის ფორმირების რთულ ისტორიაზე მიგვანიშნებს.

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Zoology

SUGAR-BEET PARASITE WEEVILS (COLEOPTERA, CURCULIONIDAE) IN SHIDA QARTLI (CENTRAL GEORGIA)

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Abstract

The paper deals with the species composition of weevils recorded on sugar-beet plants and their distribution in beet growing regions of Shida Qartli. The principal problems of weevil bioecology, trophic specialization, and areal type for every species are studied. The role of weevils in decrease of sugar-beet productivity is estimated. Preventive measures for reduction of parasite number in beet plantations are discussed.

Key words: polyphage, areal, phytolandscape, edible root, xerophilous, Chenopodiaceae

Introduction

The central part of Georgia, Shida Qartli represented mainly by xerophilous phytolandscapes to which xerophilous species of weevils are related [Miruashvili, 1970].

Among agricultural fields, such as fruit- and grape-growings, developed in Shida Qartli, beet growing is significant. Beet (Beta) is a perennial plant genus from Chenopodiaceae family. Genus involves 13 species, among them 11 species are wild and 2 – cultivated: sugar-beet – *Beta vulgaris L.ssp.esculenta* (Salisb.) *Gürke var. altissima* Rossig (=*Beta vulgaris saccharifera* Alef) and leaf-beet or chard – *Beta vulgaris L. ssp. cicla* (L.) Moq. A lot of cultivars were produced via their selection. Regional cultivar in Shida Qartli is "Goruli". Sugar-beet is very important technical culture as a rude material for sugar production. Shida Qartli sugar production factory is the only one in Georgia.

A lot of parasite insects inhabit on sugar-beet, and among them weevils are significant ones.

Materials and Methods

Material was collected during 2002-2003 in beet-growing regions of Shida Qartli (Kaspi, Gori, Qareli, Khashuri) in sugar-beet sowings. Species composition and principal problems of bioecology were studied. For field researches known methods for coleopterous collection were used [Kobakhidze, 1958]. Obtained data were examined in Gori State University and Tbilisi Institute of Zoology.

Results and Discussion

Up today only fragmentary data about weevils in the viewpoint of species belonging to various sugar-beet parasite insect groups occur [Tulashvili, 1947]. Direct investigation aiming sugar-beet parasite weevils integrated study has not been realized not only in Shida Qartli, but in Georgia on the whole.

According to our researches the following species of sugar-beet parasite weevils have been registered:

Eusomus ovulum Germ. - polyphagous species; feeds mainly by Compositae family plants, and among them by sugar-beet. Larva develops on wormwood (*Artemisia phyllotachys* (Boiss) and milfoil (*Achilea millefolium L.*). In Shida Qartli it is distributed almost everywhere. We found this species on beet, as well as on wild xerophilous plants. Western-Palaearctic-Subboreal-Subtropical species.

Parafoucartia squamulata (Herbst) = *Foucartia* – polyphagous species; damages leaves of various plants, among them of sugar-beet. Larva develops in the soil. Considerable amount was recorded on xerophilous herbaceous plants, solitary specimens – on sugar-beet. Western-Palaearctic-Boreal- Subtropical species.

Psallidium maxillosum (Fabr.). – polyphage, but mainly damages sugar-beet. Weevils gnaw newly foliated leaves and sprouts. Larva develops in the soil, feeds by plant roots. Significant amount was recorded in Qareli, Gori and Kaspi regions of Shida Qartli. Middle-Tetis species.

Tanymecus palliates (Fabr.) – wide polyphage; damages about 80 plant species, especially in Kaspi and Qareli regions. While our studies solitary specimens were registered on sugar-beet. Palaearctic-Atlantic-Continental-Boreal-Subtropical species.

Lixus subtilis Boh. – polyphage. From weeds often pass on to sugar-beet for feeding. Weevil feeds by leaves and stems. Great number of weevils is distributed almost everywhere in Shida Qartli. Trans-Palaearctic-Subboreal-Subtropical species.

Lexus sinuatus Motsch. – oligophage; damages strongly sugar-beet. Great number was recorded on wild plants of Chenopodiaceae family, solitary specimens – on beet. Western-Tetis species.

Lexus sanguineus (Rossi) – polyphage. Recorded in Khashuri region. Serious parasite of sugar-beet. Western-Palaearctic-Subboreal-Subtropical species.

Chromonotus confluens (Fabr.). – oligophage. Recorded in Kaspi region, on left slope of Kvernaqi Ridge, on field type herbaceous plants. It was not found in beet sowings, but it is very hard parasite and pass on easily from weed species of Chenopodiaceae family to sugar-beet sprouts. Middle-Tetis species.

Chromonotus vittatus (Zubk.) = *Cleonus* – oligophage. Weevils are often met on beet, but larva – on roots of various species of Chenopodiaceae family. We registered in Khashuri region. Middle-Tetis species.

Bothynodelres punetiventris (Germ.) – polyphage; distributed in every beet growing region of Shida Qartli and compared to other weevils causes damage most of all to sugar beet. Weevil feeds by aboveground parts of plant, but larva damages the root system and as a result, plant dies. Middle-Tetis species.

Chromoderus affinis Schrank (=*Cleonus fasciatus* Muller) – polyphage; hard parasite of sugar-beet. A great amount was recorded in Qareli region. Western-Palaearctic-Subboreal-Subtropical species.

Macaspis octosignatus Gyll. – widespread sugar-beet parasite of Shida Qartli. A great number was registered on sugar-beet sprouts of Gori, Qareli and Khashuri regions and on herbaceous plants of nearby virgin lands. In Georgia - found only in Shida Qartli. Anatolian-Caucasian-Atropatenian species.

Cleonus pigra (Scop.) = *Cleonus sulcirostris L. ab. scutellaris Boh., piger* Scop. – polyphage. Feeds by leaves of sugar-beet sprouts along with other plants; causes significant damage. Palaearctic-Oriental species.

Alophus triguttatus (Fabr.) – polyphage; damages leaves of beet sprouts; larva destructs root system. In Shida Qartli we found this species in Surami. Western-Palaearctic-Subboreal-Subtropical species.

Thus, 14 species of weevils were registered on sugar beet growings in Shida Qartli, which inhabit independently, as well as in complex with other groups of parasite insects. They cause significant damage decreasing the yield of sugar-beet. It should be taken into consideration the fact that wild relatives of cultivated plants, to which one or another species of weevil is related, represent their potential substantial food. According to abovementioned, the principal prophylactic action for reduction of parasite weevil number in sugar-beet plantations and nearby area lies in eradication of weeds of Chenopodiaceae family.

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შაძრის ჭარხლის მავნებელი ცხვირგრძელა ხოჭოები (**Coleoptera, Curculionidae**) შიდა ქართლში

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(მიღებულია 03.02.2006)

რეზიუმე

მოცემულია შიდა ქართლის შაქრის ჭარხალზე გამოვლენილი ცხვირგრძელა ხოჭოების სახეობრივი შემადგენლობა და გავრცელება შიდა ქართლის მეჭარხლეობის რაიონებში. შესწავლილია მათი ბიოეკოლოგიის ძირითადი მომენტები, კვებითი სპეციალიზაცია და არეალის ტიპი. შეფასებულია ცხვირგრძელა ხოჭოების მნიშვნელობა შაქრის ჭარხლის მოსავლის შემცირების საქმეში და გამოთქმულია მოსაზრება ჭარხლის პლანტაციებში მავნებელთა რიცხოვნობის შემცირების პროფილაქტიკური ღონისძიებების შესახებ.

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Zoology

EARTHWORMS OF DENDRODRILOIDES KVAVADZE, 1999 (OLIGOCHAETA, LUMBRICIDAE) GENUS FROM AJARA FAUNA

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Abstract

Anatomical, morphological and karyological investigations of the earthworm *Ddl. hydrophilica* of the genus Dendrodriloides from Ajara populations (Green Lake, villages: Chkhutuneti and Chaqvistavi) were carried out. It was established that studied populations of *Ddl.hydrophilica* is composed of, typical for the genus, amphimixis and diploid (2n=36, n=18) individuals.

Key words: *Ddl.hydrophilica,* amphimixis, diplomena, karyology

Introduction

Not so far as the genus Dendrodriloides Kvavadze, 1999 is considered as independent taxonomic unit of the Family Lumbricidae. While studying the structure and sculpture of genital setae of species of genus Eisenia it was found out that 7 species of earthworm: *E. grandis grandis, E. grandis ganjiensis, E. grandis perelae, E. grandis polysegmentica, E. grandis supsaiensis, E. hydrophilica* and *E. Thamarae*, were provided not with 3-strial genital setae, characteristic for the rest species of this genus, but with 4-strial genital setae [Svetlov, 1957; Kvavadze, 1991; Kvavadze, 1997]. Firstly, their integration in the genus Dendrobaena was considered [Kvavadze, 1991], later, according to the stria of genital setae, localization of spermathecae and other morphological characteristics, they were integrated in the new genus Dendrodriloides Kvavadze, 1999 [Kvavadze, 2000]. Hence, it is of interest to research the earthworms of the genus Dendrodriloides in various areas of their registration, namely in Ajara, where 2 species: *Ddl. polysegmentica* Kvavadze, 1973 and *Ddl. hydrophilica* Kvavadze, 1973 and 1 subspecies – Ddl.grandis perelae Kvavadze, 1973 of this genus occur [Kvavadze, 1985].

In the given paper we have settle on the species *Ddl. hydrophilica*, which is widespread in Ajara. This species belongs to ecological grouping of hydrocoles and predominantly occurs in limnetic habitats (springs, streams).

The aim of our work is anatomical-morphological and karyological study of *Ddl. hydrophilica* individuals of Ajara population and establishment of the ways of their reproduction.

Materials and Methods

Sexually mature individuals of *Ddl. hydrophilica* were collected in three regions of Ajara: Green Lake (Khulo district), village Chaqvistavi (Kobuleti district) and village Chkhutuneti

(Khelvachauri district). For anatomical-morphological study from each area 9-18 specimens were collected. To establish the ways of reproduction organs of reproduction system (seminal vesicles, spermathecae) were studied in vivo, as well as in the material fixed in 4% formalin by binocular (MBC-1, MBC-3). For karyological study 3 specimens were taken from each area. Preparations were prepared from seminal vesicles according to [Grafodatski et al., 1982; Bulatova et al., 1987] with some changes [Bakhtadze et al., 2003]. Preparations were dyed by 45% aceto-orcein. The characteristics of spermatogenesis process were studied. To establish modal number of chromosomes and polyploidy all plates of mitosis and meiosis, which provided karyotyping requirements, were analyzed.

Results and Discussion

Specimens of *Ddl. hydrophilica* collected from three populations are characterized by the following anatomical-morphological features: length of sexually mature individuals is 45-150 mm, width in the clitellum area – 4-8 mm. Number of segments – 68-173. Pigmentation – intensive purple-brown. Depigmentation of the lateral sides of the 9-11 segments is observed. Head lappet is epilobar and closed (1/3); setae approached and paired beyond clitellum – *aa:ab:bc:cd:dd*=45:9:37:9:110. Back pores began from 5/6 intersegment stria. Male pudendal fissure is located at the 15th segment, esophageal glands get partly over 16th segment. Distance, from *b* setae up to male pudendal fissure is 0.5-1.0 mm, but from pudendal fissure to *c* setae – 1.25-1.60. Female pudendal fissure is at the 14th segment, above *b* setae; distance from *b* setae to this fissure equals to 0.15-0.25 mm.

The clitellum is placed at 26-33rd or 27-33rd segments, genital protuberance – at 30-1/3 – 33 segments, spermatophores – at 28th segment. 4 pairs of seminal vesicle are located in 9-12 segments, 2 pairs of spermathecae at 9-10th segments and their ducts open on back side at the line of back pores. The distance from the spermathecae holes to the back pores equals to 0.40-0.45 mm. Seminal and infundibulum ducts are in 10-11th segments. Calciferous glands are placed in 10-13th segments. Proventriculus occupies 15-16th segments and muscle gizzard – 17-18th segments.

Results of research of reproduction system show that studied individuals of *Ddl. hydrophilica* have developed well seminal vesicles and spermathecae filled with sperm.

Analysis of preparations reveals that spermatogenesis started in seminal is continued and proceed normally in seminal vesicles. There are observed nuclei of different stages of gametogenesis, which are gathered as cysts. 18 pairs of homologous chromosomes (bivalent) are counted in the plates of meiosis diplonema stage (Fig.1). In the mitosis metaphase plates the modal number of chromosomes reaches 36.

According to the data of anatomical-morphological studies all diagnostic features of the genus Dendrodriloides are revealed in all investigated populations of Ajara. Particularly, purple-brown coloration, epilobal head lappet, paired setae, 4 pairs of seminal vesicles, 2 pairs of spermathecae, which are opened near medial line of backside. Thus, specimens of *Ddl. hydrophilica* of studied populations are the typical earthworms of the genus Dendrodriloides .

According to status of seminal vesicles and spermathecae individuals of male line belong to fertile, amphimixis forms [Muldal, 1952; Casellato et al., 1972; Perel et al., 1983]. It is noteworthy that amphimixis is revealed in all the studied populations. By karyological results, the individuals of these populations are diploids, and the basic number of chromosomes equals to 18, like other species of the genus Dendrodriloides [Bakhtadze, 2003].

Thus, specimens of *Ddl. hydrophilica* of studied populations of Ajara are presented by typical, amphimixis and diploid (2n=36, n=18) individuals of the genus Dendrodriloides.

Fig. 1. *Dendrodriloides hydrophilica*; diplomena plate of spermatogenesis with 18 bivalents, x 1250

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DENDRODRILOIDES KVAVADZE, 1999 (OLIGOCHAETA, LUMBRICIDAE) გვარის ჭიაყელები აჭარის შაუნაში

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რეზიუმე

ჩატარებულია Dendrodriloides-ის გვარის ჭიაყელა *Ddl. hydrophilica*-ს აჭარის პოპულაციების (მწვანე ტბა, სოფ. ჩხუტუნეთი და ჩაქვისთავი) ანატომიურ-მორფოლოგიური და კარიოლოგიური გამოკვლევა. დადგენილია, რომ *Ddl. hydrophilica*-ს შესწავლილი პოპულაციები შედგება გვარისათვის ტიპიური, ამფიმიქსური და დიპლოიდური (2n=36, n=18) ინდივიდებისგან.

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PRACTICAL BASES OF THE USAGE OF ANTIBIOTICS AND BACTERIOPHAGES

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Abstract

The paper deals with the potential of usage of intestinal bacteriophage, as antibiotics alternative medicine, for therapy and prophylactics of pullorosis. It was established that *S.pullorum* strains isolated from chicken have higher susceptibility (with *ce* and *oce* lysis degree) to intestinal bacteriophage, compared to antibiotics. The usage of intestinal bacteriophage in chickens increases their live mass, preserves poultry from pullorosis disease and is characterized with high therapeutic effect.

Poultry growing is related with certain difficulties concerning, mainly, feeding, drinking and prophylactic measures. Food and water of poor quality cause infective episode in chicken. Among infectious diseases of bacteriological origin pullorosis is widespread in poultry [Alexander, 1997; Bouzoubaa et al., 1992; Calnek et al., 1997; Jordan & Pattison, 1996]].

For prophylactics and therapy of infectious diseases and increasing of avian live mass antibiotics are used. From 70-80-s of 20th century data about secondary effects, due to wide and sometimes uncontrolled use of antibiotics, occurred in scientific literature. Microbial populations resistant to antibiotics appeared, dysbacteriosis developed in human and animal organisms [Santander & Robeson, 2004].

Discovery of the phenomenon of bacteriophage by Derrel (1917) became the basis of using bacterial viruses for diagnosis, prophylactics and therapy of infectious diseases.

As a result of study of various bacteriophages the positive effects of bacterial viruses compared to antibiotics have been recorded, among them: ecological harmlessness, non-allergenicity, absence of cumulative properties in the organism, reproduction in the nidus of infection, not provoking dysbacteriosis, ability of usage for prophylactic aims, etc.

Taking into consideration wide distribution of pullorosis among avian infectious pathologies, we have aimed to isolate strains of this pathogenic agent, establish susceptibility against antibiotics and intestinal bacteriophage, and also to use this phage for increase of avian live mass, prophylactics and therapy of pullorosis.

9 strains of pathogen of pullorosis were isolated in our experiments. From the practical viewpoint it is significant to study susceptibility to antibiotics of strains isolated from chicken. Experimental data concerning susceptibility to antibiotics and phages are presented in Table 1. The obtained results have shown that some isolates of pullorosis are resistant simultaneously to several antibiotics, as, for example S.pullorum-7 is resistant to erythromycin and TNF-600 (Table 1).

For usage of bacteriophages against infectious diseases establishment of range of action and the degree of lysis with regard to isolated strains is needed. The unique characteristic of

intestinal bacteriophage is its wide spectrum of action and high degree of lysis, which is mainly *ce* and *oce* (Table 1.). For some isolates degree of lysis is TV. To intestinal bacteriophage only *S.pullorum-9* revealed resistance. The wide spectrum of lysis activity of the given phage against studied cultures is convincing argument for its use in practical training.

Further investigations have shown that adding of intestinal bacteriophage in water regulates microflora of gastrointestinal tract improving vital functions of efficient bacteria, stimulates the process of fodder uptake, increases chicken weight.

Table 1. Susceptibility of *S.pullorum* strains to antibiotics and intestinal bacteriophage

№	Bacteriophage	Ampicillin	Erythromycin	Enrofloxacillin	Kanamycin	Tetracycline	TNF - 600	Cefazolin	Cefotoxin	Intestinal bacteriophage
1.	S.pullorum-1	4+	R	4+	3+	3+	3+	4+	2+	ce
2.	S.pullorum-2	3+	3+	4+	3+	4+	3+	3+	2+	oce
3.	S.pullorum-3	4+	R	4+	3+	3+	2+	4+	2+	oce
4.	S.pullorum-4	4+	3+	3+	2+	4+	R	4+	3+	ce
5.	S.pullorum-5	3+	2+	4+	3+	3+	3+	3+	3+	TV
6.	S.pullorum-6	3+	3+	4+	3+	3+	3+	3+	3+	oce
7.	S.pullorum-7	3+	R	4+	3+	4+	R	4+	2+	oce
8.	S.pullorum-8	3+	3+	3+	3+	3+	2+	3+	3+	oce
9.	S.pullorum-9	3+	3+	4+	3+	4+	3+	3+	3+	R

Note: *ce* - complete lysis; *oce* - lysis with separate plaques; TV – incomplete lysis; R – resistance;

Usage of intestinal bacteriophage in the case of infected with pullorosis chickens and healthy ones being in contact with them, have shown that its prophylactic effectiveness – 99.7%, exceeds that parameter of enrofloxacin (99%). Therapeutic effect of intestinal bacteriophage on the chickens diseased with pullorosis appeared more evident. Obtained data about therapeutic effectiveness of intestinal bacteriophage and antibiotics are presented in Table 2. Therapeutic effect of bacteriophage is 90.0%, which is considerably higher than that parameter for TNF-600 (50%). As is seen the difference comprises 40%. Therapeutic effectiveness of bacteriophage is 90.8%, which is equal to the parameter of enrofloxacin – 90.6% (Table 2.).

Table 2. Therapeutic effectiveness of intestinal bacteriophage and antibiotics

№	Preparation	Number of chicken	Result		Survival (%)
			Survive	Diseased	
1.	Intestinal bacteriophage	30	27	3	90
2.	TNF - 600	30	15	15	50
3.	Intestinal bacteriophage	250	245	5	90,8
4.	Enrofloxacin	250	240	10	90,6

Investigations carried out and obtained results enables us to conclude that the usage of intestinal bacteriophage contribute to chicken weight increasing. Preparation possesses high therapeutic-prophylactic effectiveness.

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ანტიბიოტიკების და ბაქტერიოფაგების გამოყენების პრაქტიკული საფუძვლები.

ნათიძე მ., ანთია ი.

საქართველოს სასოფლო-სამეურნეო უნივერსიტეტი

(მიღებულია 06.02.2006)

რეზიუმე

შესწავლილია ინტესტიბაქტერიოფაგის, როგორც ანტიბიოტიკების ალტერნატიული პრეპარატის გამოყენება პულოროზის მკურნალობისა და პროფილაქტიკისათვის. წიწილებიდან გამოყოფილი *S.pullorum*-ის იზოლატების შესწავლით დადგენილია, სხვადასხვა ანტიბიოტიკებთან შედარებით, მაღალმგრძნობელობა ინტესტიბაქტერიოფაგის მიმართ, რომლის ხარისხმაც ძირითადად შეადგინა *ce* და *oce*. ინტესტიბაქტერიოფაგის გამოყენება წიწილებში ზრდის ცოცხალ მასას, იცავს ფრინველს პულოროზით დაავადებებისაგან და ახასიათებს მაღალი სამკურნალო ეფექტი.

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Letter to Editor
Ecology

GLOBAL ECOSYSTEM AND ANALYSIS OF MAJOR PROCESSES IN IT

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(Received February 10, 2005)

Abstract

The work deals with pollution sources in Bolnisi District and analysis of actual status of biosphere characteristics under their impact. Using statistical data on environmental parameters the frequency of biosphere disturbances and probability of allowable concentrations of harmful substances are assessed. For estimation of environmental characteristics mathematical model competent to the law of variation of random values over time was selected. The list of control parameters, which allows for selection of carriers and limiting facilities was determined.

Mitigation of harmful impact of the wastes, development and extension of the technologies for their transformation into useful products and ensuring safe environment for the community – are the most important issues of current period.

To deal with the mentioned problem, with the purpose of contribution to the environment protection measures, Georgian Law on Environment provides for economic stimulation, which is intended for promotion of development and extension of environmentally friendly technologies and techniques providing reduced amount of wastes; usage of secondary raw materials, implementation of effective environmental projects [Georgian Law on Environment, 1997].

As a result of development of the economy and industry, volumes of both, useful products and wastes grow. Organic and inorganic harmful admixtures (including toxic ones) of the wastes spread into the biosphere in various ways. The mentioned pollutants have negative impact on flora, fauna, human beings.

Studying of the sources of generation of harmful substances and elaboration and extension of the mitigation measures is among the urgent state activities, as environmental disbalance would cause development of uncontrollable natural processes. The goals of our work are development of environmental measures, elaboration, extension and analysis of permanently improving management system on the example of Bolnisi district.

Bolnisi District is rich with metal and non-metal minerals and there are all necessary conditions for development of agriculture. Because of inadequate usage of arable lands and minerals the district currently suffers the heaviest economic, social and environmental conditions. Below we regard the methods of analysis and program control systems for dealing with this situation.

Long-term inadequate exploitation of the mines caused significant disbalance. Results of 30-year exploitation of the copper-pyrites mine should be specially admitted. Production of copper concentrate is processed with negligence to environmental requirements. This is the reason of air

pollution with low quality liquid fuel combustion products, which contain hard toxic admixtures, like benzapirene and other technological vapors of chemicals. The soil is saturated with heavy metals and toxic substances, as the arable lands are irrigated with the waters from polluter irrigation systems affecting negatively on the quality of food products.

Environmental characteristics (air, water, soil) do not require international standards. The life conditions caused baby bust, mortality of infants and dramatic increase of the cases of congenital defects and diseases. Number of healthy newborn children varies within 8-12%, what is indeed alarming.

According to data of 2003 among the teenagers (including 14 years old children) morbidity per 100 000 people achieves 9 680, what comprises 26% of ill children among Georgian population. Data for 2004 are even more alarming. Frequency of congenital defects, mortality, caused by premature birth and mortinatality achieved 12% of total birth rate and number of newborn morbidity was over 78%. Forecasting of this processes would lead to the heaviest crisis in Bolnisi District.

Analysis and study of environmental situation in Bolnisi District showed:

1. Bolnisi hydrographic network and irrigation system is polluted with the heavy metal ions and toxic substances;
2. There are not selected any control parameters for assessment of harmful impact of the sources of pollution of the arable lands and there is not provided permanent supervision for creation of the data bank;
3. There is not determined the dynamics of spreading of harmful substances in the environment over time, law of distribution of the random quantity (emission);
4. There are not studied the losses caused by environmental impact of harmful substances;
5. There are no facilities for filtration of the waste waters with polluting organic and inorganic admixtures and fecal waters;
6. There is not studied actual condition of the degraded lands used as arable ones and there are not rehabilitation and improvement measures in place.

Up to present the state authorities do not pay any attention to this situation. To solve this problem is possible only on the basis of thorough studying of the processes in the ecosystem of the District.

There are number of systematic approaches to study environmental processes, namely – the system "Human – Machine", biological "Ecosystem" (Biogeocenosis) [Khurodze et al., 1996], the system "Society and the Nature" and others. The mentioned systems are sub-systems of global ecosystem. We regard that global ecosystem consists of three elements – "Biosphere – Human – Technique" (hereinafter referred to as "BHT"), for studying of which it is convenient to apply the methods of theory of probability and mathematical statistics, as the processes in the biosphere, environmental balance and reliable functioning of the ecosystem depend on many random variables. Below is discussed the methodology for evaluation of quantitative characteristics of BHA system.

Reliability of BHA system is an ability of separate elements, to maintain major characteristics of the system, via set of features, within the allowable limits, in the process of functioning in some local conditions.

Y_i characteristics of BHA system and X_i parameters of separate elements could be considered by means of the system of interrelated equations:

$$\begin{aligned} Y_1 &= f_1(x_1, x_2, \ldots, x_n) \\ Y_2 &= f_2(x_1, x_2, \ldots, x_n) \\ &\text{----------} \\ Y_n &= f_n(x_1, x_2, \ldots, x_n) \end{aligned} \qquad (1)$$

Where Y_i – is i characteristic of BHA system; X_i – is the parameter of i unit.

In case of normal distribution of the variables frequency of disturbances of any Y_i characteristics could be calculated by means of the formula [Polovko, 1964]:

$$\mathbf{a(Yit,\xi)} = \frac{1}{\sqrt{2\pi}\sigma_{y_{i(t,\xi)}}} e^{-\frac{y_i - \overline{y}_{(t,\xi)}}{2\sigma^2 y_{i(t,\xi)}}} \qquad (2)$$

Where $\overline{y}_{(t,\xi)}$ is mathematical expectation of Y_i characteristic at t moment, in the process of functioning in environmental conditions with ξ parameters.

In the first approximation we can assume that in studying of the processes in the biosphere there is no correlative dependence between the ξ parameters. Consequently, probability that at any t moment, in the environmental conditions with ξ parameters Y_i characteristics of biosphere in BHA system are within the allowable limits, could be calculated by means of the formula [3].

$$\mathbf{P_\xi(\alpha < Yi < \beta; t, \xi) = 0{,}5\left\{ \Phi\left[\frac{\beta - \overline{Yi}_{(t,\xi)}}{\sqrt{2\pi}\sigma_{Yi(t,\xi)}}\right] - \Phi\left[\frac{\alpha - \overline{Yi}_{(t,\xi)}}{\sqrt{2\pi}\sigma_{Yi(t,\xi)}}\right] \right\}} \qquad (3)$$

Where - α, β are allowable limits of variation of Y_i characteristics.

By means of this expression it is possible to calculate value of any Y_i characteristic at t moment in the environmental conditions with ξ parameters.

As we can see, to ensure regulating of environmental balance it is necessary to know, harmful impact of which elements and substances are characteristic to given environment. ξ parameters designate such harmful substances, as heavy metals, toxic substances, harmful gases, dust, products of incomplete combustion etc.

According to the established marks, c_i – designate heavy metals; d_i – toxic substances; γ_i - harmful gases; ϑ_i - dust; q_i – soot etc.

Thus, ξ parameter of the environment will depend on the variable sum of the concentrations of harmful substances:

$$\xi = f(c_i, ..d_i, ..\gamma_i ..\vartheta_i ..q_i ...)$$

Regarding the specific substances spread in one or another environment it is necessary to select controlling carriers and for their localization – mitigation measures.

Thus, we offer methods for selection of the measures and parameters of purifying facilities, waste treatment technological lines and means for rehabilitation of degraded soils.

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გლობალური ეკოსისტემა და მასში მიმდინარე ძირითადი პროცესების ანალიზი

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[2]საქართველოს სასოფლო-სამეურნეო უნივერსიტეტი

რეზიუმე

განხილულია ბოლნისის რაიონის გარემოს გამაბინძურებელი წყაროები და მათი ზეგავლენით ბიოსფეროს მახასიათებლების ფაქტობრივი მდგომარეობის ანალიზი. გარემოს პარამეტრების შესახებ სტატისტიკური მასალის გამოყენებით შეფასებულია ბიოსფეროს შეშფოთებათა სიხშირე და მავნეობათა დასაშვებ კონცენტრაციათა ალბათობა. გარემოს მახასითებლების შეფასებათა პროგნოზირებისათვის შერჩეულია შემთხვევითი სიდიდეების დროში ცვალებადობის ნორმალური კანონის შესატყვისი მათემატიკური მოდელი. დადგენილია საკონტროლო პარამეტრების ნუსხა, რაც გვაძლევს საშუალებას შერჩეულ იქნას გადამწოდები და შემზღუდავი დანადგარები.

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Letter to editor
Ecology

BIOECOLOGICAL CHARACTERISTICS OF *PSEUDORASBORA PARVA* (SCHLEG.) "IMMIGRANT" IN BAZALETI LAKE

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Abstract

Weed "dwarf" fish *P.parva* inhabit in Bazaleti Lake. Body length, elongated enough, does not exceed 7 cm. It has straight lateral line with 37-38 dark cycloid scale of similar size almost everywhere. Population structure and dynamics character show specific relation of population with environment. Growth rate of male and female specimens is nearly similar. By pubescence age and life span Pseudorasbora of Bazaleti Lake belongs to both, I and II fish groups according to Zamakhaev (1959) classification. But we suppose that to the sexual size structural groups must be added IV group, where should be united fish with characteristics similar to Pseudorasbora.

Amur *Pseudorasbora parva* (Schleg.) is widespread Eastern-Asian species. For the first time it was found in Bazaleti Lake in 1987, further its inhabitation was shown in other fishing reservoirs of Georgia (Kumisi, Algeti, Jandara, etc.). It is a "dwarf" fish, with a maximal zoological length of 7 cm. Body is covered with wide scale, which number in lateral line is 37-38. Has small but broad mouth placed on erected snout. Nasal gaps are located near broad placed eyes. It has no whiskers; body - of dark coloration.

During 2000-2002 Pseudorasbora was caught in Bazaleti Lake. We use netting gear of the length of 6 m, height - 1 m, cell size -3 mm. Floaters are placed at every 30 cm of net top-clip, and lead weights – at every 10 cm of net bottom-clip. Obtained material was treated with 4% formalin solution. The size (mm) and weight (g) was determined for 770 specimens; gender – for 641 specimens (83.2%); the age was defined via scale for 550 specimens (71.4%) by "MBC-3" binocular.

Material was collected by seasons and selected points according to the table bellow:

	Seasons				Points					
	Spring	Summer	Autumn	Winter	I	II	III	IV	V	Total
Number of spec.	154	235	196	185	141	194	232	132	71	770
%	20.0	30.5	25.3	24.2	18.3	25.3	30.1	17.1	9.2	100

Population of small Pseudorasbora inhabited in Bazaleti Lake is characterized by simple age-size structure presented by specimens of 4 groups (O^+ - 3^+) indicating on short life span of

species: juveniles (O^+) – 218 specimens (39.6%); 1^+ - 178 specimens (32.4%); 2^+ - 107 specimens (19.4%); 3^+ - 47 specimens (8.6%).

0+ and 1+ age groups outnumber (72%) the rest groups. Age structure in both male and female specimens is similar; fish age does not exceed 3 years. Obtained results are in agreement with the data of age limit of Pseudorasbora inhibited in fishing reservoirs of Rumania [Giurca &Angelescu, 1971]. Occurrence of 3 years old individuals was shown in limnetic reservoirs of Europe [Movchan & Smirnov, 1981]. Structure of small Pseudorasbora population of Bazaleti Lake is presented by the following specimens: 1. sexually non-mature juveniles; 2. mature for the first time; 3. mature repeatedly.

In obtained material individuals without potential of reproduction, partially or entirely, were not recorded. Biological active spawning part of three age groups (1^+ - 3^+), including at least 332 specimens, is distributed by the following percentage correlation: 1^+ - 50% (167 specimens.); 2^+ - 35% (116 specimens); 3^+ - 15% (49 specimens). The similar age structure was found in Rumania fishing reservoir [Giurca & Angelescu, 1971]: 1^+ - 51%; 2^+ - 33%; 3^+ - 16%.

According to our data, the main part of spawning population is consisted in 2 years old specimens. Natural losses by age groups are: 0^+-1^+ - 10.1%; 1^+-2^+ - 24.9%; 2^+-3^+ - 39.0%.

Structure of population of *P.parva* is very dynamical and is determined by the correlation of "reserve" and "waste" specimens. Amount of fish spawning firstly is defined according to the viability, capacity and protection of reproductive biotopes of corresponding generation.

Size-weight structure is specific for the species and is under the influence of environment, but it is within usual growth range of species. In Bazaleti Lake the maximal size-weight parameters are the following: 65 mm and 3070 mg. Size-weight structure is presented in the table bellow:

Age	Length (mm)		Total weight (g)		Weight without entrails (g)	
	range	average	range	average	range	average
0^+	12-30	27	150-460	354	100-370	257
1^+	31-45	40	450-1480	965	350-1100	696
2^+	46-55	49	1460-2650	2055	1130-2230	1189
3^+	56-65	60	2470-3070	2800	1800-2300	1852

Small size and weight enable fish to inhabit in those reservoirs where big ones can not live. Amounts of biological active males and females differ in population: male – 56.4%, and females – 43.6%, in average. Increase of number of males in different age groups is the following: 55.3%, 59.9% and 65.5%, correspondingly. Size-weight parameters of males exceed of those of females [length by 12.9%, weight by 17.7% (55.7 mg)]. Size-weight parameters of *P.parva* inhabited in Bazaleti Lake are less than those of fishing reservoirs of Rumania.

Sexual dimorphism is revealed by the length of fins: length of odd fins of male is 3 mm more, and of even fins - 2.5 mm more than of female. In reproduction period males and females are not distinguished actually. Sexually mature males have sharpened asperities above eye, round upper lip, under eye vertically and along the lower jaw. Sharp, half-moon-shaped spots occur on the end of scales of males and the dorsal fin is darker as compared to females. Generally, males are of darker coloration than females.

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ბაზალეთის ტბაში "მიგრანტი" *Pseudorasbora parva* (Schleg.)-ს ბიოეკოლოგიური თვისებები

შონია ლ., ქოქოსაძე თ.

ზოოლოგიის ინსტიტუტი

(მიღებულია 20.12.2005)

რეზიუმე

ბაზალეთის ტბაში მობინადრე "ჯუჯა" სარეველა თევზი *Pseudorasbora parva*-ს ზომიერად წაგრძელებული სხეულის სიგრძე არ აღემატება 7 სმ. აქვს სწორხაზოვანი გვერდითი ხაზი, რომელზედაც აღინიშნება 37-38 ცალი მუქი ფერის, თითქმის ყველგან ერთნაირი ზომის ციკლოიდური ქერცლი. პოპულაციის სტრუქტურა და მისი დინამიკის ხასიათი ასახავს პოპულაციის სპეციპიკურ კავშირს გარემო ფაქტორებთან. მამრებისა და მდედრების ზრდის ტემპში არ აღინიშნება შესამჩნევი განსხვავება. სქესობრივი მომწიფების ასაკისა და სიცოცხლის ხანგრძლიობის მახასიათებლებით ბაზალეთის ტბის ფსევდორაზბორა მიეკუთვნება I და III ჯგუფის თევზებს (ზამახავევის კლასიფიკაციით). ჩვენი აზრით, თევზების სქესობრივ ზომით სტრუქტურულ ჯგუფებს უნდა დაემატოს IV ჯგუფი, სადაც გაერთიანდება *Pseudorasbora parva*-ს მსგავსი მახასიათებლების მქონე თევზები.

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

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

მითითებული ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

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

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