

DRUŠTVO GENETIČARA SRBIJE

SERBIAN GENETICS SOCIETY



ZBORNIK ABSTRAKATA

III KONGRESA GENETIČARA SRBIJE

Subotica, 30. novembar – 4. decembar 2004. godine

BOOK OF ABSTRACTS

OF THE III CONGRESS OF SERBIAN GENETICISTS

held in Subotica (Serbia) on November 30 – December 4, 2004.



Beograd/Belgrade
2004.

Izdavač/Publisher

Društvo genetičara Srbije, Beograd
Serbian Genetics Society, Belgrade

Urednici/Editors

Dr Janoš Berenji
Dr Snežana Mladenović Drinić

Kompjuterski prelom/Computer prepress

Nadežda Rackov

Tiraž/Number of copies printed

220

Štampa/Printing



feljton, Novi Sad
Stražilovska 17
Tel: 021/622-867

CIP - Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

575(048.3)(082)

КОНГРЕС генетичара Србије (3 ; 2004 ; Суботица)

Zbornik abstrakata (i.e. apstrakata) III kongresa genetičara Srbije : Subotica, 30. novembar - 4. decembar 2004. godine / (organizator) Društvo genetičara Srbije = Book of abstracts of the III Congress of Serbian Geneticists : held in Subotica (Serbia) on November 30 - December 4, 2004 / (organized by) Serbian Genetics Society. - Beograd : Društvo genetičara Srbije, 2004 (Novi Sad : Feljton). - 232 str. ; 24 cm

Tiraž 220. - Tekst uporedno na srp. i engl. jeziku.

a) Генетика - Зборници - Апстракти

COBISS.SR-ID 198714375

Beograd/Belgrade
2004.

III KONGRES GENETIČARA SRBIJE

Subotica, 30. novembar – 4. decembar 2004. godine

III CONGRESS OF THE SERBIAN GENETICISTS

held in Subotica (Serbia) on November 30 – December 4, 2004.

Počasni odbor/Honorary Committee

Katarina Borojević
Vukosava Diklić
Janko Dumanović
Vladimir Glišin
Dragoslav Marinković
Draga Simić
Aleksandar Tucović

Vesna Lazić Jančić
Jelena Milašin
Snežana Mladenović Drinić
Stanka Romac
Gordana Šurlan Momirović
Marina Stamenković Radak
Branka Vasiljević
Mladen Vujošević

Predsedništvo/Chairmanship

Marko Andđelković
Goran Drinić
Vasilije Isajev, *potpredsednik*
Mile Ivanović
Dražen Jelovac, *generalni sekretar*
Jelena Knežević Vukčević
Kosana Konstantinov, *predsednik*
Dragan Škorić
Ljubiša Topisirović
Nada Vučinić

Programski odbor/Scientific Committee

Milosav Babić
Janoš Berenji, *predsednik*
Miodrag Dimitrijević
Đorđe Jocković
Slobodan Jovanović
Jelena Knežević Vukčević

Organizacioni odbor/Organizing Committee

Violeta Andđelković
Goran Bekavac
Jelena Blagojević
Ninoslav Đelić
Desimir Knežević
Snežana Mladenović Drinić, *predsednik*
Mirjana Šijačić Nikolić
Ivana Strahinjić
Dragana Vasić
Nenad Vasić, *potpredsednik*
Branka Vuković Gačić

Sekretarijat /Secretariat

Vladan Ivetić
Ivana Lehocki
Vera Robović
Slaviša Stanković
Marija Živanović

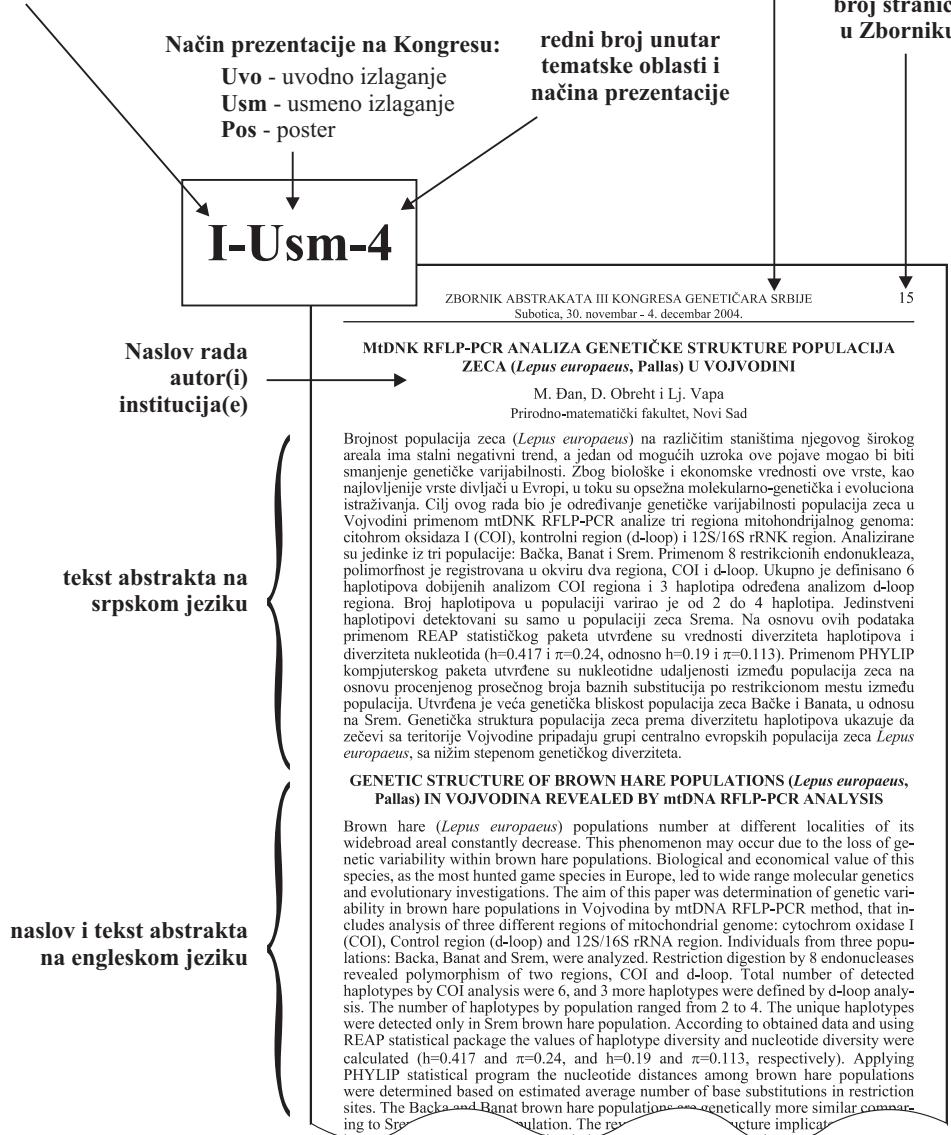
Šema štampanja abstrakata u Zborniku

Oznaka tematske oblasti:

- I Genetičke osnove i zaštita biodiverziteta
- II Struktura i funkcija genoma
- III Genetičko inženjerstvo i biotehnologija
- IV Oplemenjivanje organizama
- V Genetika čoveka i životinja

podaci o publikaciji

broj stranice
u Zborniku



Uvodno predavanje

Opening lecture

VEŠTAČKI HROMOZOM BAZIRAN NA SATELITSKOJ DNK

Gyula Hadlaczky

Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

In vivo konstrukcija animalnog veštačkog hromozoma pretstavlja ponovljivu i efikasnu tehnologiju za konstrukciju stabilnog veštačkog hromozoma baziranog na satelitskoj DNK (SATACs – Stable satellite DNA-based Artificial Chromosomes) definisanog genetičkog sadržaja. SATAC tehnologija se bazira na indukciji *de novo* formiranja hromozoma amplifikacijom na velikoj skali, koja može da se inicira cilnjom integracijom strane DNK u satelit/rDNK region hromozoma domaćina. Ko-amplifikacija sekvenci integracionog mesta ima kao rezultat *de novo* formiranje hromozomskih krakova i nove hromozome koji imaju sekvene satelitske rDNA i egzogene DNK. SATACs su heterohromatinski, ali obezbeđuju pogodnu hromozomalnu okolinu za stabilnu, trajnu ekspresiju integrisanog genetičkog materijala.

SATACs mogu biti konstruisani, prečišćeni i preneseni u ćeliju – primaoca uključujući oplodenu jajnu ćeliju. Transgene životinje su uspešno kreirane prečišćenim SATACs i utvrđena je vertikalna transmisija. Zbog osnovne sličnosti elementarnih mehanizama u ćelijama eukariota, SATAC tehnologija može da se uspešno primeni kod različitih vrsta, uključujući ljudske, animalne i biljne ćelije za *in vivo* konstrukciju veštačkog hromozoma sa odabranim sekvencama.

SATELLITE DNA-BASED ARTIFICIAL CHROMOSOMES

In vivo generation of mammalian artificial chromosomes represents a reproducible and efficient technology for construction of stable satellite DNA-based artificial chromosomes (SATACs) with defined genetic content.

SATAC technology is based on the induction of *de novo* chromosome formations via large-scale amplification, which can be initiated by targeted integration of exogenous DNA into the satellite/rDNA region of host chromosomes. Co-amplification of sequences of the integration site results in *de novo* formed chromosome arms and new chromosomes that composed of exogenous DNA and satellite/rDNA sequences. SATACs are heterochromatic, however, they provide a suitable chromosomal environment for stable, persisting expression of the integrated exogenous genetic material.

SATACs can be engineered, purified and transferred into recipient cells including fertilized eggs. Transgenic animals have successfully been generated with purified SATACs, and the transmission of artificial chromosome through generations has been demonstrated. Because of the basic similarities of the intrinsic cellular mechanisms between eukaryotes, SATAC technology can successfully be applied in different species including human, animal, and plant cells for *in vivo* construction of satellite DNA based artificial chromosomes from predictable sequences.

Due to the rapid development of SATAC technology, by the development of the Artificial Chromosome Expression System, the feasibility of the use of satellite DNA-based artificial chromosome has been established, in different fields of gene technology. Second generation artificial chromosomes represent a novel protein production platform both for cellular protein production and for production of therapeutic molecules in body fluids of transgenic animals; for development of transgenic animals with genetically modified (humanized) tissues and organs for xenotransplantation. Also, stable SATACs with practically unlimited carrying capacity may serve as potential vectors for animal breeding, and for human gene therapy.

I tematska oblast / I topic:

Genetičke osnove i zaštita biodiverziteta

Genetic basis and protection of biodiversity

Uvodna izlaganja / Introductory lectures

I-Uvo

**ZAŠTITA AGROBIODIVERZITETA JUGOISTOČNE EVROPE
– PROJEKAT SEEDNET**

V. Pekić i D. Jelovac

Institut za kukuruz «Zemun Polje», Zemun

Održivo očuvanje i korišćenje biljnih genetičkih resursa (BGR) je kako je navedeno u Konvenciji o biološkom diverzitetu (CBD), nacionalna obaveza. Primena zahteva iz CBD i ispunjavanje obaveza iz FAO globalnog akcionog plana i Međunarodnog sporazuma o BGR za ishranu i poljoprivrednu (MSBGRIP), je glavni zadatak zemalja i ostalih zainteresovanih za očuvanje BGR iz celog sveta. Nacionalni programi o genetičkim resursima čine platformu za saradnju u razmeni materijala, znanja i iskustva među zainteresovanim stranama, kao i za izgradnju kapaciteta na nacionalnom nivou. Nacionalni programi, takođe, čine osnovu regionalnih i međuregionalnih povezivanja u zajedničkim naporima za obezbeđivanje dugoročnog očuvanja BGR. Regionalne i internacionalne mreže imaju zadatak da obezbede razmenu i transfer tehnologije, materijala i informacija. Strategije čuvanja i korišćenja BGR na lokalnim, nacionalnim, regionalnim i međunarodnim nivoima su najcešći u postizanju maksimalnih efekata, kada su komplementarne i međusobno integrisane zajedničkim planiranjem i primenom. Ciljevi projekta su: doprinos uspostavljanju i jačanju nacionalnih programa u BGR u cilju očuvanja zaštite BGR regiona; promocija održivog korišćenja BGR; jačanje saradnje, povezivanja i veza između zainteresovanih učesnika na nacionalnom i regionalnom nivou kroz objedinjavanje resursa i korišćenja uporednih prednosti prisutnih kod različitih institucija i zemalja.

**SOUTH-EUROPEAN AGROBIODIVERSITY PROTECTION
– SEEDNET PROJECT**

Sustainable conservation and utilisation of plant genetic resources (PGR) is as stated in the Convention of Biological Diversity (CBD) a national undertaking. To implement the provisions of the CBD and to fulfil the commitments of the FAO Global Plan of Action (GPA) and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) is a major challenge for the countries and stakeholders of PGR all around the world. National genetic resources programmes constitute the platform for co-operation and exchange of material, knowledge and experience among the stakeholders as well as capacity building at the national level. National programmes also constitute the backbone of regional and international networking in mutual efforts to ensure the long-term conservation of PGR. The regional and international networks are the facilitators of exchange and transfer of technology and exchange of material and information. Conservation and utilisation strategies at the community, national, regional and international levels are most effective when they are complementary, and integrated with each other during planning and implementation in order to achieve maximum effect. The aims of the project are: to contribute to the establishment and strengthening of national programmes on PGR in order to secure the conservation of PGR in the region; to promote a sustainable utilisation of PGR; and to strengthen collaboration, networking and linkages among various stakeholders at both national and regional levels through pooling of resources and use of comparative advantages available in the various institutions and countries.

BIODIVERZITET DOMESTICIRANIH ŽIVOTINJA

S. Jovanović, Ružica Trailović i Mila Savić

Katedra za stočarstvo i genetiku, Fakultet veterinarske medicine, Beograd

Uprkos velike raznovrsnosti životinjskog sveta samo četrdesetak vrsta je domesticirano i gaji se u cilju zadovoljenja potreba različitih potreba čoveka. Domesticirani animalni biodiverzitet predstavlja spektar genetičke raznovrsnosti, koji uključuje preko 6000 različitih rasa od kojih svaka ima posebne karakteristike i namenu. Zbog intenzifikacije stočarske proizvodnje tokom poslednjih vekova su selekcionirane specijalizovane, visokoproduktivne rase, koje potiskuju manje selekcionirane autohtone rase, tako da je samo u poslednjih 50 godina preko 65% rasa u svetu postalo ugroženo, a mnoge su iščezle. Autohtone rase domaćih životinja, nastale u toku dugotrajnog procesa evolucije, dobro prilagođene datim uslovima gajenja, mogu u budućnosti predstavljati važan izvor varijabilnosti za poboljšanje otpornosti visokoproduktivnih rasa. Zbog toga je u okviru proučavanja i zaštite globalnog biodiverziteta, koje koordinira FAO i UNEP, uključeno i proučavanje i zaštita animalnih genetskih resursa gajenih vrsta životinja, koji mogu biti značajni u obnavljanju domesticiranog genofonda. Ključni element strategije očuvanja animalnih resursa predstavlja genetička karakterizacija rasa u cilju dobijanja kompletne slike genetičke varijabilnosti unutar svake vrste domaćih životinja. Opšte prihvaćeno mišljenje je da se merenjem genetske distance obezbeđuju trenutno najbolje informacije, koje omogućavaju rangiranje rasa u okviru vrste. Zahvaljujući brzom razvoju tehnike za mapiranje i izolaciju gena, upotreba animalnih genetskih resursa u budućnosti će biti zasnovana na identifikaciji gena koji su u vezi sa specifičnim fenotipskim karakteristikama.

DOMESTIC ANIMAL BIODIVERSITY

Although, the degree of diversity of animal species is extensive, only around 40 species had been domesticated and used to fulfill different human needs. Domesticated animal biodiversity represents a broad spectrum of genetic differences distributed among over 6000 breeds of domesticated mammals and birds, each representing a unique set of genes. The intensification of animal production during the last centuries gave force to the selective breeding which resulted in the development of specialised, highly-producing breeds. The interest to breed less productive autochthonous breeds compared to high-producing ones is lost and the trend of erosion of genetic diversity is global. Only in last 50 years around 65% of catalogized breeds became endangered or vulnerable, and some are already extinct. Autochthonous breeds of domestic animals have resulted during long evolution directed by human demands and adaptation to the local environment, environmental changes and challenges. Therefore they can serve as the source of variables for improvement of resistance in highly-selected breeds in unpredictable selective challenges in future. This was the reason that introduced the evaluation and protection of the domesticated gene pool into the programme of the protection of the global biodiversity coordinated by UNEP and FAO. The genetic characterization of the separate breeds in aim to fulfill the data of genetic variability within each domesticated species is the key element of the conservation strategy. The wide accepted opinion is that most comparable data can be obtained by evaluation of the genetic distance among breeds. Due to the development of the techniques for genetic mapping and isolation of the genes, one of the uses of domestic animal resources in the future would be based upon identification of the genes which are linked to favourable phenotypic character.

I tematska oblast / I topic:

Genetičke osnove i zaštita biodiverziteta

Genetic basis and protection of biodiversity

Usmena izlaganja/oral presentations

I-Usm

BIODIVERZITET RATARSKIH KULTURA U CRNOJ GORI

M. Dimitrijević i Sofija Petrović

Katedra za genetiku i oplemenjivanje biljaka, Departman za ratarstvo i povrtarstvo,
Poljoprivredni fakultet, Novi Sad

Intenzifikacija poljoprivredne proizvodnje, «zelena revolucija», je donela nove visoko prinosne sorte i hibride ratarskih kultura i saglasno sa tim i intenzivniji način proizvodnje. Prosečni prinosi pojedinih ratarskih kultura su povećani 2-3 puta. Međutim, gajenje intenzivnih sorti i hibrida na velikim površinama, značajno je uticalo na sužavanje biodiverziteta ratarskih kultura i spontanih srodnika. Promena strukture setve je dovela do nepovratnog gubljenja mnogih lokalnih populacija poljoprivrednog bilja, koje su generacijama gajene u pojedinim regionima. Ove populacije se nisu odlikovale visokim prinosom, često su bile dobrog kvaliteta, posebno po današnjim kriterijumima tzv. «zdrave hrane» i bile su dobro adaptirane na lokalne uslove gajenja. Ispitivanjem na teritoriji Crne Gore je ustanovljeno da se erozija biodiverziteta ratarskih i povrtarskih kultura javlja i u krajevima koji nisu izrazito poljoprivredni regioni. Stare sorte i spontani srodnici se povlače pred novostvorenim visokoprinosnim sortama i hibridima, kao i pred korišćenjem poljoprivrednog zemljišta u druge svrhe. U radu su dati rezultati ekspedicije ispitivanja biodiverziteta i sakupljanja uzoraka na teritoriji Crne Gore tokom 2004.

FIELD CROPS BIODIVERSITY IN MONTENEGRO

The advancement in agriculture, so called green revolution, has brought up a number of new high yielding field crop varieties and hybrids, as well as, remarkable changes in the intensity of agricultural production. The average yield of many crops increased 2-3 times. However, high yielding varieties and hybrids being grown on a large scale, significantly narrowed biodiversity of field crops and spontaneous relatives. That led to irreversible lost of many local populations that had been for generations in certain regions. These local populations were not high yielding, but non-rarely they had good quality, particularly according to today's criteria of «healthy food» and they were well adapted to local growing conditions. Examining biodiversity of field crops in Montenegro, remarkable erosion was noted. That trend was observed not only in agricultural areas, but also in the mountain region. Old varieties and local population had to retreat facing high yielding varieties, the products of modern breeding programs, or usage of agricultural productive land for other purposes. The article reveals the results of biodiversity examining and collecting expedition conducted in Montenegro territory in 2004.

UTVRĐIVANJE GENETIČKE DISTANCE ODABRANIH VRSTA RODA PICEA NA OSNOVU VARIJABILNOSTI TERPENOIDNOG SASTAVA ETARSKIH ULJA ČETINA

V. Isajev

Šumarski fakultet, Beograd

U cilju ispitivanja genetske distance i mogućnosti hemotaksonomskog doprinosa bližem upoznavanju međuvrsne prmenljivosti četiri vrste roda *Picea* – *Picea orientalis* L., *Picea jozoenensis* (Seib et Zucc) Carr., *Picea sitchensis* (Bong.) Carr i *Picea omorika* (Panč.) Purkyne, koje pripadaju sekcijama *Eupicea*, *Casicta*, *Omorika* obavljena je analiza terpenoidnog sastava etarskih ulja četina. Dvogodišnje četine *P. orientalis*, *P. jezoensis* i *P. sitchensis* su sakupljene sa više stabala iz kulturnih zajednica u Srbiji, a četine omorike sa šireg autohtonog staništa u Nacionalnom parku Tara. Iz sveže sakupljenih četina destilacijom vodenom parom dobijena su etarska ulja u različitim prinosima. Gasno-hromatografskom analizom primenom nepolarne kvarcne kolone DB-5, 30m; na temperaturi 65°C, utvrđen je preliminarni terpenoidni sastav isparljivih ulja iz četina. Biohemija veza između značajnih terpenskih komponenti etarskih ulja predstavljala je osnovu: za upoznavanje genetske distance između izabranih vrsta, upoznavanje mogućnosti njihovog šireg korišćenja u biotehnologiji kao i za dalja hemotaksonomska i evolutivna istraživanja roda *Picea*.

DETERMINATION OF THE GENETIC DISTANCE OF SELECTED SPECIES GENUS PICEA BASED ON THE VARIABILITY OF NEEDLE OIL TERPENE COMPOSITIONS

The aim of research in this paper was to analyze the terpene compositions of volatile oils from needles of four species *Picea orientalis* L., *Picea jezoensis* (Seib et Zucc) Carr., *Picea sitchensis* (Bong.) Carr i *Picea omorika* (Panč.) Purkyne. Selected species belong to different sections of genus *Picea* - *Eupicea*, *Casicta*, *Omorika*. The two years old needles were collected at the end of vegetation period of *Picea omorika*, from the locality in the wider native range at the National Park Tara, of *P.sitchensis*, *P. jozoenensis* and *P. orientalis*, from the culture in Serbia. The ether solutions of volatile oils were prepared and analyzed by GLC with a Perkin-Elmer Sigma 2B gas chromatograph, equipped with a FID and Shimadzu C-RIB data system.

The biosynthetic relationship of major terpenes of volatile needle oils and their significance for determination of the genetic distance of selected species, further use in biotechnology, and for the chemotaxonomy of the examined spruces were discussed.

GENETIČKA VARIJABILNOST I RAZVOJNA STABILNOST

M. Stamenković-Radak¹, G. Rašić¹, I. Tomišić-Kosić² i M. Andđelković^{1,2}

¹Biološki fakultet, Univerzitet u Beogradu, Beograd

²Institut za biološka istraživanja «S. Stanković», Univerzitet u Beogradu, Beograd

U ovom radu prikazani su rezultati dobijeni u eksperimentima sa vrstama i populacijama *Drosophila*, dizajniranim tako da otkrivaju relativni značaj tri tipa genomskog stresa i njihove moguće veze sa razvojnom nestabilnošću:

- Efekat inbridinga na varijabilnost u FA kod nekoliko morfometrijskih karaktera kod *Drosophila subobscura*
- Efekat inbridinga i homozigotnosti za određene genske aranžmane na O hromozomu kod *D.subobscura*
- Efekat interspecijske i interpopulacione hibridizacije među sestrinskim vrstama *Drosophila* na fenotipsku varijabilnost i razvojnu stabilnost hibrida.

Genetičke koadaptacije i heterozigotnost se smatraju mehanizmima koji utiču na razvojnu homeostasis. Opseg genetičke varijabilnosti pogodjene stresom je i dalje slabo poznat, a varijabilnost hromozomske inverzije *Drosophila* i interspecijske hibridizacije u odnosu na FA su slabo proučeni. Mada se eksperimentalni uslovi stresa razlikuju od onih koje poulacije sreću u prirodnim staništima, ovde dobijeni rezultati delimično osvetljavaju mehanizme putem kojih organizmi i populacije odolevaju i/ili se prilagođavaju stresnim uslovima. Uvođenje manipulisanih genoma u populacije je dodatna vrsta genomskog stresa koja može imati uticaja na genetičku strukturu populacija i uticati na biodiverzitet. U tom smislu, fokus konzervacione biologije se pomera ka populaciono genetičkim aspektima stresa.

GENETIC VARIABILITY AND DEVELOPMENTAL STABILITY

In the present study we give the results obtained using *Drosophila* species and populations in the experiments designed to reveal the importance of three kinds of genomic stress and their possible relation to developmental stability:

- The effect of inbreeding on the variability in FA in several morphometric characters in *Drosophila subobscura*
- The effect of inbreeding and homozygosity for certain gene arrangements on O chromosome of *D.subobscura* on FA in wing size parameters.
- The effect of interspecific and interpopulation hybridization among sibling *Drosophila* species on the phenotypic variability and developmental stability of hybrids.

Genetic coadaptation and heterozygosity are assumed to be the mechanisms that affect developmental homeostasis. The range of genetic variability affected by stress is still unknown and chromosome inversion variability in *Drosophila* and interspecific hybridization are rather unexplored in relation to FA. Although experimental conditions of stress differ from the ones that populations meet in natural habitats, the results obtained shed light on the mechanisms by which organisms and populations resist and/or adapt to stressful conditions. Introduction of manipulated genomes into populations is an additional kind of genomic stress which can affect the genetic structure of populations and influence biodiversity. Along these lines, the focus of conservation biology is moving toward a population-genetic aspect of stress.

**MtDNK RFLP-PCR ANALIZA GENETIČKE STRUKTURE POPULACIJA
ZECA (*Lepus europaeus*, Pallas) U VOJVODINI**

M. Đan, D. Obreht i Lj. Vapa

Prirodno-matematički fakultet, Novi Sad

Brojnost populacija zeca (*Lepus europaeus*) na različitim staništima njegovog širokog areala ima stalni negativni trend, a jedan od mogućih uzroka ove pojave mogao bi biti smanjenje genetičke varijabilnosti. Zbog biološke i ekonomske vrednosti ove vrste, kao najlovljenije vrste divljači u Evropi, u toku su opsežna molekularno-genetička i evoluciona istraživanja. Cilj ovog rada bio je određivanje genetičke varijabilnosti populacija zeca u Vojvodini primenom mtDNK RFLP-PCR analize tri regiona mitochondrialnog genoma: citohrom oksidaza I (COI), kontrolni region (d-loop) i 12S/16S rRNA region. Analizirane su jedinke iz tri populacije: Bačka, Banat i Srem. Primenom 8 restrikcionih endonukleaza, polimorfnost je registrovana u okviru dva regiona, COI i d-loop. Ukupno je definisano 6 haplotipova dobijenih analizom COI regiona i 3 haplotipa određena analizom d-loop regiona. Broj haplotipova u populaciji varirao je od 2 do 4 haplotipa. Jedinstveni haplotipovi detektovani su samo u populaciji zeca Srema. Na osnovu ovih podataka primenom REAP statističkog paketa utvrđene su vrednosti diverziteta haplotipova i diverziteta nukleotida ($h=0.417$ i $\pi=0.24$, odnosno $h=0.19$ i $\pi=0.113$). Primenom PHYLIP kompjuterskog paketa utvrđene su nukleotidne udaljenosti između populacija zeca na osnovu procenjenog prosečnog broja baznih substitucija po restrikpcionom mestu između populacija. Utvrđena je veća genetička bliskost populacija zeca Bačke i Banata, u odnosu na Srem. Genetička struktura populacija zeca prema diverzitetu haplotipova ukazuje da zecëvi sa teritorije Vojvodine pripadaju grupi centralno evropskih populacija zeca *Lepus europaeus*, sa nižim stepenom genetičkog diverziteta.

**GENETIC STRUCTURE OF BROWN HARE POPULATIONS (*Lepus europaeus*,
Pallas) IN VOJVODINA REVEALED BY mtDNA RFLP-PCR ANALYSIS**

Brown hare (*Lepus europaeus*) populations number at different localities of its widebroad areal constantly decrease. This phenomenon may occur due to the loss of genetic variability within brown hare populations. Biological and economical value of this species, as the most hunted game species in Europe, led to wide range molecular genetics and evolutionary investigations. The aim of this paper was determination of genetic variability in brown hare populations in Vojvodina by mtDNA RFLP-PCR method, that includes analysis of three different regions of mitochondrial genome: cytochrome oxidase I (COI), Control region (d-loop) and 12S/16S rRNA region. Individuals from three populations: Backa, Banat and Srem, were analyzed. Restriction digestion by 8 endonucleases revealed polymorphism of two regions, COI and d-loop. Total number of detected haplotypes by COI analysis were 6, and 3 more haplotypes were defined by d-loop analysis. The number of haplotypes by population ranged from 2 to 4. The unique haplotypes were detected only in Srem brown hare population. According to obtained data and using REAP statistical package the values of haplotype diversity and nucleotide diversity were calculated ($h=0.417$ and $\pi=0.24$, and $h=0.19$ and $\pi=0.113$, respectively). Applying PHYLIP statistical program the nucleotide distances among brown hare populations were determined based on estimated average number of base substitutions in restriction sites. The Backa and Banat brown hare populations are genetically more similar comparing to Srem brown hare population. The revealed genetic structure implicates that brown hare populations from Vojvodina belong to Central European brown hare population group, characterized by lower level of genetic diversity.

ADAPTIVNOST ILI STABILNOST Amy-GENOTIPOVA *Drosophila subobscura* U USLOVIMA KONTINUIRANIH I NAGLIH PROMENA KONCENTRACIJE SKROBA U HRANLJIVOM MEDIJUMU

Tatjana Savić¹, Marina Stamenković-Radak² i M. Anđelković^{1,2}

¹Institut za biološka istraživanja «Siniša Stanković», Beograd

²Biočistički fakultet, Univerziteta u Beogradu, Beograd

Analizom fenotipske varijabilnosti Amy-genotipova *Drosophila subobscura* na različitim koncentracijama skroba, kao model-sistema, mogu se razmotriti oblici, pravci i efekti interakcije genotip/sredina. U našim istraživanjima analiziran je uticaj postepenih i naglih promena koncentracije skroba na specifičnu aktivnost α – amilaze, dužinu razvića i preživljavanje kod jedinki *Drosophila subobscura* homozigotnih ili heterozigotnih za »spori« i »brzi« amilazni alel.

Prosečne vrednosti za specifičnu aktivnost α - amilaze, dužinu razvića i preživljavanje u uslovima postepenih i naglih promena koncentracije skroba ukazuju na opštu prosečnu genetičku varijabilnost fenotipova koji je pod značajnim uticajem sredine (različita koncentracija skroba) i posmatrane karakteristike su fenotipski plastične. Norme reakcija za prosečne vrednosti analiziranih karakteristika takođe ukazuju na postojanje značajnog sredinskog uticaja, kao i da je u njihovoj osnovi genetička varijabilnost jedinki *Drosophila subobscura*.

Rezultati dobijeni na osnovu prosečnih indeksa fenotipske plastičnosti za specifičnu aktivnost α -amilaze, dužinu razvića i preživljavanje navode na zaključak da jedinke *Drosophila subobscura* u svakoj sledećoj generaciji, odnosno sa povećanjem skroba u hranljivom supstratu, adaptibilno odgovaraju u pogledu preživljavanja, za razliku od specifične aktivnosti α -amilaze i dužine razvića čiji prosečni indeksi fenotipske plastičnosti ukazuju na stabilniji odgovor.

ADAPTABILITY OR STABILITY OF Amy-GENOTYPES OF *Drosophila subobscura* IN DISCRETE AND IMMEDIATE CHANGES OF STARCH CONCENTRATIONS IN NUTRITIVE SUBSTRATE

Through the analysis of phenotypic variability of Amy-genotypes of *Drosophila subobscura* on different starch concentrations, as a model-system, the shapes, directions and effects of genotype/environment interactions can be determined. Our research includes the study of the effect of discrete and immediate changes of starch concentrations on α – amylase specific activity, development time and viability of *Drosophila subobscura*, homozygous or heterozygous for »slow« and »fast« amylase allele.

The mean values of α – amylase specific activity, development time and viability under discrete and immediate changes of starch concentrations, show that the overall genetic variability of phenotypes is under significant environmental influence (different starch concentrations) and that studied characters show phenotypic plasticity. Norms of reactions for the mean values of analyzed traits also point towards existence of significant environmental influence and individual genetic variability of *Drosophila subobscura*.

The results obtained for the mean indices of phenotypic plasticity for α - amylase specific activity, development time and viability, suggest that *Drosophila subobscura* flies, through each succeeding generation and increased starch concentrations in substrate, show adaptive response regarding viability, but their mean indices of phenotypic plasticity for development time and specific activity of the enzyme indicate the more stable response.

VARIJABILNOST KOMPOZICIJE *Gli*-ALELA KOD PŠENICE (*Triticum aestivum L.*)

D. Knežević¹, Aleksandra Yurievna-Dragovich² i Nevena Đukić³

¹Institut za istraživanja u poljoprivredi SRBIJA, Centar za strnu žita, Kragujevac

²Institut opšte genetike, Moskva, Rusija

³Prirodno-matematički fakultet Kragujevac, Institut za biologiju, Kragujevac

Analizirana je kompozicija glijadina kod 25 sorti pšenice stvorenih u Kragujevačkom selekcionom centru. Aleli na *Gli-1* i *Gli-2* lokusima su identifikovani na bazi blokova komponenti glijadina. U proučavanjima je identifikovano ukupno 30 glijadinskih alela i to: 4 na *Gli-A1*, 6 na *Gli-B1*, 6 na *Gli-D1*, 5 na *Gli-A2*, 5 na *Gli-B2* i 4 alela na *Gli-D2* lokusu. Učestalost identifikovanih alela bila je različita. Najfrekventniji aleli bili su: *Gli-A1a* (44,00%), *Gli-B1b*, (48,00 %), *Gli-D1b* (56,00 %), *Gli-A2b* (36,00 %), *Gli-B2b* (60,00 %), *Gli-D2b* (60,00 %). Visoka učestalost verovatno je rezultat ograničene (sužene) germ-plazme korišćene pri ukrštanju a takođe i visoke adaptivnosti genotipova koji su imale ove genske alele, zbog čega su ih oplemenjivači češće odabirali u toku procesa oplemenjivanja.

VARIABILITY OF *Gli*-ALLELLE COMPOSITION IN WHEAT (*Triticum aestivum L.*)

The gliadin composition in 25 wheat cultivars created in Kragujevac's breeding Center was analyzed. Alleles at *Gli-1* and *Gli-2* loci were identified on the base of gliadin block components. The 30 alleles were identified namely: 4 at *Gli-A1*, 6 at *Gli-B1*, 6 at *Gli-D1*, 5 at *Gli-A2*, 5 at *Gli-B2*, 4 at *Gli-D2*. Frequency of identified alleles was different. The most frequent alleles have been *Gli-A1a* (44,00%), *Gli-B1b*, (48,00 %), *Gli-D1b* (56,00 %), *Gli-A2b* (36,00 %), *Gli-B2b* (60,00 %), *Gli-D2b* (60,00 %). The high value of frequency is likely results of narrow germplasm which used for cossing and higher adaptability of genotypes that carried those alleles because breeders selected frequently those genotypes during breeding process.

GLIADIN CODING GENES OF BREAD WHEAT AS GENETIC MARKERS OF ADAPTABILITY OF CULTIVAR

Dragovich-Novoselskaya A.Yu.

Institute of General Genetics RAS, Moscow, Russia

Grain storage proteins of bread wheat *Triticum aestivum*, gliadins, separated by PAAG electrophoresis are reliable genetic markers used in molecular, genetic and breeding research. There are six unlinked gliadin-codin loci. A vast multiple allelism has been described at each of these loci that permit high-precision identification of cultivar genotypes in terms of gliadin allele composition. As was shown earlier gliadins can be used as genetic markers of bread making quality, which means that they are linked to genes controlling valuable traits.

In this report we present results of the examined genetic diversity of bread wheat cultivars, bred in the Saratov plant breeding center during more than last 80 years of the scientific breeding programs. The aim of our study was to determine the genotypes most adapted to agro-ecological conditions in the region.

In the early 20th century Saratov scientific breeding was based on Poltavka, which was local cultivar-population characterized by rather high intracultivar polymorphism (table.) In the middle of 20th century on the basis of Poltavka the best Saratov cultivars (e.g. Saratovskaya 29) have been developed. About 62% of these cultivars carried biotype gained from Poltavka (f.e.a.q.s.e.).

Cultivars	Loci/ alleles					
	Gli-A1	Gli-B1	Gli-D1	Gli-A2	Gli-B2	Gli-D2
Poltavka (1930s)	o,f,c, j	e, m	a	q	o, s	e, a
Poltavka (2003)	f	E	a	q	s	e
Cultivars (1950s).	f,i	E	a	q,s	s,o, q	e
Modern cultivars*	f,i,m	E	a,k	q,o,	q,w,s,	e

*6 new alleles have been found that had not been registered earlier in a catalogue. Bold letters denote the main Poltavka biotype.

Modern Saratov cultivars, bred in the last decade, are more heterogeneous than the previous group because of extensively involving alien genetic material. This group comprises 19 alleles, 6 out of them are new, gained from foreign cultivars-donors. Frequencies of these new alleles have been increasing from year to year. However, the same Poltavka biotype (f.e.a.q.s.e.) occurs in the group of modern cultivars constituting about 50% of all biotypes (table). Nowadays, Poltavka which is no more used in breeding, has been grown as collection pattern from the 1950s till now. This pattern has become a line carrying only one biotype (f.e.a.q.s.e.), which is more frequent among different Saratov cultivar groups. Probably, this elimination of other biotypes is caused by natural selection rather than the absence of artificial selection (e.g. for bread-making). Apparently, high frequency of the biotype in all the groups can be explained by the fact that definite multilocus combinations marked by definite gliadin biotypes are selected for particular environment.

The genetic diversity in modern cultivars is twice as high as in the old ones (0.4 and 0.2, respectively). Intracultivar polymorphism of modern cultivars is 2.2 times higher. Such high heterogeneity may be attributed to the fact that the large number of genotypes increase adaptation of cultivars comprising alien (non adaptive) genetic material.

DIVERZITET I ZAŠTITA AUTOHTONIH EKOTIPOVA VRSTE *Apis mellifera carnica* U SRBIJI

Jevrosima Stevanović¹, Z. Stanimirović¹ i Slobodan Jovanović²

¹Katedra za biologiju, Fakultet veterinarske medicine, Beograd

²Katedra za stočarstvo i genetiku životinja, Fakultet veterinarske medicine, Beograd

Ovaj rad je rezultat komparativne citogenetičke, morfometrijske i bihevioralne istraživanja tri ekotipa *Apis mellifera carnica* na teritoriji Srbije. Adultne radilice uzorkovane su sa 30 različitih lokaliteta iz tri regiona u Srbiji, pri čemu su ti regioni međusobno udaljeni oko 200 km i različiti su klimatskim, geografskim i florističkim karakteristikama. Analizom varijanse (ANOVA) za svaki karakter u celokupnom uzorku utvrđene su statistički vrlo visoko signifikantne razlike kod čak 22 morfometrijska karaktera ($p<0.001$) i visoko signifikantne razlike kod 4 karaktera ($p<0.01$). Rezultati citogenetičkih istraživanja ukazali su na postojanje velikog inter- i intraekotipskog diverziteta G-traka na hromozomima ispitivanih ekotipova pčela. Najveće razlike u broju i distribuciji G-traka zabeležene su kod hromozoma 1, 2, 4, 11, 12, 13, 15 i 16. Pored toga, između ispitivanih ekotipova i unutar svakog od njih utvrđeno je postojanje razlika u ispoljenosti higijenskog i negovateljskog ponašanja, pri čemu se potencijal higijenskog ponašanja kretao u opsegu od 81.24% do 99.50%, a potencijal negovateljskog ponašanja od 20.68% do 42.14%. Na osnovu utvrđenog citogenetičkog, morfometrijskog i bihevioralnog diverziteta, može se zaključiti da analizirani ekotipovi A. m. carnica predstavljaju izuzetan genetički resurs. Da bi se sačuvalo takav diverzitet, u Srbiji je neophodna zaštita ovih ekotipova, na primer formiranjem izolovanih pčelinjaka na područjima sa kojih oni potiču.

DIVERSITY AND CONSERVATION OF INDIGENOUS *Apis mellifera carnica* ECOTYPES IN SERBIA

Comparative cytogenetic, morphometric and behavioural investigations of three honey bee ecotypes of *Apis mellifera carnica* from Serbia were performed. Adult honey bee workers were sampled from 30 different localities in Serbia, allocated in three regions, some 200 km apart, with different climatic, geographic and floristic features. The analysis of variance (ANOVA) for each character in whole specimen revealed statistically highly significant differences ($p<0.001$) in the means of 22 morphometric characters and significant differences ($p<0.01$) in the means of 4 characters. The results of cytogenetic investigations point to a great inter- and intraecotype diversity of G-bands on chromosomes of investigated honey bee ecotypes. The greatest differences in G-band number and distribution were registered in chromosomes 1, 2, 4, 11, 12, 13, 15 and 16. Moreover, differences in exertion of hygienic and grooming behaviors were affirmed within and among investigated ecotypes, with potential of hygienic behaviour ranged from 81.24% to 99.50%, and potential of the grooming behaviour ranged from 20.68% to 42.14%. Considering affirmed cytogenetic, morphometric and behavioural diversity it can be concluded that analysed indigenous ecotypes A. m. carnica represent a remarkable genetic resource. To preserve such honey bee diversity, conservation of those ecotypes in Serbia is necessary, for example by forming segregated apiaries in their original regions.

**DIVERZITET AUTOHTONIH IZOLATA *Rhizobium leguminosarum* bv. *trifolii*
IZ HUMISOLA UTVRĐEN PCR METODOM**

Dragana Jošić¹, Bogić Miličić¹, Snežana Mladenović-Drinić² i Mirjana Jarak³

¹Institut za zemljište, Beograd

²Institut za kukuruz «Zemun Polje», Zemun

³Poljoprivredni fakultet, Novi Sad

Brojne procedure bazirane na lančanoj reakciji polimeraze (PCR) korišćene su za dobijanje genomskog «fingerprinta» kod rizobia. Ove tehnike poseduju brojne prednosti u odnosu na ranije tehnike tipizacije preko korišćenja nutritivnih komponenata, rezistentnosti na antibiotike i tolerantnosti na teške metale, kao i analiza plazmidnog profila. U ovom radu korišćene su REP-PCR i RAPD metode da bi se ustanovio stepen diverziteta autohtonih izolata *Rhizobium leguminosarum* bv. *trifolii* iz humisola. Izolovano je 30 bakterijskih izolata sa markiranih lokaliteta na humisolu i testirano na IAR-HMT rezistentnost, plazmidni sadržaj i obavljene su PCR analize. Dobijeno je 12 različitih izolata. REP-PCR analiza je potvrdila da izolati pripadaju istom biovarijetu *R.leguminosarum*, a RAPD analiza je potvrdila njihove međusobne razlike. Dobijeni rezultati su pokazali visok stepen diverziteta divlje populacije *Rhizobium leguminosarum* bv. *trifolii* u humisolu.

PCR BASED DIVERSITY LEVEL ESTIMATION OF INDIGENOUS *Rhizobium leguminosarum* bv. *trifolii* FROM HUMISOL

Numerous polymerase chain reaction (PCR) based procedures are used to produce genomic fingerprints in rhizobia. These techniques possess several advantages over more common cell-typing methods such as nutritional traits, resistance to antibiotics and plasmid profiles. We used rep-PCR and RAPD to estimated biodiversity level of *Rhizobium leguminosarum* bv. *trifolii* from humisol. We isolated 30 bacterial isolates from marked site of humisol and tested to IAR-HMT resistance, plasmid contents and performed PCR analysis. The 12 different isolates were obtained. We used the ability of REP-PCR to confirm the isolates belong in the same biovar of *R. leguminosarum* and RAPD to discriminate it. The obtained resultes showed vide diversity of field population indigenous *Rhizobium leguminosarum* bv. *trifolii*.

I tematska oblast / I topic:

Genetičke osnove i zaštita biodiverziteta

Genetic basis and protection of biodiversity

Posteri / Posters

I-Pos

EX SITU KONZERVACIJA GENETIČKIH RESURSA POLJSKOG BRESTA (*Ulmus minor* Mill.) I VEZA (*U. laevis* Pall.)

Jelena Aleksić i S. Orlović

Univerzitet u Novom Sadu, Poljoprivredni Fakultet,
Institut za nizijsko šumarstvo i životnu sredinu, Novi Sad

Cilj našeg istraživanja je ex situ konzervacija genetičkih resursa poljskog bresta (*Ulmus minor* Mill.) i veza (*U. laevis* Pall.) putem osnivanja poljskih banaka gena. Obe vrste su osetljive na Holandsku bolest brestova (DED) koju izaziva gljiva *Ophiostoma novo-ulmi* Brasier, iako je druga vrsta više ugrožena narušavanjem prirodnih staništa.

Uzorkovanje je sprovedeno u po jednoj populaciji poljskog bresta i veza. Navedena populacija poljskog bresta, pored pojedinačnih, nasumično raspoređenih stabala, predstavlja jedinu grupu stabala na području Gazdinske Jedinice Vinična-Žeravinac-Puk (ukupna površina 3.552,81ha). Populacija veza se nalazi na površini od 2,43ha i predstavlja jedinu populaciju veza, pored pojedinačnih, nasumično raspoređenih stabala, na području Gazdinske jedinice Palanačke ade-Čipski poloj (ukupna površina 1.283,03ha). Biljni materijal (pupoljci) sa 9 stabala poljskog bresta i 10 stabala veza je iskorišćen za vegetativnu in vitro propagaciju. Dobijeni klonovi su iskorišćeni za osnivanje poljskih banaka gena na oglednom dobru Instituta za nizijsko šumarstvo i životnu sredinu. Mogućnost naknadne infekcije fungalnim patogenom će se izbeći sadnjom klonova na manjem rastojanju od uobičajenog, pošto je pokazano da tako sađena stabla nisu atraktivna za brestove potkornjake (*Scolytus* sp.) koji su vektori bolesti.

Rezultati istraživanja su pokazali da su *in vitro* tehnike pogodne za masovnu proizvodnju klonova obe vrste, kao i da se osnivanjem poljskih banaka gena može uspešno realizovati ex situ konzervacija genetičkih resursa navedenih vrsta.

EX SITU CONSERVATION OF GENETIC RESOURCES OF FIELD ELM (*Ulmus minor* Mill.) AND EUROPEAN WHITE ELM (*U. laevis* Pall.)

The aim of our research is *ex situ* conservation of genetic resources of field elm (*Ulmus minor* Mill.) and European white elm (*U. laevis* Pall.) through establishment of field genebank. Both species are susceptible to Dutch Elm Disease (DED) caused by the vascular wilt fungus *Ophiostoma novo-ulmi* Brasier, although the later one is more seriously threatened by the destruction of the natural habitat.

Sampling was conducted in one population of field elm and white elm, respectively. Population of field elm represents the only one cluster of elm trees, besides solitary, randomly spread trees, in whole Management Unit Vinična-Žeravinac-Puk (total area 3.552,81ha). Population of white elm is spread on 2,43ha and represents the only one population of elms, besides solitary, randomly spread trees, in whole Management Unit Palanačke ade-Čipski poloj (total area 1.283,03ha).

Plant material (buds) from 9 trees of field elm and 10 trees of white elm was used for *in vitro* production of clones. Clones were used for establishment of field genebank at Institute's of lowland forestry and environment experimental estate. The possibility of further infection with DED was pass over by planting clones in low hedges, which are unattractive for the vector of the fungal pathogen – elm bark beetle (*Scolytus* sp.).

Results pointed out that *in vitro* techniques are suitable for production of clones in short time for both elm species and that is possible to preserve elm genetic resources through establishment of field genebank.

ANTIGENOTOKSIČNI EFEKAT TERPENA IZ ŽALFIJE

T. Berić-Bjedov, J. Knežević-Vukčević, B. Vuković-Gačić, G. Joksić¹,
D. Mitić-Ćulafić, B. Nikolić, J. Stanojević, S. Stanković, O. Stajković i D. Simić
Katedra za mikrobiologiju, Biološki fakultet, Univerzitet u Beogradu, Beograd
¹Institut za nuklearne nauke «Vinča», Beograd

Istraživanja su pokazala da aktivne supstance lekovitih i aromatičnih biljaka poseduju inhibitorni ili modulatorni efekat na genotoksične agense. Cilj rada je detekcija antigenotoksičnog efekta terpena iz etarskog ulja žalfije i izučavanje mehanizama reparacije DNK koji su uključeni, pomoću prokariotskih i eukariotskih model sistema. Korišćena je baterija testova na *E. coli* K12 kojom se prati efekat na UV-indukovanu mutagenezu u reparaciono sposobnom, i ekscisiono defektnom soju; spontane mutacije; SOS indukcija i intrahromozomalna rekombinacija. Kao dodatak, urađen je mikronukleus test na humanim limfocitima ozračenim γ zracima.

Etarsko ulje žalfije ima znatno manji efekat na smanjenje broja UV-indukovanih mutanata u *uvrA*⁻ soju, nego u ekscisiono sposobnom soju. Takav efekat je nađen i kod svih testiranih monoterpena iz njegovog sastava (α + β tujon, 1,8-cineol i kamfor). U mutator *mutS* soju, nije uočen značajan efekat na broj spontanih mutanata. α + β tujon inhibira UV-indukovani SOS odgovor. Etarsko ulje žalfije, 1,8-cineol i kamfor blago stimulišu intrahromozomalnu rekombinaciju u ozračenom *recA*⁺ soju, dok α + β tujon nema značajnog efekta. Mikronukleus test je pokazao da etarsko ulje žalfije ima slab radioprotективni efekat. Rezultati dobijeni u ovom istraživanju, kao i podaci o antimutagenoj i antikancerogenoj aktivnosti monoterpena, ohrabruju dalja istraživanja etarskog ulja žalfije kao potencijalnog agensa za prevenciju kancera.

ANTIGENOTOXIC EFFECT OF TERPENOIDS FROM SAGE

It has been shown that active substances from medicinal and aromatic plants possess inhibiting or modulating effect on environmental genotoxic agenta. This work is designed to detect antigenotoxic effects of terpenes from essential oil of sage, and to study the DNA repair mechanisms involved, using pro- and eukaryotic model systems.

The battery of tests on *E. coli* K12 measuring UV-induced mutagenesis in excision repair proficient and deficient strains, spontaneous mutations, SOS induction and intrachromosomal recombination was used. In addition, micronucleus test was performed in γ -irradiated human lymphocytes.

Essential oil of sage was significantly less effective in inhibition of UV-induced mutations in *uvrA*⁻ strain than in excision repair proficient strain. Similar effect is observed with all tested monoterpenes from essential oil (α + β thujone, 1,8-cineole and camphor). No significant effect on spontaneous mutagenesis was found in mutator *mutS* strain. α + β thujone inhibit UV-induced SOS induction. Essential oil of sage, cineole and camphor mildly stimulate intrachromosomal recombination in UV-irradiated *recA*⁺ strain, while α + β thujone has no significant effect. Essential oil of sage exhibits mild radioprotective effect in micronucleus test.

Our results, as well as the data on antimutagenic and anticarcinogenic activity of monoterpenes, encourage further investigation of essential oil of sage for potential use as cancer preventing agent.

ISPITIVANJE GENOTOKSIČNIH EFEKATA UZORAKA REČNE VODE ALLIUM ANAFAZNO-TELOFAZNIM TESTOM

Jelena Blagojević, Tanja Anadević, Aleksandra Popović i M. Vujošević

Institut za biološka istraživanja «Siniša Stanković», Beograd

Allium anafazno-telofazni test je veoma pogodan test sistem za ispitivanje uzoraka vode (česmenske vode, rečne, jezerske i otpadnih voda) zato što se obavlja bez koncentrisanja uzoraka i ujedno omogućava praćenje toksičnosti i genotoksičnosti. Za test toksičnosti prati se rast korena, a za test genotoksičnosti registriraju se sve promene vezane za hromozome i vreteno tokom anafaze i telofaze. Ovim testom analizirana je potencijalna genotoksičnost uzoraka vode 5 reka u Srbiji: Dunav, Sava, Tamiš, Topčiderska i Borska reka. Iz Dunava su uzeta dva uzorka, kod Slankamena i kod Beograda. Svi uzorci pokazali su sličan nivo genotoksičnosti, koji je značajno viši od negativne kontrole, ali još uvek značajno niži od pozitivne kontrole. Samo uzorak vode Dunava kod Slankamena ne razlikuje se značajno od negativne kontrole, međutim kada se uporedi sa uzorkom vode Dunava kod Beograda vidi se jasno povećanje nivoa aberacija ($\chi^2_{(1)}=17.92$, $p<0.001$). Ovaj test se pokazao kao jednostavno i moćno sredstvo za praćenje sredinskog zagađenja.

EVALUATION OF GENOTOXIC EFFECTS OF SAMPLES OF RIVER WATER BY ALLIUM ANAPHASE-TELOPHASE TEST

Allium anaphase-telophase test is very suitable test system for examining water samples (tap water, river, lake and wastewater) because there is now need to concentrate samples and the test allow monitoring of both toxicity and genotoxicity at the same time. For testing toxicity roots growth was monitored and for genotoxicity registered are all changes connected with chromosomes and spindle during anaphase and telophase.. Using this test potential genotoxicity of water samples from 5 rivers in Serbia was analyzed: Danube, Sava, Tamiš, Topčider River and Bor River. Two samples were taken from Danube, near Slankamen and near Belgrade. All samples showed the similar level of genotoxicity, which was significantly higher than negative control but still significantly smaller than in positive control. Only the sample of Danube water near Slankamen was not significantly different from negative control, but in comparison with sample of Danube water near Belgrade clear increase in the level of aberrations was obtained ($\chi^2_{(1)}=17.92$, $p<0.001$). This test showed as simple and powerful tool for monitoring environmental pollution.

AFLP ANALIZA VARIJABILNOSTI LOKALNIH POPULACIJA KUKRUZA

D. Ignjatović-Micić, K. Marković i V. Lazić-Jančić

Institut za kukuruz «Zemun Polje», Beograd-Zemun

Karakterizacija genetičkog diverziteta lokalnih populacija kukuruza u Banci gena Instituta za kukuruz «Zemun Polje» trebalo bi da optimizuje održavanje postojeće kolekcije i olakša korišćenje datih genotipova. Primena molekularnih markera omogućava procenu genetičkog diverziteta direktno na DNK nivou prevazilazeći nedostatke morfoloških i biohemijskih markera.

Devetnaest lokalnih populacija kukuruza je analizirano korišćenjem četiri AFLP prajmer kombinacije. Primenjena je metoda grupnih uzoraka, pri čemu je svaka ispitivana populacija predstavljena sa po 30 biljaka. Nakon denaturišuće poliakrilamidne elektroforeze gelovi su obojeni srebro-nitratom. Statička analiza podataka je urađena pomoću NTSYSpc2 programske pakete, a genetička distanca je izračunata primenom Žakardovog koeficijenta na osnovu binarnog zapisa (1/0) prisustva/odsustva traka kod ispitivanih populacija.

Rezultati i diskusija će biti prikazani na posteru.

AFLP ANALYSIS OF MAIZE LOCAL POPULATION VARIABILITY

Genetic diversity characterisation of local maize populations from Maize Research Institute «Zemun Polje» genebank should optimize and facilitate their conservation and use. Molecular markers allow the estimation of genetic diversity directly at DNA level, thus overcoming the demerits of morphological and biochemical markers.

Nineteen local maize populations were analysed with four AFLP primer combinations. Thirty individual plants were bulked to form DNA pooled samples for each population. Silver staining was applied after the denaturing polyacrylamide electrophoresis. Statistical analysis was performed using NTSYSpc2 programme package and genetic distances were estimated by Jaccard coefficient based on binary codes (1/0) of the bands for their presence/absence within each population.

Results and discussion will be presented on the poster.

IZOLACIJA I KARAKTERIZACIJA BAKTERIJA MLEČNE KISELINE IZ RADANSKOG SIRA

Nataša Joković¹, Maja Vukašinović² i Lj. Topisirović³

¹Tehnološki fakultet, Leskovac

²Tehnološko-metalurški fakultet, Beograd

³Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Iz kravljeg i ovčijeg mleka kao i iz uzoraka sira tokom zrenja (5, 10, 20, 30 i 60 dana) izolovano je 118 izolata bakterija mlečne kiseline (BMK). Kao selektivna podloga korišćene su: GM17 podloga za laktokoke, MRS podloga za laktobacile i MSE podloga za leukonostoke. Na osnovu mikrobioloških i fizioloških testova izolati su identifikovani do nivoa vrste. Vrste *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* i *Enterococcus faecium* izolovane su iz uzoraka mleka i sira, dok su vrste *Lactobacillus plantarum*, *Lactobacillus paraplanarum*, *Lactobacillus brevis* i *Eterococcus fecalis* izolovane samo iz sira. Korišćenjem mikrobioloških i fizioloških testova 20% izolata nije bilo moguće identifikovati. Analiza plazmidnog profila i ispitivanje proteolitičke aktivnosti urađeno je za 73 odabранa izolata. Većina izolata *Lactococcus lactis* subsp. *lactis* kao i homofermentativni laktobacili pokazuju dobru proteolitičku aktivnost, dok su izolati iz rođiva *Leuconostoc* i *Enterococcus* slabi proteoliti. Plazmidi su detektovani kod većine izolata i plazmidni profili pokazuju varijabilnost.

ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM RADAN'S CHEESE

In this study we described characterization of 118 natural isolates of lactic acid bacteria (LAB) from cow's milk, ewe's milk and cheese (samples were taken after 5, 10, 20, 30 and 60 days of ripening). The isolates were selected using selective media for LAB, GM17 for lactococci, MRS for lactobacilli and MSE for leuconostoc. Identification of strains to the species level was performed by the analysis of their carbohydrate fermentation pattern and by their physiological characteristics. *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* and *Enterococcus faecium* were found in milk and cheese, while *Lactobacillus plantarum*, *Lactobacillus paraplanarum*, *Lactobacillus brevis* and *Enterococcus faecalis* were found only in cheese samples. Twenty percent of isolates were not determinated by used tests. Isolation of plasmids and examination of proteolytic activity were done for 73 isolates. The most strains of *Lactococcus lactis* subsp. *lactis* and homofermentative lactobacilli exhibited high proteolytic activity towards b-casein, while strains from genus *Leuconostoc* and *Enterococcus* showed weak proteolytic activity. The analysis of plasmid profiles revealed that majority of strains contain different plasmids.

**INVERZIONI POLIMORFIZAM PRIRODNIH POPULACIJA
Drosophila subobscura SA LOKALITETA GORNJEG DELA TOKA
DUNAVSKOG REGIONA U SRBIJI**

P. Kalajdžić¹, G. Živanović² i M. Anđelković^{1,2}

¹Biološki fakultet, Univerzitet u Beogradu, Beograd

²Institut za biološka istraživanja «Siniša Stanković», Beograd

Drosophila subobscura poseduje bogat inverzionalni polimorfizam na svih pet dugih akrocentričnih hromozoma. Prirodne populacije ove vrste pokazuju prostorno varijabilnost inverzionog polimorfizma koja je u izvesnoj korelaciji sa ekološkim faktorima staništa. Balkansko poluostrvo karakteriše specifičan ekološki diverzitet, naročito u regionima sa specifičnim mikroklimatskim uslovima kao što je priobalje rečnog toka Dunava. Podaci dobijeni analizom inverzionog polimorfizma prirodnih populacija *Drosophila subobscura* sa lokaliteta gornjeg dela toka Dunavskog regiona omogućavaju dodatni uvid u raznovrsnost ovog vida genetičkog polimorfizma u ekološkim staništima u regionu sa bogatim biodiverzitetom.

**CHROMOSOMAL INVERSION POLYMORPHISM OF
Drosophila subobscura NATURAL POPULATIONS FROM UPPER PART OF
THE DANUBE RIVER REGION IN SERBIA**

Drosophila subobscura possesses rich inversion polymorphism on all of its five long acrocentric chromosomes. Natural populations of this species shows clear-cut geographical variation of inversion polymorphism, in a certain degree associated with the variety and dynamics of ecological factors. Balkan Peninsula is characterized by specific ecological diversity, especially in specific microclimate regions like the Danube river region. The results derived, by analysis of inversion polymorphism of *Drosophila subobscura* natural populations from upper part of the Danube river region, are adding more information about the variety of this kind of genetic polymorphism in habitats of region with rich biodiversity.

IZOLACIJA I KARAKTERIZACIJA BAKTERIJA MLEČNE KISELINE IZ ZLATARSKOG SIRA

Katarina Krstić¹, Amarela Terzić-Vidojević¹, Maja Vukašinović²,
Jelena Lozo¹, D. Fira¹ i Lj. Topisirović¹

¹Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

²Tehnološko-metalurški fakultet, Beograd,

Sojevi bakterija mlečne kiseline (BMK) izolovani su iz svežeg mleka i sira različitog perioda zrenja (1, 10 20, 30, 45 i 60 dana). Zlatarski sir je napravljen u domaćinstvu bez dodavanja starter kulture. Ukupno je izolovano 253 isolata. Korišćenjem tradicionalnih mikrobioloških metoda determinisano je 138 isolata kao *Lactobacillus* sp. i 114 izolata kao *Lactococcus* sp. Za dalju determinaciju isolata do nivoa vrste primenićemo molekularno genetičke metode kao što su SDS-PAGE, AFLP i rep-PCR.

Analizom BMK iz ove kolekcije pokazano je da neki od izolata produkuju antimikrobne supstance, ekstracelularne proteinaze i imaju sposobnost formiranja multicelularnih agregata. Izučavanjem organizacije gena, koji kodiraju proteinaze, bakteriocine i koji agregiraju, može se omogućiti konstrukcija specifičnih starter kultura za dobijanje autohtonih fermentisanih mlečnih proizvoda, tj. sa geografskim poreklom. Intenzivnim proučavanjem molekularne genetike BMK iz prirodnih izolata otvaraju se sasvim novi potencijali u industriji hrane.

ISOLATION AND CHARACTERISATION OF LACTIC ACID BACTERIA FROM ZLATAR'S CHEESE

Strains of lactic acid bacteria (LAB) have been isolated from raw milk and cheese during different periods of ripening (1, 10, 20, 30, 45 and 60 days). Homemade cheese from Zlatar has been manufactured without addition of any starter culture. A total number of isolated LAB was 253. Using traditional microbiological methods we have determined 138 isolates as *Lactobacillus* sp. and 114 isolates as *Lactococcus* sp. For further determination of the isolates to the species level, we will apply molecular genetic methods like SDS-PAGE, AFLP and rep-PCR.

Analysis of LAB from this collection showed that some of them produced antimicrobial substances, extracellular proteinases and had ability to form multicellular aggregates. Elucidation of the organization of genes encoding proteinases, bacteriocins and aggregation factors could be used in the construction of specific starter cultures for production of autochthonous fermented milk products, i.e. products with geographical origin. The extensive studies on molecular genetics of LAB from natural sources opens new potentials in food industry.

GENETIČKA KARAKTERIZACIJA LOKALNIH POPULACIJA KUKURUZA POMOĆU SSR MARKERA

K. Marković, D. Ignjatović-Micić i V. Lazić-Jančić

Institut za kukuruz «Zemun Polje», Beograd-Zemun

Banka gena Instituta za kukuruz «Zemun Polje» poseduje preko dve hiljade lokalnih populacija kukuruza, klasifikovanih i okarakterisanih na morfološkom nivou. Da bi se omogućila preciznija karakterizacija i izbegla pojava potencijalnih duplikata u kolekciji neophodna je analiza postojećih genotipova primenom molekularnih markera.

U ovom radu analizirana je 21 lokalna populacija kukuruza pomoću 30 SSR markera. Korišćena je metoda grupnih uzoraka, gde je svaka populacija predstavljena sa po 30 biljaka. Elektroforetsko razdvajanje amplifikovanih fragmenata je rađeno na agaroznom i denaturišućem poliakrilamidnom gelu. Poliakrilamidni gelovi su bojeni srebro-nitratom. Statistička analiza dobijenih podataka je urađena pomoću NTSYSpc2 programske pakete, a za izračunavanje genetičkih distanci korišćena je «Modifikovana Rodžerova Distanca» na osnovu izračunatih alelnih frekvencija unutar populacija.

Rezultati i diskusija će biti prikazani na posteru.

GENETIC CHARACTERIZATION OF LOCAL MAIZE POPULATIONS WITH SSR MARKERS

The genebank in Maize Research Institute «Zemun Polje» maintains the collectin of over 2000 local maize populations classified and characterised at the morphological level. Molecular marker application would enable more precise characterisation and avoid occurrence of duplicate accessions, thus supporting the maintenance of the collection.

Thirty SSR markers were used to analyse twenty one local maize populations. DNA pooling strategy was done with thirty plants representing each population. Amplified fragments were separated both on denaturing polyacrylamide and agarose gels. Silver staining was performed on polyacrylamide gels. NTSYSpc2 programme package was applied for statistical analysis of the results. Genetic distance was calculated using Modified Rogers Distance based on allelic frequency scoring of the bands within accessions. Results and discussion will be presented on the poster.

POMOLOŠKE OSOBINE SUPERIORNIH SELEKCIIJA ŠUMSKE (*C. avelana L.*) I MEĆJE LESKE (*C. colurna L.*)

R. Milić, M. Žikić, Nevena Mitić i Radomirka Nikolić

Institut SRBIJA, Centar za poljoprivredna i tehnološka istraživanja, Zaječar

Izučavajući šumsku i mečju lesku u višegodišnjem periodu od 1998 do 2003. godine, na području istočne Srbije izdvojene su po osam selekcija koji se po mnogim osobinama posebno ističu. Analizirana je krupnoća, oblik i masa ploda i jezgre kao i hemijski sastav jezgre. Izučena je i potencijalna rodnost, odnosno prosečna zastupljenost plodova u cvasti leske. Prosečna krupnoća plodova odabranih selekcija šumske leske je 18,0x14,7x12,6 i mečje leske 16,3x14,0x11,0 mm, a jezgre 13,3x9,1x7,6 odnosno 13,0x9,7x6,5 mm. Masa plodova šumske leske je 1,01-1,80 g i mečje leske 1,00-1,75 g, jezgre 0,30-0,79 i 0,31-0,65 g. Sadržaj jezgre je od 27,9 do 46,2% i od 30,7 do 40,8%. Pojava šturih plodova je minimalna i to u šumske leske u šest selekcija (0,8-3,1%), a u mečje leske u pet (1,4-5,7%). Štetočina Balaninus nucum registrovana je samo u tri selekcije mečje leske u granicama od 1,0 do 1,6%. Sadržaj ulja u jezgri odabranih selekcija šumske leske je 44,6-49,9%, a u mečje leske 48,6-54,4%, sirovih proteina je 12,3-10,8 i 11,7-10,4%, a sadržaj mineralnih materija 2,8-2,4 i 2,6-2,0%. U odabranim selekcijama šumske leske u cvastima se nalazi od 4 do 6 ploda zajedno. Dominiraju dva (29,4%), jedan (26,7%) i tri (24,5%) plodova zajedno. Cvasti mečje leske su sa 8 do 13 plodova. Najviše je cvasti sa pet ploda (26,6%), četiri (21,5%) i šest (20,3%) ploda zajedno.

Prema većini pokazatelja posebno se ističu selekcije šumske leske 1, 6 i 3 i mečje leske 2, 7 i 8.

POMOLOGICAL CHARACTERISTICS OF THE SUPERIOR SELECTIONS OF THE FOREST HAZEL-NUT TREE (*C. avelana L.*) AND THE HAZEL-NUT TREE (*C. colurna L.*)

Having been examined both before mentioned hazel-nut trees cv. In the period of time from 1998 to 2003 on the territory of Eastern Serbia, there were selected eight selections, separately, that are especially distinguished by many of their characteristics. There were analyzed the coarseness, form and mass of fruit and kernel, as well as the chemical content of the kernel. The potential fruitfullness, that is the proportion of fruits in the hazel-nut inflorescence, was also being investigated. The average fruit coarseness of the chosen hazel-nut selections of the *C. avelana L.* is 18.0x 14.7x12.6 mm, and of the *C. colurna L.* it is 16.3x14.0x 11.0 mm, while of the kernel it is 13.3x9.1x 7.6 mm, that is 13.9x 9.7x 6.5 mm. The fruit mass of the former's is 1.01-1.80 g and of the latter's 1.00-1.75 g, while the kernel mass ranges from 0.30 g to 0.79 g and from 0.31 to 0.65 g. The kernel content is from 27.9 to 46.2%, and from 30.7 to 40.8 %. The occurrence of the shriveled fruits is minimum, appearing in 6 selections of the former's (0.8-3.1%) , while in 5 selections (1.4-5.7 %) of the latter's. The pest *Balaninus nucum* is registered in only three selections of the *C. colurna L.*, ranging from 1.0 to 1.6 %. The oil content of the selected hazel-nut trees of the *C. avelana L.* is 44.6-49.9 %, while in the *C. colurna L.* it is 48.6-54.4 %; the raw proteins amount is 12.3-10.8 % and 11.7-10.4 %, and the minerals matters content is 2.8-2.4 % and 2.6-2.0 %. In the chosen selections of the forest hazel-nut trees, there are 4 to 6 fruits together in a inflorescence. Two (29.4 %), one (26.7 %) and three (24.5 %) fruits together dominate. The inflorescences of the *C. colurna L.* are with 8 to 13 fruits. There are most inflorescences with 5 fruits (26.6 %), four (21.5 %) and six (20.3 %) fruits together. According to the majority of indicators, the following selections of the *C. avelana L.* : 1.6 and 3, and 2.7 and 8 of the *C. colurna L.* are especially distinguishing ones.

POMOLOŠKO-TEHNOLOŠKE OSOBINE KOLEKCIIONISANIH SELEKCIJA DŽANARIKE (*Prunus cerasifera* Erhr.)

R. Milićić, M. Žikić, Nevena Mitić i Radomirka Nikolić

Institut SRBIJA, Centar za poljoprivredna i tehnološka istraživanja, Zaječar

Kolekcioni zasad je formiran 1995. godine u okolini Svrlijiga od 32 genotipa odabranih iz spontane populacije džanarike u istočnoj Srbiji. Voćke su okalemjene na sejancu džanarike, zasađene na rastojanju 5x4 m na blagoj padini jugozapadne ekspozicije. U radu su prikazane najvažnije karakteristike za 19 kolekciionisanih selekcija i prosečni rezultati za period od 2000-2003. godine. Opisane su važnije karakteristike stabla, produktivnost i osobine plodova i koštice. Tako je prosečna krupnoća (dužina, širina, debljina) za plodove svih selekcija bila 25.0x24.4x25.0 mm, a koštica 14.5x10.3x6.6 mm. Prosečna masa plodova je 12.1 g (24.3-4.8 g), a koštica 0.85 g (2.2-0.3 g). U zavisnosti od mase ploda i koštice sadržaj mezokarpa je 93.0% (96.3-90.3). S obzirom na mogućnost korišćenja plodova u proizvodnji biološki visokovredne hrane, detaljno je obrađen hemijski sastav mezokarpa. Plodovi se pored ostalog odlikuju i povećanim sadržajem ukupnih kiselina, prosečno 3.09% (3.44-2.60%) što je bio i cilj selekcije. Sadržaj ukupno suvih materija je 13,5% (16.2-10.3%), ukupno rastvorljivih suvih materija 125% (14.5-9.5%) i ukupnih šećera 6.00% (11.45-3.14%). Izdvojene selekcije po svim osobinama zaslužuju veću pažnju kako u cilju očuvanja biodiverziteta, formiranju banke gena tako i za komercijalno gajenje u organizovanoj proizvodnji.

POMOLOGICALLY-TECHNOLOGICAL CHARACTERISTICS OF THE PLUM-TREES COLLECTED SELECTIONS OF THE *Prunus cerasifera* Erhr.

The collection plantation consisting of 32 genotypes selected from the spontaneous population of the plum-trees *Prunus cerasifera* Erhr. was established in the surroundings of the small town of Svrlijig in Eastern Serbia. The fruit-trees were budded on the *Prunus cerasifera* seedlings, planted on the distance of 5x4 on a mild slope of the south-western exposition. The study shows the most important characteristics for the 19 collected selections , as well as the average results for the period from 2000 to 2003. There are described the most significant characteristics of the tree – trunks, productivity and characteristics of fruits and bone. Therefore , the average coarseness, involving the lenght, width and thickness, of fruits is 25.0x 24.4x25.0 mm, and of the bone 14.4x 10.3 x3.6 mm. The average fruit mass is 12.1 g (24.3-4.8 g), and of bone 0.85 g (2.2-0.3 g). Depending on the fruit and bone mass, the mezocarp content is 93 % (96.3-90.3). Taking into consideration the possibility of exploiting fruits in production of biologically high-valuable food, the chemical content of the mezocarp was thoroughly examined. Among others, the fruits are distinguished by increased content of total acids, amounting averagely 3.09 % (3.44-2.60 %) , what was the aim of the selection work. The content of total amount of dry matter is 13.5 %(16.2-10.3 %) , total amount of soluble dry matters is 12.5 % (14.5-9.5 %) and total amount of sugars 6.00 % (11.45- 3.14 %) .According to all beforementioned characteristics, the picked out selections deserve greater attention, aimed to preserve the biodiversity, form the gene bank, and to be cultivated commercially in an organized production, as well.

**ANTIMUTAGENA SVOJSTVA PRIRODNIH ANTIOKSIDANATA
DETEKTOVANA *Escherichia coli* K12 TEST SISTEMOM**

B. Nikolić, J. Knežević-Vukčević, B. Vuković-Gačić, D. Mitić-Ćulafić,
T. Berić-Bjedov, J. Stanojević, S. Stanković i D. Simić

Katedra za mikrobiologiju, Biološki fakultet, Univerzitet u Beogradu, Beograd

Prirodni antioksidanti, prisutni u mnogim biljnim ekstraktima, mogu zaštiti genom od reaktivnih kiseoničnih vrsti (ROS) i time spričati nastajanje i fiksiranje oštećenja koja su najznačajniji izvor endogene mutageneze. Ispitivanje njihovog antimutagenog potencijala je značajno za prevenciju kancerogeneze, imunodeficijencije, neurodegeneracije i starenja. U cilju detekcije antimutagenih svojstava antioksidanata koristili smo *E.coli* K12 test sistem za praćenje $arg^- \rightarrow Arg^+$ reverzija. Test sistem je sastavljen iz dva testa – Testa A i B. U Testu A se prati oksidativna mutageneza indukovana t-butilhidroperoksidom (t-BOOH) na reparativno sposobnom soju. Test B se izvodi na mutator sojevima: *mutS* (defektan u ‘mismatch’ reparaciji) i *mutT* (defektan u uklanjanju 8-oxo-G); on je dizajniran u cilju praćenja efekta antioksidanta na spontanu mutagenezu, ali i bližeg određivanja molekularnog mehanizma njegovoga dejstva.

Ispitivan je potencijal vitamina E (α -tokoferola), etarskog ulja (EO) bosiljka (*Ocimum basilicum* L.) i njegovog konstituenta linaloola (čini oko 70% EO) da redukuju oksidativnu mutagenezu. Protektivni efekat model antioksidanta vitamina E je detektovan u oba testa, ali je najveća inhibicija mutageneze ostvarena u *mutS* soju (76%). EO bosiljka, kao i linalool, su pokazali umeren inhibitori potencijal u Testu A (~35%), ali je snažan efekat ostvaren u *mutS* soju (inhibicija preko 60%). Ispitivani derivati bosiljka nisu imali uticaja na mutagenezu posredovanu 8-oxo-G.

**ANTIMUTAGENIC PROPERTIES OF NATURAL ANTIOXIDANTS IN THE
Escherichia coli K12 REVERSION ASSAY**

Natural antioxidants, widely distributed in plant extracts, might protect genomic DNA from lesions induced by reactive oxygen species (ROS), a significant source of endogenous mutagenesis. The study of their antimutagenic potential can promote the prevention of carcinogenesis, immunodeficiency, neurodegeneration and aging.

In order to detect antimutagenic potential of antioxidants, we used *E.coli* K12 $arg^- \rightarrow Arg^+$ reversion assay consists of two tests, named Test A and Test B. Test A is created to evaluate the antimutagenic potential against t-butyl hydroperoxide (t-BOOH)-induced oxidative mutagenesis and is performed on repair proficient strain. Test B is performed on mutator strains *mutS* (deficient in mismatch repair) and *mutT* (deficient in removing 8-oxo-G); it is designed to elucidate the effect of antioxidants on spontaneous mutagenesis and to determine the molecular mechanism of its action.

The potential of vitamin E (α -tocopherol), essential oil (EO) of basil (*Ocimum basilicum* L.) and its major constituent linalool (~70% of EO) to reduce the oxidative mutagenesis, was studied.. The protective effect of model antioxidant vitamin E against oxidative mutagenesis was detected in both tests, but the strongest inhibition was obtained in *mutS* strain (76%). EO of basil, as well as linalool, exhibited moderate inhibitory potential in Test A (~35%), while powerful inhibition was detected with both test substances in *mutS* strain (over 60%). EO of basil as well as linalool showed no effect on mutagenesis mediated by 8-oxo-G lesions.

**MOLEKULARNA KARAKTERIZACIJA BAKTERIJA
MLEČNE KISELINE (BMK) IZ KOZIJEGL SIRA**

Milica Nikolić, Amarela Terzić-Vidojević, B. Jovčić, Lj. Topisirović i Nataša Golić
Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Klasične metode za identifikaciju i determinaciju mikroorganizama, zasnovane na kultivaciji i biohemijskim metodama, nisu uvek bile pogodne za dobijanje informacija o mikrobiološkom sastavu različitih uzorka. Analiza mikroflore klasičnim metodama je ograničena na mikroorganizme koji se lako kultivisu, tako da se ne dobija informacija o kompletnoj populaciji iz uzorka. U ovom radu su korišćene molekularne tehnike kojima je moguće okarakterisati različite bakteije mlečne kiseline (BMK) iz kozijeg sira. Totalna DNK iz sira je izolovana i korišćena za PCR reakciju, gde su kao prajmeri korišćeni U968-GC i L1401, komplementarni V6 i V8 regionima eubakterijske 16s rDNK. Sintetisani DNA fragmenati veličine 450 bp predstavljaju zbir umnoženih DNA fragmenata koji su poreklom od različitih vrsta bakterija iz sira. Fragmenti su uklonirani u plazmid pUCEry19, a ligacionom smešom je transformisan soj *E. coli* DH5 α . Transformanti su analizirani na prisustvo plazmida sa uklonanim PCR fragmentom. U daljem radu biće urađena DGGE analiza kloniranih fragmenata. Fingerprint tehnikom, kao što je DGGE, se utvrđuje genetički diverzitet mikroflore sira. Međusobno različiti fragmenti će biti sekvenciirani u cilju precizne identifikacije mikroorganizama prisutnih u siru. Dobijeni rezultati biće poređeni sa rezultatima klasične mikrobiološke analize.

**MOLECULAR CHARACTERIZATION OF LACTIC ACID
BACTERIA IN THE GOAT CHEESE**

Conventional methods for the identification and determination of microorganisms, based on cultivation and biochemical methods, usually were suitable enough for getting the information about the composition of the microorganisms present in variable environmental samples. However, the analysis of microflora by conventional methods is limited to easy cultivable microorganisms and does not give the information about the complete population in the sample. In this study molecular techniques were used to characterize different lactic acid bacteria (LAB) from a goat cheese. The first step was the isolation of total DNA from the cheese that has been used as a template for PCR reaction. PCR, by using primers U968-GC and L1401 complementary to the V6 and V8 region of eubacterial 16s rDNA was performed. Obtained DNA products of 450 bp represent the sum of amplified DNA fragments originated from different species of bacteria from the cheese. These fragments were cloned into a plasmid pUCEry19, and ligation mixture was transformed into a competent *E. coli* strain DH5 α . Transformants were screened for the presence of plasmids containing cloned PCR fragments. The aim of further research will be the DGGE analysis of the cloned fragments. DGGE, as a fingerprinting technique is suitable to describe the genetic diversity of the cheese composition. Different fragments will be sequenced and the obtained data would be used for the identification of the microorganisms present in the goat cheese. These results will be compared with results obtained by classical microbiological methods.

**STEPEN HOMOZIGOTNOSTI INVERZIJA I INVERZIONIH KOMPLEKSA
KOD *Drosophila subobscura* U USLOVIMA INBRIDINGA**Gordana Rašić¹, Marina Stamenković-Radak¹ i M. Andelković^{1,2}¹Biološki fakultet, Univerzitet u Beogradu, Beograd²Institut za biološka istraživanja «Siniša Stanković», Beograd

Genom *Drosophila subobscura* se odlikuje bogatim hromozomskim polimorfizmom paracentričnih inverzija i taj polimorfizam je najverovatnije u određenom stepenu adaptivan. U ovom radu izvršena je analiza efekata inbridinga na genetičku strukturu u okviru laboratorijskih populacija ove vrste poreklom sa različitim staništa. Cilj je bio utvrditi da li genetički sistemi ispitivanih populacija utiču na brzinu dostizanja određenog stepena homozigotnosti genskih aranžmana tokom kontinuiranog full-sib ukrštanja. Poređene su učestalosti pojedinačnih inverzija i inverzionih kompleksa između prirodnih populacija i 60 inbreedingovanih linija. Za određene aranžmane izračunate su očekivana homozigotnost i očekivana stopa fiksacije. Dobijeni rezultati tumačeni su sa ekološko-genetičkog aspekta.

**THE DEGREE OF HOMOZYGOSITY OF INVERSIONS AND INVERSION
COMPLEXES IN *Drosophila subobscura* UNDER INBREEDING**

Genome of *Drosophila subobscura* is characterized by a rich chromosomal polymorphism of paracentric inversions and this polymorphism is most probably adaptive at certain degree.

In this study, an analysis of the effects of inbreeding on genetic structure of laboratory populations of *Drosophila subobscura* from different habitats was carried out. Our aim was to determine whether the genetic systems of analyzed populations effect attainment of the degree of homozygosity of gene arrangements during continued full-sib mating.

The frequencies of inversions and inversions complexes were compared between natural populations and 60 inbred lines. Expected homozygosity and fixation indexes were calculated for certain arrangements. The obtained results were discussed from ecological and genetical aspects.

UTICAJ SEPIA MUTACIJE NA USPEH U PARENJU JEDINKI *Drosophila melanogaster*

Snežana Stanić¹, Sofija Pavković-Lucić² i V. Kekić²

¹Prirodno-matematički fakultet, Univerzitet u Kragujevcu, Kragujevac

²Biološki fakultet, Univerzitet u Beogradu, Beograd

Seksualno ponašanje *Drosophila* se nalazi pod složenom genetičkom kontrolom i sastoji se od serije specifičnih pokreta praćenih razmenom vizuelnih, akustičnih, olfaktornih i taktičnih stimuli. Uloga vizuelnih draži u reproduktivnom ponašanju *Drosophila* detektovana je, između ostalog, i izučavanjem ponašanja mutanata deficijentnih u pigmentaciji oka. Rezultati dobijeni na različitim vrstama *Drosophila* sugerisu da su mužjaci, nosioci neke od mutacija boje očiju, obično manje uspešni u parenju na svetlosti od *wt* mužjaka, kao i da ovi mutantni geni smanjuju njihovu seksualnu aktivnost. Ipak, u mnogim slučajevima nije utvrđeno postojanje seksualne izolacije između «divljih» mušica i mutanata.

Boja očiju kod *D. melanogaster* zavisi od biosinteze drozopterina (pigment crvenih tonova) i omohroma (odgovornog za nijanse braon boje). Ukoliko je jedan od gena na putu biosinteze drozopterina mutiran, kakav je *sepia*, složene oči će biti braon boje. Interesantno je, recimo, da *sepia* mutacija smanjuje seksualnu aktivnost mužjaka *D. bipectinata*, dok ista mutacija nema efekta na receptivnost ženki (Singh & Sisodia, 1995b).

U našim eksperimentima, testirali smo uspešnost u parenju *sepia* mutanata i *wt* jedinki primenom eksperimentalnih procedura koje se uobičajeno koriste u studijama seksualne selekcije kod *Drosophila* (kakve su procedure označene kao «izbor ženki» i metod «višestrukog izbora»). Mužjaci crvenih očiju su bili uspešniji u parenju od mutantnih mužjaka.

THE INFLUENCE OF SEPIA MUTATION ON MATING SUCCESS IN *Drosophila melanogaster*

Sexual behavior of *Drosophila* is under complex genetic control and it consists of series of specific movements followed by an interchange of visual, acoustic, olfactory and tactile stimuli. The role of visual cues in *Drosophila* mating behavior has been detected by studying the mating behavior of certain eye pigment deficient mutations. The results obtained from different *Drosophila* species suggest, on the whole, that eye pigment deficient mutant males are at a mating disadvantage in the light and that different mutant genes diminish the sexual activity of males. However, in most cases there is no evidence of sexual isolation between wild type flies and mutants.

The eye colors seen in *D. melanogaster* results from the interaction between two pigment pathways: the drosopterin pathway which produces pigments with red tones and the ommochrome pathway which produces brown shades of pigments. If a gene in the drosopterin pathway is mutant, like *sepia*, the eye will be more brown than the wildtype one. It is interesting that *sepia* mutation diminishes the sexual activity of *D. bipectinata* males; however, this gene has no effect on receptivity of females (Singh and Sisodia, 1995b).

Mating success of *sepia* mutant and wild type *D. melanogaster* flies was studied by usual experimental approaches in studing sexual selection in *Drosophila*, which have typically employed «choice» designs (like «female choice» and «multiple choice»). Red eyed (*wt*) males were more successful in mating than mutant ones, suggesting that *sepia* mutation affects mating success in *D. melanogaster*.

VARIJABILNOST OSOBINA CVASTI I LISTA I NASLEĐIVANJE OBLIKA LISKE SUNCOKRETA U F1 GENERACIJI

S. Terzić i Jovanka Atlagić

Institut za ratarstvo i povrtarstvo, Novi Sad

Zbog izražene varijabilnosti divlje vrste su korišćene za ukrštanja sa cms linijama gajenog suncokreta. Varijabilnost je utvrđena merenjem prečnika cvasti, broja jezičastih cvetova, dužine i širine liske. Podaci su obrađeni putem hijerarhijske klaster analize u programu SYSTAT 10 i dobijen je dendrogram na osnovu koga je tumačena divergentnost ispitivanih populacija. Način nasleđivanja je utvrđivan poređenjem 25 hibridnih kombinacija sa roditeljima. Klaster analizom su razdvojene tri grupe biljaka. Prvu čine inbred linije gajenog suncokreta. U srednjem delu dendrograma su jednogodišnje divlje vrste i treća grupa su višegodišnje divlje vrste. Razlike u srednjim vrednostima ispitivanih svojstava između roditelja su bile značajne. Najčešći je bio intermedijerni tip nasleđivanja zatim parcijalno dominantni i dominantni a u dve hibridne kombinacije manifestovao se negativni heterozis.

INFLORESCENCE AND LEAF TRAITS VARIABILITY AND INHERITANCE OF LEAF SHAPE IN F1 GENERATION OF SUNFLOWER

Populations of wild sunflower species were crossed with cms cultivated lines because of their high variability. Variability was determined by measuring inflorescence diameter, ray flower number and the leaf length and width. The data was used for hierarchical cluster analysis in the SYSTAT 10 program and the obtained dendrogram was used to interpret divergence of used populations. The modes of inheritance were tested by comparing 25 hybrid populations with parents. Cluster analysis divided plants in to three groups. The first one were inbred lines of cultivated sunflower. In the middle of the cluster tree were annual wild species and the third group were perennial wild species. The mean value differences in observed traits between parents were significant. All modes of inheritance were present in F1 generation. Most of the hybrid combinations in F1 were intermediate followed by equal number of partially dominant and dominant ones and in two hybrid combinations negative heterotic effect was scored.

RAZVOJNA STABILNOST KOD INTERSPECIJSKIH HIBRIDA *Drosophila*

I. Tomišić-Kosić¹, M. Stamenković-Radak² i M. Andelković^{1,2}

¹Institut za biološka istraživanja «Siniša Stanković», Beograd

²Biočni fakultet Univerziteta u Beogradu, Beograd

Vrste se među sobom razlikuju i po akumuliranim razlikama na genetičkom nivou, koje vode reproduktivnoj izolaciji. Međutim, ukrštanjem jedinki nekih sestrinskih vrsta nastaju interspecijski hibridi. Pretpostavka je da kod potomstva nastalog takvim ukrštanjem, može doći do poremećaja u ontogenetskoj homeostazi, odnosno razvojnoj stabilnosti jedinki. Pokazatelj promena u razvojnoj stabilnosti može biti fluktuirajuća asimetrija, tj. porast varijanse asimetrije pojedinih morfoloških, bilateralno simetričnih osobina.

U ovom radu korišćene su jedinke laboratorijskih populacija *Drosophila simulans*, *Drosophila mauritiana* i *Drosophila melanogaster*. Analiziran je stepen uspešnosti u parenju između jedinki različitih vrsta i varijabilnost fluktuirajuće asimetrije preko veličine krila u roditeljskoj generaciji i kod dobijenog hibridnog potomstva. Akumularane razlike između vrsta ispoljavaju se u različitom stepenu na nivou predzigotne reproduktivne izolacije i fenotipske varijabilnosti hibrida.

**DEVELOPMENTAL STABILITY IN INTERSPECIES
HYBRIDS OF *Drosophila***

Species differ through accumulated differences at the genetic level and which results in reproductive isolation. But, crosses of individuals of some sibling species often give hybrids. It is assumed that offspring, from those crosses, could exhibit disturbance of developmental stability, i.e. ontogenetic homeostasis. Fluctuating asymmetry can measure changes in developmental stability, by the increased variance in asymmetry of certain morphological, bilaterally symmetric traits.

In the present paper, the flies used were from laboratory stocks *Drosophila simulans*, *Drosophila mauritiana* and *Drosophila melanogaster*. The mating success is analysed between individuals of different species and variability of fluctuating asymmetry of wing size in parental generation and their offspring. The accumulated differences between species are the cause of various degree of prezygotic reproductive isolation and phenotypic variability of hybrids.

ANTIMUTAGENA SVOJSTVA PRIRODNIH ANTIOKSIDANATA DETEKTOVANA WP2 TESTOM ANTIMUTAGENEZE

B. Vuković-Gačić, J. Stanojević, J. Knežević-Vukčević, D. Mitić-Ćulafić,
T. Berić-Bjedov, B. Nikolić, S. Stanković i D. Simić

Katedra za mikrobiologiju, Biološki fakultet, Univerzitet u Beogradu, Beograd

Oksidativna oštećenja DNK, izazvana reaktivnim kiseoničnim vrstama (ROS) su veoma značajna u procesima mutageneze, kancerogeneze i starenja. Ispitivanja aktivnih supstanci lekovitim i začinskim biljaka su potvrdila da mnoge poseduju antioksidativnu aktivnost i da su potentni inhibitori mutageneze i kancerogeneze.

U ovom radu korišćen je WP2 test antimutageneze (WP2 Antimutagenicity test) za praćenje oksidativne mutageneze i kvantifikovanje antimutagenog potencijala antioksidanata. Ispitivanje je sprovedeno na dva soja *E.coli*: IC185 (WP2 *oxyR⁺*) i njegovom derivatu IC202 (WP2 *oxyR/pKM101*). Oksidativna mutageneza je indukovana *tert*-butil hidroperoksidom (*t*-BOOH), latentnim donorom RO' alkoksil radikala. Zbog defekta u OxyR funkciji, soj IC202 je mnogo osjetljiviji na oksidativna oštećenja, te je pokazao veću mutabilnost u poređenju sa OxyR⁺ sojem.

Vitamin E (α -tokoferol) je korišćen kao model antioksidanta jer je veoma efikasan sakupljač ('scavenger') ROS-a i on je testiran u oba soja. Nakon validacije test sistema vitaminom E, korišćen je samo osjetljiviji *oxyR* soj za ispitivanje antimutageneze posredovane antioksidativnim svojstvima dva derivata bosiljka (*Ocimum basilicum* L.): etarskog ulja (EO) i njegovog najzastupljenijeg monoterpena linaloola.

Vitamin E je inhibirao indukovani mutageničnost u oba soja (63% u soju IC202 i 43% u soju IC185). Oba derivata bosiljka su pokazala snažan antimutageni potencijal prema indukovanoj oksidativnoj mutagenezi (55% inhibicije je izazvalo EO, a 68% linalool).

ANTIMUTAGENIC PROPERTIES OF NATURAL ANTIOXIDANTS IN THE WP2 ANTIMUTAGENICITY TEST

Oxidative damage to DNA by reactive oxygen species (ROS) is very important in mutagenic, carcinogenic, and aging processes. The study of active substances from medicinal and aromatic plants has revealed that many of them possess antioxidative activity and may be potent inhibitors of mutagenesis and carcinogenesis.

In this work we used WP2 antimutagenicity test for detection of oxidative mutagenesis and antimutagenic potential of antioxidants. Screening was performed using *E.coli* IC185 (WP2 *oxyR⁺*) and its counterpart IC202 (WP2 *oxyR/pKM101*). Oxidative mutagenesis was induced by *t*-butyl hydroperoxide (*t*-BOOH), the latent donor of RO' alkoxyl radicals. Strain IC202, highly sensitive to oxidative damage due to its deficiency in the OxyR function, shows increased mutability with respect to the OxyR⁺ strain.

Vitamin E (α -tocopherol), potent scavenger of reactive oxygen species, was used as the model antioxidant and it was tested in both tester strains. After validation, we used highly sensitive *oxyR* strain to test essential oil (EO) of basil (*Ocimum basilicum* L.) and its major monoterpenes – linalool for antimutagenesis due to their antioxidative properties.

Vitamin E inhibited induced mutagenesis in both strains (63% in IC202 and 43% in IC185). Tested basil derivatives also exhibited strong antimutagenic potential against induced oxidative mutagenesis (55% of inhibition for EO and 68% for linalool).

DETEKCIJA ANTIOKSIDATIVNE AKTIVNOSTI SUPSTANCI PRIRODNOG POREKLA NOVIM TEST-SISTEMOM

Jasna Stanojević¹, Jelena Knežević-Vukčević¹ i G. Miloshev²

¹Katedra za mikrobiologiju, Biološki fakultet, Univerzitet u Beogradu, Beograd

²Institut za molekularnu biologiju, Bugarska Akademija Nauka, Sofija, Bugarska

Reaktivne kiseonične vrste endogenog i egzogenog porekla mogu biti uključene u proces starenja i nastanak različitih humanih bolesti kao što su ateroskleroza, kancer, dijabetes, neurodegenerativna oboljenja i sindrom stečene imunodeficiencije. Antioksidanti koji se nalaze u lekovitim i aromatičnim biljkama, voću i povrću mogu imati blagotvoran efekat na ljudsko zdravlje i mogu biti korisni za prevenciju štetnih posledica oksidativnih oštećenja izazvanih reaktivnim kiseoničnim vrstama.

U ovom radu je korišćen Comet test na kvascu (YCA), metoda razvijena od strane Miloshev et al. [1], za detekciju antioksidanata sa inhibitornim efektom na nastanak oksidativnih oštećenja indukovanih vodonik peroksidom. Vitamin E (α -tokoferol) i vitamin C (askorbinska kiselina) su korišćeni kao model antioksidanti. Nakon validacije, testiran je linalol glavni konstituent etarskog ulja bosiljka (*Ocimum basilicum* L.). Sposobnost antioksidanata da smanje nivo oksidativnih oštećenja DNK ispitivana je primenom pre-tretmana i ko-tretmana (istovremeno dodavanje antioksidanta i oksidanta).

Vitamin E koji je značajno snizio broj indukovanih oksidativnih oštećenja, jači inhibitorni efekat je pokazao u pre-tretmanu (81%) u poređenju sa ko-tretmanom (43%). Snažna sposobnost inhibicije H_2O_2 -indukovanih DNA oštećenja je detektovana i za vitamin C, ali procenat inhibicije dostiže istu vrednost (65%) i za pre-tretman i za ko-tretman.

Monoterpen linalol je takođe pokazao jaku antioksidativnu aktivnost. Detektovan inhibitorni efekat se kreće u opsegu od 55% za ko-tretman do 70% za pre-tretman. Na osnovu dobijenih rezultata linalol se može svrstati među supstance sa veoma snažnim antioksidativnim dejstvom čiji bi potencijalni antikancerogeni efekat trebalo dalje ispitati.

DETECTION OF ANTIOXIDATIVE ACTIVITIES OF NATURAL SUBSTANCES WITH A NEWLY DEVELOPED SYSTEM

Reactive oxygen species (ROS), from both endogenous and exogenous sources, may be involved in aging and different human diseases such as atherosclerosis, cancer, diabetes, neurodegenerative diseases and acquired immunodeficiency syndrome (AIDS). Natural antioxidants contained in medicinal and aromatic plants, fruits and vegetables may have beneficial health effect and may be useful in preventing the deleterious consequences of oxidative damage caused by ROS.

In this work Yeast Comet Assay (YCA), a method developed by Miloshev et al. [1], is used for the detection of antioxidative activity of different compounds against hydrogen peroxide-induced oxidative damage. Vitamin E (α -tocopherol) and vitamin C (ascorbic acid) were used as model antioxidants. After validation, we have tested linalool major constituent of basil (*Ocimum basilicum* L.). The ability of antioxidant pre-treatment and co-treatment (simultaneously with oxidant) to decrease DNA damage was assessed.

Vitamin E exhibited the protective effect against induced oxidative damage; the stronger inhibitory effect was detected in pre-treatment (81%) comparing with co-treatment (43%). Vitamin C also displayed a significant protective capability against H_2O_2 -induced DNA damage. However percentage of inhibition reached the same value (65%) both for co-treatment and pre-treatment.

Linalool, the oxygen-containing monoterpenone, showed also very strong antioxidative activity. Inhibitory effect is in the range from 55% for co-treatment to 70% for pre-treatment. These results put linalool as one of substances with very strong antioxidative acting and recommend it for further anticarcinogenic studies.

MORFOLOŠKA I MOLEKULARNA VARIJABILNOST VRSTA *Helianthus giganteus* L. I *Helianthus maximiliani* Sch.

D. Saftić-Panković, J. Atlagić, T. Miljanović i N. Radovanović

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Rod *Helianthus* se sastoji od 49 vrsta. U ovom radu ispitivane su dve vrste: *H. giganteus* L. i *H. maximiliani* Sch., koje su rasprostranjene i sakupljene u severnoj Americi. Da bi se utvrdila morfološka varijabilnost unutar/između ove dve vrste ispitivano je po petanest populacija od svake vrste. Analizirano je trideset morfoloških osobina na pet biljaka od svake populacije, koje su gajene u istim uslovima u kolekciji divljih vrsta na Rimskim Šančevima. Tri populacije iz vrste *H. giganteus* su na osnovu morfoloških osobina pokazale veću sličnost sa populacijama iz vrste *H. maximiliani*, što možda ukazuje na postojanje novog intraspecies taksona u vrsti *H. giganteus*. Da bi se proverila ova hipoteza ispitivana je i molekularna varijabilnost na istim populacijama-vrstama. Izolovana je genomska DNK iz zamrznutih listova navedenih populacija. Za ispitivanje polimorfizma genomske DNK korišteni su različiti markeri zasnovani na PCR-u (Polymerase Chain Reaction). Naročito su bili zastupljeni markeri za mikrosatelite, za koje je nedavno pokazana dosad najveća molekularna genetička varijabilnost u rodu *Helianthus*. Dobijeni rezultati potvrđuju visok stepen varijabilnosti između ispitivanih populacija, a u radu su upoređeni dendrogrami dobijeni klaster analizom morfoloških osobina i molekularnih markera.

MORPHOLOGICAL AND MOLECULAR VARIABILITY OF *Helianthus giganteus* L. AND *Helianthus maximiliani* Sch. SPECIES

Genus *Helianthus* consists of 49 species. Two species: *H. giganteus* L. and *H. maximiliani* Sch., distributed and collected in North America, were investigated. In order to determine morphological variability in/between these two species, fifteen populations of each species were used. Thirty traits were measured on five plants per species, grown in the same conditions in the wild species nursery at Rimski Šančevi. According to the investigated morphological traits, three species of *H. giganteus* were closer to *H. maximiliani* populations, which possibly indicates the existance of a new intraspecies taxon in *H. giganteus*. In order to test this hypothesis molecular variability of the same populations-species, was also investigated. The polymorphism of genomic DNA, that was isolated from frozen leaves, was investigated by PCR (Polymerase Chain Reaction) based markers. The most abundant were markers for microsatellites, recently shown to be the most powerfull for the analysis of molecular genetic variability in genus *Helianthus*. Obtained results confirm the high variability between examined populations. Dendograms constructed by cluster analysis of examined morphological traits and molecular markers are discussed.

VARIJABILNOST AMINOKISELINSKOG SASTAVA KOD SORTI JEĆMA

Nevena Đukić¹, D. Knežević², Gordana Matić³,
Marina Stamenković-Radak³ i M. Andelković³

¹Prirodno-matematički fakultet, Institut za biologiju, Kragujevac

²Institut za istraživanja u poljoprivredi SRBIJA, Centar za strna žita, Kragujevac

³Biološki fakultet, Beograd

U ovom radu ispitivano je 29 sorti ječma. Metodom hromatografije analizirano je prisustvo slobodnih aminokiselina, a spektrofotometrijski određena je njihova koncentracija. Kvalitativna analiza aminokiselinskog sastava ukazuje da su najzastupljenije među slobodnim aminokiselinama zrna ispitivanih sorti jecma: glutaminska kiselina, prolin, valin, treonin i druge. Obzirom da ječam ima svoju upotrebnu vrednost u stočnoj ishrani, u proizvodnji slada i piva, kao i za specijalne namene u ishrani ljudi, veoma je važno prisustvo esencijalnih aminokiselina. U različitim sortama ispitivanog ječma identificirali smo sedam esencijalnih aminokiselina (valin, treonin, leucin, fenilalanin, metionin, triptofan i arginin).

Kvantitativna analiza slobodnih aminokiselina ukazuje da je njihov sadržaj visok u sledećim sortama ječma: *Novosadski 519* i *Novosadski 535* (193 mg/ml), *Novosadski 703* (163 mg/ml), *Novosadski 293*, *Novosadski 309* i *Gigant* (161 mg/ml). Analiza sadržaja glutaminske kiseline u zrnu ječma pokazala je da je ona kvantitativno najzastupljenija aminokiselina. Koncentracija glutaminske kiseline kod sorte *Novosadski 319* je 86 mg/ml; a kod sorte ječma *Novosadski 529* i *Novosadski 535* koncentracija je 63,5 mg/ml, odnosno 62 mg/ml.

VARIABILITY OF AMINOACID CONTENTS IN BARLEY CULTIVARS

Study of presence of free amino acids in 29 barley cultivars have been analyzed by chromatography and their concentration were determined by spectrophotometry. Qualitative analysis of amino acid indicated that glutamic acid, proline, valine, threonine were most present among free amino acid in grain of examined barley cultivars. The presence of essential amino acid is very important, considering that barley use for feed animals, production of malt and beer, as well as for special purpose in human nutrition. In different barley cultivars was identified 7 essential amino acids (valine, threonine, leucine, phenylalanine, methionine, triptophan and arginine). Analysis of free amino acid indicated that their contents was very high in next cultivars: *Novosadski 519* and *Novosadski 535* (193 mg/ml); than *Novosadski 703* (163 mg/ml), *Novosadski 293*, *Novosadski 309* and *Gigant* (161 mg/ml). The analysis of contents of glutamic acid showed that its most present amino acid in barley grain. Concentration of glutamic acid in cultivar *Novosadski 319* is 86 mg/ml; and in barley cultivars *Novosadski 529* and *Novosadski 535* with concentration 63,5 mg/ml, and 62 mg/ml, respectively.

II tematska oblast / II topic:

Struktura i funkcija genoma

Genome structure and function

Uvodna izlaganja / Introductory lectures

II-Uvo

SINGLE CELL GEL ELECTROPHORESIS (SCGE) OR COMET ASSAY AS A METHOD FOR ASSESSING THE ROLES OF LINKER HISTONE H1 IN CHROMATIN

M. Kirilova and G. Miloshev

Yeast Molecular Genetics Laboratory, Institute of Molecular Biology,
Bulgarian Academy of Sciences, Sofia

Chromatin is the basic structure in which DNA is organized in the cell nucleus. Activity of the genes relays not only on the upstream/downstream regulating sequences and transcription factors but also is deeply affected by the way in which DNA is packaged with the chromatin proteins. Chromatin is composed predominantly of DNA and four core histones H2, H2B, H3 and H4. All four histones through their post-translational modifications play an important role in the regulation of genome function. The fifth histone, H1 is involved in the organizing the higher-order chromatin structures and presumably also in the fine regulation of gene activity. However, how and where in the cell cycle this role is accomplished remains mostly obscure.

Here we present our current results in application of the Comet assay, a method initially developed for assessing DNA damages, for the revealing differences in chromatin structure. We modified the classical Comet assay by applying it on yeast and developing the Yeast Comet Assay (YCA). Application of YCA to chromatin research demonstrated that histone H1 in yeast partially protects DNA in nucleus from digestion with nucleases and may be involved in presumable contacts of the chromatin with nuclear matrix.

**ANALIZA GENA ZA BIOSINTEZU SEKUNDARNIH METABOLITA
KOD PRIPADNIKA RODA *Streptomyces***

M. Savić, Ivana Bratić i Branka Vasiljević

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Različiti mikroorganizmi proizvode sekundarne metabolite od kojih većina pokazuju toksično delovanje na različite ćelije i organizme. Ovi metaboliti, ili prirodni proizvodi, predstavljaju izuzetno značajan resurs za otkrivanje novih jedinjenja sa specifičnim fiziološkim delovanjem. Poliketidi su velika i strukturno veoma različita grupa bioaktivnih jedinjenja za čiju biosintezu su odgovorne poliketid sintaze (PKS). Molekularna karakterizacija gena uključenih u biosintezu poliketidnih metabolita je ukazala na postojanje kompleksnih enzimskih sistema čiji lokusi obično zauzimaju desetine hiljada baznih parova na hromozomu. Kod tipa I PKS enzimatski domeni su organizovani u module i genetički redosled modula je kolinearan sa redosledom biohemijskih reakcija. Svaki modul je odgovoran za prepoznavanje i ugrađivanje specifičnog malog konstituenta u rastući poliketidni lanac, kao i za prateće reakcije redukcije. Ovakav mehanizam je u suprotnosti sa tipom II PKS gde se istovetni set proteina, svaki zasebno katalizujući različitu enzimatsku reakciju, koristi tokom nekoliko ciklusa. Analizirajući laboratorijsku kolekciju streptomiceta izabran je soj *Streptomyces* sp. MS405. Odabrani soj proizvodi bioaktivno jedinjenje mehanizma delovanja nalik na imunosupresor FK506. Poređenjem sa poznatim poliketidnim jedinjenjima, metodom tankoslojne hromatografije, kao što su FK506, FK520, rapamicin, eritromicin i rifampicin, utvrđeno je da se analizirano jedinjenje razlikuje od referentnih. Kloniranjem kratkog DNK fragmenta utvrđeno je da biosinteza ovog jedinjenja zavisi od PKS tipa I. Filogenetske analize ukazuju na visok stepen homologije između kloniranog fragmenta i *fkbA* PKS *S. hygroscopicus*, prozvođača imunosupresora rapamicina.

**ANALYSIS OF THE GENE CLUSTERS INVOLVED IN BIOSYNTHESIS
OF THE SECONDARY METABOLITES AMONG MEMBERS OF THE GENUS
*Streptomyces***

A variety of microorganisms produce secondary metabolites, many of which exhibit toxic activity against different cells and organisms. These metabolites have proved to be a valuable resource to identify compounds with specific biological effects. Polyketides are a large and structurally diverse family of bioactive compounds whose biosynthesis is catalysed by polyketide synthases (PKS). Molecular characterization of the genes involved in formation of polyketide metabolites has demonstrated the involvement of complex enzyme systems whose genetic loci usually span tens of thousands of base pairs on a chromosome. In type I PKS enzymatic domains are organized into modules and genetic order of the modules is collinear with the order of biochemical reactions. Each module is responsible for recognition and incorporation of a specific small constituent into growing polyketide chain as well as the subsequent reductive reactions. This is in contrast to type II PKS where a set of discrete proteins, each catalyzing different enzymatic reaction, is repeatedly employed. Analysing laboratory streptomycetes collection strain *Streptomyces* sp. MS405 was selected. This strain produces bioactive compound with the same mechanism of action as immunosuppressant FK506. Thin layer chromatography (TLC) studies showed that the compound is chemically different compared to referent polyketides: FK506, FK520, rapamycin, erythromycin and rifampycin. Cloned short fragment of the biosynthetical gene cluster revealed that biosynthesis of this compound is governed by type I PKS. Phylogenetical studies imply high sequence homology between cloned sequence and *fkbA* PKS of *S. hygroscopicus*, producer of polyketide immuno-suppressant rapamycin.

IDENTIFIKACIJA KONTROLNIH ELEMENATA UKLJUČENIH U REGULACIJU EKSPRESIJE SOX3 GENA RETINOIČNOM KISELINOM

Marija Mojsin, Nataša Kovačević-Grujičić, A. Krstić i Milena Stevanović

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

SOX geni pripadaju familiji gena koji pokazuju homologiju sa SRY genom (sex-determining gene) sisara. Ova homologija odnosi se na region označen kao HMG domen koji je odgovoran za vezivanje SOX proteina za specifičnu DNK sekvencu u kontrolnim regionima ciljnih gena.

NT2/D1 je permanentna ćelijska linija humanog teratokarcinoma. Ove ćelije su na stadijumu opredeljenih neuralnih progenitora, a retinoična kiselina indukuje njihovu diferencijaciju u neurone. Ova karakteristika čini NT2/D1 ćelije jedinstvenim model sistemom za proučavanje humanih gena uključenih u procese diferencijacije nervnog sistema.

Ovaj model sistem je korišćen za proučavanje transkripcione regulacije SOX3 gena i njegove ekspresije tokom neuralne diferencijacije. Pokazano je da u ranim fazama neuralne diferencijacije NT2/D1 ćelija, indukovanih retinoičnom kiselinom, dolazi do aktivacije SOX3 gena. Naš cilj bio je da identifikujemo kontrolne elemente odgovorne za vezivanje transkripcionih faktora i elemente preko kojih retinoična kiselina indukuje aktivaciju SOX3 gena. Korišćenjem nuklearnih ekstrakata izolovanih iz neindukovanih i indukovanih NT2/D1 ćelija urađene su metoda otiska proteina na DNK i metoda usporene pokretljivosti u gelu. Rezultati ovih eksperimenata ukazuju na postojanje razlika u formiranju kompleksa DNK-protein u neindukovanim i indukovanim NT2/D1 ćelijama, i omogućavaju identifikaciju kontrolnih elemenata odgovornih za aktivaciju transkripcije SOX3 gena retinoičnom kiselinom.

IDENTIFICATION OF CONTROL ELEMENTS INVOLVED IN REGULATION OF SOX3 GENE EXPRESSION BY RETINOIC ACID

SOX genes comprise a family of genes related to the mammalian sex-determining gene SRY in the region that encodes the HMG-box domain responsible for the sequence-specific DNA-binding activity. Spatial and temporal expression of SOX genes suggests they play roles in the regulation of development and differentiation. The developing brain is the major site of expression of many SOX genes. During mammalian embryogenesis, SOX3 gene is predominantly expressed in developing nervous system.

NT2/D1 is human teratocarcinoma cell line that resembles human committed neuronal progenitor cells that can be induced to differentiate into neurons by retinoic acid. Thus, retinoic acid-induced differentiation of NT2/D1 providing a unique *in vitro* model for studying human genes that promote and regulate neural differentiation.

We used this model to study transcriptional regulation of SOX3 gene expression during neuronal differentiation of NT2/D1 cells. It was shown that retinoic acid-induced differentiation of NT2/D1 cells is marked by activation of SOX3 gene during early phases of induction. In order to determine regions recognized by transcription factors and to identify control elements responsible for retinoic acid induction of SOX3 gene we performed *in vitro* DNaseI footprinting analysis and gel mobility shift assays with nuclear extracts from uninduced and induced NT2/D1 cells. These experiments showed significant difference between protein-DNA complexes in undifferentiated and differentiated NT2/D1 cells allowing us to identify control regions that mediate a stimulatory effect of retinoic acid on SOX3 gene expression.

II tematska oblast / II topic:

Struktura i funkcija genoma

Genome structure and function

Usmena izlaganja / Oral presentations

II-Usm

**ANALIZA SASTAVA MIKROBIOLOŠKIH ZAJEDNICA RIZOSFERA
Ramonda nathaliae I *Ramonda serbica***

Ivana Bratić, M. Savić, Lidija Đokić i Branka Vasiljević

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Ramonda nathaliae i *Ramonda serbica* su tercijalni relikti i endemiti Balkanskog poluostrva koji rastu u pukotinama stena na strmim krečnjačkim liticama u Sićevačkoj klisuri (Srbija). Cilj ovog rada je bio da se utvrdi sastav mikrobioloških zajednica rizosfera *R. nathaliae* i *R. serbica* metodama PCR (reakcija lančane polimerizacije) i RFLP (polimorfizam dužina restrikcionih fragmenata). Za PCR metodu je korišćen univerzalni par prajmera koji selektivno amplifikuje fragment bakterijskog 16S rRNA gena. PCR fragmenti (dužine oko 1300bp) su dobijeni iz ukupne DNA izolovane iz rizosfera *R. serbica* i *R. nathaliae*. PCR produkti su potom klonirani u pUC19 vektor i restrikciono analizirani pomoću *RsaI* enzima. Restrikpcioni profili su potvrdili prisustvo 89 različitih operativnih taksonomske jedinica (OTJ) poreklom iz rizosfere *R. nathaliae*, a samo 13 različitih OTJ poreklom iz rizosfere *R. serbica*.

**STUDY OF MICROBIAL COMMUNITY COMPOSITION FROM
RHIZOSPHERE SOILS OF *Ramonda nathaliae* AND *Ramonda serbica***

Ramonda nathaliae i *Ramonda serbica*, the Tertiary relict and the endemic species of the Balkan, grow in the rock fissures on steep limestone cliffs of the Sicevo gorge (Serbia). The aim of this study was to analyse microbial community composition of rhizosphere soils of *R. nathaliae* and *R. serbica* by PCR (polymerase chain reaction) and RFLP (restriction fragment length polymorphism) methods. A universal primer pair for PCR was designed to selectively amplify a fragment of bacterial 16S rRNA gene. PCR fragments (approximately 1300 bp) were amplified from total DNA that was isolated from rhizosphere soils of *R. serbica* and *R. nathaliae*. PCR products were cloned into pUC19 plasmid and clones were subjected to restriction analysis employing restriction enzyme *RsaI*. Restriction profiles indicated the presence of 89 different operative taxonomic units (OTU) in the rhizosphere soil of *R. nathaliae*, but only 13 different OTUs from the rhizosphere soil of *R. serbica*.

EFEKTI B HROMOZOMA KOD ŽUTOGRLOG ŠUMSKOG MIŠA, *Apodemus flavicollis* (Rodentia, Mammalia) – NIVO MORFOLOŠKE INTEGRACIJE MANDIBULE

Vida Jojić¹, Jelena Blagojević¹, Ana Ivanović² i M. Vujošević¹

¹Odeljenje za genetiku, Institut za biološka istraživanja «Siniša Stanković», Beograd

²Institut za zoologiju, Biološki fakultet, Beograd

B hromozomi (B) predstavljaju prekobrojne hromozome široko zastupljene kod mnogih biljnih i životinjskih vrsta. U pogledu efekata ovih hromozoma na fenotipskom nivou postoje velike razlike među vrstama, kao i mali broj registrovanih slučajeva gde prisustvo Bs dovodi do kvalitativnih i/ili kvantitativnih promena fenotipa. Cilj rada bio je utvrđivanje potencijalnih efekata B na morfološku varijabilnost mandibule kod jedinki populacije A. *flavicollis* sa Avala. Korишћenjem fenotipskih korelacija među mandibularnim karakterima procenjen je nivo morfološke integracije mandibule kod dve grupe jedinki (sa i bez B), kao i kod 4 grupe jedinki (mužjaci i ženke sa i bez B). Mandibula glodara predstavlja kompleksan skeletni organ sastavljen od dva funkcionalno i razvojno različita dela (corpus, koji podupire korene zuba i ascending ramus, preko koga se mišići vezuju za donju vilicu). Stepen morfološke integracije izražen preko indeksa integracije (I) procenjen je na tri nivoa - corpus, ascending ramus i čitava mandibula. Sve tri vrednosti indeksa integracije bile su veće kod grupe jedinki sa B. Najizraženije povećanje stepena morfološke integracije registrovano je kod ženki sa B. Dobijeni rezultati ukazuju ne samo da prisustvo B kod žutogrlog šumskog miša proizvodi efekte na nivou fenotipa, već i da je doprinos B povećanju nivoa morfološke integracije različit kod različitih polova.

THE EFFECTS OF B CHROMOSOMES IN YELLOW-NECKED MOUSE, *Apodemus flavicollis* (Rodentia, Mammalia) - THE LEVEL OF MORPHOLOGICAL INTEGRATION OF MANDIBLE

B chromosomes (Bs) are supernumerary chromosomes widely distributed among many species of plants and animals. From the aspect of their phenotypic effects, species possessing Bs are particularly diverse. In general, there is the scarcity of reported cases where the presence of Bs influences phenotypes qualitatively and/or quantitatively. Our goal was to search for the potential effects of Bs on morphological variation of mandible in *A. flavicollis* from Avala population. We've choosed to study phenotypic correlations among mandibular traits in order to estimate the level of their morphological integration for both groups of animals (with and without Bs), as well as for divided sample according to sex and presence of Bs (meles and females with and without Bs).. Bearing in mind that rodent mandible is a complex skeletal organ consisting of two functional and developmental units (corpus, supporting tooth roots and ascending ramus, participating in the attachments of the muscles) we estimated morphological integration by index of integration (I) on the three levels: overall mandible, corpus unit and ascending ramus unit. We found that individuals with Bs showed higher level of morphological integration for both units and for overall mandible as well. The highest increase in the level of morphological integration showed females with Bs. These results indicate that presence of Bs not only produces the effects in yellow-necked mouse, but also the contribution of Bs to increase level of morphological integration is different in different sexes.

ANALIZA PROMOTORSKOG REGIONA HUMANOG SOX3 GENA I IDENTIFIKACIJA TRANSKRIPCIONIH FAKTORA UKLJUČENIH U REGULACIJU EKSPRESIJE

Nataša Kovačević-Grujičić, Marija Mojsin, A. Krstić i Milena Stevanović

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Familija *SOX* gena kodira proteine koji funkcionišu kao klasični transkripcioni faktori i arhitektonske komponente hromatina i imaju značajnu ulogu u proliferaciji i diferencijaciji ćelija tokom embriogeneze. *Sox3* gen, smešten na X hromozomu, se eksprimira u centralnom nervnom sistemu u ranim fazama razvića i predstavlja jedan od najranijih neuralnih markera kod kičmenjaka. Cilj našeg rada bio je definisanje i karakterizacija promotor-a *SOX3* gena čoveka. Elongacijom prajmera reverznom transkripcijom određen je start transkripcije humanog *SOX3* gena. Strukturno i funkcionalno je analiziran regulatorni region odgovoran za ekspresiju *SOX3* gena u NT2/D1 ćelijskoj liniji. Koristeći promotor-reporterske konstrukte definisali smo minimalni promotorski region *SOX3* gena odgovoran za njegovu bazalnu promotorsknu aktivnost.

PROMOTER ANALYSIS OF HUMAN SOX3 GENE AND IDENTIFICATION OF TRANSCRIPTION FACTORS IMPLICATED IN REGULATION OF ITS EXPRESSION

Family of *SOX* genes encodes proteins that appear to govern cell fate decisions during embryogenesis by functioning both as classical transcription factors and architectural components of chromatin. *Sox3*, an X-linked member of the family, is expressed in the central nervous system from the earliest stages of development. It is considered to be one of the earliest neural markers in vertebrates playing the role in specifying neuronal fate. The aim of this study has been to determine and characterize the promoter of the human *SOX3* gene and to elucidate molecular mechanisms underlying the regulation of its expression. Using primer extension method, we have identified the transcription start point and carried out the structural and functional analysis of the regulatory region responsible for *SOX3* expression in NT2/D1 cell line. Using promoter-reporter constructs we have determined the minimal *SOX3* promoter region that confers the basal promoter activity.

TIAZOFURIN NE DOVODI DO OŠTEĆENJA DNK U KOMET TESTU

N. Đelić¹, Biljana Spremo-Potparević², Lada Živković² i V. Bajić³

¹Katedra za biologiju, Fakultet veterinarske medicine, Univerzitet u Beogradu, Beograd

²Institut za fiziologiju, Farmaceutski fakultet, Univerzitet u Beogradu, Beograd

³Institut za biomedicinska istraživanja, «Galenka», Beograd

Tiazofurin predstavlja nukleozid sa izraženim antitumorskim dejstvom. Intracelularno, tiazofurin se metabolički konvertuje do tiazol-4-karboksamid adenin dinukleotida (TAD), analoga nikotinamid adenin dinukleotida (NAD), koji snažno inhibira IMP dehidrogenazu. Usled inhibicije IMP dehidrogenaze kao ključnog enzima u *de novo* sintezi purinskih nukleotida, dolazi do pada guanilata i na taj način se postiže inhibicija rasta tumora kako *in vitro*, tako i *in vivo*.

Cilj ovog rada bio je da se utvrdi da li tiazofurin može da dovede do oštećenja molekula DNA u humanim limfocitima, detektabilnih Komet testom (elektroforeza DNA pojedinačnih ćelija, engl. single cell gel electrophoresis). Cela krv je izlagana eksperimentalnim koncentracijama (0,001, 0,002, 0,004 i 0,006 M) u trajanju od 30 min ili 2 sata na 37°C. Nakon tretmana i provere citotoksičnosti pomoću tripan plavog, uzorci su centrifugirani, a zatim su ćelije su resuspendovane u agarozni niske tačke topljenja, nanete na prethodno pripremljena predmetna stakla i ostavljena u rastvoru za liziranje na +4°C preko noći. Sutradan je posle denaturacije DNA u puferu sa pH>13, izvršena elektroforeza u istom puferu na 0,7 V/cm. Nakon neutralizacije alkalija, predmetna stakla su odmah bojena etidijum bromidom i obavljena je analiza dobijenih kometa. Analizirano je tačno po 200 kometa za svaku eksperimentalnu koncentraciju. Statistička analiza urađena je neparametrijskim Wilcoxon-ovim testom.

Tiazofurin u ispitanim koncentracijama nije ispoljio značajnu citotoksičnost (u svim uzorcima bilo je preko 90% vijabilnih limfocita). Pored toga, ne postoji statistički značajno odstupanje u stepenu oštećenja DNA u odnosu na kontrolne (netretirane) uzorce, kako pri tretmanu od 30 min, tako i pri tretmanu od 2 h. Na osnovu toga, može se zaključiti da je rezultat testiranja tiazofurina u *in vitro* Komet testu negativan.

TIAZOFURIN DO NOT INDUCE DNA DAMAGE IN THE COMET ASSAY

Tiazofurin is a nucleoside with a profound antitumor effects. Tiazofurin is metabolically converted intracellularly to an analogue of nicotin amide adenine dinucleotide (NAD), tiazole-4-carboxamide adenine dinucleotide (TAD), a potent inhibitor of IMP dehydrogenase. Inhibition of this rate-limiting enzyme for *de novo* purine synthesis results in reduced levels of guanylates, inhibiting tumor growth *in vitro* and *in vivo*.

The objective of the present investigation was to examine whether tiazofurin induces DNA damage in human lymphocytes detectable by single cell gel electrophoresis (Comet) assay. The whole peripheral venous blood was exposed to four experimental concentrations of tiazofurin (0,001, 0,002, 0,004 i 0,006 M) for either 30 min or 2 h at 37°C. After the treatment, we checked cytotoxicity using trypan blue dye exclusion. The samples were centrifugated, the cell pellet was resuspended in pre-heated low melting point agarose and layed on the precoated slides. Afterwards, the slides were left in lysing solution at +4°C overnight. Tomorrow, we performed DNA unwinding and electrophoresis in alkaline buffer (pH>13) at 0,7 V/cm. After the neutralisation of alkali, slides were stained with ethidium bromide and the comets were immediately scored. For each experimental concentration and exposure time we analysed exactly 200 comets. Statistical analysis was performed by non-parametric Wilcoxon rank-sum test.

Tiazofurin has not expressed significant cytotoxicity at any experimental point (lymphocyte viability was over 90%). Moreover, there is no significant departure in DNA damage

STRUKTURNA I FUNKCIONALNA ANALIZA PROMOTORA HUMANOG *SOX14* GENA

Jelena Đurović, Mina Seović, Isidora Petrović i Milena Stevanović

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

SOX14 je član *SOX* familije gena koji kodiraju transkripcione regulatore uključene u kontrolu procesa razvića. Poznato je da su mutacije u *SOX* genima odgovorne za pojavu genetičkih sindroma kod čoveka, što ukazuje na njihovu važnu ulogu u procesu razvića. Iako ekspresioni profil *SOX* gena ukazuje da je njihova ekspresija specifična za tkivo i stupanj razvića, vrlo malo se zna o mehanizmima koji regulišu ekspresiju ovih gena.

U cilju proučavanja molekularnih mehanizama koji regulišu transkripciju humanog *SOX14* gena, određen je start transkripcije metodom elongacije prajmera. Dobijena su dva produkta elongacije prajmera dužine 275 nukleotida i 194 nukleotida. Ovi rezultati ukazuju da transkripcija otpočinje od guanina na poziciji -251 bp u odnosu na start kodon, mada postojanje starta transkripcije na poziciji -170 ne može biti isključeno.

Identifikacija regiona koji pokazuje bazalnu promotorsku aktivnost kao i proksimalni enhenser, određeni su sposobnošću konstrukata sa 5' nekodirajućim regionom *SOX14* gena da indukuju ekspresiju CAT reporter gena. Ovim eksperimentima pokazano je da je region između -470 i +201 odgovoran za bazalnu aktivnost *SOX14* promotora.

NF-Y je transkripcioni faktor važan za regulaciju transkripcije gena koji ne poseduju TATA boks. U ovom radu pokazano je da se NF-Y vezuje za CCAAT motiv prisutan u promotoru *SOX14* gena. Mutacionom analizom pokazano je da je CCAAT motiv funkcionalan i značajan za regulaciju transkripcije *SOX14* gena.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE HUMAN *SOX14* PROMOTER

SOX14 is a member of *SOX* gene family of putative transcriptional regulators implicated in the control of diverse developmental processes. Mutations in *SOX* genes are known to be responsible for human genetic syndromes, demonstrating the critical role that they play in normal embryonic development. Although *SOX* gene expression patterns suggest tissue- and development stage-specific control of gene expression, little is known about mechanisms responsible for expression of these genes.

In order to elucidate the molecular mechanisms controlling the expression of the human *SOX14* gene, we have determined the transcription start site using primer extension method and carried out the structural and functional analysis of the regulatory region responsible for its expression. Two distinct primer extension products were identified: the single major product that migrates with a length of 275 nt and the minor product 194 nt in size. This result indicates that the transcription of the *SOX14* initiates at the single major site at the guanine residue 251 bp upstream of the start codon, although the existence of a minor transcription start site can not be excluded.

To identify the DNA regions responsible for the control of *SOX14* gene transcription we have analyzed the ability of truncated fragments from the 5' flanking region of the *SOX14* gene to drive expression of CAT reporter gene. Functional mapping of the 5' regulatory region revealed that the sequence between -470 and +201 bp is essential for minimal basal activity of the *SOX14* promoter.

NF-Y is a transcriptional factor involved in transcription of TATA-less genes. We have identified that NF-Y binds to the CCAAT box motif present in the regulatory region of the *SOX14* promoter. By mutation analysis we have shown that CCAAT box motif present in the *SOX14* promoter plays a functional role in the transcription of this gene.

CITOGENETIČKI EFETKI TIAMULINA S IN VIVO

Biljana Marković¹, N. Đelić², Z. Stanimirović¹, M. Anđelković² i D. Marinković³

¹Katedra za Biologiju, Fakultet veterinarske medicine, Univerzitet u Beogradu, Beograd

²Institut za zoologiju, Biološki fakultet, Univerzitet u Beogradu, Beograd

³Srpska akademija nauka i umetnosti, Beograd

U ovom radu ispitivan je citogenetički efekat antibiotskog preparata Tiamulina S (proizvođač Hemovet, Vršac), koji ima široku primenu u veterinarskoj medicini.

Eksperiment in vivo obavljen je kod laboratorijskog miša BALB/c soja praćenjem uticaja intragastrične aplikacije Tiamulina S na nastanak hromozomskih promena u ćelijama kostne srži u mitozi i semenim ćelijama u mejozi. Klastogeni efekat Tiamulina S praćen je u tri eksperimentalne doze (0,01 ml/kg, 0,2 ml/kg i 0,4ml/kg). Eksperimenti su obavljeni po smernicama OECD o testiranju na genotoksičnost. Statistička analiza urađena je Studentovim t-testom.

Eksperimentalni rezultati pokazuju da ispitivani antibiotiski preparat Timulin S ima sposobnost promene kariotipa ćelija kostne srži i testisa i indukcije numeričkih hromozomskih aberacija tipa aneuploidija i poliploidija, kao i strukturalnih hromozomskih promena tipa lezija, prekida i Robertsonovih translokacija. Pri praćenju zastupljenosti ukupnih citogenetičkih promena u ćelijama kostne srži i testisa mogu se izvesti dva zapažanja: prisustvo visoko signifikantnih razlika ($p<0,001$) između eksperimentalnih grupa tretiranih odgovarajućim dozama Tiamulina S i kontrolnih grupa, kao i postojanje korelacije između rasta jačine primenjene doze i porasta numeričkih i strukturalnih hromozomskih alteracija, u ćelijama kostne srži i semenika eksperimentalnih grupa miševa, tretiranih rastućim dozama antibiotskog preparata Tiamulina S, što ukazuje da sve ispitivane doze Tiamulina S (0,01 ml/kg, 0,2 ml/kg i 0,4ml/kg) poseduju genotoksični potencijal.

CYTOGENETIC EFFECTS OF TIAMULIN S IN VIVO

In this investigation we examined cytogenetic effects of antibiotic Tiamulin S (Hemovet, Vršac), which is intensely used in veterinary medicine.

The in vivo experiment was performed on BALB/c inbred strain of mice. The test substance was applied intragastrically. For the evaluation of genotoxic effects we monitored cytogenetic changes both in mitotic bone marrow and meiotic testicles cells. The clastogenic effects and other cytogenetic parameters were analysed following the exposure of mice to three doses of tiamulin S (0,01 ml/kg, 0,2 ml/kg i 0,4ml/kg). The experiments were performed according to OECD guidelines for genotoxicity testing. Statistical analysis was performed by Student's t-test.

The experimental results revealed that antibiotic Tiamulin S induces changes of karyotypes in both types of analysed cells (bone marrow and testicles). In addition to clastogenic effects we observed numerical (aneuploidies and polyploidies) and structural aberrations (Robertsonian translocations, acentric fragments). On the basis of the analysis of overall cytogenetic changes, we conclude that all doses applied had significant ($p<0,001$) effect on analysed karyotypes. Moreover, there is a clear dose-response relationship. Therefore, it can be concluded that all concentrations of Tiamulin S exhibited genotoxic potential.

UTICAJ TIROKSINA NA ĆELIJSKU KINETIKU HUMANIH LIMFOCITA U KULTURI

Biljana Spremo-Potparević¹, N. Đelić², Biljana Marković² i V. Bajić³

¹Institut za fiziologiju, Farmaceutski fakultet, Univerzitet u Beogradu, Beograd

²Katedra za biologiju, Fakultet veterinarske medicine, Univerzitet u Beogradu, Beograd

³Institut za biomedicinska istraživanja, «Galenka», Beograd

Cilj istraživanja u ovom radu bio je da se utvrdi da li tiroksin utiče na ćelijsku kinetiku humanih limfocita u kulturi. Analiziran je širok spektar koncentracija tiroksina, uključujući koncentracije koje odgovaraju terapijskim dozama u humanoj medicini, kao i znatno veće koncentracije od terapijskih. Identifikacija ćelija u prvoj, drugoj i trećoj mitozi obavljena je na istim preparatima upotrebljenim za analizu SCE i mikronukleusa u našim prethodnim istraživanjima. Pored genotoksičnih efekata, modulacija mitotske aktivnosti je od značaja za malignu transformaciju ćelije, tako da bi rezultati istraživanja u ovom radu mogli da dopirnesu boljem razumevanju moguće uloge tireoidnih hormona u kancerogenezi. Svi eksperimenti su obavljeni na limfocitima periferne krvi zdravih muških osoba mlađih od 35 godina. Eksperimentalna procedura obavljena je po smernicama OECD za testiranje na genotoksičnost. Za utvrđivanje kinetike proliferacije limfocita upotrebљeni su proliferacioni indeksi PI i CBPI (engl. CBPI = cytochalasine block proliferation index). Statistička analiza obavljena je χ^2 testom.

Rezultati istraživanja ukazuju da tiroksin ne dovodi do statistički značajne ($p>0,05$) modulacije mitotske aktivnosti humanih limfocita u kulturi. Mada ove analize ne predstavljaju test na kancerogenost, dobijeni rezultati sugerisu da bi tiroksin svoje efekte mogao da ostvari prevashodno na nivou inicijacije kancerogeneze.

INFLUENCE OF THYROXINE ON CELL CYCLE KINETICS OF CULTURED HUMAN LYMPHOCYTES

The aim of this investigation was to examine whether thyroxine influences cell cycle kinetics of cultured human lymphocytes. We examined a wide range of thyroxine concentrations, including those corresponding therapeutic doses in human medicine, as well as much higher concentrations. Identification of cells in the first, second and third mitosis was performed on the same slides previously used for SCE and micronuclei analysis. In addition to genotoxic effects, modulation of mitotic activity is relevant to malignant transformation. Therefore, the results of this investigation may improve our knowledge of the possible roles of thyroid hormones in carcinogenesis. The experiments were performed on human peripheral blood lymphocytes from three healthy men under 35 years. The experimental protocol was the same as recommended by the OECD guidelines for genotoxicity testing. For the evaluation of cell cycle kinetics we used proliferation index (PI) and cytochalasine block proliferation index (CBPI). Statistical analysis was performed by χ^2 test.

The obtained results have shown that thyroxine has not influenced cell cycle kinetics significantly ($p>0,05$). Although these analysis are not the test for carcinogenicity, our results suggest that thyroxine primarily acts as a tumor initiator in the process of carcinogenesis.

II tematska oblast / II topic:

Struktura i funkcija genoma

Genome structure and function

Posteri / Posters

II-Pos

γ-OZRAČENJE SUVOG BAKTERIOFAGA λ

Desanka Božin

Institut za nuklearne nauke «Vinča», Beograd

Cilj istraživanja je efekat γ-ozračivanja suvog λ1390 spi- bakteriofaga. Bakteriofag je proizведен u P2 lisogenom soju *E. coli* i prečišćen diferencijalnim sedimentacijom (centrifugiranje na 10 000 rpm, 2 puta po 10 min). Bakteriofag je sušen 170 sati na sobnoj temperaturi na vazduhu ili u visokom vakumu (10^{-5} mbar). U poređenju sa uzorkom sušenim u vakumu, dehidracija na vazduhu uzrokuje 11 puta veću inaktivaciju bakteriofaga, veoma slabi stabilnost proteina kapsida i 3 puta povećava frekvencu rekombinacije. Mutacije u DNK nisu registrovane ni u uzorku sušenom na vazduhu, niti u uzorku sušenom u visokom vakumu. Dehidrirani λ1390 spi- bakteriofagi su ozračeni γ zracima iz ^{60}Co izvora. Istu dozu γ zraka preživljava 1×10^{-1} bakteriofaga osušenih na vazduhu i 1.3×10^{-2} bakteriofaga osušenih u vakumu. U oba uzorka suvih faga, frekvencu rekombinacije je 2-3 puta veća nego u kontrolnom uzorku a DNA mutacije nisu registrovane.

γ-IRRADIATED DRY BACTERIOPHAGE λ

The effects of γ-irradiation on dry λ1390 spi- bacteriophages were investigated. Bacteriophages were preparing by confluent lysis in P2 lysogenic strain of *E. coli* and subsequently purified by differential sedimentation (centrifugation at 10 000 rpm, 2 times per 10 min). Dehydration of the bacteriophage for 170 hours was performed at room temperature on air or in high vacuum (10^{-5} mbar). Dehydration on air caused 11 times higher inactivation of bacteriophage than dehydration in high vacuum, resulting in very weakened stability of the capsid proteins and 3 times higher frequency of recombination. DNA mutations were not detected neither in the sample dried on air nor in the sample dried in vacuum. Dehydrated λ1390 spi- bacteriophages were γ-irradiated from a ^{60}Co source. For the same dose of γ-rays, survival was 1×10^{-1} for bacteriophages dried on air and 1.3×10^{-2} for bacteriophages dried in vacuum. In both dried samples, the frequency of recombination was 2-3 times higher than in the control sample and DNA mutations were not detected.

ULOGA OPŠTEG TRANSKRIPCIONOG FAKTORA *NFY* U REGULACIJI EKSPRESIJE HUMANOG *SOX3* GENA

A. Krstić, Marija Mojsin, Nataša Kovačević-Grujičić i Milena Stevanović

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Rani stadijumi neurogeneze i neuronalne diferencijacije su precizno kontrolisani serijom gena. *Sox3* gen je eksprimiran u mozgu već u najranijim fazama razvića. Smatra se da je ovaj gen jedan od najranijih neuralnih markera kod kičmenjaka, određujući sudbinu neurona.

Da bi se rasvetlili molekularni mehanizmi odgovorni za ekspresiju humanog *SOX3* gena korišćen je kompjuterski program (MatInspector) koji pretražuje baze podataka vezivnih matriksa, a sa ciljem identifikacije potencijalnih mesta vezivanja transkripcionih faktora u promotoru humanog *SOX3* gena. Niz potencijalnih vezivnih mesta za poznate transkripcione faktore je identifikovan u 5' nekodirajućem regionu humanog *SOX3* gena. Prisustvo tri evolutivno konzervisana CCAAT boksa, koji predstavlja potencijalno vezivno mesto za opšti transkripcioni faktor NFY, sugerše mogući značaj ovog faktora u regulaciji ekspresije humanog *SOX3* gena. Eksperimenti smanjene elktroforetske pokretljivosti i modifikacija ove metode uz upotrebu specifičnih antitela dokazuju specifičnost vezivanja transkripcionog faktora NFY za sva tri identifikovana CCAAT kontrolna elementa.

Da bi se ispitalo da li je potencijalni CCAAT boks funkcionalan, urađena je poziciono usmerena mutageneza. Sposobnost da utiče na ekspresiju *cat* gena u NT2/D1 ćelijskoj liniji, poređena je između mutiranog i nemutiranog konstruktta. Pokazano je da je mutageneza CCAAT boksa (-101 bp do -105 bp) umanjila ekspresiju reporter gena više od 5 puta u poređenju sa ekspresijom nemutiranog konstruktta. Ovaj rezultat ukazuje da CCAAT boks vezivni domen prisutan u promotoru *SOX3* gena ima funkcionalnu ulogu u transkripciji ovog gena.

THE ROLE OF THE GENERAL TRANSCRIPTION FACTOR *NFY* IN THE REGULATION OF THE EXPRESSION OF HUMAN *SOX3* GENE

Early stadiums of the neurogenesis and neuronal differentiation are precisely controlled by a series of genes. *Sox3* is expressed in the brain from the earliest stages of development. It is considered to be one of the earliest neural markers in vertebrates playing the role in specifying neuronal fate.

In order to elucidate molecular mechanisms underlying the regulation of the human *SOX3* gene expression, computer prediction software (MatInspector) was used to search the matrix family library database and to identify potential transcription binding sites in the human *SOX3* promoter. The number of putative consensus binding sites for known transcription factors was identified in the 5' noncoding region of the human *SOX3* gene. The presence of three evolutionary conserved CCAAT boxes, representing the putative binding sites for the general transcription factor NFY, suggests the potential importance of this factor in the regulation of the human *SOX3* gene expression. EMSA and «supershift» experiments are performed to prove the specificity of the NFY binding to all three identified CCAAT control elements.

To examine whether the putative CCAAT box is functional, site directed mutagenesis was done. The ability of the mutant and its wild-type counterpart to drive expression of the *cat* reporter gene was compared in NT2/D1 cell line. We have shown that mutagenesis of the CCAAT box (-101 bp to -105 bp) reduced the expression of the reporter gene more than 5-fold when compared to the wild-type expression. This result indicates that the CCAAT box motif present in the *SOX3* promoter plays a functional role in the transcription of this gene.

USPOSTAVLJANJE VEĆE EFIKASNOSTI TRANSFEKCIJE HUMANIH ĆELIJA U KULTURI

Gordana Nikčević, Nataša Kovačević-Grujičić, Isidora Petrović i Milena Stevanović
Institut za molekularnu genetiku and genetičko inženjerstvo, Beograd

Transfer rekombinantnih gena u različite eukariotske ćelije u kulturi je široko rasprostranjena metoda za proučavanje genske ekspresije. Pošto je potreba za brzom, visoko efikasnom transfekcijom postala sve izraženija, razvijen je veliki broj produkata, uključujući lipozome, neliposomalne lipide, sintetičke polimere, i dr., koji omogućavaju transport gena u ćelije. Međutim, problem kod većine ovih, tzv. ne-viralnih agenasa je njihova niska efikasnost transfekcije.

U cilju izučavanja regulacije gena uključenih u neuralnu diferencijaciju, bilo nam je neophodno da privremenom (transient) transfekcijom uvedemo rekombinantnu plazmidnu DNK u humanu embrionalno-karcinomsku ćelijsku liniju (NT2/D1) sa visokom efikasnošću. U tu svrhu, najpre smo testirali dva transfekciona sistema: LIPOFECTAMINE™ (Life Technologies) i EffecteneTM (QIAGEN) koristeći pCH110 eukariotski vektor koji sadrži *lacZ* reporter gen.

Rezultati našeg istraživanja pokazali su da za NT2/D1 i HeLa ćelije (model sistem koji se često koristi kao kontrola u eksperimentima transfekcije), efikasnost transfekcije EffecteneTM reagensom, može jednostavno biti uvećana dodavanjem 1.5-3 puta veće količine plazmidne DNK od one koja je preporučena od strane proizvođača. Koristeći LIPOFECTAMINE™ reagens dobili smo optimalnu efikasnost transfekcije za obe ćelijske linije sa preporučenim koncentracijama, ali dodajući maksimalne količine plazmidne DNK. Zaključak našeg istraživanja je da prilikom optimizacije procesa transfekcije treba testirati i koncentracije plazmidne DNK značajno veće od onih koje su preporučene od strane proizvođača u cilju postizanja visokog nivoa efikasnosti transfekcije humanih ćelija u kulturi.

IMPROVED TRANSFECTION EFFICIENCY OF CULTURED HUMAN CELLS

The transfer of recombinant genes into variety of eukaryotic cultured cells is an extensively used approach in gene expression research. Since demand for rapid, high efficiency transfections become more intense, a number of products, including liposomes, non-liposomal lipids, synthetic polymers, etc., have been developed that mediate transport of genes into cells. However, problem associated with majority non-viral gene-delivery agents is their relatively low transfection efficiency.

In order to study regulation of genes involved in neuronal differentiation, it was essential for us to transiently introduce recombinant plasmid DNA into NT2/D1 human embryonal carcinoma cell line with the high efficiency. For that purpose we first evaluated two transfection systems: LIPOFECTAMINE™ (Life Technologies) and EffecteneTM (QIAGEN) Transfection Reagents using pCH110 eukaryotic assay vector which contains functional *lacZ* reporter gene.

We found out that under our culture conditions for NT2/D1 and HeLa cells (commonly used in transfections as a control), EffecteneTM transfection efficiency could simply be augmented by increasing the amount of plasmid DNA 1.5-3 times above recommended concentration without any visible cytotoxicity. With LIPOFECTAMINE™ reagent we obtained optimal transfection efficiency for both cell lines within the recommended concentrations, but with the highest point. Our conclusion is that optimization of transfection process should include plasmid DNA concentration above the level suggested by manufacturers in order to accomplish the highest transfection efficiency of human cells in culture.

EKSPRESIJA I STRUKTURNA I FUNKCIONALNA ANALIZA PROMOTORA HUMANOG *SOX18* GENA

Isidora Petrović, Jelena Đurović, Gordana Nikčević i Milena Stevanović

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Familija *SOX* gena sadrži veliki broj gena koji se eksprimiraju tokom embriogeneze, a koji su u srodstvu sa sisarskim genom koji determiniše pol, označenim kao SRY gen. Iako je samo nekoliko *SOX* gena detaljno analizirano, sve dosadašnje studije ukazuju na njihovu ulogu u regulaciji razvića, a mutacije u nekim *SOX* genima su povezane sa pojavom oboljenja kod čoveka.

Humani *SOX18* gen je identifikovan pretraživanjem cDNK biblioteke napravljene iz mozga humanog fetusa, korišćenjem specifične *SOXA* probe. Mapiranjem pozicije gena utvrđeno je da se *SOX18* gen nalazi na hromozomu 20 u regionu q13,3. Pokazano je da mutacije u *SOX18* genu dovode do pojave recessivne i dominantne forme sindroma koji obuhvata hipotrihozu, limfedem i teleangiekaziju. Pored poznate funkcije u razvoju dlake i krvnih sudova, ovo ukazuje i na važnu ulogu *SOX18* transkripcionog faktora u razvoju limfnih sudova.

U cilju određivanja molekularnih mehanizama odgovornih za kontrolu ekspresije humanog *SOX18* gena, analizirane su različite ćelijske linije koje bi mogle da se koriste kao model sistem za buduće eksperimente. Ekspresija *SOX18* gena je analizirana u HUV-EC i EAhyb926 ćelijskim linijama i detektovan je transkript dužine 1,8 Kb.

Karakterizacija *SOX18* promotora obuhvatala je određivanje starta transkripcije metodom elongacije prajmera, kao i strukturne i funkcionalne analize regulatornog regiona odgovornog za ekspresiju *SOX18* gena. Analizirana je sposobnost različitih fragmenata 5' nekodirajućeg regiona da utiču na ekspresiju cat reporter gena, a u cilju identifikacije DNA regiona odgovornog za ekspresiju *SOX18* gena.

EXPRESSION AND STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE PROMOTER OF HUMAN *SOX18* GENE

The *SOX* gene family consists of a large number of embryonically expressed genes that are related to the mammalian sex determining gene SRY. Although only few *SOX* genes have been characterized in detail, all studies indicate that these genes have important roles in the regulation of development and mutations in several *SOX* genes have been implicated in human diseases.

Human *SOX18* gene has been identified by screening human foetal cDNA library with specific *SOXA* probe. Mapping analysis has revealed that *SOX18* gene is located on human chromosome 20q13.3. It has been shown that *SOX18* mutations in human cause both recessive and dominant hypotrichosis- limphedema and telangiectasia, suggesting that, in addition to its established role in hair and blood vessel development, the *SOX18* transcription factor plays a role in the development and/or maintenance of lymphatic vessels. In order to elucidate the molecular mechanisms controlling the expression of the human *SOX18* gene, we have analyzed different cell lines that could be used as a model system for further experiments. The expression of *SOX18* was studied by Northern blot analysis in HUV-EC and EAhyb926 cell lines and a single transcript of about 1.8 kb was detected. In order to characterize promoter of the *SOX18* gene we have determined the transcription start site by primer extension analysis and carried out structural and functional analysis of the regulatory region responsible for *SOX18* expression. To identify the DNA regions responsible for the control of the *SOX18* transcription, we have analyzed the ability of truncated fragments from the 5' flanking region to drive expression of cat reporter gene.

**MUTACIONA ANALIZA POTENCIJALNOG MESTA VEZIVANJA RXRa
PROTEINA U REGULATORNOM REGIONU SOX3 GENA**

Tijana Savić, Gordana Nikčević i Milena Stevanović

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

SOX3 gen je član SOX familije gena koji ima značajnu ulogu u procesima neurogenese i diferencijacije neurona. Poznato je da je ekspresija humanog *SOX3* gena indukovana retinoičnom kiselinom (RA). U cilju definisanja elemenata odgovrnih za ovu indukciju napravljena je serija delecionih konstrukata u okviru 5' nekodirajućeg regiona *SOX3* gena. Funkcionalna analiza pokazala je da je najmanji od ovih konstrukata dužine 31 bp zadržao sposobnost inducibilnosti RA. Na osnovu rezultata dobijenih u eksperimentima kotransfekcije sa ovim regionom zaključeno je da je dejstvo RA na ekspresiju *SOX3* gena posredovano RXRa nuklearnim receptorom. U eksperimentima smanjene elektroforetske pokretnjivosti pokazano je da se rekombinantni humani RXR α protein vezuje za ovaj region. Mutacionom analizom ovog regiona tj. nizom eksperimenta smanjene elektroforetske pokretnjivosti u kojima je poređen afinitet vezivanja RXR α nuklearnog receptora za mutirane probe u odnosu na wild type, definisan je RXR α vezivni region. Analiza je pokazala da se ovaj vezivni region sastoji od 2 polumesta razdvojena sa 4bp. RXR α vezivni region okarakterisan u ovom radu razlikuje se po svojoj sekvenci kao i po strukturi od RXR vezivnih mesta već opisanih u literaturi.

**MUTATION ANALYSIS OF THE POTENTIAL RXRa RESPONSE ELEMENT
WITHIN REGULATORY REGION OF SOX3 GENE**

SOX3 gene is member of *SOX* gene family which has an important role in the process of neurogenesis and neuronal differentiation. It is known that the expression of the human *SOX3* gene is induced by retinoic acid (RA). In order to define elements responsible for this induction a series of various deletion constructs of the 5' noncoding region of the *SOX3* gene has been made. Functional analysis has showed that the shortest of these constructs 31 bp in length is able to hold RA inducibility. Cotransfection experiments have indicated that the effect of RA on *SOX3* gene expression is mediated by RXR α nuclear receptor. Gelshift and supershift experiments have shown that the human recombinant RXR α protein binds to this region. By comparison of the ability of RXR α nuclear receptor to bind to wild type and serious of mutated oligonucleotides using gelshifts experiments, the RXR α response region has been determined. Detailed analysis has shown that this response region consists of two halfsites separated with 4 bp. RXR α response region, characterised in this study differs by its sequence, as well as its structure, from already described RXR response elements.

NALIZA INTRONA U SOJEVIMA *Bacillus* spp.

S. Stanković¹, V. Lazarević², Blaženka Soldo², Tanja Berić-Bjedov¹,
Jelena Knežević-Vukčević¹ i Draga Simić¹

¹Katedra za mikrobiologiju, Biološki fakultet, Univerzitet u Beogradu, Beograd

²Département de Microbiologie Fundamental, Faculté de Biologie et de Médecine,
Université de Lausanne, Lausanne, Suisse

Kolekcija sojeva *Bacillus* spp. izolovanih iz prirodnih staništa je pretraživana pomoću PCR na prisustvo umetnutih sekvenci (introna i intein-kodirajućih sekvenci) u tandem genima *bnrdE/bnrdF* za ribonukleotid reduktazu SPβ profaga. Sekvenciranje PCR produkata je pokazalo tri nove konfiguracije u *bnrdE-bnrdF* tandemu koje se sastoje od introna grupe I u kodirajućim regionima i ORF u intergenskom regionu. Sva tri soja pripadaju vrsti *B. subtilis* što je utvrđeno pomoću API 50 CHB sistema za identifikaciju. Analiza genetičkih determinanti ćelijskog zida, kao i inkorporacija radioaktivno obeleženog glicerola u ćelijski zid, omogućila je kategorizaciju osam do sada nađenih sojeva *B. subtilis* koji sadrže umetnute sekvence u profagnim genima za ribonukleotid reduktazu u dve podvrste. Sojevi koji pripadaju subsp. *subtilis* sadrže tri umetnute sekvence, introne grupe I i inteine, od kojih mnoge sadrže ORF za pretpostavljenu «homing» endonukleazu. Sojevi koji pripadaju subsp. *spizizenii* sadrže jedan ili dva introna grupe I koji ne sadrže ORF.

ANALYSIS OF Collection of *Bacillus* spp. strains isolated from natural habitats was screened by low stringency PCR for the presence of intervening sequences (introns and intein-coding sequences) in the SPβ prophage-related ribonucleotide reductase gene tandem *bnrdE/bnrdF*. The subsequent sequencing of the PCR products revealed three novel configurations within the *bnrdE-bnrdF* tandem based on the presence of the group I introns in protein coding sequences and an ORF in the *bnrdE-bnrdF* spacer. The three relevant strains belong to the species *B. subtilis* as determined by the API 50 CHB identification system. Analysis of the cell wall genetic determinants as well as the incorporation of radio-labeled glycerol into cell wall allowed categorization of eight *B. subtilis* strains known to contain intervening sequences in the prophage ribonucleotide reductase genes into two subspecies. Strains apparently belonging to the subsp. *subtilis* contain three intervening sequences, group I introns and inteins, many of which contain an ORF for the putative homing endonuclease. Strains of the subsp. *spizizenii* contain only one or two ORF-less group I introns.

**INTRA-INDIVIDUALNI MOZAICIZAM U BROJU B HROMOZOMA KOD
ŽUTOGRLOG MIŠA, *Apodemus flavicollis* (Rodentia, Mammalia)**

M. Vujošević i Jelena Blagojević

Institut za biološka istraživanja «Siniša Stanković», Beograd

Populacije žutoglog miša, *Apodemus flavicollis*, karakteriše često prisustvo B hromozoma. Frekvenca intra-individualnog mozaicizma B hromozoma ispitana je kod 995 životinja sakupljenih na 6 lokaliteta u Srbiji. Nađeno je da 33.06% životinja ima B hromozome. Među njima je 26.44% bilo mozaično. Ukupno 32 mozaične životinje, koje su imale više od jednog B hromozoma, analizirane su u odnosu na distribuciju B hromozoma. Utvrđeno je da je distribucija B hromozoma različita među grupama životinja sa različitim brojem B hromozoma i raste sa brojem B hromozoma. Frekvenca mozaika je u rasponu od 0.22 do 0.25 i razlikuje se među lokalitetima.

**INTRA INDIVIDUAL MOSAICISM IN THE NUMBER OF B CHROMOSOMES
IN YELLOW-NECKED MOUSE, *Apodemus flavicollis* (Rodentia, Mammalia)**

Populations of yellow-necked mouse, *Apodemus flavicollis* are characterized by frequent appearance of B chromosomes (Bs). Frequency of intra individual mosaicism of Bs was studied in 995 animals collected at six localities in Serbia. It was found that 33.06% were with B chromosomes. Among them 26.44% were mosaics. Total of 32 mosaic animals with more than one B chromosome were analyzed for distribution of Bs and it was found that distribution of mosaics is quite different between groups of animals with different number of Bs and is increasing with number of Bs. Frequency of mosaics differ between localities and is in the range from 0.22 to 0.55.

III tematska oblast / III topic:

Genetičko inženjerstvo i biotehnologija

Genetic engineering and biotechnology

Uvodna izlaganja / Introductory lectures

III-Uvo

GENETIČKO INŽENJERSTVO - OSNOVA MOLEKULARNE BIOTEHNOLOGIJE

Lj. Topisirović

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Istraživanja u proteklih dvadeset godina nedvojbeno su pokazala da je genetičko inženjerstvo otvorilo perspektivu razvoja molekularne biotehnologije. Genetičko inženjerstvo omogućava (a) izučavanje strukture i funkcije gena bilo kog organizma, (b). izazivanje mutacija na specifičnom mestu u genu (dirigovana mutageza) koja za posledicu može imati povećanje sinteze produkta za koga taj gen nosi informaciju, (c) prevazilaženje uskih grla u biosintetskim putevima različitih metabolita od koristi, kao i (d) dobijanje proizvoda koje je na drugi način teško, skupo ili nemoguće dobiti. Svakako da je najveći doprinos genetičkog inženjerstva molekularnoj biotehnologiji upravo u ovom domenu. Naime, genetičko inženjerstvo omogućava manipulaciju genima i prebacivanjem tih gena iz jedne biološke vrste u drugu, što se u prirodi ne može spontano dešavati. S druge strane, moguće je konstruisati himerne gene *in vitro*) i tako konstruisane gene ubaciti u živi organizam.

Sve svetske prognoze oko osnova tehnologije 21. veka se slažu u tome da će upravo molekularna biotehnologija biti pokretačka snaga razvoja savremene civilizacije. Uvođenjem genetičkog inženjerstva u cilju unapređivanja biotehnoloških procesa, dobio se jedan sasvim novi kvalitet. Sada je moguće od mikroorganizama, biljnih ili životinjskih ćelija napraviti «biološke fabrike» koje će proizvoditi veliku količinu ekonomski vrednih jedinjenja, odnosno moguće je konstruisati transgene organizme koji mogu biti osnova za proširenje palete biotehnoloških proizvoda.

GENETIC ENGINEERING – THE BASIS OF MOLECULAR BIOTECHNOLOGY

Results obtained by using genetic engineering in the research of living systems in the past twenty years indisputably showed that genetic engineering opened real perspective for the development of molecular biotechnology. The application of genetic engineering in the research allowed (a) investigation of structure and function of genes of any organism, (b) direct introduction of mutations into the gene of interest, (c) overcoming the bottle-necks in biosynthetic pathways as well as (d) production of products that are very hard or at all impossible to get in traditional processes. By all means, the greatest contribution of genetic engineering in molecular biotechnology lies in this domain. This is because, genetic engineering enable the gene manipulation and transfer of gene(s) from one into another species, what is impossible to happen in nature. On the other hand, it is possible to construct chimerical genes *in vitro* and introduce such genes into chosen host organism.

According to general prognosis, the molecular biotechnology will be a basis for 21st century technologies and that it would be a moving force for development of modern civilisation. Introduction of genetic engineering for an improvement of biotechnology processes opens qualitatively new approaches. Molecular biotechnology facilitate the using of micro-organisms, plant and animal cells as «biological factories» that are able to produce higher quantities of high value-added compounds, that is, it would be possible to construct transgenic organisms that could be a basis for broadening of biotechnology products.

POREĐENJE AKTIVNOSTI RAZLIČITIH *kgmB::lacZ* GENSKIH I OPERONSKIH FUZIJA

Sandra Vojnović, Nataša Milojević i Branka Vasiljević

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

KgmB (rezistencija na kanamicin-gentamicin) metilaza iz soja *Streptomyces tenebrarius* modifikuje G-1405 u okviru 16S rRNK unutar sekvene CGUCA i na taj način štiti bakteriju od sopstvenog toksičnog proizvoda. Pentanukleotid CGCCC je prisutan u okviru istog regiona 16S rRNK pri čemu se i preklapa sa CGUCA sekvenom. Pokazano je da su obe sekvene prisutne i u uzvodnom regionu *kgmB* gena, ispred RBS-a, ali su ovde razdvojene. Predložili smo regulatorni model po kome *KgmB* metilaza može da se veže za sopstvenu iRNK, u okviru ove dve potencijalne regulatorne sekvene, i na taj način spreči dalju translaciju, nakon što je modifikovala sve ribozome.

Sa ciljem izučavanja translacione autoregulacije *kgmB* gena konstruisali smo različite *kgmB::lacZ* genske i operonske fuzije i pratili uticaj *KgmB* metilaze na aktivnost tih fuzija kako *in-cis* tako i *in-trans*. Pokazali smo da čak i kada se PCR-om mutira CGTCA pentanukleotid, aktivnost fuzionog proteina opada u prisustvu funkcionalne *KgmB* metilaze. Paralelno smo uradili i eksperimente sa *KgmB* proteinom inaktiviranim u okviru BglII restripcionog mesta. Uprkos činjenici da inaktivirani *KgmB* protein ima samo 66 originalnih amino kiselina od ukupno 135, uticaji skraćenog *KgmB* proteina i originalne *KgmB* metilaze na aktivnost različitih *kgmB::lacZ* fuzija su vrlo slični. Iz ovih rezultata proizilazi da bi *KgmB* protein mogao da ima dva odvojena domena, obzirom da je regulatorna funkcija skraćenog *KgmB* proteina očuvana, a rezistencija na kanamicin i gentamicin narušena. Eksperimenti sa *kgmB::lacZ* operonskim fuzijama su pokazali da *KgmB* protein, kompletan ili skraćen, nema uticaja na ekspresiju *lacZ* gena.

COMPARATIVE ANALYSIS OF DIFFERENT *kgmB::lacZ* GENE AND OPERON FUSIONS

The *KgmB* (kanamycin-gentamicin resistance) methylase from *Streptomyces tenebrarius* acts at G-1405 of 16S rRNA within the sequence CGUCA and thus protects bacteria from its own toxic product. The pentanucleotide CGCCC is present in the same region of 16S rRNA overleaping with the CGUCA sequence. It was shown that both of these sequences are present in the upstream region of the *kgmB* gene, in front of RBS but, they are separated. We proposed a regulatory model in which the *KgmB* methylase can bind to its own mRNA, within these two potential regulatory sequences, preventing further translation once all the ribosomes are modified.

To study translational autoregulation of the *kgmB* gene different *kgmB::lacZ* gene and operon fusions were constructed and the effect of overexpression of *kgmB* gene to these fusions was analyzed *in-cis* as well as *in-trans*. We showed that even if CGTCA pentanucleotide is mutated by PCR, there is still decrease in the activity of the fusion protein. We performed analogous experiments with the *kgmB* gene inactivated in BglII restriction site. Despite the fact that inactivated *KgmB* protein has only 66 sense amino acids out of 135, the effect of truncated *KgmB* protein to different *kgmB::lacZ* fusions was similar. These results suggested that the *KgmB* protein might have two separate domains since regulatory function of the truncated *KgmB* protein is preserved in spite of the fact that resistance to kanamycin and gentamicin was lost. The experiments with *kgmB::lacZ* operon fusions showed that the *KgmB* protein, wild type or truncated, had no effect on the expression of the *lacZ* fusions.

III tematska oblast / III topic:

Genetičke inženjerstvo i biotehnologija

Genetic engineering and biotechnology

Usmena izlaganja / Oral presentations

III-Usm

PRIMENA GENETIČKOG INŽINJERINGA KOD OTPORNOSTI BILJAKA PREMA PATOGENIMA

Jelena Bošković¹, Željana Prijić¹ i M. Bošković²

¹Megatrend univerzitet primenjenih nauka, Beograd

²Poljoprivredni fakultet, Novi Sad

Genetički inžinjering najavljuje novu eru za naučnike, koji se bave istraživanjem održavanja zdravih biljaka, sa optimalnim prinosima useva i minimalnom primenom pesticida. Naučnici iz celog sveta istražuju biohemiju prirode i važnost biljnih reakcija prema patogenima i njihovom razvoju. Geni otpornosti kod biljaka i oni koji su odgovorni za reakcije otpornosti, identificuju se i inžinjeringom prenose u biljne vrste u cilju njihove zaštite prema patogenima. Određivanje ovih gena stvara mogućnosti za postepeno vrednovanje njihovih specifičnih uloga i važnosti u reakciji patogeneze prema parazitima upotreboom transgenih biljaka, koje su dobijene tehnikom genetičkog inžinjeringa. Poslednje primenjene tehnike molekularne biologije kod biljaka i genetički inžinjering za studije interakcija domaćina i patogena su rezultirale za identifikacije kloniranja brojnih gena uključenih u reakciju odbrane biljaka posle infekcije sa patogenima. To obuhvata gene sa ekspresijom proteina, peptida ili antimikrobnih jedinjenja koji su direktno toksični prema patogenima. Genetički produkti neposredno sprečavaju proizvode virulentnosti patogena ili podstiču strukturalne gene odbrane, koji direktno ili indirektno aktiviraju opšte reakcije odbrane biljke, i gene otpornosti kod hipersenzitativnih reakcija u interakcijama sa avirulentnim faktorima. U ovom radu se pregledno iznose ostvareni rezultati kod korišćenja širokog spektra kloniranih gena u povećanju otpornosti prema gljivičnim patogenima i virusima u transgenim biljkama i upućuju na buduća osporovanja i perspektive. Koncept programiranog izumiranja ćelija (PCD) i fitoaleksini imaju posebnu ulogu kod razjašnjenja molekularne osnove induciranih reakcija odbrane biljke od patogena.

APPLICATIONS OF GENETIC ENGINEERING IN RESISTANCE TO PLANT PATHOGENS

Genetic engineering ushers in new era for plant scientists working to maintain healthy plants, optimize crop yields, and minimize pesticide usage. Scientists from all over the world are investigating the biochemical nature of, and the signals involved in, a plants reactions to pathogens invasion and pathogens development. Plant resistance genes and genes involved in resistance reactions are being identified and engineered into crop plants to protect them against plant pathogens. The identifications of these genes have made it possible to subsequently evaluate their specific roles and importance in disease response pathways using transgenic plants developed with genetic engineering techniques. Recent applications of technique in plant molecular biology and genetic engineering to study of host pathogen interactions have resulted in the identifications on cloning of numerous genes involved in the defense responses of plants following pathogen infection. These include genes that expresses proteins, peptides or antimicrobial compounds that are directly toxic to pathogens. Genes products directly inhibit pathogen virulence products or enhance plant structural defense genes, that directly or indirectly activate general plant defense response, and resistance genes in the hypersensitive response and in the interactions with avirulence factors. In this paper is reviewed advances made in utilizing a broad range of cloned genes to enhance disease resistance against fungal pathogens and viruses, in transgenic plants and address future challenges and prospect. The concept of programmed cell death (PCD) and phytoalexins played a special role in the molecular basis of inducible defense reactions

ANALIZA SKRAĆENOG *KgmB* PROTEINA IZ *S. tenebrarius*

Sandra Marković, Sandra Vojnović i Branka Vasiljević

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

kgmB gen iz proizvođača nebramicinskog kompleksa antibiotika, *Streptomyces tenebrarius*, kodira KgmB metilazu koja obezbeđuje rezistenciju na kanamycin i gentamicin. KgmB metilaza modifikuje ciljno mesto na 16S rRNK i na taj način štiti proizvođača od sopstvenog toksičnog produkta. Ekspresija *kgmB* gena je autoregulisana na nivou translacije. Regulatorni model je najpre testiran in vivo korišćenjem *kgmB-lacZ* genskih fuzija. U cilju proučavanja ovog procesa *in vitro*, KgmB protein je eksprimiran u *E. coli* i precišćen pomoću QIAexpress sistema. Nedavno je pokazano da inaktivirani *kgmB* gen (inaktivacija u BglII restripcionom mestu koja dovodi do sinteze skraćenog KgmB proteina sa samo 66 originalnih aminokiselina) smanjuje aktivnost *kgmB-lacZ* genske fuzije. Ovaj rezultat ukazuje da je N-terminalni region KgmB metilaze zadužen za regulaciju t.j. za vezivanje za iRNK. Da bi se ispitale osobine i funkcionalnost N-terminalnog regiona KgmB metilaze inaktivirani *kgmB* gen je kloniran u pQE30 ekspresioni vektor. Eksprimirani protein obeležen histidinskim repičem je precišćen metal afinitetnom hromatografijom. Precišćeni skraćeni KgmB protein je analiziran elektroforetski i Western blotom. Funkcionalnost skraćenog KgmB proteina biće ispitana eksperimentima RNK-gel šifta i upoređena sa intaktnom KgmB metilazom.

ANALYSIS OF TRUNCATED *KgmB* METHYLASE FROM *S. tenebrarius*

The *kgmB* gene from *Streptomyces tenebrarius*, producer of nebramycin complex of antibiotics, encodes for kanamycin-gentamicin resistance methylase, *KgmB*. *KgmB* methylase modifies the target site on 16S rRNA and thus protect producer against its own toxic product. The *kgmB* gene is autogenously regulated at translational level. A regulatory model was first tested *in vivo* using *kgmB-lacZ* gene fusions. In order to study this process *in vitro* *KgmB* protein was overexpressed in *E. coli* and purified using QIAexpress System. Recent results showed that inactivated *kgmB* gene (inactivation in BglII restriction site which gives truncated protein with only 66 original amino acids) is able to repress activity of *kgmB-lacZ* gene fusions. This finding suggests that N-terminal region of KgmB methylase is responsible for regulation i.e. for binding to mRNA. With the intention of analyzing properties and functionality of N-terminal region of KgmB protein inactivated *kgmB* gene was cloned in pQE30 expression vector to make a construct that places the 6xHis tag at the N-terminus of the protein. For purification of 6xHis-tagged protein metal affinity chromatography was used. Purified truncated *KgmB* protein was analyzed electrophoretically and on Western blot. Functionality of truncated KgmB protein would be examined by RNA-gel shift experiments and compared with intact KgmB methylase.

PREČIŠĆAVANJE SGM METILAZE IZOLOVANE IZ *Micromonospora zionensis* I DETERMINACIJA SEKUNDARNE STRUKTURE

Tatjana Ilić-Tomić, M. Savić i Branka Vasiljević

Institut za molekularnu genetiku i genetsko inženjerstvo, Beograd

Iz *Micromonospora zionensis*, proizvođača antibiotika G-52, kloniran je *sgm* gen koji kodira metilazu koja modifikuje 16S rRNK u okviru male ribozomalne subjedinice i tako štiti bakteriju od sopstvenog toksičnog proizvoda.

U cilju prečišćavanja Sgm proteina, *sgm* gen je ukloniran u *Qiaexpress pQE* vektor, pod promotor-operatorski element T5 faga, koji se sastoji od promotora koga prepoznaće *E. coli* RNK polimeraza i dve *lac* operatorske sekvene koje povećavaju vezivanje *lac* represora. Ovim konstruktom se repiće od 6 histidina pozicionira na N terminus proteina. Aktivnost His₆-Sgm proteina je potvrđena *in vivo*.

Prečišćavanje His₆-Sgm proteina je obavljeno na Ni-NTA koloni afinitivnom hromatografijom pod nativnim uslovima i prisustvo proteina je detektovano na SDS poliakrilamidnom gelu.

Snimljen je CD spektar (cirkularni dihroizam) prečišćenog Sgm proteina. Prema podacima dobijenim iz CD spektra utvrđeno je da su α heliksi i zavojnice predominantni elementi sekundarne strukture što je u saglasnosti sa kompjuterski zasnovanim predikcijama. Sekundarna struktura Sgm proteina određena CD spektroskopijom pokazuje visok stepen homologije sa β -laktamazom *S. aureus* iako na aminokiselinskom nivou nema homologije.

PURIFICATION OF THE SGM METHYLASE ISOLATED FROM *Micromonospora zionensis* AND DETERMINATION OF THE SECONDARY STRUCTURE

The *sgm* gene, coding for specific 16S rRNA methylase that modifies the target site on small ribosomal subunit and thus protects the producer against its own toxic product, was cloned from *Micromospora zionensis*, producer of antibiotic G52.

In order to purify the Sgm protein, the *sgm* gene was cloned under optimized promoter-operator element consisting of phage T5 promoter (recognized by the *E. coli* RNA polymerase) and two *lac* operator sequences which increase *lac* repressor binding, in *QIAexpress pQE* vectors. This construct places the 6xHis tag at the N-terminus of the protein. The functional activity of His₆-Sgm fusion protein was confirmed *in vivo*. Purification of His₆-tagged protein by Ni-NTA affinity chromatography was carried out under native conditions and the protein was detected on sodium dodecyl sulphate polyacrylamide gel.

Purified protein was used in circular dichroism (CD) spectroscopy studies. According to CD spectral data protein was mostly α helically structured with a lot of amino acid residues placed in turns connecting α helices that is in agreement with results based on computer secondary structure prediction. The closest match in secondary structure database was β -lactamase from *S. aureus*. Protein sequences alignment showed no significant sequence homology between this two proteins.

III tematska oblast / III topic:

Genetičke inženjerstvo i biotehnologija

Genetic engineering and biotechnology

Posteri / Posters

III-Pos

NASLEDIVANJE *in vitro* ODGOVORA KOD PŠENICE

Nevena Mitić i Radomirka Nikolić

Institut «Srbija», Centar za poljoprivredna i tehnološka istraživanja, Zaječar

Ispitivanje je nasleđivanje *in vitro* odgovora nezrelih embriona kod pet kultivara pšenice i njihovih recipročnih hibrida. *In vitro* odgovor je ocenjivan na osnovu procenata formiranih kalusa, regenerativnih kalusa i broja biljaka dobijenih po embrionu. Ukrštanjem sorte 'Vesna' ('VS') sa najvišim regenerativnim potencijalom sa dvema sortama sa najnižim potencijalom 'Zajecarska 65' ('ZA') i 'Leda' ('LD'), pokazano je da je potencijal za regeneraciju nasledna osobina. 'VS' kao ženski roditelj uslovio je povećanje regenerativnog odgovora kod hibrida 'VS'x'LD' i 'VS'x'ZA', dok 'VS' kao muški roditelj nije ispoljio isto dejstvo. Međutim, 'LD' i 'ZA' kao muške komponente su uticale na smanjenje regenerativnog potencijala hibrida sa 'VS', u odnosu na samooprašenu 'VS'. Rezultati ukazuju na postojanje klase ne-jedarnih činilaca u kultivaru 'VS'. Oni značajno doprinose relativno višoj regenerativnoj sposobnosti kalusa hibrida gde je 'VS' ženski roditelj, u poređenju sa onima kod kojih je 'VS' muški roditelj.

INHERITANCE OF *in vitro* RESPONSE IN WHEAT

The inheritance of *in vitro* culture response was studied by using immature embryos from five wheat cultivars and their reciprocal hybrids. *In vitro* culture response was evaluated according to callus formation, percentage of regenerative calli and the number of plants per embryo. By crossing the cultivar 'Vesna' ('VS') with highest tissue culture response and the two cultivars with lowest response 'Leda' ('LD') and 'Zajecarska 65' ('ZA'), it was demonstrated that the regeneration potential was heritable. 'VS' as female parent, enhanced regeneration response in hybrids 'VS'x'LD' and 'VS'x'ZA', while as a male parent, 'VS' did not affect the regeneration ability of hybrids 'LD' and 'ZA'. However, hybrids having 'LD' and 'ZA' as a male parents exhibited a decreased regeneration potential, as compared to self-pollinated 'VS'. The results suggest the presence of a class of extra-nuclear factors in the 'VS' cultivar. They significantly account for relatively higher regeneration capacity in the hybrids having this cultivar as a female parent than in those where the 'VS' was male parent.

**MORFOGENETSKI POTENCIJAL ZA MIKROPROPAGACIJU
HAPLOIDA ŠEĆERNE REPE**

Nevena Nagl, Snežana Mezei, L. Kovačev i N. Čačić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

U radu je predstavljeno ispitivanje potencijalnih razlika u morfogenetskom potencijalu za mikropropagaciju osam haploidnih genotipova šećerne repe. Haploidi su dobijeni kulturom neoplodenog semenog zametka i nakon razvoja prvog para listova postavljeni na podlogu za mikropropagaciju. Pod uticajem citokinina u podlozi kod svih genotipova došlo je do formiranja aksilarnih pupoljaka dok su se kod pojedinih genotipova razvili i adventivni pupoljci. Intenzitet multiplikacije nije bio ujednačen i menjao se nakon svake subkultivacije, iako je kod svih genotipova dolazilo do povećanja broja novostvorenih biljaka. Razlike u porastu broja biljaka su se počele uočavati nakon četvrte subkultivacije, da bi u sladaća dve postale još uočljivije. Testiranjem značajnosti razlika između koeficijenata regresije, odnosno intenziteta porasta broja novonastalih biljaka tokom šest subkultivacija, ustanovaljeno je da se oni između pojedinih genotipova značajno razlikuju.

**MORPHOGENETIC POTENTIAL FOR MICROPROPAGATION
OF SUGAR BEET HAPLOIDS**

Here are presented results of investigation about potential differences between morphogenetic potential for micropropagation of eight sugar beet haploids. Haploids were obtained by ovule culture and after development of first leaves put on micropropagation medium. The presence of cytokinin in medium stimulated development of axillary bud, while in some genotypes adventitious bud developed as well. Multiplication rate was not consistent, although number of developed plants grew after each subcultivation. Differences in plant multiplication started to differ after four subcultures. By testing of differences between correlation coefficients, i.e. multiplication rate during six subcultivations, it was determined that they significantly differ between tested genotypes.

**ANALIZA MORFOLOŠKIH KARAKTERISTIKA BILJAKA ŽUTOG
ZVEZDANA cv. BOKOR TRANSFORMISANIH SA *Agrobacterium rhizogenes***

Radomirka Nikolić i Nevena Mitić

Institut «Srbija», Centar za poljoprivredna i tehnološka istraživanja, Zaječar

Uspostavljen je efikasan metod za transformaciju žutog zvezdana cv. Bokor pomoću *A. rhizogenes*. Transformisani pupoljci su regenerisani na segmentima adventivnih korenova u visokoj frekvenci. Posle ožiljanja i aklimatizacije transformisane T₀ biljke su gajene na eksperimentalnom polju. Izvršena je analiza morfoloških karakteristika deset slučajno odabralih T₀ biljaka žutog zvezdana (genotipovi br. 2 i br. 5). One su upoređivane sa istim osobinama kontrolnih, netransformisanih biljaka. Osobine kao što su broj stabala po biljci, dužina internodija najdužeg stable i broj cvetova po biljci su bile signifikantno različite od istih osobina kontrolnih biljaka, dok nisu utvrđene značajne razlike u površini listova. Nije bilo znakova prisustva genotipa sa *rol* genima. Transformisane biljke su imale povećan sadržaj celuloze, dok je sadržaj proteina i azota bio na novou kontrolnih biljaka.

**ANALYSIS OF MORPHOLOGICAL TRAITS OF BIRD'S FOOT TREFOIL
PLANTS cv. BOKOR TRANSFORMED WITH *Agrobacterium rhizogenes***

An efficient method for genetic transformation and shoot regeneration was achieved in bird's foot trefoil cv. Bokor using *A. rhizogenes*. The transformed shoots were regenerated on hairy root segments in high frequency. After rooting and acclimation, transformed T₀ plants were grown in experimental field. Analysis of morphological traits and chemical content in ten unintentionally chosen T₀ bird's foot trefoil plants (genotypes no. 2 and no. 5) was performed. They were compared to those of control non-transformed plants. The traits as a number of stems per plant, length of internodes in longest stem, number of flowers per plant and plan high were very significant differed than the same traits in control plants, while there were no significant differences in the leaf area. No signs of the *rol* genes genotype were present. The transformed plants had significantly higher content of cellulose, while the protein and nitrogen contents of are in the range of control plants.

ALELNA VARIJABILNOST U LOKUSU *RHT8* GENA U NOVOSADSKOJ KOLEKCIJI PŠENICE I NJEN ZNAČAJ U OPLEMENJIVAČKIM PROGRAMIMA

J. Pilipović, B. Kobiljski, D. Obreht i Vladislava Galović

Naučni institut za ratarstvo i povtarstvo, Zavod za strana žita, Novi Sad

Visina stabljike pšenice je složena osobina uslovljena velikim brojem gena kao i uticajima spoljašnje sredine. Korišćenje gena koji redukuju visinu stabljike, a povećavaju prinos je jedna od glavnih strategija u dobijanju visoko prinosnih sorti hlebne pšenice. Do sada je identifikovano 21 major gena koji redukuju visinu pšenice, a od kojih se Rht8 gen najčešće koristi u oplemenjivačkim programima u južnoj Evropi.

Sa ciljem da se utvrdi odgovor 350 genotipova pšenice iz novosadske kolekcije na egzogeno dodatu giberelinsku kiselinu, primenjen je GA test. Priblizno 22% od 350 genotipova nije reagovalo izduživanjem ponika na egzogeno dodatu giberelinsku kiselinu, a 78% jeste. Alelna varijabilnost na Rht8 lokusu ovih genotipova utvrđena je analizom mikrosatelitnog lokusa MR 21, koji je blisko vezan sa Rht8 lokusom. Nakon toga, ispitivan je efekat različitih alela na agronomске karakteristike i utvrđena je značajna veza između alelne varijabilnosti na Rht8 lokusu sa jedne strane i visine stabljike i komponenti prinosa sa druge strane.

U radu su dati rezultati GA testa i molekularne analize, sa specijalnim naglaskom na potencijalnoj upotrebi ovih podataka u oplemenjivačke svrhe.

ALLELIC VARIATION AT THE DWARFING GENE *RHT8* LOCUS IN NOVI SAD WHEAT COLLECTION AND ITS SIGNIFICANCE IN BREEDING PROGRAMMES

The control of plant height in wheat is known to be complex, being determined by many genes, and subject to environmental effects. The use of dwarfing genes to reduce plant height and improve yield potential has been one of the major strategies in breeding modern, high yielding bread wheat cultivars. So far, 21 major genes for reducing plant height have been determined, among which Rht8 is most frequently used in wheat breeding programs in Southern Europe.

In order to determine the respond of 350 genotypes from Novi Sad Wheat Core Collection to exogenously applied Gibberellic Acid, GA test was performed. Approximately, 22% of 350 genotypes have shown to be GA-insensitive and 78% to be GA-sensitive. A wheat microsatellite locus MR 21, which is closely linked to Rht8 locus, was used to screen these wheat genotypes to assess the variation at Rht8 locus. Afterwards, effects of different alleles on agronomic traits were analyzed and significant relation was detected between allelic variation at Rht8 locus at one side and plant height and grain yield and its components on the other side.

For both GA test and molecular screening, obtained results are discussed in this paper, with special emphasis on potential use of the data for breeding purposes.

**INAKTIVACIJA GENA ZA PROTEINAZU A U rPAC
PROIZVODNOM SOJU LN5.5 P. pastoris**

Lidija Šenerović, Nada Stanković i [G. Ljubijankić]

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Pored velike količine aktivne PAC u medijumu utvrđeno je Western blot analizom i prisustvo degradacionih produkata ovog proteina, nastalih delovanjem vakuolarnih proteaza proizvedenih od strane ćelije domaćina. Osim smanjenja ukupne količine intaktnog enzima, usled proteolize otežano je i prečišćavanja rekombinantnog produkta. Rešenje problema nespecifične degradacije predstavlja zato značajan korak u daljoj optimizaciji proizvodnje rPAC. Kvaščeva proteinaza A (PrA), vakuolarna aspartična proteaza, kodirana je *PEP4* genom i sintetiše se u obliku propeptida. Sposobna je za samoaktivaciju i odgovorna za sazrevanje bar pet vakuolarnih proteaza - proteinaze B (PrB), karboksipeptidaze Y (CPY), RNKaze, velike aminopeptidaze i nespecifične alkalne fosfataze. Zbog toga inaktivacija *PEP4* gena ima plejotropni efekat: mutantne ćelije nemaju PrA, CPY i aktivnost alkalne fosfataze, dok je PrB delimično inaktivirana. U cilju inaktivacije *PEP4* gena *P. pastoris*, konstruisan je disruptivni vektor pNat *PEP4*. pNat *PEP4* nosi nat1 dominantni marker koji obezbeđuje rezistenciju na antibiotik nurseotricin i središnji fragment *PEP4* gena veličine 400bp, za koji se očekivalo da će putem homologne rekombinacije omogućiti ugradnju inaktivacione kasete u *PEP4* lokus. Zbog nepostojanja metode kojom bi se direktno merila PrA aktivnost u ćelijama, urađen je esej koji omogućuje indirektno utvrđivanje inaktivacije *PEP4* gena preko praćenja aktivnosti CPY (APNE overlay assay). Nekoliko kolonija koje su pokazale smanjenu aktivnost CPY proverene su dalje Southern blot analizom. Od 8 izabranih kolonija 4 su pokazale uspešnu ugradnju inaktivacione kasete u *PEP4* lokus.

**INACTIVATION OF A PROTEINASE A GENE
IN *P. pastoris* LN5.5 STRAIN PRODUCING rPAC**

The highest yield of rPAC was obtained in LN5.5 strain with four copies of pac gene integrated into genome. Apart from the high quantity of secreted active rPAC in medium considerable amount of degradation products created by the host cell vacuolar proteases was detected by Western blot. In addition to reducing amount of the intact product in the medium, proteolysis also complicates the recovery process. Therefore, in further optimization of the expression of rPAC the issue of nonspecific proteolysis is an important factor. Proteinase A (PrA), yeast vacuolar aspartyl protease, encoded by *PEP4* gene is synthesized as a propeptide. It is capable of self-activation, as well as subsequent activation of additional five vacuolar proteases, such as proteinase B (PrB), carboxypeptidase Y (CPY), RNase, large aminopeptidase and nonspecific alkaline phosphatase. The inactivation of *PEP4* gene results in a pleiotropic phenotype: mutant cells lack PrA, CPY, alkaline phosphatase activities and partially PrB activity. In order to inactivate *PEP4* gene, integrative vector pNat *PEP4* was constructed. pNat *PEP4* contains nat1 dominant marker gene conferring resistance to antibiotic nurseothricin and a 400bp portion of *PEP4* gene for the integration of inactivation cassette by homologous recombination into host chromosome. Since a satisfactory plate assay that directly measures PrA activity in colonies is not available, we performed APNE overlay assay which enables detection of *PEP4* inactivation by following CPY activity. Colonies that appeared to have low CPY activity were selected for Southern blot hybridization analysis. Four of eight selected colonies showed successful integration of inactivation cassette in *PEP4* locus.

**REGENERACIJA BILJAKA IZ PROTOPLASTA DOBIJENIH IZ
HIPOKOTILA SUNCOKRETA (*Helianthus annuus* L.)**Ksenija Taški¹ i Dragana Vasic²¹Nacionalna laboratorija za ispitivanje semena, Novi Sad²Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Protoplasti dobijeni iz etioliranih hipokotila gajenog suncokreta su postavljeni u kapljice agaroze i gajeni prema protokolu Trabace et al. (1995). Dobijeni mikrokalusci su oslobođeni iz kapljica agaroze i prebačeni na čvrste regeneracione podloge.

Regeneracija biljaka je dobijena na MSE regeneracionoj podlozi (Wingender et al., 1996) nakon kratkotrajnog tretmana sa 2,2 µM Thidiazuron (TDZ). Indukcija korena je postignuta potapanjem izdanaka u IBA rastvor. Uspešno ukorenjene biljke su cvetale.

**PLANT REGENERATION FROM HYPOCOTYL PROTOPLASTS OF
SUNFLOWER (*Helianthus annuus* L.)**

Hypocotyl protoplasts of sunflower were cultured in agarose droplets according to the protocol of Trabace et al. (1995). Resulting micro calluses were released from agarose and transferred onto different solid regeneration media.

Shoot regeneration was achieved by culture of calluses onto MSE regeneration medium (Wingender et al., 1996) after treatment with 2,2 µM Thidiazuron (TDZ) for a limited period. Freshly excised shoots were induced to root by IBA treatment. Regenerated plants were flowered.

KONSTRUKCIJA MOBILIZACIONOG VEKTORA ZA TRANSFER HETEROLOGIH GENA U LAKTOKOKAMA

Maja Tolinački, Ivana Strahinić, Jelena Begović, Lj. Topisirović i M. Kojić

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Soj *Lactococcus lactis* subsp. *lactis* biovar. diacetylactis S50 je prirođni izolat poreklom iz maslačne maje. Soj S50 poseduje pet plazmida označenih kao pS50-140, pS50-80, pS50-7a, pS50-7b i pS50-6. Određena je kompletanu nukleotidna sekvenca dva mala plazmida pS50-7a i pS50-7b koji pokazuju 99% homologije među sobom (deo plazmida na kome se nalaze ORF1, ORF2, ORF3 i oriT pokazuje 100% identičnosti). Analizom nukleotidne sekvene pokazano je da plazmidi pS50-7a i pS50-7b pripadaju familiji laktokokalnih theta replicirajućih plazmida. Zahvaljujući stabilnom održavanju unutar jedne ćelije, kao i posedovanju mobilizacionih sposobnosti, plazmidi pS50-7a i pS50-7b predstavljaju dobru osnovu za konstrukciju mobilizacionog vektora uz pomoć koga bi konjugacijom mogli biti preneti heterologi geni u netransformabilne sojeve. U cilju konstrukcije vektora za transfer heterologih gena iskorišćen je vektor za selekciju oridžina replikacije pIS1. Za konstrukciju mobilizacionog vektora bilo je neophodno klonirati fragment poreklom iz plazmida pS50-7a, koji sadrži esencijalne elemente za replikaciju, kao i oriT sekvenu, neophodnu *in cis* za transfer plazmida iz jedne ćelije u drugu. Na osnovu analize nukleotidne sekvene utvrđeno je da EcoRI fragment, veličine 3392 bp, obuhvata minimalni replikacioni region kao i oriT sekvenu. Ovaj EcoRI fragment kloniran je u pIS1 vektor (konstrukt pISE3392) i uspešno eksprimiran u laktokokalnom soju VEL1122.

CONSTRUCTION OF A MOBILIZABLE VECTOR FOR TRANSFER OF HETEROLOGUS GENES IN LACTOCOCCI

Strain *Lactococcus lactis* subsp. *lactis* biovar. diacetylactis S50 is a natural isolate from starter for butter production. The strain S50 contains five different plasmids, designated as: pS50-140, pS50-80, pS50-7a, pS50-7b and pS50-6. The nucleotide sequence of both plasmids (pS50-7a and pS50-7b) was determined and data analysis revealed that the homology between them is 99%, (regions of plasmids containing ORF1, ORF2, ORF3 and oriT show 100% identity). Nucleotide sequence analysis of small plasmids (pS50-7a and pS50-7b) showed that they belong to a family of lactococcal theta replicons. The identification of mobilization ability of small plasmids and their stability in the cell would be beneficial in the generation of mobilizable vectors for conjugative transfer of heterologous genes in non-transformable lactococci. For construction of mobilizable vector we used origin probe vector pIS1 (pUC19 derivate carrying gene conferring resistance to erytromycin, and without replication ability in lactococci). The mobilizable vector shoud contain fragment from the plasmid pS50-7a, which carrying essential elements for replication and oriT sequence (*in cis* acting sequence, necessary for plasmid transfer from one to another cell). The sequence analysis of EcoRI fragment of 3392 bp revealed the presence of oriT sequence and minimum region involved in plasmid replication. This EcoRI fragment was cloned into pIS1 vector (construct pISE3392) and successfully replicated in lactococcal strain VEL1122.

**TESTIRANJE REGENERACIONE SPOSOBNOSTI INBRED
LINIJA GAJENOG SUNCOKRETA**

Dragana Vasić, Ksenija Taški i D. Škorić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Sposobnost regeneracije *in vitro* je kod suncokreta (*Helianthus annuus* L.) ograničena na samo mali broj genotipova. Kako je krajnji cilj kulture *in vitro* regeneracija biljaka, poželjno je testirati regeneracionu sposobnost genotipova pre njihovog uvođenja u eksperiment.

Ispitivana je regeneraciona sposobnost 27 inbred linija gajenog suncokreta upotrebom metode Paterson i Everett (1985). Prečen je broj regenerisanih izdanaka iz eksplantata hipokotila. Genotipovi sa najvećim brojem regeneranata su dalje korišćeni u eksperimentima sa fuzijom protoplasta, kao roditelji recipienti.

**TESTING OF REGENERATION CAPACITY OF
CULTIVATED SUNFLOWER INBRED LINES**

In sunflower (*Helianthus annuus* L.), only small number of genotypes possesses a capacity to regenerate *in vitro*. As plant regeneration is the ultimate goal of *in vitro* culture it is necessary to test genotype regeneration capacity before its introduction into the experiment.

Regeneration capacity of 27 cultivated sunflower inbred lines was tested using the method of Paterson and Everett (1985). Number of regenerated shoots from hypocotyl explants was observed. Genotypes with the highest number of regenerants were further used in the experiments with protoplast fusion as recipient parents.

TESTIRANJE TOLERANTNOSTI PŠENICE PREMA BORU PRIMENOM KULTURE ZIGOTNOG EMBRIONA

Milica Marjanović, Ankica Kondić-Špika, B. Kobiljski i Marija Kraljević-Balalić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Problem toksičnosti bora se javlja na prirodno zaslanjenim zemljištima, na zemljištima dobijenim povlačenjem mora ili isparavanjem morske vode. Bor je verovatno i najtoksičniji elemenat koji je nađen u slatinama. Amelioracija zemljišta sa visokim koncentracijama bora je veoma složen proces, ali kao jedno od mogućih rešenja jeste stvaranje biljaka tolerantnih na povišene koncentracije ovog elementa. Stoga je i cilj ovog rada bio utvrđivanje razlika u tolerantnosti sorti pšenice prema ovom elementu. Analiziranje četrnaest domaćih sorte pšenice vršeno je *in vitro* metodom kulture zigotnog embriona. Embrioni su gajeni na modifikovanoj MS (Murashige and Skoog, 1962) hranljivoj podlozi, kojoj je borna kiselina dodata u dve različite koncentracije (15 mM i 30 mM). Dobijeni rezultati su poređeni sa rezultatima kontrolne grupe, koja je gajena na podlozi bez borne kiseline u suvišku. Nakon dva meseca porasta merena je sveža masa kalusa. Utvrđene su značajne razlike između ispitivanih sorti u pogledu tolerantnosti na povišene koncentracije bora. Pri koncentraciji od 15 mM bora najtolerantnije su bile sorte Sonata, Simfonija i Rapsodija, kod kojih nije utvrđena značajna razlika u pogledu sveže mase kalusa u odnosu na kontrolu. Sorta Balada je pokazala najviši nivo tolerantnosti pri koncentraciji od 30 mM, jer je imala najveći porast sveže mase kalusa na ovoj podlozi. Najosetljiviji genotipovi su bili Ljiljana, Vila i Kantata, jer su pokazali nagli pad u porastu kalusa već na 15 mM (67,3-79,1% u odnosu na kontrolu), koji je nastavljen i na 30 mM (78,2-82,3% u odnosu na kontrolu). Kod ostalih ispitivanih sorti uočeno je postepeno smanjivanje sveže mase kalusa sa porastom koncentracije borne kiseline u hranljivoj podlozi.

TESTING OF WHEAT TOLERANCE TO BORON USING ZYGOTIC EMBRYO CULTURE

Problem of boron toxicity is occurring on saline soils, on soils derived by retirement of the sea or from marine evaporation. Boron is probably the most toxic element present in saline soils. Amelioration of high boron soils is extremely difficult process but breeding of plants that are tolerant to high external B concentrations is possible. The objective of this paper was to determine differences in tolerance to high boron concentrations among wheat cultivars. The *in vitro* method of mature embryo culture was used for analysing 14 leading domestic wheat cultivars. Isolated embryos were grown on a modified MS (Murashige and Skoog, 1962) nutrient medium, to which boron acid was added in two different concentrations (15 mM and 30 mM). The results were compared with the results of control group of calluses, which were grown on a boron-free MS medium. After two months of growing callus fresh weight was determined. Considerable variations in response to high boron concentrations have been identified among the cultivars. At the concentration of 15 mM of boron the most tolerant cultivars were Sonata, Simfonija and Rapsodija, which had no significant differences in callus fresh weight in relation to the control. Cultivar Balada was the most tolerant one at the concentration of 30 mM of boron, because it had the highest callus growth on this medium. The most sensitive genotypes were Ljiljana, Vila and Kantata because they had sudden reduction (67,3-79,1% in relation to the control) of callus fresh weight on medium with 15 mM of boron, as well as on 30 mM of boron (78,2-82,3% in relation to the control). The rest of the cultivars had gradual reduction in callus growth in response to the increase of boron concentrations in nutrient media.

IN VITRO OCENA TOLERANTNOSTI PŠENICE NA SUŠU

Vladislava Galović, Zorana Kotaranin i S. Denčić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

U radu je analiziran *in vitro* efekat stresa suše na 17 različitih genotipova pšenice, različitog geografskog porekla. Kalusno tkivo je indukovano iz nezrelih zigotnih embriona (10-15 dana posle polinacije) na modifikovanoj MS (MURASHIGE and SKOOG, 1962) hranljivoj podlozi. Nakon dve nedelje kalusno tkivo je presađeno na istu hranljivu podlogu obogaćenu 5% visokomolekularnim polyethylene glycol-om (PEG 6000) koji je upotrebljen kao agens stresa za postizanje hemijskog efekta suše. Kontrolna grupa kalusa gajena je na istoj hranljivoj podlozi bez PEG-a. Nakon četiri nedelje gajenja na ovim podlogama ocenjena je sposobnost preživljavanja kalusne mase kod posmatranih genotipova do presadijanja kao i procenat smanjenja sveže kalusne mase nakon presadijanja na hranljivu podlogu sa 5% PEG-om. Utvrđeno je postojanje statistički značajne razlike između genotipova u odgovoru na indukovani stres. Minimalno sniženje vrednosti sveže mase kalusa u odnosu na kontrolu ocenjeno je kod genotipa Rozofskaja (14,4%) a maksimalno kod genotipa Miranovska (58,4%) ukazujući na nivo tolerantnosti posmatranih genotipova na stres suše.

Rezultati su pokazali da se novosadski genotipovi, između ostalih, odlikuju srednjom tolerantnošću na vodni deficit indukovani u *in vitro* uslovima. Genotipovi Slavija, Košuta i Rapsodija su nešto tolerantniji na stres suše od genotipova Venera, NS55/25 i Odisej.

IN VITRO ASSESSMENT OF WHEAT TOLERANCE TO DROUGHT

In vitro effect of drought stress was analyzed in 17 wheat genotypes of different geographic origins. Callus tissue was induced from immature zygous embryos (10-15 days after pollination) on a modified MS (MURASHIGE and SKOOG, 1962) nutritive medium. After two weeks, the callus tissue was transplanted to the same medium previously enriched with 5% high-molecular polyethylene glycol (PEG 6000) which served for induction of drought stress. The control group of calli was grown in the medium without PEG. After four weeks of cultivation, the genotypes were assessed for survival ability of callus mass before transplanting and the percentage of reduction of fresh callus mass after transplanting to the medium with 5% PEG. Statistically significant differences were registered among the genotypes in the response to the induced stress. The lowest reduction of fresh callus mass in relation to the control was observed in the genotype Rozofskaja (14.4%), the highest in the genotype Miranovska (58.4%), indicating differences in drought tolerance among the genotypes.

The analyzed NS genotypes had medium tolerance to the water deficit induced under *in vitro* conditions. The genotypes Slavija, Košuta and Rapsodija were slightly more tolerant to drought stress than the genotypes Venera, NS55/25 and Odisej

UTVRĐIVANJE POLIMORFIZMA MIKROSATELITSKIH PRAJMERA KOD HEKSAPLOIDNE PŠENICE

Vladislava Galović¹, S. Denčić¹ i D. Jelovac²

¹Naučni institut za ratarstvo i povrтарstvo, Novi Sad

²Institut za kukuruz «Zemun polje», Beograd-Zemun

Ukupna genomska DNK izolovana je iz sorti/linija po modifikovanoj metodi PLASCHKE et al. (1995). Kao predstavnici Gatersleben wheat microsatellites (GWM) korишћena su dva seta mikrosatelitskih prajmera, GWM165 i GWM539, koji su opisani po RODER et al. (1998a, 1998b). Kineska sorta «Chinese Spring» je izabrana kao standard za analizu parametara kao što su: GWM designacija, veličina fragmenata po "CS" standardu, rang veličine alela i lokacija na hromozomu. PCR umnožavanje željenih fragmenata je izvedeno u zapremini od 30ul u Eppendorf PCR mašini po metodi Roder et al. (1998b).

Procedura provere PCR produkata na agaroznom gelu, priprema PCR produkata za nanošenje na PAA (polyacrylamide gel) gel, uslovi elektroforeze kao i bojenje PAA gela sprovedena je po GALOVIĆ et al. (2004). Statistička obrada podataka je rađena u programu NTSYSpc, modulom SIMQUAL za izračunavanje genetičke distance. Izračunata je genetička distanca po proceduri DICE (NEI and LI, 1979; DICE, 1945). Prikaz UPGMA dendrograma urađen je modulom SAHN (Sequential agglomerative hierarchical nested cluster analysis) po metodi SNEATH and SOKAL (1973). Oba, za pšenicu specifična prajmer seta umnožila su očekivane fragmente (prema podacima o DNK sekvenci). Polimorfnost oba primenjena prajmer seta potvrdila je ocenjena alelna varijabilnost čime je detektovano ukupno 10 različitih alela za oba gwm lokusa. Na osnovu dobijenih rezultata može se zaključiti da je prajmer set gwm539 sa 7 različitih alela pokazao veću polimorfnost od prajmer seta gwm165 sa 3 različita alela kod posmatranih genotipova.

DETERMINATION OF POLYMORPHISM OF MICROSATELLITE PRIMERS IN HEXAPLOID WHEAT

Total genomic DNA was isolated from the cultivars and lines using a modification of the method by PLASCHKE et al. (1995). The Gatersleben wheat microsatellites (GWM) were represented by two sets of microsatellite primers, GWM165 and GWM539, which were described according to RODER et al. (1998a, 1998b). The cultivar Chinese Spring was used as the standard to analyze parameters such as GWM designation, fragment size according to the CS standard, allele size rank and location on the chromosome. PCR multiplication of desired fragments was performed at 30ul volume on an Eppendorf PCR machine according to the method by Roder et al. (1998b).

The checking of the PCR products on agarose gel, their preparation for transfer onto PAA (polyacrylamide gel) gel, electrophoresis conditions, and staining of the PAA gel were all carried out according to GALOVIĆ et al. (2004). Statistical data processing was done using the NTSYSpc program and the SIMQUAL module for genetic distance calculation. Genetic distance was calculated according to DICE (NEI and LI, 1979; DICE, 1945). The UPGMA dendrogram was presented using the SAHN module (Sequential Agglomerative Hierarchical Nested Cluster Analysis) according to SNEATH and SOKAL (1973). Both of the wheat-specific sets of primers multiplied the expected fragments (according to data on DNA sequence). The polymorphism of both primer sets was confirmed by the estimated allelic variability and a total of 10 different alleles were detected for the two gwm loci. The results showed that the gwm539 primer set with seven different alleles exhibited a higher level of polymorphism than the gwm165 one with three different alleles.

IV tematska oblast / IV topic:

Oplemenjivanje organizama Breeding of organisms

Uvodna izlaganja / Introductory lectures

IV-Uvo

MOGUĆNOST GENETIČKE KONTROLE OTPORNOSTI PREMA VOLOVODU (*Orobanche cumana* L.) NA SUNCOKRETU KOD NAS I U SVETU

D. Škorić i S. Jocić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Suncokret napada volovod (*Orobanche cumana* L.). Glavni centri napada volovoda na suncokretu su zemlje oko Crnog Mora (Rusija, Ukrajina, Moldavija, Rumunija, Bugarska i Turska). Drugi centar gde volovod napada suncokret i nanosi značajne ekonomski štete je Španija, a treći Izrael. U predhodne dve godine volovod je pronađen na suncokretu i u zapadnom delu Kine. Volovod je prenet u našu zemlju najverovatnije iz Bugarske ili Rumunije, gde je *Orobanche cumana* L. znatno ranije bio prisutan.

Orobanche cumana L. je stranooplodna parazitna cvetnica i pojava novih rasa je stalno prisutna.

Izvori otpornosti prema volovodu se nalaze u više divljih vrsta suncokreta, ali je najveća frekvencija gena za otpornost prema volovodu prisutna u *H. tuberosus*.

U Evropi je dugo bila prisutna samo jedna rasa volovoda (rasa A) koju kontroliše jedan dominantni gen Or_1 . Polovinom 20.-og veka naglo se proširila rasa B. Otpornost kod suncokreta prema rasi B kontroliše jedan dominantni gen (Or_2). U drugoj polovini 20.-og veka došlo je do pojave novih rasa C (Or_3), D (Or_4) i rase E (Or_5).

Dugo godina u našoj zemlji je bila dominantno prisutna rasa B prema kojoj su otporne ruske sorte i novosadski hibridi. Pred kraj 20.-og veka kod nas se takođe proširila rasa E (Bačka i Banat). Prema rasi E su otporni hibridi Bačvanin i Perun i velik broj novih inbred linija.

POSSIBILITIES FOR GENETIC CONTROL OF SUNFLOWER RESISTANCE TO BROOMRAPE (*Orobanche cumana* L.) AT DOMESTIC AND INTERNATIONAL LEVELS

The sunflower is attacked by broomrape (*Orobanche cumana* L.). The main centers of broomrape attacks against sunflower are Black Sea countries such as Russia, the Ukraine, Moldova, Romania, Bulgaria and Turkey. The second major region where broomrape attacks this crop species and causes significant economic damage is Spain, while the third is Israel. Over the last two years, broomrape infestations of sunflower have been reported in western China as well. Broomrape was most probably introduced to Serbia and Montenegro from Bulgaria or Romania, where it had been present for quite some time before that.

Orobanche cumana L. is an open pollinated floriferous parasite and new races of it appear all the time.

Sources of resistance to broomrape can be found in several wild sunflower species, but the greatest frequency of these genes is found in *H. tuberosus*.

For a long time, Europe had only one broomrape race (A), resistance to which is controlled by a single dominant gene called Or_1 . In the mid 20th century, however, a new race (B) appeared and spread rapidly. Sunflower resistance to race B is controlled by the dominant gene Or_2 . In the latter part of the 20th century, several more races of this parasite appeared, namely races C (Or_3), D (Or_4) and E (Or_5).

For many years, Serbia and Montenegro was dominated by broomrape race B. Resistance to this race is present in Russian cultivars and Novi Sad hybrids. At the close of the 20th century, race E also appeared in the country and spread across the regions of Bačka and Banat. Resistance to race E exists in the hybrids Bačvanin and Perun and a large number of new inbred lines.

UTICAJ RAZLIČITE PROPORCIJE EGZOTIČNE GERMPLASME U OČINSKOJ KOMPONENTI HIBRIDA KUKURUZA NA NJIHOVE OSOBINE

M. R. Ivanović¹, N. J. Vasić¹ i M. Zorić²

¹Institut za ratarstvo i povrтарstvo, Novi Sad

²Univerzitet u Novom Sadu, Novi Sad

Cilj ovog rada bio je da se uporedi uticaj različite proporcije egzotične germplasme (preko inbred linije NC298) u očinskoj komponenti eksperimentalnih hibrida kukuruza na prinos zrna, sadržaj vlage u zrnu, stabilnost pojedinačnih genotipova i interakciju genotipa i spoljne sredine. Pet grupa očeva je formirano sa 0, 25, 50, 75 i 100% tropske germplasme (ili sa 1/2 manje u njihovim odgovarajućim hibridnim kombinacijama) koristeći direktna i povratna ukrštanja između NC298 inbred linije (100% tropska germplasma) i Mo17 i NS796 linija očeva (0% tropske germplasme). Svaka grupa očeva je testirana sa 3 inbred testera koji pripadaju Reid yellow dent heterotičnoj grupi da bi se stvorili eksperimentalni hibridi sa različitim procentom egzotične germplasme koji su bili predmet ovog istraživanja. Signifikantost razlika između srednje vrednosti eksperimentalnih hibrida sa različitom proporcijom NC298 inbred linije u očinskoj komponenti je ocenjivana pomoću F vrednosti koristeći Repeated-Measure Design (RMD).

Stabilnost pojedinačnih genotipova u odnosu na posmatrane spoljašnje sredine je određivana regresionim koeficijentom (b_i) i R^2 regresionom vrednošću. Interakcija genotipa i spoljašnje sredine za prinos zrna i sadržaj vlage u zrnu određivana je AMMI (Glavni aditivni efekti i višestruke interakcije) metodom. Stabilnost pojedinačnih genotipova nije ograničavajući faktor izbora poželjnih genotipova kako između tako i unutar grupa sa različitom proporcijom tropske germplasme, i nemože se utvrditi sigurna veza između stablinosti genotipa i proporcije tropske germplasme. Inbred linija NC298 sa 100% tropskom germplasmom može se uspešno inkorporirati u temperirani oplemenjivački materijal ukoliko njen udio u početnim populacijama ne prelazi 25%, kako za prinos zrna tako i sadržaj vlage u zrnu.

EVALUATION OF MAIZE HYBRIDS CONTAINING DIFFERENT PROPORTION OF TROPICAL GERMPLASM IN THEIR MALE PARENTS

The objectives of this study were to compare effects of the different proportions of tropical maize germplasm (*via* inbred NC298) in hybrids male parent, on grain yield, grain moisture content, cultivars stability performance and genotype-by-environment interaction. Using direct crosses and backcrosses (between NC298, Mo17 and NS796 male lines) five male groups were formed containing 0, 25, 50, 75 and 100 percent of tropical germplasm, respectively (or one half in their corresponding hybrids). Each male parent group was tested with three female inbred-testers that belong to the Reid yellow dent heterotic group. The level of significance between mean values of hybrids containing the different proportion of NC298 line in male parent was estimated by F value using Repeated-Measure Design (RMD). Individual genotype stability over environments for grain yield was determined by regression coefficient (b_i) and R^2 regression value. Genotype-by-environment interaction for grain yield and grain moisture content was estimated by AMMI (Additive main effects and multiplicative interaction) method. Individual genotypic stability did not restrict the choice of desirable genotypes between and/or within groups containing different proportion of tropical germplasm, and no association between genotype stability and the proportion of tropical germplasm could be defined. The NC298 tropical germplasm inbred line of maize, could be incorporated into temperate breeding material comprising 25% of the foundation population proportion, for both grain yield and grain moisture content.

NALAŽENJE KANDIDATNOG GENA ZA OTPORNOST JEČMA NA BYMOVIRUSE (*BaMMV*, *BaYMV* i *BaYMV-2*) PRIMENOM HROMOZOMSKOG ŠETANJA I KOMPARATIVNOG MAPIRANJA *Rym4/5* LOKUSA

D. Perović^{1,3}, N. Stein^{1,3}, S. Streng¹, B. Pellio², Jelena Perović¹,
S. Stracke¹, F. Ordon^{2,3} i A. Graner¹

¹Institute of Plant Genetics and Crop Plant Research (IPK), Germany

²Institute of Crop Science and Plant Breeding I, Justus-Liebig-University, Germany

³Institute of Epidemiology and Resistance, Federal Center
for Breeding Research on Cultivated Plants, Germany

Jedini ekonomičan način zaštite ječma od kompleksa žutog virusa, u zemljama centralne Evrope, koji izazivaju pojedinačno ili zajedno *Barley mild mosaic virus* (*BaMMV*), *Barley yellow mosaic virus* (*BaYMV*) ili *BaYMV-2*, je stvaranje i gajenje otpornih sorata. Recesivni lokus na dugom kraku trećeg hromozoma ječma, predstavljen sa dva gena *rym4* i *rym5*, korišćen je u oplemenjivačkim programima kao izvor otpornosti. U cilju pronađenja funkcionalne osnove recesivne otpornosti na virus započeto je hromozomsko šetanje po *Rym4/5* lokusu. Izolovano je 650 kb kontinuirane BAC sekvene iz neotporne sorte Morex. Tri četvrtine fizičke dužine sekvene kosegregira sa lokusom za otpornost. Identifikovan je transkribovani homolog gena ORF1 kod eukariota. Homolozi ovog gena kod paprike i salate su nosioci otpornosti na potyviruse, pa prema tome ovaj gen je perfektni kandidat za recesivnu otpornost prema *BaMMV/BaYMV*. Ortologni gen je prisutan i na dugom kraku prvog hromozoma piroča. Dijagnostički sekvenčni tačkasti polimorfizmi su nađeni u diferencijalnoj kolekciji ječmova i svi od njih izazivaju aminokiselinsku zamjenu kod odgovarajućeg proteina. Iz dobijenih rezultata može se reći da je u ovom radu identifikovan kandidatni gen za recesivnu otpornost ječma prema *BaMMV/BaYMV*, a sve ovo nas upućuje na moguću funkcionalnu osnovu *rym4/5* otpornosti.

CHROMOSOME WALKING AND COMPARATIVE MAPPING OF THE BARLEY *Rym4/5* LOCUS REVEALS THE *ORF1* AS A CANDIDATE GENE FOR BYMOVIRUS RESISTANCE

Breeding for resistance is the only strategy for protecting barley against barley yellow mosaic disease, which is caused in central Europe by single or combined infections with *Barley mild mosaic virus* (*BaMMV*), *Barley yellow mosaic virus* (*BaYMV*) or *BaYMV-2*. One recessive locus present on the long arm of chromosome 3H exhibiting the two genes *rym4* and *rym5* has been used as the source of resistance in European breeding programs. In order to elucidate the functional basis of recessive virus resistance, chromosome walking towards the *Rym4/5* locus was initiated. A 650 kb BAC contig of the susceptible cultivar Morex was established co-segregating with three quarters of its physical extension with the resistance locus. A transcribed gene homologous to the *ORF1* was identified. It provides a perfect candidate gene for recessive resistance to *BaMMV/BaYMV*, since homologues of this gene are involved in recessive potyvirus resistance in pepper and lettuce. An orthologous gene is present on rice chromosome 1L. Diagnostic single nucleotide polymorphisms in the candidate gene were identified among a differential set of barley genotypes all leading to amino acid changes in the deduced protein, which implies a possible function in *rym4/5* mediated virus resistance in barley.

ORGANSKO OPLEMENJIVANJE BILJA

J. Berenji

Institut za ratarstvo i povrtarstvo, Novi Sad

Propisi koji regulišu oblast organske proizvodnje navode posebne zahteve prema semenu i sadnom materijalu. Na ovaj način certifikacija organskih proizvoda je striktno uslovljena pridržavanjem zahteva prema semenu i sadnom materijalu. Upotreba genetički modifikovanih (transgenih) sorti kao i primena savremene biotehnologije u oplemenjivanju bilja je zabranjena, osim upotrebe genetičkih markera u selekciji. U praksi organske proizvodnje se razlikuju tri faze po pitanju sorti i soznog semena. U prvoj fazi (koja je zvanično trajala do 1. januara 2004) bila je dozvoljena upotreba sortnog semena bilo koje sorte stvorene konvencionalnim putem ukoliko je takvo seme umnožavano bar jedne godine (kod višegodišnjih vrsta dve godine) u organskim uslovima. U drugoj fazi, koja je sada aktuelna, javlja se zahtev da se uz umnožavanje i samo održavanje sorte vrši u organskim uslovima najmanje tri godine. Treća faza će zahtevati da se koriste samo tzv. organske sorte koje su nastale u procesu organskog oplemenjivanja bilja. Neki od najvažnijih genetičkih principa organske proizvodnje su: (1) da farmeri sami mogu umnožavati sortno seme za svoje potrebe (s tim u vezi ostalo je otvoreno pitanje statusa F_1 hibrida koje je nemoguće dalje umnožavati bez gubitka genetičkog identiteta hibrida); (2) postojanje genetičke varijabilnosti unutar sorte (u cilju bolje adaptabilnosti i da se uspori širenje bolesti i štetočina); (3) otpornost (po mogućnosti horizontalna) na bolesti i štetočine; (4) razvijen i efikasan korenov sistem i sposobnost potiskivanja korova.

ORGANIC PLANT BREEDING

The legislation that regulates organic agriculture enforces special requirements regarding seed and planting material. Certification of organic produce is strict with respect to the adherence to these requirements. Use of genetically modified (transgenic) varieties and application of modern biotechnology, with the exception of the use of genetic markers for selection, are prohibited in plant breeding. Organic production distinguishes three phases that concern varieties and certified seed. In the first phase (which officially lasted till 1 January 2004), it was permitted to use certified seed of any conventionally developed variety if that seed had been multiplied for at least one year (two years in the case of perennial species) in organic production. In the second phase, which is presently in due course, variety multiplication and maintenance under organic conditions must last for at least three years. The third phase will require an exclusive use of the so-called organic varieties, those that shall have been developed in the process of organic plant breeding. These are the major genetic principles of organic production: (1) farmers are permitted to multiply their own seed (the question of F_1 hybrids which cannot be multiplied without the loss of genetic identity remains open); (2) genetic variability exists within the variety (aimed at improved adaptability and slower distribution of diseases and pests); (3) resistance (horizontal if possible) to diseases and pests; (4) well-developed and efficient root system and capacity of weed repellence.

IV tematska oblast / IV topic:

Oplemenjivanje organizama Breeding of organisms

Usmena izlaganja / Oral presentations

IV-Usm

**UTICAJ TIPOA OTPORNOSTI PREMA RIZOMANIJI NA
KVALITATIVNA SVOJSTVA ŠEĆERNE REPE**

L. Kovačev, Snežana Mezei, N. Čačić i Nevena Nagl

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Rizomanija je u svetskim razmerama jedno od najdestruktivnijih oboljenja šećerne repe koje je do sada ustanovljeno i opisano. Obolenje izaziva virus nekrotičnog žutila nerava šećerne repe (BNYVV), a prenosi ga parazitna gljiva *Polymixa betae*. Da bi se postigla visoka i stabilna proizvodnja šećerne repe novi genotipovi pored dobrih kvantitativnih svojstava (prinos korena, sadržaj šećera i nešećera) treba da poseduju tolerantnost ili otpornost prema Rizomaniji. Pet različitih tipova tolerantnosti – otpornosti (Alba, Rizor, Holly, WB42 i *Beta maritima*) korišteni su kao donori gena u procesu povratnog ukrštanja sa domaćom osetljivom populacijom NS-56 2n MM. Različiti izvori otpornosti prema Rizomaniji, posle pet povratnih ukrštanja, kod near-isogenic populacija uske genetičke osnove, nisu značajno uticali na njihove najvažnije kvantitativne osobine. Ovo ukazuje da geni koji određuju tolerantnost-otpornost nisu povezani sa genima koji su odgovorni za ispoljavanje najvažnijih kvantitativnih svojstava šećernere repe.

**SUGAR BEET QUALITATIVE TRAITS AS AFFECTED
BY TYPE OF RESISTANCE TO RHIZOMANIA**

Rhizomania is globally one of the most destructive sugar beet diseases ever recorded and described. The disease is caused by the beet necrotic yellow vein virus (BNYVV) and is transmitted by the parasitic fungus *Polymixa betae*. For high and stable sugar beet production, it is necessary for new genotypes of this species to have good quantitative traits (root yield, sugar content, levels of nonsugars) as well as tolerance or resistance to rhizomania. Five different types of tolerance/resistance to this disease (Alba, Rizor, Holly, WB42 and *Beta maritima*) were used as gene donors in the process of backcrossing with the susceptible domestic population NS-56 2n MM. After five backcrosses, the different resistance types had no significant effect on the major quantitative traits of near-isogenic lines with a narrow genetic base. This indicates that genes controlling this tolerance/resistance are not linked with genes that are responsible for the expression of major quantitative traits in sugar beet.

KARAKTERIZACIJA REPETITIVNE SEKVENCE JEĆMA SA HROMOZOM SPECIFIČNIM PCR PRODUKTIMA

Gordana Šurlan-Momirović¹, D. Perović² i A. Graner²

¹Poljoprivredni fakultet, Katedra za genetiku i oplemenjivanje biljaka, Zemun

²Institut za genetiku i oplemenjivanje biljaka (IPK), Odeljenje genbanke,
AG MOM, Gatersleben, Nemačka

Transpozoni su samoreplikirajući elementi koji su odgovorni za veći deo repetativne DNA u genomu biljaka. Oni se mogu klasifikovati u tri grupe: dugi terminalno ponovljivi (LTR) retrotranspozoni, ne-LTR retroranspozoni i minijaturni invertovano-ponovljivi transpozoni (MITE). Mada je većina transpozona determinisana, još nije u potpunosti poznata njihova biološka funkcija i regulacija njihove aktivnosti. Međutim, specifični transpozoni se mogu koristiti za genetičku analizu, kao što su: u determinaciji genetičke raznovrsnosti i filogenije, kao molekularni markeri, itd. Veličina genoma jećma (*Hordeum vulgare*) iznosi 4800 Mb (Arumuganathan and Erle, 1991), od čega 80% DNA čine različite vrste transpozona. BARE-1 transpozon jećma je nabolje proučen i zauzima 2,9% njegovog genoma (Vicient et al., 1999), dok su drugi tipovi retrotranspozona manje ispitani. Cilj ovog rada je bio da se izvrši karakterizacija repetativnih sekvenci DNA jećma, utvrdi njihva lokacija i sekvencionira 9 različitih klonova. Korišćena je RFLP I FISH tehnika da bi se utvrdio broj kopija retrotranspozona u genomu jećma.

CHARACTERIZATION OF BARLEY REPETITIVE SEQUENCE WITH CHROMOSOME SPECIFIC PCR PRODUCTS

Transposable elements are self-propagating elements, which account for major repetitive DNAs in plant genome. They can be generally classified in three groups: long terminal repeat (LTR) retrotransposons, non-LTR retrotransposons and miniature inverted-repeated transposable elements (MITEs). Although many transposable elements have been reported, biological function and regulation of their activities remain unknown. However, unique properties of transposable elements can be exploited for genome analysis such as: determining genetic diversity and phylogeny, development of molecular markers, gene tagging etc. Barley, *Hordeum vulgare* contains 4800 Mb DNA (Arumuganathan and Erle, 1991) and more than 80% are different transposable elements. The barley transposable element BARE-1 (Vicient et al., 1999) accounts for 2.9% of the genome and is very well characterized; others have been reported and may be less-well described. The aim of this research was to characterize chromosome specific *Hordeum vulgare* repeats by determination of sequence redundancy, chromosomal localization and sequence analysis of nine cloned products. Two hybridization methods RFLP and FISH were applied in order to define the number of copies in the barley genome.

**INTERSPECIES HIBRIDIZACIJA I CITOGENETSKA
ISTRAŽIVANJA U OPLEMENJIVANJU SUNCOKRETA**

Jovanka Atlagić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Mnoštvo i raznolikost vrsta roda *Helianthus* pruža velike mogućnosti oplemenjivačima suncokreta. S druge strane divergentnost i heterogenost prisutna u rodu *Helianthus* nosi niz teškoća, pre svega «cross» inkompatibilnost, abortivnost embriona, sterilnost i smanjenu fertilnost interspecies hibrida. Za detektovanje uzroka ovakvih pojava, pa i njihovo prevazilaženje koriste se citogenetska istraživanja. Određivanje broja i strukture hromozoma, analiza mejoze-mikrosporogeneze, vitalnosti polena, omogućuje utvrđivanje filogenetskih veza između divljih vrsta i gajenog suncokreta, a time i mogućnost njihovog korишћenja u oplemenjivanju. Razvoj citogenetskih istraživanja na suncokretu je tekao od citologije preko citotaksonomije i klasične citogenetike do citogenetsko-molekularnih istraživanja. Poseban razvoj citogenetskih istraživanja je vezan za primenu interspecies hibridizacije u oplemenjivanju suncokreta.

**ROLES OF INTERSPECIFIC HYBRIDIZATION AND
CYTOGENETIC STUDIES IN SUNFLOWER BREEDING**

The abundance and diversity of species within the genus *Helianthus* offer numerous and rewarding possibilities to sunflower breeders. On the other side, the divergence and heterogeneity of the genus cause considerable difficulties, such as cross-incompatibility, embryo abortiveness, sterility and reduced fertility in interspecific hybrids. Cytogenetic studies are employed to detect and overcome such undesirable phenomena. Determinations of chromosome number and structure and analyses of meiosis - microsporogenesis and pollen viability make it possible to establish phylogenetic relations between wild sunflower species and the cultivated sunflower, enabling the use of the former in sunflower breeding. Cytogenetic studies of the sunflower have evolved from cytology, through cytobotany and classic cytogenetic to cytogenetic-molecular studies. Most intensive progress of cytogenetic studies has been associated with the use of interspecific hybridization in sunflower breeding.

STRUKTURA PRINOSA PASULJA PRIKAZANA METODAMA MULTIVARIJACIONE ANALIZE

Mirjana Vasić¹, Jelica Gvozdanović-Varga¹ i Marija Kraljević-Balalić²

¹Naučni institut za ratarstvo i povrтарstvo, Novi Sad

²Poljoprivredni fakultet, Novi Sad

Pasulj se gaji zbog korišćenja zrna, te tako masa zrna po biljci i broj biljaka po jedinicima površine čine prinos. Masa zrna po biljci, kao glavni i krajnji cilj selekcije, u centru je pažnje oplemenjivača. Direktna selekcija na prinos je neprecizna, pa se moraju razmotriti neki važniji elementi strukture prinosa kao i njihove međuzavisnosti. Značaj pojedinih komponenti se menja u zavisnosti od ekoloških činilaca. U selekciji za određene uslove uspevanja velika je važnost poznavanja i upotrebe lokalnih populacija jer su kod njih odnosi komponenata prinosa izbalansirani i usklaćeni sa dejstvom konkretnih klimatskih i edafskih faktora. U radu je analizirana genetička divergentnost genotipova pasulja iz kolekcije pasulja Naučnog instituta za ratarstvo i povrтарstvo iz Novog Sada i Banke biljnih gena Jugoslavije. Analizirane su direktnе komponente prinosa (broj zrna i mahuna po biljci, broj zrna po mahuni, masa 1000 zrna ili apsolutna masa zrna i masa zrna po biljci). Korišćeno je nekoliko metoda multivarijacione analize. Analiza glavnih komponenata (PCA) prikazuje koja komponenta prinosa na koji način vrši podelu genotipova u okviru kolekcije. PCA je bazirana na Pirsonovoj korelacionoj matrici (prikazanoj u radu) i Euklidijanskim rastojanjima. U radu je prikazana povezanost ispitivanih osobina sa glavnim komponentama i učeće pojedinačnih glavnih komponenata u ukupnoj varijabilnosti skupa, nerotiranih i rotiranih vrednosti. Za rotaciju glavnih komponenata korišćen je Varimax metod. Konstruisan je biplot grafikon preko izračunavanja vektorskih (za sve ispitivane osobine) i objekatskih (za sve ispitivane genotipove) koordinata u faktorskoj analizi. Ovakav način prikazivanja podataka omogućava uspešno sagledavanje međusobnih odnosa između pojedinačnih genotipova i njih sa komponentama prinosa.

STRUCTURE OF BEAN YIELD AS SHOWN BY MULTIVARIATE ANALYSIS

Bean is grown for grain, so grain mass per plant and plant number per unit area constitute the yield of this crop. Grain mass per plant is the focus of bean breeders' attention and the main and ultimate goal of bean selection. As direct breeding for yield is not precise enough, some major elements of yield structure and their interdependence need to be considered. The importance of individual components varies according to environmental factors. When breeding for a particular set of growing conditions, it is highly important to know and use the local populations, since in them the relationships among yield components are balanced and in harmony with the effects of the specific climatic and edaphic factors.

We studied the divergence of the bean collection of the Institute of Field and Vegetable Crops in Novi Sad. Genotypes were analyzed for direct yield components. The study included several methods of multivariate analysis. Principal component analysis (PCA) showed which of the traits were decisive in genotype differentiation. The principal components analysis was based on Pearson's correlation matrix and Euclidean distances. The Warimax method was used for the rotation of principal components. The percentage contribution of particular main components to total variability was shown, as was the accumulation of variability. The variability of the collection was interpreted based on the two principal components. Vector coordinates for all traits and object coordinates for all genotypes were shown by row biplot graphs. This enables us to overview interactions of individual genotypes with traits in factor analyses.

ANALIZA DOMESTIFIKACIONOG SINDROMA CRNOG BORA NA PODRUČJU SCG I BIH

A. Tucović¹, M. Mataruga² i Mirjana Šijačić-Nikolić¹

¹Šumarski fakultet, Beograd

²Šumarski fakultet, Banja Luka

Na osnovu istraživanja prirasta prirodnih, gajenih i oglednih stabala, može se zaključiti da je crni bor, posebno u našim uslovima, vrsta čiji genetički potencijal nije dovoljno upoznat, s tim ni dovoljno iskorišćen. Međuprovenjenična i unutarprovenjenična promenljivost osobina uzoraka semena, jednogodišnjih i dvogodišnjih sadnica odgajenih na raznim supstratima u BIH, pokazuje da je unutarprovenjenična varijabilnost veća od međuprovenjenične varijabilnosti. Ekonomski značajana svojstva, npr. otpornost na sušu su kompleksnog karaktera i zavise od velikog broja osobina. Krupan doprinos je u razradi postupaka za izradu modela sadnica otpornih na sušu, uz postupani izbor značajnih parametara stanja vodnog režima.

Izražen morfo-anatomski i fiziološki varijabilitet crnog bora uslovljen je visokim stepenom heterozigotnosti tj. specifičnom genetičkom strukturom lokalnih populacija i pripada tzv. ekstremnom balansnom tipu genetičke strukture njegovih populacija. Prema iznetoj hipotezi lokusi u hromozomima nisu zastupljeni dominantno-recessivnim alalima već brojnih serijama multiplnih alela, što je jedna od osnova za brži i uspešniji rad na daljem oplemenjivanju vrste u funkciji proizvodnje kvalitetnog sadnog materijala i namenskih kultura ove vrste. Crni bor obezbeđuje značajne izvore korisnih informacija koje se odnose na prirodne i gajene sastojine, kao i onih koje se tiču funkcionalisanja genetskih mehanizama, te ulazi u red vrsta pogodnih za izučavanje tzv. domestifikacionog sindroma.

ANALYSIS OF DOMESTICATION SYNDROME OF AUSTRIAN PINE IN THE AREA OF SERBIA AND MONTENEGRO AND BOSNIA AND HERZEGOVINA

Based on the increment study of natural, cultivated and test trees, it can be concluded that Austrian pine, especially in our conditions, is the species whose genetic potential is not sufficiently elucidated, and also not sufficiently utilised. Inter- and intra-provenance variability of the characters of seed samples, one-year-old and two-year-old seedlings cultivated in different substrates in BIH, shows that intra-provenance variability is higher than inter-provenance variability. Economically significant properties, e.g. resistance to drought, are complex and depend on a great number of characters. A large contribution is the working out of the procedures for the development of the seedling model resistant to drought, with a gradual selection of the significant parameters of water regime conditions.

A high morpho-anatomical and physiological variability of Austrian pine is conditioned by a high degree of heterozygosity, i.e. specific genetic structure of the local populations, and it belongs to the so-called extreme balance type of its population genetic structure. According to the hypothesis, the loci in the chromosomes are not represented by dominant-recessive alleles, but by numerous series of multiple alleles, which is one of the bases for the faster and more successful work on further improvement of species in the function of production of good quality planting material and specific purpose plantations. Austrian pine ensures significant sources of useful information referring to natural and cultivated stands and information on the function of genetic mechanisms, so it belongs to the species favourable for the study of the so-called domestication syndrome.

OPLEMENJIVANJE PŠENICE NA OTPORNOST PREMA NISKIM TEMPERATURAMA

N. Hristov i N. Mladenov

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Otpornost prema niskim temperaturama od velikog je značaja u proizvodnji pšenice. To se posebno ističe u godinama kao što je bila 2002/03, kada su značajne površine pod žitaricama izmrzle. Spuštanjem temperature ispod -10°C bez snega, javlja se opasnost od oštećenja lisne mase. Ukoliko golomrazica traje duže od 48 časova, a temperatura se spusti ispod -15°C , može doći i do izmrzavanja čvora bokorenja, čak i kod tolerantnih genotipova. Imajući u vidu stalni napredak u oplemenjivanju biljaka, u ovom radu je analizirano 23 sorte pšenice iz različitih etapa oplemenjivanja. Testiranje je izvršeno u hladnim komorama na temperaturi -15°C u trajanju od 24, 36, 48 i 60 časova. Sve sorte su podeljene u tri grupe u zavisnosti od godine priznavanja. U prvoj grupi, u kojoj se uglavnom nalaze introdukovane sorte, procenat preživelih biljaka na svim tretmanima iznosio je u proseku 91%. Utvrđeno je da se u okviru ove grupe, sorte međusobno razlikuju po otpornosti na niske temperature u zavisnosti od porekla. U drugoj grupi, koju čine domaće sorte koje su gajene na velikim površinama 70-ih godina, procenat preživelih biljaka iznosio je 90,8%. Ovaj podatak ukazuje da je i kod domaćih sorti postignut izuzetan napredak u pogledu ovog svojstva. Treću grupu čine sorte novijeg datuma, koje ujedno predstavljaju i aktuelni sortiment novosadskih sorti pšenice. Kod ovih sorti, otpornost prema niskim temperaturama se nalazi na nivou prethodne grupe, pri čemu je procenat preživelih biljaka iznosio 89,2%. U procesu oplemenjivanja važno je zadržati određena svojstva na zadovoljavajućem nivou, a pri tome unaprediti pre svega ekonomski važna svojstva, kao što su prinos i kvalitet, što je upravo i ostvareno kod sorti koje se trenutno nalaze u proizvodnji.

WHEAT BREEDING FOR RESISTANCE TO LOW TEMPERATURE

Resistance to low temperature is important in wheat production, especially in years such as 2002/03, when winterkill occurred at a large acreage. When temperature drops below -10°C and there is no snow blanket, leaf mass becomes prone to damage. If such conditions persist longer than 48 hours and the temperature drops below -15°C , tillering nodes may freeze even in the case of tolerant genotypes. Twenty-three wheat cultivars from different breeding cycles have been tested for reaction to exposure to the temperature of -15°C for 24, 36, 48 and 60 hours. The cultivars were divided in three groups, in dependence of year of registration. In the first group, consisting mostly of introduced cultivars, the average percentage of survival in all treatments was 91%. The cultivars in this group were found to differ in resistance to low temperature in dependence of origin. In the second group, consisting of the domestic cultivars commercially grown in the 1970's, the percentage of survival was 90.8%. This was an indication that the domestic breeding program had been quite successful with regard to this trait. The third group comprises new cultivars, those which are currently in the domestic commercial production. Their resistance to low temperature was 89.2%, at the level of the second group. In breeding process it is important to maintain certain traits, such as yield and quality, at an appointed level. This has been fully achieved with the cultivars from the third group.

UTICAJ KOMPOZICIJE GLUTENINA I PUROINDOLINA NA KVALITET SORTI HLEBNE PŠENICE

D. Obreht¹, B. Kobiljski², S. Denčić², M. Đan¹ i Ljiljana Vapa¹

¹Prirodno-matematički fakultet, Novi Sad

²Institut za ratarstvo i povrtarstvo, Novi Sad

Jedinstvene karakteristike testa hlebne pšenice (*T. aestivum* L.) ogledaju se prvenstveno kroz elastičnost i viskoznost glutena. Kvantitativni i kvalitativni sastav glutena, koji je u visokoj meri definisan kompozicijom *Glu-1* lokusa, osnovna je karakteristika pri određivanju tehnološkog kvaliteta hlebne pšenice. Utvrđeno je da sorte koje nose subjedinicu $Bx7^{OE}$ u *Glu-B1* lokusu poseduju poboljšane performanse testa. 1BL/1RS traslokacija između pšenice i raži široko se koristi u programima oplemenjivanja pšenice. Prisustvo 1RS hromatina može imati negativan efekat na osobine testa i kvalitet pšenice. Kako tekstura endosperma zrna pšenice predstavlja važnu karakteristiku tehnološkog kvaliteta utvrđivanje alelne kompozicije puroindolinskih lokusa takođe se primenjuje u oplemenjivačkim programima. U ovom radu analizirana je varijabilnost HMW glutenina, prisustvo 1BL/1RS translokacije i kompozicija puroindolina u kolekciji sorti hlebne pšenice, kao i veza ovih karakteristika endosperma pšenice sa tehnološkim kvalitetom. Osnovni ciljevi istraživanja su ispitivanje uticaja mutacija puroindolinskih gena na osobine hlebnog testa i definisanje efekata koje HMW GS i 1BL/1RS translokacije imaju na tehnološki kvalitet pšenice.

INFLUENCE OF GLUTENINS AND PUROINDOLINES COMPOSITION ON THE QUALITY OF BREAD WHEAT CULTIVARS

Dough of bread wheat (*T. aestivum* L.) has unique properties, the most important of which are elasticity and viscosity of gluten. High gluten strength is used as a predictor of good quality bread wheat and it has been attributed largely to the composition of alleles at the *Glu-1* loci. It has been observed that cultivars with over expressed subunit $Bx7^{OE}$ at the *Glu-B1* have enhanced dough strength. The 1BL/1RS wheat-rye translocation has been used extensively in wheat breeding programs. Rye 1RS chromatin can negatively impact wheat dough and bread quality. Since grain texture is also important for milling and bread making properties analysis of puroindoline loci allelic variability is also included in molecular evaluation of breeding germplasm. In this study we analyse the HMW glutenin variability, the presence of 1BL/1RS translocation and puroindoline composition in a collection of bread wheat cultivars, and its relationship with technological quality parameters. Our main objectives are to clarify if there is any relationship between puroindoline mutations and dough properties, and to compare the effects of HMW GS and 1BL/1RS translocation in wheat technological quality.

PRAVCI I METODE OPLEMENJIVANJA STOČNOG GRAŠKA (*Pisum sativum* L.) U NAUČNOM INSTITUTU ZA RATARSTVO I POVRTARSTVO U NOVOM SADU

V. Mihailović, A. Mikić, S. Katić, I. Pataki i Đ. Karagić

Naučni institut za ratarstvo i povrтарstvo, Zavod za krmno bilje, Novi Sad

Primenom masovne, a potom i individualne selekcije iz prirodnih populacija, nastale su prve jugoslovenske sorte ozimog krmnog graška, NS pionir i NS dunav, od kojih je prva i dalje najraširenija u domaćoj proizvodnji. Prateći trendove u oplemenjivanju stočnog graška u SAD, Kanadi, Zapadnoj Evropi i Sovjetskom Savezu, tokom poslednje dve decenije prošlog veka započet je rad na nastanku domaćih sorti jarog stočnog graška, namenjenog kombinovanom iskorišćavanju, kao i za zrno bogato proteinima. Ukrštanjem postojećih domaćih i inostranih genotipova sa poželjnim agronomskim svojstima i odgovarajućim metodama selekcije, prvenstveno pedigree metodom, metodom F₂ potomstva i metodom povratnog ukrštanja, stvorene su najpre sorte stočnog graška za kombinovano iskorišćavanje, NS-lim i NS-junior, a potom, istim metodama selekcije uz unošenje gena za skraćenje stabla i afila tip lista, i sorte namenjene isključivo za visoke i kvalitetne prinose zrna, kao što su moravac, jezero i javor.

Mikroogled sa genotipovima stočnog graška, stvorenim u Naučnom institutu za ratarstvo i povrтарstvo u Novom Sadu, bio je postavljen na oglednom polju Instituta na Rimskim Šančevima i trajao je od 2002. do 2004. godine. Prvi deo ogleda je bio namenjen ispitivanju genetičke varijabilnosti komponenti prinosa zelene krme i uključivao je ozime sorte NS pionir i NS dunav, jare sorte NS-lim, NS-junior i moravac i jare linije P-823, P-824 i P-342. Drugi deo ogleda je bio namenjen određivanju genetičke varijabilnosti komponenti prinosa zrna i uključivao je ozime linije L-013, L-015 i L-016, jare sorte NS-lim, NS-junior, moravac, jezero i javor i jare linije P-823, P-824 i P-342.

DIRECTIONS AND METHODS OF FODDER PEA (*Pisum sativum* L.) BREEDING AT THE INSTITUTE OF FIELD AND VEGETABLE CROPS IN NOVI SAD

By means of massive and individual selection from local landraces, the first Yugoslav winter forage pea cultivars were created, NS Pionir and NS Dunav, the first still being the most widely distributed in the domestic production. Following the trends in fodder pea breeding in the USA, Canada and the Soviet Union, during two last decades of the last century a work on the creation of domestic spring fodder pea cultivars for combined use, as well as for protein-rich grain has been commenced. Crossings between domestic and introduced genotypes with desirable agronomic characteristics and appropriate methods of selection, mainly pedigree, F₂ and backcross method, led to the development of the combined-use spring pea cultivars, NS-Lim and NS-Junior, and, by the same methods and introduction of genes for shortened stems and afila leaf type, to the cultivars solely for high and quality grain yields, such as Moravac, Jezero and Javor.

A small-plot trial with Novi Sad fodder pea cultivars was carried out from 2002 to 2004 at the Rimski Šančevi Experiment Field. One part of the trial was established to study the genetic variability of forage yield components and therefore included winter cultivars NS Pionir and NS Dunav, spring cultivars NS-Lim, NS-Junior and Moravac and spring lines P-823, P-824 and P-342. The other part of the trial was aimed at the study of genetic variability of grain yield components and therefore included winter lines L-013, L-015 and L-016, spring cultivars NS-Lim, NS-Junior, Moravac, Jezero and Javor and spring lines P-823, P-824 and P-342.

TESTIRANJE NOVIH SELEKCIJA VIŠEGODIŠNJIH LEGUMINOZA

Z. Tomić, Z. Nešić i M. žujović

Institut za stočarstvo, Zemun-Beograd

U radu su prikazani rezultati testiranja novih selekcija četiri najvažnijih i najzastupljenijih vrsta leguminoza koje se kod nas koriste za stočnu hranu. Lucerka, crvena detelina, žuti zvezdan i esparzeta pripadaju ujedno i najkvalitetnijim biljkama kako u pogledu hranljivih materija, tako i po prinosu suve materije. Ispitivane su tri nove selekcije lucerke, dve crvne deteline, dve žutog zvezdana i jedna esparzete u odnosu na standarde. Ogled je trajao dve godine (2003-2004), postavljen po standardnim metodama za testiranje i priznavanje novih sorti. Utvrđena je produkcija zelne mase kroz četiri otkosa po godini, obračunat je i koeficijent varijacije između sorti. U drugoj godini u uzorcima drugog otkosa utvrđeni su parametri kvaliteta standardnim laboratorijskim analizama. Sadržaj proteina kod sve tri nove selekcije lucerke je viši od standarda (16,01%), dok je standard crvena detelina sa 20,03% bolja od novih selekcija. Nove selekcije žutog zvezdana su kvalitetnije od standarda (22,08%), kao i esparzete (19,71%). Na osnovu dobijenih parametara produkcije i kvaliteta sortiment višegodišnjih leguminoza biće obogaćen novim sortama.

TESTING THE NEW SELECTIONS OF PERENNIAL LEGUMES

This paper presents the results obtained in testing the new selection of four most important and most common species of legumes used for animal feeds in Serbia. Lucerne, red clover, bird's trefoil and sainfoin are also high quality plants regarding both nutritive substances and yield of dry matter. We have investigated three new selections of lucerne, two selections of red clover, two selections of bird's trefoil and one of sainfoin in relation to standard ones. Trial lasted two years (2003-2004), designed according to standard methods for testing and recognition of new sorts. The production of green mass through four cuts per year was determined, together with the variation coefficient among sorts. In the cuts of the second year the quality parameters were determined by standard lab analyses. The content of protein in all three new selections of lucerne was higher in relation to standard (16,01%), while the standard of red clover with 20,03% was better than new selection. New selections of bird's trefoil are of better quality than standard (22,08%), which is the case also with sainfoin (19,71%). On the basis of obtained production and quality parameters the assortment of perennial legumes will be enriched by new sorts.

**MEĐUZAVISNOST S₁ LINIJA I NJIHOVIH TEST UKRŠTANJA U
SINTETIČKIM POPULACIJAMA KUKURUZA**

G. Bekavac, N. Vasić, Božana Purar i Aleksandra Nastasić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Sa uvođenjem prvih single cross hibrida u proizvodnju kukuruza, međusobna povezanost linija per se i njihovih test ukrštanja je postala predmet interesovanja oplemenjivača. Simultana procena vrednosti linija per se i test ukrštanja istih sa inbred testerom je opšte prihvaćen metod u stvaranju visoko produktivnih hibrida. Dve sintetičke populacije kukuruza (NS Syn 88/12 and NS Syn 88/14) korišćene su u half-sib odnosno S₁ programu rekurentne selekcije. Nezavisni ogledi sa S₁ i HS potomstvima postavljeni su po nekompletnom blok dizajnu.

Niske korelacije između linija per se i njihovih test ukrštanja za prinos zrna (<0.5) ukazuju na preovlapajuću determinisanost prinosa zrna neadditivnim genima, dok visoke korelacije za neka druga agronomski važna svojstva (>0.8) omogućuju visoku pouzdanost predikcije istih u oplemenjivačkim programima.

**INTERRELATIONSHIP BETWEEN S₁ LINES AND THEIR
TESTCROSSES IN MAIZE SYNTHETIC POPULATIONS**

The relationship between line per se and their test crosses have become important to breeders since the first single-cross hybrid have been introduced in maize production. A method used in developing high-yielding genotypes is simultaneous assessment of the performances of lines per se and testcrosses of these lines with an inbred tester. Two synthetic maize populations (NS Syn 88/12 and NS Syn 88/14) were used in a half-sib followed by an S₁ recurrent selection program. Independent trials with S₁ and HS progenies were set up according to an incomplete block design.

Low correlations (<0.5) between line per se and testcross performance for grain yield indicate large amount of nonadditive gene action, while correlations for some other agronomically important traits were sufficiently large (>0.8) to have predictive value in a breeding program.

**PRIMENA MARKER ASISTIRANE SELEKCIJE (MAS) U
OPLEMENJIVANJU PŠENICE U NOVOM SADU**

B. Kobiljski¹, D. Obreht², A. Kondić¹, S. Denčić¹, Lj. Vapa², J. Pilipović¹,
R. Barjaktarović², M. Marjanović¹ i M. Davidović²

¹Naučni Institut za ratarstvo i povrтарstvo, Novi Sad

²Prirodno-matematički fakultet, Novi Sad

U radu su analizirani rezultati dobijeni u poslednje dve godine primenom molekularnih markera – mikrosatelita, u Zavodu za strna žita, Naučnog Instituta za ratarstvo i povrtarstvo u Novom Sadu. Jedan od segmenata rada bio je detekcija alelnog polimorfizma u lokusu Rht8 gena i utvrđivanje međuzavisnosti alelnog skora i fenotipske ekspresije važnih svojstava za oplemenjivanje pšenice (ranostasnost, visina biljke, broj i masa zrna po klasu). Drugi deo molekularne i fenotipske evaluacije odnosio se na genetsku determinaciju mirovanja semena pšenice. Deo rada sa molekularnim markerima obuhvatio je i utvrđivanje alelnog polimorfizma gluteninskih subjedinica i prisustva 1B/1R translokacije kod većeg broja genotipova pšenice iz kolekcije Zavoda. Konačno, u radu su tumačeni rezultati dobijeni primenom mikrosatelita u detekciji alela koji uslovjavaju tolerantnost pšenice na suvišak B. Dobijeni rezultati jasno ukazuju na se MAS sa uspehom može primeni kao izuzetno moćna, precizna i korisna metoda u oplemenjivanju pšenice. Trenutno, najveći značaj metode je, što se na osnovu dobijenih rezultata, mogu sa sigurnošću odabratи genotipovi koji poseduju poželjne gene (sekvence) i da se, kao takvi, koriste kao roditeljske komponente u hibridizaciji. U bliskoj budućnosti, pored daljeg rada na detekciji molekularne i fenotipske varijabilnosti, primena molekularnih markera će obuhvatiti i analizu jednog dela generacijskog materijala, kao i linija u komparativnim ogledima, kako za gore navedena svojstva, tako i za druga: otpornost na niske temperature, tolerantnost na fusarijum, zaštitu autorskih prava sorti pšenice Srbije i Crne Gore itd.

**APPLICATION OF MARKER-ASSISTED SELECTION (MAS) IN WHEAT
BREEDING IN NOVI SAD**

This paper analyses the results obtained by using molecular markers – microsatellites over last two years at the Small Grains Department of Institute of Field and Vegetable Crops in Novi Sad. One part of the research was detection of allelic polymorphism at the dwarfing gene Rht8 locus and determination of relation between allelic score and phenotypic expression of traits important for wheat breeding (earliness, plant height, grain number and grain weight per spike). Another part of the molecular and phenotypic evaluations has been related to genetic determination of seed dormancy in wheat. The part of the work with molecular markers comprised the identification of allelic polymorphism of glutenin subunits and the presence of the 1B/1R translocation in genotypes from the Departmental Core Collection. Finally, the paper interprets the results obtained using microsatellites in detection of the alleles determining wheat tolerance to increased B concentration. The obtained results clearly show that MAS can be successfully applied as an extremely powerful, precise and useful method in wheat breeding. At present, the main advantage of the method is that, on the basis of its results, genotypes with desirable genes (sequences) can be determined and used as parents in hybridization. In very near future, in addition to molecular and phenotypic variability determination, application of molecular markers will be expanded to include the evaluation of segregation generations and advanced lines for both the traits mentioned above and other ones such as the tolerance to low temperatures, *Fusarium*, fingerprinting of wheat varieties from Serbia and Montenegro for breeders' rights protection, etc.

NOVIJI REZULTATI I NEKI BUDUĆI PRAVCI OPLEMENJIVANJA TRITIKALEA U SCG

M. Milovanović

IIP «SRBIJA», Centar za strna žita, Kragujevac

Prve sorte ozimog i jarog tritikalea na tadašnjim Jugoslovenskim prostorima su stvorene u Kragujevcu. Pored kragujevačkog programa pažnju zavređuju i rezultati postignuti u Novom Sadu i Zaječaru. Do sada priznat broj sorti i njihove poželjne osobine omogućili su da ova nova gajena vrsta dostigne danas u SCG površine od oko 60.000 ha sa tendencijom daljeg porasta. U našoj zemlji se tritikale uglavnom seje u ozimoj setvi (oko 98%). Danas su vodeće sorte tritikalea u proizvodnji KG. 20, Knjaz i Rtanj. Pored njih zastupljene su i sorte Goranac i Novosadski tritikale. U proizvodnju se uvode sorte ozimog tritikalea Favorit i Trijumf koje ispunjavaju većinu zahteva proizvođača, pa se očekuje još veći budući porast površina pod ovom vrstom. Novije sorte tritikalea su dostigle prinose vodećih komercijalnih sorti ozime pšenice i značajno nadmašuju prinose sorti ozimog ječma, što doprinosi da su sve više zastupljene u proizvodnji zamenjujući ječam, raz i pšenicu prvenstveno po pitanju proizvodnje stočne hrane. Kod najnovijih sorti ozimog tritikalea postignuti su zavidni rezultati po osobinama ranostasnosti, niske stabljike, rodnosti, produktivnog bokorenja, tolerantnosti na kiselo zemljишte, nalivenosti i kvaliteta zrna, što doprinosi da ove sorte bivaju atraktivne i za šire (Evropske) okvire gajenja. Pored rezultata oplemenjivanja u radu su prezentirani i neki važniji problemi u dosadašnjem radu i predloženi budući pravci i strategije u cilju njihovog prevazilaženja i budućeg još uspešnijeg istraživanja, širenja i unapređenja ove nove vrste. Posebna pažnja poklonjena je budućim metodama za stvaranje nove varijabilnosti i proširenju biodiverziteta tritikalea.

RECENT RESULTS AND SOME FUTURE PROSPECTS OF TRITICALE BREEDING IN SCG

First cultivars of winter and spring triticale on former Yugoslavia spaces were created in Kragujevac. Besides of KG program, noticeable results were achieved in Novi Sad and Zajecar, too. Recognized number of cultivars until now days and their desirable traits were made possible that this grown species attain growing areas of about 60.000 ha this year in SCG, with ascending further tendency. In SCG triticale is grown in generally in winter sowing terms (about 98%), with major cultivars in production KG. 20, Knjaz and Rtanj. Besides of those there are present cultivars Goranac and Novosadski tritikale, too. New cultivars of winter triticale Favorite and Triumph, which satisfy majority of producers needs, are in phase of introduction to practice, so, the further even greater growing areas under this species in the future could be expected. Some of new cultivars attained grain yield of leading commercial cultivars of winter wheat and significantly surpassed grain yield of winter barley cultivars, which contributes that they are more and more present in practice on the place of barley, rye and wheat, for feed production purpose on the first place. At the most new cultivars of winter triticale were achieved desirable results according to traits of: earliness, short stem, high yield, productive tillering, tolerance to acid soil, kernel filling and quality, which contribute that these cultivars becomes attractive for broader (European) frames of growing, too. Besides of results of breeding, also, in the paper were presented some important problems in the past work and proposed future prospects and strategies in the goal of theirs overcoming, as well as even more successful future research, broadening and improvement of this new species. Special attention was directed to future methods for creation of new variability and broadening of triticale biodiversity.

REKURENTNA SELEKCIJA NA POVEĆAN SADRŽAJ ULJA KOD KUKURUZA

G. Saratlić¹, Violeta Andelković¹, Nada Lečić², M. Babić¹, N. Delić¹,
G. Stanković¹ i K. Lemkey³

¹Institut za kukuruz «Zemun Polje», Beograd

²Naučni institut za ratarstvo i povrтарstvo, Novi Sad

³USDA-ARS, Poljoprivredni fakultet, Ajova, SAD

Rekurentna selekcija na povećan sadržaj ulja primenjena je tokom sedam ciklusa fenotipske masovne selekcije kod dve sintetičke populacije kukuruza. Izučavani su direktni i indirektni odgovor na selekciju, kao i korelativne promene sadržaja ulja i drugih agronomskih svojstava, koje će biti prikazane u ovom radu. Sadržaj ulja u zrnu određen je NMR spektroskopijom.

Povećan sadržaj ulja bio je u negativnoj korelaciji sa prinosom zrna u populaciji DS7u *per se*, dok je u populaciji YUSSSu *per se*, sa povećanjem sadržaja ulja došlo i do povećanja prinosa zrna. Sličan trend zabeležen je i kod međuzavisnosti sadržaja ulja u zrnu i visine biljke, dok je procenat poleglijih biljaka bio u signifikanto negativnoj korelaciji sa sadržajem ulja u zrnu kod populacije YUSSSu, dok je međuzavisnost ovih svojstava bila nesignifikantna unutar populacije DS7u.

Prema ovim i drugim do sada objavljenim rezultatima, može se zaključiti da prilikom selekcije na povećan sadržaj ulja obavezno treba uzeti u obzir korelativne promene prinosa zrna i drugih značajnih svojstava.

RECURRENT SELECTION FOR INCREASED OIL CONTENT IN MAIZE

Recurrent selection for increased oil content was conducted on maize populations over seven cycles of phenotypic mass selection. The objectives were to evaluate direct and indirect responses to selection and correlative changes between oil content and other agromomic traits. Oil content in individual kernels was determined by nuclear magnetic resonance spectroscopy.

The increased oil content was associated with grain yield reduction in DS7u *per se* while grain yield increased significantly in YUSSSu *per se*. Similar trend was detected in correlation between oil content and plant height; percentage of lodged plants has significant and negative correlation with oil content in YUSSSu, while correlation among these traits was not significant in DS7u.

Due to our and previous results, it could be recommended that selection for increased oil content in the populations should consider correlative changes in grain yield and other important traits.

EFEKAT HETEROZISA ZA PRINOS SEMENA I KOMPONENTE PRINOSA SUNCOKRETA

Nada Hladni¹, D. Škorić¹, Marija Kraljević-Balalić²

¹Naučni institut za ratarstvo i povrтарство, Novi Sad

²Poljoprivredni fakultet, Novi Sad

Stvaranje novih visoko prinosnih i stabilnih hibrida suncokreta na bazi interspecies hibridizacije zahteva posedovanje informacije o efektu heterozisa za agronomski važna svojstva u F₁ generaciji. Efekat heterozisa za prinos semena, visinu biljke i prečnik glave proučavan je kod interspecies hibrida stvorenih metodom linija x tester. Inbred linije majke nastale su interspecies hibridizacijom, a restorer inbred linije oca dobrih kombinacionih sposobnosti korišćene su kao testeri u formi restauratora fertilitnosti. Hibridi F₁ generacije nastali su ukrštanjem svakog testera sa svakom inbred linijom majke. Između inbred linija i njihovih hibrida postoje značajne razlike u srednjim vrednostima za ispitivanja svojstva.

Vrednosti heterozisa za prinos semena po biljci bile su pozitivne i visoko značajne u odnosu na roditeljski prosek (98.4-274.13%) kao i u odnosu na boljeg roditelja (54.8-223.2%). Znatno niži efekat heterozisa ustanovljen je za visinu biljke (19.0-66.0%) u odnosu na roditeljski prosek i u odnosu na boljeg roditelja (-3.9-51.6%). Kod prečnika glave heterozis se kretao od 19.0 do 55.6% u odnosu na roditeljski prosek i od 7.8 do 36.6% u odnosu na boljeg roditelja. Ova istraživanja mogu biti od značaja za stvaranje novih visokoprinosnih genotipova suncokreta na bazi interspecies hibridizacije.

HETEROSIS FOR SEED YIELD AND YIELD COMPONENTS IN SUNFLOWER

The development of new high-yielding and stable sunflower hybrids based on interspecific hybridization requires information on the heterotic effects for agronomically important traits in the F₁ generation. Heterotic effects for seed yield, plant height and head diameter were studied in interspecific sunflower hybrids developed by the line x tester method. The female inbred lines were developed by interspecific hybridization, while the male restorer inbreds with good combining abilities were used as testers in the form of fertility restorers. F₁ hybrids were obtained by crossing each tester with each female inbred. The inbred lines and their F₁ hybrids differed significantly in their mean values of the traits under study.

Heterosis values for seed yield per plant were positive and highly significant relative to both the parental mean (98.4-274.1%) and the better parent (54.8-223.2%). Significantly less heterosis was recorded in the case of plant height relative to parental mean (19.0-66.0%) and better parent (-3.9-51.6%). With head diameter, the heterotic effect ranged from 19.0 to 55.6% (parental mean) and from 7.8 to 36.6% (better parent). The results of this study may be used for the development of new high-yielding and stable sunflower hybrids based on interspecific hybridization.

GENETIČKA ANALIZA RANOSTASNOSTI U GENERACIJAMA RAZDVAYANJA PAPRIKE (*Capsicum annuum* L.)

B. Zečević, M. Mijatović, Ž. Marković i Ž. Radošević

Institut «SRBIJA», Centar za povrтарstvo, S. Palanka

Cilj ovoga istraživanja bio je da se odrede genetičke vrednosti za ranostasnost F_1 i F_2 generacije dialelno ukrštenih genotipova paprike, kao i da se ocene efekti primene *pedigree* i *bulk* metoda selekcije kod F_3 hibridnih populacija.

Hibridne populacije su formirane od ukrštanja divergentnih genotipova koji pripadaju različitim varijetetima vrste *Capsicum annuum* L. Uzimajući sve dobijene kombinacije ukrštanja u obzir ocenjeno je da je efekat heterozisa u F_1 generaciji za ispitivanu osobinu bio mali. Genetička analiza F_2 generacija pokazala je da je superdominacija način na koji se nasleđuje ranostasnost.

U F_3 generaciji hibridne populacije nastale primenom *pedigree* metoda selekcije ocenjene su kao oplemenjivački vrednije po osnovu ispitivane osobine u odnosu na hibridne populacije dobijene modifikovanom *bulk* metodom.

GENETIC ANALYSIS OF EARLINESS IN SEGREGATED GENERATIONS OF PEPPER (*Capsicum annuum* L.)

The goal of this researching was determination of genetic values for earliness F_1 and F_2 generations of diallel crossing pepper genotypes, and also it was estimated the effects of applying of pedigree and bulk methods of selection in F_3 hybrid populations.

The hybrid populations were formed from crossing of divergent genotypes, which belong to different varieties of species *Capsicum annuum* L. Considering all combinations of crossing it was estimated that the effect of heterosis in F_1 generation for analysed trait was low. The genetic analysis of F_2 generation showed that superdominance is the mode of inheritance for the earliness.

In F_3 generation the hybrid populations which formed by the pedigree method of selection were estimated as better for all traits in compare to the hybrid populations from modified bulk method.

STVARANJE HIBRIDA KUKURUZA TOLERANTNIH NA RAZLIČITE GUSTINE GAJENJA

M. Rošulj, Jelena Vančetović, Zorica Pajić i G. Todorović

Institut za kukuruz «Zemun Polje», Zemun Polje, Beograd

Prinosi modernih hibrida kukuruz (*Zea mays L.*) zavise uglavnom od velikih gustina gajenja. Pri niskim gustinama gajenja razlike između novih i starih hibrida su vrlo male i postaju sve veće kako se gustina gajenja povećava. U Srbiji i Crnoj Gori proizvođači kukuruza oduvek su više voleli hibride čiji prinos nije uslovljen gustinom gajenja zbog čestih suša, poleganja, bolesti i cene semena.

Cilj ovog istraživanja je bio da se utvrdi mogućnost stvaranja hibrida tolerantnih na različite gustine gajenja putem rekurentne half – sib selekcije sa inbred testerom. Pet elitnih inbred linija je odabранo sa formiranje početnog ciklusa. Biljke su samooplodene i ukrštene sa opozitnim elitnim inbred testerom. Potomci su testirani u dve gustine (10.428 i 90.533 biljaka ha^{-1}).

Najboljih pet potomaka iz obe gustine uzeti su za formiranje sledećeg ciklusa selekcije. Rezultati nakon prvog ciklusa selekcije pokazuju da je moguće stvoriti hibride tolerantne na gustinu gajenja.

DEVELOPING MAIZE HYBRIDS TOLERANT TO DIFFERENT PLANT DENSITIES

Yield of modern maize (*Zea mays L.*) hybrids depends heavily on high plant densities. At lower plant densities, differences between older and newer hybrids were small, becoming greater as plant density increased. In Serbia and Montenegro farmers have always favored density independent hybrids because of very common drought, seeding cost, lodging, diseases etc.

The objective of this study was to determine the possibility to develop density independent maize hybrids trough recurrent half – sib selection with inbred tester. Five elite inbred lines were chosen to form cycle 0. Plants were selfed and crossed to opposite elite inbred tester. Progenies were tested at two densities (10.428 and 90.533 plants ha^{-1}).

Best five progenies from both densities were taken to form next cycle. Results after one cycle of recurrent selection show that is possible to develop density tolerant maize hybrids.

GENETIČKA DIVERGENTNOST EKSPERIMENTALNIH HIBRIDA SUNCOKRETA

D. Jovanović, R. Marinković i D. Škorić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Povećanje prinosa semena je glavni cilj u programima oplemenjivanja suncokreta. Određivanje i poznavanje genetičke varijabilnosti olakšava izbor superiornih hibridnih kombinacija. Svake godine ulaze se veliki napor u pronaalaženju najboljih hibrida između velikog broja testiranih. Prinos i kvalitet semena analiziran je kod 114 eksperimentalnih hibrida suncokreta nastalih ukrštanjem inbred linija različitog genetičkog porekla. Kontrole su bili standardni NS hibridi suncokreta (Krajišnik, Velja i NS-H-111). Ocena hibrida urađena je na osnovu prinosa i sledećih kvalitetnih svojstava semena: sadržaj ulja i proteina u semenu, masa 1000 semena i sadržaj ljuske. U cilju sagledavanja odnosa između eksperimentalnih i standardnih hibrida suncokreta, kao mera sličnosti korišćena je kvadrirana Mahalanobisova mera odstojanja. Prosečan prinos semena kontrole i eksperimentalnih hibrida suncokreta varirao je od 3075 do 3712 kg ha^{-1} .

GENETIC DIVERGENCE OF EXPERIMENTAL SUNFLOWER HYBRIDS

Increase in seed yield is the main objective of sunflower breeding programs. Determination and knowledge of genetic variability facilitate the selection of superior experimental hybrids. In breeding programs, much effort is expended each year to find superior hybrids among a large number of tested. Analyzed in the present study was the yield and quality of seed of 114 experimental sunflower hybrids developed by crossing inbred lines of different genetic origin. The standard NS hybrids (Krajišnik, Velja and NS-H-111) were used as controls. The hybrids were evaluated based on seed yield and the followingÂ quality seed traits: oil and protein content in seeds, 1000-seed mass, hull contents and kernel to hull ratio. Squared Mahalanobis distance was used as the similarity measure among the standard and experimental sunflowers in the study. Average yield of kontral and experimental hybrids of sunflower varied from 3075 to 3712 kg ha^{-1} .

PROMENLJIVOST BROJA I MORFOLOGIJE HROMOZOMA I OPLEMENJIVANJE GOLOSEMENICA

Mirjana Šijačić-Nikolić, A. Tucović i Dragica Vilotić
Šumarski fakultet, Beograd

Rezultati obavljenih citogenetskih analiza vrsta golosemenica, prikazuju da četinare karakteriše evolucija na diploidnom nivou, što svedoči o stabilnosti kariotipova i idiograma ove grupe drveća i žbunja. Kod golosemenica, prirodno visok osnovni broj hromozoma poznat je samo za tri od 359 vrsta. Golosemenice su grupe drveća, redje žbunja sa relativno krupnim hromozomima sličnim kariotipovima i idiogramima. Osnovna pažnja usmerena je na unutarvrsnu citogenetsku diferencijaciju politipskih vrsta (13%) u odnosu na osnovni broj, kao značajnu osnovu za bržu determinaciju homologih hromozoma, izradu kariotipova, idiograma i kartiranje gena u kombinaciji sa različitim tipovima ukrštanja odabralih individua. Uočene specifične unutarvrsne karakteristike (različiti osnovni broj hromozoma, aneuploidiji, dopunski B-hromozomi, pojedinačna pojava poliploidije, prvenstveno na mlađim oglednim biljkama) nisu unikalne jer su opisane kod veoma udaljenih rodova. Uverenje o evolucionoj stabilnosti osnovnih diploidnih brojeva hromozoma protivreče, upravo, specifične citogenetske karakteristike 47 tzv. politipskih (polimorfnih) vrsta golosemenica.

Prema evidentiranim tipovima vrsta golosemenice (monotipske i politipske po osnovnim brojevima hromozoma) karakteriše drevna (spora) evolucija hromozoma, kariotipova i idiograma, za razliku od vrsta skrivenosemenica koju karakteriše manje-više brza citogenetska evolucija. Citogenetska, kariološka i druga istraživanja rodova i vrsta osetno olakšavaju oplemenjivanje ekonomski značajnih vrsta golosemenica.

VARIABILITY OF CHROMOSOME NUMBER AND MORPHOLOGY AND IMPROVEMENT OF GYMNOSPERMS

The results of the published cytogenetic analyses of gymnosperm species show that conifers are characterised by the evolution at a diploid level, which proves the stability of karyotypes and idiograms of this tree and shrub group. In gymnosperms, the naturally high basic number of chromosomes is known only in three of 359 species. Gymnosperms are groups of trees, more rarely shrubs, with relatively large chromosomes similar to karyotypes and idiograms. The attention is focused to intraspecific cytogenetic differentiation of polytypic species (13%) compared to the basic number. This is a significant base of a faster determination of homologous chromosomes, development of karyotypes, idiograms and gene mapping in combination with different types of crossing of the selected individuals. The specific intraspecies characteristics (different basic number of chromosomes, aneuploidy, accessory B-chromosomes, individual occurrence of polyploidy, primarily in younger test plants) are not unique since they are described in very distant genera. The belief on evolution stability of the basic diploid chromosome numbers is contradicted by the specific cytogenetic characteristics of 47 so-called polytypic (polymorphic) species of gymnosperms.

Based on the recorded types, gymnosperm species (monotype and polytypic by the basic numbers of chromosomes) are characterised by the ancestral (slow) evolution of chromosomes, karyotypes, idiograms, while angiosperm species are characterised by more or less fast cytogenetic evolution. The cytogenetic, karyologic and other study of genera and species support the breeding of economically significant species of gymnosperms.

GENETIČKI PARAMETRI PRINOSA I MORFOLOŠKIH OSOBINA PLODA I KOŠTICE KAJSIJE

Evica Mratinić, Vera Rakonjac i D. Milatović
Poljoprivredni fakultet, Beograd

Tokom četiri godine, kod 24 sorte kajsije, utvrđeni su prinos, masa i dimenzije (visina, širina i debljina) ploda i koštice. Ogleđ je postavljen na OD «Radmilovac» Poljoprivrednog fakulteta po potpuno slučajnom planu u tri ponavljanja. Rezultati analize varijanse su pokazali da je varijabilnost svih proučavanih osobina bila veoma značajno uslovljena i genetičkim i ekološkim faktorima, kao i njihovom interakcijom. Analizom komponenti ukupne varijabilnosti ustanovljeno je da su genetičke razlike među sortama kajsije u najvećem procentu uslovile varijabilnost mase koštice ($S^2_g=47\%$), visine ploda ($S^2_g=45\%$), visine koštice ($S^2_g=55\%$), širine koštice ($S^2_g=62\%$) i debljine koštice ($S^2_g=4\%$), dok je variranje prinosa ($S^2_y=70\%$), mase ploda ($S^2_y=44\%$), širine ploda ($S^2_y=43\%$) i debljine ploda ($S^2_y=49\%$) bilo u najvećem procentu uslovljeno ekološkim faktorima. Vrednosti koeficijenata heritabilnosti u širem smislu bile su relativno visoke za masu i dimenzije ploda i koštice ($h^2=88,5-93,0\%$), a srednja za prinos ($h^2=48,3\%$). Korelacionom analizom ustanovljeno je da su svi koeficijenti genetičke i fenotipske korelacije, između mase i dimenzija ploda i koštice, bili pozitivni i statistički veoma značajni. Prinos je bio u veoma značajnoj, pozitivnoj, genetičkoj korelaciji sa većinom proučavanih osobina, osim sa širinom ploda. Kao posledica uticaja faktora spoljašnje sredine, genetička korelacija prinosa sa većinom osobina ploda i koštice nije se ispoljila na fenotipu, jer fenotipski koeficijenti korelacije između ovih osobina nisu bili statistički značajni.

GENETIC PARAMETERS OF YIELD AND MORPHOLOGICAL FRUIT AND STONE PROPERTIES IN APRICOT

During four years, in 24 apricot cultivars, yield, fruit and stone weight and dimensions (height, breadth and thickness) were studied. The experiment was established in Experimental estate of Faculty of Agriculture «Radmilovac» as completely random plan in three repetitions. The results of analysis of variance showed that the variability of all studied properties very significantly was determined by both genetic and ecological factors, as well as their interaction. By the results of analysis of the components of total variance it was found out that genetic differences between cultivars in the highest percentage determined variability of stone weight ($S^2_g=47\%$), fruit height ($S^2_g=45\%$), stone height ($S^2_g=55\%$), stone breadth ($S^2_g=62\%$) and stone thickness ($S^2_g=54\%$), whereas the variation of yield ($S^2_y=70\%$), fruit weight ($S^2_y=44\%$), fruit breadth ($S^2_y=43\%$) and fruit thickness ($S^2_y=48\%$) was determined by ecological factors. The values of coefficients of heritability in a broader sense were found to be relatively high for the fruit and stone weight and dimensions ($h^2=88.5-93.0\%$), and medium for the yield ($h^2=48.3\%$). By the means of correlation analysis it was established that all genetic and phenotypic coefficients of correlation between fruit and stone weight as well as fruit and stone dimensions were positive and statistically very significant. The yield was in very significant, positive correlation with the majority of studied properties except of fruit breadth. Under the influence of environmental factors, genetic correlation between yield and the majority of fruit and stone properties did not appear on phenotype, because phenotypic coefficients of correlation between that properties were not statistically significant.

VARIJABILNOST SVOJSTAVA OŽILJAVANJA REZNICA CRNIH TOPOLA U USLOVIMA VODENE KULTURE

B. Kovačević¹, V. Guzina¹, Marija Kraljević-Balalić² i M. Ivanović³

¹IRC Institut za topolarstvo i vanšumsko zelenilo, Poljoprivredni fakultet, Novi Sad

²Poljoprivredni fakultet, Novi Sad

³Naučni institut za ratarstvo i povrtarstvo, Novi Sad

Na osnovu trogodišnjih ogleda u polu-kontrolisanim uslovima u vodenoj kulturi ($t = 25 \pm 2^{\circ}\text{C}$ i 16/8h režim dan / noć) sa 13 genotipova američke crne topole, 3 genotipa eurameričke topole i 2 genotipa iz familija u polusrodstvu američke crne topole, ispitana je varijabilnost 45 merenih i izvedenih morfometrijskih, fenoloških i masenih svojstava ožiljavanja reznica. Ogledi su postavljeni u četiri roka tokom perioda mirovanja: druga polovina decembra, druga polovina januara, kraj februara i sredina marta. Uticaj genotipa je bio značajan za svojstva korenčića u drugom i četvrtom roku, za svojstva izbojka, pozicije korenčića i fenološke parametre u trećem roku, a za masena svojstva u prva tri roka. Uticaj godine je bio značajan za svojstva korena u drugom i četvrtom roku, izbojka u drugom i trećem roku, za fenološka svojstva u svim osim u trećem roku, a za svojstva mase u četvrtom roku. Interakcija je značajna za većinu svojstava korenčića u prva tri roka, u četvrtom za masena i svojstva korenčića rane, za parametre izbojka u prvom, a za fenološka svojstva u trećem roku. Jasne razlike u varijabilnosti ispitivanih svojstava tokom perioda mirovanja upućuju na značaj roka postavljanja selekcionih testova u vodenoj kulturi. Heritabilnosti u širem smislu, ispitane na osnovu genotipova američke crne topole, ukazuju na značaj drugog i četvrtog roka. Za potpunu ocenu značaja pojedinih rokova i svojstava u oplemenjivačkom procesu, neophodno je proučiti i njihovu vezu sa preživljavanjem reznica u poljskim uslovima.

VARIABILITY OF CHARACTERS OF CUTTING ROOTING IN BLACK POPLARS IN THE CONDITIONS OF WATER CULTURE

At the base of three-annual exams in the semi-controloled conditions of water culture ($t = 25 \pm 2^{\circ}\text{C}$ and 16/8h day/night) with 13 genotypes of *Populus deltoides*, 3 genotypes of *Populus x euramericana* and 2 genotypes from half-sib families of *Populus deltoides* the variability of 45 measured and derived morphometric, phenology and dry-mass character was examined. The experiments were established in four terms during the period of dormancy: second half of December, second half of January, the end of February and middle of March. The influence of genotype was considerable for root characters in second and fourth term, for shoot characters, characters of roots position and phenology in third, and for mass characters in first three terms. The influence of year on variation was considerable in second and fourth term for root characters, in second and third term for shoot characters and in third term for characters of phenology. The influence of genotype x year interaction for root characters in first three terms, in fourth for the mass and characters of roots on basal cut, while for shoot characters in first term. Clear differences in influence of examined sources of variation among examined terms suggest the importance of proper time of establishment for selection tests in water culture. The heritabilities in broad sense, examined for genotypes of *Populus deltoides*, favourise second and fourth term. For complete evaluation of the significance of terms and characters for their use in breeding process it is necessary to examine thei relationship with the survival of cuttings in field condititions.

GENETIČKA DIVERGENTNOST KLONOVA OBLAČINSKE VIŠNJE (*Prunus cerasus L.*)

D. Nikolić, Vera Rakonjac, M. Milutinović i Milica Fotirić

Poljoprivredni fakultet, Beograd-Zemun

Oblačinska višnja predstavlja sortu populaciju koja obuhvata veliki broj formi različitih po bujnosti, rodnosti, vremenu sazrevanja, krupnoći, boji i kvalitetu plodova. Pošto Oblačinska višnja nije jedan klon (genotip) već smeša velikog broja klonova (genotipova) nameće se potreba izdvajanja genetički divergentnih formi za eksploraciju i dalji oplemenjivački rad. U ovom radu prikazani su rezultati analize 10 selekcionisanih klonova Oblačinske višnje. Ogled je izveden na OD «Radmilovac» Poljoprivrednog fakulteta u Beogradu. Tokom trogodišnjeg perioda istraživanja analizirane su sledeće osobine: vreme sazrevanja, prinos, masa ploda, masa koštice, randman, dužina peteljke ploda, sadržaj rastvorljivih suvih materija, sadržaj ukupnih kiselina, sadržaj ukupnih šećera i sadržaj invertnih šećera. Genetička divergentnost između proučavanih klonova ustanovljena je primenom hijerarhijske klaster analize. Korišćen je UPGA metod, pri čemu je razlika između grupa izražena preko Euklideanovog rastojanja. Na dobijenom dendrogramu izdvojeno je četiri grupe srodnih klonova. Razdvajanje klonova u grupe bilo je prvenstveno u funkciji njihovog prinosa, mada su uticaj na razdvajanje imale i ostale proučavane osobine.

GENETIC DIVERGENCE OF OBLAČINSKA SOUR CHERRY CLONES (*Prunus cerasus L.*)

Oblačinska sour cherry represents the population of a cultivar encompassing a great number of forms different on vigor, yielding, ripening time, size, color and quality of fruits. The need arises to separate the genetically divergent forms for further exploitation and breeding considering the fact that Oblačinska sour cherry is actually a mixture of a great number of clones (genotypes). The results of the analysis of 10 selected clones of Oblačinska sour cherry are given in this paper. The experiment was conducted at the Experimental Station «Radmilovac» of the Faculty of Agriculture in Belgrade. A three-year trial was carried out in order to test the following properties: ripening time, yield, fruit weight, stone weight, randman, stalk fruit length, soluble solid content, total acid content, total sugar content and invert sugar content. The genetic divergence between the analyzed clones was determined using the hierarchical cluster analysis. The UPGA method was used and the Euclidean distance in order to determine the difference between the groups. Four similar clone groups were obtained on the dendrogram. The objective of clone differentiation was primarily yield, although other properties were taken into account as well.

**MORFO-ANATOMSKA SVOJSTVA ČETINA SPONTANIH MUTANATA
BELOG BORA (*Pinus silvestris* L.) SA ŽUTIM ČETINAMA**Dragica Vilotić¹, M. Tošić² i Gordana Radošević¹¹Šumarski fakultet, Beograd²Dimitrija Tucovića 41, Užice

Borovi kod kojih je žuta boja uslovljena genetskom konstitucijom, poznati su kao «zlatni borovi» i odavno su predmet interesovanja, posebno stručnjaka za pejzažnu arhitekturu. U radu su prikazani uporedni rezultati morfoloških i anatomskih svojstava dvogodišnjih četina tri stabla belog bora sa žutim četinama na Zlatiboru: *P. silvestris* L. var. *zlatibirica* Omanović (1937), *P. silvestris* L. var. *aurea zlatiborensis* Tošić (1995) i novootkrivenog stabla sa osobinama himere (Tošić M. 2002) koje ima posebne grane sa zelenim, sa izrazito žutim i sa žuto-zelenim (panaširanim) četinama, kada je jedna strana četine zelena, a druga žuta po celoj svojoj dužini. Analizirani su: veličina, brojnost i boja četina, kao i: broj slojeva hipodermalnih ćelija i broj njihovih grupacija, slojevi sklerenhimskih ćelija u zoni sprovodnih snopića i broj smolnih kanala. Četine proučavanih mutanata bitno se razlikuju po svojim morfološkim osobinama i anatomskoj gradi, kako od tipičnog varijeteta belog bora, tako i međusobno. Proučavanje spontanih mutanata šumskog drveća, kao i ovih «zlatnih borova» zaslužuje punu pažnju, jer su prirodne retkosti sa specifičnim osobinama, koje ih čine atraktivnim za korišćenje u hortikulturi. U cilju trajnog očuvanja i proizvodnje ukrasnih kultivara, njihovi genotipovi su fiksirani heterovegetativnim razmnožavanjem.

**MORPHO-ANATOMIC PROPERTIES OF THE NEEDLES OF SCOTS PINE
(*Pinus silvestris* L.) SPONTANEOUS MUTANTS WITH YELLOW NEEDLES**

The pines in which yellow colour is conditioned by genetic constitution, are known as «golden pines» and they have been subjects of interest, especially of landscape architects, for a long time. This paper presents the comparative results of morphological and anatomical characters of second-year needles of three Scots pines with yellow needles on Mt. Zlatibor: *P. silvestris* L. var. *zlatibirica* Omanović (1937), *P. silvestris* L. var. *aurea zlatiborensis* Tošić (1995) and the newly discovered tree with chimera characteristics (Tošić M., 2002), with individual branches with green, with explicitly yellow and with yellow-green needles, when one side of the needle is green, and the other yellow, along the whole length. The analysed characters are: needle size, number and colour, number of layers of hypodermic cells and number of their groups, layers of sclerenchymous cells in the vascular bundle zone and the number of resin channels. The needles of the study mutants differ essentially by their morphological characters and anatomic structure, both from the typical Scots pine variety, and among themselves. The study of spontaneous mutants of forest trees, and also these «golden pines» deserves full attention, because they are natural rarities with specific characteristics, which make them attractive for amenity horticulture. In the aim of permanent conservation and production of ornamental cultivars, their genotypes have been fixed by heterovegetative reproduction.

**KORELACIONA POVEZANOST PRINOSA I KVALITETA
PLODA BRESKVE SA NEKIM FENOLOŠKIH FAZAMA**

Vera Rakonjac

Poljoprivredni fakultet, Beograd-Zemun

Vreme sazrevanja sorti breskve koje se gaje u našoj zemlji proteže se gotovo na četiri meseca. I pored toga, jedan od važnih ciljeva u oplemenjivanju ove vrste voćaka je stvaranje sorti veoma ranog ili veoma kasnog vremena sazrevanja. Dosadašnje iskustvo pokazalo je da se sorte breskve ekstremne po vremenu sazrevanja odlikuju slabijim kvalitetom plodova. Upravo stoga, cilj ovog rada je bio da se utvrde relacije i priroda zavisnosti vremena cvetanja ili vremena sazrevanja s jedne strane i prinosa, komponenti prinosa (broj zametnutih i broj ubranih plodova, masa ploda i masa koštice) i hemijskog sastava plodova (sadržaj ukupnih i rastvorljivih suvih materija, sadržaj ukupnih invertnih i redukujućih šećera i sadržaj ukupnih kiselina) s druge strane. Kao materijal korišćeno je 20 sorti breskve različitog vremena sazrevanja. Povezanost osobina utvrđena je na osnovu vrednosti koeficijenata genetičke i fenotipske korelacije. Koeficijenti genetičke i fenotipske korelacije izračunatu su na osnovu rezultata analize kovarianse, gde su godine uzete kao ponavljanje. Ustanovljeno je da je vreme cvetanja bilo pozitivno i veoma značajno genetički i fenotipski korelisano sa brojem zametnutih plodova ($r_g=0.90^{**}$; $r_f=0.75^{**}$) i brojem ubranih plodova ($r_g=0.87^{**}$; $r_f=0.67^{**}$). Postojanje značajnih genetičkih i fenotipskih korelacija između vremena sazrevanja i mase ploda ($r_g=0.66^{**}$; $r_f=0.60^{**}$), vremena sazrevanja i sadržaja ukupnih suvih materija ($r_g=0.75^{**}$; $r_f=0.59^{**}$), vremena sazrevanja i sadržaja rastvorljivih suvih materija ($r_g=0.89^{**}$; $r_f=0.56^{**}$) predstavlja teškoću u stvaranju sorti breskve ranog vremena zrenja sa poboljšanim kvalitetom ploda.

**CORRELATIVE RELATION OF YIELD AND FRUIT QUALITY
WITH SOME PHENOLOGICAL PHASES IN PEACH**

Ripening time of peach cultivars that are grown up in our country is extended over a time period of almost four months. In addition to that, one of more important objectives of the peach breeding is creation of the cultivars having either very early or very late ripening time. Our so far experience is that the peach cultivars which ripening time is in the extremes is of lower fruit quality. The aim of this study, therefore, was to determine relationship and the nature of the dependence between flowering time or ripening time from one side and yield, yield components (initial and final fruit set, fruit weight and stone weight) and chemical composition of fruit (content of total dry matter, content of soluble solids, content of total, invert and reduced sugar and content of total acid) on the other side. Twenty peach cultivars each having a different ripening time were using as material. The relationship between characteristics was determined on the basis of values of genetic and phenotypic correlation coefficients. Genetic and phenotypic coefficients of correlation were calculated based on the results of analysis of covariance where the years considered as replications. It was confirmed that flowering time was positive genetic and phenotypic correlated with initial fruit set ($r_g=0.90^{**}$; $r_f=0.75^{**}$), and with final fruit set ($r_g=0.87^{**}$; $r_f=0.67^{**}$). The existence of significant genetic and phenotypic correlation between ripening time and fruit weight ($r_g=0.66^{**}$; $r_f=0.60^{**}$), between ripening time and dry matter content ($r_g=0.75^{**}$; $r_f=0.59^{**}$) and between ripening time and soluble solid content ($r_g=0.89^{**}$; $r_f=0.56^{**}$) represented difficulty in creation of new peach cultivars of early ripening time and improved fruit quality.

REZULTATI ČETRNAESTOGODIŠNJE PROVENIJENIČNOG TESTA SMRČE U SRBIJI

V. Ivetić, V. Isajev i M. Šijačić-Nikolić
Šumarski fakultet, Beograd

Rad predstavlja kontinuitet analiza svojstava osam odabranih provenijencija smrče (*Picea abies* /L./ Karst.). Test je osnovan od tri provenijencije ove vrste iz Slovenije – Mašun, Menina i Jelovica; i pet provenijencija iz Srbije – Golija, Zlatar, Čemerno, Radočelo i Kopaonik. Monokulture kod Ivanjice, osnovane su sadnjom četvorogodišnjih sadnica na tri lokaliteta, nadmorske visine od 600, 1100 i 1600 m. Istraživanja koja su u njima sprovedena imala su za cilj proučavanje diferencijalnih svojstava i varijabilnosti smrče u delu njenog prirodnog rasprostranjenja na jugoistoku Evrope, upoznavanje proizvodnih sposobnosti i razlika između pojedinih provenijencija u manje-više istim i različitim ekološkim uslovima u Srbiji.

Ovim radom obuhvaćeno je više morfometrijskih svojstava juvenilnih biljaka, za svaku godinu starosti, od 7. do 14. godine. Merene su visine i visinski prirast na godišnjem nivou, prečnici i prirast prečnika biljaka u korenovom vratu i broj grana. Primenom standardnih statističkih metoda, dobijeni rezultati metričkih svojstava su analizirani.

Obavljena komaprativna istraživanja sa osam provenijencija, pokazala su da se provenijencije Golija i Zlatar odlikuju izraženom adaptabilnošću i dinamikom rasta, te bi ih u budućim aktivnostima na osnivanju kultura ili pri pošumljavanju goleti trebalo više koristiti.

RESULTS OF FOURTHEEN YEARS OLD NORWAY SPRUCE PROVENENCE TEST IN SERBIA

This study is the continued analyses of eight selected spruce (*Picea abies* /L./ Karst.) provenances. The test consists of three provenances of this species from Slovenia – Mašun, Menina and Jelovica; and five provenances from Serbia – Golija, Zlatar, Čemerno, Radočelo and Kopaonik. The monocultures near Ivanjica were established by planting four-year old seedlings at three localities, at the altitudes of 600, 1100 and 1600 m. The aim of the research was to identify the spruce differential characters and variability in the part of its natural range in the south-east Europe, to study the production capacity and the differences between individual provenances in more or less identical and different ecological conditions in Serbia.

This study includes several morphometric characters of juvenile plants for 14 years of age. The measured characters are height and height increment, root collar diameter and diameter increment. The study results of metric properties were analysed by standard statistical methods.

PROMENE U HROMOZOMIMA GOVEDA NA FARMAMA U SRBIJI

Slavica Košarčić¹, A. Kovacs², D. Košarčić³, Ljiljana Suvajdžić¹, Mira Kovačević¹,
M. Kapetanov¹ i Dubravka Milanov¹

¹Institut za veterinarstvo, Novi Sad

²Research Institute for Animal Breeding and Nutrition, Herceghalom, Hungary

³Institut za reprodukciju i veštačko osemenjavanje domaćih životinja, Temerin

U radu su prezentovana petogodišnja istraživanja numeričkih i strukturnih promena u kariotipu goveda na pet farmi i dva centra za reprodukciju u Srbiji. Analizirana je 371 priplodna životinja i to 215 muških i 156 ženskih grla, od toga, Holstein Friesian rase 267 (193 m i 74 ž), Simmental 62 (17 m i 45 ž) i Grei Steppe rase 42 (5 m i 37 ž). Korišćen je uobičajen metod za uspostavljanje kulture limfocita i analizu kariotipa prema međunarodnim standardima za karotipizaciju domaćih životinja. Cilj ovih istraživanja je provera genetičkog materijala na hromozomskom nivou grla, koja se uvode u reprodukciju. Otkrivene su sledeće promene u kariotipu: 6 grla sa himerizmom $2n=60XX/XY$ Holstein Friesian rase i jedna Robertsonova translokacija u Simmental-skoj rasi. Strukturne promene tipa prekida i ringa otkrivene su kod dve životinje Holstein Friesian i Simmental-ske rase, koje su bile u zoni dejstava za vreme bombardovanja Srbije. Sva grla Grei Steppe rase imala su normalan kariotip. Ukupno je 9 životinja sa promenama ili 3 %, dok normalan kariotip imaju 362 ili 97 %. Prema dobijenim rezultatima možemo zaključiti da je neophodno nastaviti citogenetičku attestaciju priplodnih životinja iz ekonomskih razloga i u cilju zaštite genofonda na farmama goveda.

CHROMOSOME CHANGES IN CATTLE ON THE FARMS IN SERBIA

In the paper we presented five-year investigations of numeric and structural changes in cattle karyotype on five farms and two centers for reproduction in Serbia. There were 371 breeding animals (215 male and 156 female), out of which 267 Holstein Friesian breed (193 male and 74 female), 62 Simmental (17 male and 45 female) and 42 Grey Steppe breed (5 male and 37 female). Cultivating of lymphocytes and karyotype analyses, according to the international standards for karyotypization of domestic animals, were applied. The aim of the investigation was to test genetic material on chromosome level of animals introduced into reproduction. The following changes were discovered in the karyotype: 6 animals of Holstein-Friesian breed were with chimeras $2n=60XX/XY$ and there was one Robertson's translocation in Simmental breed. Structural changes as breakage and a ring were discovered in two animals of Holstein-Friesian and Simmental breed raised in the area of bombing in Serbia. The animals of Grey Steppe breed had normal karyotype. There is a total of 9 animals with changes (3 %), while 362 were with normal karyotype, total (97%). According to the results, it may be concluded that cytogenetical attestation of the breeding animals will have to be continued for the purpose of protecting the genofond on cattle farms.

HERITABILNOST HIGIJENSKOG PONAŠANJA SJENIČKO-PEŠTERSKOG EKOGENOTIPA MEDONOSNE PČELE (*Apis mellifera carnica*)

Z. Stanimirović¹, Jevrosima Stevanović¹ i D. Ćirković²

¹Katedra za biologiju, Fakultet veterinarske medicine, Beograd

²Ministarstvo poljoprivrede i vodoprivrede Republike Srbije, Okrug Novi Pazar

Obavljeno je istraživanje higijenskog ponašanja autohtonog sjeničko-pešterskog ekogenotipa medonosne pčele *Apis mellifera carnica* na 11 lokaliteta područja Sjeničko-pešterske visoravni, Podpešterja, istočnih padina Golije i jugozapadnih padina Rogozne. Potencijal higijenskog ponašanja praćen je kod 440 pčelinjih zajednica primenom modifikovane pin-killed tehnike koju su postavili Kefuss i sar. (1996) a modifikovali Stanimirović i sar. (2001). Rezultati su ukazali na veliku interpopulacijsku varijabilnost potencijala higijenskog ponašanja koji se kretao u opsegu od 81.24% do 99.50% zavisno od jačine društva i starosti matica. Odabrano je 8 matica iz najboljih pčelinjih zajednica koje su služile kao izvorni materijal u daljem programu selekcije medonosne pčele u odnosu na higijensko ponašanje. Heritabilnost je praćena u tri generacije (baba, majka, unuka) kod ukupno 512 matica i njihovih zajednica. Izračunavanje heritabiliteta obavljeno je metodom regresije roditelj-potomak. Dobijeni rezultati ukazuju na činjenicu da je higijensko ponašanje kod sjeničko-pešterskog ekogenotipa medonosne pčele visoke heritabilnosti $h^2 = 0.61$. Ovi naši nalazi u skladu su sa rezultatima Harbo-a (1995) koji su ukazali da heritabilnost za ovo svojstvo dostiže vrednost od $h^2 = 0.65$, uz napomenu da je sposobnost otklapanja i uklanjanja lutki iz otklopljenog legla, prema Milne-u (1985) niže heritabilnosti, $h^2 = 0.144$ za otklanjanje, odnosno $h^2 = 0.022$ za uklanjanje lutki iz otklopljenih ćelija legla. Naši rezultati ukazuju na opravdanost sprovođenja daljih mera selekcije matica na higijensko ponašanje obzirom na visoki genetski stepen determinacije ovog svojstva.

HERITABILITY OF HYGIENIC BEHAVIOUR OF SYENICHKO-PESHTERSKI HONEY BEE ECOTYPE (*Apis mellifera carnica*)

Hygienic behaviour of indigenous Syenichko-Peshterski honey bee ecogenotype *Apis mellifera carnica* was investigated at 11 localities on the territory of Syenichko-Peshterski plateau, Podpeshterje, Golija Mt. and Rogozna Mt. Potential of hygienic behaviour was monitored in 440 honey bee colonies using pin-killed technique established by Kefuss et al. (1996), modified by Stanimirović i sar. (2001). The obtained results revealed great interpopulation variability of hygienic behaviour ranged from 81.24% to 99.50%, depending on colony strength and queen age. Eight queens were selected from best colonies which served as source material in further honey bee selection program regarding hygienic behaviour. Heritability was monitored in three generations (grandmother, mother, granddaughter) in 512 queens and their colonies. Heritability was calculated using regression method parent-offspring. The results of the study pointed at great heritability of hygienic behaviour in Syenichko-Peshterski honey bee ecogenotype $h^2 = 0.61$. Such results are in accordance with those of Harbo (1995) which revealed heritability for monitored behaviour $h^2 = 0.65$, with remark that Milne (1985) found lower heritability for ability of uncapping and removal of pupae from uncapped cells, $h^2 = 0.144$ for uncapping and $h^2 = 0.022$ for removal of pupae from uncapped cells. Having in mind high degree of genetic determination of hygienic behaviour, our results signify justification for performing further selection procedures of honey bee queens regarding investigated quality.

NANOMOLEKULSKI PREKIDAČI I STARENJE SEMENA HIBRIDA KUKURUZA I NJIHOVIH RODITELJSKIH KOMPONENTI

V. Dragičević¹, S. Sredojević¹, M. Spasić², M. Srebrić¹, M. Ivanović³ i M. Vrvić²

¹Institut za kukruz «Zemun Polje», Zemun Polje

²Hemijski fakultet, Beograd

³Institut za ratarstvo i povrtarstvo, Novi Sad

Od presudnog značaja za održanje životnih funkcija živih sistema je postojanje nanomolekulskih prekidača koji modulišu njihov redoks ekvilibrijum. Bazu tog mehanizma čine jedinjenja tipa proteinskih sulfhidril (PSH) i glutationa (GSH), kao i njihovi oksidovani oblici (PSSP i GSSG). Praćenjem promena u koncentracijama navedenih jedinjenja tokom ubrzanog starenja semena dva hibrida kukuruza iz grupe zrenja 500: ZP SC 580-H1 i ZP SC 504_{su}-H2 i njihovih roditeljskih komponenti: ZP PL 175 (♀)-L1, ZP PL 188 (♂)-L2 i ZP PL 51 (♀)-L3, ZP PL 67 (♂)-L4, konstatovano je postojanje dva tipa nanomolekulskih prekidača: tip I, koji paralelno uključuje oksidaciju GSH i PSH i tip II koji u ključuje oksidaciju GSH, uz povećanje nivoa PSH. Potrebno je naglasiti da su kod svih ispitivanih linija i hibrida bila prisutna oba tipa prekidača, s tim što je dominantnost svakog od njih bila karakteristika pojedinog hibrida. Kod H1 bio je prisutan pad nivoa GSH i PSH, uz povećanje GSSG, što je karakteristično za prekidač tipa I, dok je kod H2 bio prisutan pad nivoa GSH, uz povećanje GSSG i PSH, a što je karakteristično za prekidač tipa II. Kod L1 i L2 bio je dominantan tip I prekidača, kao kod H1, dok je kod L3 bio dominantan tip II prekidača, kao kod H2. Izneta razmatranja bi mogla da posluže za klasifikaciju genotipova prema dominantnom nanoprekidaču, uz otvaranje pitanja njihovog uticaja na mehanizme otpornosti.

THE NANOMOLECULAR SWITCHES AND AGEING OF MAIZE HYBRID SEEDS AND THEIR INBREDS

The vital property of living systems conditioning is connected to existence of nanomolecular switches, via modulation of redox equilibrium. The noted mechanism is based on presence of sulfhydryl proteins (PSH) and glutathione (GSH), together with their oxidised forms (PSSP and GSSG). The seeds of two maize hybrids (FAO 500): ZP SC 580 - H1 and ZP SC 504_{su} - H2 and their inbreds: ZP PL 175 (♀)-L1, ZP PL 188 (♂)-L2 and ZP PL 51 (♀)-L3, ZP PL 67 (♂)-L4, were subjected to accelerated ageing treatment up to 12 days. The changes in concentration of related compounds are evidence for existence of two types of nanomolecular switches: type I – includes oxidation of GSH and PSH and type II – includes oxidation of GSH, with increase of PSH level. It is important to underline that all examined hybrids and their inbreds had both types of nanoswitches, while genotypes differ by domination of one of them. There is decrease in GSH and PSH and raise in GSSG content in H1, what is characteristic for type I of switch, whereas the lower GSH, increased PSH and GSSG content in H2 is typical for type II. The dominance of type I of switch was obtained in L1 and L2, as in H1, but the domination of type II was achieved in L3, correspondingly to H2. The noted sentences could be used for classification of genotypes in mode of dominated nanoswitch with opening of question of its influence on resistance mechanisms.

IV tematska oblast / IV topic:

Oplemenjivanje organizama

Breeding of organisms

Posteri / Posters

IV-Pos

OSOBINE I VARIJABILNOST KLJAVACA ZELENE DUGLAZIJE
(*Pseudotsuga menziesii* (Mirb.) Franco)

N. Anastasijević, Mirjana Ocokoljić i Vesna Vratuša

Katedra za podizanje i održavanje zelenih površina, Šumarski fakultet, Beograd

Na osnovu komparativne morfofiziološke analize kljavaca *Pseudotsuga menziesii* (Mirb.) Franco, i to tri linije polusrodnika sa staništa šume sladuna i cera i tri linije polusrodnika sa staništa vrba i topola, iznose se podaci o osobinama i promenljivosti kljavaca ove gajene vrste. U radu se takođe konstatiše promenljivost svojstava kljavaca koja utiču na selekciju materinskih stabala i tehnologiju proizvodnje sadnog materijala sa željenim osobinama, za primenu u šumarstvu i pejzažnoj arhitekturi i hortikulturi.

PROPERTIES AND VARIABILITY OF DOUGLAS-FIR SEEDLINGS
(*Pseudotsuga menziesii* (Mirb.) Franco)

On the basis of comparative morphophysiological analysis of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings, three half-sib lines from *Quercetum farneto-cerris* sl. site, and three half-sib lines from *Saliceto-Populetum* sl. site, data regarding characteristics and variability of seedlings of this cultivated species are discussed. Paper also ascertains the variability of seedling properties that affect mother tree selection and production technology of plant material of desired characteristics for application in forestry and landscape architecture and horticulture.

**FENOTIPSKA I MOLEKULARNA EVALUACIJA
MIROVANJA SEMENA PŠENICE**R. Barjaktarović¹, B. Kobiljski², D. Obreht¹ i Lj. Vapa¹¹Prirodno-matematički fakultet, Novi Sad²Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Mirovanje semena je fiziološko svojstvo u čijoj ekspresiji učestvuje genotip i faktori spoljašnje sredine. Evaluacija nivoa mirovanja semena 78 genotipova pšenice izvršena je pomoću fenotipskih i molekularnih analiza. Materijal je poreklom iz genetičke kolekcije pšenice Naučnog instituta za ratarstvo i povrтарstvo u Novom Sadu. Tokom leta 2003. godine mirovanje semena ovih genotipova je procenjivano testovima germinacije i određivanjem broja padanja (*falling number*-FN). Boja zrna za sve genotipove je određena vizuelno. Ispitivana je varijabilnost mikrosatelitskog markera MR 26, koji se nalazi na 3D hromozomu u blizini lokusa za mirovanje semena. Utvrđeno je prisustvo šest alela (označenih kao: a, b, c, d, e, g). Urađena je korelacija između rezultata dobijenih putem testova germinacije, vrednosti broja padanja i boje zrna sa rezultatima dobijenim molekularnim analizama sa ciljem utvrđivanja moguće primene molekularnog markera u selekciji genotipova na svojstvo mirovanje semena.

FENOTYPIC AND MOLECULAR EVALUATION OF WHEAT DORMANCY

Dormancy is a physiological trait influenced by genotype and environmental conditions. Evaluation of the general level of dormancy in 78 wheat genotypes using phenotypic and molecular analyses was performed. The material was originated from Wheat Core Collection of the Institute of Field and Vegetable Crops, Novi Sad. During the summer of 2003 these genotypes were evaluated for dormancy using germination tests and falling number (FN) determination. Grain color of all genotypes was determined visually. Allelic variability of microsatellite MR 26, located on the chromosome 3D, which is close to the locus for seed dormancy, was analyzed. Six alleles were scored (designated as: a, b, c, d, e, g). Results from germination tests, falling number determination and evaluation of grain color were correlated with results of molecular analyses in order to estimate the microsatellite marker validity in marker assisted selection of seed dormancy.

PRODUKTIVNOST NEKIH GENOTIPOVA PŠENICE

M. Biberdžić¹, M. Đorđević², S. Barac¹, N. Deletić¹ i S. Stojković¹

¹Poljoprivredni fakultet Univerziteta u Prištini, Lešak

²Zavod za poljoprivredu, Leskovac

Pšenica je naša najvažnija biljna vrsta, a pšenično zrno osnovni elemenat ishrane ljudi. S obzirom na stalni rast ljudske populacije i potrebom za hranom, nameće se potreba za obezbeđenjem dovoljnih količina zrna ove biljne vrste. Prinosi koje sorte postižu su različiti i zavise od više faktora, a najviše od genotipa, agroklimatskih uslova i primenjene tehnologije proizvodnje. Stoga je cilj ovoga rada bio da se utvrdi produktivnost više genotipova pšenice u južnom delu Srbije, putem makroogleda. Tako je u 2000. i 2001. godini u makroogledu bilo uključeno 20 genotipova pšenice. Rezultati istraživanja pokazuju da su se prinosi znatno razlikovali po godinama i ta razlika je u proseku iznosila oko 1300 kg/ha. Prosečni dvogodišnji rezultati pokazuju da su najveće prinose ostvarile sorte Toplica, Stamena, Evropa, NS-rana 5 i Renesansa. Najniže prinose imale su sorte Tiha, Sara, Sreća i Mina.

PRODUCTIVITY OF SOME WINTER WHEAT GENOTYPES

Winter wheat is the most important plant species in our country, and wheat grain is a basic element of human diet. There is a need for sufficient supplies increase of human population. Yields given by different genotypes are affected by many factors, but mostly by genotype, climatic conditions and applied growing practice. Therefore, the aim of this study was to establish productivity of many winter wheat genotypes in southern Serbia, through macro trials. A set of 20 wheat genotypes was studied in 2000 and 2001. The results showed the difference of grain yield mean between the investigated seasons, and that difference amounted about 1300 kg/ha. The average yield was the highest in cultivars Toplica, Stamena, Evropa, NS-rana 5, and Renesansa. The lowest grain yield was given by cultivars Tiha, Sara, Sreća, and Mina.

**EKSPEKMENTALNO UTVRĐENA DUŽINA TRAJANJA, PROSEČNI I
MAKSIMALNI INTENZITET NALIVANJA ZRNA GENOTIPOVA PŠENICE
RAZLIČITE RANOSTASNOSTI**

Milka Brdar, Marija Kraljević-Balalić i B. Kobiljski

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Prinos zrna pšenice (*Triticum aestivum L.*) je delom uslovljen krajnjom masom suvog zrelog zrna, koja je u velikoj meri određena intenzitetom i dužinom trajanja procesa nalivanja zrna. Cilj istraživanja je poređenje eksperimentalno utvrđene krajnje mase suvog zrelog zrna 5 grupa genotipova pšenice različite ranostasnosti (ekstra rane, srednje rane, srednje kasne, kasne i kontrolna grupa najprinosnijih NS sorti) sa eksperimentalno utvrđenim prosečnim i maksimalnim intenzitetom, te dužinom trajanja nalivanja zrna u različitim uslovima sredine. Korelacioni koeficijenti su upotrebljeni u pokušaju da se odredi koji parametar nalivanja zrna ima veći uticaj na krajnju masu suvog zrelog zrna. U kod nas uobičajenim uslovima sredine (2002.) je krajnja masa suvog zrelog zrna bila u jakim pozitivnim vezama sa prosečnim i maksimalnim intenzitetom i u jakoj negativnoj vezi sa dužinom trajanja nalivanja zrna. Najveće krajnje mase suvih zrelih zrna postigli su srednje kasni i genotipovi kontrolne grupe. Korelacije između krajnje mase suvog zrelog zrna i dužine trajanja, te prosečnog intenziteta nalivanja zrna su bile značajno pozitivne u nepovoljnim uslovima sredine (2001.). Novosadske sorte i ekstra rani genotipovi su imali najveće mase suvih zrelih zrna. Intenzitet i dužina trajanja nalivanja zrna su obično u negativnoj korelaciji. Uticaj parametara nalivanja zrna na krajnju masu suvog zrelog zrna nije jednak u različitim uslovima sredine, tako da bi mogla biti bitnija sposobnost genotipa da u nepovoljnim uslovima sredine niske intenzitete nalivanja zrna nadomesti produženjem akumulacije suve materije. Za opisivanje ovih procesa bi eksperimentalno utvrđeni prosečni intenzitet nalivanja zrna mogao biti važniji parametar od maksimalnog.

**OBSERVED DURATION AND AVERAGE AND MAXIMUM GRAIN FILLING
RATES IN WHEAT GENOTYPES OF DIFFERENT EARLINESS**

Grain yields of wheat (*Triticum aestivum L.*) are influenced partly by final grain dry weight, which is largely determined by the rate and duration of the grain filling process. A study was undertaken to compare the observed final grain dry weight of five groups of wheat genotypes differing in earliness (extra early, medium early, medium late, late and a control group of high-yielding NS cultivars) with the observed duration and average and maximum rates of grain filling in two different environments. Correlation coefficients were used to determine which grain filling parameter had more influence on final grain dry weight. In an environment common for our country (2002), final grain dry weight was strongly positively correlated with the average and maximum rates and strongly negatively correlated with the duration of grain filling. The medium late and control groups had the highest final grain dry weights. Correlations between final grain dry weight and the duration and average rate of grain filling were positive in an unfavorable environment (2001). The NS cultivars and extra early genotypes had the highest final grain dry weights. The rate and duration of grain filling are usually negatively correlated. The influence of grain filling parameters on final grain dry weight is not the same in different environments, so the ability of the genotype to compensate for the low grain filling rate with grain fill prolongation in unfavorable environments might be more important. The observed average grain filling rate is probably more important as a parameter for describing these processes than the maximum one.

**UTICAJ VISOKOG INTENZITETA SELEKCIJE NA PROMENU
ADITIVNE VARIJANSE KOMPONENTI PRINOSA KUKRUZA**

N. Deletić, S. Stojković, M. Biberdžić, V. Đurić i S. Gudžić

Poljoprivredni fakultet Univerziteta u Prištini, Lešak

U radu je ispitivana 31 SSD linija iz *ZP-Syn-1 C₀* i 37 iz *ZP-Syn-1 C₃* populacije kukuruza. Nakon odabira i umnožavanja semena linija u 2000, ogledi su obavljeni u periodu 2001-2002 u Kruševcu i Zemun Polju, po RCB metodi sa tri ponavljanja. Nakon tri ciklusa rekurentne selekcije došlo je do značajnog sniženja srednje vrednosti homozigotnih potomstava za procenat poleglih i slomljenih biljaka, visinu biljke i klipa, ali i za broj redova zrna. Povećane su srednje vrednosti za broj zrna u redu i masu 1000 zrna. Razlike u dužini klipa i prinosu zrna nisu bile značajne. Nakon tri ciklusa je došlo do značajnog suženja aditivne varijabilnosti za procenat poleglih i slomljenih biljaka, dužinu klipa i broj redova zrna. Smanjenje vrednosti za ostala svojstva nije bilo statistički značajno. Utvrđene su i značajne vrednosti heritabilnosti u užem smislu.

**THE EFFECT OF A HIGH SELECTION INTENSITY ON THE CHANGE OF
MAIZE YIELD COMPONENTS' ADDITIVE VARIANCE**

A set of 31 SSD lines from *ZP-Syn-1 C₀* and 37 from *ZP-Syn-1 C₃* maize population was studied in this paper. After line selection and seed multiplication in 2000, the trials were carried out in 2001-2002, at Krusevac and Zemun Polje, in RCB design. After three cycles of recurrent selection we observed a significant decrease of homozygous progenies' means of root and stalk lodged plants percent, plant and ear height, but also of row number per ear. The means of grain number per ear and 1000 grain mass were increased, and the differences in ear lenght and grain yield were not significant. After three cycles, a significant narrowing of additive variance happened for root and stalk lodged plant percent, ear lenght, and row number per ear, but this narrowing was not significant for the other traits. We also found significant values of narrow-sense herutability.

**PHENOTYPE STABILITY OF YIELD COMPONENTS
OF PROTEIN SUNFLOWER**

Dijana Dijanović, Vesna Stanković i I. Mihajlović
IIP «SERBIA», Centar za poljoprivrednu i tehnološka istraživanja, Zaječar

The study analyses the phenotype stability of the components of three inbred lines of sunflower (*Helianthus annuus L.*) in the third, fourth and fifth year of self-pollination. The stability parameters have been counted by application the *Eberhart and Russel* (1966) model. According to the regression coefficient, the investigated lines showed satisfactory stability referring to the content of proteins in seed and 1000 seeds mass. The most stable line proved to be the D4441 line in the third year of self-pollination. The best genotype stability for the 1000 seeds mass was achieved by the line Rs 4 I 10 in the fourth year of self-pollination.

NASLEĐIVANJE VISINE STABLJIKE I DUŽINE KLASA KOD PŠENICE

Biljana Gorjanović i Marija Kraljević-Balalić
Poljoprivredni fakultet, Novi Sad

Izbor roditelja za ukrštanje je veoma važan zadatak u programima oplemenjivanja biljaka. Analiza kombinacionih sposobnosti je jedan od metoda koji se koriste za određivanje roditelja koji će u ukrštanju sa drugim roditeljima dati superiorno potomstvo. Koristeći linija x tester analizu (Singh i Choudhary, 1979) proučavane su kombinacione sposobnosti, efekti gena i način nasleđivanja visine stabljične i dužine klasa, koristeći pet majki, tri testera i 15 F₁ hibrida pšenice.

Analiza komponenti genetičke varijanse kao i odnos OKS/PKS ukazuju da su u nasleđivanju visine stabljične u prvoj godini i dužine klasa u drugoj godini najveći značaj imali aditivni geni. Neaditivni geni su preovlađivali u nasleđivanju visine stabljične u drugoj godini i dužine klasa u prvoj godini ispitivanja. Način nasleđivanja ispitivanih svojstava zavisio je od kombinacije ukrštanja i godine ispitivanja. U većini slučajeva nasleđivanje je bilo dominantno. Analizom opštih kombinacionih sposobnosti (OKS) može se zaključiti da je najbolji opšti kombinator za visinu stabljične u prvoj godini bila linija NS 31/96, dok je u drugoj godini najbolji kombinator bila sorta Fundulea 490. Za dužinu klasa najbolji opšti kombinator u obe godine ispitivanja bila je linija NS 31/96. U većini slučajeva hibridi sa najboljim posebnim kombinacionim sposobnostima (PKS) nastajali su ukrštanjem jednog dobrog i jednog lošeg opštег kombinatora. Linija NS 31/96 je generalno bila najbolji opšti kombinator zbog čega se preporučuje za korišćenje u programima oplemenjivanja pšenice.

INHERITANCE OF PLANT HEIGHT AND SPIKE LENGTH IN WHEAT

The choice of parents is a very important task in a breeding program. Combining ability studies are used by plant breeders to select parents with maximum potential of transmitting desirable genes to the progenies. Using the line x tester analysis (Sing and Choudhary, 1979), we studied the combining ability, gene effects and mode of inheritance of plant height and spike length, using 5 females, 3 testers and 15 F₁ hybrids of wheat.

Estimation of the genetic components of variation as well as ratio of GCA/SCA showed that plant height in the first year and spike length in the second year of research were predominantly controlled by additive gene action. Non-additive gene effects have been found to be more important than additive ones in the inheritance of plant height in the second year and spike length in the first year of research. The mode of inheritance of characters under study depended on the cross combination and the year of growing. In most cases the mode of inheritance was dominant. The estimates of general combining ability (GCA) pointed out that the best general combiner for the plant height in the first year was NS 31/96, while in the second year the best combiner was Fundulea 490. For the spike length the best general combiner in the both years was NS 31/96. In majority of the cases good specific combining ability (SCA) were usually associated with crosses of two genetically divergent parents having at least one parent as a good general combiner. The line NS 31/96 was the best general combiner in this research and it can be used in wheat breeding.

OCENA POGODNOSTI MONOGERMNIH LINIJA ŠEĆERNE REPE ZA STVARANJE HIBRIDA TOLERANTNIH PREMA RIZOMANIJI

N. Čačić, L. Kovačev, Snežana Mezei i Nevena Nagl

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Prvi podaci o prisustvu rizomanije u našoj zemlji datiraju iz 1971. godine i od tog vremena ovo obolenje se raširilo na čitavo proizvodno područje šećerne repe. Kao posledica širenja ovog, veoma destruktivnog, obolenja u proizvodnju su se uvodile sorte šećerne repe tolerantne prema rizomaniji, a u poslednje dve godine gaje se skoro stoprocentno tolerantne sorte. Imajući ovu činjenicu u vidu 2002. godine izvršili smo ukrštanje 250 monogermnih cms linija, koje ne poseduju gene tolerantnosti prema rizomaniji, sa multigermnom diplidnom populacijom koja poseduje rizor gen tolerantnosti prema ovom obolenju. Tokom 2003. godine na zemljisu zaraženom rizomanjom, izvršena je provera proizvodnih svojstava dobijenih hibridnih kombinacija. Za ispitivanja u 2004. godini odabранo je 40 hibridnih kombinacija stavljajući pri tome naglasak na sadržaj šećera i tehnološke karakteristike. U monogermne linije koje pokažu dobre kombinacione sposobnosti za najvažnija proizvodna svojstva uneće se holly odnosno wb gen otpornosti, a potom će se ove linije koristiti za stvaranje hibrida šećerne repe visoko tolerantnih prema rizomaniji i dobrih proizvodnih svojstava.

EVALUATION OF SUITABILITY OF MONOGERM SUGAR BEET LINES FOR THE DEVELOPMENT OF HYBRIDS TOLERANT TO RHIZOMANIA

The first reports of rhizomania in our country date back to 1971. Since then, the disease has spread to all sugar beet-growing areas of the country. In response to the spread of this highly destructive disease, sugar beet cultivars tolerant of rhizomania were introduced increasingly into commercial production, and in the last two years virtually all sugar beet cultivars grown commercially have tolerance to rhizomania. With this in mind, in 2002 we crossed 250 monogerm cms lines that have no genes for tolerance to rhizomania with a multigerm diploid population that has the Rizor gene for tolerance to this disease. During 2003, hybrid combinations obtained in this manner were evaluated for their production characteristics on rhizomania-infected soil. Forty of the combinations were then chosen for testing in 2004, with emphasis on sugar content and technological characteristics. Monogerm lines with good combining abilities for the most important production characteristics will be identified and the Holly and WB genes for rhizomania resistance will be incorporated into them. These lines will then be used to develop sugar beet hybrids that have high tolerance towards rhizomania and good production characteristics.

GENETIČKA KARAKTERIZACIJA RANIH HIBRIDA KUKURUZA PRIMENOM PROTEINSKIH I RAPD MARKERA

I. Erić, S. Mladenović-Drinić i K. Konstantinov

Institut za kukuruz «Zemun Polje», Zemun-Beograd

Poznavanje genetičke varijabilnosti germplazme kukuruza je važno zbog planiranja programa selekcije, konzervacije germplazme *per se*. Proizvođači kukuruza daju prednost uniformnosti useva koja se u većini slučajeva postiže gajenjem inbred linija i dvolinijskih hibrida. Genetička uniformnost uključuje rizik od velikih gubitaka u prinosima zbog izuzetne osetljivosti ovakvih useva na stresne faktore sredine. Ovaj rizik se može smanjiti gajenjem nesrodnih dvolinijskih hibrida. Zbog toga je postojanje informacije o genetičkoj varijabilnosti hibrida koji se gaje na poljima je takođe vrlo važno. U ovom radu ispitivali smo informativnost proteininskih i RAPD markera za genetičku karakterizaciju hibrida i utvrđivanje njihove genetičke srodnosti. Kao materijal je izabранo 11 ranih hibrida kukuruza FAO 100-200 sa najvišim prinosom koji su pogodni za gajenje u brdsko-planinskim područjima. Protein klice rastvorljivi u solima izolovani su i razdvojeni PAA elektroforezom. Amplifikacija genomske DNK ovih genotipova urađena je sa 15 RAPD prajmera. Dobijeni rezultati kao prisustvo/odsustvo proteinске frakcije ili RAPD fragmenta prevedeni su u binarni kod (1,0). Za izračunavanje koeficijenata sličnosti i UPGMA klaster metodu korišćen je NTSYS-pc programski paket. RAPD markeri su detektovали visok nivo polimorfizma među ispitivanim hibridima i informativniji su za procenu genetičke srodnosti od proteininskih markera.

GENETIC CHARACTERIZATION OF EARLY MATURING MAIZE HYBRIDS OBTAINED BY PROTEIN AND RAPD MARKERS

Knowledge on genetic diversity of maize germplasm is important for planning breeding programmes, germplasm conservation *per se* etc. The maize farmers prefer uniform crop fields, which in the majority of cases is attained by using inbred lines and single cross hybrids. This genetic uniformity may cause losses in yield because of genetic susceptibility of material to environmental stress factors. This risk may be reduced by the use of unrelated single cross hybrids, so information on genetic variability of maize hybrids is very important. In our study we examined usefulness of protein and RAPD markers for characterization of maize hybrids and evaluation of their genetic relatedness. 11 early maturing ZP maize hybrids from groups FAO 100-200 were chosen for the analysis on the basis of their superior yield. The salt soluble proteins from embryo tissue were isolated and separated by gel electrophoresis (SDS-PAGE). The amplification of genomic DNA from embryo tissue was performed with 15 RAPD primers. Presence/absence of protein fractions and DNA fragments were transformed to binary data (1,0). The computing of binary data was performed using NTSYS-pc software including coefficients of similarities and UPGMA clustering. RAPD markers detected high level of polymorphism among hybrids and were more informative in assessing genetic relatedness between them.

**PRINOS ZRNA ZP HIBRIDA KUKRUZA RAZLIČITIH FAO GRUPE
ZRENJA U ZAVISNOSTI OD GUSTINE SETVE**

Branka J. Kresović, Ž. V. Videnović, M. M. Tolimir i Lj. M. Prijić
Institut za kukuruz «Zemun Polje», Zemun-Beograd

U periodu 1998-2000 u Zemun Polju na černozemu obavljena su proučavanja uticaja navodnjavanja i gustine setve na genotipove kukuruza različitih FAO grupa zrenja (ZP 360, ZP 580 i ZP 704). Ogled je postavljen po split-plot metodi, u četiri ponavljanja, sa varijantama sedam gustina setve (40816, 50125, 59523, 69686, 79365, 89286 i 98522 bilj./ha). Rezultati prinosa zrna kukuruza obrađeni su metodom analize varijanse, uz korišćenje LSD testa. Regresionom analizom utvrđene su gustine pri kojima se postižu maksimalni prinosi zrna kukuruza.

U uslovima prirodnog vodnog režima, dobijeni rezultati pokazuju da su, u godinama proučavanja, između ispitivanih hibrida i gustina ostvarene statistički veoma značajne razlike prinosa zrna kukuruza. U relativno povoljnijim godinama za gajenje kukuruza ispitivani hibridi ostvarili su najbolje rezultate prinosa u sledećim gustinama: ZP 360 – 80-90.000 bilj./ha; ZP 580 – 70-80.000 bilj./ha; ZP 704 – 70-80.000 bilj./ha. U uslovima izrazitog deficita vode tokom vegetacije najpovoljnije gustine bile su: ZP 360 – 70.000 bilj./ha; ZP 580 – 50-60.000 bilj./ha; ZP 704 – 60-70.000 bilj./ha. U uslovima navodnjavanja svi hibridi ostvarili su veoma značajno niže prinose u gustinama setve sa oko 50.000 bilj.·ha⁻¹. Najbolje rezultate prinosa u navodnjavanju hibridi ZP 360 i ZP 580 ostvarili su u gustinama 80, 90 i 100.000 bilj.·ha⁻¹, a ZP 704 u gustinama 70, 80, 90 i 100.000 bilj.·ha⁻¹.

**YIELD OF ZP MAIZE HYBRIDS OF DIFFERENT MATURITY
GROUPS IN VARIOUS SOWING RATES**

Investigations were carried out on chernozem at Zemun Polje location in the period 1998 – 2000. The aim was to determine effects of irrigation and sowing rate on yield of different FAO maturity group maize (ZP 360, ZP 580 and ZP 704). The trial was set up using split-plot method in four replications. Seven sowing rate variants: 40816, 50125, 59523, 69686, 79365, 89286 and 98522 plants per hectare were applied. Analysis of variance method with LSD test was used to analyze the maize grain yield data. Crop density variants with maximal yields were determined by regression analysis.

Obtained results indicated that very significant maize yield differences between investigated hybrids and densities were achieved under dryland farming. Investigated hybrids, grown in relatively favourable years, showed the best yield results in the next densities: 80-90,000 plants per ha (ZP 360), 70-80,000 plants per ha (ZP 580 and ZP 704). The most favourable densities under extreme water deficiency during growing period were: 70,000 plants per ha (ZP 360), 50,000-60,000 (ZP 580) and 60,000-70,000 (ZP 704). All hybrids produced very significantly lower yields, when grown under irrigation and crop density around 50,000 plants per ha. The best yield results under irrigation with hybrids ZP 360 and ZP 580 were attained in crop densities of 80, 90 and 100,000 plants ha⁻¹.

**EFEKAT GENA I KOMBINACIONE SPOSOBNOSTI ZA VISINU BILJKE
I PREČNIK GLAVE KOD SUNCOKRETA**

Sandra Gvozdenović, J. Joksimović i D. Škorić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Visina biljke i prečnik glave su značajni parametri koji utiču na visinu prinosa kod suncokreta. Metodom linija x tester ispitano je šest restorer linija, tri tester A linije i njihovih F_1 hibrida. Utvrđene su značajne razlike između restorer linija i testera i njihovih F_1 hibrida za visinu biljke i prečnik glave. U nasleđivanju ispitivanih svojstava ispoljile su se superdominacija i dominacija boljeg roditelja, ali i intermedijarnost. Najveću srednju vrednost za visinu biljke su imali linija RHA-SELEUS (172,7 cm) i F_1 hibrid HA-48A x RUS-RF-OL-168 (237,4 cm), a najmanju linija RHA-BRE-1 (105,35 cm), odnosno hibrid L-19A x RHA-BRE-1 (147,9 cm). Kod prečnika glave najveću srednju vrednost su imali linija RUS-RF-OL-168 (16,45 cm) i F_1 hibridi HA-48A x RHA-TR-20 i L-19A x RHA-TR-20 (24,55 cm), a najmanju linija RHA-BRE-1 (13,10 cm) i hibrid HA-26A x RHA-M-72 (20,25 cm). Na osnovu dobijenih rezultata može se zaključiti da najbolje OKS imaju linije RHA-BRE-1 i RHA-TR-20, a najbolje PKS, hibridi L-19A x RHA-BRE-1 i HA-26A x RHA-SELEUS. Najveći prosečni doprinos u ekspresiji visine biljke dale su A-tester linije 83,17%, a za prečnik glave najveći uticaj su imale restorer linije 58,13%. Analiziranjem komponenti genetske varijanse, neaditivna komponenta je imala najveći uticaj na nasleđivanje visine biljke, a aditivna na prečnika glave.

**GENE EFFECT AND COMBINING ABILITIES FOR PLANT HEIGHT
AND HEAD DIAMETER IN SUNFLOWER**

Plant height and head diameter are important parameters which effect on a yield in sunflower. Six restorer Rf lines, three tester A lines and their 18 F_1 hybrids were studied, using line x tester method. Significant differences were found between restorer lines and testers and their F_1 hybrids for plant height and head diameter. Regarding the inheritance of examined characteristic, super dominance and dominance of better parent occurred, and the intermediary too. Line RHA-SELEUS (172.7 cm) and F_1 hybrid HA-48A x RUS-RF-OL-168 (237.4 cm) had the highest mean value for plant height, and the lowest value had line RHA-BRE-1 (105.35 cm) and hybrid L-19A x RHA-BRE-1 (147.9 cm). For head diameter, highest mean value had line RUS-RF-OL-168 (16.45 cm) and F_1 hybrids HA-48A x RHA-TR-20 and L-19A x RHA-TR-20 (24.55 cm), and the lowest line RHA-BRE-1 (13.10 cm) and hybrid HA-26A x RHA-M-72 (20.25 cm). Based on the results, the following conclusion is that lines RHA-BRE-1 and RHA-SELEUS have the best GCA, and best SCA have hybrids L-19A x RHA-BRE-1 and HA-26A x SELEUS. The largest average contribution in the expression of plant height had the tester A-lines (82.88%) and for head diameter Rf lines had the greatest influence (47.15%). Analyzing components of genetic variance, the non additive component played the main role in the inheritance of plant height and the additive of head diameter.

VARIJABILNOST AKTIVNOSTI PEROKSIDAZA I SADRŽAJA TEŠKIH METALA U ČETINAMA LINIJA POLUSRODNIKA CRNOG BORA (*Pinus nigra Arn.*)

V. Isajev¹, Ksenija Radotić² i M. Dučić¹

¹Šumarski fakultet, Beograd

²Centar za multidisciplinarnе studije, Univerzitet u Beogradu, Beograd

Ekstrakcija enzima je rađena najpre homogenizacijom u 5 mL 0.1 M TRIS-HCl puferu pH 7.6 koji sadrži 1 mM DTT i 1 mM EDTA na ledenom kupatilu po proceduri Mocquot et al. (1996) i Weckx i Clijsters (1996). Koncentracija proteina je urađena po metodi Bradford (Bradford, 1976). Za određivanje koncentracije metala u četinama crnog bora, uzorci su pripremani prema proceduri za «suvo spaljivanje». Uzorak je očitavan na apsorberu *Varian AA-10*. Detekcioni limit za Cd je od 0.02 do 3 µg/mL, za Cu je 0.03-10 µg/mL i za Ni 0.1-20 µg/mL. U cilju utvrđivanja koliki je uticaj genetičke raznolikosti linija polusrodnika na jedan od ključnih enzima uključenih u odgovor na stres, i zaduženog za proces lignifikacije u ćelijskom zidu, praćena je aktivnost enzima peroksidaza. Takođe je praćena aktivnost ovog enzima u zavisnosti od kiselosti medijuma. Izoelektrofokusiranjem utvrđeni su izoenzimi koji su prisutni u četinama analiziranih osam linija crnog bora.

Rezultati ovog rada pokazuju prisustvo izraženog varijabiliteta u koncentraciji ukupnih proteina, sadržaju teških metala i aktivnosti enzima peroksidaza kod analiziranih linija polusrodnika crnog bora. Ovo govori da proteini imaju različit nivo ekspresije u pojedinih linijama crnog bora. Takođe enzim peroksidaza pokazuje različitu aktivnost koja zavisi od genetske konstitucije stabala iz analiziranih linija polusrodnika, jer se, na osnovu veličine plantaže i šeme sadnje biljaka, može smatrati da je uticaj spoljašnjih faktora sveden na minimum.

VARIABILITY OF PEROXIDASE ACTIVITY AND HEAVY METAL CONTENT IN THE NEEDLES OF AUSTRIAN PINE (*Pinus nigra Arn.*) HALF-SIB LINES

Enzyme extraction was performed first by homogenisation in 5 mL 0.1 M TRIS-HCl buffer pH 7.6 containing 1 mM DTT and 1 mM EDTA in ice bath, by procedure Mocquot et al. (1996) and Weckx and Clijsters (1996). The concentration of proteins was determined by Bradford (Bradford, 1976) method. For the measurement of metal concentration in Austrian pine needles, the samples were prepared by the procedure for «dry burning». The sample was read off on the absorber *Varian AA-10*. Detection limit for Cd is from 0.02 to 3 µg/mL, for Cu 0.03-10 µg/mL and for Ni 0.1-20 µg/mL. The enzyme activity of peroxidases was monitored in order to determine the effect of genetic variability of half-sib lines on one of the key enzymes included in the response to stress, and responsible for the process of lignification in the cell wall. Also, the activity of this enzyme was monitored depending on the medium acidity. The isoenzymes present in the needles of the analysed eight Austrian pine lines were determined by isoelectric focusing.

The results of this study show the pronounced variability of the concentration of total proteins, heavy metal concentration and enzyme activity of peroxidases in the study Austrian pine half-sib lines. This infers that proteins have different levels of expression in individual Austrian pine lines. Also the enzyme of peroxidases shows different activity depending on the genetic constitution of the trees of the analysed half-sib lines, because based on he seed orchard size and the planting pattern, it can be assumed that the effect of external factors is reduced to a minimum.

**MEĐUZAVISNOST USLOVA OPRAŠIVANJA, OPLODNJE
I PRINOSA SEMENA SUNCOKRETA**

J. Joksimović, J. Atlagić, V. Miklič, N. Dušanić i Z. Sakač

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Kod četiri komercijalno važna hibrida suncokreta (NS-H-45, NS-H-111, NS-H-702 i Velja) i njihovih roditeljskih komponenti (Ha-74B, Ha-98B, CMS-3-8B, Ha-26B, RHA-583, RHA-R-Pl-2/1 i RHA-113N) u dve godine su ispitivana sledeća svojstva: dužina krunice cevastog cveta, sadržaj nektara, vitalnost polena, poseta pčela, % oplodnje i prinos semena. Međuzavisnost ispitivanih svojstava je utvrđena Path coefficient analizom.

Prosti koeficijenti korelacije su pokazali da su % oplodnje i poseta oprašivača imali visoko značajan pozitivan uticaj na prinos semena. Značajan pozitivan efekat je imala dužina krunice na sadržaj nektara, a značajan negativan efekat je imao sadržaj nektara na vitalnost polena. Najveći značajan direktni efekat na prinos semena je imao procenat oplodnje, dok je negativan, ali ne i značajan efekat imao sadržaj nektara na prinos semena. Koeficijent determinacije je iznosio 0,8071.

**INTERDEPENDENCE OF POLINATING CONDITIONS, SEED SET
AND SEED YIELD OF SUNFLOWER**

Corolla length, nectar content, pollen viability, honey bee visitation, seed set and seed yield have been observed on four commercially important sunflower hybrids (NS-H-45, NS-H-111, NS-H-702 and Velja) and their parental components (Ha-74B, Ha-98B, CMS-3-8B, Ha-26B, RHA-583, RHA-R-pl-2/1 and RHA-113N) in the course of two years. Interdependence of tested traits has been determined using path coefficient analysis.

Simple correlation coefficients have shown that seed set and visiting of the pollinators had highly significant, positive influence on the seed yield. Corolla length had significant positive effect on the nectar content while the nectar content had negative significant influence on the pollen viability. Seed set shown the biggest significant direct effect on the seed yield while the nectar content had negative, but not important effect on the seed yield. Coefficient of determination was 0.8071.

GENETIČKA VARIJABILNOST I KOEFICIJENTI KORELACIJE NAJAVAŽNIJIH AGRONOMSKIH SVOJSTAVA SELEKCIJONISANIH POPULACIJA CRVENE DETELINE (*Trifolium pratense L.*)

Z. Lugić, J. Radović i G. Jevtić

Institut za istraživanja u poljoprivredi «SRBIJA», Centar za krmno bilje, Kruševac

U radu su prikazani osnovni parametri varijabilnosti, kao i genetički i fenotipski koeficijenti korelacija dve selekcionisane populacije crvene deteline (A i B) za sledeće osobine: prinos suve mase po biljci, visinu biljke, broj izdanaka po biljci, broj internodija na glavnom izdanku, sadržaj sirovih proteina (SSP), sadržaj sirove celuloze (SSC) i sadržaj ukupnih šećera (SUŠ). Varijabilnost pojedinačnih biljaka za sva ispitivana svojstva je bila visoka i najveća za prinos suve mase po biljci (CV_F 17,78-22,42%) i broj izdanaka po biljci (CV_F 20,74-21,21%). Najniža varijabilnost je konstatovana za visinu biljke i SSP. Populacija A je ostvarila veće srednje vrednosti i intervale varijacije za sve ispitivane osobine. Pozitivne, jake i vrlo značajne genetičke korelacije kod populacije A zabeležene su za prinos suve mase, kao najvažniju osobinu, visinu biljke i SSC (0,776** i 0,754**). Srednje jake, pozitivne korelacije su konstatovane za prinos suve mase po biljci i visinu biljke, kao i za SSC i visinu biljke. Korelativni odnos SSP i SUŠ je sa ostalim svojstvima bio slabo pozitivan do jako i značajno negativan. Negativne genetičke korelacije dobijene su za visinu biljke, broj izdanaka po biljci i SUŠ, dok su visina biljke i SSP, kao i SSP i SSC bili u značajnom negativnom korelativnom odnosu (- 0,404* i -0,832**). Kod populacije B su dobijene niže vrednosti genetičkih koeficijenata korelacije, ali su odnosi ispitivanih osobina bili slični kao kod populacije A. Visoka varijabilnost pojedinačnih biljaka za sve ispitivane osobine ukazuje na to da su proučavane populacije perspektivne za dalje programe oplemenjivanja.

GENETIC VARIABILITY AND COEFFICIENTS OF CORRELATION FOR AGRONOMIC MOST IMPORTANT TRAITS OF SELECTED POPULATIONS OF RED CLOVER (*Trifolium pratense L.*)

The objective of this work was investigation of two selected population of red clover (A and B). The traits dry matter yield (DMY), plant height, number of tillers per plant, number of internodes on main shot, crude protein content (CPC), crude fiber content (CFC) and total sugar content (TSC) was determined during this research. Average values, interval of variation, coefficient of variation and genetic and phenotypic coefficient of correlations were calculated for all investigated traits. Plant variability of all traits was very high and highest for DMY per plant and number of tillers per plant (CV_F 17,78-22,42% and 20,74-21,21%, respectively). Lowest variability is detected for plant height and CPC. Highest averages and interval of variation were detected for population A. Positive, strong and high significant genetic coefficient of correlations for population A are estimated between DMY (most important agronomic trait) and plant height (0,776**) and between DMY and CFC (0,754**). Correlation coefficients of medium intensity were calculated for DMY and plant height and for CFC and plant height. Negative genetic correlations were detected for plant height, number of tillers per plant ant TSC, while plant height and CPC and CPC and CFC show significant negative coefficient of correlation (- 0,404* i -0,832**, respectively). For population B significantly lower coefficients of correlation were calculated, but relationships among traits were similar to population A. High variability of all plants for all examined traits shows that populations are very perspective for further breeding process.

UTICAJ KOLHICINA NA REGENERACIONU SPOSOBNOST KALUSA U KULTURI ANTERA PŠENICE

Branka Ljevnać¹, Ankica Kondić-Špika², B. Kobiljski² i S. Denčić²

¹Poljoprivredni fakultet, Novi Sad

²Naučni institut za ratarstvo i povrtarstvo, Novi Sad

Cilj istraživanja je bio da se ispita uticaj kolhicina na regeneracionu sposobnost kalusa u kulturi antera pšenice. Kao materijal korišćena je F₂ generacija 14 različitih kombinacija ukrštanja pšenice (*Triticum aestivum* L.). Od ukupnog broja izolovanih antera, polovina je bila izložena delovanju kolhicina u indukcionoj podlozi (300mg/l) u trajanju od tri dana. Druga polovina antera gajena je na modifikovanoj Potato-2 indukpcionoj hranljivoj podlozi bez kolhicina. Rezultati su pokazali da je kolhicin kod većine kombinacija značajno povećao regeneracionu sposobnost kalusa. Kombinacija Banks/F53-70 pokazala se kao najosetljivija na prisustvo kolhicina u podlozi. Kod ove kombinacije, ukupna regeneraciona sposobnost kalusa iznosila je 40,9% na podlozi bez kolhicina i 15,0% na podlozi sa kolhicinom. Pozitivan uticaj kolhicina na regeneraciju zelenih biljaka bio je najviše izražen kod kombinacije Prima/Bezostja-1. Kod ove kombinacije svaki kalus je dao zelenu biljku (100,0%) na podlozi sa kolhicinom, dok je na podlozi bez kolhicina 37,5% kalusa regenerisalo zelene biljke. Na podlozi bez kolhicina, u proseku za sve ispitivane kombinacije, 18,1% kalusa je regenerisalo biljke (13,9% zelenih i 4,2% albino) dok je na podlozi sa kolhicinom 30,3% kalusa regenerisalo biljke (18,2% zelenih i 12,1% albino). Kolhicin je kod većine kombinacija ukrštanja smanjio broj formiranih kalusa, ali je njihova regeneraciona sposobnost značajno povećana.

EFFECT OF COLCHICINE ON REGENERATION ABILITY OF CALLI IN WHEAT ANTHON CULTURE

The aim of this study was to investigate effect of colchicine on regeneration ability of calli in wheat anther culture. F₂ generations of 14 different wheat (*Triticum aestivum* L.) combinations were used as a material. A half of isolated anthers were exposed to colchicine effect in induction medium (300mg/l) for three days. The other half were grown on a modified Potato-2 induction medium without colchicine. The results have shown that colchicine in most of the combinations significantly increased regeneration ability of calli. The combination Banks/F53-70 was the most sensitive one to the presence of colchicine. In this combination, whole regeneration ability of calli was 40.9% and 15.0%, on media without and with colchicine, respectively. The positive effect of colchicine was the most expressed at Prima/Bezostja-1 combination. In this combination, every callus regenerated green plants (100.0%) on medium with colchicine, while 37.5% of calli regenerated green plants on medium without colchicine. In average 18.1% of calli regenerated plants (13.9% green and 4.2% albino) on medium without colchicine, while on medium with colchicine 30.3% of calli regenerated plants (18.2% green and 12.1% albino). Colchicine has decreased a number of formed calli in most of the studied combinations but their regeneration ability was significantly increased.

PRIMENA SSR MARKERA RADI UTVRĐIVANJA PRISUSTVA QTL-OVA ZA UKORENJAVAњE I SADRŽAJ ABA U LISTU KOD KOMPOZITNIH POPULACIJA KUKURUZA

V. Lazić-Jančić, D. Ignjatović-Micić i K. Marković

Institut za kukuruz «Zemun Polje», Beograd-Zemun

Tolerantnost kukuruza na sušu je kompleksna osobina, čija je ekspresija uslovljena aktivnošću većeg broja gena. Sadržaj ABA u listu i ukorenjavanje su neke od fiziološko-morfoloških osobina koje utiču na ispoljavanje ove tolerantnosti, a čiji su QTL-ovi mapirani kod različitih genotipova kukuruza. Tako je, na primer, u segregujućoj populaciji ukrštanja Os420xIABO78, QTL sa major efektom za sadržaj ABA u listu identifikovan na hromozomu 2 (bin 2.04), a za ukorenjavanje na hromozomu 1 (bin 1.06).

Cilj ovog rada je bio da se utvrdi da li se QTL-ovi sa major efektom detektovani u jednom genetipu mogu detektovati u nekom drugom genetičkom okruženju primenom molekularnih markera datog hromozomskog regiona. Za realizaciju navedenog cilja analizirano je 10 tolerantnih i 10 netolerantnih populacija kukuruza, korišćenjem 12 SSR prajmer kombinacije – dve na bin 1.05/ 1.06, a 10 na bin 2.04/2.05. Elektroforeza je rađena na denaturišućem poliakrilamidnom gelu, a nakon razdvajanja amplifikovanih fragmenata DNK je bojena srebro-nitratom. Analizom elektroforegrama, na osnovu prisustva/odsustva traka, izračunata je razlika u frekvenciji alela između tolerantnih i netolerantnih populacija. Rezultati i diskusija će biti prikazani na posteru.

SSR MARKER APPLICATION FOR DETERMINING PRESENCE OF QTLs FOR ROOTING AND LEAF ABA CONTENT IN COMPOSITE MAIZE POPULATIONS

Maize drought tolerance is a complex trait with its expression determined by many genes. Leaf ABA content and rooting are some of physiological-morphological traits influencing drought tolerance, whose QTLs were mapped in different maize genotypes. For example, in a segregating population, derived from the cross Os420xIABO78, a QTL with a major effect for leaf ABA content was identified on chromosome 2 (bin 2.04) and for rooting on chromosome 1 (bin 1.06).

The objective of this study was to determine if the QTLs with major effects detected in one genotype could also be detected in some different genetic background using molecular markers within a chromosome region harboring the QTLs in question. In order to fulfill the objective 10 drought tolerant and 10 drought non-tolerant maize populations were analyzed with 12 SSR primer combinations – two on bin 1.05/1.06 and 10 on bin 2.04/2.05. Electrophoresis was run on a polyacrylamide denaturing gel and then silver stained. Allele frequency differences between tolerant and non-tolerant populations were calculated based on presence/absence of the bands on the gel. Results and discussion will be presented on the poster.

GENETIČKA VARIJABILNOST LUCERKE (*Medicago sativa* L.) ZA PRODUKCIJU I VAŽNIJE MORFOLOŠKE OSOBINE

Jasmina Radović, Z. Lugić, D. Sokolović i Tanja Vasić

IIP «SRBIJA», Centar za krmno bilje, Kruševac

Cilj rada je određivanje obima i strukture genetičke varijabilnosti poboljšane populacije lucerke. Istraživanja su obavljena u Centru za krmno bilje u Kruševcu. Potomstvo je dobijeno ručnim ukrštanjem slučajno odabranih biljaka po proceduri koju su za ksenogamne biljke predložili Comstock i Robinson. Ogled je postavljen po Nested dizajnu I sa setovima u okviru ponavljanja. Proučavano je ukupno 48 half-sib (HS) potomstava podeljena u dva seta. U toku trogodišnjeg perioda iskorišćavanja na pojedinačnim biljkama su praćene sledeće osobine: prinos suve mase po biljci, visina biljaka, broj izdanaka po biljci, broj internodija po stabljici, debljina stabljike i odnos list: stablo.

Rezultati ukazuju na visoku varijabilnost ispitivanih osobina u sve tri godine istraživanja. Najveće vrednosti za skoro sve osobine su zabeležene u drugoj godini istraživanja. Utvrđeni intervali variranja ukazuju na visok genetički potencijal ispitivane populacije. Najveća varijabilnost je dobijena za prinos suve materije po biljci (CVg 25%) i broj stabljika (CVg 30%), dok je niži koeficijent varijacije zabeležen za visinu biljaka (9%), broj internodija (5%) i ostale praćene osobine. Varijabilnost pojedinačnih biljaka u okviru HS potomstava je takođe bila visoka. Utvrđen je jak uticaj visine biljaka i broja stabljika na prinos suve materije. Rezultati ukazuju na značajnu genetičku varijabilnost unutar populacije.

GENETIC VARIABILITY FOR PRODUCTION AND MORPHOLOGICAL TRAITS OF ALFALFA (*Medicago sativa* L.)

The aim of this investigation is to determined genetic variability for production and morphological traits of alfalfa. The experiment was realized in the Center for forage crops in Krusevac. The progeny used for examining genetic variability were obtained by hand polination of randomly chosen plants from population according to a procedure for xenogam plants suggested by Comstock and Robinson (1948). There have been studied 48 half-sib (HS) progenies separated in two sets. During three years period the following characteristic have been examined on spaced plants: dry matter yield per plant, plant height, number of steam per plant, number of internodium on main steam, width of steam and leaf-steam ratio.

Results showed a high variability for examined traits in all three years of investigation. The higher values for almost all traits was noticed in second years of utilization. Determinated levels of mean values and intervals of variability indicate high genetic potential of evaluated population for all examined traits. The highest variability was obtained for dry matter yield (CVg 25%) and number of steam (CVg 30%), while the lower coefficient of variation was noticed for number of internodium (5%) and plant height (9%). Variability of individual plants within HS progeny was higher for all traits than variability between HS. The strong correlation was assessed for dry matter yield and plant height. The high variation of examined traits represent on significant genetic variability inside the population.

**OCENA KVALITETA KLIJAVACA BAGRENCA (*Amorpha fruticosa* L.)
METODOM P. S. WELLINGTONA**

Radmila Knežević i A. Tucović
Šumarski fakultet, Beograd

Objekat istraživanja su odabrana stabla bagrenca (*Amorpha fruticosa* L.) odgajana u Beogradu i okolini. Ukupno je sakupljeno seme sa: 12 stabala iz slobodnog opršivanja, tri prostorno izolovana stabala i tri stabla nakon manje-više ukrštanja u srodstvu. U uzrastu klijavaca od 2 do 4 lista mereno je više osobina i utvrđen je procenat anormalnih klijavaca za svaki od tretmana. Za analizirana kvantitativna svojstva izračunate su srednje vrednosti, standardna devijacija, varijacioni koeficijent i njihove greške. Statistička opravdanost između srednjih vrednosti određivana je pomoću Studentovog t-parametra.

Normalnim klijavcem, se prema kriterijumima međunarodne kontrole, smatra klijavac koji je sposoban za tipičan rast pri gajenju na standardnoj podlozi, odgovarajućoj vlažnosti, temperaturi i osvetljenosti. Dosadašnje informacije o karakteristikama klijavaca bagrenca nas ne zadovoljavaju s obzirom da se u stručnoj literaturi daju samo podaci na nivou vrste, a svera raznih kategorija anormalnih klijavaca izlazi iz granica običnog laboratorijskog ispitivanja.

U radu se iznose uporedne karakteristike klijavaca u linijama polusrodnika od slobodnog opršivanja, opršivanja u manje-više srodstvu i samooplodnji. Najviše vrednosti za četiri analizirana svojstva klijavaca, evidentirana su kod potomaka od slobodnog opršivanja, dok kod opršivanja u manje-više srodstvu i samoopršivanja, vrednosti osetno opadaju. Utvrđene razlike su statistički opravdane. Udeo više kategorija anormalnih klijavaca se osetno uvećava pri opršivanju u srodstvu i samooplodnji. Utvrđene razlike u analiziranim tretmanima dokazuju da je njihova pojava pod genetičkom kontrolom odabranih test stabala.

**QUALITY ASSESSMENT OF AMORPHA (*Amorpha fruticosa* L.) SEEDLINGS
BY P. S. WELLINGTON'S METHOD**

The study was focused on the selected amorpha (*Amorpha fruticosa* L.) trees cultivated in Belgrade and its surroundings. The seed was collected from 12 trees from free pollination, three spatially isolated trees and three trees after more or less crossing of related trees. At the seedling stage of 2 to 4 leaves, several characters were measured and the percentage of abnormal seedlings for each treatment. Mean values, standard deviation, variation coefficient and their errors were calculated for the analysed quantitative characters. Statistical justification between mean values was determined by Student t-parameter.

Normal seedling, according to the criteria of international control, is the seedling capable of typical growth under cultivation in standard substrate, adequate moisture, temperature and light. Previous information on amorpha seedling characteristics are not satisfactory because professional literature gives only the data at the level of the species, and the sphere of different categories of abnormal seedlings exceeds the limits of the common laboratory analysis.

This paper presents the comparative characteristics of seedlings in half-sib lines of free pollination, pollination of more or less related trees and self-fertilisation. The highest values for the four study seedling characters were recorded in the progeny of free pollination, while in the pollination of more or less related trees and self-pollination the values decrease considerably. The differences are statistically justified. The percentage of several categories of abnormal seedlings increases under the pollination of related trees and self-fertilisation. The differences of the study treatments prove that their occurrence is under genetic control of the selected test trees.

UTICAJ OPRAŠIVAČA NA FORMIRANJE HAPLOIDNIH EMBRIONA U UKRŠTANJIMA PŠENICE I KUKURUZA

Ankica Kondić-Špika i B. Kobiljski

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Metod ukrštanja pšenice i kukuruza predstavlja veoma efikasan i često korišćen sistem za proizvodnju haploida pšenice. U cilju daljeg povećanja efikasnosti ove metode, u ovom radu ispitana je uticaj polinatora (kukuruz) na frekvenciju oplodnje (br. stranooplodnih zrna na 100 oprašenih cvetova) i frekvenciju haploidnih embriona (br. haploidnih embriona na 100 oprašenih cvetova) u ukrštanjima pšenice i kukuruza.

Trinaest kombinacija pšenice (*Triticum aestivum L.*) ukršteno je sa dva različita genotipa kukuruza (*Zea mays L.*), Kelvedon Glory (V. Britanija) i ZPSC-213 (SCG). U pogledu frekvencije oplodnje značajne razlike između ovih oprašivača utvrđene su kod tri kombinacije pšenice (Banks/Rodna, Banks/NS-205-98 i Lira/Sonata). Kod sve tri navedene kombinacije postignuti su značajno bolji rezultati u oplodnji sa oprašivačem Kelvedon Glory. Prednost ovog oprašivača naročito je ispoljena u pogledu frekvencije formiranih haploidnih embriona, koja se kretala od 0,2-3,6% u odnosu na ukupan broj oprašenih cvetova. U ukrštanjima pšenice sa oprašivačem ZPSC-213 frekvencija haploidnih embriona se kretala od 0-1,2%. Samo kod kombinacije Banks/Rodna nije utvrđena značajna razlika između polinatora u pogledu ovog svojstva, dok su sve ostale kombinacije ukrštanja pšenice dale značajno veći broj haploidnih embriona u ukrštanjima sa polinatom Kelvedon Glory.

EFFECT OF POLLINATOR ON HAPLOID EMBRYO FORMATION IN WHEAT X MAIZE HYBRIDIZATION

Wheat x maize hybridization method is an efficient and frequently used system for production of haploids in wheat. With the intention of further improving the efficiency of this method, the aim of this work was to analyze the influence of pollinator (maize) on fertilization frequency (no. of open-pollinated seeds per 100 pollinated florets) and frequency of haploid embryos (no. of haploid embryos per 100 pollinated florets) in wheat x maize hybridization.

Thirteen wheat (*Triticum aestivum L.*) combinations were crossed with two different maize (*Zea mays L.*) genotypes, Kelvedon Glory (GB) and ZPSC-213 (S&M). Significant differences in fertilization frequency were found between pollinators in three wheat combinations (Banks/Rodna, Banks/NS-205-98 and Lira/Sonata). In these combinations, significantly better results were obtained in the crosses with Kelvedon Glory pollinator. The advantage of this pollinator was specially expressed in the frequency of haploid embryos, which ranged from 0.2 to 3.6% in relation to the total number of pollinated florets. In wheat crosses with ZPSC-213 pollinator, the frequency of haploid embryos ranged from 0 to 1.2%. No significant difference between the pollinators in regard of this trait was found in combination Banks/Rodna, while all other wheat combinations had significantly greater number of haploid embryos in the crosses with Kelvedon Glory pollinator.

**OCENA KOMPONENTA VARIJANSE MASE 1000 SEMENA KOD
SUNCOKRETA (*Helianthus annuus* L.)**

R. Marinković

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Kod deset F_1 hibrida nastalih ukrštanjem između pet inbred linija suncokreta po dialelnoj šemi analiziran je uticaj gena sa aditivnim i dominantnim efektima kao i njihovih interakcija u nasleđivanju mase 1000 semena. Povezanost između očekivanih srednjih vrednosti potomstava proverena je primenom metode scaling testova (Mather, 1949), a procena genskih efekata i način nasleđivanja urađeni su po metodi *Generation Mean Analysis* (Mather i Jinks, 1982). Aditivno-dominantan model nije bio adekvatan kod svih ukrštanja u obe godine ispitivanja. Bio je adekvatan kod ukrštanja C_1 , C_2 , C_3 i C_5 , u prvoj i kod ukrštanja C_3 , C_8 , C_9 i C_{10} u drugoj godini ispitivanja. Pored glavnih genskih efekata, aditivan i dominantan, u nasleđivanju ovog svojstva veliki značaj imali su i epistatični genski efekti. Duplikatni tip epistatze između dominantnih gena sa negativnim predznakom nađen je kod ukrštanja C_1 , C_4 , C_5 , C_6 i C_8 u prvoj godini i kod ukrštanja C_4 , C_9 i C_{10} u drugoj godini ispitivanja. Komplementarna epistaza između dominantnih gena sa negativnim predznakom napena je kod ukrštanja C_{10} u prvoj, a duplikatna epistaza između dominantnih gena sa pozitivnim predznakom je nađena u ukrštanju C_5 u drugoj godini ispitivanja.

**ASSESSMENT OF COMPONENTS OF GENETIC VARIANCE OF MASS 1000
SEEDS IN SUNFLOWER (*Helianthus annuus* L.)**

Seven F_1 hybrids obtained by crossing five sunflower inbred lines were used to analyze the impact of genes with additive and dominant effects and their interactions on the inheritance of mass 1000 seeds. The linkage among the expected progeny means was tested using the scaling tests method (Mather, 1949), while the estimates of gene effects and mode of inheritance were made by Generation Mean Analysis (Mather i Jinks, 1982). The additive-dominant model was not proved adequate in all crosses in both year investigation. It was adequate in crosses C_1 , C_2 , C_3 and C_5 in first and in crosses C_3 , C_8 , C_9 and C_{10} in second year investigation. Besides the main gene effects (additive and dominant), epistatic gene effects were also of great importance in the inheritance of this trait. Duplicate epistasis between dominant decreasers was found in C_1 , C_4 , C_5 , C_6 and C_8 in first year and in crosses C_4 , C_9 and C_{10} in second year investigation. Complementary epistasis between dominant decreasers was found in cross C_{10} in first, and duplicate epistasis between dominant increasers in cross C_5 in second year investigation.

**VARIJABILNOST PROTEINA U SEMENU I KLIJAVCIMA 40 LINIJA
POLUSRODNIKA CRNOG BORA (*Pinus nigra* Arn.)**

M. Mataruga¹, V. Isajev^{1,2}, Snežana Mladenović-Drinić³,
Kosana Konstantinov³ i Vanja Daničić¹

¹Šumarski fakultet, Univerzitet u Banja Luci, Bosna i Hercegovina

²Šumarski fakultet, Beograd

³Institut za kukuruz «Zemun Polje», Zemun

Istraživanjima je obuhvaćeno 40 linija crnog bora slobodnog opršavanja koje potiču iz 5 provenijencija: Sutjeska, Višegrad, Tara, Teslić i Durmitor. U svakoj provenijenciji izdvojene su dve populacije i to: 5 linija slobodnog opršavanja predstavlja populaciju sa stena i 3 linije slobodnog opršavanja populaciju na najboljem staništu crnog bora. Specifičnost ispitivanih provenijencija, populacija, kao i linija slobodnog opršavanja dokazana je preko proučavanja osobina semena, dinamike klijanja i usvajanja vode, te osobina klijavaca, a potvrđena putem analiza u solima rastvorljivih proteina. Identičan proteinski sastav u ponavljanjima iste linije slobodnog opršavanja je dokazan u malom broju slučajeva, što govori u prilog velike unutarlinijske varijabilnosti koja može biti posledica uticaja oca, kao i heterozigotnosti materinskih stabala. U analizama proteinskog sastava semena konstatovane su najznačajnije razlike na nivou provenijencija. Kod klijavaca starih 9 dana, isklijalih u uslovima indukovane suše i u standardnim uslovima utvrđene međulinjske, populacione i provenjenične razlike. Korelacionim analizama utvrđeno je postojanje značajnih međuzavisnosti osobina klijavaca i kasnijeg razvoja sadnica.

**VARIABILITY OF THE SALT-SOLUBLE PROTEINS IN SEED AND
SEEDLING OF 40 HALF-SIB LINES OF AUSTRIAN PINE**

The study includes 40 lines of free pollination originating from 5 provenances: Sutjeska, Višegrad, Tara, Teslic and Durmitor. Two populations were selected from each provenance, i.e.: 5 lines of free pollination represent the population growing on the cliffs and 3 lines of free pollination represent the population growing at the best site of Austrian pine. The specificities of the study provenances, populations, and free pollination lines were proved by the study of seed properties, dynamics of germination and water uptake, and by the seedling properties and it was confirmed by the analyses in the salt-soluble proteins. The identical protein composition in the replicates of the same free pollination line was proved in a small number of cases, which indicates a high intra-line variability which can be the result of the effect of father, as well as of heterozygosity of mother trees. While the analyses of protein composition of seed show the most significant provenance differences, inter-line, population and provenance differences can be recorded at the level of 9-day old seedlings germinated in drought stress conditions and in normal conditions. The correlation analyses show significant correlation of seedling properties and subsequent seedling development. The results of morpho-physiological properties of one-year old seedlings in nursery conditions also point to the wide modification variability of the tested planting stock.

ANALIZA EFEKTA GENA U NASLEĐIVANJU BROJA ZRNA PO KLASU KOD HIBRIDA JECMA

Milomirka Madić, M. Kuburović i Marija Kraljević-Balalić¹

Agronomski fakultet, Čačak

¹Poljoprivredni fakultet, Novi Sad

U dialelnom ukrštanju, bez recipročnih pet divergentnih genotipova ječma (Vada, Đerdap, NS-293, Jagodinac i Sladoran) regresionom analizom varijanse i kovarijanse proučavan je način nasleđivanja i efekat gena za broj zrna po klasu.

Regresionom VrWr i WrW' analizom utvrđeno je odsustvo interallelne interakcije, što opravdava primenu odabranog modela. U nasleđivanju broja zrna po klasu regresija je ukazala na dominantan efekat gena, kao i na nejednak raspored dominantnih i recesivnih gena kod odabralih genotipova. Najveći broj dominantnih gena za broj zrna po klasu imaju genotipovi Jagodinac, Vada i NS-293, dok recesivnih Đerdap i Sladoran.

Dobijeni rezultati ukazuju da kod dvorednih genotipova ječma veći broj dominantnih gena dovodi do povećanja broja zrna po klasu, a povećan sadržaj recesivnih gena ima suprotan efekat.

THE ANALYSIS OF GENE EFFECT IN THE INHERITANCE OF KERNEL NUMBER PER SPIKE IN BARLEY HYBRIDS

In diallel crossing excluding reciprocal ones, the five divergent genotypes of barley (Vada, Đerdap, NS-293, Jagodinac and Sladoran) were involved and the mode of inheritance and gene effect studied using the regression analysis of variance and covariance for the number of kernels per spike.

The absence of interallel interaction was established through the regression analysis VrWr and WrW' which justified the application of the model chosen. In the inheritance of the number of kernels per spike, the regression pointed to the dominant gene effect as well as to the unequal distribution of the dominant and recessive genes in the genotypes chosen. While the genotypes Jagodinac, Vada and NS-293 had the highest number of the dominant genes , those of Đerdap and Sladoran had the highest number of the recessive ones for the number of kernels per spike.

The obtained results denoted that, in two-rowed genotypes, the higher number of dominant genes had led to a larger number of kernels per spike, whereas the higher number of the recessive genes had brought about the opposite effect.

**PRIMENA KULTURE CVASTI *in vitro* U
OPLEMENJIVANJU ŠEĆERNE REPE**

Snežana Mezei, L. Kovačev, N. Čačić i Nevena Nagl

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Obzirom da kod šećerne repe (*Beta vulgaris* L.) nisu razdvojene faze izduživanja aksilarnih pupoljaka od faze umnožavanja, giberelin se dodaje zajedno sa citokininom i neophodnim mikro i makro elementima u podlogu. Već u prvoj subkulturi može da se dobije desetak biljčica koje su spremne za fazu ožiljavanja. U fazi mikropropagacije iz eksplantata cvasti dovoljne su koncentracije od svega 0,3 mg/l BA da se dobije zadovoljavajuća stopa umnožavanja u periodu od nekoliko subkulturna. Podloga za ožiljavanje šećerne repe ne sadrži fitohormone, a poznato je da se rizogeneza sastoji od faze indukcije, inicijacije i elongacije, te se smatra da je de novo inicijacija korena stimulisana postojanjem endogenih auksina i citokinina u optimalnom odnosu u tkivu repe. Na osnovu kombinacionih sposobnosti utvrđenih za potomstva proizvedena iz semena pojedinačnih biljaka dobijenog u slobodnoj oplodnji, odabrani su genotipovi iz zasnovane klonske populacije i ožiljeni *in vitro*. Nakon toga su biljke aklimatizovane u staklari a u hladnoj komori je izvršena fotermalna indukcija. Seme dobijeno od odabranih klonova predstavlja rekurentnu populaciju oprasivača sorte Lara.

**APPLICATION OF INFLORESCENCE *in vitro* CULTURE
IN SUGAR BEET BREEDING**

Since in sugar beet (*Beta vulgaris* L.) elongation of axillary buds in not independent from multiplication, gibrerelins are added to multiplication media with citokynines, micro- and macro- elements. Already in the first subculture few plantlets could be obtained, which could be put on media fro rhizogenesis. For the micropropagation from inflorescence, concentration of 0,3 mg/l BA is enough to obtain satisfying multiplication rate in few subcultivations. There is no need to add phytohormones in rhizogenesis medium, since it was proved that induction, initiation and elongation of sugar beet roots is spontaneous and result of optimal ratio of endogenous auxines and citokynines. According to combining abilities determined for offspring produced from seed of single plants in free pollination, number of genotypes were selected and served as a base for formation of clone population *in vitro*. The plants were put on rooting medium, after that on acclimatization in the greenhouse and then in cold chamber for photothermal induction. Seed of selected genotypes presents recurrent population of pollinator for variety Lara.

GENETIČKA VARIJABILNOST SORTI LUCERKE RAZLIČITOG GEOGRAFSKOG POREKLA

S. Katić, V. Mihailović, D. Milić, S. Vasiljević i A. Mikić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Cilj rada je bio da se utvrdi genetička varijabilnost lucerke u i između sorti različitog geografskog porekla. Ogled je vođen od 2002-2004 godine sa 12 sorti lucerke (*M. sativa*): dve iz Novog Sada, 4 iz Grčke, 5 iz Bolivije, 1 iz Ukrajine i 3 sorte *M. varia* iz Estonije. Analizom dvogodišnjih rezultata (2003. i 2004), dobijeno je najveće variranje između sorti različitog geografskog porekla odnosno između *M. sativa* i *M. varia* sorte. Takođe bilo je veće variranje između sorti sa udaljenim poreklom (Srbija - Bolivija). Najmanje variranje zapaženo je između sorti bliskog geografskog porekla i ekoloških uslova (Srbija - Grčka). Uočeno je veće variranje između sorti različitog geografskog i biološkog porekla, nego između sorti zajedničkog porekla i sličnih ekoloških uslova. Najveće genetičko variranje zapaženo je kod osobina: prinos suve materije (CV_g 27,8 %) i brzina regeneracije (CV_g 24,0 %). Najmanje genetičko variranje između sorti zapaženo je kod udela lišća (CV_g 8,4 %) i sadržaja suve materije (CV_g 2,4 %).

GENETIC VARIABILITY IN LUCERNE CULTIVARS OF DIFFERENT GEOGRAPHIC ORIGIN

The aim of this paper was to analyze genetic variability within and between lucerne cultivars of different geographic origin. A trial was conducted during 2002-2004 with 12 *M. sativa* cultivars: two from Novi Sad, Serbia, four from Greece, five from Bolivia, one from Ukraine and three cultivars of *Medicago varia* from Estonia. The results of 2003 and 2004 have shown that the greatest variation was between cultivars of different geographic origin, as well as *M. sativa* and *M. varia*. There was also a great variation between cultivars of distant origin (Serbia - Bolivia). The smallest variation was found in cultivars of close geographic origin and environmental conditions (Serbia-Greece). There was also a greater variation between cultivars of different geographic and biological origin than between cultivars of common origin and similar ecological conditions. The greatest genetic variation was found in dry matter yield (CV_g 27.8 %) and regeneration rate (CV_g 24.0 %). The smallest genetic variation was found in portion of leaves (CV_g 8.4 %) and (CV_g 2.4 %).

GRUPE VEZANIH GENA I AGRONOMSKA SVOJSTVA STOČNOG GRAŠKA
(*Pisum sativum* L.)V. Mihailović¹, A. Mikić¹, Marija Kraljević-Balalić², S. Vasiljević¹ i D. Milić¹¹Naučni institut za ratarstvo i povrtarstvo, Zavod za krmno bilje, Novi Sad²Univerzitet u Novom Sadu, Poljoprivredni fakultet, Novi Sad

Iako je grašak (*Pisum sativum* L.) jedna od najčešće i najduže genetski i citogenetski proučavanih biljnih vrsta, tek je relativno nedavno došlo do nastanka celovite slike graškovog genoma, kako u smislu kariotipa, tako i u pogledu organizacije grupa vezanosti, odnosno grupa vezanih gena (*linkage groups*). Grašak ima nevelik broj hromozoma ($n=7$), od kojih su pet akrocentri (3, 4, 5, 6 i 7) i dva submetacentri (1 i 2), ali, usled veličine biljaka i složenosti genoma, nije mnogo omiljen u genetičkoj analizi. Sa druge strane, bogat genom graška, od oko $4 \cdot 10^9$ bp i uz prosecni CG sadržaj od 37,4 %, zajedno sa visokim stepenom samooplodonje i izraženom i lako uočljivom morfološkom varijabilnošću, može da pruži izuzetnu osnovu za temeljno i uspešno proučavanje građe genoma.

Od sedam grupa vezanih gena graška, prva se vezuje za drugi, druga za šesti, treća za peti, četvrta za četvrti, peta za treći, šesta za prvi i sedma za sedmi hromozom. Ogroman doprinos razvijuću genetskih mapa graška dalo je korišćenje genetskih markera, u prvom redu onih koji su zasnovani na tehnikama AFLP, RAPD, retrotranspozona ili EST. Zahvaljujući tome, došlo je do objedinjavanja različitih mapa u jedinstvenu, usaglašenu mapu vezanosti za grašak. Poslednjih godina, veliki podsticaj fundamentalnim istraživanjima daju saznanja o genomima novijih model biljaka, *Medicago truncatula* i *Lotus japonicus*, uz određivanje stepena sintenije između njih i ostalih mahunarki, u prvom redu graška, sočiva i nauta. Istovremeno, primena molekularnih markera postaje neprocenjiva u pronalaženju lokusa za kvantitativna svojstva (QTL), što je od izvanrednog značaja za efikasno oplemenjivanje.

**LINKAGE GROUPS AND AGRONOMIC CHARACTERISTICS
OF FODDER PEA (*Pisum sativum* L.)**

Although pea (*Pisum sativum* L.) is one of the most frequently and most extensively studied plant species, it is only relatively recently that a coherent picture of pea genome has emerged, both in terms of karyotype and the organisation of the linkage groups. Pea has only seven chromosomes, five acrocentrics (3, 4, 5, 6 and 7) and two submetacentrics (1 and 2), but is not very popular in genetic analysis, due to plant size and complex genome. However, the richness of pea genome, with about $4 \cdot 10^9$ bp and the average CG content of 37.4 %, together with high level of self-pollination and noticeable morphological variability, provides an excellent basis for universal study of genome architecture.

Pea has seven linkage groups, the first being linked with the second chromosome, the second with the sixth, the third with the fifth, the fourth with the fourth, the fifth with the third, the sixth with the first and the seventh group linked with the seventh chromosome. Genetic markers, especially by those AFLP, RAPD, transposone or EST based, gave an immense contribution to genetic maps development and their integration into a consensus linkage map for pea. During last few years, the fundamental research in pea was given a great stimulus by knowledge on genomes of novel model plants, *Medicago truncatula* and *Lotus japonicus*, along with the determination of level of synteny among these two and other legumes, especially pea, lentil and chickpea. At the same time, the exploitation of genetic markers becomes irreplaceable in finding out QTL, gaining an extraordinary importance in efficient breeding.

UTICAJ DIAZOTROFA NA NEKE FIZIOLOŠKE PARAMETRE PŠENICE

Danica Micanović, Veselinka Zečević, D. Knežević, M. Pavlović i D. Urošević

Institut za istraživanja u poljoprivredi «SRBIJA», Centar za strnu žita, Kragujevac

Istraživanja su izvedena u laboratorijskim uslovima sa tri divergentna genotipa pšenice (DK-1, DK-2, DK-3). Seme je površinski sterilisano, a zatim naklijavano šest dana. Biljke su inokulisana sa suspenzijom od 0,5 ml sojeva diazotrofa (Kg-10, Kg-11 Kg-67, Kg-73, Kg-74, Kg-75 i Az-zp) u koncentraciji 10^8 ćelija po biljci. Kao kontrola su služile neinokulisane biljke. Biljke su gajene u *in vitro* uslovima 42 dana, u bezazotnom rastvoru Reid Yorka za pšenicu. Izolati su determinisani molekularno genetičkom metodom PCR kao *nif* bakterije. Uticaj inokulacije je praćen preko sadržaja suve materije korena i nadzemnog dela biljke, visine biljke, dužine korena kao i sadržaja azota u korenu i nadzemnom delu biljke. Dobijene su statistički visoko značajne razlike u masi suve materije i sadržaju azota, kako u nadzemnom delu tako i u korenu, kod svih genotipova, dok razlike kod ostalih ispitivanih parametara nisu bile statistički opravdane.

Najveća masa suve materije u nadzemnom delu i korenu biljaka dobijena je kod sorte DK-1 (18.59 mg/biljkci; 10.14 mg/biljkci). Najveći sadržaj azota u nadzemnom delu biljke dobijen je takođe kod genotipa DK-1 (0.455 mg/biljkci), a u korenu kod genotipa DK-3 (0.170 mg biljka⁻¹). Najveći uticaj na većinu ispitivanih parametara imali su sojevi Kg-74 i Kg-75, prosečno za sve genotipove.

INFLUENCE OF DIAZOTROPHS ON PHYSIOLOGICAL PARAMETERS IN WHEAT

The experiment was done in semi control conditions with three different wheat genotypes (DK-1, DK-2, DK-3). Grain surface of each cultivars were sterilized and after that were germinate during six days. Inoculation has been made with seven different strains (Kg-10, Kg-11 Kg-67, Kg-73, Kg-74, Kg-75 and Az-zp) with 0,5 ml 10^8 cell per seed. Control plants were grown in tubes without inoculation. Plants were grown *in vitro* in nitrogen free solutions, during 42 days. Pure isolates determined by PCR as *nif* bacteria. Influence of inoculation was analyzed on the base of value of assessed traits (dry matter content of above ground part of plant and root, height of plants, length of root, nitrogen concentration and nitrogen content of above ground part of plant and root). Variability was found in majority investigation parameters. Influence on inoculation with diazotrophs indicated very significant differences of dry matter content of above ground part of plant and root and nitrogen content in above part of plant and root, but differences in height of plants and length of root were not significant.

The highest content of dry matter of above ground part of plant and root were found in cultivar DK-1 (18.59 mg plant⁻¹; 10.14 mg plant⁻¹). The highest nitrogen content of above ground part of plant else were found in cultivar DK-1 (0.455 mg plant⁻¹) but in root in cultivar DK-3 (0.170 mg plant⁻¹). The highest increasing in most investigation parameters was influenced by strains Kg-74 and Kg-75 in average for all cultivars.

POTENCIJALNA I STANDARDNA KLJAVOST KOD SEMENA VOĆARSKIH I ŠUMSKIH VRSTA

Marija Milivojević¹ i D. Poštić²

¹Institut za kukuruz «Zemun Polje», Beograd-Zemun

²Institut za zaštitu bilja i životnu sredinu Topčider

Tetrazolium test (TTZ) predstavlja osnovni metod za ispitivanje kvaliteta semena voćarskih i šumskih vrsta. Velika prednost TTZ testa u odnosu na test kljavosti jeste brzo dobijanje rezultata (2 dana) što je izuzetno značajno kod kratkih rokova pri uvozu i izvozu semena. Za ispitivanje kljavosti kod većine voćarskih vrsta je potrebno i do 4 meseca. Predtretman u vidu prethodnog hlađenja traje 2-3 meseca nakon čega sledi mesec dana naklijavanja u optimalnim uslovima. Kada se to ima u vidu jasno je zbog čega je tetrazolium test postao dominantan test u ispitivanju kvaliteta semena voćarskih i šumskih vrsta. Ipak treba imati u vidu da test kljavosti i tetrazolium test nisu identični po svom karakteru. Tetrazolium test daje vrednost potencijalne kljavosti. Vrednosti standardne kljavosti će dostići taj potencijal ukoliko nema nikakvih prepreka za klijanje kao što je na primer bilo koji vid mirovanja semena. Cilj ovog rada je utvrđivanje razlika između potencijalne i standardne kljavosti semena vinogradarske breskve, džanarike, bagrema, crnog bora, smrče i divlje jabuke. U radu su korišćene procedure propisane u ISTA Pravilima (2003). Rezultati su statistički obrađeni i utvrđeno da postoje značajne razlike između vrednosti potencijalne i standardne kljavosti kod ispitivanog uzorka vinogradarske breskve, bagrema i smrče. Osim navedena dva testa (standardna kljavost i tetrazolium test) u ISTA Pravilima za neke biljne vrste preporučuje se i test izdvojenog embriona (EET) koji predstavlja jedan vid ubrzanih testa kljavosti. Ovaj test takođe poseduje specifičnosti. Primjenjen je kod breskve, džanarike i divlje jabuke. Dobijene vrednosti EET testa se statistički razlikuju od vrednosti potencijalne i standardne kljavosti samo kod vinogradarske breskve.

POTENTIAL AND STANDARD GERMINATION OF SEEDS OF FRUIT AND FOREST SPECIES

Tetrazolium test (TZ) is the basic test for testing tree seeds. The main reason for that is the fact that only two days are required for conducting TZ test. On the other hand germination test can last even four months. Pretreatment is very often required (2-3 months stratification) and final evaluation of germination takes place after one month under optimal conditions. Having that in mind it is quite clear why TZ test become predominant in testing tree and shrub seeds. Still it must be underlined that TZ test and germination test reveal different characteristics of seed. Tetrazolium test determines the potential germination. Standard germination will reach the value of the potential in case the seed dormancy is not present. The objective of this work was to examine the difference between potential and standard germination of several tree species (*Prunus cerasifera*, *Prunus persica vulgaris*, *Malus silverstis*, *Picea abies*, *Pinus nigra*, *Robinia pseudoacacia*). The tests were conducted according to ISTA Rules (2003). Significant differences between germination and tetrazolium values were found in *Prunus persica vulgaris*, *Robinia pseudoacacia* and *Picea abies*. Beside these two tests (TZ and germination) ISTA Rules for some species recommend extracted embryo test (EET). It can be said that EET test is an accelerated germination test and it also has specific characteristics. This test was used for testing *Prunus* and *Malus* seeds. Only obtained EET values for *Prunus persica vulgaris* significantly differed from potential and standard germination values.

VARIJABILNOST VELIČINE PLODOVA I SJEMENA DIVLJE TREŠNJE (*Prunus avium* L.) SA PODRUČJA BOSNE I HERCEGOVINE

T. Mikić¹, D. Ballian² i S. Orlović³

¹Samostalni istraživač, Karađorđevo,

²Šumarski fakultet u Sarajevu, Sarajevo

³Poljoprivredni fakultet, Institut za nizijsko šumarstvo i životnu sredinu, Novi Sad

U ovom radu su prikazani rezultati istraživanja varijabilnosti divlje trešnje (*Prunus avium* L.) iz istočnog dijela Bosne i Hercegovine. Plodovi su sakupljeni iz četiri populacije, Kalinovik, Sokolac, te Petrovići i Jasik kod Sarajeva.

Istraživala se masa plodova potrebna za jedan kilogram sjemena i broj plodova u jednom kilogramu, te apsolutna masa (težina 1000 sjemenki).

Masa plodova potrebna za jedan kilogram se kretala kod populacije Kalinovik od 4,28 kg do 7,73 kg, kod populacije Sokolac od 4,52 kg do 8,57 kg, kod populacije Jasik od 5,45 kg do 7,97 kg i kod populacije Petrovići od 4,48 kg do 10,00 kg. Broj plodova u jednom kilogramu kretao se kod populacije Kalinovik od 663 do 2051, kod populacije Sokolac od 736 do 1270, kod populacije Jasik od 795 do 1281 i kod populacije Petrovići od 745 do 1489 plodova. Apsolutna težina se kretala od 78,39 gr do 245,46 gr kod populacije Kalinovik, kod populacije Sokolac od 119,67 gr do 249,16 gr, kod populacije Jasik od 122,42 gr do 195,74 gr i kod populacije Petrovići od 101,77 gr do 197,97 gr.

Sva istraživana svojstva pokazuju veliku individualnu unutar populacijsku i među populacijsku varijabilnost.

Istraživana svojstva su u korelacionoj vezi sa nadmorskom visinom.

U populacijama divlje trešnje veliko je učešće stabala koja su nastala spontanom hibridizacijom sa sortama pitome trešnje. Te se to može primijetiti i iz dobivenih rezultata, male težine plodova za jedan kg sjemena, malog broja plodova u jednom kg, velike apsolutne težine sjemena.

VARIABILITY OF WILD CHERRY (*Prunus avium* L.) FRUIT AND SEED SIZES IN THE REGION OF BOSNIA AND HERZEGOVINA

This paper presents the study results of wild cherry (*Prunus avium* L.) variability in the east part of Bosnia and Herzegovina. The fruits were collected in four populations, Kalinovik, Sokolac, and Petrovići and Jasik near Sarajevo.

The study included fruit mass needed for 1 kilogram of seed and the number of fruits per 1 kilogram, as well as the absolute mass (weight of 1000 seeds).

Fruit mass needed for 1 kilogram ranged in population Kalinovik from 4.28 kg to 7.73 kg, population Sokolac from 4.52 kg to 8.57 kg, populations Jasik from 5.45 kg to 7.97 kg and population Petrovići from 4.48 kg to 10.00 kg. Fruit number per 1 kilogram was as follows: population Kalinovik from 663 to 2051, population Sokolac from 736 to 1270, population Jasik from 795 to 1281 and population Petrovići from 745 to 1489 fruits. Absolute weight was from 78.39 g to 245.46 g in population Kalinovik, population Sokolac from 119.67 g. to 249.16 g, population Jasik from 122.42 g to 195.74 g and population Petrovići from 101.77 g to 197.97 g.

All the study characters show a high individual inter population and intra population variability.

The study characters are in correlation with the altitude.

In the populations of wild cherry there is a high percentage of trees generated by the spontaneous hybridisation with the varieties of domestic cherry. This can also be concluded from the study results, low fruit weight per 1 kg of seed, low number of fruits per 1 kg, and high absolute weight of the seed.

MORFOANATOMSKE KARAKTERISTIKE ČETINA RAZLIČITIH FENOGRUPA OMORIKE

Jelena Milovanović, Mirjana Šijačić-Nikolić, Dragica Vilotić, V. Ivetić
Šumarski fakultet, Beograd

Pančićeva omorika (*Picea omorika* Panč./Purkyine), kao endemska vrsta Balkanskog poluostrva i tercijarni relikt, veoma je značajna za istraživanja u oblasti šumarstva i pejzažne arhitekture. U cilju proizvodnje selekcionisanog semena ove vrste, 1986. godine podignuta je generativna semenska plantaža iznad sela Godovik, SO Požega, koja obuhvata 50 linija polusrodnika razvrstanih u sedam fenogrupa. U ovom radu, izvršena je analiza morfoanatomskih karakteristika četina, na uzorku od po pet stabala u okviru pet fenogrupa, što predstavlja korak napred u istraživanjima vezanim za Pančićevu omoriku. Rezultati ovog rada predstavljaju značajnu polaznu osnovu za dalja istraživanja, u cilju utvrđivanja varijabilnosti strukture četina različitih fenogrupa i mogućnosti primene morfoanatomskih karakteristika četina kao parametra pri proceni obilnosti uroda i produkcije biomase četina za potrebe farmaceutske i kozmetičke industrije.

MORPH - ANATOMICAL CHARACTERISTICS OF SERBIAN SPRUCE NEEDLES FROM DIFFERENT PHENOGROUPES

Serbian spruce (*Picea omorika* Panč./Purkyine), Balkan endemic species and tertiary relict, have a great significance in forestry research and landscape architecture. Serbian spruce seed orchard at Godovik was established in 1986, from 50 half-sib lines divided in seven phenogroupes. On phenogroupes level, within five genotypes sample, applied method in this study was to analysis morph - anatomic characteristics of needles, which is a step forward in Serbian spruce research. The results of this study made very important starting point for further research aimed to estimate variability of needles structure from different phenogroupes, with possible application of needles morph - anatomical characteristics as parameter for estimate crop and needles biomass production for pharmaceutical and cosmetics industry needs.

SELEKCIJA *Azotobacter chroococcum* – PUT DO MIKROBIOLOŠKOG ĐUBRIVA ZA ŠEĆERNU REPУ

Nastasija Mrkovački, Snežana Mezei, L. Kovačev i N. Čačić

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

Značajan doprinos plodnosti zemljišta i održivoj poljoprivrednoj proizvodnji potiče od slobodne fiksacije atmosferskog azota. Više vrsta bakterija, slobodnih azotofiksatora, a među njima i vrste *Azotobacteria* su dale pozitivan odgovor inokulisanih biljaka. Kod različitih biljnih vrsta – pšenice, kukuruza, sunčokreta, šećerne repe, paradajza inokulacija *Azotobacterom* uticala je ne samo na povećanje prinosa već i na poboljšanje njegovog kvaliteta. Rezultati ovih istraživanja otvorili su pitanje mogućnosti selekcije sojeva za određenu biljnu vrstu.

U ovim ispitani su sojevi *Azotobacter chroococcum* u čistoj kulturi. Testirano je niz osobina: aktivnost nitrogenaze, rezistentnost na različite pesticide i njihove različite koncentracije, zatim uticaj temperature na rast sojeva, uticaj piletne šećerne repe na rast, uticaj različitih nivoa azota u podlozi kao i uticaj sojeva na kaluse šećerne repe. Ispitane osobine sojeva omogućile su izbor – selekciju dobrih sojeva (od 20 do 12, i od 8 do 5 sojeva) koji su se pokazali kao najbolji za buduću uspešnu zajednicu sa šećernom repom. U daljim ispitivanjima sojeva *Azotobacter chroococcum* u zajednici sa biljkom šećerne repe u staklari i u polju odabrali smo tri soja *Azotobacter chroococcum* – kao najefektivnije za gajenje šećerne repe. Ovi sojevi čine bazu za proizvodnju mikrobiološkog đubriva za šećernu repu.

SELECTION OF *Azotobacter chroococcum* – A WAY TO MICROBIOLOGICAL FERTILIZER FOR SUGARBEET

Fixation of atmospheric nitrogen contributes significantly to soil fertility and indirectly to sustainable agriculture. Several free nitrogen-fixing bacteria including *Azotobacter* species have been observed to elicit a positive response of inoculated plants. In different plant species – wheat, corn, sunflower, sugarbeet, tomato – inoculation with *Azotobacter* increased not only crop yield but also crop quality. This study has been initiated to assess possibilities of selecting bacterial strains for specific crops.

In this study, *Azotobacter chroococcum* strains have been tested in pure culture, for the following characteristics: nitrogenase activity, resistance to various pesticides and their different concentrations, effect of temperature on bacterial growth, effect of sugarbeet pelleting mass on bacterial growth, effect of different nitrogen levels in the substrate and the effect of bacterial strains on sugarbeet calli. Based on their performance, good strains have been selected (from 20 to 5 strains) for combining with sugarbeet. Of the *Azotobacter chroococcum* strains that were subsequently grown in association with sugarbeet plants in the greenhouse and in the field, three were selected as most effective for combining with sugarbeet. These strains will be used for the production of a microbiological fertilizer for sugarbeet.

**NASLEĐIVANJE OSOBINA RAZVIJENOG LISTA
U POTOMSTVU VINOVE LOZE DOBIJENOM UKRŠTANJEM SORTI
MUSKAT HAMBURG I VILLARD BLANC**

D. Nikolić

Poljoprivredni fakultet, Beograd-Zemun

Od 21 morfološke osobine koje za opis i determinaciju sorti vinove loze preporučuje Internacionallni institut za biljne genetičke resurse šest su osobine razvijenog lista. To su: veličina, broj režnjeva, oblik zubaca, oblik peteljkinog sinusa, gustina poleglih malja i gustina uspravnih malja između nerava na naličju razvijenog lista. Varijabilnost i način nasleđivanja navedenih osobina ispitivani su kod 90 sejanaca F₁ generacije iz kombinacije ukrštanja Muskat hamburg x Villard blanc. Kategorizacija proučavanih osobina izvršena je pomoću sistema šifri OIV, a određivanje načina njihovog nasleđivanja pomoću χ^2 testa. Dobijeni rezultati pokazuju da je u hibridnom potomstvu ispoljena znatna varijabilnost svih proučavanih osobina. Veličina razvijenog lista, broj režnjeva razvijenog lista, oblik zubaca razvijenog lista i oblik peteljkinog sinusa razvijenog lista najverovatnije su uslovljeni većim brojem genetičkih faktora, jer je pri proučavanju načina njihovog nasleđivanja ustanovljeno odstupanje od testiranih, monogenskih odnosa razdvajanja. Za gustinu poleglih malja i gustinu uspravnih malja između nerava na naličju razvijenog lista utvrđeno je monogensko nasleđivanje.

**INHERITANCE OF MATURE LEAF PROPERTIES IN GRAPEVINE
PROGENY OBTAINED BY CROSSING MUSCAT HAMBURG AND VILLARD
BLANC CULTIVARS**

Of the 21 morphological property recommended by the International Plant Genetic Resources Institute for description and determination of grapevine cultivars six properties are those of the mature leaf, e.g. size, number of lobes, shape of teeth, shape of petiole sinus, density of prostrate and erect hairs between the veins on lower side. Variability and inheritance mode of these properties were analyzed in 90 seedlings of the F₁ generation from the crossing combination of Muscat Hamburg x Villard Blanc. The properties analyzed were classified according to the OIV system of classification and χ^2 test was used to determine the inheritance mode. A substantial variability of the analyzed characteristics was recorded in the hybrid progeny. Mature leaf size, number of mature leaf lobes, shape of mature leaf teeth and shape of mature leaf petiole sinus were most probably affected by a great number of genetic factors because there were divergences with regard to inheritance in relation to the tested monogenic relationships of separation. Monogenic inheritance was determined with regard to the density of prostrate and erect hairs between the veins on lower side of the mature leaf.

ADAPTABILNOST I STABILNOST GENOTIPOVA PŠENICE U VOJVODINI

Sofija Petrović, M. Dimitrijević i Marija Kraljević-Balalić

Katedra za genetiku i oplemenjivanje biljaka, Departman za ratarstvo i povrtarstvo,
Poljoprivredni fakultet, Novi Sad

Adaptabilnost i stabilnost genotipova pšenice, kao i ostalih kultura, je posledica interakcije genotipa i spoljne sredine. Oplemenjivanje na visok prinos dobija na efektu tek ako je taj visok prinos praćen i dobrom stabilnošću tj. ako se visok nivo prinosa održava, ili što manje varira iz godine u godinu. Posmatrajući prinos kao rezultat ukupne genetičke osnove i uslova života, u radu je praćena interakcija genotipa i uslova gajenja za komponente prinosa u poljoprivredno naprednom regionu Vojvodine.

**ADAPTABILITY AND STABILITY OF WHEAT
GENOTYPES IN VOJVODINA**

Adaptability and stability of the wheat genotypes are the results of genotype by environment interaction. The full effect of breeding for high yield is achieved when is followed by a good stability when high yield shows as low variation as possible through seasons. Considering yield as a result of total genetic background and growth conditions, genotype/environment interaction of the yield components in developed agricultural region of Vojvodina has been followed in the article.

**VARIJABILNOST I HERITABILNOST NEKIH OSOBINA GENOTIPOVA
ENGLESKOG LJULJA (*Lolium perenne* L.)**

D. Sokolović, Z. Lugić i J. Radović

Institut «SRBIJA», Centar za krmno bilje, Kruševac

U ovom radu su predstavljena istraživanja pokazatelja varijabilnosti genotipova engleskog ljulja (komponenti varijansi i koeficijenata varijacije) i heritabilnosti za neke agronomski vrlo bitne osobine. Takođe su predstavljene međuzavisnosti najbitnijih osobina odabralih genotipova.

Genotipovi engleskog ljulja koji potiču od divljih populacija su proučavani u ogledu sa pojedinačnim biljkama i praćene su sledeće osobine: vreme klasanja (izraženo brojem dana od 1. aprila), visina biljaka, širina bokora, broj izdanaka, prinos suve materije i sadržaj sirovih proteina. Najveća razlika između genetičkih i fenotipskih komponenti varijanse, odnosno najmanja heritabilnost u širem smislu je utvrđena za osobine visina biljaka (16,4%) i prinos suve materije (25,4%). Najveću heritabilnost su pokazali genotipovi za vreme klasanja (83,26%) i širinu bokora (79,87%) tako da se oplemenjivanjem ovih osobina očekuju vrlo brzi rezultati. Takođe vrlo visoko nasleđivanje imaju osobine broj izdanaka i sadržaj sirovih proteina. Upoređenjem relativnih pokazatelja varijabilnosti utvrđeno je da se genotipovi najviše razlikuju po broju izdanaka i prinosu i da je kod broja izdanaka od ukupno 15% variranja čak za 80% odgovoran genotip biljaka. Kod vremena klasanja čak 91% varijabilnosti potiče od genotipa, ali je ukupna varijabilnost relativno mala (3,44%). Najveći koeficijenti korelacije utvrđeni su između širine bokora i visine biljke (0,86), odnosno visine biljke i sadržaja sirovih proteina (-0,87).

**VARIABILITY AND HERITABILITY OF SOME TRAITS OF PERENNIAL
RYEGRASS (*Lolium perenne* L.) GENOTYPES**

Investigations of variability parameters (variance components and coefficients of variation) and heritability of perennial ryegrass genotypes for agronomic most important traits are shown in this article. Interdependence of those traits is estimated, too.

Perennial ryegrass genotypes originated from wild populations were investigated in space-plant design. Time of tillering, plant height, sward diameter, number of tillers per plant, dry matter yield and crude protein content were investigated. The highest differences between genetic and phenotypic variance components were determined for plant height and dry matter yield. Lowest heritability in broad sense is estimated for those traits (16,4% and 25,4%), respectively. Highest heritability is determined for time of tillering (83,26%) and sward diameter (79,87%). According those data, breeding of genotypes for improvement of those traits could provide effective results in short period. Also high heritability is detected for number of tillers per plant and crude protein content. It was determined in comparison of variability parameters that main difference between genotypes came from number of tillers per plant and dry matter yield. For number of tillers, genotype of plants is responsible for 80% of variations, from 15% of total variation. Also, 91% of variability for time of tillering originated from genotype, although total variability is relative low (3,44%). Highest coefficients of correlation were estimated between sward diameter and plant height (0,86), and between plant height and crude protein content (-0,87), respectively.

VARIJABILNOST POKAZATELJA AKUMULACIJE I ISKORIŠĆAVANJA SUVE MATERIJE U BILJCI PŠENICE

S. Stojković¹, N. Deletić¹, M. Biberdžić¹ i Jovanka Stojanović²

¹Poljoprivredni fakultet Univerziteta u Prištini, Lešak

²Institut za istraživanja u poljoprivredi «SRBIJA», Centar za strnu žita, Kragujevac

U ogledu sa 20 sorata ozime pšenice izučavani su neki pokazatelji akumulacije i iskorišćavanja suve materije u biljci i njihov uticaj na prinos zrna. Utvrđeno je da vrednosti pokazatelja (biološki prinos, žetveni indeks zrna, reutilizacija suve materije, broj zrna po klasu, masa 1000 zrna, visina biljaka i dr.) variraju u zavisnosti od genotipske specifičnosti kao i od uslova spoljne sredine. Prinos zrna nije zavisio samo od ukupne akumulacije suve materije u biljci tj. biološkog prinosa, već i od odnosa ove akumulacije u periodu do cvetanja i posle cvetanja. Na formiranje prinosa uticalo je više pokazatelja, redosled sorata po visini pojedinih pokazatelja se menjao. Najbolji prinosi su dobijeni u sorata kod kojih je veći broj pokazatelja imao povećane vrednosti. Od ispitivanih sorti najveći prosečan prinos zrna ostvarila je sorta Tiha, a sorte koje su se još istakle po prinosu su: Gruža, Nevesinjka i Toplica.

VARIABILITY OF DRY SUBSTANCE ACCUMULATION AND UTILIZATION PARAMETERS OF WINTER WHEAT PLANT

Some dry substance accumulation and utilization parameters of wheat plant and their influence on grain yield have been studied through a field trial with 20 winter wheat cultivars. The studied parameters value (biological yield, dry substance reutilization, number of grains per spike, 1000 grain mass, plant height, etc.) varried depending of genotypic specificity and environmental conditions. Grain yield was influenced not only by the total dry substance accumulation in plant (i.e. biological yield), but also by this accumulation amount before and after flowering. Yield forming was affected by many parameters, and cultivar range was different for various parameters. The best grain yield was observed in cultivars having elevated values of more than few parameters. The highest mean grain yield was observed in cultivar Tiha, and also high grain yield values were found in cultivars Gruza, Nevesinjka, and Toplica.

POJAVA POLUPATULJAKA U SUBSPONTANOM POTOMSTVU I IZGRADNJA KULTIVARA PATULJASTOG RASTA SEVERNOG AMERIČKOG HRASTA

Mirjana Šijačić-Nikolić, A. Tucović i Dragica Vilotić
Šumarski fakultet, Beograd

Uporodna morfo-fiziološka analiza više osobina obavljena je na generativnom potomstvu tri manje-više prostorno izolovana stabala na Adi Ciganlji. Kvantitativna svojstva su statistički obređena. Opravданost razlika između srednjih vrednosti određivana je Studentovim t-testom.

U radu je evidentirana i opisana pojava polupatuljaka u generativnom potomstvu tri, manje-više prostorno izolovana stabala na Adi Ciganlji. Transgresivno cepanje svojstava i izražena segregacija na dva alternativna fenotipa, prema rastu u analiziranom potomstvu, od 27 potomaka (25/2) proizilazi iz genetske divergentnosti roditeljskih stabala. Kako je problematika usmerenog, planskog oplemenjivanja na patuljast rast, samo zacrtana, ali nedovoljno razrađena, ovom pravcu oplemenjivanja treba obezbediti obim i kontinuitet. Da bi se ostvario brži napredak za potrebe urbanog šumarstva, trebalo bi više menjati gajenu vrstu. Jedan od takvih pravaca je sinteza patuljastih kultivara, ukrštanjima polupatuljaka u manje-više srodstvu, što obezbeđuje veće mogućnosti za racionalnije korišćenje urbanog prostora i u oplemenjivanju gradske sredine. Specifičnost rada zahteva: (1) definisanje željenog tipa patuljastog kultivara, (2) izradu modela, kao i (3) planiranje genetičke prirode zamišljene sorte. Orientacija na doslednu primenu ovih zahteva ima bitan uticaj na unapređenje proizvodnje namenskih sadnica *Quercus borealis* Michx., pa joj treba pokloniti punu pažnju.

THE OCCURRENCE OF SEMI-DWARFS IN SUB-SPONTANEOUS PROGENY AND THE DEVELOPMENT OF AMERICAN RED OAK CULTIVARS WITH DWARF GROWTH

The comparative morpho-physiological analysis of several characters was performed on the generative progeny of three more or less spatially isolated trees on Ada Ciganlja. The quantitative results were statistically processed. The justification of differences between mean values was determined by Student t-test.

This paper records and describes the occurrence of semi-dwarfs in the generative progeny of three more or less spatially isolated trees on Ada Ciganlja. The transgressive separation of characters and the expressed segregation into two alternative phenotypes of growth in the study progeny of 27 offsprings (25/2) results from the genetic divergence of parent trees. As the issues of directed, planned breeding on dwarf growth is only intended, but insufficiently developed, this direction of breeding should be more complete and continual. To achieve a faster progress for the demands of urban forestry, the cultivated species should be more changes. One of such directions is the synthesis of dwarf cultivars, by crossing of semi-dwarfs of more or less related trees, which ensures greater possibilities for the more rational use of urban space and the improvement of urban environment. The specific work requires to: (1) define the desired type of the dwarf cultivar, (2) develop the model, and (3) plan the genetic nature of the conceived variety. The orientation to the consistent application of the above has a significant effect on the improvement of production of specific purpose seedlings of *Quercus borealis* Michx., so it should be paid full attention to.

KVALITET I PRODUKCIJA DOMAČIH SORTI LUCERKEZ. Nešić¹, Z. Tomicić¹, M. Žujović¹, I. Đalović²¹Institut za stočarstvo, Zemun-Beograd,²Agronomski fakultet, Čačak

Visoki prinosi krme, ideo i kvalitet proteina, dužina života, prinos semena i druga agronomski važna svojstva svrstavaju lucerku u red nezamenljivih biljaka u proizvodnji kvalitetne voluminozne stočne hrane. Na oglednim poljima Instituta za stočarstvo u periodu od 2002–2004. godine ispitivana su važnija agronomска svojstava (prinos zelene krme i sadržaj suve materije) sedam genetički divergentnih genotipova lucerke u poređenju sa sortom NS-Medijana koja je korišćena kao standard. U drugoj i trećoj godini, ispitivanjem utvrđeni su i osnovni parametri kvaliteta standardnim labaratorijskim metodama (sadržaj suve materije, sirovi proteini, sirove masti, sirova celuloza, mineralne materije, BEM). Komponente prinosa lucerke su u značajnim genetičkim korelacijama sa prinosom suve materije. Analizom kvaliteta, komponenti prinosa i sadržaja suve materije nije utvrđena značajna genetska varijabilnost između ispitivanih sorti. Dobijeni rezultati pokazuju da sve proučavane sorte nisu ispoljile statistički značajne razlike, a po osnovnim parametrima kvaliteta i prinosa zelene mase i suve materije nalaze se na nivou standarda.

QUALITY AND PRODUCTION OF DOMESTIC SORTS OF LUCERNE

High yields of fodder, yield and quality of proteins, longevity, yield of seeds and other agronomically important properties make lucerne irreplaceable in the production of quality voluminous animal fodder. On the experimental fields of the Institute for Animal Husbandry in the period from 2002–2004 we have investigated more important agronomical traits (yield of green fodder and content of dry matter) of seven genetically divergent genotypes of lucerne in comparison with sort NS-Medijana which was used as standard. In the second and third year of investigation we have determined also the major quality parameters by standard lab methods (content of dry matter, crude protein, crude fat, crude fibre, mineral matters, BEM). The components of lucerne yield are in significant genetic correlations with the yield of dry matter. By the analysis of quality, components of yield and content of dry matter no significant genetic variability among investigated sorts was observed. The results obtained show that all investigated sorts expressed no statistically significant differences, while according to major parameters of quality and yield of green mass and dry matter they are at the standard level.

**VARIJABILITET SADNICA *Ginkgo biloba* L. PROIZVEDENIH
IZ SEMENA KINESKE PROVENIJENCIJE U RASADNIKU TAMARIS
COMPANY U BANJA LUCI**

M. Tošić¹ i Dragica Vilotić²

¹Dimitrija Tucovića 41, Užice

²Šumarski fakultet u Beogradu

Ginkgo biloba, najstarija i najotporna drvenasta vrsta na planeti, endemit istočnog dela Kine, ali od čoveka unet u mnoge zemlje sveta u kojima, kao «živi fosil» sa izvanrednim estetskim i značajnim lekovitim svojstvima, veoma dobro uspeva. Na Balkan je verovatno donet iz parkova evropskih zemalja, gde se nalazi još od davne 1730. godine (Vilotić D. 2004). U radu se daju preliminarni podaci o varijabilitetu 5000 kom. sadnica u rasadniku *TAMARIS COMPANY* u Banja Luci, proizvedenih iz semena uvezenog iz Kine. Utvrđena je znatna fenotipska raznovrsnost analiziranih svojstava (rasta i boje dugorasta, oblika i boje listova u toku leta i dr.) koja je, svakako, samo deo genetičkog varijabiliteta. Prisustvo individua sa estetskim osobinama nekih već poznatih ukrasnih kultivara ginka, objašnjava se činjenicom da strane vrste u uslovima kultivisanja, zbog izmenjenih uslova nove sredine, mogu obrazovati u kratkom periodu niz novih mutanata (Tucović A. & Ocokoljić M. 2002) saglasno zakonu M. I. Vavilova, o paralelnim serijama nasledne promenljivosti. Utvrđeni varijabilitet je osnova za sintezu atraktivnih kultivara ginka za potrebe pejzažne arhitekture i proizvodnju sve potrebnijih lekovitih supstanci, a ostvariće se primenom selekcije, generativnog i vegetativnog razmnožavanja.

**VARIABILITY OF *Ginkgo biloba* L. SEEDLINGS PRODUCED FROM
THE SEEDS OF CHINESE PROVENANCE IN THE NURSERY TAMARIS
COMPANY IN BANJA LUKA**

Ginkgo biloba, the oldest and the most resistant woody species on the planet, endemic of east China, but anthropogenically introduced to many parts of the world where, as a «living fossil» with extraordinary esthetical and significant medicinal properties, succeeds very well. It has probably been brought to the Balkans from the parks of the European countries, where it occurs since 1730 (Vilotić D., 2004). This paper presents the preliminary data on the variability of 5,000 seedlings in the nursery *TAMARIS COMPANY*, Banja Luka, produced from the seeds imported from China. The assessed considerable phenotypical diversity of the study properties (long shoot growth and colour, leaf shape and colour during summer, etc.) is by all means only a part of its genetic variability. The presence of the individuals with aesthetic properties of some known ginkgo ornamental cultivars is explained by the fact that exotic species in the conditions of cultivation, due to the changed conditions of the new environment, can form a series of new mutants in a short period (Tucović A. & Ocokoljić M., 2002) consistent with the law by M. I. Vavilov, on the parallel series of inherited variability. The assessed variability is the base of the synthesis of attractive ginkgo cultivars for landscape architecture and of the production of increasingly demanded medical substances and it is going to be realised by selection and by generative and vegetative reproduction.

**GENETIČKA ANALIZA NASLEĐIVANJA I MEĐUZAVISNOST
KOMPONENTI PRINOSA, MORFOLOŠKO-BIOLOŠKIH KARAKTERISTIKA
I PRINOSA ZELENE MASE CRVENE DETELINE (*Trifolium pratense* L.)**

Sanja Vasiljević¹, Gordana Šurlan-Momirović², T. Đivanović², M. Ivanović¹,
V. Mihailović¹, S. Katić¹ i A. Mikić¹

¹Naučni institut za ratarstvo i povrтарstvo, Novi Sad

²Poljoprivredni fakultet, Novi Sad

U radu je korišćeno pet divergentnih genotipova crvene deteline (dve sorte: Junior, Diana i tri populacije: Vlaška, BL-3, M-11) koje su dialelno ukrštene. Eksperimentalni deo ogleda je bio urađen na oglednom polju Zavoda za krmno bilje Naučnog Instituta za ratarstvo i povrтарstvo u Novom Sadu. Tokom 1999 i 2000 godine su bila sprovedena dialelna ukrštanja između odabralih genotipova crvene deteline u svim kombinacijama, nakon čega je u dvogodišnjem periodu (2001-2002) izvršeno testiranje dobijenog potomstva u odnosu na ispitivane osobine (dužina stabljike, debljina stabljike, dužina centralne liske, prinos zelene mase po biljci, vreme početka cvetanja, persistencija i forma rasta). Proučavani su: varijabilnost, način nasleđivanja, efekat gena, kombinirajuće sposobnosti, kao i heritabilnost, ispitivanih osobina crvene deteline. Analizom fenotipske varijanse može se zaključiti da je aditivan način delovanja gena imao dominantnu ulogu u nasleđivanju: dužine stabljike, prinos zelene mase po biljci, forme rasta, a kao najčešći način nasleđivanja ispoljila se parcijalna dominacija. Veće učešće dominantne varijanse u ukupnoj genotipskoj varijansi, kao i niske vrednosti heritabilnosti u užem smislu (h^2_n) su dobijene za: debljinu stabljike (16,67 % i 23,07 %). Tokom obe godine (2001-2002) analizom path koeficijenta utvrđen je značajan direktni efekat dužine stabljike na prinos zelene mase po biljci crvene deteline (9,09*, odnosno 6,23*). Direktni efekat debljine stabljike na prinos zelene mase po biljci u 2001. godini je bio izrazito negativan ($r = -0,141$), međutim njegov pozitivan ukupni efekat je rezultat pozitivnih indirektnih efekata dužine stabljike ($r = 0,379$) i forme rasta ($r = 0,164$).

**GENETIC ANALYSIS OF INHERITANCE AND MUTUAL RELATIONSHIPS
AMONG YIELD COMPONENTS, MORPHOLOGICAL-BIOLOGICAL TRAITS
AND YIELD OF GREEN MASS OF RED CLOVER (*Trifolium pratense* L.)**

A diallel cross including five divergent red clover genotypes (two varieties: Junior and Diana, and three populations: Vlaška, BL-3 and M-11) has been used in this study. Experiments were conducted at the experiment field of Forage Crops Department, Institute of Field and Vegetable Crops, Novi Sad. In the course of 1999 and 2000, a diallel cross was made with the selected red clover genotypes. In the period 2001-2002, the obtained progenies were tested for stem length, stem thickness, length of central lamina, yield of green mass per plant, beginning of flowering, persistence and growth habit of red clover. These traits were analyzed for variability, mode of inheritance, gene effects, combining ability and heritability. The analysis of phenotypic variance showed that the additive gene action played the dominant role in the inheritance of stem length, yield of green mass per plant and growth habit. Partial dominance was the most frequent mode of inheritance. Increased proportion of dominant variance in the total genotypic variance and low values of narrow-sense heritability (h^2_n) were obtained for stem thickness (16.67% and 23.07%, respectively). In both study years (2001-2002) by path coefficient analysis we obtained significant direct effect of stem length on yield of green mass per plant of red clover (9,09* and 6,23* respectively). The direct effect of stem thickness on green mass per plant in 2001 was expressly negative ($r = -0,141$), but positive value of total effect of stem thickness on green mass per plant is result of positive values of indirect effects of stem length ($r = 0,379$) and growth habit ($r = 0,164$).

VARIJABILNOST I FAKTORSKA ANALIZA MORFOLOŠKIH I PRODUKTIVNIH OSOBINA VRSTA IZ RODA *Amaranthus*

Vesna Vujačić¹, Marija Bodroža-Solarov², T. Đivanović¹ i V. Pešić¹

¹Poljoprivredni fakultet, Beograd

²Tehnološki fakultet, Novi Sad

U trogodišnjem radu je ispitivano deset genotipova amarantusa. Ispitivane su morfološke i produktivne osobine - visina biljke, broj listova po biljci, dužina srednjeg lista, širina srednjeg lista, masa lista po biljci, masa zrna po biljci i ukupan prinos zrna amarantusa. Analizirana je varijabilnost navedenih osobina i izvršena klasifikacija metodom glavnih komponenti.

Varijabilnost u okviru pojedinih osobina je značajna i iznosi za visinu biljke od 93,18 cm (genotip 9 - *A. cruentus*) do 160,78 cm (genotip 1 - *A. mantegazzianus*); broj listova po biljci od 12,89 (genotip 10 - *A. cruentus*) do 23,46 (genotip 1 - *A. mantegazzianus*); dužinu srednjeg lista od 14,77 cm (genotip 9 - *A. cruentus*) do 26,72 cm (genotip 1 - *A. mantegazzianus*); širinu srednjeg lista od 6,30 cm (genotip 9 - *A. cruentus*) do 14,46 cm (genotip 1 - *A. mantegazzianus*); masu lista po biljci od 94,05 g (genotip 3 - *A. molleros*) do 246,81 g (genotip 1 - *A. mantegazzianus*), masu zrna po biljci od 45,56 g (genotip 3 - *A. molleros*) do 67,55 g (genotip 1 - *A. mantegazzianus*) i za ukupan prinos zrna od 2,22 t/ha (genotip 3 - *A. molleros*) do 3,20 t/ha (genotip 1 - *A. mantegazzianus*).

VARIABILITY AND FACTOR ANALYSIS OF MORPHOLOGICAL AND PRODUCTIVE CHARACTERISTICS OF SPECIES OF THE GENUS *Amaranthus*

Through out three years of scientific researches, ten genotypes of amaranth were studied. Morphological and productive characteristics - plant height, foliage per plant, medium foliage length, medium foliage width, mass per plant, seed mass per plant were the subject of researches. Variability of these features was analyzed and classification by the method of major components conducted.

Variability within specific features is significant and for the plant height it ranges between 93.18 cm (genotype 9 - *A. cruentus*) and 160.78 cm (genotype 1 - *A. mantegazzianus*); foliage per plant between 12.89 (genotype 10 - *A. cruentus*) and 23.46 (genotype 1 - *A. mantegazzianus*); medium foliage length between 14.77 cm (genotype 9 - *A. cruentus*) and 26.72 cm (genotype 1 - *A. mantegazzianus*); medium foliage width between 6.30 cm (genotype 9 - *A. cruentus*) and 14.46 cm (genotype 1 - *A. mantegazzianus*); foliage mass per plant between 94.05 g (genotype 3 - *A. molleros*) and 246.81 g (genotype 1 - *A. mantegazzianus*) and seed mass per plant between 45.56 g (genotype 3 - *A. molleros*) and 67.55 g (genotype 1 - *A. mantegazzianus*).

NASLEĐIVANJE VISINE STABLJIKE OZIME PŠENICE

Veselinka Zečević, D. Knežević, Danica Micanović, M. Pavlović i D. Urošević

Institut za istraživanja u poljoprivredi «SRBIJA», Centar za strna žita, Kragujevac

U radu je ispitivan način nasleđivanja, efekat gena, kombinacione sposobnosti i komponente genetičke varijanse za visinu stabljkice kod četiri sorte ozime pšenice (Srbijanka, Partizanka, KG-56 i PKB-111). Ukrštanje sorti je urađeno po metodi dialela, a ispitivanja nasleđivanja izvršena su na biljkama F_2 generacije na uzorku od 60 biljaka. U nasleđivanju visine stabljkice preovladavala je superdominacija i parcijalna dominacija. Utvrđene su visoko signifikantne razlike za opšte i posebne kombinacione sposobnosti, što znači da u nasleđivanju ovog svojstva značajnu ulogu imaju aditivna i neaditivna komponenta genetičke varijanse. Najbolje opšte kombinacione sposobnosti za visinu stabljkice ispoljila je sorta KG-56, a najbolje posebne kombinacione sposobnosti pokazali su hibridi KG-56 x PKB-111, Srbijanka x PKB-111 i Partizanka x KG-56.

Ustanovljeno je da glavni deo genetičke varijanse čini dominantna komponenta u nasleđivanju visine stabljkice. U ekspresiji ovog svojstva preovladavali su dominantni u odnosu na recessivne gene. Ovo je potvrđeno frekvencijom dominantnih alela i odnosom ukupnog broja dominantnih prema recessivnim alelima. Genetička analiza je pokazala da je u nasleđivanju visine stabljkice došlo do superdominacije.

THE INHERITANCE OF PLANT HEIGHT IN WINTER WHEAT

Four winter wheat cultivars (Srbijanka, Partizanka, KG-56 and PKB-111) have been selected for diallel crossing in order to study the mode of inheritance, gene effect and genetic variance components for the plant height in F_2 generation. Sixty plants of parents and F_2 generation were used for analysis. The mode of inheritance was done on the basis of the significance of components of genetic variance and the regression analysis.

The inheritance of plant height in the most crossing combinations was over dominant and partial dominance. The combining ability analysis was found to be highly significant, which means both additive and non-additive type of gene actions. The best general combining ability manifested KG-56 cultivar, and the best specific combining ability have shown hybrids KG-56 x PKB-111, Srbijanka x PKB-111 and Partizanka x KG-56.

The genetic variance components, average degree of dominance and regression line indicated over dominance in the inheritance of plant height. The dominant alleles frequency was higher than recessive alleles frequency, which confirmed the ratio of dominant/recessive alleles.

**KOMBINACIONE SPOSOBNOSTI NASLEĐIVANJA VISINE PRVE MAHUNE
NEKIH LINIJA BORANIJE (*Phaseolus vulgaris L.*)**

M. Zdravković, Jasmina Zdravković, Ljiljana Stanković i N. Pavlović

Institut «SRBIJA», Centar za povrтарstvo, Smederevska Palanka

Visina formiranja prve mahune je važna sortna osobina od koje zavisi mogućnost mehanizovanog ubiranja useva boranije, što je osnova proizvodnje ove biljne vrste za prehrambenu i konzervnu industriju. Sorte koje nisko formiraju prve mahune, pri mehanizovanoj žetvi, usled nedovoljne visine, bivaju oštećene.

U ispitivanje su uključene se 6 divergentne roditeljske linije za proces selekcije na povećanje visine formiranja prve mahune. Za utvrđivanje opštih (OKS) i posebnih kombinacionih sposobnosti (PKS) pojedinih linija korišćen je matematički metod 2, model 1 (Griffing 1956). Vrednost parametra su utvrđivani u F_1 i F_2 generaciji potomstva. Analiza kombinacionih sposobnosti pokazuje delovanje aditivnih i dominantnih genskih efekata. Šumadinka x Zorka predstavljuju najbolju kombinaciju za dalji proces selekcije na povećanje visine prve mahune.

**COMBINING ABILITIES OF INHERITING FIRST POD HEIGHT OF SOME
FRENCH BEAN LINES (*Phaseolus vulgaris L.*)**

First pod height is an important characteristic of a cultivar, especially for French bean since it determines the mechanical harvesting. Cultivars with low first pod height may be cut, damaged or not harvested by mechanical harvesting.

In our investigation six different genotypes were used for examination of the first pod height on French beans and diallel analysis. The result was obtained by using method 2 model 1 (Griffing 1956) for analysis of combining abilities. The mode of inheritance was different, as the results showed - from intermediate to super dominance, for both generations (F_1 and F_2). Analysis of combining abilities showed both influence of additive and dominant gene effects. Combination Šumadinka x Zora is suitable for further breeding for first pod height.

SAZREVANJE I STARENJE-PROPADANJE PLODOVA PARADAJZA *RIN, U I RIN/U GENETSKE KONSTITUCIJE*

Jasmina Zdravković, Ž. Marković, Ljiljana Stanković, M. Zdravković i M. Damjanović
Institut «SRBIJA», Centar za povrтарstvo, Smederevska Palanka

Inhibitor sazrevanja (*rin*) gen predstavlja spontani recesivni mutant koji menja aspekte sazrevanja ploda, a najznačajniji, sintezu karotenoida posebno likopina. Takođe usporava starenje plodova. Paradajz spada u biljke koje imaju karakteristični vrhunac (pik) prilikom sazrevanja plodova. Genotipovi paradajza homozigoti za *rin* gen nemaju ovaj vrhunac u sazrevanju, tako da izostaje produkcija etilena i crvena likopinska boja.

Za potrebe ispitivanja procesa sazrevanja, korišćeni su materijali iz završnih ciklusa selekcije paradajza: čista linija S-49 (genetske konstitucije *u/u*, uniformnog sazrevanja), linija hom 4 (*rin/rin*, homozigot za *ripening inhibitor*) i hibridna kombinacija 449 F1 (*u/rin*, heterozigot za *ripening inhibitor*). Plodovi sa uniformnim sazrevanjem i hibridi sazrevali su isto vreme dok homozigoti za *rin* gene nisu sazrevali. Etilen stimuliše razvoj žute boje u *rin* plodovima stimuliše pojavu likopina. Posle tretmana etefonom (0.1%) (etilen) od plodova sa uniformnim sazrevanjem utvrđeno je brže propadanje, kod hibrida je utvrđen duži period izdržljivosti, odnosno sporije propadanje. Kod *rin* homozigota nije dobijena promena boje, ali su plodovi propadali ubrzani u odnosu na netretiranu varijantu. Tretman etilenom ubrzavao je proces sazrevanja, ali i starenja plodova paradajza.

Prolongacija sazrevanja kao posledica delovanja *rin* gena svrstava dobijene hibride u kasniju grupu sazrevanja, čime se gubi na ranostasnosti. S druge, strane dobija se na čvrstini plodova i na «shelf life», koji omogućava duži transport i skladištenje, a ukoliko se ubiraju zeleni plodovi i na koordiniranom sazrevanju prema potrebama tržišta.

Cilj ispitivanja je bio da se utvrdi ponašanje u sazrevanje *rin* heterozigota, kao i reakcija genotipova na tretman etrelom u cilju ublažavanja ekstremnih vrednosti kasnog sazrevanja.

MATURING AND SENESCENCE OF *RIN, U* AND *RIN/U* TOMATO FRUITS

Ripening inhibitor (*rin*) gene is spontaneous recessive mutant which changes fruit ripening aspects (most important synthesis of carotene, especially lycopene). It also delays fruit senescence. Tomato is a vegetable crop with specific maturing climax (pik). Tomato genotypes homozygote for *rin* gene does not have this maturing climax, so the ethylene production and red, lycopene colour does not appear.

In order to research the maturing process material from the final tomato selection cycle was used: pure line S-49 (genetic constitution *u/u*, with uniform ripening), line hom 4 (*rin/rin*, homozygote with *ripening inhibitor*) and hybrid combination 449 F1 (*u/rin*, heterozygote for *ripening inhibitor*). Fruits with uniform ripening and hybrids ripened simultaneously, while homozygote with *rin* gene did not ripe at all. Ethylene stimulates the appearance of yellow colour in *rin* fruits and the lycopene production. After treatment with ETEFON (0.1%) (Ethylene) fruits with uniform ripening senescence more quickly, while hybrids senescence slowly. *Rin* homozygotes did not change colour, but the fruits senescence more quickly comparing to control. Ethylene treatment speeded the maturing and senescence process in tomato fruits.

Extended maturing process as a result influence of *rin* gene, puts the created hybrids in late maturing tomatoes. On the other hand, the firmness of fruits is improved as well as the «shelf life», which enables longer transportation and storing, coordinated maturing according to market demands.

The aim was to research the maturing process of *rin* heterozygote, and the reaction of some genotypes to treatment with ETREL (Ethylene) in order to decrease extremely late maturing.

OCENA LINIJA PARADAJZA KAO DONORA POŽELJNIH ALELA ZA POPRAVKU KOMPONENTI PRINOSA ELITNOG HIBRIDA

T. živanović¹, S. Krstanović², Gordana Šurlan-Momirović¹ i A. Arsenović³

¹Poljoprivredni fakultet, Univerzitet u Beogradu, Zemun

²INI PKB Agroekonomik Padinska Skela, Beograd

³PIK «13 maj» Zemun, Beograd

Cilj istraživanja je identificirati linija koje mogu biti korišćene kao donori poželjnih alela za poboljšanje kvantitativnih osobina elitnog hibrida paradajza. Izučavane su sledeće osobine: dužina ploda, prečnik ploda, broj komora ploda, debljina perikarpa, broj plodova po biljci, masa ploda i masa ploda po biljci.

Komponente prinosa šest linija (B-99, Ma-127, M-29, ZJ-17, Kz-13 i Au-09) i njihovih 15 hibrida su izučavane na bazi poljskih ogleda u tri ponavljanja (2000. godine) po slučajnom blok sistemu.

Linija Kz-13 je pokazala pozitivne ili negativne i znacajne μG vrednosti za sve osobine. Ova linija, Kz-13 je ispoljila najviše i najznačajnije vrednosti ovog parametra za broj komora ploda, broj plodova po biljci i masu ploda. Srodnja je sa Au-09 roditeljem elitnog hibrida. Poboljšanje ovih osobina zajedno može biti izvedeno sa povratnim ukrštanjem hibrida B-99xAu-09 sa P_2 (Au-09) ili donorima (Ma-127, M-29, ZJ-17, Kz-13).

Ovi rezultati ukazuju da linije mogu biti korišćene kao donori poželjnih alele za poboljšanje komponentata prinosa elitnog hibrida. Na bazi rezultata linija pronadeni su najbolji potencijalni donori za poboljšanje elitnog hibrida B-99 x Au-09.

THE EVALUATION OF LINES OF TOMATO AS DONORS OF FAVORABLE ALLEGES FOR THE IMPROVEMENT OF YIELD COMPONENTS OF AN ELITE HYBRID

The aim of this study is to identify which of different lines may be useful as donor of favorable alleles in the improvement of the quantitative traits of the elite single cross hybrid. Fruit length, fruit diameter, number of locules per a fruit, pericarp thickness, number of fruit per plant, fruit weight and weight fruit per plant were investigate.

Yield components in six lines and their 15 hybrids were determined on the basis of field trials set up in three replications (2000. year) in a randomized block design.

Line, Kz-13 showed positive or negative and significant μG values of all traits. This line, Kz-13, expressed the highest significant value of this parameter for number of locules per a fruit, number of fruit per plant and fruit weight and could be useful in the improvement of these traits. This line was more closely related to the parent of the elite hybrid Au-09. Improvement of these traits should be carried out together by backcrossing the hybrid B-99xAu-09 to the P_2 (Au-09) or the donors (Ma-127, M-29, ZJ-17, Kz-13).

These results suggest that lines could be used as donor of favorable alleles for improvement of yields components of an elite hybrid. On the bases of results of lines were found to be the best potential donor for improvement of the elite hybrid B-99 x Au-09.

**VARIJABILNOST SADRŽAJA ETARSKIH ULJA U ČETINAMA DUGLAZIJE
(*Pseudotsuga menziesii* /Mirb./ Franco) IZ RAZLIČITIH PROVENIJENCIJA**Vera Lavadinović¹ i V. Isajev²¹Institut za šumarstvo, Beograd²Šumarski fakultet, Beograd

Analiza promenljivosti sadržaja etarskih ulja u četinama duglazije su među prvim ogledima u složenom sistemu komparativnih ispitivanja koja se sprovode pri introdukciji. U radu su predstavljeni rezultata analiza varijabiliteta, kvantiteta i kvaliteta sadržaja etarskih ulja dobijenog iz svežih četina duglazije poreklom iz šest Kanadskih provenijencija čije se prirodne populacije nalaze od $49^{\circ} 10'$ do $51^{\circ} 35'$ geografske širine, i u visinskom dijapozanu od 600 do 1070 m. Sadržaj etarskih ulja u četinama aliziran je metodama gasne hromatografije (GH) i kombinacije gasne hromatografije-masene spektrometrije (GH/MS). Monoterpeni su utvrđeni kao glavne komponente etarskih ulja. Razlike u sadržaju terpenskih komponenata razvrstalo je provenijencije u dve grupe, u jednu one koje su bogatije sa α -pinenom, kamfenom i bornil acetatom i u drugu one gde je veći sadržaj α - pinena, β -pinena and citronenela.

Poznavanje promenljivosti ispitivanih komponeneta etarskih ulja iz četina, od značaja je za upoznavanje genetskog potencijala selekcionisanih provenijencija, kao jednog od parametara bitnog za introdukciju duglazije na alohtona staništa u Srbiji.

**VARIABILITY OF THE CONTENT OF ESSENTIAL OIL IN THE NEEDLES
OF DOUGLAS FIR (*Pseudotsuga menziesii* /Mirb./ Franco) FROM DIFFERENT
PROVENANCES**

Analyses of the variability of the essential oils in needles of douglas fir, are among the first experiments in complex system of comparative research conducted in addition to introduction. Paper presents the results of the variability of quantity and quality content of essential oil in the fresh needles from six Canadian provenances of Douglas fir, originated from $49^{\circ} 10'$ to $51^{\circ} 35'$ latitude, and from 600 to 1070 m altitude. The essential oils were analysed by GC and GC-MS. Monoterpenes, α -pinen, camphene, β -pinen, citronenal and bornyl acetate were identified by mass spectra and retention time correlations, as major constituents. The differences in percentages of terpene components indicate division of six populations in two groups, one of them richer in α -pinen, camphene and bornyl acetate and the other in α - pinen, β -pinen and citronenal.

Knowledge about variability of investigated parameters, is of great significance for knowing about genetic potential of selected provenances, as one of essential parameter for introduction of Douglas fir on allohtoneus sites in Serbia.

MEĐUZAVISNOST KOMPONENTI VISINE U GERMPLAZMI SIRKA METLAŠA [*Sorghum bicolor* (L.) Moench]

V. Sikora

Naučni institut za ratarstvo i povrтарstvo, Novi Sad

U radu je izvršeno ispitivanje mepuzavisnosti komponenti visine (visina biljke, visina stabla, dužina metlice, dužina peteljki, dužina drške metlice, dužina rukavca lista zastavičara i eksponiranost metlice) u germplazmi sirkra metlaša. Dvogodišnji ogled se sastojao od kolekcije koja broji 450 genotipova. S obzirom na njeno poreklo i obim može se tvrditi da se radi o svetskoj kolekciji sirkra metlaša, koja obuhvata lokalne autohtone populacije, priznate i perspektivne sorte različitog porekla, kao i materijal proizšao iz programa oplemenjivanja.

Od svih komponenti visine pozitivne, visoko signifikantne genetičke korelacije su zabeležene između visine biljke i visine stabla ($r_g=0,986^{**}$) i dužine drške i eksponiranosti metlice ($r_g=0,910^{**}$). Vrednosti koeficijenata fenotipske korelacijske takope ukazuju na to da visina biljke u najvećoj meri zavisi od visine stabla ($r_f=0,984^{**}$), odnosno eksponiranost metlice od dužine drške ($r_f=0,887^{**}$). Značajne korelacije su još zabeležene između dužine drške i visine biljke ($r_g=0,702^*$; $r_f=0,646^*$), dužine drške i visine stabla ($r_g=0,693^*$; $r_f=0,614^*$) i dužine peteljki i dužine metlice ($r_g=0,673^*$; $r_f=0,677^*$). Dužina peteljki je osobina koja je u negativnoj korelaciji sa visinom biljke, visinom stabla, dužinom drške i eksponiranošću. Pored toga, u negativnoj korelaciji su još i eksponiranost metlice i dužina rukavca. Pošto se u praksi metlice sirkra metlaša skidaju ručno, koncepcija selekcije se uglavnom bazira na stvaranju niskih sorti sa eksponiranom metlicom.

INTERRELATIONSHIP COMPONENTS OF HEIGHT IN BROOMCORN [*Sorghum bicolor* (L.) Moench] GERMPLASM

The examination of interrelationship component of height (plant height, stalk height, lenght of panicle, fiber lenght, lenght of peduncle, flag leaf sheat lenght and panicle exsseration) in broomcorn germplasm was carried out. Two years experiment include collection of 450 broomcorn genotypes. Consider on its origin and volume it represent world broomcorn collection, which include local populations, grown and perspective varieties from different origin, as well as selection material.

High significant positive genetic correlation was record between plant height and stalk height ($r_g=0,986^{**}$) as well as lenght of peduncle and plant exsertion ($r_g=0,910^{**}$). Coefficients of phenotypic correlation also shows that plant height is mostly depend on stalk height ($r_f=0,984^{**}$) and panicle exsertion on lenght of peduncle ($r_f=0,887^{**}$). Significant correlation was record between lenght of peduncle and plant height ($r_g=0,702^*$, $r_f=0,646^*$), lenght of peduncle and stalk height ($r_g=0,693^*$, $r_f=0,614^*$) and fiber lenght and lenght of panicle ($r_g=0,673^*$, $r_f=0,677^*$). Fiber lenght is characteristic which is in negative correlation with plant height, stalk height, peduncle lenght and panicle exsertion. Negative correlation was also record between panicle exsertion and flag leaf sheat lenght. In praxis broomcorn panicle is harvested manauly, so the concept of selec-tion is based on low hights varieties with exsertion panicle.

GENI VIRULENTNOSTI I AVIRULENTNOSTI U POPULACIJI PARAZITA *Puccinia coronata avenae*

Mirjana Staletić, S. Stojanović i M. Milovanović

Institut za istraživanja u poljoprivredi «SRBIJA», Centar za strna žita, Kragujevac

Ispitivanja su obavljena u 1999. i 2000. godini u Centru za strna žita u Kragujevcu. Uzorci uredospora su prikupljeni sa različitim sorti ovsu u 23 lokaliteta na području Srbije. U laboratoriji su dobijene čiste kulture, kojima je inokulisan set izogenih linija sa Pc genima: Pc38, Pc39, Pc48, Pc50, Pc50-2, Pc54-1, Pc54-2, Pc55, Pc56, Pc58, Pc59, Pc60, Pc61, Pc62, Pc63, Pc64, Pc67 i Pc68. Geni virulentnosti *P. coronata avenae* su identifikovani na osnovama uzajamnih odnosa gena po teoriji «gen za gen» (Flor, 1955). Utvrđeno je postojanje 23 različite formule virulentnosti. Broj gena virulentnosti u identifikovanim patotipovima se kreće od 2 do 12. Najvirulentniji je bio patotip sa genima virulentnosti V48, V50, V50-2, V55, V56, V58, V59, V60, V61, V62, V64 i V67, dok je sa dva gena virulentnosti (V63+V68) bio samo jedan patotip. Najveći broj patotipova imao je 5, 6, 7 i 8 gena virulentnosti, dok je ukupan odnos virulentnih prema avirulentnim genima odgovarao teoretskom odnosu 1:2. Najveću frekvenciju su imali geni virulentnosti V54-2, V64, V67, V61, V50-2, V62, V60 i V50, a najmanju V63, V58, V54-1, V59, V38, V48, V55, V56, V39 i V68.

Dobijeni rezultati pokazuju da je genetička varijabilnost virulentnosti *P. coronata avenae* u Srbiji u 1999. i 2000. godini bila visoka, što upućuje na neophodnost iznalaženja novih donora Pc gena otpornosti radi njihovog korišćenja u oplemenjivanju ovsu na otpornost prema ovom patogenu.

THE VIRULENCE AND A VIRULENCE GENES IN POPULATION OF *PARASITE Puccinia coronata avenae*

Investigations were performed during 1999. and 2000. years in Center for small grains in Kragujevac. The samples of uredo-spores were collected from different oats cultivars in 23 locations of Serbia. In laboratory were obtained pure cultures, and by them was inoculated the set of isogenic lines with Pc genes: Pc38, Pc39, Pc48, Pc50, Pc50-2, Pc54-1, Pc54-2, Pc55, Pc56, Pc58, Pc59, Pc60, Pc61, Pc62, Pc63, Pc64, Pc67, and Pc68. The virulence genes of *P. coronata avenae* were identified on the basis of mutual relations of genes according to theory «gene for gene».

The existence of 23 different formulas of virulence was established. The number of virulence genes in identified patho-types was 2 to 12. The most virulent was patho-type with genes of virulence V48, V50, V50-2, V55, V56, V58, V59, V60, V61, V62, V64, and V67, until with two virulence genes (V63+V68) was only one patho-type. The greatest number of patho-types had 5,6,7 and 8 virulence genes, until the total relation of virulent to a virulent genes was in accordance with theoretic relation 1:2. The most frequent were virulence genes V54-2, V64, V67, V61, V50-2, V62, V60, and V50, and the least frequent were V63, V58, V54-1, V59, V38, V48, V55, V56, V39 and V68.

Obtained results point out that in 1999. and 2000. seasons, the genetic variability of virulence of *P. coronata avenae* in Serbia was high, which demonstrates on necessity of search of new donors of Pc genes of resistance for theirs future usage in oat breeding for resistance to this pathogen.

KORELACIJA PRINOSA I BROJA DANA IZMEĐU METLIČENJA I SVILANJA (ASI) KAO POKAZATELJA TOLERANTNOSTI PREMA SUŠI KOD HIBRIDA KUKURUZA

D. Bošev¹, Sofija Pekić² i Violeta Anđelković³

¹Fakultet za poljoprivredne nauke i hranu, Univerzitet «Čirilo i Metodije», Skoplje, R. Makedonija

²Poljoprivredni fakultet, Univerzitet u Beogradu, Beograd

³Institut za kukuruz «Zemun Polje», Beograd

Trogodišnje ispitivanje vršeno je u poljskim uslovima u sušnom regionu Ovčeg Polja - Makedonija. Ispitivano je osam komercijalnih hibrida selekcionisanih u Institutu za kukuruz «Zemun Polje» i kompanije «Pioneer». Postavljena su dva poljska ogleda, u uslovima suvog ratarenja (suše) i navodnjavanja tokom vegetativnog perioda. Cilj je bio utvrđivanje korelacije prinosa zrna i broja dana između metličenja i svilanja (ASI).

Prosečan prinos suvog zrna po hektaru u uslovima navodnjavanja bio je od 7.37 t/ha do 12.1 t/ha. U sušnim uslovima, bez navodnjavanja, najviši prinos zrna iznosio je 4.58 t/ha, a najniži 3.27 t/ha.

Prinos zrna ZP hibrida u uslovima navodnjavanja bio je u pozitivnoj korelaciji sa ASI ($r = 0.91$), a u uslovima suše u negativnoj ($r = -0.97$). Hibridi «Pioneer» imali su takođe pozitivnu međuzavisnost prinosa zrna i ASI u uslovima navodnjavanja ($r = 0.83$) i negativnu u uslovima suše ($r = -0.89$), što je u saglasnosti sa drugim istraživanjima u uslovima suše.

Dobijeni rezultati ukazuju na karakteristike i potencijal ispitivanih hibrida za uslove sušnih regiona.

THE CORRELATION YIELD - ASI AS INDICATOR OF THE DROUGHT TOLERANCE OF SEVERAL MAIZE HYBRIDS

In the period of three years, there has been established field investigation in the drought region of Ovce Pole - Macedonia. The research has been performed in eight commercial hybrids of maize, selected by Maize Research Institute «Zemun Polje» and «Pioneer». We used two field trials, one with irrigation and another without irrigation (drought) during vegetative period. The goal was to find out correlation between yield and ASI (Anthesis Silking Interval).

Average yield of dry seed per hectare in the irrigated location was from 7.37 t/ha, up to 12.1 t/ha. In the drought location, the highest yield was performed by ZP360 (4.58 t/ha), and the lowest by ZP599 (3.27 t/ha).

The yield of ZP hybrids planted in the irrigated location, was in positive correlation to ASI ($r = 0.91$), but in the drought conditions, the correlation was negative ($r = -0.97$). The Pioneer hybrids had also positive correlation ($r = 0.83$) in irrigated conditions, and negative ($r = -0.89$) in drought conditions, so the relation to ASI is in accordance to previously reported results in drought conditions.

These results indicate performance and potential of the investigated maize hybrids in the drought regions.

HEMIJSKI SASTAV ZRNA KUKURUZA RAZLIČITIH GRUPA ZRENJA I USLOVA GAJENJA

D. Bošev¹, G. Vasilevski¹ i Z. Bošev²

¹Fakultet za poljoprivredne nauke i hranu, Univerzitet «Ćirilo i Metodije», Skoplje, R. Makedonija

²Ministarstvo za ekologiju i fizičko planiranje, Skoplje, R. Makedonija

Ovo istraživanje je obavljeno tokom tri godine u dva poljska ogleda, u uslovima sa i bez navodnjavanja. Ispitivano je osam hibrida kukuruza koji pripadaju različitim FAO grupama zrenja. Cilj istraživanja bio je utvrđivanje razlike u dužini vegetacije u različitim uslovima gajenja i u hemijskom sastavu zrna kukuruza.

Najveće promene u hemijskom sastavu zrna, u zavisnosti od grupe zrenja i navodnjavanja, utvrđene su za sadržaj proteina i šećera. Sadržaj proteina bio je najviši za hibrid ZP480 i u uslovima suše (13.6%) i u navodnjavanju (12.2%). Sadržaj šećera u navodnjavanju bio je najniži kod hibrida ZP360 (67.23%), a najviši kod hibrida ZP599 (69.3%). U uslovima suše najviši sadržaj šećera bio je kod hibrida ZP360 (69.69%), a najniže vrednosti kod ZP599 (65.72%).

Ovakva variranja u sadržaju šećera i proteina su posledica reakcije hibrida na različite uslove gajenja i osetljivosti prema suši. Rezultati mogu biti od značaja za preporuku hibrida u različitim regionima u Republici Makedoniji.

CHEMICAL CONTENT OF THE MAIZE KERNEL IN DIFFERENT MATURITY GROUPS AND GROWING CONDITIONS

This investigation has been established in two trial fields, during three years, where have been used irrigated field and non-irrigated field. The research has been performed in eight hybrids of maize (ZP360, ZP480, ZP599, ZP677, Stira, Colomba, Cecilia, and Constanza), which belongs to different maturity groups.

The aim of this research was to identify duration of vegetative period in different growing conditions and to investigate chemical content of kernels.

Regarding chemical content of basic metabolites into maize seed, the biggest variations were detected for protein and sugar content.

The percent of proteins was highest in ZP480, in both the irrigated (12,2%) and drought (13,6%) location. The content of sugars in irrigated locations was lowest in ZP360 (67,23%) and highest in ZP599 (69,3%). In the drought location highest percent of sugars was noted in ZP360 (69,69%), while lowest figure noted in ZP599 (65,72%).

These variations are results of reaction of the hybrids to different cultivated conditions and their susceptibility to drought. The results of this paper could help to recommend hybrids for different regions of the agricultural complex in Republic of Macedonia.

**PATERN ANALIZA U OPLEMENJIVANJU KUKRUZA 1.
- PRINOS ZRNA**

M. Babić, Violeta Andelković, N. Delić i G. Stanković

Institut za kukuruz «Zemun Polje», Beograd-Zemun

Ogledi u pojedinačnim spoljašnjim sredinama ne omogućuju donošenje opštih zaključaka koji se tiču testiranih genotipova. Zato se ogledi izvode u više spoljašnjih sredina što je najčešće praćeno pojavom značajne interakcija genotipa i spoljne sredine za kvantitativna svojstva. Sa povećanjem razlika između genotipova i spoljašnjih sredina, interakcija genotipa i spoljašnje sredine se povećava i postaje značajnija. Značajna interakcija genotipa i spoljne sredine za kvantitativna svojstva, kao što je prinos zrna, umanjuje, kod superiornih genotipova, upotrebljivost srednje vrednosti za sve lokalitete.

Združene tehnike klasifikacije i ordinacije omogućuju grupisanje genotipova na osnovu sličnosti koju pokazuju u ogledima izvedenim u više spoljašnjih sredina (najčešće lokacija) što je od izuzetne vaznosti u uslovima izrazene interakcije genotipa i spoljašnje sredine kada sama prosečna visina prinosa najčešće nije dovoljno pouzdan parametar. Ovaj vid analize nije dovoljno primenjen u uobičajenom oplemenjivačkom radu delimično iz razloga što se kod svake od integrisanih tehnika (klasifikacija i ordinacija) pruža više mogućnosti, tako da novim korisnicima često izgleda teško napraviti pravi izbor metoda, dijagnostike i grafike, kako kada se radi o tehnikama klasifikacije tako i kada se radi o tehnikama ordinacije.

**PATERN ANALYSIS IN MAIZE BREEDING 1.
- GRAIN YIELD**

Experiments in single environment (location or year) do not allow drawing a general conclusion regarding the tested genotypes. Breeders want to know how the genotype reacts in wide range of environments. So, Multi Environment Trials are essential for breeding process. Most frequently, multi environment trials (MET) are followed by significant genotype by environment interaction. Significant Genotype by Environment interaction for quantitative traits, such as grain yield, reduces the usefulness of genotype means, over all environments, for selecting superior genotypes. As the range of genotypic and environmental differences widens, Genotype by Environment interaction often become large and more apparent.

Because proximity measures occur in pairs where both, similarity and dissimilarity measures exploit the same type of informations, companion classification and ordination techniques can be achieved. They complement each other in analysis of GE data, and combined analysis is more valuable, especially if GxE interaction is significant (usefulness of genotype means, over all environments, for selecting superior genotypes is reduced).

This analysis seems to be not enough exploited in routine breeding work partly because within both of integrated techniques the choice of method, diagnostics and graphics is required, which seems to be problem for new user.

**PATERN ANALIZA U OPLEMENJIVANJU KUKRUZA 2.
ASI (BROJ DANA IZMEĐU METLIČENJA I SVILANJA)**

Violeta Andelković, M. Babić, N. Delić, G. Saratlić i G. Stanković

Institut za kukuruz «Zemun Polje», Beograd

Kukuruz, kao i većina drugih žitarica, najveće potrebe za vodom ima u vreme cvetanja, kada se formira broj zrna. Vodni deficit u vreme cvetenja može uticati na smanjenje prinosa zrna koje 2-3 puta veće u odnosu na sušu u drugoj vegetativnoj fazi.

Broj dana između metličenja i svilanja (ASI) je značajan pokazatelj vodnog statusa biljke, koji je u negativnoj korelaciji sa prinosom zrna u uslovima suše. Oplemenjivanje kukuruza u pravcu povećanja tolerantnosti prema suši u vreme cvetanja, je veoma kompleksan proces.

Primena novih analitičkih metoda omogućava grupisanje i izdvajanje genotipova sa određenim karakteristikama. Klaster analiza je veoma pogodna i za organizovanje genotipova sličnih u odnosu na ASI. Kombinacija rezultata klastera za ASI i prinos zrna u različitim sredinama, može biti veoma korisno u procesu oplemenjivanja kukuruza.

**PATERN ANALYSIS IN MAIZE BREEDING 2.
ASI (ANTHESIS-SILKING INTERVAL)**

Maize, like other crops, has the biggest water deficit at flowering time during grain yield formation. Drought at flowering could decrease grain yield 2-3 fold more than drought in other vegetative phases.

ASI is very important indicator of water status in maize, which is negatively correlated with grain yield during drought. Breeding maize for improved drought tolerance at flowering time is very complex.

Application of new analytical methods allows to group together genotypes with similar characteristics. Cluster analysis is very effective in organizing genotypes regarding to ASI. Combination of clustering results for ASI and grain yield in different environments could be very useful in breeding programmes in maize.

NOVA SORTA OZIMOG TRITIKALEA TRIJUMF

M. Milovanović, V. Perišić i Mirjana Staletić
IIP «SRBIJA», Centar za strna žita, Kragujevac

U radu su prikazani rezultati ispitivanja (ogledi sortne komisije SCG i Centra za strna žita u Kragujevcu) novopriznate KG sorte ozimog 6x tritikalea (linija KG. 64/3) koja je dobila ime Trijumf. Sorta je priznata 2003. godine od strane sortne Komisije Republičkog Ministarstva Poljoprivrede i Vodoprivrede RS, autor Dr Milivoje Milovanović. Po razviću spada u prave ozime tipove tritikalea, srednje je kasna sorta i izuzetno dobre otpornosti prema važnijim bolestima i štetočinama u uslovima SCG. Srednje je visoke stabljike (105-115 cm), veoma elastične, čvrste i otporne prema poleganju. Odlikuje se izuzetno visokim koeficijentom produktivnog bokorenja i moćnim korenovim sistemom, što doprinosi da postiže veoma dobar sklop, dobro podnosi sušu i marginalna zemljišta. Po prosečnim prinosima je visoko značajno nadmašila standardnu sortu tritikalea KG. 20. Pored visokih i stabilnih prinosa odlikuje se i veoma dobrim parametrima kvaliteta zrna. Ima viši sadržaj proteina od standarda KG. 20 i komercijalnih sorti ozime pšenice. Krupno-zrna je sa masom 1000 zrna iznad 40 g i prosečnom hektolitarskom masom oko 78 kg, što čini da je skoro dostigla po nalivenosti i kvalitetu zrna komercijalne sorte hlebne pšenice. Optimalno vreme za setvu ove sorte je u prvoj polovini meseca oktobra, upotreboom 500-650 kljavih zrna po m² (200-250 kg/ha), pri čemu za žetvu stiže u prvoj dekadi jula. Najbolje rezultate postiže na srednje plodnim i plodnim zemljištima upotreboom 100-120 kg/ha čistog N i odgovarajućih kolicina P i K hrani.

NEW CULTIVAR OF WINTER TRITICALE TRIUMPH

In the paper were shown results of investigation (trials of cultivars approval Commission of SCG and Center for Small Grains in Kragujevac) of new recognized KG cultivar of winter 6x triticale (line KG. 64/3), which obtained name Triumph. This cultivar was recognized in 2003rd by the cultivar approval Commission of Republic Ministry of Agriculture RS, and author is dr Milivoje Milovanovic. According to development it belongs to real winter types of triticale, medium late, with exceptionally high resistance to the most important diseases and pests in conditions of SCG. Its medium high stem (105-115 cm) is very elastic, strong and resistant to lodging. It distinguishes with exceptionally high coefficient of productive tillering and powerful roots system, which contributes to its achieving of very good stand of plants, as well as high drought and marginal soils tolerance. According to its average grain yield, Triumph surpassed standard triticale cv. KG. 20 high significantly. Besides of high and stable grain yield, also it distinguishes with very good parameters of kernel quality. Its content of protein is higher than in standard cv. KG. 20 and commercial winter wheat cultivars. Its 1000 kernels weight is above 40 g with mean mass of hectoliter about 78 kg, which makes that it nearly attained commercial (bread) winter wheat cultivars according to its kernel filling and quality. Optimal sowing term for this cultivar in conditions of SCG is in first half of October, by applying 500-650 sprouting seeds per m² (200-250 kg/ha), in which case its harvest is usually in the first decade of July. This cultivar achieves the best results on moderate fertile and fertile soil types by applying 100-120 kg/ha of pure N and adequate quantities of P and K fertilizers.

GENETIČKA SLIČNOST GENOTIPOVA SOJE DOBIJENA NA OSNOVU GENETIČKIH MARKERA

A. Nikolić, M. Srebić i S. Mladenović-Drinić

Institut za kukuruz «Zemun Polje», Beograd-Zemun

Genetička osnova soje je veoma uska. Tradicionalno, genetički diverzitet soje procenjivao se na osnovu morfoloških i agronomskih svojstava ili na informacijama o poreklu. Cilj ovog istraživanja je bio da se proceni informativnost izabranih genetičkih markera da bi se utvrdio genetički diverzitet između 20 genotipova soje, kao i svrstavanje genotipova soje u određene grupe u zavisnosti od genetičke sličnosti.

U ovom radu korisitli smo morfološke, proteinske i RAPD markere za utvrđivanje genetičke sličnosti. Proteini semena su izolovani i razdvojeni PAA elektroforezom. Genomska DNK iz ovih genotipova karakterisana je RAPD markerima korišćenjem 20 izabranih prajmera. Na osnovu prisustva/odsustva traka na proteinskim gelovima/amplifikovanih DNK fragmenata izračunati su koeficijenti sličnosti između parova genotipova. Za klaster analizu korišćen je UPGMA metod, a za neophodna proračunavanja korišćen je NTSYS-pc program.

Prema dobijenim rezultatima, genotipovi soje su svrstani u odgovarajuće grupe. RAPD markeri su detektovali viši nivo polimorfizma nego proteinski markeri. DNK markeri kao što su RAPD markeri su informativniji u proceni genetičkog diverziteta nego proteinski markeri.

GENETIC SIMILARITY AMONG SOYBEAN GENOTYPES REVEALED BY GENETIC MARKERS

The genetic diversity of cultivated soybean is very narrow. Traditionally, its estimation has been based on the differences in morphological and agronomic traits or pedigree information.

The objective of this research were to evaluate informativeness of chosen genetic markers for estimating genetic diversity among 20 soybean genotypes, and assigning soybean genotypes into groups according to genetic similarity.

In this study, we used morphological, protein and RAPD markers to validate their genetic relationship. Seed proteins were isolated and separated by PAA electrophoresis. Genomic DNA from these genotypes was characterized by RAPD markers with 20 selected decamer primers. On the basis of the presence/absence of bands in protein gels/amplified DNA fragments, coefficients of similarity were calculated between pairs of genotypes. The similarity matrix was submitted for hierachial cluster analysis of unweighted pair group using arithmetic average (UPGMA) method and necessary computation were performed using NTSYS-pc program.

According to obtained results, soybean genotypes were assigned to corresponding groups. RAPD markers detected higher level of polymorphism in soybean than protein markers. DNA markers such as RAPDs are more informative in estimating genetic diversity compared with protein markers.

GENETIČKE OSNOVE EKSPRESIJE ADAPTIVNOSTI PŠENICE NA USLOVE SPOLJAŠNJE SREDINE

D. Knežević¹, Ana Pešić² i Vesna Stevanović³

¹Institut za istraživanja u poljoprivredi «SRBIJA», Centar za strnu žita, Kragujevac

²Prirodno-matematički fakultet, Institut za biologiju, Kragujevac

³Agronomski fakultet, Čacak.

Proučavane su komponente prinosa za 2 genetički divergenci sorte pšenice (KG-35265 i KG-52) gajene u poljskim uslovima zalivanja vodom različitog stepena čistoće (kontrola=bez zalivanja, voda sa česme, voda čistih ekosistema, poluzagađena voda i zagađena voda). Reakcija proučavanih genotipova je ocenjena na osnovu analiziranih njihovih morfoloških i produktivnih osobina. Efekat zalivanja je analiziran. Takođe je analizirana razvijenost i masa korena kod proučavanih genotipova. Najveći prinos zrna dobijen je na varijantama zalivanim vodom sa česme i vodom iz čistih ekosistema a najmanji na varijanti bez zalivanja.

EXPRESSION OF WHEAT GENETIC ADAPTABILITY ON ENVIRONMENTAL CONDITIONS

Yield components of wheat were studied in two genetic divergent cultivars (KG-35265 and KG-52) which grown under irrigation condition. Water was from source with different degree of purity (control = without, common water, water from pure ecosystem, half polluted an polluted water). Reaction of investigated genotypes was estimated on the base of analyzed morphological and productive traits. Effect of water was analyzed. At the investigated genotypes the mass of root was analyzed, too. The highest value of grain yield was obtained on variant irrigated by pure water in compare with control.

**GENETIČKA OSNOVA KUKURUZNOG OKLASKA – MORFOLOŠKA
GRAĐA, PROIZVODI I UPOTREBA**

Milica Radosavljević

Institut za kukuruz «Zemun Polje», Beograd-Zemun

U Institutu za kukuruz razvijen je originalni tehnološki proces prerade kukuruznog oklaska kojim se dobijaju različiti lignocelulozni proizvodi namenjeni za upotrebu u različitim granama industrije, poljoprivredi i zaštiti životne sredine. Za kukuruzni oklasak kao vrednu prirodnoubovnovljivu polaznu sirovinu za industrijsku preradu, kao i za ostale namene veoma je bitna njegova morfološka građa i zahteva se njegovo fizičko prilagođavanje, odnosno mehaničko usitnjavanje, granuliranje i otprašivanje.

U ovom radu su date morfološka građa oklaska dvadeset različitih ZP hibrida kukuruza, kao i fizičkohemijske karakteristike i hemijski sastav različitih frakcija oklaska dobijenih po originalnoj ZP tehnologiji. Pored toga, detaljno su opisane i zbirno prikazane mogućnosti korišćenja kukuruznog oklaska.

**GENETIC BASE OF THE MAIZE COB - MORPHOLOGICAL
STRUCTURE, PRODUCTS AND UTILISATION**

The original technological process of maize cob processing was developed at the Maize Research Institute, Zemun Polje. This process provides the production of different lignocellulose products intended for utilisation in various industrial sectors, agriculture and environmental protection. A morphological structure of maize cob as a valuable naturally renewable raw material for industrial processing and other purposes is very important and therefore it is necessary to physically adopt the cob for different purposes, i.e. its mechanical pulverisation, pelleting and air separation are required.

This paper presents the morphological structure of cobs of twenty different ZP maize hybrids, as well as, physical and chemical properties and the chemical composition of various cob fractions produced by the original ZP technology. Besides, possibilities of maize cob utilisation are described in detail and summary presented.

**POLIMORFIZAM MOLEKULARNIH MARKERA KOD INBRED
LINIJA KUKURUZA I HETEROZIS**

G. Drinić, J. Srdić, S. Mladenović-Drinić i K. Konstantinov

Institut za kukuruz «Zemun Polje», Beograd

U radu je proučavana genetička divergentnost inbredovanih linija kukuruza proteinskim i RAPD markerima i korelacija dobijenih podataka sa ispoljenim heterozisom za prinos zrna u njihovim hibridnim kombinacijama. Deset inbredovanih linija, različitog porekla i dužine vegetacije dialelno su ukrštene i dobijene hibridne kombinacije su ispitane u tri lokacije. Najveći heterotični efekat je ispoljila kombinacija ZPPL15 i ZPPL 200. Ukupno je detektovano 42 proteinske frakcije od kojih je 76% polimorfno. Sa RAPD markerima je utvrđen viši nivo polimorfizma (81%). Klaster analiza na osnovu genetičke distance sa oba tipa markera kao i heterotičnog efekta za prinos zrna grupisala je ispitivane genotipove u dve grupe. Grupisanje na osnovu klaster i PCA analize odgovaralo je podacima o njihovom poreklu. Značajna korelacija utvrđena je između genetičke distance roditeljskih linija u njihovim hibridnim kombinacijama i heterozisa za prinos zrna tih hibrida.

**POLYMORPHISM OF MOLECULAR MARKERS OF MAIZE
INBRED LINES AND HETEROZIS**

The objective of the present study was to evaluate genetic diversity among maize inbred lines using polymorphism of protein and RAPD markers and to correlate single cross heterosis, to the genetic distance of the parental lines. Ten maize inbred lines of different origin and maturity were crossed diallelly without reciprocal crosses and their combinations were tested at three location. The highest heterosis for yield was detected in the combination ZPPL15 x ZPLL 200. The analysis of embryo salt soluble proteins showed that all studied genotypes have a specific protein pattern. Totally 42 protein fractions of different molecular weight were observed, from which 76% of protein fractions were polymorphic. With RAPD markers higher polymorphism was detected (81%). The cluster analysis based on genetic distance computed from protein and RAPD data as well as heterosis classifies each of 10 inbreds into two groups. It could be concluded from the present study that grouping of inbred lines by Cluster and PCA generally agree with the pedigrees of these lines. The correlation coefficient between heterosis for grain yield and genetic distances based on molecular markers are positive and mainly significant.

EFEKAT MUTANATA ENDOSPERMA NA KLIJAVOST SEMENA KUKURUZA

Zorica Pajić, Lana Đukanović i U. Erić

Institut za kukuruz «Zemun Polje», Zemun

Ispoljavanje genetičkog potencijala rodnosti i kvaliteta određenog genotipa zavisi, pored drugih faktora, i od kvaliteta semena. Seme je veoma važno kako za reprodukciju određene biljne vrste tako i za savremenu biljnu proizvodnju.

Svaki od delova semena kukuruza, perikarp, endosperm i klica, ima određenu funkciju u kompleksnom procesu kljanja i nicanja.

Ispitivana su tri genotipa različitog tipa endosperma: ZPSC 42A, hibrid standardnog kvaliteta zrna (zuban), ZPSC 504 *su* (hibrid šećerca sa *sugary* genom) i ZPSyn.II sh2 (sintetička populacija sa *shrunken2* genom).

Za određivanje vitalnosti semena navedenih genotipa korišćene su priznate ISTA metode: standardni metod, ubrzano starenje i cold test. Rezultati pokazuju da postoje razlike u kapacitetu kljanja ispitivanih genotipova. Najveće smanjenje kapacitet kljavosti i brzine nicanja ispoljeno je primenom metode ubrzanog starenja. Nastale razlike su verovatno posledica građe (tipa) endosperma, težine zrna, sadržaja šećera i debljine i sastava perikarpa.

EFFECT OF ENDOSPERM MUTANTS ON MAIZE SEED GERMINATION

The expression of genetic potential of yielding and quality of a certain genotype depends among other factors on seed quality. Seed is very important not only for the reproduction of the particular plant species, but also, for the contemporary plant production. Each part of maize seed (pericarp, endosperm and germ) has a specific function in the complex process of germination and emergence.

The following three genotypes of different endosperm types were observed: ZPSC 42A (standard grain quality dent hybrid), ZPSC 504 *su* (sweet corn hybrid with a *sugary* gene) and ZPSyn.II sh2 (synthetic population with a *shrunken2* gene).

Seed vitality of the stated genotypes was determined by the accepted ISTA methods: standard method, accelerating age and cold test. Obtained results point out to differences in the germination capacity of the observed genotypes. The greatest reduction of the germination capacity and the emergence rate was expressed by the application of the accelerating ageing method. Appeared differences are probably a result of the endosperm texture (type), grain weight, sugar content and pericarp thickens and composition.

SELEKCIJONO-GENETIČKA KARAKTERISTIKA SORTE KUKURUZA UZBEKSKA BELA

L. Kojić¹, Ajgozina Dillyara Serikbaevna²

¹Institut za kukuruz «Zemun Polje», Beograd

²OAO «PRODRESURS», Alma-Ata, Kazahstan

Pri otpočinjanju novih programa selekcije kukuruza (Stvaranje hibrida kukuruza visokog genetičkog potencijala rodnosti i kvaliteta zrna, Kazahstan, 1993) izučavanje početnog materijala je od presudne važnosti za uspešnu realizaciju samog programa.

Tako smo za ispitivanja izabrali sortu Uzbekska bela (Uz.b.) i ukrstili je sa devet inbred linija. Tri inbred linije potiču iz Amerike (FR 809 i B 73 tipa BSSS, i Mo 17 tipa Lancaster, 2 iz Jugoslavije – P 3/72-22 dobijene iz ukrštanja domaćih populacija Konjski zub x Domaći tvrdunac, L 17/17 dobijena iz sintetičke populacije Syn 6/9 (L) C0 tipa Lancaster i četiri inbred linije iz Zambije – L 7, N 3, L 9-2 i L 12-7). Uporedno sa ispitivanjem dobijenih kombinacija, koje smo ispitivali 1995 i 1996 godine u Alma-Ati, procesom samooplodnje dobili smo iz Uz.b. 6 inbred linija, ukrstili sa Mo 17 i ispitivali u uporednim ogledima tokom 2003. godine.

Sorta Uz.b. (subtropskog porekla) pokazala je prosečnu rodnost zrna za 2 godine ispitivanja od 8.045 kg/ha. To je manje za 45,9% od rodnosti hibrida B73 x Mo 17. Rodnost hibridnih kombinacija Uz.b. x P 3/72-22, Uz.b. x FR 809, Uz.b. x B 73 i Uz.b. x Mo 17 je na nivou hibrida B 73 x Mo 17 (razlike u prinosu nisu statistički značajne). Procenat heterozisa (izračunat je u odnosu na zajedničkog roditelja – Uz.b.) je visok i iznosi: Uz.b. x P 3/72-22 – 151,2%, Uz.b. x FR 809 – 148,2%, Uz.b. x B 73 – 146,5% i Uz.b. x Mo 17 – 146,4%. Hibridna kombinacija Uz.b. x L 17/17 dala je manji prinos zrna od B 73 x Mo 17 za 10,7% iako je procenat heterozisa iznosi 139,2%.

BREEDING-GENETIC CHARACTERISTICS OF THE UZBEKSKA BELA (UZBEKH WHITE) MAIZE VARIETY

When launching new maize breeding programs (Development of maize hybrids of high genetic potential for yield and grain quality, Kazakhstan, 1993) the study of initial material is of crucial importance for the successful realization of the programme.

Hence, we selected for the experiment the Uzbekska Bela (Uz.b.) variety and crossed it with nine inbred maize lines. Three inbred lines have their descent from the US (FR 809 and B 73 type BSS, and Mo 17 type Lancaster, 2 from Yugoslavia – P 3/72-22 that was obtained from a cross between the domestic populations Konjski zub (Horse tooth) x Domaći tvrdunac (Domestic flint), L 17/17 that was obtained from synthetic population Syn 6/9 (L) C0 type Lancaster and four inbred lines from Zambia – L 7, N 3, L 9-2 and L 12 - 7). Along with the testing of the offspring from the aforementioned combinations, which were tested in 1995 and 1996 in Alma Ata, we also obtained 6 inbred lines from Uz.b. by selfing, crossed them with Mo 17 and tested in parallel trials during 2003.

The Uz.b. variety (of subtropical origin) demonstrated average grain yield of 8,045kg/ha for 2 years of examinations. This is 45.9% less than the yield performance of the B 73 x Mo 17 hybrid. Yields of the hybrid combinations Uz. B. x P 3/72-22, Uz.b. x FR 809, Uz.b. x B 73 and Uz.b. x Mo 17 is on the level of the B 73 x Mo 17 hybrid (yield differences are not statistically significant). The heterosis ratio (calculated in relation to the common parent – Uz.b.) is high and amounts to: Uz.b. x P 3/72-22 – 151.2%, Uz.b. x FR 809 – 148.2%, Uz.b. x B 73 – 146.5% and Uz.b. x Mo 17 – 146.4%. The Uz.b. x L 17/17 hybrid combination had a 10.7% lower yield than the B 73 x Mo 17 combination, despite the fact that the heterosis ration amounted to 139.2%.

PRIMENA NEPARAMETARSKE STATISTIKE U OCENI INTERAKCIJE GENOTIP X SPOLJNA SREDINA

N. Delić, M. Babić, V. Andelković, G. Stanković i G. Saratlić

Institut za kukuruz «Zemun Polje», Beograd-Zemun

Spoljašnja sredina odnosi se na set negenetičkih faktora koji afektiraju fenotipsku vrednost varijeteta koje ispitujemo u našim ogledima. Ukoliko se genotipovi razlikuju u njihovim vrednostima za određene osobine u različitim spoljnim sredinama to ukazuje na postojanje interakcije genotip x spoljna sredina. Statistička analiza interakcije je od izuzetnog interesa u programima selekcije. Različiti statistički pristupi u oceni interakcije genotip x spoljna sredina su u primeni: komponente varijanse, multivariaciona analiza, regresioni metodi, klaster tehnike...

Istraživači u praktičnim situacijama nisu prvenstveno zainteresovani za numeričku kvantifikaciju interakcije genotip x spoljna sredina *per se* već samo za rekognosciranje iste.

Klasični parametrijski pristupi za analizu interakcije genotip x spoljna sredina počivaju na nekoliko prepostavki (normalitet distribucije, homogenost varijansi, aditivnost efekata). Ukoliko neka od ovih prepostavki nije ispunjena, validnost ovih metoda može biti narušena. Primenom neparametrijskih metoda, jednostavnih i lакih za primenu i analizu, izbegavaju se ove prepostavke.

U ovom radu, primenom nekoliko metoda neparametrijske ocene, analizirana je interakcije genotip x spoljna sredina za prinosa zrna 24 hibrida kukuruza (*Zea mays L.*) u šest lokaliteta. Primjenjena je korelacija ranga po Spearman-u i Kendallov koeficijent saglasnosti u smislu: 1) kvantifikovanja relacije genotip x spoljna sredina i 2) grupisanja lokaliteta i genotipova.

NON PARAMETRIC STATISTICAL APPROACH IN ESTIMATION OF GENOTYPE X ENVIROMENT INTERACTION

An environment is represented by whole set of nongenetic factors that affect the phenotypic value of varieties under investigation in our trials.

If the genotypes values for the particular trait differ through locations that indicate presence of the genotype x environment interaction. The statistical analysis of genotype x environment is of the outstanding interest in breeding programs. An different approaches in estimation of genotype x environment interaction are developed: variance components, multivariate analysis, regression methods, cluster techniques...

In many practical situations the breeders are not just interested to quantify the genotype x environment interaction *per se*, but to recognize presence or absence of the same one.

The mostly used, classical parametric approaches for an analysis of genotype x environment interaction are based on several assumptions: normality of the distribution, homogeneity of variances, additivity...

If some of mentioned assumptions are not fulfilled, the validity of these methods may be questionable. By use of nonparametric methods, which are simple and easy for analysis, all of the mentioned assumptions are avoided.

In this paper we used several of non parametric techniques for an analysis of genotype x environment interaction for grain yield of 24 maize (*Zea mays L.*) hybrids through six locations. The procedures proposed by Spearman (rank correlation) and Kendall (coefficient of concordance) are used in order of: 1) quantification of relation of genotype x environment 2) comparison and grouping of locations and genotypes.

**PROMENE VARIJABILNOSTI PRINOSA ZRNA
PRI SESTRINSKOM UKRŠTANJU SOJE**

Mirjana Srebić

Institut za kukuruz «Zemun Polje», Beograd – Zemun

Proučavali smo promene varijabilnosti prinosa zrna po biljci soje kod potomstava sestrinskih ukrštanja u odnosu na potomstva njihovih majki. Kao roditelji za sestrinska ukrštanja korišćene su tri najprinosnije F_3 linije razvijene iz ukrštanja sorti KUNITZ x KADOR. Svaka od ovih linija korišćena je po jednom kao materinska komponenta.

Testiran je prinos zrna po biljci potomstava sestrinskih ukrštanja i potomstava njihovih majčinskih linija. Promene varijabilnosti prinosa zrna su ocenjene preko koeficijenata varijacije (genotipskih i fenotipskih). Varijabilnost prinosa zrna po biljci je uglavnom bila blago povećana kod potomstava sestrinskih ukrštanja u odnosu na potomstva njihovih majki.

**THE CHANGES OF SEED YIELD VARIABILITY
IN SOYBEAN SISTER CROSSINGS**

The objective of experiment was the changes of seed yield per plant in progenies to F_3 soybean sister crosses in compare to progenies of their maternal lines. The three lines with the highest yield, derived from crossing KUNITZ x KADOR cultivars were utilized as parents. Each derived line was used as a maternal component once.

The grain yield per plant of both, lines and sister crosses of three combinations was tested. The changes of grain yield variability were evaluated by using coefficients of variation (genetic and phenotypic). The variability of soybean seed yield per plant was mostly delicate increased in progenies of sister crossings when compared to their maternal lines progenies.

NASLEĐIVANJE PRINOSA ZRNA SILAŽNOG KUKURUZA

M. Sečanski¹, T. živanović², G. Todorović¹ i Gordana Šurlan-Momirović²

¹Institut za kukuruz «Zemun Polje», Zemun Polje

²Poljoprivredni fakultet Univerziteta u Beogradu, Zemun

Cilj ovog istraživanja je bio da se za prinos zrna silažnog kukuruza procene: varijabilnost inbred linija i njihovih dialelnih hibrida, heterozis u odnosu na boljeg roditelja i komponente genetičke varijabilnosti i heritabilnosti na bazi dialelnog seta. Ogled je postavljen po slučajnom blok sistemu u četiri ponavljanja tokom dve godine na lokaciji Zemun Polje. Utvrđeno je da na varijabilnost ove osobine značajno utiču genotip, godina i njihova interakcija. Inbred linija silažnog kukuruza ZPLB402 je imala prosečno najveći, a ZPLB405 najmanji prinos zrna kukuruza u obe godine ispitivanja. Analiza komponenti genetičke varijanse za prinos zrna pokazuje da je aditivna komponenta (D) bila manja od dominantne (H_1 i H_2) genetičke varijanse, a komponenta F koja je bila pozitivna i frekvencija dominantnih gena (u) i recessivnih gena (v) za ovu ispitivanu osobinu ukazuju da dominantni geni preovlađuju nad recessivnim. Takođe ovo potvrđuje i odnos dominantnih prema recessivnim genima kod roditeljskih genotipova za prinos zrna ($Kd/Kr > 1$) koji je veći od jedinice u obe godine ispitivanja. Izračunata vrednost prosečnog stepena $\sqrt{H_1}/D$ dominacije je veća od jedinice, što pokazuje da se u nasleđivanju ove osobine radi o superdominaciji u obe godine ispitivanja. Rezultati Vr/Wr regresione analize ukazuju na superdominaciju u nasleđivanju prinosa zrna. Takođe je ustanovljeno i prisustvo nealelne interakcije, što ukazuje na potrebu proučavanja efekta epistaze, jer može imati veći značaj kod pojedinih hibrida. Veća vrednost dominantne od aditivne varijanse uticala je da se dobije visoka heritabilnost u širem smislu za prinos zrna u obe godine ispitivanja (98.71% (1997) i 97.19% (1998), a niska u užem smislu (11.9% (1997) i 12.2% (1998)).

INHERITANCE OF GRAIN YIELD OF SILAGE MAIZE

The aim of the present study was to evaluate the following parameters for the grain yield of silage maize: variability of inbred lines and their diallel hybrids, superior-parent heterosis and components of genetic variability and heritability on the basis of the diallel set. The two-year four-replicate trial was set up according to the randomised complete-block design at Zemun Polje. It was determined that a genotype, year and their interaction significantly affected variability of this trait. The highest, i.e. the lowest grain yield, on the average for both investigation years, was recorded in the silage maize inbred lines ZPLB402 and ZPLB405, respectively. The analysis of components of genetic variance for grain yield shows that the additive component (D) was lower than the dominant (H_1 and H_2) genetic variance, while a positive component F and the frequency of dominant (u) and recessive (v) genes for this observed trait point to prevalence of dominant genes over recessive ones. Furthermore, this is confirmed by the ratio of dominant to recessive genes in parental genotypes for grain yield ($Kd/Kr > 1$) that is greater than unity in both years of investigation. The estimated value of the average degree of dominance $\sqrt{H_1}/D$ exceeds unity, pointing out to superdominance in inheritance of this trait in both years of investigation. Results of Vr/Wr regression analysis indicate superdominance in inheritance of grain yield. Moreover, a registered presence of non-allelic interaction points out to the need to study effects of epistasis, as it can have a greater significance in certain hybrids. A greater value of dominant than additive variance resulted in high values of broad-sense heritability for grain yield in both investigation years (98.71%, i.e. 97.19% in 1997, i.e. 1998, respectively), and low values of narrow-sense heritability (11.9% in 1997 and 12.2% in 1998).

**NASLEĐIVANJE NEKIH MORFOLOŠKIH OSOBINA PRI HIBRIDIZACIJI
SORTI VINOVE LOZE DRENAK CRVENI I AFUZ-ALI**

Z. Bešlić, Slavica Todić i Vera Rakonjac

Poljoprivredni fakultet, Univerzitet u Beogradu, Beograd

Praćene su varijabilnost i način nasleđivanja morfoloških osobina vrha mladog lastara, lista, grozda i bobice u F₁ generaciji nastaloj ukrštanjem sorti Drenak crveni i Afuz-ali. Na osnovu opisa sejanaca izvršeno njihovo grupisanje po posmatranim obeležjima primenom propisane metode O.I.V-a. Zaključci o načinu nasleđivanja posmatranih osobina doneti su na osnovu rezultata χ^2 testa. U hibridnom potomstvu ispoljena je znatna varijabilnost svih proučavanih osobina. Za osobine vrha mladog lastara (intenzitet obojenosti vrha antocijanima, gustina dugih malja i kratkih dlačica), kao i za osobine razvijenog lista (veličina, oblik, izdeljenost, oblik zubaca, forma ureza) utvrđeno je odstupanje od odnosa razdvajanja karakterističnog za monogensko nasleđivanje. Za osobine lista kao što su gustina dugih malja i kratkih dlačica utvrđeno je da se nasleđuju monogenski. Od osobina grozda i bobice monogenski način nasleđivanja nije potvrđen kod veličine grozda, zbijenosti bobica, dužine peteljke, veličine i ujednačenosti veličine bobica, oblika i ukusa, dok se boja pokožice nasleđuje monogenski.

**INHERITANCE OF SOME MORPHOLOGICAL CHARACTERISTICS IN
HYBRIDIZATION OF GRAPEVINE CULTIVARS DRENAK CRVENI AND
AFUZ-ALI**

The variability and mode of inheritance of young shoot tip, leaf, grape and berry morphological characteristics were investigated in F₁ generation obtained by crossing of Drenak crveni and Afuz-ali. Based on seedlings description, considering investigated characteristics, grouping was done by applying O.I.V. method. The conclusions about inheritance of characteristics were obtained based on results of χ^2 test. The considerable variability was expressed in hybrid population. For properties of young shoot tip (intensity of anthocyanins, hairiness), and leaf characteristics (largeness, shape, dividuity, shape of tooth and incisions), exception from typical monogenic ratio was determined. The monohybrid inheritance was determined in density of leaf hairs and downs. The monohybrid mode of inheritance was not confirmed in grape characteristics (largeness, compactness and stem length) and berry characteristics (largeness, shape and taste), while skin color is monogenic propertie.

V tematska oblast / V topic:

Genetika čoveka i životinja
Human and animal genetics

Uvodna izlaganja / Introductory lectures

V-Uvo

DETEKCIJA MIKRODELECIIONIH SINDROMA PRIMENOM FLUORESCENTNE *in situ* HIBRIDIZACIJE

Danijela Drakulić, Gordana Nikčević i Milena Stevanović

Institut za molekularnu genetiku and genetičko inženjerstvo, Beograd

Mikrodelecioni sindromi obuhvataju heterogenu grupu poremećaja nastalih usled delecija specifičnih regiona hromozoma koje se ne mogu detektovati primenom standarnih teknika za analizu hromozoma. Gubitak genetičkog materijala može se analizirati primenom Fluorescentne *in situ* Hibridizacije (FISH). FISH je tehnika koja je bazirana na hibridizaciji fluorescentno obeležene probe specifične za određeni region hromozoma sa ciljnom DNK pacijenata prisutnom u metafaznim hromozomima. Pri tome odsustvo hibridizacije potvrđuje mikrodelecioni sindrom. Kao izvor materijala za pripremu hromozomskih preparata može se koristiti periferna krv, amnionska tečnost, horionske čupice, kao i uzorci tkiva.

Mikrodelecioni sindromi su vrlo često povezani sa pojavom specifičnih poremećaja, tako da klinička slika nosioca ukazuje na prisutvo specifične mikrodelekcije. Mikrodelekcija Del(22)(q11.2) je najzastupljenija kod mikrodelecionih sindroma i nju karakteriše prisustvo srčanih malformacija, poremećaja glave i lica, rascep nepca, kao i hipoplazija timusa i paratireoidnih žlezda. Zavisno od težine kliničke slike ova mikrodelekcija se može povezati sa nastankom nekoliko različitih sindroma koji uključuju DiGeorge sindrom, CTAF (Conotruncal Anomaly Face Sindrom) kao i velokardiofacijalni sindrom. U ovom radu prikazana je detekcija mikrodelekcije 22q11.2 kod pacijenata primenom mešavine proba koja se sastoji od Spectrum Orange TUPLE1 (HIRA) probe kao i Spectrum Green LSI ARSA kontrolne proba koja je mapirana na telomernom regionu duggog kraka hromozoma 22 u regionu 22q13.3.

DETECTION OF MICREDELETION SYNDROMES BY FLUORESCENT *in situ* HYBRIDIZATION (FISH)

Microdeletion syndromes are a heterogeneous group of disorders caused by deletion of specific regions of chromosomal DNA that are not visible using standard chromosome analysis. The loss of these genomic sequences can be demonstrated using FISH (Fluorescent *in situ* Hybridization) techniques. FISH is a technique that can be used to hybridize fluorescently labeled DNA probes to a specific chromosomal region, the loss of which results in a microdeletion syndrome. The probe for the region of interest and a control probe to the same chromosome are hybridized to metaphase chromosomes. Chromosomes from various sources including amniotic fluid, chorionic villi, peripheral blood, and tissue samples can be used for FISH to rule out microdeletion syndromes.

Specific abnormalities are often associated with microdeletion syndromes and the phenotype of the carriers of such chromosomal changes frequently give clues to the underlying deletion. Del(22)(q11.2) microdeletion is among the most common of the microdeletion syndromes and is characterized by cardiac malformations, craniofacial features, cleft palate, thymic hypoplasia, and hypoparathyroidism. The severity of the condition can be very variable and is now recognized as the basis of several independently described syndromes: DiGeorge syndrome, conotruncal face anomaly syndrome, and velo-cardiofacial syndrome.

Here we present detection of 22q11.2 microdeletion in the carriers using two color probe mixture that contains the Spectrum Orange TUPLE1 (HIRA) probe and the Spectrum Green LSI ARSA gene control probe that maps very close to the telomeric end of 22q at 22q13.3.

CITOGENETSKI I MOLEKULARNI PROFIL HRONIČNE MIJELOIDNE LEUKEMIJE

Biljana Todorović-živanović, Dragana Stamatović, Milica Strnad,

¹Koviljka Krtolica i Z. Magić

Vojnomedicinska Akademija, Beograd

¹Institut za nuklearne nauke «Vinča», Beograd

Hronična mijeloidna leukemija (CML) predstavlja klonalnu bolest matične ćelije hematopoeze. U preko 90% slučajeva karakteriše se prisustvom Philadelphia (Ph) hromozoma nastalog recipročnom translokacijom t(9;22)(q34;q11). Ph hromozom predstavlja prvu hromozomsku aberaciju povezану sa malignim oboljenjem kod ljudi. U 5-10% slučajeva Ph hromozom nastaje rearanžmanima drugačijim od klasične t(9;22). To su varijantne Ph translokacije. U 5-10% bolesnika Ph hromozom nije prisutan. Translokacijom t(9;22)(q34;q11) ćelijski protoonkogen c-abl smešten u 9q34 spaja se sa bcr genom smeštenim u 22q11 i formira himerni bcr-abl gen koji kodira protein sa povećanom tirozin kinaznom aktivnošću. Smatra se da upravo ovaj protein ima centralnu ulogu u patogenezi CML. U najvećem broju slučajeva formira se jedan od dva tipa bcr-abl rearanžmana b3a2 ili b2a2. Cilj ovoga rada bio je da se citogenetski ispitaju bolesnici sa CML kao i da se utvrdi učestalost formi bcr-abl gena. Citogenetski je ispitana grupa od 141 bolesnika sa CML. Nakon preparacije hromozoma iz koštane srži direktnom metodom i/ili nakon 24h kulture, citogenetski je analizirano 20 metafaza. RNK je izolovana iz mononuklearnih ćelija periferne krvi. RT-PCR metoda rađena je sa prajmerima za bcr-abl sekvencu. Klasična t(9;22)(q34;q11) detektovana je kod 122 (86.5%) bolesnika. Varijantne Ph translokacije kod 6 (4.3%) bolesnika. Normalan kariotip kod 13 (9.3%), 1 bolesnik je bio Ph negativan i bcr-abl negativan. U grupi od 47 bolesnika sa CML kod koje je utvrđeno prisustvo bcr-abl gena, b3a2 forma detektovana je kod 36 (76.6%) a b2a2 forma kod 11 (23.4%). Podaci dobijeni citogenetskim i molekularnim ispitivanjima naše grupe bolesnika poklapaju se sa podacima iz literature.

CYTOGENETIC AND MOLECULAR PROFILE OF CHRONIC MYELOID LEUKEMIA

Chronic myeloid leukemia (CML) is a clonally myeloproliferative disease of the pluripotent stem cell. Characteristic of the disease is a presence of Philadelphia (Ph) chromosome produced by reciprocal translocation t(9;22)(q34;q11) in more than 90% of the cases. Ph chromosome was the first consistent chromosome abnormality detected in a human malignancy. The Ph chromosome originates through other rearrangements than the classic t(9;22) in 5-10% of CML cases. Those are variant Ph translocations. The Ph chromosome is absent in 5-10% of cases. Cell proto-oncogene c-abl located at 9q34 is fused with bcr located at 22q11 by this translocation t(9;22)(q34;q11). Chimeric bcr-abl gene is coding protein with elevated tyrosine kinase activity. It is taught that this protein plays central role in pathogenesis of the CML. In the largest number of cases one of the two types of bcr-abl gene is formed: b3a2 and b2a2. The aim of this study was cytogenetic investigation of the group of CML patients and molecular investigation of the frequency of bcr-abl forms. We cytogenetically investigated the group of 141 patients (pts) with CML. Chromosomes were prepared from bone marrow aspirates by the direct method or/and after 24h culture. We analyzed 20 metaphases of each patient. RNA was isolated from peripheral blood mononuclear cells. RT-PCR method was done with primers for bcr-abl sequence. Classic t(9;22)(q34;q11) was detected in 122 (86.5%) pts, variant Ph translocation in 6 (4.3%) pts, normal karyotype in 13 (9.3%), and one patient was Ph negative and bcr-abl negative. In the group of 47 CML pts who were bcr-abl positive, b3a2 form was detected in 36 (76.6%) pts and b2a2 in 11 (23.4%). Results from this study are in correlation with results obtained from the literature.

V tematska oblast / V topic:

Genetika čoveka i životinja

Human and animal genetics

Usmena izlaganja / Oral presentations

V-Usm

ANALIZA KLONALNOSTI T-ĆELIJA U TUMORSKIM UZORCIMA BOLESNICA SA KARCINOMOM DOJKE

Bojana Cikota¹, Mirjana Branković-Magić² i Z. Magić¹

¹Institut za medicinska istraživanja, Vojnomedicinska akademija, Beograd

²Institut za onkologiju i radiologiju Srbije, Beograd

Rezultati brojnih istraživanja su pokazali da različiti tumori, uključujući i tumore dojke, mogu stimulisati specifični T-ćelijski antitumorski odgovor. Prepoznavanje tumor-specifičnog antiga (kojeg tumorska ćelija eksprimira endogeno ili egzogeno kao peptid prezentovan putem antigen-prezentujućih ćelija) rezultuje klonalnom ekspanzijom i diferencijacijom naivnih CD8⁺ T-ćelija u aktivirane efektorske ćelije koje učestvuju u ubijanju tumorskih ćelija. U ovom istraživanju je analizirana klonalnost T-ćelija u tumorskim uzorcima 54 bolesnice sa karcinomom dojke. Na osnovu prisustva/odsustva metastaza u regionalnim limfnim čvorovima bolesnice su klasifikovane u grupu N⁺ (prisutne metastaze; n=25) ili N₀ (bez metastaza; n=16). Analiza klonalnosti T-ćelija se zasnivala na PCR amplifikaciji dela rearanžiranog gena za lanac TCR-a i PAGE.

Prisustvo monoklonalne/oligoklonalne (M/O) populacije T-ćelija je detektovano u 9 bolesnica iz N⁺ i 6 bolesnica iz N₀ grupe. U svim analiziranim grupama (N₊ + N₀, N⁺, N₀) nije uočena statistički značajna razlika u učestalosti relapsa između bolesnica sa M/O u odnosu na bolesnice sa poliklonском T-ćelijskom populacijom. Poredjenjem dužine perioda bez bolesti (DFI) tokom prve dve godine u bolesnica sa M/O T-ćelijama u odnosu na bolesnice sa poliklonском populacijom je dobijena statistički značajna razlika granične vrednosti samo u N⁺ grupi. Bolesnice sa M/O T-ćelijama su imale kraći DFI od bolesnica sa poliklonском populacijom. Ova razlika nije uočena kada se analiza zasnivala na periodu praćenja dužem od 2 godine.

Dobijeni rezultati ukazuju da su populacije tumor infiltrirajućih limfocita uglavnom poliklonalne.

ANALYSIS OF T-CELL CLONALITY PATTERN IN TUMOR SAMPLES OF BREAST CANCER PATIENTS

Numerous experimental evidences confirm that different tumors, including breast carcinomas, can stimulate specific T-cell mediated immune response. Recognition of tumor-associated antigen (expressed endogenously by tumor cells or exogenously as peptide presented by antigen-presenting cells) results in the clonal expansion and differentiation of naive CD8+ T-cells into activated effector cells that mediate tumor cell killing. In this study we have analyzed pattern of T-cell clonality in tumor samples of 54 breast cancer patients classified as lymph node-negative – N₀ (n=16) or lymph node-positive – N⁺ (n=25). Analysis of T-cell clonality was based on PCR amplification of rearranged TCR γ chain gene followed by PAGE.

Monoclonal/oligoclonal (M/O) T-cell populations were found in 9 of N₊ and 6 of N₀ breast cancer patients, respectively. In all analyzed groups (N₊ + N₀, N⁺, N₀), incidence of relapse was not significantly different between patients with M/O T-cells vs patients with polyclonal T-cells. Comparison of disease free interval (DFI) between the patients divided according to the presence of TCRγ monoclonality/oligoclonality showed board-line significant difference only in the group of N⁺ patients within first 24 months of follow-up. Patients with M/O T-cell population had shorter DFI than patients with polyclonal T-cell population. Concerning the complete follow-up in the same group of patients, this difference was not observed.

Our results imply that tumor infiltrating T-cells are usually polyclonal.

MULTIPLEKS PCR KAO BRZ, SENZITIVAN I SPECIFIČAN METOD ZA DETEKCIJU PATOGENIH MIKROORGANIZAMA

Nataša Golić, L. Ranin, M. Kojić, Ivana Strahinić, Đ. Fira, Maja Vukašinović,
Amarela Terzić-Vidojević, Jelena Begović, Jelena Lozo, Katarina Krstić, B. Jovčić,
Maja Tolinački, Milica Nikolić i Lj. Topisirović

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

U poslednjih deset godina su razvijene različite metode za identifikaciju i tipizaciju prokariotskih i eukariotskih organizama na DNK nivou. PCR metod omogućava brzu i specifičnu detekciju širokog spektra bakterijskih vrsta. Multipleks PCR je brz, senzitivan i specifičan metod za detekciju patogenih mikroorganizama u jednoj PCR reakciji. Metod je zasnovan na PCR reakciji u kojoj se koristi nekoliko parova prajmera. Jedan set prajmera je najčešće specifičan za vrstu i potiče iz regiona 16S rDNK, te se na osnovu dobijenog produkta nedvosmisleno detektuje prisustvo specifičnog mikroorganizma. Drugi set prajmera je obično specifičan za gene koji su prisutni samo kod patogenih serotipova. PCR metod je izuzetno senzitivan i može da detektuje vrlo mali broj patogenih ćelija u uzorku (oko 10 ćelija). Štaviše, ovaj metod je brz i rezultati se dobijaju u toku jednog radnog dana. Dobijeni rezultati su nedvosmisleni. Prednost multipleks PCR metoda je da omogućuje detekciju specifičnih mikroorganizama odmah nakon infekcije, pre pojave prvih simptoma i stvaranja antitela, kao i nakon infekcije kada je titar antitela blizu normalnih vrednosti (ELISA test). U ovom radu predstavljamo multipleks PCR, kao metod za detekciju *Listeria monocytogenes*, *Yersinia enterocolitica*, *Helicobacter pylori*, *Mycoplasma pneumoniae*, *Chlamidia trachomatis*, *Chlamidia pneumoniae*, *Campilobacter jejuni*, *Borrelia burgdorferi*, i *Brucella sp.* iz kliničkih uzoraka.

MULTIPLEX PCR AS A RAPID, SENSITIVE AND SPECIFIC METHOD FOR DETECTION OF PATHOGEN MICROORGANISMS

In the past decade, various methods have been developed for the identification and typing of prokaryotic and eukaryotic organisms at the DNA level. The PCR approach allows rapid and specific detection of a wide range of bacterial species. Multiplex PCR is rapid, sensitive and specific method for detection of pathogen microorganisms in one PCR reaction. The method is based on PCR reaction in which several sets of primers are used. One set of primers is usually species specific, originated from 16S rDNA, that unambiguously detect the presence of a specific microorganism. The other set of primers is commonly specific for the genes that are predominantly present in pathogen serotypes. PCR method is extremely sensitive and very low amount of pathogen cells in the sample could be detected (approximately 10 cells). Moreover, this method is fast and the analysis could be completed in one working day. The results obtained by this method are unambiguous. The most important is that by using the multiplex PCR method it becomes possible to detect the presence of the specific microorganism immediately after infection, before the appearance of the first symptoms and the antibody generation, as well as after the infection when the titer of antibodies is close to referent values (ELISA test). In this work we describe the multiplex PCRs that are used for detection of *Listeria monocytogenes*, *Yersinia enterocolitica*, *Helicobacter pylori*, *Mycoplasma pneumoniae*, *Chlamidia trachomatis*, *Chlamidia pneumoniae*, *Campilobacter jejuni*, *Borrelia burgdorferi*, and *Brucella sp.* from the clinical specimens.

VARIJANTNE TRANSLOKACIJE I TIPOVI BCR/abl GENSKOG REARANŽMANA U HRONIČNOJ MIJELOIDNOJ LEUKEMIJI

Vesna Đorđević¹, Jelica Jovanović¹, Biljana Todorčić², Z. Magić²,
Koviljka Krtolica³ i Darinka Bošković¹

¹Institut za hematologiju Kliničkog centra Srbije, ²Vojnomedicinska akademija,

³Institut za nuklearne nauke «Vinča», Beograd

U radu je predstavljeno 19 bolesnika sa dijagnozom CML kod kojih je potvrđeno prisustvo varijantne translokacije u kariotipu. Citogenetska analiza urađena je direktnom preparacijom ćelija iz kostne srži bolesnika, a analiza hromozoma vršena je modifikovanom metodom – HG tehnikom traka. Kod dva (2/19) bolesnika detektovan je Ph hromozom nastao jednostavnim, varijantnim translokacijama i to: t(10;22)(q25;q11) i t(12;22)(p11;p12). U kariotipu preostalih 17 (17/19) bolesnika otkrivene su varijantne translokacije kompleksnog tipa.

Reverzna transkripcija-lančana reakcija polimeraze (RT-PCR) korišćena je za detekciju ekspresije *BCR/abl* fuzionisanih gena kod osam (8/19) bolesnika. Registrovan je *BCR/abl* genski rearanžman tipa b3a2 kod šest (6/8) bolesnika, dok je genski rearanžman tipa b2a2 bio prisutan kod jednog (1/8) bolesnika. Kod jednog (1/8) bolesnika sa jednostavnom, varijantnom translokacijom, ekspresija *BCR/abl* gena nije detektovana.

Rezultati citogenetskih i molekularnih ispitivanja CML bolesnika sa «varijantnim» Ph hromozomom prikazani u ovom radu, jasno ukazuju da varijantne translokacije hromozoma 9 i 22 predstavljaju veliki istraživački izazov, pogotovo ako se ima u vidu činjenica da tipovi rearanžmana *BCR/abl* fuzionisanog gena mogu biti različiti i drugačiji od specifičnih rearanžmana (b2a2 i b3a2) za CML.

VARIANT TRANSLOCATIONS AND THE FORMS OF BCR/abl REARRANGEMENT IN CHRONIC MYELOID LEUKEMIA

In this study, variant translocation in 19 patients with newly diagnosed CML, was presented. Cytogenetic analysis was performed on bone marrow cells after direct preparation. Chromosomal analysis was performed according to our modified method of HG-banding. Two (2/19) patients had simple variant Ph-producing translocations, and other (17/19) patients had complex variant translocations.

Reverse transcription-polymerase chain reaction (RT-PCR) for detection of the expression of *BCR/abl* sequence in eight CML patients was performed. *BCR/abl* rearrangement was found to be expressed in b3a2 form in six (6/8) patients, but in b2a2 form in one (1/8) patient. Using RT-PCR analysis in one (1/8) CML patient with simple variant translocation, we found that *BCR/abl* rearrangement was not expressed.

Results of cytogenetic and molecular investigations in cases of «variant» Ph chromosome CML reviewed in this study clearly indicate that variant translocations of chromosomes 9 and 22 are great research challenge, especially in case of rearrangement forms of *BCR/abl* hybrid gen that can be different from each other and different from specific rearrangements (b2a2, b3a2) for CML.

PROGNOŠTIČKI ZNAČAJ GENETIČKIH PROMENA DETEKTOVANIH KOD PACIJENATA SA NEUROBLASTOMOM

M. Đurišić¹, M. Guć-Šćekić¹, D. Đokić², D. Vujić³, I. Milović⁴, S. Đuričić⁵,
D. Radivojević¹, T. Lalić¹ i M. Đurić⁶

¹Laboratorija za medicinsku genetiku, ²Odeljenje hematoonkologije, ³Odeljenje za transplantaciju kostne srži i kriobiologiju, ⁴Odeljenje za kliničku patologiju, ⁵Hirurška klinika, ⁶Odeljenje za neurologiju, Institut za zdravstvenu zaštitu majke i deteta Srbije «Dr Vukan Čupić», Beograd

U periodu od januara 1997. do juna 2003. godine u Institutu za zdravstvenu zaštitu majke i deteta Srbije «Dr Vukan Čupić» u Beogradu je dijagnostikovano 47 pacijenata sa NB. Među njima je dvanaestoro (25,53%) bilo uzrasta ispod 1 godine. Citogenetička analiza je urađena kod 34 (72,34%) pacijenta. Kod 16 pacijenata bez infiltracije tumorskih ćelija u kostnu srž nađen je normalan kariotip. Kod 18 pacijenata sa diseminovanim tumorom rezultati citogenetičke analize su bili sledeći: 8 (44,4%) pacijenata je imalo normalan kariotip, a kod 10 (29,4%) pacijenata je nađena poliploidija u mozaiku sa normalnim ćelijama. Detektovani su sledeći aberantni kariotipovi: «near»-diploidija (± 46) u mozaiku (2 slučaja-stadijum IV), «near»-triploidija (± 69) u mozaiku (1 slučaj-stadijum IV), «near»-tetraploidija (± 92) u mozaiku (4 slučaja-stadijum IV), homogeno obojeni regioni (HSRs) i «double» minutni hromozomi (DMs)(1 slučaj-stadijum III), delecija 1p36 (1 slučaj-stadijum IV) i kompleksni kariotip (1 slučaj-stadijum IV). FISH u analizi hromozoma 1p36 je primenjen u 19 slučajeva, a PCR u 4 slučaja. MYCN analize su sprovedene na 27 tumora FISH metodom. Sedam od 27 NB tumora (25,92 %) (3 u stadijumu III, 3 u stadijumu IV i jedan u stadijumu IVs) je imalo amplifikovani MYCN.

PROGNOSTIC VALUE OF GENETIC FINDINGS IN NEUROBLASTOMA PATIENTS FROM SERBIA AND MONTENEGRO

In the present study 47 patients with NB were diagnosed at the Mother and Child Health Institute of Serbia «Dr Vukan Cupic», between January 1997. and June 2003. Among 47 NB patients 12 children (25,53%) were under the age of 1 year. Cytogenetic analysis was performed on 34 (72,34%) patients. In 16 patients with no bone marrow tumor cell infiltration normal karyotype was found. In 18 patients with disseminated disease cytogenetic results were as follows: 8 (44,4%) patients showed normal karyotype and in 10 (29,4%) patients poliploidy with normal cells were observed. The following aberrant karyotypes were detected: near diploidy (± 46) in mosaic (2 cases-stage IV), near- triploidy (± 69) in mosaic (one case-stage IV), near-tetraploidy (± 92) in mosaic (4 cases-stage IV), homogeneously staining regions (HSRs) and double minute chromosomes (DMs)(one case-stage III), deletion 1p36 (one case-stage IV) and complex karyotype (one case-stage IV). Chromosome 1p36 analysis was performed on twenty three patients using FISH (19 cases) and PCR (4 cases). MYCN analyses were performed on 27 tumors by FISH. Seven of 27 NB tumors (25,92%)(3 at stage III, 3 at stage IV and one at stage IVs) were with amplified MYCN.

ODREĐIVANJE UČESTALOSTI I ZNAČAJA POLIMORFIZMA GENA ZA ANGIOTENZINOGEN U RAZVOJU DIJABETESNE NEFROPATIJE

Vesna Ilić¹, M. Ilić² i Z. Magic¹

¹Institut za medicinska istraživanja VMA, Beograd

²Institut za endokrinologiju, dijabetes i bolesti metabolizma, KCS, Beograd

Hipertenzija, jedan od najvažnijih činilaca u razvoju nefropatije, vezuje se za hiperaktivnost sistema renin-angiotenzin (RAS) i povišenja intraglomerulske pritiska što predstavlja jedan od najznačajnijih faktora oštećenja glomerula. Kako se aktivnost renin-angiotenzin sistema smatra genetski određenom, polimorfizam gena za angiotenzinogen (AGT) i angiotenzin konvertujući enzim (ACE) predstavlja molekularni marker hipertenzije kao i marker sklonosti prema razvoju dijabetesne nefropatije.

Ispitivanje distribucije polimorfizma AGT gena u grupi pacijenata obolelih od dijabetesa tipa 1 u odnosu na stepen razvoja nefropatije.

Polimorfizam gena za angiotenzinogen (AGT) detektovan PCR-RFLP metodom korišćenjem restriktione endonukleaze Psy I (Tth 111I). Ispitivanje je obuhvatilo 42 pacijenta, starosti 20-40 godina sa tipom 1 dijabetesa, svrstanih u tri grupe. Prvu grupu je činilo 15 normoalbuminuričnih dijabetičara bez nefropatije (mikroalbuminurija $30\mu\text{g}/24\text{h}$), drugu grupu, 13 mikroalbuminuričnih dijabetičara odnosno pacijenata sa početnom, incipientnom nefropatijom (mikroalbuminurija $30-300\mu\text{g}/24\text{h}$) i treću sastavljenu od 14 pacijenata sa manifestnom nefropatijom (proteinurija veća od $300\mu\text{g}/24\text{h}$).

Učestalost MT i TT genotipa koji nose veliki rizik za razvoj hipertenzije i nefropatije kod dijabetičara sa tipom 1 dijabetesa je bila u grupi normoalbuminuričnih 67% (10 od 15 ptc.), u grupi mikroalbuminuričnih 92,8% (12 od 13 ptc.) i u grupi proteinuričnih 87% (12 od 14 ptc.).

Prisutan značajan porast zastupljenosti MT i TT genotipa kod ispitanika sa mikro i proteinurijom, ukazuje na vezu između ovih alelskih formi i progresije dijabetesne nefropatije kao kasne komplikacije dijabetesa.

DETERMINATION OF FREQUENCY AND IMPORTANCE OF AGT GENE POLYMORPHISM IN DIABETES NEPHROPATHY DEVELOPMENT

Hypertension, as the best predictor of high risk of developing diabetic nephropathy, is connected with hyperactivity of renin-angiotensine system inducing progression in intraglomerular hypertension as the leading cause of glomerular failures. While it is proved that the renin-angiotensine system activity is genetical determined, AGT and ACE gene polymorphism could be molecular marker for hypertension and diabetes nephropathy development.

To assess a distribution of the AGT gene polymorphism in a group of patients with diabetes type I according to the increase of diabetes nephropathy level.

Polymorphism was detected by polymerase chain reaction and restriction fragment length polymorphism using restriction enzyme Psy I (Tth 111 I).

The study group consisted of 42 patients (20-40 years old) divided in three groups: 15 normoalbuminuric patients without nephropathy (microalbuminuria $< 30\mu\text{g}/24\text{ h}$), 13 microalbuminuric patients with incipient nephropathy and 14 with manifesting nephropathy (proteinuria over $300\mu\text{g}/24\text{ h}$). The frequency of MT and TT genotip assosiated with increasing risk of hypertension and nephropathy in patients with diabetes type I was 67% (10/15) in patients with normoalbuminuria, 92,8% (12/13) in microalbuminuric patients and 87% (12/14) in patients with proteinuria.

A significant increase of MT and TT genotype in group of patients with micro and macroproteinuria, show the association between the presence of these alel forms and progression of diabetes nephropathy as the latest complication of diabetes.

MOLEKULARNA OSNOVA FENILKETONURIJE U SRBIJI

J. Jovanović¹, B. Petručev¹, M. Đurić³, N. Tošić¹, M. Čvorkov-Dražić², M. Đorđević²,
Lj. Stojanov² i S. Pavlović¹

¹Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

²Institut za zdravstvenu zaštitu majke i deteta «Dr Vukan Čupić», Beograd

³Institut bezbednosti, Beograd

Fenilketonurija (PKU) je najčešći urođeni poremećaj u metabolizmu aminokiselina kod Evropljana. Uzrokovanja je autozomno recesivnom deficijencijom hepatičnog enzima fenilalanin hidroksilaze (PAH). Nemogućnost konverzije fenilalanina u tirozin dovodi do povećanja fenilalanina u telesnim tečnostima i teške mentalne retardacije ukoliko se ne ograniči unos fenilalanina ishranom. U većini evropskih zemalja PKU se kompletno dijagnostikuje neonatalnim skriningom. Do sada je u PAH genu identifikovano više od 400 mutacija koje leže u osnovi PKU. Nedavno je pokazano da se na osnovu PAH genotipa može predvideti biohemski fenotip kod većine pacijenata sa PKU. Iako se biohemski neonatalni skrining sprovodi u Srbiji od 1980. godine, ovo je prva studija o molekularnoj osnovi PKU u našoj populaciji. Ispitano je 35 pacijenata, odnosno 68 nezavisnih hromozoma. Kod svih pacijenata testirano je prisustvo mutacija u egzonima 7 i 11 PAH gena (sa egzon/intron granicama) elektroforezom na gelu sa gradijentom denaturišućeg agensa (DGGE) i automatskim sekvenciranjem. Neke česte mutacije u drugim egzonima identifikovane su kombinacijom lančane reakcije polimeraze i digestije restripcionim enzimima (PCR-RFLP). Detektovano je devet različitih mutacija na 42 od 68 analiziranih hromozoma (procenat detekcije 62%). Šest mutacija nalazi se na ~60% mutiranih alela: L48S (22.06%), R408W (14.71%), P281L (5.88%), E390G (5.88%), R261Q (4.41%), R158Q (4.41%). Genetska osnova PKU u Srbiji je umereno heterogena. Sve detektovane mutacije su prethodno opisane u drugim evropskim populacijama koje su istorijski i geografski povezane sa srpskom populacijom. Relativno je velika učestalost mutacija sa blagim fenotipskim efektom. Definisanje profila PKU mutacija u Srbiji biće iskorišćeno za predviđanje težine bolesti, izbor odgovarajućeg dijetetskog režima i davanje genetičkog saveta.

GENSKA TERAPIJA KANCERA

Tatjana Mitrović

Prirodno-matematički fakultet, Odsek za biologiju sa ekologijom, Niš

Genska terapija kancera bi se moglo definisati kao transfer nukleinskih kiselina u tumorske ili normalne ćelije sa ciljem eliminacije ili redukcije tumorske mase direktnim ubijanjem ćelija, stimulisanim imunim sistemom ili korekcijom genetičkih grešaka i reverzijom malignog stanja. Trenutno se u svetu odvija 608 kliničkih protokola genske terapije kancera (66.2% od ukupnog broja tekućih protokola genske terapije).

Strategije koje su na raspolaganju u areni za borbu protiv kancera su: 1) terapija «genom samoubicom», 2) imunogenska terapija, 3) korekcija mutiranog tumor supresor gena i/ili blokada onkogena i proinflamatornih gena i 4) antiangiogenska terapija. Iako je pokrenuta sa dosta optimizma i entuzijazma, genska terapija kancera je do sada dala ograničene rezultate. Osim nekoliko slučajeva terapeutskih odgovora kod pacijenata, genska terapija kancera još uvek nije pokazala kliničku efikasnost što je uzrokovano pre svega veoma niskom transdukcijom i efikasnošću ekspresije *in vivo* trenutnih vektora. «Magični» vektor za gensku terapiju tumora bi trebao da poseduje sledeće karakteristike: da bude primjenjen neinvazivnim putem, da pogoda ne samo primarni tumor, nego i rasejane tumorske ćelije i mikrometastaze na udaljenim i nepristupačnim mestima i da nosi terapeutski gen sa tumor-restiktivnom, vremenski-regulisanom i postojanom ekspresijom. Ovaj rad daje pregled trenutnih poteškoća i beskrajnih nada i mogućnosti genske terapije.

CANCER GENE THERAPY

Cancer gene therapy can be defined as transfer of nucleic acids into tumor or normal cells with aim to eradicate or reduce tumor mass by direct killing of cells, induction of immune-mediated destruction or correction of genetic errors and reversion of malignant status. Presently, there are 608 cancer gene therapy clinical trials worldwide (66.2% of total number ongoing gene therapy clinical trials).

Available strategies in cancer gene therapy arena are following: 1) suicide gene therapy, 2) immunogene therapy, 3) tumor suppressor gene replacement and/or oncogene and proinflammatory gene blockage and 4) antiangiogenic therapy. Initially started with lots of optimism and enthusiasm, cancer gene therapy has shown limited success so far. Despite few reports of therapeutic responses in some patients, there is still no proof of clinical efficacy of most cancer gene therapy approaches, primarily due to very low transduction and expression efficacy *in vivo* of available vectors. «Magic» gene therapy vector should: be administrated through a noninvasive route, target not only primary tumor mass, but disseminated tumor cells and micrometastasis at distanced and unreachable sites as well, and carry therapeutic gene with tumor-restricted and time - regulated and sustained expression. This review highlights current hurdles and endless hopes and possibilities of cancer gene therapy.

VARIJABILNOST GENA ZA TIOPURIN S-METILTRANSFERAZU U SRBIJI – FARMAKOGENETIKA U KLINIČKOJ PRAKSI

B. Petručev¹, J. Jovanović¹, N. Tošić¹, D. Janić²,
L. Krivokapić-Dokmanović² i S. Pavlović¹

¹Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

²Univerzitetska dečja klinika, Beograd

Ova studija predstavlja jedno od pionirskih farmakogenetičkih istraživanja u našoj populaciji. Tiopurin S-metiltransferaza (TPMT) je citoplazmatski enzim koji katalizuje S-metilaciju (inaktivaciju) tiopurinskih lekova kao što su antitumorski agensi i imunosupresanti. Smanjena aktivnost TPMT enzima se manifestuje sa teškom hematopoetskom toksičnošću posle primene standardnih doza ovih lekova. Aktivnost TPMT enzima zavisi od genetske varijabilnosti. Nekoliko mutacija u genu za TPMT utiče na smanjenu fenotipsku aktivnost enzima. Nemutirani alel je označen kao TPMT*1, a mutirani aleli su: TPMT*2 (G238C), TPMT*3A (G460A i A719G), TPMT*3B (G460A) i TPMT*3C (A719G). Genotipovi su određivani kod 70 dece sa akutnom limfoblastnom leukemijom (ALL) u Srbiji, korišćenjem lančane reakcije polimeraze i analize polimorfizama dužine restrikcionih fragmenata (PCR-RFLP) i alel-specifičnog PCR-a. 60 pacijenata je imalo normalan genotip (85,8%), devet pacijenata TPMT*2/TPMT*1 genotip (12,8%) i jedan pacijent TPMT*3A/TPMT*1 genotip (1,4%). Da bismo odredili učestalosti TPMT alela u srpskoj populaciji, 100 zdravih davalaca krvi je takođe analizirano. 96% analiziranih je imalo normalan TPMT genotip, 3% TPMT*1/TPMT*3A genotip i jedan TPMT*1/TPMT*3B genotip. Naši rezultati su pokazali da je učestalost mutiranih alela TPMT*3A veoma visoka kod dece sa ALL. Uočena je statistički značajna razlika između učestalosti mutiranog alela TPMT*3A kod dece sa ALL i kod zdravih osoba. Svi pacijenti sa genetskim varijacijama razvili su neutropeniјu tokom primene standardnih doza tiopurina.

THIOPURINE S-METHYLTRANSFERASE GENETIC VARIATION IN SERBIA – PHARMACOGENETICS IN CLINICAL PRACTICE

This study represents one of the pioneer pharmacogenetic research in our population. Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes S-methylation (inactivation) of thiopurine drugs such as anticancer and immunosupressant agents. Decreased activity of TPMT is associated with severe hematopoietic toxicity after using standard doses of these drugs. Activity of TPMT enzyme depends on genetic background. There are several mutations in TPMT gene which give rise to low phenotypic activity. The wild type allele is designated as TPMT*1 and the mutant alleles are: TPMT*2 (G238C), TPMT*3A (G460A and A719G), TPMT*3B (G460A) and TPMT*3C (A719G). Genotypes were determined in 70 Serbian children with acute lymphoblastic leukemia (ALL), using polymerase chain reaction (PCR)-restriction fragment length polymorphism and allele-specific PCR assays. 60 patients had wild type genotype (85,8%), nine patients TPMT*3A/TPMT*1 genotype (12,8%) and one patient TPMT*2/TPMT*1 genotype (1,4%). In order to determine the frequencies of TPMT alleles in Serbian population, 100 volunteer blood donors were analysed as well. 96% of them had wild type TPMT genotype, 3% TPMT*1/TPMT*3A genotype and 1% TPMT*1/TPMT*3B genotype. Our results revealed that the frequency of mutated allele TPMT*3A is very high in children affected with ALL. Statistically significant difference between the frequency of mutated allele TPMT*3A in children affected with ALL and in healthy individuals was observed. All the patients with genetic variations revealed neutropenia during full dose of thiopurine administration.

MOLEKULARNA OSNOVA I POREKLO TALASEMIJSKIH SINDROMA U SRBIJI

S. Pavlović¹, J. Jovanović¹, B. Petručev¹, N. Tošić¹, L. Krivokapić-Dokmanović²,
D. Janić², M. Čvorkov-Dražić³ i G. Bunjevački³

¹Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

²Univerzitetska dečja klinika, Beograd

³Institut za zdravstvenu zaštitu majke i deteta «Dr Vukan Čupić», Beograd

Talasemijski sindromi su grupa naslednih oboljenja koja se karakteriše poremećajem u sintezi jednog ili više polipeptidnih lanaca hemoglobina (Hb). Obuhvataju talasemije i talasemijske strukturne hemoglobinske varijante. Posledica su mutacija u globinskiim genima. Ovaj rad predstavlja prvu sistematsku studiju molekularne osnove talasemijskih sindroma u Srbiji. Otkriveno je osam različitih b-talasemijskih mutacija (kodon 39 (C→T), IVS-I-110 (G→A), IVS-II-745 (C→G), kodon 44 (-C), - 87 (C→G), IVS-II-1 (G→A), IVS-I-6 (T→C), IVS I-1 (G→A)) kod 70 pacijenata iz 29 porodica. Metodologija kojom su okarakterisane mutacije je obuhvatala metode molekularne biologije bazirane na lančanoj reakciji umnožavanja DNK (PCR): reverzni dot blot (RDB), alel-specifični PCR (ARMS), delecioni PCR (gap PCR), sekvenciranje amplifikovanog fragmenta DNK. Četiri mutacije (Hb Lepore, kodon 39, IVS-I-110, IVS-II-745) su detektovane u 80% analiziranih pacijenata. Najčešći uzrok talasemijskog fenotipa u našoj populaciji (30 %) je talasemijska hemoglobinska varijanta, Hb Lepore. Dijagnostikovani su i nosioci retke hemoglobinske varijante Hb Sabine, kao i pacijenti oboleli od a-talasemije. Pored toga, urađena je detaljna analiza diverziteta haplotipova β-globinskog lokusa kod nosilaca najčešćih β-talasemijskih mutacija i zdravih betaA/betaA osoba u Srbiji. Identifikovan je novi haplotip asociiran sa Hb Lepore genom u srpskoj populaciji. Ovi podaci idu u prilog hipotezi o multicentričnom poreklu ove mutacije. Mutacija je nastala *de novo* u hromozomskom kontekstu koji je karakterističan za populaciju u Srbiji. Takođe je pokazano da su dve najčešće mediteranske mutacije, β⁰39 i β⁺IVS-I-110, verovatno «uvezenе» u populaciju Srbije iz Italije, odnosno Turske, istorijskim migracijama i seobama naroda.

MOLECULAR BASIS AND ORIGIN OF THALASSEMIA SYNDROMES IN SERBIA

Thalassemia syndromes are a group of hereditary disorders comprising thalassemias and thalassemic hemoglobin (Hb) variants. They are generally caused by mutations in globin genes resulting in reduced amount of globin produced. This study represents the first systematic molecular characterization of thalassemia syndromes in Serbian populations. We have identified eight b-thalassemia mutations (codon 39 (C→T), IVS-I-110 (G→A), IVS-II-745 (C→G), codon 44 (-C), - 87 (C→G), IVS-II-1 (G→A), IVS-I-6 (T→C), IVS I-1 (G→A)) in 70 members of 29 families using polymerase chain reaction (PCR), reverse dot blot (RDB), amplification refractory mutation system (ARMS) and direct sequencing analysis. Among all detected mutations, 4 (Hb Lepore, codon 39, IVS-I-110, IVS-II-745) accounted for more than 80%. Hemoglobin (Hb) Lepore is found to be the most common cause of thalassemia phenotype (30%). Hb Sabine and a thalassemia were detected as well. We have also studied β-globin gene cluster haplotypes and their association with the most common mutations. A novel haplotype associated with Hb Lepore gene was identified. These data support the hypothesis of multicentric origin of this mutation. The mutation has arised *de novo* in the chromosomal background characteristic for Serbian population. Additionally, we have shown that two most common Mediterranean mutations, β⁰39 and β⁺ IVS-I-110, have probably been introduced into Serbian population from Italy and Turkey, respectively, through historically documented migrations and settlements.

PREVENCIJA RAĐANJA DECE SA HROMOZOMSKIM ANOMALIJAMA NA TERITORIJI VOJVODINE

J. Jovanović-Privrodska, I. Kavečan, A. Krstić, Lj. Gaćina, R. Madžar, J. Rudež,
V. Manasijević, M. Obrenović i T. Tarasenko

Institut za zdravstvenu zaštitu dece i omladine, Novi Sad

U proteklih 14 godina u Centru za medicinsku genetiku (Institut za zdravstvenu zaštitu dece i omladine) u Novom Sadu urađeno je 15171 analiza prenatalne dijagnostike (kariotip iz amnionske tečnosti, fetalne krvi i horionskih čupica). Iz amnionske tečnosti bilo je 13224 analiza kariotipa, iz fetalne krvi bilo je 1947 analiza, a iz horionskih čupica bilo je 264 analiza kariotipa. Patoloških kariotipova iz amnionske tečnosti bilo je 287/13224 (2.17%), iz fetalne krvi 70/1947 (3.59%), a iz horionskih čupica 34 (12.88%). Indikacija za prenatalnu dijagnostiku postavljena je u Genetskom savetovalištu Centra za medicinsku genetiku. Tokom navedenog perioda bilo je 30120 genetskih konsultacija. Indikacije su postavljene na osnovu genetičke anamneze (rođoslova), uzrasta trudnice (granica 32 godine) i supruga (granica 41 godina), biohemičkih markera (PAPPa, free beta HCG i AFP), na osnovu nalaza ekspertnog ultrazvuka, i nalaza fetalne ehokardiografije.

PREVENTION OF BORNING CHILDREN WITH CHROMOSOMAL ABBERRATION IN VOJVODINA

In Medical Genetics Center in Novi Sad were done 15171 prenatal analyses (amniotic fluid karyotypes, fetal blood karyotypes and chorionic villi karyotypes), in last fourteen years. There were 13224 amniotic fluid analyses, 1947 fetal blood analyses and 264 chorionic villi analyses. There were found 287 (2,17%) pathologic karyotypes from amniotic fluid, 70 (3,59%) pathologic karyotypes from fetal blood and 34 (12,88%) from chorionic villi. Indications for prenatal diagnostics were made in Genetic Counselory of Medical Genetics Center in Novi Sad. Indications were based on genetic anamnesis (family tree), woman age (up 32 years), husband age (up 41 years), biochemical markers (PAPPa, free ??hCG and AFP), expert ultrasound and fetal echocardiography.

NIVO HOMOCISTEINA I POLIMORFIZMI MTHFR GENA KOD ANTIFOSFOLIPIDNOG SINDROMA

D. Popović-Kuzmanović¹, I. Novaković², Lj. Stojanović¹, D. Mirković³,
Lj. Luković², I. Aksentijević⁴ i M. Krajinović²

¹Kliničko bolnički centar «Bezanijska Kosa», Beograd

²Institut za biologiju i humanu genetiku Medicinskog fakulteta, Beograd

³Institut za biohemiju, KCS, Beograd

⁴National Institutes of Health, Arthritis and Rheumatism Branch, Bethesda, USA

Antifosfolipidni sindrom (AFS) je autoimuna bolest udružena sa trombozama, gubitkom ploda i prisustvom antifosfolipidnih antitela. Hiperhomocisteinemija (HHcy) i genski polimorfizam Metilentetrahidrofolat reduktaze (MTHFR) su poznati faktori rizika za nastanak tromboembolija. Iz tog razloga smo ispitivali nivo plazma homocisteina (pHcy) i polimorfizme C677T i A1298C MTHFR gena kod pacijenata sa AFS.

Ispitivali smo 27 pacijenata: 14 sa primarnim i 13 sa sekundarnim AFS. Dijagnoza je postavljena na osnovu kliničke slike, imaging metoda i prisustva anti-kardiolipinskih (aKL) i anti-β2 glikoprotein I (a β2 gpI) antitela. Polimorfizme smo detektovali PCR/RFLPS metodom, a za digestiju smo koristili restrikcione enzime Hinf I i Mbo II. Nivo Hcy smo merili HPLC metodom.

Genotip MTHFR 677TT je detektovan kod 1/14 bolesnika u grupi pAFS (7,1) i kod 1/13 bolesnika sa sAFS (7,7). Ista je učestalost genotipova MTHFR A1298C. Prevalanca HHcy je dva puta veća kod sAFS nego kod pAFS, ali je bila niska u obe grupe (1/14 i 2/13). Dva od tri pacijenta sa hiperhomocisteinjom su bili homozigoti 677 TT, jedan sa pAFS, a drugi sa sAFS. Treći pacijent je bio dvostruki heterozigot CT/AC sa sAFS. Genotipovi MTHFR 1298CC nisu bili praćeni hiperhomocisteinjom.

Niska prevalanca polimorfizama MTHFR C677T i A1298C kod pacijenata sa AFS ukazuje na to da ove genetske promene nisu značajan faktor rizika kod AFS.

PLASMA HOMOCYSTEIN LEVEL AND MTHFR GENE POLYMORPHISM IN ANTIPHOLIPID SYNDROME

Antiphospholipid syndrome (APS) is autoimmune disease associated with thrombosis, pregnancy morbidity and presence of anticardiolipin antibodies. Hyperhomocysteinemia (HHcy) and methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms are known risk factors for thromboembolism. For this reasons we analyzed plasma homocysteine (pHcy) level and MTHFR C677T and A1298C genotype in APS patients. In this study we evaluated 27 patients with APS: 14 primary (p) and 13 secondary (s). The diagnosis was established by clinical signs, imaging methods and presence of anti cardiolipin (aCL) and β2 glicoprotein I (a β2gp I) antibodies. MTHFR C677T and A1298C polymorphisms were analyzed by PCR/RFLPS and PCR followed by restrictive enzyme Hinf I and Mob II digestion. Plasma Hcy was measured by HPLC method.

Genotype MTHFR677 TT was found in 1/14 of pAPS (7,1%) and in 1/13 among sAPS (7,7%) patients. The same frequency was observed for MTHFR1298CC genotype. Prevalence of HHcy in sAPS was twice times higher than in pAPS, but it was low in both groups (1/14 and 2/13). Two of three patients with HHcy were homozygous MTHFR677TT, one with pAPS and another with sAPS. The third patient was double heterozygote CT/AC with sAPS. Patients with genotypes MTHFR1298CC had not HHcy.

Low prevalence of MTHFR C677T and A1298C polymorphisms in APS patients suggests that these genetics alterations are not prominent risk factors in APS.

EVALUACIJA CITOTOKSIČNIH I GENOTOKSIČKIH EFEKATA ESTRADIOLA PRIMENOM IN VITRO MIKRONUKLEUS TESTA

N. Đelić¹, Biljana Spremo-Potparević², Dijana Đelić³ i V. Bajić⁴

¹Katedra za biologiju, Fakultet veterinarske medicine, Univerzitet u Beogradu, Beograd

²Institut za fiziologiju, Farmaceutski fakultet, Univerzitet u Beogradu, Beograd

³Katedra za anatomiju, Fakultet veterinarske medicine, Univerzitet u Beogradu, Beograd

⁴Institut za biomedicinska istraživanja «Galenka», Beograd

Hormoni predstavljaju jedan od glavnih faktora rizika za nastanak malignih oboljenja, naročito u organima pod snažnim uticajem hormona (dojka, endometrijum, prostate). Najbolje proučena grupa hormona su seksualni steroidi, posebno estrogeni. Pokazano je da prirodnji i sintetički, steroidni i nesteroidni estrogeni mogu da ostvare stimulaciju mitotičkih deoba delujući na taj način kao tumor-promotori. Međutim, sve je više podataka da estrogeni mogu da deluju i na nivou inicijacije kancerogeneze. Naime, pri metaboličkoj transformaciji estrogena u ćeliji dolazi do stvaranja oksidativnog stresa praćenog oštećenjima molekula DNK, uključujući kovalentne modifikacije azotnih baza. Praćen je širok spektar koncentracija estradiola, uključujući koncentracije koje odgovaraju terapijskim dozama u humanoj medicini, kao i znatno veće koncentracije od terapijskih. *In vitro* mikronukleus test urađen je po odgovarajućim smernicama OECD-a o testiranju na genotoksičnost. Korišćena je periferna venska krv tri zdrave muške osobe mlade od 35 godina. Za statističku analizu upotreljen je Studentov t-test.

Dobijeni rezultati ukazuju da tek pri koncentracijama 30 i 100 puta većim od maksimalnih terapijskih doza u humanoj medicini estradiol značajno povećava učestalost mikronukleusa. Prema tome, rizik od izraženih genotoksičnih efekata estradiola postoji u slučaju predoziranja estradiola i, verovatno, pri dugotrajnoj terapiji. Pored toga ove dve najviše ispitivane koncentracije dovele su do usporavanja progresije kroz ćelijski ciklus i do smanjenja procenta binukleisanih limfocita verovatno usled citotoksičnih efekata.

EVALUATION OF CYTOTOXIC AND GENOTOXIC EFFECTS OF OESTRADIOL IN CYTOKINESIS BLOCK MICRONUCLEUS ASSAY

Hormones can be considered as the major risk factor for the development of cancer, especially in organs under strong hormonal influence (breast, endometrium, prostate). The best studied group of hormones are sexual steroids, especially oestrogens. It has been revealed that oestrogens (natural and synthetic, steroid and nonsteroidal) can stimulate mitotic divisions and, therefore, act as tumor promoters. However, there is increasing evidence of the genotoxic effects of oestrogens (tumor initiation). According to the modern standpoint, metabolic conversion (redox cycling) of oestrogens leads to oxidative stress and DNA damage, including formation of DNA adducts.

We examined a wide range of oestradiol concentrations, including those corresponding therapeutic doses in human medicine, as well as much higher concentrations. *In vitro* micronucleus assay was performed according to the standard genotoxicity OECD guidelines. Peripheral venous blood from three healthy men younger than 35 was used in these experiments. Statistical analysis was performed by Student's t-test.

The obtained results showed that only the concentrations of 30 and 100 fold maximal therapeutic doses have caused a significant increase of micronucleus frequency. Therefore, there is some genetic risk if oestradiol is overdosed and, possibly, after a long term therapy. In addition, the two highest concentrations caused cell cycle delay and decrease of binucleated lymphocytes which probably resulted from cytotoxic effects.

**ANALIZA EKSPRESIJE GENA PRIMENOM NERADIOAKTIVNE
RNK-RNK *IN SITU* HIBRIDIZACIJE**

Gordana Nikčević, Danijela Drakulić i Milena Stevanović

Institut za molekularnu genetiku and genetičko inženjerstvo, Beograd

RNK-RNK *in situ* hibridizacija je pogodna metoda za izučavanje specifične ekspresije gena u tkivima i ćelijama, kojom je omogućena vizualizacija obeležene antisens RNK probe hibridizovane za specifičnu iRNK. U ovom saopštenju biće prikazano detektovanje ekspresije SOX gena u tumorskim ćelijskim linijama primenom neradioaktivne RNK-RNK *in situ* hibridizacije koristeći RNK probe obeležene biotinom ili digoksigeninom. Koristeći ovaj pristup potvrdili smo rezultate dobijene Northern blot analizom, tj. pokazano je prisustvo *SOX2* iRNK u NT2/D1 i *SOX14* iRNK u HepG2 ćelijama. Cilj ovog rada bio je uspostavljanje RNK-RNK *in situ* hibridizacije na *in vitro* kultivisanim ćelijama da bi metodu primenili pri proučavanju ekspresije SOX gena u različitim normalnim i tumorskim tkivima.

**GENE EXPRESSION ANALYSIS BY NON-RADIOACTIVE
RNA-RNA *IN SITU* HYBRIDIZATION TECHNIQUES**

RNA-RNA *in situ* hybridization is reliable method for studying tissue and cell specific gene expression, which enables visualization of labeled antisense RNA probe hybridized to specific mRNA. In this study we have employed non-radioactive RNA-RNA *in situ* hybridization using biotin- or digoxigenin-labeled RNA probes in order to detect *SOX* gene expression in carcinoma cell lines. By applying this approach we confirmed results obtained by Northern blot analysis, where presence of *SOX2* mRNA in NT2/D1 and *SOX14* mRNA in HepG2 cells has been shown. Our aim was to set up RNA-RNA *in situ* hybridization method in *in vitro* cultured cells in order to further analyze *SOX* gene expression on various normal and cancer tissues.

DETEKCIJA MINIMALNE REZIDUALNE BOLESTI KOD BOLESNIKA SA HRONIČNOM MIJELOIDNOM LEUKEMIJOM (CML) LEČENIH IMATINIB MESYLATOM

Anka Radović, Biljana Todorić-živanović, Milica Strnad, Dragana Stamatović,
Koviljka Krtolica¹ i Z. Magić¹

Vojnomedicinska Akademija, Beograd

¹Institut za nuklearne nauke «Vinča», Beograd

Detekcija i praćenje minimalne rezidualne bolesti (MRB), zaostalih malignih ćelija nakon terapije, postalo je jedno od najvažnijih zadataka kad su u pitanju bolesnici sa CML lečeni imatinib mesylatom. Postizanje kompletne hematološke i citogenetske remisije kod određenog broja bolesnika sa CML a zatim relaps bolesti objašnjavaju se upravo postojanjem malog broja malignih ćelija rezistentnih na primjenjenu terapiju. Zbog toga njihovo otkrivanje daje važnu informaciju o efektu terapije kao i mogućnost da terapeut pravovremeno odreaguje pre nego što dođe do relapsa bolesti. RT-PCR tehnika se pokazala kao najsenzitivnija, omogućujući detekciju jedne maligne ćelije na 10^5 - 10^6 normalnih. Kao pozitivni marker za detekciju MRB koristi se BCR-ABL fuzioni gen koji ima ključnu ulogu u patogenezi CML. Detekcija prisustva minimalne rezidualne bolesti bolesnika sa CML koji su postigli kompletan citogenetski odgovor (0% ćelija sa Philadelphia hromozomom) nakon primjenjene terapije imatinib mesylatom. Prisustvo rezidualnih ćelija koje eksprimiraju BCR-ABL himerni gen analizirano je u uzorcima periferne krvi kod pet bolesnika (od dvadeset na imatinib terapiji) koji su postigli kompletan citogenetski odgovor. Detekcija je vršena RT-PCR i «nested» RT-PCR tehnikom. Kod tri bolesnika RT-PCR tehnikom detektovano je prisustvo rearanžmana b3a2 tipa. Kod dva bolesnika je po dobijanju negativnog rezultata nakon RT-PCR tehnike primenjena «nested» RT-PCR metoda.

Ovom metodom utvrđeno je prisustvo BCR-ABL rearanžmana i to kod jednog bolesnika b3a2 tip a kod drugog b2a2 tip.

DETECTION OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA (CML) TREATED WITH IMATINIB MESYLATE

Detection and monitoring of minimal residual disease (MRD), malignant cells remained after the therapy, has become one of the most important tasks when it comes to CML patients treated with imatinib mesylate. The fact that certain number of patients suffering from CML first achieve complete hematologic and cytogenetic remission which is afterwards followed by relapse of disease could actually be clarified through the existence of small number of malignant cells that are resistant to the applied therapy. Therefore, detection of these cells will provide important information on the effect of therapy as well as offer a therapist an opportunity to react promptly before the relapse of disease takes place. RT-PCR technique proved to be the most sensitive one enabling the detection of one malignant cell in 10^5 - 10^6 normal ones. BCR-ABL fusion gene that has a crucial role in pathogenesis of CML is used as positive marker for detection of MRD. Detection of the presence of minimal residual disease in CML patients who after the application of imatinib mesylate therapy reached complete cytogenetic response (0% cells with Philadelphia chromosome). Presence of residual cells that express BCR-ABL chimerical gene was analysed in the samples of peripheral blood from five patients (out of twenty undergoing imatinib therapy) who had reached complete cytogenetic response. Detection was performed by the means of RT-PCR and nested RT-PCR technique. In three patients b3a2 rearrangement type was detected by RT-PCR technique. In two remaining patients after negative RT-PCR results nested RT-PCR was applied. By this method BCR-ABL rearrangement was detected in both patients, one of them having b3a2 type and the other b2a2 type.

ISTOVREMENA METILACIJA $p16^{INK4A}$ I $MGMT$ GENA JE POVEZANA SA POVOLJNIJIM TOKOM BOLESTI KOD OBOLELIH OD KOLOREKTALNOG KARCINOMA

Milena Krajanović¹, Koviljka Krtolica¹, Slavica Knežević-Ušaj² i B. Dimitrijević¹

¹Laboratorija za radiobiologiju i molekularnu genetiku, Institut za nuklearne nauke «Vinča», Beograd

²Institut za patologiju, VMA, Beograd

Da bismo ispitali eventualnu povezanost između navedenih parametara, analizirali smo 37 isečaka tkiva tumora obolelih od kolorektalnog karcinoma. Za utvrđivanje metilacionog statusa $p16^{INK4A}$ i $MGMT$ gena je primenjena metoda bisulfite modifikacije DNK, kojom se pod odgovarajućim uslovima svi nemetilovani, ali ne i metilovani citozini prevode u uracil, a željeni fragmenti ovako modifikovane DNK su zatim amplifikovani posebno dizajniranom, za metilaciju specifičnom PCR tehnikom-MSP. Istovremena metilacija $p16^{INK4A}$ i $MGMT$ gena je u našem radu detektovana kod 27% (10 od 37) ispitanika, a korelacija između ovog događaja i kliničko-patoloških karakteristika, kao što su pol, starost, Đukovi stadijumi, histološki tip, diferenciranost i lokacija tumora, nije uočena. Međutim, u analizi kliničkog toka i ishoda bolesti u dvogodišnjem periodu, istovremena metilacija navedenih gena je statistički značajno ($p < 0.05$) korelirala sa manje agresivnim postoperativnim tokom bolesti i dužim preživljavanjem obolelih od kolorektalnog karcinoma. Utvrđeno je da je do pojave metastaza i smrtnog ishoda u toku dve godine došlo kod svega 30% (3 od 10) ispitanika sa istovremenom metilacijom oba gena, dok je to bio slučaj kod 72% (18 od 25), odnosno, 68% (17 od 25) obolelih bez istovremene metilacije analiziranih gena. Dobijeni rezultati sugerisu da bi istovremena metilacija $p16^{INK4A}$ i $MGMT$ gena mogla da predstavlja povoljniji prognostički parametar kod obolelih od kolorektalnog karcinoma, a identifikacija ovih molekularnih markera bi omogućila dizajniranje specifičnih terapijskih pristupa.

SIMULTANEOUS METHYLATION OF $p16^{INK4A}$ AND $MGMT$ GENES IS ASSOCIATED WITH LESS AGGRESSIVENESS OF THE DISEASE AND LONGER SURVIVAL IN COLORECTAL CANCER

To explore the possible association between these parameters we analysed tumour tissue samples from 37 patients with colorectal cancer. DNA methylation patterns were determined by chemical bisulphite modification of unmethylated, but not methylated cytosines to uracil and subsequent PCR amplification, using primers specific for either methylated or the unmethylated DNA. Simultaneous methylation of $p16^{INK4A}$ tumour suppressor and $MGMT$ genes was found in 27% (10 of 37) of samples and there was no correlation between this event and any clinicopathological characteristics including age, gender, Dukes' stage, histological type, differentiation and tumour location. However, in the two-years survival analysis, simultaneous methylation of these two genes was significantly associated ($p < 0.05$) with less aggressiveness of the disease and longer survival in patients with colorectal cancer who underwent curative surgery. In this period, the progression of the disease and the death occurred in only 30% (3 of 10) patients with simultaneous methylation of two examined genes, while that was the case with 72% (18 of 25) and 68% (17 of 25) patients without simultaneous methylation of analysed genes, respectively. Our results suggest that simultaneous methylation of $p16^{INK4A}$ and $MGMT$ genes could be the factor of better prognosis in patients with colorectal cancer and that identification of these molecular markers may be of use for the patient specific design of antitumour therapy.

**IN VITRO ANALIZA MIKRONUKLEUSA I ĆELIJSKE KINETIKE U
HUMANIM LIMFOCITIMA POD DEJSTVOM ADRENALINA**

Biljana Marković i N. Đelić

Katedra za biologiju, Fakultet veterinarske medicine, Univerzitet u Beogradu, Beograd

Upotrebljeno je osam različitih koncentracija adrenalina (opseg 5×10^{-10} M do 5×10^{-5} M), od one koja odgovara fiziološkom nivou ovog hormona u plazmi, preko koncentracija uporedljivih sa minimalnim, srednjim i maksimalnim terapijskim dozama u humanoj medicini, sve to 10, 30 i 100 puta jačih koncentracija od terapijskih doza u humanoj medicini. Eksperimentalna procedura u potpunosti je odgovarala smernicama OECD za *in vitro* mikronukleus test. Upotrebljena je krv tri zdrave muške osobe mlade od 35 god. Statistička analiza dobijenih rezultata urađena je Studentovim *t*-testom i χ^2 testom. Dobijeni rezultati ukazuju da adrenalin značajno smanjuje kinetiku proliferacije humanih limfocita praćenu CBPI indeksom (engl. cytochalasine block proliferation index) pri koncentraciji uporedljivoj sa maksimalnom terapijskom dozom, kao i pri svim višim koncentracijama adrenalina. Smanjena ćelijska kinetika pod uticajem adrenalina u skladu je sa literaturnim podacima da povećana količina cAMP može da uspori deobu ćelija. S druge strane, u svim primjenjenim koncentracijama adrenalin nije doveo do statistički značajnog porasta učestalosti mikronukleusa u humanim limfocitima. Odsustvo aneugenih i klastogenih efekata moguće je objasniti ili nedovoljnim metaboličkim kapacitetom limfocita za konverziju kateholamina, ili nedovoljnom senzitivnošću samog mikronukleus testa.

**IN VITRO ANALYSIS OF MICRONEUCLEI AND CELL CYCLE KINETICS IN
HUMAN LYMPHOCYTES EXPOSED TO EPINEPHRINE**

We used eight experimental concentrations of epinephrine in a range from 5×10^{-10} M to 5×10^{-5} M, starting with a concentration which corresponds to physiological level of this hormone in human plasma, through concentrations corresponding to minimal, average and maximal therapeutic doses in human medicine, and finally 10-, 30- and 100-fold max. therapeutic doses. The experimental procedure was entirely according to OECD *in vitro* micronucleus assay guidelines. Three healthy men under 35 donated blood for these experiments. Statistical analysis was performed by Student's *t*-test and χ^2 test.

Epinephrine significantly decreased progression through the cell cycle measured by CBPI (cytochalasine block proliferation index) at the concentration comparable to maximal therapeutic dose, as well as all higher concentrations. Cell cycle delay is consistent to literature data that increased level of cAMP can suppress mitotic activity. On the other hand, there is no significant change in micronucleus frequencies at all concentrations analysed. The absence of aneugenic and clastogenic effects may have resulted from poor metabolic capacity of human lymphocytes, or undersensitivity of micronucleus assay itself.

ANALIZA KARIOTIPA SVINJE SA HERMAFRODITIZMOM

Jelena Armuš¹, Z. Stanimirović², P. Sikimić² i Jevrosima Stevanović²

¹Student IV godine Fakulteta veterinarske medicine, Univerzitet u Beogradu, Beograd

²Katedra za biologiju, Fakultet veterinarske medicine, Beograd

U radu je opisan jedan slučaj pravog hermafroditizma kod svinje *Sus scrofa domestica* gajene na Fakultetu veterinarske medicine. Opservacija spoljašnje morfologije navela nas je na pretpostavku da je reč o pravom hermafroditizmu obzirom da je konstantovano prisustvo muških i ženskih polnih organa (vulva sa hipertrofiranim klitorisom, testisi i penis). Patomorfološkim analizama takođe je potvrđen dualizam muških i ženskih polnih organa, pa je odlučeno da se pristupi hromozomskim analizama ove životinje primenom modifikovane metode Evans-a i O'Riordan-a (1975). Analizom kariotipa limfocita periferne krvi svinje utvrđen je mozaicizam polnih hromozoma tipa $2n=38,XY/XX$ uz otkriće određenog procenta aneuploidnih ćelija. S obzirom na izgled spoljašnjih genitalnih organa (prisutni su vulva sa hipertrofiranim klitorisom, testisi i penis) i unutrašnjih genitalnih organa (smanjeni testisi, prisutni ovarijumi i uterus) i hromozomske analize možemo prepostaviti da postoje dva moguća uzroka hermafroditizma kod ove životinje: mozaicizam i himerizam.

KARYOTYPE ANALYSIS OF HERMAPHRODITE SWINE

A case of hermaphrodite swine *Sus scrofa domestica* housed in the Faculty of Veterinary Medicine was described. After observation of outer morphology we supposed that it is a question of true hermaphroditism because the swine had male and female reproductive organs (vulvae with hypertrophed clitoris, testes and penis). Pathomorphologic analyses also affirmed dualism of male and female reproductive organs, so we decided to perform chromosome analyses using modified method of Evans-a i O'Riordan-a (1975). Karyotype analyses of lymphocytes from peripheral blood revealed mosaicism of gonosomes $2n = 38,XY/XX$ and certain presence of aneuploid cells. Having in mind the view of outer genital organs (vulvae with hypertrophed clitoris, testes and penis) and internal genital organs (reduced testes, appearance of ovaries and uterus) we can assumed that there are two possible causes of hermaphroditism in this animal: mosaicism and chimerism.

INICIJALNA DIJAGNOZA I PRAĆENJE MINIMALNE REZIDUALNE BOLESTI KOD DEČJIH AKUTNIH LEUKEMIJA UZ POMOĆ PCR METODOLOGIJE

N. Tošić¹, J. Jovanović¹, B. Petručev¹, L. Krivokapić-Dokmanović², D. Janić² i S. Pavlović¹

¹Institut za molekularnu genetiku i genetički inženjerинг, Beograd

²Univerzitetska dečja klinika, Beograd

U našem istraživanju koristili smo multipleks RT-PCR tehniku za detekciju četiri najčešće translokacije kod dece obolele od ALL; t(9; 22) BCR-ABL (p190) fuzioni transkript (prisutan u 15% slučajeva), t(1; 19) E2A-PBX1 fuzioni transkript (4%), t(12; 21) TEL-AML1 fuzioni transkript (8%) i t(4; 11) MLL-AF4 fuzioni transkript (0%). Ukoliko nije detektovana nijedna od ovih translokacija MRB je praćena analizom klon-specifičnih regiona spajanja rearanžiranih gena za IgH i T-ćeljkog receptora (TCR). Za detekciju tri najčešće genetičke abnormalnosti u AML, PML/RAR fuzioni transkript za t(15;17) (prisutna u 13% slučajeva), AML1/ETO fuzioni transkript za t(8;21) (0%) i CBFbeta/MYH11 fuzioni transkript za inv(16) (13%), koristili smo nested RT-PCR esej. AML pacijenti kod kojih nisu detektovane ove translokacije testirane su na mutacije u Flt3 genu. Mutacije u Flt3 genu su analizirane PCR-RFLP analizom za D835 tačkastu mutaciju (prisutna u 4% pacijenata) i PCR analizom za Flt3/ITD (internal tandem duplication) (8%). Metodologija bazirana na PCR analizi predstavlja osetljiv test za dijagnostikovanje akutnih leukemija i obezbeđuje dodatne markere za praćenje MRB.

INITIAL DIAGNOSIS AND FOLLOW-UP OF MINIMAL RESIDUAL DISEASE IN CHILDHOOD ACUTE LEUKEMIA USING PCR-BASED METHODOLOGY

In our investigation we used multiplex RT-PCR technique for detection of four most common translocations in children affected with ALL; t(9; 22) BCR-ABL (p190) fusion transcript (present in 15% of cases), t(1; 19) E2A-PBX1 fusion transcript (4%), t(12; 21) TEL-AML1 fusion transcript (8%) and t(4; 11) MLL-AF4 fusion transcript (0%). If none of the most common translocations in ALL had been available for MRD detection, the analysis of clone-specific junctional regions of rearranged genes for both IgH and the T-cell receptor (TCR) was used for the follow up of MRD. For identification of 3 most common genetic abnormalities in AML, PML/RAR fusion transcript for t(15;17) (present in 13% of patients), AML1/ETO fusion transcript for t(8;21) (0%) and CBFbeta/MYH11 fusion transcript for inv(16) (13%), we used nested RT-PCR assay. AML patients lacking the most common translocations were tested for the mutations of Flt3 gene. Mutations of Flt3 gene were analyzed by PCR-RFLP for D835 point mutation (present in 4% of patients) and by PCR method for Flt3/ITD (internal tandem duplication) (8%). By using PCR analysis we have provided a sensitive test for the diagnosis of acute leukemias and additional markers for MRD follow up.

V tematska oblast / V topic:

Genetika čoveka i životinja
Human and animal genetics

Posteri / Posters

V-Pos

HETEROGENE ABERACIJE 11q23 U HEMATOLOŠKIM MALIGNITETIMA

Sandra Bižić, Vesna Đorđević, Marija Denčić-Fekete, Jelica Jovanović,
Mirjana Gotić i Darinka Bošković

Institut za hematologiju, Klinički centar Srbije, Beograd

Aberacije na hromozomu 11 u regionu q23 mogu se uočiti citogenetskom analizom u svim hematološkim malignitetima sa učestalošću do 10% kao solo aberacija ili udružene sa drugim hromozomskim promenama. U regionu 11q23 mapiran je MLL gen, čiji je produkt važan regulacioni faktor procesa hematopoeze, tako da sve promene u ovom regionu dovode do maligne transformacije ćelija različitih krvnih loza. Zbog činjenice da su aberacije u ovom regionu hromozoma 11(11q23/MLL) veoma različite i nisu u korelaciji sa morfologijom trasformisanih ćelija, klasifikovane su kao poseban entitet malignih hemopatija (WHO classification). Posebno su interesantne translokacije koje dovode do fuzije MLL gena sa oko 50 različitim «partner» gena. Bolesnici kod kojih je utvrđeno prisustvo aberacije 11q23/MLL imaju lošu prognozu bez obzira na tip promene. Kod 11 bolesnika promena u regionu 11q23 je bila kao solo aberacija, a kod 9 bolesnika udružena sa drugim aberacijama. Promene 11q23 su bile tipa: delecija, inverzija, adicija hromatiniskog materijala nepoznatog porekla i translokacija. Najčešća translokacija bila je sa partner genom na hromozomu 4(q21) kod 7 bolesnika, po jednog bolesnika sa genom na hromozomu 2(p21), na hromozomu 17(q12) i na hromozomu 19(p13). Posebno je interesantna kompleksna translokacija u kojoj pored hromozoma 11 učestvuju hromozomi 7(q22) i 12(p13).

Aberacije 11q23 u grupi naših bolesnika bile su heterogene po tipu promena, vrsti «partner» gena uključenih u fuziju, što nije imalo značaja za dalji tok bolesti kao ni odgovor na terapiju.

**HETEROGENOUS ABNORMALITIES OF 11q23
AT HEMATOLOGICAL MALIGNANTS**

Chromosome 11 abnormalities at q23 region can be observed by conventional cytogenetic analyses at all hematological malignants with frequency up to 10%, as solo abnormality or in combination with other chromosomal abnormalities. Product of MLL gene, which is mapped at 11q23 region, is important hematopoiesis regulation factor. Therefore, all abnormalities at this region lead to malignant blood cell transformations. As abnormalities at this region of chromosome 11 (11q23/MLL) are heterogeneous and they are not in correlation with morphology of transformed cells, these aberrations were classified as a separate group of malignant hemopathy (WHO classification). Translocations, which lead to fusion of MLL gene with about 50 different «partner» genes, are especially interesting. Patients with 11q23/MLL abnormality have poor prognosis for all types of abnormalities.

It was observed that in 11 patients abnormalities at 11q23 region occurred as a solo abnormalities, but in 9 patients this abnormality was associated with other abnormalities. Types of 11q23 abnormalities were: deletions, inversions, additions of chromatin material of unknown source and translocations. The most abundant translocation was with the partner gene at chromosome 4(q21) – 7 patients. Other translocations were with partner gene: at chromosome 2(p21) – 1 patient, at chromosome 17(q12) – 1 patient and at chromosome 19(p13) – 1 patient. A complex translocation of chromosome 11 with chromosome 7(q22) and chromosome 12(p13) was especially interesting.

11q23 aberrations, in our group of patients, were heterogeneous by type of aberration, kind of partner genes included in fusion, but without importance for the course of a disease and outcome to therapy.

THE IMPOIRTANCE OF ENZYMATIC ANALYSIS IN THE CELLS FOR DETERMINATION OF SPECIFIC TYPES OF MUCOPOLYSACCHARIDOSESJ. Durković¹, I. Kremensky² and I. Sinigerska²¹Health Center Subotica, Department of Medical Genetics, Subotica²Laboratory of Molecular Pathology, University Hospital, Sofia, Bulgaria

Mucopolysaccharidoses are recessive very heterogenic group of diseases caused by storages in lysosomes. The substances stored are partially degraded mucopolysaccharides. Degradation of acid mucopolysaccharides begins by separation of glucosaminoglycans (GAG) from the proteins. Due to the inherited defectiveness of specific lysosomal hydrolase enzymes, incompletely hydrolyzed molecules are accumulated in tissues and they are increasingly excreted in urine. The detection of urinary GAG excess is the first step in diagnostic work-up of a patient with such clinical features. In case the increased excretion of GAG is confirmed, examination directed to discriminate the types of the disease is necessary and that is very important in terms of prognostics and further treatment of the diseased.

In our case report clinically suspected mucopolysaccharidosis called for metabolic screening of first morning urine and the positive toluidine blue test result indicated the increased excretion of mucopolysaccharides. The electrophoretical fractionation of excreted GAG showed, that heparan sulphate was the predominant component. One dimensional barium acetate electrophoresis is more suitable than thin layer chromatography for separation of heparan sulphate. Blood sample was tested for specific enzyme deficiency in white blood cells. Since the enzyme analysis indicated deficiency of heparin sulphamidase, MPS III the A type was diagnosed. This enzyme is a specific hydrolase involved in separating sulphate bonds from amino groups of glucosamines. Heparin sulfamidase enzyme activities of patient with MPS IIIA in leukocytes is 0,38 nmol/17 h/mg and control subjects is 1,8-6,8 nmol/17 h/mg.

The gene for this enzymes has not been cloned yet. Mucopolysaccharidoses are of special genetic interest since they show mutation in various loci as well as more than one mutation in one single locus. Multiple alleles give a similar phenotype but different enzyme deficiency that results in different products of hydrolysis. This means that DNA diagnosis (PCR) is made more difficult and therefore making diagnosis of mucopolysaccharidosis is based on enzyme analyses.

TEACHERS COLLINS SYNDROME KLINIČKE KARAKTERISTIKE I PREPORUKE DALJEG TRETMANA

Slobodanka Grković, M. Ješić i G. Čuturilo

Univerzitetska dečja klinika, Beograd

Teacher Collins sindrom je retko autosomno dominantno oboljenje. U literaturi, ovaj sindrom je poznat i kao mandibulofacialna dozostozna. Približna učestalost kod novorođene dece je 1/25.000-1/50.000.

Glavne karakteristike kliničke slike ovog sindroma su: hipoplazija srednjeg dela lica, hipoplastična mandibula, nerazvijene ušne školjke, progresivni razvoj konduktivne gluvoće i rascep nepca. Takođe prisutne su i sledeće karakteristike: palpebralni prorezi antimongoloidno ukošeni, kolobom donjeg kapka, delimično ili totalno dsustvo donjih trepavica. Druge anomalije su redje prisutne.

Kliničke karakteristike Teacher Collins sindroma su obično bilateralne i simetrične i postoje značajne fenotipske varijacije unutar jedne familije. Oko 60% slučajeva je posledica de novo mutacije. TCS gen (TCOF 1) je lokalizovan na hromosomu broj 5_{q32-q33.1}.

Prikaz slučaja. Prvo dete iz prve uredne trudnoće primljeno je radi ispitivanja etiologije prisutnih kongenitalnih nomalija.

Na prijemu muško novorođenče sa fenotipskim karakteristikama Treacher Collins-ovog sindrom: hipoplazija zigomatičnih kostiju, hipoplazija mandibile, hipoplastične ušne školjke sa zatvorenim ušnim kanalima, kao i drugim prisutnim anomalijama koje odgovaraju fenotipu ovog sindroma.

U daljem praćenju predviđena je mogućnost razvoja konjuktivne gluvoće kao i eventualne korektivne hirurške intervencije.

TEACHERS COLLINS SYNDROME CLINICAL CHARACTERISTICS AND GUIDELINES FOR FURTHER TREATMENT

Treachers Collins Syndrome is a rare autosomal dominant disorder of craniofacial development with variable expressions. The incidence of this syndrome is about 1/25.000 - 1/50.000 live newborns. Treacher Collin Syndrome is alternatively called mandibulofacial dysostosis.

The main clinical characteristics of this syndrome include: midface hypoplasia, micrognathia, microtia, conductive hearing loss and cleft palate. Antimongoloid palpebral fissures, coloboma of lower eyelids, eyelash malformations, preauricular hair displacements are also obligatory features. There are other abnormalities, which are infrequent.

The clinical features of this syndrome are usually bilateral and symmetrical, and marked variation of the phenotype can be observed in family members. About 60% of cases arise as a result of a de novo mutation. The TCS gene (TCOF 1) is localized on the human chromosome 5_{q32-q33.1}.

Case Report. A newborn male baby was admitted to our hospital because of congenital malformation.

The baby was born from the first uncomplicated pregnancy. Family background was normal.

Clinical findings on admission: The baby had a typical face appearance for Treacher Collin Syndrome. These face features included: hypoplastic mandible and zygomatic bones, antimongoloid fissures, hypoplastic auricles and other.

Future studies should be focused on a possibility for the development of conductive hearing loss and the possibility of surgical treatment.

TEACHERS COLLINS SYNDROME KLINIČKE KARAKTERISTIKE I PREPORUKE DALJEG TRETMANA

Slobodanka Grković, M. Ješić i G. Čuturilo

Univerzitetska dečja klinika, Beograd

Teacher Collins sindrom je retko autosomno dominantno oboljenje. U literaturi, ovaj sindrom je poznat i kao mandibulofacialna dozostozna. Približna učestalost kod novorođene dece je 1/25.000-1/50.000.

Glavne karakteristike kliničke slike ovog sindroma su: hipoplazija srednjeg dela lica, hipoplastična mandibula, nerazvijene ušne školjke, progresivni razvoj konduktivne gluvoće i rascep nepca. Takođe prisutne su i sledeće karakteristike: palpebralni prorezi antimongoloidno ukošeni, kolobom donjeg kapka, delimično ili totalno dsustvo donjih trepavica. Druge anomalije su redje prisutne.

Kliničke karakteristike Teacher Collins sindroma su obično bilateralne i simetrične i postoje značajne fenotipske varijacije unutar jedne familije. Oko 60% slučajeva je posledica de novo mutacije. TCS gen (TCOF 1) je lokalizovan na hromosomu broj 5_{q32-q33.1}.

Prikaz slučaja. Prvo dete iz prve uredne trudnoće primljeno je radi ispitivanja etiologije prisutnih kongenitalnih nomalija.

Na prijemu muško novorođenče sa fenotipskim karakteristikama Treacher Collins-ovog sindrom: hipoplazija zigomatičnih kostiju, hipoplazija mandibile, hipoplastične ušne školjke sa zatvorenim ušnim kanalima, kao i drugim prisutnim anomalijama koje odgovaraju fenotipu ovog sindroma.

U daljem praćenju predviđena je mogućnost razvoja konjuktivne gluvoće kao i eventualne korektivne hirurške intervencije.

TEACHERS COLLINS SYNDROME CLINICAL CHARACTERISTICS AND GUIDELINES FOR FURTHER TREATMENT

Treachers Collins Syndrome is a rare autosomal dominant disorder of craniofacial development with variable expressions. The incidence of this syndrome is about 1/25.000 - 1/50.000 live newborns. Treacher Collin Syndrome is alternatively called mandibulofacial dysostosis.

The main clinical characteristics of this syndrome include: midface hypoplasia, micrognathia, microtia, conductive hearing loss and cleft palate. Antimongoloid palpebral fissures, coloboma of lower eyelids, eyelash malformations, preauricular hair displacements are also obligatory features. There are other abnormalities, which are infrequent.

The clinical features of this syndrome are usually bilateral and symmetrical, and marked variation of the phenotype can be observed in family members. About 60% of cases arise as a result of a de novo mutation. The TCS gene (TCOF 1) is localized on the human chromosome 5_{q32-q33.1}.

Case Report. A newborn male baby was admitted to our hospital because of congenital malformation.

The baby was born from the first uncomplicated pregnancy. Family background was normal.

Clinical findings on admission: The baby had a typical face appearance for Treacher Collin Syndrome. These face features included: hypoplastic mandible and zygomatic bones, antimongoloid fissures, hypoplastic auricles and other.

Future studies should be focused on a possibility for the development of conductive hearing loss and the possibility of surgical treatment.

GENOTIP-FENOTIP KORELACIJE KOD PACIJENATA SA DUCHENNE-OVOM I BECKER-OVOM MIŠIĆNOM DISTROFIJOM

T. Lalić¹, D. Radivojević¹, M. Đurišić¹, M. Guć-Šćekić¹ i D. Zamurović²

¹Laboratorija za medicinsku genetiku, ²Odeljenje za neurologiju,
Institut za zdravstvenu zaštitu majke i deteta Srbije «Dr Vukan Čupić», Beograd

Klinička ekspresija bolesti kod oko 60% pacijenata sa Duchenne-ovom i Becker-ovom mišićnom distrofijom nastaje kao posledica delecija u genu za dystrofin. Kod ovakvih slučajeva je na osnovu tzv. «frame-shift» hipoteze ustanovljeno da delecije koje dovode do narušavanja translacionog okvira čitanja onemogućavaju produkciju stabilnog proteina i za posledicu imaju ekspresiju DMD fenotipa. Nasuprot tome, delecije koje zahvataju integralan broj kodona zadržavaju odgovarajući okvir čitanja, nastali protein je stabilan i delimično funkcionalan i eksprimira se klinički blaža forma bolesti-Becker-ova mišićna distrofija.

U cilju ispitivanja validnosti ove teorije kod pacijenata Neurološke službe Pedijatrijske klinike Instituta za zdravstvenu zaštitu majke i deteta analizirano je 90 bolesnika kod kojih je detektovano 39 različitih delecija u DMD/BMD genu.

Rezultati ovih analiza pokazali su da je kod 87,8% bolesnika korelacija kliničke ekspresije bolesti i delecija u genu za dystrofin bila u skladu sa očekivanom na osnovu «frame-shift» hipoteze.

Ovakvi podaci imaju veliki prognostički značaj za individualne pacijente čak i za sporadične slučajeve sa negativnom familijarnom anamnezom.

CORRELATION OF CLINICAL AND DELETION DATA IN YUGOSLAV DUCHENNE/BECKER MUSCULAR DYSTROPHY PATIENTS

About 60% of both Duchenne's muscular dystrophy (DMD) and Becker's muscular dystrophy (BMD) cases is due to deletions of dystrophin gene. For cases with deletion mutations the «reading frame» hypothesis predicts that deletions which result in disruption of the translation reading frame prevent production of stable protein and are associated with DMD. In contrast, intragenic deletions that involve exons encoding an integral number of triplet codons maintain proper reading frame. The resulting abnormal proteins are stable and partially functional, resulting in a milder and more variable BMD phenotype.

To test the validity of this theory, we analyzed 90 patients-39 independent deletions at the DMD/BMD locus.

Our results showed that in this sample the correlation between deletion and clinical severity of the disease was as predicted in 87,8% of cases.

This data should be useful in establishing the prognosis in individual patients even in sporadic cases with no affected relatives.

UČESTALOST ABERANTNIH KARIOTIPOVA I NJIHOV PROGNOSTIČKI ZNAČAJ U UZORKU OD 146. DECE SA ALL

N. Lakić¹, A. Krstić¹, S. Ćirković¹, M. Guć-Šćekić¹, G. Bunjevački² i D. Mićić²

¹Laboratorija za medicinsku genetiku, ²Odeljenje za hematoonkologiju,
Instituta za zdravstvenu zaštitu majke i deteta Srbije «Dr Vukan Čupić», Beograd

Od ukupnog broja analiziranih pacijenata, kod 79 (54%) je dijagnostikovan normalan kariotip dok je aberantni uočen kod 67. (46%) dece; numerički hromozomski rearanžmani detektovani su kod 44 (65%) pacijenta, a prisustvo strukturalnih rearanžmana uočeno je kod njih 23. (35%). U grupi pacijenata sa aberantnim kariotipom analiza je pokazala prisustvo specifičnih i nespecifičnih hromozomskih rearanžmana. Od specifičnih aberacija dijagnostikovane su: hiperdiploidija kod dece sa ALL (L1 ili L2), hiperhaploidija kod 1 pacijenta sa ALL (L1), translokacija 9/22 [t(9;22)(q34;q11)] kod 4 (6%) pacijenta sa ALL (L1 ili L2), translokacija 4/11 [t(4;11)(q21;q23)] kod jednog deteta sa ALL (L1) i translokacija 11/14 [t(11;14)(p13;q11)] takođe kod jednog pacijenta sa L1 akutnom limfoblastnom leukemijom. Učestalost specifičnih hromozomskih rearanžmana u obrađenoj grupi bolesnika, kao i njihov prognostički značaj slažu se sa podacima sličnih studija u literaturi. Citogenetska analiza ćelija kosne srži pacijenata u našem uzorku, pokazala je i prisustvo sledećih nespecifičnih rearanžmana: hipodiploidije kod 6 (9%) pacijenata sa ALL (L1 ili L2), pojedinačne slučajeve translokacija 6/9 [t(6;9)(q23;p24)] i 13/19 [t (13;19)(q14,p13)], delecije hromozoma 7 [del(7)(q22)], inverzije hromozoma 1[inv (1)(p34;q21)], duplikacije hromozoma 1 [dupl(1)(q21;q32)], kod pacijenta sa ALL (L1), kao i delecije hromozoma 2 [del(2)(q33)] i hromozoma 8[del (8)(p11)] detektovanih kod pacijenata sa ALL (L2).

FREQUENCY OF ABERRANT KARYOTYPES AND THEIR PROGNOSTIC VALUE IN CHILDREN WITH ALL

Normal karyotype was found in 79 (54%) of our patients. Chromosomal abnormalities were seen in 67 (46%) children; among them 44 (65%) have numerical and 23 (35%) structural rearrangements. Structural abnormalities were presented in a form of specific and nonspecific chromosomal aberrations. Among specific chromosomal disorders cytogenetic analysis revealed: hyperdiploidy in 37 (55.7%) children with ALL L1 or L2, hyperhaploid in one patient with ALL (L1), translocation 9/22 [t(9;22)(q34;q11)] in 4 (6%) patients with ALL (L1 or L2), translocation 4/11 [t(4;11)(q21;q23)] in one patient with ALL (L1) and translocation 11/14 [t(11;14)(p13;q11)] in one patient with the same type of ALL. The frequency of specific chromosomal aberrations in our group of patients and their prognostic value is in agreement with the results of similar studies in the literature. Nonspecific chromosomal abnormalities detected in our group of patients were: hypodiploidy (6 patients (9%) with ALL (L1 or L2)); translocations: t(6;9)(q23;p24) (1 patient with ALL (L1)), t (13;19)(q14;p13) (1 case with ALL (L1)); deletions : del(7)(q22) (1 patient with ALL (L1)) , del (8)(p11) (1 case with ALL (L2)), del(2)(q33) (1 patient with ALL (L2)) and inversion of chromosome 1[inv (1)(p34;q21)] in one patient with ALL (L1).

TRANSLOKACIJA $t(8;21)(q22;q22)$ I NJENE VARIJANTE U AKUTNOJ MIJELOIDNOJ LEUKEMIJI

Jelica Jovanović, Vesna Đorđević, Marija Denčić-Fekete, Sandra Bižić,
Mirjana Gotić i Darinka Bošković

Institut za hematologiju, Klinički centar Srbije, Beograd

Translokacija $t(8;21)(q22;q22)$ je jedna od najčešćih citogenetskih abnormalnosti u akutnoj mijeloidnoj leukemiji. Preko 90% slučajeva korelira sa dijagnozom AML M2, a redje se nalazi u AML M1 i AML M4. Na hromozomu 8 prekid se dešava u okviru ETO gena a na hromozomu 21 u okviru AML1 gena pri čemu se formira fuzioni AML1/ETO gen koji je lociran na derivatu hromozoma 8. Posebno su interesantne kompleksne translokacije gde pored hromozoma 8 i 21 učestvuje i treći hromozom.

U Laboratoriji za citogenetiku Instituta za hematologiju KCS u periodu od januara 1990. do septembra 2004. godine, bilo je 47 AML pacijenata sa $t(8;21)(q22;q22)$. Među njima je svega 3 pacijenta sa kompleksnim translokacijama gde su, pored hromozoma 8 i 21 uključeni sledeći regioni: 3p11, 5q13 i 9q34.

Od 47 pacijenata sa $t(8;21)(q22;q22)$ 40 je bilo sa dijagnozom AML M2, 5 sa AML M4 i 2 sa AML M1. 24 pacijenta su imala $t(8;21)$ kao solo aberaciju a kod ostalih su detektovane dodatne citogenetske aberacije. Najčešća dodatna aberacija je gubitak Y hromozoma i ona je prisutna kod 15 pacijenata.

Iako je kod pacijenata sa varijantnim tipom translokacije $t(8;21)$ došlo do kompleksnijih promena na nivou hromozoma, derivat hromozoma 8 je na citogenetskom nivou ostao intaktan. Kako se kod ovih pacijenata razvio isti tip hematološkog maligniteta kao i kod standardne $t(8;21)(q22;q22)$, najverovatnije struktura fuzionog AML1/ETO gena, koji je glavni leukemogeni faktor, nije narušena.

TRANSLOCATION $t(8;21)(q22;q22)$ AND ITS VARIANTS IN ACUTE MYELOID LEUKEMIA

Translocation $t(8;21)$ is one of the most common cytogenetic abnormalities found in AML. More than 90% of all cases found are in correlation with AML-M2, rarely with AML-M1 and M4. Breakpoint on chromosome 8 is in the region of ETO gene, while on chromosome 21 it is in the region of AML1 gene. As a result of translocation, new fusion gene called AML1/ETO is formed, and located on derivate of chromosome 8. Specially interesting are complex translocations in which besides chromosomes 8 and 21, third chromosome is involved.

Since January 1990. until September 2004. in cytogenetic laboratory of Institute of hematology KCS we had 47 patients with AML who had $t(8;21)$. Among them only three patients had complex translocations, which involved following chromosomes and their regions: 3p11, 5q13 and 9q34.

In this group of 47 patients with $t(8;21)$, 40 had diagnosis of AML-M2, five had AML-M4 and two had AML-M1. Twenty four patients had $t(8;21)$ as the only aberration, while the other in this group had additional cytogenetic aberrations. The most common additional aberration, found in 15 patients, was the lost of chromosome Y.

Despite the fact that patients with variable type of translocations had more complex changes at chromosomal level, on cytogenetic level derivate of chromosome 8 stayed intact. Most probably the structure of fusion gene AML1/ETO, which was the main leucogenic factor, was not changed. That could be the explanation why these patients had the same type of hematological malignancy as the one with standard translocation $t(8;21)$.

TRANSLOKACIONI OBLIK DAUNOVOG SINDROMA (PRIKAZ SLUČAJA)

I. Kavečan, J. Jovanović-Privrodska, M. Obrenović i Lj. Gaćina

Daunov sindrom, najčešće se javlja u klasičnoj formi u oko 95% slučajeva, translokacijski tip se javlja u učestalosti u 4-5%, a mozaični tip 1-2%. Na hromozomu 21 nalazi se region pod nazivom «Daun kritični region», lokalizovan na 21q22.1-q22.3, odgovaran za fenotipske karakteristike Daunovog sindroma. Mozaični tip Daunovog sindroma nastaje postzigotično, i u tom slučaju su slabije izražene fenotipske karakteristike Daunovog sindroma.

Prikaz slučaja: drugo dete iz pete nekontrolisane trudnoće 41- godišnje majke (1. zdravo dete, 3 spontana pobačaja). Po rođenju, uočene fenotipske karakteristike Daunovog sindroma: koso postavljeni očni otvori, hipertelorizam, epikantusi, širok koren nosa, nosni otvori okrenuti put napred, uške loše modelirane, asimetrične, usne tanke, nepce višje postavljeno, blag pectus infundibuliforme, klinodaktilia V prsta na rukama, sandalske brazde na stopalima, niže postavljen umbilikus, izražena hipotonija, atrezija anusa (hirurški korigovana), urođena srčana mana (trikuspidualna regurgitacija, mitralna regurgitacija, ductus arteriosus). Minor malformacioni skor: 6 Kariotip deteta: 46,XY,-21,+t(q21;q21) – translokacijski tip Daunovog sindroma.

Obzirom da se radi o translokacijskom tipu Daunovog sindroma kod deteta, urađen je kariotip roditeljima. Majka se leči na Klinici za psihijatriju, završila osnovnu školu, domaćica. Do rođenja deteta nije upućivana kod genetičara. Kariotip majke: 46,XX/46,XX,-21,+t(q21;q21) 98:2, mozaična forma Daunovog sindroma, nebalansirana translokacija, u procentu od 2%.

U ovom slučaju, majka ima nebalansiranu translokaciju i mali procenat ćelija (2%) sa translokacionim oblikom Daunovog sindroma, što predstavlja redak oblik hromozomske aberacije.

DOWN SYNDROME: TRANSLOCATION TYPE (CASE REPORT)

Downs syndrome (DS) appears as 21. chromosome trisomy (95% cases), translocation type (4-5% cases) and mosaic type (1-2% cases). Part of 21. chromosome that deals with phenotypic features of Down syndrome is called «Down Critical Region» (DCR).

DCR is located on 21q22.1-22.3. Mosaic type appears postzygotically and phenotypic features are weaker.

Case Report: second child from fifth non-controlled pregnancy (1 healthy child, 3 miscarriages), mothers age is 41. Phenotypic features of Down Syndrome on birth: slanted palpebral fissures, hipertelorism, epicantic folds, flattened nasal root, anteverted nostrils, abnormal auricles, asymmetric auricles, thin lips, high palate, mild pectus excavatum, 5th finger clinodactyly on hands, plantar crease between 1st and 2nd toes, distally displaced umbilicus, serious hipotony, imperforated anus (surgically corrugated), congenital heart disease (tricuspidal regurgitation, mitral regurgitation, ductus arteriosus). Minor malformation score: six. Karyotype: 46, XY, -21, +t(q21;q21).

Since translocation type is estimated, parents karyotypes were done. Mother is hospitalized on Psychiatry, elementary school, and housewife. First genetic counsel was done after this child had been born. Mothers karyotype: 46, XX/ 46,XX, -21, +t(q21;q21), 98 versus 2 analyzed cells. This is mosaic form of Down syndrome with unbalanced translocation in 2%.

In this case mother has nonbalanced translocation and low percent (2% cells) with translocation type of Down Syndrome versus child who has 100% translocation type. Both cases are rare.

VARIJABILNOST FREKVENCE MIKRONUKLEUSA U LIMFOCITIMA PERIFERNE KRVI ZDRAVIH OSOBA

Olivera Milošević-Dordević¹, D. Grujičić¹, S. Arsenijević² i D. Marinković³

¹Prirodno-matematički fakultet Univerziteta u Kragujevcu

²Ginekološko-Akušerska Klinika KBC, Kragujevac

³Srpska Akademija Nauka i Umetnosti , Beograd

Analizirani uzorak, koji su sačinjavale 142 fenotipski zdrave osobe, podelili smo u četiri starosne grupe, I uzorak-novorođenčići (N=25); II uzorak- osobe starosti 19 do 28 godina (N=52); III uzorak- osobe starosti 29 do 39 godina (N=30); IV uzorak-osobe starosti 40-53 godina (N=35). Ukupna prosečna frekvencu MN na 1000 analiziranih binuklearnih limfoblasta u uzorku od 142 fenotipski zdrave osobe, iznosila je 6.18 ± 3.59 , sa opsegom variranja 0-17 MN/1000 analiziranih limfocita. Najnižu frekvencu MN (5.25 ± 2.67) zapazili smo u uzorku osoba starosti 40-53 godine (IV uzorak), a najvišu u uzorku osoba starosti 29-39 godina-III uzorak (7.27 ± 4.51). U uzorku novorođenčadi zabeležili smo 5.53 ± 3.02 MN/1000 analiziranih limfoblasta, a u uzorku osoba starosti 19-28 godina (II uzorak) 6.50 ± 3.65 MN/1000. Mada postoji razlika u prosečnim frekvencama MN između analiziranih uzoraka, primenom Student-ovog t-testa statistički značajnu razliku konstatovali smo jedino između III i IV uzorka (osobe starosti 29-39 i 40-53 godina), sa verovatnoćom $p < 0.05$. Primenom analize varijanse (ANOVA) za frekvencu MN unutar i između svakog analiziranog uzorka, pokazali smo da između međugrupne i unutargrupne varijanse ne postoji statistički značajna razlika ($p > 0.05$), a da je međugrupna varijansa ($S^2_{BG} = 27.20$) daleko veća od unutargrupne varijanse ($S^2_{WG} = 12.54$). Dobijeni rezultati sugeriju da je variabilnost frekvence MN u limfocitima periferne krvi analiziranih uzoraka zdravih osoba u najvećoj meri uslovljena jednim od genetičkih faktora, starenjem.

VARIABILITY OF FREQUENCY OF MICRONUCLEI IN PERIPHERAL BLOOD LYMPHOCYTES OF HEALTHY PERSONS

The analyzed sample which comprised 142 phenotypically healthy persons, we separated in four groups depending on the age of tested individuals: I sample-newborns (N=25); II sample-persons from 19 to 28 years of age (N=52); III sample-persons from 29 to 39 years of age (N=30); IV sample-persons from 40 to 53 years of age (N=35). The total average MN frequency on 1000 analyzed binucleated lymphoblasts in a sample of 142 phenotypically healthy persons was 6.18 ± 3.59 , with the range of variation from 0 to 17 MN/1000 analyzed lymphocytes. The lowest MN frequency (5.25 ± 2.67) we observed in a sample of persons aged from 40 to 53 years (IV sample), but the highest in a sample of persons aged from 29 to 39 years-III sample (7.27 ± 4.51). In a sample of newborns we noted 5.53 ± 3.02 MN/1000 analyzed lymphocytes, and in a sample of persons aged from 19 to 28 years (II sample) 6.50 ± 3.65 MN/1000. Although difference in MN frequencies between tested samples exist, by using of Student,s t-test we recorded statistically significant difference only between III and IV samples (persons aged from 29 to 39, and 40 to 53 years), with probability $p < 0.05$. By applying of analysis of variance (ANOVA) for MN frequency within and between each analyzed sample, we showed that statistically significant difference between intergroup and intragroup variance do not exist ($p > 0.05$), and that the intergroup variance ($S^2_{BG} = 27.20$) so far greater than intragroup variance($S^2_{WG} = 12.54$). The obtained results indicate that variability of MN frequency in peripheral blood lymphocytes of analyzed samples of phenotypically healthy persons in the greatest level is caused by one of genetic factors, by ageing.

**MTHFRE GENOTIP I NIVO HOMOCISTEINA KOD
BOLESNIKA NA HEMODIJALIZI**

O. Mladenović¹, I. Novaković¹, S. Simić-Ogrizović², D. Mirković³, B. Popović⁴,
B. Jekić¹, T. Damnjanović¹, Lj. Luković¹, Lj. Đukanović² i M. Krajinović¹

¹Institut za biologiju i humanu genetiku Medicinskog fakulteta, Beograd

²Institut za nefrologiju KCS, Beograd

³Institut za biohemiju KCS, Beograd

⁴Stomatološki fakultet, Beograd

Metilentetrahidrofolat reduktaza (MTHFR) je jedan od ključnih enzima u metabolizmu homocisteina (Hci), koji učestvuje u remetilaciji Hci u metionin. Snižena aktivnost MTHFR je povezana sa povišenim nivoom Hci u plazmi, što je od posebnog značaja zbog dokazanog toksičnog delovanja ove amino kiseline na vaskularni endotel. Utvrđeno je da je aktivnost MTHFR genetički determinisana. Postojanje polimorfizma MTHFR C677T uslovljava sintezu termolabilne forme enzima sa smanjenom aktivnošću. Učestalost ovog polimorfizma kod bolesnika sa vaskularnim poremećajima je predmet brojnih studija. Ova ispitivanja su od interesa i za nefrološke bolesnike, kod kojih se postavlja pitanje da li je hiperhomocisteinemija posledica isključivo bubrežne insuficijencije, ili na nju ima uticaja i genetička osnova. Cilj našeg istraživanja je da se utvrdi učestalost MTHFR C677T polimorfizma kod bolesnika na programu hronične hemodialize (HD), i da se izvrši korelacija dobijenih genotipova sa nivoom homocisteina u plazmi (pHci). Istraživanjem je obuhvaćena grupa od 81 bolesnika u terminalnoj bubrežnoj insuficijenciji. Analiza genotipa je vršena PCR/RFLPs metodom a pHci je određivan metodom HPLC sa fluorescentnom detekcijom. Genotip CC je utvrđen kod 42% bolesnika, 46,9% bolesnika je imalo CT a 11,1% TT genotip. Nije utvrđena statistički značajna razlika između frekvence genotipova i alela u odnosu na kontrolnu grupu zdravih osoba. Kod bolesnika je nađena povišena prosečna vrednost pHci, a distribucija srednjih vrednosti prema prema genotipovima je bila: 26,9 mmol/l za CC, 28,39 µmol/l za CT i 27,2 µmol/l za TT genotip. Nivoi pHci nisu bili u korelaciji sa MTHFR677 genotipom, već sa primenom suplementacione terapije vitaminima i folatima.

**MTHFR GENOTYPE AND HOMOCYSTEINE LEVEL
IN HEMODIALYSIS PATIENTS**

Methylenetetrahydropholate reductase (MTHFR) is an important factor in homocysteine (Hcy) metabolism, playing a role in the remethylation of Hcy to methionine. MTHFR activity has genetic determination: C677T polymorphism of MTHFR gene, causes synthesis of thermolabile form of enzyme, with decreased enzyme activity and consecutive hyper-Hcy-emia. Number of studies analyzed C677T frequency in vascular diseases, due to established toxicity of the Hcy to vascular endothelium. These investigations are important for nephrological patients too, trying to give answers about a role of genetic factors in hyper-Hcy-emia in renal failure. The aim of our study was to establish MTHFR C677T frequency in hemodialysis (HD) patients, and to make correlation between MTHFR677 genotypes and plasma Hcy (pHcy) levels. Our group consisted of 81 patients with terminal renal failure. Genotype analysis was performed by PCR/RFLPs method, and pHcy was determined by HPLC with fluorescence detection. The frequencies of detected genotypes were: 42%, 46.9% and 11.1% for CC, CT and TT genotype, respectively. There was no significant difference of genotype and allele frequencies between HD patients and control group. Mean level of pHcy in patients was elevated with following distribution by genotypes: 26.9µmol/l for CC, 28.39 µmol/l for CT and 27.2mmol/l for TT genotype. Hcy levels in our group of HD patients were in correlation rather with vitamin and folate supplementation than with MTHFR genotypes.

UČESTALOST *F508del* I MUTACIJA U EGZONU 11 CFTR GENA U ZDRAVOJ JUGOSLOVENSKOJ POPULACIJI

M. Stanković, A. Divac, A. Nikolić i D. Radojković

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

Cistična fibroza (CF) je jedno od najčešćih naslednih monogenetskih oboljenja u populaciji belaca. Učestalost CF varira među različitim populacijama i kreće se od 1 na 2000-4000. Učestalost heterozigotnih nosilaca mutiranog gena je 1 na 20-25 individua. Gen odgovoran za nastanak cistične fibroze označen je kao transmembranski regulator provodljivosti u cističnoj fibrozi, tj. CFTR gen (Cystic Fibrosis Transmembrane Conductance Regulator). Do danas je otkriveno preko 1000 mutacija i preko 200 polimorfizama u CFTR genu. Od do sada otkrivenih mutacija samo osam je zastupljeno u populaciji belaca sa učestalošću većom od 0.5%, a mnoge mutacije su opisane samo jednom. Najčešća mutacija u CFTR genu je *F508del*, delecija tri bazna para u desetom egzonu, prisutna na oko 70% hromozoma koji nose mutirani gen. Raspodela mutacija u okviru CFTR gena nije ravnomerna. Pored *F508del* mutacije u egzonu 10, velika gustina mutacija karakteriše egzon 11.

Cilj ovog istraživanja bio je određivanje učestalosti mutiranog CFTR gena i određivanje učestalosti nosilaca CFTR mutacije u zdravoj jugoslovenskoj populaciji.

Studija je obuhvatila 69 dobrovoljnih davalaca krvi. Mutacija *F508del* detektovana je PSM metodom (PCR-mediated Site-directed Mutagenesis), dok su mutacije u egzonu 11 detektovane SSCP metodom (Single Strand Conformation Polymorphism).

U ovom istraživanju ukupno je detektovano 3 mutirana alela na 138 analiziranim hromozoma. Učestalost mutacija u CFTR genu u zdravoj jugoslovenskoj populaciji je 2.2%, dok je učestalost heterozigotnih nosilaca mutiranog alela 1/23. Dobijene učestalosti koreliraju sa do sada objavljenim podacima za evropske populacije. Na taj način dobijen je prvi podatak o učestalosti nosilaca mutiranog CFTR gena kod nas.

PCR DETEKCIJA GENOMA RAZLIČITIH MIKROORGANIZAMA U SINOVII BOLESNIKA SA REITER-OVIM SINDROMOM TRETIRANIH AZITROMICINOM

N. Strelić¹, Lj. Pavlica² i Z. Magić¹

¹Institut za medicinska istraživanja, Beograd

²Klinika za reumatologiju, Vojnomedicinska akademija, Beograd

Bakterijska infekcija zglobo je ozbiljan problem čiji ishod zavisi od pravilnog tretmana. Mali broj intra-artikularnih bakterija se može detektovati ultraosetljivim molekularnim tehnikama, baziranim na lančanoj reakciji polimeraze (PCR). Brza identifikacija bakterijske infekcije je neophodna za odgovarajući tretman inficiranih pacijenata.

Cilj ovog istraživanja je bio detekcija bakterijske DNK u tkivu sinovije, sinovijskoj tečnosti i perifernoj krvi bolesnika sa Reiter-ovim sindromom tretiranih azitromicinom. Tkivo sinovije, sinovijska tečnost i mononuklearni periferne krvi su korišćeni kao izvori DNK u PCR amplifikaciji bakterijskog 16 S rRNA gena. PCR prajmeri za specifičnu amplifikaciju DNK fragmenta 16S rRNA gena različitih bakterija (*Chlamydia trachomatis*, *C. pneumoniae*, *Mycoplasma hominis*, *Ureaplasma urealyticum*) su bili visoko specifični. Uzorci su dobijeni od 20 bolesnika sa Reiter-ovim sindromom. DNK je izolovana fenol-hloroform ekstrakcijom i posle PCR amplifikacije bakterijska DNK je detektovana na 10% PAGE.

Bakterijska DNK je detektovana u tri uzorka sinovijske tečnosti (15%), 11 uzoraka tkiva sinovije (55%) i 17 uzoraka periferne krvi (80%). Nakon tretmana azitromicinom, bakterijska DNK je detektovana u tri uzorka sinovijske tečnosti (15%), 7 uzoraka tkiva sinovije (35%) i 10 uzoraka periferne krvi (50%).

Najbolji rezultat terapije azitromicinom je dobijen u krvi posebno za *Mycoplasma hominis*.

PCR DETECTION OF GENOME OF DIFFERENT MICROORGANISMS IN SYNOVIA OF PATIENTS WITH REITER'S SYNDROME TREATED WITH AZITHROMYCYIN

Bacterial joint infection is a serious problem, the outcome of which depends on appropriate treatment. A small number of intra-articular bacteria can only be detected by using ultrasensitive molecular techniques, mainly those based on the polymerase chain reaction (PCR). The rapid identification of bacterial infection is essential for the proper treatment of infected patients.

The aim of this study was to detect bacterial DNA in synovial tissue, synovial fluid and peripheral blood of patients with Reiter's syndrome treated with azithromycin.

Synovial tissue, synovial fluid and peripheral blood mononuclear cells were used as the source of DNA for PCR amplification of bacterial 16 S rRNA gene. PCR primers for the specific amplification of DNA fragment of the 16 S rRNA gene of different bacteria (*Chlamydia trachomatis*, *C. pneumoniae*, *Mycoplasma hominis*, *Ureaplasma urealyticum*) were highly specific. Samples were obtained from 20 patients with Reiter's syndrome. DNA was isolated by phenol/chloroform extraction and after PCR amplification bacterial DNA was detected by 10% PAGE.

Bacterial DNA was detected in 3 samples of the synovial fluid (15%), 11 samples of the synovial tissue (55%), and 17 samples of peripheral blood (80%). After the treatment with azithromycin, bacterial DNA was detected in 3 samples of the synovial fluid (15%), 7 samples of the synovial tissue (35%), and 10 samples of peripheral blood (50%).

The best result with azithromycin therapy was obtained in blood especially for *Mycoplasma hominis*.

NIVO HOMOCISTEINA I *MTHFR C677T* GENOTIP KOD BOLESNIKA SA KARDIOVASKULARnim POREMEĆAJIMA

V. Šango¹, I. Novaković², N. Antonijević³, D. Mirković⁴, Lj. Luković²,
T. Damnjanović², M. Stanojević³, J. Peruničić³, Z. Vasiljević³ i M. Krajinović²

¹Laboratorija za medicinsku genetiku, ZC Jagodina, Jagodina

²Institut za biologiju i humanu genetiku Medicinskog fakulteta, Beograd

³Institut za kardiovaskularne bolesti KCS, Beograd

⁴Institut za medicinsku biohemiju KCS, Beograd

Metilentetrahidrofolat reduktaza (*MTHFR*) je jedan od značajnih enzima u metabolizmu homocisteina (Hci) koji katalizuje remetilaciju Hci u metionin. Hiperhomocisteinemija je mogući faktor rizika za kardiovaskularne bolesti (KVB), uključujući aterosklerozu i arterijske i venske tromboembolije, ali uloga *MTHFR C677T* polimorfizma u ovim poremećajima nije razjašnjena i brojne studije daju oprečne rezultate. Naše istraživanje smo preduzeli sa ciljem da se ispita udruženost *MTHFR C677T* genotipa i nivoa Hci kod bolesnika sa infarktom miokarda (IM) i plućnom embolijom (PE).

U istraživanje je uključeno 43 bolesnika sa IM (prosečna dob 42 god.) i 36 bolesnika sa PE (prosečna dob 51 god.). Kontrolnu grupu je činilo 38 zdravih osoba. Analiza *MTHFR* genotipa je vršena PCR/RFLPs metodom, a nivo Hci u plazmi je određivan metodom HPLC sa fluorescentnom detekcijom.

U grupi bolesnika sa IM bilo je 55.81% osoba sa C/C genotipom, 32.56% osoba sa C/T genotipom i 11.63% osoba sa T/T genotipom. Kod bolesnika sa PE učestalost genotipova je bila: .45.71% za C/C, 42.85% za C/T i 11.42% za T/T genotip. Razlika u učestalosti genotipova između dve grupe bolesnika sa KVB i kontrolne grupe nije bila statistički značajna. Prosečan nivo Hci u plazmi bolesnika sa IM bio je u okviru normalnih vrednosti: 11.93 μ mol/l za C/C, 10.58 μ mol/l za C/T i 9.68 μ mol/l za T/T genotip. Kod bolesnika sa PE nađene su blago povišene prosečne vrednosti Hci u sve tri grupe genotipova: 15.25 μ mol/l za C/C, 15.81 μ mol/l za C/T i 14.07 μ mol/l za T/T genotip. U našem istraživanju nije utvrđena povezanost između *MTHFR 677* genotipa i nivoa Hci kod bolesnika sa IM i PE.

HOMOCYSTEINE LEVELS AND *MTHFR C677T* GENOTYPES IN PATIENTS WITH CARDIOVASCULAR DISEASES

Methylentetrahydropholate reductase (*MTHFR*) is one of the important enzymes involved in homocysteine (Hcy) metabolism controlling remethylation Hcy to methionine. Hyperhomocysteinemia is a possible risk factor in cardiovascular diseases (CVD) including atherosclerosis and arterial and venous thrombosis, but role of *MTHFR C677T* polymorphism in CVD is not clearly understood, and results of number of studies are controversial.

We assessed the association between *MTHFR C677T* genotypes and homocysteine levels in patients with myocardial infarction (MI) and pulmonary embolism (PE).

Our group involved 43 patients with acute MI (mean age 42 y) and 36 patients with PE (mean age 51 y). Control group had 38 healthy subjects. *MTHFR* genotypes were analyzed using PCR/RFLPs method. Total plasma Hcy concentration was measured using HPLC with fluorescence detection. In a group of patients with MI we found 55.81% C/C homozygous genotypes, 32.56% C/T heterozygous genotypes and 11.63% T/T homozygous genotypes. In a PE group we detected 45.71% C/C, 42.85% C/T and 11.42% T/T genotypes. There was no significant difference in *MTHFR* genotype frequencies between two group of CVD patients and control subjects. Mean Hcy levels in MI patients were within normal range: 11.93 μ mol/l, 10.58 μ mol/l and 9.68 μ mol/l for C/C, C/T, and T/T genotype, respectively. In PE patients mean Hcy levels for all of three genotypes were slightly elevated: 15.25 μ mol/l, 15.81 μ mol/l and 14.07 μ mol/l for C/C, C/T and T/T group, respectively. We did not find association between *MTHFR 677* genotypes and homocysteine levels in patients with MI and PE.

UTICAJ PROFESIONALNE EKSPOZICIJE JONIZUJUĆIM ZRAČENJIMA NA UČESTALOST HROMOZOMSKIH PREDJELA

Snežana Tomanović, Dušanka Mirković, Branka Đurović, M. Mišović i Z. Bošković
Institut za medicinu rada, Vojnomedicinska akademija, Beograd

Upotreba rendgen aparata u dijagnostičke svrhe jedan je od najznačajnijih veštačkih izvora jonizujućih zračenja. U ukupnom ozračivaju populacije u medicinske svrhe rendgen dijagnostika učestvuje sa 90-95%. Pri svakoj primeni jonizujućih zračenja, pored pacijenata, malim dozama jonizujućih zračenja (MĐZ) izloženo je i zdravstveno osoblje, koje čini najbrojniju grupu lica profesionalno eksponiranih.

Rezultati analize hromozomskih aberacija najpouzdaniji su pokazatelj oštećenja nastalih usled delovanja MĐZ na organizam, zbog čega se ova analiza sprovodi u okviru zdravstvenih pregleda profesionalno eksponiranih lica.

U radu su prikazani rezultati citogenetskih ispitivanja 25 radiologa i rendgen tehničara. Kod svakog ispitanika izvršena je analiza 200 metafaznih hromozoma limfocita periferne krvi dobijenih klasičnom Morchaed-ovom metodom. Prosečan ekspozicioni staž iznosio je 13.52 ± 7.52 godina. Prema podacima lične dozimetrije za period od decembra 2002. godine do avgusta 2004. godine doze jonizujućih zračenja kretale su se u intervalu od 0.00-3.01 mSv, što je znatno ispod zakonom utvrđene granične doze od 20 mSv/god. Promene na hromozomima su konstatovane kod 28% ispitanih. Kod 16% su konstatovane promene tipa hromatidnih prekida i gapova, dok su kod 12% registrovane strukturne hromozomske aberacije (delekcija, translokacija, dicentrični hromozom i acentrični fragment). Istovremeno su u kontrolnoj grupi, koju su činila 33 medicinska radnika van zone MĐZ, promene uočene kod 12% ispitanih. U ovoj grupi dominante su promene tipa hromatidnih prekida i gapova, dok strukturne hromozomske aberacije nisu uočene.

Komparacijom ove dve grupe ispitanih uočavamo kako kvantitativne tako i kvalitativne razlike u pogledu konstatovanih hromozomskih aberacija.

INFLUENCE OF OCCUPATIONAL EXPOSITION TO IONIZING RADIATION ON THE FREQUENCY OF CHROMOSOME ABERRATIONS

Application of X-ray generators in diagnostic purposes is one of the most important artificial sources of ionizing radiation. X-ray diagnostic represents 90-95% of total population exposition from medical sources. With every application of ionizing radiation besides the patients, medical staff is exposed to low doses of ionizing radiation too. They represent the most abundant occupationally exposed group.

The results of chromosome aberration analysis represent one of the most reliable indicator of radiation damages. Thus, this analysis is included in health surveillance of radiation workers.

In this paper the results of citogenetical investigations for 25 radiologists and technicians are shown. For each person analysis of 200 metaphase chromosomes from peripheral blood lymphocytes was done. The average duration of occupational exposition was 13.52 ± 7.52 years. All radiation workers have used TL dosimeters. During the period: December 2002 - August 2004 the total doses varied between 0.00 and 3.01 mSv, which were significant below limited annual doses of 20 mSv. Chromosome changes were found at 28 % of investigated persons. Chromatide breaks and gaps were found at 16 %, and 12 % had structural chromosome aberrations (deletions, translocations, dicentric chromosome and acentric fragments). At the same time, in the control group of 33 medical workers, who were not exposed to ionizing radiation, chromosome changes were found at 12% of them. Chromatide breaks and gaps were dominant aberrations in this group, while structural chromosome aberrations weren't found.

Comparation between these two groups showed differences in quantity and quality of the recorded chromosome aberrations.

MUTACIJE C-MYC I C-ERB2 GENA KOD PLANOCELULARNOG KARCINOMA USNE DUPLJE

B. Popović¹, J. Milašin¹, B. Jekić², I. Novaković² i Lj. Luković²

¹Stomatološki fakultet, Institut za Biologiju, Univerzitet u Beogradu, Beograd

²Medicinski fakultet, Institut za biologiju i humanu genetiku, Univerzitet u Beogradu, Beograd

Planocelularni karcinomi, kao i većina malignih tumora, rezultat su akumulacije molekularno-genetičkih lezija, koje obuhvataju protoonkogene i tumor supresorske gene. Najčešće aktivirani geni, odgovorni za gubitak kontrole tokom transdukcije mitogenog signala, kod velike grupe humanih tumora, uključujući i planocelularni karcinom su protoonkogeni c-erb2 i c-myc. Produkt c-erb2 gena je transmembranski glikoproteinski receptor sa tirozin kinaznom aktivnošću, dok je produkt c-myc protoonkogena, transkripcioni faktor uključen u kontrolu ćelijske proliferacije. Osnovni mehanizam njihove onkogene aktivacije je genska amplifikacija.

U našoj studiji, genska amplifikacija c-myc i c-erb2 gena ispitana je metodom diferencijalnog PCR-a, a zatim je stopa amplifikacije korelisana sa kliničko-patološkim parametrima. Kod 14/70 (20%) PCR analiziranih parafinskih uzoraka, pokazana je amplifikacija c-erb2 gena, dok je kod 12/70 (17%) uzoraka utvrđena amplifikacija c-myc gena. Iako se procenat genske amplifikacije povećava sa stadijumom bolesti, korelacija između prisustva ovih genetičkih markera i kliničkih parametara nije najjasnija. Tumori sa amplifikacijom, bez obzira na stadijum i mesto nastanka predstavljaju podgrupu sa lošijom prognozom, ali je ipak potrebno pratiti pacijente u dužem vremenskom periodu, da bi mogli pouzdani zaključci da se donesu o korelaciji genotip-fenotip.

C-MYC AND C-ERB2 MUTATIONS IN ORAL SQUAMOUS CELL CARCINOMA

Oral squamous cell carcinomas (OSCC), like most other malignancies, result from an accumulation of molecular lesions in proto-oncogene and tumour suppressor genes. The most commonly activated genes responsible for the loss of control in signal transduction pathways in a wide range of human tumours including OSCC are proto-oncogenes - c-erb2 and c-myc. The product of c-erb2 is a transmembrane glycoprotein receptor with tyrosine kinase activity while the product of c-myc is a transcriptional factor which is involved in the control of cell proliferation. The principle mechanism of their oncogenic activation is gene amplification.

In our study, amplification status for c-myc and c-erb2 was evaluated using double differential PCR and the level of amplification was correlated with clinicopathological markers. 14 out of 70 PCR analyzed paraffin embedded samples, showed amplification of c-erb2 (20%) and 12 (17%) of the c-myc gene. Although the percentage of gene amplification tended to increase in high grade, high stage tumours, the correlation between the presence of amplification and tumour staging was not so clear. Tumours with amplification, regardless of their stage and site represent a subgroup with worse prognosis but a longer follow-up of the patients is still necessary.

FRIDRAJHOVA ATAJSIJA: ANALIZA MITOTIČKE I MEJOTIČKE NESTABILNOSTI FRDA LOKUSA

V. Dobričić, D. Savić, D. Keckarević i S. Romac

Biološki fakultet, Univerzitet u Beogradu, Centar za razvoj i primenu PCR-a, Beograd

Fridrajhova ataksija je autozomalno recessivno obolenje i predstavlja najčešću formu naslednih ataksija koja se javlja sa učestalošću od 1 u 50 000 u kavkazoidnoj populaciji. Osnovni klinički simptomi Fridrajhove ataksije su: ataksičan hod, ataksija ekstremiteta, areflexija donjih ekstremiteta, odsutni mišićni tetivni refleksi, dizartrija, oslabljen vibracioni senzibilitet, ekstenzioni plantarni odgovor, kardiomiopatija, pes cavus i skolioza.

U 96% slučajeva uzrok bolesti je homozigotna ekspanzija GAA ponovaka u prvom intronu gena za frataksin (*FRDA*). U 4% slučajeva bolest je izazvana GAA ekspanzijom na jednom i tačkastom mutacijom na drugom hromozomu. Normalni aleli sadrže od 5 do 33 GAA ponovaka. Aleli veličine od 34 do 65 ponovaka nisu asocirani sa bolešću, ali imaju veću verovatnoću ekspanzije prilikom intergeneracijskog prenošenja u odnosu na normalne alele. Mutirani aleli sadrže od 66 do 1700 ponovaka i odlikuju se somatskom nestabilnošću.

Rutinska molekularna dijagnostika Fridrajhove ataksije vrši se PCR amplifikacijom regiona FRDA gena sa GAA ponovcima i analizom amplifikovanih fragmenata na 1% agaroznom gelu.

U našoj laboratoriji uspostavljena je znatno preciznija i senzitivnija metoda (eng. *small pool, long range PCR based Southern blot*) za određivanje broja GAA ponovaka u FRDA genu, u cilju izučavanja intra- i intertkivnog somatskog mozaicizma mutiranog alela, kao i praćenja intergeneracijske nestabilnosti istih.

FRIEDREICH'S ATAXIA: ANALYSIS OF MITOTIC AND MEIOTIC INSTABILITY

Friedreich ataxia is an autosomal recessive disease and it is the most common of the inherited ataxias, with a frequency of 1 in 50 000 in the Caucasian population. Friedreich's ataxia is characterized by ataxic gait, limb ataxia, lower limb areflexia, absent tendon reflexes, dysarthria, decreased vibration sense, extensor plantar response, cardiomyopathy, pes cavus and scoliosis.

Friedreich's ataxia is primarily caused by an homozygous GAA repeat-expansion mutation within intron 1 of the FRDA gene (96% of patients). Approximately 4% of patients are compound heterozygotes for this expansion and a point mutation within the same gene. The number of GAA repeats in normal alleles range from 5 to 33. Alleles with 34-65 GAA repeats are not associated with an abnormal phenotype but they have significant propensity to expand during parental transmission. Disease-causing alleles are characterised by the huge number of GAA repeats (66-1700) and somatic instability.

Routine genetic testing is performed by PCR based GAA repeat length analysis.

In our laboratory we established more sensitive and precise method: *small pool, long range PCR based Southern blot method*. It is used for study of intra- and intertissue mosaicism and intergenerational instability of disease-causing allele.

DA LI SU CAG EKSPANZIJE UZROK NASTANKA AUTOZOMALNO DOMINANTNIH CEREBELARNIH ATAKSIJA NEPOZNATE ETIOLOGIJE?

Miljana Stevanović, Slobodanka Vukosavić i Stanka Romac

Biološki fakultet Univerziteta u Beogradu, Centar za primenu i razvoj PCR-a, Beograd

Autozomalno-dominantne cerebelarne ataksije (ADCA) predstavljaju heterogenu grupu poremećaja koji su asocirani sa različitim neurološkim poremećajima. Grupa ADCA za koju je genetička osnova i ova grupa bolesti označena je i terminom spinocerebelarne ataksije (SCA). Iako je u ovim bolestima očigledna genetička heterogenost koja se ogleda u činjenici da su ekspanzije u različitim lokusima odgovorne za pojavu bolesti, klinički simptomi su ipak veoma slični, što može biti posledica sličnog tipa mutacija (dinamičke mutacije) koji je u osnovi ovih poremećaja. Ove mutacije podrazumevaju, u najvećem broju slučajeva, ekspanzije CAG trinukleotidnih ponovaka koji se prepisuju u niz poliglutamina (poliQ).

Molekularno-genetičkom analizom odrepenog broja ADCA pacijenata, nije utvrđeno prisustvo ekspandovanih alela u do sada poznatim SCA lokusima. S obzirom da je kod tih pacijenata uočen fenomen genetičke anticipacije, pretpostavljeno je da se radi o istom tipu mutacija ali u do sada nepoznatim lokusima. Analiza veličine proteina sa poliQ nizovima iz proteinских ekstrakata izolovanih iz periferne krvi ovih pacijenata, uz pomoć visoko specifičnih poliQ antitela, može se koristiti kao pristup za definisanje novih lokusa koji sadrže CAG ponovke, a čija ekspanzija može biti asocirana sa određenim tipovima ADCA.

ARE CAG EXPANSIONS CAUSE OF AUTOSOMAL-DOMINANT CEREBELLAR ATAXIAS UNKNOWN ETIOLOGY?

Autosomal-dominant cerebellar ataxias (ADCA) are heterogenous group of disorders associated with wide array of neurological abnormalities. Genetic background for most of ADCA is known and this group of diseases is called spinocerebellar ataxias (SCA). Beside the fact that expansions in different loci are responsible for different type of disease, clinical symptoms are very similar, which is probably result of similar type of mutation (dynamic mutations) that are base of these disorders. These mutations are in the most of cases, result of expansion of CAG trinucleotide repeats that translate into tract of polyglutamine (polyQ).

Genetic analysis of some ADCA patients, showed no presence of expanded alleles in loci known so far. Since the phenomenon of genetic anticipation is observed, it was assumed that same type of mutations, but in some other loci, is responsible for these clinical symptoms. Analysis of protein polyQ length from protein extracts isolated from peripheral blood samples from these patients, using highly-specific polyQ antibodies, can be used as an approach to define new loci with CAG triplets, which expansion could be associated with certain ADCA types.

MOGUĆNOSTI MOLEKULARNE DIJAGNOSTIKE HEREDITARNE HEMOHROMATOZE TIP1 U SRBIJI I CRNOJ GORI

Lj. Zamurović¹, M. Šarić¹, M. Keckarević-Marković¹, B. Čuljković¹, J. Jović², S. Romac¹

¹Biološki fakultet Univerziteta u Beogradu

²Vojnomedicinska akademija, Beograd

Hereditarna hemohromatoza (HH) je čest, genetski uzrokovana poremećaj metabolisma gvožđa. Nasledjuje se autozomalno recessivno. Najčešći tip bolesti, HH tip1, je posledica mutacija u *HFE* genu (6p21.3). Do sada su opisane 3 *missense* mutacije: tranzicija G u A na poziciji 845 *HFE* gena (C282Y), transverzija C u G na poziciji 187 (H63D) i transverzija A u T na 193 poziciji (S65C). U 83% slučajeva oboleli su homozigoti za C282Y mutaciju. Druge dve mutacije se kod pacijenata javljaju znatno ređe, uglavnom u obliku kombinovanih heterozigota sa prvom mutacijom (C282Y/H63D i C282Y/S65C). U našoj laboratoriji uspostavljena je molekularna dijagnostika HH bazirana na umnožavanju željenog regiona *HFE* gena PCR metodom, restrikcionom digestijom PCR produkata i njihovom analizom na 3% agaroznom i 6% denaturišućem poliakrilamidnom gelu.

Značaj uvođenja ove analize je u obezbeđivanju dopunske dijagnostičke metode koja povećava pouzdanost dijagnostike, olakšava dijagnostiku u ranim fazama bolesti, kada se adekvatnim lečenjem mogu izbegići irreverzibilna oštećenja organa, kao i u otkrivanju grupa sa povećanim rizikom od oboljevanja. Pored toga, analiza *HFE* gena može dati značajne podatke o učestalosti mutacija u ovom genu u populaciji SCG.

POSSIBILITIES OF MOLECULAR DIAGNOSTIC OF HEREDITARY HEMOCHROMATOSIS TYPE1 IN SERBIA AND MONTENEGRO

Hereditary hemochromatosis is a common, autosomal recessive genetic disorder of iron metabolism. The most common form (HH type1) is caused by the mutations in *HFE* gene (6p21.3). Three missense mutations have been described so far: transition G to A at position 845 of *HFE* gene (C282Y), transversion C to G at position 187 (H63D) and transversion at position 193 (S65C). 83% of patients are homozygous for the C282Y mutation. The other two mutations are less common and mostly in form of compound heterozygots with the first mutation in phenotypic HH patients.

In our laboratory, molecular diagnostic of HH has been established, based on amplification of the selected region of *HFE* by PCR, restriction enzyme digest and analysis on 3% agarose and 6% denaturing polyacrylamid gel.

The importance of its application is in developing an additional diagnostic method which increases the liability of diagnosis, provide diagnosis in the early phases of illness, when is possible with appropriate treatment to avoid irreversible organ damage, and is also helpful as a screening to identify high-risk group. In addition, the *HFE* gene analysis can provide important data about prevalence of mutations in this gene in the population of Serbia and Montenegro.

MOGUĆNOSTI MOLEKULARNE DIJAGNOSTIKE HEREDITARNE HEMOHROMATOZE TIP1 U SRBIJI I CRNOJ GORI

Lj. Zamurović¹, M. Šarić¹, M. Keckarević-Marković¹, B. Čuljković¹, J. Jović², S. Romac¹

¹Biološki fakultet Univerziteta u Beogradu

²Vojnomedicinska akademija, Beograd

Hereditarna hemohromatoza (HH) je čest, genetski uzrokovana poremećaj metabolisma gvožđa. Nasleđuje se autozomalno recessivno. Najčešći tip bolesti, HH tip1, je posledica mutacije u *HFE* genu (6p21.3). Do sada su opisane 3 *missense* mutacije: tranzicija G u A na poziciji 845 *HFE* gena (C282Y), transverzija C u G na poziciji 187 (H63D) i transverzija A u T na 193 poziciji (S65C). U 83% slučajeva oboleli su homozigoti za C282Y mutaciju. Druge dve mutacije se kod pacijenata javljaju znatno ređe, uglavnom u obliku kombinovanih heterozigota sa prvom mutacijom (C282Y/H63D i C282Y/S65C). U našoj laboratoriji uspostavljena je molekularna dijagnostika HH bazirana na umnožavanju željenog regiona *HFE* gena PCR metodom, restrikcionom digestijom PCR produkata i njihovom analizom na 3% agaroznom i 6% denaturišućem poliakrilamidnom gelu.

Značaj uvođenja ove analize je u obezbeđivanju dopunske dijagnostičke metode koja povećava pouzdanost dijagnostike, olakšava dijagnostiku u ranim fazama bolesti, kada se adekvatnim lečenjem mogu izbeći irreverzibilna oštećenja organa, kao i u otkrivanju grupa sa povećanim rizikom od oboljevanja. Pored toga, analiza *HFE* gena može dati značajne podatke o učestalosti mutacija u ovom genu u populaciji SCG.

POSSIBILITIES OF MOLECULAR DIAGNOSTIC OF HEREDITARY HEMOCHROMATOSIS TYPE1 IN SERBIA AND MONTENEGRO

Hereditary hemochromatosis is a common, autosomal recessive genetic disorder of iron metabolism. The most common form (HH type1) is caused by the mutations in *HFE* gene (6p21.3). Three missense mutations have been described so far: transition G to A at position 845 of *HFE* gene (C282Y), transversion C to G at position 187 (H63D) and transversion at position 193 (S65C). 83% of patients are homozygous for the C282Y mutation. The other two mutations are less common and mostly in form of compound heterozygots with the first mutation in phenotypic HH patients.

In our laboratory, molecular diagnostic of HH has been established, based on amplification of the selected region of *HFE* by PCR, restriction enzyme digest and analysis on 3% agarose and 6% denaturing polyacrylamid gel.

The importance of its application is in developing an additional diagnostic method which increases the liability of diagnosis, provide diagnosis in the early phases of illness, when is possible with appropriate treatment to avoid irreversible organ damage, and is also helpful as a screening to identify high-risk group. In addition, the *HFE* gene analysis can provide important data about prevalence of mutations in this gene in the population of Serbia and Montenegro.

CITOGENETSKA EVOLUCIJA HRONIČNE MIJELOIDNE LEUKEMIJE

Milica Strnad¹, Biljana Todorić-Živanović¹, Željka Tatomirović¹ i Dragana Stamatović²

¹Zavod za patologiju i sudsku medicinu, VMA, Beograd

²Klinika za hematologiju, VMA, Beograd

Hronična mijeloidna leukemija (CML) je maligna, mijeloproliferativna bolest sa klonalnom proliferacijom matične ćelije hematopoeze. Centralnu ulogu u patogenezi CML ima Philadelphia hromozom (Ph) koji nastaje kao rezultat recipročne translokacije t(9;22)(q34;q11). Ph hromozom je uglavnom jedinstvena hromozomska aberacija u hroničnoj fazi CML ali su dodatne hromozomske promene uočene u 75-80% slučajeva u fazi akceleracije i blastne transformacije bolesti. Najčešće detektovane sekundarne hromozomske aberacije su prisustvo dodatnog Ph hromozoma, trizomija 8, trizomija 19 i i(17q). Citogenetska analiza je rađena na metafaznim hromozomima dobijanim iz aspirata kostne srži 30 bolesnika sa kliničkom dijagnozom CML (7-blastna transformacija, 23-hronična faza). U okviru analizirane grupe evolucija kariotipa je uočena kod 11(37%) bolesnika. Kod 8 bolesnika su uočene mitoze sa preko 60 hromozoma, kod dva bolesnika strukturne hromozomske aberacije [46,XX,t(3;21)(q21;p11),t(9;22)(q34;q11);46,XX,t(6;9)(q15;p24),t(9;22)(q34;q11)] i kod jednog numerička aberacija sa prisustvom dodatnog marker hromozoma (47,XX, t(9;22)(q34;q11),+mar). Evolucija kariotipa pretstavlja bitnu karakteristiku neoplastično transformisanih CML ćelija. Uočavanje sekundarnih hromozomske promene je važno jer uglavnom prethode hematološkoj i kliničkoj manifestaciji blastne transformacije bolesti zbog čega se smatraju negativnim prognostičkim parametrom u lečenju CML.

PROCENA GENOTOKSIČNOG OŠTEĆENJA KOD RADNIKA PROFESIONALNO IZLOŽENIH NAFTINIM DERIVATIMA

J. Mrđanović, D. Jakimov, S. Turšijan i G. Bogdanović

Institut za onkologiju, Sremska Kamenica

Cilj ovog istraživanja je da se proceni stepen genotoksičnog oštećenja kod radnika koji su profesionalno izloženi isparenjima naftinih derivata i da se utvrdi uticaj pušenja kao kofaktora koji učestvuje u oštećenju genoma.

Biomonitoring je izvršen na limfocitima periferne krvi radnika NIS-Rafinerije Novi Sad primenom testova mikronukleusa (MN) i razmene sestrinskih hromatida (SCE). Ispitivanjem je obuhvaćeno 30 radnika (13 pušača i 17 nepušača) i 15 kontrolnih osoba (7 pušača i 8 nepušača).

Učestalost SCE i MN kod radnika je bila povećana u odnosu na kontrolnu grupu, dok je index proliferacije pokazivao nižu vrednost. Pušači u grupi profesionalno izloženih radnika, imali su najveću vrednost SCE u poređenju sa radnicima nepušačima kao i sa kontrolnom grupom. Pušači u grupi radnika imali su veću vrednost MN od nepušača iz iste grupe, dok su u kontrolnoj grupi nepušači imali višu vrednost MN. Proliferacioni index je pokazivao najveću vrednost u grupi kontrolnih nepušača, a najmanji u grupi nepušača radnika.

Preliminarni rezultati ukazuju na porast genotoksičnog oštećenja kod radnika koji su profesionalno izloženi isparenjima naftinih derivata. Pušenje kao kofaktor u genotoksičnom delovanju hemijskih agenasa doprinosi oštećenju genoma.

EVALUATION OF GENOTOXIC DAMAGES IN WORKERS PROFESSIONALLY EXPOSED TO OIL EVAPORATION

The aim of this study was to estimate genotoxic damages in workers occupationally exposed to oil evaporation, and to evaluate the influence of smoking as confounding factor in genome damaging.

Biomonitoring was performed on peripheral blood lymphocytes of the workers employed in NIS-Rafinerija Novi Sad using micronuclei analysis (MN) and sister chromatide exchanges (SCE). Thirty workers (13 smokers and 17 nonsmokers) and 15 control persons (7 smokers and 8 nonsmokers) were included in the study.

Frequency of SCE and MN in workers was higher in comparison to control group, while proliferation index showed lower value. Smokers in the exposed group of workers had the highest values of SCE compared with workers-nonsmokers and control group. Smokers in group of workers showed higher incidence of MN compared to nonsmokers in the same group, while control-nonsmokers had the higher value of MN. Proliferation index was the highest in the group of control-nonsmokers and the lowest in the group of workers-nonsmokers.

Preliminary results demonstrate the increased incidence of genotoxic damage in workers professionally exposed to oil evaporation. Smoking as cofactor in genotoxic effect of chemical agents contributes to genome damage.

REZULTATI OSMOGODIŠNJEK ISKUSTVA U DETEKCIJI MUTACIJA U GENU ZA CISTIČNU FIBROZU U SRBIJI I CRNOJ GORI

D. Radivojević¹, M. Đurišić¹, T. Lalić¹, M. Guć-Šćekić¹, P. Minić²,
A. Sovtić², Lj. Stojanov³ i ž. Puzigaća⁴

¹Laboratorija za medicinsku genetiku, ²Odeljenje za pulmologiju,

³Odeljenje za metabolizam i kliničku genetiku, ⁴Ginekološka klinika,

Institut za zdravstvenu zaštitu majke i deteta «Dr Vukan Čupić», Beograd

Šest mutacija (F508del, G542X, 621+1G>T, 2789+5G>A, R1070Q and S466X) su bile zastupljene kod 79.89% CF alela, od kojih je najveću učestalost imala F508del mutacija (72,35%). Drugih 12 mutacija (R334W, 2184insA, I507del, 1525-1G>A, E585X, R75X, M1I, 457TAT>G, 574delA, 2723delTT, A120T and 2907delTT) je nađeno kod 3.36% CF alela. Kod jednog bolesnika je identifikovana nova mutacija (2723delTT). Korišćenjem pomenutih metoda, ukupno je detektovano 18 različitih mutacija koje su bile zastupljene kod 82.41% CF alela.

Analiza haplotipa je urađena kod 23 porodice kod kojih su jedan ili oba CF alela ostala neidentifikovana. Analiza je urađena za 6 dialelnih polimorfizama i 1 tetranukleotidne ponovke (XV2C-KM19-MP6D9-J44-IVS6a(GATT)-M470V-T854T), na 102 CF i 54 normalna hromozoma. Haplotip 1-2-2-1-6-1-1 je bio najčešće vezan za F508del mutaciju (tzv. linkage disequilibrium), dok su normalni hromozomi uglavnom bili vezani za 1-1-2-1-6-1-2 haplotip.

Ovi rezultati ukazuju da je molekularna osnova cistične fibroze u Srbiji i Crnoj Gori dosta heterogena. Š obzirom da je u toku ove studije identifikovano vise od 80% CF alela, dobijeni podaci se mogu iskoristiti za pravljenje strategije o mogućnostima skrininga u našoj zemlji i pružanje odgovarajućeg genetičkog saveta visoko rizičnim porodicama.

RESULTS OF EIGHT YEARS EXPERIENCE IN CYSTIC FIBROSIS MUTATION TESTING IN SERBIA AND MONTENEGRO

Six different mutations (F508del, G542X, 621+1G>T, 2789+5G>A, R1070Q and S466X) accounted for 79.89% of CF alleles, with F508del mutation showing a frequency of 72.35%. Another 12 mutations (R334W, 2184insA, I507del, 1525-1G>A, E585X, R75X, M1I, 457TAT>G, 574delA, 2723delTT, A120T and 2907delTT), covered an additional 3.36%. A novel mutation (2723delTT) was found in one CF patient (F508del/2723delTT). Thus, a total of 18 mutations cover 82.41% of CF alleles.

Haplotype analysis was done in 23 families where one or both CF alleles remain uncharacterized. Analysis was done for 6 diallelic sites and one tetranucleotide repeat (XV2C-KM19-MP6D9-J44-IVS6a(GATT)-M470V-T854T) on 102 CF and 54 normal chromosomes. Strong linkage disequilibrium was observed for F508del mutation and one haplotype (1-2-2-1-6-1-1), while normal chromosomes mostly were associated with another one (1-1-2-1-6-1-2).

These results imply that the molecular basis of cystic fibrosis in Serbia and Montenegro is heterogeneous. Since we detected more than 80% of CFTR alleles, results could be used for making strategy for future screening and appropriate genetic counseling programs in our country.

KORELACIJA INFEKCIJE VISOKORIZIČNIM HUMANIM PAPILOMA VIRUSIMA SA TOKOM BOLESTI U BOLESNIKA SA ORALNIM KARCINOMU USNE DUPLJE

Z. Magić¹, N. Jović², R. Kozomara² i J. Stojanović¹

¹Institut za medicinska istraživanja, Vojnomedicinska akademija, Beograd

²Klinika za maksilofacijalnu hirurgiju, Vojnomedicinska akademija, Beograd

Analizirano je 50 pacijenata u II i III stadijumu OSCC jezika i poda usne duplje (histološki i nuklearni gradus 1, 2 i 3). Starost pacijenata se kretala od 45 do 72 godine, 97% su bili pušači dok je 93% konzumiralo alkohol. DNK iz tumorskog tkiva je izolovana fenol-hloroformskom ekstrakcijom. Prisustvo visokorizičnih tipova HPV (16, 18, 31 i 33) je detektovano PCR-om i PAGE-om. HPV infekcija je detektovana kod 64% pacijenata. HPV16 je detektovan kod 10 pacijenata (31.2%), HPV18 i HPV31 kod 6 (18.7%), dok HPV33 nije detektovan. Kod 3 pacijenta (9.3%) su detektovani istovremeno HPV16 i HPV31 a HPV18 i HPV31 kod 7 (21%). Tumori u III kliničkom stadijumu su u 58% slučajeva sadržali neki od ispitivanih virusa. Umereno-diferentovani OSCC sa srednjim nuklearnim gradusom su najčešće bili inficirani sa HPV (46.8%), dobro diferentovani 40.6%, a slabo-diferentovani svega 12.5%. Distribucija HPV infekcije u tumorima niskog nuklearnog gradusa (28.1%) i visokog nuklearnog gradusa (25%) je bila slična. Pušenje i konzumacija alkohola su zavisni prediktivni faktori HPV infekcije. Period bez pojave bolesti (DFI) u ispitivanoj grupi pacijenata se kretao od 4 do 36 meseci. DFI i ukupno preživljavanje (OS) kod pacijenata sa HPV infekcijom su bili značajno kraći u odnosu na pacijente bez HPV infekcije (log rank test). Karcinomi usne duplje su često inficirani kancerogenim tipovima HPV. U ispitivanoj grupi pacijenata napena je značajna povezanost infekcije tumora kancerogenim tipovima HPV sa tokom i prognozom bolesti.

CORRELATION OF HIGH RISK HUMAN PAPILLOMAVIRUS INFECTION WITH DISEASE PROGRESSION IN PATIENTS WITH ORAL SQUAMOCELLULAR CARCINOMA

We analysed 50 patients with stage II and III of histologically confirmed OSCC of the tongue or oral floor (histological and nuclear grade 1, 2 or 3). Patients were 45-72 years old, 97% were heavy smokers, 93% were alcohol consumers. Genomic DNA was isolated according to standard procedure with phenol/chloroform/isoamylalcohol. Presence of HPV types 16, 18, 31 and 33 was detected by PCR/PAGE. HPV infection was detected in 64% of patients. HPV16 was found in 10 pts (31.2%), HPV18 and HPV31 in 6 (18.7%) and none with HPV33. Double positive HPV infection HPV16+31 was detected in 3 patients (9.3%) and HPV18+31 in 7 patients (21%). Tumors in clinical stage III was more likely to contain HPV (58%). Moderately-differentiated OSCC with medium-nuclear grade were more likely to contain HPV (46.8%), well-differentiated to (40.6%) and poorly- differentiated in only (12.5%). Low-nuclear grade was detected in 28.1% and high-nuclear grade in 25% HPV positive tumors. Tobacco usage and alcohol consumption were an independent predictor of HPV risk for OSCC. Disease-free interval (DFI) of patients ranged from 4 to 36 months. DFI and overall survival (OS) in pts with HPV infection compared to the pts without HPV infection was significantly shorter (log rank test). Infection with oncogenic HPV is frequent in oral cancers. Viral oncogene expression and viral integration suggest firm correlation between HPV infection and clinical course of disease in the examined group of patients.

ZAVRŠNO PREDAVANJE
CLOSING LECTURE

MOLEKULARNA BIOLOGIJA U VREMENU PRED NAMA

V. Glišin

Institut za molekularnu genetiku i genetičko inženjerstvo, Beograd

U drugoj polovini prošlog veka molekularna biologija se afirmisala kao vodeća biološka naučna disciplina koja objašnjava na jedan racionalan i naučno zasnovan način bez izuzetka sve osobine nekog živog bića. Bez obzira na to da li su u pitanju bakterije, biljke ili životinje. Svakim danom sve veći i veći broj bioloških fenomena dobijaju svoje molekularno objašnjenje. Rađanje, starenje, smrt, evolucija, kognicija kod čoveka, emocije, talent, bolesti, lečenje organskih i infektivnih bolesti, sve se danas može da objasni i objašnjava sa tačke gledišta molekularne biologije. Jasno je onda zašto smatram da je tzv. Redukcionistički pristup interpretacije života uopšte, jedino valjan. Za takvu moju argumentaciju dovoljno je pročitati samo naslove poglavlja u knjizi Bruce Albertsa i koautora, «*Molecular Biology of the Cell*», pa da se utvrdi da je osim molekularnog, svako drugo objašnjenje svih bioloških fenomena bliže religiji nego nauci.

Prva faza molekularne biologije je otpočela negde oko 1945. godine i možemo reći da je trajala negde do početka 1970-tih godina. U ovom periodu bitna otkrića uključuje saznanje, prvo, da su u svim živim organizmima geni smešteni u velikim molekulima nazvanim DNK (deoskribonukleinska kiselina); drugo, opis hemijske strukture DNK, i treće, na koji način hemijska struktura DNK određuje specifične individualne osobine. Drugi period, koji počinje početkom sedamdesetih i traje do danas, počeo je razvojem tehnologije rekombinantne DNK, popularnije, genetičkog inženjerstva. Tehnologija rekombinantne DNK nam je omogućila da razumemo na koji način funkcionišu složeni genetički sistemi. Od tih dana naši pogledi na strukturu, organizaciju i funkcionisanje gena su dramatično modifikovani. Imajući u vidu ogromno znanje i bezmalo neograničene mogućnosti da analiziramo, intervenišemo i menjamo po sopstvenoj želji bilo koju osobinu bilo kog živog bića, otvorilo nam je mogućnost da u sledećem vremenskom razdoblju vrlo aktivno i prioritetsno, sa tačke gledišta molekularne biologije, pristupimo objašnjenju i odgometanju porekla naše SVESTI.

THE FUTURE OF MOLECULAR BIOLOGY

During second half of last century molecular biology has been successful as leading scientific discipline explaining through rationally and scientific based way, without exception, all traits of living organisms, including bacteria, plants and animals. Number of biological processes explained at the molecular level is increasing day by day. Birth, apoptosis, death, evolution, human cognitive processes, emotions, gifts, diseases, treatment of organic and infective illness, all mentioned above have explanation by molecular biology today. Reduction approach to the life explanation, according it my opinion is only valid during these days. Arguments could be find in the Bruce Alberts publication «*Molecular Biology of the Cell*» which are giving more chance to scientific molecular explanation of the life than religious one.

First phase of molecular biology development started around 1945 and could be considered as ended by 1970. During this period the main discoveries are: genes in all living organisms are in big molecules –DNA (deoxyribonucleic acid); determination of DNA chemical structure and finally way of DNA controlling individual specificity of living organism. Second period started after 1970. And is lasting up to actual days. First step has been developing of recombinant DNA technology, popularly called genetic engineering. This technology enables scientists to understand the function of complexes genetic systems. Knowledge about the structure, organization and gene function has been dramatically modified. Using enormous knowledge we are able for the analyses, interventions and, according to own will changing any behavior of living organisms.

LISTA AUTORA / AUTOR INDEX

A

- Aksentijević I. 194
Aleksić J. 22
Anađević T. 24
Anastasijević N. 118
Andželković M. 14, 16, 27, 34, 37, 41, 53
Andželković V. 102, 164, 166, 167, 175
Antonijević N. 216
Armuš J. 200
Arsenijević S. 212
Arsenović A. 160
Atlagić J. 36, 40, 92, 130

B

- Babić M. 102, 166, 167, 175
Bajić V. 51, 54, 195
Ballian D. 145
Barac S. 120
Barjaktarović R. 100, 119
Begović J. 78, 185
Bekavac G. 99
Berenji J. 87
Berić-Bjedov T. 23, 32, 38, 61
Bešlić Z. 178
Biberdžić M. 120, 122, 151
Bižić S. 204, 210
Blagojević J. 24, 49, 62
Bodroža-Solarov M. 156
Bogdanović G. 224
Bošev D. 164, 165
Bošev Z. 165
Bošković D. 186, 204, 210
Bošković J. 68
Bošković M. 68
Bošković Z. 217
Božić M. 223
Božin D. 56
Branković-Magić M. 184
Bratić I. 45, 48
Brdar M. 121
Bunjevački G. 192
Bunjevački G. 209

C

- Cikota B. 184

Ć

- Ćirković D. 115
Ćirković S. 209

Č

- Čačić N. 73, 90, 125, 140, 147
Čuljković B. 221
Čuturilo G. 206
Čvorkov-Dražić M. 189, 192

D

- Damjanović M. 159
Damjanović T. 213, 216
Daničić V. 138
Davidović M. 100
Deletić N. 120, 122, 151
Delić N. 102, 166, 167, 175
Denčić S. 81, 82, 96, 100, 132
Denčić-Feketa M. 204, 210
Dijanović D. 123
Dillyara Serikbaevna A. 174
Dimitrijević B. 198
Dimitrijević M. 12, 149
Divac A. 214
Dobričin V. 219
Dragičević V. 116
Dragovich-Novosalenskaya A. Yu. 18
Drakulić D. 180, 196
Drinić G. 172
Dučić M. 129
Durković J. 205
Dušanić N. 130

Đ

- Đalović I. 153
Đan M. 15, 96
Đelić D. 195
Đelić N. 51, 53, 54, 195, 199
Đivanović T. 155, 156
Đokić D. 187
Đokić L. 48
Đorđević M. 120, 189, 207
Đorđević V. 186, 204, 210
Đukanović L. 173
Đukanović Lj. 213
Đukić N. 17, 41

Đurić M. 187, 189
Đurić V. 122
Đuričić S. 187
Đurišić M. 187, 208, 225
Đurović B. 217
Đurović J. 52, 59

E

Erić I. 126
Erić U. 173

F

Fira Đ. 28, 185
Fotirić M. 110

G

Gaćina Lj. 193, 211
Galović V. 75, 81, 82
Glišin V. 228
Golić N. 33
Gorjanović B. 124
Gotić M. 204, 210
Graner A. 86, 91
Grković S. 206
Grujičić D. 212
Gué-Šćekić M. 187, 207, 208, 209, 225
Gudžić S. 122
Guzina V. 109
Gvozdanović-Varga J. 93
Gvozdenović S. 128

H

Hadlaczky G. 6
Hladni N. 103
Hristov N. 95

I

Ignjatović-Micić D. 25, 29, 133
Ilić M. 188
Ilić V. 188
Ilić-Tomić T. 70
Isajev V. 13, 113, 129, 138, 161
Ivanović A. 49
Ivanović M. 109, 116, 155
Ivanović M. R. 85
Ivetić V. 113, 146

J

Jakimov D. 224
Janić D. 191, 192, 201
Jarak M. 20
Jekić B. 213, 218
Jelovac D. 8, 82
Ješić M. 206
Jevtić G. 131
Jocić S. 84
Jojić V. 49
Joković N. 26
Joksić G. 23
Joksimović J. 128, 130
Jošić D. 20
Jovanović D. 106
Jovanović J. 186, 189, 191, 192, 201, 204, 210
Jovanović S. 9, 19
Jovanović-Ćupin S. 223
Jovanović-Privrođski J. 193, 211
Jovčić B. 33, 185
Jović J. 221
Jović N. 226

K

Kalajdžić P. 27
Kapetanov M. 114
Karagić Đ. 97
Katić S. 97, 141, 155
Kavečan I. 193, 211
Keckarević D. 219
Keckarević-Marković M. 221
Kekić V. 35
Kirilova M. 44
Knežević D. 17, 41, 143, 157, 170
Knežević R. 135
Knežević-Ušaj S. 198
Knežević-Vukčević J. 23, 32, 38, 39, 61
Kobiljski B. 75, 80, 96, 100, 119, 121, 132, 136
Kojić L. 174
Kojić M. 78
Kondić A. 100
Kondić-Šipka A. 80, 132, 136
Konstantinov K. 126, 138, 172
Košarčić D. 114
Košarčić S. 114
Kotaranin Z. 81

- Kovačev L. 73, 90, 125, 140, 147
 Kovačević B. 109
 Kovačević M. 114
 Kovačević-Gruičić N. 46, 50, 57, 58
 Kovacs A. 114
 Kozomora R. 226
 Krajinović M. 194, 198, 213, 216
 Kraljević-Balalić M. 80, 93, 103, 109, 121, 124, 139, 142, 149
 Kremensky I. 205
 Kresović B. 127
 Krivokapić-Dokmanović L. 191, 192, 201
 Krstanović S. 160
 Krstić A. 46, 57, 193, 209
 Krstić K. 28, 185
 Krtolica K. 181, 186, 197, 198
 Kuburović M. 139
- L**
- Lakić N. 209
 Lalić T. 187, 208, 225
 Lavadinović V. 161
 Lazarević V. 61
 Lazić-Jančić V. 25, 29, 133
 Lečić N. 102
 Lemkey K. 102
 Lozo J. 28, 185
 Lugić Z. 131, 134, 150
 Luković Lj. 194, 213, 216, 218
- Lj**
- Ljevnaić B. 132
 Ljubijankić G. 76
- M**
- Madić M. 139
 Madžar R. 193
 Magić Z. 181, 184, 186, 188, 197, 215, 226
 Manasijević V. 193
 Marinković D. 53, 212
 Marinković R. 106, 137
 Marjanović M. 80, 100
 Marković B. 53, 54, 199
 Marković K. 25, 29, 133
 Marković S. 69
 Marković Ž. 104, 159
- Mataruga M. 94, 138
 Matić G. 41
 Mezei S. 73, 90, 125, 140, 147
 Micanović D. 143, 157
 Mićić D. 209
 Mihailović V. 97, 141, 142, 155
 Mihajlović I. 123
 Mijatović M. 104
 Mikić A. 97, 141, 142, 155
 Mikić T. 145
 Miklič V. 130
 Milanov D. 114
 Milašin J. 207, 218
 Milatović D. 108
 Miletić R. 30, 31
 Milić D. 141, 142
 Miličić S. 20
 Milivojević M. 144
 Miljanović T. 40
 Milojević N. 65
 Milošević-Đorđević O. 212
 Miloshev G. 39, 44
 Milovanović J. 146
 Milovanović M. 101, 163, 168
 Milović I. 187
 Milutinović M. 110
 Minić P. 225
 Mirković D. 194, 213, 216, 217
 Mišović M. 217
 Mitić N. 30, 31, 72, 74
 Mitić-Ćulafić D. 23, 32, 38
 Mitrović T. 190
 Mladenov N. 95
 Mladenović O. 213
 Mladenović-Drinić S. 20, 126, 138, 169, 172
 Mojsin M. 46, 50, 57
 Mratinić E. 108
 Mrđanović J. 224
 Mrkovački N. 147
- N**
- Nagl N. 73, 90, 125, 140
 Nastasić A. 99
 Nešić Z. 98, 153
 Nešković G. 223
 Nikčević G. 58, 59, 60, 180, 196
 Nikolić A. 169, 214
 Nikolić B. 23, 32, 38

Nikolić D. 110, 148
Nikolić M. 33, 185
Nikolić R. 30, 31, 72, 74
Novaković I. 194, 213, 216, 218
Nožić D. 223

O

Obadović M. 193
Obreht D. 15, 75, 96, 100, 119
Obrenović M. 211
Ocokoljić M. 118
Ordon F. 86
Orlović S. 22, 145

P

Pajić Z. 105, 173
Pataki I. 97
Pavković-Lučić S. 35
Pavlica Lj. 215
Pavlović M. 143, 157
Pavlović N. 158
Pavlović S. 189, 191, 192, 201
Pekić S. 164
Pekić V. 8
Pellio B. 86
Perišić V. 168
Perović D. 86, 91
Perović J. 86
Peruničić J. 216
Pešić A. 170
Pešić V. 156
Petrović I. 52, 58, 59
Petrović S. 12, 149
Petručev B. 189, 191, 192, 201
Pilipović J. 75, 100
Popović A. 24
Popović B. 213, 218
Popović-Kuzmanović D. 194
Poštić D. 144
Prijić Lj. 127
Prijić Ž 68
Purar B. 99
Puzigaća Ž 225

R

Radivojević D. 187, 208, 225
Radojković D. 214
Radosavljević M. 171

Radošević G. 111
Radošević Ž 104
Radotić K. 129
Radovanović N. 40
Radović A. 197
Radović J. 131, 134, 150
Rakonjac V. 108, 110, 112, 178
Ranin L. 185
Rašić G. 14, 34
Romac S. 219, 220, 221
Rošulj M. 105
Rudež J. 193

S

Saftić-Panković D. 40
Sakač Z. 130
Saratlić G. 102, 167, 175
Savić D. 219
Savić M. 9, 45, 48, 70,
Savić T. 16, 60
Sečanski M. 177
Seović m. 52
Sikimić P. 200
Sikora V. 162
Simić D. 23, 32, 38, 61
Simić-Ogrizović S. 213
Simonović J. 223
Sinigerska I. 205
Sokolović D. 134, 150
Soldo B. 61
Sovtić A. 225
Spasić M. 116
Spasojević-Tišma V. 223
Spremo-Potparević B. 51, 54, 195
Srđić J. 172
Srebrić M. 116, 169, 176
Sredojević S. 116
Stajković O. 23
Staletić M. 163, 168
Stamatović D. 181, 197, 222
Stamenković-Radak M. 14, 16, 34, 37, 41
Stanić S. 35
Stanimirović Z. 19, 53, 115, 200
Stanković G. 102, 166, 167, 175
Stanković Lj. 158, 159
Stanković M. 214
Stanković N. 76
Stanković S. 23, 32, 38, 61
Stanković V. 123

- Stanojević B. 223
 Stanojević J. 23, 32, 38, 39
 Stanojević M. 216
 Stein N. 86
 Stevanović J. 19, 115, 200
 Stevanović M. 46, 50, 52, 57, 58, 59,
 60, 180, 196, 207, 220
 Stevanović V. 170
 Stojanov Lj. 189, 207, 225
 Stojanović J. 151, 226
 Stojanović Lj. 194
 Stojanović S. 163
 Stojković S. 120, 122, 151
 Stracke S. 86
 Strahinić I. 78, 185
 Strand M. 181, 222
 Strelić N. 215
 Streng S. 86
 Strndar M. 197
 Suvajdžić Lj. 114
- Š**
- Šango V. 216
 Šarić M. 221
 Šenerović L. 76
 Šijačić-Nikolić M. 94, 113, 107, 146, 152
 Škorić D. 79, 84, 103, 106, 128
 Šurlan-Momirović G. 91, 155, 160, 177
- T**
- Tarasenko T. 193
 Taški K. 77, 79
 Tatomirović Ž 222
 Terzić S. 36
 Terzić-Vidojević A. 28, 33, 185
 Todić S. 178
 Todorić B. 186
 Todorić-Živanović B. 181
 Todorović G. 105, 177
 Todorović-Živanović B. 197, 222
 Tolimir M. 127
 Tolinački M. 78, 185
 Tomanović S. 217
 Tomić Z. 98, 153
 Tomišić-Kosić I. 14, 37
 Topisarević Lj. 26, 28, 33, 64, 78, 185
 Tošić M. 111, 154, 189
 Tošić N. 191, 192, 201
 Trailović R. 9
- Tucović A. 94, 107, 135, 152
 Turšijan S. 224
- U**
- Urošević D. 143, 157
- V**
- Vančetović J. 105
 Vapa Lj. 15, 96, 100, 119
 Vasić D. 77, 79, 93
 Vasić M. 93
 Vasić N. 99
 Vasić N. J. 85
 Vasić T. 134
 Vasilevski G. 165
 Vasiljević B. 45, 48, 65, 69, 70
 Vasiljević S. 141, 142, 155
 Vasiljević Z. 216
 Videnović Ž 127
 Vilotić D. 107, 111, 146, 152, 154
 Vojnović S. 65, 69
 Vratuša V. 118
 Vrvić M. 116
 Vujačić V. 156
 Vujić D. 187
 Vujošević M. 24, 49, 62
 Vukašinović M. 26, 28, 185
 Vukosavić S. 220
 Vuković-Gačić B. 23, 32, 38
- Z**
- Zamurović D. 208
 Zamurović Lj. 221
 Zdravković J. 158, 159
 Zdravković M. 158, 159,
 Zečević B. 104
 Zečević V. 143, 157
 Zorić M. 85
- Ž**
- Žikić M. 30, 31
 Živanović G. 27
 Živanović T. 160, 177
 Žvković L. 51
 Žujović M. 98, 153
- Y**
- Yurievna-Dragovich A. 17

S P O N Z O R I
III KONGRESA GENETIČARA SRBIJE

S P O N S O R S
OF THE III CONGRESS OF SERBIAN GENETICISTS

Ministarstvo za nauku i zaštitu životne sredine Republike Srbije, Beograd

Naučni institut za ratarstvo i povrtarstvo, Novi Sad

Istraživački institut za kukuruz «Zemun Polje», Zemun

Biološki fakultet Univerziteta u Beogradu, Beograd

Institut za istraživanja u poljoprivredi «SRBIJA», Beograd

Semenarstvo, Novi Sad

L.K.B., Beograd

SUPERLABORATORY, Beograd

Zavod dezinfekciju, dezinsekciju i deratizaciju «Zavod DDD», Beograd

Uprava šuma Srbije, Beograd

UNI-CHEM, Beograd

Semenarnacoop, Petrovaradin

BB Minakva, Novi Sad

EkoSeed, Subotica

Port, Subotica

BASF Jugoslavija, Beograd

Alfatrade enterprise , Beograd

Agromarket, Kragujevac