BIOLOGIJA. 2012. Vol. 58. No. 3. P. 123–231

Dear Colleagues,

It is my great pleasure to welcome you at the 5th Baltic Congress of Genetics and hope you will have an exciting meeting in Kaunas, Vytautas Magnus University, Lithuania on October 19–22, 2012.

It is very important that the Baltic Congress is becoming a nice tradition that occurs every three years in different Baltic country. I believe that this Congress will further stimulate collaboration and warm relationships not only between the Baltic countries but also between other European countries. The Baltic Congress of Genetics has developed a tradition of promoting an exchange of ideas and methods between scientists working with mammals, plants, microorganisms and human. Each year a different country welcomes researchers who gather aiming to present and discuss the most recent results in the area of genetics. I believe that this meeting will stimulate further collaboration and warm relationship between alle scientists. In the present congress in Kaunas, 109 participants from 9 countries (Estonia, Latvia, Lithuania, Denmark, Germany, Netherlands, Norway, Finland, Scotland) will give 23 oral and 69 poster presentations.

Our last milestone, but certainly not the least, has been the growing trend of a better involvement of the students into professional genetical research. We have no statistics relating to the number of students of each Baltic country that have participated in every meeting, but we can be sure it is many. Through their involvement, we go from strength to strength and it is with pride that I always keep my eye on the reinforcement of young genetists.

Before finishing off, let me wish you a lot of useful bring-back-home ideas in promoting advance in your research and best impressions of staying together in Kaunas.

Sincerely yours,
Chair of the host team of the meeting
Professor Algimantas Paulauskas

5th BALTIC CONGRESS OF GENETICS

Kaunas, Vytautas Magnus University, Lithuania

October 19–22, 2012

Part 1
Abstracts of Oral Presentations

POPULATION GENETIC ANALYSIS OF ALIEN SPECIES IN BALTIC COUNTRIES

Algimantas Paulauskas*

Faculty of Natural Sciences, Vytautas Magnus University, Lithuania

** Corresponding author. E-mail: a.paulauskas@gmf.vdu.lt*

The study of genetic diversity to invasiveness and patterns of adaptation and naturalisation of diverse alien organisms (viruses, plankton algae, plants, animals and their parasites), and pathways of the distribution and origin of the alien species were analised. The results of 24 research projects (2010–2011) from The National Research Program "Ecosystems in Lithuania: climate change and human impact", approved by the Act V-951 of the Lithuanian Minister of Education and Science on June 19, 2010 are present. Population genetics can answer questions about the demographic and geographic dynamics of recent biological invasions. To identify the geographic source of introduced alien populations and determine the number of introductions, the native range of the invasive species must be thoroughly sampled and potential source populations must be sufficiently differentiated using molecular population genetics. Detecting bottlenecks in recent invasions seems to be a common component of attempting to assess genetic structure of invasive species. Investigators are interested in understanding the initial conditions of the invasion in order to better control and predict future invasions. Population genetics can characterizing the processes that have caused and maintain the population structure of an invasive species, significant migration occurs among established populations. Such information might be used to intuit the factors that either inhibit, or facilitate, gene flow among populations, useful for understanding the factors that influence the rate of spread, or the location of the initial introduction, much as the field of phylogeography aims at understanding the history of native taxa and hybridization with native species.

References

1. Butkauskas D, Švažas S, Tubelytė V, Morkūnas J, Sruoga A, Boiko D, Paulauskas A, Stanevičius V, Baublys V. Coexistence and population genetic structure of the whooper swan *Cygnus cygnus* and mute swan *Cygnus olor* in Lithuania and Latvia. Centr Eur J Biol 2012; 7(5): 886–94.

2. Patamsytė J, Čėsnienė T, Naugžemys D, Kleizaitė V, Vaitkūnienė V, Rančelis V, Žvingila D. Genetic diversity of warty cabbabe (*Bunias orientalis* L.) reveald by RAPD and ISSR markers. Žemdirbystė=Agriculture 2011; 98(3): 293–300.

3. Vyšniauskienė R, Rančelienė V, Žvingila D, Patamsytė J. Genetic diversity of invasive alien species Lupinus polyphyllus populations in Lithuania, Žemdirbystė=Agriculture 2011; 98(4): 383–90.

4. Staniulis J, Zitikaitė I, Žižytė M, Jackevičienė E, Urbanavičienė L, Šneideris D. Detection and molecular identification of alien viruses of stone fruit, sugar beet and tomato. Žemdirbystė=Agriculture 2012; 99(1): 85–92.

5. Tunaitienė V, Patamsytė J, Čėsnienė T, Kleizaitė V, Naugžemys D, Rančelis V, Žvingila D. Comparison of genetic diversity in two alien plant species Vergleich der genetischen Vielfalt von zwei nicht-einheimischen Pflanzenarten. Julius-Kühn-Archiv 2012; 434: 679–85.

6. Zybartaitė L, Jodinskienė M, Žukauskienė J, Janssens SB, Paulauskas A, Kupčinskienė E. RAPD analysis of genetic diversity among Lithuanian populations of *Impatiens glandulifera*. Žemdirbystė=Agriculture 2011; 98(4): 391–8.

IDENTIFICATION AND PHYLOGENETIC RELATIONSHIPS OF *SARCOCYSTIS* SPECIES PARASITIZING WILD BIRDS AND MAMMALS

P. Prakas[1*], D. Butkauskas[1], L. Kutkienė[1], A. Sruoga[2]

[1] *Nature Research Centre, LT-08412 Vilnius, Lithuania*

[2] *Vytautas Magnus University, LT-44248 Kaunas, Lithuania*

* *Corresponding author. E-mail: petrasprakas@yahoo.com.*

Introduction. Representatives of the genus *Sarcocystis* are cyst forming protozoa parasites of mammals, birds and reptiles, characterized by obligatory prey-predator two-host life cycle. At present over 220 named *Sarcocystis* species are known. Asexual stages (sarcocysts) develop in the muscle tissues of the intermediate hosts and sexual multiplication takes place in the small intestine of the definitive host. Some of *Sarcocystis* species are pathogenic organisms dangerous to humans, domestic and wild animals. The aim of this study was to identify *Sarcocystis* species isolated from muscles of wild birds and mammals combining morphological and DNA analysis methods and to investigate phylogenetic relationships of examined species within genus *Sarcocystis*.

Methods. During the period 2005–2012, muscle tissues of hunted or found dead animals were tested for *Sarcocystis* cysts. *Sarcocystis* spp. were preliminary characterized based on morphological features of sarcocysts, sarcocysts wall and cystozoites by light and electron microscopy. DNA was extracted from the sarcocysts found in wild boar (*Sus scrofa*), European roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), moose (*Alces alces*), barnacle goose (*Branta leucopsis*), mallard (*Anas platyrhynchos*), great white-fronted goose (*Anser albiforns*), common woodpigeon (*Columba palumbus*), hooded crow (*Corvus cornix*), Eurasian jackdaw (*Corvus monedula*), common blackbird (*Turdus merula*), herring gull (*Larus argentatus*) and great black-backed gull (*Larus marinus*). 7 primer pairs for ITS-1 region, 18S rRNA gene and variable fragment of 28S rRNA gene were designed for diagnostics of *Sarcocystis* species. The phylogenetic trees were constructed using the Bayesian method.

Results. *Sarcocystis* species isolated from mammals were genetically characterized using 18S rRNA gene, while resolution power of this genetic marker alone was insufficient for bird *Sarcocystis* species diagnostic. Therefore, more rapidly evolving 28S rRNA gene and ITS-1 region were used in separating closely related *Sarcocystis* species parasitizing birds. Eight *Sarcocystis* species were identified in game mammals i. e. *S. miescheriana* in the wild boar, *S. hjorti* in the moose, *S. hjorti*, *S. hofmanni*-like and *Sarcocystis* sp. in the red deer, *S. capreolicanis*, *S. gracilis*, *S. oviformis*, *S. silva* and *S. hofmanni*-like in the European roe deer. According to the cyst wall ultrastructure in combination with results of DNA analysis five new bird *Sarcocystis* species were described: *S. albifronsi* from great white-fronted goose, *S. anasi* from mallard, *S. cornixi* from hooded crow, *S. turdusi* from common blackbird and *S. wobeseri* parasitizing barnacle goose and mallard. Based on the DNA sequences analysis two known species i. e. *S. rileyi* and *S. columbae* were identified in mallards and common woodpigeons, respectively. Furthermore, *Sarcocystis* sp. from the Eurasian jackdaw and *Sarcocystis* sp. from the great black-backed gull significantly genetically differed from all other previously studied *Sarcocystis* species. *Sarcocystis* species are grouped in the phylogenetic trees according to affinity of definitive and intermediate hosts and the similarity of the morphological characteristics of sarcocysts. The branch length in phylograms were significantly shorter between *Sarcocystis* species whose intermediate hosts are birds comparing with *Sarcocystis* species whose intermediate hosts are mammals or reptiles.

CONTRIBUTION OF THE LATVIAN GENETICIST JANIS LUSIS INTO THE ANIMAL GENETICS RESEARCH

Jekabs Raipulis
Riga Teacher Training and Educational Management Academy, Latvia
E-mail: jekabs.raipulis@rpiva.lv

It has been 115 years since the birth of the most prominent Latvian researcher of the classical genetics, professor Janis Lusis (Jānis Lūsis) on December 05, 1897. Jānis Lūsis was the student of the Biology Department, Faculty of Mathematics and Natural Sciences at the Petrograd University during the World War I and the Civil War of Russia. His interest in genetics and genetic research has been incited by the founder of the fundamentals of that new science of that age in Russia, Yuri Philipchenko. As a student J. Lusis got actively involved in the scientific research and upon the graduation from the university continued working with the first genetics and experimental zoology department in Russia while being an active participant in the organization of the department. He performed the first experimental studies on the regeneration of the *Planaria* and from 1926 started the research on the two-spotted ladybug (*Adalia bipunctata*) systematization and genetics research which he pursued for the rest of his life. Starting with 1926 he participated and later on managed the domestic animal resource study expeditions in the southern republics of the Soviet Union and the People's Republic of Mongolia.

In 1933 he became the head of the domestic animals genetics and evolution department at the Genetics Institute of the Academy of Science of the USSR led by the academic N. Vavilov. The centers of origin for the domestic animals were studied under the guidance of L. Lusis which to a great extent coincided with the centers of origin of the cultivated plants as described by N. Vavilov. He studied the ways to use the distanced hybridization in the selection of the domestic animals, and in 1933 produced the plan and started on the practical crossbreeding of the wild ram – arhar first with the fatty tail sheep, later-with the household fine fleece merino sheep. Unique work for the creation of the inter-species breed was concluded in 1950 with the development of a breed suitable for the highland conditions of the arharomerinos. J. Lusis though has not been mentioned among the architects of the aforementioned breed as he refused to give up his conviction of the geneticist. While running the department of the Genetics Institute J. Lusis continued the research into the genetic structure of domestic animals and production of plans to improve the stock in various locations throughout the USSR.

In 1941 the new head of the Genetics Institute T. Lisenko dismissed J. Lusis for the latter's unwillingness to take part in the anti-scientific experiments of T. Lisenko. J. Lusis became the associate of the Zoology Institute at the Academy of Sciences of the USSR, later renamed as the A. Severcov Institute of Evolutionary Morphology. During the WW2, while evacuated to the Central Asia, he produced a plan of an improved Kirgiz horse breed. In 1948 after the genetics research has been prohibited in the USSR, J. Lusis found himself without a job.

In 1949 he was hired as a zoology lecturer at the Department of Biology of the Latvian State University, and in 1951 he became the head of the Zoology Department. Beside the official themes of the scientific work like "Biological fundamentals to improve the fish productivity in internal water reservoirs in the Latvian SSR" he continued the research on the two-spotted ladybug genetics and microevolution having carried out the observations both

in natural surroundings and the crossbreeds of the genetic analyses in the laboratory he set up in his apartment. In 1964, after the resumption of the genetic research J. Lusis started organizing the genetics classes at the Biology Department and established the Department of Zoology and Genetics. In 1967 he established the N. Vavilov all-Union geneticist and breeder association and became its president. While working as the lecturer J. Lusis brought up a whole generation of Latvian zoologists and geneticists having led the geneticist and breeder association, organized conferences and schools on genetics. The vast material of experimental research devoted to the two-spotted ladybugs by the professor Janis Lusis has not been surveyed thoroughly and published so far. His fundamental work "Taxonomic relations of the *Adalia* genus and its geographic distribution" is still being quoted in the publications of various researchers worldwide. J. Lusis died on August 10, 1979.

THE STUDY OF GENETICS OF FRESHWATER ANIMAL ORGANISMS' POPULATION IN LATVIA

Natalja Shkute

Institute of Ecology, Daugavpils University,
Vienības iela 13, LV-5401 Daugavpils, Latvia
E-mail: natalja.skute@du.lv

Introduction. There are many water bodies in Latvia: rivers, lakes, and ponds. Freshwater animal organisms play a very important role in environment, economics, and tourism. Many of freshwater animal organisms are protected species. Finfishes are investigated by Institute of Biology (pikeperch) and Institute of Food Safety, Animal Health and Environment (salmon). Institute of Ecology and Daugavpils University studied freshwater organisms very widely. A special attention was paid to the rare and protected species. Such hidrobionts' organisms as different zooplankton groups, different finfish, and amphibian were studied.

Methods. We investigated different zooplanktons: *Daphnia, Bosmina* that are unique parthenogenetic organisms and indicators of water systems and, different finfishes: *Salmonidae*: vendace (*Coregonus albula* (L.), sea trout (*Salmo trutta* L.), salmon (*Salmo salar*) and others: pikeperch (*Sander Lucioperca*); amphibians – fire-bellied toad *Bombina bombina.* For the genetic analysis we used the finfish and amphibian and non-destructive methods of DNA extraction (from scale and buccal swabs). For the study of the genetic variability and differentiation of some Latvian populations of hidrobionts' organisms and influence of some water environment factors on the genetic structure of these populations such nuclear DNA markers as allozymes, RAPD, microsatellites were used. For the study of the origin of the Latvian population for subsequent sequencing and phylogenetic analyses there were used the fragments of three mitochondrial DNA loci: cytochrome *b (cyt b)*, cytochrome *c* oxidase I *(cox1)* and nicotinamide adenine dinucleotide dehydrogenase subunit 4 *(nad4)*.

INVESTIGATION INTO INTRASPECIFIC GENETIC DIVERSITY OF CAPERCAILLIE (*TETRAO UROGALLUS*) POPULATION IN BELARUS USING MITOCHONDRIAL DNA CONTROL REGION AND MICROSATELLITE DNA MARKERS

Simona Stropaitytė[1]*, Dalius Butkauskas[1], Aniolas Sruoga[2], Saulius Švažas[1]

[1] *Institute of Ecology of Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania*

[2] *Vytautas Magnus University, K. Donelaičio 58, LT-44248 Kaunas, Lithuania*

* *Corresponding author. E-mail: simona.str@gmail.com*

The present study has evaluated the intraspecific genetic diversity of Capercaillie (*Tetrao urogallus*) population in Belarus, which was studied for the first time based on mitochondrial and nuclear DNA markers. It was determined that population is characterized by high diversity of haplotypes identified after sequencing of mtDNA control region 355 bp length fragments. Based on haplotypic network it was shown that phylogenetic relationships of haplotypes found in Belarus population indicate its close relations with Russian and Finnish populations. Extensive gene flow between investigated population and neighbouring populations as well as inside the country helps to sustain genetic diversity, which suggests that habitat conditions for this species in the northern, central and eastern parts of the country, despite occuring fragmentation, are still sufficient to sustain effective gene flow. Although birds investigated in Brest region were defined by unique haplotype composition, collected genetic data are still insufficient to confirm this feature as the consequence of increasing isolation and decreasing population size. Brest region is situated in the western part of the country and characterized by severely fragmented landscape, which inhabits *T. u. major* subspecies. The distinguishing between different Capercaillie subspecies considering genetic methods, implied in current study still has not enough basis, as even thought it was observed, that each of three distinguished mtDNA control region haplogroups were genetically related to certain subspecies, significant genetic differentiation, inferred by using seven hypervariable microsatellite DNA markers, was not determined. It is likely, that increased homozygosity, which was observed within the studied population is caused by the specific mating strategy, and such non-random mating may also be the reason of noted deviation from Hardy-Weinberg equilibrium. More genetically related were those population individuals, that, at least in theory, represent *T. u. pleskei* and *T. u. major* subspecies, described in the country, considering data, obtained by both mitochondrial DNA and microsatellite DNA markers.

CRANIOFACIAL PECULIARITIES IN PARENTS WHO HAVE NONSYNDROMIC CLEFT CHILDREN

Erika Nagle[1]*, Ieva Grīnfelde[3], Dzintra Kažoka[2], Baiba Lāce[3]

[1] *Riga Stradins University, Department of Biology and Microbiology*

[2] *Institute of Anatomy and Anthropology*

[3] *Children's Clinical University Hospital, Clinics of Medical Genetics, Riga, Latvia*

* *Corresponding author. E-mail: erikanagle@gmail.com*

Aim and methods. The aim of this study was to identify specific craniofacial characteristics in parents with cleft children that would assist in the identification of the "cleft" genotype. For this purpose two approaches – anthropometry and somatoscopy were used in evaluation craniofacial morphology. Anthropometric measurements were compared between not affected parents (cleft group) and controls separately for males and females due to strong sexual dimorphisms in the craniofacial region.

Results. Results presented in the table give clear evidence that in cleft parents several characteristics are distinctive from those obtained in control groups. Fathers in cleft group could be characterized by wider and longer heads, and greater head circumferences, as well as wider faces and mandibles in comparison with control male group. All differences are statistically significant ($p < 0.05$). The same tendency is observed in cleft mothers group – all mentioned parameters are greater in cleft group in comparison with the controls, but measurements of the head length and head circumference are not significant. Our results allow determine a set of craniofacial parameters that characterize cleft parents – they have wider and longer heads as well as wider faces and mandibles. In addition association of minor anomalies in cleft parents were evaluated by clinical somatoscopy. The most common minor anomalies in craniofacial region in cleft parents were high arched palate (24%), hypoplasia and dislocation of incisors (13%), and diastema (7%). Several other researches have also shown distinct phenotypes of cleft patients family members [1–3]. The set of specific phenotypic characteristics (minor anomalies along with the specific craniofacial parameters) can allow restrict number of individuals subjected for genetic testing. Our results show that differences do exist and could be used in discriminating susceptible genotypes for genetic testing.

Table. Basic craniofacial parameters (cm) in cleft parents and control groups

Craniofacial parameter	Clefts fathers		Control fathers		Clefts mothers		Control mothers	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Head width	15.97	0.52	15.42	1.01	15.04	0.62	14.58	0.59
Head length	19.60	0.68	19.31	0.68	18.41	0.68	18.33	0.66
Head circumference	58.16	1.43	57.37	1.49	55.52	1.60	55.22	1.87
Mandible width	11.19	0.83	10.54	0.63	10.27	0.53	9.69	0.75
Face width	14.36	0.68	13.31	0.98	13.37	0.65	12.24	0.80

References

1. Mossey PA, Batra P, Grant T. McIntyre GT. The parental dentocraniofacial phenotype – an orofacial clefting microform. Cleft Palate-Cran J 2010; 479(1): 22–34.

2. Gorantla S. Facial form as a subclinical phenotype of nonsyndromic orofacial clefting: an anthropometric analysis. University of Pitsburg; 2012.

3. Wu TT, Chen PK, Lo LJ, Cheng MC, Ko EW. The characteristics and distribution of dental anomalies in patients with cleft. Chang Gung Med J 2011; 34(3): 306–14.

THE MOLECULAR BASIS OF BREAST CANCER METASTASIS

R. Ugenskiene[1]*, E. Juozaityte[2]

[1] Oncology Institute of Lithuanian University of Health Sciences, Oncology Research Laboratory

[2] Oncology Institute of Lithuanian University of Health Sciences

** Corresponding author. E-mail: rasaugenskiene@gmail.com*

The aim of the paper is to highlight some of the newest research data and to present a summary of tissue-specific genes involved in breast cancer metastasis. Breast cancer spreads to different distinct organ, preferentially to bone, lung, liver and brain. Tumor cell invasion, migration and colonization require a successful cascade of molecular events where different gene mutations and altered expression play an important role. The molecular basis of breast cancer metastatic process, especially the mechanism of organ-specific metastasis, is poorly understood and is extensively studied. Recent research data suggest that complexity of tumor biology and tumor heterogeneity are important in determining the response to targeted therapy and chemotherapy. Advanced knowledge and better understanding of metastatic process should help to tailor treatment directed towards preventing or delaying metastasis formation.

PREVALENCE OF C282Y, H63D, AND S65C MUTATIONS IN HEREDITARY HFE-HEMOCHROMATOSIS GENE IN LITHUANIAN POPULATION

L. Kucinskas, S. Juzenas, J. Sventoraityte, R. Steponaitiene*, A. Vitkauskiene, L. Kupcinskas

Institute for Digestive Research, Lithuanian University of Health Sciences

** Corresponding author. E-mail: r.steponaitiene@gmail.com*

Introduction and aim. HFE-hemochromatosis is a common autosomal recessive disease caused by HFE gene mutations and characterized as iron overload and failure of different organs. The aim of this study was to determine the prevalence of C282Y (c.845 G>A), H63D (c.187 C>G), and S65C (c.193A>T) alleles of HFE gene in the Lithuanian population.

Methods and results. One thousand and eleven healthy blood donors of Lithuanian nationality were examined in four different ethnic Lithuanian regions to determine HFE gene alleles and genotype frequencies. The samples of DNA were analyzed for the presence of restriction fragment length polymorphism and validated by DNA sequencing. Among 1,011 blood donors tested, the frequency of C282Y, H63D, and S65C alleles were 2.6%, 15.9%, and 1.9%, respectively. One third of the tested subjects (n = 336) had at least one of the C282Y or H63D HFE gene mutations. The screening of Lithuanian blood donors has detected 13 (1.3%) subjects with a genotype C282Y/C282Y or C282Y/H63D responsible for the development of HFE-hemochromatosis. The prevalence of C282Y mutation was significantly higher among the inhabitants of Žemaitija (Somogitia) at the Baltic Sea area (5.9%) in comparison to the regions of continental part of Lithuania (2.4% in Dzūkija, 2.3% in Aukštaitija, and 2% in Suvalkija, $p < 0.05$). The data support the hypothesis that the p.C282Y mutation originated from Scandinavia and spread with the Vikings along the Baltic Sea coast. The first epidemiological investigation of HFE gene mutations in ethnic Lithuanians showed that the frequencies of H63D, C282Y, and S65C of HFE gene alleles are similar to other North-eastern Europeans, especially in the Baltic region (Estonia, Latvia), Poland, and part of Russia (Moscow region).

MOUSE STRAINS SELECTED FOR DIVERGENT GROWTH: A MODEL FOR MAPPING GENES UNDERLYING MUSCLE MASS

A. Kilikevicius[1]*, C. Meharg[2], R. Zelniene[1], L. Bünger[3], A. Lionikas[4]

[1] *Lithuanian Academy of Physical Education, Sports and Movement Science Centre, Kaunas, Lithuania*

[2] *Bioinformatics Group, Max Planck Institute for Biology of Aging, D-50931, Germany*

[3] *Animal & Veterinary Sciences, SAC, Midlothian, Scotland, UK*

[4] *University of Aberdeen, College of Life Sciences and Medicine, School of Medical Sciences, Foresterhill, Aberdeen, Scotland, UK*

* *Corresponding author. E-mail: audrius.kilikevicius@gmail.com*

Background. Declining muscle mass due to natural aging processes or disease impairs muscular function and can lead to disability. Development of muscle mass and function and their maintenance through the active life style can have favorable effects, however, hereditary factors also play an important role (1) in the mouse model showed that muscle weight, an important determinant of muscle function, is a polygenic trait (2, 3). Therefore, better understanding of genetic mechanisms underlying variation of this phenotype may reveal novel pharmacological targets for therapy of the muscle atrophy. To fill this gap in knowledge we initiated the phenotypic screening of skeletal muscle and the search for genetic and gene expression differences between BEHi and BELi mouse strains selected for divergent growth (4).

Methods. Skeletal muscle mass and histological parameters of soleus muscle were measured in 10-14 week old male mice from each strain (n = 6-to-16 samples per strain). The trascriptome of the *gastrocnemius* muscle was analyzed using RNA-Seq (n = 3 per strain).

F1 (n = 31) and F2 (n = 291) intercross populations were generated and the calf muscles of mice from both populations were weighed at the age of 4 weeks.

Results. BEHi mice possess up to 8-fold larger muscle mass than BELi strain. For instance, the weight of *soleus* muscle was 16.6 ± 2.2 mg and 2.1 ± 0.8 mg, respectively. Histological analysis of *soleus* demonstrated that this is due to a 2-fold difference in the number of muscle fibers (1063 ± 54 in BEHi *vs*. 497 ± 122 in BELi) and >2-fold difference in their cross section area (1673 ± 158 μm^2 *vs*. 678 ± 115 μm^2 for type I, and 1932 ± 139 μm^2 *vs*. 812 ± 180 μm^2 for type IIa muscle fibers). Genetic factors explained half of the phenotypic variation in muscle mass ($h^2 = 0.50$).

Transcripts of nearly 16,000 genes have been identified in gastrocnemius muscle and ≈2,300 genes were differentially expressed (adjusted $p < 0.1$) between BEHi and BELi strains. In addition, >45,000 SNPs were found and ≈2,300 of them were non-synonymous.

Conclusions. The BEHi and BELi strains provide a powerful tool for identification of genes underlying skeletal muscle mass. We are currently using the genomic data to initiate genotyping and mapping of quantitative trait loci (QTL) affecting muscle mass differences between the two strains. The transcriptome information will facilitate nomination of the candidate genes underlying QTL effects.

References

1. Arden NK, Spector TD. J Bone Miner Res 1997; 12(12): 2076–81.
2. Brockmann GA, Kratzsch J, et al. Genome Res 2000; 10(12): 1941–57.
3. Lionikas A, Cheng R, et al. Physiol Genomics 2010; 42A(1): 33–38.
4. Bünger L, Laidlaw A, et al. Mamm Genome 2001; 12(9): 678–86.

HIGH FREQUENCY OF THE C.3207C>A (P.H1069Q) MUTATION IN ATP7B GENE OF LITHUANIAN PATIENTS WITH HEPATIC PRESENTATION OF WILSON'S DISEASE

L. Kucinskas*, A. Vitkauskiene, V. Petrenkiene, L. Kupcinskas

Institute for Digestive Research,
Lithuanian University of Health Sciences

** Corresponding author. E-mail: kucinskas@vsion.kmu.lt*

Aim. To investigate the prevalence of the ATP7B gene mutation in patients with hepatic presentation of Wilson's disease (WD) in Lithuania.

Methods. Eleven unrelated Lithuanian families, including 13 WD patients were tested. Clinically WD diagnosis was established in accordance to the Leipzig scoring system. Genomic DNA was extracted from whole venous blood using a salt precipitation method. Firstly, the semi-nested polymerase chain reaction (PCR) technique was used to detect the c.3207C>A (p.H1069Q) mutation. Patients not homozygous for the c.3207C>A (p.H1069Q) mutation were further analyzed. The 21 exons of the WD gene were amplified in a thermal cycler (Biometra T3 Thermocycler, Gottingen, Germany). Direct sequencing of the amplified PCR products was performed by cycle sequencing using fluorescent dye terminators in an automatic sequencer (Applied Biosystems, Darmstadt, Germany).

Results. A total of 21 WD patient (mean age 256.4 years; range 17–56 years; male / female 5/16) presented with hepatic disorders and 24 their first degree relatives (including 19 siblings) were studied. Some of WD patients, in addition to hepatic symptoms, have had extrahepatic disorders (hemolytic anemia 5; Fanconi syndrome 1; neurophsychiatric and behavioural disorder 4). Liver biopsy specimens were available in all of 21 WD patients (16 had cirrhosis; 2-chronic hepatitis; 3-acute liver failure, 2-liver steatosis). Nineteen of 21 (90%) WD patients had the c.3207C>A (p.H1069Q) mutation, 11 of them in both chromosomes, 8 were presented as compound heterozygotes with additional c.3472-82delGGTTTAACCAT, c.3402delC, c.3121C>T (p.R1041W) or unknown mutations. For 2 patients with liver cirrhosis and psychiatric disorder (Leipzig score 6), no mutations were found. Two patients with fulminant WD died from acute liver failure and 11 are in full remission under penicillamine or zinc acetate treatment. Five women with WD successfully delivered healthy babies.

Conclusion. The c.3207C>A (p.H1069Q) missense mutation is the most characteristic mutation for Lithuanian patients with WD. Even 90% of WD patients with hepatic presentation of the disease are homozygous or compound heterozygotes for the p.H1069Q mutation.

SIGNIFICANCE OF THIOPURINE METHYLTRANSFERASE POLYMORPHISM IN LITHUANIAN INFLAMMATORY BOWEL DISEASE PATIENTS

R. Steponaitiene*, M. Kasperaviciene, J. Kupcinskas, L. Jonaitis, A. Zvirbliene, G. Kiudelis, J. Skieceviciene, L. Kupcinskas

Institute for Digestive Research, Lithuanian University of Health Sciences, Kaunas, Lithuania

** Corresponding author. E-mail: r.steponaitiene@gmail.com*

Introduction. Inter-individual drug metabolism variability can influence treatment outcome. Genetic polymorphisms in thiopurine methyltransferase gene (*TPMT*) are known to correlate with the toxicity of azathioprine (AZA). Patients with low TPMT activity (poor metabolizers) are at high risk of developing severe haematopoietic toxicity. *TPMT* genetic polymorphisms were not previously investigated in Lithuanian IBD patients.

Aims and methods. The aim of this study was to investigate frequencies of *TPMT* polymorphisms and their association with adverse events during AZA therapy in the Lithuanian IBD patients'. The genotyping of *TPMT*2* (rs1800462*), TPMT*3B* (rs1800460*)* and *TPMT*3C* (rs1142345) was performed using allele-specific PCR or restriction fragment length polymorphism analysis methods. In total 460 consecutive IBD patients, referred to two university hospitals in Lithuania, were genotyped for *TPMT*2* (G238C)*, TPMT*3A* (G460A and A719G)*, TPMT*3B* (G460A) and *TPMT*3C (*A719G) mutations. The use of AZA and its' side effect was assessed retrospectively according to the data of hospital medical records during six year period before genetic testing.

Results. Among 460 IBD patients the frequency for the *TPMT*1* (wild-type), *TPMT*3A* and *TPMT*3B* alleles were 96.63%, 3.04% and 0.33%, respectively. The frequency of the *TPMT* genotypes were 93.48% for *TPMT*1/*1*, 5.87% for *TPMT*1/3A*, 0.43% for *TPMT*1/3B,* and 0.22% for *TPMT*3A/*3B*. No significant differences between experimental and expected genotypic frequencies were observed by Hardy-Weinberg equilibrium. Prescription of AZA provoked neutropenia, which required adjustment of the dose or discontinuation of the drug, in 15.4% of cases bearing heterozygous (*TPMT*1/3A* or *TPMT*1/3B*) genotype, whereas patients with wild-type (*TPMT*1/*1*) genotype experienced this side effect only in 2% of cases ($p < 0.05$). Only one patient had high-risk compound heterozygous genotype (*TPMT*3A/*3B*) and an alarming experience of severe neutropenia after prescription of AZA.

Conclusions. *TPMT*3A* is the most prevalent variant allele in Lithuanian IBD patients. The estimated frequency of variant alleles in the study group was similar to that observed in Caucasian populations of Northern and Eastern Europe. Our work supports the strong evidence that patients with TPMT genotype *TPMT*3A/*3B* are at high risk of severe myelosuppression at standard doses of AZA. *TPMT* heterozygotes also experienced risk of neutropenia in comparison to control.

GENE POLYMORPHISMS OF MICRORNAS IN *HELICOBACTER PYLORI* INDUCED HIGH RISK ATROPHIC GASTRITIS AND GASTRIC CANCER

Juozas Kupcinskas[1]*, Thomas Wex[2], Ruta Steponaitiene[1], Simonas Juzenas[1], Marcis Leja[3], Gediminas Kiudelis[1], Laimas Jonaitis[1], Jurgita Skieceviciene[1], Peter Malfertheiner[2]

[1] Institute for Digestive Research, Lithuanian University of Health Sciences, Kaunas, Lithuania

[2] Clinic of Gastroenterology, Hepatology and Infectious Diseases, Otto von Guericke University, Magdeburg, Germany

[3] Faculty of Medicine, University of Latvia, Digestive Diseases Center, Hospital Lizeners, Riga, Latvia

** Corresponding author. E-mail: j_kupcinskas@yahoo.com*

Background and aims. Recent discovery of microRNAs (miRNAs) has shed new insights in biomarker field with diagnostic and prognostic implications. Various studies have shown that miRNA are deregulated in gastric cancer (GC) and atrophic gastritis patients. Several single nucleotide polymorphisms (SNPs) of genes related to miRNAs have been linked with different types of cancer and premalignant lesions. The data onthepotential association between miRNA SNPs and the risk of GC or *Helicobacter pylori* induced atrophic gastritis, however, are scarce and partially conflicting.The aim of our study was to evaluate potential associations between the presence of gastric cancer and high risk atrophic gastritis (HRAG) and SNPs of genes related to mir-146a, mir-149, mir-196a-2, mir-379, mir-499a and mir-608.

Methods. Gene polymorphisms were analyzed in 538 subjects (GC: n = 106; HRAG: n = 222, controls: n = 210) of Caucasian origin. Mir-146a C>G (rs2910164), mir-149 T>C (rs2292832), mir-196a-2 C>T (rs11614913), mir-379 A>G (rs61991156), mir-499a A>G (rs3746444) and mir-608 C>G (rs4919510) SNPs were genotyped by RT-PCR, using predesigned Taqman primers.

Results. Frequencies of genotypes in our study are similar to the data reported on subjects of Caucasian ethnicity. Analysis of data revealed that the frequencies of SNP genotypes are in line with Hardy-Weinberg equilibrium. There was a tendency for mir-196a-2 C/C genotype to be associated with lower incidence of HRAG (49.0% in controls *vs.* 41.4% in HRAG group, $p = 0.079$). Allele C of mir-196a-2 SNP was also more frequent in controls when compared to HRAG group, 67.8% and 60.1% respectively, however it failed to reach significance level ($p = 0.087$). We did not find any significant associations for all examined miRNA polymorphisms in relation to GC or HRAG.

Conclusions. Mir-146a, mir-149, mir-196a-2, mir-379, mir-499a and mir-608 SNPs are not linked with gastric carcinogenesis in Caucasians, and therefore they do not appear as potential biomarkers for identifying individuals with higher risk for GC.

HOMOLOGOUS RECOMBINATION AND MUTAGENIC PROCESSES IN *PSEUDOMONAS PUTIDA*

K. Tavita[1], K. Mikkel[1], R. Teras[1], M. Tark-Dame[1, 2], H. Jerabek[2], J. Sidorenko[1], T. Juurik[1], H. Ilves[1], T. Ilmjärv[1], K. Ukkivi[1], A. Teppo[1], R. T. Dame[3], M. Kivisaar[1]*

[1] Department of Genetics, Institute of Molecular and Cell Biology, Tartu University and Estonian Biocentre, 23 Riia Street 51010 Tartu, Estonia

[2] Institute for Theoretical Physics, Philosophenweg 19, D-69120 and Interdisciplinary Center for Scientific Computing (IWR), Im Neuenheimer Feld 368 69120 Heidelberg, Germany

[3] Leiden Institute of Chemistry, Gorlaeus Laboratories, Laboratory of Molecular Genetics and Cell Observatory, Leiden University, Leiden, The Netherlands

** Corresponding author. E-mail: maia.kivisaar@ut.ee*

Introduction. Homologous recombination (HR) has a major impact in bacterial evolution. Most of the knowledge about the mechanisms and control of HR in bacteria has been obtained in fast growing bacteria. However, in their natural environment bacteria frequently meet adverse conditions that restrict the growth of cells.

Methods. We constructed a test system to investigate HR in populations of the soil bacterium *Pseudomonas putida* under carbon starvation conditions. This assay system enables to monitor HR events between a non-conjugative plasmid and bacterial chromosome which result in restoring the expression of the phenol monooxygenase gene *pheA*.

Results. Our results show that HR taking place between the *pheA* alleles locating on the plasmid and on the *P. putida* chromosome is elevated during the prolonged starvation of bacteria in the presence of phenol. Study of the mechanisms of HR revealed that HR is suppressed by DNA mismatch repair enzymes and stimulated by reactive oxygen species. The fact that the presence of phenol facilitates HR in starving populations of *P. putida* could be of significance to understand mechanisms of rapid evolution of catabolic pathways for the degradation of xenobiotic aromatic compounds in soil bacteria. Since toxicity of several aromatic compounds could be associated with elevated production of ROS, the exposure of bacteria to such compounds may induce DNA damage resulting in an increase of genetic changes in bacteria. Importantly, our assay system enables to study the effect of local genome accessibility on HR. Specifically we observed that the location of the chromosomal target of the test system influences the frequency of HR. *In silico* analysis revealed that chromosomal DNA regions which flanked the test system in the strains exhibiting lower HR frequency were enriched in binding sites for the Nucleoid-associated proteins (NAPs) compared to those which expressed higher frequency of HR. This indicates that the structural organization of the bacterial chromosome is determinative for the local frequency of HR in bacteria. The occurrence of point mutations in various chromosomal sites of *P. putida* was also investigated. Our results suggest that both the mutation rate and the spectrum of mutations vary at different chromosomal positions. Taken together, our results indicate that individual chromosomal regions are not equally accessible to HR and mutagenic processes. This may play an important role in divergence of bacterial populations in nature. Depending on the location of the potential target genes in the chromosome some mutational pathways may prevail over the others in the evolution of bacteria.

INVESTIGATION OF *BORRELIA* GENOME

Jana Radzijevskaja[1]*, Algimantas Paulauskas[1], Olav Rosef[1, 2], Eglė Galdikaitė[1]

[1] *Faculty of Natural Sciences, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania*

[2] *ATP-Innovation AS, Rokkemakarven 40, 3800 Bø i Telemark, Norway*

E-mail: j.radzijevskaja@bs.vdu.lt

Spirochetes of the genus *Borrelia* are unique among bacteria in that they have one linear chromosomes and carry a large number (at least 21) of linear and circular plasmids. In 1997 the first genome of *B. burgdorferi* s. s. (strain B31) has been completely sequenced by Fraser et al. (1997). It was the first sequenced genome of a parasite that infected both invertebrates and vertebrates. Plasmids encode 535 genes, and 90% of the genes have no similarity to genes outside *Borrelia* genus, suggesting that they perform specialized functions possibly related to spirochete adaptation (Pal, Fikrig, 2003). *B. burgdorferi* has the largest number of plasmids known for any bacterium. Genes related with pathogenicity including outer surface protein genes (*osp*) are primarily located on the plasmids.

Data from a number of molecular and phenotypic studies resulted in subdivision of the *B. burgdorferi* s. l. complex that causes Lyme disease into different taxonomic entities, named genospecies or genotypes. Although the *B. burgdorferi* sensu lato complex now comprises up to 18 *Borrelia* species, only three of them are clearly pathogenic for humans, namely *B. afzelii, B. burgdorferi*, and *B. garinii* (Stanek, Reiter, 2011). The studies on different chromosomal and plasmid genetic loci discovered that each *Borrelia* species comprises a variety of strains (Margos et al., 2009), and questions remain about how these variations correlate with different clinical manifestations. The complete *B. burgdorferi* genome sequence provided a new potential for the study of the molecular pathogenesis, prevention and treatment of Lyme disease. Up to now, genomes of several strains of *B. burgdorferi* s. s. *B. afzelii*, and *B. garinii* have been fully sequenced (Casjens et al., 2011). These genome sequencs provided a new potential for understanding *B. burgdorferi* sensu lato diversity and evolution, as well as the development of species- and group-specific diagnostics and vaccines.

As habitat type and climatic conditions influence presence and distribution of paticular *Borrelia* genospecies in hosts, we performed several studies for identification of *Borrelia* genospecies and strains distributed in different ecological and biogeographical zones in Baltic countries and Norway.

References

1. Casjens SR, et al. Whole genome sequence of two *Borrelia afzelii* and two *Borrelia garinii* Lyme Disease agent isolates. J Bacteriol 2011; 193(24): 6995–6.

2. Fraser CM, et al. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature 1997; 390: 580–6.

3. Margos G, et al. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. Appl Environ Microbiol 2009; 75: 5410–6.

4. Pal U, Fikrig E. Adaptation of *Borrelia burgdorferi* in the vector and vertebrate host. Microb Infect 2003; 5: 659–66.

5. Stanek G, Reiter M. The expanding Lyme *Borrelia* complex – Clinical significance of genomic species? Clin Microbiol Infect 2011; 17(4): 487–93.

SUCROSE SYNTHASE AND AQUAPORIN-LIKE GENE EXPRESSION DURING EARLY WOOD AND LATE WOOD FORMATION IN SCOTS PINE (*PINUS SYLVETRIS* L.), AND CORRELATION WITH WOOD DENSITY

Krista Kānberga-Siliņa[1], Āris Jansons[2], Dainis Ruņģis[1]*

[1] *Genetic Resource Centre, Latvian State Forest Research Institute "Silava", Rigas 111, LV-2169 Salaspils, Latvia*

[2] *Forest Tree Breeding, Latvian State Forest Research Institute "Silava", Rigas 111, LV-2169 Salaspils, Latvia*

* *Corresponding author. E-mail: dainis.rungis@silava.lv*

Introduction. Wood formation is one of the most important processes that occur in forest trees, both physiologically and economically. However, expression of genes involved in wood formation has primarily been studied in model plants, and for many forest tree species there is relatively little data available. For this research, expression of the candidate genes sucrose synthase and aquaporin-like gene was determined in early and late wood formation. The sucrose synthase gene (*Susy*) is involved in sucrose metabolism and provides precursors for cellulose biosynthesis (Nairn et al., 2007; Coleman et al., 2009). The aquaporin-like gene (*Aqual*) is involved in water transport across cell membranes and cell elongation (Johansson et al., 2000). Genes involved with these important development processes were selected in an attempt to identify a correlation between candidate gene expression and wood density and to estimate natural gene expression variation in trees from open pollinated tree families growing in natural conditions. Wood density was chosen as it is one of the most important wood quality parameters and it can be measured in a non-destructive manner using a Pilodyn instrument.

Materials and methods. Samples for gene expression analysis were collected from 50 29 year old trees in spring and autumn. Wood density of these trees was measured using the Pilodyn instrument. Gene expression analyses were performed with real time PCR, using the relative standard curve method.

Results. There were large differences in average gene transcript abundance between families, reflecting the natural variation of gene expression between individuals and families (Figure).

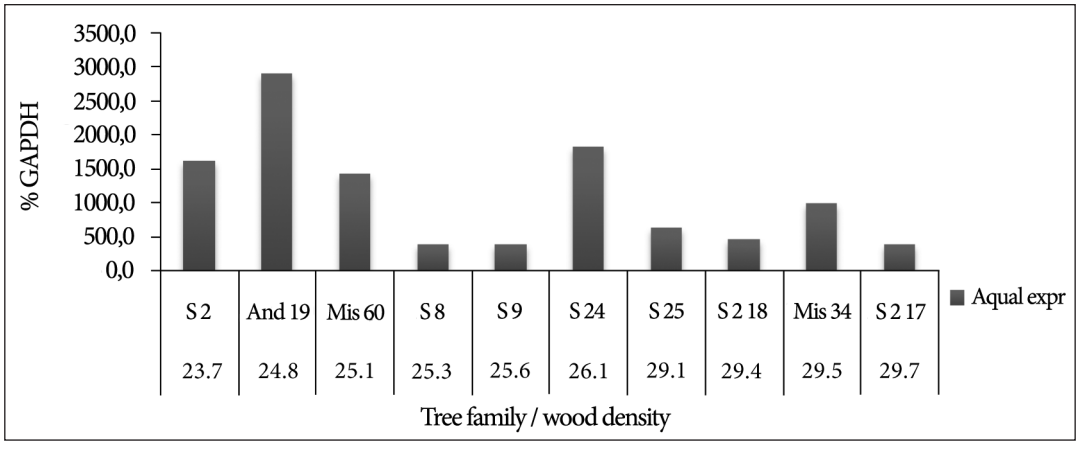

Figure. Average expression of the *Aqual* gene during early wood formation in different tree families

A significant positive correlation was found between wood density and *SuSy* gene expression during early wood formation. There was a negative correlation between the *Aqual* gene expression and wood density during early wood formation. No significant correlations were found between candidate gene expression during late wood formation and wood density. The knowledge gained will be used to develop molecular tools directed toward improving wood properties of Scots pine.

References

1. Johansson IM, Karlsson U, Johanson C, Larsson, Kjellbom P. The role of aquaporins in cellular and whole plant water balance. Biochem Biophys Acta 2000; 1465: 324–42.

2. Nairn CJ, Lennon DM, et al. Carbohydrate-related genes and cell wall biosynthesis in vascular tissues of loblolly pine (*Pinus taeda*). Tree Physiol 2008; 28: 1099–110.

3. Coleman HD, Yan J, Mansfield SD. Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. Proc Natl Acad Sci USA 2009; 106: 13118–23.

DEVELOPMENT OF ISSR MARKERS IN PLANT GENETIC ANALYSIS

Izolda Pašakinskienė

Faculty of Natural Sciences Vilnius University,
M. K. Čiurlionio 21/27, LT-03101 Vilnius, Lithuania

Botanical Garden of Vilnius University,
Kairėnų 43, LT-10239 Vilnius, Lithuania

E-mail: izolda.pasakinskiene@gf.vu.lt

Inter-simple sequence repeats (ISSRs) are regions between microsatellite loci (Zietkiewicz et al., 1994). When SSRs are used as PCR primers for generating DNA profiles, numerous loci throughout the genome are generally targeted. In a single primer reaction, closely spaced reverse oriented SSRs on the DNA template serve as primer binding sites to initiate amplification of the intervening DNA sequences. The result is a mix of a variety of amplified DNA fragments which vary in size between ≤100 and 3 000 bp; about 10–60 fragments from multiple loci are generated simultaneously. ISSR markers can reveal plant genetic diversity and identify individual genotypes. The use of ISSRs was efficient in genotyping breeding germplasm of grasses and cereals (Pašakinskienė et al., 1999; Pašakinskienė et al., 2000). ISSRs were found widely dispersed in all linkage groups of perennial ryegrass, both in intergenic regions and in gene loci; and the identification and characterization of these sequences were useful for the enrichment of the *Lolium perenne* genetic map (Pivorienė et al., 2008). These studies were extended in evaluation of genetic polymorphism within a wide set of plant species including *Lolium* spp., *Festuca* spp., *Trifolium* spp., and *Taxus baccata*.

References

1. Pašakinskienė I, Brazauskas G, Paplauskienė V, Sasnauskas K. Amplification of DNA markers in cereals using primers of anchored simple sequence repeats. Biologija 1999; 3: 64–6.

2. Pašakinskienė I, Griffiths CM, Bettany AJE, Paplauskienė V, Humphreys MW. Anchored simple sequence repeats as primers to generate species-specific DNA markers in *Lolium* and *Festuca* grasses. Theor Appl Genet 2000; 100: 384–90.

3. Pivorienė O, Pašakinskienė I, Brazauskas G, Lideikytė L, Jensen LB, Lübberstedt T. Inter-simple sequence repeat (ISSR) loci mapping in the genome of perennial ryegrass. Biologija 2008; 54(1): 17–21.

4. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 1994; 20: 176–83.

COPY NUMBER VARIATION ANALYSIS OF RESISTANCE GENES IN SCOTS PINE

Vilnis Šķipars, Viktorija Beļeviča, Baiba Krivmane, Dainis E. Ruņģis*

Genetic Resource Centre
Latvian State Forest Research Institute "Silava",
** Corresponding author. E-mail: dainis.rungis@silava.lv*

Introduction. While DNA segment copy number variations have been studied extensively in humans and model organisms, the first study on gene copy number variation (CNV) in conifers was published only last year (Šķipars et al., 2011).

Materials and methods. Here we present our data about CNVs of four defence-related Scots pine genes: *Pinus sylvestris* thaumatin-like protein (*TLP*) gene, defensin 2 gene (*Def2*), *Pinus sylvestris* pinosylvin synthase gene (*PsBBs*), and *PsACRE* (an *Avr9/Cf-9* rapidly elicited (*ACRE*) gene homolog (Li & Asiegbu, 2004)). Gene copy numbers were determined by use of real-time PCR. 29 samples were analysed for all genes except *PsBBs*, for this gene 27 samples were analysed.

Results. Frequencies of CNVs are shown in Figure. Elevated copy number of the *TLP* gene was demonstrated to coincide with a higher increase of *TLP* gene expression in mature Scots pine trees inoculated with *Heterobasidion annosum* compared to individuals with normal *TLP* gene copy number. After initial CNV determination experiments a larger set of samples was checked for CNVs of *TLP* (n = 54) and *PsBBs* (n = 86) genes providing indication of frequency of CNV occurrence. The results of these studies indicate that CNV analyses may potentially be of use as criteria for selection of individuals in Scots pine breeding programs. Two strategies of molecular genetics assisted selection towards *H. annosum* resistant Scots pines – use of gene copy number variation and gene expression analyses, are compared.

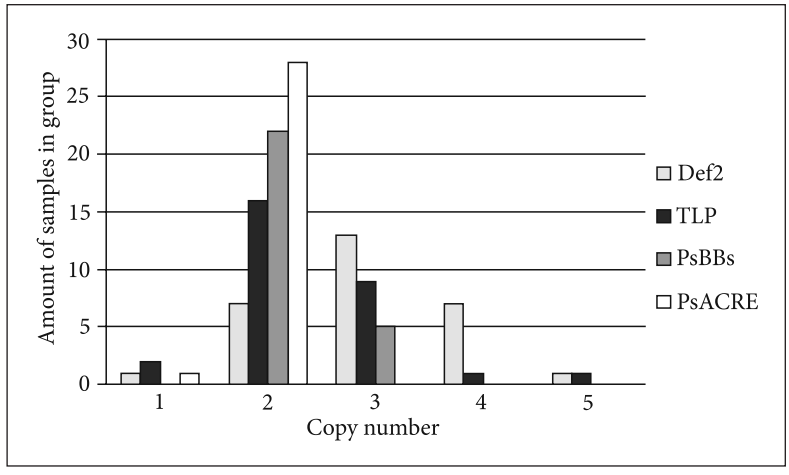

Figure. Frequency of CNVs of four resistance genes in Scots pine

References

1. Li G, Asiegbu FO. Induction of *Pinus sylvestris PsACRE*, a homology of *Avr9/Cf-9* rapidly elicited defense-related gene following infection with root rot fungus *Heterobasidion annosum*. Plant Sci 2004; 167(3): 535–540 doi:10.1016/j.plantsci.2004.04.025

2. Šķipars V, Krivmane B, Ruņģis D. Thaumatin-like protein gene copy number variation in Scots pine (*Pinus sylvestris*). Environ Exp Biol 2011; 9: 75–81.

IDENTIFICATION OF RETROTRANSPOSON-LIKE SEQUENCES IN *PINUS SYLVESTRIS* ACTIVATED IN RESPONSE TO STRESS CONDITIONS

Angelika Voronova[1]*, Āris Jansons[2], Dainis Ruņģis[1]

[1] *Genetic Resource Centre, Latvian State Forest Research Institute "Silava", Rigas 111, LV-2169 Salaspils, Latvia*

[2] *Forest Tree Breeding, Latvian State Forest Research Institute "Silava", Rigas 111, LV-2169 Salaspils, Latvia*

* *Corresponding author. E-mail: angelika.voronova@silava.lv*

Introduction. This study examines retrotransposon-like sequences in the expression profile of stressed Scots pine trees. Retrotransposon activity can be a major factor in genome instability, rearrangements and therefore also plasticity of the genome and adaptation to changing environmental conditions. Naturally occurring stresses such as heat shock and insect infection as well as treatment with specific chemicals like abscisic acid and salicylic acid were tested.

Materials and methods. Two year old Scots pine ramets were used in this study to minimise genetic diversity between individuals. To discover transcriptionally active mobile genetic elements non-specific inter Primer Binding Site (iPBS) reactions were performed as described by Kalendar et al. (2010). Differentially expressed fragments amplified from stressed tree samples, but not from control tree samples, were excised, purified, and sequenced. Sequence analyses and mobile element identification were performed by searching within several nucleotide and protein databases. All fragments that were similar to known TEs in databases could be classified to at least an order or superfamily according to the classification proposed by Wicker et al. (2007).

Results. Our first results suggest the existence of several groups of active retrotransposons in the Scots pine genome, which share differing similarity levels with known elements from other plant species (Voronova et al., 2011). The expressed retrotransposon-like sequences isolated after exposure to different stresses were often found to belong to different families of mobile elements. Specific primers were used to evaluate expression of retrotransposon-like sequences between samples subjected to various stresses. Retrotransposon-like sequences are widely transcribed as many are also present in EST (Expressed Sequence Tag) databases of various plant species. Slight sequence variations were observed within a single isolated fragment, which could be explained by the origin of observed elements from multiple loci in the pine genome. Searches of nucleotide sequence databases reveals several elements that have characteristic structural features of full-length LTR (Long Terminal Repeat) retrotransposons in other pine species where more sequence information is available (*Pinustaeda, Pinusradiata*). Nine specific LTR primers were designed using retrotransposon-like sequences obtained from heat shock and insect infestation experiments. These primers were used to study 150 individuals growing in differential conditions in one natural pine stand using IRAP (Inter-Retrotransposon Amplified Polymorphisms). Results indicated a substantial increase in the number of amplification fragments in trees growing in predominantly dry conditions. While the level of genetic diversity was similar if compare with Simple Sequence Repeat marker data for same populations. It has not been clarified how many copies from the identified retrotransposon-like sequences are activated in the complex pine genome, as translation of even a single element can cause transcription of

many more non-autonomous retrotransposons with truncated sequences. Further studies of retrotransposon activation in pine could increase understanding of genomic rearrangements in response to stress conditions.

References

1. Kalendar R, Antonius K, Smykal P, Schulman AH. iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theor Appl Genet 2010. DOI 10.1007/s00122-010-1398-2.

2. Voronova A, Jansons A, Ruņģis D. Expression of retrotransposon-like sequences in Scots pine (*Pinus sylvestris*) in response to heat stress. Environ Exp Biol 2011; 9: 121–7.

3. Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P, Morgante M, Panaud O, Paux E, SanMiguel P, Schulman AH. A unified classification system for eukaryotic transposable elements. Nature Rev Gen 2007; 8: 973–82.

GENETIC AND BIOTECHNOLOGY METHODS FOR FLAX AND HEMP BREEDING ACCELERATION IN LATVIA

Dace Grauda[1]*, Andra Miķelsone[1], Veneranda Stramkale[2], Reinis Ornicāns[1], Nadežda Ļisina[1], Karīna Latiševa[1], Isaak Rashal[1]

[1] *Institute of Biology, University of Latvia, LV-2169 Salaspils, Latvia*

[2] *Agricultural Science Centre of Latgale, Vilani, LV-4650 Rezekne distr., Latvia*

* *Corresponding author. E-mail: dace@email.lubi.edu.lv*

Introduction. Presently, there are no flax and hemp varieties suitable for Latvian weather and agronomical conditions. The main drawback of the best foreign varieties is too long vegetation period. For breeding adapted varieties collecting and evolution of Latvian flax and hemp genetic resources became a very important task.

Methods and results. More than 90 flax and 20 hemp accessions of the Latvian origin, mostly created before the Second World War, were collected and evaluated for important agronomic traits in field trials during 2007–2011. Several appropriate accessions were selected and used for crosses. Because the quality of flax fibre and seeds is affected by diseases, especially by microscopic fungus, analysis of presence of effective alleles of flax rust resistance genes was performed for 50 best Latvian flax accessions and standard varieties 'Lirina' and 'Vega'. Most accessions contain resistance allele *L9*. Only three accessions (landrace 'Blue di Riga', lines N32 and S53) possessing *M* allele. In two lines (S53 and N32) were found both mentioned resistance alleles *L9* and *M*. For obtaining additional flax breeding initial material two *in vitro* (anther and callus cultures) methods were applied. Androgenic response of used hybrids ranged from 1 to 11% and was depending from genotype and growing conditions. Regeneration was observed only from diploid callus. By molecular markers was found out increased genetic variability in flax plants-regenerants (somaclonal variation). To start hemp breeding very important was to find out optimal conditions of cloning and cultivating hemp breeding material. The best medium for seeds shooting *in vitro* was medium with a half of MS (Murashige and Skoog) medium salts, but for plant cultivation the best was MS medium with activated carbon. SCAR (Sequenced Characterized Amplified Region) marker method was used for early hemp female plants identification.

5th BALTIC CONGRESS OF GENETICS

Kaunas, Vytautas Magnus University, Lithuania

October 19–22, 2012

Part 2
Abstracts of Poster Presentations

GENETIC ANALYSIS OF WILD BOAR (*SUS SCROFA*) BY USING RAPD MARKERS

Vykintas Baublys[1]*, Algimantas Paulauskas[1], Zenonas Janulaitis[2], Rauno Veeroja[3], Jolita Bakanauskaitė[1]

[1] *Vytautas Magnus University, Vileikos str. 8, LT-44404 Kaunas, Lithuania*

[2] *Kaunas Forestry and Environmental Engineering College, Liepų str. 1, Girionys, LT-53101 Kaunas, Lithuania*

[3] *Estonian Environment Information Centre, Roomu tee 2, 51013 Tartu, Estonia*

* *Corresponding author. E-mail: v.baublys@gmf.vdu.lt*

Introduction. In last decades population size of wild boar (*Sus scrofa*) has been increasing dramatically. Since 1934 (280 individuals) the population density of wild boar in Lithuania has increased 195 times. With the increasing and spreading of wild boar populations the damage to agricultural fields is rising. Tethered of diseases like swine fever, trichinosis are on the rise. Wild boar is a major reservoir host for pathogens that affect humans and domestic animals. The wild boar is a flexible species, shown by high variation of all observed results between individual groups. There are very few population genetics studies done in Europe (Gimenez et al., 2003; Kaller et al., 2007; Perez et al., 1998). And there were no wild boar populational genetics studies done in Lithuania at all. There is a need to investigate genetic structure of wild boar in Baltic countries.

Aim and methods. The aim of this study was to evaluate genetic variability of wild boar populations in different Baltic countries and populations in different Lithuania regions separately. Total 189 samples were examined from different Lithuania regions, Estonia and Belarus. Heart muscle and liver samples were collected and stored in ethanol until use. RAPD analysis was made with eight primers to estimate genetic variation of wild boar.

Results. Our investigated RAPD markers revealed high polymorphism of wild boar (*Sus scrofa*) in Baltic countries. From all our investigated markers no one is suitable for good within population analysis, because no one reveals high molecular variance. Genetic differences among all five investigated Lithuanian regions were small. But location specific alleles were detected in all these regions. Most distinct populations were found in Central Lithuania and Varėna region. We found that from all used markers most useful for among population analysis is Roth-180-06. It showed the largest molecular variance among populations. Comparing populations of Lithuania, Estonia and Belarus we detected quite large differences. In Lithuania wild boar average polymorphism was bigger (55%) than in Belarus (33%) and in Estonia (48%). We concluded what intensive hunting has a marked impact on wild boar genetic structure and can maintain high polymorphism.

References

1. Gimenez DL, Mota LSLS, Curi RA, Rosa GJM, Gimenes MA, Lopes CR, Lucca EJ. Análise cromossômica e molecular do javali europeu Sus scrofa scrofa e do suíno doméstico Sus scrofa domesticus. Brazil J Vet Res Anim Sci 2003; 40: 146–54.

2. Kaller MD, Hudson III JD, Achberger EC, Kelso WE. Feral hog research in western Luisiana: expanding populations and unforseen consequences. Human–Wildlife Conflicts 2007; 1(2): 168–77.

3. Perez T, Albornoz J, Dominguez A. An evaluation of RAPD fragment reproducibility and nature. Mol Ecol 1998; 7: 1347–57.

D-LOOP SEQUENCE VARIATION AND PHYLOGEOGRAPHIC RELATIONSHIPS OF PERCH POPULATIONS IN THE BALTIC REGION

Dalius Butkauskas[1]*, Adomas Ragauskas[1], Aniolas Sruoga[2], Linas Ložys[1], Isaak Rashal[3]

[1] Nature Research Centre, Akademijos str. 2, LT-08412 Vilnius, Lithuania

[2] Vytautas Magnus University, K. Donelaičio str. 58, LT-44248 Kaunas, Lithuania

[3] Institute of Biology, University of Latvia, Miera str. 3, LV-2169 Salaspils, Latvia

** Corresponding author. E. mail: dalius@ekoi.lt*

Partial D-loop sequences of the perch samples collected in Lithuanian and Latvian inland water bodies were analyzed. Nine new haplotypes were identified from a total of 140 fish collected in Lithuania and six new haplotypes were found among 48 fish collected in Latvia. A total of 55 different haplotypes were ascertained out of 733 individuals investigated after newly detected sequences were combined together with D-loop sequences obtained by other authors. It was revealed that genetic diversity of the perch population distributed in the coastal and inland water bodies of the Baltic States is rich and unique. Significant genetic differentiation was found between perch populations representing the inland water bodies and territorial waters of the coastal zone in Lithuania and Latvia, respectively. Phylogeographic relationships of the perch populations of different parts of the Baltic region are revealed on the basis of the results of the current study.

GENETIC DIVERSITY IN PERCH (*PERCA FLUVIATILIS*) POPULATION OF LAKE KALEZERS (LATVIA)

Oksana Fokina*, Dace Grauda, Isaak Rashal

Institute of Biology, University of Latvia,
Miera Street 3, LV-2169 Salaspils, Latvia

** Corresponding author. E-mail: oksanafokina@inbox.lv*

Introduction. Different types of retrotransposons-based molecular markers are universal and very informative therefore they became very popular for analysis of genetic diversity in populations of animals, yeasts and plants. For each of the type of those markers it is crucial to find specific primers which reveal maximum of genetic polymorphism of particular species. Last years rather wide application has got method which reveals inter-retrotransposons amplified polymorphisms (IRAP). Till now, information is absent in the literature concerning use of IRAP method for genetic analysis of fish populations.

Aim and methods. The goal of the study was to adjust IRAP method for genetic analysis of perch (*Perca fluviatilis*) populations and to determine the genetic diversity of perch population of Latvian lake Kalezers. To find most appropriate primers for analysis of perch populations 26 PCR primers, which were previously successfully used in analysis of different animal and plant species, were tested. DNA samples were extracted from blood of 40 specimens caught in the lake Kalezers (Latvia). The PCR reaction mix consisted of 0.5 µl primer, 2.5 µl 10 × Dream Taq buffer, 0.5 µl dNTP Mix, 0.25 µl Dream Taq DNA polymerase, 0.025 µl Pfu DNA polymerase, 17.225 µl molecular water and 2.0 µl DNA. The reaction mix volume per each sample was 25 µl. PCR reaction had 32 cycles: 1 cycle at 95 °C for 3 min, 30 cycles with 95 °C for 30 s, 50 °C for 40 s, and 68 °C for 1 min, and the last cycle at 72 °C for 10 min). PCR products were tested in 1.7% agarose gel for 4.5 hours (80 V). Three primers (2080, 2081 and 2239) with high level of polymorphisms were chosen for further analysis. PCR products were analysed in agarose gel for 15 hours (50 V). PopGene software for population genetic analysis was used. Selected primers showed good applicability for analysis of perch genetic polymorphisms: 50 loci were revealed by the primer 2080, 42 loci – by primer 2081, and 35 loci – by primer 2239.

Results. High genetic diversity was found in lake Kalezers, 79 (67%) loci were polymorphic. Perch population of the lake is isolated from other perch populations in range. Comparison of genetic diversity of perch in the lake Kalezers with other Latvian lakes is in the progress. The IRAP method is useful for genetic analysis of perch populations. Loci numbers produced by selected primers were close to the loci number that was established with retrotransposons-based molecular markers in plants.

SPECIES IDENTIFICATION OF GENUS *OSMODERMA* (COLEOPTERA: SCARABAEIDAE: CETONIINAE) IN LATVIA THROUGH GENETIC ANALYSIS USING UNIVERSAL MTDNA PRIMERS

Inese Gavarāne*, Inese Kokina, Kristīna Aksjuta, Uldis Valainis, Māris Nitcis, Arvīds Barševskis

Daugavpils University, Institute of Systematic Biology, Vienības 13, LV-5401 Daugavpils, Latvia

** Corresponding author. E-mail: inese.gavarane@biology.lv*

The genus *Osmoderma* Lepeletier & Serville, 1828 is represented in Latvia. All known populations of *Osmoderma* are small and there is a little information available about genetic variation and population isolation. For population analysis, it is necessary to be sure about species identification. In this study, specimens collected by using pheromone trap from variable parts of Latvia were analyzed. A molecular analysis was carried out on twenty seven *Osmoderma* individuals for species identification. Sequences were obtained by using universal primers of mtDNA cytochrome C oxidase I gene (COI). For DNA isolation by using membrane spin-column method the molecular protocol (DNeasy Blood and Tissue, Qiagen, Germany) was modified. PCR amplification products were visualized by submarine gel electrophoresis. Sequences were obtained by using Sanger method. According to analyzed sequences it is possible to use obtained results for further species identification and population analysis.

Financial support for this study was provided by the LIFE-Nature program of European Commission EREMITA MEADOWS (No LIFE09/NAT/LV/000240).

INVESTIGATION OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF RACCOON DOGS IN LITHUANIA BY USING MICROSATELLITE ANALYSIS

Loreta Griciuvienė[1]*, Algimantas Paulauskas[1], Jana Radzijevskaja[1], Simona Juknelytė[1], Vaclovas Gedminas[2]

[1] Faculty of Natural Sciences, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

[2] Kaunas Tadas Ivanauskas Zoological Museum, Laisvės 106, LT-44253 Kaunas, Lithuania

** Corresponding author. E-mail: l.griciuviene@gmf.vdu.lt*

Introduction. In Europe, the raccoon dog (*Nyctereutes procyonoides*) is one of the most successful alien carnivores with a wide distribution, significant ecological impact and remarkable dynamics of spread over the continent (Nowak, 1973; Kauhala, 1996). In Lithuania the raccoon dog has been first observed in 1948 in the eastern part of the country, and since 1970 declared as invasive species (Kauhala, 2011). However, no genetic study on raccoon dog in Lithuania was carried out, and the genetic structure of population is not clear.

Aim and methods. The objective of this study was to assess the levels of genetic diversity and population structure of raccoon dog in Lithuania by using microsatellite analysis.

The tissue samples of the raccoon dogs were collected by hunters during period 2007–2011 from different locations in Lithuania. Genomic DNA was extracted from frozen muscles using "Genomic DNA purification kit" (Thermo scientific, Lithuania). We screened 15 canine microsatellite loci on 50 randomly selected samples of raccoon dogs from 10 different regions of Lithuania.

Results. Six from tested loci (FH-2096, FH-2054, FH-2010, FH-2004, PEZ-17) were found to be polymorphic, one (VWF.X) monomorphic. Total number of alleles at loci ranged from 4 to 9. Altogether, there were 35 alleles identified. The mean number of alleles in all the loci in various raccoon dog groups ranged from 3,167 to 4,333. All six microsatellite loci were found to be highly variable with observed heterozygosity values ranging from 0.49 to 0.78. Grouping of the individuals from different localities was carried out by a principal coordinate analysis (PCA) performed on Nei's genetic distance matrix. Analysis of molecular variance (AMOVA) showed that genetic diversity of raccoon dog within sampling locations yielded 91%, and among locations 9% of the total genetic diversity. The present data revealed a high level of genetic variation in six microsatellite loci and showed high heterogeneity and weak population structure of raccoon dog in Lithuania.

Financial support for this study was provided by the Research Council of Lithuania (grant. No. LEK – 14/2012).

References

1. Kauhala K. Introduced carnivores in Europe with special reference to central and northern Europe. Wildlife Biol 1996; 2: 197–204.

2. Nowak E. Ansiedlung und Ausbreitung des Marderhundes (Nyctereutes procyonoides Gray) in Europa. Beitr Jagd-und Wildforschung 1973; 8: 351–84.

3. Kauhala K, Kowalczyk R. Invasion of the raccoon dog Nyctereutes procyonoides in Europe: History of colonization, features behind its success, and threats to native fauna. Curr Zool 2011; 57: 584–98.

APPROBATION OF MTDNA AMPLIFICATION CONDITIONS BY REAL-TIME PCR FOR *OMOPHRON* LATREILLE, 1802 GENUS (COLEOPTERA: CARABIDAE) FROM AIR-DRIED SYSTEMATIC COLLECTIONS

Inese Kokina*, Kristīna Aksjuta, Ilze Rubeniņa, Uldis Valainis, Arvīds Barševskis, Inese Gavarāne

Daugavpils University, Institute of Systematic Biology, Vienības 13, LV-5401 Daugavpils, Latvia

** Corresponding author. E-mail: inese.kokina@biology.lv*

The genetic information from different organisms is now an indispensable starting point for molecular biology research. Approbation of various molecular protocols is a powerful tool for obtaining genetic information. Many traditional methods in molecular biology have now been superseded by PCR. However, there has been increasing interest about using real-time PCR techniques. In its simplest form a real-time PCR is set up that includes a DNA-binding cyanine dye such as SYBR green. Nevertheless, PCR conditions are one of the significant provisions of genetic information acquisition. *Omophron aequale aequale* Morawitz, 1863 and *Omophron aequale jacobsoni* Semenov, 1922 subspecies from Japan were used for obtaining nucleotide sequences. In our study, isolated from air-dried collection material DNA was used. In most cases it was a miserable amount of DNA in the final elution volume. To obtain PCR products it was necessary to optimize amount of DNA template and primer annealing temperature for each reaction. Universal mtDNA primer pair LCO1490/HCO2198 annealing temperature range is 45–52 °C. It gradually increases the temperature of each tube until the double-stranded PCR product denatures or melts and allows a precise although not definitive determination of the product. Confirmation of the product was obtained by DNA sequencing Sanger method.

Financial support for this study was provided by the project of European Social Fund (No. 2009/0206/1DP/1.1.1.2.0/09/APIA/VIAA/010).

GENETIC FACTORS INFLUENCING CATTLE MILKING CHARACTERISTICS

Kristina Liucvaikienė, Rita Kairytė, Ilona Miceikienė, Kristina Morkūnienė, Lina Baltrėnaitė

Lithuanian University of Health Sciences, Veterinary Academy
Tilžės str. 18, LT-47181 Kaunas, Lithuania

** Corresponding author. E-mail: genetikalab@lva.lt*

Aim. The aim of the study was to investigate the genetic factors influencing cattle milking genetic characteristics.

Methods. The investigation was undertaken in a group of 163 selected cows belonging to Danish Black and White, Danish Red, Holstein, Lithuanian Black and White, Old genotipe of Lithuanian Black and White and Swedish Black and White breeds. Milking data was collected from Windows ALPRO system which automatically records milking data during the milking process: milk yield per day (kg), fat yield per day (kg), the average milk yield of 7 days (kg), the maximum milk flow (kg/min), milking speed (kg/min), milking time (min). Productivity data was taken from Agricultural informatikon centre: day of milking, milk yield (kg), percentage of fat, fat (kg), percentage of protein, protein (kg).

Results. The results show that in the study the milk flow was on average 4.08 kg/min, milking speed was average 2.36 kg/min, milking time was on average 6.36 min. The maximum milk flow was from the Danish Black and White breed cattle – 4.60 kg/min, the largest milking speed was from Lithuanian Black and White – 2.63 kg/min and the shorest milking duration – 5.42 kg/minutes had Old genotipe Black and White breed cattle. The genetic factor – breed – statistically significantly affected the production traits of milk ($p < 0.001$). It influenced 10.9% yield (kg) variation, 23% variation of the amount of milk produced per day, 19.7% milk fat and 13% indicators of a variation in milk protein. Milking characteristics were effected by the genetic factor – breed- as well ($p < 0.001$). Breed influenced 13.1% variation of the maximum milk flow rate, 22% variation of the milking speed and 13.3% milking time variation. There is a strong positive correlation between milk yield (kg) and the highest milk flow (kg/min) (0.37; $p < 0.001$), milk yield (kg) and milking speed (kg/min), milk yield (kg) and milking time (min) (0.32; $p < 0.001$). It was found that increasing yield, increases the maximum milk flow, milking rate and milking time. The maximum milk flow (6,150 kg/min) and the highest milking speed (3,775 kg/min) was in the most productive cows (over 13,000 kg).

GENETIC VARIABILITY IN LOCAL POPULATIONS OF VENDACE (*COREGONUS ALBULA* (L.) FROM SOME LATVIAN LAKES BASED ON MICROSATELLITE MARKERS

Jeļena Oreha, Natalja Shkute

Institute of Ecology, Daugavpils University,
Vienības iela 13, LV-5401 Daugavpils, Latvia

E-mail: jelena.oreha@du.lv; natalja.skute@du.lv

Vendace (*Coregonus albula*) is a representative of the genus *Coregonus* and can be found in many Latvian lakes. Though its share in the fishery is not large, the catch is insignificant and unstable. This species is included in the list of specially protected species with limited use in Latvia. Taking into account the high degree of variability of the vendace and the fact that it belongs to valuable marketable fishes presents a scientific interest to make an attempt to determine the major factors that impact its variability. A lack of precise scientific data regarding the local vendace populations and its biology hinders rational exploitation of this fish and does not allow an opportunity for its reproduction in Latvian lakes.

Hence, there is a need for a cost effective and powerful genetic tool in the monitoring, protection and management of this species. For this reason 111 individuals of vendace from Lake Svente, Razna, Nirza, Dridzas, Stirnas, Ezezers were analysed using microsatellite markers. The genetic diversity and variability, mean number of alleles on locus, expected and observed heterozygosity were analyzed at six microsatellite loci.

The study was supported by the project No. PD1/ESF/PIAA/04/NP/3.2.3.1/0003/0065 and ESF projects: No. D1/ESF/PIAA/04/NP/3.2.3.1/0003/0065, No. 2009/0214/1DP/1.1.1.2.0/09/APIA/VIAA/089.

GENETIC DIVERSITY OF ROE DEER (*CAPREOLUS CAPREOLUS* L.) IN THE LITHUANIAN POPULATION

I. Pūraitė*, A. Paulauskas, A. Sruoga

Faculty of Natural Sciences, Vytautas Magnus University,
Vileikos str. 8, LT-44404 Kaunas, Lithuania

** Corresponding author. E-mail: i.puraite@gmf.vdu.lt*

Introduction. European roe deer (*Capreolus capreolus* L.) are very common all over Europe. It is relatively small, reddish and grey-brown, and well-adapted to cold environments. Their distribution ranges from southern Spain to northern Scandinavia. As generalist herbivores, roe deer are able to feed on a wide variety of plants and thus live in several kinds of habitats. Roe deer are known to migrate more than 100 km (Linnell et al., 1998), distances up to a few kilometres are usual for large parts of continental Europe (Vor et al., 2010). In Lithuania as in many European countries (Poland, Germany, Czech Republic, Slovakia, Hungary, Romania, Denmark) two roe deer (*Capreolus capreolus*) ecotypes can be distinguished: forest roe deer and field roe deer (Reasfeld et al., 1985).

Aim and methods. In this study 22 individuals were tested to investigate genetic diversity in Lithuanian population. Individuals from 5 different localities of Lithuania were analyzed by microsatellites (SSR) polymerase chain reaction (PCR) using six primers. Data from electrophoresis gels were loaded to GenAlEx program. This program calculated effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He).

Results. According to Nei evaluation, the highest Genetic Distance (D) was 0,236 between Ukmerge and Cinkiskes subpopulations. The lowest Genetic Identity (I) was between Ukmerge and Cinkiskes subpopulations 0,790. Using GenAlEx 6.41 program were calculated genetic diversity among and within populations (AMOVA). As a result 26% difference in genetic diversity was determined between Lithuanian roe deer subpopulations and 74% genetic variability between individuals in all Lithuanian population was established.

References

1. Linnell JDC, Wahlström K, Gaillard JM et al. From birth to independence: birth, growth, neonatal mortality, hiding behaviour and dispersal. In: Andersen R et al. (eds.). The European roe deer: the biology of success. Oslo: Scandinavian University Press 1998: 257–84.

2. Reasfeld F, Neahaus AH, Schaich K. The roe deer. Hamburg und Berlin: Paul Parey, 1985.

3. Vor T, Kiffner C, Hagedorn P, Niedrig M, Ruhe F. Tick burden on European roe deer (*Capreolus capreolus*). Exp Appl Acarol 2010; 51: 405–17.

MICROSATELLITE ANALYSIS OF THE GENETIC STATUS OF RED DEER (*CERVUS ELAPHUS*)

I. Pūraitė[1], J. M. Gibiežaitė[1], A. Paulauskas[1]*, A. Sruoga[1], O. Rosef[2]

[1] Faculty of Natural Sciences, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

[2] ATP-innovation AS, 3800 Bø i Telemark, Norway

** Corresponding author. E-mail: a.paulauskas@gmf.vdu.lt*

Introduction. Red deer (*Cervus elaphus*) is the most widespread and best known deer species in the world (Ludt et al., 2004). The European red deer has been undergoing human influences such as translocations, habitat fragmentation and selective hunting throughout Europe for many centuries (Lowe, Gardiner, 1974; Hartl et al., 2003). Study of Cervids genetic diversity was launched recently and is still not much data on the genetic variability of hoofed animals. Genetic testing helps to identify the genetic differences between animals in the deer population also to assess the specificity of different populations, and identify individual animals.

Aim and methods. In year 2010 there were around 150 deer farms with about 4 000 deers held in them in Lithuania. Sometimes animals can escape from the enclosure and start interbreed with native deer, and thus affect their genetics. It is very important to observe and analyse these genetic changes in populations of wild and domestic animals and to use the results in order to avoid infest. In this study 20 individuals from Lithuania and Norway were analysed by six SSR loci. Genetic distance (D) and genetic identity (I) were calculated according to Nei evaluation.

Results. AMOVA results showed 15% difference in genetic diversity between Lithuanian and Norwegian deer populations and 85% genetic variability within populations was established.

References

1. Hartl GB, Zachos F, Nadlinger K. Genetic diversity in European red deer (*Cervus elaphus* L.): antropogenic influences on natural populations. CR Biologies 2003; 326: S37-S42.

2. Lowe VPM, Gardiner AS. A re-examination of the subspecies of Red deer (*Cervus elaphus*) with particular reference to the stocks in Britain. J Zool (London) 1974; 174: 185–201.

3. Ludt ChJ, Schroeder W, Rottman O, Kuehn R. Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*). Mol Phylogen Evol 2004; 31: 1064–83.

MICROSATELLITE MARKERS FOR SOLITARY TRAP-NESTING WASP *ANCISTROCERUS TRIFASCIATUS* (MÜLLER, 1776): RESULTS OF CROSS-SPECIES AMPLIFICATION EXPERIMENTS

Rita Radzevičiūtė[1, 2]*, Jurga Turčinavičienė[1], Eduardas Budrys[1, 2]

[1] *Department of Zoology, Vilnius University, M. K. Čiurlionio 21/27, LT-03101 Vilnius, Lithuania*

[2] *Institute of Ecology of Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania*

* *Corresponding author. E-mail: rita_radzeviciute@yahoo.de*

Solitary cavity-nesting Hymenoptera constitute a group of important bioindicators of terrestrial habitats. Some of them, like caterpillar-hunting potter wasp *Ancistrocerus trifasciatus* (Hymenoptera: Vespidae: Eumeninae), are quite abundant in both continuous and fragmented habitats and might be a promising model species for studying the impact of habitat fragmentation and landscape connectivity on genetic diversity of entomophagous insects. Highly polymorphic microsatellites are a powerful molecular tool for intraspecific studies but the development of this marker system *de novo* is especially laborious and time-consuming. An alternative time- and effort-effective approach to establish the microsatellite markers for the species of interest is the cross-species amplification of loci already isolated in related species. Here we present a panel of five polymorphic microsatellite loci for *A. trifasciatus* developed by cross-species amplification of twenty-nine microsatellite markers published so far for nearctic potter wasps.

GENETIC AND MORPHOLOGICAL VARIABILITY OF SEA TROUT (*SALMO TRUTTA* L.) POPULATION USED FOR ARTIFICIAL REPRODUCTION IN GAUJA RIVER BASIN (LATVIA)

Natalja Shkute*, Arturs Karklinsh, Andrej Solomenikov

Institute of Ecology, Daugavpils University,
Vienības 13, LV-5400 Daugavpils, Latvia

** Corresponding author. E-mail: natalja.skute@du.lv*

Introduction. Sea trout (*Salmo trutta* L.), with other representatives of the Salmonidae fish, is among the most valuable biological resources in Latvia. The growth of salmonid aquaculture has raised concerns about the possibility of detrimental effects on the genetic integrity and diversity of wild population. Indigenous salmonid fish gene pools are affected by domesticated conspecifics, derived from aquaculture escapes and deliberate releases. Ecogenetic criteria are usually associated with such genetic structure of population, which is close to the genetic structure of populations evolutionarily established in nature. Sustainability of the sea trout population is ensured by artificial reproduction. For many years the fish has been artificially reproduced in Latvian fish farms "Brasla" (Gauja basin).

Materials and methods. The diversity of wild sea trout population from Gauja basin was investigated. Twelve morphological parameters were analysed and Pearson correlation was calculated. The fish non-destructive methods of DNA extraction (from scale) were used for genetic analysis. The population genetic structure was analysed by four microsatellite primers. Genetic parameters of the populations, such as the percentage of polymorphic loci, level of polymorphism, level of heterozygosity, and number of private alleles per locus were calculated.

Conclusions. Information of the genetic structure of Sea trout (*Salmo trutta* L.) wild population may be useful for sustainable development and preservation of genetic diversity of fish populations.

THE MICROSATELLITE-BASED GENETIC RELATIONSHIP AMONG HORSE BREEDS AND THE EVALUATION OF THE GENETIC STRUCTURE OF THE ESTONIAN HORSE POPULATION

Erkki Sild[1]*, Krista Rooni[1], Sirje Värv[1], Juha Kantanen[2], Haldja Viinlass[1]

[1] *Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia*

[2] *Biotechnology and Food Research, MTT Agrifood Research Finland, FI-31600 Jokioinen, Finland*

* *Corresponding author. E-mail: erkki.sild@emu.ee*

Introduction. The three local Estonian horse breeds (Estonian Native, Estonian Heavy Draught, Tori) were compared with the local breeds of neighbouring countries (Latvia and Finland) as well as with the international transboundary breeds raised in Estonia (the Akhal-Teke, Arabian, Hanoverian, Standardbred, and Trakehner).

Materials and methods. A total of 155 horses of the Estonian local breeds and 192 horses from the other breeds were randomly sampled. All 347 individuals were characterized by 16 microsatellites recommended by ISAG/FAO (FAO, 2011). The genetic relationship between breeds was estimated using Reynolds distances. Bayesian clustering of the breeds and individuals within each breed was carried out. In addition, the Tori horses were analysed following the stud book registration: the O-type (Old Tori) and A-type (conservation population), representing the endangered-maintained population, and the B-type, which is open to gene introgression.

Results. The between-breed distances ranged from 0.027 (between the Tori and Latvian Horse) to 0.161 (the Estonian Heavy Draught and Trakehner). Bayesian clustering at k = 2 revealed two main genetic clusters: the breeds defined as local breeds (Estonian Native, Estonian Heavy Draught, and Finnhorse), and the group of international breeds. Characteristically to this breeds' classification, the within-breed variation showed more diversity in the local breeds in terms of allelic richness (ranging from 6.0 to 6.4 in local, and from 4.8 to 5.5 in transboundary breeds). The highest likelihood for population structuring was found for k = 10 partition. The Tori and Latvian Horse, demonstrating a close relationship, tended to cluster with transboundary breeds, although statistically it formed a weaker structure than the local breeds' group. In recent decades upgrading of the Tori breed has been used to achieve the changed breeding goal(s). The gene flow into the population as well as limited number of horses in the types A and O have resulted in significant breed differentiation. The emergence of two genetic clusters within the Tori was clearly visualized constructing the breeds' NJ tree. The individuals in the Tori O- and A-type formed a homogenous cluster whereas the upgraded-type (B-type) and Latvian Horse demonstrated an admixed pattern of the population. Our results confirmed that the local breeds are valuable genetic resources. Due to the changing nature of Estonian horse population it is advisable to monitor the local breeds regularly to preserve the unique gene pool.

References

FAO. 2011. Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome.

LITHUANIAN AND NORWAY *APODEMUS* GENUS GENOME SEQUENCE COMPARISON

Karolis Sivickis, Algimantas Paulauskas*

Faculty of Natural Sciences, Vytautas Magnus University,
Vileikos 8, LT-44404 Kaunas, Lithuania

** Corresponding author. E-mail: a.paulauskas@gmf.vdu.lt*

Introduction. Mice of genus *Apodemus* are widely spred in Europe, western Asia and North Africa. Twenty one species is now recognized, but morphological similarity makes difficulties to recognize these species. Molecular markers to clear species identification are required.

Aim and materials. The aim of our investigation was to perform for suitable primers for *Apodemus* genus getic variable studies. For DNA research there were captured 45 mices from Kaunas district, 13 from Žemaitija National Park, 74 rodents from Neringa. Also for research were used other mice from previous expeditions from Norway of Vytautas Magnus University, Falculty of Natural Sciences. In Norway 15 *Apodemus* genus mice were captured.

Methods. Using chloroform method "Genomic DNA Purification Kit" (Fermentas, K0512). DNA was extracted from ears and muscles for genome studies. For research control mtDNA region sequence was chosen. Chosen primers: **AP-1** (5'-(CAG)5GC-3'), **AP-2** (5'-(CAG)4AC-3'), **AP-3** (5'-(CAA)5GC-3'), **AP-4** (5'-(CAA)4AC-3'), **AP-5** (5'-(GA)6GC-3'), AP-6 (5'-(GA)8AC-3'), **c-myc** (5'-GCTCCAAGACGTTGTGTGTTCG-3'), **E8S** (5'-TAAAT-GGGACAGGTAGGACC-3') and **Ep8-1** (5' CCTTACTGCCTCTTGCTTC-3') (2, 3). For PCR data analysis horizontal 1.5% agarose gels were used.

Results. DNA was extracted from all mice, accomplished all PCR reactions with chosen primers and multiply fragments for sequencing. Positive results were obtained by using AP-1, AP-5, AP-6, c-myc-1 and E8S primers. Using the primers *A. flavicollis*, *A. sylvaticus* was identified for morphological difficulties specimen of *Apodemus* mice.

References

1. Michaux JR, et al. Mitochondrial phylography of the woodmouse (*Apodenys Sylvaticus*) in the western Paleartic region. Mol Ecol 2003; 12: 685–97.

2. Bugarski-Stanojevic V, et al. Molecylar phylogeny and distribution of three *Apodemus* species (Muridae, Rodentia) in Serbia, Zool Syst Evol Res 2008; 46(3): 278–86.

3. Bugarski-Stanojevic V, et al. Comparative study of the phylogenetics structure in six Apodemus (Mammalia, Rodentia) inferred from ISSR-PCR data. Systemat Biodiver 2011; 90(1): 95–106.

POLYMORPHIC MICROSATELLITE MARKERS FOR THE MUSKRAT *(ONDATRA ZIBETHICUS)*

Gintarė Skyrienė*, Algimantas Paulauskas

Faculty of Natural Sciences, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

** Corresponding author. E-mail: ginsky2@gmail.com*

Introduction. The muskrat (*Ondatra zibethicus*) is a widespread semi-aquatic rodent in North America. In 1954 these mammals were released into several rivers in Lithuania.

Methods. The microsatellite markers were used only for the Canadian muskrat populations (Laurence et al., 2009). Consequently, 12 microsatellite primers (Oz06, Oz08, Oz16, Oz17, Oz22, Oz27, Oz30, Oz32, Oz34, Oz41, Oz43, Oz44) were used in this study for the aim to comparing the genetic differentiation between native (Canada) and introduced (Lithuania) populations of muskrats. But only 7 of these 12 microsatellite loci were used in subsequent analyses in case 5 of them (Oz06, Oz16, Oz27, Oz32, Oz34) were not informative or showed inconsistent peak morphology.

Results. Population analysis showed high levels of genetic diversity (in Lithuania: H_e = 0.47–0.91, H_o = 0.13–1.00; in Canada: H_e = 0.68–0.96, H_o = 0.48–0.89). The numbers of polymorphic loci were 100% and size ranged from 196 to 276 base pairs in Lithuania and 174 to 278 in Canada. The numbers of alleles per locus ranging from 2 to 14 in the introduced populations of muskrat; in native populations – from 8 to 22, respectively. Each locus was tested for departure from Hardy-Weinberg equilibrium (HWE) and 2 loci exhibited significant in native populations and all loci in introduced populations (Table).

Analysis of Molecular Variance (AMOVA) showed that the genetic differences within populations (80% of the total genetic diversity) and among populations (20% of the total genetic diversity) were significant in Lithuania. The results of principal coordinate analysis (PCA) by genetic similarity showed that the population of muskrat in the western parts of Lithuania was furthermost from the population of the middle part of Lithuania. The middle and eastern parts of muskrat populations are the most related by genetically; PCA by populations showed that all populations from different parts of Lithuania moved a similar distance.

Table. Genetic diversity of muskrat populations in Lithuania and Canada

Locus	Lithuania				Canada			
	Size range (bp)	N_A	H_e	H_0	Size range (bp)	N_A	H_e	H_0
Oz08	228–237	5	0.75	0.46*	231–251	8	0.82	0.79
Oz17	204–212	2	0.47	0.13*	174–228	22	0.96	0.89*
Oz22	210–244	3	0.61	0.39*	207–241	15	0.90	0.48*
Oz30	227–246	8	0.76	0.60*	212–254	19	0.95	0.74
Oz41	196–240	9	0.85	1.00*	180–248	21	0.95	0.83
Oz43	244–276	14	0.91	0.93*	230–278	15	0.91	0.83
Oz44	223–236	6	0.72	0.71*	215–257	11	0.68	0.50

*– significant deviation from Hardy-Weinberg equilibrium

These markers will be useful for further studies and will provide more details about the genetic structure of population in muskrat.

Acknowledgement. This research was funded by Research Council of Lithuania, grant No. LEK-14/2012.

References

1. Laurence S, Bewick AJ, Coltman DW, Davis CS, Elsasser SC, Kidd AG, Lesbarrères D, Schulte-Hostedde AI. Isolation and characterization of polymorphic microsatellite loci in muskrat, *Ondatra zibethicus*. Mol Ecol Res 2009; 9: 654–7.

MODIFYING EFFECT OF VITAMIN E ON ADRIAMYCIN AND CYCLOPHOSPHAMIDE INDUCED GENOTOXICITY AND ANTIOXIDANT STATUS IN RATS

G. Slapšytė[1]*, J. Mierauskienė[1], V. Morkūnas[1], J. Didžiapetrienė[2]

[1] *Department of Botany and Genetics, Vilnius University, Lithuania*

[2] *Institute of Oncology, Vilnius University, Lithuania*

* *Corresponding author. E-mail: grazina.slapsyte@gf.vu.lt*

Introduction. Cancer chemotherapy is often associated with oxidative stress and fall in plasma concentrations of various antioxidants in treated patients. This may trigger various physiological side-effects and certain toxicities including DNA damage in normal tissues. One of the most promising strategies to reduce oxidative stress and thus prevent or attenuate the subsequent side-effects is the combination of the drug delivery together with an antioxidant. Vitamin E is considered to be one of the most important antioxidants to prevent oxidative injury of DNA. The present study was undertaken to investigate the possible beneficial effect of vitamin E against adriamycin- and cyclophosphamide- induced genotoxicity and changes of antioxidant status in rats.

Methods. Wistar rats were treated with a single dose of adriamycin (AD, 5 mg/kg b. w.) or cyclophosphamide (CP, 30 mg/kg b. w.) by the intraperitoneal route. Vitamin E (VE) was administered via gavage at a dose of 250 mg/kg once a day for 3 consecutive days before or after drug treatment. The cytogenetic endpoints screened were chromosome aberrations and micronuclei in rat bone marrow cells. Parameters of the oxidant / antioxidant status in rats included the activities of catalase (CAT) and superoxide dismutase (SOD), and the level of lipid peroxidation product malondialdehyde (MDA).

Results. Significant decrease of chromosome aberration frequency was determined in animals pre-treated with VE before AD injection (9.57 *vs*. 5.5, $P < 0.02$). Micronuclei assay revealed protective effect of VE against both CP and AD induced chromosome damage, which was significant against CP-induced damage in animals post-treated with VE (0.66 *vs.* 0.31, $P = 0.0028$) and against AD-induced damage in animals pre-treated with VE (1.51 *vs.* 0.72, $P = 0.0129$). Besides, VE revealed protective effect against drug induced bone marrow toxicity: the proportion of immature polychromatic erythrocytes to total erythrocytes significantly increased in all VE-treated groups when compared to the adequate drug-treated animals ($P < 0.0001$).

Significantly higher MDA levels were determined in all VE co-treated animal groups when compared with the controls and adequate drug-treated groups (with the only exception of AD group post-treated with VE). Pre-treatment of CP or AD injected animals with VE significantly decreased SOD activity. On the contrary, a slight though insignificant increase of SOD activity was determined after VE post-treatment of AD or CP injected rats. Similar though insignificant results were obtained with CAT activity but only in the AD injected animals. No obvious effect of VE treatment was determined in CP injected animals.

Conclusions. In conclusion, our results revealed antimutagenic potential of vitamin E against adriamycin- and cyclophosphamide- induced chromosome damage and modifying effect on SOD and CAT activities in rats, which were dependent on the treatment schedule and the drug used.

OBESE GENE POLYMORPHISM AND IT'S INFLUENCE ON THE VIABILITY OF BORN PIGLETS

I. Šatrovskaja*, I. Miceikienė, L. Baltrėnaitė, H. Žilinskas, V. Riškevičienė

Lithuanian University of Health Sciences,
Veterinary Academy, Tilžės str. 18, Kaunas, Lithuania
** Corresponding author. E-mail: irina11@inbox.lt*

Aim. The aim of present study was to identify polymorphism in the *Obese* gene of 115 crossbred sows and determine influence on born piglets viability. In pigs, the *Obese* gene (or *leptin* gene) is considered to be an eligible gene to follow traits of economic importance, such as pig fat thickness, growth, and reproduction (Lagonigro et al., 2003). But the contribution of individual genes for such reproductive trait number as dead piglets is still unknown.

Methods. Blood samples were collected from jugular vein. DNA was extracted by chloroform / phenol method. Genotyping *Obese* of gene was perfomed by polymerase chain reaction – restriction fragment length polymorphism (PCR/RFLP) method (Stratil et al., 1997). PCR amplicon was digested with 2 U *Hin*fI restriction enzyme, genotyping on a 3.5% agarose gel and stained with ethidium bromide. Two alleles of Obese gene were identified: T contained one 152 bp fragment, C had two fragments (84 and 68 bp).

Results. The genotype frequencies observed were 65.5% TT genotype, 29.5% TC genotype and 5% of sows had CC genotype. The C allele was with frequency 0.2, T allele with frequency 0.8 in the studied population of sows. The same allele frequencies have been found by other researchers (Szydlowski et al., 2004; Silveira et al., 2008). TT genotype sows had on average 9,255 alive piglets, respectively TC genotype sows had 9,776, CC genotype 10,433. CC genotype pigs had 0,265 dead piglets less than TC genotype sows and 0,241 piglet less than TT genotype sows. The results primarily show the influence of Obese gene on reproduction properties of sows – the number of piglets born dead and alive – which is very important in pig reproduction. For giving recommendations to farmers for use of *Obese* gene test as important marker for reproduction traits the number of tested pigs as well as breed influence should be increased.

References

1. Lagonigro R, Wiener P, Pilla F, Woolliams JA, et al. A new mutation in the coding region of the bovine leptin gene associated with feed intake. Anim Genet 2003; 34: 371–4.

2. Silveira A, Antunes R, Almeida J, Braga T, et al. *Obese* gene polymorphism in Pietrain and Large White pigs after a divergent selection. Genet Mol Res 2008; 7(4): 1217–22.

3. Stratil A, Peelman L, Van Poucke M, Cepica S. A *Hin*fI PCR-RFLP at the porcine leptin (LEP) gene. Anim Genet 1997; 28: 371–2.

4. Szydlowski M, Stachowiak M, Mackowski M, Kamyczek M, et al. No major effect of the leptin gene polymorphism on porcine production traits. J Anim Breed Genet 2004; 121: 149–55.

INVESTIGATION OF THE GENETIC ORIGIN OF THE FIRE-BELLIED TOAD BOMBINA BOMBINA POPULATION IN LATVIA BY ANALYZING THREE MITOCHONDRIAL DNA LOCI

Dmitijs Uzameckis, Natalja Shkute*

Ecology institute, Daugavpils University,
Vienības Str. 13, Daugavpils, Latvia

** Corresponding author. E-mail: natalja.skute@du.lv*

Introduction. The fire-bellied toad *Bombina bombina* is highly endangered European species with the northern border of its distribution in Latvia. Recently, two new subpopulations of this anuran were discovered in Latvia for the first time, widening the international distribution area of this amphibian far to the north, but their genetic origin, intrapopulational genomic variability and genetic differences from other *Bombina bombina* world populations are still unknown.

Methods. To investigate *Bombina bombina* genetic origin, mitochondrial DNA polymorphism was studied. The fragments of three *Bombina bombina* mtDNA loci – cytochrome *b (cyt b)*, cytochrome *c* oxidase I *(cox1)* and nicotinamide adenine dinucleotide dehydrogenase subunit 4 *(nad4)* were successfully amplified from two toad individuals' DNA samples (representing Latvian subpopulations from Medumi and Demene municipalities) and used for subsequent sequencing and phylogenetic analyses.

Results. The genetic origin of these local *B. bombina* clusters was clarified by comparing the obtained multilocus single nucleotide polymorphism (SNP) data with previously published mtDNA haplotypes. Nevertheless, to develop a more comprehensive phylogeographic reconstruction, as also to maximally characterise the genetic diversities and possible gene flow between and within these Latvian fire-bellied toad subpopulations, an application of several unlinked DNA loci such as microsatellites and nuclear markers is necessary.

THE LANDSCAPE OF CHROMOSOME PATHOLOGY IN LITHUANIAN POPULATION

Irena Andriuškevičiūtė*, Algimantas Sinkus, Lina Jurkėnienė, Loreta Šalomskienė, Loreta Kalasūnienė

Lithuanian University of Health Sciences, A. Mickevičiaus 9, Kaunas, Lithuania

** Corresponding author. E-mail: ireandr@vision.kmu.lt*

Here are given the results of 10,147 karyotype investigations performed in our laboratory of cytogenetics. The standard clinical contingent which is usually sent for karyotype analysis and genetic counselling was investigated: children with multiple congenital malformations, patients with severe mental retardation, also males and females with disturbances of generative functions. We have also paid special attention to specific contingents in which one could predict low frequencies of chromosome aberrations: unselected newborns (4,032 individuals), the patients suffering from mild mental retardation (2,209 individuals), inmates of schools for blind children (326 individuals), couples who gave birth to babies with malformations or suffered from abortions (612 individuals). Considering the size of Lithuanian population, demographic situation, the lifespan of chromosome patients, one can assume that about 15,000 chromosome patients are living in our country. The forecast of chromosomal anomalies in Lithuanian population for the future seems doubtful and complicated because of diverse influences, – either demographic or mutagenic. The situation is clearer only for balanced chromosomal translocations (reciprocal and Robertsonian). At present time in Lithuanian population we are approaching the familial model "only one but a healthy baby", and therefore the frequency of balanced chromosome aberrations will accumulate.

DNA METHYLATION STABILITY OF CULTURED ADIPOSE TISSUE AND SYNOVIAL FLUID-DERIVED STEM CELLS

Kristina Daniunaite[1]*, Ausra Girdziusaite[1], Karolis Matjosaitis[1], Saule Valiuniene[2], Eiva Bernotiene[2], Ausra Unguryte[2], Sonata Jarmalaite[1]*

[1] Faculty of Natural Sciences, Vilnius University, Vilnius, Lithuania

[2] State Research Institute Center for Innovative Medicine, Vilnius, Lithuania

** Corresponding author. E-mail: vailaomalyn@yahoo.com*

Background. Mesenchymal stem cells (MSCs) from different sources, including those from adipose tissue and synovial fluid, are currently being tested for their potential application in treatment of numerous human diseases. However, the effect of cell culture conditions must be carefully assessed before therapeutic usage of cultured MSCs.

Methods. MSCs derived from adipose tissue (ADSCs) were cultured with fetal calf serum (FCS) and allogeneic human serum (AHS) until early or late passages and processed for epigenetic analysis of several regulatory genes by methylation-specific PCR (MSP). DNA methylation in promoters of several regulatory genes was also studied in cultured synovial fluid mesenchymal stem cells (SFSCs) from patients with juvenile idiopathic arthritis (JIA). In addition, DNA methylation profile of ADSCs and SFSCs differentiated into osteogenic and adipogenic lineages were also investigated.

Results. Normal methylation status was retained in 7 (*p16*, *p14*, *RARB*, *RASSF1*, *GSTP1*, *DAPK1*, and *ZAC1*) out of 10 tumor suppressor genes during ADSCs growth in cell culture and at differentiation. Three genes, including the telomerase gene (*TERT*), exhibited irregular DNA methylation status of their promoters, with slightly better epigenetic stability observed in ADSCs propagated with AHS than with FCS. *TERT* was partially methylated in all osteogenic and adipogenic lineages of SFCs and ADSCs, but methylation status was irregular in undifferentiated MSCs irrespective of the serum used. Epigenetic instability was more frequently observed in SFSCs from patients with JIA. DNA methylation occurred in significant regulatory genes, such as *p16, DAPK*, *GSTP1*, and *ESR1*.

Conclusions. Mesenchymal stem cells from healthy tissue possess higher epigenetic stability than mesenchymal stem cells from patients with rheumatic disorders. Usage of allogeneic human serum for cultivation of somatic stem cells assists in improved maintenance of epigenetic stability.

A FAMILY WITH WHOLE ARM TRANSLOCATION T(14;18)(Q10;P10) REVEALED DUE TO PRENATAL DIAGNOSIS

Aigars Dzalbs*, Agnija Stamere, Gita Taurina, Gunta Kalnberza, Irena Teilane, Ieva Grinfelde, Janis Bars, Rita Lugovska

Medical Genetics Clinic,
University Children's Hospital,
Juglas str. 20, LV-1079 Riga, Latvia

** Corresponding author. E-mail: aigars.dzalbs@inbox.lv*

Introduction. Reciprocal translocations are quite common in population. It is well known that chromosomal reciprocal translocations are associated with an increased risk of miscarriges, stillbirths, and abnormal offsprings of the translocation carriers. The translocation carrier may have a risk to have a child who would have a partial trisomy or partial monosomy. Whole arm translocations (WAT) are rare constitutional chromosome abnormalities. This type of chromosome aberration results from centromeric fission or juxtacentromeric breaks and reciprocal exchange of entire arms of two chromosomes.

Case report. A 40-year-old pregnant woman referred to the prenatal diagnostics because of an increased age risk for chromosomal abnormalities. This was her fourth pregnancy. From the first pregnancy she had a daughter, but the next two pregnancies ended with early spontaneous abortions.

In 16 weeks of pregnancy amniocentesis was performed, and an abnormal fetal karyotype was revealed: monosomy 18p (18p-) resulting from unbalanced whole arm translocations between chromosomes 14 and 18: 46,XX,+14,der(14;18)(p10;q10). The standard GTG-banding analysis was confirmed by fluorescent *in situ* hybridization method (FISH) with subtelomeric probes. The detected chromosomal rearrangement may lead to multiple congenital abnormalities and mental retardation.

Parental karyotypes were analysed. The father's karyotype was normal, but the mother's karyotype showed balanced translocation 46,XX,t(14;18)(q10;p10). Her 9-year-old daughter had clubfoot and speech delay. Her karyotype analysis revealed the unbalanced translocation 46,XX,+18,der(14;18)(p10;q10) that results in partial trisomy of short arm of chromosome 18 (18p+). Based on the literature, the phenotype correlates with genotype.

Chordocentesis showed mosaic fetal karyotype contained two cell lines – balanced reciprocal translocation like the mother's and abnormal with monosomy 18p 46,XX,t(14;18)(p10;q10)[23]/46,XX,+14,der(14;18)(p10;q10)[2]. The pregnancy was terminated, and non-mosaic abnormal karyotype 46,XX,+14,der(14;18)(p10;q10) with monosomy 18p was demonstrated in cardiocentesis. No abnormalities of fetus were observed in visual inspection.

Conclusions. We present difficult prenatal genetic counseling situation with discrepancy of investigation results of amniocentesis (amniotic fluid) and chordocentesis (fetal blood). The placental mosaicism, fetal sample contamination with maternal blood and chimerism are under the discussion to explain these findings.

Also the familiy with balanced karyotype of mother was discovered, which is important because of the possibility of transmission the unbalanced translocation to offsprings.

ANALYSIS OF ASSOCIATION OF SNPS IN *RNASEL*, *LEPR*, AND *CRY1* GENES WITH BIOCHEMICAL RECURRENCE IN PROSTATE CANCER

Ž. Kapustina, G. Trakanavičiūtė, J. Lazutka*

Department of Botany and Genetics, Vilnius University, M. K. Čiurlionio 21/27, LT-03101 Vilnius, Lithuania

** Corresponding author. E-mail: Juozas.Lazutka@gf.vu.lt*

Aim. The aim of this work was to analyze single nucleotide polymorphisms (SNPs) of *RNASEL* (*rs627839*), *CRY1* (*rs10778534*), and *LEPR* (*rs1137100*) genes in peripheral blood samples from prostate cancer patients.

Materials and methods. Frequency of polymorphisms was then analyzed in relation with the duration of biochemical recurrence-free period. Genotyping was done using TaqMan SNP assay kits (Applied Biosystems) and protocol with STRATAGENE Mx3005P QPCR system. *RNASEL*, *CRY1* and *LEPR* genes were genotyped using DNA from blood samples of 225 prostate cancer patients. Each sample was analyzed twice.

Results. For each gene, allele frequencies were determined. Statistical analysis was performed by means of Caplan-Meier analysis. There was no correlation between specific genotypes and the duration of biochemical recurrence-free period. Our results do not confirm previously reported findings (Lin et al., 2011) about good prognostic value of SNPs in prostate cancer. Instead we suggest combining SNP analysis with the analysis of gene expression and miRNA biomarkers.

OPTICO-ANATOMICAL AND NEUROPHYSIOLOGICAL ASPECTS OF PREMATURITY CHILDREN EYES

Loresa Kriaučiūnienė[1]*, Rimtautė Aukštikalnienė[2], Alvydas Paunksnis[2], Valerijus Barzdžiukas[1]

[1] *Lithuanian University of Health Sciences, Institute of Neurosciences, Kaunas, Lithuania*

[2] *Eye Clinic, Kaunas*

* *Corresponding author: E-mail: loresa@takas.lt*

Purpose. The aim of our work has been to determine changes in optico-anatomical elements and neurophysiological aspects of accommodation of pre-maturity children myopic eyes using precise ultrasonic biometry.

Materials and methods. Research has been done on healthy full-term children eyes with emmetropic refraction (first group n = 20); full-term 1st degree myopic children eyes with refraction from –1.0D to –3.0D (second group, n = 16), and pre-maturity children myopic eyes with refraction –1.0D to –3.0D (third group, n = 12). The age of children ranged from 6 to 15 years old. Gestagetion ages in the pre-term group ranged from 25 to 34 weeks. All were checked in the clinic due to the risk of the developing genetical diseases. Precise ultrasonic biometry was done by the ultrasonic measuring system.

Results. Ultrasonic biometry evaluates optical-anatomical parameters changes in pre-maturity children myopic eyes. Axial length was longer than in healthy and full-term myopic children eyes, average 24.09 ± 0.69 mm), and lens thickness was bigger (average 3.35 ± 0.14 mm). In healthy children group respectively axial length was 23.49 ± 0.48 mm, and lens thickness was 3.00 ± 0.07 mm and in full-term myopic children group the axial length was 23.79 ± 0.59 mm, lens thickness was 3.24 ± 0.14 mm. No changes in the size of the eye optico-anatomical elements were found in the neurophysiological process of accommodation for pre-maturity children slight myopic eyes.

Conclusions. In the group of pre-maturity children myopic eyes longer axial length and bigger size of lens thickness were observed (it was 24.09 ± 0.69 mm and 3.35 ± 0.14 mm respectively). In the group of pre-maturity children myopic eyes changes in optico-anatomical elements parameters were more active than in group of full-term children myopic eyes and it can lead to higher myopia and low vision development.

The ultrasonic biometry is an effective method in evaluating the activity of eye accommodation apparatus neurophysiology and possibilities of the eye accommodation. Precise ultrasonic biometry can reveal early disturbances in pre-maturity children eyes accommodation and evaluate the level of myopia.

Eyes of prematurity children need a more detailed examination of genetically determinated eye disturbances.

References

1. Charman WN, Radhakrishnan H. Accommodation, pupil diameter and myopia. Ophthalmic Physiol Opt 2009; 29(1): 72–9.

2. Xu D, Chen X, Yan J, Jiang J, Qu J, Lü F. Accommodation response fluctuation in myopia during sustained near work. Zhonghua Yan Ke Za Zhi 2009; 45(1): 50–5.

3. Hsieh CJ, Liu JW, Huang JS, Lin KC. Kaohsiung refractive outcome of premature infants with or without retinopathy of prematurity at 2 years of age: a prospective controlled cohort study. J Med Sci 2012; 28(4): 204–11.

4. Chen TC, Tsai TH, Shih YF, Yeh PT, Yang CH, Hu FC, Lin LL, Yang CM. Long-term evaluation of refractive status and optical components in eyes of children born prematurely. Invest Ophthalmol Vis Sci 2010; 51(12): 6140–8.

GENE POLYMORPHISMS OF MICRORNAS IN *HELICOBACTER PYLORI* INDUCED HIGH RISK ATROPHIC GASTRITIS AND GASTRIC CANCER

Juozas Kupcinskas[1]*, Thomas Wex[2], Ruta Steponaitiene[1], Simonas Juzenas[1], Marcis Leja[3], Gediminas Kiudelis[1], Laimas Jonaitis[1], Jurgita Skieceviciene[1], Peter Malfertheiner[2]

[1] Institute for Digestive Research, Lithuanian University of Health Sciences, Kaunas, Lithuania

[2] Clinic of Gastroenterology, Hepatology and Infectious Diseases, Otto von Guericke University, Magdeburg, Germany

[3] Faculty of Medicine, University of Latvia, Digestive Diseases Center, Hospital Lizeners, Riga, Latvia

** Corresponding author: E-mail: j_kupcinskas@yahoo.com*

Background and aims. Recent discovery of microRNAs (miRNAs) has shed new insights in biomarker field with diagnostic and prognostic implications. Various studies have shown that miRNA are deregulated in gastric cancer (GC) and atrophic gastritis patients. Several single nucleotide polymorphisms (SNPs) of genes related to miRNAs have been linked with different types of cancer and premalignant lesions. The data on the potential association between miRNA SNPs and the risk of GC or *Helicobacter pylori* induced atrophic gastritis, however, are scarce and partially conflicting. The aim of our study was to evaluate potential associations between the presence of gastric cancer and high risk atrophic gastritis (HRAG) and SNPs of genes related to mir-146a, mir-149, mir-196a-2, mir-379, mir-499a and mir-608.

Methods. Gene polymorphisms were analyzed in 538 subjects (GC: n = 106; HRAG: n = 222, controls: n = 210) of Caucasian origin. Mir-146a C>G (rs2910164), mir-149 T>C (rs2292832), mir-196a-2 C>T (rs11614913), mir-379 A>G (rs61991156), mir-499a A>G (rs3746444) and mir-608 C>G (rs4919510) SNPs were genotyped by RT-PCR, using predesigned Taqman primers.

Results. Frequencies of genotypes in our study are similar to the data reported on subjects of Caucasian ethnicity. Analysis of data revealed that the frequencies of SNP genotypes are in line with Hardy-Weinberg equilibrium. There was a tendency for mir-196a-2 C/C genotype to be associated with lower incidence of HRAG (49.0% in controls*vs.*41.4% in HRAG group, $p = 0.079$). Allele C of mir-196a-2 SNP was also more frequent in controls when compared to HRAG group, 67.8% and 60.1% respectively, however it failed to reach significance level ($p = 0.087$). We did not find any significant associations for all examined miRNA polymorphisms in relation to GC or HRAG.

Conclusions. Mir-146a, mir-149, mir-196a-2, mir-379, mir-499a and mir-608 SNPs are not linked with gastric carcinogenesis in Caucasians, and therefore they do not appear as potential biomarkers for identifying individuals with higher risk for GC.

BODY MASS INDEX IN TWINS

A. Raskilienė*, I. Andriuškevičiūtė, L. Šalomskienė

Lithuanian University of Health Sciences,
A. Mickevičiaus 9, Kaunas, Lithuania

** Corresponding author. E-mail: raskiliene@vision.kmu.lt*

The objective of this study was to analyze the association of physical activity and genetic factors with body mass index of twins. For this study, an original questionnaire was developed. The questionnaire was send by email to twins which were registered in the Twin Center of Lithuanian University of Health Sciences. Respondents were 10–60 years old. The data of 248 (124 pairs) twins were analyzed. The body mass index was calculated by using twins' weight and height. Obesity was defined as body mass index ≥25 kg/m^2. Physical activity was assessed by taking into account the frequency of physical activity and the duration of going on foot / by bike. Most of twins had normal weight (57.5% of MZ and 50.9% of DZ twins). There were not overweight twins in younger age group, but in older – 50% of twins were overweight. Differences of BMI and physical activity were bigger in DZ twins pairs, compared with MZ. In 10–18 years old age group, the different BMI were in 5.9% pairs of MZ twin and 26.3% of DZ. Physical activity decreased with age and it was relative to BMI. In twins pairs which had a similar behavior of physical activity differences of BMI were 1.49 kg/m^2, in pairs where physical activity behavior was different – 1.64 kg/m^2. Behavior and genetic influence body mass index, but influence of genetic could be modified by physical activity.

MOLECULAR CYTOGENETIC INVESTIGATION AND GENETIC COUNSELING OF TWO PATIENS WITH SEX CHROMOSOME – AUTOSOME TRANSLOCATION

Agnija Stamere*, Aigars Dzalbs, Gita Taurina, Gunta Kalnberza, Ieva Grinfelde, Rita Lugovska

Medical Genetics Clinic, University Children's Hospital, Juglas Street 20, LV-1079 Riga, Latvia

** Corresponding author. E-mail: agnija.stamere@gmail.com*

Introduction. The constitutional sex chromosome – autosome translocations are rare in humans. The sex chromosomes (X and Y) in human are unique among other chromosomes. The X chromosome is capable of going to "transcriptional silencing" or lyonization, but the Y chromosome composed of chromatin is, in large part, permanently inert. These sex chromosome properties need to be taken in account in genetic counseling of individuals with chromosome aberrations involving X and / or Y chromosome.

Case 1. 19-year-old women with congenital cleft palate and lip and mental retardation referred to genetic counseling. Conventional cytogenetic analysis on peripheral lymphocytes revealed additional genetic material attached to chromosome 21 short arm. The fluorescent in situ hybridization (FISH) studies demonstrated the presence of the subtelomeric probe DXYS129 on the derivative chromosome 21 short arm, indicated unbalanced translocation with primary pseudoautosomal region 1 (PAR1). The karyotype was interpreted as 46,XX,der(21)t(X;21)(p22.1;p11.2). The patient had trisomy of Xp22.1→pter segment.

Case 2. A couple with infertility (15 years) referred to genetic counseling. The woman had unsuccessful *in vitro* fertilisation (IVF) and intra cytoplasmatic sperm injection (ICSI) procedures. Her karyotype was normal. The husband had teratoastenozoospermia. His karyotype analyses revealed the unbalanced translocation between Y chromosome and chromosome 22: 45,X,der(Y;22)(q11.23;q11.2). The FISH investigation showed that the testis – determining region of Y chromosome, containing the *SRY* gene, and DYZ3 locus (Y hromosome centromere) was translocated onto the chromosome 22. The microdeletions of azoospermia loci AZFa, AZFb, AZFc and AZFc/AZFd in Yq11 were not detected by Multiplex-PCR. The patient had deletion of chromosome Y long arm (Yq11.23→qter).

Conclusions. We demonstrate two rare constitutional chromosome aberrations involving sex chromosome – autosome translocation, which are leading to mental retardation, congenital anomalies or reproductive failure.

The first case is the young women with mental retardation and congenital anomalies. She had partial trisomy of chromosme X, that would be the reason for her clinical condition. The mechanism of chromosome imbalances could be functional disomy of small part of X chromosome (Xp22.1→p22.3) or functional trisomy of PAR1, also nonskewed X chromosome inactivation can not be excluded.

The second case is the individual, phenotypically male, who had 45 chromosomes, including the Y;22 translocation. By the data of literature this type of chromosome rearrangement may be of no phenotypic or reproductive effect. Our patient azoospermia region had preserved, but morphology and motility of sperm cells were disturbed. It could be explained by compromise of formation of the sex vesicle in spermatogenesis. Theoretically he would have offsprings, but segregation of chromosomes in meiosis might have led to 45,X Turner syndrome and 46,XX,t(Y;22) Klinefelter syndrome. However phenotypically normal girl with karyotype 46,XX is possible outcome.

HIGH-RESOLUTION MELTING-BASED QUANTITATIVE ANALYSIS OF *RASSF1* METHYLATION IN BREAST CANCER

Kristina Stuopelyte[1], Kristina Daniunaite[1], Aida Laurinaviciene[2], Valerijus Ostapenko[3], Sonata Jarmalaite[1]*

[1] *Faculty of Natural Sciences, Vilnius University, Lithuania*

[2] *National Center of Pathology, Affiliate of Vilnius University Hospital Santariškių Clinics, Lithuania*

[3] *Institute of Oncology, Vilnius University, Vilnius, Lithuania*

* *Corresponding author. E-mail: sonata.jarmalaite@gf.vu.lt*

Background and objective. Breast cancer (BCa) is the most prevalent and the leading cause of death from cancer among women worldwide. Aberrant promoter methylation of tumor suppressor genes is a typical epigenetic alteration for BCa and can be detected in early carcinogenesis. High-throughput and cost-effective methods are needed for early and sensitive detection of epigenetic changes in clinical material. The main purpose of our study was to optimize high-resolution melting (HRM) assay for reliable and quantitative assessment of the *RASSF1* gene methylation – one of the earliest epigenetic alterations observed in BCa.

Material and methods. 76 breast carcinomas and 10 non-cancerous breast tissues were studied by means of HRM, and compared to the results obtained by means of quantitative methylation-specific polymerase chain reaction (QMSP) and methylation-specific polymerase chain reaction (MSP).

Results. Both quantitative methods, HRM and QMSP, showed similar specificity and sensitivity for *RASSF1* methylation detection in BCa (about 80% and 70%, respectively). In BCa, the mean methylation intensity of *RASSF1* was 42.5% according to HRM, and 48.6% – according to QMSP. Both methods detected low levels of methylation (less than 5%) in non-cancerous breast tissues. The highest frequency of *RASSF1* methylation in BCa was identified by non-quantitative MSP method. However, in a set of the MSP-positive cases the levels of methylation were very low and similar to that observed in non-cancerous breast tissues, as specified by HRM and QMSP evaluation.

Conclusion. HRM is as a simple, cost-effective method for reliable high-throughput quantification of DNA methylation in clinical material.

CLINICAL PRESENTATION OF TWO CHILDREN WITH PARTIAL TRISOMY OF CHROMOSOME 21

Gita Taurina*, Aigars Dzalbs, Agnija Stamere, Gunta Kalnberza, Daiga Bauze, Rita Lugovska

Medical Genetics Clinic, University Children's Hospital, Juglas Street 20, LV-1079 Riga, Latvia

** Corresponding author. E-mail: gita.taurina@gmail.com*

Introduction. Down syndrome (trisomy of chromosome 21) is the most frequent liveborn human aneuploidy, occurring in approximately one out of 800–1 000 live birth, whereas partial trisomy 21 is a rare event. Karyotype analyses provide rapid confirmation of Down syndrome clinical diagnosis, but in small proportion of patients further detailed genetic investigations is still needed. Fluorescent *in situ* hybridization method (FISH) has provided significant improvement in the diagnosis in individuals with developmental delay and multiple congenital anomalies.

Clinical case 1. A 3-year-old girl was referred to genetic counseling because of developmental delay, facial dysmorphism. She was the first child of healthy non-consanguineous parents. She had muscular hypotonia, motor developmental delay, stereotypic hand movements, and no speech. At the age of 5 months she had seizures. Physical examination revealed that her weight was around 50th centile, height – 90th centile. Also she had several *cafe au lait* spots, atypical dermatogliphics, flatfoot. Her dysmorphic facial features included broad nasal bridge, upward nose tip and high arched and cleft palate. Karyotype analysis revealed additional material on the short arm of chromosome 21. The FISH analysis with subtelomeric probes precised the origin of the additional material, which had been derived from the long arm of chromosome 21: 46,XX,add(21)(p11.2).ish (21)(qter+). The additional material did not include critical region for Down syndrome (DSCR). The parental karyotypes were normal. Dysmorphic features and clinical manifestation have been described in other similar cases of trisomy 21qter.

Clinical case 2. A boy was born in 40 weeks of gestation from the uncomplicated fourth pregnancy as the third child in family. Mother was 31 year old and suffered from syphilis, and father was 27 years old and otherwise healthy. Prenatal biochemical screening for Down syndrome was not done in pregnancy. Mother was smoking one pack per day throughout pregnancy. Birth weight was 2 970 g, height 49 cm, and head circumference – 35 cm. The boy had dolychocephaly, broad nasal bridge, macroglossia, upslanting palpebral fissures, single transverse plamar crease on right hand, *foramen ovale apertus*. Cytogenetic analyse was performed. Karyotype revealed interstitial duplication of chromosome 21 leading to partial trisomy of chromosome 21. It was confirmed by FISH: 46,XY,dup(21)(q21q22.2).ish dup(21)(q21q22.2) (DSCR1+,AML+). The duplicated region included DSCR.

Conclusions. We demonstrate two patients with partial trisomy of chromosome 21. One of them (Case 2) had typical Down syndrome phenotype, and duplication included DSCR at 21q22.1. The other patient (Case 1) had only trisomy of subtelomeric region of chromosome 21 and did not have typical Down syndrome phenotype. FISH method with locus specific and subtelomeric probes provided additional useful information for clinical diagnosis and genetic counseling.

ABERRANT PROMOTER DNA METHYLATION IN GLIOBLASTOMA: IMPACT ON PATIENT OUTCOME

Paulina Vaitkienė*, Daina Skiriutė, Giedrius Steponaitis, Arimantas Tamašauskas

Laboratory of Neurooncology and Genetics, Neuroscience Institute,
Medical Academy, Lithuanian University of Health Sciences,
Eivenių str. 4, LT-50009 Kaunas, Lithuania

** Corresponding author. E-mail: p.grigaite@gmail.com*

Background. Aberrant promoter DNA methylation of genes involved in key biological pathways frequently has been reported in brain glioblastomas. The survey on epigenetic alterations could help to decipher aggressive phenotype of tumor and consequently patient postoperative outcome. Promoter DNA methylation of multiple genes was analyzed and its combined impact on glioblastoma patient survival evaluated.

Material and methods. Methylation of 15 genes was investigated in 100 WHO grade IV GBM. Promoter methylation was detected using bisulfite modified DNA by methylation-specific PCR (MSP). Tumor specificity of DNA methylation was identified through the comparison of glioblastoma tissue to normal brain.

Results. Methylation frequencies in *AREG, CASP8, CD81, DcR1, DR4, GATA4, GATA6, hMLH1, HOXA9, HOXA11, MGMT, NDRG2, NPTX2, TES* and *TFPI2* promoter were 58.6%, 55.0%, 48.0%, 27.6%, 43.4%, 22.7%, 68%, 2%, 58%, 52%, 54.5%, 47%, 53.1%, 66.7% and 27.1%, respectively. Kaplan-Meier log-rank test identified significant association between unmethylated *AREG* (hazard ratio = 1.8), unmethylated *CASP8* (hazard ratio = 1.6), unmethylated GATA6 (hazard ratio = 1.7), unmethylated TFPI-2 (hazard ratio = 1.7), methylated *MGMT* (hazard ratio = 0.6) and better patient survival. The higher number of concomitantly methylated genes (0–2 genes *versus* 3–6 genes) per tumor was significantly associated with poor patient outcome (hazard ratio = 2.1). In the same tumor tissue concomitantly hypermethylated *AREG, CASP8, GATA6* and *TFPI-2* were significantly associated with poor patient outcome (hazard ratio = 2.0).

Conclusion. The methylation status of *AREG, CASP8, GATA6, TFPI-2* and *MGMT* genes and the higher number of concomitantly methylated genes was significantly associated with patient outcome.

GENETIC DIVERSITY OF *IXODES RICINUS* AND *IXODES PERSULCATUS* TICKS IN BALTIC COUNTRIES

Asta Banaitytė, Algimantas Paulauskas*, Jana Radzijevskaja

Faculty of Natural Sciences, Vytautas Magnus University,
Vileikos 8, LT-44404 Kaunas, Lithuania

** Corresponding author. E-mail: a.paulauskas@gmf.vdu.lt*

Introduction. *Ixodes ricinus* and *Ixodes persulcatus* ticks are members of *Ixodes ricinus* complex composed of 14 species distributed worldwide (Xu et al., 2003). *I. ricinus* and *I. persulcatus* are involved in the transmission of a number of diseases to animals and humans, in particular Lyme borreliosis, tick-borne encephalitis, anaplasmosis and babesiosis, in Eurasia. The application of molecular markers to the study of ticks has recently yielded new insights into their population structures and taxonomic relationships (Navajas, Fenton, 2000). Ticks have been studied at individual, population and species level. In previous our study genetic diversity of *I. ricinus* was investigated at two distinct spatial scales: geographically separate regional populations and local populations within Norway and Lithuania using random amplified polymorphic DNA (RAPD) method (Paulauskas et al. 2006). The aim of this study was to evaluate genetic diversity of *I. ricinus* and *I. persulcatus* ticks in Baltic countries using RAPD and microsatellites markers.

Methods and results. We screened 12 oligonucleotides primers in RAPD-PCR, and reproducible amplification patterns were obtained with two primers OPA-04 and OPA-09. In RAPD analysis were involved 150 *I. ricinus* and 48 *I. persulcatus* ticks from 12 localities of Baltic countries. The number of fragments and the amount of polymorphism varied between the primers, between the species and between the countries. The results of analysis showed that *I. ricinus* ticks have a higher genetic diversity than *I. perculcatus* ticks. The percentage of polymorphic loci in the Lithuanian population of *I. ricinus* was 94.44%, in Latvian – 88.89%, and in Estonian – 83.33%. The higher percentage of polymorphic loci was observed in *I. persulcatus* from Latvia (66.67%), compared with *I. persulcatus* from Estonia (44.44%). Analysis of molecular variance (AMOVA) showed that genetic diversity of *I. ricinus* within populations yielded 81%, and among populations 19% of the total genetic diversity.

We used six microsatellite loci, of which five polymorphic (IRN4, IRN7, IRN8, IRN28, IRN30) were further characterized among 55 *I. ricinus* and 10 *I. persulcatus* ticks, collected from 13 locations: in Lithuania (5 locations), Latvia (6) and Estonia (2). The numbers of alleles per locus, allele frequencies, observed and expected heterozygosity at the locus were estimated. The number of alleles per locus in *I. persulcatus* was 2, and in *I. ricinus* varied from 3 to 6. The highest number of alleles were observed in *I. ricinus* ticks from Latvia (24 allele), followed by in Lithuania (17 allele), and the lowest number in Estonia (14 allele).

References

1. Navajas M, Fenton B. The application of molecular markers in the study of diversity in acarology: a review. Exp Appl Acarol 2000; 24: 751–74.

2. Paulauskas A, Radzijevskaja J, Rosef O, Turčinavičienė J, Ambrasienė D, Makarevičiūtė D. Genetic variations of ticks (*Ixodes ricinus* L.) in the Lithuanian and Norwegian populations. Exp Appl Acarol 2006; 40(3–4): 259–70.

3. Xu G, Fang Q, Keirans J, Durden L. Molecular phylogenetic analyses indicate that the *Ixodes ricinus* complex is a paraphyletic group. J Parasitol 2003; 89(3): 452–7.

GENETICAL VARIATION OF *DAPHNIA CUCULLATA* IN SOME LATVIAN LAKES

Aija Brakovska, Natalja Škute*

Institute of Ecology, Daugavpils University, Daugavpils, Latvia

** Corresponding author. E-mail: natalja.skute@du.lv*

Introduction. Cladocera are of great importance in the aquatic food chains. They mostly feed on algae, detritus or both and are in turn consumend by planktivorous fish and invertebrate predators. Some cladoceran genera (e. g. *Daphnia*) have been unilized as a model organisms in ecological genetic. During most of the year, a population consists of females that reproduce partenogenesis and can be considered as a conglomerate of many clones we studied the genetic structure of *Daphnia cucullata* population from the deepest places of Latvian lakes: Svente, Riča, Dridzis and Geraņimovas-Ilzas.

Methods and results. Molecular genetic technique was used to find out genetic diversity and plasticity of *Daphnia cucullata* in the lakes. The genetic structure of *Daphnia cucullata* population of Lake Svente, Riča, Dridzis and Geraņimovas-Ilzas was studied based on nuclear DNA polymorphism. DNA polymorphism was studied by randomly amplified sequences (RAPD method). 55 Randomly primers from A, B, C and F sets (Carl Roth, Germany) were tested for this investigation. 14 primers from set A, 10 primers from set B, 5 primers from set C and 5 primers from set F were used for study population genetic of *Daphnia cucullata* of Lake Svente, Riča, Dridzis and Geraņimovas-Ilzas. The size of scored polymorphic DNA fragments ranged from 400 bp to 3 000 bp. The number of polymorphic loci, polymorphic level and genetical diversity were detected in each investigated populations. The number of polymorphic DNA band of *Daphnia cucullata* in the three investigated lakes is different.

Research was supported by the ESF project *Formation of Interdisciplinarity Research Group for Securing the Sustainability of Salmonid Lakes in Latvia* No. 2009/0214/1DP/1.1.1.2.0/09/APIA/VIAA/089.

IXODES RICINUS GENETIC VARIABILITY USING 3 MITOCHONDRIAL GENES

E. Galdikaitė[1], A. Paulauskas[1]*, A. Slettan[2], J. Radzijevskaja[1], L. Korslund[2]

[1] *Vytautas Magnus University, Kaunas, Lithuania*

[2] *University of Agder, Kristiansand, Norway*

* *Corresponding author. E-mail: a.paulauskas@gmf.vdu.lt*

Introduction. *Ixodes ricinus* is the most widely spread tick species in Europe, that transmits number of patogenic microorganisms. In order to better understanding the epidemiology of *I. ricinus* transmitted diseases, disease and vector, vector and host evolutionary dynamics, it is essecial to get more information about genetic structure of tick populations in Europe. Genetic variability of *I. ricinus* ticks previously was analysed using alozymes (Delaye et al., 1997; Estrada-Peña et al., 1996; Radulović et al., 2006), RAPD (Paulauskas et al., 2006), restriction fragment length polymorphism (RFLP) (Chitimia et al., 2009), microsatellite analysis (Meeüs et al., 2002), nuclear gene (Rumer et al., 2011; McLain, 2001; Noureddine et al., 2011) and mitochondrial gene (Chitimia et al., 2010; Casati et al., 2008) analysis.

Aim and materials. The aim of the study was to determine mtDNA haplotypes of 3 mitochondrial genes sequenced in ticks, collected from vegetation and rodents in Norway. Target genes were cytochrome *b* oxydase, 16 S rRNA and control region. In total 80 ticks were investigated.

Results. The most variable gene was *16S rRNR* (15 haplotypes, Hd – 0.7342, 14 (3.8%) variable sites). *cytb* gene had lowest variability (4 haplotypes, Hd – 0,4117, 4 (2.3%) variable sites). Haplotype and nucleotide diversity was higher in ticks collected from rodents. Both 16S and cytb genes showed no phylogenetic structure in Europe after comparing Norwegian tick gene sequences with ones in GeneBank. However control region showed differentiation in Norwegian tick population: big part of norvegian ticks formed a clade with other skandinavian ticks compared to other locations.

References

1. Delaye C, Béati L, Aeschlimann A, Renaud F, De Meeûs T. Population genetic structure of *Ixodes ricinus* in Switzerland from allozymic data: No evidence of divergence between nearby sites. Int J Parasitol 1997; 7: 769–73.

2. Estrada-Peña A, Gray JS, Kahl O. Variability in cuticular hydrocarbons and phenotypic discrimination of ixodes ricinus populations (acarina: Ixodidae) from Europe. Exp Appl Acarol 1996; 8: 457–66.

3. Radulović Ž, Milutinović M, Anđelković M, Vujčić Z, Tomanović S, Božić N, Marinković D. Allozyme polymorphism of *mdh* and α-*gpdh* in *Ixodes ricinus* populations: Comparison of borreliae-infected and uninfected ticks. Exp Appl Acarol 2006; 2: 113–21.

4. Paulauskas A, Radzijevskaja J, Rosef O, Turcinaviciene J, Ambrasiene D, Makareviciute D. Genetic variation of ticks (*Ixodes ricinus* L.) in the Lithuanian and Norwegian populations. Exp Appl Acarol 2006; 3: 259–70.

5. Chitimia L, Lin R, Cosoroaba I, Braila P, Song H, Zhu X. Molecular characterization of hard and soft ticks from Romania by sequences of the internal transcribed spacers of ribosomal DNA. Parasit Res 2009; 4: 907–11.

6. Casati S, Bernasconi MV, Gern L, Piffaretti J. Assessment of intraspecific mtDNA variability of European *Ixodes ricinus* sensu stricto (Acari: Ixodidae). Infect Genet Evol 2008; 2: 152–8.

7. Meeüs Td, Béati L, Delaye C, Aeschlimann A, Renaud F. Sex-biased genetic structure in the vector of Lyme disease, *Ixodes ricinus*. Evolution 2002; 9: 1802–7.

8. McLain DK. Evolution of transcript structure and base composition of rDNA expansion segment D3 in ticks. Heredity Pt 2001; 5: 544–57.

9. Rumer L, Sheshukova O, Dautel H, Donoso Mantke O, Niedrig M. Differentiation of medically important Euro-Asian tick species *Ixodes ricinus*, *Ixodes persulcatus*, *Ixodes hexagonus*, and *Dermacentor reticulatus* by polymerase chain reaction. Vector borne and zoonotic diseases (Larchmont, N. Y.) 2011; 7: 899–905.

10. Noureddine R, Chauvin A, Plantard O. Lack of genetic structure among Eurasian populations of the tick *Ixodes ricinus* contrasts with marked divergence from North-african populations. Int J Parasitol 2011; 2: 183–92.

11. Chitimia L, Lin RQ, Cosoroaba I, Wu XY, Song HQ, Yuan ZG, Zhu XQ. Genetic characterization of ticks from southwestern Romania by sequences of mitochondrial cox1 and nad5 genes. Exp Appl Acarol 2010; 3: 305–11.

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN OILSEED RAPE – *LEPTOSPHAERIA* SPP. INTERACTION

Agnė Piliponytė-Dzikienė[1]*, Elena Andriūnaitė[2], Irena Brazauskienė[1], Eglė Petraitienė[1], Gintaras Brazauskas[1]

[1] Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto a. 1, Akademija, LT-58344 Kėdainiai, Lithuania

[2] Vytautas Magnus University, Vileikos str. 8, LT-44404 Kaunas, Lithuania

** Corresponding author. E-mail: agne.piliponyte@lzi.lt*

Introduction. Stem cancer or blackleg is one of the most destructive diseases of Brassicaceae. The disease is caused by *Leptosphaeria* spp. complex. *L. maculans* develops stem base cancer and can potentially cause total crop loss, while *L. biglobosa* is associated with upper stem lesions.

Aim. The aim of this study is to identify oilseed rape genes which are differentially expressed in host-pathogen interaction. The inoculation experiment was done in a controlled environment glasshouse.

Materials and methods. Plant leaves of oilseed rape cultivars 'Jet Neuf' (*Rlm4*), '02-22-2-1' (*Rlm5*), and 'Westar' (no resistance genes) were inoculated with *L. maculans* or *L. biglobosa* conidia suspension. Leaves were sampled 3, 5 and 7 days after inoculation. Total RNA was extracted from a total of 81 inoculated samples and subjected to complementary DNA synthesis. cDNA-AFLP was used for differential profiling of the samples with a total of 176 primers combinations.

Results. Four primer combinations (Oligo48 × Oligo78, Oligo46 × Oligo78, Oligo50 × Oligo70, Oligo50 × Oligo69) yielded specific fragments for cultivar '02-22-2-1'. Oligo48 × Oligo78 specific fragment (220 bp) was expressed 3 days after inoculation with *L. maculans* and 3, 5 and 7 days after inoculation with *L. biglobosa*. Oligo50 × Oligo69 specific fragment (260 bp) was detected 3 and 5 days after inoculation with *L. maculans* and 5 days after inoculation with *L. biglobosa*. These fragments will be cloned and sequenced. Identification of differential expressed genes will lead to better understanding of *Leptosphaeria* spp. infection genetic regulation and will give useful insights into the disease control improvement.

THE CREATION OF TILLING POPULATION IN WINTER WHEAT AND SCREENING FOR MUTATIONS BY HIGH RESOLUTION MELTING ANALYSIS

Rita Armonienė*, Gintaras Brazauskas

Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture
Instituto al. 1, LT-58344 Akademija, Kėdainiai distr., Lithuania

** Corresponding author. E-mail: rita.armoniene@lzi.lt*

Introduction. Freezing temperatures is one of the most severe abiotic stresses limiting wheat growth, productivity, and distribution. Frost tolerance can be defined as the ability of plants to survive freezing temperatures, prevent damage to the vegetative tissues and minimize other negative effects of freezing temperatures on future yield potential (Reinheime, 2004). In winter cereals, frost tolerance is associated with the occurrence of a cold-hardening or cold acclimation, which is triggered by induction of the battery of *Cor* (cold responsive) genes after exposure of plants to low but non-freezing temperature (LT) for certain periods of time (Winfield et al., 2010). TILLING (Targeting Induced Local Lesions IN genomes) is a powerful tool for reverse genetics, combining traditional chemical mutagenesis with high-throughput PCR-based mutation detection to discover induced mutations that alter protein function (Dong et al., 2009).

Aim. In this study we are looking for genes showing expression at various time-points of cold-acclimation in winter wheat and subsequently try to develop mutant forms for these genes to verify their role in freezing-tolerance formation.

Methods and results. Firstly, we optimized the dose of a mutagen ethyl methanesulfonate (EMS) to achieve a substantial mutation rate while avoiding serious defects in germination and plant development. Seeds of two winter wheat genotypes (genotype '5450-1' and '5899-16') were soaked in 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% EMS solutions for 18 h, then washed extensively for 4 h and subsequently sown in 64 cm^3 germination pots filled with peat-sand (1 : 1) substrate. Seeds were allowed to germinate in the greenhouse and the number of germinating seeds was scored daily. Percentage survival of wheat seedlings was estimated after five weeks. Analysis of the results revealed significant differences ($p < 0.01$) of percentage survival between EMS concentrations and wheat genotypes. The most appropriate concentrations of EMS solution for two winter wheat genotypes were determined and a total of 2147 M2 lines were produced. A High Resolution Melting (HRM) analysis as one of mutation detection technique that measures the disassociation of double-stranded DNA at high temperatures resolution (Martino et al., 2010) was tested on the gene *SBEIIa-B* to estimate the mutation rate in our material. Further identification and analysis of the new genes will provide means for better understanding of the genetic regulation of cold acclimation and freezing tolerance formation in winter wheat.

References

1. Reinheimer LJ, Barr AR, Eglinton JK. QTL mapping of chromosomal regions conferring reproductive frost tolerance in barley (*Hordeum vulgare* L.). Theor Appl Gen 2004; 109: 67–74.

2. Dong C, Vincent K, Sharp S. Simultaneous mutation detection of three homoeologous genes in wheat by High Resolution Melting analysis and Mutation Surveyor. BMC Plant Biol 2009; 9: 143.

3. Winfield MO, Lu C, Wilson ID, Coghill JA, Edwards KJ. Plant responses to cold: transcriptome analysis of wheat. Plant Biotechnol J 2010; 8: 749–71.

4. Martino A, Mancuso T, Rossi AM. Application of high-resolution melting to large-scale, high-throughput SNP genotyping: A comparison with the TaqMan® method. J Biomol Screen 2010; 15: 623–9.

GENETIC DIVERSITY OF LATVIAN *LIPARIS LOESELII* POPULATION

Inta Belogrudova[1, 3]*, Dace Grauda[2], Lita Lapina[3], Gunta Jakobsone[1], Daina Roze[1, 3], Isaak Rashal[2]

[1] *National Botanic Garden, Miera Street 1, LV-2169 Salaspils, Latvia*

[2] *Institute of Biology, University of Latvia, Miera Street 3, LV-2169 Salaspils, Latvia*

[3] *Daugavpils University, 13 Vienības Street, LV-5401 Daugavpils, Latvia*

* *Corresponding author. E-mail: inta.belogrudova@nbd.gov.lv*

Introduction. Global strategy for plant conservation (GSPC) has been added to the Rio de Janeiro Convention on Biological Diversity (CBD) in 2002, and later (2010) in Nagoya (Japan) an updated version of the strategy was adopted. Latvia is among 180 countries that are involved in this activity. According the strategy 75% of endangered species should be preserved in *ex situ* collections till 2020. *Liparis loeselii* is a rare and endangered orchid species occurring in Europe. In Latvia *L. loeselii* is classified as the third threat category of protected species. To develop best conservation strategy knowledge concerning genetic differences of protected plants in particular area is crucial.

Methods and results. For this reason, genetic diversity of *L. loeselii* populations from different Latvian habitats was tested. Inter-retrotransposon amplified polymorphism (IRAP) method was used for genotyping. Totally, 54 accessions from 9 habitats were collected and analyzed. *L. loeselii* plants have high level of phenols in leaves that decreases the quality of extracted DNA. Therefore modified Frier (2005) DNA extraction method with double precipitation of DNA in ice-cold 95% ethanol for obtaining of large amount of high quality DNA was used. It was found that percentage of polymorph loci varied among the populations of *L. loeselii* grown in different habitats, some of populations were genetically homogeneous. Genetic diversity level of *L. loeselii* populations is related with age of population and growing conditions.

DIVERSITY AND EVALUATION OF PERENNIAL GRASS WILD POPULATIONS IN LATVIA

Peteris Berzins, Vija Stesele, Ieva Dzene, Sarmite Rancane*

Agency of LLU Research Institute of Agriculture,
Zemkopibas instituts 7, LV-5125 Skriveri, Latvia

** Corresponding author. E-mail: sarmite.rancane@inbox.lv*

Introduction. Grasslands are species richest ecosystems of agricultural lands. Economic activities are a constant subject of biodiversity loses. Genetic resources are the wealth and the property of the country where they are located (Jansone, 2002).

Materials. From 2000 to 2007 together with the Institute of Biology of LU due to the financial support of the Ministry of Agriculture in 14 scientific expeditions there were gathered 446 population examples of 18 wild grasses species, that were afterwards evaluated or evaluation still continues. The most valuable material after multiplying seeds is given to the Gene Bank of Latvia for preserving. Collected samples represent all regions of Latvia with various soil and agroclimatic conditions. Most of all collected samples are for forage uses like cocksfoot (*Dactylis glomerata*), timothy (*Phleum pratense*), red fescue (*Festuca rubra*), meadow foxtail (*Alopecurus pratensis*), and reed canarygrass (*Phalaris arundinacea*). A part of the collected cocksfoot and some timothy seed material could be originated from the sorts of cultivated plants distributed once. The major diversity in population between the species and even between one type of species was observed in hilly conditions. In low lands was observed homogeneity of sorts and populations, one population at times occupied notable areas for example fowl bluegrass (*Poa palustris*) in the flood lands of river of Kuja (GPS LAT 56°45'38"N; LONG 26°19'17"E; 90 m above sea level). Many species like cocksfoot, timothy, meadow foxtail and reed canarygrass are widespread in all regions of Latvia but a few of them are growing only in fixed areas like fowl bluegrass in the area of Lubana lake (GPS LAT 56°46'49"N; LONG 26°46'57"E; 89 m above sea level) or false oatgrass (*Arrhenatherum elatius*) in the warmest south-east part of Latvia.

Methods and results. All the collected grass samples were sowed in Skriveri, mostly all of them were suitable for the local agro-climatic conditions and have shown good winter hardiness. Only the sample of perennial ryegrass *(Lolium perenne*) from the Dobele area, which practically did not wintered in first winter and a sample of tall fescue (*Festuca arundinacea*) from coastal region of Liepaja wintered poorly were exceptions. Many of cocksfoot, timothy, meadow fescue (*Festuca pratensis*), meadow foxtail, reed canarygrass, red fescue and smooth meadow-grass (*Poa pratensis*) forms can be successfully used in breeding work for forage uses. Some reed canarygrass and tall fescue forms are suitable in breeding work for energocultures. Between red fescue, smooth meadow-grass and flattened meadow-grass (*Poa compressa*) forms are many decorative forms that is useful for making lawn varieties. In our comparisons of varieties best meadow fescue forms from Aiviekste river (GPS LAT 56°42'40"N; LONG 26°10'19"E; 90 m above sea level) surrounding an average of 3 years gave dry matter yield 5.8–6.2 tha^{-1}, or 94–96% of our meadow fescue standard variety Silva overcome their resistance to disease and ability to remain in the mixture. In comparisons of meadow foxtail varieties some samples in dry matter yield were 11% higher than the Czech variety Vulpina yield. Currently in our breeding program are included several forms of cocksfoot from Vidzeme highlands with softer leaves and good winter hardiness. Interesting forms for cutting and grazing is between the timothy

and meadow fescue samples, which are permanent and resistant to disease, but smooth meadow-grass forms are significant by a large number of straws that could potentially provide higher seed yields (Chapman, 1996; Barones, etc., 2003).

Conclusion. The role of false oat-grass and fowl bluegrass in forage production is not fully evaluated. Our wild perennial grass forms conceal a great potential for the improvement of forage production, for better environment, perennial lawn establishment and landscaping.

References

1. Jansone B. Genetic Resources of forage grasses and legumes in Latvia. Proceedings in agronomy. Jelgava, LLU. 2002; 4: 176–180 p. (In Latvian).

2. Chapman, GP. 1996. The biology of grasses. Wallingford, UK: CAB International.

3. Barones RF, Nelson CJ, Collins M, Moore KJ. Forages. Vol. I. Structure and Morphology of Grasses. Chapter 2. Iowa State Press, USA. 2003: 25–49.

GENETIC DIVERSITY EVALUATION OF KAUNAS BOTANICAL GARDEN BLUEBERRY (*VACCINIUM* L.) COLLECTION

Sigita Bogačiovienė[1], Judita Žukauskienė[1]*, Algimantas Paulauskas[1], Eugenija Kupčinskienė[1], Laima Česonienė[2], Remigijus Daubaras[2]

[1] Faculty of Natural Sciences, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

[2] Kaunas Botanical Garden of Vytautas Magnus University, Ž. E. Žilibero 6, LT-46324 Kaunas, Lithuania

** Corresponding author. E-mail: j.zukauskiene@gmf.vdu.lt*

Introduction. Blueberry (*Vaccinium corymbosum* L.) is a special plant, because it is a lengthy selection of fruit. Humans created hybrids from several types of blueberry. According to Longley (1927), *Vaccinium corymbosum* basic chromosome number is 12. Blueberry has three ploidy levels: 2x (2n = 24), 4x (4n = 48), 6x (2n = 72). Berries contained about 15% of sugar, organic acids, there are many other valuable nutrients: vitamins A, B1, B2, B6, C, K, PP, carotenoids and minerals. Microsatellite markers are widely used for many plants species – building gene maps, gene searches, genotype identification, population genetic diversity and phylogenetic analysis. For blueberry searches these markers show excellent promise for further use in germplasm identification, in genetic studies of wild *Vaccinium* L. populations, and for constructing linkage maps.

Materials and methods. DNA analysis using SSR method: we had 40 blueberry species from VMU Kaunas Botanical garden (highbush, lowbush and selection numbers blueberry). DNA amplification reaction was performed by modifications of Boches et al., (2005) recommendations. Reaction mix made of: 12.5 µl 2x PCR Master Mix (0.05 U/µl Taq DNA polymerase; 4 mM MgCl2; 0.4 mM each dNTP), 0.5 µl each primer (concentration pmol/µl), 7.5 µl twice distilled water and 4 µl (5 ng) DNA. PCR reaction was performed in a thermocycler (Mastercycler, Eppendorf, Germany) according to Boches et al., (2005). Calculation of genetic distances and UPGMA cluster analyses were performed with the TREECON program for Windows V 1.3b (Nei, Li, 1979).

Results. Using 5 selected microsatellite markers were explored highbush, lowbush and Lithuanian blueberries (selection numbers – 11, 16, 17) from VMU Kaunas Botanical Garden. In total, were studied 40 different species. After genetic analysis the most unique alleles have highbush blueberies – 50 alleles, blueberries of selection numbers don't have unique alleles. The most unique alleles detected with the primer CA421F (17 alleles) at least with primer CA169F (7 alleles). Analyzed blueberry groups using 5 microsatellite primers in UPGMA dendrogram showed that all genotypes were divided into two groups – highbush and lowbush blueberries. And blueberries of selection numbers (No. 11, No. 16, No. 17) belong to lowbush blueberry.

Financial support for this study was provided by the Research Council of Lithuania by project "Change of bioactive compounds in underutilised berry species depending on genotype and environment" (No. SVE-04/2011).

BREEDING OF HIGHBUSH BLUEBERRY IN LITHUANIA

Laima Česonienė*, Remigijus Daubaras

Kaunas Botanical Garden of Vytautas Magnus University,
Ž. E. Žilibero 6, LT-46324 Kaunas, Lithuania

** Corresponding author. E-mail: laimac@hotmail.com*

Growing of half highbush blueberry in Lithuania has been limited up till the present time. One of the main reasons for such situation was absence of local cultivars. The collection of half highbush blueberry germplasm and respective breeding programme were started at Kaunas Botanical Garden of Vytautas Magnus University in 1993. Fifty seedlings were examined in 1993–2010. The seedlings No. 11, No. 16, and No. 17 were selected for further cultivar testing. The results were compared with the same characteristics of the cultivar Putte. Fruiting potential of Lithuanian half highbush blueberry accessions was determined, taking into account these following characteristics: number of berries and number of simple clusters in a composite cluster, average berry mass and volume. Productivity of investigated seedlings was determined too. The seedling No. 17 was distinguished for the biggest clusters with the largest number of berries and the largest number of simple clusters in a composite cluster. The average berry mass of selected seedlings was smaller than berry mass of the cultivar 'Putte'. Ripening period of all investigated seedlings was long. Berries of the seedlings No. 11 and No. 16 were harvested completely during 2–3 pickings, whereas harvesting berries of the seedling No. 17 was completed in 3–4 pickings. The No. 17 appeared the most productive, its yield reached from 1.8 to 3.3 kg in different years. Yields of the seedlings No. 11, No. 16 were significantly lower.

INVESTIGATION OF KOLOMIKTA KIWI (*ACTINIDIA KOLOMIKTA*) PHENOTYPIC AND GENETIC DIVERSITY

Laima Česonienė*, Remigijus Daubaras

Kaunas Botanical Garden of Vytautas Magnus University,
Ž. E. Žilibero 6, LT-46324 Kaunas, Lithuania

** Corresponding author. E-mail: laimac@hotmail.com*

Introduction. *Actinidia kolomikta* Maxim. has been gradually introduced into culture in Lithuania, along with successive assessment of its possibilities to adapt to the country's climatic conditions. *A. kolomikta* possesses exceptionally decorative properties and produces valuable berries. Therefore it may supplement the assortment of berry plants.

Aim and methods. The aim of this study was to distinguish the informative phenotypic characteristics and to evaluate genetic diversity of *A. kolomikta* germplasm collection at Kaunas Botanical Garden of the Vytautas Magnus University. The following traits were the most informative for phenotypic characterization of cultivars and clones: variegation intensity of leaves, size and distribution of flowers as well as berry size and shape. Female cultivars differed in the total number of fruiting shoots per metre length of two-year-old shoots. DNA investigations by RAPD method defined significant genetic diversity of *A. kolomikta* accessions and the level of their relationship.

Results. The percentage of polymorphism ranged from 55.6 to 80.0%. The highest genetic identity have been established for the female clones F2 and F4 (GD_{xy} = 0.059 and for the male clone M1 and female clone F2M2 (GD_{xy} = 0.094). Two specific markers were identified with the primers OPC-02 and 2B for the cultivar Laiba and the female clone F4M4. The dendrogram grouped the accessions by UPGMA method and revealed two main clusters. 'Laiba' proved to be the most divergent cultivar and joined to the other accessions at the 0.824 genetic distance.

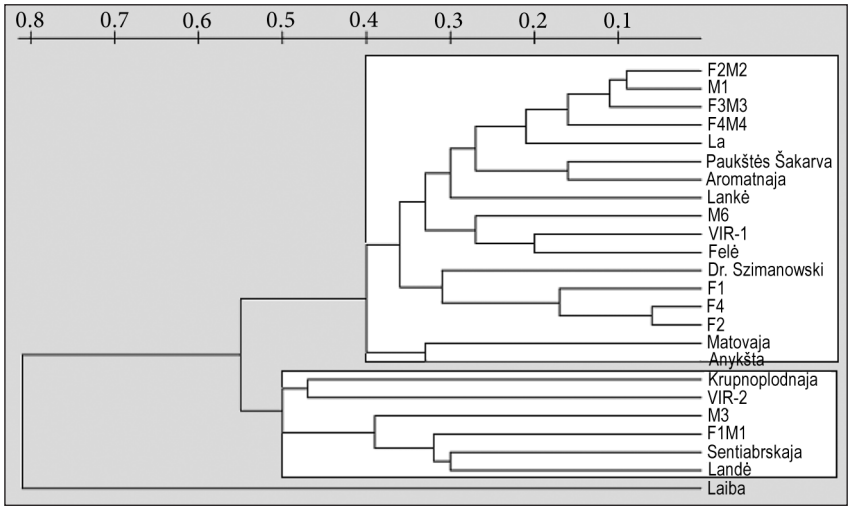

Figure. UPGMA dendrogram of *A. kolomikta* accessions

References

1. Česonienė L. Assessment of phenological and microphenological timing of *Actinidia kolomikta*. Sodininkystė ir daržininkystė 2004; 25(1): 82–91.

2. Česonienė L, Daubaras R, Gelvonauskis B. Characterization of kolomikta kiwi (*Actinidia kolomikta*) genetic diversity by RAPD fingerprinting. Biologija 2005; 3: 1–5.

RESEARCH INTO THE EFFECTS OF POLYPLOIDY-INDUCING AGENTS ON TETRAPLOID YIELD IN RYEGRASS

Giedrė Dabkevičienė

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry

** Corresponding author. E-mail: genetik@lzi.lt*

Introduction. Experimental polyploidy in combination with various *in vitro* techniques can significantly contribute to the enhancement of genetic diversity and development of novel varieties.

Aim. The research was aimed to estimate the efficacy of different ploidy-inducing agents in ryegrass.

Materials. The study objects were populations of perennial ryegrass (*Lolium perenne* L.) cat. Nos. 3548, 3532, 3722, 3569, Italian ryegrass (*Lolium multiflorum* Lamk.) cat. Nos. 316, 314, 112, 323, annual ryegrass (*Lolium multiflorum Lam. Var. Westerwoldicum*) varieties Witesse, Varpė, Druva, Weldra, and populations cat. Nos. 287, 299, 120.

Methods. The embryos were isolated from mature seeds after soaking them for 24 h in sterile water. The excised embryos were transferred on 2/3 strength Linsmaier & Skoog medium supplemented with 0.2 mg IAR, 0.25 mg kinetin, 1 mg thiamine, 0.1 mg nicotinic acid 0.1 mg pyridoxine. The embryos were cultured for 3–5 days until 8–14 mm long coleptile formed. After cell division synchronization (48 h, 2–4 °C), the embryos were transferred for 4 hours into flasks with sterile polyploidy-inducing agents. Polyploidization was discontinued by removing the solution and rinsing the embryos in sterile water. The treated embryos were cultured on a new medium of the same composition and were stored for 1.5–2 months in a cultivation room (24 °C, photoperiod 16 h, 9000 lux). The regenerants were transferred into the soil and cultivated in the greenhouse (20–24 °C). After rooting, the plants were tested for ploidy level by establishing chromosome number in rootlets or ovaries.

Apart from conventionally used colchicine 0.4% /KOLCH/, polyploidization was performed with oryzalin 50 μM /ORYZ/, trifluralin 50 μM /TRIF/, amiprofos-methyl 100 μM /APM/ application. The efficacy of polyploidization was determined by analysing the three indicators: survival of explants after treatment with ploidy-inducing agents, tetraploid yield and formation of chimeras.

Results. The polyploidy-inducing agents used in this study exhibited different efficacy. Tetraploid yield depended not only on the polyploidy-inducing agent applied but also on the plant species. Embryo treatment with KOLCH gave a tetraploid yield ranging from 54.2% (Italian ryegrass) to 70.1% and 78.3% (annual and perennial ryegrass). APM induced the highest tetraploid yield in perennial ryegrass plants (76.3%) and the lowest yield in Italian ryegrass (39.5%). An especially high tetraploid percentage variation from 1.0% (Italian ryegrass) to 81.3% (perennial ryegrass) was observed under the effect of TRIF.

The highest polyplody effect was exerted by ORYZ – 100% of perennial ryegrass plants were tetraploid after embryo treatment. However, the effect of ORYZ on embryos was more toxic – tetraploids accounted for as little as 1.7–15.1% of the total number of treated embryos. The lowest yield of chimeric individual was produced having treated ryegrass with TRIF (19.1%), while the highest yield (27.7%) resulted from APM treatment.

The success of polyploidization depended not only on the material used but also on the populations' genetic make-up (i. e. ability to respond to the polyploidy-inducing agents). Moderate variation of tetraploid yield value (CV% = 17.6–21.6) was established having treated annual and perennial ryegrass with KOLCH and having treated Italian ryegrass with APM. In other treatments, CV% value within populations varied within even a wide range 22.3–91.3.

References

1. Yemets AI, Blume YaB. Progress in plant polyploidization based on antimicrotubular drugs. Open Horticult J 2008; 1: 15–20.

2. Rey HY, Sansberro PA, Collavino MM, Davina JR, Mroginski LA. Colchicine, trifluralin, and oryzalin promoted development of somatic embryos in Ilex paraguariensis (Aquifoliaceae). Euphytica 2002; 123: 49–56.

CLONING, DNA SEQUENCE ANALYSIS AND *IN VITRO* EXPRESSION OF THE TLP PROTEIN FROM *PINUS SYLVESTRIS* L. AND STUDY OF ITS ANTIMICROBIAL ACTIVITY

Ilze Gaile, Dainis Ruņģis

Genetic Resource Centre, LSFRI Silava,
Rīgas 111, LV-2169 Salaspils, Latvia
E-mail: ilze.gaile@silava.com; dainis.rungis@silava.lv

Introduction. One of the most important inducible defense mechanisms is the biosynthesis of pathogenesis – related (PR) proteins. Members of the PR – 5 groups are called thaumatin – like proteins (TLPs). Many PR – 5 proteins are induced in plants in response to infection by pathogens, osmotic stress, treatment with abscisic acid, ethylene, salicylic acid, methyl jasmonate and wounding (Eyles et al., 2010). Members of this group have been shown to have antifungal activity against a broad spectrum of fungal pathogens (Jayaraj et al., 2004; Mohamed et al., 2011). Previous results from our laboratory have shown that TLP gene expression increases in *P. sylvestris* after inoculation with *Heterobasidion annosum*; therefore the effect of TLP protein on growth of *H. annosum* was studied.

Materials and methods. The *Pinus taeda* TLP gene sequence was used to design primers for amplification of the full-length TLP gene from *P. sylvestris* (PsTLP). The full-length PsTLP has an open reading frame of 606 bp, encoding a protein of 202 amino acids, molecular weight approximately 22.5 kDa, including 16 cysteine residues which are characteristic of L-type TLPs. The disulfide bridges formed by these cysteine residues have important role in maintaining the protein stability and correct folding and preserving high activity under extreme temperatures and pH conditions. In addition, the deduced protein has a thaumatin family signature G-x-[GF]-x-C-x-T-[GA]-D-C-x(1,2)-[GQ]-x(2,3)C (Liu et al., 2010). Also sequence has 5 conserved amino acids residues responsible for the acidic cleft with known antifungal activity. This conserved acidic cleft is comprised of five amino acids (R, E, D, D, D) and is believed to be involved in binding to β-1,3-glucan on the fungal cell membrane, which leads to permeabilization of the fungal cell membrane and disruption of the osmotic balance inside hyphal cells, resulting in cell rupture (Osmond et al., 2001).

Results. To study the effect of the *Pinus sylvestris* TLP protein on fungal growth, the gene was cloned into *Escherichia coli*, then subcloned into expression vector pET100 using BL21 Star bacteria. The protein was present in the soluble fraction. The presence of a faint band of the same size protein even in the non – induced culture indicated some background protein expression in non-induced cultures. The presence of a faint band of the recombinant PsTLP protein in the induced culture and reduced growth of *E. coli* (measured optical density at 600 nm) indicate that PsTLP protein expression in BL21 cells was toxic to bacteria cells (Mohamed et al., 2011). Optimization of the recombinant PsTLP protein was achieved by cold induction through decreasing both expression temperature and IPTG concentration resulted in a lowered expression levels of the recombinant protein in *E. coli* cells, which reduced the toxicity to the host cells. Also 1% glucose was added to the bacterial culture medium to repress basal expression of T7 RNA polymerase and to stabilize the construct. In order to examine the effect of the *in vitro* expressed PsTLP protein, an agar plug containing mycelia of *H. annosum* was placed in the center of agar plates and incubated for one week. The various

supernatant containing proteins (soluble fraction) from induced and non-induced cultures (with and without glucose) were applied in wells that were punched in the agar plate surrounding the plugs 1cm away from the rim of the mycelia colony. After 2 weeks incubation at 25 °C, clear inhibition zones were seen around the wells with PsTLP protein (from induced and non – induced cultures) (Figure).

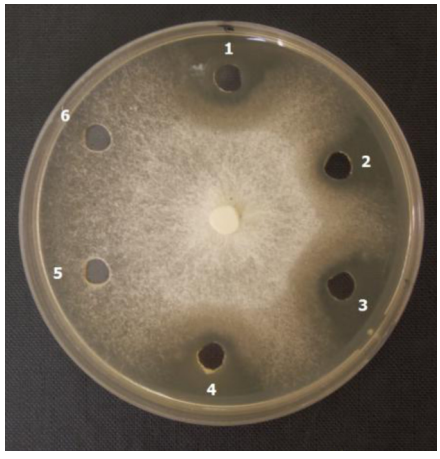

Figure. Test for the antimicrobial activity of the recombinant *P. sylvestris* TLP protein. (1) PsTLP protein (plasmid containing PsTLP gene, IPTG, 1% glucose), (2) PsTLP protein (plasmid containing PsTLP gene, IPTG, without 1% glucose), (3) NK (plasmid containing PsTLP gene, 1% glucose), (4) NK (plasmid containing PsTLP gene, without 1% glucose), (5) 1% glucose, (6) empty well

References

1. Eyles A, Bonello P, Ganley R, Mohammed C. Induced resistance to pests and pathogens in trees. New Phytologist 2010; 185: 893–908.

2. Jayaraj J, Velazhahan R, Fu D, Liang GH, Muthukrishnan S. Bacterially produced rice thaumatin-like protein shows in vitro antifungal activity. J Plant Dis Protect 2004; 111(4): 334–44.

3. Liu JJ, Sturrock R, Ekramoddoullah AK. The superfamily of thaumatin-like proteins: its origin, evolution, and expression towards biological function. Plant Cell Report 2010; 29(5): 419–36.

4. Mohamed MS, El-Soud WA, Mohamed MF. Cloning and expression of the recombinant NP24I protein from tomato fruit and study of its antimicrobial activity. Afr J Biotechnol 2011; 10(65): 14276–85.

5. Osmond RIW, Hrmova M, Fontaine F, Imberty A, Fincher GB. Binding interactions between barley thaumatin-like proteins an (1, 3)-b-D-glucans. Eur J Biochem 2001; 268(15): 4190–9.

GENETIC DIVERSITY OF LATVIAN AND ESTONIAN *SAUSSUREA ESTHONICA* POPULATIONS AND PHYLOGENETIC COMPARISON WITH *S. ALPINA* AND *S. DISCOLOR*

Agnese Gailite[1, 2*], Dainis Rungis[1]

[1] *Genetic Resource Centre, Latvian State Forest Research Institute "Silava", Rigas st. 111, LV-2169 Salaspils, Latvia*

[2] *Faculty of Biology, University of Latvia, Kronvalda Bulv. 4, LV-1586 Riga, Latvia*

* *Corresponding author. E-mail: agnese.gailite@silava.lv*

Introduction. *Saussurea esthonica* belongs to the genus *Saussurea* which is one of the largest in the family *Compositae* and is defined either as a separate species or a subspecies of *S. alpina* (Narits et al., 2000). Four subspecies of *S. alpina* have been reported: *S. alpina* subsp. *alpina*, *S. alpina* subsp. *depressa* and *S. alpina* subsp. *macrophylla*, *S. alpina* subsp. *esthonica* (Kell et al., 2005). *Saussurea esthonica* is an endangered species in Latvia and distributed in Latvia, Estonia and the Leningrad region (Ingelög et al., 1993).

Methods. One of the factors affecting species viability is reduction of genetic diversity. Therefore the information about genetic variability is necessary for species conservation management. To analyse threats to population stability and long term survival in Latvia, genetic diversity studies of Latvian and Estonian populations were performed by using PBS DNA markers. Most of genetic variation was found within populations (85%). The iPBS method showed differentiation of the Latvian and Estonian populations (10%) and only 5% variation between the populations within Latvia and Estonia. The Nei's genetic distances were lower between the sampled Estonian populations than between the Latvian populations. This might be due the higher gene flow between the Estonian populations because in Estonia *S. esthonica* populations are found throughout the country, while in Latvia there are only two known populations located about 95 km from each other. Mean expected heterozygosity was higher in Latvian populations as well. Based on these results we concluded that Latvian populations have a relatively high level of genetic diversity.

Results. For investigation of the phylogenetic relationship between *S. esthonica*, *S. alpina* and *S. discolor*, ITS sequencing was used. Four *S. esthonica* populations were compared with three *S. alpina* populations (from Norway, Austria and Italy) and one *S. discolor* (Austria) population. *S. esthonica* clustered together with the Italian and Norwegian *S. alpina* populations, but differed from Austrian *S. alpina* population. The results indicated that *S. alpina* has genetically distinct populations and that possibly different subspecies were analysed, as the accessions that were analysed were not designated as belonging to a *S. alpina* subspecies. Therefore for further phylogenetic studies, detailed description and sub-species designation of accessions is needed.

References

1. Ingelög T, Andersson R, Tjernberg M. Red Data Book of the Baltic Region. Part 1. Swedish Threatened Species Unit, Uppsala. 1993.

2. Kell SP, Knüpffer H, Jury SL, Maxted N, Ford-Lloyd BV. Catalogue of Crop Wild Relatives for Europe and the Mediterranean. University of Birmingham, Birmingham, UK. 2005. Available online via the Crop Wild Relative Information System (CWRIS – http://www.pgrforum.org/cwris/cwris.asp)

3. Narits A, Leht M, Paal J. Taxonomic status of *Saussurea alpina* subsp. *esthonica* (Asteraceae): phenetical analysis. Ann Bot Fennici 2000; 37: 197–206.

THE EVALUATION OF RED CLOVER (*TRIFOLIUM PRATENSE* L.) VARIETIES AND HYBRIDS ACCORDING TO THE DESCRIPTORS

Biruta Jansone, Sarmīte Rancāne*, Aldis Jansons

LLU Agency Research Institute of Agriculture,
Zemkopības institūts 7, LV-5125 Skrīveru novads, Latvia

** Corresponding author. E-mail: sarmite.rancane@inbox.lv*

Introduction. Describing of crop varieties after a certain set of features is an important step that facilitates the activities of breeders for achieving a specific task and provide performance accuracy of gene bank.

Trifolium genus is very large, it is represented by more than 300 different species, but economically the most valuable and widely cultivated in Latvia is red clover (*Trifolium pratense* L.). Diploid (2n) red clover cultivars with chromosome number 14 is widely used for the meadows and pastures herbages formation (Jansone et al., 2008). However, increasing attention has been given to tetraploid varieties (4n = 28 chromosomes), which are characterized by a greater height, wider leaves, higher green mass and dry matter yields.

During the period from 2007 by 2010 at the Research Institute of Agriculture in Skriveri using descriptors were evaluated 12 diploid (2n) and 14 tetraploid (4n) red clover breeding numbers and varieties. Each number was sown in 3 replicates in two 2 m long rows, 60 cm between rows. As a standard variety was selected 'Skriveru agrais' for 2n varieties and 'Skriveru tetra' for 4n varieties. The assessment period lasted for 3 years.

Materials and methods. Samples evaluation using descriptors allows to easily and quickly distinguish between different phenotypes features (Andersen, Davies, 1984). They are mainly inherited, it is easy to see visually, and they are similar and explicit at different growth conditions.

Altogether 19 features for all samples were evaluated on the basis of UPOV and IPGRI developed methodology. The key indicators of the best varieties are summarized in Table.

Table. The most valuable red clover varieties and breeding lines characteristics

Variety / hybrid	Winter damages in 2nd year (1–9 points*)	Green mass yield in 1st year of use (1–9 points**)		Central leaf length / width (1–9 points***)	Seed yield g 2 m^{-2}
		1st cut	2nd cut		
Skriveru agrais	5	8	8	5/5	119.2
Arija	7	9	9	5/5	122.3
Stendes agrais	5	9	8	5/5	158.1
Raunis	3	9	7	5/6	201.0
Dižstende	3	9	8	8/5	112.1
Jancis	3	9	8	5/7	164.2
Stendes velais II	3	9	4	9/9	105.4
Skriveru tetra	8	7	8	9/9	210.0
Kaive	7	8	8	9/9	275.0
Nr. 1577	6	7	8	7/9	225.0
Nr. 1619	5	4	7	9/9	260.0
Nr. 1509	7	7	8	9/9	280.0
Divaja	5	8	3	9/9	285.0

*1 – very few, 9 – very strong; **1 – very low, 9 – great; ***1 – very short / narrow, 9 – long / wide

In the first year of use almost all diploid varieties showed good winter hardiness, in winter damage scale gained only 1 point. In the next wintering cycle with a better results stood out medium late and late ripening varieties: 'Dizstende', 'Raunis', 'Jancis', and 'Stendes velais II' (Table).

Important features are the clover central leaf length and width, which characterize the variety and its economic properties, because leaves have a higher protein content. Large leaves and rapid re-growth in the spring and after cutting is characterized by tetraploid varieties. The central leaf length reaches 6–7 cm, width 3–4 cm.

Results. 8 to 9 June noted the beginning of flowering for early samples. With the higher green mass and DM yields both the first and second mowing stood out tetraploid varieties: 'Kaive', 'Skriveru tetra', No. 1577, but among diploid a more productive were 'Arija', 'Stendes agrais', 'Dizstende'.

An important indicator of forage legumes is seed yield also. Higher seed yield obtained from 'Raunis', 'Kaive', No. 1509, No. 1577, No. 1619, and 'Divaja', which average from 2 m long line gave more than 200 g seed, it means – the biological seed yield of these samples exceeded 1 t ha^{-1}.

Evaluated red clover samples are characterized by a wide variety of features and valuable commercial properties.

References

1. Andersen S, Davies EW. Descriptor list for forage legumes. CEC Secretariat, Brussels, IPGRI Secretariat, Rome, 1984: 28.

2. Jansone B, Jansons A, Tiltina L, Rancane S. Wild populations of red clover and their agro-morphological characteristics in Latvian climate conditions. Modern variety breeding for present and future needs. Proceedings of the 18th Eucarpia General Congress, Valencia, Spain, 2008: 143–7.

IDENTIFICATION OF FUNCTIONAL MARKERS FOR DROUGHT AND COLD TOLERANCE IN PERENNIAL RYEGRASS

K. Jonavičienė[1]*, T. Asp[2], G. Brazauskas[1]

[1] Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto al. 1, LT-58344 Kėdainiai distr., Lithuania

[2] Department of Genetics and Biotechnology, Faculty of Agricultural Sciences, Research Center Flakkebjerg, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark

** Corresponding author. E-mail: kristinaja@lzi.lt*

Introduction. The high forage quality makes perennial ryegrass (*Lolium perenne* L.) the predominant forage grass used in temperate agriculture. However, abiotic stresses such as drought and cold not only limit the geographic distribution of green crop production but also adversely affect green crop development and yield. Thus, ryegrass varieties with improved tolerance to cold and drought are appreciable in the countries with unpredictable climate. Since the genome data in perennial ryegrass have been rapidly accumulated over the recent years (Studer et al., 2010) this species can be used as a model for studying and improving tolerance to abiotic stresses in genetically more complex grassland species. A large number of genes have been implicated in the drought response, but identification of the promising targets for breeding drought- and / or cold-resistant crop varieties remains a significant technical challenge.

Methods and results. The main objective of this project is to develop and validate functional markers conferring ryegrass tolerance to drought and cold through candidate gene–based association studies with two phenotyping platforms, controlled and field. Seven candidate genes (Jonavičienė et al., 2012) were examined for gene expression levels during the cold and drought treatment in two parental genotypes of perennial ryegrass VrnA mapping population (Jensen et al., 2005). Selected candidate genes will be further used for identification of Single Nucleotide Polymorphisms (SNPs) in ryegrass association (AS) panel as well as for association studies in order to validate SNP molecular markers for drought and cold tolerance. Drought and / or cold tolerance markers will be integrated in the VrnA genetic linkage map.

References

1. Jensen LB, Andersen JR, Frei U, Xing Y, Taylor C, Holm PB, Lübberstedt T. QTL mapping of vernalization response in perennial ryegrass (*Lolium perenne* L.) reveals co-location with an orthologue of wheat *VRN1*. Theor Appl Genet 2005; 110: 527–36.

2. Jonavičienė K, Studer B, AspT, Jensen LB, Paplauskienė V, Lazauskas S, Brazauskas G. Identification of genes involved in a water stress response in timothy and mapping of orthologous loci in perennial ryegrass. Biol Plantarum 2012; 56: 473–83.

3. Studer B, Kölliker R, Muylle H, Asp T, Frei U, Roldán-Ruiz I, Barre P, Tomaszewski C, Meally H, Barth S, Skøt L, Armstead IP, Dolstra O, Lübberstedt T. EST-derived SSR markers used as anchor loci for the construction of a consensus linkage map in ryegrass (*Lolium* spp.). BMC Plant Biol 2010; 10: 77–187.

THE RESEARCH OF *ARABIDOPSIS THALIANA* CYTOCHROME P450 GENES

I. Jonuškienė[1]*, S. Kuusienė[2]

[1] *Kaunas University of Technology*

[2] *Lithuanian Research Centre for Agriculture and Forestry*
Liepų str. 1, LT-53101 Girionys, Kaunas distr., Lithuania

** Corresponding author. E-mail: ilona.jonuskiene@ktu.lt*

Introduction. Plants produce a huge array of compounds used for foods, medicines, flavors and industrial materials. These plant metabolites are synthesized by the networks of proteins encoded in the genome of each plant. However, even after the completion of the genome sequencing of *Arabidopsis thaliana* function of these genes and networks of gene-to-metabolite are largely unknown. To reveal the function of genes involved in metabolics-based approach is regarded as a direct way (1).

Cytochrome P450 enzymes (CYPs) constitute a large superfamily of heme-containing monooxygenases that are widely distributed in all kingdoms of life. CYPs are involved in the metabolism of a wide variety of endogenous and xenobiotic compounds by catalyzing regio- and stereospecific monooxygenation with an oxygen atom generated from molecular oxygen (2). Glucosinolates are amino acid-derived natural products that, upon hydrolysis, typically release isothiocyanates with a wide range of biological activities. The first committed step in the biosynthesis of the core glucosinolate structure is the conversion of amino acids to the corresponding aldoximes. This reaction is catalyzed by the substrate-specific cytochromes P450 from CYP79 family. The *Arabidopsis thaliana* genome contains seven CYP79 genes, of which five have been characterized with respect to substrate specificity. CYP79A2 converts Phe to phenylacetaldoxime, CYP79B2 and CYP79B3 metabolize Trp, and CYP79F1 and CYP79F2 metabolize all chain-elongated Met derivatives (3).

Methods and results. Particular emphasis in this research was put on genes *CYP79C1* and *CYP79C2* of cytochromes P450 as well as new glucosinolate biosynthesis-related functions. There were selected homozygous knockout mutant lines from SALK collection line of *CYP79C2*. It was carried out genotyping of *CYP79C2* SALK collection line by PCR approach. The crossbreeding of homozygous *CYP79C2* and *CYP79C1* plants was performed and glucosinolates were identified. T-DNA studies were performed for the attainment of evaluation of quantitative and qualitative variation of glucosinolates in different organs of *Arabidopsis thaliana* of homozygous of *CYP79C2* and *CYP79C1*, knockout mutants and of (*CYP79C2 X CYP79C1*) F2 generation recombinants.

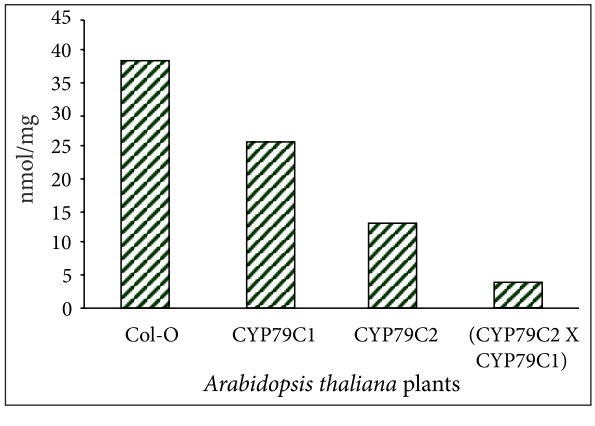

Figure. The total amount of glucosinolates in seeds of *Arabidopsis thaliana* of the ecotype 'Col-O', of *CYP79C1* and *CYP79C2* knockout mutants, of (*CYP79C2 X CYP79C1*) F2 recombinants

On analyzing the study results (Figure), it has been established that the lowest amount of total glucosinolates was found to be accumulated in seeds of *Arabidopsis thaliana* (*CYP79C2 X CYP79C1*) F2 generation recombinants.

References

1. Hirai MY, Yano M, Goodenowe DB, Kanaya S, Kimura T, Awazuhara M, Arita M, Fujiwara T, Saito K. Integration of transcriptomics and metabolomics for understanding of global responses to nutritional stresses in *Arabidopsis thaliana*. PNAS 2004; 101(27): 10205–10.

2. Nakamura R, Kondo M, Shen H, Ochiai S, Hisamatsu S, Sonoki S. Identification of cytochrome P450 monooxygenase genes from the white-rot fungus *Phlebia brevispora*. AMB Express 2012; 2: 8.

3. Mikkelsen MD, Halkier BA. Metabolic engineering of valine and isoleucine-derived glucosinolates in *Arabidopsis* expressing CYP79D2 from Cassava. Plant Physiol 2003; 131: 773–9.

ALTERED PHYTOHORMONE CONTENT AND RESPONSE IN *ARABIDOPSIS* MUTANT *DND2*

Liga Kale*, Anete Keisa, Laura Kunga, Nils Rostoks

University of Latvia

** Corresponding author. E-mail: Liga.kale@gmail.com*

Plant lesion mimic mutants have a potential for understanding the mechanisms of plant development and immunity. Interestingly, several plant cyclic nucleotide-gated ion channels (CNGC) encoded by a 20 member gene family have been implicated in altered hypersensitive response and disease resistance in *Arabidopsis thaliana*. *Arabidopsis* mutants *dnd2* (*hlm1*) have mutations in *CNGC4* gene and display reduced hypersensitive response, while simultaneously exhibiting broad-spectrum disease resistance. In addition, *dnd2/hlm1* plants exhibit reduced size and enhanced branching compared to wild-type plants suggesting a link to auxin responses. It has been also shown that auxin can restore apical dominance in mutant plants. Orthologous mutant *nec1* in barley comprises constitutively induced systemic acquired resistance markers. Recently we showed that apart from induction of SAR markers *nec1* also affects auxin related response of barley. Here we characterize changes in auxin response in *Arabidopsis dnd2* mutant. Several physiological tests, such as root elongation, stomatal aperture and dehydration rate measurements were used. IAA and ABA accumulation were detected by HPLC. Comparative analysis of *Arabidopsis dnd2* mutant and parent line Columbia will be discussed.

TOWARDS MOLECULAR CLONING OF THE BARLEY NEC3 GENE

A. Keisa, D. Tupina, L. Kunga, N. Rostoks*

Faculty of Biology, University of Latvia,
4 Kronvalda Blvd., LV-1586 Riga, Latvia

** Corresponding author. E-mail: nils.rostoks@lu.lv*

Introduction. Studies of disease resistance in *A. thaliana* have significantly benefited from identification and characterization of mutations conferring lesion mimic phenotype. This allows for assumption that studies of barley lesion mimic mutants (lmm) can help in identification and characterization of molecular components of barley disease resistance. Although lmm class is usually well represented in barley mutant collections only several mutations conferring lesion mimic phenotype have been identified or characterized in barley so far. Here we describe application of forward genetics approach (transcript based cloning and positional cloning) to identification of mutation underlying necrotic phenotype of barley lmm *nec3*.

Methods. Fast neutron irradiation is known to cause large deletions in plant genomes which may cause complete or partial deletion of one or several genes and, consequently, lack the corresponding mRNA in the plant. Thus, comparison of transcriptome between mutant and wt plant may identify mutation underlying mutant phenotype. We used Affymetrix Barley GeneChip1 for transcriptome analysis of two allelic fast neutron irradiated recessive mutants with necrotic spots FN362 (*nec3.l*) and FN363 (*nec3.m*) and a parental cv. Steptoe to identify mutation responsible for the necrotic phenotype in *nec3*. Taking into account late development of necrotic spots on *nec3* leaves transcriptome analysis of *nec3* was performed with 10 day and 7 week old *nec3* plants. PCR based screen of genes significantly down-regulated in both mutants failed to identify *nec3* candidate gene since none of the genes appeared to be deleted to a detectable extent in the FN362 and FN363. Transcriptome of *nec3* mutants showed characteristics of barley under conditions artificially triggering senescence. Physiological characterization of *nec3* under conditions triggering senescence confirmed results of transcriptome analysis.

Results. Since transcript based cloning failed to identify *nec3* mutation we applied positional cloning for *nec3* identification. *nec3* mapping was done in two F_2 mapping populations: FN338 × GSHO2423(*nec3*.e) and GSHO2423(*nec3*.e) × GSHO1284 (*nec1.c*). Based on data from both mapping populations *nec3* position was delineated to 13 cM region on 6HS between CMWG652a and HARV32_5771. Syntenic region in rice contains 55 genes (Os02g0114200 to Os02g0124700). Functional annotation of rice gene set revealed several genes potentially involved in necrotic phenotype development or early senescence. PCR based screen of 19 homologous barley genes from FN362 and FN363 did not reveal large deletions suggesting that these genes are unlikely to be *nec3* candidates.

Conclusion. In conclusion, transcriptome analysis of fast neutron barley lmm *nec3* using Affymetrix Barley GeneChip1 failed to identify *nec3* mutation. However, cluster analysis and annotation of differentially expressed gene set from *nec3* revealed that *nec3* transcriptome shares significant overlap with barley uder conditions artificially triggering senescence. Map-based cloning has so far delineated *nec3* position to 13 cM region of 6HS. Fine scale mapping of *nec3* is required to identify mutation uderlying *nec3* phenotype.

MONITORING OF THE POPULATIONS OF *BLUMERIA GRAMINIS* F. SP. *HORDEI* IN LATVIA AND LITHUANIA

Inese Kokina[1], Izaks Rashal[2]

[1] Daugavpils University, Institute of Systematic Biology, Vienības 13, LV-5401 Daugavpils, Latvia

[2] University of Latvia, Institute of Biology, Miera 3, LV-2169 Salaspils, Latvia

** Corresponding author. E-mail: izaks@email.lubi.edu.lv*

Introduction and aim. Barley (*Hordeum vulgare* L.) is worldwide growing cereal crop including Baltic States. Powdery mildew is one of the most destructive foliar diseases of barley. The goal of this study was to characterize *B. graminis* f. sp. *hordei* populations in Latvia and Lithuania.

Materials and methods. Random samples of the causal agent of barley powdery mildew were collected in different locations of Latvia: Daugavpils (South-Eastern part of Latvia), Stende (North-Western part of Latvia), Priekuļi (North-Eastern part of Latvia) and in Dotnuva-Akademija (Lithuania). Both in conidia and cleistothecia, for isolation and multiplication of single colonies the first leaves of universally susceptible barley variety 'Otra' were used. For virulence testing of each single colony a set of differentials (Kølster et al., 1986) comprised 10 near-isogenic Pallas lines, supplemented by barley line *SI1* and three barley varieties 'Steffi', 'Goldie' and 'Meltan' with different powdery mildew resistance genes, was used. Virulence frequency, complexity and pathotypes were calculated by the computer programme RASA. For characterization of the diversity within populations and the distance between populations, Kosman indices were used (Kosman, Leonard, 2007).

Results. All diversity parameters were computed by using KOIND package (Kosman, 2002), which is based on the bootstrap method. Virulence frequencies of *Va6, Va7, Va9, Va12, Vk* and *VLa* were similarly high in all locations. Genes *Mla6, Mla7, Mla9, Mla12, Mlk* and *MlLa* can be nominating as a typical unnecessary resistance genes. Significant differences of the pathogen virulence frequencies between different samples were detected for *Va1, Va3* and *Va13.* A clear tendency to increase of frequency of mentioned virulence genes was observed in some previous years in South-Eastern part of Latvia. During the last decade considerable increasing of virulence frequency of genes *V(Me), V(St)* and *V(Go)* was detected both in Latvia and Lithuania. The presence of the above mentioned virulence in both populations of the pathogen can be explained by the fact that the correspondent pathotypes have spread from Western Europe, where the tendency to increasing was detected already in 2000. Not a single isolate with the virulence to *SI1* was detected in Latvia and Lithuania. The Daugavpils population of *Blumeria graminis* f. sp. *hordei* is especially characterised by high diversity level. Based on the investigation data it is possible to choose the most appropriate strategy for resistance breeding under both Latvian and Lithuanian conditions.

PENETRATION OF NANOPARTICLES IN FLAX (*LINUM USITATISSIMUM* L.) CALLI AND REGENERANTS

I. Kokina[1]*, V. Gerbreders[2], E. Sledevskis[2], A. Bulanovs[2], M. Jermaļonoka[1], K. Valaine[1], I. Gavarāne[1], D. Grauda[1]

[1] Daugavpils University, Institute of Systematic Biology, Vienības 13, LV-5401 Daugavpils, Latvia

[2] G. Liberts' Innovative Microscopy Centre, Daugavpils University, Vienības 13, LV-5401 Daugavpils, Latvia

** Corresponding author. E-mail: inese.kokina@biology.lv*

Introduction. There are few studies regarding the use of nanoparticles (NPs) in plant cell and tissue cultures. Uptake mechanisms and distribution of Ag NPs and Au NPs in cells are still insufficiently explored.

Aim. The aim of our research was to obtain hormone-derived transport of Ag NPs and Au NPs and demonstrate their uptake in flax regenerants.

Materials and methods. Latvian origin flax (*Linum usitatissimum* L.) accession 'Blue di Riga' was used for calli formation. The stem segments of *in vitro* grown seedlings were used as a source of explants, which were placed onto MS medium supplemented with 1 mg/l of 2.4D (2,4-Dichlorophenoxyacetic acid) and 1 mg/l of BAP (6-Benzyl-aminopurine). Previously, BAP crystals were coated with Ag NPs and Au NPs by the method of magnetron deposition in vacuum in the compact turbomolecular-pumped coating system Q150T ES. Explants cultured at 24 °C, 2 Lx, 18/8 h (day/night) photoperiod and 80% humidity for six weeks. The induced calli were transferred onto regeneration medium (MS medium with 1 mg/l of BAP). Approximately after two months of calli cultivation, regenerants observed were placed on the root growth medium.

Results. The uptake of Ag NPs and Au NPs in calli and regenerants was visualized by SEM. The sections both of calli and regenerants were placed directly in QuantomiX QX-capsules bounded by electron-transparent and pressure-resistant membrane and imaged in a conventional SEM. Absorption spectra of control calli and samples which were grown on metal nanoparticles was detected. On a medium containing Au and Ag nanoparticles, we identified increasing of optical absorption at 400–450 nm as compared to the absorption spectra in control samples. In recent studies, results of the investigations with the use of optical spectroscopy in the UV and visible ranges and transmission microscopy showed that the spectrum obtained testifies to the presence of metal nanoparticles in calli cells. Presence of nanoparticles close together in regenerants obtained from somatic embryos which were grown on a medium completed by Au and Ag nanoparticles was detected. In addition, scanning electron microscopy showed distribution of gold and silver particles inside plant cells at low magnification. At high magnification, we monitored numerous particles in organellar region. Thus, the method stated above allows to transport metal particles in calli cells, zones of regeneration and in regenerants. The fact that metal nanoparticles uptake in cells open up the possibility to apply this technology to targeted delivery in plants for general plant research and breeding (e. g. for increasing of somaclonal variation).

This work was supported by the Project of the European Regional Development Fund No. 2010/0321/2DP/2.1.1.1.0/10/APIA/VIAA/144.

PYROSEQUENCING FOR METHYLATION FEATURES DETECTION IN FLAX CALLI CULTURE OBTAINED ON MEDIUM SUPPLEMENTED WITH SILVER NANOPARTICLES

I. Kokina[1]*, V. Gerbreders[2], E. Sledevskis[2], A. Bulanovs[2], M. Jermaļonoka[1], K. Valaine[1], I. Gavarāne[1], D. Grauda[1]

[1] Daugavpils University, Institute of Systematic Biology, Vienības 13, LV-5401 Daugavpils, Latvia

[2] G. Liberts' Innovative Microscopy Centre, Daugavpils University, Vienības 13, LV-5401 Daugavpils, Latvia

** Corresponding author. E-mail: inese.kokina@biology.lv*

Introduction. For determination of DNA methylation in calli cells of *Linum usitatissimum* L., genes rich with CpG sites were chosen: complete sequence of 26S ribosomal RNA gene, 26S–18S ribosomal RNA intergenic spacer, 18S ribosomal RNA gene, internal transcribed spacer 1, 5, 8 S ribosomal RNA gene, and internal transcribed spacer 2.

Methods. PCR primers and sequencing primer were designed using PyroMark® Assay Design SW 2.0 from Qiagen. The first step for methylation analysis was sodium bisulfite conversion of unmethylated cytosines in DNA according to EpiTect® Bisulfite protocol (Qiagen, Hilden, Germany) by using EpiTect Bisulfite Kit® (Qiagen, Germany). The bisulfite-treated DNA was amplified by PCR technique. Amplification reaction was carried out on a Veriti Thermal Cycler GeneAmp© PCR System (Applied Biosystems, CA, USA) following the protocol of the PyroMark PCR Master Mix (Qiagen, Hilden, Germany). Two modification of the protocol were used: with and without Q solution (Qiagen, Hilden, Germany) in the reaction mix. The reaction was performed using the following protocol: 95 °C for 15 min, followed by 45 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s with the final extinction at 72 °C for 10 min. The sequence created by PyroMark® Assay Design SW was used for creation of the assay setup with PyroMark Q24 Application Software. Pyrosequencing was done using Pyromark Q24 and data analysis was performed by using PyroMark Q24 Software (Qiagen, Hilden, Germany).

Results. The methylation level of each CpG site in percentage was calculated by the software as the peak height of cytosine, divided by the sum of cytosine and thymine peak heights multiplied by 100. Significant differences between control and calli, grown on medium with different concentrations of nanoparticles were found. In calli grown on medium with nanoparticles the methylation level was significant higher than it was found in control calli ($p < 0.05$). Increase of the concentration of nanoparticles arised increasing of the methylation level practically in all CpG sites.

This study is supported by the Project of the European Regional Development Fund No. 2010/0321/2DP/2.1.1.1.0/10/APIA/VIAA/144.

INFLUENCE OF DIFFERENT NANOPARTICLES ON RED CLOVER CALLI CULTURE

I. Kokina*, M. Jermaļonoka, K. Valaine, I. Gavarāne, V. Gerbreders, E. Sledevskis, D. Grauda

Daugavpils University, Institute of Systematic Biology, Vienības 13, LV-5401 Daugavpils, Latvia

** Corresponding author. E-mail: inese.kokina@biology.lv*

Introduction. Red clover is the most popular fodder legume suitable for growing in agro-ecological conditions of Latvia. The somaclonal variation *in vitro* systems gives the possibility to obtain new breeding material without time-consuming hybridization. For the first time the response of calli culture of red clover on different nanoparticles (NPs) is reported.

Materials and methods. For calli culture obtaining, donor plants of red clover variety 'Skrīveru agrais' were grown on MS medium. Leaf petiols segments (5 mm) were used as explants for calli formation, which were cultivated from 3 to 5 weeks on basal MS medium with 1 mg/l of 2.4D (2,4-Dichlorophenoxyacetic acid). For further cultivation, calli were transferred on the MS medium with 2.4D supplemented with 10^{-3} g/l of different NPs (Ni, ZnO, Au and Ag). For calli ploidy detection, Partec Flow Cytometer was used. Approximately 10,000 cells were measured with a minimum of double analyses performed for each sample.

Results. Analysis of variance showed that calli size was significantly depending on the type of NPs used. The largest calli were obtained on Au and Ag NPs, as well as the most number of tetraploid cells were detected in calli which were grown on medium with NPs mentioned above. In calli which were grown on medium with Ag NPs, regeneration ability was considerable higher than in control, through embryogenesis and these calli produced more embryos per explant. Calli which were grown on medium with Au NPs were embryogenic as well, nevertheless the ratio of embryogenesis was lower compared with the control. Calli on medium with ZnO NPs, formed abnormal embryous only. Embryo-like structures were not visible on calli grown on medium with Ni NPs. Our study of red clover calli showed that there are differences in development of calli, ploidy changes in calli cells and somatic embryogenesis, which are caused by different NPs on the cultivation medium.

This study is supported by the Project of the European Regional Development Fund No. 2010/0321/2DP/2.1.1.1.0/10/APIA/VIAA/144.

THE STUDY OF DNA POLYMORPHISM OF DIFFERENTLY DAMAGED COMMON ASH INDIVIDUALS USING RAPD

R. Lukšienė, S. Kuusienė*

Lithuanian Research Centre for Agriculture and Forestry
Institute of Forestry, Laboratory of Wood Plants Biotechnologies
** Corresponding author. E-mail: biotec@mi.lt*

Introduction. Currently, massive dieback of common ash (*Fraxinus excelsior* L.) is observed in many Europien countries. Starting from 1996, large-scale dieback of ash stands has been increasingly observed in Lithuania and Poland (2). According to the forest monitoring data, until the last decade of 20th century, common ash was one of the healthienest species of forest trees in Lithuania (4). Recently, particularly in Europe, the genetic research of forest trees expand using conventional methods (1) and also molecular marker methods (3, 5).

Aim. The aim of this research was to evaluate genetic diversity of healthy and desiccated common ash trees using RAPD method.

Materials and methods. In total 30 individuals characterized by different defoliation degrees have been studied for the DNA polymorphism of common ash (*Fraxinus exelsior* L.) by analyzing 181 clearly reproducible RAPD fragment, obtained with 9 informative random primers of 13 used for the research. Eight genotype-specific bands were revealed by the primers Roth 370-05, Roth 370-06, Roth 370-10 and A07.

Results. The size of the fragments ranged from 200 to 3250 bp. Most of the fragments were amplified with the primers Roth 370-5 and Roth 370-04. The primers Roth 170-03 and Roth 170-4 were previously found to be among the most informative ones in the study on genetic diversity of 10 populations of common ash (5). Dendrogram obtained using UPGMA method showed the genetic relationship between tested individuals. All individuals do not cluster by their defoliation degree and do not show clear separate groups according to the degree of damage. The largest genetic distance (0.90 was determined between M-1 and M-7 moderately damaged individuals.

This could be explained suggesting either that only resistant individuals remain in this stand from where the leaves were picked, or that the markers studied are not associated with gene related to resistance / health.

References

1. Baliuckas V, Lagerström T, Eriksson G. Within-population variation in juvenile growth rhythm and growth in *Fraxinus excelsior* L. and *Prunus avium* L. Forest Genetics 2000; 7(3): 193–202.

2. Juodvalkis A, Vasiliauskas A. The extent and possible causes of dieback of ash stands in Lithuania. LZUU Mokslo Darbai. Biomedicinos Mokslai 2002; 56: 17–22 (in Lithuanian).

3. Ferrazzini D, Monteleone I, Belletti P. Genetic variability and divergence among Italian populations of common ash (*Fraxinus excelsior* L.). Ann Forest Sci 2007; 64: 159–68.

4. Ozolinčius R, Stakėnas V. Lietuvos miškų būklės monitoringas: vizualiai identifikuojami medžių pažeidimai ir jų dinamika. Miškininkystė 1998; 1(41): 59–71.

5. Žvingila D, Verbylaitė R, Baliuckas V, Pliūra A, Kuusienė S. Genetic diversity (RAPD) in natural Lithuanian populations of common ash (*Fraxinus excelsior* L.). Biologija 2005; 3: 46–53.

PHENOTYPIC DIVERSITY AND GENETIC POLYMORPHISM OF SEVEN CARAWAY (*CARUM CARVI* L.) GENOTYPES

Nijolė Maršalkienė*, Liuda Žilėnaitė

Aleksandras Stulginskis University,
Studentų 11, LT-53361
Kaunas district, Lithuania

** Corresponding author. E-mail: nijole.petraityte@delfi.lt*

Introduction. Caraway is the most widely grown medicinal and aromatic plant in Lithuania, its fruits are exported to the other EU countries. Caraway seeds are rich sources of essential oils and have been actively researched for their chemical composition and biological activities. Little is known about caraway genetic diversity and population structure despite its importance as an aromatic and medicinal plant.

Materials. In 2000–2004 during field trials were evaluated 107 *Carum carvi* samples from all territory of Lithuania. Qualitative and quantitative traits of caraway samples were performed. Investigation results show high variation of *Carum carvi* samples in morphological and productivity parameters.

Methods. After establishment of wide diversity of caraway characteristics, for molecular analyses were selected 6 geographically remote cenopopulations varying in phenotypic traits and Lithuanian variety 'Gintaras'. To assess *Carum carvi* genetic diversity among and inside populations by employing the random amplified polymorphic DNA (RAPD) method was used. DNA was identified by plant DNA identification package Macherey–Nagel according to the producer's methodology (Genomic DNA from Plant, 2002). OPC02 primer was employed in PRC reaction to obtain DNA fingerprints from different caraway genotypes. For some genotypes were obtained DNR profiles, recurring within cenopopulations of different origin.

Results. By molecular analyses it was found that for most individuals of the investigated cenopopulations irrespective of their place of origin 2250; 1200, 950, 740, 550 bp DNA fragment was characteristic (Figure). Only one individual had 1500, 1000, 875 and 375 bp DNA fragment and only one had 500 bp DNA.

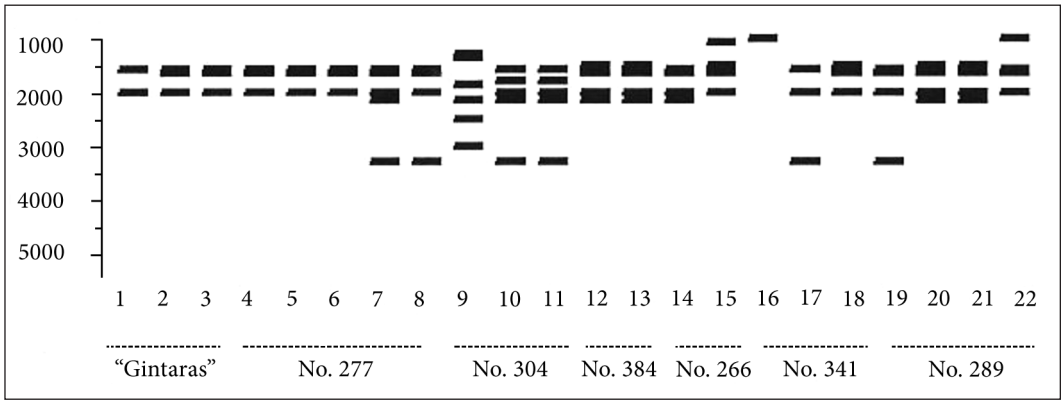

Figure. DNA fingerprints of different caraway cenopopulations

Specific DNA profiles can be suitable for estimating genomic differences between genotypes as well as inside of cenopopulations.

References

1. Johri RK. *Cuminum cyminum* and *Carum carvi*: An update. Pharmacogn Rev 2011; 5(9): 63–72.

2. Petraitytė N, Dastikaitė A. Agrobiological assessment of wild *Carum carvi* cenopopulations biodiversity. *Ex situ*. Biologija 2007; 53(4): 74–9.

3. Laribi B, Zoghlami N, Lamine M, Kouki K, Ghorbel A, Mougou A. 1RAPD-based assessment of genetic diversity among annual caraway (*Carum carvi*) populations. Eurasia J Biosci 2011; 5(5): 37–47.

FUNDAMENTAL STUDY OF SMALL RNA METHYLTRANSFERASE HEN1

Aleksandr Osipenko*, Simona Jachimovičiūtė, Giedrius Vilkaitis

Institute of Biotechnology, Vilnius University, Vilnius, Lithuania

** Corresponding author. E-mail: aleks.o@inbox.ru*

Introduction. Small RNAs are 21–30 nt long non-coding RNAs found in most eukaryotic organisms. There are two groups of these molecules in plants – microRNAs (miRNAs) that play important role in gene regulation and also small interfering RNAs (siRNAs) mostly known for transposon activity repression. In *Arabidopsis thaliana* both miRNAs and siRNAs are methylated by S-adenosylmethionine-dependent and dsRNA-specific methyltransferase HEN1 on its 2'-O-hydroxy position of 3'-end nucleotide. Latter modification is important for stability of small RNAs *in vivo*.

Materials and methods. Kinetic parameters of miRNA modification by HEN1 methyltransferase were studied in our laboratory for better understanding of this reaction. During *in vitro* experiments synthetic miR173/miR173* duplexes with radiolabelled strand of interest were modified by recombinant HEN1 protein for specified reaction time, after what methylation was stopped by sodium dodecyl sulfate and Proteinase K in the rapid quench-flow system "*KinTek RQF-3*". During subsequent sample treating with sodium periodate non-modified RNAs were shortened by one 3'-end nucleoside due to beta-elimination reaction allowing to distinguish them from full-length methylated RNAs in denaturating polyacrylamide gel electrophoresis. Modified part of the samples was measured and the rate of methylation of each strand in miRNA duplex under steady state conditions was calculated.

Results. After series of experiments was proposed two-step two-way model of miRNA duplex methylation by HEN1. According to it full miRNA duplex modification appears in two steps via intermediate hemimethylated duplex in which each of the strands can be modified first. Due to this model it was found out that HEN1 exhibits preferability to one of the strands and miR173* is methylated first about five times more often than miR173. It also revealed that methyl group on one of the duplex strands does not influence methylation kinetics of the second one.

THE EVALUATION OF RAMAN SPECTRA OF NUCLEAR PLANT DNA AT DIFFERENT CONDITION

A. Petjukevičs, N. Škute*, A. Lukaševiča

Daugavpils University, Faculty of Natural Sciences and Mathematics, Institute of Ecology, Vienības 13, LV-5400 Daugavpils, Latvia

** Corresponding author. E-mail: natalja.skute@du.lv*

Introduction. The goal of this preliminary study was to establish the ability of Raman scattering spectroscopy for high throughput express analysis of the structure of DNA.

Materials and methods. The nuclear DNA from different plants (*Triticum* spp., *Nicotiana tabacum*, *Lemna minor* and *Hydrocotyle vulgaris*) was investigated. Quality of the DNA samples was analyzed by spectrophotometry (A260/280) and by agarose gel electrophoresis (1.8%). In this study also was found the effect of quenching of luminescence in adsorption on the surface of silver, which had made it possible to obtain more precise spectra. Spectra of DNA were obtained on Raman microscope instrumentation employing 514.5 nm (air cooled Argon Ion laser) and 785.0 nm (diode laser) excitations, respectively without the addition of reagents. Renishaw inVia system, which we are using in this analysis, was equipped with the Leica DM 2500 researcher grade optical microscope. This highly collimated, monochromatic light illuminates on a sample through a Leica objective and the Raman scattered light from the sample is collected by an objective, analyzed through inVia Spectrometer, and focused on a Renishaw air cooled high sensitivity ultra-low noise *RenCam* CCD array detector. The Raman scattering was collected with a 50X short distance objective. The radiant power of the laser was maintained at or below 200 mW at the laser head (<15 mW at the sample).

Results. Raman spectra were collected over the 200–1 800 cm^{-1} region and 100–3 200 cm^{-1} for comparing. The data are unsmoothed averages of 5–10 exposures obtained with an integration time of 50 and 100 s/exposure and the spectral resolution was less than 2 cm^{-1}. Data collection was accomplished with *WiRE 3.0 Raman software,* spectrometer scans, data collection and processing were controlled by a personal computer. Processing of the spectral data, including decomposition of complex bandshapes, was accomplished with *WiRE 3.0 Raman software* for curve fitting. The pure nuclear DNA free from contaminants and enzyme inhibitors was prepared with Qiagen *DNeasy 96 Plant* Kit and by traditional DNA extraction protocol. Laser radiation was focused by the lens on the quartz glass cuvette with dissolved plant DNA (high and *low molecular weight DNA*, fragmented DNA). As a result of this research there have been discovered, analyzed and found patterns in change of Raman spectra depending on different condition of DNA. A detailed analysis of changes in the Raman shift peaks, and the intensity of the peaks in the future will be able to expand the application of Raman Spectroscopy in the express diagnostics of plant DNA.

YELLOW RUST (*PUCCINIA STRIIFORMIS* F. SP. *TRITICI*) IN LATVIA IN 2012

Ilze Priekule[1]*, Vija Strazdina[2], Kerstin Gillen[3]

[1] *Latvian Plant Protection Research Centre, Struktoru 14a, LV-1039 Riga, Latvia*

[2] *Department of Cereal Breeding, State Stende Cereal Breeding Institute*

[3] *Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences*

* *Corresponding author. E-mail: ilze.priekule@laapc.lv*

Introduction. Yellow rust caused by *Puccinia striiformis* f. sp. *tritici* is very important disease of wheat. Serious outbrakes of disease were observed in early 1990s when in BBCH 75–77 a severity of disease reached 50–70% of some varieties. A period with very rare reports about a yellow rust infection in wheat in Latvia lasted till 2012 when signs of increasing of disease spreading were observed. In 1980–1990 effective resistance genes in Eastern Europe were Yr6–Yr8. In 2009–2010 a serious outbreak of yellow rust was reported in many countries of the North Africa and East Asia, where the resistance of Yr9 was overpowered. There are also reports from Europe, for example from South Sweden about a serious yellow rust infection in wheat in 2008–2010. Therefore a question about a possible genetic variation of *P. striiformis* population in Latvia arose.

Materials and methods. In 2012 the following research was done: 1) evaluation of winter wheat collection items determination yellow rust severity in % at BBCH 73–75; 2) comparison and analyzing of data given by the same collection items in early 1990ies and 2012; 3) analyzing of Latvian yellow rust population (Stende collection) by multiplex PCR genotyping using microsatellite primers and comparison with populations of South Sweden and Estonia.

Results. Results of field assessments of yellow rust severity on winter wheat collection varieties and lines showed a variation from 0 to 55%, the severity of the disease for majority of varieties was 0–10%. Comparison of the yellow rust infection level of varieties evaluated in early 1990thies and 2012 showed similar results, therefore, it can be assumed that there are no significant changes in a genetic structure of the yellow rust population at least in one locality in Latvia, namely in Stende.

Results of multiplex PCR genotyping of yellow rust populations from Stende, South Sweden and Estonia will allow a better understanding of the situation with this population. Data will be presented in poster presentation at the congress.

THE SYNTHESIS OF DIFFERENT ISOFORMS OF ANTIOXIDANT ENZYMES AS A REACTION ON HEAT STRESS IN DIFFERENT WHEAT ORGANS (*TRITICUM AESTIVUM* L.)

Marina Savicka*, Natalja Shkute

Ecology Institute, Daugavpils University,
Vienības 13, LV-5400 Daugavpils, Latvia

** Corresponding author. E-mail: marina.savicka@du.lv*

Introduction. Heat stress adversely affects plant growth and development and induces oxidative stress in plants. To understand the effect of high-temperature stress on plant defence system, it is necessary to study gene expression of the antioxidant systems of whole plants and their organs.

Materials and methods. Impact of elevated temperature on some antioxidant (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD)) electrophoretic activity in organs of etiolated wheat seedlings was examined for characterizing the response of wheat (*Triticum aestivum* L., cv. Harmonia) seedlings to heat stress. The seedlings were exposed to 42 °C for 15 min, 30 min, 60 min and 24 h, and first leaves, coleoptiles and roots were taken immediately after high-temperature exposure and at twenty-four hour intervals for measurement of antioxidant (SOD, CAT, POD) electrophoretic activity.

Ingel assays indicated variation in intensities of SOD, POD and CAT during stresses. CAT isozymes showed reduced intensity during temperature stress in the root of wheat seedlings. Native PAGE of the crude extracts showed the presence of a single isoform of CAT in the first leaf, but one more isoform of CAT appeared in the coleoptile after 24 h high temperature exposure.

Results. The POD intensity decreased in the first leaf and root after long-term high-temperature exposure. Inhibition of activity of some POD isoforms after long-term (24 h) high-temperature exposure was reported in roots and leaves of wheat seedlings. An insignificant increase in POD electrophoretic activity was indicated in leaves after heat stress. Electrophoresis indicated two SOD isoforms (CuZnSOD and MnSOD) isoforms. The electrophoretic activity of SOD in the wheat organs increased during the development and did not change after short-term high-temperature exposure. An insignificant increase in SOD activity was indicated in leaves after heat stress. However, inhibition of CuZnSOD activity after long-term (24 h) high-temperature exposure was reported in roots of wheat seedlings. Cu, Zn-SOD and Mn-SOD found in the coleoptile of wheat seedlings were more active in heat-treated plants than in control.

This study was supported by ESF project No. 2009/0205/1DP/1.1.1.2.0/09/APIA/VIAA/152.

EVALUATION OF LUMINOMETRIC METHODS FOR STUDY OF THE GC AND GNC METHYLATION IN NUCLEAR DNA OF SOME PLANTS

Natalja Shkute*, Dmitrij Uzhameckis

Ecology Institute, Daudagpils University,
Vienības 13, LV-5400 Daugavpils, Latvia

** Corresponding author. E-mail: natalja.skute@du.lv*

Enzymatic methylation of nuclear DNA with creation of 5-methylcytosine (5mC) is one of major mechanisms of epigenetic modification. Change in overall DNA methylation pattern involving both hypomethylation and hypermethylation, is frequently observed in normal and pathological cellular processes, contributing both to development and differentiation. The regulatory effects of DNA methylation could be divided in two broad categories: specific, when a particular gene activity is directly influenced by methylation of its regulatory regions, and general, when methylation causes changes in the chromatin structure. In plants, 5mC comprises up to 30%. The cause of this phenomenon and role of nuclear DNA methylation in plants are still not clear. Analysis of global DNA methylation may be a powerful tool to provide basic information about these mechanisms. Pyrosequencing is the method of choice whenever short or medium DNA sequences need to be analyzed with high precision and in a quantitative manner. It was the first attempt to quantitatively analyze plant global genomic DNA methylation, using a luminometric technology (LUMA) coupled with methylation sensitive restriction analysis. The LUMA method is based on digestion of genomic DNA with methylation sensitive and insensitive restriction enzyme, followed by a quantification of the resulting number of cut sites using a luminometric polymerase extension platform for the experimental read out. We used the CpG methylation sensitive restriction enzyme HpaII and its CpG methylation insensitive isoschizome MspI and AjnI and PSp6I restriction enzyme also in parallel reactions. EcorRI was included in all reactions as a normalization reference. For each assay, thease can thus be filled in by separated polymerase extension through the sequental addition of dNTPs and individual assesssment of each restriction overhang can therefore be separately determined. Following the successful extension of a dNTP, inorganic pyrophosphate (PPi) is released and converted to ATP by ATP-sulfurylase and adenosine-5 phosphorisulfate. Luciferin is subsequently converted by luciferase to oxyluciferin using the formed ATP, to produce a proportional amount of visible light, which is detected. The light peacs produced by dATP and dTTP addition both represent EcoR I cleavage and are therefore expected to be equal to one another. The assay was successfully applied for studying of intra-individual changes of global DNA methylation in different zones of a cultivated tobacco (*Nicotiana tabaccum* L.) and wheat (*Triticum aestivum* L.) leaf during its senescence. High sensitivity and versatility to perform accurate DNA methylation studies in a large number of plant tissue samples have been shown.

GENETIC AND BIOTECHNOLOGICAL ADVANCES IN BREEDING OF RESISTANCE TO APPLE SCAB AT THE INSTITUTE OF HORTICULTURE, LRCAF

S. Sikorskaite, D. Gelvonauskiene, P. Haimi, G. Staniene, A. Sasnauskas, V. Stanys, D. Baniulis*

Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai LT-54333 Kaunas district, Lithuania

** Corresponding author. E-mail: d.baniulis@lsdi.lt*

Apple fruits constitute the main part of horticultural production in Lithuania. Demand for high quality and organic production requires development of scab resistant apple cultivars based on natural plant protection technologies. A qualitative and quantitative resistance to apple scab (*Venturia inaequalis*) is characteristic to *Malus* sp. plants, therefore identification of resistance genes and efficient introduction of the genes to apple cultivars is crucial for apple breeding. In addition, the importance of pyramidization of resistance genes is emphasized in the breeding programme at the IH, LRCAF due to the genetic variability of the scab pathogen and the risk of loss of resistance in widely grown apple cultivars. Apple accessions featuring complex monogenic resistance to apple scab were obtained from crosses of cultivars with monogenic resistance traits during last decade. Current research incorporates application of genetic and biotechnological approaches for identification of new sources of resistance to apple scab. Over 40 accessions of the traditional cultivars and cultivars derived from crosses with the traditional cultivars are deposited at the collection of genetic resources of the Institute of Horticulture, LRCAF. A study on genetic polymorphism and morphological traits of these cultivars revealed new genetic sources for apple scab resistance breeding. Further, a biotechnological method of screening for apple scab resistance at embryonic stage using apple seed cotyledons is employed to resolve a complexity of genetic background of resistance of the identified genotypes. In addition to identification and pyramidization of major resistance genes, an emphasis is laid on understanding of the mechanistic basis of the regulation of the apple scab induced resistance response in apple. Therefore an experimental approach involving proteomics analysis of *Malus* sp.–*V. inaequalis* interaction has been established to reveal genetic constituents of the regulation of resistance response. This study will lead to development of molecular markers important for the disease resistant breeding in apple and related plant species of the Rosaceae family.

ASSESSMENT OF GENOTOXIC PROPERTIES OF HIGHBUSH BLUEBERRY LEAF EXTRACTS ISOLATED FROM VARIOUS CULTIVARS

G. Slapšytė[1]*, V. Dedonytė[1], J. Mierauskienė[1], P. R. Venskutonis[2]

[1] *Department of Botany and Genetics, Vilnius University, Lithuania*

[2] *Department of Food Technology, Kaunas University of Technology, Lithuania*

* *Corresponding author. E-mail: grazina.slapsyte@gf.vu.lt*

Introduction. The highbush blueberry (*Vaccinium* × *covilleanum* Butkus et Pliszka) berries are widely consumed, they are very rich in phenolic phytochemicals which are known to have strong antioxidant properties and potential health benefits. Recently it was estimated that concentration of antioxidants in leaves is even higher then in berries. However, studies show that many antioxidants including phytophenolics can also exhibit prooxidant and cytotoxic properties under certain conditions. Besides, chemical composition of plants as well as amount of phenolic compounds is conditioned both by genetic (cultivar) and environmental (weather, agronomic practices) factors.

Aim and methods. The aim of this study was to evaluate the genotoxic potential of highbush blueberry leaf extracts isolated from seven cultivars: 'Gila', 'Gretha', 'Northblue', 'Northland', 'Bluecrop', 'Dixi', and 'Putte'. The total phenolic contents in methanolic extracts was in a range of 102.85 to 237.89 mg gallic acid equivalents in 1 g. Genotoxicity was investigated using Ames Salmonella/microsomal test, alkaline single-cell gel electrophoresis (comet) assay and cytokinesis-block micronucleus test in human blood cells *in vitro*. The micronuclei (MN) frequency was expressed as MN/1 000 binucleated cells, and the considered parameters for comet analysis were tail length (TL), percentage of DNA in the tail (%TDNA), tail moment (TM). The ranges of exposure concentrations were between 50 and 300 μg/ml.

Results. Ames test was carried out using *Salmonella typhimurium* strains TA98 and TA100, with or without S9 metabolic activation. All investigated extract samples showed negative results in the Ames test, indicating that they do not produce reverse mutation in bacterial cells. In the micronucleus test, only a slight (not significant) increase of micronuclei frequency was determined after treatment with single doses of blueberry leaf extracts. The results of the comet assay revealed different genotoxic potential of the extracts as well as different susceptibility of the donors to the extracts tested. Statistically significant genotoxicity with a clear dose-dependent effect (e. g. for %TDNA) was determined for 'Northblue' ($r = 0.9535$; $P = 0.032$), 'Northland' ($r = 0.8765$; $P = 0.0219$) and 'Putte' ($r = 0.8398$; $P = 0.0364$) extracts in the lymphocytes of the first donor, and 'Bluecrop' ($r = 0.8810$; $P = 0.0204$) and 'Gretha' ($r = 0.9168$; $P = 0.0101$) extracts in the lymphocytes of the second donor. Extract of 'Dixi' cultivar had little or none genotoxic effect on both donors cells.

Conclusions. Thus, the data of the present study show that highbush blueberry leaf extracts are not genotoxic in the Ames and micronucleus tests, but can induce genetic damage evaluated by the comet assay. The determined variation in response was due to the cultivar tested and difference in susceptibility between donors.

This research was funded by Research Council of Lithuania, grant No. SVE-04/2011.

IDENTIFICATION OF INTERSPECIFIC HYBRIDS BETWEEN *LILIUM CANDIDUM* L. AND ASIATIC HYBRID LILIES BY INHERITANCE OF DNA MARKERS

Žilvinas Sliesaravičius[1]*, Juozas Proscevičius[2]

[1] *Nature Research Centre, Institute of Botany, Akademijos 2, LT-08412 Vilnius, Lithuania*

[2] *Faculty of Natural Sciences, Lithuanian Educological University, Studentų str. 39, LT-01806 Vilnius, Lithuania*

* *Corresponding author. E-mail: zilvinass@gmail.com*

Introduction. The lilies *Lilium* are used widely as model crop in solving problems of pre- and post- zygotic isolation barriers during inter-specific hybridization, as well as in investigation of interaction between alien genomes in hybrids. Reciprocal interspecific hybrids are valuable as model systems in studying epigenetic phenomena. However, obtaining reciprocal interspecific hybrids often is problematic. Previously we received interspecific hybrids between lilies from horticular group Asiatic Hybrids (AH) and species *Lilium candidum*. To overcome pre-zygotic isolation barriers and to receive reciprocal hybrids *L. candidum* × AH method of pollination by mixed incongruous pollen was applied. Culture of isolated immature embryos *in vitro* was performed to overcome post-zygotic isolation barrier. 7 progenies were received after pollinatin of female *L. candidum* with mixed pollen of AH cultivar *Red Carpet* and *L. longiflorum*. Hybrids *L. candidum* × AH (*Red Carpet*) were screened by inheritance of DNA markers characteristic of *Red Carpet.*

Methods and results. After testing nucleotide primers OPA1, OPA4, OPA5, OPA10, OPA11 for amplification of genomic DNA by PGR the primer OPA11 and OPA10 were used to detect random amplified polymorphic DNA (RAPD) characteristic to cultivar *Red Carpet*. Among 7 tested offsprings originated from random paternity 4 inherited DNA fragment of 400 bp characteristic of *Red Carpet* after amplification by primer OPA11, however only 3 of them inherited DNA fragment of 280 bp characteristic to *Red Carpet* after amplification by primer OPA10. Only hybrids which inherited both these fragments were selected as *L. candidum* × AH hybrids.

References

1. Proscevičius J, Kleizaitė V, Dambrauskaitė D. Screening of hybrid progeny derived from *Lilium candidum* L. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture 2007; 26: 187–93.

2. Mc Rae A. Lilies: a guide for growers and collectors, Timber Press, Portland, Oregon 1998, 392.

3. Van Tuyl JM, Van Dien MP, Van Criej MGM, Kleinwee TCM, Franken J, Bino RJ. Application of in vitro pollination, ovary culture, ovule culture and embryo rescue for overcoming incongruity barriers in inter-specific *Lilium* crosses. Plant Sci 1991; 74: 115–26.

DEVELOPMENT OF FUNCTIONAL MARKERS FOR PLANT ARCHITECTURE GENES (FUMAG)

Gražina Statkevičiūtė[1]*, Andrius Aleliūnas[1], Vilma Kemešytė[1], Izolda Pašakinskienė[2], Gintaras Brazauskas[1]

[1] Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto a. 1, Akademija, LT-58344 Kėdainiai district, Lithuania

[2] Faculty of Natural Sciences, Vilnius University, M. K. Čiurlionio 21, LT-03101 Vilnius, Lithuania

** Corresponding author. E-mail: grazinastat@lzi.lt*

Introduction. Plant architecture describes tri-dimensional structure of the plant. Genetic control of this complex system of traits plays an important role in crop productivity regulation, however only a limited number of functional mutations in plant architecture genes have been identified so far. The best known examples come from wheat stem length and maize tillering genetic regulation. Plant architecture control in perennial grasses is less understood despite high levels of variation and versatile application of these plants as traditional fodder and amenity grasslands, as well as bioenergy biomass producers.

Aim and methods. The aim of this project is to develop functional markers for perennial plant architecture genes. DNA polymorphism in five perennial ryegrass genes will be studied, which orthologs in related species were shown to control plant architecture traits. Functional markers will be identified in those genes by association analysis combining data set of DNA and phenotypic variation in a panel of diverse ryegrass genotypes. Preliminary results of genetic variation and population structure in a set of 200 perennial ryegrass genotypes, based on AFLP markers, will be presented at the Congress.

FLOWER STRUCTURE OF BARLEY HYBRIDS BETWEEN *TWEAKY SPIKE* AND *HOODED*-TYPE MUTANTS DEPENDS ON THE PECULIARITIES OF SPIKE MORPHOLOGY

R. Šiukšta[1, 2*], V. Vaitkūnienė[1], J. Žukauskaitė[2], I. Fiodorovas[1], V. Rančelis[1]

[1] *Department of Botany and Genetics, Vilnius University, M. K. Čiurlionio 21, LT-03101 Vilnius, Lithuania*

[2] *Botanical Garden of Vilnius University, Kairėnų 43, LT-10239 Vilnius, Lithuania*

* *Corresponding author. E-mail: Raimondas.Siuksta@gf.stud.vu.lt*

Introduction. Pleiotropic barley mutant *tweaky spike 2* (tw_2) is the unique developmental mutant having unfixed homeotic transformations of lodicules to stamens and / or pistils accompanied by alteration of the number of flower organs. Mutant tw_2 also has well expressed gradient of spike development characterised by sterile flowers at the lower two-row part of the spike and overdeveloped multi-row upper part (a "crown"). Hybridization of mutant tw_2 with various six-row *Hooded*-type mutants, having normal basic flower and ectopically developed extra flower of inverse polarity instead of the lemma awn or on it, led to the great diversity of hybrids with differentially expressed parental features.

Results. The analysis showed the correlation between the *tw*-type spike phenotype and the level of variation in basal flower structure: the more attributes of *tw* spike it has, the more abnormal basic flowers it contains, i. e. the highest variation in the basal flower structure was revealed in two-row or *intermedium*-type hybrids having "crown" and / or flowerless breaks in spike. In addition, a broad spectrum of awn and extra flower alterations was identified not only among different hybrids but also along the same spike. The proportion of extra flowers with generative organs depends mainly on the distance from extra flower to basal flower: extra flower developed directly on lemma is more likely to have sexual organs while extra flower developed at the distal part of the awn typically consists of sterile flower organs e. g. lodicules. Further observations also showed the dependence of variation in basal and additional flower structure on the growing conditions of plants.

COMPARISON OF GEOGRAPHICALLY REMOTE POPULATIONS OF INVASIVE PLANT SPECIES *ERIGERON ANNUUS*

Virginija Tunaitienė*, Jolanta Patamsytė, Tatjana Česnienė, Violeta Kleizaitė, Donatas Naugžemys, Virginija Vaikūnienė, Vytautas Rančelis, Donatas Žvingila

University of Vilnius, Lithuania

** Corresponding author. E-mail: virginija.tunaitiene@gf.vu.lt*

Introduction. *E. annuus* (Asteraceae) is a winter annual, triploid and apomictic plant native in the eastern parts of the United States. In the 17th century this species was introduced to Europe. During the 20th century, *E. annuus* spread in many parts of the continental Europe, especially on roadsides and in ruderal places (Edwards et al. 2006). At the end of 19th century *E. annuus* reached Lithuania and now is widely spread in the south and southeastern part of the country and has the potential to be serious environmental weed (Gudžinskas, 1997).

Methods and results. We used DNA molecular markers to provide estimates of the comparative genetic variation within and among populations of *E. annuus* from Lithuania and some other countries, where this exotic species was introduced earlier. Analysis of DNA markers conducted using AMOVA showed high genetic differentiation among populations of *E. annuus*. Breeding system had a very strong impact on the level of genetic variability. Identical molecular phenotypes were established among plants of different populations of this apomictic species. Cluster analysis showed a moderately expressed regional grouping of the studied populations. The impact of genetic structure on the further spreading of *E. annuus* is discussed.

References

1. Edwards PJ, Frey D, Bailer H, Baltisberger M. Genetic variation in native and invasive populations of *Erigeron annuus* as assessed by RAPD markers. Int J Plant Sci 2006; 167: 93–101.

2. Gudžinskas Z. Conspectus of alien plant species of Lithuania. Botanica Lithuanica 1997; 3: 335–66.

COMPARISON OF SOME ASPECTS OF GENETIC DIVERSITY BETWEEN TWO ALIEN SPECIES OF FABACEAE IN LITHUANIA

R. Vyšniauskienė*, V. Rančelienė

Institute of Botany, Nature Research Centre, Vilnius, Lithuania

** Corresponding author. E-mail: regina.vysniauskiene@botanika.lt*

Introduction. Alien plants of the *Fabaceae* family are found in Lithuania since the first half of the 20th century. Alfalfa (*Medicago sativa*) was started to grow in cultural areas as a protein-rich animal feed, and large-leaved lupine (*Lupinus polyphyllus*) was primarily sown as forage for wild animals in the forests and for prevention of fires. In Lithuania lupine is regarded as invasive species that should be eradicated in certain ecosystems. The species spread from the forest outskirts to the abandoned cultivated fields; the plants are frequent and abundant on roadsides, along railway lines. Due to favourable climatic conditions alfalfa is starting to spread from cultivated fields into natural ecosystems.

Aim and methods. The aim of the present work was to compare some major aspects of genetic diversity of these alien *Fabaceae* plants of unequal spread and to determine whether potential of *Medicago sativa* genetic diversity favours its invasiveness increase in the future. Eight *Lupinus polyphyllus* and six *Medicago sativa* populations from different regions of Lithuania were studied. Investigations were carried out by random amplified polymorphic DNA (RAPD) method.

Results. A significant DNA polymorphism was recorded in populations of both species. Molecular genetic diversity analysis (AMOVA) showed that populations of both species are characterized by high genetic differentiation. Higher genetic differentiation and greater genetic distances were revealed among *Medicago sativa* populations. Molecular studies showed that populations of both *Lupinus polyphyllus* and *Medicago sativa* possess high potential of genetic variability, which can lead to rapid expansion of these alien species into natural ecosystems as well as require a wider range of measures in order to stop their penetration, especially that of *Lupinus polyphyllus*.

References

1. Gudžinskas Z. Conspectus of alien species of Lithuania. 10. Fabaceae, Bot Lithuanica 1999; 5: 103–14.

2. Lambdon PW, Pyšek P, Basnou C, Hejda M, Arianoutsou M, Essl F, Jarošík V. et al. Alien flora of Europe: species diversity, temporal trends, geographical patterns and research needs, Preslia 2008; 80: 101–49.

3. Riday H, Brummer EC, Campbell TA, Luth D, Cazcarro P. Comparisons of genetic and morphological distance with heterosis between *Medicago sativa* subsp. *sativa* and subsp. *falcata*, Euphytica 2003; 131: 37–45.

4. Tucak M, Popovič S, Čupič T, Grljušič S, Bolarič S, Kozumplik V. Genetic diversity of alfalfa (*Medicago* spp.) estimated by molecular markers and morphological characters, Period Biol 2008; 110: 243–9.

GENETIC DIVERSITY OF *IMPATIENS GLANDULIFERA* POPULATIONS IN LITHUANIA USING MOLECULAR MARKERS

Lina Zybartaitė, Kristė Stravinskaitė, Judita Žukauskienė, Daiva Ambrasienė, Algimantas Paulauskas, Eugenija Kupčinskienė*

Department of Biology, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

** Corresponding author. E-mail: e.kupcinskiene@gmail.com*

Introduction. In the beginning of 19th century introduced from Himalayas Mountains to Europe, *I. glandulifera* rapidly became favorite ornamental plant (Morgan, 2007). Nowadays it is among the most invasive plants in almost whole Europe including Lithuania where it might be found throughout all the country. Concerning Baltic States, *I. glandulifera* data mainly refer to geography of distribution (Priede, 2008). In this region no accounts exist for genetic differentiation of the species.

Aim. The objective of this study was to evaluate genetic variability of different in geography and habitats populations of *I. glandulifera* growing in Lithuania.

Methods. Simple Sequence Repeats (SSR) and Randomly Amplified Polymorphic DNA (RAPD) methods were chosen. A total of 20 populations (15 individuals in each) of *I. glandulifera* were sampled in various places of Lithuania. For molecular analyses 6 SSR markers (IGNSSR101 / EF025990, IGNSSR104 / EF025992, IGNSSR106 / EF025993, IGNSSR203 / EF025994, IGNSSR210 / EF025995, IGNSSR240 / EF025997) suggested by Provan et al. (2007) were used.

Results. Each primer pair generated two different alleles, fragment size ranged from 100 to 156 bp. Genetic parameters of Lithuanian populations of *I. glandulifera* were as following: observed heterozygosity (H_o) ranged from 0 to 1, mean being 0.5. Respectively expected heterozygosity (H_e) ranged from 0.2 to 0.5, the mean being 0.4. For RAPD analyses OPA-20, OPD-20, 222, 250, 269, 340, 474, 516 markers were used. Selected RAPD markers generated between 18 and 30 bands each, in total 188 bands were recorded and all of them were polymorphic. Among populations of *I. glandulifera* genetic parameters ranged in the following intervals: percentage of polymorphic DNA bands was between 40 and 56%, Nei's gene diversity interval was 0.115–0.165 and Shannon's information index ranged between 0.179–0.255. Pairwise genetic distances between populations were 0.088–0.259. The UPGMA dendrogram revealed clear differentiation between the populations, PhiPT value was 0.511 ($p \leq 0.01$).

Conclusions. Our RAPD analyses indicate multiple introduction of this species in Lithuania. In Lithuania ditches, backyards, abandoned estates or landfills are the most common surroundings for *I. glandulifera* while wet natural sites such as riverbanks or lakesides are less frequent habitats. Presumably various ways of expansion of *I. glandulifera* take place, among them the prevailing one is gardening and removal of excessive seedlings unintentionally throwing them away from allotments. According to our habitat analyses, the most successful *I. glandulifera* invasion occurs from the yards along roads with excavated in parallel dikes.

References

1. Morgan RJ. Impatiens: the Vibrant World of Busy Lizzies, Balsams, and Touch-me-nots. Portland, USA 2007, 219.

2. Priede A. Distribution of some invasive alien plant species in riparian habitats in Latvia. Botanica Lithuanica 2008; 14(3): 137–50.

3. Provan J, Love HM, Maggs CA. Development of microsatellites for the invasive riparian plant *Impatiens glandulifera* (Himalayan balsam) using intersimple sequence repeat cloning. Mol Ecol Notes 2007; 7: 451–3.

EVALUATION OF DIVERSITY OF *IMPATIENS PARVIFLORA* POPULATIONS GROWING IN LITHUANIA

Lina Zybartaitė, Rasa Janulionienė, Ramūnas Vilčinskas, Judita Žukauskienė, Daiva Ambrasienė, Algimantas Paulauskas, Eugenija Kupčinskienė*

Department of Biology, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

** Corresponding author. E-mail: e.kupcinskiene@gmail.com*

Introduction. In 19th century small balsam (*Impatiens parviflora*) was introduced from Asia to Europe. Within several decades it has been recorded as naturalized and has spread through most countries of Europe. Presently it is considered being among the most invasive species in Central and Western parts of the continent. In Northern Europe such process has been speeded only within recent decades. In addition to human settlements *I. parviflora* very often grows along forest roads most intensively crowded by transport. It starts to be a very common herbaceous plant species in main nature tourism centers. This species is affecting ground-layer vegetation displacing native community components having similar biology. Very frequent neighbour of *I. parviflora* is related species *I. noli-tangere* naturally growing in wet deciduous forests. In Lithuania *I. parviflora* was introduced into Vilnius Botanical Gardens and in 1934 it was recorded as an escape in suburbs of the city. It has gradually been recognized that ecological attributes alone are insufficient to explain why some plant species become invasive.

Aim. Our study aimed at evaluation of genetic variability and habitat features of *I. parviflora* populations growing in Lithuania.

Methods. Interrelations between habitat features and genetic variability of *I. parviflora* were tested employing two molecular methods: RAPD (Randomly Amplified Polymorphic DNA) and SSR (Simple Sequence Repeats). Twenty one population of *I. parviflora* from different parts of Lithuania was selected for genetic studies. In total 315 individuals were examined employing RAPD and SSR markers. For RAPD analysis 30 primers were tested and 8 out them (222, 250, 269, 340, 474, 516, OPA-20, OPD-20) appeared to be the most valuable for genetic diversity studies. For SSR analysis 7 microsatellite primers (INGSSR101 EF025990, IGNSSR103 EF025993, IGNSSR203 EF025994, IGNSSR210 EF025995, IGNSSR240 EF025997) designed for *I. glandulifera* by Provan et al. (2007) were tested.

Results. Natural and anthropogenic habitat features of selected sites of *I. parviflora* were classified according to the water source and its proximity in these categories: 1) overmoistured, parallel to the dike / groove / stream, 2) in a 50–100 m distance from the river bank, 3) no water basin in the vicinity. Light intensity was characterized estimating life form of neighboring plants: 1) open place with shrubs / individual trees, 2) park / forest edges of the woods, 3) roads, paths inside wood). Sites were subdivided taking into account traffic intensity and road type also vicinity: 1) along blacktop road with intensive traffic, 2) along blacktop road of the city / town with low intensity traffic, 3) along the road without blacktop or along footpath in the forest. Proximity to any type of the buildings was recorded: backyards of the estates / houses / farms. According to size (estimated based on the length of transect through the population) populations were divided into following groups: small size (<100 m), intermediate size (100–500 m), big size (500–1 000 m), very big size (>1 000 m).

Conclusions. Data show that in Lithuania *I. parviflora* occurs abundantly in disturbed by human localities – urban sites, roadsides, parks or forest edges. Molecular diversity will be discussed in relation to habitat types.

References

1. Provan J, Love HM, Maggs CA. Development of microsatellites for the invasive riparian plant *Impatiens glandulifera* (Himalayan balsam) using intersimple sequence repeat cloning. Mol Ecol Notes 2007; 7: 451–3.

DESCRIPTION OF GENETIC RESOURCES OF OATS ORIGINATED IN LATVIA

Sanita Zute*, Zaiga Vicupe, Linda Brunava

State Stende Cereal Breeding Insitute, Dižzemes, p.o. Dižstende, LV-3258 Talsi Region, Latvia

** Corresponding author. E-mail: sanita.zute@stendeselekcija.lv*

Introduction. Oats as one of most economically valuable species of cereals are highly diverse on a world-wide scale less investigated till now compared to wheat, barley, maize and other species. Genetic resources of local origin oats have gradually investigated since 1923, when oat breeding was started in Latvia (Lielmanis, Berzins, Garbars, 1947). The State Stende Cereals Breeding Institute is considered as oat breeding centre of Latvia, where over a period of ninety years 16 oat varieties have been developed (Rashals, Zute, Marga, 1998; Zute, Belicka, Kalmanis, 2012).

Materials and methods. Seed samples of 11 oat varieties of Latvian origin, 13 local oat populations and 53 valuable breeding lines currently are in preservation in Latvia Crop Plant Gene bank. Investigation results of genetic resources of Latvian origin oats have been summed up in this report from 2007 to 2009. Field trials were established in Talsi region at the State Stende Cereal Breeding Institute. This research consisted of 11 oat varieties (Table) and 53 breeding lines developed by Latvian breeders. Description of oats includes assessment of morphological and biological traits: plant growth habit; time of panicle emergence and ripening, length of plant, lodging resistance, grain husk and color of lemma, tendency to be awned etc., agronomic and biochemical traits: grain yield, hull content in yield, grain test weight, 1000-grain weight, crude protein, crude fat and β-glucan content in grain.

Table. Oat varieties origined in Latvia and used in the research, 2007–2009

No.	Varieties	Information on origin	Year of registration
1.	Stendes Dārta	PCU- 32 /3/ Panther // Sang / Parsival	2005
2.	Arta	Hja71801 / Kurokaura	1993
3.	Laima	Pol 1712 / D-104	1992
4.	Stendes Līva	Dzintara // Sovetskij / Premis /3/ Ebequeit	1986
5.	Stmara	Astor // Stendes vēlās / Premis	1985
6.	Māra (light)	Astor // Stendes vēlās / Premis	1985
7.	Santa	Blenda / Kijev	1978
8.	Stendes vēlās	Stendes Dzintara / Sovetskij	1960
9.	Stendes dzeltenās	Stendes Dzintara / Svalöf Uzvara // Sovetskij	1959
10.	Līgo	SW Ligovo2 / Stendes mazās agrās	1936
11.	Stendes mazās agrās	Selection from local population from Nereta (Latvia)	1930

Results. Results of these investigations are important because in order to describe genetic resources of oats of Latvian origin by unified methods and these results present comprehensive information regarding economic importance of oat varieties and breeding lines accepted for inclusion in gene bank.

References

1. Lielmanis J, Bērziņš E, Garbars J. [25 working years of Stende breeding station]. Stendes selekcijas stacija. 1947, 354 (in Latvian).

2. Zute S, Belicka I, Kalmanis Z. State Stende Cereal Breeding institute: from the origin to the present. Proceedings of the Latvian Academy of Sciences. Section B: Natural, Exact and Applied Sciences 2012; 66(1/2): 59–70.

3. Rashals I, Zute S, Marga G. Status of oat collections in Latvia. Report of a Working Group on Avena, 5th meeting ECP/GR. 1998 May 7–9; Vilnius, Lithuania 1998; 48.

STUDY OF GENETIC VARIABILITY BY RETROTRANSPOSONS-BASED MOLECULAR MARKERS IN WHEAT CALLI CULTURE

Kārlis Žagata, Dace Grauda, Isaak Rashal*

Institute of Biology, University of Latvia,
Miera 3, LV-2169 Salaspils, Latvia

** Corresponding author. E-mail: izaks@email.lubi.edu.lv*

Introduction. Hereditary changes in plants obtained from tissue culture are defined as somaclonal variation. After cultivation of plant tissues *in vitro*, especially in calli cultures, high frequency genetic changes have occurred. Thus, somaclonal variation could serve as an alternative source of genetic diversity and provide additional raw material for breeding without hybridization. In some cases somaclonal variation can be related with retrotransposons activity in plant tissue cultures under stress conditions during *in vitro* cultivation.

Aim and methods. The aim of the work was detection of genetic changes in wheat calli culture and plants-regenerants. Latvian spring wheat variety *Robijs*, created on the base of a doubled haploid line, was used in the experiment. To induce calli formation embryos of immature seeds were placed on Petri dishes with MS media, enriched by 2,4-D (2 mg/l), and cultivated in thermostat (+27 °C, darkness). After 60 days of the cultivation on induction media calli were transferred to the regeneration media (MS media supplemented by NES 2 mg/l and kinetin 2 mg/l) and cultivated in thermostat (+27 °C, 16 h day/8 h night). DNA was isolated from leaves of mother plants, dried calli, and from leaves of plants-regenerants. Genetic diversity analysis was carried out by IRAP (inter-retrotransposons amplified polymorphisms) method. Altogether 31 primer that had previously shown a high level of polymorphism in genetic analysis of different types of organisms were tested. Obtained PCR products were analysed by 1.7% agarose gel electrophoresis (70 V, 4 h). Two primers (2377, 2386) that showed the highest number of loci, were used for followed genetic diversity analysis.

Results. All 250 explants formed calli. Among them were 32 calli with regeneration zones, 180 only root-forming calli, and 38 calli without signs of organogenesis. Reduction of regenerative capacity of calli, observed after 60 days of calli cultivation on regeneration media, could be associated with genetic changes in DNA. Totally, 26 plants-regenerants were obtained. Analysis of allelic polymorphism showed that all plants of variety *Robijs*, used as mother plants, were identical. Plants-regenerants and calli showed genetic changes in comparison with mother plants. Altogether in plants-regenerants and in calli 46 loci were revealed, primer 2 377 produced 24 loci, among them polymorphic were 20, primer 2 386 produced 22 loci, all of them polymorphic. Using high-quality DNA samples, retrotransposons-based IRAP method is suitable for analysis of genetic changes in spring wheat calli cultures. Genetic variability caused by changes of retrotransposons activity during cultivation *in vitro* plant tissue cultures can be observed.

RESEARCH ON THE VARIETY OF LITHUANIAN PAEONY BREEDS USING RAPD METHOD

J. Žukauskienė*, J. Varkulevičienė, A. Paulauskas

Faculty of Natural Sciences of Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

** Corresponding author. E-mail: j.zukauskiene@gmf.vdu.lt*

Introduction. While expanding the variety of physical features in Paeonies, it is importaint to choose genetically and morphologically as distant as possible individuals. Comparing Paeonies only by their external features does not let to evaluate how much genetically distant are species and breeds which are having similar morphological features.

Aim. Paeonies studied in this work were varying morphologically by their height, type of blossom, shape and color, aromatic features, number and strongness of masts, density and color of foliage, time, length and abundance of blossoming.

Methods and results. Genetical differencies between 13 different chosen Paeonies, which are grown in VDU Kaunas Botanical garden, were compared by using RAPD method. To make genetical analysis 9 RAPD primers were chosen. Number of DNA fragments was varying from 1 to 15, and their length from 100 to 1 800 np. Most of polymorphic DNA fragments were amplified with four chosen primers MP-8, OPB-7, OPA-18 and OPB-8. Forthcoming common amount of different size DNA fragments was varying from 1 to 15, and their size from 100 to 1 800 np. The most genetically distant Paeony breeds are 'Skeivienės vėlyvasis' and 'Garbė Motinai'. Genetically most common Pagony breeds are 'General MacMahon' and 'Germaine Burgos'. Genetically most common breed to its parental forms is 'Freda', and the most distant are 'Darius-Girėnas' and 'Skeivienės vėlyvasis'.

ASSESSMENT OF VIBURNUM (*VIBURNUM*) GENETIC DIVERSITY USING MICROSATELLITE MARKERS

J. Žukauskienė[1]*, A. Paulauskas[1], S. Bogačiovienė[1], E. Kupčinskienė[1], L. Česonienė[2], R. Daubaras[2]

[1] *Faculty of Natural Sciences of Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania*

[2] *Kaunas Botanical Garden of Vytautas Magnus University, Ž. E. Žilibero 6, LT-46324 Kaunas, Lithuania*

Introduction. The genus *Viburnum* includes more than 160 species of shrubs and small trees distributed widely throughout the Northern hemisphere into the Southern hemisphere (Winkworth and Donoghue, 2004). *Viburnum* species is an ornamental plant which provides many ideal year-round qualities (Dean et al., 2011). Over years molecular tools have become a superior method for hybrid analysis and cultivar identification of ornamental plants (Dean et al., 2011). Vytautas Magnus University's Kaunas Botanical Garden contains a rich collection of planted more than 20 *Viburnum* species. With this *Viburnum* gene collection there has not been done any molecular research until now.

Materials and methods. Using 11 selected microsatellite markers *Viburnum* collection from VMU Kaunas Botanical Garden was explored In total, 20 different species were studied.

Results. After genetic analysis there were 2 to 10 alleles per primer, amplitude of allele's size was 81 to 178(bp). There were 24 unique fragments detected. The most unique alleles were detected with the primer VD012. Eight species out of 20 did not have any unique snippets. The most polyphormic was 'Zarnice' species, and the less polyphormic was 'Leningradskaja otbornaja' species. AMOVA results show that intraspecific and interspecies *Viburnum* diversity is respectively 42% and 58%.

Financial support for this study was provided by the Research Council of Lithuania by project "Change of bioactive compounds in underutilised berry species depending on genotype and environment" (No. SVE-04/2011).

References

1. Dean D, Wang X, Klingeman WE, Ownley BH. Screening and characterization of 11 Novel Microsatellite Markers from *Viburnum dilitatum.* Hort Sci 2011; 46(11): 1456–9.

2. Winkworth RC, Donoghue MJ. *Viburnum* phylogeny: Evidence from the duplicated nuclear gene GBSSI. Mol Phylogenet Evol 2004; 33: 109–26.