WELCOME ADDRESS OF THE 7th BALTIC GENETICS CONGRESS

Dear participants of the VII Baltic Genetics Congress. I am glad to welcome you in Rīga just a few weeks before Latvia's 100th anniversary, which will be celebrated on November 18. The idea of Baltic Genetics Congresses first arose in 1991 when the three Baltic States restored their independence. The necessity for closer connections in all areas between these countries was obvious, and in such an atmosphere the wish to bring together geneticists from all the Baltic States was very timely.

The first Baltic Genetics Congress was held in Vilnius in 1992. Since then, Congresses have been organised regularly in all Baltic States, and specialists from different areas of theoretical and applied genetics, including breeding, have been involved. These events saw the participation not only of scientists from the Baltic States but also of our colleagues and friends from other countries. Among the participants, experienced, grey-haired scientists are encountered as well as enthusiastic young scientists who are making their first steps on their prospectively long-term scientific way. Notably, such events help to create a bridge between scientists of different generations from different countries working in various areas of genetics.

The VII Baltic Genetics Congress has gathered more than 100 participants from 9 countries. I wish all the participants a successful exchange of new scientific ideas as well as enjoy staying in our nice city of Rīga.

Professor Isaak Rashal
Chairman of the Organising Committee of the VII Baltic Genetics Congress

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ABSTRACTS
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Effect of ploidy level on drought stress response in annual ryegrass

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Key words: *Lolium multiflorum*, morphological traits, polyploidization.

Drought is one of the major abiotic stresses that affect the growth and development of plants. Understanding the way in which plant responds to drought at the physiological and molecular level is essential for developing improved cultivars that are well suited for growth and development in regions that have limited water. Baltic countries fall under the region with relatively high summer soil moisture, however the occurrence of drought spells during growing season is increasing, leading to substantial losses in food and feed production. Plant polyploidization has been utilized in forage grass breeding to develop cultivars with high herbage yield production. Genome duplication and consequently gene redundancy can cause transcriptomic changes leading to higher adaptability compared to diploids. The studies of model plants suggest that increase in ploidy level leads to higher adaptability to adverse environments. However, various studies of *Lolium* diploids and tetraploids subjected to drought stress yielded contrasting results, leaving the question whether genome duplication in ryegrasses leads to superior resistance to abiotic stress still unanswered.

The aim of this study was to induce tetraploid lines of commercially available diploid annual ryegrass (*Lolium multiflorum* subsp. *multiflorum*) cultivars and evaluate the effect of genome duplication on drought stress response. The approach of comparing induced tetraploids with the parental diploid lines was chosen to minimize the effect genetic background and thus reveal the effect of plant ploidy level. Tetraploid induction was accomplished using various antimitotic agents and treatment procedures. The highest efficacy was achieved using colchicine solution. To understand the role ploidy level plays in the physiological response to drought, field trials and drought simulation experiments under the controlled conditions were performed.

The results from field trials indicated that the tetraploids exhibited superior morphological traits than their diploid counterparts. In the controlled experiments, variations were found in the physiological response to drought between the induced tetraploids and their diploid progenitors as well as between the different cultivars regardless of the ploidy level. The lines exhibiting contrasting drought stress response will be used in further experiments set up to evaluate candidate gene expression patterns.

Molecular detection and characterization of vector-borne pathogens in small rodents

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Key words: *Bartonella, Borrelia, Rickettsia*, rodents.

In the ecosystem rodents play an important role as they are the main carriers of various infectious diseases. In the course of climate change and with increasing anthropogenic effects, increases in rodent populations and increasing interactions between humans and rodents are observed. Rodents are considered to be the main reservoir hosts of vector (ticks, fleas) pathogens transmitted. As an object of research were used spleen tissues obtained from 182 individuals belonging to nine wild-living small rodents species trapped in Lithuania, Ukraine and Norway during 2017. Two molecular detection methods were used to detect the infection in rodents: dot-blot reverse hybridization and multiplex RT-PCR with bacterial specific primers. The results showed that investigated rodents were infected with *Bartonella* spp., *Rickettsia* spp. and *Borrelia* spp. pathogens. Positive samples were examined by one-step and nested PCR reactions using different targets (*rpoB, gltA* genes) and intergenetic species regions (ITS). Sequence analysis of *Bartonella, Rickettsia* and *Borrelia* isolates showed the presence of *Borrelia afzelii, Borrelia miyamotoi, Rickettsia helvetica* and multiple *Bartonella* strains, which were associated with different host species.

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In vitro competence of some barley varieties with different parameters of grain quality

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Key words: barley, callusogenesis, grain quality, immature embryo, *in vitro* morphogenesis.

In Azerbaijan, where nine out of 11 existing climate zones are present, the yield of barley grown in each individual region varies to a certain extent, but never has high rates. Reduction of yield and deterioration of the grain quality of cereals is explained by sharp changes in climatic conditions over the last years. In this regard, conditions for the production of varieties characterized by adaptive abilities to specific growing conditions, high yield and good quality of grain are developed in the country. In order to solve this problem, *in vitro* methods are used, which makes possible to model the necessary cultivation conditions. However, the response of plants with different origins to cultivation under the conditions of the nutrient medium of the same phytohormonal composition is different.

Therefore, in order to carry out selection at the cellular level, it is necessary to select those varieties which competence is manifested in the conditions necessary for the experimenter. To reach this goal, Gudratly 48, Dayanatly and Jalilabad 19 (used as a standard), zoned barley varieties of Azerbaijan selection, were used as the research material.

Unripe embryos of these varieties isolated at the stage of wax ripeness were used as explants to introduce into *in vitro* culture. Before isolating the embryos the seeds were successively sterilized in ethyl alcohol and sodium hypochlorite and in each shift were washed with distilled water. To obtain a callus culture the embryos were planted on Gamborg B_5 nutrient medium with the same phytohormonal composition. Cultivation of explants was carried out: (i) in the dark at a temperature of 26 °C; (ii) in the light at a temperature of 34 to 36 °C. Two weeks later, the samples of the second variant of the experiment were placed to the conditions of scattered light. The following were determined: number of embryos with direct germination, number of embryos that formed callus, morphogenetic potential of callus. This series of experiments was conducted in the Laboratory of Plant Biotechnology of the Institute of Molecular Biology and Biotechnology of the National Academy of Sciences of the Republic of Azerbaijan.

For each of the above-mentioned varieties, as well as for varieties Garabag 33, Sadig and Zami, the following grain quality indicators were determined: total protein content, starch content, grain moisture, weight of 1000 grains, grain water absorption capacity, husk weight. The work was carried out at the Grain Quality Laboratory of the Research Institute of Crop Husbandry under the Ministry of Agriculture of the Republic of Azerbaijan.

The obtained data revealed that variety Gudratly 48 turned out to be more stable, thus ability to form callus and regeneration potential changed insignificantly in both variants of the experiment.

Response of juveniles of different forest tree species and populations to the complex of simulated climate change-related stressors: spring-frost, heat, drought, increased UV radiation and ozone concentrations under elevated CO_2 level

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Key words: climate change, elevated CO_2, forest tree species, stressors, UV radiation.

The aim of the present study was to assess response of different juvenile progeny of seven forest tree species, P*inus sylvestris, Picea abies, Betula pendula, Alnus glutinosa, Populus tremula, Quercus robur* and *Fraxinus excelsior*, and their populations to different combinations of climate change-related stressors, simulated in a phytotron under elevated CO_2 concentration: (i) heat + elevated humidity (HW); (ii) heat + frost + drought (HFD); (iii) heat + elevated humidity + increased UV-B radiation doses + elevated ozone concentration (HWUO); and (iv) heat + frost + drought + increased UV-B radiation doses + ozone (HFDUO).

Effects of the complex treatments on sapling growth, physiological and biochemical traits were significant. Species effect and species-by-treatment interaction were highly significant in most of the traits studied, indicating species-specific reactions to the applied treatments. For deciduous trees, height increment was much higher under HW treatment than in ambient conditions indicating a positive effect of elevated temperature and better water availability. HFD treatment caused reduction of height increment in comparison to HW treatment in most species except *Q. robur* and *F. excelsior* which benefited from lower humidity. Treatment HWUO and HFDUO have caused substantial damages to leaves and partial defoliation, although height increment following both these treatments was the same or even higher than that in ambient conditions. This was likely due to a positive compensatory effect of increased CO_2 concentration and temperature; meanwhile, all treatments had little effect on height increment of *P. abies* and *P. sylvestris*.

Rates of photosynthesis in most of the tree species were greatest in HFD treatment. A lower photosynthetic rate (compared to control) observed in *B. pendula, P. tremula* and *F. excelsior* in HW treatment, and in most species – in HWUO treatment, indicates that heat waves can cause stress on certain tree species even at good availability of water and may negatively affect physiology, although this does not necessarily reduce tree growth. Compared to ambient conditions, intrinsic water use efficiency in all treatments was significantly lower in *P. tremula, A. glutinosa* and *F. excelsior*, at similar levels in *Q. robur* and *B. pendula*, and substantially higher in conifers *P. syvestris* and *P. abies*. Concentrations of malondialdehyde and hydrogen peroxide varied a lot across treatments showing variable tree species' responses to stress, but hydrogen peroxide concentrations in all deciduous species were substantially lower than in ambient conditions and were not affected by treatments in coniferous species .

Significant population effect and population-by-treatment interactions found for most traits showed different plasticity and patterns of response of populations to the treatments. Highest ecovalency was found in populations of *P. tremula* and *F. excelsior*. The observed reactions may not be adaptive acclimation, but indicate deteriorating performance of some populations which may lead to changes in species competitiveness thus compromising regeneration, persistence of natural successions and sustainability of future forest ecosystems.

Genetic and agronomic analysis of Latvian fescue (*Festuca* spp.), ryegrass (*Lolium* spp.) accessions and their hybrids

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Key words: Festulolium, fodder crops, hybridization, SSR markers.

The aim of this research was to characterize the main agronomic properties of Latvian fescue and ryegrass accessions and their hybrids, and to obtain data on the genetic relationships of these accessions. This information can be utilized in breeding efforts, and to analyse the correlation of genetic and agronomic properties.

Agronomic traits were characterised by breeders and experts from the LLU Institute of Agriculture. The data on analysed accessions were collected from multi-year trials, genetic resource characterization and evaluation, fodder quality analyses, and field observations in both cultivated and natural fields and pastures.

DNA for genetic analysis was extracted from plants or germinated seeds using a modified CTAB method. Genotyping was done using the SSR markers G03_020, G05_033, G07_037, G01_053, G07_065, G05_071, G05_088, G05_099 (Studer et al. 2008). PCR reaction conditions: DNA: 2 µL, 5x HOT FIREPol® Blend Master Mix with 10 mM $MgCl_2$ (Soltis Biodyn, Tartu, Estonia): 4 µL, PCR primers (4 mM): 1 µL each, H_2O to 20 µL. PCR program: 95 °C 15 min; (95 °C 20 s, 54 °C 30 s, 72 °C 45 s) × 40; 72 °C 5 min. PCR products were analysed on a ABI Prism 3130xl Genetic Analyzer (Applied Biosystems), and genotyped using GeneMapper 3.5. Analysis of genetic data was done using GenAlEx 4.1.

Tall fescue (*Festuca arundinacea*) accession and Festulolium hybrids derived from these were characterized by high productivity, winter hardiness and persistence under normal moisture conditions. Red fescue (*Festuca rubra*) accessions also had high persistence, but were not above average in yield and quality of feed. Unfortunately, the feed quality of tall fescue accession and the Festulolium hybrid varieties derived from them were also not satisfactory. The winter hardiness of meadow fescue (*F. pratensis*) accession was satisfactory, better than perennial ryegrass (*Lolium perenne*) and derived Festulolium hybrid varieties, however, they did not have increased persistence compared to perennial ryegrass and derived hybrid varieties.

Genetic analyses revealed a high level of diversity in the analysed material, with the number of alleles amplified by the markers ranging from four (G05_099) to 16 G01_053). Genetic diversity within accessions varied according to the pedigree and type of accession, but was considerably lower than the total genetic diversity (mean number of alleles per locus over all analysed accessions ranged from 2.4 to 4.6. The analysed accessions were genetically differentiated (F_{st} –0.209, $p < 0.001$), particularly between species, but within species, genetic differentiation was lower due to the outcrossing nature of these species.

These forage grass varieties and accessions, developed from related species and their hybrids, have both positive and negative agronomic properties, therefore breeding objectives are to combine favourable agronomic traits, while reducing the negative effects as much as possible. The integration of agronomic and genetic data and analyses can increase the efficiency of fodder and forage grass breeding programs.

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Genetic diversity of *Anaplasma phagocytophilum* in Red Deer (*Cervus elaphus*) from Lithuania and Norway

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Key words: *Anaplasma phagocytophilum, ankA, msp4*, red deer, 16S rRNA.

Anaplasma phagocytophilum is the causative pathogen of granulocytic anaplasmosis in many species. To assess the role of the red deer as possible reservoirs for this zoonotic infection in Lithuania and Norway, we have investigated the prevalence of *A. phagocytophilum* in free-ranging and captive red deer. In this study, spleen samples from 143 red deer (*Cervus elaphus*) individuals, collected from 12 different farms in Norway and samples of 110 free ranging red deer individuals from Lithuania, were analysed for presence of *A. phagocytophilum* DNA by real-time, nested and single PCR, using *msp2, msp4*, *16S rRNA* and *ankA* genes. Different *A. phagocytophilum* variants were identified.

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The performance and stability for agronomic and grain quality traits of Latvian spring barley varieties

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Key words: agronomic traits, grain quality, spring barley, stability, variety.

The objective of Latvian spring barley (*Hordeum vulgare* L.) breeding program is to create new varieties suitable for the Latvian agro-climatic conditions and different agricultural systems that are characterized by high and stable yield, resistance to lodging and diseases, and grain quality appropriate for different directions of use. Analysis of genotype interaction with seasons and other agro-ecological conditions would help to get information on the adaptability and stability performance of genotypes. The stability of agronomic and grain quality traits of genotype in different environmental conditions is very important for barley varieties recommendation. This study aimed to evaluate the performance of agronomic and grain quality traits and their stability of 11 spring barley varieties from Latvian breeding program grown in two locations and four years.

The study was carried out for Latvian spring barley varieties including nine two-row covered varieties 'Abava', 'Ansis', 'Austris', 'Didzis', 'Gate', 'Idumeja', 'Jumara', 'Kristaps', 'Saule PR' and two two-row hulless varieties 'Irbe' and 'Kornelija'. Varieties were grown in the field trials of Institute of Agricultural resources and Economics, Priekuli Research Centre and Stende Research Centre during 2014 – 2017. The field trials at each location were arranged by a randomized complete block design grown in three replicates in 6.6 to 10 m2 field plots.

The mean performance and dynamic concept of stability was evaluated for grain yield where genotype × environment interaction from analysis of variance was portioned into heterogeneity of regression coefficient (b_i) and the sum of squares deviation from regression (s^2d). Phenotypic stability according to the static concept by using environmental variance (s^2_E) was measured for agronomic traits such as resistance to lodging and diseases (*Pyrenophora teres, Blumeria graminis*), and grain quality traits (1000 grain weight, test weight, crude protein and β-glucan content).

Average grain yield in eight environments for Latvian spring barley varieties varied from 3.90 to 6.63 t ha^{-1}. Varieties 'Didzis', 'Jumara' and 'Ansis' yielded significantly above the grand mean (5.70 t ha^{-1}; LSD = 0.476 t ha^{-1}). Among high yielding varieties, 'Didzis' showed relatively high dynamic yield stability and wide adaptability to all environments (b_i = 0.83; b = 1; s^2d = 0.10). Grain yield for variety 'Jumara' showed above average stability and specific adaptability to unfavorable environments (b_i = 0.48; b < 1; s^2d = 0.07). Variety 'Ruja' with average grain yield (5.92 t ha^{-1}) have the highest dynamic grain yield stability (b_i = 0.95; b < 1; s^2d = 0.06). Hulless barley 'Irbe' and 'Kornelija' showed significantly lower mean yield and lower yield stability comparing to other Latvian covered varieties. Among covered spring barley these were considered the best varieties within eight environments with stable high 1000 grain weight ('Austris' 53.3 g; 'Idumeja' 51.5 g), test weight ('Gate' 717 g L^{-1}; 'Jumara' 708.0 g L^{-1}; 'Austris' 705.3 g L^{-1}), lodging resistance ('Austris' 9.0 scores, 'Jumara' 9.0 scores, 'Didzis' 8.9 scores), resistance to *Pyrenophora teres* ('Didzis' 2.6 scores, 'Saule PR' 3.1 scores) and *Blumeria graminis* ('Saule PR' 0.6 scores; 'Didzis' 0.8 scores). Similarly, one of the newest covered barley variety 'Saule PR' and both hulless barley varieties 'Irbe' and 'Kornelija' were distinguished for heightened and phenotypic stability of crude protein (12.1, 12.4 and 14.9% respectively) and β-glucan content (4.4, 4.6 and 4.8% respectively); these varieties could be suitable as grain raw material for food uses. Overall current commercially available Latvian spring barley varieties are showed the different reaction across changing growing conditions in respect of performance of agronomic and grain quality traits.

Restoration of Baltic Sturgeon population in Lithuania and its characterisation using DNA markers

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Key words: *Acipenser oxyrinchus*, D-loop, DNA microsatellites, genetic diversity.

Following works on the restoration of the Baltic sturgeon population that are conducted in the Oder and the Vistula basins the stockings of the Nemunas River with the sturgeon fry were started in 2011. It is contemplated that the sturgeon stockings of the main rivers of the Baltic Sea will enable to restore local sturgeon populations. Till the beginning of the XXI century it was supposed that the European sturgeon (*Acipenser sturio*) had inhabited the Baltic Sea, but recent genetic analyses of the sturgeon remains obtained from the archeological findings have revealed that the Baltic Sea more than 2000 years ago had been inhabited with another species Atlantic sturgeon (*Acipenser oxyrinchus*). As some wildlife populations of Atlantic sturgeon still inhabit several rivers in Canada it became possible to get material for creation of the Baltic sturgeon broodstock for restoration of extinct population. The program of sturgeon restoration in Lithuania was launched in combination with molecular investigations including sequencing of mtDNA fragments and microsatellite DNA analysis of museum specimens and representative samples of sturgeons reared as broodstock and released in Nemunas River. Molecular data were collected in order to evaluate the genetic background of historic population of sturgeons and to initiate genetic monitoring of the restored population.

Fin clips of 50 *A. oxyrinchus* individuals were collected from fry representing two distinct hatcheries. At hatchary "First" sturgeons were reared from fertilized eggs received from Acadian Sturgeon and Caviar Inc, Canada. At hatchery "Second" larvae provided by Regional Research Institute for Agriculture and Fisheries (Germany) were reared for several months until were released in rivers. Fish tissue samples were preserved in 70% ethanol and used for DNA extraction and molecular analysis, including sequencing of mtDNA D-loop fragments and genotyping of individual fishes at 13 microsatellite loci. Obtained molecular data of 22 and 28 specimens representing two distinct hatcheries named "First" and "Second", respectively, were compared with homological DNA sequences and molecular data of some microsatellite loci derived from two museum specimens of sturgeons from Tadas Ivanauskas Museum of Zoology, Kaunas, Lithuania. Both museum specimens were caught in 1960 in the Baltic Sea, near Nida (Lithuanian territorial waters) and by previous investigators were identified as representatives of *A. sturio* species. Signs of possible introgressive hybridization between *A. sturio* and *A. oxyrinchus* in two museum specimens were checked by comparison of alleles at loci *AoxD161, AoxD188, AoxD297, AoxD242*, and *AoxC30*, as the allele range sizes of Atlantic and European sturgeons did not overlap at these loci. Possibility to assign any sturgeon released in Lithuanian rivers in 2015 to one of two samples representing hatchery "First" or "Second" was estimated based on multi-locus genotyping data using Structure Version 2.3.4 (Pritchard et al. 2000).

Comparison of sequencing data of two museum specimens representing extinct Baltic population and individuals of *A. oxyrinchus* collected in both hatcheries revealed that the same D-loop haplotype H1 is characteristic for museum specimens and for sturgeons reared in hatcheries and representing contemporary population. Possible hybridisation between *A. sturio* and *A. oxyrinchus* was not confirmed. First results of genetic application undertaken in Lithuania also revealed possibility to discriminate individuals obtained from different hatcheries based on the set of 13 microsatellite loci *Aox45, AoxD54, AoxD161, AoxD297, AoxD188, AoxD234, AoxD242, AoxC45, AoxC30, AoxD241, AoxD64, AoxD186, Ls-68*. Analysis of *A. oxyrinchus* specimens representing F1 generation of parental individuals reared in two separate hatcheries in Canada and Germany disclosed significant differences in allele composition and high probabilities for individuals released into natural environment to be assigned to hatchery of their origin. The results of molecular analysis will be useful for identification of age, pedigree and for initiation of genetic monitoring of the restored population of the Baltic sturgeon.

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Mutations in genes causing monogenic hepatic diseases in patients with chronic virus hepatitis C

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Key words: chronic C hepatitis, cirrhosis, *HFE*, *UGTA1*.

Hepatitis C is highly spread throughout the world. According to WHO, about 1 to 2.4% of people, inhabiting Belarus, are infected by hepatitis C virus. Among them, some people possess complex pathology, such as mixed hepatitis: genetic hepatic defects and hepatitis C infection. In such cases interference of internal and external defects can occur that aggravate the symptoms and complicate therapy. That is why early DNA-diagnostics of definite mutations leading to hepatic defects is important for the hepatitis patients.

Our aim was to elaborate the convenient protocols of detecting mutations in four genes, causing hepatic defects and to screen the group of patients with chronic C hepatitis for the presence of these mutations. Considering the population rate of various mutations and their clinical significance, we selected four genes: *UGT1A1* (**28*), *HFE* (*H63D* and *C282Y*), *Pi* (*PiS* and *PiZ*), *ATP7B* (*H1069Q*), which cause Zilber's syndrome, hereditary hemochromatosis, alpha 1 antitrypsin deficiency and Wilson-Konovalov, respectively.

Phenol-proteinase K method was used for DNA extraction from buccal cells of 247 patients with chronic hepatitis C (CHC). Adequate methods were chosen: real-time PCR with TaqMan probes (*HFE, Pi, ATP7B*), PCR using fluorescent labeled primers with detection in a capillary sequencer (*UGT1A1*). The results are summarized in the Table 1.

For *Pi* gene, as expected, the rate of mutant alleles was quite low, we found only five patients: two with *PiS* heterozygote genotype without cirrhosis and three with *PiZ* heterozygote genotype with A (one patient) and B (two patients) grades of cirrhosis. There are not enough patients for searching any correlations between mutations leading to alpha 1 antitrypsin deficiency and hepatitis C severity.

We did not found any persons with *H1069Q* (*ATP7B*) mutation among 247 patients with CHC.

Mutations of *HFE* gene are very common, both among patients (Table 1) and in Belarus population (Сивицкая, Кушнеревич 2007). Among 247 patients, 16 (6.5%) were *C282Y* heterozygotes, 60 (24.3%) *H63D* heterozygotes and 7 (2.8%) *63D* homozygotes. Comparing with published Belarus population data, we did not find that patients with HFE mutations are more vulnerable to chronic hepatitis C.

The most common among all studied is the *UGT1A1 *28* mutant allele, detected in 60% patients with CHC that is close to its rate in population: 58% of control group of native Belarus inhabitants carry *UGT1A1 *28* (Синявская 2018).

While analyzing the CHC progression to cirrhosis we found that among CHC patients carrying three or more pathogenic alleles of the genes studied, only 44% do not have cirrhosis during the observation period. At the same time in the group of CHC patients without mutations, 66% did not have cirrhosis (Fig. 1). Among eight patients

Table 1. Rate of *UGT1A1* (**28*), *HFE* (*H63D* and *C282Y*), *Pi* (*PiS* and *PiZ*), *ATP7B* (*H1069Q*) mutations in patients with chronic hepatitis C

Degree of cirrhosis	Patients with liver cirrhosis			Patients without liver cirrhosis
	C	B	A	
Total number of patients	8	28	67	144
Patients with *HFE* (*H63D* + *C282Y*) mutations (%)	4 (50%)	8 (28.6%)	20 (29.9%)	5 (35.4%)
Patients with *UGT1A1 *28* (%)	6 (75%)	14 (50%)	47 (70%)	82 (57%)
Patients with mutations *Pi* (*S, Z*)	0	2 (Z) (8%)	1 (Z) (1.5%)	2 (S) (1.4%)
Patients with *ATP7B* (*H1069Q*) mutation	0	0	0	0
Patients without any of specified mutations (%)	1 (12.5%)	8 (28.6%)	13(19.4%)	34 (23.6%)

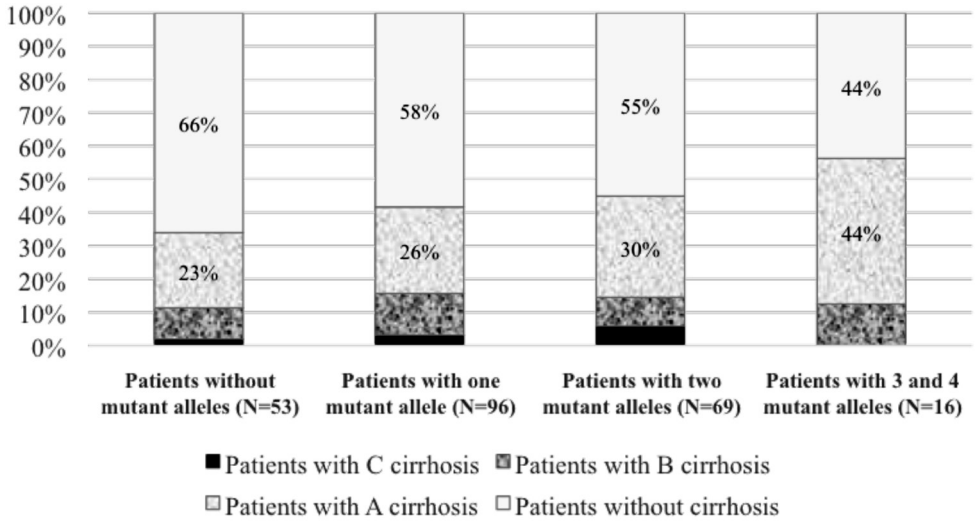

Fig. 1. Rate of patients with hepatic cirrhosis detected in CHC groups with varying number of pathogenic alleles of *HFE, UGTA1* and *Pi* genes.

with the most severe C grade of cirrhosis, only one person (12.5%) was without mutations in the four genes studied (Table 1).

The presented data demonstrate that the mutant alleles of genes *UGT1A1* (**28*), *HFE* (*H63D* and *C282Y*), *Pi* (*PiS* and *PiZ*) probably can accelerate the progressing of fibrosis in chronic C hepatitis and provoke, along with other factors, the development of hepatic cirrhosis. Definitely, detailed analysis both of the patient and environmental factors will help to get the full picture.

Acknowledgements

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Molecular mechanisms of adaptation of eukaryotes in the conditions of global warming

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Key words: enzymes, DNA, global warming, RNA, RNA-thermometers.

Global warming is an irreversible process that leads to a deterioration in the living conditions of living organisms, including the main agricultural species that play a significant role in human nutrition. There are so-called factors σ32 in *E. coli*, which are embedded in the RNA-thermometer in the *λ cIII* gene, this factor plays a significant role in the regulation of bacteria at elevated temperatures. The expression of heat and cold shock and some virulence genes is coordinated by the genome in response to a change in temperature. A number of RNA-thermometers are known that differ in structural and functional control of a variety of cellular processes. The most common RNA-thermometer is the ROSE element, which suppresses the expression of heat shock genes. A common feature of all ROSE elements is the presence of the G residue of the opposite SD sequence, since this nucleotide is functionally important, and its elimination makes the thermometer irresponsive to high temperatures. A sequence of molecular level (RNA-thermometers) has been established whose chemical compounds influence the regulation of the homeostasis temperature, namely enzymes. Although the results obtained for RNA-thermometers were on microorganisms, there is a real prospect at the molecular level to alter the genome of the animal, namely the insertion of these sequences, or the cultivation of symbiotic microorganisms that can be used in biotechnologies for the production of biologically active substances that, at elevated ambient temperatures can reduce the negative impact of high temperatures on living organisms.

Genetic aspects of lactase persistence in the Eastern Ukraine population

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Key words: hypolactasia, lactase persistence, Ukraine, 13910C-T, 22018G-A.

Lactose tolerance or lactase persistence (LP) is an example of selection-based evolutionary change in humans from milk-drinking cultures (Feldman, Cavalli-Sforza 1989). The geographic distribution of LP matches the distribution of dairy farming. The highest frequencies are observed in North Western and Northern Europe (Krūmiņa 2018). In European Caucasian populations two SNPs: 13910C-T (rs4988235) and 22018G-A (rs182549) located in the *MCM6* gene, but with influence on the lactase *LCT* gene, are regulating lactose tolerance and hypolactasia. (Enattah 2002; Bersaglieri 2004). Described polymorphisms are associated with multifactorial diseases such as osteoporosis, with calcium metabolism, differences in serum calcium levels and calcium intake (Lee, Krasinski 1998; Koek 2010). European lactase regulation genotypes show evidence of association with increase in body (Kettunen 2010), lipid pathway (Wagh 2012), abdominal obesity, overweight/obesity, obesity-related variables (Albuquerque 2013; Hartwig 2016; Manco 2017), metabolic syndrome (Friedrich 2014), cardiovascular diseases (Silander 2008) in different ethnic groups. In Ukraine, the genetic aspects of lactase deficiency and its relationship with human pathologies have not been studied, which was the aim of our investigation.

Population study included persons living in Eastern Ukraine aged from 17 to 91 years (n = 570). Patients which hospitalized in trauma department of Kharkiv clinical multi-field hospital No. 17 with fractures of the proximal femur, coxarthrosis (n = 38), were examined by internal medicine specialist and different types of analyses. Bone mineral density (BMD) of patients was estimated by ultrasonic densitometry. Genotyping of SNPs 13910C-T and 22018G-A was made by PCR-RFLP. The linkage disequilibrium (LD) was estimated by D', r^2. Statistical analysis had been carried out using Shapiro-Wilk test for normality, Mann-Whitney U test, Chi-square test, Spearman correlation.

Lactase nonpersistence phenotype was found in approximately 7.0% of European Ukrainians in the 21.3 ± 0.2 age group (n = 157), 9.9 % in the 45.9 ± 0.4 (n = 192) and 4.1% in the 69.5 ± 0.5 (n = 221) groups. It is known that the correlation of phenotype, the results of functional tests for lactose intolerance and DNA testing results is quite high (Kozlov 2004; Enattah 2005; Prasolova 2015). The analysis of the phenotypes of the patients (n = 38) genotyped by us confirmed this. Therefore, in general group the C allele frequency of 13910C-T could be represented as 0.265, T allele frequency as 0.735, but group was not in Hardy-Weinberg equilibrium. The literature describes that a haplotype 13910T/-22018A conferring lactase persistence has a tightly clustered microsatellite allele distribution and show lack of recombination (Coelho et al. 2005). According our results the linkage disequilibrium was estimated, D' (r^2) for SNPs analyzed were 0.209 (0.554).

In patients group allele frequencies were as follows: 13910T 0.30, 13910C 0.70, 22018A 0.33, 22018G 0.67. Distribution of genotypes was 0.0/60.5/39.5% for TT/CT/CC; 2.6/60.5/36.9% for AA/GA/GG. Deviation from the Hardy-Weinberg equilibrium was observed (p = 0.027, p = 0.050). When analyzing genotypes for both SNPs, we found the ratio CTAA/CTGA/CCGA/CCGG as 2.6/58.0/2.6/36.8%. More than half of the patients had osteoporosis or osteopenia (52.6%), but no one with the TTAA genotype was found. The parameters BMD of CTGA patients were 4047.1 ± 37.0 and 4116.1 ± 28.4 for CCGG patients (p = 0.358). A significant correlation between the number of low-functional alleles and the values of BMD of patients have not been revealed (r = 0.23, p = 0.172).

The obtained genetic parameters of the Ukrainian population makes it possible to analyze the role of lactose metabolism in the multifactorial pathology development.

Genetically determined differences in microclonal propagation of Silver birch (*Betula pendula*)

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Key words: clonal plantations, genetic gain, selection differential, silver birch, vegetative propagation.

Silver birch is the economically important tree species in Latvia. It is grown for plywood production with rather high requirements for timber quality. Most of the quality traits (like branch angle, forking) are to large degree genetically determined. Therefore tree breeding is carried out to ensure selection and propagation of superior genotypes via the nurseries. However, largest practical gain from tree breeding would be achieved, if best clones would be vegetatively propagated. Such approach would ensure, that the stands can reach target diameter, required for final harvest, notably earlier than minimal harvesting age (71 years). It is important both from forest owner perspective (potential to harvest the stand within the lifetime of the owner) as well as from state perspective: due to un-even age structure of silver birch, shortage of raw material supply can be predicted c.a. 40 years from now, that could be minimized, if the best genotypes would be intensively used for planting. Silver birch is less affected by biotic and abiotic (like wind) damages, so comparatively safe choice for forest owners in context of increasing risks related to climate change. Microclonal propagation of birch had been done in Latvia and elsewhere, also such material had been used in practical forestry in Finland. However, differences can be expected between the clones in the traits important for efficiency of large-scale (practical) application of this method. Therefore aim of this study was to determine factors affecting *in vitro* culture initiation of silver birch and to determine the optimal cultivation media for different birch genotypes. The reproduction capacity of 50 birch clones selected from progeny trials was tested and technology for their cultivation *in vitro* was developed.

Significant differences in growth capacity, vitality and multiplication rate of *in vitro* shoots of different silver birch genotypes were found. The results of *in vitro* propagation is also affected by macronutrients and cytokinins in the medium. For all clones, shoot multiplication was more successful using Murashige-Skoog rather than woody plant medium, supplemented with natural cytokinin zeatin, compared to synthetic cytokinin BAP, which induced vitrification. Faster growing clones from first group show higher multiplication rate and relatively small concentration of zeatin (0.1 mg L^{-1}) are required for the best results; clones with average multiplication rate require higher concentration of zeatin (1.0 mg L^{-1}) for the best results. Clones with low growth capacity show low multiplication rate also on different macronutrient medium and cytokinins, which could be due to low tissue responsiveness to cytokinins.

Acknowledgements

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Genetic characteristic of *Dermacentor reticulatus* in the Baltic countries

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Key words: *Dermacentor reticulatus*, phylogenetics, variability.

Ornate meadow tick, *Dermacentor reticulatus* (Fabricius, 1794) is an important tick-borne disease vector in Europe. It transmits bacterial and viral diseases dangerous to humans, livestock and wild animals. The expansion of *D. reticulatus* tick to the new areas has been observed in the Central and Northern Europe explained by climate change. Distribution range of these ticks are discontinuous with existing gap in the Central Poland. For this study we used various genetic markers to investigate *D. reticulatus* genetic diversity in the Baltic states. Ticks were collected using flagging method in a timeline of 2012 and 2017 from Lithuania, Latvia and several sites in surrounding countries. *12S*, *16S rRNA* and *ITS2* gene fragments were successfully amplified and sequenced using specific PCR and Sanger sequencing. Acquired data showed that *D. reticulatus* in the Baltic states lack haplotype polymorphism but presents a separate group if compared to individuals collected in Poland and other regions of Europe.

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Effect of various concentrations of NaCl on the growth and photosynthesis of seedlings of bread wheat (*Triticum aestivum*) genotypes with contrasting productivity and drought tolerance

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Key words: chlorophyll, germination, photosystem II, stress, wheat varieties.

Soil salinization is one of the significant environmental factors that limit the growth, development and productivity of plants. Currently, about 20% of all irrigated areas of the world are saline. Salinity is the major factor affecting plant metabolism, thereby causing changes in morphological, anatomical structure, physiological and biochemical conditions of plants. The study of salt effects on plant growth and development, evaluation of plant adaptation mechanisms to salt stress are very important issues for the effective use of saline soils. Considering the above-mentioned issues, the main purpose of the presented work was the comparative study of salt tolerance of bread wheat genotypes with contrasting productivity, drought tolerance and height based on their morphophysiological indices and establishing changes in leaf water regime, amounts of photosynthetic pigments and PSII activity. The objects of the study were bread wheat (*Triticum aestivum* L.) genotypes: high productive Gobustan, low productive 12nd FAWWON No 97, drought tolerant Pirshahin-1 and drought sensitive Tale-38, tall Daghdash-94 and short Gyrmyzygul-1. For the assessment of the morphometric and physiological parameters of drought tolerance, seeds of bread wheat varieties were germinated at various NaCl concentrations (0, 150, 200 mM) using the roll method. Germination ability of the wheat embryo was examined during 7 days. Based on some morphophysiological indices such as average root length, RWC, concentration of photosynthetic pigments and chlorophyll fluorescence indices, salt tolerance of the studied varieties were assessed on the 10th day of the germination stage. A decreasing trend in germination ability was observed in the all wheat genotypes germinated at various salt concentrations. Germination ability of the studied varieties changed in the following ranges: 100 to 92% in the control variants, 100 to 75% at 150 mM NaCl and 83 to 33% at 200 mM NaCl. In 3-day-old wheat seedlings treated with NaCl, germination energy changed in the ranges: 92 to 58% in the control variants, 58 to 33% at 150 mM NaCl and 83 to 33% at 200 mM NaCl. However, maximum germination percentage was observed in both variants of the all studied varieties. Maximum germination showed the varieties 12nd FAWWON No 97, Daghdash-94 and Gyrmyzygul-1. Germination energy was relatively low (16 to 17%) only in high productive Gobustan and drought tolerant Pirshahin at 200 mM concentration of NaCl. Thus, on the first 10 days the development of the studied wheat genotypes continued and then a decline relative to the control occurred. The development of roots and shoots of the all varieties was retarded as the concentration of NaCl increased. Thus, two fold decline was observed in the length of shoots and three to four fold decline in the length of roots relative to the control. However, the varieties did not significantly differed in the lengths of roots and shoots. The variety Daghdash-94 was found to be tall in both variants. RWC was found to decrease significantly as salt concentration increased. RWC changed in the ranges 99 to 86%, 96 to 79% and 94 to 66% in the control variant, and at 150 mM and 200 mM concentrations of salt, respectively. There was no pronounced difference in the dynamics of the changes in RWC in the wheat varieties Daghdash-94 and Gyrmyzygul-1 depending on NaCl concentrations. A marked negative impact of 200 mM NaCl was observed in the variety 12nd FAWWON No 97. The highest chlorophyll content was observed in the variety Gyrmyzygul-1.

Trichinella spp. identification by molecular biology methods and larvae examination by fluorescence microscopy techniques with developed benzanthrone luminophores

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Key words: benzanthrone dyes, fluorescence microscopy, *Trichinella* species.

Nematoda worms of the genus *Trichinella* are important worldwide distributed zoonotic parasites and are known as causative agents of human trichinellosis, a disease that is a public health hazard. *Trichinella* is a complex of at least 12 species and genotypes. Species identification is mainly based on molecular biology methods. However, one of the powerful techniques that is successfully applied for the studying the external and internal anatomy of different parasites is fluorescence microscopy. Staining with appropriate dyes is promoted the fluorescence of examined specimen. During this study *Trichinella* larvae samples were identified by multiplex-polymerase chain reaction using species specific primers. Additionally, confocal laser scanning microscopy and fluorescence in situ hybridization without probe hybridisation were applied. For staining procedure two novel benzanthrone luminophores AZM and P13 were used. The parasites were fixated in four different fixatives: Bouin's solution, 70% ethanol, AFA and Carnoy's solution. The fluorescence intensity was evaluated in particular organ to assess efficacy of staining protocols. For both microscopy methods similar staining protocols were developed: parasites were washed with 70% ethanol, placed into the lactic acid to get appropriate transparency, dehydrated in ethanol, stained up to 15 min and washed in 70 % ethanol. Obtained results showed that novel luminophores could be applied to examine external and internal structures of larvae by confocal microscopy. FISH method visualized cuticula and the region of rectum that allowed to determine the sex of the larvae. Developed benzanthrone luminophores AZM and P13 are appropriate for *Trichinella* larvae investigation by fluorescence microscopy and in usage with molecular biology techniques enhance knowledge about *Trichinella* species.

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Influence of SiO_2 nanoparticles on immature gametic cells of lime trees from Riga urban area

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Key words: flow cytometry, fluorescence, SiO_2 nanoparticles, UV irradiation, *Tilia* sp.

Silica or silicon dioxide (SiO_2) nanoparticles (SiNPs) are one of the most widely common nanoparticles in the environment particularly in urban areas, nevertheless their influence on plant cells, including toxicity, is unclear. The goal of the study was to test a hypothesis that plant cell relative fluorescence and SiNPs nanotoxsicity differs depending on plant properties and environmental conditions and is related to plant adaptation to the environment. In this study relationship of tree growth conditions, genotype and reaction of tree gametic cells (in one nucleus stage) on SiNPs and UV irradiation on observed by means of relative fluorescence of cells after influence of laser.

BD FACSJazz® cell sorter (BD Biosciences, USA) with flow cytometer function was used to test the relative fluorescence of plant cells. The excitation of the cell fluorescence was made by 488 nm Coherent Sapphire Solid State (blue) laser.

The genetic analysis of used lime trees population showed that trees used in the study are a mixture of Tilia sp. hybrids, for that reason the genetic factor may potentially have the influence on reaction to stressof individual plants. It was found that the young pollen cells (mid to late one-nucleate developmental stage) were most sensitive (had highest relative fluorescence) to influence of SiNPs and UV irradiation. The cultivation of young pollen cells of lime trees in the medium supplemented with SiNPs had influence on relative fluorescence of cells after UV irradiation. Significant linkage of tree genotype and growth conditions with alteration of relative fluorescence was observed: the young pollen cells of greenhouse-grown trees were more sensitive than the same type of cells from trees grown in urban area of Riga. It was shown that changes of relative fluorescence of cells from trees grown in the urban area of Riga were different but all of them had much lover reaction rate on SiNPs in comparison to cells from trees grown in greenhouse. This phenomenon could be explained by presence of high amount of different size SiNPs in environment of urban area of Riga and, consequently, SiNPs accumulate in the tree cells and might be associated with formation of conglomerates that caused loss of the toxic activity of used SiNPs, in turn, formed SiNPs conglomerates affected the cells as stress inhibitors and are linked to epigenetic changes in those trees cells caused by prolonged influence of urban environment. The lime trees grown for a long time in urban area had complex adaptability to changing environment and might be a good source material for tree nurseries for propagation of lime trees for growing in urban areas.

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Mitochondrial DNA sequence variation in the European bison (*Bison bonasus*) population from Lithuania

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Key words: *Bison bonasus*, genetic variation, Lithuania, mtDNA.

The European bison (*Bison bonasus* Linnaeus, 1758) is the largest herbivore in Europe. The historical distribution of bison was in western, central, and south-eastern Europe and the Caucasus. As a result of reintroductions and introductions, it now occurs in free-ranging and semi-free herds in Poland, Lithuania, Belarus, Russian Federation, Ukraine, and Slovakia. Levels of mitochondrial DNA (mtDNA) variation were examined to investigate the population structure of the European bison population in Lithuania. A 884-bp region of hypervariable domain of the mitochondrial control region was analyzed in 29 individuals and revealed only two distinct haplotypes. Current genetic diversity study revealed low mtDNA control-region sequence variation in the European bison population from Lithuania.

Antipsychotic-induced extrapyramidal symptoms: pilot preventing search of potential genetic predictors in Belarusian population

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Key words: antipsychotic-induced parkinsonism and akathisia, dopamine and glutamate candidate genes, pharmacogenetics.

The efficiency and safety of antipsychotic treatment for schizophrenia patients is still a challenge in clinical psychiatry. Investigation of genetic predictors which are involved in antipsychotic response will help treatment optimization and personalization.

Our sample was obtained from an observational, cross-sectional trial of patient diagnosed with paranoid schizophrenia and assessed for antipsychotic-induced parkinsonism and akathisia symptoms using the Extrapyramidal Symptom Rating Scale. Patients were divided into three clinical groups: (i) akathisia side effects only (n = 127), (ii) parkinsonism side effects only (n = 115), (iii) no extrapyramidal side effects (n = 91). Statistical analyses were conducted in SPSS 22.0. The aim was to investigate association of gene polymorphism of *DRD2* (rs1800497), *CYP2D6*4*, *GSTM1, GSTT1, SLC6A4* (*5HTTLPR*), *COMT* (rs4680), *MDRI* (rs1045642) with extrapyramidal side effects induced by antipsychotics.

There was a significant association of antipsychotic-induced parkinsonism with *DRD2* rs1800497 (χ^2 = 24.319; p = 0.001), *CYP2D6* rs3892097 (χ^2 = 8.961, p = 0.011) and *MDR1* rs1045642 (χ^2 = 6.46; p = 0.04). Patients with parkinsonism demonstrated a higher frequency of the *DRD2* rs1800497 A2/A2, *CYP2D6* rs3892097 A/G and *MDR1* rs1045642 T/T genotypes. Patients with akathisia demonstrated a higher frequency of the *GSTM1* del/del (χ^2 = 30.577; p = 0.0003) and and *GSTT1* del/del (χ^2 = 7.101; p = 0.008) genotypes.

The mechanisms to develop the antipsychotic-induced extrapyramidal symptoms such as akathisia and parkinsonism may differ and, therefore, separate genetic predictors need to be evaluated to understand their genetic basis and to facilitate successful treatment.

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Leaf growth response to environmental restrictions: how to distinguish the performance of various perennial ryegrass genotypes?

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Key words: leaf elongation rate (LER), leaf growth, phenotyping, perennial ryegrass (*Lolium perenne* L.), TriPhase function, water deficit.

Due to an increase in the consumption of food, feed, fuel and to meet global food security needs for the rapidly growing human population, there is a necessity to breed for high yielding crops that can adapt to future climate changes. As the grasslands play a major role in milk and meat production,breeding of perennial ryegrass (*Lolium perenne* L.) for increased biomass, which is the primary yield target, is very important. Given that biomass accumulation is largely determined by leaf growth, understanding the effect of water limitation on leaf growth could be used as a diagnostic tool to assess the plants' response to drought and to improve this trait through breeding. However, to non-invasively determine, when a plant perceives drought, is challenging. Therefore, a novel, non-destructive, largely automated phenotyping platform for the real time analysis of the leaf elongation rate (LER) in perennial ryegrass under water limiting conditions is presented. The data obtained were integrated into an R function to investigate the response of the LER to temperature and soil moisture. The function is developed for use in a dynamic environment and takes into account environmental variables to describe leaf elongation in grasses. According to it, plant growth in response to water deficit is not linear but has three phases demarcated by growth reduction and growth arrest. The first phase depicts "normal" growth, when water in soil is freely available and the growth is mainly governed by temperature, followed by the second phase "decrease", which is attributable to temperature and soil moisture and the terminal phase "arrest", where leaf growth has stopped. The results were highly reproducible and revealed large differences in a diverse panel of perennial ryegrass genotypes.

The ability to dissect complex data into quantitative parameters and to pinpoint growth reduction and arrest allows for direct selection of these traits in breeding programs and further enables association analysis in genetically characterized populations leading to identification of QTLs underpinning the phenotype. The versatility of the method enables its adaptation for studying other abiotic (salinity, osmotic, heavy metal) and chemical (fertilizer, pesticide) stresses of perennial ryegrass and other graminoid species. Therefore, the method described here, has a strong application for both fundamental and applied research to improve crop productivity.

Genetic diversity of blackcurrant reversion virus

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Key words: BRD, BRV, gall mite, phylogenetics.

Blackcurrant reversion virus (BRV) is the first identified mite-transmitted member of the genus *Nepovirus* (family Comoviridae). Biological vector of BRV is gall mite (*Cecidophyopsis ribis*) and both pathogens are widespread in all countries where blackcurrants are cultivated commercially. BRV is the agent of blackcurrant reversion disease (BRD), which is economically the most significant virus disease in *Ribes* species. Two forms of the BRD have been described: the common European form (E) which often did not causes visible symptoms on blackcurrants, and the more severe R form with malformed flowers and leaves.

The viral genome is composed of two polyadenylated single stranded RNA species: RNR1 7700 nt and RNR2 6400 nt. The 3′ untranslated regions (UTR) of BRV RNA1 and RNA2 are highly similar and most stable parts of genome. These parts are involved in the strand synthesis initiation, regulation and translation.

The aim of the research was to identify the diversity of blackcurrant reversion virus in cultivar 'Gojai' using molecular methods.

Research was carried out in Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Department of Orchard Plant Genetics and Biotechnology in 2018.

The BRV virus was detected by PCR method on the infected leaves of the cultivar 'Gojai' showing symptoms of blackcurrant reversion disease. The amplified cDNA fragments of the virus were ligated to the vector p*JET* 1.2 and sequenced. Sequences of blackcurrant reversion virus RNR2 3′ UTR from one host plant were compared to each other by the KALIGN programme, and with virus sequences available in the NCBI GeneBank by Clustal W programme.

Heterogenous infection of BRV in the same plant of cultivar 'Gojai' was detected. Leaves were determined to have been infected with three different BRV isolates with various nucleotide's mutations from RNA2 3′ untranslated region (1366 nt). Homology among sequences from virus partial genome from 94.6 to 99.6 % was found. Sequences identified in Lithuania were uploaded into the NCBI GeneBank, accession numbers: MH891843, MH891844, MH891845.

Three isolates from virus RNR2 3′ UTR were selected with restriction enzymes *Apa*I, *Hha*I and *Sca*I, which are suitable for assessing diversity of BRV mutations and cut sequences two or three times.

Phylogenetic dendrogram was constructed in order to determine the viral infection affinity using sequences identified in Lithuania and sequences submitted in NCBI GeneBank. BRV isolates divided into two branches at 100% bootstrap. First branch consisted of 70% isolates from Finland, Poland, Scotland and Lithuania and they were inoculated by BRV virus, which causes R form or reversion disease. Thirty percent of investigated sequences were from Russia, Finland, Sweden and New Zealand. These sequences belonged to type of BRV that causes E form of BRD.

BRV_1_18_LT and BRV_7_18_LT were located in one phylogenetic dendrogram's branch at 100% bootstrap. These sequences were genetically close to each other but significantly genetically diverse from other BRV sequences worldwide. The highest homology of sequence BRV_3_18_LT was determined with isolate from Poland (AF321570) at 94% bootstrap from isolates identified in other countries.

Genetic characterization of *Rickettsia* in mites (Dermanyssoidea)

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Key words: *Rickettsia* spp., Laelapidae, mites, Lithuania.

Currently, genus *Rickettsia* comprises 31 recognized species and numerous uncharacterized strains causing diseases in both humans and domestic and wild animals. The development of molecular tools over recent decades has resulted in reorganizations in the rickettsiae taxonomy. *Rickettsia* spp. are vectored mainly by hematophagous arthropods. Mesostigmatid mites, which feed on very different species of small rodents, may be reservoirs as well as vectors of some pathogenic rickettsiae. The aim of this study was to investigate the presence of *Rickettsia* spp. in mites parasitizing small rodents and to genetically characterize rickettsiae strains obtained from different mites species. A total of 550 parasitic mites of five species belonging to Laelapidae family were collected from small rodents in Lithuania during 2013 – 2014. *Rickettsia* DNA was detected in four mite species *Laelaps agilis, Hyperlaelaps microti, Eulaelaps stabularis* and *Myonyssus gigas*. PCR and sequence analysis of the partial *17kDa* and *gltA* genes revealed the presence of three *Rickettsia* species *Rickettsia helvetica, Rickettsia felis*, and unidentified *Rickettsia* sp. Phylogenetic analysis of *Rickettsia* strains isolated from different mite species revealed genetic heterogeneity and provides evidence for host-specific strain variation. To our knowledge, this is the first evidence of the presence of *R. felis* in *L. agilis* and *H. microti* mites, and the first report of the occurrence and molecular characterization of *Rickettsia* spp. in Mesostigmata mites in the Baltic region.

Creation of chimeric genetic constructions of plant protein kinase IREH1 from *Arabidopsis thaliana*

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Key words: *Arabidopsis thaliana*, gateway-cloning, genetic vector, MAST2, protein kinase.

In spite of the fact that protein kinase genes form a significant part of plant genome (about 1024 genes in A*rabidopsis thaliana*), our knowledge regarding the role in regulation of plant cytoskeleton phosphorylation is still fragmentary. In particular, special attention is concentrated on regulatory role of protein kinase MAST2, since it is considered to be one of the important targets of mitogenic effects on animal cell cytoskeleton. Recently, our studies have proven possible existence of protein genes in *A. thaliana* genome that are very close to the sequences of human MAST protein kinase.

Protein kinase IREH1 (At3g17850) from *A. thaliana* was identified as the most probable functional homolog of animal MAST2 and potential regulator of plant cytoskeleton. To experimentally study these plant enzyme and clarify its role in regulation of cytoskeleton structure, isolation of mRNA sequence and synthesis of cDNA library of plant protein kinase from *A. thaliana* (*Arabidopsis* MAST-like Kinase, AMLK/IREH1) was conducted. To prove the role of IREH1 in phosphorylation of microtubular proteins, *A. thaliana* has been transformed with genetic constructions enabling expression of a hybrid protein containing BFP (blue fluorescent protein) and a catalytic domain AMLK/IREH1 (At3g17850).

Genomic DNA sequences (AB019230 and CP002686) and corresponding mRNAs were obtained from GeneBank (AK117224) and EMBL-EBI (European Nucleotide Archive, ENA: AEE76015.1). Isolation of mRNA NMR1M12666.3 from *A. thaliana* seedlings was performed according to standard protocol by using TRIzol (Thermo Fisher Scientific, USA). Set of primers for cloning protein kinase IREH1A has been developed for sequence AK117224.1 (GenBank).

With the help of oligonucleotide primers containing *Bam*HI and *Sac*I endonuclease recognition sites, sequences at3g17850.1 were obtained for cloning in genetic vector Gateway® TagBFP-AS-C entry clone and Gateway® TagBFP-AS-N entry clone (Evrogen). Three fragments of protein kinase IREH1 (IREL, At3g17850): N (192-1689 bp), M (1601-2798 bp) and C (2639-4183 bp), that overlap each other in the corresponding ending sequences have been cloned. The resulting fragments were inserted in pEGFP-M + C plasmid and a cDNA library was obtained.

By using created cDNA library the genetic constructions for expression a fused chimeric protein containing BFP fragment (blue fluorescent protein) and product of gene *at3g17850.1* have been obtained. Restriction analysis, PCR and DNA sequencing of the resulting constructions confirmed successful cloning of *at3g17850.1* gene. The obtained genetic tools will be applied to conduct transient transformation of *A. thaliana*. The resulting lines, capable to express fluorescent chimeric products IREH1 (IREL, At3g17850) and certain components of the plant cytoskeleton (α-tubulin, MA4), allow co-localization studying of cytoskeleton structures and obtained fluorescence constructs IREH1 (IREL, At3g17850). Hopefully applying of resulting lines will confirm, or vice versa, participation of protein kinase IREH1 in regulation of the *A. thaliana* microtubular cytoskeleton.

Results of breeding program of the new fruit crop Japanese quince

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Key words: biochemical content, *Chaenomeles japonica*, fruit weight, plant habit, self-compatibility, yield.

Plants of *Chaenomeles* sp. as ornamentals are known in different countries of the world. More than 200 cultivars are grown for this purpose. However, *Chaenomeles japonica* as a fruit crop is well known only in Baltic countries, more or less in Scandinavia and Poland. Fruits of it are interesting raw material for food industry because of their nutritive value: organic acids, vitamin C, pectic polysaccharides, aroma components, phenolic compounds.

In Latvia, breeding of *C. japonica* as fruit crop was initiated in 1950-ies, and first large plantations were established in the 1970-ies. The interest for cultivation of *C. japonica* increased in Latvia in the 1990-ies when the area of plantations reached approximately 300 ha. All these commercial plantations were established by plants propagated from seeds, which are very heterogeneous. Because of this reason seedling plantations cannot be as profitable as those where vegetative propagated special cultivars would be grown. At Dobele Horticultural Plant Breeding Experimental Station (now Institute of Horticulture, Latvia University of Agriculture, further Institute) breeding of *Chaenomeles* was started in 1990-ies with the aim to obtain local cultivars adapted to Latvian climate. For breeding only one species – *C. japonica* – was used, because the others are not winter hardy in the Northern Europe.

C. japonica is very diverse in plant and fruit characters, and many important traits are controlled by additive as well as non-additive genes, so breeding of new cultivars took many years.

The main goal of the breeding program was to obtain cultivars with high yielding, locally adapted plants, resistant to frost and diseases, erect, not too dense and without thorns, early ripening, with good pollination, high content of biologically active compounds.

Breeding strategy was based on extensive test crosses and progeny tests in field trials. The main methods were controlled crosses choosing genotypes with desirable traits as parents. Performing hybridization, selection and evaluation in several populations, promising genotypes were obtained. In 2012 the first cultivar 'Rasa' was registered in Latvia. Also, two another cultivars – 'Darius' and 'Rondo' – were created in the common program together with Lithuanian and Swedish breeders.

All these cultivars are thornless, productive, total yield during 5 years can reach 20 to 28 kg per bush (maximal in 1 year: 8 kg per bush), resistant to leaf spot and fruit rotting. Fruits are yellow, weight 40 to 50 g, contain vitamin C 60 to 95 mg% and phenolic compounds 520 to 740 mg% in average, ripen at the beginning or middle of September. Since 80 to 90% of all studied genotypes are self-fertile, partial self-fertility of cv. 'Rasa' is a positive trait.

All three cultivars have been handed over for trials at five commercial farms in different regions of Latvia (various soil and climatic conditions), as well as at the Institute of Horticulture in Lithuania and at a commercial plantation in Estonia.

Since *Chaenomeles* is relatively resistant to diseases and pests, it can be grown in environmentally friendly way, and interest in it as a commercial fruit crop increases. The research activities in ERDF project Nr. 1.1.1.1/16/A/094 will develop new cultivars of this crop.

Determination of self-incompatibility alleles of sweet cherry (*Prunus avium*) cultivars in Estonia

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Key words: consensus primers, *Prunus avium*, S-alleles, self-incompatibility, sweet cherry.

Sweet cherry (*Prunus avium* L.), like most flowering plants, is a self-incompatible species. Determining suitable pollinators empirically involves deliberate pollination experiments over several years and counting the number of formed fruits which is very time and resource consuming. Genetically determining sweet cherry self-incompatibility groups is a more cost-effective alternative.

A wide range of local and foreign sweet cherry cultivars are grown in Estonia. Although extensive compatibility experiments have been done, it is not possible to use genetic background of the varieties to deduce possible S-alleles. This is because parentage of most Estonian sweet cherry varieties is unknown.

More than 16 S-alleles have been found for sweet cherry, but alleles S1-S6 are most common in the Nordic countries. In this study, we have used two sets of universal primers. The first set was primer pairs SI-19, SI-20 and SI-31, SI-32 that amplify S1-S6, S9 (Wiersma et al. 2001). The second set was primer pairs PaConsI and PaConsII that amplify S1-S16 (Sonneveld et al. 2001). Additionally, allele-specific primer pairs for the distinct identification of sweet cherry S-alleles S1 to S16 were used (Sonneveld et al. 2003).

Our objective is to reliably determine self-incompatibility alleles of sweet cherry varieties grown in Estonia and to find the most suitable method for genotyping sweet cherry S-alleles for advancement of sweet cherry breeding in Estonia. In this study we show the difference between two sets of universal primers and indicate possible challenges with universal praimers.

Acknowledgements
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Combining the cohort based biobank and health care system resources for effective study of pharmacogenomics of type 2 diabetes

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Key words: Genome Database of Latvian Population, type 2 diabetes.

Genome Database of Latvian Population (LGDB) is a national biobank that gathers, maintains and process health information data and genetic material collection of Latvian population, that serves for research, prevention and therapeutic purposes. LGDB comprises biosamples and associated phenotypic and clinical information of over 34 000 participants, that together with other biobanks in Latvia constitutes more than 2% of Latvian population. Among population based and hospital initiated recruitment segments the disease specific longitudinal cohorts are the most valuable for biomedical studies. Some of such cohorts have provided a unique opportunity to study the complexity of antidiabetic drug responses in healthy people and patients of type 2 diabetes. This study goes beyond the pharmacogenomics and demonstrates the system based approach involving investigation of microbiome and epigenetic factors determining the response of metformin, a widely used antihyperglycaemic agent in terms of efficacy and intolerance.

Nanoparticles as gene silencing tool in yellow alfalfa *Medicago falcata*

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Key words: *Medicago falcata*, microRNA, nanoparticles, pectin methylesterase, stress response.

Since the population grows continuously, an increasing food supply is necessary, while crop and herbage productivity decreases due to constantly changing environment. Plants are unable to move away from environmental stresses and are susceptible to various stress factors that affect growth, development and productivity. Abiotic stress such as nanopaticle pollution can negatively affect plant growth and yield, leading to yield decrease and economic losses. Therefore, understanding of plant stress response and adaptation mechanisms are fundamental biological questions with extreme importance for breeding stress tolerant crops.

Medicago falcata L. or yellow alfalfa is economically and ecologically important plurannual forage legume that is commonly distributed throughout the world. Moreover, *M. falcata* is one of the best stress tolerant plant species and has variety of genotypes that makes plant agriculturally important.

As a sessile organisms plants have developed complicated mechanisms for perception and responding to environmental stresses. To turn on regulatory mechanisms, they initialize a network of genetic regulations by transciptional or translational changes. Recent research shows that to continue growth under stress conditions, plants utilize microRNA (miRNA) as key post-transcriptional gene expression regulators. These small endogenous RNAs regulate gene expression via target mRNA cleavage and translational repression. Generally, stress-upregulated miRNAs down-regulate their target mRNA, while their reduction result in accumulation of positive proteins.

Pectin methylesterase is a group of enzymes that are coding by *Pme* gene family, disintegrate pectin structure in plant cell wall. *Pme3* gene expression is related with enzyme activity that acts as a factor for cell wall weakening, consequently causing easier entry of pathogens, resulting in plant damage and death.

The aim of this study was to detect the amount of plant resistance-specific miRNA in plants under nanoparticle stress conditions. To experimentally detect specific miRNA, *M. falcata* was used as a model plant. Obtained yellow alfalfa seedlings were grown in hydroponics supplemented with various concentration of Fe_3O_4 nanoparticles. Alfalfa *pme3* gene-specific primer with locked nucleic acid for real-time PCR reactions was designed. Results indicated that low concentration of iron oxide nanoparticles can affect amount of specific miRNAs. Nevertheless, further investigations are needed to deeper understand mechanisms of plant stress response and to examine method for improving the ability of plants to withstand against biotic and abiotic environmental stresses. Understanding stress responses will increase our capability to improve stress resistance in crops and achieve agricultural sustainability for a growing world population.

Cellular and molecular technologies for creation of intergeneric hybrids of Festulolium

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Key words: Festulolium, genotyping, hybridization, intergeneric hybrids, micropropagation.

The biotechnology contribution to agricultural production is to facilitate the traditional methods of plant breeding. New molecular and cellular technologies allow to improve the effectiveness of agriculture. To create Festulolium intergeneric hybrids we used biotechnological methods of distant hybridization, the method of embryo culture from an immature caryopsis, microclonal micropropagation *in vitro* and DNA genotyping.

Hybridization was carried out under phytotro-greenhouse complex (FGC) conditions and in field conditions on plants that started flowering in the early and late periods. In FGC conditions, the stamens were removed in the panicles before pollinating. On the 17th day after pollination the immature caryopsis were derived from the panicles of the mother plant. In field conditions at ear formation phase before flowering the main shoots of the mother plant were isolated. Pollination was carried out during full flowering of panicles without pollinium castration. Late-ripening biotype hybridization was carried out a week after the early ripening biotypes of Festulolium.

To overcome the pro-and post-gamma incompatibility more than 300 embryos of intergeneric Festulolium hybrids were cultivated on nutrient medium with specially selected composition. In the course of intergeneric hybridization an intensive morphogenetic process takes place as a result of gene recombination that allowed to accelerate the selection of perspective forms. This forms integrates genomes in Festulolium hybrids.

Viable Festulolium plants which were obtained as a result of biotechnological works were planted in soil conditions of FGC. Form bushed plants will undergo jarovization to obtain seed grains from hybrid plants.

For micropropagation of Festulolium hybrids the method of axillary meristem activation was used. For forthputting activation we used Murashige and Skoog medium containing various concentrations of benzylaminopurine. It has been shown that for cutting cultivation of Festulolium hybrids Murashige and Skoog medium with benzylaminopurine addition in concentrations 0.5 and 1.0 mg L^{-1} for different lines should be preferably used.

Then we carried out the hybrid genotyping. Five RAPD primers were selected for the Festulolium hybrid genotyping. The maximum number of loci (17) was identified using SCoT-02 primer, minimum (12) with SCoT-23 primer. A total amount of identified loci was 73, 69 markers were polymorphic. The applied marker system made it possible to reveal a high level of polymorphism in the studied genotypes of Festulolium, 94.52% on average.

Based on 73 SCoT-markers for Festulolium hybrids the genetic passports have been composed, the genetic distances have been calculated with genotype clustering by Neighbor joining method and separate dendrograms for each primer have been designed.

So the biotechnology methods makes it possible to shorten the period of intergeneric hybrids creation with economically valuable characteristics.

Modern possibilities of genetics and genomics in cereal breeding

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Key words: cereals, genetics, genomic selection, genomic, molecular breeding.

At the beginning of the 21st century, mankind faces the dual challenges of providing enough food for a growing population with a background of reduced resources and more variable climatic conditions. In this context, genomics and associated molecular marker technology must play a key role in developing new varieties better adapted to address these challenges. During the last decade, molecular marker technology has provided a wide range of novel approaches to improve selection strategies and together with the rapid accumulation of genomics tools and the emergence of high throughput technologies has facilitated practical implementation into cereal breeding.

The availability of new molecular tools and technologies is beginning to filter through the breeding process to have a significant impact on plant variety development and is proving to be the essential element required to accelerate breeding process. The results of specific applications of molecular markers, potential of genomic selection and the application of genomics in cereals will be demonstrated and discussed.

Linking organismal growth, coping styles, stress reactivity, and metabolism via responses against a selective serotonin reuptake inhibitor in an insect selected for developmental speed

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Key words: behaviour, developmental speed, metabolism, selection lines, selective serotonin reuptake inhibitor.

Evidence suggests that brain serotonin (5-HT) is one of the central mediators of different types of animal personality. We tested this assumption in field crickets *Gryllus integer* using a selective serotonin reuptake inhibitor (SSRI). Crickets were selected for slow and rapid development and tested for their coping styles under non-stressful conditions (time spent exploring a novel object). Resting metabolic rate, maximum metabolic rate and latency to resume activity were measured under stressful conditions (stress reactivity). Measurements were taken (i) before and (ii) during the SSRI treatment. Before the SSRI treatment, a strong negative correlation was observed between coping style and stress reactivity, which suggests the existence of a behavioral syndrome. After the SSRI treatment, the syndrome was no longer evident. The results of this study show that 5-HT may be involved in regulating behavior not only along a stress reactivity gradient but also along a coping styles axis. The relationship between personality and the strength and direction of 5-HT treatment on observed behaviors indicates trait-like individual differences in 5-HT signaling. Overall, these findings do not support recent ideas arising from the pace-of-life syndrome (POLS) hypothesis, which predict higher exploration and metabolic rates in rapidly developing bold animals.

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Genetic variation of *Astrantia major* population using for restoration of natural Belarus coenopopulations

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Key words: AFLP, *Astrantia major* L., critically rare species, restoration of natural coenopopulation.

Astrantia major L. is critically rare species of the category I. The natural range of this species covers mainly the mountainous regions of Central Europe. Individual locations are known in Eastern Europe: Lithuania, Latvia, Ukraine and Moldova. The only one location in the Belovezhskaya Pushcha National Park (BPNP) coenopopulation is known in Belarus at the time of the examination (May 23, 2018), which is located in the grove of the hornbeam and consisting of 14 individuals developing into a right-sided type populations with no signs of generation. This site of growth in BPNP was described for the first time in the early 1970-ies and this population was quite numerous having at about 120 individuals, although even then it was represented by underdeveloped plants with irregular flowering. Based on the results of the age spectrum studies of this coenopopulation conducted over a number of years, a trend toward its slow extinction has been found. In order to preserve the biodiversity of BPNP and restore this population, protection translocation measures are planning to implement according to the task "Foundation of scientific base for the formation of a national reserve gene pool of rare and endangered plant species of Belarus natural flora and identify ways of their conservation and repatriation" within the framework of the State Nature Management and Ecology Program.

A sample of about 20 plants obtained from seeds of BPNP coenopopulation is growing in the collection of rare and endangered plant species of Belarus natural flora of the Central Botanical Garden (CBG) of the National Academy of Sciences of Belarus. Comprehensive ecological, morphological, anatomical and carpological studied have been carried out of this sample during the last five years. The aim of this investigation was to perform comparative DNA analysis of CBG sample to ensure possibility of its use for restoration natural coenopopulation in BPNP.

To analyse genetic variation of *A. major* population planning for restorating natural coenopopulation, the following samples were included in AFLP analyses: (i) BPNP natural coenopopulation, (ii) BPNP artificial population used for restoration in 1980 (origin of the plants used is unknown), (iii) CBG collection of rare and endangered plant species, (iv) sample from National Botanical Garden (Ukraine, Kyiv) and (v) *A. major* commercial variety using for estimation of possible range of species genetic diversity. Leaf material was collected in summer 2018. The leaves were dried in silica gel and DNA was extracted using QIAGEN DNeasy 96 Plant Kit to maintain the quality of DNA as high as possible. 300 ng of genomic DNA was digested with *Eco*RI and *MseI*, and double-stranded *Eco*RI and *Mse*I adapters were ligated to the ends of the fragments. Six primer combinations were used in the last amplification using C1000 TouchTM Thermal Cycler (BioRad). Fragment analysis was performed on GenomeLab GeXP (Beckman Coulter) using 3` fluorescently labeled primers. Genetic distances was estimated using GenAlEx ver. 6.502. A dendrogram was created using unweighted pair group method with arithmetic mean.

The results obtained proved genetic closeness of BPNP natural coenopopulation and CBG collection sample planning for restoring. Some genetic difference in these samples could result from species allogamous mechanism leading to intrapopulation genetic variation rather than evolution (population) changes during cultivation in CBG conditions. It was detected that plants used for restoration of BPNP coenopopulation in 1980 were not closed to natural *A. major* population. The commercial variety significantly differs from natural samples as it was predicted to estimate possible range of species genetic diversity. The closeness of Kyiv sample to BPNP and CBG population was detected that needs to be additionally investigated as could result from collecting near BPNP or common genetic structure of Belarus and Ukraine *A. major* populations.

Variation in DNA polymorphism of hybrid aspen F2 progeny *in vitro* system

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Key words: aspen, gene, hybrid, offspring, SSR markers.

Selected hybrid aspen trees are promising in Lithuanian conditions for planting of short term tree plantations. The biomass from fast growing trees provides a renewable feedstock for biofuels, limber, pulp, paper and other uses.

The selection of individuals with specific genotype and phenotype at early stage of development could be very useful for research and breeding purposes.

The aims of this study were to perform in vitro system initial estimation of F2 progeny variation in DNA polymorphism after lineal hybridization selected trees 51DhPL009 ♀ (*Populus tremuloides* × *Populus tremula*) × (*P. tremuloides* × *P. tremula*) ♂ 51DF1001. The 11 microsatellite (SSR) primers of specific loci were used for identification to specific trait, linked region in progeny F2 genotypes. It was fixed different variability of the selected microsatellite loci in DNA of 38 individuals. The five SSR markers of *Pto CesA4* gene were identified associated with cellulose and lignin components. The indications of polyploidy were found in several genotypes by three SSR markers. The obtained results show that combining two methods of plant biotechnology as tissue culture and molecular markers are promising tool for improving hybrid aspen breeding program at early stage of tree development *in vitro* system after hybridization under controlled conditions.

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Investigation of the content of polyfructans in plants of buckwheat with different flower color

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Key words: *Fagopirum esculentum*, polysaccharides.

Buckwheat (*Fagopirum esculentum* Moench) is one of the most valuable pseudocereal crops due to the unique combination of positive therapeutic and dietary properties, which improves functioning of the gastrointestinal, blood and hormonal systems of humans. The seed of this crop is rich in vitamins B1, B2, B6, B9, E, PP, P, has valuable mineral elements (iron, copper, manganese, calcium), as well as organic acids (citric acid, malic acid etc.). Buckwheat seed contains 10 to 18% protein, in which the content is dominated by the essential amino acids lysine and tryptophan, which are low in other cereals and pseudocereals. In addition to protein, buckwheat seed contain about 70 to 85% of carbohydrates (mainly starch) and 2.5 to 4% of oils with high content of linoleic and linolenic acids, resistant to oxidation during storage. The starch of buckwheat is more slowly digested than the starch of cereals, so it is an indispensable product for diabetics.

Growing and breeding of buckwheat has a long history in Ukraine. For many years breeding work on buckwheat with involvement of local genetic material created high-yielding varieties. However, obtaining high and stable buckwheat yields over the years is still a difficult task due to low adaptability of this culture and sensitivity to such abiotic stressors as lack of moisture or high temperatures.

One of the probable ways for breeding improvement of buckwheat is the creation of varieties that are resistant to the influence of adverse factors during germination. Changes in physiological processes that lead to adaptation of plants to germination in certain areas can have an impact, both positive and negative. For example, the spectrum of fatty acids of membrane lipids may change, and there may be an increase or decrease in the accumulation of polysaccharides, which also causes changes in the organoleptic properties of the product. The purpose of the study was to investigate differences in the amount of polysaccharides between the genotypes of buckwheat seeds of different origin.

For this purpose, the total amount of polysaccharides per 100 mg of buckwheat mass has been investigated. The experiment used varieties of different origin (Ukraine, Russia, Belarus) and different ecological groups, which varied by a color of flowers. Higher polysaccharides are polymers consisting of a number of structural units, monosaccharide residues. Polysaccharide molecules consist of numerous residues of monosaccharides (glucose, fructose, galactose, mannose, etc.).

During the study it was found that the largest amount of polysaccharides are in seeds of plants with white flowers and the least in plants with red flowers. As for data on plants with green flowers, there is no clear picture on them, but there is a certain tendency to reduce the amount of accumulation of complex sugars compared to plants with white flowers.

First attempt to appreciate genetic structure of river lamprey population in Latvia, Kurzeme region

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Key words: lamprey, population genetics.

Lampreys are jawless fishes evolutionarily positioned between invertebrates and vertebrates. In Latvia three species of lampreys are recorded: river lamprey (*Lampetra fluviatilis* L.), brook lamprey (*Lampreta planeri* Bloch) and sea lamprey (*Petromyzon marinus* L.) (Kļaviņš 2018). These species can be distinguished by size and specific colouration only in adult form, but in younger stages only during their freshwater life period by DNA analyses. Adult sea lampreys are the largest ones (~120 cm), river lampreys are ~51 cm, but brook lampray's length is only ~32 cm. River lamprey is a commercially important species and its population is continuously declining. All lamprey species require good water and substrate quality, so they are threatened throughout their range by habitat degradation. Physical barriers in rivers are also a threat to lampreys as they migrate upstream to spawn. Latvia is one of few EU countries that still breeds and recycles river lamprey on a large commercial scale.

Microsatellite analysis and mitochondrial DNA (mtDNA) sequences have been applied previously to explore genetic diversity of different lamprey species and reconstruct evolutionary events and migratory patterns. Different sets of microsatellite loci have been used for river lamprey and brook lamprey. Multiple mitochondrial gene segments have also been used to discriminate between these two closely related lamprey species. Some studies using mtDNA have failed to distinguish both species genetically. On the other hand, microsatellite data have shown genetic differences across species and ecological groups within the same species. Overall, there are quite a few genetic studies in North American and Western European lamprey populations, however, no assessment of the structure of Latvian lamprey population has been done so far. This indicates the urgent need foor such studies in local populations as they might be prone to decline.

Here we present planned activities of the recently launched LAMPREY project. One of the objectives is to appreciate genetic structure of local population by microsatellite analysis and mtDNA sequencing. These data will allow to evaluate level of natal homing in migratory river lamprey populations in the Region clarifying whether river lampreys should be treated as one or separate management units. As well as will help to appreciate efficiency of existing lamprey restocking methods. Genetic data also give some insights into speciation of river lamprey and brook lamprey as there is an unsolved global debate whether they are the same species or not. Currently only river lamprey is commercially most exploited in the programme area. There are concerns in appreciation of lamprey stock due to the lack of speciation between river and brook lamprey. Within project Cross-Boundary Evaluation and Management of Lamprey Stocks in Lithuania and Latvia (LAMPREY, 2019 – 2020) river lamprey population will be analyzed using genetic differentiation at microsatellite DNA loci and mtDNA in catchment area of Kurzeme region.

Character of inheritance of flower color of hybrids F1 of winter rape (*Brassica napus*)

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Key words: dominance, hybridization, marker trait, winter rape.

Rape (*Brassica napus* L.) is an important and perspective oil crop in Ukraine and world. The quick growth of sown area of rape to 1.1 – 1.5 million ha, in the last 10 to 15 years in Ukraine, is associated with an increase in demand for rapeseed oil and seeds in international markets. The main factor contributing to this was the targeted breeding work on the introduction into the rapeseed production of varieties with low content of erucic acid in oil and glucosinolates, and in the future – non-erucic and low-glucosinolates varieties (two low types, "00") that guarantee the production of oils and edible products that are safe for health of man or animals.

The importance for breeding work is selection of parental components with distinct genetically determined marker traits for crossings, based on their previous studies and evaluation of received hybrids for complex of traits. Such signs can be biochemical features: the content of erucic acid in the oil, the content of glucosinolates in the meal; signs of the structure of the yield: the weight of 1000 seeds, the number of pods per plant, the number of branches and phenotypic markers: the shape of the leaf plate, the presence of specific features of the flower – the length of pollen or stamens, the color of the petals. It is the study of the inheritance of the white color of flower petals devoted to the work, since this feature is promising in view of the use in the breeding process. In the State Register of Plant Varieties of Ukraine there is one variety of white-flowered rape, indicating the possibility of using such genotypes when developing a new initial breeding material of rape.

The color of the rape flower can be yellow, creamy white, orange and white. This is largely due to gene alleles that control the synthesis of xanthophyll and other yellow pigments. It is the minimal synthesis of these pigments that determines the white color of the flower petals. Such a color of a flower is a mutation found not only in rape but also other species of the Brassicaceae family such as *Brassica juncea* and *Brassica carinata*, etc. The cytological and genetic studies of the white color of the flower in the rape determined that this trait could be transmitted to the rape genus of the wild radish (*Raphanus sativus* L.). In other studies, the authors point to ambiguous genetic control of the white color of the rape flower: from one semi-dominant gene with multiple alleles to three genes with a different inheritance character.

In generations of hybrids of winter rape, ten parental components differing in their flower color were distinguished by their economically valuable features: BK-1, BK-2 (with a white flower), GK-1, GK-2, GK-3, GK-4 (with a yellow flower), LK-1, LK-2 (with lemon flower) and KK-1, KK-2 (with a creamy white flower). Hybridization was carried out by isolating individual plants with gauze insulators, castration of flowers with further interbreeding. The first generation hybrids were sown in separate rows in the conditions of the Chabany, Kyiv-Sviatoshinsky district of Kyiv region of Ukraine in 2017.

In selected hybrids of rape from combinations of crosses, which inherited the white color of the flower, maternal forms were white flowered lines, and the parents were genotypes with white flowers and lemon color of flowers. This indicates a certain possible recessive nature of the inheritance of the sign of white petals color. In the first generation of hybrids, from the crossing of the yellow flower and white flower rapeseed genotypes, the yellow color of the flower revealed the dominant nature of the inheritance.

Use of *Solanum verrucosum* and *SvSv*-lines for overcoming unilateral incompatibility in crosses with wild allotetraploid potato species

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Key words: interspecific hybridization, potato, unilateral incompatibility.

Wild allotetraploid potato species (4x, 2 EBN) are considered as a valuable source of genes that are of interest for breeding aimed at resistance to a wide range of pests, diseases and abiotic stresses. Nevertheless, allotetraploid species are rarely used in breeding since they practically do not cross with cultivated potatoes *Solanum tuberosum* (4x, 4 EBN) due to difference in their endospem balance number (EBN). In addition, seed formation hardly occurs when allotetraploid species are used as a male parent because of unilateral incompatibility. Sparse cases of successful gene introgression of allotetraploid species into breeding material are accompanied by male sterility of interspecific hybrids associated with cytoplasm of wild species.

The aim of the research was to use wild diploid self-compatible potato species *Solanum verrucosum* and *SvSv*-lines to overcome unilateral interspecific incompatibility in crosses with wild allotetraploid potato species. It was demonstrated earlier that self-compatibility of *S. verrucosum* is caused by the lack of stylar S-RNases that inhibit pollen tube growth (Eijlander 1998). *SvSv*-lines produced in our laboratory represent F2 *S. tuberosum* dihaploids × *S. verrucosum*. It was anticipated that in *SvSv*-lines S_t-alleles of *S. tuberosum* have been substituted for S_v from *S. verrucosum*. Thus, *SvSv*-lines have the same ability for elimination of prezygotic incompatibility in interspecific hybridization as *S. verrucosum* (Polyukhovich et al. 2010). They have the markers of D/γ type of cytoplasm and produce functionally fertile pollen.

We were the first who produced hybrid seeds and viable hybrids in crosses between *S. verrucosum* and allotetraploid potato species *Solanum stoloniferum* and *Solanum acaule* as a male parent (Polyukhovich et al. 2013). In 2013 six *SvSv*-lines were pollinated by 26 accessions of *S. stoloniferum*. Crosses with three *SvSv*-lines resulted in production of 1893 hybrid seeds. In spite of poor seed germination (1.9% on the average), 36 viable seedlings were produced. Chromosome counting in 13 seedlings revealed that all of them were triploids. Experiment on hybridization between *SvSv*-lines and *S. stoloniferum* has been reproduced in 2015 with the accession of wild species PI205522 that had, according to our data, DNA markers of potato virus Y (PVY) resistance genes *Rysto, Ryf-sto, Ryadg*, as well as the gene *Rpi-sto1* of high resistance to the late blight (LB). It also had markers of "sterile" type cytoplasm W/γ. Five hundred forty eight hybrid seeds and 12 viable seedlings were produced. Genome of some of these seedlings was doubled by colchicine treatment. We were able to cross them as females with fertile cv. Katahdin (about 100 seeds have been obtained). Pentaploid hybrids (BC1) formed viable seeds when were pollinated by cv. Quarta. The hybrids of BC2, selected for markers of PVY and LB resistance genes were successfully involved in crosses with some potato cultivars as female and as male parents. Considerable part of hybrids F1 (hexaploid), BC1 and BC2 were male fertile. Now we study the peculiarities of introgression of the above mentioned genes into breeding material during backcrossing the hybrids with potato cultivars.

Thus, the use of *S. verrucosum* and *SvSv*-lines made it possible to overcome unilateral incompatibility in crosses with allotetraploid species *S. stoloniferum* and *S. acaule*. With the use of *SvSv*-lines we were able to perform marker assisted introgression into breeding material of valuable resistance genes of the particular *S. stoloniferum* accession PI205522. The hybrids between *SvSv*-lines and *S. stoloniferum* had cytoplasm of "fertile" type and therefore many of them as well as their backcrosses to cultivated potatoes had good male fertility. It substantially extends the sphere of their use in breeding.

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APOA1, *LPA* and *PLA2G7* gene polymorphism in patients with subclinical atherosclerosis and ischemic heart disease

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Key words: *APOA1*, cardiovascular pathology, *LPA*, *PLA2G7*.

The frequency of ischemic heart disease (IHD) in people under 45 significantly increased during last decades. Together with the fact that in more than 50% cases the first symptom to be manifested is acute myocardial infarction it makes the search of potential IHD risk markers extremely important. It was shown that high-density lipoproteins blood concentration could affect the predisposition to cardiovascular pathology.

Therefore, we have studied polymorphism in three loci, associated with high-density lipoproteins metabolism (rs670 in *APOA1* gene, rs1805017 in *PLA2G7* gene and CNV in KIV-2 locus of *LPA* gene), in 39 individuals with ISD, 81 individuals with subclinical atherosclerosis (SA) and 40 individuals without known cardiovascular pathology. Genotyping was performed using self-designed Taq-Man probes.

The rs670 genotypes distribution in patients with IHD was found to be significantly different from that in controls ($p = 0.019$). Heterozygote genotype GA frequency was more than twice higher in control group (47.5 and 20.5%, OR = 0.29, 95% CI 0.11 – 0.77), so this genotype is supposed to be protective against IHD. Genotypes frequencies in patients with SA and controls were almost similar ($p = 0.998$).

The average numbers of KIV2 repeats in *LPA* gene were 20.47 ± 8.87 in controls, 26.94 ± 11.04 in patients with SA and 27.43 ± 12.27 in patients with IHD. Patients in both groups had significantly higher numbers of KIV2 repeats compared to individuals without cardiovascular pathology ($p = 0.003$ and $p = 0.009$ for SA and IHD, respectively). These results let us assume that CNV polymorphism of KIV-2 locus of *LPA* gene could be associated not only with IHD risk, but also with asymptomatic blood vessels disease.

No significant differences in *PLA2G7* genotypes distribution between groups were found.

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Metabolomics of *Avena* species as a resources for new directions in plant breeding

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Key words: *Avena*, avenanthramide, FHB, metabolom, mycotoxins.

Complex field and laboratory evaluation of accessions of wild species of the genus *Avena* collection at VIR were conducted. The metabolomic analysis of oat cultivars (*Avena sativa* L.) and accessions of *Avena* wild species resulted in the identification of metabolites the content of which tends to change along with the 'domestication' process and differentiates wild oat species from oat cultivars. The range of content of some acids was narrower in *A. sativa* compared to that observed in wild oat accessions. The concentration of malic acid in the seeds varied within the 1.5 to 4.5 mg 100 g^{-1} range in cultivars, while in wild oat accessions it was from 1.18 to 10 mg 100 g^{-1}; saccharose ranged from 3 to 3000 mg 100 g^{-1} in wild species and from 800 to 2800 mg 100 g^{-1} in cultivars. Wild oats contained 0.8 to 86 mg 100 g^{-1} of monoacylglycerol (MAG, C16:0), while its concentration in cultivars was 6 to 20 mg 100 g^{-1}. For MAG-2 (C18:2) these values were 78 to 695 mg 100 g^{-1} and 18 to 46 mg 100 g^{-1}, respectively. Our opinion is that such compounds as MAG 16:0 and MAG-2-18:2 may have some relation to adaptability, in particular to resistance to biotic and abiotic environmental factors. The analysis of the micronutrient composition in *A. sativa* accessions showed genotypes with a high content in the groat of such elements as Fe, Zn and Mn. A study of 15 landraces from Mongolia, China and Russia and 100 cultivars and 30 accessions of wild *Avena* species with differing ploidy levels showed them to differ much in terms of avenanthramide content in the kernel. This is to be further examined in detail by metabolomic screening of oat accessions in which we found some degree of resistance to *Fusarium* head blight. Positive correlations were found between *Fusarium* head blight resistance and mycotoxin accumulation. It was also found that the concentration of sucrose and fructose inversely correlated positively with grain affection with *Fusarium* head blight and mycotoxins content. It was established that an improvement of the studied quality parameters of the kernel (content of protein and oil) leads to a decrease in avenanthramides. The oil content showed a positive correlation with content of protein in the groat with the exception of avenanthramides content. Besides, a weak link with increased avenanthramide content in kernels infected with *Fusarium* head blight was established. Therefore, the global oat collection at VIR is an essential and valuable source of material for ensuring food, bioresource and ecological security, as well as for the stable supply with high quality food and feed. It supports a sustainable development of ecologically safe agriculture.

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Challenges to crop breeding and production to tackle climate change

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Key words: climate change, crop production, plant breeding.

According to IPCC modelling results, future climatic conditions in northern Europe, including Latvia, will be warmer and wetter, and temperature increase will be higher in northern than in southern Europe. Since the summer precipitation may increase only slightly, increasing temperature stress and early summer droughts, which are considered as one of the main causes of low crop yields, may become more common. Moreover, climate change also implies increasing frequency of rainy days and heavy rainfalls. Increases in atmospheric CO_2 concentration will also impact on crop growth by increasing the resource (radiation, water and nitrogen) efficiency. Despite for most crops grown in northern Europe (i.e. in Latvia) some favourable growing conditions are projected, however negative impact and consequences are also indicated.

Breeding efforts that focus on yield increase have gradually minimized or eliminated the plasticity for stress response, and exposing the crop production vulnerable to climatic changes. It is essential to understand how plants respond to different abiotic stresses in order to improve crop performance.

Taking into account the above mentioned considerations, the aim of the study is to identify main issues and to determine appropriate options, including plant breeding, for crop production adoption to climate change.

The principal materials used for the studies are as follows: various sources of literature, e.g. scholars' articles, research papers and the reports, *inter alia* institutions. The suitable qualitative research methods have been used: monographic, analysis and synthesis; logical and abstractive constructional; data grouping and comparing. The results of study are grouped and presented further in the text.

The preliminary results provided by scholars have evidence suggesting necessity to identify potential candidate genes for crop improvement.

The both climatic conditions and soil characteristics could limit crop production. The land and soil suitability must be assessed, and some factors could be used to distinguish between unsuited and suitable land.

Adaptations to climate change could be explored using process-based models. The crop-level adaptations of existing cropping systems could change crop varieties and crop management.

Robustness has also been discussed in the context of cropping systems exposed to climatic or biotic perturbations. Increasing diversity and adaptive capacity of agricultural systems are key drivers for increasing the ability of agricultural systems to tackle different types of perturbation. The level of sensitivity of a crop to drought depends to soil characteristics. Similarly, increasing diversity at the species level by using crops characterized by different exposure periods allows the spreading of risks through the entire cropping rotation.

Increasing landscape diversity minimizes the impacts of perturbations on agricultural systems. There is strong evidence that diversity in farm size and intensity (e.g., variety choice, fertilizer and pesticide use) reduces the vulnerability to climate change. Land use diversification positively correlates with the resilience. Besides, agricultural biodiversity is essential to the climate change resilience, which is supported by strategies: protection and restoration of ecosystems, sustainable use of soil and water resources, farming systems diversification, various alterations in cultivation practices, use of stress-tolerant crops and crop improvement.

In crop management decision tools are helpful to prevent abiotic or biotic risks. Several tools are available (pest outbreaks, soil water availability or nitrogen nutrition index).

Induction of polyploidy in giant miscanthus (*Miscanthus* × *giganteus*)

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Key words: antimitotic agents, cell culture, dinitroanilines, *Miscanthus* × *giganteus*, polyploidization.

Utilization of renewable energy sources, such as wind, solar irradiation and biomass is one of the main components of sustainable economics. Moreover, growing of technical crops for biomass production solves the problem of contaminated and lands unsuitable to plant food crops (Tytko, Kalinichenko 2010). Today, the giant miscanthus (*Miscanthus* × *giganteus* Greef et Deu.) is considered to be one of the most promising energy crops due to its low requirement to soil and high biomass yield, the latter can reach up to 39.89 t per ha of dry matter (Bilandžija 2018).

Miscanthus × *giganteus* is a sterile allotriploid emerged as a result of combining of *Miscanthus sacchariflorus* and *Miscanthus sinensis* genomes (Rayburn et al. 2009). Thus, there is no breeding programs to be applied for *M. giganteus*, and according to the results of the DNA chloroplast study, only one haplotype represents all its commercial plantations (de Cesare et al. 2010). One of the methods to restore fertility in such cases is obtaining of polyploid forms. In addition, polyploidization proved to be an indispensable method for obtaining new genotypes of plants that have a larger cell size and higher productivity of biomass accumulation compare to the original forms (Kim et al. 2003).

Recently, for the production of plant polyploids, besides colchicine, more effective antimitotic agents became popular, namely representatives of dinitroaniline compounds: oryzalin and trifluralin. It should be noted, that these classical representatives of this compound class have a significant level of phytotoxicity which negatively affects the effectiveness of polyploidization of plants when applied. Therefore, the aim of the work was to develop methods for effective polyploidization of *M. giganteus*. Modern methods of plant biotechnology, and particularly, application of bioinformatics allows to conduct *in silico* screening of compounds on their affinity to tubulin, the main microtubules protein which are the most important intracellular cell division elements, for selecting the most promising compounds for polyploidization *in vitro*. Thus, six new compounds, derivatives of 2-nitro- and 2,6-dinitroaniline have been used in the work (Melnychuk et al. 2016).

According to the results of the experiments, all compounds applied in the work proved to be capable to induce polyploidization. At the same time, their low phytotoxicity, unlike classical dinitroanilines, led to a decrease in deaths of cultivated explants, which positively affected the effectiveness of their use. As results of the study effective protocols for *in vitro* culture establishment have been developed, micropropagation techniques have been worked out and effective concentrations of dinitroanilines to conduct *in vitro* polyploidization of *M. giganteus* have been determined. Obtained lines of miscanthus have been adapted for growing in open soil conditions. Currently, the analysis of the obtained lines is ongoing in order to study their morphometry and economic value as raw materials for biofuel production.

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Effect of farming system on genetic diversity of spring barley populations

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Key words: composite cross populations, genetic adaptation, *Hordeum vulgare* L., microsatellite markers, natural selection

During the last century a great progress of crop yield and stability has been achieved in high-input, conventional agriculture partly due to creating of innovative homogeneous varieties. However, crop cultivation in fluctuating environments such as organic farming system and crop response to biotic and abiotic stressors still remains a challenge. Plant populations in contrast to the pure lines are expected be adapting to environmental conditions. Increasing genetic diversity in crops and subjecting them to natural selection can improve such traits as disease severity, competitive ability with weeds and nutrient uptake efficiency (Döring et al. 2011).

To test for the effect of the farming system – conventional and organic – on genetic diversity of three barley composite cross populations (CCP, bulked dialell crosses among group of 10 parents) and one complex cross population (CPOP, four parents combined in one cross) were used. The breeding aims were following: yield and its stability (CCP-1), hulless barley for food use with high β-glucan content (CCP-2), yield, its stability and quality (CCP-3) and hulless barley for organic farming containing resistance to loose smut (CPOP).

In 2014, 96 individuals from CPOP (F5) and all CPPs (F3) were genotyped and cultivation in two farming systems was initiated (each population divided in two subpopulations for growing in a respective environment). After two year cultivation, in 2016 genotyping of subpopulations took place. Eight simple sequence repeat (SSR) markers were multiplexed in three marker sets: set A (Bmac0067, Bmac0032), set B (Bmag0135, WMC1E8, Bmag0173) and set C (Bmag0353, Bmac0093, Bmac0156) (Macaulay et al., 2001). PCR products were separated on an ABI 3100 × l capillary sequencer.

In 2014 the mean number of alleles in CCP populations ranged from 4.3 to 4.8, but CPOP had lower average number of alleles – 3.9 – as expected by having less parental lines involved in the cross. After two years of cultivation the number of alleles in CPOP had decreased to 3.5 in organic conditions and to 2.9 in conventional conditions, but in CCP number of alleles fluctuated by less than 10% around the initial mean allele number. Principal coordinate analysis showed that cultivation of populations for two years in two different growing systems did not result in division of distinct sub-populations.

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Why gout develops in some people but in others it does not?

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Key words: *ABCG2* gene, 'Genome Database of the Latvian Population' (LGDB), gout, podagra, risk factors.

Gout (greek *podagra*) is a severe, extremely painful form of inflammatory arthritis, which affects a significant proportion of the community.

Gout is the result of excess uric acid in the body, a condition known as hyperuricemia. Several reasons can lead to hyperuricemia: the overproduction of uric acid, an excess of purines in food, or a reduction of urate transportation in the kidneys. If urate levels are persistently above a critical level (> 7 mg dL^{-1}), urate crystal formation is triggered in and around the joints. Both genetic and environmental factors are involved in the aetiology of gout. Amongst genetic studies that have already been carried out, most evidence is reported in reference to the *ABCG2* gene. Genetic variants in the *ABCG2* gene influence the functions of the ABCG2 protein, which results in a reduction of the urate transportation rate. The most significant evidence regarding the relationship between the genetic variant in this gene and gout is already known for rs2231142 (which has a traditional name of Q141K). Although the role of genetic factors in the aetiology of gout are well documented, demography data such as education level, BMI, or blood pressure are important risk factors in relation to gout. In addition, in the aetiology of gout, the sex of an individual plays a crucial role. Males are more commonly affected.

However, not all people with hyperuricemia manage to develop gout. Several reasons are known for this phenomenon. One of these could be the "risk" genotype. In this study we were analysing whether rs2231142 in the *ABCG2* gene is risk factor for gout in Latvia's inhabitants; in addition, the role of demographic data was also considered.

A total of 43 patients with gout were analysed, along with 99 healthy individuals, from the 'Genome Database of the Latvian Population'. Genotyping was carried out using a TaqMan probe.

In this study, the T allele as a risk allele for the development of gout was confirmed: *OR* = 3.73, *CI* 95% = 1.77 – 7.85, $p = 0.0003$.

From the demographic data, the strongest association with gout can be observed in terms of the sex of the individual. According to the obtained *OR* risk levels for males when it comes to developing gout, they are 3.15 times higher when compared to the risk levels for females. In order to weight up the educational level in our results we can see that individuals with a higher level of education (tertiary) are less commonly affected by gout in comparison with those with a lower level (secondary) education: *OR* 0.32 and 1.02 respectively. A strong influence on the development of gout was also identified in the BMI of individuals (*OR* = 1.133). A high BMI indicates the absolute importance of good dietary habits in a patient who is suffering with gout.

This study indicates that development of gout depends on "risk" genotypes, as well as on factors such as age, sex, BMI, lifestyle habits, and even education level.

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Deschampsia antarctica as a model for studying the microevolutionary processes in marginal populations

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Key words: chromosomal polymorphism, *Deschampsia antarctica*, genetic variation, maritime Antarctic, marginal populations.

According to the concept of 'species range' peripheral populations exhibit a lower genetic diversity and higher genetic differentiation than central ones. In the marginal populations, which are under influence of unusual ecological conditions, intense processes of speciation may occur; genetic changes combined with karyotype repatterning enable some portion of a species to survive as a new form or even as a new species under strong environmental pressure. *Deschampsia antarctica* (Poaceae) is a plant species native for maritime Antarctic region and an promising model for studying the microevolutionary processes. Therefore, the aim of our research was to study genome variations in *D. antarctica* populations from the southern range limit in Western Antarctic (Argentine Islands region) using the methods of cytogenetics and molecular genetic analysis.

The study of individual plants from different island populations revealed that most of them were diploids with 26 chromosomes. In addition, new forms of chromosomal polymorphism were found, namely a hypotriploid (2n = 36 – 38) and a genotype with an occasional occurrence of B chromosome (2n = 26 + 0-2B). FISH analysis demonstrated that genotypes with different chromosome numbers vary in the number of 5S rDNA and 35S rDNA sites. Furthermore, the molecular genetic analysis performed using ISSR- and IRAP-PCR demonstrated the low level variation among the plants: the genetic distances between the plants with different chromosome numbers fall within the range of distances between the diploids only. These results may be interpreted as follows. Periodic climatic fluctuations result in development of stressful environmental conditions that lead to marked reductions in the size of populations or their complete disappearance and to repeated cycles of colonization and extinction. In this way, periodically stressful conditions may reduce the genetic variation within the populations. On the other hand, the environmental stress may induce chromosomal rearrangements that result in the appearance of plant forms with atypical karyotype.

The results of this study suggest that *D. antarctica* plants from the southern range limit in maritime Antarctic show molecular cytogenetic heterogeneity combined with a relatively low molecular genetic variation. The ecological and adaptive significance of chromosomal forms that were discovered in our study is still uncertain, but it seems that the period of their existence at the current time is not sufficient for the formation of new races or cytotypes. Thus, their appearance can be considered as an initial stage of microevolution, which occurred in marginal populations.

Use of circulating cell-free DNA of human pituitary adenoma

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Key words: ccfDNA, exome sequencing, non-invasive tumor detection, pituitary adenoma.

Pituitary adenoma (PA) is a non-metastatic tumor in pituitary gland which is associated with increased mortality and disability. In population clinically significant PA affects one out of 1000 individuals. The discoveries of new non-invasive diagnostic methods could significantly improve wellbeing of patients and tracking of disease.

Circulating cell free DNA (ccfDNA) is approximately 165 bp long DNA fragments which are released in bloodstream during cell death. Lately, it has been used as a non-invasive method in cancer diagnostics as the concentrations of ccfDNA in blood of cancer patients is noticeably higher than in healthy individuals, furthermore the tumor derived ccfDNA can provide accurate reflection of the genome of the tumor. Currently there have been a very few studies on adenoma derived ccfDNA detection as it is considerably more difficult to detect tumor derived ccfDNA in adenoma patients than in cancer patients due to lower ccfDNA concentrations.

The primary objective of this study was to conclude if it is possible to detect PA ccfDNA based on known mutations found previously in PA exome, by performing deep IonTorrent semiconductor sequencing on ccfDNA extracted from the plasma samples of the same patients.

Blood and tumor samples were obtained in cooperation with Pauls Stradins Clinical University Hospital from fiveNFPA patients. Primers were designed with primer3plus to create amplicons containing previously detected mutations.

ccfDNA samples were extracted from patients frozen plasma samples using QIAamp Circulating Nucleic Acid Kit (50) (Qiagen, Germany). Concentrations and length of extracted ccfDNA samples were analysed with 2100 Bioanalyzer (Agilent Technologies, US). PCR with ccfDNA was done with HOT FIREPol® (Solis BioDyne, Estonia). Libraries for NGS were prepared with Ion Plus Fragment Library Kit (ThermoFisher, US). Size selection and clean-up between library preparation in all stages, except during end repair, was performed with NucleoMag® NGS Clean-up and Size beads (Macherey-Nagel, Germany). During End repair sephadex was used. Prepared libraries were sequenced on Ion Proton semiconductor sequencing system (ThermoFisher, USA).

Sequences were aligned to GRCh37 – hg19 using Burrow-Wheeler Aligner and stored in BAM format. Base call summarization and format change was done with Samtools. Results were visualized with Integrative Genomics Viewer.

In total NGS was performed on 16 amplicons from ccfDNA samples of five PA patients. Sequencing yielded 6191 reads on average (range 444 – 13026). In the sequencing results five out of 16 amplicons alternate allele showed up in close to 50% of reads. In two out of 16 samples alternate allele was in less than 10% of reads. In six out of 16 samples alternate allele was in less than 0.1% of reads. In three of 16 samples alternate allele showed up in no reads. The background alternate allele frequency (sequencing error rate) in non-mutation sites also was below 0.1%.

It is not possible to detect PA derived ccfDNA using semiconductor sequencing because error rate of base calling is comparable to expected amount of PA derived ccfDNA molecules. It is possible that this method can be used to reduce the number potential PA driver mutations discovered in whole exome sequencing data of PA as the results with alternate allele in close to 50% of reads indicate that the mutation is not PA origin.

Acknowledgements
This research was supported by the European Regional Development Fund Measure 1.1.1.1 "Industry-Driven Research" within the project "Molecular markers of pituitary tumour development, progression and therapy response" (Project no. 1.1.1.1/16/A/066). The authors acknowledge the Latvian Biomedical Research and Study Centre and the Genome Database of the Latvian Population for providing infrastructure, biological material and data.

Production of *Camelina sativa* plants bearing the genes for trehalose biosynthesis from yeast *Saccharomyces cerevisiae*

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Key words: drought, TPS1, TPS2, trehalose, Tre6P.

A large area of arable land in Ukraine is located in areas of insufficient and unstable hydration, so often drought tolerance is a critical factor in increasing the harvesting of agricultural plants. One of the approaches that can provide the growth of plant tolerance to drought is genetic transformation.

Trehalose is a non-renewable glucose disaccharide. Through participation in the regulation of the stomata's operation, trehalose promotes to the effective use of water by plants and is involved in responses to salinity and low temperature.

Synthesis of trehalose in higher plants is carried out by two consecutive reactions involving trehalose-6-phosphate synthase (TPS1) and trehalose-6-phosphate phosphatase (TPS2). The amount of the intermediate product of synthesis [trehalose-6-phosphate (Tre6P)] positively correlates with the amount of sucrose, which is the main product of photosynthesis and the main transport sugar in plants. There is a point of view that the effect of Tre6P is aimed at maintaining the concentration of sucrose at the optimal level.

Camelina sativa (camelina), a member of the Brassicaceae (Cruciferae) family, is an oilseed crop that has received increased interest because of the relatively high oil content of its seeds (≤ 43% oil per seed weight) and its high content of the omega-3 fatty acid linolenic acid, which composes up to 40% of the total oil. In addition, camelina is a productive crop with low inputs of soil fertility and rainfall. These properties have made camelina attractive for cultivation in underutilized lands, particularly dry land regions. Vegetable oil derived from camelina seeds is a demonstrated feedstock for biojet fuel.

The aim of our work was producing of *C. sativa* plants with improved tolerance to water deficit due to expression of the genes for trehalose biosynthesis from yeast *Saccharomyces cerevisiae*. The line FEORZhYaF-1 was used as an initial material for transgenesis using floral deep transformation. This line was previously selected out of six spring camelina lines (FEORZhYaF-1 and FEORZhYaF-3) and cultivars (Euro-12, Peremoga, Myrazh, and Klondike) from Gryshko National Botanical Garden collection as the one with the highest superoxide dismutase activity under aseptic both normal conditions and water withdrawal. Mannitol (up to 300 mM) was added to culture media as an osmotic stress modulating agent. The genetic constructs bearing *ScTPS1* and *ScTPS2* genes from the trehalose-overproducing strain of *S. cerevisiae* were created. Both *ScTPS1* and *ScTPS2* genes were cloned from yeast DNA through BP-recombination and Entry clones creation following transfer into the destination vectors pGW2 and pBract124 (target gene under control of 35S or Ubi promoter, respectively) through LR-recombination. The constructs were cloned into *Agrobacterium tumefaciens* strains GV3101 and AGL1. The *HPT* gene for hygromycin phosphotransferase was used as selective one in all constructs. At the stage of floral buds, camelina plants were cocultivated with agrobacteria suspension without vacuum infiltration. Seeds obtained were surface sterilized and germinated on the agar solidified hormone-free Murashige and Skoog medium containing hygromycin (25 mg L^{-1}) for transgenic seedlings selection. T_1-green seedlings (5 to 6-week-old) with roots were transferred into pods with soil to produce seeds (T_2-generation). Molecular biological and biochemical analyses of camelina transgenics are in progress.

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Grain quality in bread wheat lines with introgression of genetic material

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Key words: bread wheat *Triticum aestivum*, grain quality, high molecular weight glutenin subunits, species of the genus *Triticum*.

The grain of bread wheat *Triticum aestivum* L. is widely used for the production of bakery products. Therefore, the possibility of improved biological value and baking properties of this crop due to the gene pool of wheat relatives generates interest. We evaluated the effect of the genetic material of tetraploid *Triticum dicoccoides, Triticum dicoccum* and hexaploid *Triticum spelta, Triticum kiharae* species of the genus *Triticum* on the grain quality of 16 introgression lines of spring bread wheat.

The baking properties of *T. aestivum* are largely determined by its protein content and the gluten amount correlating with this indicator. The samples of tetraploid species *T. dicoccoides* (24.56%), *T. dicoccum* (26.21%) and the hybrids produced with their involvement (17.73 to 27.61%) were characterized by the highest protein content in the grain. Most introgression lines were superior to the parent varieties of bread wheat or were characterized by close values against this indicator. The protein content decreased in comparison with the parent variety only in 5 lines out of 16 studied. The gluten content of introgression lines was more than 36%, which corresponds to the highest class of food grains. Only one line showed a low gluten percentage, 23.5%.

For characterizing the baking properties of grains, the gluten quality determined by its physico-chemical characteristics is of main importance. According to the value of gluten deformation index, the increased gluten quality was found for six out of 16 introgression lines in comparison with the parent wheat variety and its reduction for four. It is known that high molecular weight glutenin subunits are encoded by *Glu-1* loci and play an essential role in the formation of wheat baking properties. Comparative analysis of the high molecular weight glutenin subunits' composition in the introgression lines of bread wheat and their parental forms allowed to separate the lines with *Glu-1* loci alleles from wheat relatives, which are not characteristic of *T. aestivum*. It was found out that the lines with *Glu-A1* loci alleles from *T. dicoccoides* and *Glu-B1* from *T. dicoccum* were at the level of a parent wheat variety or of higher gluten quality. The presence of high molecular weight glutenin subunits from *T. spelta* and *T. kiharae* in introgression wheat lines reduced their gluten quality when compared with the parent wheat variety.

As a result of the research, the new lines of bread soft wheat with high grain quality were found and can be used in the crop breeding.

Investigation of parasite genetics in the Baltic countries

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Key words: bacteria, genomes, helminth, parasite, population genetics, protozoa.

We present a review studies of investigation of parasite genetics in the Baltic countries. Parasite genetics is the study of the genetic material of parasites, including the distribution, function and evolution of genes and genomes. Topics include species identification, phylogeography, host specificity and speciation, population genetic structure, and searching for loci under selection.

Genome of cultured pituispheres represents genome of pituitary adenoma

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Key words: pituispheres, pituitary adenoma, tumor sequencing, whole exome sequencing.

Pituitary harbors up to 15% of all intracranial neoplasms. The most common type of pituitary neoplasms is benign pituitary adenomas (PA). Although non-metastasizing they still cause increased mortality and morbidity. Clinically significant PAs affect around 0.1% of population during their lifetime. Currently there is no established PA cell culture line available and when cells from primary surgery material are cultured they form two distinct populations. The first population is free floating aggregates also known as pituispheres and the second population is cells adhering to the surface of petri dish and displaying multipotency: these cells are usually called mesenchymal cells.

In this study we investigated genetic relationship between germline DNA (extracted from white blood cells), somatic DNA extracted from primary surgery material, DNA of pituispheres and mesenchymal cells obtained from culture of primary surgery material.

Five PA patients were enrolled to Genome Database of Latvian Population from Pauls Stradins Clinical University Hospital where transsphenoidal surgery of PA was performed for all patients. DNA was extracted using standard DNA biobank protocol. Primary cell cultures were obtained from fresh surgery material by mechanical cutting and washed in DMEM with 1x Antibiotic-Antimycotic solution (Thermo Fisher Scientific, USA). Followed by enzymatic dissociation with Accutase solution (Thermo Fisher Scientific, USA) for 20 min at 37 °C on a rotating platform in a humidified atmosphere maintained at 5% CO_2. Cells were grown in DMEM-F12 (Thermo Fisher Scientific, USA), containing 1% penicillin/streptomycin (GIBCO, USA), 0.1% EGF (Sigma-Aldrich, Germany), 0.02% FGF (Sigma-Aldrich, Germany) and 2% B27 supplement (GIBCO, USA). The cell culture media was changed once in three days. Exome of four samples of four PA patients and three samples of one PA patient was sequenced (total 19 samples) using Illumina NextSeq machine. Obtained reads were aligned to human HG19 reference genome using Isaac, variants called with Starling algorithm and variants annotated with Illumina Annotation Engine.

On average 97% of exome sequence were obtained from all DNA samples. Variation analysis revealed that somatic mutations (range 1 – 5) of primary surgery material can be detected in DNA of pituispheres, but not in DNA of mesenchymal cells.

PAs contain low amount of somatic mutations in their exome. Genetic analysis indicates that pituispheres represent PA and can be used as a model to study this tumor type. Mesenchymal cells which are derived from the same primary surgery material, does not contain PA specific mutations therefore representing normal cells of pituitary or surrounding tissues.

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SSR markers for cpDNA on exploring germplasm diversity to understand the gene flow process in red clover

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Key words: biodiversity, climatic adaptation, genetic depletion.

Red clover (*Trifolium pratense*) is characterized as ecologically and economically important fodder crop. Unfortunately changes in natural habitats cause a decline of diversity between red clover populations. Usually in clover population studies, only morphological and agronomic traits are being assessed, and there is not enough studies aimed to understand gene flow between natural and domesticated clover populations. Due to the fact that there is a high variation in morphology of red clover, which might not correlate with genetic diversity due to G × E interaction, there is a need to use methods of molecular biology. For years researchers have been using different methods, such as RFLP, AFLP, ISSR or SSR, to identify genetic diversity in red clover. All of these methods are based on PCR by using nuclear DNA. In this study we are going to use chloroplast DNA (cpDNA), because there are some reports that chloroplast genome sequences have considerable variation within and between species of Fabaceae family. In this case, by using microsatellites (SSR), we will be able to identify polymorphic differences between individuals based on the fact that the inherited polymorphism could only be received from the maternal line.

T. pratense chloroplast genomic sequence (unverified) KJ788290 is available in the NCBI database. Perfect simple sequence repeats in *T. pratense* chloroplast genome were identified by using Simple Sequence Repeat Identification Tool (SSRIT). Analysis has identified 67 dimer and three trimer motifs of SSR with at least four repeats of each motif independently. The primers were constructed for those motifs by using Basic Local Alignment Search Tool (BLAST), 10 primer pairs were tested on red clover and similar Fabaceae family species *Trifolium medium* and Poaceae family species *Triticum aestivum* and *Lolium multiflorum* to ensure their specificity and efficiency.

All primers amplified fragments in red clover cpDNA, meanwhile no amplification was achieved when *T. medium, T. aestivum* and *L. multiflorum* DNA was used. The size of amplified bands varied from 75 bp (primer pair: cpRDct-32-10F, cpRDct-32-10R) to 150 bp (primer pair: cpRDga-33-3F, cpRDga-33-3R) as we expected. More than 30 different populations and 10 cultivars of red clover will be screened with these primers in near future.

This study will enable us to evaluate the genetic structure of the natural red clover populations, detect possible gene flow from the locally grown red clover cultivars and select the most exclusive red clover populations as germplasm to preserve for future generations in GeneBank.

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Ergot alkaloids in Estonian rye-containing foods

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Key words: Estonia, ergot alkaloids, food, monitoring.

Ergot alkaloids (EAs), secondary metabolites of fungus *Claviceps purpurea*, are natural habitants of cereals, mostly rye and triticale, but also wheat, oats etc. However sorting and sieving after harvesting eliminate most ergots, EFSA opinion stated an increase in *C. purpurea* infections due to extensive use of hybrid varieties in 40 to 50% of all investigated rye samples in Germany (EFSA 2012). Although EAs are present only in minor concentrations, they can be toxic to humans and animals. EFSA has established acute reference dose of 1 μg kg^{-1} body weight (b.w.) and tolerable daily intake of 0.6 μg kg^{-1} b.w. for sum of EAs in food and strongly recommended monitoring of marketed food in EU member states.

The main goal of the present study was to monitor rye containing foodstuffs available and consumed in Estonia. The products (grains, flours, flakes, breads, chips; in total 59 samples) were collected in 2016 and 2017 from Estonian supermarkets. The sampling points, manufacturers and foods were chosen randomly and analyses of six prevalent ergot alkaloids found in sclerotia [ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and their related epimers (-inines)] were ordered from Eurofins WEJ Contaminants certified laboratory.

EAs were detected in five of 39 conventional (rye wholemeal flours, rye flakes and multi-cereal flours and flakes in range of 4.2 to 190 μg kg^{-1}) and in one of 20 (300 μg kg^{-1} in rye wholemeal flour) organic products. In all breads, crispbreads and rye flours EAs content was under limit of quantification (*LOQ* = 3 μg kg^{-1}). These data will be compared with analogous studies carried out in UK, Belgium, Albania, Canada, etc. Availability of EAs to consumers will be given comparing the analytical data with dietary from starchy foods food group (rye and whole grain bread, fine rye bread, porridge flakes and other cereal based side dishes and breakfast cereals) in published tables of Estonian National Dietary Survey carried out in 2013 – 2015.

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Molecular investigations on *Sarcocystis* parasites in the Baltic States

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Key words: game animals, intraspecific variability, phylogeny, *Sarcocystis*, species identification.

Members of the genus *Sarcocystis* are common, worldwide distributed protozoan parasites of reptiles, birds, and mammals. They are characterized by an obligatory two-host prey-predator life cycle. *Sarcocystis* are formed mainly in striated muscles of intermediate host, while oocysts/sporocysts develop in the small intestine of definitive host. Some *Sarcocystis* species are pathogenic to humans, domestic and wild animals. The objective of the study was to investigate *Sarcocystis* infection rates, species diversity, intraspecific variability in game animals from Latvia and Lithuania.

Between 2012 and 2018, various muscles types, i.e. diaphragm, hind leg, heart and tongue of 452 red foxes (*Vulpes vulpes*), 163 wild boars (*Sus scrofa*), 60 moose (*Alces alces*), 104 red deer (*Cervus elaphus*) and 41 roe deer (*Capreolus capreolus*) from different regions of Latvia and Lithuania were microscopically examined for the presence of sarcocysts. Infection prevalence was evaluated in approximately one gram of muscle pieces stained with methylene-blue. Single sarcocysts were excised from fresh-squashed muscle samples for morphological and molecular analysis. Isolated sarcocysts were characterised and identified according to the size and shape of the cyst and bradyzoites, and the structure of the cyst wall. Sarcocysts isolated form red foxes were characterised at 18S rDNA, 28S rDNA, ITS1, cox1 and rpoB; cysts isolated from wild boars were examined using 18S rDNA, ITS1 and cox1; while cysts isolated from cervids were described using 18S rDNA and *cox1*.

Lowest infection prevalence was established in red fox (3.1%), whereas in other game animal species it exceeded 80% (81.7% in moose, 85.6% in red deer, 85.9% in wild boar and 95.1% in roe deer). It was not detected a significant difference in infection rates between Latvia and Lithuania when comparing infection prevalence among the same hosts.

Based on the sequence comparison, two species were identified in red foxes (*Sarcocystis arctica* and *Sarcocystis lutrae*), one in the wild boars (*Sarcocystis mieshceriana*), five in moose (*Sarcocystis alces, Sarcocystis hjorti, Sarcocystis linearis, Sarcocystis ovalis* and *Sarcocystis silva*), six in roe deer (*Sarcocystis capreolicanis, Sarcocystis entzerothi, Sarcocystis gracilis, S. linearis, S. silva* and *Sarcocystis oviformis*) and seven in red deer (*Sarcocystis frondea, S. hjorti, Sarcocystis iberica, S. linearis, Sarcocystis pilosa, Sarcocystis truncata* and *S. silva*). *S. arctica* was predominant species in red foxes and demonstrated 100% sequence identity within 18S rDNA, 28S rDNA, ITS1; whereas two and four haplotypes were found within *cox1* and *rpoB*, respectively. Based on the results obtained it was suggested the existence of two genetic lineages of *S. arctica*, and such divergence relies on its geographical distribution but not on their intermediate host species. For the differentiation of *S. miescheriana* from *S. suihominis* 18S rDNA primers were designed and the following species, pathogenic to humans was not determined in the muscles of wild boars. It emerged that ITS1 is not suitable for the intraspecific genetic researches of *S. miecheriana*. By contrast, huge population genetic variability of *S. miecheriana* was found using *cox1*, i.e. each isolate of *S. miescheriana* (n = 23) represented unique haplotypes. It was shown that some Sarcocystis species derived from cervids are not strictly intermediate hosts specific and new host records of *S. linearis, S. frondea* and *S. pilosa* were presented within current investigation. The 18S rDNA, in contrast to the *cox1*, was not sufficiently variable to discriminate some closely related *Sarcocystis* species employing cervids as intermediate hosts. *Sarcocystis* species from the same host of the family Cervidae displayed different levels of the intraspecific variability. Results of phylogenetic relationships are helpful predicting possible definitive hosts of *Sarcocystis* species detected.

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Deciphering the genetic architecture of intellectual disability and congenital anomalies by the use of microarray, next generation sequencing methods and functional genomics analysis

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Key words: congenital anomalies, functional analysis, intellectual disability, microarrays, next generation sequencing.

Intellectual disability and congenital anomalies are significant causes of chronic illness and constitute a leading socio-economic problem of health care. The evaluation of the genetic causes of intellectual disability and congenital anomalies is challenging because these conditions are genetically heterogeneous with many different genetic alterations resulting in clinically indistinguishable phenotypes. Current worldwide research in identification of genetic causes of intellectual disability and congenital anomalies has been significantly accelerated by the advent of genome wide molecular technologies. Nevertheless, the molecular basis of more than 700 congenital disorders is still unknown and awaits elucidation.

In recent years many studies have shown that molecular karyotyping is an effective diagnostic tool in individuals with intellectual disability/ congenital anomalies. The information about the origin of the copy number variant (CNV), size, and gene content in the chromosomal alteration allow identification of the known and new microdeletion/microduplication syndromes, narrowing the critical regions of known syndromes or even identifying critical genes for syndromes. Whole exome/ genome sequencing of affected individuals for whom previous detection of CNVs testing has proven negative, enables identification of DNA sequence variation in the form of DNA base-pair substitutions and short indels and significantly contributes to the rapid delineation of multiple new syndromes. Functional genomic approaches such as RNA expression analysis and genome editing tools provide unique possibilities to identify the underlying molecular basic of many diseases.

The application of genome wide molecular technologies allows to identify causal variants in many individuals with intellectual disability/congenital anomalies regardless of frequency, heterogeneity, and inheritance. Therefore, every single individual with unrecognized clinical condition becomes the main unit in identifying new candidate genes for Mendelian disorders. Discovery of novel genes associated with intellectual disability/congenital anomalies contributes to deeper understanding of etiology and pathophysiology of congenital disorders.

The second intron length polymorphism of β-tubulin genes of *Aegilops biuncialis*

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Key words: *Aegilops*, intron length polymorphism, plant genotyping, tubulin base polymorphism, β-tubulin.

The genus *Aegilops* L. is the closest relative of the genus *Triticum* L. and presents a natural pool of useful traits for the improvement of wheat agronomic characteristics. It is established that *Aegilops biuncialis* Vis. usually has genotypes with better drought resistance than in wheat. In addition, some accessions are highly resistant to barley yellow dwarf disease or to cereal rust. Cheap and simple methods based on the study of structural and functional organization of genes are constantly searched during collections making, assessment of genetic diversity and population resources of wild species in their natural condition. In this connection, the relatively new evolving method, using intron length polymorphism of β-tubulin genes (tubulin-based polymorphism, TBP) is perspective (Bardini et al. 2004). The TBP method is based on the fact due to the key role of the β-tubulin in the cell life, the sequence of its gene exons (in contrast to introns) is rather conservative in all eukaryotic organisms. There are several variations of this method: the original TBP (using the length of the first intron of the β-tubulin genes), horse-TBP (h-TBP, allows the amplification of the gene sites that contains first and second introns with second encoding exon) and combinatorial-TBP (c-TBP, using length polymorphism of the second intron of the β-tubulin gene). Using TBP and h-TBP we have already analyzed several genotypes *A. biuncialis*, and a sufficiently high level of the polymorphism and high efficiency of the method for differentiating the genotypes of *A. biuncialis* were detected (Rabokon et al. 2017). Therefore, the goal of this work was to evaluate the possibility of using the length of the second intron of β-tubulin genes (cTBP) in genetic studies of the *A. biuncialis*; and also to compare the differentiating ability of this variant of the analysis with other modifications of the method using this object of study.

The fifteen different Crimean populations of *A. biuncialis* were investigated. cTBP analysis was performed according to a previously developed protocol (Breviario et al. 2004; Rabokon 2017). Based on the results of the c-TBP analysis of the samples, 42 reproducible clear fragments were detected in the range from 395 bp up to 2880 bp, 29 of which were polymorphic. Monomorphic 14 bands have an approximate molecular weight of 360 bp, 365 bp, 375 bp, 378 bp, 475 bp, 595 bp, 620 bp, 875 bp, 1280 bp, 1865 bp, 2000 bp, 2230 bp and 2550 bp. On the basis of the obtained sample profiles, a UPGMA denrogram was constructed. Almost all samples differentiate from each other with a high percentage of bootstrap support. In general, the dendrogram based on cTBP analysis is very similar to the h-TBP dendrogram (Rabokon et al. 2017). All samples are divided into three large groups. The first one (with 100% bootstrap) is formed by three samples of NK 4N2, NK 6-2 and NK B-1, as in the case of hTBP. Second group is formed by NK 02 NK 1-I and NK MMB-1, as in the case of the hTBP tree, but with a smaller bootstrap support 63%. Third group includes all other samples, but they differentiate well from each other. The Nei and Lee similarity coefficient is maximal (1.0) only between the samples NK 11-2, NK 13-1, NK OZ-2 and NK 10-3. The minimum value of the coefficient (0.6) is observed between the two samples NK B1-1 and NK 02. The average PIC (Polymorphism Information Content) is 0.231. This is somewhat lower than the PIC values obtained with the TBP analysis (Rabokon et al. 2017), but it is explained by the fact that considerably more fragments (42) are formed in the c-TBP analysis, both polymorphic and monomorphic.

Thus, the results demonstrate a good differentiating ability of this modification of the TBP-method and the possibility of using it alone or in combination with the study of the first intron length polymorphism to assess genetic polymorphism of plants and fingerprinting of cereal populations, in particular *A. biuncialis*.

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Genetic characterization of *Bartonella* spp. from rodents

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Key words: *Bartonella*, genetic variability, rodents.

Bartonella are facultative intracellular, fastidious, gram-negative bacteria that are transmitted to mammals and humans by bloodsucking arthropod vectors. Currently, 45 official and candidate *Bartonella* species have been detected in vertebrates, and at least twenty of them have been detected in rodents. Several rodent-associated *Bartonella* species have been related to human diseases. However, there is a lack of studies on the presence and diversity of *Bartonella* spp. in rodents from Baltic region. We analyzed 580 individuals belonging to eight small rodent species, trapped in Lithuania during 2015 – 2016. The presence of *Bartonella* DNA was examined by real-time PCR targeting the *ssrA* gene. Species identification and molecular characterization of bacteria strains were based on sequence analysis of two housekeeping genes (*rpoB, groEL*) and the intergenic species region. Sequence analysis reveal that rodents harbor multiple *Bartonella* species belonging to six clades, including *Bartonella grahamii, Bartonella taylorii, Bartonella tribocorum, Bartonella coopersplasensis, Bartonella doshiae* and *Bartonella rochalimae*. Phylogenetic analysis showed the presence of different *B. grahamii, B. taylorii* and *B. tribocorum* genotypes associated with different host species and demonstrated that in rodent communities circulate more than one bacteria genetic variant, and multiple *Bartonella* genotypes found in the same reservoir species. Our study represents the first genetic characterization of *Bartonella* strains circulating in rodents in the Baltic countries.

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First results of investigation into genetic diversity of Eurasian perch (*Perca fluviatilis*) mtDNA *ATP6* gene

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Key words: ATP6, genetic diversity, mtDNA, perch, population.

In order to study the genetic structure of freshwater fish populations affected by anthropogenic activities related to power plants it is necessary to use both neutral and adaptive molecular markers. While most regions of mtDNA genome are treated as neutral markers *ATP6* gene is considered as adaptive marker. The primer pair ATP-PCR suitable for amplification of mtDNA *ATP6* gene of the Eurasian perch was designed in the laboratory of Molecular Ecology of Nature Research Centre using Primer3 program. PCR was conducted using following conditions: 95 °C 2 min, 94 °C 30 s, 52 °C 45 s, 72 °C 45 s, 72 °C 5 min; 35 cycles. DNA from perch samples collected in Lithuania (Siesartis River and Lake Drūkšiai) and Ukraine (Desna River, Chernobyl) was used. Amplified fragment was 890 bp length but sequence analysis was carried out with trimmed 710 bp fragments. Obtained 17 sequences of partial mtDNA *ATP6* gene were used for construction of haplotype network. Six haplotypes were separated by 1 to 2 mutational steps and could be grouped to two main haplogroups A and B. Perch of Ukraine population represented both haplogroups while samples from Siesartis River were attributed to haplogroup A. Based on this preliminary study it could be concluded that mtDNA *ATP6* gene is suitable molecular marker for investigations of population genetic structure of the perch at least at the macrogeographic scale.

Is there a revival of Lysenkoism?

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Key words: causes of erroneous ideas, genetics, incorrect concepts of heredity, Lysenkoism.

Along with the significant discoveries in genetics and their comprehensive practical applications in medicine, agriculture, production, etc., scientifically unfounded theories and assertions (Lysenkoism) are revived, and new ones are also emerging.

Expressing opinions contrary to genetic principles. Knowledge of properties that are realized only in specific environmental conditions, immunity to all infections, perception of the existence of good and evil in a child from the moment of birth (indigo and crystal children); the future generations would inherit the positive characteristics and attributes acquired during their lifetime; denial of the balance in conservative and variable, recognizing only the adaptive processes of the changing organisms; purposefully directed mutation and expression of genes development process (epigenetic); denial of the existence of the gene as an essential element of the hereditary information medium ("no one has seen gene"); gender, gender identity change due to social factors in human life instead of being determined by sex chromosomes X and Y ("the gender is predominantly shaped by society's norms and beliefs"); denying heterosexual relations as the only type of sexual relationship for the purpose of human reproduction, acknowledging genders diverging from male or female sex (hermaphroditism, testicular feminization, etc.), social constructs (eunuchs, hijas, skopci, etc.); segregation of biological and social gender; a person's opposition to the rest of living nature (outside the laws of living nature); human brain progressive evolution and predicting the loss of gender (Y chromosome); etc.

Causes of various misleading ideas. First of all, it is the lack of knowledge and oversimplification of genetic relationships. The attainment of the gene and gene heredity marks is understood only in its monogenic manifestation. If there is a brown eye gene, there will be brown eyes (monogenic determination). However, in most cases, several genes are identified to determine hereditary features (polygenycal determination), and genes are determined only as a potential influence in the development, whether they occur are determined by specific conditions (multifactorial determination), various genetic interactions, and so on, genetic legalities whose significance within the meaning of heredity is not known or ignored in false theories. Considering social factors as more significant in the concept of human being in comparison to biological ones, above scientific experiments and theories.

In the 40-ies and 50-ies of the last century, the Soviet Union was combating and banning genetics, as philosophers and sociologists "proved" that Genetic legitimacy was not in line with the Soviet communist ideology, and therefore not correct. Even today, liberal and post-liberal sociologists and philosophers ignore many biological and genetic associations with the argument that geneticists have no understanding of social connections that are not related to biological ones. However, genetic relationships should not be ignored as they have a limiting role of what is socially acceptable. Human behavior and values are based on three foundations – genetically determined – of biological determinants, the so-called "eternal values", paradigms accumulated and tested during the existence of humanity, and paradigms of the particular time period. Human history holds many examples where socially-selected models did not fit the biological nature of human beings and it has led to tragic consequences. Currently, as a warning sign that some of the biological principles (such as reproduction) are being ignored could be recognized in demographic statistics that show that there is no positive population growth in any of the European Union countries.

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Provenance differences in browsing damage in Lodgepole pine stands: case study in two experimental sites in Latvia

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Key words: clonal plantations, genetic gain, selection differential, vegetative propagation.

Population densities of cervids are increasing in Latvia since middle of 1990-ies, but are still lower than those observed in Western Europe. Therefore it is likely, that they will remain unchanged or even increase in future, thus, there will be notable browsing pressure in young forest stands. Browsing damages by cervids can significantly affect survival, increment and quality of trees. Lodgepole pine (*Pinus contorta*) is an introduced species in Latvia that in sandy, unfertile soils can ensure better productivity than Scots pine, being a potential alternative for forest regeneration in such forest types. However, in contrast to Scots pine, it has thinner bark and consequently is vulnerable to browsing damages also when exceeding the age of 15 to 20 years. Therefore aim of our study was to assess the provenance differences in frequency of cervid damage in young Lodgepole pine stands.

The study was carried out in two experimental plantations, located in central part of Latvia. Stands were established with initial density 5000 trees ha^{-1}, thinned 2 years before the final measurement at the age 28 and 36 years and included 8 provenances.

Frequency of damaged trees was 9.4% before thinning and 2.6% in final measurement in younger stand; corresponding figures were 24.4 and 6.4% in older one. The proportion of the browsing damages at second assessment was higher for trees that were affected already at the time of first assessment, than for trees that were not: 17.5 vs 1.3% and 18.5 vs 5.3% in younger and older stand, respectively. Differences in frequency of browsed trees between provenances were notable, but not statistically significant. There were no significant difference in breast height diameter or height between affected and un-affected trees (in any of the assessments) at the stand as well as at the provenance (within stand) level. Results indicate, that selection or silvicultural treatment aimed at improving growth of trees most likely will not affect the frequency of browsing damages by cervids in Lodgepole pine stands.

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Development and characterization of genome-edited blueberry (*Vaccinium corymbosum*) cultures for production of high-value secondary metabolites

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Key words: blueberry, CRISPR/Cas9, genome editing, plant tissue culture, *Vaccinium corymbosum*.

Plants naturally produce secondary metabolites used in medicine, nutrition and as industrial precursors. Harvesting chemical compounds from wild or cultivated plants comes with a high environmental impact, depends on seasonal availability and, in the case of endangered species, may not be feasible. Biotechnological solutions, such as plant tissue cultures can provide the required plant material, if appropriate culture techniques are used and metabolite levels are sufficiently high. Blueberry (*Vaccinium corymbosum* L.) is a woody perennial crop species rich in anthocyanins, resveratrol and lutein with established tissue culture and Agrobacterium-mediated transformation protocols, but lacking genome-editing protocols. Recently funded Latvian Council of Science project aims to develop CRISPR/Cas system to edit blueberry genome for selected genes to redirect flux of precursors towards specific secondary metabolites of interest. To achieve this aim, we propose (i) to use genome-scale and pathway-specific metabolic modelling to identify target genes for editing; (ii) to adapt existing protocols for blueberry tissue cultures; (iii) to develop workable DNA-free and *Agrobacterium*-mediated protocols for CRISPR/Cas-mediated genome-editing in blueberry; (iv) to monitor levels of target compounds, their precursors and major competing metabolites using LC-MS. Overall, the project will contribute to the Latvian RIS3 investment priorities (1) high added value products and (2) productive innovation system, as well as to RIS3 knowledge specialization areas (1) knowledge-intensive bio-economics and (3) bio-pharmacy and biotechnologies. Additionally, it aligns with the National research priorities, and will strengthen the leading role of University of Latvia in the field of agricultural biotechnology. The major outcome will be proof-of-principle for establishing of genome-edited blueberry tissue cultures that produce target metabolites, as well as improved understanding of biochemical pathways leading to production of key high-value metabolites.

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Genome database of Latvian population – national biobank for genetic studies

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Key words: genetic studies, Latvian biobank, research infrastructure.

The Genome Database of the Latvian Population (LGDB) is a national biobank that collects, maintains and processes health information, data and biospecimens collected from representatives of the Latvian population. Since the establishment LGDB is comprised of biosamples, associated phenotypic and clinical information from over 33 000 participants constituting approximately 1.5% of the Latvian population. LGDB stores DNA, plasma and serum from all recruited participants and for specific research segments tissue biopsies, microbiome and urine samples are collected. Participants are recruited via different research projects and collection comprises both general population and disease specific cohorts.

The LGDB have served as infrastructure for many genetic research projects. The specific cohorts have been used in phylogenetic study for the comparison of genetic structure of different European populations, included in international consortium for breast cancer, schizophrenia, type one and two diabetes and other studies. The replication and validation have been carried out of different genetic factors for diabetes, vein thrombosis, hemochromatosis, obesity, cardiovascular and other diseases using LGDB resources. Many genetic tests have been developed and used for diagnostics including Celiac disease, HLA, hemochromatosis, thrombosis and phamacogenetic markers.

Currently, LGDB has started to redesign its infrastructure to meet modern research needs on using the large massive genome and medical information data. The pilot study has been conducted where state medical records of 4056 type two diabetes patients have been obtained. These data included diabetes registry data, all state funded doctor visits, manipulations and medication and death registry data. Extensive study of linking this information to LGDB phenotype and genotype information have been carried out and results help evaluate efficacy of diabetes treatment in Latvia an discover novel findings in the disease pathogenesis. The use of state medical information will be included in other LGDB projects. LGDB has intentionally involved in several wide genome association studies to obtain genotypes for the recruited participants, these involve diabetes pharmacogenetics, type one diabetes, gastric cancer and flu etiology studies. The obtained large-scale genotype data can now be used in other research projects and is bringing more added value to the biobanked samples.

As the overall recruitment of LGDB has previously been project specific, the emphasis of LGDB now is to promote and continue the high-quality strategic cohorts, these currently include OPTIMED, longitudinal cohort of type two diabetes patients with prospective design; Latvian pituitary adenoma cohort, rare endocrinological disease collection; LatDiane, prospective type one diabetes complications study and several other significantly valuable collections. The further development of these collections is planned as enrichment of relevant medical and clinical information and obtainment of large scale -omics data to set ground for valuable studies and international collaboration.

As national biobank LGDB collaborate with largest research, education and health organizations on national level in fields of molecular biology, medicine, pharmacology. Since 2016, the Republic of Latvia has been approved the admission as a member of the BBMRI-ERIC (European Research Infrastructure Consortium) and Latvian Biomedical Research and Study Centre appointed as National node. LGDB coordinate BBMRI-ERIC activities on national and international level and have participated in projects: ADOPT BBMRI-ERIC and BBMRI-LPC. The activities are focused to promote biobanking in Latvia to correspond to European requirements to increase potential to participate in international research projects. This is attained via communication and education in fields of ethics and legislation, IT and quality assurance.

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Association of 4q25 variants and echocardiographic parameters in patients with atrial fibrillation

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Key words: atrial fibrillation, common genetic variants, echocardiography.

Atrial fibrillation (AF) is the most common cardiac arrhythmia. AF has a heritable component. Variants at locus 4q25 are the most significantly associated with the risk of AF development in genome-wide association studies. Heart failure with both preserved and reduced left ventricle ejection fraction often co-exists with AF as a risk factor, as well as complication of the arrhythmia. Furthermore, left atrial volume is an independent predictor of recurrence of AF after successful sinus rhythm restoration. The aim of our study was to investigate potential association between genetic variants at 4q25 locus and left ventricle ejection fraction and left atrial volume index.

We included 137 persistent and long-standing persistent AF patients into the study. All participants were admitted for an elective electrocardioversion in Arrhythmology Department of Cardiology centre. Left ventricle ejection fraction (LVEF, %) and left atrial volume index (LAVI, mL m^{-2}) were assessed by transthoracic echocardiography during outpatient visits prior the procedure. We selected five 4q25 genetic variants (rs6825911, rs1126483, rs10004516, rs6838973, rs2200733) for the analysis. Selection of variants was based on minor allele frequency (MAF > 10%) and possible pathogenic implication in AF development. Variants rs1126483, rs6838973 and rs2200733 were genotyped by high-resolution melting analysis, rs10004516 variation was analysed by RFLP assay and rs6825911 genotyping was performed using TaqMan assay C_29321008_10 (ThermoScientific, USA). The results for all variants were confirmed by bidirectional automated sequencing for five to 10 randomly selected samples with different genotypes; in all cases the genotypes were identical. IBM SPSS v.20.0 software was used for statistical analysis.

Eighty (58.4%) patients were male. Mean age was 64.4 ± 10.3 years. Median for LAVI was 39.0 mL m^{-2} (IQR = 10.0) and 56.0% (IQR = 13.0) for LVEF. Statistically significant association was observed only between LAVI and variation rs1126483 in dominant model of inheritance (median LAVI in CC vs. CT+TT – 38 mL m^{-2} vs. 40 mL m^{-2}, U = 1602.5, p = 0.032). No signifficant association was found for LVEF and analysed genotypes.

CT and TT genotypes of rs1126483 variation are associated with greater left atrial volume index. No relation was observed between LVEF and inluded variants.

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Genomic and genetic studies of Latvian forest species

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Key words: adaptation, DNA fingerprinting, population genetics, resistance.

The molecular genetic laboratory in the Latvian State Forest Research institute "Silava" (LSFRI Silava) is involved in a wide range of genomic and genetic studies of forest species and ecosystems. The research and analyses can be broadly divided into three directions: (i) DNA fingerprinting of forest tree species' breeding and reproductive material, (ii) population genetic studies of Latvian forest species, and (iii) fundamental research on the pine genome.

(i) DNA fingerprinting of forest tree species' breeding and reproductive material. DNA fingerprinting protocols have been developed for species in the Latvian forest tree breeding program – Scots pine, Norway spruce, birch, hybrid aspen, black alder, larch, willow. DNA fingerprinting is used for clonal identification/confirmation in seed orchards and vegetatively reproduced reproductive material.

(ii) Population genetic studies of Latvian forest species. DNA markers (mainly microsatellite or SSR markers) are utilised to investigate the genetic diversity and structure of a wide range of forest species endemic to Latvia, including conifer and broadleaf tree species, as well as wolf, lynx and capercaillie. Results are utilised for identification and characterization of forest genetic resources and population monitoring. The result of these population studies can be utilised to assess the need for supplementing existing forest genetic resource stands, as well as identifying possible introduced material, which may have differing adaptive properties or other traits. Genetic monitoring of wildlife populations provides additional information for the implementation of conservation and management action plans. In addition, high throughput barcode sequencing is utilised to investigate microbial diversity in forest ecosystems, and to link this with ecosystem function and services.

(iii) Fundamental research on the pine genome. Molecular responses to stress are being studied in the Scots pine genome. Resistance mechanisms to infection with the fungal pathogens *Heterobasidion annosum* and *Lophodermium seditiosum* are the primary targets, however other stress conditions are also being investigated. Transcript profiling of expressed genes and short non-coding RNAs are being used to identify stress response pathways and processes. Gene copy number variation (CNV) analyses can identify polymorphism between individuals and are also linked to differential gene expression. Pine genomes contain a high proportion of repetitive sequences, including partial and complete transposable element (TE) sequences. TEs are activated under a range of stress conditions, resulting in genomic rearrangements, and TE composition varies considerably between individuals. TEs can influence gene regulation by integration within or near coding sequences. Pine genome sequences are being analysed to identify genes in proximity to TEs, and to investigate their influence on gene expression and genetic diversity.

Analysis of molecular genetic disorders of *EGFR* and *KRAS* in patients with lung adenocarcinoma in Belarusian patients using Next Generation Sequencing

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Key words: *EGFR* gene, *KRAS* gene, lung adenocarcinoma, next generation sequencing, somatic mutations.

Non-small cell lung cancer (NSCLC) constitutes 85% of all lung cancers. Non-small cell lung cancer includes such morphological types as squamous, adenocarcinoma, large cell lung cancer and each of them requires a different approach to treatment and is characterized by different susceptibility to progression and metastasis. Histological types of non-small cell lung cancer have a different molecular genetic profile and therefore there is a need for a comprehensive study of molecular changes in a tumor, taking into account different morphotypes. Data on molecular disorders in genes associated with biologically important signaling pathways of lung carcinogenesis are important for predicting the course of a disease and correcting the ongoing treatment.

The study aims to analyze molecular disorders in the *EGFR* and *KRAS* genes in patients with adenocarcinoma using Next Generation Sequencing.

The study included 44 patients (18 women and 26 men) with a histologically and morphologically confirmed diagnosis of lung adenocarcinoma. DNA was isolated from lung tumor tissue samples. Molecular genetic research was carried out using the TruSeq Amplicon Sequencing Cancer Panel (Illumina) at the MiSeq (Illumina) device. Processing of primary data, obtaining reads with their further alignment to target regions of the reference genome (hg19) was carried out using the sequencer software (MiSeq Reporter). Subsequent filtration, annotation, verification and interpretation of options (work with vcf- and bam-files) was performed using ANNOVAR, VariantStudio, and Integrative Genomics Viewer (IGV) software. The identified deletions were verified using the direct Sanger sequencing method; the method of direct sequencing with preliminary cloning of a fragment by vector was used to verify the insertions. Statistical processing of the material was carried out using the program GraphPad InStat 3.05.

In the examined cohort of patients, synonymous substitutions in the 15th exon (A613A) and in the 20th exon (Q787Q) were detected in the *EGFR* gene. The polymorphic variant Q787Q (rs1050171) is also detected in DNA isolated from the non-tumor tissue and the blood of patients, and it has recently been seen as a potential marker in predicting the course and response to treatment of various oncological diseases. In four patients (9.1%), a substitution in the 18th intron (p.2184 + 19G>A) was found. In the 19th intron, the substitutions c.2284-34G>A (2.3%) and c.2284-60T>C (38.6%) were detected.

Somatic mutations in the *EGFR* gene were identified in 11 patients with adenocarcinoma (25.0%) and women predominate among the carriers of *EGFR* mutations: the mutation rate in women was 55.6% and among men only 3.8% (*OR* = 31.25; 95% *CI* 3.44 – 283.43; $p < 0.0002$). Most common deletions occur in the 19th exon (in six patients, 13.6%) and insertions in the 20th exon (in three patients, 6.8%). In one patient with bronchioloalveolar adenocarcinoma, a combination of the p.771insN insertion and the p.Pro772Arg missense mutation was found. This combination was identified for the first time and is not described in the COSMIC database.

Somatic mutations in the *KRAS* gene were detected in eight patients (18.2%), found only in men and did not occur in women (*OR* = 17.00, 95% *CI* 0.91 – 316.71; $p < 0.014$). The most common in this sample of patients is the somatic mutation c.34G> T (p.Gly12Cys).

Detection of molecular disorders in *EGFR* and *KRAS* genes will allow to identify groups of patients with adenocarcinoma, sensitive and non-sensitive to the ongoing therapy.

The second system of steroid hormonal regulation in higher plants: progesterone as a very ancient bioregulator of plant cells

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Key words: biotic and abiotic stresses, brassinosteroids, *CYP11A1*, phytohormones, progesterone.

The initial stage of steroid hormone biosynthesis in animals occurs in the mitochondria of steroidogenic tissues, where cytochrome P450scc (side chain cleaving), encoded by the *CYP11A1* gene, catalyzes the side chain cleavage of cholesterol with its transformation into pregnenolone, a common precursor of all animal steroid hormones starting with progesterone. This first stage of phytosterol (β-sitosterol, campesterol, stigmasterol, cholesterol) hydroxylation can not be exactly repeated in plants, as genes encoding mitochondrial cytochrome P450 ('mito CYP' clan), in their genomes are still not found. For a more detailed comparison of steroidogenic systems of Plantae and Animalia, we have obtained and comprehensively characterized transgenic tobacco (*Nicotiana tabacum* L.), foxglove (*Digitalis purpurea* L.) and tomato (*Solanum lycopersicum* L.) plants, effectively expressing *CYP11A1* cDNA from the bovine adrenal cortex.

As a result, the compatibility *in vivo* of even the most specific components of biosynthesis systems of steroid hormones in Plantae and Animalia was demonstrated for the first time. By increasing the level of the endogenic progesterone in the aforementioned *CYP11A1* transgenic tobacco and tomato plants, we were able to accelerate processes of growth and development and enhance plant resistance to biotic (*Botrytis cinerea, Oidium neolycopersici* and *Cladosporium fulvum*) and abiotic (water shortage, drought, high salinity) stresses. The formation of the above-noted successful phenotypes of transgenic Solanaceae and Scrophulariaceaae plants, expressing mammalian cytochrome P450scc (*CYP11A1*) cDNA, implies that in addition to already well-known brassinosteroids, higher plants have a second system of steroid hormonal regulation in which progesterone plays the most prominent role. Taken in the evolutionary context, our data provide strong support to the idea that progesterone can be considered as very ancient bioregulator of plant cells and the first true hormone common to plants and animals. The results indicate a definite similarity of the steroid compound biosynthesis and steroid regulatory systems of plants and animals and can be used in new biotechnologies for agriculture and pharmacology.

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The influence of various factors on the phenotypic variability of medicinal plants *Calendula officinalis* and *Nigella sativa*

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Key words: aminolevulinic acid, *Calendula officinalis*, electromagnetic radiation, *Nigella sativa*, phenotypic variability.

The studies aimed at the spectrum of plant variability on the resistance to various environmental parameters at the level of family, genus, species, and variety are of great interest because the results comprise the basis for understanding the genetic determination of the resistance of cultivated plants to various factors and developing the principles of ecological plant breeding. Nevertheless, the data on medicinal plants are very limited. The influence of such factors as agro-climatic conditions, pre-sowing effects of physical and chemical nature of varying intensity, including ultra-low doses, on the phenotypic variability, morphological and biochemical parameters of *Calendula officinalis* and *Nigella sativa*, medicinal plants grown in Belarus have been evaluated.

The experiments were laid out in areas with different agrochemical and mineral composition of the soil. The years in which research was conducted in Belarus differed in the level of moisture, average temperature and solar radiation. Pre-sowing seeds treatment of physical and chemical nature was used. Microwave electromagnetic radiation (EMR) of the millimeter wavelength range was selected as the physical impact on the seeds in the modes: Mode 1 (processing frequency 53.57 – 78.33 GHz) with processing exposure of 20 min, Mode 2 (64.00 – 66.00 GHz) with exposure of 12 min, Mode 3 (64.00 – 66.00 GHz) with exposure of 8 min. The seeds treatment was carried out at the Institute of Nuclear Problems of Belarusian State University on the laboratory equipment for microwave treatment in a wide frequency range (from 37 to 120 GHz) with continuously adjustable power from 1 to 10 mW. For chemical treatment, aminolevulinic acid (ALA) and epin were used in concentrations of 10^{-6} to 10^{-11}%. The parameters of germination, germination energy, height, parameters of productivity elements, the state of the photosynthetic apparatus, the qualitative and quantitative composition of phenolic compounds, including flavonoids, carotenoids, fatty acids, were studied. Changes in the state of the antioxidant system, as well as the hormonal status of plants as a result of the exposure were evaluated.

The species and variety specificity of realization of genetic potential of the studied medicinal plants in small-plot field experiments in 2011 – 2017 has been found. The influence of the studied factors on the state of the phenotype of medicinal plants has been shown. The productivity of *N. sativa* was significantly affected by the number of sunny days during the growing season. Also for this plant, the intensity of the effect of pre-sowing treatment was closely related to weather conditions. The EMR treatment led to an increase in productivity parameters, such as the number of seeds in the padding, the weight of 1000 seeds, while the use of ALA contributed to the change in the number of shoots of the 1st order, and due to this, an increase in the number of seed heads on the plant. Changes in the qualitative composition of *N. sativa* oil were also observed. The EMR treatment resulted in an increase in the unsaturation of *N. sativa* fatty acids, but a decrease in the amount of timoquinone. At the same time, pre-sowing treatment with ALA and epin led to an increase in the content of timoquinone by more than 20%.

The conditions of the growing season were more important for *C. officinalis*; in years with a higher temperature, the maximum accumulation of carotenoids was observed. In years with relatively unfavorable conditions, the pre-treatments led to the most pronounced positive effect in the accumulation of biologically active substances. The agrochemical composition of the soil was not the limiting factor for *C. officinlis* under the experimental conditions, while for *N. sativa* this factor was significant.

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The c.1397C>G and c.3209G>A mutations in exon 10 of CFTR gene in an infertile men with oligoastenozoospermia

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Key words: *CFTR* gene. male infertility, NGS.

Cystic fibrosis and congenital bilateral absence of the vas deferens (CBAVD) are autosomal recessive diseases that caused by *CFTR* gene mutations. Recent literature findings claim a strong association between *CFTR* gene variations and CBAVD, but the role of this gene in male infertility is still unclear. Heterozygous two different point mutations in *CFTR* gene are identified in an infertile men with non-obstructive oligoastenozoospermia in the current results.

We tested 45-year-old infertile man who admitted to the Medical Genetics laboratory of Canakkale Onsekiz Mart University. Peripheral blood-EDTA sample was used for genomic DNA isolation and the sequenced for all exonic-intronic regions of CFTR gene was assessed by NGS gene panel on Ion Torrent S5 platform (Thermo Fischer Scientific). The NGS data outputs and all intronic variations were analysed by Ion Reported 5.6 and IGV softwares.

He had low sperm count (10×10^6 mL^{-1}, $N > 15 \times 106$ mL^{-1}), motility (25%, N > 40%) and volume (1.5 mL, N 2 – 6 mL) at spermiogram analysis. Here we detected heterozygous c.1397C>G(p.Ser466Ter) and c.3209G>A(p.Arg1070Gln) missence mutations in exon 10 of *CFTR* gene in the current infertile man.

Based on the present case specific findings, the CFTR gene mutations may be corelated with nonobstructive oligospermia and possible some forms of male infertility. NGS is a versatile technique in the detection of such a mutated carriers and it was suggested that cases need to be given genetic counseling before IVF.

The molecular identification of *Borrelia* bacteria in red squirrels and their ectoparasites in Lithuania

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Key words: *Borrelia*, ectoparasites, infection, red squirrel.

Borrelia is a genus of bacteria of the spirochete phylum. It causes borreliosis, a zoonotic, vector-borne disease transmitted primarily by ticks and by lice, depending on the species. Infected ticks can transmit *Borrelia* to a large group of vertebrates such as reptiles, birds, and small mammals. The aim of this study was to carry out molecular identification of *Borrelia* bacterium in red squirrels and their ectoparasites in Lithuania. DNA from red squirrels was extracted by using a Genomic DNA Purification Kit, according to the manufacturer's instructions. DNA from fleas and ticks was extracted by using 2.5% ammonium hydroxide. *Borrelia* DNA in samples was detected using a RT-PCR and nested-PCR of the *18S rRNR* gene. A total of 39 red squirrels (*Sciurus vulgaris*), victims of road traffic, were found. Squirrels were found to be infected with *Ixodes ricinus* ticks (191) and *Ceratophyllus sciurorum* fleas (70). *Borrelia* spp. DNA was detected in 8 (20.51%) samples of squirrels and 27 (10.34%) sample of tick. None of flea samples were infected with *Borrelia*. The *18S rRNR* gene sequences showed that *Borrelia afzelii, Borrelia burgdorferi* and *Borrelia miyamotoi* were detected in squirrels and their ticks. The results of this study suggest that *I. ricinus* ticks may be substantial vector for transmitting of different *Borrelia* species in red squirrels in Lithuania.

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Next-generation sequencing study of barley plastid genome expands the knowledge about the molecular evolution of *Hordeum vulgare*

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Key words: alloplasmic, barley, chloroplast DNA, NGS, phylogeny.

Chloroplasts are essential organelles in plant cells and play an important role in sustaining life. The chloroplast genome is a suitable object for phylogeny studies in plants. The maternal inheritance, low rate of mutations, and practically lack of recombinations make it indispensable for studying the processes of molecular evolution of species and genera in different taxa.

Formerly, the study of the variability of plastid genomes, the establishment of phylogenetic links were conducted mainly on the basis of the SNP (single nucleotide polymorphism) in distinct chloroplast DNA loci: *matK*, *rbcL* etc., or microsatellite repeats in individual genes and intergenic regions. It was a relatively small (in %) part of plastid genome.

Next-generation sequencing (NGS) of the full chloroplast genomes from different matrices (whether total DNA, or a mixture of organelle DNA, or pure plastid DNA) allows us to simultaneously explore the large number of samples, to obtain qualitatively new comparative data on the variability of chloroplast genomes. Complete chloroplast genome sequences are essential for realizing the phylogenetic relationships between closely related taxa and for improving our understanding of the evolution of plant species.

The objective was to perform the comparative study of the plastid genomes variability in alloplasmic barley lines (differing in origin of the cytoplasm donor) and their euplasmic analogues.

We performed NGS (Illumina, MiSeq) analysis of 12 organelle DNA samples (plastid fraction) from the collection of alloplasmic barley lines created and maintained in the Lab of Cytoplasmic Inheritance of the Institute of Genetics and Cytology NAS Belarus. Peculiar bioinformatics' approaches for the processing of the "raw" data after sequencing of chloroplast DNA have been developed. Twelve new full sequences of the plastid genomes were compiled, comparative analysis among themselves and with the available in Pubmed (GeneBank) sequences of H*ordeum vulgare* and related specimens was carried out.

More than 80 sites of polymorphism of plastid DNA were revealed for the investigated set of barley samples. So, we identified many new loci of variability in the plastid genomes of barley, that could be valuable for broadening our knowledge about the molecular evolution of barley and other cereals. Based on our data and the complete cpDNA sequences available in the PubMed, a tree of barley cognate relationships (*Hordeum vulgare*) has been constructed. Two main branches are distinguished by 22 polymorphic sites: one contains all the available in PubMed sequences and the majority of cpDNA nucleotide sequences analyzed by us, and the second includes a single sample with the cytoplasm W4 and the nucleus of the Vezha variety.

The branch, which included most cpDNA sequences of barley, was differentiated into three clusters: the first was represented by the barley *Hordeum vulgare* subsp. *spontaneum* from Israel [this DNA sequence was taken for comparison from the GeneBank (Pubmed)], the second included the predominant majority of the barley samples (nine specimens), mainly with the cytoplasm from *H. vulgare* subsp. *spontaneum*, and the third cluster consisted of two cvs. Roland and Visit (*H. vulgare* subsp. *vulgare*) and several cpDNA sequences of barley from GeneBank (Pubmed): cv. Morex (*H. vulgare* subsp. *vulgare*) and *H. vulgare* subsp. *spontaneum* from Turkey.

During this study twelve cpDNA complete sequences of *H. vulgare* were obtained, comparative genomic analysis with previously published cp genome sequences of *H. vulgare* was performed. The results provide a basis for future studies of molecular evolution and population genetics in *Hordeum*.

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New mutation in the *LAMP2* gene causing Danon syndrome phenotype

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Key words: cardiomyopathy, Danon disease, LAMP2, NGS, X-chromosome inactivation.

Danon disease (DD), a rare X-linked genetic disease with poor prognosis, was described in 1981 by Danon; up to 2016 already 124 proved DD cases were reported (Botillo 2016). Three main clinical features of the pathology are cardiomyopathy, skeletal myopathy and mental retardation. In women DD can manifest as phenocopy of dilated cardiomyopathy (DCM). DD is caused by a mutation in the gene of lysosomal-associated membrane protein 2 (LAMP2, Xq24). Most DD mutations create premature stop codons, resulting in the decrease or absence of LAMP2 protein, the situation more problematic in males who are hemizygous for *LAMP2*. Reduction in LAMP2 disrupts intracytoplasmic trafficking and leads to accumulation of autophagic material, besides often glycogen in skeletal muscle and cardiac muscle cells. Combined somatic-germinal mosaicism of *LAMP2* mutations has been suggested to contribute to a clinical diversity among female DD patients. The prevalence of Danon disease is unknown, it's considered as less than one case per million.

We used targeted next-generation sequencing while looking for a mutation in of the 34-year-old woman B., suffering from DCM. The initial symptoms of this disease (swelling, shortness of breath and weakness) appeared during the last trimester of the third pregnancy. Dilatation of the heart chambers and systolic left ventricular dysfunction were established. Delivery was carried out on the maturity of the fetus. During the next 18 months after delivery, B. heart failure symptoms progressed rapidly despite the medical therapy. Proband underwent clinical investigations in the Scientific and Practical Center of Cardiology (Belarus). Dilated cardiomyopathy was diagnosed according to established criteria of the ESC Cardiology Working Group on Myocardial and Pericardial Diseases. The physical examination revealed no specific abnormalities, especially no muscle weakness. In 6 months the patient underwent heart transplantation. The family history reconstruction showed a sudden cardiac death of proband's mother at the age of 30.

We identified a novel 2 bp-deletion in exon 3 of the *LAMP2* gene – c.190_191delAC (NM_001122606). It is not detected in the publicly available population databases. This mutation results in a reading frame shift and premature terminating codon creation – p.V64NfsX11. The total length of truncating protein is predicted 74 aa instead of 410 aa. It means the mutant protein lacks the transmembrane domain and most part of the luminal domain. Such rearrangement leads to loss of function of the LAMP2 protein. We consider the variant c.190_191delAC as likely pathogenic based on a strong association between loss-of-function variants in LAMP2 and DD.

Proband's family genotyping by Sanger sequencing revealed c.190_191delAC in two sons. One of them doesn't show any cardiac or neurological involvement due to his young age (three years old). The investigation of a 7-year-old boy, who is the mutation carrier as well, demonstrated a high ECG voltage, signs of mild left ventricular hypertrophy.

We estimated the X-chromosome inactivation (XCI) pattern in the heart muscle of patient B. It was evaluated by methylation of Hin6I sites in the androgen receptor gene in three independent experiments. This gene is reliably methylated when inactivated and correlated with X-inactivation. As a result, the patient has an XCI rate 33:66, that suggested a random distribution. It means that only 33% cardiomyocytes carry active X-chromosome with normal LAMP2. This amount is not enough for the provision of intracytoplasmic trafficking in heart tissue. Importantly, X-inactivation is known to influence the clinical manifestation of a number of X-linked diseases. We assume there is a relationship between XCI and clinical involvement in DD. Additional investigations are required.

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Scots pine transcriptome dynamics in response to inoculation with *Heterobasidion annosum*: initial results

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Key words: *Heterobasidion annosum, Pinus sylvestris,* plant pathogen, RNA-seq, transcriptome..

As a species of high economic and ecological significance for the Baltic states, the genetic potential of Scots pine has been successfully utilised in breeding programs to select faster-growing, higher economic value material. Yet root rot, the economically most significant conifer disease, continues to present a problem.

A detailed analysis of transcriptome dynamics of a single genotype (grafted clones) challenged with wounding or inoculation with *Heterobasidion annosum*, the causative pathogen of root rot, will provide targets for further research of genetic aspects of defence responses of Scots pine against root rot. In the current study, biological replicates representing different conditions (two untreated controls, two wounded and three inoculated samples) were used for transcriptome sequencing. Transcriptome analysis revealed a number of differentially expressed genes, the treatment causing the highest number of genes to be differentially expressed (330 in total) was wounding which caused over-expression of 168 genes and down-regulation of 162 genes while inoculation with *H. annosum* changed the expression of, correspondingly, 124 and 200 genes. When compared to wounded samples, inoculation up-regulated the expression of 66 genes and down-regulated 28 genes. Although annotation of the differentially expressed genes relies on data available about functions of *Arabidopsis thaliana* genes, general trends could be detected. Inoculation and wounding caused down-regulation of photosynthesis-related genes. Genes down-regulated in response to wounding or inoculation (12 and 13% respectively), were linked to photosynthetic processes while almost no such genes were up-regulated.

Defence-related genes represented 22% of the genes up-regulated after inoculation, in case of wounding it was 26%. Defence-related genes were also down-regulated after inoculation or wounding (16 and 18% of the total number, respectively). Comparison of transcription levels after inoculation vs wounding revealed that a high proportion of up-regulated genes were defence-related, or genes with functions in transport, signalling and transcription (18, 17, 11 and 11%, respectively). Among down-regulated genes, the largest proportion were linked to transcription, defence, metabolism, plant structure development, signalling and transport (18, 14, 11, 7, 7, 7%). The functions of 18% of up-regulated and 14% of down-regulated genes could not be categorised.

Genes which are up-regulated after inoculation compared to wounding could provide information on specific defence processes. Five genes which show the highest positive differential expression are *WSD1*, a calcium binding protein gene, a stress-response A/B barrel domain-containing protein HS1 gene, a mitochondrial 3-hydroxyisobutyryl-CoA hydrolase-like protein gene and a 60S ribosomal protein L13E gene. *WSD1* is probably involved in plant defence as it encodes a wax ester synthase with a possible role in cuticle formation. Calcium is a secondary messenger in many signalling cascades so proteins with the capacity to bind calcium could be either responsive to changes in intracellular calcium levels or could be involved in regulation of calcium levels. The HS1 protein is a heat stable protein involved in defence against fungal pathogens in *Arabidopsis thaliana*. In *A. thaliana,* 3-hydroxyisobutyryl-CoA hydrolase 1 is involved in valine catabolism, and may be indirectly involved in benzoic acid biosynthesis, cold signalling and cold tolerance. The 60S ribosomal protein L13E is a ribosomal structural component.

Further analysis of these and other differentially expressed genes will enable better understanding of defence processes in Scots pine and improved characterisation of breeding material.

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Implementation of advanced methods in potato breeding program

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Key words: biologically active compounds, MAS, potato breeding, resistance, trait stability.

The potato breeding program, carried out at AREI Crop Research department in Priekuli, was improved by implementation of advanced methods for assessment of potato breeding clones in the past ten years. The goal of the breeding program is to develop of varieties suitable to organic growing conditions. To reach the goal evaluation of potato clones were carried out in two locations and farming systems: integrated and organic. For organic farming it is essential to have varieties with high stability of traits and adaptability to different growing conditions. The application of dynamic concept of stability – regression coefficient (b_1) has been applied for evaluation of tuber yield stability of clone. Additionally, several static concepts were applied for estimation of quality trait such as stability of starch content. Determination of biologically active compounds in tubers and development of calibration model for near infrared spectroscopy (MAS) with aim to asses breeding material has been worked out. This approach is important for selection of outstanding genotypes for functional food: tubers with high concentration of vitamins C and B, anthocyanins, carotenoids etc. Resistance to most dangerous pests and diseases in Latvia have been detected using marker assisted selection and compared with evaluation of resistances in artificial and natural conditions. Molecular markers were used for detection resistant alleles of genes conferred resistance to potato cyst nematode (*Globodera rostochinensis* Ro1) and late blight (*Phytophthora infestans*). Application of advanced methods in potato breeding to evaluate the clone provides selection of most valuable new potato varieties. Superior potato varieties: early variety 'Rigonda' and 'Jogla' variety with high content of starch were registered in 2018. .

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Association of polymorphisms of milk protein genes with milk quantity and quality traits in Latvian native dairy cow breeds

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Key words: association, cows, Latvian Blue (LZ), Latvian Brown (LB), milk protein polymorphism.

There are two Latvian native breeds of dairy cattle (Bos taurus) Latvian Blue (Latvijas zilā; LZ) and Latvian Brown (Latvijas brūnā; LB), respectively. Historically both breeds have been created in territory of Latvia, but for the improvement they have been mixed with other breeds of dairy cows. Few genetic studies have been dedicated to Latvian native breeds in Latvian population of dairy cows. The aim of this work was to explore and to compare the populations of Latvian Blue and Latvian Brown breeds in Latvia, by analyzing six polymorphisms of five milk protein genes, g.4646092A>G (rs109817504) in *CSN1S1* gene, c.4451A>C (rs43703011) in *CSN2* gene, c.11625C>T (rs43703015) and c.11661A>C (rs43703016) in *CSN3* gene, c.15A>G (rs209045823) in untranslated region of *LAA* gene and c.3106T>C (rs109625649) in *LGB* gene, respectively. Association analysis for each breed was performed between alleles/genotypes of proteins and indicators of milk quantity and quality (milk yield, protein and fat amount and content). Differences were found in phenotypes as well as in distribution of alleles/genotypes of three proteins in LZ and LB breeds. Statistically significant associations between *CSN2, CSN3, LAA* and *LGB* and analyzed indicators in LB breed and between whey proteins and same indicators in LZ breed were found.

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Phylogenetic study of the fire-bellied toad (*Bombina bombina*) population on northern border of areal (Latvia)

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Key words: *Bombina bombina*, fire-bellied toad, phylogenetyc analysis, sequencing.

The fire-bellied toad (*Bombina bombina* L.) is endangered European amphibian species with the northern border of its distribution areal in Latvia. The phylogenetic study of the animal populations at the border of the areals is important for understanding their evolution and adaptation. There is an ongoing discussion about whether populations at the edge of a distribution range are specifically adapted to the presumably suboptimal conditions or whether these areas constitute a sink in a metapopulation dynamic. Mitochondrial DNA (mtDNA) is a model molecule in evolutionary, systematic and conservation study. Samples of fire-bellied toads were obtained from two places of Latvia: Demene and Medumi. Mitochondrial cytochrome b, cytochrome oxidase 1 and NADH dehydrogenase subunit 4 gene sequences were sequenced, compiled and searched for similarity using NCBI databasis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and CLC Genomics workbench software 10.0. *B. bombina* sequences from NCBI databasis were selected and compared to *B. bombina* sequences from Latvia and phylogenetic analyses were performed using CLC Genomics workbench softwere using Neighbor Joining tree construction method and Jukes-Cantor nucleotide distance measurment.

There were observed 99.5% identity between genes sequences of Demene and Medumi and 100% identity between *B. bombina* isolates obtained from Latvia and from Poland, Sedranki (accession nr EF212624) and they formed common branch in the phylogram. Next, the most homologous sequences were observed from other reģions from Poland: Ksiaz Wielki (JF898321), Strui (EF212509), Olszanica (EF212547). There were observed that isolates from Russia (JF898336, JF898334), Romania (EF212505, EF212503), Slovakia (JF898345), Germany (EU531242), Hungary (JF898363), Turkey (JX893173), Austria (JF898358) and Bulgaria (JF898352) formed the different branch in the phylogram. These results indicated that *B. bombina* isolates from Latvia forms the common genetic population with Northern area.

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Genetic and epigenetic monitoring of some fish populations in Latvia

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Key words: epigenetic, fish populations, genetic monitoring.

Genetic monitoring is long-term surveillance over the state of population gene pools, intended to evaluate and predict their temporal and spatial dynamics and to determine the limits of permissible changes. The populations of local Salmonidae fish (vendace *Coregonus albula* L. and lake smelt *Osmerus eperlanus spirinchus*) in this region are residues of Arctic freshwater faunal complex, and can be considered as glacio relicts – an indicator species of Lake ecosystem status. Vendace and lake smelt are widespread in Europe. But in Latvia their share in the fishery is not large, the catch is insignificant and unstable, and these species are included into the list of specially protected fish species with limited use. It was determined the genetics structure and differentiation of local Salmonidae populations. To evaluate the influence of economic activity on freshwater ecosystem, the genetic structure of Eurasian perch *Perca fluviatilis* (L.) populations was studied.

The genetic structure of the perch, vendace and lake smelt populations from Daugava River and Lielupe River and 11 Latvian lakes was investigated using nine DNA microsatellites in each fish population. The genetic diversity, the level of polymorphism, means of alleles on locus, observed and expected heterozygosity, population structuring (F_{ST} and R_{ST}), Bayesian approach, gene flow (*Nm*) were analysed in fish populations. The genetic differentiation among the populations was estimated according to the principal component analysis, F_{ST} value, the coefficient of genetic differentiation of the populations (G_{st}). With the help of the computer program Bottleneck 1.2.02 it was verified whether the studied populations had passed the bottleneck effect. In order to estimate and visualize the genetic structure and differentiation of the studied fish populations (according to the microsatellite data), the computer programs STRUCTURE and STRUCTURE HARVESTER were used. STRUCTURE displayed that the individuals in the studied populations of Eurasian perch could be partitioned in three genetic groups, separating perch populations from Tome (Daugava), Kegums (Daugava) and Griva (Lielupe) in one group, Voleri (Daugava) and Jelgava (Lielupe) into the other groups. STRUCTURE displayed that the individuals in the studied populations of vendace could be partitioned in three genetic group ($K = 3$), separating Lake Nirzas and Lake Raznas in one group, Lake Aluksnes and Lake Ezezers into the other groups.

Amur sleeper (*Perccottus glenii* Dybowski) is a freshwater invasive fish species, which has rapidly spread during the last two decades in many European countries. The total registered areal of *P. glenii* covers at least the central and eastern part of Latvia also. The study of invasion success must be considered complete with the evolutionary genetics, as it might be correlated with the genetic and epigenetic polymorphism of populations. Epigenetics, particularly a noticeable shift in DNA methylation status, is often associated with the process of colonization of new environments. Different types of stressing environmental conditions may alter global DNA methylation levels. For study of influence of environmental factors of epigenetic changes of Amur sleeper (total DNA methylation) LUMA method was applied in *P. glenii* samples from ecological different aquatic ecosystems. It was shown that in the same age, sex and size of *P. glenii* samples the total DNA methylation levels are different in ecological different lakes and under the anthropogenic influence the global relative DNA GC-methylation level was increased to 40%.

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Genetic resources of Jerusalem artichok (*Helianthus tuberosus*) in VIR collection

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Key words: *Helianthus tuberosus*, Jerusalem artichoke, morphological features.

Jerusalem artichoke, *Helianthus tuberosus* L., is a valuable forage, food, technical and medicinal plant. The study of biological, geographical, environmental, taxonomic and other features of the species is relevant and necessary, first of all, to identify the variability of the complex features, the development of intraspecific system and clarify the characteristics of species distribution in Russia and neighboring countries. One of the basic components of such research is the analysis of morphological features of plants.

The material for the research was the collection of Jerusalem artichoke at the branch of VIR "Maikop experimental station" (223 cultivated varieties, 98 hybrids). 100 samples of different geographical origin were measured: plant height, length of leaf blade with petiole, length of leaf blade, width of leaf blade. Five qualitative traits of leaf were evaluated: leaf shape, form of leaf base, the pubescence of the leaf plate, type of the edges of the leaf form, the top of the leaf. Inflorescences of 58 samples were analyzed for eleven characters. For statistical analysis, the samples were grouped by historical and geographical origin into nine groups. Statistical analysis was carried out using StatSoft Statistica 13.0 package.

A number of related issues were identified: characteristics of leaves: the index of the leaf (leaf length/ length of leaf plates) - with the length of the petiole; index of the leaf blade (leaf length plates/width of the leaf blade) - with the width of the leaf blade and paired with a lanceolate leaf shape; etc.

The most differentiating characters of studied fragment of the collection were: length of leaf plates and petiole; leaf blade width; index of the leaf; leaf shape and edge of the leaf. These characters can be used for the purposes of systematics. The maximum differences between the groups by geographical origin were revealed by the number of inflorescences on the plant and the number of lingual flowers in the inflorescence. The length of tubular flowers was constant within the species and was 1.4 cm; the specific feature was the most constant and probably can be used as a diagnostic for this species. According to the complex morphological features were contrasting groups: Caucasus; Asia; Japan. The accession 'Sakhalin red 4' was the most original on the aggregate characters (Sakhalin population, country of origin Japan).

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Impact of ecological factors on development of *Linum usitatissimum*

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Key words: diseases, flax, growth stages, humidity, yield.

Flax (*Linum usitatissimum* L.) is a multiple purpose crop valued for its fibre and seed properties. It is adapted to different environmental and agroecological conditions. Variable environmental factors have different impacts on yield formation of flax and can cause favorable conditions for development of flax diseases. A detailed understanding of the impact of humidity on flax development and disease spread can be used for more accurate predicteion of flax yield.

The aim of this study was to clarify possibilities of development flax genotypes with important agronomic traits at different humidity conditions and to investigate spread of fungal diseases in dependence on flax growth stages.

In this study 25 fibre flax genotypes were evaluated in field conditions on the background of natural infection. Agronomically important traits, such as stem and seed yield, total plant height, technical plant height, fibre content, 1000 seed weight, number of seed vessels per plant, seed number per seed vessel and incidence to different diseases were evaluated. Relationship between agronomically important yield traits and hydrothermal coefficient as well as between incidence of fungal diseases and growth stages of flax was analyzed.

The correlation found confirmed that most genotypes had highest total plant height, technical plant height, stem yield, a number of seed vessels per plant and oil content in high humidity conditions. However, seed yield, seed number per seed vessel as well as 1000 seed weight were highest in dry conditions for most genotypes. The correlation between flax fibre content differed in dependence on genotype. Among all studied flax genotypes, the genotype 'L11-11/11-97' had the highest average stem yield (840.00 g m^{-2}) and seed yield (168.68 g m^{-2}).

Disease incidence showed significant differences between vegetative and reproductive stages of flax development. Statistically significant ($p \leq 0.05$) higher summary infection level was during early yellow stage for all diseases except flax wilt. Statistically significant positive ($p \leq 0.01$) correlation confirmed the highest progress of anthracnose ($r = 0.85$), stem break, browning of flax ($r = 0.87$) and Fusarium browning ($r = 0.86$) during all growth stages, but development of pasmo ($r = 0.95$) and powdery mildew ($r = 0.95$) during reproductive stages of flax. The incidence of flax wilt was lowest and was observed at vegetative stages of flax development.

Knowledge about impact of humidity on yield development and spread of different diseases allows for development of effective management systems for flax breeding.

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The project "Preservation of gene pool of flax" was financial supported by the Ministry of Agriculture of Latvia.

Search for pre-breeding material with combined resistance to late blight (*Phytophthora infestans*) and nematode (*Globodera rostochiensis*) within interspecific potato hybrids

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Key words: *Globodera rostohiensis*, host plant resistance, interspecific hybrids, *Phytophthora infestans*, potato.

Potato cyst nematode (*Globodera rostochiensis*) and late blight (agent *Phytophtora infestans*) are economically important potato pathogens. Nowadays the potato breeding requires basic material with resistance to two and more pathogens. Resistance to nematode and late blight is one of the mostly desirable traits in developed potato cultivars. Solanum species are intensively used in the potato breeding as a source of resistance to many diseases and pests. Application of validated DNA markers for resistance breeding saves time and efforts and permits efficiently select genotypes with desirable traits (Gebhardt et al. 2006). Most of the current potato varieties are resistant to *G. rostochiensis* pathotype Ro1 as they have *H1* resistance gene. However, bioassays are still basic approach to select resistant plants.

The goal of our research was to identify the interspecific hybrid progenies combining resistance to the both pathogens and reveal an ability of markers associated with resistance to G. rostochiensis of pathotype Ro1 to identify resistant plants. Plants of eleven original interspecific potato hybrids obtained in crosses with cultivated (*Solanum tuberosum* spp. *tuberosum* (tub), *S. tuberosum* group *Andigena*, *S. tuberosum* group *Phureja* (phu)) and wild (*Solanum guerreroense* (grr), *Solanum microdintum* (mcd), *Solanum kurtzianum*, *Solanum tarijense* (tar) and *Solanum neoantipoviczii*) potato species were screened in bioassays (resistance to late blight and nematode) as well as in molecular tests (nematode).

Nine hybrid clones were found as resistant or moderately resistant to nematode. Marker CP113 of gene H1 adapted by Skupinová et al. (2002) was amplified in all 11 accessions and marker of gene *Gro1-4* described by Gebhardt et al. (2006) in seven accessions. Using the marker CP113 no association between the resistance levels and the presence of gene *H1* resistance allele was found for number of genotypes. Results obtained have established the desability of marker CP113 to distinguish resistant/susceptible to *G. rostochiensis* of pathotype Ro1 plants among a set of interspecific hybrids of divers genetic background. Among the tested accessions the resistance to both pathogens was found in several plants of hybrids cv. Aurora × (tub × phu), grr × Black differential genotype R5 and mcd × tar. Marker *Gro1-4* was detected only in nematode resistant plants.

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RAPD analysis of the lady fern (*Athyrium filix-femina*) from industrial and natural recreational areas in Lithuania and Norway

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Key words: biomonitoring, DNA polymorphism, heavy metals, technogenic pollution.

An unfortunate consequence of industrialization and urbanization is the growing environmental pollution. Hazardous compounds enter the environment from various sources such as discharged wastewater, air emissions, domestic and industrial waste sites. Anthropogenic emissions of metals cause considerable pollution problems due to their persistency. Mining activity is perhaps the most important source of discharges of metals into the environment of all land releases of toxic pollutants. Metals present in excess may exert various toxic effects upon biota.

A widespread species, like the lady fern, that is native throughout most of the temperate Northern Hemisphere, can be utilized in ecotoxicology studies to search for genotoxic compounds in environmental compartments. Measuring the accumulation of pollutants in plant tissue is a common method that is used to assess the effects of many pollutants for in situ toxicity studies and for biomonitoring purposes. Concentrations of metals as a bioindicator of contamination integrated with the genetic damage indicators is a favoured strategy, and the combination of assays is vital in risk assessment.

Our aim was to assess genetic diversity in *Athyrium filix-femina* populations in relation to the extent of technogenic pollution mainly heavy metals.

Twelve ferns were collected at each of the 23 sampling sites that included (i) unpolluted control (natural recreational areas); (ii) possibly polluted (roadsides); and (iii) heavily polluted (industrial, mining/smelter areas) sites located in Lithuania and Norway. Concentration of 11 metals (Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Zn) were determined in leaflets and petioles by atomic absorption spectroscopy. Frond tips were used for standard RAPD analysis. Logit regression and multivariate analysis were applied to data.

Ferns at the control areas had higher concentrations of macronutrients and the multifold lower levels of heavy metals as compared to ferns at polluted areas. Excessive and toxic concentrations of Cu, Mn, Ni and Zn were determined in several cases but mostly in ferns at industrial, mining/smelter sites in Norway. Overall metal content significantly differed in plant material at all sampling sites. Metal content in different organs differed as well except for Cu. Translocation factor showed effective translocation of Ca, Fe, Mg, Mn, Ni, Pb and Zn from basal to the apical fern parts.

Ten RAPD primers generated 480 polymorphic loci in a range of 0.38 to 5 kbp. The average percentage of polymorphic loci for each population was 42.88 and 33.51% in Lithuania and Norway, respectively. A significant correlation was determined between fern geographic and genetic distances (r = 0.25, p = 0.001). Ferns from the unpolluted, and waste sites formed large distinct clades in genetic distance dendrogram while the rest ferns grouped into smaller clades.

The total molecular variance of the lady fern depends exceedingly on the variation within populations (70%), much less on variation among populations (28%) and only 2% of it depends on variation among regions, Lithuania and Norway. Population analysis, in relation to low–high contamination level, revealed that metal pollution accounted for 1% of the total molecular variance in ferns. Comparison of fern genetic diversity at habitats of different pollution degree implies for the response to toxins present in the environment and the likely impact of habitat local ecological conditions to fern genetic differentiation. A logit model revealed 19 RAPD loci which profiles were best predicted (up to 85%) by the metal content in plant tissue, and these loci will be reamplified for sequencing and homology analyses.

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Comparison of genetic structure of Lithuanian *Impatiens parviflora* populations according to several multilocus dominant DNA markers

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Key words: Balsaminaceae, genetic diversity, invasive species, molecular markers.

Nowadays invasive annual herb *Impatiens parviflora*, which can easily adapt to different habitats and outcompete native plant species, spreads rapidly in Lithuanian parks and forest edges. The aim of our study was to investigate genetic diversity of *I. parviflora* growing in Lithuania according to several multilocus dominant DNA markers. Twenty one populations were chosen from different regions of Lithuania on purpose to cover all the territory of the country. Up to 16 plants were sampled per population, in total more than 300 individuals. Four inter-simple sequence repeat (ISSR), eight randomply amplified polymorphic DNA (RAPD) markers and eight amplified fragment length polymorphism (AFLP) marker pairs were used in the research. Agarose gel and capillary (ABI 3130 genetic analyzer) electrophoreses were used for fractionation of DNA fragments. Populations of *I. parviflora* from Central Lithuania had the highest and North-West Lithuania populations showed the lowest polymorphism according to all three types of dominant DNA makers. Analysis of molecular variance (AMOVA) revealed that highest genetic variably within populations was estimated using AFLP (88%) makers, using ISSR (23%) and RAPD (21%) makers this value was much lower. Significant correlations between genetic and geographic distances of *I. parviflora* populations were found for AFLP and RAPD markers, but no significance between these parameters were found for ISSR makers. Comparing Bayesian clustering data of AFLP, ISSR and RAPD markers, high numbers of clusters were obtained (respectively 17, 11, 13). These results suggest that *I. parviflora* might be introduced to Lithuania multiple times from neighbouring countries.

Acknowledgements

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Allelic variation at HMW-GS loci (*Glu-1*) and baking quality-related characteristics of Estonian spring wheat cultivars and breeding lines

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Key words: baking quality, *Glu-1* quality score, HMW-glutenin subunits, spring wheat.

The important direction of the spring wheat (*Triticum aestivum* L.) breeding in Estonia is improving of baking quality of wheat varieties. Studies done by Payne et al. (1980; 1987) and numerous other authors provided evidence of a strong association between the presence of certain alleles of *Glu-1* loci coding for high molecular weight glutenin subunits (HMW-GS) and bread-making quality.

The aim of the present study was to identify *Glu-1* loci allelic composition of new spring wheat cultivars and breeding lines obtained in Estonian Crop Research Institute (ECRI) using DNA markers and to investigate the relation of the quality scores of HMW-GS with dough and bread quality traits.

Genotypes of three Estonian cultivars and seven breeding lines were analysed with 12 DNA markers for their *Glu-A1, Glu-B1* and *Glu-D1* loci alleles detection and with one marker for detection of 1RS.1BL rye translocation. HMW glutenin subunit quality scores (*Glu-1* QS) were calculated for the cultivars according to the method described by Payne et al. (1987) by adding together the scores of individual subunits.

The following quality parameters of 2015 and 2016 years were measured for this study flour water absorption at 15% moisture content (WA), farinograph dough stability (DS), valorimetric value (VV), gluten index (GI) and loaf volume (LV). Spring wheat cultivar Manu (Finland) was used as a quality standard.

The identified alleles (subunits) were: *Glu-A1* (a (2*), b (1) and c (n)), *Glu-B1* (b (7+8), c (7+9), a (7+0)) and *Glu-D1* (d (5+10), a (2+12)) (Table 1). Translocation 1RS.1BL was not found. *Glu-1* score ranged from 7.5 to 10.

Values of investigated parameters close to or higher than standard cultivar Manu, were revealed mostly in samples with *Glu-1* QS 10, as cv. Hiie had the highest WA, line 161.1.6 had highest DS, VV and LF, line 720 had the strongest gluten. Also cultivars with *Glu-1* QS 8.5 had high

Table 1. Characterisation of spring wheat cultivars and breeding lines. Quality parameter values was the average of 2015 and 2016

Cultivar/ breeding line	HMW-GS A1/B1/D1	Glu-1 QS	WA (%)	DS (min)	VV	GI	LV (cm^3)
Manu	2*/7+8/5+10	10	54.5	7.5	58.5	53	1670
161.1.6	1&2*/7+8/5+10	10	53.9	6.1	55.5	43	1730
Hiie	1/7+8/5+10	10	56.7	5.6	51.5	64	1550
720	1&2*/7+8/5+10	10	53.3	5.9	49.5	88	1490
Means of Glu-1 QS 10			54.6	6.3	53.8	62	1610
Mooni	2*/7+8&7+9/2+12&5+10	8.5	56.0	3.1	49.0	38	1430
Voore	2*/7+8&7+9/2+12&5+10	8.5	51.7	6.1	49.5	61	1605
Means of Glu-1 QS 8.5			53.9	4.6	49.3	50	1518
373.1	1/7/5+10	8	53.9	4.9	47.5	53	1460
392	1&2*/7+9/5+10	8	52.8	4.7	47.0	66	1440
450-2	2*/7+8/2+12	8	54.4	3.0	44.5	21	1540
453	1&2*/7+9/2+12&5+10	8	52.0	3.4	44.0	54	1510
Means of Glu-1 QS 8			53.3	4,0	45.8	49	1488
721	2*/7+8&7+9/2+12	7.5	51.8	3.8	48.5	36	1600

values of some traits: cv. Mooni WA, cv. Voore DS, GI and LV. Low values were detected mainly in breeding lines with *Glu-1* QS 8, particularly line 453 showed the lowest values of WA, VV, line 450-2 DS, VV and GI in different years. Line 721 with *Glu-1* QS 7,5 had one of lowest WA values on the average of two years, and low GI. However, LF of line 721 was quite high and close to standard: 1500 cm^3 in 2015 (cv. Manu 1560 cm^3), 1700 in 2016 (cv. Manu 1780), and on the average (Table 1). On the contrary, cultivars with higher *Glu-1* QS (8.5) had the lowest values of WA (Voore), DS and LF (Mooni). GI value of cv. Mooni was also well below optimum (60 – 90).

We demonstrate that mean values of investigated quality parameters tend to decrease with the decrease of *Glu-1* QS. However, there are exceptions, such as breeding line 721 with *Glu-1* QS 7.5, which had VV and LF higher than lines with *Glu-1* QS 8.

Despite baking quality is being influenced also by factors that were not discussed in current research, such as weather conditions, used agricultural practice, markers of HMW-GS alleles and *Glu-1* QS can be used as one of preliminary criterion for prediction bread-making quality of wheat varieties in conjunction with experimental parameters.

Results of wheat breeding in Latvia

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Key words: varieties, quality, yield, wheat.

The aim of the current wheat breeding is creating and introducing into commercial production varieties adapted both for the conventional and the organic management. The main goal of Latvian wheat breeders is to create the new varieties, suitable for the Baltic climatically conditions; high yielding, resistant to lodging and diseases, with grain quality appropriate for producers.

In Latvia the beginning of wheat breeding was in 1922. Since this the large-scale of wheat germplasm was collected and applied for hybridization and selection. In each breeding program specific collection of winter and spring wheat lines and varieties suitable for local conditions were used. Several wheat varieties were created by traditional breeding method: hybridization and subsequent selection. Since 2000 collaboration with the Plant Genetics Laboratory of the Institute of Biology, University of Latvia allowed the application of biotechnology methods in wheat breeding programs.

Varieties 'Fredis' and 'Edvins' developed at Stende are popular winter wheat in all Baltic States. There are registered in Latvian, Estonian and Lithuanian Catalogue of Plant Varieties. Also spring wheat 'Uffo' and 'Robijs' (result of collaboration with the Plant Genetics Laboratory of the Institute of Biology, University of Latvia) are grown in Latvia and Estonia even now.

The newest winter wheat varieties registered in Latvian Catalogue of Plant Varieties are "Talsis" (registered 2015) and 'Brencis' (registered 2018). The new winter wheat hybrid line 94-5 –N ('Reinis') most suitable for organic farming is under the DUS and VCU test now.

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Meta-analysis of male tumour karyotypes points towards an association between extra X-chromosome acquisition and para-triploidy

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Key words: cancer, karyotype, Mitelman database, sex chromosomes, triploidy.

Tumour aggression is often associated with a para-triploid karyotype (62-76 chromosomes). However, the origin of this para-triploidy still remains unclear. Bioinformatical meta-analysis of 11 cancer types (of which two were germ cell tumours and nine somatic), comprising a total of 1754 karyotypes, was conducted using male cancer patient data from the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. The most notable features were the karyotypes' heterogeneity (varying mostly between 2n and 4n) and acquisition of an extra X-chromosome. For both germ cell and somatic tumours, a high positive correlation between extra X-chromosome acquisition (XXY or XX,-Y karyotype) and para-triploidy of the whole karyotype was revealed. Moreover, the acquisition of an extra X-chromosome had a strong negative correlation with hyperdiploidy. The proportion of XXY and XX,-Y triploid karyotypes was particularly high in germ cell tumours. We conclude that the acquisition of the X-chromosome in male tumour karyotypes is primarily associated with whole-genome rearrangements („meio-mitosis"). However, the results also point towards a combination of whole-genome alterations and chromosomal rearrangements (which are more pronounced in somatic cancers) that likely favour beneficial chromosome gains and losses by clonal selection during mutagenic tumour progression. Moreover, according to the ergodic theorem, the summary karyotype histogram of any given tumour type (if sampled from a large number of cases) might reflect, in fact, a complex process of reproduction (including vertical, horizontal, and reciprocal steps of genetic transfer), the details of which should be further researched.

Developing of DNA-based human eye and hair color prediction model for forensic proposes in Belarus

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Key words: DNA-phenotyping, eye and hair color, forensic.

Forensic DNA-phenotyping refers to the prediction of human physical appearance, especially eye, hair and skin color prognosis. Genome-wide association study detected candidate genes for eye and hair color. Among several DNA-based phenotype predictive tools, SNapShot and HIrisPlex were successfully validated by numerous studies. This tools could be applied not only for forensic purposes but also for genogeography, population dynamic studies etc.

This research is conducted for the purposes of scientific and technical program of Commonwealth of Independent States «Development of innovative genogeographic and genomic technologies for identifying an individual and personality traits on the basis of studying the gene pools of the regions of the Union State» (DNA-identification), 2017-2021. Project «Development of the technology for identifying of visible characters of unknown individuals based on DNA-features».

In this study, we have collected of phenotyping data and DNA samples at least 500 individuals of Belarusian population. Now we try to reflect the spectrum of phenotypic variability of our citizens. Native Belarusians belong to the Central European type of the Europeoid race. In the population mixed shades of hair and eyes predominate (dark-blond, light and dark-brown hair, gray or green eyes). Among Belarusians of the north-east region of the country, which is located closer to the distribution area of the North-European race, a light pigmented population is more common. The inhabitants of Polesye (south region) often have darker eyes and darker hair.

Each DNA sample from our collection is accompanied of individual questionnaire, eye photo and a hair strings scan. To now, we worked out on the test-system of individuals phenotyping. For initial digitization, we used: (i) ultra-close high-resolution shots (Canon 750d completed by macrocamera and circular flash-light, with manual standard of exposition and lighting); (ii) color-fundus photography; (iii) digital scanning for individual hair strings.

All images were run in Matlab script R2018a as described by Wollstein et al. (2016). The original RGB image (5184 × 3456 pixels) was translated into the HSV model. To identify the three main segments (unpigmented section, pheomelanin and eumelanin) digital standards were selected. For quantitative pigmentation phenotyping Matlab (script R2018a) automatically segmented iris image and measure of the digital equivalents of pigmented and unpigmented area under research.

Actually, our database consists of 500 individual eye and hair digital images, questionnaires and DNA samples. We intend to enlarge the number of test individual up to 1000. For further genotyping, we suppose to use well-established tools like HIrisPlex and «Human phenotyping» NGS panels.

Marker-assisted identification of the sources of PVY-resistance genes in the initial material originated from interspecific potato hybrids

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Key words: extreme resistance genes (ER-genes), marker-assisted selection (MAS), potato, PVY-resistance.

Potato virus Y (PVY) is one of the most devastating potato pathogen causing great yield losses and worsening its quality and the quality of seed tubers. The most promising approach for preventing virus diseases is use of potato varieties carrying genes of extreme resistance (ER) to viruses that were introgressed from wild and cultivated related species. Application in breeding of DNA-markers of dominant genes of ER to PVY, opens novel possibilities in improving efficacy of breeding. For example, marker assisted selection (MAS) makes it possible to scan the collections for the sources of certain resistance genes and to trace their introgression into breeding material. It simplifies the determination of allelic status of the resistance genes in parental lines and to identify multiplex ones which use in crosses raises the efficiency of selection on corresponding characters. In addition, identification of DNA-markers is less laborious and expansive than phenotypic assessment of the hybrids for PVY-resistance and can be applied in any season of the year.

The aim of our research was screening the collection of breeding material produced in Research and Practical Centre of Potato Growing for DNA-markers of four genes of ER to PVY. The collection contained more than three hundred tetraploid clones originated from interspecific hybrids of some potato species, in particular, *Solanum tuberosum* subsp. *andigenum*, *Solanum stoloniferum* and *Solanum chacoense*.

As it was expected, a lot of genotypes carried the marker RYSC3 used for identification of the gene *Ryadg* (from *S. tuberosum* subsp. *andigenum*). It was revealed in 68.7% of the collection clones. The frequency of the marker Yes-3-3A of the gene *Rysto* (from *S. stoloniferum*) was found to be even higher – 70.5%. It testifies to the appreciated value of this material for breeding varieties with ER to PVY. The gene *Rysto* is considered to be effective against all currently known PVY strains, including highly pathogenic necrotic strain PVY^{TNT}. The markers RAPD38-530 and Ry-364 used for the identification of the *Rychc* (from *S. chacoense*) also had good presentation in the collection – 55.8 and 42.3%, respectively. However, both these markers of the gene were present in only 21.8% of collection clones that indicated with high probability on the availability of full-size dominant allele of the gene *Rychc* in these clones. All the four markers of the three studied resistance genes were found in 36 genotypes (11.3%). In addition, 20 clones combined markers of genes *Ryadg* and *Rysto* with at least one of the markers of the gene *Rychc*. We were not able to reveal any genotype with the marker $GP122/EcoRV_{780}$ of the gene *Ryf-sto* (from *S. stoloniferum*) as well as with marker R-186 of the genes *Rychc* (from *S. chacoense*) and *Ny* (from *Solanum tuberosum* subsp. *tuberosum*).

Analysis of segregation on markers in populations of hybrids of some of the above mentioned clones has shown that they contained, as a rule, the corresponding genes in simplex condition. Nevertheless, we had revealed one parental clone with duplex of *Ryadg* (according to analysis of two cross combinations), as well as two clones with duplex on *Rysto* or *Rychc*.

Evaluation of gene networks formed by prescence of similar transposable elements in gene flanking regions and introns

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Key words: gene networks, gene regulation, retrotransposons, transposable elements.

Plant genomes contain a diverse and widespread fraction of mobile genetic elements or transposable elements (TEs). Rapid transcription and also transposition of TEs is induced by stress conditions. Elevated frequencies of TE-induced mutations in plant genomes can result in rapid genotype variation and may reflect an adaptive mechanism to changing environmental conditions. TE insertions in gene regions could form regulative gene networks as TEs contain Transcription Factor Binding Sites and other cis-acting regulatory elements. TEs have been reported to influence genes in various ways by disrupting gene sequences, changing gene expression, introducing new regulatory motifs by insertion into promoter regions, facilitating gene duplication and sub-functionalization, induction of gene function and stress sensitivity, epigenetic silencing by initiation of antisense gene transcription etc. Current investigation is focused on identification of *Pinus taeda* genes associated with TE-derived structural variations and their possible influence on gene expression and networking.

The conifer TE database PIER v.2.0. (Pine Interspersed Element Resource) was used to identify TEs in gene introns and flanking regions (0-5kb, 5'and 3') in reference genomes (*P .taeda* v. 2.0. and v. 1.0, *P. lambertiana* v. 1.01.). Less than 50% of the *P. taeda* genes (15534 of 36730) could be categorized to Gene Ontology terms. BINGO v. 3.0.3 (Maere et al. 2005) was used for overrepresentation tests of GO categories using custom annotation. Cytoscape v.3.3.0. (Smoot et al. 2011) was used for gene network visualisation. Comparison of the two versions of *P. taeda* genome revealed differences in TE representation that are due to technical issues of genome sequencing and assembly methods, TE database quality and the properties of TEs in conifer genomes. To overcome these problems, shorter TE fragments were used in further analysis, rather than full length TE sequences. This strategy allowed us to identify families of repeats that are distributed in gene flanking regions or introns; however, the structure of the identified TEs should be further investigated.

An unequal distribution of repeats was found in gene flanking regions with significant enrichment of TEs in 0 – 1 kB gene flanking regions. Forty repeats were enriched in the 0 – 1 kB region compared to similar regions up to 5 kB from genes with a high level of significance ($p = 0.001$), and 29 REs were enriched with a lower significance level ($p = 0.05$), two-tailed *t*-test. It was estimated that about 14 to 17% of all genes contain TE insertions (from enriched families) in its 0 – 1 kB flanking regions. TE distribution in gene introns revealed that approximately 11 to 26% (with different significance level) of all *P. taeda* genes contain TEs, that are present at least in 100 other gene introns. GO analysis of proteins encoded with these genes and presence of functional motifs in TEs could help to distinguish TE candidates that could be involved in network formation. GO network analysis of genes with similar TE families in their vicinity revealed some gene groups that could be advantageous in response to stress conditions. Comparison of TE sequences enriched in gene flanking regions with those found in introns revealed that only two common repeat families were distributed in both regions with hits to more than 100 genes. Different genes contained similar TEs in flank regions or in introns, but these genes were involved in similar processes. For example PtRLG_885 was found in the introns (200 genes) and flank regions (354 genes) of genes involved in stress response: cell wall biogenesis, auxin mediated signalling pathway, immune response, transmembrane transport etc. PtRLG_885 contains a W-box motif, which is often found in plant stress-inducible promoters and is recognized by WRKY-family transcription factors, which can result in coordinated expression of genes with this motif in their vicinity.

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Polymorphism of *Anthocyanin 1* gene orthologs in the Solanaceae family

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Key words: anthocyanin accumulation, eggplant, DNA-markers, pepper, tomato.

Anthocyanins are high value plant antioxidants, which not only give specific coloration to fruits and seeds, but also determine biotic and abiotic stress resistance. This work is devoted to the study of the polymorphism of alleles affecting flavonoid accumulation in fruits and vegetative parts of plants in Solanaceae species (tomato, pepper and eggplant).

The research was carried out on tomato and pepper collection samples from the Institute of Genetics and Cytology of the National Academy of Sciences of Belarus and the Belarusian State Academy of Agriculture, as well as eggplant varieties from the N. I. Vavilov All-Russian Institute of Plant Genetic Resources.

It is known that the tomato gene *Antocyanin 1* (*Ant1*) encodes a *Myb* transcription factor directly regulating flavonoid accumulation in fruits. We successfully tested the CAPS marker Ant1-NcoI for the detection of the allele Ant1C (mRNA EF433417) (Patent WO 2008/096354 A2, I. Levin et al.) forming the Aft (Anthocyanin fruit tomato) phenotype with a characteristic coloration of the vegetative mass and dark blue coloring of fruits.

The search for orthologic genes to the tomato allele *Ant1* (EF433416) in the GeneBank database revealed the following sequences closest by the nucleotide sequence: myb113-like transcription factors in *Capsicum annuum* (mRNA XM_016689227, mRNA NM_001324618) and the myb1 transcription factor in *Solanum melongena* (DNA KT27965 or mRNA KF72747 or mRNA KT259043). The similarity level in homologous regions was 80 to 90%. Based on the Solgenomics database on the genomic DNA structure of *C. annuum* (Pepper1.55ch10) and *S. melongena* (Sme2.5_05099.1), the primers completely overlapping the exons of the above genes were selected. Using these primers in PCR on genomic DNA, amplicons were obtained and then sequenced in pepper and eggplant collection samples with a contrasting anthocyanin fruit coloration.

Comparative analysis of *C. annuum* sequences under study allowed to identify the following polymorphism: 4 SNP and single nucleotide deletion in the 3rd exon of myb113-like factor (XM_016689227) (in Belosnezhka, L160-10 varieties with a white coloration of fruits without anthocyanins' accumulation) and 2 SNP in the 4th exon of myb113-like factor (NM_001324618). This 1Indel leads to a shift in the reading frame and the truncated protein synthesis. The CAPS marker (Myb 113-AccI) was developed to detect deletion in varieties without anthocyanin accumulation in fruits (Belosnezhka, L-160-10) and successfully tested on our pepper collection. However, in one of the forms with a white fruit coloration (WF4-18), the DNA sequences completely coincided with XM_016689227 and NM_001324618. No anthocyanin accumulation in WF4-18 is probably associated with polymorphisms in other genes.

Comparative analysis of *myb1* sequences in *S. melongena* collection forms revealed varying polymorphism: the deletion (6 bp) at the end of exon 1 leading to the loss of two amino acids in the protein (in Zelenenkiy variety characterized by the absence of anthocyanin coloration in vegetative organs and green fruits), or the longer deletion (26 bp) at the end of intron 1, the beginning of exon 2 and 11 SNP (Snezhnyj and Pelikan varieties characterized by the absence of anthocyanin coloration in vegetative organs and white fruits). Most likely, the deletion (26 bp) leads to disturbances during the mRNA maturation and impossibility of functional protein synthesis. The effect of this deletion on mRNA splicing requires further study. Based on the identified polymorphism, the SCAR marker MybMel and the CAPS marker Mybmel-Pst1 were developed and tested to detect deletions of 26 bp and 6 bp, respectively, in different eggplant varieties. These markers allow selecting eggplant forms with no anthocyanin accumulation in fruits.

Selection samples of tomato, sweet pepper and eggplant with different alleles associated with anthocyanin accumulation were chosen for further study of genetic polymorphism associated with pigment accumulation in fruits and to develop appreciable varieties for agriculture.

Marker-assisted parental line breeding of potatoes at the diploid level

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Key words: MAS, parental lines, potato, selection at the diploid level.

The use in crosses of specially bred parental lines makes it possible to greatly improve the efficacy of potato breeding due to production of more uniform hybrid populations not segregating into the complex of useful characters. It raises the probability of selection of valuable genotypes. In our laboratory we conduct the work on breeding the following kinds of parental lines: tetraploid lines (mitotically duplicated dihaploids) with a good combining ability on yield characters, having a set of genes of disease and pest resistance in duplex or quadruplex states; diploid lines that form fertile FDR 2n pollen, have a good combining ability on yield characters and a set of resistance genes.

As a result of a long term research we were able to solve the main problems of diploid potato breeding: sterility or low male fertility of *Solanum tuberosum* dihaploids and low frequency of 2n gamete formation. The initial material necessary for the reliable marker assisted selection (MAS) at the diploid level has been developed. It includes:

(i) Collection of initial dihaploids of potato varieties selected for high viability, tuber productivity and a set of DNA-markers of resistance genes. These dihaploids are being used in a breeding program as the donors of characters of cultivar appearance, high productivity and known pest resistance genes.

(ii) The diploid lines that can serve as donors of genes of high male fertility. These lines carry the genes favorable for pollen fertility. They can act in homozygous, as well as in heterozygous states. Use of these lines in crosses with initial dihaploids made it possible to produce fertile hybrids suitable for crosses at the diploid level. Hybridization between such hybrids (initial dihaploid × donor of fertility) produces the diploid hybrid populations, which are suitable for selection of highly fertile genotypes.

(iii) The diploid lines that are donors of genes of male fertile FDR 2n-gamete formation. They were produced by crossing between the lines-donors of fertility and the lines bred for high frequency of FDR 2n-gamete formation. Hybridization between initial dihaploid × donor of fertile 2n gametes hybrids gives rise to diploid hybrid populations, in which it is easy to select genotypes, producing fertile FDR 2n gametes with high frequency (i.e. suitable for crossing to tetraploid varieties).

(iv) Diploid breeding material with a wide range of late blight resistance genes and genes of resistance to viruses. This material originated from different interspecific hybrids produced in our laboratory as a result of our own innovative methods of overcoming interspecific reproductive barriers. In particular, it contains the resistance genes introduced from 1 EBN diploid potato species *Solanum bulbocastanum, Solanum pinnatisectum, Solanum polyadenium, Solanum commersonii*, as well as from 2 EBN allotetraploid species *Solanum stoloniferum, Solanum fendleri, Solanum polytrichon* and *Solanum acaule* that are rarely used in breeding because of the lack of crossability with cultivated potatoes.

As a result of MAS at the diploid level, diploid hybrids were produced that have cultivar phenotype, set of resistance genes, male fertility, ability to form 2n pollen with high frequency. Some of them produce equal or better yield than standard potato varieties. The best of them have been mitotically doubled. Produced tetraploid lines, as well diploid lines forming fertile 2n pollen, had been crossed to tetraploid testers (potato varieties) to estimate their combining ability.

The diploid initial material is of interest for promising directions of breeding potatoes: breeding diploid potato varieties as well as parental lines for production of hybrids and hybrid populations for true potato seed technology.

Level of methylation of promoter of the *petE* gene is not related to formation of green plants *in vitro*

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Key words: barley, DNA methylation level, doubled haploids.

The low production of green plants-regenerants is one of the problems of obtaining doubled haploids from cereals (wheat, barley): the number of albino plants is significantly higher than the number of green plants-regenerants. The presence of copper ions (Cu) in the media during *in vitro* cultivation promotes formation of green plants in barley anther culture, nevertheless still proportion of albino plants-regenerants is much higher. The process of photosynthesis is closely related to the Cu ion-containing protein plastocyanine, which is responsible for the transfer of electrons from cytochrome-f to the reaction centre P700. Synthesis of the plasticyanine is controlled by nuclear gene *petE*. DNA methylation is one of the mechanisms of gene regulation, therefore the methylation of CpG regions in the gene promoter sequences may cause blocking of the gene transcription. It can be hypothesized that even if Cu ions necessary for the synthesis of plasticyanine are available in the medium, transcription of the gene and, as result, synthesis of the protein *in vitro* conditions is blocked by the methylation of the *petE* gene promoter, what, finally, manifested in developing albino plants.

Two barley (*Hordeum vulgare* L.) doubled haploid lines $6IL_3$ ('Primus' × 'Anni') and 7Ip10109 ('Abava' × 'Aura') with previously established contrasting genotypic response to embryogenesis were selected for experiment: $6IL_3$ ('Primus' × 'Anni') – high responsive, and 7Ip10109 ('Abava' × 'Aura') – non-responsive. For intact plants level of methylation in the region of the promoter of the *petE* gene was determined in germinated mature embryos, as well in etiolated and green seedlings of both genotypes. In anther culture the level of methylation was determined for pollens, induced embryos and for albino plants-regenerants of genotype 6IL3. For bisulfide conversion of the DNA of samples Qiagen EpiTect® Plus Bisulfite Conversion Kit was used, followed by DNA purification with the Qiagen EpiTect Plus DNA Bisulite Kit. Qiagen PyroMark® PRC Kit and Qiagen PyroMark® Q24 Advanced CpG Reagent was used for analysing level of methylation. All reactions were made according to the manufacturer's protocol. Used PCR primer sequences were: forward primer <ATGGAGAGGAGGTAGTTAAT>, reverse primer <TACTACCTACTTAAATTTATACCCA TCAA>, as biotinylated primer was used reverse primer, sequencing primer sequence was <AGTTAGGTTATA GTTTATTTTTAT>.

The level of methylation of petE gene promoter region of the line 6IL3 varied in embryos in range 1 to 12%, in etiolated seedlings 0 to 18%, in green seedlings 1 to 14%; in the line 7Ip10109 methylation varied in embryos in range 0 to 17%, in etiolated seedlings 0 to 13%, in green seedlings 1 to 14% . The level of methylation in the pollen of the genotype 6IL3 ranged 1 to 27%, in embryos 1 to 62%, in plant-regenerants 2 to 30%. Obtained results shown that the methylation level of the *petE* gene promoter region of embryos, etiolated and green seedlings is similar for both genotypes. After anther culture some increase of variation of methylation level was observed but it was not statistically significant. Most probably, the level of methylation of promoter of the *petE* gene is not related to formation of green plants *in vitro*.

Is it sufficient with direct sequencing of *ATP7B* gene coding regions to diagnose Wilson disease?

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Key words: *ATP7B* gene, copper metabolism, Wilson disease.

Wilson disease (WD) is an autosomal recessive disorder of hepatocellular copper metabolism caused by homozygous or compound heterozygous pathogenic variants in copper-transporting gene *ATP7B*. More than 700 pathogenic variants in *ATP7B* have been identified, with single-nucleotide missense and nonsense variants as the most common, followed by insertions/deletions, and, rarely, splice site allelic variants. Pathogenic variants in the *ATP7B* gene have been reported in all exons. The c.3207C>A; p.H1069Q variant is one of the most common allelic variants among Caucasians, it is found in 30 to 70% of WD alleles. Other rare genetic mechanisms include whole-exon deletions, pathogenic variants in promoter region, and uniparental disomy.

WD shows very wide spectrum in age of onset and clinical presentation, which in combination with allelic heterogeneity makes both – clinical and molecular diagnostics of WD – challenging. Direct sequencing of the *ATP7B* gene is the most sensitive and widely used confirmatory testing method, and concurrent biochemical testing improves diagnostic accuracy. For large gene rearrangements other methods like MLPA (Multiplex Ligation-dependent Probe Amplification) are used, that could improve the pathogenic causative variant detection rate.

Study included 66 unrelated patients with clinically confirmed Wilson disease (all patients had at least four points according to the WD scoring system). The most common allelic variant p.H1069Q was tested by PCR-BiPASA. All of patients confirmed to be homozygous for this variant were excluded for the further investigation, which included direct DNA sequencing of coding parts in the gene *ATP7B* (all 21 exons). Afterwards, patients with clinically confirmed or suspicious WD and only one or none identified variant in the gene *ATP7B* underwent sequencing of the promoter region in the gene *ATP7B* and the last step involved MLPA for the patients with unidentified *ATP7B* pathogenic variants. Results of MLPA were validated by quantitative PCR (qPCR).

Wilson disease was genetically confirmed in 51 patients: 38 patients were homozygous for the most common variant p.H1069Q, 13 patients were compound-heterozygous, six patients were heterozygous for a pathogenic variant, but nine patients did not have any pathogenic variants in coding parts of gene *ATP7B*. In total 81.82% of WD patient alleles were identified, 18.18% of the alleles remained unidentified. In promoter region of *ATP7B* gene six benign variants were found: rs28362532, rs9563084, rs145371060, rs74962976, rs1055659322 and rs1386687836. By MLPA the data in two patients were suspicious about two exon duplications, one for exons 16 and 18, the second for exons 14 and 19. Further investigation by qPCR did not confirm the duplications.

Adding the direct sequencing of *ATP7B* gene promoter region and MLPA, followed by qPCR, to the diagnostics of WD did not increase the number of identified pathogenic variants in WD patients, suggesting that pathogenic variants in the promoter region are not very common among Latvian WD patients, and for daily molecular diagnostics direct sequencing of *ATP7B* gene coding parts is sufficient. MLPA method can give false positive results, making validation of MLPA results by other method (e.g. qPCR) essential.

Changes of leukocyte mtDNA copy number in glaucoma patients and different age groups of healthy individuals

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Key words: ageing, glaucoma, mitochondrial DNA, PBMC, WBC.

Glaucoma is the most common cause for irreversible blindness worldwide. Glaucoma is a chronic neurodegenerative disease characterized by the progressive loss of retinal ganglion cells (RGCs), and the risk for glaucoma increases with age. Mitochondrial DNA (mtDNA) alterations have been associated with many neurodegenerative disorders and aging. There is a study showing reduction of an expression of mtDNA repair/replication enzymes and decrease of mtDNA copy number (CN) in the RGCs of Wistar rats.

The aim of this study was to investigate changes of mtDNA amount in glaucoma patients compared to healthy individuals.

Twenty six healthy young (average age 29) and 26 healthy elderly (average age 65) volunteers, as well as 18 glaucoma patients (average age 72) participated in this study. Peripheral blood mononuclear cells (PBMC) were obtained from 5 mL blood by using ACCUSPIN System-HISTOPAQUE-1077 tubes. Genomic DNA was extracted simultaneously from the PBMC and WBC (whole blood cells) samples using the phenol–chloroform method. The relative mtDNA CN was measured simultaneously in all samples using qPCR with the Maxima Probe qPCR Master Mix.

In healthy individuals mtDNA amount in PBMC was significantly greater than in WBC ($P < 0.0001$); in PBMC mtDNA amount increased for the elderly age group ($P = 0.0088$) but in WBC did not ($P = 0.1201$).

Glaucoma patients had significantly less mtDNA CN both PBMC and WBC samples comparing with the healthy elderly volunteers ($P < 0.0001$ and $P < 0.0001$, respectively). Glaucoma patients also did not have any difference between mtDNA CN in PBMC and WBC sample types ($P = 0.9437$), as it was within the healthy control group ($P < 0.0001$), meaning that there could be some disturbance in mtDNA CN maintenance or replication of mtDNA in these patients' blood cells especially in PBMC cells.

No statistically significant correlation of mtDNA CN between PBMC and WBC was observed in healthy individuals (Pearson $r = 0.03743$, $P = 0.7923$) and in glaucoma patients (Pearson $r = –0.2124$, $P = 0.3975$).

It is clear that in glaucoma patients leucocyte mtDNA amount was reduced both in PBMC and WBC compared to healthy individuals, and it seems like aging processes may affect PBMC and WBC cell types differently. While in healthy individuals mtDNA amount in PBMC increased at greater age, possibly because of some compensating mechanisms or loss of mitophagy, those mechanisms do not work in glaucoma patients leading to massive mtDNA reduction in PBMC.

Genomic characterization of novel Bacillaceae bacteriophage Mimir87

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Key words: DNA bacteriophages, genomics, *in silico, Virgibacillus halotolerans*.

We would like to present completely sequenced and annotated genome of yet unpublished and locally isolated Bacillaceae phage, named by us as Mimir87 from the Siphoviridae family along with its morphological and microbiological characterization.

BLASTn (Altschul et al.) analysis has shown that Mimir87 shares a very little sequence similarity to other objects available in the GenBank (highest query cover being 6% as of September 2018).

16S rRNA sequencing of the host bacteria revealed it to be a strain of *Virgibacillus halotolerans* (Seiler, Wenning 2013), which makes Mimir87 a first known *Virgibacillus* phage being sequenced and annotated up to date.

By *in silico* (Garneau et al. 2017) analysis and RFLP experiments (Karlene et al. 2012) we found that Mimir87 has 3' cos physical ends at its 48007bp long DNA molecule. As it was expected, Mimir87 genome is composed of both "core" and "accessory" (Cazares et al. 2014) elements and many of the ORFs found encode hypothetical proteins. In total, 67 open reading frames have been identified in the Mimir87 genome.

Novelty of the studied phage makes it hard to plausibly predict functions for nearly half of the Mimir87 encoded gene products. Furthermore, analysis of putative functions for some of the products revealed extraordinary features of genomic composition of bacteriophage Mimir87.

Some of Mimir87 genes encode proteins that contain evolutionary conserved domains with yet unknown function (DUFs) which opens new possibilities for further structural and functional studies using this particular object in the future.

At present, six bacteriophage Mimir87 genes are considered to be of interest for further studies of gene products.

Therefore, Mimir87 is an another example adding to the big diversity of bacteriophages [2200 complete bacteria virus genomes at NCBI database as of 25th of September 2018 (NCBI 2018)].

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Performance of faba bean (*Vicia faba*) varieties in Latvia

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Key words: composition, faba beans, varieties, yield.

The European Union (EU), including Latvia, recently stressed the importance of increasing its own production of grain legume crops. Two main reasons were outlined by researchers and officials. The first reason is the necessity to reduce EU's dependency on soybean meal imports, mainly from the United States; as well as due to the soybean meal became more expensive and in most cases is genetically modified. This has forced the farmers in animal nutrition to find and use alternative sources of vegetable protein on a larger scale, which could be a valuable tool in lowering feed cost of animal feeds. The second is to reduce negative environmental impacts associated with intensive crop in particular cereal production. The crops, which accumulate the greatest amount of protein are legumes, because in symbiosis with bacteria they binding atmospheric nitrogen. Accordingly, legumes do not require intensive nitrogen fertilization, and could be successfully cultivated in conventional or organic farming systems. Besides, faba bean as an important representative of legumes, helps to reduce phytosanitary development of crop diseases, and improves the physical and chemical properties of soil, as well as promoting the development of desirable soil microorganisms.

In Latvia, in recent years, the sowing areas of legumes have increased rapidly, especially for faba bean, which are cultivated in more than 42 000 hectares. The identification of faba bean's varieties or cultivars, which are most appropriate and suitable for particular region and its agro-climatic conditions, are an important topic for legumes cultivation and breeding.

The aim of the study was to determine the effect of faba bean (*Vicia faba* L.) varieties and the impact of climate conditions (production year) on faba bean seeds yield and chemical composition (content of crude protein and crude fibre).

The five-year field trials with 17 faba bean varieties were conducted at the Institute of Agricultural Resources and Economics (Stende Research Centre). For assessment of faba bean varieties performance or its variability, nine varieties or cultivars and the data (yield, content of crude protein and crude fiber) of the last three years (2015 to 2017) was chosen. The varieties "Boba", "Boxer", "Fuego", "Granits", "Isabella", "Laura", "Lielplatone", "Olga" and "Vertigo" were selected for assessment, because these varieties were the object of field trials during all three years.

The comparisons between varieties and years were performed, measuring the mean values by one-way analysis of variance (ANOVA) from SPSS. The Scheffe's criterion or test was used to compare the pairs, which corrects alpha for all pair-wise or simple comparisons of means, but also for all complex comparisons of means as well. Complex comparisons involve contrasts of more than two means at a time.

The results show that the yield variability among faba beans varieties is the smallest and is not significant during a three year period. The climatic conditions have the least effect on the content of crude protein for the variety "Lielplatone". In addition, it retains the highest percentage. However, the variety "Olga" has the greatest changes of crude protein content. The results show that during three years the amount of crude fiber is the least variable for the "Lauma" variety, which has the highest content of fiber.

Population structure and genetic diversity of the non-native plant species *Bunias orientalis* from the Baltic Countries

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Invasive alien species play detrimental impact on indigenous biota changing natural and cultivated ecosystems. Alien species can also alter the structure and species composition of ecosystems by reducing or eliminating native species. Genetic studies of biological invasions may help to reveal the causes and consequences of this process and develop effective control measures. We studied genetic variability within and among populations of warty cabbage (*Bunias orientalis* L.), which is an alien species in Lithuania and other neighbor countries. This perennial cruciferous plant is geographically widespread: nonnative populations are found in large parts of Europe and Asia.

The genetic variability and differentiation among 698 samples from 47 populations collected in three Baltic countries were accessed using inter-simple sequence repeat (ISSR) markers. A total of 64 polymorphic loci were detected. Our analysis revealed structured populations (AMOVA $F_{ST} = 0.35$; $P = 0.001$) in the Eastern Baltic region. A hierarchical AMOVA revealed 12% genetic variation between countries and 38% between populations within countries. An equal proportion of the genetic variation occurred within populations (50%). AMOVA revealed the highest percentage of between-population variation in Lithuania (48%) and the lowest in Latvia (38%). For the Estonian populations this value was 43%. Population structure was also analyzed using a Bayesian clustering approach, which was implemented in the program STRUCTURE. We identified several main groups of *B. orientalis* genotypes in the Eastern Baltic region.

No isolation by distance between populations was detected within each of the tested countries, and these results together indicate that there is no correlation between genetic and geographical distances at the scale of this study. Analysis of population genetic structure of *B. orientalis* in the Baltic countries indicates human impact on spreading of this species and multiple sources of propagules for non-native populations.

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