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Книга содержит материалы 2-й международной конференции "Генетика, геномика и биотехнология растений", состоявшейся 30 июля – 3 августа 2012 г. в г. Иркутске. Конференция была организована Сибирским институтом физиологии и биохимии растений СО РАН, Институтом цитологии и генетики СО РАН, Сибирским отделением Российской академии наук, Вавиловским обществом генетиков и селекционеров, Иркутским научным центром СО РАН и Обществом физиологов растений России. На конференции были представлены результаты новейших исследований в области генетики, геномики и биотехнологии растений, обсуждались вопросы организации и эволюции генома растений, физиологической и экологической генетики растений, генетики и селекции в изменяющихся условиях окружающей среды, генетической инженерии, хромосомной и клеточной биотехнологии.
Для генетиков, селекционеров, молекулярных биологов, биохимиков и физиологов растений, экологов, а также для студентов и аспирантов биологических специальностей высших учебных заведений.

The book of proceedings contains abstracts of the 2nd International Conference "Plant Genetics, Genomics, and Biotechnology" held on July 30 – August 3, 2012 in Irkutsk, Russia. The Conference has been organized by Siberian Institute of Plant Physiology and Biochemistry SB RAS, Institute of Cytology and Genetics SB RAS, Siberian Branch of the Russian Academy of Sciences, Vavilov Society of Geneticists and Breeders, Irkutsk Scientific Center SB RAS, Russian Society of Plant Physiologists. The state-of-the-art results in the field of plant genetics, genomics and biotechnology were presented, the problems of plant genome organization and evolution, plant physiological and ecological genetics, genetics and breeding in a changing environment, problems of genetic engineering, chromosome and cell biotechnology were discussed.
The target audience for the proceedings may include geneticists, breeders, molecular biologists, plant physiologists and biochemists, ecologists, and also postgraduate students and students of biological specialties.

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COMPUTER APPROACHES TO WHEAT HIGH-THROUGHPUT PHENOTYPING

***D. Afonnikov*[1,2]*, *M. Genaev*[1], *A. Doroshkov*[1], *V. Denisyuk*[2], *E. Morozova*[1], *A. Simonov*[1], *T. Pshenichnikova*[1]

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The growing need for rapid and accurate approaches for large-scale assessment of phenotypic characters in plants becomes more and more obvious in the studies looking into relationships between genotype and phenotype. This need is due to the advent of high throughput methods for analysis of genomes. Nowadays, any genetic experiment involves data on thousands and dozens of thousands of plants. Traditional ways of assessing most phenotypic characteristics (those with reliance on the eye, the touch, the ruler) are little effective on samples of such sizes. Modern approaches seek to take advantage of automated phenotyping, which warrants a much more rapid data acquisition, higher accuracy of the assessment of phenotypic features, measurement of new parameters of these features and exclusion of human subjectivity from the process. Additionally, automation allows measurement data to be rapidly loaded into computer databases, which reduces data processing time.

In this work, we present the WheatPGE information system designed to solve the problem of integration of genotypic and phenotypic data and parameters of the environment, as well as to analyze the relationships between the genotype and phenotype in wheat. The system is used to consolidate miscellaneous data on a plant for storing and processing various morphological traits and genotypes of wheat plants as well as data on various environmental factors. The system is available at www.wheatdb.org. Its potential in genetic experiments has been demonstrated in high-throughput phenotyping of wheat leaf pubescence.

The work is supported by State Contract No. 07.514.11.4052, RFBR grant 11-04-91397.

IMPROVEMENT OF TOMATO SPOROPHYTE SELECTION FOR SPECIAL HYDROPONIC CONDITIONS

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Gamete selection technologies are widely applied in breeding of adaptive forms. Effect of gamete and zygote selection is conditioned by correlation links between genes expression on the stage of gamete/zygote and genes expression on the stage of sporophyte. Establishment of such links is the first and necessary step of gamete selection. Division of gametophyte and sporophyte cycles in the evolution of *Eucarpia* ensured significant adaptive advantages for higher plants. But this division conditioned limitations related with separate selection of gametes. And if the direct microspores screening at regulated environment conditions is usual, zygote selection at the same conditions is very difficult. We think that the special selection of female plants could be used for this purpose. The idea of the special selection of female plants has been used for the improvement of tomato gamete selection to obtain of tomato forms could be cultivated under special hydroponic conditions (on narrow benches). Hydroponic technology on narrow benches allows increasing of tomato yield to 100-250 kg/m^2 (by the data of originator). This has been shown for one tomato variety ‘Plamia’. But we haven't enough varieties or hybrids for this technology now, because the model of tomato variety for this purpose must have many advantages, such as high productivity, short height of the stem, early ripening and resistance to main diseases. We analysed the heritability of these traits in F_1 hybrid progenies of tomato and showed, that such traits of productivity as "the mass of one fruit" and "the quantity of fruits on plant" could be inherited by female line. So, plants with good characteristics of productivity can be used as female forms in hybridization. We evaluated 65 samples from the collection of our laboratory and selected of 24 samples for crossings. Hybrid progenies from these female forms have been obtained.

miRNA BINDING SITES IN GENES OF *ARABIDOPSIS THALIANA* ARE CONSERVATIVE IN ORTHOLOGOUS PLANT GENES

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Expression of many plant microRNAs (miRNAs) depends on plant response to abiotic and biotic stress. For understanding the mechanisms of plant response to stress it is necessary to identify target genes of each miRNA and relationship between miRNA and target gene. The complexity of the problem is that one miRNA has several or dozens of target genes and for one gene there are multiple binding miRNAs. Most plant miRNAs bind to the protein-coding region of mRNA and it is important to determine the stability of these bonds in the orthologous genes of plants. Nucleotide sequences of genes and miRNAs were obtained from Genbank (http://www.ncbi.nlm.nih.gov) and miRBase (http://www.mirbase.org) respectively. The free energy value (ΔG) of hybridization of miRNA with studied mRNAs was calculated using RNAHybrid 2.1 program (http://sites.google.com/site/malaheenee/software/). The diagrams of nucleotide and amino acid sequences variability were visualized by WebLogo program. We have revealed binding characteristics of miR414 with 98 genes in chromosome 4 of *A. thaliana*. The protein-coding sequence (CDS) of *AT4G12610* gene (transcription initiation factor IIF subunit alpha) has four miR414 binding sites with confidence level from $p<0.001$ to $p<0.0001$. There are three sites in CDS of *AT4G31420* gene (zinc finger protein 622) and two sites in CDS of *AT4G26600* gene (S-adenosyl-L-methionine-dependent methyltransferase-like protein). Consequently the expression of these genes is under strong control by miR414. miR414 target genes encode transcription factors, DNA repair enzymes, ubiquitin, enzymes involved in cell proliferation, development and differentiation, response to stress and pathogens. One miR414 binding site with high affinity ($p<0.0001$) in *AT4G05410* gene (YAO transducin/WD40 domain-containing protein) was established. Orthologous gene family of *AT1G48400* (F-box/RNI-like/FBD-like domain-containing protein) in *Arabidopsis lyrata, Ashbya gossypii, Ricinus communis, Vitis vinifera* contains from one to nine binding sites for miR414. Each binding site contains GAYGAYGAYGAYGAYGAYGAY polynucleotide which encodes highly conservative heptapeptide DDDDDDD. Orthologous genes for *AT1G48400* of *A. thaliana* are contained in cells of unicellular fungi (*Neosartorya fischeri, Meyerozyma guilliermondii* etc.). Bacteria (*Herpetosiphon aurantiacus*) also encode similar heptapeptide. Amino acids that are located before and after this heptapeptide are variable. It was found that targets for ath-miR171a are GRAS transcription factors gene family (*NAM1, NAM2, NAM3*) of *A. thaliana*. Binding sites for ath-miR171a localized in CDS contain GAUAUUGGCGCGGCUCAAUCA polynucleotide which encodes ILARLN hexapeptide in corresponding proteins. ath-miR171a:mRNA interaction sites in orthologous genes of *A.lyrata, Brachypodium distachyon, Glycine max, Medicago truncatula, Oryza sativa, Physcomitrella patens, R. communis, Sorghum bicolor, Selaginella moellendorffii, V. vinifera, Zea mays* also contain conservative polynucleotide. Orthologous proteins *NAM1, NAM2, NAM3* have conservative ILARLN hexapeptide but amino acids located before and after the hexapeptide are variable. Thus, the interaction of miRNA with protein-coding region of many genes has appeared long time ago and has been preserved in evolution process.

GENETICS AND GENOMICS OF PLANT GENETIC RESOURCES

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Plant genetic resources play a major role for global food security. The most significant and widespread mean of conserving plant genetic resources is ex situ conservation. Most conserved accessions are kept in specialized facilities known as genebanks maintained by public or private institutions. World-wide 7.4 million accessions are stored in about 1,500 *ex situ* genebanks.
In addition, series of genetic stocks including chromosome substitution lines, alloplasmic lines, single chromosome recombinant lines, introgression lines, etc. have been created. Analysing these genetic stocks many qualitative and quantitative inherited traits were associated to certain chromosomes, chromosome arms or introgressed segments. Today, genetic stocks are supplemented by a huge number of genotyped mapping populations. Beside progenies of bi-parental crosses (doubled haploid lines, recombinant inbred lines, etc.) panels for association mapping were created recently.
In our presentation we give examples for the successful utilisation of genebank accessions and genetic stocks for genetic and genomic studies. Using both segregation and association mapping approaches, data on mapping of loci/marker trait associations for a range of different traits are presented.

IMPROVING WHEAT *TRITICUM AESTIVUM* L. BY INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION WITH POACEAE FAMILY SPECIES

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The related species of the family Poaceae (Triticeae) are the source of unprecedented new genes that allow the extension of genetic variation of common wheat *Triticum aestivum* L. These species have similar homoeologous chromosomes and rDNA sequences very similar to *T. aestivum* L. [1-3]. This allows the introgression of alien genes and their incorporation into the genomes A, B and D of wheat, where they can function permanently in the wheat genetic systems. Many of them have already been transferred to the varieties of *T. aestivum* L. [4].

The experimental material consisted of 28 lines of winter wheat obtained using the interspecific and intergeneric hybridization of *T. aestivum* L. with alien species *T. durum* Desf., *T. timopheevii* Zhuk., *Lolium perenne* L. and *Aegilops speltoides* Taush. Among them, 15 lines were developed from the cross-combination with tetraploid species (AABB) *T. durum* Desf., 4 lines from the combination with other tetraploid species of different genome composition (AAGG) *T. timopheevii* Zhuk., 4 lines from cross with *L. perenne* L. and 5 lines were the double hybrids (three-generic) derived with two related species, *T. durum* Desf. (AABB) and *Ae. speltoides* Taush (BB).

The anther culture method was used for obtaining DH lines from these interspecific and intergeneric hybrids. In *in vitro* culture 124 green plants were regenerated. The method of cluster analysis grouped hybrids in terms of comprehensive general similarity of the studied traits.

1. Frederiksen et al. (1992) Hereditas 116, 15-19.
2. Sasanuma et al. (2008) Euphytica 127, 81-93.
3. Zhang et al. (2008) Plant Biol 10, 635-642.
4. Pilch et al. (2011) Biuletyn IHAR 262, 3-24.

DESCRIPTION OF THE SPIKE SHAPE USING COMPUTED FEATURES IN IMAGES

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The morphology of the ear is an important trait of cereal plants. It defines characteristics such as productivity and ease of threshing. The size and shape of an ear of wheat varies widely. Identification of the morphological characteristics of grains spike is an actual problem of cereal genetic research. The mathematical description makes it possible to compile a detailed picture of the structure of the object. The calculated characteristics can be used to identify genes influencing the shape of the spike. In the source image processing improving the characteristics of the source image binarization and filters is proposed to use. To gather information about the form of spikes we used method for defining the contour and the midline of the object. Characteristics of the spike obtained by plotting the number of points on the spike depend on the location on the stem. The resulting plot is approximated by a combination of sinusoidal and gaussian functions. The parameters of the approximating functions form feature vector, which is characteristic of the computing form of the spike Developed algorithm, was tested on the set of images of several cultivars of bread wheat. This led us to the conclusion that the images of objects of different shapes can be obtained by different numerical characteristics. Image classification of cultivars with differences in the morphology of spike was carried out. Based on the results we can conclude that the spike image provides detailed information about a structure of the spike and can be used in large-scale genetic studies. The results showed that the proposed method makes it possible to construct a vector of characteristics describing the shape of the spike. The data obtained can be used to describe varieties for further classification and identification of the sample.

DNA CONTENT IN NUCLEI OF SEED ROOT EMBRYOS AS A MOLECULAR MARKER FOR PRIMING

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Advancing osmotic treatments of seeds (priming) lead to the activation of biochemical processes in seeds allowing repair of damage in embryo cells and resulting in completion of all essential pre-germination processes. It was shown that priming induces cell cycling in embryos of sugar beet. The changes in DNA content in nuclei under different levels of priming are analyzed. Effect of the same priming conditions is not identical for various sugar beet hybrids, and number of cells accumulated in G2 phase at the end of treatment differs. This also leads to the different viability of treated hybrid seeds after storage. It was shown that under optimal level of priming number of cell in G2 with 4C DNA content should not exceed 15%. Number of cells in G2 phase of cell cycle can be used as a molecular marker for testing priming levels in sugar beet.

EFFECT OF CHRONIC RADIATION ON PLANT-PATHOGEN INTERACTIONS IN 30-KM CHERNOBYL ZONE

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It was established in pot experiments that infection with powdery mildew (*Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal) and brown rust (Puccinia triticana Erikss. & Henn.) of three wheat (*Triticum aestivum* L.) cultivars ('Mironovskaya 808', 'Polesskay 70', and 'Kiyanka') grown from seeds, collected in the Chernobyl exclusion zone, was 1.5–2.0 times higher than that of plants grown from control seeds. On filed plots in the Chernobyl zone, wheat plant resistance to biotic stress was reduced. At artificial infection with brown rusts, the disease development was enhanced on plots with increased radiation background. One of the mechanisms of declined phytoimmunity potential under the action of low doses of chronic irradiation is evidently a reduced activity of plant proteinase inhibitors. Thus, in wheat and rye (*Secale cereale* L., cv. 'Saratovskaya') kernels, their activity reduced by 35–60% as compared to control. Active form and race formation in the population of the grass stem rust causal agent (*Puccinia graminis* Pers.) was observed in the Chernobyl zone. A "new" population of this fungus with high frequency of more virulent clones than in other Ukraine regions was distinguished. The results obtained independently in greenhouse and field trials performed in the Chernobyl zone demonstrated radiation stress influence on the pathogen–plant system. They indicate a necessity of monitoring the microevolutionary processes occurring in both plants and their pathogens under conditions of technogenic stresses.

CHARACTERIZATION OF THE *WFZP* HOMOEOLOGOUS GENES IN BREAD WHEAT

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Grass species show unique feature of inflorescence development, they are characterized by the spikelet, a reduced branch that gives a rise to floral meristems. Spikelet meristem identity is regulated by the action of the orthologous genes, *BRANCHED SILKLESS 1 (BD1)* in maize and *FRIZZY PANICLE (FZP1)* in rice (Chuck et al., 2002; Komatsu et al., 2003). *BD1* and *FZP1* belong to the *APETALA2 (AP2)* transcription factor family. The *fzp* and *bd1* mutants exhibit defects in the floral meristems and development of branch structures. The *FZP/BD1* gene is one of the key regulators of the spikelet development in small grain cereals; this gene has not been cloned in Triticeae species until recently. In this study, the three homoeologous genes *WFZP (WHEAT FRIZZY PANICLE), WFZP-A, WFZP-B,* and *WFZP-D,* were isolated in *T. aestivum* for the first time. BAC clones, spanning the *WFZP* gene of the A, B, and D genomes of *T. aestivum* were screened from cv. 'Renan' and cv. 'Chinese Spring' BAC libraries using PCR primers developed on conservative region of the *FZP/BD1* orthologs. Homoeologous specific features have been identified in resulted sequences. Comparison of the three homoeologous genes was used as a tool to study wheat genome evolution and the mutation rate associated to the three *WFZP* genes. The *WFZP-A, WFZP-B, WFZP-D* homoeologous genes were physically and genetically mapped on the short arms of chromosomes 2A, 2B and 2D, respectively. On the genetic map of chromosome 2DS, the *WFZP-D* gene is co-localized with the *Mrs1* gene (Dobrovolskaya et. al., 2009), whose mutation results in development of additional spikelets at the rachis node.

GENETIC VARIABILITY OF CULTURED PLANT TISSUES UNDER NORMAL CONDITIONS AND UNDER STRESS

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The genetic variability induced by *in vitro* conditions known as somaclonal variation is of practical interest due to its potential uses in plant breeding but, on the other hand, if clonal propagation or transformation is main goal, it becomes an unwelcome phenomenon. Thus, it is important to know frequency, the genomic distribution, the mechanisms and factors influencing somaclonal variation. We studied variability of PCR-based DNA markers of cultured tissues and regenerated plants of maize and bread wheat. The original A188 line of maize and the somaclones obtained were tested using 38 RAPD and 10 ISSR primers. None of the A188 plants showed variation in the RAPD and ISSR spectra for any of the primers used. However, the PCR spectra obtained from the somaclones demonstrated some variations, i.e., 22 RAPD primers and 6 ISSR primers differentiated at least one somaclonal variant from the progenitor line. Six SCAR markers were developed based on several RAPD and ISSR fragments. The inheritance of these SCAR markers was verified in the selfing progeny of each somaclone in the R_1–R_4 generations and in the hybrids, with A188 as the parental line in the F_1 and F_2 generations. These markers were sequenced and bioinformatic searches were performed to understand the molecular events that may underlie the variability observed in the somaclones. All changes were found in noncoding sequences and were induced by different molecular events, such as the insertion of long terminal repeat transposon, precise miniature inverted repeat transposable element (MITE) excision, microdeletion, recombination, and a change in the pool of mitochondrial DNA. In two groups of independently produced somaclones, the same features (morphological, molecular) were variable, which confirms the theory of 'hot spots' occurring in the genome. The presence of the same molecular markers in the somaclones and in different non-somaclonal maize variants suggests that in some cases, the same mechanisms determine both *in vitro* and *in vivo* variability. Stress during tissue culture can induce somaclonal variation. For example during cryopreservation the callus cells experience stress caused by exposure to a complex of various factors, which may induce free radical formation and provide conditions for the appearance of genetic changes. ISSR and retrotransposon-microsatellite amplified polymorphism (REMAP) markers were applied to study the influence of individual steps of dehydration cryopreservation technique on DNA in calli and regenerated plants of bread wheat. The precultivation with sucrose and freezing had no influence on the genetic stability of plant material. After the dehydration step, a new fragment appeared in the REMAP profiles for one DNA sample in calli of one line. The most likely cause of the this change is triggered by the stress experienced by cells during dehydration, insertion of a new copy of retrotransposon close to the microsatellite sequence complementary to the ISSR primer.

PREDICTION OF THE STRUCTURE AND LOCALIZATION OF GENES CONTROLLING LEAF PUBESCENCE IN WHEAT *TRITICUM AESTIVUM* L. AND BARLEY *HORDEUM VULGARE* L. BASED ON *ARABIDOPSIS THALIANA* (L.) HEYNH. GENES DATA

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Investigation of the formation of leaf hairs in cereals is important both for practical and fundamental points of view. Trichomes have a number of important functions related to protection from pests and counteracting to environmental factors. On the other hand trichomes are interesting from the plant development standpoint. However, information about the molecular organization of these genes and their interactions, and localization is incomplete. Large genome size of *T. aestivum* and *H. vulgare* makes finding and mapping the genes responsible for leaf pubescence enormously resource-intensive task. On the other hand, control of leaf pubescence in a representative dicotyledonous *A. thaliana* is well researched. According to modern ideas, higher plants are descended from the one common ancestor, i.e. they are monophyletic. That allows, based on genes known to one well-studied species, to predict the structure, as well as to predict the location of functionally similar genes in other species. Preliminary analysis and prediction of localization can significantly reduce the work on the mapping of genes controlling pubescence. In our work, on the basis of literary sources, as well as information from databases, the gene network for trichome formation was reconstructed. We used KEGG (http://www.genome.jp/kegg/), NCBI: Gene (http://www.ncbi.nlm.nih.gov/gene) and PLAZA 2.5 databases (http://bioinformatics.psb.ugent.be/plaza/). We performed a phylogenetic analysis of genes belonging to this network. The analysis suggests the presence of functionally similar genes from the reconstructed gene network for a wide range of higher plants. Based on the representation of the genes we have identified conserved part of gene network controlling leaf pubescence of *A. thaliana*. With service KEGG SSDB (http://www.kegg.jp/kegg/ssdb/), we found the genes most similar functionally to the current ones in *Oryza sativa* L. Then, using the comparative chromosome maps we evaluated the localization of the leaf hairiness genes on *T. aestivum* and *H. vulgare* chromosomes. Interestingly that three of the predicted genes colocalized with the known genetic markers, modifying the pubescence in *H. vulgare*. Thus, these data allow us to predict the location of genes controlling leaf pubescence on the *H. vulgare* chromosomes and in *T. aestivum* homoeologous group of chromosomes. Phenotypic effects of the predicted genes may also be predicted.

QUANTITATIVE CHARACTERISTICS OF LEAF PUBESCENCE IN BREAD WHEAT CULTIVARS

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Plant leaf pubescence (hairiness) plays an important biological role in adaptation to the environment and displays a wide phenotypic variation. Pubescence in wheat is due to unicellular unbranched epidermal outgrowths (trichomes). Trichomes of wheat vary in size and number on the leaf surface. Different forms of this trait are various effective in counteracting environmental factors and protection from pests. We used leaf fold photomicrographs as a source to describe the numerical characteristics of leaf hairiness. LHDetect2 algorithm allowed us to extract a number of leaf hairiness characteristics, such as the number of trichomes in the image (it tells about trichome density); trichome length and its distribution (it tells about trichome size); average trichome length (it tells about average trichome size). We carried out a detailed study of leaf pubescence morphology in cultivars 'Rodina', 'Diamant 2', 'Chinese Spring', 'Yanetskis Probat', 'Golubka', 'Saratovskaya 29' and 'Novosibirskaya 67'. These cultivars vary in origin and adapted to different growing conditions suggesting that the quantitative characteristics of the leaf pubescence may also vary. The cultivars revealed large differences in the trichome length and density. However, we identified a linear correlation between these parameters. Analysis showed that these varieties can be divided into 2 groups according to degree of pubescence of the leaf blade. 'Rodina', 'Diamant 2', 'Chinese Spring', 'Yanetskis Probat' varieties form the group with low expression of this trait (low density and length of trichomes). 'Golubka', 'Saratovskaya 29', 'Hong-mang-mai' and 'Novosibirskaya 67' varieties are in the group with a pronounced leaf pubescence. The results proved for a complex mechanism of the genetic control of the pubescence in the varieties under study including interplay between genes that affect both the length and density of trichomes and the genes that modify hairiness length or density.

MOLECULAR CYTOGENETIC ANALYSIS OF TRITICALE RECOMBINANT LINES

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Triticale combines genetic potentials of wheat and rye and frequently exceeds both parents in such important parameters as productivity and nutritive value of a product. Along with this, this crop is not devoid of some shortcomings, which include, in particular, low bread-making quality, inclination to lodging, pre-harvest sprouting of grain. Gradual reduction in resistance of this crop to the influence of biotic and abiotic stresses is observed too. In this connection broadening and qualitative change of spectrum genotypic variability of triticale is particularly urgent. Introgression of high breeding value alien genetic material into triticale karyotype is a promising solution of the given problem. We have tested two approaches for achieving this goal. The first one consists in introgression of *Aegilops* L. genetic material into genome of hexaploid triticale using genome-substitution forms of common wheat as mediators in which the genome D is substituted by genomes of diploid *Aegilops*. The second approach is based on substitution of a part of chromosomes in the wheat A- and B-genomes by chromosomes of the D-genome under keeping a complete set of rye chromosomes. This research work presents results of the genomic structure analysis of recombinant triticale lines included in investigations for working out a technology marker-assistant selection of triticale for semidwarfness (two lines from crossing triticale cultivars 'Ugo' and 'Idea' (AABBRR) with a genome-substitution form of wheat 'Avrolata' (AABBUU) and five lines from crossing *8x*- × *4x*-triticale containing 2D(2A)-, 2D(2B)- and 4D(4B)-chromosome substitutions in their karyotypes). The analysis was carried out using methods of C-banding, genomic *in situ* hybridization (GISH), fluorescent in situ hybridization (FISH) as well as microsatellite markers. GISH with DNA of *S. cereale* and *Ae. umbellulata* has shown that in the line selected from the crossing combination 'Idea' × 'Avrolata', one pair of rye chromosomes was substituted by a pair of *Ae. umbellulata* chromosomes. The molecular-genetic analysis of this line by using microsatellite markers has revealed substitution of 2R chromosome by 2U chromosome of *Ae. umbellulata*. Similar data were obtained when using the C-banding method. The C-banding analysis showed that a pair of rye chromosomes in the line, selected from the crossing combination 'Ugo' × 'Avrolata' was substituted with pair of 3D chromosomes. These results were verified by FISH with probes pSc119.2 and pAs1 and they are indicating cytological instability of genome-substitution forms of common wheat used in crosses. The study of the genome structure of recombinant forms from crossing *8x*- × *4x*-triticale by GISH with DNA of *S. cereale* and of FISH with probes pSc119.2 and pAs1 has confirmed the data concerning group belonging of introduced chromosomes of the wheat D-genome obtained earlier by C-banding and has shown that chromosomes of wheat and rye were not subjected to structural changes during triticale karyotype reconstruction. On the whole, the results of the molecular-cytogenetic analysis of triticale lines developed by the remote hybridization method indicate that recombinant events with involvement of Aegilops, rye or wheat chromosomes took place in all the studied triticale lines that makes it possible to recommend the approaches devised by us for extension of the crop gene pool. Overall, the results of molecular-cytogenetic analysis of triticale lines created by distant hybridization indicate that all the lines studied recombination events have occurred involving chromosome *Aegilops*, rye or wheat. It allows recommending the approaches designed by us for extension of the crop gene pool.

EXPRESSION OF CALCIUM-DEPENDENT PROTEIN KINASE (CDPK) GENES IN *VITIS AMURENSIS* UNDER ABIOTIC STRESS CONDITIONS

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Abiotic stresses, such as extreme temperatures, soil salinity, or water deficit, are one of the major limiting factors of crop productivity worldwide. Examination of molecular and genetic mechanisms of abiotic stress tolerance in plants is of great interest to plant biologists. Calcium-dependent protein kinases (CDPKs), which are the most important Ca^{2+} sensors in plants, are known to play one of the key roles in plant adaptation to abiotic stress. CDPK is a multigene family of enzymes. Analysis of *CDPK* gene expression under various abiotic stress conditions would help identify those *CDPKs* that might play important roles in plant adaptation to abiotic stress. We focused on studying *CDPK* gene expression under osmotic, water deficit, and temperature stress conditions in a wild-growing grapevine *Vitis amurensis* Rurp., which is native to the Russian Far East and is known to possess high adaptive potential and high level of resistance against adverse environmental conditions. Healthy *V. amurensis* cuttings (excised young stems with one healthy leaf) were used for the treatments. For the non-stress treatment, we placed the cuttings in distilled water for 12 h at room temperature. For the water-deficit stress, detached cuttings were laid on a paper towel for 12 h at room temperature. For osmotic stress treatments, the cuttings were placed in 0.4 M NaCl and 0.4 M mannitol solutions for 12 h at room temperature. To examine temperature stress tolerance, the *V. amurensis* cuttings were placed in a growth chamber at $+10^{o}$C and $+37^{o}$C for 12 h. The total expression of *VaCDPK* genes was examined by semiquantitative RT-PCR with degenerate primers designed to the *CDPK* kinase domain. The total level of *CDPK* gene expression increased under salt and decreased under low temperature stress conditions. We sequenced 300 clones of the amplified part of different *CDPK* transcripts obtained from the analyzed cDNA probes. Analysis of the cDNA sequences identified 8 different *CDPK* genes (*VaCDPK1a, 1e, 1d, 2a, 3a, 3b, 3c, 3d*). We sequenced full cDNA sequences of the genes and analyzed their expression levels by real-time PCR and FAPP method, which has been recently developed by our research group. The prevalent *CDPK* transcript was *VaCDPK3a* under both non-stress and abiotic stress conditions. Under high-salt conditions, *VaCDPK1d, 1e, 3b, and 3d* transcripts were up-regulated. Under high mannitol conditions, expression of *VaCPK1e* and *3b* was up-regulated, while expression of *VaCDPK1d, 3c*, and *3d* was only slightly induced. Under water-deficit, expression of only *VaCDPK3b* and *3c* genes was induced. Cold stress induced expression of *VaCDPK2a* and *3d* genes; while hot stress induced expression of *VaCDPK1a, 1d, 1e, 2a, 3a,* and *3c* genes. Taken together, the data show that the *VaCDPK* genes are transcriptionally regulated by osmotic, water-deficit, and temperature stresses. The differential expression of the *VaCDPK* genes during osmotic, water-deficit, and temperature stresses is suggestive of their involvement in the underlying signal transduction pathways.

AGROBACTERIUM-MEDIATED GENETIC TRANSFORMATION OF SORGHUM USING TISSUE CULTURE-BASED AND POLLEN-MEDIATED APPROACHES

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Genetic transformation is a powerful tool for genetic improvement of arable crops. Genetic engineering approaches are especially important for modification of starch and protein contents, vitamin and micronutrient concentration, improvement of nutritive value of protein fractions, and increase tolerance to environmental stresses. Application of transgenic technologies for genetic improvement of sorghum, a highly productive heat tolerant and drought resistant crop, is extremely important since climate aridization in many regions all over the globe hampers sustainable production of traditional cereals, such as wheat, maize and barley. However, sorghum, in spite of great number of investigations, is one of the most recalcitrant crop species to genetic modification. The most frequently reported problems are a low frequency of transformation and silencing of transgenes. Using the *A. tumefaciens* strain AGL0/p35SGIB with the bar and gus-intron genes under the nos and CaMV35S promoters, respectively, we studied different methods of *Agrobacterium*-mediated genetic transformation of the grain sorghum: *in vitro* culture-based techniques, by inoculation of immature embryos or embryo-derived calli, and pollen-mediated approach, by inoculation of flowering panicles. Four lines of grain sorghum – Milo-10, [9E] Milo-10 (CMS-line), KVV-114, and KVV-45 – were used. In both approaches, for activation of vir-genes agrobacterial cell suspension was grown in the AB or modified AB media with acetosyringone at room temperature. *In vitro* culture approach was effective for obtaining transgenic plants in the lines Milo-10 and KVV-45, which were able to produce embryogenic callus from immature embryos after their co-cultivation with agrobacterial cell suspension. Callus cultures tolerant to glufosinate ammonium (GA) and capable to plant regeneration were obtained. The frequency of immature embryos producing PCR-positive transgenic plants varied in different experiments from 4.5% to 5.4%. Cultivation conditions increasing embryogenic potentials of cultured tissues were the key factors for obtaining of transgenic plants. In the Milo-10, transgenic plants were regenerated also from established embryogenic cultures after their co-cultivation with agrobacterial cell suspension, their frequency was 1.7%. Immature embryos of KVV-114 did not produce embryogenic callus, and in this line transgenic plants were obtained by inoculation of flowering panicles at anthesis. In the progeny of each inoculated panicle the frequency of fertile PCR-positive transgenic plants survived BASTA application was approx. 1%. In the progeny of the [9E] Milo-10 panicle, which was obtained by its pollination with the Milo-10 pollen following agrobacterial inoculation, the frequency of PCR-positive plants survived BASTA application was 3.4%. In the self-pollinated progeny (T1) of KVV-114 and Milo-10 transgenic plants (T0), the seedlings that grew on the GA-containing medium were found, while the leaves of adult plants were sensitive to BASTA application. Nevertheless, PCR analysis confirmed the inheritance of the transgene.

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GENETIC AND CYTOLOGICAL CHARACTERIZATION OF MALE STERILITY MUTATIONS INDUCED BY SODIUM ASCORBATE IN SORGHUM TISSUE CULTURE

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It is generally accepted that oxidative stress is a strong mutagenic factor affecting genome of plant cell. As far as mitochondria play a leading role in maintenance of plant cell redox status, the mitochondrial genome is a primary target of oxidative stress. Assuming that numerous mitochondrial genes are involved in genetic control of pollen development in plants, in order to obtain male-sterile sorghum mutants we used treatment of callus cultures with sodium ascorbate, which is known to be an efficient inducer of oxidative stress. Few plants regenerated from callus cultures of cv. 'Milo-10' treated with sodium ascorbate (1 g/l) were found to contain mutations of male sterility. These mutations were characterized by complete (*ms-asc1*) or partial male sterility (*ms-asc2*, *ms-asc3*). As far as the primary target for sodium ascorbate is plant mitochondria, the male-sterile phenotype of these mutants was expected to be the consequence of cytoplasmic mutation(s). However, the F_1 hybrids obtained by crossing these mutants with the original line were completely fertile; in the F_2, semi-sterile plants were segregated-out. The *ms-asc2* mutation was transferred through the pollen by crossing emasculated plant of original line with restored F_1 hybrids that pointed to nuclear location of this mutation. Cytological analysis of *ms-asc1* revealed significant spectrum of abnormalities during microsporogenesis and pollen maturation, such as cytomixis, chromosomal laggards, chromosome disjunction, adhesion of chromosomes, disturbed cytokinesis, and others. In tapetum, the cells with one nucleus, with unequal nuclei, and with micronuclei have been observed. In mature anthers, a variety of pollen grain (PG) types has been revealed: fertile, of irregular shape, incompletely filled with starch, PGs delayed at the uni-nucleate or bi-nucleate gametophyte stages, with partially or fully degenerated contents, and with abnormal coloration. Significant variation of spectrum and a frequency of disturbances not only between the plants, but also between the flowers from the same panicle was revealed, the frequency of abnormalities varied considerably (1.0-80.0%) resulting in semi-sterile phenotype. Being self-pollinated semi-sterile plants produced in their progeny fertile, semi-sterile and sterile plants in variable ratios. In *ms-asc2* and *ms-asc3* families, alongside with male sterility multiple genetic instabilities were found (dwarfness, awnless, precocious leaf drying, *virescent* mutations). It is possible that action of the sodium ascorbate resulted in activation of a transposon as it took place in our experiments with treatment of sorghum callus cultures by ethidium bromide. In these experiments, similar mutations were found also after several cycles of self-pollination of semi-sterile plants. Perhaps, these treatments having a common target – the mitochondrial genetic system – might cause a retrograde signal to nuclear genome resulting in activation of a mobile genetic element(s).

SEQUENCES OF PLANT TRANSFORMATION: THE RESULT OF THE INSERTED FOREIGN GENE EXPRESSION OR THE STRESS REACTION?

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The gene technology using in plant investigations came to the better understanding of plant physiological processes. At the same time unclear knowledge about transgenic plant physiology may occur a source of incorrect interpretation of obtained results and, consequently, wrong conclusions. In addition, the causes and mechanisms of pleiotropic effects associated with transgenic insertion and gene silencing are remaining unexplained. To solve the problem of transgenic plant physiology it is necessary to pay a close attention to physiological and biochemical peculiarities of plant-agrobacterium symbiosis, because it is a base of plant transformation. It was assumed earlier that agrobacterial transformation is a complex biotic stressing factor and transgenic plant is a long-term stressed organism. We suppose that physiological consequences of plant transformation are determined not only by foreign gene insertion, but largely by stress reaction of plant cells on agrobacterium transformation. Foreign DNA insertion to the plant recipient results in cascade of response reactions remarkably changing metabolism. The degree of such response is supposed to be in dependence on phylogenetic relations of gene donor and recipient. Cell cultures were obtained from tobacco plants (*Nicotiana tabacum* cv.'Samsung') transformed by following *Agrobacterium tumefaciens* strains: disarmed 669 one and LBA4400 one with hsp 101 in sense or antisense orientation. These cell cultures were used for investigations of the stress-reactions on biotic (bacterial infection agent *Clavibacter michiganensis* subsp. *Sepedonicus*) and abiotic (high temperature, potassium fluoride) factors. It was revealed that "sense" culture was superior to normal and "699" ones in tolerance to pointed stressing factors. Similar results were obtained for "antisense" culture, nevertheless it was *a priori* not expected to be tolerant. So, to assess the transformation consequence is necessary to take into account that observed effects may not result from action of the invected gene only.

Conclusions:

1. To consider the transgenesis to be completed solely if expression of transferred gene is present – is methodologically incorrect way. The transferred genes could be silenced because of the response defense reaction likely as under a "pathogen attack". So the absence of transferred gene expression doesn't mean the absence of transformation as fact. Moreover the deletion of inserted construction could take place but physiological trace of the insertion nevertheless can be noticeable.
2. The assessment of physiological consequences of transgenesis when using the plants transformed by disarmed constructions and the plants transformed by constructions including foreign geterological genes should be carried out carefully because of these systems are different. The process of transformation by disarmed constructions is very similar with natural agrobacterial infection where plants and bacteria have been coadapted during evolution, so the transformation by insertion of foreign genes leads to forming much more unstable systems.

THE MODEL FOR AUXIN REGULATED *AtPIN1* EXPRESSION IN THE ROOT APICAL MERISTEM

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Plant hormone auxin regulates many aspects of plant growth and development. PIN-FORMED (PIN) gene family encodes transmembrane proteins, which mediate auxin efflux. PIN proteins are asymmetrically localized within cells, thereby forming in tissue auxin concentration gradients and maxima. Auxin has various effects on PIN1 expression in a cell providing for both positive and negative feedbacks on its own transport [1]. Earlier we proposed that this dual regulation determines stem cell niche maintenance in root apical meristem [2].

Using two reporter lines of *Arabidopsis thaliana* we investigated dose-response auxin regulation of PIN1 expression at the levels of RNA and protein. PIN1::PIN1-GFP containing part of PIN1 coding region reveals both transcriptional and posttranscriptional regulation, whereas pPIN1::GUS displays only transcriptional regulation. The reporter line pPIN1::GUS[-1388;+82] was created by authors; PIN1::PIN1-GFP was provided by Alexis Peaucelle (INRA, France). PIN1::PIN1-GFP and pPIN1::GUS seedlings were grown in a 16 hours light/8 hours dark cycle at 25/22°C on 1/2MS with sucrose. Before microscopic analysis 3 dag seedlings were incubated for 24 h in liquid 1/2MS supplemented with different IAA concentrations. The experimental images were analyzed using ImageJ program.

We found the following changes in PIN1 expression pattern in the root for both lines under low and moderate auxin treatments: (1) increased domain of PIN1 expression in the root meristem; (2) ectopic expression in epidermis and cortex, (3) increased level of PIN1 expression in provascular cells. However, we observed differences in PIN1 expression between the lines: in columella and under high auxin concentrations. The experimental data suggests posttranslational PIN1 regulation by high auxin concentrations. A mathematical model [2] was extended to describe the observed phenomena. The model simulation well agrees with the experimental data and predicts new aspects on the mechanisms of auxin transport in the root meristem.

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TRACKING THE PRESENCE *WIS-2-1A* RETROTRANSPOZONS IN WHEAT GENOMES (*TRITICUM AESTIVUM* L.)

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WIS-2-1A elements were the first retrotranspozons identified in a high molecular weight glutenin subunit (HMW-GS) of wheat. *WIS-2-1A* belong to the *Ty-1 Copia* family. They occur in very different copy numbers, ranging from tens of thousands to just a few copies per genome. They are characterized by a great diversity, while most of the sequences of those retrotranspozons are inactive. It has been proven that a much larger number of genetic mobile elements are contained in the genome A than in the other two (B and D) genomes of *Triticum aestivum* L. The results obtained and presented in this study confirm the presence of *WIS-2-1A* retrotranspozons in the genome of common wheat *(Triticum aestivum).* In this study, the integrase gene for *WIS-2-1A* occupying locus *X63184.1* was used. This gene was identified in 16 of 26 cultivars of common wheat *(Triticum aestivum L.)* in the area 171 bp. Analysing the amplification products of *WIS-2-1A* using Gel Doc^{XR} software, the amount of DNA bound integrase in ng per 1 ml of the examined varieties was determined. It seems therefore feasible to analyze *WIS-2-1A* in common wheat, as it is yet another example that in trying to predict gluten quality, one should not limit one's research to only studying glutenin units and their genetic basis, but one should examine the expression of moving parts whose presence may influence the modification the glutenin loci.

ANTIMUTAGENIC COMPOUNDS OF *CHAMAENERION ANGUSTIFOLIUM* AND *CHAMAENERION LATIFOLIUM* - FROM GENOME TO BIOTECHNOLOGY

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The Onagraceae family comprises 17 genera and about 675 species. The genera are grouped in seven tribes; the tribe Epilobieae has two genera (*Boisduvalia* Spach, and *Epilobium* L.). Some scientists mark out the *Chamaenerion* genus. *Chamaenerion* differs sharply from *Epilobium* in multiple features. Molecular analysis provides strong support (100% BS) for Chamaenerion as a monophyletic group separate from *Epilobium*. Chemotaxonomic relationships in the family Onagraceae have been extensively studied, especially in respect of leaf flavonoids, leaf phenolics and flower chalcones. Phytochemical investigations on the aerial part lipophilic extracts have been mainly restricted to the analysis of the fatty acid (FA) composition of the seed oil. The chemotaxonomic potential of FA and triterpenic acids (TTA) in the family Onagraceae has not been evaluated. The objective of this study was to analyze a collection of 6 representatives of Onagraceae for total lipophilic extracts content, FA and TTA composition, and to evaluate the chemotaxonomic potential of FA and TTA in this family. A quantitative comparison of the acid lipophilic compounds in different botanical parts of widely distributed and commonly used in folk medicine fireweed (*Chamaenerion angustifolium*) was carried out. Some distinguishes on the percentage and on composition of the components were discovered. The investigation of the acid fractions was carried out after full methylation by diazomethane. The GC oven temperature was programmed from 50º to 300. 22 FA fatty acids and 6 TTA were determined by GC-MS method for the first time. These acids such as pomolic, ursonic, oleanonic, acetylursolic, acetyloleanolic and betulinic were determined for the first time. Many compounds of this group are reported to have various interesting biological, pharmacological, or medicinal activities including antiinflammatory and anticarcinogenic activities. Our own results show the great potential of TTA as antimutagenic compounds. *Chamaenerion angustifolium* (CA) is especially prospective as raw material for TTA isolation. The literature data concerning phylogenetic features of Onagraceae show the similarity of CA to *Chamaenerion latifolium* (CL). Our chemotaxonomic results confirm the supposition about availability of CL as useful raw material for drug creation. The other samples of Onagraceae are more pure of biologically active compounds and less prospective.

REGULATION OF *ARABIDOPSIS GDH2* NUCLEAR GENE EXPRESSION DEPENDS ON FUNCTIONAL STATE OF MITOCHONDRIA AND CHLOROPLASTS

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The results of recent studies indicate that expression of some plant nuclear genes depends on functional state of mitochondria and chloroplasts. We have demonstrated that expression of *gdh2* gene encoding beta-subunit of mitochondrial glutamate dehydrogenase depends on a redox state of mitochondrial respiratory chain. Treatment of *Arabidopsis thaliana* cell culture with respiratory complex III inhibitor antimycin A or complex IV inhibitor KCN led to rapid increase of *gdh2* transcript content. Complex I inhibition by rotenone had no influence on the transcript level. We suggest that *gdh2* expression responds to changes of redox state of the respiratory chain segment located between complex I and complex III. We suppose that the revealed effect is not due to elevated generation of reactive oxygen species occurring upon the electron transport chain blockage, because cell treatment with hydrogen peroxide and paraquat did not lead to induction of *gdh2* expression. Experiments with *Arabidopsis* green seedlings have demonstrated that *gdh2* gene expression and GDH2 enzyme activity decrease strongly in the normal and high light conditions and increase in darkness. Resuming our experiments on different *Arabidopsis* organs and cell types we generalize that *gdh2* expression is maximal when both respiratory and photosynthetic electron transport chains are inhibited and minimal when both of the electron transport chains are highly active. There are a number of hypotheses which would explain such a regularity. The first one proposes an energetic deficit as a regulatory factor initiating *gdh2* gene induction. We assume, however, that sugar starvation or ATP depletion cannot be the main factors in regulation of *gdh2* expression, because oxidative phosphorylation uncoupling by FCCP did not mimic the effects of antimycin A or prolonged dark treatment on the *gdh2* gene expression. The second hypothesis is developed for chloroplast-to-nucleus signaling and proposes that the regulatory signal can be initiated by redox state of plastoquinone pool and mediated by thylakoid membrane-bound protein kinases. We assume that similar mechanism would exist also in mitochondria-to-nucleus signaling, so that *gdh2* expression would depend on redox state of both ubiquinone and plastoquinone pools. This is confirmed by our experiments, in which the involvement of serine/threonine protein kinases in the antimycin-related *gdh2* induction was demonstrated as an ultimate step in transduction of the regulatory signal to the nucleus. Abscisic acid and/or pyridine nucleotides ratio changes can also participate in the retrograde regulation of *gdh2* gene expression. Thus, we have demonstrated that regulation of *gdh2* gene expression depends on mitochondrial and chloroplast functional state, and it can be considered as another example of retrograde regulation. The redox states of ubiquinone/plastoquinone pools are the most likely primary factors of this regulation type, which also involves serine/threonine protein kinases in signal transduction.

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THE ALKALOID CYTISINE IN THE CELL CULTURE

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Alkaloids are vegetative establishments of complex and original structure with nitrous heterocycles in the basis. For a long time they drew researchers' attention because of their unique and specific physiological effect on alive organisms. Not all the representatives of the globe's flora contain these unique substances. Alkaloid cytisine is to be found mainly in the plants of the fabaceous family - Fabaceae. For the cytisine production the seeds of *Thermopsis lanceolata* R.Br (*T. lanceolata* R.Br) and *Cytisus laburnum* (*C. laburnum*) are used as a raw material. The object of the research is *T. lanceolata* cell culture. Sterile sprouts are used at the first stage of the experiment. Callus genesis is accompanied with dedifferentiation. It leads to the cellular organization simplification. Based on an important property of a plant cell, such as totipotency, there appears the formation of the "de novo" biosynthetic device. The cultivation algorithm consists of two basic stages: (i) the cultivation conditions optimization of callus with a high level of the primary metabolites biosynthesis (Aspartat – lysine); (ii) the research of cultivation chemical and physical factors influence on the secondary metabolite (cytisine) biosynthesis and accumulation. During the cultivation the Murashige and Skoog classical recipe of nutrient medium will be used. Optimization of the cultivation conditions will concern the phytohormones, macro- and micronutrients content, as the purpose of optimization is the production of the determined high-level competence embriogenical callus. The main problem is genetic heterogeneity of a cellular population and instability of morpho-physiological processes. The correct management of higher plants cells population is possible at the synchronization of a cellular cycle phases. The references analysis has shown that it is almost impossible to synchronize cellular cycles in the culture of plant tissue. The application of chemical inhibitors allows achieving sufficiently high level of SPCC. Their use also results in the rise of a mitotic index level. Another method of SPCC is based on the effect of limiting factors, such as, the reduction of phytohormones level and carbohydrates and nitrogen sources in a nutrient medium. The cells accumulation in the mitosis phase will allow getting a cellular mass ready to adequate response to chemical and physical influences. It promotes the formation of the "de novo" biosynthetic device according to genetic conditionality. The following receptions will be used for the cells genetic potential activation: (i) optimization of organic additives concentration; (ii) optimal physical parameters installation. The process of plant cells cultivation is carried out according to the researcher's objectives. In one case it is a biomass accumulation. In the second one it is the reception of producers' strains. Carrying out the research within the limits of the first task, it is important to optimize a nutrient medium composition at the level of macro-micro elements and phytohormones. To increase the cellular clusters productivity the work is carried out at the organic additives level.

EXTRACTION OF QUANTITATIVE CHARACTERISTICS DESCRIBING WHEAT LEAF PUBESCENCE WITH A NOVEL IMAGE PROCESSING TECHNIQUE

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Leaf pubescence (hairiness) in wheat plays an important biological role in adaptation to the environment. However, this trait has always been methodologically difficult to phenotype. An important step forward has been taken with the use of computer technologies. In the present work, computer analysis of a photomicrograph of a transverse fold line of a leaf is proposed for quantitative evaluation of wheat leaf pubescence.

We are presenting a novel algorithm, which allows such images to be used for rapid quantitative evaluation of leaf pubescence. This algorithm has been implemented as the LHDetect2 software program, which comes in two flavors: as a console application and as a Web service. We provide an example of analysis of leaf pubescence in wheat. The results demonstrate that the proposed method is rapid, adequately assesses leaf pubescence density, the length distribution of trichomes, and the data obtained using this method are significantly correlated with the density of trichomes on the leaf surface. Thus, the proposed method is efficient for high-throughput analysis of leaf pubescence morphology in cereal genetic collections and mapping populations.

THE CHANGES OF GENE EXPRESSION, PROTEIN CONTENT AND ALTERNATIVE AND CYTOCHROME PATHWAYS CAPACITY IN THE WINTER WHEAT MITOCHONDRIA UNDER COLD HARDENING

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The expression of genes, encoding number of cytochrome electron transport pathway components, alternative cyanide-resistant oxidase (AOX) and uncoupling protein, protein content and capacity of cytochrome and alternative pathways in the mitochondria from shoots of winter wheat etiolated seedlings (*Triticum aestivum* L., cv. 'Irkutskaya') under low (7 days, 2 - 3 ºC) and subzero (2 days, -2 – -3 ºC) temperatures were studied. These temperatures are necessary for increasing of winter cereals frost-resistance. The capacity of mitochondria to oxidize the different substrates and possible mechanisms of AOX activity regulation were examined. It was shown that to 7 days of cold hardening the participation of cytochrome pathway into respiration was decreased but the participation of alternative pathway and AOX protein content was increased. The AOX activity was related to free fatty acids content. Using qRT-PCR with SYBR Green I the changes of gene expression of mitochondrial proteins under cold hardening were detected, differential expression of *ucp1a* and *ucp1b* and coordinated expression of *ucp1a* and *aox1a* were observed. Accumulation of *aox1a* transcripts, increase of AOX protein content and activation of alternative pathway capacity under cold hardening were accompanied the decreasing of antimycine A-dependent and increasing of benzhydroxamic acid-dependent of reactive oxygen species (ROS) production by mitochondria from hardened seedlings. Cold hardening of winter wheat seedlings was accompanied with maintaining of outer mitochondrial membrane intactness, the decreasing of ROS content and lipid peroxidation products in mitochondria under following cold shock (-8 ºC, 6 h). Thus cold hardening of winter wheat seedlings caused coordinated expression of genes related to non-phosphorylating electron transport pathways that led to changes of energetic cell metabolism, aimed at increasing of adaptive possibilities of plant organism.

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BACTERICIDE IMPACT OF POLYMER-STABILIZED MULTI-FUNCTIONAL NANO-COMPOSITES

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Synthesis on the basis of natural matrices in order to acquire products with the desired properties is one of the promising trends of modern science. Using polysaccharides as a matrix allowed to generate derivatives with diverse structures and new properties. Growing interest towards anti-microbe effect of selenium-containing nano-composites is induced by the phenomenon of antibiotic-resistance of contemporary pathogenic microorganisms.

Clavibacter genus bacteria are the most significant and widely spread among gram-positive bacteria. Bacteria cells are static pleimorphous rods, normally singular, sometimes coupled or joined in short chains, strict anaerobes in need of certain growth factors, non-sporogenous. *Clavibacter michiganensis* subsp. s*epedonicus* cause potato ring rot. At the tuber slice the damage is shaped as a ring; growing bacteria are accumulated in the conducting vessels causing their occlusion and therefore gradual withering of leaves and stem. This disease is distributed at all the continents including Australia. Harvest loss through ring rot damage may reach 10-45%.

Our work was aimed at the study of complex interaction between microbe cultivar and selenium-based nanocomposites.

Bacterial strain As1405 was acquired from the All-Russia collection of microorganisms, IMBP RAS. This genus is not included in the classification of pathogenic microorganisms by pathogenic groups of Sanitary-Epidemiological Rules SP 1.3.2322-08. The present study was focused on characteristics of the acquired strain.

Fluorescent and electronic-scanning microscope was used to acquire photographs of bacterial cells. Pathogen was identified by PCR-analysis, which confirmed the presence of DNA of desired size. The extracted DNA was sequenced with the sequenced sequence added to Gen Bank under the number HQ394204. Cellulolytic and phytotoxic activity of this strain was determined.

Chemistry Institute named A.E. Favorsky provided water-soluble nano-composites containing selenium stabilized by various polymers. Nano-composites anti-microbe activity was studied on the investigated strain of potato ring rot. Nano-composites of elementary selenium (3.4% Se) and Se with arabinogalactan acquired from SeO_2 (1.23% Se) were found to demonstrate anti-microbe effect increasing with the rise of selenium content. The work enumerates various conditions and time periods of cultivation and determination of the influence of the given water-soluble nano-composites on bacterial cells survivability.

GENE TRANSFER IN TOBACCO MITOCHONDRIA *IN VITRO* AND *IN VIVO*

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Earlier, we had showed that isolated mitochondria from different organisms can import DNA. Exploiting this mechanism, we assessed the possibility of genes transfer in tobacco mitochondria *in vitro* and *in vivo*. Whereas homologous recombination is a rare occasion in higher plant nuclei, recombination between the large direct repeats in plant mitochondrial genome generates its multipartite structure. Following transfection of isolated organelles with constructs composed of a partial *gfp* gene flanked by mitochondrial DNA fragments, we showed the homologous recombination of imported DNA with the resident DNA and the integration of the reporter gene. The recombination yielded an insertion of a continuous exogenous DNA fragment including the *gfp* sequence and at least the 0.5 kb of the flanking sequence on each side. Using of transfection constructs carrying multiple sequences homologous to mitochondrial DNA could be suitable for insertion of a target gene into any region of the mitochondrial genome, which turns this approach to be of a general and methodical importance. Usually mitochondrial reactive oxygen species (ROS) level is under strict control of the antioxidant system including the Mn-containing superoxide dismutase (MnSOD). MnSOD is presented in multiple forms encoded by several genes in plants. Possibly, this enzyme, beside its catalytic function, fulfills as well some unknown biochemical functions. Thus, one of maize SOD enzymes (SOD3.4) could bind with mitochondrial DNA. Another SOD form (SOD3.1) is located in close proximity to mitochondrial respiratory complexes, where ROS are generated. To study possible physiological functions of this enzyme, we cloned the maize SOD3.1 gene. Compared to the SOD3.4, this enzyme didn't demonstrate DNA-binding activity. At the same time, SOD3.1 didn't show non-specific DNA-hydrolyzing activity as Cu/ZnSOD does. It means that this enzyme might have some DNA protective function. We made NtPcob-sod3.1-IGR-cob* genetic construct with integrative properties. It contains the selective gene and the gene of interest under control of the 5'-regulatory regions of Arabidopsis *orf262* gene and the tobacco *cob* gene. We used modified variant of the tobacco apocytochrome b gene as a gene for selection with the nucleotide substitution G128T (G43V) which results in antimycin A resistance. The maize *sod3.1* gene was used as a gene of interest. The construct was delivered into tobacco callus cells and leaf disks by biolistic method. The callus lines demonstrating the high growth rates in the presence of antimycin A in comparison with the non-transformed control lines were selected. PCR analysis of transformed callus lines revealed the presence of heterologous maize *sod3.1* sequence and the integration of the construct elements in tobacco mitochondrial genome. The work was supported by grants from Russian Fund for Basic Research (09-04-00992, 12-04-01400).

FUNCTIONAL SPECIALIZATION OF DUPLICATED FLAVONOID BIOSYNTHESIS GENES IN WHEAT

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Gene duplication followed by subfunctionalization and neofunctionalization is of a great evolutionary importance. In plant genomes, duplicated genes may result from either polyploidization (homoeologous genes) or segmental chromosome duplications (paralogous genes). In allohexaploid wheat *Triticum aestivum* L. (*2n=6x=*42, genome BBAADD), both homoeologous and paralogous copies were found for the regulatory gene *Myc* encoding MYC-like transcriptional factor in the biosynthesis of flavonoid pigments, anthocyanins, and for the structural gene *F3h* encoding one of the key enzymes of flavonoid biosynthesis, flavanone 3-hydroxylase. From the 5 copies (3 homoeologous and 2 paralogous) of the *Myc* gene found in *T. aestivum*, only one plays a regulatory role in anthocyanin biosynthesis, interacting complementary with another transcriptional factor (MYB-like) to confer purple pigmentation of grain pericarp in wheat. The role and functionality of the other 4 copies of the *Myc* gene remain unknown. From the 4 functional copies of the *F3h* gene in *T. aestivum*, three homoeologues have similar function. They are expressed in wheat organs colored with anthocyanins or in the endosperm, participating there in biosynthesis of uncolored flavonoid substances. The fourth copy (the B-genomic paralogue) is transcribed neither in wheat organs colored with anthocyanins nor in seeds, however, it's expression has been noticed in roots of aluminium-stressed plants, where the three homoeologous copies are not active. Functional diversification of the duplicated flavonoid biosynthesis genes in wheat may be a reason for maintenance of the duplicated copies and preventing them from pseudogenization.

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DNA MUTAGENESIS IN *PANAX GINSENG* CELL CULTURES

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At the present time, it is well documented that plant tissue culture induces a number of mutations and chromosome rearrangements termed "somaclonal variations". However, little is known about the nature and the molecular mechanisms of the tissue culture-induced mutagenesis and the effects of long-term subculturing on the rate and specific features of the mutagenesis. The aim of the present study was to investigate and compare DNA mutagenesis in different genes of *Panax ginseng* callus cultures of different age. It has previously been shown that the nucleotide sequences of the *Agrobacterium rhizogenes rolC* locus and the selective marker *nptII* developed mutations during long-term cultivation of transgenic cell cultures of *P. ginseng*. In the present work, we analyzed nucleotide sequences of selected plant gene families in a 2-year-old and 20-year-old *P. ginseng* 1c cell culture and in leaves of cultivated *P. ginseng* plants. We analysed sequence variability between the *Actin* genes, which are a family of house-keeping genes; the phenylalanine ammonia-lyase (*PAL*) and dammarenediol synthase (*DDS*) genes, which actively participate in the biosynthesis of ginsenosides; and the somatic embryogenesis receptor kinase (*SERK*) genes, which control plant development. The frequency of point mutations in the *Actin*, *PAL*, *DDS*, and *SERK* genes in the 2-year-old callus culture was markedly higher than that in cultivated plants but lower than that in the 20-year-old callus culture of *P. ginseng*. Most of the mutations in the 2- and 20-year-old *P. ginseng* calli were A↔G and T↔C transitions. The number of nonsynonymous mutations was higher in the 2- and 20-year-old callus cultures than the number of nonsynonymous mutations in the cultivated plants of *P. ginseng*. Interestingly, the total number of N→G or N→C substitutions in the analyzed genes was 1.6 times higher than the total number of N→A or N→T substitutions. Using methylation-sensitive DNA fragmentation assay, we showed that the level of methylcytosine was higher in the DNA of the 20-year-old *P. ginseng* calli that than that in the DNA of the 2-year-old calli. Taken together, the data obtained demonstrate that both 2- and 20-year-old subculturing of *P. ginseng* tissues *in vitro* increased the number of point mutations, the diversity of mutation types, and the number of potential DNA methylation sites in the analyzed gene regions. It is possible that these mutation processes is the main reason underlying the decline in the vigor and regenerability of *P. ginseng* tissue culture over time.

IDENTIFICATION OF PHOTOPERIOD-INSENSITIVE *PPD-B1* ALLELE USING NEAR-ISOGENIC LINES OF *TRITICUM AESTIVUM* L.

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Understanding of genetic mechanisms underplaying photoperiod sensitivity of hexaploid wheat is important for modern plant breeding. Discovering of new insensitive alleles of *Ppd* genes may significantly increase productivity of newly developed wheat varieties. It was previously shown, that mutation in *Ppd-D1* gene promotor leads to alteration of photoperiodic sensitivity in wheat (Beales et al., 2006). Similarly, we suppose that alterations in *Ppd-B1* locus can lead to the same effects. Two pairs of near-isogenic lines (NILs: Ppd-m, Ppd-0m and Ppd-w, Ppd-0w) different in their photoperiodic sensitivity were recently developed at VIR (Koshkin et al., 2009). Using gene-specific primers we confirmed that all the lines contain the recessive allele of *Ppd-D1* gene. Genotyping of the lines with the representative number of SSR-markers showed that they have practically identical genome, except for chromosome 2B. Thus, the different alleles of *Ppd-B1* gene could be a reason for the different photoperiod sensitivity of the NILs. At the first step we focused on the possible nucleotide polymorphism of the *Ppd-B1* locus that may cause different reaction of the NILs on the day length. To design primers we used public available sequences of *Ppd* genes in 'Chinese Spring' wheat variety: DQ885753, DQ885757, DQ885766 assigned to the genome A, B and D, respectively (Beales et al., 2006). The alignment was obtained using clustalw algorithm of program Unipro UGENE. Based on the alignment, specific primers to *Ppd-B1* gene sequence were constructed avoiding possible amplification from orthological loci on 2A and 2D chromosomes. Totally 13 primer pairs were designed to cover whole *Ppd-B1* gene sequence (about 15 kb). For the primers design Unipro UGENE, OligoAnalyzer 3.1 (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/), and Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) software were employed. With all the 13 pairs of primers PCR were successfully performed and results were estimated via standard agarose gel electrophoresis. In most cases, PCR-products showed the expected sizes without obvious polymorphisms except for primer pairs № 8 and №9. The PCR fragments obtained with the primers were observed only in the lines representing recessive sibs 0m and 0w carrying recessive *Ppd-B1* allele. The photoperiodic sensitive parental line “Fchl2” also showed obvious amplification. The results suggest that primer pairs № 8 and №9 might be not complementary to the *Ppd-B1* sequence in photoperiod insensitive lines Ppd-m and Ppd-w preventing successful PCR. Hence we hypothesize that unknown alterations can occur in the *Ppd-B1* alleles presented in the NILs with low photoperiodic sensitivity. Recently Diaz et al. (Dıaz et al., 2012) has shown that altered photoperiod sensitivity caused by *Ppd-B1* alleles may depend on copy number variation (CNV) of the gene. Future investigations will be performed to understand whether the alteration of photoperiod sensitivity in the NILs are determined by CNV or other unknown mutation.

THE INFLUENCE OF THE COLD HARDENING ON THE FROST TOLERANCE OF ARABIDOPSIS (*ARABIDOPSIS THALIANA* (L.) HEINH.) AND THELLUNGIELLA (*THELLUNGIELLA SALSUGINEA* (PALL.) O.E. SCHULZ)

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With extensive research focused on molecular-genetic mechanisms of higher *T. salsuguinea* (TEL) resistance to salination, mechanisms of its cold resistance have been described in a limited number of publications. Subjection to cold was shown to be accompanied by the change in transcription of a larger amount of genes related to cold adaptation. Virtually none of the known publications cover the change in TEL proteome in response to cooling. It remained unclear to what extent the changes observed determine higher resistance of TEL to cold stress, as there was no comparison with *A. thaliana* (ARA). Besides there is no clarity concerning correlation between these changes and frost resistance. In the present work we compared frost resistance of non-hardened and hardened at 4ºC ARA and TEL plants and confronted it with the changes in composition and content of two groups of protective proteins – dehydrins (dhns) and heat shock proteins (Hsps). The study was based on ARA seeds (Columbia race) and TEL seeds of Shandong ecotype (courtesy of V. A. Moffat, Canada). Growing was conducted during two (ARA) and four (TEL) weeks (23°C with 16 hours photoperiod per day). The grown plants were transferred to a climatic chamber (16 hours photoperiod, 4°C) for one and two weeks for hardening. After 7 days of hardening some of the plants were subjected to 2-hours freezing in liquid thermostat. Total protein was extracted from the rosette leaves according to the usual method and subjected to electrophoresis and Western Blott. To detect dhns and Hsps there were used primary antibodies against Hsp101, Hsp17,6 (class II) (Agrisera), Hsp60, Hsp70, Hsc70 (cognate protein) and dehydrins (StressGen). Non-hardened plants of ARA were damaged by 2 hours of frost of –10°C and –15°C. 7-days hardening removed damaging effect of the frosts (-10 or -15°C, 2 hours) on ARA. Non-hardened plants of TEL were not damaged by freezing at -10 and -15°C, therefore non-hardened and hardened plants of TEL did not differ after freezing at -10 and -15°C. Non-hardened plants of TEL were damaged by 2 hours of frost of -18°C, but hardening induced protective effect. According to immunoblotting data, non-hardened TEL plants contained more Hsp101, Hsp60 and Hsp70 and had lower content of Hsc70 than ARA plants. Hardening resulted in significant increase of heat shock protein content in ARA, while TEL demonstrated insignificant rise of their content under hardening. Dehydrin spectrum in TEL was more multivarious than in ARA. TEL plants, unlike ARA plants may be supposed to be ready to counteract frost even without hardening, with the latter enhancing their resistance to even more intense frost. Heat shock proteins and dehydrins may play an important role in higher frost resistance of TEL.

THE SIBERIAN LARCH COMPLETE *DE NOVO* GENOME SEQUENCING PROJECT AT THE SIBERIAN FEDERAL UNIVERSITY GENOME RESEARCH CENTER

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A new Genome Research Center was established in April 23, 2012 at the Siberian Federal University (Krasnoyarsk, Russia). One of the Center's main projects is to completely sequence, assemble and annotate *de novo* the Siberian larch (*Larix sibirica* Ledeb.) genome (1C = 12.03 Gbp). The larch genome is four times larger than the human genome (1C = 3.20 Gbp) that remains the largest genome completely sequenced so far. Meanwhile, larch is one of the most important key elements of Siberian boreal forests having a great economic and ecological value. However, the study of larch and other closely related important conifer forest tree species is hindered by an almost complete lack of data on its genome structure and genes that control important adaptive and selective traits. Complete larch genome sequence would allow us to obtain such data and effectively use them for studying conifer forests genetic variation, genetic adaptation to global climate change and for creating conservation and breeding programs. It should also help us sequence other important conifer species, such as, for instance Siberian stone pine (*Pinus sibirica* Du Tour) that has an even larger genome (23.62 Gbp) – almost twice as large as the larch genome. The gigantic genome size and high allelic variation of conifers impede their complete genome sequencing and assembling. The conifer genomes are not only extremely large, but also contain a great number of repetitive elements and large gene families with high similarity in nucleotide sequences. To overcome these problems and facilitate assembling we apply an innovative unique approach via using haploid tissue cultures developed from haploid immature megagametophytes (female gametophytic tissue). The haploid nature of tissue cultures or calluses obtained from megagametophytes has been confirmed by genotyping their nuclear genomic DNA with informative SSR markers that are heterozygous in the diploid tissue of the parent tree. After fragmentation a fraction of nuclear genomic DNA within 550-600 nucleotide base pairs size range has been used for paired-end sequencing with 101 cycles and four lanes of a flow cell of the Illumina HiSeq 2000 sequencer that should give an expected ~12X genome coverage. The preliminary results based on these sequence data will be demonstrated and represent the first step in the multi-disciplinary integrative innovative international project on complete *de novo* larch genome sequencing that is planned to be done at the Genome Research Center.

This work has been supported by the grant «Genetic research of the Siberian larch» awarded to the Siberian Federal University by the Russian Government Department of Science and Education.

EFFECT OF AUXIN PULSE TREATMENT, GELLING AGENTS AND AGNO3 ON ADVENTITIOUS ROOT FORMATION OF SCOTS PINE *IN VITRO*

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Scots pine (*Pinus sylvestris* L.) is one of the most widespread conifers in the world and in Russia. This species has great economic value as a timber tree for logs, lumber, and pulpwood. For establishment of forest plantations the superior genotypes (plus trees) must be clonally propagated. However, vegetative propagation of Scots pine, especially that of mature trees, is quite problematic. In the process of clonal micropropagation of Scots pine, rooting is still the limiting step. We investigated influence of various factors, such as basal nutrient media, sucrose, auxins, ethylene precursor and inhibitors, gelling agents and pulse treatment on rooting of shoots obtained through micropropagation of young seedlings of Scots pine. Preliminary experiments demonstrated the advantages of DCR medium over other media and NAA over IBA. The best sucrose concentration for rooting was 30 g/l. Frequency of root formation was higher on Gelrite (up to 38%) in comparison with Gelzan and three types of agar. Addition of 40 μM AgNO3 some increased rooting whereas $CoCl_2$ and ethephon decreased it. The dark treatment of shoots during 1-2 weeks did not have a significant effect on rooting. Shoots were pulsed for 6 or 24 hours with NAA and IBA at 50 or 100 mg/l, then transferred to medium without growth regulators. The treatment with 50 mg/l NAA during 6 hours resulted in 44% rooted shoots, whereas pulse treatments with IBA resulted in 13-19% root formation. Rooted plantlets were successfully acclimatized in the greenhouse.

EVALUATION OF TRANSGENIC ASPEN AND BIRCH PLANTS WITH GLUTAMINE SYNTHETASE GENE OVER THREE SEASONS

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Availability of inorganic forms of nitrogen is frequently a limiting factor for productivity of plants. To improve growth of forest trees we cloned the glutamine synthetase gene (*GS*) from Scots pine and transferred it into aspen (*Populus tremula*) and birch (*Betula pubescens*) plants by agrobacterial transformation. Three-year trial was performed for evaluation of transgenic plants. Six lines (nontransgenic control and five lines with the *GS* gene) were grown in pots under greenhouse (2009-2010) and open air (2011) conditions. Transgenic aspen plants were similar to each other. Their height varied from 91 to 123% and volume of wood varied from 114 to 141% to control plants. Two lines with decreased growth and one line with highly enhanced growth were identified within birch plants. Compared with the control plants, the height was reduced by 25-31% (2b and 2c lines) or increased by 41% (8b line), respectively. The wood volumes of these lines were 26-49% smaller or 74% greater than control plants. Automated analysis of aspen and birch leaves by LAMINA program showed no differences between plants of leaf-shape parameters such as leaf area, perimeter, circularity, length, width, horizontal and vertical symmetry. Enzyme activities in soil for evaluation the potential risk of transgenic trees were analyzed annually at the end of the growing season. Results of analysis did not differ significantly between transgenic and control plants.

CYTOGENETIC FACTORS AND MECHANISMS OF FORMATION OF TRITICALE GENOME WITH RYE CYTOPLASM (*SECALOTRITICUM*, RRAABB, *2n=6x=*42)

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Triticale with rye cytoplasm (*Secalotriticum*) are developed for a strengthening of rye genome expression, an increasing of adaptive potential, an extension of triticale genofund and distribution area. Analysis of cytogenetic factors of formation and stabilization of rye-wheat amphiploid genome has allowed making the theoretical and experimental substantiation of the efficient secalotriticum development methodology: Rye (RRRR, *4x*=28) × Triticale (AABBRR, *6x*=42) → F_1 (RRABR, *5x*=35) × Triticale (AABBRR, *6x*=42) → *Secalotriticum* (RRAABB, *6x*=42) (C-banding identification). Gametogenesis specificity of rye-triticale F_1 hybrids is the key stage in secalotriticum genome formation because of interaction of genetic control meiosis systems in initial species – wheat (Ph, I/Edu and others) and rye (Sy, P/Edu and others). Meiosis analysis of pentaploids F_1 (RRABR, *5x*=35) has allowed finding the main cytogenetic factors and mechanisms of secalotriticum genome formation: (i) the polygenome of pentaploids F_1 (RRABR, *5x*=35), including homo- and homeologous monogenomes of initial species; (ii) promoter effect of threefold dose of a rye genome on the chromosomes conjugation (12-14 bivalents instead of 7); (iii) diploid basic RR-genome, as the factor for meiosis normalization and functionality of gametes with a different chromosomal set; (iv) joint control of the meiosis stages and genotypic specificity of expression rye and wheat genetic systems in the rye cytoplasm; (v) features of segregation and elimination of chromosomes from the initial genomes, caused by different types of polar orientation of centromeres because of genotypic specificity their pairing in meiosis prophase; (vi) partial non-reduction of gametes in meiosis with heightened frequency depending on genotype (to 35%).
The rye-triticale hybrids F_1 with the dominance of wheat genes controlling meiosis (Ph, I/Edu and others) formed the partial non-reduction gametes of two types - RR (*2x*=14) and RAB (*3x*=21). At the prevalence of the rye genes (Sy, P/Edu and others) the homeologs synapsis (A, B, R), the uniform distribution of chromosomes and the formation of the gametes with different chromosomal set occurred. Utilization of the RAB-gametes at the backcross of pentaploids F_1 on triticale results to formation the balanced genome of hexaploid secalotriticum (RRAABB, *2n*=42). Stabilization of the secalotriticum genome in F_{1-3} correlated with the decrease of anomalies frequency in the second meiosis division, the increase of the plant fertility, the fuller expression of rye genome (ω2,3,4-secalines). The stability genotypes of secalotriticum occur in F_{5-7}. Positive correlation of fertility with a frequency of infringements in AII meiosis (r=0,96) and with presence (r=0,63) and quantity (r=0,63-0,74) of microkernels on the tetrad microspores stage was established. Stability of the heteroplasmatic triticale genome was defined by the nature of univalents. Desynaptic univalents (pseudounivalents) typical for secalotriticum, destabilized genome to a lesser degree, than asynaptic univalents in triticale. Detection of the cytogenetic factors and mechanisms of formation and stabilization of secalotriticum genome dilates the possibility of reconstruction of hybrid genomes of crops.

TRANSGENIC TOBACCO PLANTS IN SUCCESSIVE GENERATIONS: THE TRACE OF TRANSFORMATION

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Although there are numerous investigating of the post-transgenesis effects, much less is known about ones after transformation with disarmed vector which sometimes reveals unexplainable results. Frequently, presented data are limited to cell cultures or to early stages of T0-T1 generations without detailed description of development, so the ontogenesis and physiology of mature transgenic plant in many instances are unclear till now. The question how long transgenesis induced alterations are being demonstrated in a succeeding generations remains of great interest. We assessed some morphochronometric characteristics of the development of successive T1-T5 generations of *Nicotiana tabacum* L. transformed by *Agrobacterium tumefacience* strain 699 with disarmed plasmid, where vector pCNL 65 with the *npt* gene was used. The procedure of transformation carried out according to widely known Draper's method. To make sure of transgenic status of kanamycin selected tobacco T0 lines the PCR analysis was performed. Tobacco plants were grown in the same environmental conditions. Following plant characters were measured: leaf area (weekly); number of flowers per plant (daily); stem and internodes length (after harvesting); flower parts. To test the statistical significance of the differences, Wilcoxon test was used ($p<0.05$). Transgenic plants (TP) flowered earlier, produced longer stems and had increased total leaf area 2-3 times greater compared to NP. Therefore, to provide the earlier development and increased vegetative characteristics, TP must have an enhanced biosynthesis of hormones, proteins, lipids, hydrocarbons *etc.*, and have an additional pool of constituents; in other words, they will have enhanced metabolism. It is known that plants have a great potential for yield that is commonly unrealized because of insufficient adaptation to unfavorable environment. But our results were obtained at the same environmental conditions. Disarmed agrobacterial strain used for transformation doesn't contain any sense sequences directly associated with plant metabolism. So there should be another cause for such events. Many authors point to the elevations of antioxidant content, POL, activity of oxidative enzymes in transgenic plants that like nonspecific stress response. We interpret these responses as a consequence of contact with *Agrobacterium* and a transformation procedure. Agrobacterial transformation is considered to be a complex multilevel biotic stress factor. Thus, relevant alterations in phenotypes of the transgenic plants are assumed to be more likely related to the stress-reaction after agrobacterial transformation. Our results showed that first T-generations demonstrated clearly increasing plant size but then tended to decrease, returning to origin; T5 was generally similar to control plants. Based on these results, it was suggested that effect of growth and development stimulation after agrobacterial transformation had similarity with I st phase of the adaptation (according Selye), and also is accompanied by releasing of hidden metabolic reserves. Causes and details of this phenomenon are appeared to be of great interest and further investigations of the plant metabolism potential need to be continued.

CYTOGENETICS STUDIES ON SOME *MATRICARIA CHAMOMILLA* SPECIES IN IRAN

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This study was performed to identify karyotype differences between chamomile species (*Matricaria* sp.). Seeds that prepared by Forests and Pastures research Institute were 'Salmas' (cities of West Azerbaijan province, *Matricaria chamomilla*), 'Qom' (*M. chamomilla*), 'Golestan' (*M. chamomilla*), 'Baneh' (Kurdistan province, *M. talyschensis*), 'Urmia' (West Azerbaijan province, *M. tinctoria*) and an unknown region of (*M. chamomilla*). The ploidy levels were found between the masses. Basic number of chromosomes in these masses counted was $x=n=9$. Karyotype studies with measured characters such as length of long arm, length of short arm, total chromosome length, arm ratio and centromer index were measured by MicroMeasure software. Results indicate significant differences between studied masses chromosomal characters. Long arm and short arm length were two traits that determine the difference between the masses. Comparison of chromosomal traits (based on the long arm) by Duncan's test divide masses to three groups, including 'Golestan', 'Salmas' (West Azeri), 'Urmia' (West Azerbaijan) in group 'a' and 'b', respectively, and the mass of unknown masses of 'Qom' and 'Baneh' (Kurdistan) were classified in category 'c'.

EFFECT OF SALINITY ON VIRAL DISEASE SPREAD IN PLANTS

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Salt stress is an important factor affecting the quality and quantity of crop yields. The total area of the world's land exposed salinity increased to 15% in 2011 compared to 7% in 2001. In addition, crops are susceptible to disease, which strongly affects the yield. Thus, viral diseases reduce crop yield, sometimes up to 80-100%, for example Eggplant mottled crinkle virus (EMCV) can infect up to 100% yield of eggplant. Taken together, these two stress factors can cause enormous economic damage to agriculture. Despite of the importance, the effect of salinity on plant virus disease has not been well studied.

In our study, we investigated the effect of high concentrations of salt (150mM NaCl) on the systemic viral disease caused by EMCV. The virus causes the systemic necrosis in *Nicotiana benthamiana*. Systemic accumulation of virus at high concentrations of NaCl was drastically reduced. In the plants exposed to salt stress (100mM and 150mM NaCl) for 21 days before infection systemic symptoms were significantly delayed. The relationship between plant responses to biotic and abiotic stress factors may indicate the existence of universal defensive pathways of plant adaptation to unfavorable conditions.

PLANT ANTIMICROBIAL PEPTIDES

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Plants are constantly exposed to numerous pathogens, however diseases occur seldom. To cope with infection, plants have evolved a sophisticated multi-level immune system that enables them to perceive and ward off pathogen attack. Among them, antimicrobial peptides (AMPs) that inhibit growth of pathogens play an important role. In recent years they have attracted considerable interest due to their possible application in engineering pathogen-resistance in crops and development of novel pharmatheuticals. The objective of our work was to study AMPs of wild species and related forms, which display enhanced resistance to pathogens. A highly efficient procedure has been developed to isolate AMPs from *Triticum kiharae* Dorof.et Migusch., a synthetic allopolyploid produced by crossing *Triticum timopheevii* with *Aegilops tauschii*, and a cosmopolitan weed species *Stellaria media*. Wheat and chickweed belong to the families Poaceae and Caryophyllaceae, respectively. In these species, several novel AMPs were identified, their amino acid sequences determined, and for some of them three-dimensional structure was elucidated. A novel family of 4-Cys antimicrobial peptides was discovered. Their genes were shown to encode multidomain precursor proteins containing several homologous peptides both in *T. kiharae* and chickweed. Wheat genes were shown to be upregulated by pathogens and abiotic stress via activation of salicylic-acid-mediated signaling pathway. Sequences homologous to 4-Cys peptide genes were found in related *Triticum* and *Aegilops* species, however, they differ in the number of peptide modules: *Ae. tauschii* gene encodes 5 peptide domains, both *T. boeoticum* and *T. monococcum* - 6 domains. Among AMPs of *S. media*, 4-Cys peptides with potent antimicrobial activity were also discovered. They are also synthesized as long precursor proteins containing 12 homologous 4-Cys peptides. *S. media* 4-Cys peptide genes display tissue-specific expression pattern. Among AMPs of *T. kiharae*, a novel hevein-type peptide WAMP was discovered. Similar genes were discovered in Poaceae species belonging to *Triticum* and *Aegilops* genera. It is produced as a precursor protein, which consists of a signal peptide, a mature peptide domain and a C-terminal prodomain. WAMP cDNA shows sequence similarity with class 1 chitinase genes suggesting origin of WAMP genes from the chitinase genes.

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BIOLOGICAL FUNCTION OF TOMBUSVIRUS-ENCODED SUPPRESSOR OF RNA SILENCING IN PLANTS

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RNA interference (RNAi) plays multiple biological roles in eukaryotic organisms to regulate gene expression. RNAi also operates as a conserved adaptive molecular immune mechanism against invading viruses. The antiviral RNAi pathway is initiated with the generation of virus-derived short-interfering RNAs (siRNAs) that are used for subsequent sequence-specific recognition and degradation of the cognate viral RNA molecules. As an efficient counter-defensive strategy, most plant viruses evolved the ability to encode specific proteins capable of interfering with RNAi, and this process is commonly known as RNA silencing suppression. Virus-encoded suppressors of RNAi (VSRs) operate at different steps in the RNAi pathway and display distinct biochemical properties that enable these proteins to efficiently interfere with the host-defense system.

Tombusvirus-encoded P19 is an important pathogenicity factor, required for symptom development and elicitation of a hypersensitive response in a host-dependent manner. Protein plays a crucial role of TBSV P19 in protecting viral RNA during systemic infection on *Nicotiana benthamiana.* The X-ray crystallographic studies conducted by two independent groups revealed the existence of a P19-siRNA complex; a conformation whereby caliper tryptophan residues on two subunits of P19 dimers measure and bind 21-nt siRNA duplexes. These structural studies provided the first details on the possible molecular mechanism of any viral suppressor to block RNAi. The association between P19 and siRNAs was also shown to occur in infected plants These and related studies revealed that in general the ability of P19 to efficiently sequester siRNAs influences symptom severity, however this is not a strict correlation in all hosts.

The current working model is that during TBSV infection of plants, P19 appropriates abundantly circulating *Tombusvirus*-derived siRNAs thereby rendering these unavailable to program RISC, to prevent degradation of viral RNA and thus permit maintenance of viral RNA for systemic invasion. Evidence in support of this notion is that infection of *N. benthamiana* with P19-deficient tombusviral mutants was associated with the assembly of a discrete, high molecular weight RISC-like complex, which contains virus-derived siRNAs and exhibits specific ribonuclease activity.

INTEGRATIVE COMPUTER ANALYSIS OF ANTISENSE TRANSCRIPTS AND miRNA TARGETS IN PLANT GENOMES

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Non-coding RNA, including small interfering RNAs (siRNAs), are important components of gene expression in eukaryotes, forming a regulatory network. miRNAs are expressed through nucleolytic maturation of hairpin precursors transcribed by RNA Polymerase II or III. Such transcripts are involved in post-transcriptional gene regulation in plants, fungi and animals. miRNAs bind to target RNA transcripts and guide their cleavage (mostly for plants) or act to prevent translation. siRNAs act via a similar mechanism of cleavage of their target genes, but they also can direct genomic DNA methylation and chromatin remodeling. It is estimated that large fraction, up to 30% of all human genes also may be post-transcriptionally regulated by miRNAs. For plant genomes numbers could be higher depending on quality of sequencing and genome annotation. Due to availability of genome and mRNA sequences genome-wide searches for sense-antisense transcripts have been reported, but few plant sense-antisense transcript pairs have been studied. Integration of these data in specialized databases is challenging problem of computer genomics. We have developed set of computer programs to define antisense transcripts and miRNA genes based on available sequencing data. We have analyzed data from PlantNATsDB (Plant Natural Antisense Transcripts DataBase) which is a platform for annotating and discovering Natural Antisense Transcripts (NAT) by integrating various data sources [1]. NATs can be grouped into two categories, cis-NATs and trans-NATs. Cis-NAT pairs are transcribed from opposing DNA strands at the same genomic locus and have a variety of orientations and differing lengths of overlap between the perfect sequence complementary regions, whereas trans-NAT pairs are transcribed from different loci and form partial complementarily. The database contains at the moment 69 plant species. The database provides an integrative, interactive and information-rich web graphical interface to display multidimensional data. Available information for the transcription factors (TF) for each species was retrieved from the Plant Transcription Factor Database. We have compared gene structure for wheat, rice and related plant genomes. The phenomenon of antisense transcription and miRNA interference need further annotation in new sequenced genomes. GO annotation and high-throughput small RNA sequencing data currently available will be integrated to investigate the biological function of such transcripts.

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1. Chen et al. (2012) Nucleic Acids Res, 40(Database issue): D1187-93.

CHROMOSOME LOCATION OF GENETIC FACTORS DETERMINIG PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES ASSOCIATED WITH DROUGHT TOLERANCE IN WHEAT *TRITICUM AESTIVUM* L.

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Drought tolerance is characterized as the most recalcitrant trait to improve for its complexity and considered target for genomic-assisted improvement. A profitable genetic strategy lies in the discovery and exploitation of quantitative trait loci (QTL) involved in determining tolerance to water deficit at the cellular level. Enzymes of the antioxidant system participating in detoxification of reactive oxygen species accumulating under stress are the essential component of the common protective systems in cell. The same is lipoxygenase – a key enzyme of jasmonate-dependent signaling pathway initiating the development of adaptive programs in cell. Understanding of the genetic basis of wheat drought tolerance as a polygenic trait and identification of the QTL is facilitated by the availability of a number of sets of inter-varietal single chromosome substitution lines (ISCSLs) in bread wheat *Triticum aestivum* L. Two sets of bread wheat ISCSLs were used in this study. In the first set, 'Saratovskaya' 29 (S29) / 'Janetzkis Probat' (JP), the recipient was a drought tolerant cultivar and the donor of individual pairs of homologous chromosomes was a sensitive one. In the second set, 'Chinese Spring' (CS) / 'Synthetic 6x' (Syn 6x), the donor of separate chromosomes was a synthetic hexaploid wheat (*T. dicoccoides* × *Ae. tauschii*). In the set S29/JP the chromosomes of the second homoeological group and 4D chromosome were found to be critical for drought tolerance. A decrease of tolerance correlated with decreasing of antioxidant enzymes cumulative activity in leaves. In the set CS/Syn, chromosomes 4B and 4D were found to be critical for drought tolerance. The levels of LOX activity in leaves of both sets differently correlated with grain productivity but influenced positively on retaining a grain size under drought. Besides the structural genes for LOX biosynthesis situated on chromosomes of 4 and 5 homoeological groups, in both sets, the genetic factors on chromosomes 1D and 3A were associated to a large extent with the regulation of enzyme activity under water deficit. Using recombinant introgression lines developed on the base of D-genome CS/Syn ISCSLs QTL were mapped on 4D and 5D chromosomes associated with LOX activity. Study of the genetic basis of wheat drought tolerance will accelerate the development of wheat cultivars with high yield in water – deficient environment.

GENETIC DIVERSITY OF RUSSIAN ADVANCED WHEAT CULTIVARS REVEALED WITH SSR MARKERS

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A quick and reproducible tool for cultivar identification is useful to assess in certified seed production programs and to resolve legal conflicts over the recognition of a seed stock (Mangini et al., 2009). In order to obtain plant breeders' rights (PBR) the new variety has to pass the distinctness-uniformity-stability (DUS) criteria in which the candidate breeding lines are compared with existing cultivars on the basis of a series of morphological traits. Although these traits are informative and practical, they exhibit a polygenic control and are subject to environmental influences. Seed storage protein electrophoresis is included in the DUS testing guidelines, but the low level of polymorphism limits the ability to distinguish different genotypes. The resolving power of DNA markers is significantly higher allowing to prove that a new variety is unique from all other varieties that have already been described and that all individuals are as identical as possible.
As a part of research project initiated in 2011 we are developing a molecular identification key for 320 wheat cultivars registered in Russian Federation and the Republic of Belarus using SSR markers. A subset of 24 cultivars was randomly selected from the 320 cultivars and screened with 84 genomic SSR primers (Xbarc, Xgwm). At the present stage of project the SSRs with high discriminating ability were further analysed on a set of 96 genotypes. The power of each primer to distinguish among the studied genotypes was estimated by Polymorphism Information Content and the Resolving power. With the selected markers 96 genotypes were easily discriminated. A molecular identification key to distinguish Russian advanced wheat cultivars using SSR-profiling is discussed. We suggest the reproducible fingerprint system for the identification of Russian hexaploid wheat cultivars that could be employed in certified seed production programs to identify sources of seed contamination, and to distinguish cultivars with similar phenotype.

THE STUDY OF ROOT SYSTEM IN INTER-VARIETAL SINGLE CHROMOSOME SUBSTITUION AND INTROGRESSION LINES 'CHINESE SPRING/SYNTHETIC 6X' OF BREAD WHEAT

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Root length and weight was studied in the set of inter-varietal single chromosome substitution lines 'Chinese Spring/Synthetic 6x' (CS/Syn) where the donor of separate chromosome pairs was a synthetic hexaploid AABBDD (*Triticum dicoccoides* × *Aegilops tauschii*). These parameters were studied under normal and restricted water supply. It was found that 1A substitution resulted in a substantial reduction of root size in plant. Substitution of 5D chromosome, on contrary, led to its significant increase comparing to the recipient and donor. On the next stage of the work, a set of genotyped introgression recombinant lines CS/Syn 5D (Pestsova et al. 2001) was studied under the same conditions. The lines were studied after 30-days vernalization as the donor of 5D chromosome, Synthetic 6x, has a winter growth habit. The greatest root weight and length as well as a number of days till flowering comparable to 5D substitution line was detected in the introgression lines 5D-5, 5D-6 и 5D-10. These lines has a common introgression fragment in the long arm of 5D chromosome marked with the molecular marker *Xgwm292* and the gene *Vrn-D1* (Pestsova et al. 2006). The lines were additionally studied under 45- and 60-day vernalization. It was found that in both cases these three lines had the most developed root system. Generally, a drought effected depressingly on root development in all lines but the greatest root weight and length was detected in the substitution line for 5D chromosome and in the introgression lines 5D-5, 5D-6 и 5D-10. The data obtained may prove the existence of the locus responsible for traits under study in this region of 5D chromosome. Seeds of the lines were kindly provided by Andreas Börner (IPK, Gatersleben, Germany).

AMIODARONE INDUCES THE SYNTHESIS OF HSPS IN *SACCHAROMYCES CEREVISIAE* AND *ARABIDOPSIS THALIANA* CELLS

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Many biotic and abiotic stresses cause an increase of cytosolic Ca^{2+} level in cells. Calcium is one of the most important second messengers, regulating many various activities in the cell and was known to affect expression of stress activated genes. Mild heat shock induces the expression of heat shock proteins (Hsps) which protect cell from drastic heat shock exposure. There are some literature data permitting to suggest that transient elevation of cytosolic Ca^{2+} level in plant cells is important for activation of Hsps expression. On the other hand mitochondria are known to regulate the intracellular calcium and reactive oxygen species signaling. It has been shown recently that mild heat shock induces hyperpolarization of inner mitochondrial membrane in plant and yeast cells and this event is critically important for activation of Hsps expression. To reveal the relationship between mitochondrial activity, intracellular calcium homeostasis and Hsps expression an antiarrhythmic drug amiodarone (AMD) have been used. AMD is known to cause transient increase of cytosolic Ca^{2+} level in *Saccharomyces cerevisiae*. Obtained results have showed that AMD treatment induced the synthesis of Hsp104p in *S. cerevisiae* cells and Hsp101p in *A. thaliana* cell culture. Induction of Hsp104p synthesis leads to enhanced yeast capability to survive lethal heat shock exposure. Development of *S. cerevisiae* thermotolerance depended significantly on the presence of Hsp104p. Elevation of Hsp104p level in the result of AMD treatment was shown to be governed by activity of Msn2p and Msn4p transcription factors. Deletion of the *MSN2* and *MSN4* genes abrogated the AMD ability to induce Hsp104p synthesis. Mild heat shock and AMD treatment induced the hyperpolarization of the inner mitochondrial membrane in yeast and Arabidopsis cells which accompanied by HSP synthesis and development of thermotolerance. It was suggested that increase of cytosolic Ca^{2+} level after AMD treatment directly or indirectly causes the activation of mitochondrial activity which leads to hyperpolarization of the inner mitochondrial membrane and production of reactive oxygen species (ROS). Modulation of cellular Ca^{2+} and ROS signals by mitochondria is assumed to play a prominent role in activation of Hsps expression in yeast and plant cells.

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TRANSGENIC PLANTS OF RAPE (*BRASSICA NAPUS* L.) WITH GENE *OSMYB4* HAVE INCREASED RESISTANCE TO SALTS OF HEAVY METALS

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This work aims to study the response of the transgenic spring rape plants (*Brassica napus* L. var. 'Westar') with the rice transfactor-encoding gene *Osmyb4* to treatment with salts of heavy metals (HM) CuSO4 or ZnSO4 and accumulation in the leaves of biomass, metals, photosynthetic pigments, lipid peroxidation, and antioxidant compounds: total phenols, anthocyanins, and antioxidant enzyme activity superoxide dismutase (SOD) and guaiacol peroxidase (POX) were determined. Vegetatively propagated transgenic plants and wild-type plants were grown on Hoagland-Snyder medium at 24°C, then at the 5-6th leaves-stage, CuSO4 (in concentration 25-150 mM) or ZnSO4 (500 - 5000 mM) were added to the growth medium, and plants were exposed to the salts for 15 days. Under the action of small concentrations of salts, the results obtained for the transgenic and untransformed plants did not differ, but at high concentrations strong differences between transgenic and untransformed plants were observed. In transgenic plants, accumulation of biomass was greater. Carotene and xanthophyll were destroyed in transgenic plants less than in the untransformed plants. They have accumulated in their leaves more metal, especially Zn, reaching almost to the accumulation of 7 mg per g of dry biomass, bringing these plants to the hyperaccumulation of Zn. In the tissues of transgenic plants exposed to high concentrations of salts, the content of total phenols, anthocyanins, and low molecular weight compounds, that are responsible for protection against ROS, increased significantly. All these results indicate a greater stability of the transgenic plants to the action of heavy metals, as evidenced also by less activity of lipid peroxidases in their tissue: at high salt concentrations, malondialdehyde (MDA) accumulated significantly less in transgenic plants than in non-transformed plant tissues. The greater stability of transgenic plants to stressful effect of HM is also evidenced by the fact that at very high concentrations of salts non-transformed plants died after 12-13 days, whereas the transgenic oilseed rape remained alive long enough time. Thus, the incorporation of the plant gene transcription factor OSMYB4 increased the resistance of transgenic plants to the stress effect of HM.

THE EXPERIENCE OF THE TRANSFORMATION OF SOME CULTIVATED PLANTS WITH THE GENE *UGT* ENCODING THE SYNTHESIS OF UDPG-TRANSFERASE IN ORDER TO CHANGE THE HORMONAL STATUS

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The gene *ugt/iaglu* was isolated from cDNA library obtained from seedlings of *Zea mays* L. Positive clones prepared by Lambda ZAPII (Stratagene, USA) procedure were screened via western blot with antibodies to UDPG-transferase from corn endosperm raised in rabbit serum. The plasmid pBluescript harboring the gene *ugt/iaglu* was placed into *Escherichia coli* (*E.coli*) DH5a under T7/T3 promoter. The gene *ugt/iaglu* was sequenced and the size was determined as much as 1740 bp. The UDPG-transferase or by trivial name Indoleacetic acid (IAA) - glucose synthase (IAGlu-synthase) binds IAA with glucose from UDPG thereby making the temporary inactivation and storing of this phytohormone which is capable to be released after the demand from cells. Several cultivated plants were used for transfromation with the gene *ugt/iaglu* from corn: tomato, potato, lettuce, egg-plant, pepper, strawberry, cucumber, squash, aspen, poplar, pine and others. All plants transformed with the gene *ugt/iaglu* showed fast growth, better flowering and harvest. The insertion and expression of the gene *ugt/iaglu* was confirmed in transformed tomato, potato and aspen with PCR, RT-PCR, southern and northern blottings. The contents of free IAA and its bound form IAGlu were higher as much as twice in tomato, potato and aspen transformed with the gene *ugt/iaglu*. The harvest of tomato was 3-4 times higher in transgenic tomato. The amount of potato tubers and their whole masses were 1.5 - 2 times higher in transgenic potato of several varieties in comparison to control.

EFFECT OF LOW TEMPERATURE AND CADMIUM ON THE GENE EXPRESSION OF ATP-DEPENDENT PROTEASES IN LEAVES OF WHEAT AND CUCUMBER SEEDLINGS

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Proteases are involved in proteins degradation and prevention against the accumulation of damaged proteins and peptides in the cell, thus play an important role in plant responses to various unfavorable environmental conditions. Considering that the affect of stress factors on plants is accompanied by significant changes in protease activity, it is logical to expect changes in the corresponding gene expression. However, the proteases gene expression in plants under the influence of unfavorable environments is poorly studied. The goal of our study was to analyze the effect of low temperature and cadmium on the expression of the genes encoding ATP-dependent proteases of chloroplasts and mitochondria. The experiments were performed on seedlings of winter wheat (*Triticum aestivum* L.) cv. 'Moskovskaya 39' and cucumber (*Cucumis sativus* L.) cv. 'Zozulya' grown for 7 days. Seedlings were subjected to hardening low-temperature treatment (4ºC for wheat and 10ºC for cucumber) during 7 days. Wheat seedlings also were subjected to cadmium sulphate (100 μM) treatment for 7 day as well as the combined (simultaneous) action of low temperature and cadmium. The level of the *Lon1* and *ClpP* genes expression in leaves was analyzed by Real-Time PCR. The obtained results showed that the level of the *Lon1* and *ClpP* gene expression in the leaves of cold tolerant wheat plants significantly increased in the initial period (1–5 h) of cold hardening, reached its maximum after 2 days and retained practically the same until the end of the experiment. The content of transcripts of the *ClpP* gene in leaves of cold sensitive cucumber plants altered to less degree. In the initial period (first few hours) of wheat plant treatment with cadmium the expression of the *Lon1* and *ClpP* genes increased, during following 1–2 days it gradually decreased and after 3 days returned to high level. The combined (simultaneous) impact of low hardening temperature (4^{o}C) and cadmium showed already after 0.5 h from its beginning the significant increase in the level of *Lon1* and *ClpP* transcripts. At a later time (5–7 days) the additive effect of both factors on proteases gene expression was shown. Owerall, the increase in gene expression of ATP-dependent *Lon1* and *ClpP* proteases in wheat seedlings leaves under the influence of low hardening temperature and cadmium as well as combined impact of chilling and cadmium supposed their participation in nonspecific protective responses of plants to different environments.

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REARRANGEMENT IN THE B-GENOME FROM DIPLOID PROGENITOR TO WHEAT ALLOPOLYPOLID

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Three key periods that were accompanied by considerable rearrangements in the B genome of wheat and its progenitor can be considered. The first period covers the period from the divergence of diploid *Triticum* and *Aegilops* species from their common progenitor (2.5–6 million years ago) to formation of the tetraploid *T. diccocoides* (about 500 thousand years ago). Significant genomic rearrangements in the diploid progenitor of the B genome, *Ae. speltoides* (SS genome), involved a considerable amplification of repeated DNA sequences, which led to an increase in the number of heterochromatin blocks on chromosomes relative to other diploid *Aegilops* and *Triticum* species. Our analysis has demonstrated that during this period the Spelt1 repeats intensively amplified as well as several mobile elements proliferated, in particular, the genome-specific gypsy LTR-retrotransposon Fatima and CACTA DNA-transposon Caspar. The second period in the B-genome evolution was associated with the emergence of tetraploid (BBAA genome) and its subsequent evolution. The third most important event leading to the next rearrangement of the B genome took place relatively recently, 7000–9500 years ago, being associated with the emergence of hexaploid wheat with the genomic formula BBAADD. The evolution of the B/S genome involved intergenomic and intragenomic translocations and chromosome inversions. So far, five rearrangements in the B-genome chromosomes of polyploid wheats has been observed and described; the majority of them took place during the formation and evolution of tetraploid species. The mapping of the S-genome chromosomes and comparison with the B-genome chromosome maps have demonstrated that individual rearrangements pre-existed in *Ae. speltoides*; moreover, *Ae. speltoides* is polymorphic for these rearrangements.

Chromosome 5B is nearly 870 Mbp (5BL = 580 Mbp and 5BS = 290 Mbp) and is known to carry important genes controlling the key aspects of wheat biology, in particular, *Ph1*, critical for correct mitosis and meiosis in the allopolyploid nucleus; *Kr1*, controlling interspecific incompatibility; the genes controlling hybrid necrosis and response to vernalization, *Ne1* and *Vrn-B1*; and genes controlling resistance to various pathogens and bread-making quality. The translocations and inversions of chromosome 5B/5S, which could have taken place in the evolution of *Ae. speltoides* and allopolyploid wheats, yet has not been detected so far. On the other hand, the changes in chromosome 5B that had brought forth the locus *Ph1* took place due to certain yet unknown mechanisms. Construction of the physical map for chromosome 5B and determination of its primary structure are in progress now.

PERSPECTIVES OF THE DEVELOPMENT OF MUCOSAL VACCINES AGAINST DANGEROUS INFECTIONS ON THE BASE OF TRANSGENIC PLANTS

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Mucosal vaccines created on the base of transgenic plants reacting with mucosal layers of the intestines and other organs are considered to be the perspective method of the vaccination. These vaccines induce both mucosal and general humoral immunogenicity after the peroral administration. The folding of antigenic proteins synthesizing in plants occurs via eukaryotic type and has advantages before yeast and prokaryotic platforms. This feature results to more adequate synthesis of antibodies against pathogens and to the interaction with effector molecules of complement. Earlier we together with The State Scientific Center "Vector", Institute of chemical biology and fundamental medicine SB RAS and Dr R.Hammond from Laboratory of Plant Pathology (Maryland, USA) created two candidate vaccines : one of them against AIDS (HIV-1) and hepatitis B on the base of the chimeric gene TBI-HBS, encoding simultaneously 9 antigenic determinants of HIV-1 and the main surface antigen of hepatitis B (HBsAg). The second candidate vaccine was created against hepatitis B on the base of the genetic construct with the gene preS2-S encoding the synthesis of two subunits of the main surface antigen of hepatitis B and the signal peptide HDEL which directed antigens for the accumulation on ER. Both vaccines were tested on mice and confirmed their immunogenicity as the pronounced antibodies response. Twice vaccinated mice maintained the antibodies response during 11 months after there was little tendency to lowering. It was established that transgenic plants – vaccines (tomato) kept the capability to the synthesis of antigenic determinants in seven seed generations during 7 years. The results of the development of the mucosal vaccine against cervical carcinoma (carcinoma of uterine cervix) evoked by human papillomaviruses of high oncogenic risks were presented in this report. We created the genetic construct consisting of 35S CaMV promoter, Ω (omega) leader of TMV, the target gene HPV16 L1 and the nos terminator. The target gene HPV16 L1 of the most oncogenic type 16 of human papillomavirus was choosen as the object. Different procedures of the plant transformation were elaborated and the transgenic plants synthesizing the antigenic protein L1 of human papillomavirus of type 16 were obtained. The insertion and the expression of the target gene were controlled by northern blotting, the synthesis of antigenic protein HPV16 L1 was determined by ELISA and western blot. The antigenic protein of HPV16 L1 was synthesized in amount of 20 – 50 ng/mg of total soluble proteins in tomato transgenic plants. The results of the examination of the immunogenicity of the vaccine obtained by means of the peroral immunization of mice were showed in the report. Therefore it was demonstrated the principal opportunity of the creation of mucosal vaccines on the base of transgenic plants against several dangerous diseases.

SUPEROXIDE DISMUTASE ACTIVITY IN *A. THALIANA* LEAVES DURING ACCLIMATION TO HYPOTHERMIA

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It is known that low temperature damages both cold-sensitive and cold-tolerant plants particularly due to oxidative stress, i.e. increase in reactive oxygen species (ROS) content. Oxidative stress leads to lipid peroxidation damaging membrane structures. The initial stage in ROS generation is a formation of superoxide anion (O_2^-). One of the most important antioxidant enzymes is superoxide dismutase (SOD) neutralizing superoxide anion. Usually plant cells contain several SOD types. They differ in their structures and molecular masses and contain different metal ions. The goal of the present work was to study the SOD types present in the Arabidopsis cells and changes in their activity levels during acclimation to hypothermia. *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia plants were grown in compost at 22ºC and 8-h photoperiod to a fully expanded rosette stage (6 weeks). Plants were exposed to 2ºC at 16-hour light for 5 days. Samples were collected daily. Control plants were not exposed to low temperature. Protein extracts from plant leaves were purified by centrifugation and gel-filtration on a PD-10 column. Then the samples were subjected to non-denaturing electrophoresis in polyacrylamide gel as described by Ornstein and Davis (1964). The SOD activity was detected by staining with nitroblue tetrazolium. The SOD types were identified using inhibitory analysis: Cu/Zn- and Fe-containing SOD activities are inhibited with hydrogen peroxide, Cu/Zn-containing SOD activity is inhibited with KCN, while Mn-containing SOD activity is not inhibited by mentioned substances. In *A. thaliana* cells, we observed three types of SOD, namely: Mn-SOD, Fe-SOD and Cu/Zn-SOD. Mn-SOD had the highest activity of about 88% out of total SOD activity but we did not observe any change in its activity within acclimation period. Activity levels of Cu/Zn- and Fe-SOD were about 2% and 1% out of total SOD activity, respectively. There was no change in Fe-SOD activity but Cu/Zn-SOD activity increased transiently. It reached maximum on the 2nd day (nearly 10 times higher comparatively to initial) and then decreased gradually by the end of acclimation period. We also found a polypeptide possessing SOD activity with mol wt higher than proposed Mn-SOD which was not inhibited neither with H_2O_2 nor with KCN and its activity did not change during acclimation. Contribution of specific SODs to oxidative stress tolerance requires further studies.

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THE APPROACHES TO THE ANALYSIS OF ORGANIZATION OF 5BS CHROMOSOME OF *TRITICUM AESTIVUM* L.

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Triticum aestivum (bread wheat) is the allohexaploid with genomic formula BBAADD, (*2n = 6x =* 42) and the large genome size of 17 000 Mbp. Approximately 90% of wheat genome accounts for repetitive sequences of different origin and degree of reiteration that makes the wheat genomic sequencing very difficult and expensive. At the present day the wheat genome sequencing is in progress, coordinated by International Wheat Genome Sequencing Consortium (IWGSC), and we take an effort by the physical mapping of the 5B chromosome.

Physical mapping is the essential stage of wheat genomic sequencing, and includes the fingerprinting of chromosome-specific BAC-library followed by selection of minimal set of overlapping clones, and anchoring of mapped molecular markers to the BAC-clones. The anchoring includes the PCR-screening of BAC-library with selected and mapped markers. We performed the fingerprinting and the subsequent analysis of 5BS chromosome-specific BAC library (contains 43776 clones, 15-fold chromosome coverage). For fast and efficient PCR screening we pooled the clones of the library. For mapping and screening we used the already published markers (SSRs, sequences of the known genes) as well as newly developed ones. The crucial point for physical mapping is to obtain the set of markers distributed uniformly along the chromosome. The 454-sequencing technology allows us to develop such a kind of PCR markers named ISBP (Insertion Site Based Polymorphism). We have 39695 454-reads with total length 16183252 bp (5.5% of chromosome) and 1302 markers obtained with ISBPFinder program. For reduce the number of markers to analyze, we performed the BLAST analysis of amplicon specificity against the wheat genomic sequences at NCBI database. 12% of ISBP-amplicons showed >90% identity with published sequences; among them 4% strongly matched with 5BS chromosome, 58% had no data on chromosomal localization, 38% matched with another chromosomes. We effectively used some of the selected markers for the mapping of deletion lines and screening of 5BS-specific BAC-library. The obtained results will contribute to the successful physical mapping and sequencing of the 5B chromosome of the bread wheat.

GENETIC DIVERSITY AND ITS RELATIONSHIP TO HYBRID PERFORMANCE IN WHITE CABBAGE

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Breeding cabbage is mainly aimed at creating hybrids. This is due to the economic value of heterosis, as an effective mechanism for increasing yield of many crops. In accordance with the needs of practical breeding, we tried to find the tests for effective mating design and to predict end-productive effect of F_1 white cabbage hybrids. In this study the divergence of white cabbage collection (31 lines) based on RAPD-and ISSR-screening and the predictive potential of DNA markers for hybrid breeding have been evaluated. Polymorphic genotypes were selected and diallel crosses (5×5) were conducted. We carried out analysis of hybrid performance (F1P), mid- (MPH) and high-parents heterosis (HPH), and then correlations were investigated. The study of associations of genetic distances (GD) and heterosis has found that ISSR-GD, MPH and HPH don't have close relations for the majority traits, except the cabbage head density, for which positive correlation (r=0.4*) exist. Using of Euclidean distances which were calculated from quantitative considerations was the most successful for the prediction of the hybrid vigor. Thus, 'vegetative mass of plants' and 'mean weight of loaf' had positive associations with values 0.44 and 0.56, respectively. Relations of GD with hybrid performance (F1P) were higher than with heterosis. ISSR GD were significantly and positively associated with such traits expression as 'weight of external heart of cabbage head' (r=0.79) and its ratio to 'weight of head' (r=0.66). Likewise average correlation is observed for ISSR GD and F1P for 'number of leaves' (r=0.45) and 'diameter external heart of cabbage' (r=0.51). In contrast Euclidean distances were negatively associated with such traits as 'average weight' and 'volume of head'. In addition there are no significant correlations between RAPD, GD and F1P. Taking into account our results we can conclude the analysis of genetic heterogeneity of white cabbage can be useful, along with classical breeding approaches, because it allows allocate variability in breeding collections, which has no phenotypic expression and can be associated with valuable traits. It should be borne in mind that selection will be more effective for hybrid populations or for creating synthetic variety, where average expression of the trait is more important.

HVP10 (V-PPase), A CANDIDATE GENE FOR *HvNax3* CONTROLLING SODIUM EXCLUSION AND SALINITY TOLERANCE IN BARLEY: MAPPING, SEQUENCE ANALYSIS AND GENE EXPRESSION

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Salinity is a major abiotic stress limiting the production of agricultural plants in Australia and in other countries across the world. Wild relatives of cultivated barley have wider diversity in tolerance to salinity. We previously reported the identification of a major QTL for sodium exclusion (*HvNax3*) on chromosome 7HS, in a barley mapping population originating from a cross between the Australian feed barley Barque-73 and a *Hordeum spontaneum* accession, CPI-71284. Initial analysis of an AB-QTL population and F_2 recombinants reduced the interval containing *HvNax3* from 15.0 cM to 1.3 cM. For fine mapping of this region, four F_3 progenies (60-100 individuals in each) with different recombination events were genotyped with various CAPS markers and phenotyped for sodium exclusion. The interval was further reduced to 0.4 cM, limiting the number of candidate genes based on rice-barley synteny to five, with the most promising candidate encoding a vacuolar pyrophosphatase proton pump, V-PPase (*HVP10* gene). The protein encoded by this gene has been shown to be responsible for establishing an electrochemical gradient across the tonoplast that allows other transporters such as Na^+/H^+ antiporters to transport sodium into the vacuole, thereby reducing toxic effects of excess Na^+ in the cytosol. BLAST analysis of sequences of the complete *HVP10* gene from both parents indicated the presence of eight exons and seven introns, with an open reading frame of 4,356 bp. The eight exons were well-conserved with only seven SNPs in the coding regions identified between the two parents but none of the SNPs altered the amino-acid sequence. The differences in Na^+ accumulation between the two parents is, therefore, not related to the coding sequence of the *HVP10* gene. However, Q-PCR experiments showed that expression of the gene in shoots and in roots of CPI-71284 was two-fold and 24%, respectively, higher than in Barque-73 on the third day following exposure to salt stress. The *HVP10* gene may be related to differences in the promoter region. To clone the promoter of the *HVP10*, a barley BAC clone library (cv. Morex) was screened using the *HVP10*-specific primers to identify positive clones. Sequencing of one positive BAC clone (0262-H05) has allowed primers to be designed approximately 2 – 2.5 kb upstream of the start codon of the *HVP10*, so that the promoter from both Barque-73 and CPI-71284 can be isolated. In combining these data, we expect to obtain a complete picture of the sequence and functional differences in the *HVP10* gene between the two parents, lines from AB-QTL population and in the segregating progenies in the response to salt stress. This will help us to better understand the sequence structure and role of the favourable allele of the *HVP10* gene originating from wild barley, *H. spontaneum*, with an introgression of the gene into commercial cultivated barley for improvement of salinity tolerance.

A WIDE DISTRIBUTION OF A NEW *VRN-B1c* ALLELE OF WHEAT *TRITICUM AESTIVUM* L. IN RUSSIA, UKRAINE AND ADJACENT REGIONS: A LINK WITH THE HEADING TIME AND ADAPTIVE POTENTIAL

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The adaptation of common wheat (*T. aestivum* L.) to diverse environmental conditions is greatly under the control of genes involved in determination of vernalization response (*Vrn-1* genes). It was found that the variation in common wheat heading time is affected not only by combination of *Vrn-1* homoeoalleles but also by multiple alleles at a separate *Vrn-1* locus. Previously, we described the *Vrn-B1c* allele from *T.aestivum* cv. 'Saratovskaya 29' and found significant differences in the structure of the first (1st) intron of this allele when compared to another highly abundant *Vrn-B1a* allele, specifically, the deletion of 0.8 kb coupled with the duplication of 0.4 kb. We suggested that the changes in the intron 1 of *Vrn-B1c* allele caused earlier ear emergence in the near-isogenic line and cultivars, carrying this allele. In this study we investigate the distribution of the *Vrn-B1c* allele in a wide set of spring wheat cultivars from Russia, Ukraine and adjacent regions. The analysis revealed that 40% of Russian and 53% of Ukrainian spring wheat cultivars contain the *Vrn-B1c* allele. The high distribution of the *Vrn-B1c* allele can be explained by a frequent using of 'Saratovskaya 29' in the breeding process inside the studied area. From the other hand, the predominance of the *Vrn-B1c* allele among cultivars cultivated in West Siberia and Kazakhstan may be due to the selective advantage of this allele for the region where there is a high risk of early fall frosts.

GENETIC DETERMINATION OF THE NITROGEN SUPPLY OF SPRING WHEAT (*TRITICUM AESTIVUM* L.)

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The maximum grain productivity can be achieved only taking into account plant biological needs. The need in mineral nutrients depends on the plant hereditary nature and environmental conditions. The greatest demand for nitrogen is characteristic in cereals for spring and winter wheat, the lowest one for barley and rye. The use of mineral nutrients in amounts exceeding plant needs does not result in yield increasing and can worsen the production quality. We were studying reaction of spring bread wheat (*Triticum aestivum* L.) genotypes to changes in nutrition soil conditions. In the experiment, ITMI mapping population consisting of 110 recombinant inbred lines was evaluated for a number of morphological, biological and economically important traits under different levels of the nitrogen supply. To create different soil nutrition level and to prevent leaching of fertilizers during the plant vegetation season we prepared trenches with depth 0.4 m, width 1 m and length 20 m; the bottoms of which were covered with plastic films. The trenches were filled with soil from the lower soil horizons. In first variant of the experiment, nutrient mixture on the basis of the physiological rate for cereals (N – 0.15 g, P - 0.1 g, K - 0.1 g of active substances per 1 kg of dry soil) was applied. In the second variant, nitrogen dose was reduced half with the same phosphorus and potassium doses. Variant without fertilizers applying was used as a control. Thirty nine characters were analyzed during the all growing season. The combination of field and vegetation experiment conditions allowed approximating maximally to real conditions of the experiment and at the same time to control strictly plant vegetation. QTLs identified in our study can be differentiated as dependent and independent on environmental conditions. For example, some QTLs controlling such traits as a wax bloom, phenological phases, *etc.* are stable under different conditions of soil nutrition. QTLs of traits determining the yield structure were unstable and changed their locations on chromosomes under different conditions of nitrogen supply. The most of identified QTLs changed their locations at different nitrogen doses, and in some cases additional QTLs were found which also influenced to a particular trait expression. The activity of genes blocks determining the physiological and morpho-agronomical and biological quantitative traits was experimentally shown to depend on the mineral nitrogen doses. Identification of chromosomal loci involved in the nitrogen metabolism allows planning more accurately breeding programs directed to increase the plants productivity.

VARIATION IN THE INTERGENIC SPACERS OF RIBOSOMAL DNA OF RYE SPECIES

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The non-coding rDNA spacers (IGS) and the internal transcribed spacer (ITS) could be substantially variable in size due to differences in the number of repetitive elements among the closely related species. The repetitive and highly variable nature as well as the fast evolution rate allowed the rDNA spacers to be considered valuable alternative molecular marker systems. Three pairs of universal primers were used for amplification of non-coding regions of ribosomal (rRNA) IGS. The IGS amplified products obtained from 20 Secale accessions, consisting of cultivated and non-cultivated rye, and represent three species and four subspecies of rye genus, showed a high level of polymorphism. The primers for IGS PCR resulted in multiple bands (2 – 13), different size (59.9 bp-3249.7 bp) and polymorphism average 81.3%.

Cluster analysis by the Neighbor-Joining method in the Molecular image Gel DocTM XR (Bio-Rad) program on the basis of the Dice's coefficient of genetic similarity indicated a division of the species studied into three groups of similarity. *S. vavilovii* (Hungary) *S. vavilovii* (Russia) made a separate group. The second group includes nine species: *S. strictum* (Turkey), *S. strictum* (Australia), *S. ancestrale* (Japan), *S. afganicum* (Armenia), *S. segetale* (Azerbaijan), *S. anatolicum* (Canada), *S. ancestrale* (Turkey), *S. cereale* (Macedonia), *S. cereale* (USA). The third – the remaining species of rye.

Highly inter specific polymorphisms for rRNA IGS region suggesting the IGS will be a useful molecular marker for studies of *Secale* species.

SYNTHESIS OF THE MAIN ANTIGENE PROTEIN (HBsAg) OF A VIRUS OF HEPATITIS B IN TOMATO PLANTS TRANSFORMED WITH THE preS2-S-HDEL GENE

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We received tomato plants, transgenic on the preS2-S-HDEL gene encoding the main hepatitis B virus (HBV) surface antigen protein HBsAg. The choice of the preS2-S gene is reasoned by the stronger immune answer at a joint expression of genes of S and preS2. The alarm sequence HDEL (histidine-aspartat-glutamate-leucine) was used for accumulation of the preS2-S gene expression products in endoplasmic reticulum that should have positive effect on the antigen level. Availability of the main antigen protein of HBV in fabrics of the transformed plants was confirmed by means of the enzyme-linked immunosorbent assay (ELISA). It has been shown by researches that in the transformed plants of generation T1 the amount of antigen HBsAg protein reaches high values comparable with positive control containing 20±10 ayw2 subtype ME/ml HBsAg. Processing of results revealed that level of antigen protein in fruits of the transformed plants is rather lower than that in leaves, nevertheless it considerably exceeds level of negative control. For additional verification of the data obtained the competitive ELISA was carried out, which showed that the optical density of control samples at noncompetitive and competitive ELISA practically doesn't change. The investigation of samples obtained from the transformed plants showed that signal suppression exceeds 50 % indicating reliability of the results of the noncompetitive ELISA and the presence of the HBsAg protein in fruits of T1 plants. The data we obtained demonstrate successful integration and expression of the target gene preS2-S-HDEL in the transformed plants. These results open the possibility for further work on the creation of a candidate edible vaccine against hepatitis B on the basis of transgenic tomato plants.

PHOSPHORYLATION/DEPHOSPHORYLATION OF MITOCHONDRIAL PROTEINS IN REDOX-SIGNALLING OF HIGHER PLANTS UNDER ABIOTIC STRESS CONDITIONS

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We studied an impact of the widely spread intra-cellular signals Ca^{2+} and cAMP on activity of the protein phosphorylation in maize mitochondria. The use of the isolated mitochondria is a convenient model system for investigation of the different physiological processes, for example for simulation of the different stress conditions. The treatment of maize mitochondria with high concentration of calcium ions which mimics the initial stage of apoptosis led to an increase of the phosphorylation level of some proteins and to an additional phosphorylation of the 59 and 66 kDa proteins. The treatment of the mitoplasts, i.e., the mitochondria devoid of the outer membrane with calcium ions insignificantly induced the activity of protein phosphorylation. It is assumed that the outer membrane is essential for Ca^{2+} signal transduction to plant mitochondria. We also identified a 94 kDa protein involved in phosphorylation of the mitochondrial proteins. This protein might be a single-subunit protein kinase or one of the subunits of the protein kinase complex. Antimycin A and KCN which are the inhibitors of mitochondria respiration increased the phosphorylation activity of the mitochondrial polypeptides. The effect of this inhibitors was similar both in *in organello* system and at the level of the whole plant. It should be noticed that at the level of the whole plant the effect of KCN on activity of the mitochondrial protein phosphorylation was more essential. Some considerable differences were found both at the level of protein phosphorylation and in electrophoresis patterns representing the intact mitochondria, the mitoplasts and the outer membrane fraction. The activity of protein phosphorylation in mitoplasts and the outer membrane fraction was extremely high compared to the phosphorylation activity of the mitochondrial proteins. This could be explained by the higher level of "substrate phosphoprotein phosphatase" in the outer membrane of mitochondria. This phosphoprotein phosphatase primarily dephosphorylates the protein of the inner membrane. When the proteins of the outer membrane were added together with the phosphorylated mitoplast proteins, the activity of phosphorylation effectively decreased. Thus, the phosphoprotein phosphatases of the outer membrane could dephosphorylate the inner membrane proteins and vice versa, the inner membrane phosphatases dephosphorylate the outer membrane proteins. Mn-SOD is the first line protection enzyme of the mitochondrial DNA, as this enzyme scavenges superoxide anion and severely reduces the toxic influences of ROS. Till now, the regulation of this enzyme activity is not clear. One of such mechanisms can be the phosphorylation of serine and threonine residues. We studied the effect of different redox agents on the phosphorylation of the mitochondrial Mn-SOD by using the recombinant Mn-SOD protein. It was found out that the oxidizing agent $K_3[Fe(CN)_6]$ led to dephosphorylation of the Mn-SOD, while, at the same time, such reducing agents as sodium dithionite and GSH did not exert any effect on phosphorylation.

DISRUPTION OF *ARABIDOPSIS* RETICULON GENE *RTNLB16* RESULTS IN CHLOROPLAST DYSFUNCTION AND OXIDATIVE STRESS

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Reticulons (RTNs) are endoplasmic reticulum (ER)-localized proteins that have recently attracted much attention. RTNs are ubiquitous proteins present in all eukaryotic organisms examined so far. In animal and yeast, in which knowledge of this protein family is more advanced, RTNs are involved in numerous cellular processes such as apoptosis, cell division and intracellular trafficking. Up to now, a little attention has been paid to their plant counterparts, RTNLBs. Meanwhile, gene search across sequenced genomes revealed that the RTN gene family is more diverse and numerous in plants than in animals and yeasts, which possibly suggests existence of functions specific for plant RTNs. Recently, the localization in different ER regions was shown for two members of plant reticulon family. The location in close proximity to chloroplast membrane was revealed for one of RTNLBs, which is argument in favor of its role in interorganellar interactions. In spite of growing interest towards to plant RTNs, there are no investigations devoted to insertion mutagenesis of genes encoding these proteins. We have genotyped an Arabidopsis line containing T-DNA insertion in *RTNLB16* gene encoding uncharacterized member of RTNLB family. The obtained homozygous plants have marked phenotype expressed in a decreased growth rate and a pale-green leaf color. The leaf total chlorophyll content as well as the chlorophyll a/b ratio was significantly lower in mutant plants. It is interesting to note that the extent of phenotypic expression depended on a light intensity. The growth rate of wild-type and mutant plants was the same in low light conditions. The growth rate was significantly decreased and chlorophyll content was 3-5-fold lower in mutant plants growing under moderate light conditions. The growing of plants under high light conditions led to halted growth and death of mutants on the seedling stage. The demonstrated phenotype probably points out to a chloroplast dysfunction and resembles the phenotype of plants with inactivated genes encoding chloroplast proteins. The study of reactive oxygen species (ROS) level revealed the significantly elevated superoxide content in the mutant plant leaves. Moreover, the measurement of enzymatic activity of different superoxide dismutase isoforms showed an increased level of CuZnSOD which is localized predominantly in chloroplasts. At the same time, the level of mitochondria-localized MnSOD remained unchanged. This fact also points to chloroplasts as a potential source of increased ROS content in mutant plants. To test this hypothesis, we studied the ROS level in the guard cells of mutant and wild-type plants. As a result, the significant increase of chloroplast-derived ROS content in guard cells of mutant plants was showed. Therefore, we conclude that an inactivation of the *RTNLB16* gene leads to severe defects in chloroplast functioning and assotiated oxidative stress. We suppose that RTNLB16 protein participates in interactions between chloroplasts and other intracellular structures. The work was supported by RFBR (12-04-01027-a) and SB RAS Integration project 59.

ANTHOCYANIN PIGMENTATION IN *TRITICUM AESTIVUM* L.: GENETIC BASIS AND ROLE UNDER ABIOTIC STRESS CONDITIONS

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Anthocyanins are secondary metabolites of plants. They have a wide range of biological activity such as antioxidant, photoprotection, osmoregulation, heavy metal ions chelation, antimicrobial and antifungal activities, which help plants to survive under different stress conditions. Bread wheat (*T. aestivum* L.) can have purple pigmentation provided by anthocyanin compounds in different organs, such as grain pericarp, coleoptile, culm, leaf blades, leaf sheaths, glumes and anthers. However, the genetic mechanisms underlying formation of these traits as well as contribution of the pigmentation to stress tolerance have not been widely studied in wheat. The aim of the current study was to investigate molecular-genetic mechanisms underlying anthocyanin pigmentation in different wheat organs and to estimate the role of the pigmentation under different abiotic stress conditions in wheat seedlings. In the current study, near-isogenic lines (NILs): cv. 'Saratovskaya 29' ('S29') and lines i:S29Pp1Pp2^{PF} and i:S29Pp1Pp3^{P} developed on the 'S29' background but having grain pericarp coloration (genes *Pp*) and more intense coleoptile (*Rc*), culm (*Pc*), leaf blade (*Plb*), leaf sheath (*Pls*) pigmentation in comparison with 'S29', were used. Comparative transcriptional analysis of the five structural genes *Chs*, *Chi*, *F3h*, *Dfr*, *Ans*, encoding enzymes participating in the anthocyanin biosynthesis, was performed in different organs of NILs. It was shown that the presence of the *Rc*, *Pc*, *Plb*, *Pls* and *Pp* alleles conferring strong anthocyanin pigmentation induced more intense transcription of the structural genes, suggesting the genes *Rc*, *Pc*, *Plb*, *Pls* and *Pp* to play a regulatory role in anthocyanin biosynthesis network. To evaluate the role of anthocyanins in stress response at the seedling stage, growth ability of the NILs and anthocyanin content in their coleoptiles were assessed after treatments with NaCl (100 and 200 mM), $CdCl_2$ (25 and 50 μM) and 15% PEG 6000 (polyethylene glycol 6000), simulating salinity, heavy metal and drought stress, respectively. Under salinity and drought stress, the level of anthocyanins increased significantly in all three NILs in comparison with untreated control, whereas under $CdCl_2$ treatment anthocyanin content increased significantly in 'S29' only. The tendency of the lines having more intensive anthocyanin pigmentation to have better growth ability under stress conditions was observed. Taken together the results obtained it may be suggested that anthocyanin production in wheat seedlings is tightly related with the response to abiotic stress.

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THE BIOTECHNOLOGY OF EMBRYOGENIC CELL LINES OBTAINING AND PLANTLETS OF CONIFEROUS SPECIES IN SIBERIA IN CULTURE *IN VITRO*

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Experiments of culturing the immature isolated embryos and megagamethophytes of Siberian coniferous species were carried out on different modified media: ½ LV medium for *Pinus sibirica* and *Pinus pumila*, MSG and AI media (patent № 2431651) for *Larix sibirica* and *Larix gmelinii*, DCR medium for *Picea ajanensis*. For induction of embryogenic callus every species needs the optimized medium supplemented with L-glutamine, casein hydrolysate, ascorbic acid and hormones with different concentrations and their different proportions. The active proliferation of embryonal masses is observed on the same medium with reduced concentration of cytokinins. The proliferation of embryonal masses was significantly improved when they were subcultured after dispersing in liquid medium. The somatic embryos from embryonal masses are matured on basal medium with ABA (60-120 mM) and PEG. In spite of species specificity the embryogenesis of morphogenic structures had the same scheme: elongation and asymmetric division of somatic cells, formation of initial cells and embryonal tubes, development of globular, torpedo and bipolar somatic embryos, embryos maturation and germination. However, not all donor-plants of coniferous species can form the embryogenic cell lines and somatic embryos. The active development of embryonal masses and formation of somatic embryos are observed from zygotic embryo of hybrid seeds of *P. sibirica* and *L. sibirica*. The obtained embryogenic lines were characterized by different proliferative activity. During 10 months cultivation the value of embryonal masses in different lines was 140-570 g. The number of somatic embryos varies from 210 to 410 per 100 mg of callus fresh weight. Decreasing proliferation activity did not observed during 24-45 months cultivation. However, development of somatic embryos in long cultivated lines decreased. Maturation of somatic embryos and development of plantlets were established in *L. sibirica* and *P. pumila* 50-60 somatic embryos were matured per 1g of callus fresh weight. Somatic embryogenesis passes over the strong genetic control. Only donor tree genotypes with high reproductive potential form embryogenic cell lines and somatic embryos. The maternal affect was very strong relative to paternal and other effects. The studying molecular mechanisms involved in the control regulation of embryo development (embryo maturation, desiccation and germination) allows to understand many aspects of molecular biology of gymnosperms.

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ACQUISITION OF ASPEN TRANSGENIC PLANTS WITH *UGT* GENE ENCODING UDPG-TRANSFERASE

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One of important object of up-to-dateness is preservation of forest as unique ecosystems which supply of atmosphere by oxygen and create comfort conditions for residence of people. Therefore it is advisable both to preserve arboreous massiv and to create artificial ecosystem desirable with good consumer characteristics. We were undertaken attempts to use the *ugt* gene encoding enzyme UDPG-transferase wich accomplishes a reversible binding of growth hormone IAA with glucose and forms a reserve pool which can to be used in periods of plants rapid growth and after stopping of action of stress factors. Major task of this investigation is a transformation one of perspective in economic purpose arboreous plants – aspen Populus tremula L.. Freshly collected seeds of aspen were surface sterilized and placed on agar media of Murashige and Skoog. Germination and tree seedling growth were carried in station of artificial climate Phytotron. Aspen cuttings were propagated in vitro on MS agar media supplemented with 0,6 mg/l IBA. Then cuttings were infected by pricking meristematic zones of dormant axillary buds by transconjugates of triparental mating of the following bacteria: *Agrobacterium tumefaciens* 699 (nptII, gus-Int) or A.t. AA (acb), *Escherichia coli* XL1-Blue (*ugt*), *E.coli* K802 (gus). The event of transformation was assayed by placing cuttings on media containing 50 mg/l kanamycin and gus-reaction. Evaluation of *ugt* and acb genes integration carried out by PCR and Southern blot hybridization. These experiments verified successful transformation. In aspen plants grown in vitro were also determined the activity of *ugt* target enzyme UDPG-transferase by measuring the IAA-glucose bound and the content of free IAA. The elevated content of ester bound IAA in transgenic plants of aspen is correlated with higher activity of target enzyme UDPG-transferase. Therefore, this bound IAA increase is due to the expression of the *ugt* gene, which also contributes to increase the fresh biosynthesized IAA required as a substrate for UDPG-transferase. In progress of work were watched metrical parameters of plant growth. Certainly in vitro aspen plants had a accelerate growth. After carrying over of transgenic plants in soil vessels they considerably outrun control. Perhaps so far as transgenic plants had the higher IAA content they had also better rootage. When later plants were transplanted of on field a more intensive growth is preserved and is constitute about 30 %. Transgenic plants of aspen hold an advantage in height during 4 years till liquidation of planting for exclusion of transgenic plants dispersion via pollen in an environment. Accordingly this investigation demonstrates a principled chance to use the *ugt* gene for acceleration of aspen growth for creation of quick growth trees planting.

THE ROLE OF EXTRACELLULAR NUCLEASES AND RNA-BINDING ACTIVITY IN THE RESISTANCE OF HIGHER PLANTS TO PHYTOPATHOGENS

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A detailed understanding of plant antiviral immune function will underpin crop improvement for food, fibre and biofuel production. In plants, RNA silencing is an efficient antiviral system, and therefore successful virus infection requires suppression of silencing. Although many viral silencing suppressors have been identified, the common molecular basis of silencing suppression is poorly understood. It was proposed that dsRNA binding is a general silencing suppression strategy. In the case any additional dsRNA binding activity would reduce plant resistance to viral pathogens. At the same time, transgenic wheat plants expressing mutant intracellular ribonuclease lacking hydrolytic activity but capable of binding to the dsRNA were resistant to the barley stripe mosaic virus. We decided to examine whether extracellular RNA binding or only binding plus hydrolytic activities have effect upon plant virus-resistance. For this aim we have created several vectors bearing a native, chimeric and mutant *Serratia marcescens* nuclease gene. This enzyme strongly prefers dsRNA and was widely used as antiviral agent for plant and insect protections. We have created transgenic tobacco plants expressing native, chimeric and mutant *S. marcescens* extracellular nuclease. Some of these plants have demonstrated significant delay of the appearance of typical mosaic symptoms and the retarded accumulation of viral antigen.

FIRST ESTIMATIONS OF PLANT ACRIDONE ALKALOID IMPLEMENTED IN MUSHROOM CULTURE: GROWTH PROMOTER VERSUS ENVIRONMENTAL STRESSOR

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Acridones (=acridin-9(10H)-ones) are ketones of tricyclic molecular-skeleton parents having an N-atom at the 10-position and the keto group at the 9-position. Acridone derivatives are widespread in nature, commonly occurring in a number of genera of the family *Rutaceae*. Any information on the acridone series compounds in relation to higher fungi, including edible mushrooms, is not available in literature at all. Acridones are known to be capable of having a variety of biological activities, e.g., antiviral, antitumor, antimalarial, and antibiotic properties. Thus, arborinine (1-hydroxy-2,3-dimethoxy-N-methylacridone) and normelicopicine (1-hydroxy-2,3,4-trimethoxy-N-methylacridone) display antiplasmodial activity. The related 1-hydroxyacridones from *Boronia* species are inhibitors of *Staphylococcus* and *Salmonella* growth. *Citropsis gabunensis* has been revealed to be a plant producer of novel acridone alkaloid citropridone, the instances of such kind being numerous and to be continued. The perspective goal of our work is to obtain the initial data on the general principles of structure (functional groups) of polycyclic compounds - mushroom growth promoting substances, as well as to conclude on the potentialities of research into the enhancement of bioavailability of the pharmacologically important acridone derivatives related to their biotransformation in the edible mushrooms mycelia. At the current step of that work, for studying firstly the effect of acridone-N-acetic acid (AAA) on mushrooms, the culture of basidiomycete *Lentinula edodes* (shiitake) has been used. The influence of AAA upon the fungal culture growth on liquid media was examined within the AAA concentration range of $1.0 \cdot 10^{-6}$-$1.0 \cdot 10^{-3}$ mol/l, and on agar media the corresponding range was $1.0 \cdot 10^{-6}$-$5.0 \cdot 10^{-4}$ mol/l. Differentiation in the above values of initial concentration of acridone additive is caused by different extent of the shiitake's growth inhibition manifested under the conditions of liquid-phase and solid-phase culture. Estimations of the basidiomycete growth on agar media and on submerged cultivation were performed using the commonly accepted procedures, by measuring the sizes of colonies and the dry biomass weight, respectively. The culture age and AAA concentration corresponding to the most intense radial growth of mycelium on agar media or to the greatest biomass of submerged mycelium were the experimentally determined parameters to be assessed. Obviously, AAA plays a role of stressing factor at $5.0 \cdot 10^{-4}$ mol/l or higher, and when this acridone derivative at lower levels enters the composition of culture medium, the enhanced mushroom development takes place. The results obtained in respect to influence of exogenic compounds of acridone series on the mushroom culture testify to the relative ecological safety of this substance for mushroom organism, and to the mycelial growth promoting capability of AAA at favourable concentrations, both under the solid-phase and liquid-phase culture conditions. In fact, the very first step toward the investigation into the systems "macromycete - acridone series compound" has been made.

APPLICATION OF SOMACLONAL VARIABILITY TO PRODUCTION OF FAST-GROWING TREES AS A RAW MATERIAL FOR BIOFUEL

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Wood is one of the most widespread materials on the Earth. Wood raw materials may be used either to generate energy directly or to produce biofuel. The demand for sufficient amount of wood for these purposes necessitated establishment of arboreal plantations. Poplar тополь (*Populus sp.*) is the most promising object to produce wood from plantations.

In the last few years the Institute has been involved in the studies of potential of somaclonal variability in tissue cultivars to select plants with high growth indices. Berlin polar (*P.* × *berolinensis*), has been selected for this purpose, as its pyramid-shaped crown allows to place more plants on a plantation square area unit. It easily propagates by grafts and coppice shoots and is fairly resistant to leaf rust. Fast-growing trees may be acquired due to somaclonal variability and genetic transformation. In both cases an indispensable preliminary condition is a well-tested protocol of acquiring regenerates and their propagation in the cultivar of isolated tissues. Such a protocol has been worked out by now. It comprises the following phases: 1) isolation of stem tops in the first half of summer ant their sterilization; 2) cutting of explantates and their cultivation on the medium to induce regeneration; 3) propagation of regenerates on propagation medium; 4) elongation of regenerants; 5) rooting of regenerants; 6) acclimation and transfer of plants to the field for growth. At phases 3, 4 and 5 plants with desirable somaclonal changes are selected. Somaclonal varieties are selected on the basis of a large number of regenerants. For their further growth prior to planting on the Institute test site hydroponic units installed at the artificial climate station are used. This ensures equal conditions of nutrition and moisturizing for all the regenerants planted. Little plants cultivated *in vitro*, which are acquired at this stage of the work, will be further used for genetic transformation. A gene regulating plant growth has been extracted by now; it is currently subjected to sequenation and is being prepared for embedding in agrobacterium *Ti*-plasmid. Once such bacterium is acquired, poplar genetic transformation will be conducted. In the near future transgenic poplar plants are intended to be acquired. Protocol of regenerants acquisition and propagation *in vitro* was also developed for aboriginal Siberian poplar species - *P. laurifolia* и *P. suaviolens* in order to further select promising somaclones.

Based on somaclonal variability the Institute staff has selected several clones of Berlin poplar with the highest growth speed. They will be used to create transgenic poplar plants with embedded genes controlling plant growth.

SELECTION OF MYCORRHIZAL MUTANTS IN BLACK MEDIC (*MEDICAGO LUPULINA*)

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Arbuscular mycorrhiza (AM) is a widespread plant-microbial symbiosis formed by Glomeromycota and the majority of land plant species. AM-fungi increase macro- and microelements uptake in plants, especially phosphorus, optimize hormone balance of plants, enhance plant resistance to root pathogens and increase beneficial plant interactions with nitrogen-fixing microorganisms. Despite intensive research of AM-symbiosis, numerous mechanisms controlling AM-development and symbiotic efficiency are not described because applied cultivars usually have a reduced ability to mycotrophic nutrition. The problem is the absence of suitable plants selected under low level of soil phosphorus (P) available for plant nutrition. The model plant in our research is a new obligate mycotrophic black medic MlS-1 line selected from primitive cultivar ARFI32 (*Medicago lupulina* L. *var. vulgaris* W.D.J.Koch). MlS-1 is characterized by dwarf symptom without AM (uninoculated with strain CIAM8 *Glomus intraradices*) under condition of low level of available P in soil or other media. The aim of this investigation is to obtain mycorrhizal mutants from a population of mutagenized M2 plants with defects in AM-development and symbiotic efficiency. For this purpose we carried out the mutagenesis of the black medic MlS-1 line by ethylmetanesulfonate. Basic morphological characteristics of individual mutants are dwarf symptoms (small leaf discs, short stem, etc.), variation of leaf and stem form and colour, variation of internodes length. We selected 15 morphological mutants with defects in development of AM-structures including 6 mutants characterized by stable inheritance up to the 7^{th} progeny. These mutants and the wild type MlS-1 line can be used to explore the mechanisms controlling development and efficiency of arbuscular mycorrhiza.

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Electronic supplementary materials

CHEMOTAXONOMIC ASPECTS OF FIVE-NEEDLE PINES HYBRIDIZATION

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The plants of *Pinaceae* family are native throughout the temperate Northen hemisphere, including large parts of the boreal forests. Forests of Russia are represented by coniferous species up to 75 %. Three species of five-needle pines (*Pinus* L. subsection *Cembrae* Loudon) are naturally distributed in Russia: *Pinus sibirica* Du Tour, *P. koraiensis* Sieb. *et* Zucc. and *P. pumila* (Pall.) Regel. The natural ranges of Siberian stone pine (*Pinus sibirica* Du Tour) and Japanese stone pine (*P. pumila* Regel) overlap in the Baikal region of Siberia. The occurrence of natural hybridization between these species has been the subject of debate since putative hybrids were described 70 years ago. Genetic structure of sympatric *Pinus sibirica* Du Tour and *P. pumila* (Pall.) Regel populations and putative interspecific hybrids between them were analyzed in the Baikal Lake region by professors Goroshkevich and Politov with colleagues. Diversity structure of 5-needles pines from North and East Asia was studied with classical (anatomical, morphological) and current (molecular genetic) traits. The chemotaxonomic feature of parents species are known from literature. Our purpose was determination of qualitative and quantitative composition of hybrid sample in comparison to parent's species. Our own data concerning 30 main components showed the differences in occurrences and quantities of aliphatic and diterpenic constituents. Samples of Siberian stone pine, Japanese stone pine and hybrid between *P. sibirica* and *P. pumila* needles were collected in August of 2011 in permanent establishment of IMCES to exclude the influence of ecological factors. The samples of row materials were extracted by hexane and MTBE. Samples of extracts were investigated by GC-MS, oven temperature was kept at 50º C for 2 min and programmed to 300º as a rate 4 º/min and then kept constant at 300º C for 30 min. As a result of our research we identified the aliphatic acids with the chain-lengths from 12 up to 34 C-atoms on free and bonded form and diterpenic acids, hydrocarbons and alcohols including chemotaxonomic markers cembrene, lambertianic, isocupressic, succinylagatholic, agathic and anticopalic acid. Sufficient viability and fertility of natural hybrids between *P. sibirica* and *P. pumila* and their aptitude to cross among themselves permit to consider hybrids as a promising evolutional new formation that can result in novel species with interesting BAC composition.