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NEW ORIGINAL FORM OF COTTON PLANT WITH DETERMINANT TYPE OF GROWTH

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Genus *Gossypium* L. (cotton) includes 50 species, among them 45 diploid (2n = 2x = 26, genomes A, B, G, C, D, E, F, K; mainly wild species) and tetraploid (2n = 4x = 52, genome AD) species. Tetraploid species are allopolyploids with mainly polygenetic trait control. By the type of growth of the main stem, all cotton species are indeterminate - tree-type, arboreous. Procumbent forms among cultivated species of *G.hirsutum* L. and *G.barbadense* L. as well as stunted and dwarf forms exist. In the process of genetic analysis of leaf forms, sympodial branches of the *G.hirsutum* L. species, playing role in determining of the structure and shape of the bush, the gene *Inl* and gene *S* controlling fruit bearing branches were identified. Dominant homozygote *InlInl* confers whole leaf type. Recessive homozygote *ss* is responsible for marginal type of sympodium. Plants having their combination *InlInlss* are phenotypically determinant. According to the literature data, integrifolia species combine xerophytes characteristics. In plants having this genotype 3-4 fruits are formed on the apical part of the stem at the budding phase end. In the 4-5th node of the stem, two fruit elements with zero type are formed in the *G.barbadense* L. species. Plants with determinant type possess 3-4 bolls of raw cotton (2-3 g in each box). Weight of raw cotton needs increase up to 3.0-3.5 g. The forms having 3-4 boxes with 3 g of raw cotton each (hence up to 12 g per plant) at the density of 300,000 result in yield 36 metric centner / ha. With a weight 4-4.5 g per box, yield increases up to 48 metric centner/ha. This form is new not only for *G.hirsutum* L. species, but also for the whole *Gossyrium* L. genus. It can serve as an initial form in the breeding of varieties adapted to dense and overdense planting of cotton crops, contributing to high yield per unit area. Currently, a number of lines (with determinant 1, 2, 3, 4) are selected as initial material for practical breeding.

COMPUTER HIGH-THROUGHPUT APPROACHES TO WHEAT PHENOTYPING: APPLICATION TO LEAF PUBESCENCE ANALYSIS

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Rapid and accurate approaches for large-scale assessment of phenotypic characters in plants needed to study relationships between genotype and phenotype. Automated phenotyping is a plausible solution providing 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. We present the high-throughput approach to wheat leaf pubescence phenotyping based on image analysis of the folded leaf. Our method estimates the trichome number and trichome length distribution. 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. Using our technique we estimated the phenotypic effect of known bread wheat leaf pubescence genes. Our results demonstrated that these genes differed in their effect on trichome growth, initiation and patterning. A model of the action and interaction of these genes has been proposed to explain the genetic basis of trichome length and number. The work supported by RSF grant 14-14-00734.

APPLICATION OF MOLECULAR MARKERS FOR IDENTIFICATION OF GENOME CONSTITUTION AND STUDY OF PHYLOGENETIC RELATIONSHIPS IN PERENNIAL GRASSES OF THE GENUS *ELYMUS* (TRITICEAE: POACEAE)

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Elymus L. is a genus of the Poaceae family which includes allopolyploid species only. Taxa of the genus are widespread over all continents, at least half of them have been occurred in Asia, and this continent is considered to be its motherland. However, the diversity, genetic characteristics, and evolutionary relationships among *Elymus* species of some regions of Asia are still vague. Genome constitution is not identified in the majority of taxa described from Northern Eurasia, which is absolutely necessary for creating phylogenetically oriented taxonomical system of the genus. It is especially important, because a series of new independent genera are recognized on the basis of the genome constitution, such as *Roegneria* Koch (StY genome), *Campeiostachys* Drobov (StHY genome) which traditionally were a part of the genus *Elymus sensu lato*. For the territory of Asian part of Russia the most actual task is delimitation of the StH-genome species and StY-genome ones, and the later group should be allocated, at least, in separate section or a subgenus. It was shown that low-copied nuclear genes are more suitable to reconstruct phylogeny in interspecies level, as phylogenetic tree reconstructed from ITS sequences mostly has low length of branches and low values of bootstrap support. Furthermore, a specific site was found within eleventh intron of the *waxy* gene, which contains motives that are highly conservative within sequences from one haplome but vary between sequences from different haplomes and thus can be used to verify presence of H haplome in genome constitution of novel *Elymus* species. Availability of St haplome which is present in all *Elymus* species was confirmed in *E. kamczadalorum* by sequencing of ITS1-5,8S-ITS2 genes. Thus, taxonomical rank of *E. kamczadalorum* as a species was proven valid and it was shown to have genomic constitution StStHH.

HAPLOID BIOTECHNOLOGY OF WHEAT: PRACTICAL RESULTS

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Modern developments of the haploid biotechnology directions the basis of the method of isolated anthers and microspores culture *in vitro*, allow to create the constant homozygous double haploid lines from plant hybrid populations during 1-2 years while a traditional breeding method of stable lines reception demands about 10-12 years. Wheat genotypes monitoring on rust diseases resistance was carried out in Southern Kazakhstan conditions in natural and infectious nursery. Races *Puccinia graminis*, *Puccinia striiformis*, *Puccinia recondita* were used as an infecting agent. The following genotypes have shown a high stability to rust diseases: *Triticum kihara*, *Triticum diccocum*, *Triticum thimofeevi*, Almaly, Naz, Taza and others. The selected steady genotypes have been used for crossing and breeding of perspective inter-cultivar and inter-species remote hybrids. More than 120 hybrid combinations were created. Received hybrids were genetically stabilized by haploid biotechnology method on the basis of isolated anthers and microspores culture *in vitro*. The structures of nutrient mediums on Blaydes and №6 basis were modified through activated coal and amylodextrine addition. In the following experiments series rust- disease steady and rust-disease susceptible parental forms, bred hybrid forms and new haploid lines were analyzed at DNA level using molecular markers method. As a result of the SSR-analysis and isogenic lines on the Thatcher basis it was established that rust-disease steady genotypes and lines have *Lr24* gene. In a final stage new perspective DH lines were tested on stability to rust diseases in natural and artificial conditions in two areas of Southern Kazakhstan (Almaty and Zhambyl region). Among investigated DH lines the numbers DHL 1257, DHL 1250, DHL 1245 and DHL 1227 were allocated. They appeared rust-diseases steady and are characterized with high productivity and grain quality. The selected perspective DH lines were used for production of new wheat cultivar resistant to rust diseases. This cultivar was introduced in the region of Southern Kazakhstan.

INTROGRESSION OF ALIEN GENETIC MATERIAL IN INTERSPECIFIC SOMATIC HYBRIDS OF POTATO AND THEIR BACKCROSS PROGENIES

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The introgression of wild species germplasms into the cultivated potato genome may provide a source of durable resistance to pathogens and pests. Wild

Mexican potato species *Solanum bulbocastanum*, *S. cardiophyllum*, *S. pinnatisectum*, *S. tarnii* (*2n=2x*=24, BB genome) are resistant to late blight, Potato virus Y (PVY) and to Colorado potato beetle. The wild non-tuber-bearing species *S. etuberosum* (*2n=2x*=24, EE genome) is extreme resistant to PVY. The protoplast fusions technique was used to produce the interspecific somatic hybrids in five interspecific combinations - between sexually incompatible species: cultivated potato *S. tuberosum* (*2n=4x*=48, AAAA genome) and the resistant accessions of wild species mentioned above. Fertile somatic hybrids and their backcross progenies derived from sexual crosses with cultivated potato segregated for resistance to pathogenes. This hybrid material was screened using molecular markers and GISH. To select the markers which are able to distinguish the wild species genetic material in interspecific hybrids 173 loci with known chromosome locations were examined. From 36 to 50 polymorphic markers (nSSR, STS, CAPS) were developed for each interspecific combination. Somatic hybrids of different combinations and the most BC1 lines retained all wild species specific markers. Molecular marker analysis of 180 BC3 and BC4 hybrids from the most successful combinations *S. tuberosum* (+) *S. tarnii* revealed from 0 to 5 alien chromosomes. These results showed concordance with data of GISH analysis. BC lines are used for mapping of the PVY resistance gene and hybrid material resistant to different pathogens are involved into pre-breeding programs.

INTROGRESSIVE FORMS OF COTTON DONOR WITH HIGH QUALITY FIBER

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Creation of cotton varieties with high fiber quality and resistant to diseases, insect pests and unfavorable condition of environment is considered as important problem for Uzbekistan. Wild and semi-wild cotton species possess mentioned above traits. Using these species in fundamental genetic-breeding researches can provide many valuable fertile synthetic introgressive cotton forms combining positive economic traits of wild and cultivated polyploid species. Synthetic introgrossive forms of cotton were created by hybridization with wild diploid species of *Gossypium trilobum* Skovsted. (such as IL-296, IL-1378, IL-32) with cultivated cotton species (Korotkostebelniy-1, Akademiya Nauk-14). Valuable perspective introgressive hybrid families (C-85A, C-88A, C-215A/1, C-214A/1, C-209A/3, C-65A, C-109A, C-76A/1, C-16 etc.) possessing high productivity, with good technologic fiber properties, resistant to diseases, insect pests and other stresses during hybridization were obtained. Data obtained from Center of Technological Properties of Fiber "Sifat" of Republic of Uzbekistan demonstrated coefficient of fiber micronaire (Mic) to be 4.0-5.4; relative breaking load of fiber (Str) – 31.2-41.1; index of fiber length (Len) - 1.06-1.21 inches. These hybrids can be used in cotton breeding programs as initial forms for creation of new varieties and as donors of important traits.

PECULIARITIES OF CAROTENOID ACCUMULATION IN TOMATO FORMS WITH A DIFFERENT COMBINATION OF FRUIT QUALITY GENES

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Development of hybrids with a high fruit quality, including an increased carotenoid content and long fruit shelf life is one of the urgent trends in tomato breeding. We have previously developed techniques for DNA identification of fruit quality genes *nor*, *rin*, *norA* (long shelf life), *og*, *Del*, *r*, *hp-1*, *hp-2^{dg}*, *B*, *og^{c}*, *t*, *gf-3* (increased carotenoid content). The breeding material was identified according to the allele composition. A number of hybrids combining alleles of the genes under study were developed. The forms with desirable allele combinations were selected in the F_2 generation using MAS methods. The goal of this research was to study carotenoid accumulation in tomato fruit depending on the combination of fruit quality genes. The research material was represented by the forms with different gene combinations: double homozygotes, homozygotes by the carotenoid content genes and heterozygotes by the genes extending fruit shelf life, hybrid combinations with two carotenoid content genes and one long shelf life gene. Pigment composition of tomato fruit was analysed by spectrophotometric and HPLC methods. As a result of the analysis the following peculiarities of carotenoid accumulation were revealed: despite the reduction in carotenoid accumulation in tomato fruit under the influence of genes *rin*, *nor*, *norA*, their

combination in the heterozygous genotype with genes *B, og^c, t, hp* in the homo- and heterozygous genotypes restores carotenoid accumulation in tomato fruit to the standard level – forms without long shelf life genes; use of the accessions with the *hp-2^{dg}* gene as a maternal form in hybrid development provides a larger lycopene amount as compared to the accessions with the *hp-2^{dg}* gene as a paternal form. Forms providing high accumulation of different carotenoids and long fruit shelf life were selected: β-carotene – accessions with combination of genes nor and B, rin and B; ζ-carotene - *nor* and *t*; lycopene - *nor* and *og^c*.

CHROMOSOMES IN THE ANALYSIS OF DOMESTICATION AND SPREADING OF EMMER WHEAT

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Emmer wheat *Triticum dicoccum* is one of the earliest domesticated plants. It was first domesticated in the Fertile Crescent 12,000-10,000 years ago, and from there it spread outward being accompanying human migration. Emmer was broadly cultivated in the past, until the third millenium BP, when it began to be replaced by free-thresheing bread and durum wheat. Emmer was not affected by severe genetic bottlneck effect and can provide a rich source of genetic variation for breeding. Karyotypic information obtained from 421 emmer wheat lines from 47 countries of Europe, Asia and Africa suggests that chromosomal characteristics are associated with geographic origin. Statistical analysis of chromosomal passports obtained for all lines by comparing their karyotypes with generalized idiogram of the A and B genome chromosomes discriminated four major karyotypic groups within *T. dicoccum*, each showing characteristic C-banding patterns and translocation spectra: the Balkan, Asian, European, and Ethiopian in accordance with taxonomical treatment of the species. The Moroccan emmer did not appear as a separate group and was clustered together with the European emmer, which can be due to the small number of accessions studied (only 2% of the whole sample) and hybrid nature of Moroccan forms. The Asian and Balkan groups were cytogenetically most similar with wild emmer lines from southeastern Turkey and probably represent the early diffusion out of the Fertile Crescent. A second diffusion via Mediterranean to Western Europe gave rise to European group and probably started in southern Levant. The Ethiopian group could have originated from the estern Fertile Crescent probably from a secondary hybridization with wild emmer. Our data suggest that emmer was introduced into Russia by two diffusion routes: from Transcaucasia via rival trade and from the Balkans.

GLUME VENATION IN *FESTUCA ARUNDINACEA* (POACEAE): SYSTEMIC AND FUNCTIONAL APPROACHES

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Phenotypic and genotypic variability in botany (linnean) and genetics (mendelian) are traditionally considered without relationship. For instance, in grasses, in particular in species of the genus *Triticum*, according to the ideas of Mendel's genetics, genes *B1*, *B2*, *b2*, *ta* and *ga* determine «awnless» and «awned» glumes. However, when traits are analysed by using the systemic and functional approaches, actually, they show genetic control of forms of glumes, differing in the functional state of veins. Data on functional status of a trait in structural units of plant shoots, probably, can be «the tool»for predicting and comparison them with the same data on status of genes controlling the trait of the organ, but gained by using methods of genetics. The aim of our study is diagnostics of the functional status of «venation» (VEN) trait in glumes of *Festuca arundinacea* Schreb. (*2n*=42), domesticated in West Siberia, determination of phenotypic classes of forms and grouping elements of the trait structure. Functional state of the trait structural elements was studied by using modified method of structural analysis and stereomicroscope Carl Zeiss Stereo Discovery V12. The lower glume (LG) has 3 structural elements (se) of VEN trait – midvein (mv) and 2 lateral veins (lv) – left and right. Five forms of LG are identified. The forms with mv have the highest frequency (p = 85%).The original forms (p = 4%) have 3 veins – mv and 2lv. One of the two lv in full reduction is identified for 10% of forms. Lateral veins are reduced in 2 ways: parallel and in series. The frequency of forms with reducing mv is 1%. The upper glume (UG) has 6 se of VEN trait – mv, 4lv and transversal anastomoses (ta). We have found 6 forms of UG. The original forms (p = 2%) of the series have 4 veins. Its ta connects 2lv with mv. The forms with 3 veins (p = 90%) have parallel reduction

in lv. Three veins (mv, 2lv) of intermediate forms (p = 4%) are connected with ta. The forms with 4 veins (p = 4%) have no ta. Thus, one and two groups of se respectively in LG and UG are established. UG have a stable (17%) and reducing (83%) se but in LG all elements (100%) are reduced. The results obtained, on the one hand let predict the inhibiting status of prognostic gene *VEN*, and on the other hand correlate data of population, organismic and gene (prognostic) levels.

INHERITANCE "THE DWARF"-TRAIT, CONTROLLED BY THE *d*-GENES IN *SOLANUM LYCOPERSICUM* L., STUDIED IN THE SPECIAL BREEDING PROGRAM

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Regular supplying with fresh vegetables could be ensured with new module technologies, such as new hydroponic technology on narrow benches. But requirements for tomato forms will be cultivated by that technology are very strict. Plant's model represents the dwarf plant with high productivity, early ripening and resistance to main stresses of greenhouses. So, the goal of new breeding program in our Institute is obtaining new tomato forms, which could be cultivated under special hydroponic conditions (on narrow benches). The first step of this program was the breeding of dwarf plants (30-35cm) with good productivity and big fruits. Using of gamete's selection approaches and 2-factorial dispersion analysis we show, that "the dwarf"-trait (h^2=0,83) and "the early ripening"-trait (h^2=0,60) could be inherited by father's line. But main traits of productivity - "the mass of one fruit" (h^2=0,99) and "the number of fruits on the plant" (h^2=0,96) - could be inherited by mother's line. We selected 9 mother and of 7 father forms with needed parameters and developed strategies of selection and of hybridization. Morphologic markers of d-genes can to appear at seedling stage, so, the selection of dwarf forms from populations is easy. But the obtaining of hybrids is connected with some problems. The *d*-genes are recessive and will appear in F_2 progeny. So, we crossed mother forms with good productivity and big fruits with "dwarf" father forms, obtained of F_1 progenies and F_2 progenies from self-pollinating of F_1 progenies. F_2 progenies segregated in the expected ratio: 3 parts of "high" plants and 1 part of "dwarf" plants. We selected some of early ripening plants with good productivity and big fruits from "dwarf" plants and studied F_3 progenies of these "dwarf" plants. It was confirmed, that the strategy of hybridization was correct: the height of the plant was lowered to the level of the "dwarf" father. The mass of one fruit and the productivity of plant were increased in 2 times.

ADAPTIVE SELECTION OF BREAD SPRING WHEAT IN SIBERIA

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The development of new spring varieties combining high yield with increased tolerance to abiotic and biotic stress is the most cost-effective way to decrease yield losses and to increase average crop capacity of wheat. For this purpose, a complex of advanced field and laboratory methods are used to estimate plant material for stress environmental factors, testing in vitro. The number of varieties included in Stage Registry from 1971 has shown the progress in breeding work, which effectiveness has been increased in the last 25 years. Thirty five varieties were sent to variety trial, 18 of them were included in Stage Registry, 5 are in variety trial, and 12 are cultivated in Republic of Kazakhstan. The main strategy to create adaptive varieties is the involvement of new gene sources. By means of purposeful selection of parental genotypes, intergeneric crosses and selection in breeding nursery promising forms with increased yielding capacity, high seed quality and tolerance to abiotic and biotic stress were selected. The development of varieties resistant to leaf diseases are based on selection of genetically different resistant pairs for crossing, evaluation of lines and varieties under natural and infectious conditions, and evaluation of seedlings resistance to virulent pathogenes. The involvement in crossing of varieties carrying alien germplasm enabled the creation of varieties Omskaya 29, Omskaya 37, Omskaya 38, Omskaya 41 and promising lines resistant to leaf pathogenes. Evaluation of breeding material for resistance to Ug99 stem rust race was carried in Kenya. The improvement of the breeding effectiveness resulted

from collaboration with Institute of Cytology and Genetics, N.I.Vavilov Research Institute of Plant Industry, All-Russian Research Institute of Crop Protection, Joint-Stock Company "Kurgansemena", Tatar Institute of Agriculture, Institute of Plant Cultivation Ukraine and CIMMYT. The result of collective work are varieties Kazanskaya Ubileinaya, Omskaya 36, Boevchanka, Omskaya 38, Gerakl, Omskaya 35, Uralosibirskaya, Omskaya Krasa, Pamyati Maystrenko, Sigma, Sigma2.

EXPRESSION OF *GDH1* AND *GDH2* GENES IS REGULATED BY REDOX SIGNALS OF PLASTOQUINONE POOL

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The *Arabidopsis thaliana gdh1* and *gdh2* transcript levels are higher in dark and lower at light. Hypothetically these genes are regulated by sugar signaling. We investigated a role of sugar signaling mediated by glucose sensor hexokinase 1 in the regulation of *gdh1* and *gdh2* genes expression. Our results suggested that the sugar regulation of *gdh1* and *gdh2* genes expression is not transmitted through hexokinase 1. A light-depended retrograde signaling mediated by changes in redox state of the plastoquinone pool (PQ-pool) could be involved in regulation of *gdh1* and *gdh2* genes expression. The change of the PQ pool redox state can be caused by treatment of electron transport inhibitors. The treatment with DCMU results in oxidation of the PQ pool. The treatment with DBMIB results in reduction of the PQ pool. It is known that redox signals from PQ pool can regulate the expression of photosynthesis-associated nuclear genes. We have shown that light-depended redox signals from PQ pool regulate the *gdh1* and *gdh2* genes expression. The decrease of *gdh1* and *gdh2* transcript levels at light could be mediated by reactive oxygen species (ROS). Our experiments indicate that ROS can not be the reason for the light-depended repression of the investigated genes. Thus the *gdh1* and *gdh2* genes expression is regulated by both sugar signaling and light-depended redox signaling. The work has been financially supported by the RFBR grant 14-44-04001.

ASSEMBLY AND ANNOTATION OF SIBERIAN LARCH (*LARIX SIBIRICA* LEDEB.) CHLOROPLAST GENOME AND THE SEARCH FOR POLYMORPHIC GENETIC MARKERS (SSRs AND SNPs)

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The main objectives of this study were assembling and annotation of chloroplast genome of Siberian larch (*Larix sibirica* Ledeb.), and detection of polymorphic genetic markers – simple microsatellite repeats (SSRs) and single nucleotide polymorphisms (SNPs). We used data of the whole genome sequencing of three Siberian larch trees from different regions - Urals, Krasnoyarsk, and Khakassia, respectively. Sequence reads were obtained using the Illumina HiSeq2000 in Laboratory of Forest Genomics at the Genome Research and Education Center of the Siberian Federal University. The assembling was done using the Bowtie2 mapping program and the SPAdes genomic assembler. The genome annotation was performed using the RAST service. For SSRs search we used the SciRoKo program, for SNPs detection – the Bowtie2 and UGENE programs. Length of the assembled chloroplast genome was 122,561 bp, which is close to 122,474 bp in closely related European larch (*Larix decidua* Mill.). As a result of annotation and comparison of the data with existing data available only for two larch species - *L. decidua* and *L. occidentalis* (partial genome of 119,680 bp), we identified 121 coding regions, 34 of which represented tRNA and 87 - CDS genes. Total 26 SNPs were detected, 5 of them were in the coding regions of the genome: *PsbZ, cell-surface adhesin2C putative, FtsH, gene trnK*. The presented study was a part of the project "Genomic studies major boreal coniferous forest tree species and their most dangerous pathogens in the Russian Federation" funded by the Government of the Russian Federation (contract № 14.Y26.31.0004).

POLYMORPHISM OF *SUS* HOMOLOGUES IN WILD AND CULTIVATED ROSACEAE SPECIES

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Sucrose synthase (Sus) catalyses reversible conversion of sucrose and UDP to UDP-glucose and fructose and is one of the key enzymes of carbohydrate metabolism involved in fruit growth and development in plants. Accumulation of sugars in fruit determines its edible quality in Rosaceae fruit tree species. Sucrose synthase is also involved in cell wall synthesis and abiotic stress response. Still there is limited data on sucrose synthase genes polymorphism in Rosaceae. In this study we characterize the variability of *Sus* homologues within 13 Rosaceae species: *Chaenomeles japonica*, *Cydonia oblonga*, *Mespilus germanica*, *Aronia melanocarpa*, *Pyrus elata*, *Crataegus monogyna*, *Sorbaria sorbifolia*, *Malus domestica* var. Skala, *Malus baccata*, *Malus orientalis*, *Malus sylvestris*, *Malus coronaria*, *Malus pumila*. Selective primers were designed which enabled to discriminate and amplify full coding sequences of *Sus* homologues. Amplified fragments were cloned, sequenced and analyzed. 13 cloned *Sus* sequences varied in length from 3401 bp (*Pyrus elata*) to 3514 bp (*Cydonia oblonga*), and contained both SNPs and indels. In total 551 SNPs were identified in analyzed sequences. Several SNPs found in exons were species-specific. Total exon polymorphism rate was rather high (11.9%). Several indels detected in introns were species-specific or specific for groups of species.132 out of 290 SNPs that have been identified in analyzed *Sus* exon sequences resulted in amino acid substitutions. Within the studied sequences two conserved motives were detected, in exon V and X corresponding to sucrose synthase and glucosyltransferase domains. The study is supported by RSF project № 14-16-00121.

PLANT GENETIC RESOURCES – THE PREREQUISITE FOR FUTURE GENETIC STUDIES AND PLANT BREEDING

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Plant genetic resources play a major role for global food security. The most significant and widespread mean of preserving plant genetic resources is ex situ conservation. The majority of 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 1,750 ex situ genebanks. One of the ten largest ex situ collections of our globe is located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, conserving about 150,000 accessions from 3,212 plant species and 776 genera. Since the majority of genebank holdings globally are stored as seed, seed storability is of exceptional importance for germplasm conservation. At IPK research on seed longevity was initiated for a range of crops stored over decades. Variation between and within crop species was detected and genetic analyses were initiated using long term stored and experimental aged materials. The complex trait seed longevity was studied exploiting classical quantitative trait locus (QTL) analysis and association genetics. We present results obtained for wheat, oilseed rape and tobacco. In addition examples are given for an efficient exploitation of the germplasm collections for genetic studies of traits important in plant breeding.

ANALYSIS OF MAIZE GAMETE FUSION GENES

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The molecular-genetic aspects of double fertilization in angiosperms plants, discovered by S. Navashin over a hundred years ago, still remains poorly understood. Gametes fusion is a central step for the double fertilization process. It is believed HAP2/GCS1 protein is required for Arabidopsis gamete cells fusions, however, the precise functions

of HAP2/GCS1 protein is unknown. We searched for *hap2/gcs1* gene (generative cell specific 1) homology in maize genome using BLAST program, and set of maize transcript with high homology to *hap2/gcs1* gene was observed. In particular, the ZM_BFb0162K03 maize transcript with 67% identity to the *hap2/gcs1* gene and conserved region (100 % similarity) to the *hap2/gcs1* gene fragment was found. Using the Blastx program for translated protein sequences searching, the ZmGEX2 gene in maize genome was found also, while genes homologues to *Arabidopsis* THAT and TET12 was not observed. We supposed the haplo-inducing ability of maize lines (Germ Marker Saratov Purple (GMSP)), may be due to *hap2/gcs1* mutation, violation of the sperm cells function. In DNA and RNA samples isolated from pollen, embryos, leaf of the haplo-inducing (Germ Marker Saratov Purple (GMSP)) and control (Sweet) maize lines, a PCR to the *hap2/gcs1* conservative domain was observed for the first time. We registered of 228 bp PCR product from *hap2/gcs1* conservative domain in all plant samples, and did not found any differences between GMSP and control *hap2/gcs1* conservative domain sequences. We assume that the haploinducing capacity of the ZMSP line possibly is not associated with improper functioning of the *hap2/gcs1* gene. This research was supported in part by a grant 15-04-08413 from the Russian Foundation for Basic Research.

IDENTIFICATION THE CHROMOSOME COMPOSITION OF COMMON WHEAT-ALIEN CHROMOSOME SUBSTITUTION LINES AND GENETIC EFFECTS OF ALIEN CHROMOSOMES

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Wild and cultivated species of cereals are widely used to increase the genetic diversity and to improve the agronomic and adaptive traits of common wheat. Alien chromosomes of cereal species may be introgressed into wheat in the process of production the substitution or translocation lines. On the basis of cytological analysis of metaphase I of meiosis were isolated stable 42-chromosome wheat-rye lines. Using genomic in situ hybridization (GISH), was demonstrated the presence of rye pair chromosome in the lines with 5R(5A) and 5R(5D) chromosome substitution on varieties Mironovskaya Krupnozernaya and Pyrothrix 28. In line 5R(5D) × L2075, the presence of chromosome 5R and the 1RS.1BL translocation was identified. In another line, 5R(5A)×L2075, new Robertsonian translocation T5AS.5RL in addition to T1RS.1BL was discovered. This translocation appeared as a result of fusion of the short arm of chromosome 5A and the long arm of chromosome rye 5R. A line with two translocations, T1RS.1BL+T5AS.5RL, appeared to be more productive as compared to the line carrying T1RS.1BL in combination with the 5R(5D) substitution. In addition, using molecular markers the presence of genes *Lr26* and *Lr19* in these lines, which provide resistance to leaf rust in conditions of Novosibirsk, was demostrated. It is shown that using BAMR marker for *β-amylase* rye gene allows quick and reliable identification of the presence of the long arm of chromosome 5R in wheat-rye lines with chromosome substitutions and translocations. Cytologically stable 42-chromosome ditelosomic wheat-barley (*Hordeum marinum* subsp. *gussoneanum* Hudson *4x*) lines with substitutions of homoeologous group 7 chromosomes were isolated. The identification of telocentric barley chromosome was performed using GISH. The features of the transmission of barley and wheat chromosomes through the gametes in the progeny of reciprocal hybrids [21 "× F_1 (20" + I'w + t'7H)] of wheat-barley ditelosomic lines were studied, and the better transmission of the alien barley chromosome 7HL through pollen it was shown. A relatively high competitive ability of 21-chromosome gametes (20 + t7H) was revealed.

USE OF DNA MARKERS FOR TRANSFER OF LEAF RUST RESISTANCE GENES *Lr9* AND *Lr35* FROM *AEGILOPS UMBELLULATA* AND *AEGILOPS SPELTOIDES* TO COMMON WHEAT

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The leaf rust of wheat (*Puccinia triticinia* Erikss.) is one of the most wide spread and harmful diseases of common wheat (*Triticum aestivum* L.). The most effective and ecological safe way of protection is production of resistance cultivars. Today there is a basic source of effective genes of resistance to leaf rust, as well as to other diseases in the gene pool of

wild relatives of wheat. The high stock of such genes is concentrated in the *Aegilops* species. For transfer of the leaf rust resistance genes from *Ae. umbellulata* and *Ae. speltoides* to common wheat synthetic forms Avrolata (BAU) and Avrodes (BAS) were used. Marker-assisted screening of the previously obtained resistant introgressive lines did not reveal resistance genes *Lr9* and *Lr35*. At the same time these genes have been identified in synthetic forms Avrodes and Avrolata. For targeted transfer of genes *Lr9* and *Lr35* new crossings synthetic forms with cultivars of common wheat susceptible to leaf rust have been made. Since generations F_4 and BC_2 selection of resistant plants has been assisted with DNA-markers specific to *Lr9* and *Lr35* genes. 126 plants obtained with participation of form Avrolata were analyzed. The marker linked to gene *Lr9* was detected in 62 plants. Screening of 64 resistant plants, obtained on the basis of synthetic Avrodes, for the presence of the *Lr35* gene, has detected this gene in 31 plants. The selected plants were backcrossed with susceptible cultivars of common wheat Krasnodar 99 and GROM. Also the works on combination of the resistance genes *Lr9* and *Lr35* with other *Lr* genes are started. The study is supported by the Russian Foundation for Basic Research and administration of Krasnodar territory (project № 13-04-96545).

USE OF THE SYNTHETIC FORM *TRITICUM MIGUSCHOVAE* FOR COMMON WHEAT IMPROVEMENT

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At present the genetic variability of cultivated wheat has been greatly eroded. The introduction of genetic material from alien species has been a valuable method for increasing the genetic diversity of common wheat. Synthetic hexaploid *T. miguschovae*, which includes genomes of *T. militinae* (AG) and *Ae. tauschii* (D), is a unique source of wheat resistance to diseases and other valuable characters. Using these synthetics a high number of introgressive lines was produced. The evaluation of lines on resistance to a leaf rust, yellow rust and powdery mildew has revealed lines with resistance to two and three diseases. Cytological analysis established that the majority of lines have a stable meiosis (21^{II}). Transfer of genetic material from *T. miguschovae* into common wheat basically occurs through translocations, less often through recombinations and substitutions of the whole chromosomes. Such form of transfer is possibly connected with the presence in synthetics of genomes completely (A and D) or partially (G) homologous to common wheat subgenomes (A, D or B, respectively). The lines with translocation 5BS.5BL-5GL and substitution of chromosome 1D of common wheat by 1D of *T. miguschovae* (1D-1DMig) were identified using C-banding method. The hybridological and PCR analysis indicated that leaf rust resistance genes of the lines differ from each other and from the known genes *Lr39* and *Lr50* transferred from *T. timopheevii* and *Ae. tauschii*. The lines obtained demonstrate high polymorphism by other characters. Lines with high protein and gluten content were selected. The analysis of gliadin indicated that the lines differ from recipient cultivars to the gliadin formula. Introgressive lines with genetic material of *T. miguschovae* are successfully used in breeding. Six common winter wheat cultivars have been developed with using these lines. The study is supported by the Russian Foundation for Basic Research and administration of Krasnodar territory (project №13-04-96545).

GENETIC CONTROL OF WHEAT INFLORESCENCE DEVELOPMENT

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Wheat species (*T. aestivum* L. (BBAADD), *T. durum* L. (BBAA) etc.) present the most important food crops in the world, and the yield of grain from these crops is largely dependent on inflorescence architecture. Studies about the genetic regulation of inflorescence development can allow scientists to design new spike architectures with the goal of enhancing grain production. The inflorescence of wheat is a spike with the main axis (spike rachis)

carrying lateral sessile spikelets that are directly attached to the rachis and a terminal spikelet. The spikelet is a reproductive unit unique to the grass inflorescence, which is comprised of florets and is encompassed by two small bract leaves, glumes. A wheat spike normally bears one spikelet per rachis node, which arises directly on the main inflorescence axis, and the formation of supernumerary spikelets (SS) is rare. Wheat mutants associated with the development supernumerary spikelets constitute key resources in understanding the genetic echanisms underlying wheat inflorescence architecture and, ultimately, yield components. Here, we present the characterization of genetically unrelated mutants that led to the identification of the wheat *FRIZZY PANICLE* gene, which drives the SS traits in wheat. Structural and functional characterization of the three wheat *FRIZZY PANICLE* homoeologous genes (*WFZP*) was performed, in which both the coding and null-mutations of *WFZP-D* are associated with the SS phenotype with the most severe effect when associated with a frameshift mutation in *WFZP-A*. Evolutionary history of *WFZP* in wheat species is discussed. The work is supported in part by RFBR (N15-04-05371 A)

CLE PEPTIDES: MOBILE, DIVERSE, UNIVERSAL

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The balance of proliferation and differentiation of plant stem cells depends on interactions of WOX genes encoding homeodomain-containing transcription factors, and s CLAVATA system, controlling their expression. In the last decade, the attention of many researchers focused on CLE-peptides (CLAVATA3/ENDOSPERM SURROUNDING REGION) - the family of short signal peptides, which play a central role in controlling the fate of stem cells in all types of meristems, as mobile components of CLAVATA systems. CLE peptides are widely distributed in all land plants: in the genome of *Arabidopsis* identified 32 of the gene encoding the peptides of this class, and in the rice genome - 47 CLE genes. Certain members of the family of CLE-peptides (CLV3 in the SAM, CLE40 in the RAM, and CLE41/44 in the cambium) are central regulators of development in the primary meristems. Recent years the role of CLE-peptides in the development of secondary meristems such as meristem nodules in legumes, as well as abnormal meristems – tumors was revealed. The role of CLE-peptides in plant development, apparently, is not limited by meristem: so, was the role of peptide CLE8 in controlling the expression of one of the *WOX* genes in early embryogenesis. A unique event was the discovery of CLE-peptides outside of the plant kingdom – in parasitic root-knot nematodes. However, in the study CLE-peptides, a number of "white spots" such as the study of their interaction with other plant hormones. The study was supported by grants from the University 1.38.229.2014, 1.38.676.2013 and RFBR 14-04-a

THE EFFECT OF STRESSORS ON GENETIC VARIABILITY OF PLANT TISSUE CULTURES

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Adverse environmental conditions have a negative influence on many life processes in plants. Although physiological effects of stresses are actively investigated, the effects of adverse factors on the plant genome remain insufficiently clarified. Various stress factors induce different epigenetical changes which may become an indirect cause of mutations. However, direct data on the origin of stress-related genetic variability are scarce, and effects of stresses on the plant genome were not quantitatively assessed. The aim of this study was to compare the levels of genetic variability of DNA-markers in *Zea mays* and *Arabidopsis thaliana* tissue cultures cultivated under standard and stress conditions: culturing the calli in the medium with copper ions; incubation at high temperature or incubation under anoxic conditions. Maize cells cultured under standard conditions were characterized by a high level of variability RAPD- and ISSR- markers: the percentage of polymorphic DNA bands was about 15 %. Under the influence of stress factors no new polymorphic bands in the spectrum of DNA markers were revealed. On the contrary, a decrease in genetic diversity, probably due to selection of the most viable cells under stress, was observed. No differences were found when samples of *A. thaliana* cells cultured under standard and stress condition were investigated by RAPD and ISSR method despite the large sample of DNA and primers used. However, the more global AFLP method detected 5% polymorphic amplicons. In stressful conditions, the degree of genetic diversity callus cells did not increase. There were no specific amplicons induced by stressors. Induction of culture and culturing *in vitro* was shown to have a stronger destabilizing effect on plant genome than other

stressors used. The action of stressors revealed itself mainly at the population level and resulted in the reduction of genetic diversity, probably, due to selection of the most viable cells. This work was supported by the Russian Foundation for Basic Research, project № 13-04-00950.

THE EFFECT OF PLANT HORMONES ON THE MANIFESTATION OF LEAF PUBESCENCE GENES IN BREAD WHEAT

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One of the adaptation mechanisms in bread wheat plants is associated with the formation of leaf pubescence. This trait is known to make a significant contribution to the protection from pests and adaptation to environmental factors. Currently Catalogue of Gene Symbols for wheat contains only two loci associated with this trait: the gene *Hl1* in 4B chromosome and the gene $Hl2^{aesp}$ in 7B chromosome. Molecular function and regulation of these genes are currently not known. In this study, the effect of phytohormone treatment on the phenotypic expression of bread wheat leaf pubescence genes was investigated. The method of high-throughput leaf hairiness phenotyping (wheatdb.org/lhdetect2) was used. This method allows to obtain rapidly quantitative characteristics of leaf pubescence (length of individual trichomes and their number) among many plants. The effects of phytohormones on trichome cell growth and initiation were explored. The effects of auxin (IAA), gibberellic acid (GA3), cytokinins (6-BAP, Kinetin), metyl jasmonate (MeJa), ethylene (ACC) have been investigated and described. Our data revealed a key role of cytokinin signaling pathway in *Hl1* and *Hl2* gene manifestation. This work was supported by Russian Science Foundation (RSCF) grant № 14-14-00734.

EVOLUTIONARY RELATIONSHIPS IN HEXAPLOID WHEATS AS UNCOVERED WITH BARE-1 AND JELI LTR RETROTRANSPOSON-BASED SSAP MARKERS

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Integration site polymorphism of the LTR retrotransposons (LTR-RTs) Jeli and BARE-1 was studied in 48 accessions of seven hexaploid wheat species (forms) by SSAP analysis and revealed some specifics of genetic differentiation for the wheat group. The genetic pool of the hexaploid species was found to be split into two main groups, one including European spelts (*Triticum spelta* ssp. *spelta* L.) and the other combining *T. aestivum* L., *T. compactum* Host., and *T. sphaerococcum* Percival with the Asian spelt *T. spelta* spp. *kuckuckianum* Gokg. The speltoid species *T. macha* Dekapr. and Menabde, *T. vavilovii* Tumanian and Jakubz., and *T. petropavlovskyi* Udaczin and Migush were intermediate between the two groups, but their position was uncertain. Georgian endemic *T. macha* proved to be genetically close to European spelt. Subgroups formed by the geographical origin of the accessions were observed in the main groups. For instance, spelt accessions of Asian and European origins grouped separately from each other, European spelts formed two distinct independent subgroups, Central European and Spanish Based on the difference in genomic prevalence between Jeli and BARE-1, the genetic relatedness of hulled *T. macha* and European *T. spelta* was attributed mostly to the A genome, while differentiation of Bavarian and Spanish spelts was determined by all of the three subgenomes (A, B, and D). The markers proved to be highly efficient to employ in studying the phylogenetic relationships among species and accessions of the genus *Triticum*.

MICROEVOLUTIONARY DIFFERENTIATION OF CEREAL TETRAPLOID SPECIES BY FORMATION OF RECOMBINANT GENOMES

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The process of microevolutionary differentiation of cereal tetraploid species by formation of recombinant genomes using tetraploid wheat-rye amphidiploids as a model system was reproduced in the experiment and studied in dynamics (F_1 - F_{17}). It was shown that hybridization of tetraploid amphidiploids having one common (pivotal) genome in their composition and differing in secondary (differential) genomes leads to formation of hybrid forms with a wide range of variability caused by different combinations of chromosomes and chromosome segments in differential genomes while maintaining the same structure of the pivotal genome. It was revealed that

the process of chromosome intersubstitution in differential genomes does not take place randomly but is subject to selection pressure during formation of the recombinant genome. Selection proceeds at the level of homeologues, selective advantages of which are determined by genotype-environment interactions. With evident selective advantages of the gomeologue, stabilization of the chromosome composition of the corresponding homeological group ends quickly (in $F_2 – F_3$) forming intergenomic recombinations at the level of whole chromosomes. In the case of equal competitiveness of homeologues, the stabilization rate of the corresponding group composition becomes slower. Dominance of regulatory genetic systems of the pivotal genome provides a high pairing level of homeologues in meiosis in differentiated genomes followed by formation of crossover exchanges whereby intergenomic recombinations occur at the level of chromosome segments. Experimental data were obtained that newly developed tetraploid forms interbreed easily forming a single hybrid zone, where permanent redistribution of genetic material of differential genomes and further range expansion of genotypic variability available to selection, take place during alternation of generations whereby such a zone becomes a potential centre of speciation. Subsequent adaptive radiation of hybrid material in ecologically separated environment is carried out by selection of forms with different variants of the recombinant genome in various ecological niches. It promotes rapid divergence of species and emergence of new taxonomic units on their basis.

USING THE COMMON WHEAT SUBSTITUTION AND NEAR ISOGENIC LINES TO STUDY CHARACTERS THAT DETERMINE ADAPTATION AND RESISTANCE TO STRESS

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The effects of individual chromosomes of common wheat on the expression of the important adaptive and agronomic traits were investigated based on established substitution and introgression lines. To create substitution lines methods of rapid and exact transfer of the whole chromosome or the chromosome fragments of the related and alien species into genotypic environment of different recipient varieties were exploited. We used *in situ* hybridization and PCR analysis with chromosome-specific molecular markers to identify alien rye, barley and *Agropyron* chromosomes. In the process of creation of the wheat-alien substitution lines laws and features of intergenomic substitutions and translocations between chromosomes of rye, barley and common wheat were studied. In lines with the replacement of individual chromosomes new genes or alleles determining the time of flowering and response to vernalization, resistance to stressful environmental influences (wintering/frost resistance, resistance to fungal diseases) were identified. The new genetic models were created on the basis of common wheat isogenic and substitution lines having the different alleles *VRN-1* loci to study the genetic mechanisms of regulation the length of growing season. The influence of *Vrn-B1a* and *Vrn-B1c* alleles on the length of developmental phases in lines with intervarietal substitution of chromosomes 5B and near-isogenic lines with these loci were studied. We have confirmed that the effects of *Vrn* genes appeared on the tillering phase and response to vernalization and shortening of day length can change the duration of this phase. We have shown that the *Vrn-B1a* allele has a strongest effect on the length of tillering after vernalization and short-day conditions. The combinations of alleles of the *Vrn-A1*, *Vrn-B1*, *Vrn-D1* genes were analyzed with allele-specific primers in 150 spring wheat varieties from Siberia. Winter 5R(5A) wheat-rye substitution lines were obtained which will be used as a genetic models to study wheat winter hardiness. This will enable a further use of the genetic potential winter rye, which has the largest wintering/frost resistance among other cereals for creation the original forms with increased resistance to abiotic stresses.

EVALUATION OF SOYBEAN REGENERANTS WHICH ARE TOLERANT TO THE IONS OF COPPER (Cu^{2+})

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Biotechnology and bioengineering plays special role in solution of the problem of increasing the efficiency of agricultural production in our country. Recent years the heavy metals’ ions are recognized as a mutagenic factor and are used in the process of regeneration *in vitro* to increase additionally genetic diversity. However, there should be noted a narrow range of research on soybean in the described

direction, which proves necessity of the experiments performance using heavy metals as selective background in vitroin the research with soybean tissue culture. The objects of our research were the following 222 regenerant lines of soybean from 10 initial forms (Primorakaya81, Primorskaya13, Primorskaya301, Primorskaya69, Primorskaya28, Hodson, Lidia, R1, R362, R565), developed on the selective mediums with addition of copper ions (Cu^{2+}) and ions of zink (Zn^{2+}) as a mutagenic factor. As a result of research on the seeds productivity from one plant in the selection nursery there were distinguished 19 regenerant lines, which reliably exceed the initial forms. According to the results of the study in the control nursery seven lines exceeded standard on yield by 3,0-19,7%. Maximum increase of yield by 19,7% and 15,4% was marked on lines R944 and R793. According to biochemical indices, there were three lines which differed by high content of oil and histidine in the seeds when there was a low index of linolenic acid (R 1485, R 1357, R 1490). Regenerant line R1357 was distinguished on the complex of reliably exceeded biochemical comopnents. The relative electrophoretic mobility of peroxidase with area of 0,36-0,40 Rf was found in the forms developed on the medium containing ions of Cu^{2+}: R 1357, R 1496, R 1524, R 109 and R 1490. More mobile electrophoretic spectra were defined for R 1496 (0,43-0,45 Rf), R 1490 (0,48-0,50 Rf), and R 1518 (0,42 Rf; 0,47 Rf). While creating soybean somaclones, tolerant to abiotic factors of the environment, it will be more effective to use copper ions (Cu^{2+}) as a selective factor in the nutrient medium.

EPIGENETIC REGULATION OF CYTOPLASMIC MALE STERILITY IN SORGHUM

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Restoration of male fertility in plants with cytoplasmic male sterility (CMS) is a complex phenomenon that is conditioned by interaction of nuclear fertility-restoring genes with genetic factors of specific CMS-inducing cytoplasms. In many CMS types, environmental factors can disturb this interaction and cause male sterility in the F1 hybrids. We found that in the '9E' CMS-inducing cytoplasm of sorghum, water availability of plants and relative humidity of air at flowering period are the key factors governing restoration of male fertility of the F1 hybrids. DNA of male-sterile and male-fertile hybrids grown, respectively, in the "dry plot" and irrigated plot, and DNA of male-fertile revertants developed from male-sterile hybrids in the greenhouse, were used in comparative MSAP-analysis. MSAP-analysis carried out with *Hpa*II/*Msp*I restrictases and primers to the genes involved in pollen development and anther dehiscence revealed differences in the number and the length of amplified fragments associated with restoration of male fertility. These data suggest that drought conditions may down-regulate expression of fertility-restoring genes by changes in methylation of their nucleotide sequences. This observation favors understanding unusual phenomenon in the '9E' cytoplasm, when fertility-restoring genes are stably function in fertile lines obtained by self-pollination of restored F1 hybrids but poorly function in test-crosses of these fertile lines to CMS-lines with the same cytoplasm type. Perhaps, certain genes involved in formation of fertile pollen are repressed by methylation in genome of F_1 hybrids in the '9E' cytoplasm; in conditions of high water availability the repression is removed, and fertile pollen is formed. As a result of self-pollination in heterozygous plants, homozygotes arise; they may be less sensitive to drought conditions and are fertile under both high and low water availability. These data demonstrate that methylation of nuclear genes in sterile cytoplasm may be one of mechanisms causing the CMS phenomenon. This work was supported by the RFBR, grants 13-04-01404, 14-0497089 r_povolzh'e_a.

CONSTRUCTION OF EXPRESSION VECTOR SYSTEMS FOR TANDEM EXPRESSION OF TWO OR MORE GENES IN PLANTS

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Nowdays, plant genetic engineering often requires co-expression of two or more genes in one cell of modified organism. The successful gene co-expression can be obtained by using 2A-sequence, which mediates the effective cleavage of peptide products co-translationally. Compared to other co-expression systems the 2A- one has such advantages as: production of complex proteins consist of several

subunits in equimolar proportion without proteolytic enzymes; subsequent targeting of translation products to one or different cell compartments. Currently, the wide range of expression vectors is used. For instance, plant transient expression vector pXSN is convenient due to capability of TA-cloning and existence of suicide gene *ccdB* for self-ligation control. Besides, virus based vectors are commonly used due to high level of expression. One of them - pJL-TRBO vector based on tobacco mosaic virus genome do not require co-transformation with gene silencing suppressor p19. The purpose of our research is to create plant expression vectors with polypeptide autoproteolysis for tandem expression of two reporter genes *gfp* and *bfp* under one promoter. Such constructs would be an effective tool for simultaneous production of two or more heterologous proteins in one plat cell. At first, by molecular cloning methods the *gfp-2a-bfp* encoding green and blue fluorescent proteins divided by autoproteolysis 2A-sequence was constructed. The correction of this *gfp-2a-bfp* construction was proved by sequencing analysis. Secondly, obtained sequence was cloned in expression vectors pJL-TRBO and pXSN, thus pJL-*gfp-2a-bfp* and pXSN-*gfp-2a-bfp* have been obtained. Also the control vectors pJL-*bfp* and pJL-*gfp* was constructed. Thirdly, all these vectors were transformed into *Agrobacterium tumefaciens* cells, which subsequently were infiltrated into *Nicotiana benthamiana* plants leaves. Efficiency of the work of all vectors was detected visually and by immune hybridization analysis.

THE STUDY OF THE LENGTH OF VEGETATION PERIOD OF SPRING OCTOPLOID AND HEXAPLOID TRITICALES

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Under artificial growth conditions octoploid (8x) triticales with dominant *Vrn* genes headed in the order, established earlier for the NILs of common wheat: 8xVrn-A1 (53 days) ≥ 8xVrn-D1 (60 days) ≥ 8xVrn-D4 (63 days) ≥ 8xVrn-B1 (76 days). In the same row they are ranged according to the length of period "tillering – the first node": 8xVrn-A1 (3 days) ≥ 8xVrn-D1 (8 days) ≥ 8xVrn-D4 (18 days) ≥ 8xVrn-B1 (22 days). Under natural conditions the number of days before heading of different families of 8x triticales was within the interval 60-77 days (for 8xVrn-A1), 72-94 days (for 8xVrn-B1), 70-97 days (for 8xVrn-D1), 68-103 days (for 8xVrn-D4). These differences on heading time of families within a Vrn triticale group may be explained by rye gametes heterogeneity within a cross combination in the process of triticale making, or by aneuploidy, specific for 8x triticales. Under natural conditions all 8x triticales headed a few days later, than under artificial ones. 32-days vernalization differently influenced the 8x triticales. After vernalization two families of 8xVrn-A1 group headed 2-3 days later, than the non-vernalized control. One family of 8xVrn-B1 group headed 6 days earlier, the other family – 6 days later, than the control. The response to vernalization of one family of 8xVrn-D1 group was 10-days acceleration of heading. 8xVrn-D4 family headed 2 days later, than the non-vernalized control. Under natural conditions hexaploid (6x) triticale samples headed earlier, than 8x triticales – from 2 to 29 July. In addition, periods "shoots – the first node" and "stem elongation – heading" in 6x triticales were shorter, than in 8x triticales, which, probably, caused earlier heading of 6x triticales. The earliest 6x triticales ripened at the end of August, the latest – at the beginning of October. At the second term of sowing, 1-2 weeks later the first one, the majority of 8x and 6x triticales shortened the period "shoots – the 3rd leaf", and lengthened the period "tillering – the first node".

WOX-CLAVATA SYSTEM IN SECONDARY GROWTH OF ROOT IN RADISH (*RAPHANUS SATIVUS* L.) INBRED LINE

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CLE peptides are small proteins which are important in regulating plant meristematic activity. The targets of CLE in the shoot apical meristem are WUSCHEL (WUS)-RELATED HOMEOBOX (WOX) family transcription factors. WOX-CLAVATA regulatory system might control cell proliferation and differentiation not only in the root and shoot apical meristems but also in cambium, nodule meristems and meristem-like structures, such as galls and tumors. We identified 19 radish RsCLE genes: 17 RsCLE genes homologous to A-group AtCLEs, and 2 RsCLE genes homologous to B-group AtCLEs. The expression of some *RsCLEs* changed upon radish root and hypocotyl thickening. Furthermore, qRT-PCR analysis detected significant differences on *RsCLEs*

and *WOX* expression levels in different part of radish root. We suggested that CLE peptides encoded by these genes regulate root thickening. To check this hypothesis we carried out the experiments on overexpression and exogenous CLE peptide treatment. Also we have analyzed *RsCLE* and *WOX* expression in the response of exogenous CLE peptide treatment. This work was supported by the St. Petersburg State University research grants 1.38.229.2014 and 1.38.676.2013, RFBR grant 14-04-00591a.

THE LIGHT-DEPENDED REGULATION OF GLUTATHIONE REDUCTASE GENES IN *ARABIDOPSIS THALIANA*

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Glutathione reductase (EC 1.8.1.7) is a FAD-containing antioxidant enzyme responsible for the glutathione conversion from oxidized to reduced form in the glutathione-ascorbate cycle. There are two nuclear genes in *Arabidopsis thaliana*, *gr1* and *gr2*, coding for cytosolic and plastidic/mitochondrial forms of the enzyme, respectively. The enzymatic activity of glutathione reductase is stimulated by a number of biotic and abiotic stresses. The transcript levels of both *Arabidopsis* glutathione reductase genes are higher at light compared to dark, but the mechanisms of this regulation are studied poorly. We had demonstrated that the main increase of *gr1* and *gr2* transcript levels in the light shift experiments occurs when the light conditions change from dark to low light (up to 10-20 mcmol*m^{-2}*s^{-1}), and the further light escalation has no pronounced effect. Using photosynthetic electron transport inhibitors we had showed that expression of *gr2* gene (but not *gr1*) can depend on plastoquinone pool redox state. We propose that the light-related induction of glutathione reductase genes in *Arabidopsis* depends on the events related to the state transitions in the photosynthetic electron transport chain. The work has been financially supported by the RFBR grant 14-44-04001.

FORMING OF CHINESE WHEAT SPECIES

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Since the middle of the last century the information on discovering and describing of new polyploidy wheat species in China has appeared. Hexaploid wheat species was discovered in Xinjang in 1948 and was described as *T.petropavlovskyi* Udacz. et Migusch. One more endemic Chinese species – *T.yunnanense* King was described in 1959 and *T.tibetianum* Shao - in 1970-s. Only one of them (*T.petropavlovskyi*) had included in genus *Triticum* L. taxonomy by Dorofeev et al. (1979). *T.petropavlovskyi* is hybrid species carrying the *T.polonicum* gene *P1* for long glumes. It should be placed in *T.aestivum* according to our results of studying as ssp. *petropavlovskyi* (Udacz. et Migusch.) N.P.Gontsch. More detailed research on *T.aestivum* ssp. *tibetanum* Shao и *T.aestivum* ssp. *yunnanense* King ex S.L.Chen. is required. The latter subspecies doesn't differ from *T.spelta* subspecues according to its agronomic traits [Dong Y. et al., 1981]. The situation with *T.aestivum* ssp. *tibetanum* is also more difficult because its taxon status isn't strictly determined by authors. According to Tsunewaki et al. [1990], its including in common wheat causes some bewilderment because the type is described as hulled speltoid. However, a gene, which control hulled in it and that in T.spelta, are non allelic. Hence, ssp. *tibetanum*, most probably is also artificial amphyploid. Endemicity of all three hexaploid wheat subspecies is relative, because all Chinese tetraploid wheats are borrowed from other areas. The results of studying of ssp. *tibetanum* and some other hexaploid wheats confirm the probable artificial origin of ssp. tibetanum [Cao et al., 2000]. According to our results in spite of the fact that spelt phenotypes in *T.spelta*, *T tibetanum* and *T.yunnanense* is controlled by non-allelic genes, we believe that to add symmetry and logicality to genera *Triticum* taxonomy it is necessary to describe two latter species as subspecies of *T.spelta*, because description of species (subspecies) in any «Keys…» (for example, see Gonharov 2011), based on morphological characters, is made according to the characters which are typical for species (subspecies) and genes are important only for describing phylogeny and relative relationships in genera. The place of this and other subspecies in the genera *Triticum* taxomony is discussed.

LOW-DOSE IRRADIATION EFFECT ON SEEDLING GROWTH PARAMETERS IN BREAD WHEAT LINES DIFFERING BY ANTHOCYANIN CONTENT

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Anthocyanins are biologically active compounds of plant origin. As natural antioxidants, anthocyanins may scavenge free radicals generated in plants under unfavorable environmental conditions, including excessive UV, cold, drought, salinity, heat, heavy metal toxicity or irradiation, thus preventing cell destruction. Since anthocyanin pigmentation can be favorable for germination of seeds exposed to damaging factors (in particular, the maintenance of seed viability after irradiation exposure is one of the tasks important in the course of space exploration projects), we analyzed in the current study seedlings' growth parameters of wheat near-isogenic lines (NILs) differing by anthocyanin content in grains and coleoptiles. The dry seeds of 8 NILs were exposed to 50, 100 or 200 Gy, and then germinated along with unirradiated control under 12-h photoperiod with the temperature 20°C. At the fourth day after germination the length of the longest root and that of the first leaf as well as root and shoot weights were measured. Tolerance indices were calculated as the ratio of the parameter value under irradiation to the corresponding value of unirradiated control. Statistical analysis was performed using Statistica 6.0 software. Multiple comparisons carried out using Kruskal-Wallis test showed significant positive effect of the coleoptile coloration, grain coloration and combination of these two traits on tolerance indices under 50 Gy irradiation. Similarly, grain coloration and combination of grain and coleoptile coloration positively affected tolerance indices under 200 Gy irradiation. In addition, Spearmen test revealed positive correlation between anthocyanin content in the coleoptile and tolerance indices under each dose 50, 100 or 200 Gy. We also estimated dose effect on the change of relative anthocyanin content in the coleoptile. In genotypes with weak *Rc* (red coleoptile) allele the dose-dependent decrease of the relative anthocyanin content was observed, while in the lines with strong *Rc* allele irradiation exposure did not result in significant changes in anthocyanin production. Thus, it is concluded that intensive anthocyanin pigmentation of coleoptiles and its combination with grain coloration can increase vigor of seedlings germinated from low-dose irradiated wheat grains. This study was partially supported by the State Budget Programme (Project No. VI.53.1.5.) and the Integration project SBRAS/NAS Belarus. We thank Galina Generalova and Olga Zakharova for technical assistance.

DNA ELIMINATION IN RYE TETRAPLOID FORMS

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The research carried out in recent years demonstrates that polyploidy is more than a summation of the genomes. The study on various plant systems by using molecular technologies has shown that genesis of polyploid forms is accompanied by genomic transformations and modifications. The data obtained in synthetic polyploids verify the genomic changes during polyploidization: rearrangement of entire DNA sequences, DNA elimination, gene diversification, DNA methylation changes, activation of mobile genetic elements, SNP-polymorphism, etc. We have transferred 5 commercial cultivars of winter diploid rye (*Secale cereale* L., 2n =14) to a tetraploid level (*2n* = 28) using the nitrous oxide (N_2O) technique for genome duplication (polyploidization) in zygote. It has been revealed that the DNA amount does not increase in direct proportion to the ploidy level. A part of DNA was found to eliminate in subsequent generations after polyploidization. To determine the DNA amount in rye tetraploid forms as against the original diploid cultivars cytophotometric measurements of the DNA content in their nuclei were made. The DNA content was determined by fluorochrome - ethidium bromide, fluorescence of which is proportional to the DNA content in the nucleus. The nuclei were analysed by computer processing of microphotographs obtained by the fluorescence microscope. It was established that the amount of eliminated DNA vaied from 10,0 to 28,0% and made up on the average 20.2% in the obtained rye tetraploids in 4-7 generations after genome duplication. The dependence of the eliminated DNA amount on the tetraploid generation after genome duplication was revealed. The amount of eliminated DNA made up 10-19% in the 4^{th} -6^{th} generation and 25,0-28,0% - in the 7^{th} one. A number of researchers have shown that noncoding sequences, entire

chromosome segments, highly repetitive DNA sequences, and telomere heterochromatin eliminate in eukaryotes after genome duplication. The DNA elimination can be caused by unequal and "illegitimate" homologous recombinations, retrotransposons activation and natural selection, favouring species with a reduced DNA amount.

GENETIC DIVERSITY OF NATURAL POPULATIONS OF *P. SIBIRICA* AND *P. PUMILA* AND THEIR HYBRIDS SHED A LIGHT ONTO THE ORIGIN OF THE WIDESPREAD NONMONOPHYLY IN *PINUS*

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Pinus is among the most numerous genera of conifers; it is comprised of more than 100 species subdivided into two subsections: *Pinus* and *Strobus*. Studies of the phylogenetic relationships with various molecular markers in *Pinus* have been shown that sequences of some species could be clustered into the several paralagous groups, which was interpreted as nonmonophyletic origin of these species. There is seems to be one possible and the most plausible explanation of the discrepancies observed by the suggestion of frequent interspecific hybridization in *Pinus*. Numerous cases of natural interspecific hybridization were described for different *Pinus* species, and successful artificial hybridization experiments were also carried out. *Pinus sibirica* and *Pinus pumila* are among the most widespread species in the *Strobus* subsection. According to the last phylogenetic studies of *Pinus*, these species are clustered separately, and no cases of nonmonophyly for them were observed. *P. sibirica* and *P. pumila* have overlapping ranges over a large area in East Siberia, and in overlapping regions their interspecific hybrids were found and morphologically described. To evaluate the impact of interspecific hybridization to the observed cases of the nonmonophyly, the analysis of the genetic diversity of *P. sibirica* and *P. pumila* and their hybrids both in the places of overlapping ranges and outside of them is required. In the present study 11 specimens of *P. sibirica*, 7 specimens of *P. pumila* and 13 specimens of their morphological hybrids from natural populations ranging from Urals to the Far East were analyzed using the three unlinked molecular nuclear markers: LEA (Late Embryogenesis Abundant (LEA)-like gene, linkage group 3), 4CL (4-coumarate: CoA ligase, linkage group 7), and AGP6 (Arabinogalactan-like protein 6, linkage group 5). Comparative and phylogenetic sequence analyzes revealed two species-specif alleles for each marker studied, which are the characteristics for *P. sibirica* and *P. pumila*, respectively. Those specimens which were described as hybrids have been shown simultaneous presence alleles of both from *P. sibirica* and *P. pumila*, and in all possible combinations, for all the three genes studied (F_1, F_2, … F_n generations of the hybrids), that speaks in favor of multiple events of hybridization of the hybrids to themselves and/or with *P. pumila* and *P. sibirica*. Thus, indeed, the interspecific hybridization in *Pinus* takes place and could be easily detected using standard set of unlinked genetic markers. The existence of interspecific hybrids of various generations may explain the origin of the complicating pattern of nonmonophyly, which is often occurs in phylogenetic analyzes of the *Pinus* species.

POTENTIAL OF MISEQ (ILLUMINA) PLATFORM FOR FLAX WHOLE-TRANSCRIPTOME ANALYSIS

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Next-Generation Sequencing is the approach that made the revolution in molecular biology, including plant biology. However, its application to study transcriptome of multicellular organisms faces the challenge of sample heterogeneity: as a rule, tissue mixture is analyzed, giving average values for cells of different types and at various stages of development. We used platform MiSeq Illumina for whole-transcriptome analysis of certain plant cell type at certain developmental stage. Such experiment was performed on the developing in planta phloem fibers of flax (*Linum usitatissimum* L.) at the stage of tertiary cell wall formation. Such cell wall type is rich in cellulose (up to 90%) and lacks detectable xylan and lignin; the mechanisms of tertiary cell wall formation are largely unclear. Presence of thick cell wall gave possibility to isolate fiber bundles by

washing stem tissues in 80% ethanol. For comparison, stem portions containing fibers with only primary cell wall were used. A cDNA library for each sample was sequenced using single-end read sequencing on a MiSeq System. The reads (75bp) were mapped to the flax genome sequence using TopHat. Reference-based assembly of the aligned reads and the estimation of transcript abundance in terms of Fragments Per Kilobase of exon per Million mapped fragments (FPKM) was performed using the Cufflinks protocol. From 19,3 to 24,2 million reads per sample were acquired, having quality scores Q30. About 95% of reads were successfully mapped to the reference sequence. The mean gene expression levels indicated that 62.6% had FPKM values between 1 and 100, 5.4% of genes had the expression value above 100. A total of 32098 from 43484 protein-coding genes were expressed, of which 1124 differentially expressed genes (DEGs) were detected with a q-value less 0,05. Validation of transcriptome profiling results using qRT-PCR was performed (23 genes). DEG characterization would be given in presentation.

ABSCISIC ACID-DEPENDENT GENE EXPRESSION IN BOTH THE HOST-PLANT AND PHYTOPATHOGENIC BACTERIUM AS AN IMPORTANT ASPECT OF PLANT-MICROBE INTERACTIONS

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Abscisic acid (ABA) is a well known phytohormone that coordinates abiotic stress responses, water balance and ontogenetic programs in plants. Wherein, ABA was also shown to determine several aspects of plant-microbe interactions. The pathogenesis induced by representatives of *Soft-Rot Enterobacteriaceae* (*SRE*), including *Pectobacterium atrosepticum* (*Pba*) is known to depend on water and ontogenetic status of the host-plant. However, the roles of ABA and ABA responses in plant-*SRE* interactions are unknown. The aim of the present study was to explore the possible roles of ABA in plant-*Pba* interactions. Gene expression analysis was used to monitor ABA-signaling in infected tobacco plants and to check the possibility of ABA perception by *Pba* within the frameworks of plant-pathogen cross-talk. The level of ABA signaling was assessed by the expression of ABA marker-genes, including ABA-responsive ones and those that encode ABA biosynthetic enzymes and proteins involved in ABA-signal transduction. Expression of all the marker genes selected was down-regulated during *Pba*-induced infection, indicating the repression of ABA-pathway in the host plant. The responsiveness of *Pba* cells to ABA was analyzed using in vitro model by means of Next-Generation Sequencing (Illumina Miseq Platform). Many *Pba* virulence-related genes, including those that encode effector proteins of type III secretion system, pectate lyases, regulatory and transport proteins, were shown to be ABA-repressed. This is in agreement with our finding on ABA-dependent repression of activity of key *Pba* virulence factors – pectate lyases – in bacterial cultures. Thus, our data indicate on ABA-mediated suppression of Pba virulence; in turn, the progression of infection is related to the repression of ABA-pathway in the host plant. The obtained results are in accordance with the well-known observations: *Pba*-induced disease symptoms are not expressed under low humidity when the concentration of ABA is elevated in plants. This study was supported by the RFBR, research project No. 14-04-01750_A.

ANTHER TECHNOLOGY *IN VITRO* IN THE DEVELOPMENT OF A WINTER RYE (*SECALE CEREALE L.*) DOUBLE HAPLOIDS.

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The success of hybrid rye cultivars development is defined first of all by presence of a wide gene-pool of inbred lines. Traditionally rye lines development consists of consecutive self-pollination of plants with use of self-compatibility genes (Sf). This process occupies 4-5 years, thus it is impossible to reach 100 % homozygous lines. Development of double haploid plants is the most effective way of constant homozygous samples development. For a rye there are no big successes in the given direction, so working off of all stages of an anther culture *in vitro* is necessary: definition of an optimum stage of anther development, methods of preprocessing of anthers, type of medium, conditions of an artificial climate for a callus development, plants regeneration, methods of doubling of chromosomes number. The aim of present researches is working off of anther culture *in vitro* technology for a rye. Mediums “Murasige-Skuga” and “Potato” were used. The planted material

during 4-5 weeks has been stored at t=25-28 °C without illumination before occurrence of calluses. In total it has been planted 21900 anthers. It was formed 204 calluses. All calluses were replaced on the medium "Murasige-Skuga". From 204 calluses new growths were recycled at 123 that, including risogenesis, white plants and green plants. Green plants are developed on the genetic basis of the tetraploid rye variety "Belaya Vezha" on the first medium "Potato", as were not exposed polyploidization by colchicine since tetraploid number of chromosomes allows to exclude this procedure. The use of diploid samples did not allow to receive any plant. From 28 green plants at various stages of germination was lost 15 that because of various infringements (aneuploid genotypes, etc.). Seed yield was obtained from 7 plants in an artificial climate conditions. Seed-set was 12,6% - 54,5% depending on genotype. The developed material will be comprehensively studied on a complex of traits with next selection of genetic stable samples.

OPTIMIZATION OF PROTOCOL OF ANTHER CULTURE TECHNIQUE *IN VITRO* FOR PRACTICAL RICE BREEDING PROGRAMME IN RUSSIAN FAR EAST

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Anther culture technique is widely used for practical rice breeding in many counties. But varieties of rice received through biotechnological methods are absent in Russian Far East. Anther culture can be valuable method for improvement of rice cultivars in Primorsky Region (Russia). Purpose of investigation is optimization of protocol of anther culture technique *in vitro* for Far Eastern hybrid rice lines. Four F_2 hybrids of rice *Oryza sativa* L. subspecies *japonica* were used for experiment. Donor rice plants were cultivated during summer 2013 in three types of environment. Panicles were stored in the refrigerators at 5°C or 10°C for 7 days. The anthers were cultured on 8 variants of nutrient medium with different mineral and hormonal composition. Calli were transferred for differentiation on identical regeneration medium. 8226 immature anthers of rice were inoculated on different mediums (from 20 to 128 explants on each variant). The callus induction frequencies varied from 0 to 40,0%. Two medium variants chose with maximum average callus induction (14,9% and 18,5%). There was no difference in effect between low-temperature pretreatment of panicle for callus induction. The higher frequency of plant regeneration and ratio green shoots/albino was observed in the low-temperature pretreatment 5°C. The release of microspores from the anther and their subsequent divisions leading to plant regeneration often depend on the conditions under which the donor plants were grown in a particular environment. In our experiment best callus induction frequency was recorded for hybrids 2-1 and 7-1 for donor rice plants cultivated at t=20°C. But for 13-3 hybrids open space for vegetation donor plants was preferable for callus induction and regeneration. Plant regeneration on callus was twice and more higher on open space rather at the other environments. So it is difficult to unify conditions for donor plants, each genotype requires unique environment. As the result of work, 264 rice regenerant lines with seeds were utilized for selection in practical rice breeding programme in Primorsky Region.

ARG-X PROCESSING IN INTERPHASE CHROMATIN REORGANIZATION IN WINTER WHEAT

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Plants have developed a number of monitoring systems to feel the environment, to coordinate their growth and development respectively. Vernalization is an example of in which the flowering happened after the plants were exposed to long period of cold temperature. The cold period of plant life leaves a certain pattern for its vegetative morphogenetic period. The corresponding to these factors genes of vernalization and photoperiod are closely related and form a single dynamic network in the organization of proteome processing of chromatin matrix. The biochemical effect of such influence could be an anchored pattern of molecular morphogenesis. Features of the biochemical adaptation, that are taking place in the cell nucleus can be incorporated into molecular mechanisms of intranuclear *Arg-X* proteolysis on the chromatin matrix during the

induction of growth processes of vegetative growth phase that formed the mature embryos of spring and winter wheat. The aim of this work was the analysis of localization of *Arg-X* protease-sensitive zones in non-histone and histone blocks of suprastructures (nucleoplasm, chromatin, the nuclear matrix), as possible zones affecting to the conformational rearrangements of total interphase chromatin in the G1/S-transitional phase of the cell cycle, during the induction of vegetative growth phase of morphogenesis mature embryos of spring and winter wheat. For the investigation have been selected elite seeds of wheat (*Triticum aestivum L.*) varieties Artemovka (spring), transformed from it Mironovskaya 808 (winter) and transformed from last Mironoskaya spring. From hatched embryos was isolated the cell nuclei and their suprastructures. Our data showed that the trypsin-like zones that are sensitive to *Arg-X* proteolysis of winter varietity are located in non-histone proteins and H2A + H2B core histones in chromatin supra-structure that tightly bound to the nuclear matrix. We consider that *Arg-X* hyper-sensitive trypsin-like zone may be one of the elements of LCR (locus control regions).

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INTROGRESSION OF RYE CHROMOSOME 1R LEADS MODIFICATIONS IN WHEAT SUBGENOMES

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To increase the adaptive properties of wheat as a source of traits rye *Secale cereale* L. is used. To date, wheat varieties carrying wheat-rye 1RS.1BL translocation are widely spread. 1RS.1AL translocation and wheat-rye 1R(1B) substitution are less spread. During the transmission of rye chromosomes in wheat genome their modification occurs, however, the information about the changes in the structure of wheat subgenome chromosomes is rather limited. We conducted karyotyping self-pollinated progeny of F_2 on the model of wheat-rye 1Rv-1A dimonosomic. Various chromosomes aberrations of wheat and rye were identified. In addition to the telocentric 1RS chromosome, the second product of the misdivision univalent 1R chromosome - telocentric 1RL was identified in two plants. The karyotype of one plant contained telocentric chromosome 1DL, another - 2BS. The modifications of such type could arise due to cross-division in the centromere region (misdivision) of univalent chromosomes of rye and wheat in the meiosis of F_1 hybrids. In the analysed progeny wheat chromosomal aberrations as deletions of large fragments of one of the arms were noted. Introgression of chromosome 1R caused a monosomy 3D, 4A, 6A chromosomes in the individual plant, and trisomy 7D chromosome in two plants. Asynapsis can be a possible reason for the elimination of chromosomes. However, the line 1Rv(1A) is cytologically stable, indicating a compensatory ability of rye chromosome. Average number of univalents on the cell did not exceed values 2.01 - 2.05 in the 1Rv-1A meiosis. Overall, 42.8% of the analyzed plants contained either aberrant chromosomes or they were unstable due to chromosome composition. Thus, the results showed that the selection of individual genotypes of alien-substituted forms is necessary for purposeful reorganization of wheat genome. Transmission of single rye chromosome, for example, 1R in the wheat genome, results in structural modifications both a rye chromosome itself and chromosomes of wheat subgenomes too.

THE INFLUENCE OF WHEAT-RYE CHROMOSOME SUBSTITUTION ON THE FORMATION OF HYBRIDS *TRITICUM AESTIVUM* L. x *SECALE CEREALE* L.: MORPHOLOGY, PRODUCTIVITY, KARYOTYPES

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Introgressive forms of bread wheat with rye genetic material are widely used in breeding programs. The formation of new hybrids is accompanied by genome reorganization resulting from intergeneric hybridization, during which cytological and genetic stability are achieved in diploid progeny. Stabilization of hybrid genomes is accompanied by phenotypic and meiotic instability, changes in

morphology of wheat and rye chromosomes. Molecular analyses showed large DNA sequences elimination in triticale. Most changes are related to the rye genome. In this work the formation of hybrids C29xR, 1Rv(1A)xR, 6R(6A)xR was studied in self-pollinated progenies F_2 and F_3. The analysis of karyotypes of F_2 hybrids was conducted using C-banding and genomic *in situ* hybridization. The results showed differences in the chromosomal composition of genome: the presence of complete set of rye chromosomes and absence of centric breaks on octoploid hybrids 6R(6A)xR, elimination of rye chromosomes and centric breaks on hybrids 1Rv(1A)xR. Analysis of fertility showed a large variability in the number of grains per plants in all hybrids. Also a large variability found according to morphological characteristics such as the shape of the spike, the shape and color of the grains. The plants were selected with high grain number per spike among C29xR, 1Rv(1A)xR and 6R(6A)xR for the analysis of forming hybrids in F3 generation. The progeny of these plants were fertile, but it differed by the number of grains, 1000 grain weight, the shape and color of the grains. Hybrids 6R(6A)xR was characterized by larger grains. 1000 grain weight amounted to 46.11 grams. White grains had an elongated shape. Hybrids 1Rv(1A)xR had smaller grains with red color. The weight of 1000 grains amounted to 33.56 grams. Plants 6R(6A)xR and 1Rv(1A)xR differed also by the length of the vegetation period and morphotype. Therefore, the genotypes of lines 6R/6A and 1Rv/1A make an individual contribution to the formation of hybrids.

FLAVONOID BIOSYNTHESIS REGULATORY NETWORK IN TRITICEAE

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Flavonoids are plant phenolic compounds related with plant adaptation to unfavourable environment conditions. The enzymes participated in synthesis of flavonoid compounds are encoded by the flavonoid biosynthesis structural genes (FBSGs), while FBSGs are activated by transcriptional factors (TFs) encoded by regulatory genes (FBRGs). Unlike FBSGs, the regulatory FB genes encoding TFs of MYB, MYC or WD40 type belong to multigene families. Isolation of FBRGs sequences is complicated (especially in allohexaploid wheat *Triticum aestivum* L.) and is usually based on forward genetics approaches. Among the first FBRGs isolated in Triticeae are the genes determining synthesis of flavonoid pigments. The *TaMyc1* (*Pp3 – purple pericarp*) gene encoding MYC-factor necessary for synthesis of flavonoid pigments anthocyanins in pericarp as well as the *HvMpc1* (*Ant1*) gene of barley *Hordeum vulgare* L., encoding R2R3 MYB-type protein (a part of the regulatory machinery governing anthocyanin synthesis in the leaf sheath), were isolated and characterized in the current study. Using wheat near isogenic lines (NILs) differing by FBRGs allelic composition, the regulatory interaction between MYC- and MYB-encoding FBRGs was demonstrated. In addition, analysis of these NILs revealed the key point of regulation of the anthocyanin biosynthesis in wheat: it is activation of *F3h*, the gene encoding flavanone 3-hydroxylase. Species-specific regulatory features of flavonoid synthesis are discussed. This study was partially supported by RFBR (grant no 14-04-31637).

THE ROLE OF CALCIUM-DEPENDENT PROTEIN KINASE GENE *VaCPK21* IN THE ABIOTIC STRESS RESISTANCE OF WILD-GROWING GRAPEVINE *Vitis amurensis* Rupr.

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Calcium-dependent protein kinases (CPKs) are known to play important roles in plant adaptation to abiotic stresses. We aimed to investigate the role of *VaCPK21* gene in the adaptation to abiotic stresses of wild-growing grapevine *Vitis amurensis* Rupr. We studied *VaCPK21* expression under abiotic stress treatments using healthy *V. amurensis* cuttings. qRT-PCRs revealed that mRNA levels of *VaCPK21* were significantly up-regulated under high salt, high mannitol, and high temperature stresses. We obtained six transgenic cell lines of *V. amurensis* overexpressing the *VaCPK21* gene and a control cell line transformed with the "empty" vector using Agrobacterium-mediated transformation. We examined the effect of salinity, heat, and cold stresses on growth of the cell lines. We also obtained three transgenic homozygous *Arabidopsis* plant lines

overexpressing the *VaCPK21* gene. We analyzed the effect of salinity, heat, cold, and drought stresses on the survival rates of the *Arabidopsis* plants. The data obtained revealed that *VaCPK21* overexpression increased resistance of the transgenic cell cultures of *V. amurensis* and transgenic plants of *A. thaliana* to salt stress. To test whether the salt resistance induced by overexpression of the *VaCPK21* is also effective at the seed germination stage, the response of young *Arabidopsis* transgenic seedlings to salt stress was analyzed. We found that overexpression of the *VaCPK21* gene conferred improved salt resistance of the seedlings. To evaluate the molecular effects of *VaCPK21* gene in plants subjected to salt stress, we monitored the expression of stress-responsive genes *(DREB2A, KIN1, LEA, NHX1, P5CS, RD22, RD26, RD29A, RD29B, SOS1)* in the transgenic *Arabidopsis* using qRT-PCR. This work was supported by: the Russian Scientific Foundation (RSF, 14-14-00366) and the Dynasty Foundation (DF). RSF supported binary vector construction, grape cell culture transformations, and selection; DF supported work related to abiotic stress tolerance assays and transgenic *Arabidopsis*.

CALLUS INDUCTION, REGENERATION AND *AGROBACTERIUM*-MEDIATED TRANSFORMATION OF *WOLFFIA ARRHIZA*

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Vaccines creation based on transgenic plants may be considered as a groundbreaking technology in modern vaccinology with the advantages as compared to bacterial and yeast systems, such as the lack of common human and animal pathogens, and high level expression of heterologous proteins.To date the development of tissue culture systems in duckweeds is limited to species of the genus *Lemna* and *Spirodella*. Yet more promising target for biopharming is *Wolffia arrhiza* as an object for submerged cultivation in a fermenter. We have developed a two-step procedure of callus induction in *Wolffia*. At the first stage cluster structures are induced in the presence of 2,4-D and BA during 16 weeks. At the second stage BA in the medium for callus induction is replaced by PCL over a period of 4 weeks. The resulting callus can be maintained in vitro for a long time or it is possible to regenerate the whole plants of *Wolffia*. The created protocols for callus induction and regeneration allow to achieve not only the high efficiency at each stage, but also proceed to the development of a protocol for *Wolffia arrhiza* stable transformation.The most efficient transgenesis and selection of the transgenic lines occures in the presence of hygromycin B. The successful transformation requires the presence both of 2,4-D and BA in the cultivation medium within 15 days. As a result of investigations 84 transgenic lines of *Wolffia* harbouring both reporter [*gfp* (1 lines) and *gus* (3 lines)] and target [*desulfatohirudin-1* (46 lines) and *granulocyte colony-stimulating factor* (34 lines)] genes were obtained. Integration of heterologous DNA was proved by molecular-biological analyzes.

GENETIC DETERMINANTS OF PHOTOPERIOD INSENSITIVITY ON 2B CHROMOSOME OF WHEAT NEAR-ISOGENIC LINES

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Photoperiod sensitivity (PS) is a key agronomic trait determining productivity and adaptivity of wheat crops. A number of modern varieties reveal photoperiod insensitive (PI) phenotype. Previously, it was shown that indel mutations or copy number variation (CNV) in *Ppd-1* genes cause PI phenotype. The *Ppd-1* genes encode a regulatory proteins involved in the circadian rhythms regulation. Discovering new insensitive alleles of *Ppd-1* genes may significantly increase productivity and adaptive potential of newly developed wheat varieties. We examined two pairs of Near-Isogenic Lines (NILs) that differ in PS, to detect genetic determinants of their phenotype. The results of SSR analysis demonstrated that introgression of the chromosome 2B fragment from the Sonora variety is reason for PI of NILs. The chromosome region between the *Xgwm388* and *Xgwm148* markers was a possible intersection area of two introgressions (in different lines). Therefore, the *Ppd-B1* was the most probable genetic determinant of the PI of the lines. However, the *Ppd-B1a.1* allele with insertion in promoter region was not found in the NILs. The qPCR showed increased copy number of *Ppd-B1* gene in early flowering lines and Sonora. We found that this allele

had the same junction between copies like Sonora64 variety. Thereafter, we investigated possible nucleotide polymorphisms of *Ppd-B1* copies. Some polymorphisms were detected. The indel in promoter region distinguished the lines under investigation from Sonora64 allele and the exon 3 SNP from Chinese Spring allele. The promoter SNP confirmed difference between NILs and their sibs. The intron 4 SNP pointed to some *Ppd-B1* copies in Sonora, as cloned sequences with A or G in this position were revealed. Sequencing of this area with B-genome specific primers confirmed presence of both variants (A/G double peak). None of previously described CNV alleles revealed difference between copies. Moreover, some extra copies with nucleotide polymorphisms were found in Sonora. Identification of these copies in PI lines is in progress. This study was supported by Russian Scientific Foundation (14-14-00161)

MOLECULAR GENOTYPING OF BREAD WHEAT CULTIVARS FOR SEPTORIA RESISTANCE

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Septoria blotch is one of the most economically important diseases of wheat in countries with temperate climate. The hampering breeding for resistance to septoria in combination with wheat monoculture involving cultivation of susceptible cultivars has resulted in frequent and wide onset of septoria blotch in every region of Russia specialized on spring and winter wheat growing. Selection of resistant cultivars is based on screening of donors for resistance genes to *Septoria tritici* Stb 1-8. We used molecular markers to identify the resistance genes *Stb1*, *Stb2*, *Stb4*, *Stb5*, *Stb7* and *Stb8* in 46 lines of spring bread wheat which characterized with low infection types under high disease pressure. The similar amplification products of PCR with *Xgwm335* linked to *Stb1* were shown for 21 tested lines, with *Xgwm111* linked to *Stb-4* - for 16 tested lines, with *Xgwm44* linked to *Stb-5* – for 25 tested lines, *Xwmc313* linked to *Stb7* – for 11 tested lines. The four lines were suggested to carry the resistant gene *Stb-2* along with the positive signals from the three molecular markers *Xgwm389*, *Xgwm533.1*, *Xgwm493*. The molecular markers *Xgwm146* and *Xgwm577* both linked to *Stb8* were amplified together on seven wheat tested lines. The polygenic inheritance of resistance to *S. tritici* involving up to 5 *Stb* genes was revealed for tested bread wheat cultivars. Among the identified *Stb*-genotypes there were 10 lines with 2 resistance genes, 16 lines with 3 genes, 2 lines with 4 and a single line carrying all five genes *Stb1*, *Stb2*, *Stb4*, *Stb5*, *Stb7* and *Stb8*. To recommend for utilization in different regions of Russia several *Stb*-genotypes were selected: 8916-CG09-S, P8917-B4D4 (Canada), Mult 760, Mult 7 (Peru), 16-52-2, BH 2845, TRAREANO, ITAPEVA (Brazil), additionally they carried up to four durable resistance genes to septoria bloth.

SEEDCOUNTER – MOBILE APPLICATION FOR GRAIN PHENOTYPING

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Grains morphometry in cereals is an important step in selecting new high-yielding plants. The manual assessment of parameters such as the number of grains per ear and their size is laborious. The solution to this problem is image-based analysis, which can be performed using a desktop PC. The effectiveness of this analysis in the field can be improved through the use of mobile devices. In this work, we propose a method for the automated evaluation of phenotypic parameters of grains using mobile devices running the Android operational system. The experimental results show that this approach is efficient and sufficiently accurate for the large-scale analysis of phenotypic characteristics in wheat grains. This work was in part supported by the RFBR (project 14-07-31226) and budget project VI.61.1.2.

STUDYING OF MECHANISM(S) OF NATURAL COMPETENCE FOR DNA UPTAKE IN PLANT MITOCHONDRIA

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DNA import into mitochondria is a firmly established process in plant cells but little is known on the involved membrane translocation mechanism(s). Using DNA as a substrate for in vitro uptake assays, we show that there are definite differences in the translocation process into isolated mitochondria for DNA of various lengths and structures. Addition of the terminal inverted repeats from the Brassica napus 11.6 kb linear plasmid to large (> 9 kb) DNA fragments leads to greater import efficiency in Solanum tuberosum mitochondria. The uptake of large DNA shows similarities with that of DNA with medium size (0.7-3 kb) and involves the voltage-dependent anion channel (VDAC) and the adenine nucleotide translocator (ANT). DNA fragments of small (0.1-0.3 kb) and medium (0.7-3 kb) size are imported into mitochondria apparently by partly overlapping but independent mechanisms. The interrelationships of DNA with a small or medium size are non-competitive in the import. Transport of small size DNA is little affected by ANT and VDAC inhibitors, but is sensitive to inhibitors of the ADNT1 transporter or of the phosphate carrier, as well as to the presence of tRNA. With the help of the *A.thaliana* mutant lacking one subunit of the respiratory complex I, a Cu-binding protein (CuBP), it was demonstrated the probable involvement of this protein in the inner mitochondrial membrane in forming of alternative channels for the DNA import. It was found that the absence of the CuBP protein in the mitochondrial membrane affected the activity of the DNA import in comparison with the import into the mitochondria of wild-type plants reducing it for the DNA molecules of small, average and large lengths.We propose a model of transport into plant mitochondria based on the idea of alternative mechanisms for the import of DNA with different lengths and structures. This work is supported by Russian Foundation for Basic Research (grants 12-04-01400, 15-04-05046).

INVESTIGATION OF PHENOTYPIC DIVERSITY FOR TECHNOLOGICAL PROPERTIES OF GRAIN AND FLOUR AMONG SPRING AND WINTER CULTIVARS OF DIVERSE ORIGIN

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The aim of this work was to evaluate technological properties of grain and flour in two groups of spring and winter wheat cultivars of diverse origin. The groups were presented by cultivars of domestic and foreign breeding. The phenotypic diversity in groups was studied. For this, the technological characteristics of quality traits, such as milling parameters, gluten content in grain and rheological properties of grain were determined. The data obtained during processing of results of field and green-house seasons of several years were statistically analyzed. In the group of spring wheat cultivars the higher flour strength, dough stiffness and extensibility were found and as a rule, a flour higher particle size. These cultivars also possessed significantly higher gluten content in grain comparing to winter cultivars. The winter cultivars had a higher thousand grain weight and a higher stiffness/extensibility ratio. Generally, the Russian cultivars overcome foreign cultivars for grain quality.

THE COMPENSATORY MECHANISM PROVIDING FOR AUXIN TRANSPORT IN *PIN* MUTANTS OF *ARABIDOPSIS THALIANA* L.

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Auxin is the main regulator of the root patterning. Various auxin concentrations determine root cell growth, division and differentiation. Auxin distribution in the root with a maximum in stem cells is mainly formed by active transport through PIN family carriers. Despite the key role of PINs in the auxin gradients establishment, single and even some double *pin* mutants have normal root morphology. In this study we investigated the mechanisms underlying this phenomenon using the *in vivo/in silico* experiments. *In vivo*. We studied PINs expression patterns in *wild type* and *pin* mutants using the specific PIN antibodies. Analyzing the *wild type* data, we noticed the correlation between the auxin level in a cell with this cell ability to express an exact PIN set. This was supported in the experiments on PIN::PIN-GFP plants treatments by exogenous auxin in different dosage. PIN expression in *pin1*, *pin2*, *pin3* and *pin4* mutants confirmed the previously shown evidences that PIN paralogs adjust their expression domains to compensate an absence of one

of them. We used all these data to test the mathematical model. *In silico*. We studied the feedbacks between auxin distribution pattern and PINs expression domains in a mathematical model. For this purpose we modified the auxin transportation model with the new experimental data on auxin regulation of PINs expression. As a result, for wild type and each of *pin* mutants we received the stationary solutions in which the auxin maximum self-organizes in the root tip altogether with PIN domains in accordance with the experimental data. Our results show that auxin-dependent PIN expression provides for both: (1) tissue-specific PINs localization; (2) compensation of an absence of one of PINs in *pin* mutants. The work was supported by RSF (14-14-00734) and Dynasty Foundation.

SEASONAL LIFE HISTORY OF SEVERAL *TRITICUM AESTIVUM* AND TRITICALE WINTER CULTIVARS

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Common winter wheat gives higher grain yield than spring one. Winterhardy cultivars give rise grain production by planting them instead of spring wheat in regions with hardy winter. 25 cultivars of common winter wheat, 6 intercultivar wheat hybrid populations and 3 triticale winter cultivars were chosen to develop cultivars suitable to Siberian environment. Seeds were kindly provided by Dr.Ludmila A. Bespalova (Krasnodar Lukyanenko Research Institute of Agriculture, Krasnodar, Russia). After overwintering in Novosibirsk region environment (West Siberia, Russia) under thick snow cover plant survival on plots varied from 5 to 95%. To check seasonal life history plasticity of plants survived their offsprings were sowed individually in spring. In autumn main part of plants of wheat cultivars Aivina, Grom, Proton and triticale cultivar Dozor were at the tillering and small part of plants demonstrated bolting or flowering. In another group of 12 wheat and 2 triticale cultivars main part of plants reached full ripeness, the rest of plants demonstrated tillering. In third group wheat cultivars and in all hybrid populations main part of plants stopped its development at the tillering, the rest of plants was distributed from bolting to ripeness. So, offsprings of winter plants survived after overwintering demonstrated in spring sowing inter- and intracultivar variability in ability and timing of transition to generative development, to develop in spring manner or stop its development because of absence vernalization influence . Continuous manner of this variability was observed even in separate offsprings. It says about its regulatory, epigenetical nature and about it possible common presence in winter cereals. A broad variability in number of productive ears, in number of grains per plant, their weight and size was observed between plants that developed grains. It opens possibility to selects spring cultivars, which can be used also in genetical and molecular investigations as isogenic lines in life style.

EVALUATION OF THE LEVEL OF POLYMORPHISM OF *TULIPA GESNERIANA* L. IN SARATOV REGION

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Tulipa gesneriana L. (*Liliaceae*) is a highly decorative protected species. It tends to reduction in the number within the territory of Saratov region. The aim of this study was to estimate *T. gesneriana* intraspecific polymorphism level in order to select and to preserve the plants in the germplasm bank. 26 primers were tested by ISSR analysis; 10 of them producing sufficient quantity of clear polymorphic amplicons were selected for further work. Amlificated fragment size ranged from 100 to 1000 bp. The average percentage of polymorphic loci was 50.9%. According to data received from the UPGMA, based on distances of Nei (1978), PCoA, based on Jaccard coefficient, and clustering by the Neighbor Net, it has been discovered that populations under study can be united into two main clusters by genetic proximity. The AMOVA results have shown significant differences between these groups. Percentage of variation among groups was 42.12%, among populations within groups – 23.59%, within populations – 34.28%. If groups of populations were composed according to geographical location (right and left banks of Volga river), the AMOVA results were not significant. The Mantel test confirmed these data (P = 0.797000; Regression coefficient = -0.000270; Correlation coefficient = -0.117851; Determination = 0.013889). Fixation indices were followed: Øsc = 0.40763, Øct = 0.42123, Øst = 0.65715. Øst was very great and it shows that all the populations are genetically isolated from each other.

It is supposed that genetic proximity and difference level is determined by the time the plants of species populated the mentioned territories and by features of their seed reproduction. To solve these questions further investigations including interpretation of sequence data are very important.

DE NOVO SEQUENCING OF CONIFER MEGAGENOMES

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The latest achievements in whole genome *de novo* sequencing of conifer megagenomes will be presented including Siberian larch (*Larix sibirica* Ledeb.) and Siberian pine (*Pinus sibirica* Du Tour.) that are being sequenced in the Laboratory of Forest Genomics at the Genome Research and Education Center of the Siberian Federal University. This study is supported by Research Grant No. 14.Y26.31.0004 from the Government of the Russian Federation.

STUDY OF TRANSCRIPTOME RESPONSES TO CADMIUM IN PEA (*P. SATIVUM* L.) ROOTS WITH THE USE OF MACE-SEQUENCING

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One of the factors that have a significant impact on most plant organisms is the presence of cadmium in the soil. Transfer of heavy metal in the food chains causes serious disorders in many living organisms. Issues related to the signaling of stress factors, the expression of certain groups of genes and subsequent synthesis of proteins still remain poorly understood. In the present work we analyze the effect of cadmium on the changes in the pea transcriptome. A Digital Gene Expression Profiling Technique MACE (MAssive Analysis of cDNA Ends) consists of sequencing only one representative fragment per transcript and therefore achieves ultra deep analyses to include the rare transcripts at about 20 times lower sequencing depth as RNAseq. An interesting problem is the use of this method for studying the transcriptome of non - model objects. This approach was used to analyze transcriptome responses to cadmium in roots of two lines of pea: SGE (sensitive to cadmium) and mutant SGECd^t (resistant to cadmium, showed increased cadmium concentrations). RNA was isolated from control and treated with cadmium roots, cDNA libraries were prepared and sequenced with the Illumina HiSeq2000. For data analysis we used several approaches. Reads were collected in contigs using assembler Trinity. In summary, we obtain 37216 contigs, some of them were annotated relative to Swiss-Prot and TrEMBL databases. Analysis of differential gene expression pointed to different ways of forming a response to the action of cadmium in two lines of pea. Most of SGE transcripts were associated with catalytic activity, whereas SGECd^t transcripts were associated with the process of binding of various substances. Coexpression analysis showed an association of some transcription factor with genes, whose expression changes under the cadmium stress in mutant and wild type genotype. These studies are important for understanding the mechanisms of cadmium resistance in plant organisms. This work was supported by Russian Scientific Fund [grant number 14-24-00135].

DISTRIBUITON AND GENETIC DIVERSITY OF FOUR APPLE VIRUSES IN OLD AND MODERN ORCHARDS IN BELARUS

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Apple is one of the most widely grown and economically important fruit crop in Belarus. Apple mosaic virus (ApMV), apple chlorotic leaf spot virus (ACLSV), apple stem grooving virus (ASGV) and apple stem pitting virus (ASPV) are common in many cultivars. Infected plants often remain symptomless. However, apple viruses can cause

significant yield reduction, up to 60%. To evaluate the occurrence and genetic diversity of apple viruses in old and modern cultivars 260 plant samples were tested for infections by multiplex RT-PCR. Among them 130 were more than 40 years old and the other 130 were collected in modern commercial orchards on the territory of Minsk, Brest and Grodno regions. All tested trees had no symptoms of viral infections. In old apple trees no viral infections were detected. In modern orchards 9,2% of tested trees were infected by ASGV, 1,5% - by ApMV and 1,5% - by ASPV. Some of RT-PCR products were cloned and sequenced. 19 isolates with nucleotide identities of 96-100% in fragment of coat protein gene were obtained from 11 infected with ASGV trees. Evolutionary relationships were inferred using neighbor-joining method. According to the phylogenetic network the sequences segregates into 4 major phylogenetic groups regardless of the geographical region from which the samples were collected. Most of isolates from the same trees were determined in the same clusters, but some were in different clusters. 5 isolates with nucleotide identities of 87,8 - 99,7% in fragment of coat protein gene were obtained from 2 infected with ASPV trees. 3 isolates with nucleotide identities of 98,9 - 99,6% in fragment of coat protein gene were obtained from 2 infected with ApMV trees.

GENOMIC APPROACHES TO IMPROVE CEREAL PRODUCTION IN LOW YIELDING ENVIRONMENTS

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Abiotic stresses including high and low temperatures, low water, intense light, salinity, and deficiencies or toxicities for a range of nutrients can severely reduce crop plant productivity. Multiple abiotic stresses will frequently challenge crop plants simultaneously. Higher plants have evolved multiple, interconnected strategies that enable them to survive unpredictable environmental fluctuations. However, these strategies are not always well developed in the cereal cultivars grown by grain producers and most of the strategies are focused on plant survival at the expense of yield. For the cereals wheat and barley the genetic control of traits determining yield in water limited and low yielding environments are generally expected to be of low heritability, polygenic and many of the key loci will show epistatic rather than additive effects. Current breeding and mapping techniques make it very difficult to detect and select for these types of loci. Know confounding factors, such as maturity, height, resistance or tolerance to soil diseases, and tolerance to related stresses such as boron, acidity, salinity and nutrient deficiencies must be taken into account. In many cases the genetic control of tolerance to these factors is known so that they could be fixed in both breeding and mapping populations. Results now coming out of genomics studies are providing new insights into stress responses and provide novel strategies to improve stress tolerance. A broad approach to using genomics techniques to tackle abiotic tress tolerance in wheat and barley will be presented with some specific examples of how these results can influence crop improvement.

FROM NEW SOURCES TO NEW INITIAL MATERIAL OF SPRING COMMON WHEAT RESISTANT TO STEM RUST (UG99)

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Preventive breeding wheat for resistance to race of stem rust Ug99 is very important because of possible spread of this pathogen via the Middle East countries to the RF regions. Six sources of resistance of wheat to stem race Ug99 were selected from VIR and "Arsenal" collections. Identification of 17 known Sr genes by molecular markers recommended for MAS was carried out in the sources of resistance to stem rust, including Ug99. Cultivar of winter wheat Donskaya Polukarlikovaya (*Sr2*, *Sr44*), winter wheat line from Bulgaria GT96/90 (*Sr24*, *Sr36*, *Sr40*, *Sr47*), winter line 119/4-06rw (*Sr22*, *Sr32*, *Sr44*) and spring line 113/00i-4 (*Sr2*, *Sr36*, *Sr39*, *Sr40*, *Sr44*, *Sr47*) were crossed. The obtained hybrids were backcrossed by donor 113/00i-4 with target genes for pyramiding genes of resistance. 129 individual plants with homozygote state of alleles of two, three, four and five target genes were selected in hybrid combinations of spring wheat from F_4-F_5 and backcross progeny BC_1F_3-BC_2F_2-BC_3F_2. They are plants with genes *Sr2*, Sr36, *Sr44*, *Sr40*, *Sr32*, *Sr39*, *Sr47* and *Sr22* in different combinations. The presence of a recessive gene of adult plant resistance *Sr2* to all virulent races of stem rust in combination

with other genes must guarantee durable resistance to this pathogen. We have selected plants 70—100 cm high with ear productivity 1.4-3.3 g, mass of 1,000 grains 40-52g with amber-colored and dark-colored (presence of anthocyan on pericarp) grains. The progenies of individual plants with identified genes of resistance undergo tests for resistance to the West Siberian, Central Region and North Caucasian populations of the pathogen. According to the results of evaluations of resistance to diseases and line productivity evaluation, recommendations will be given for their further tests and use in breeding program. Investigation was conducted with the support of the Russian Foundation for Basic Research (Project No. 13-04-00922).

COMPARATIVE ANALYSIS OF GENOME CONTENT OF CULTIVATED WHEAT VARIETIES FROM DIFFERENT RUSSIAN REGIONS

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Molecular markers are widely used for the analysis of genomic composition of wheat cultivars, tagging of agronomic valuable genes and monitoring of selection processes. Eleven winter and nine spring wheat cultivars were analyzed by molecular-genetic and cytogenetic approaches. Molecular analysis was performed by means of SSR (simple sequences repeats) and ISBP (insertion site-based polymorphism) markers. Thirty-one SSR markers specific for chromosomes of common wheat generated 162 alleles in the genomes of all analyzed cultivars. For ISPB analysis it was used 97 markers developed for 5B chromosomes of common wheat. Phylogenetic investigation revealed that all wheat varieties are combined into two clusters, one of which includes winter wheats, regardless of their origin, the other spring varieties. According to SSR and ISBP data differences was found between varieties that was included into the subclusters, which probably reflects the different level of divergence of individual chromosomes in sites of marker localizations. Comparative analysis conducted by C-banding and in situ hybridization revealed chromosomal rearrangements in 8 of 21 cultivars. The greatest numbers of translocations (5BS.5GL, 6BS.6GL, 1D/1Dt, 6D/6Dt) were detected in cv. Fisht. Translocation 1RS.1BS was found in cv. Filatovka, Tanya, and Vassa. Translocation 2DS.2SL from Ae. speltoides was detected in cv. Chelyaba 75. Chromosome 6D of Tulaikovskaya 100 was found to be substituted by the Th. intermedium homoeologous chromosome 6Ai. Wheat cultivar Bezenchukskaya 98 has pericentric inversion of chromosome 2B. We also found changes in C-banding patterns of the long arm of chromosome 5B in cv. Novosibirskaya 15. Comparative analysis of data obtained from two approaches allowed us to estimate the formation of genomic composition of the studied cultivars in the selection process and the effect of individual translocations/rearrangements on agronomic valuable traits of common wheat. The work was supported by RFBR (project № 14-04-00297).

GENETIC DIVERSITY OF *Picea abies* (L.) H. Karst. POPULATIONS IN THE EAST EUROPEAN PLAIN REVEALED BY MARKERS OF MITOCHONDRIAL DNA

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Relatively long-living organisms such as conifers have mark of postglacial migrations history in their areal and in population genetic structure. *P. abies* is one of the first tree species which colonized Central Europe after last glaciation. It is deemed that Norway spruce survived because of several refugia. One of supposed refugia is located in territory of so-called «Moscow basin» in central part of European Russia. From here so-called «northern family» of Norway spruce may migrate after withdrawing Scandinavian glacial sheet in west and north-west directions. It was previously suggested that highest level of genetic diversity might be observed in populations closely located to former postglacial refugia. On the other hand, the spike of genetic diversity could be found in zone of secondary contact of populations that survived the glaciation in different refugia. From this point of view the nucleotide diversity of several mitochondrial genes in the natural populations of Norway spruce has been studied. Heteroplasmy of mitochondrial *Nad1* gene was discovered in the populations, located in hypothetical zone of introgressive hybridization of Norway spruce and Siberian spruce (*P. obovata* Ledeb.). It has been previously hypothesized that recurrent hybridization events, such as those observed in the zones of contact

between closely related species, could provide the opportunity for heteroplasmy and recombination to occur.

MEIOSIS IN WHEAT-RYE HYBRIDS WITH POLYHAPLOID GENOMES: THE FERTILITY PATHWAY

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Allopolyploidy is a successful mechanism of speciation, which is characterized by a combination of two or more genomes in one nucleus through interspecific / intergeneric hybridization followed by chromosome doubling. When such hybrids are first formed, conflicts arise due to differences in genome size, genome composition, regulatory mechanisms, cell cycle duration, genetic and epigenetic modifications and so on. A critical aspect of allopolyploid formation is to overcome the sterility of the first-generation hybrids. Only gametes with unreduced chromosome number may be viable. Unreduced gamete formation in wheat intergeneric hybrids is the result of meiotic cycle abnormality. Here, we identified and characterized two cytogenetic mechanisms of unreduced gamete formation in wheat-rye hybrids 5R(5D)xR, 1Rv(1A)xR, 6R(6A)xR и C29xR with the molecular cytogenetic methods: 1) the first division blocking and sister chromatid separation in the second meiosis; 2) sister chromatid separation in anaphase I and the second division blocking; meiosis ends with the dyad formation in late telophase I. In the first case, monopolar spindle and point sites of antiCENH3 hybridization are revealed with immunostaining with antibodies against α-tubulin, also centromere-specific probe pAet-06 hybridization pinpoint sites are revealed with the *in situ* hybridization. Microtubules formed radial cytoskeleton at telophase I, and then bipolar spindle formed in the second division and sister chromatids separated to the opposite poles. Phosphorylation of histone H3 at Ser10 residues in the centromeric region served as an indicator of the second division. In the second case, divergent bipolar spindle was formed, the antiCENH3 hybridization sites visualized as two points, and the centromere-specific probe pAet06 hybridization signal looked like a diffuse stretched structure. However, immunostaining with antibodies to phH3Ser10 showed that sister chromatids separated to the poles evenly painted along its entire length, as the rye and wheat chromosomes in the first meiotic division. Therefore, sister chromatids separation in the first division and subsequent blocking of the second division, probably, was not true mitosis.

MOLECULAR-GENETIC CONTROL OF TUMOR GROWTH IN PLANTS

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Growth and development of plants is determined by the balance of processes of cell proliferation and differentiation. Coordination of these processes is part of a systemic control; the output of even a single cell from this control causes its uncontrolled proliferation, promoting tumor growth. The control of cell proliferation and differentiation is carried out by phytohormones and transcription factors (TFs) which regulate expression of many genes. In plants several examples of spontaneous tumor formation (genetic tumors), and induced tumor formation, caused by pathogens, are described. A common feature of plant tumors of different origin is the alteration of hormonal balance, which is the initial mechanism of tumor formation. The expression of genes controlling cell proliferation (regulators of the cell cycle and regulators of meristem development) depends on the concentration of phytohormones and the efficiency of transduction of their signal in plant cells. We assume that the development of tumors of any type associated with a pathological cell proliferation can be regulated by the same set of TFs that normally regulates the activity of meristems. The report will focus on the role of meristem-specific TFs (WOX, KNOX and others) in the development of plant tumors. We used as a model certain inbred lines from the genetic collection of radish (Raphanus sativus), some lines of which are capable of spontaneous tumor formation. Other model is tumors induced by Agrobacterium tumefaciens. Comparative histological analysis of spontaneous and induced tumors in radish and expression analysis of meristem-specific genes were carried out. We have shown that both types of tumor formation in plants of are accompanied by an activation of meristem-specific genes expression. The study was supported by grants from the University 1.38.229.2014, 1.38.676.2013 and RFBR 14-04-00591a.

SEGREGATION ANALYSIS OF ANTHOCYANINLESS MUTATIONS AND MOLECULAR MARKERS IN RYE

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A high level of the environmental and genotypic variability of anthocyanin coloration was described under study of rye inbred lines (Smirnov, Sosnihina, 1984). Six non-allelic recessive mutations (*vi1-vi6*) leading to the absence of anthocyanins in all parts of plant were described in segregation analysis. However, position of corresponding genes on genetic maps and their molecular function are not known. Gene *vi1* was previously localized on 7R chromosome in accordance to the linkage with isozyme loci. For now, based on results of microsatellite markers segregation, *vi1* gene was mapped in previously published molecular maps in 7R chromosome in following order *Xrems1135-vi1-Xrems1188-Xgwm1302*. Genes encoding key enzymes of anthocyanin biosynthesis one may regard as candidates for anthocyaninless genes. In order to develop intralocus markers for anthocyanidin synthase gene (*Ans*) a fragment of this gene about 500 bp was sequenced in all anthocyaninless mutants and three colored lines. Primers for amplification were constructed by alignment of *Ans* genes of bread wheat. They have following sequences: F gggaagagggagtgggaggactacctgt; R gcgaagacgacccaggagacgcgcacgg. Four individually isolated DNA preparations for each line were amplified and corresponding products were purified and sequenced in both directions. Multiple alignments revealed single nucleotide substitutions and insertion/deletion from 3 to 35 bp. One colored line is distinguished by one of such deletions from all anthocyaninless lines. Primers flanking this deletion were used for segregation analysis of corresponding fragments. No cosegregation was observed between coleoptile color and length of DNA fragments in studied cross combination. Thus, anthocyaninless mutations *vi1-vi6* are not related to *Ans* gene.

FEATURES OF CELL DEATH, CAUSED BY FREEZING AND HIGH TEMPERATURES INFLUENCES IN SUGAR AND WINTER WHEAT SUSPENSION CULTURES

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Temperature is one of the most important environmental factors limiting the growth, development and productivity of plants. Using suspension cultures allows to viewing the processes occurring in plant cells during the destruction under influences of extreme temperatures. In the present study, we used suspension cultures derived from plants with varying degrees of resistance to high and low temperatures: winter wheat (*Triticum aestivum*) and sugar cane (*Saccharum officinarum*). Temperatures cause long-term process of cell death in both cultures, were -8 and 50 °C. It was shown that the time of death process in the suspension cultures depends on the temperature and resistance degree of plants from which the cultures were derived. Negative temperature induced cell death process occuring for 10 days after treatment in wheat culture, whereas in the sugar cane culture 80% of cells have died within 18 hours after this exposure. High temperature (50 °C) activated cell death in cultures within the next 2 days. However, despite the differences in speed realizing, the death processes caused by the action of both temperatures in winter wheat and sugar cane suspension cultures are carried out involving mitochondria. It is evidenced of inner mitochondrial membrane hyperpolarization and release of cytochrome c from mitochondria to cytosol. Hyperpolarization of mitochondrial membrane caused by the negative temperature maintained for several hours, at processing and thereafter. At the same time, the inner mitochondrial membrane hyperpolarization induced by the action of 50 °C is replaced by depolarization with increasing treatment period up to 30 min. We can conclude that despite the similarity of the death processes caused by temperatures of -8 and 50 °C, and the significant role of mitochondria in both cases, regulatory and signaling pathways that are activated by the action of high and low temperatures are different. This work was supported by the RFBR grant №14-04-32126.

IN VITRO EVALUATION OF CELLS PROLIFERATIVE CAPACITY OF *ARABIDOPSIS THALIANA* MUTANT WITH DISTURBED SHOOT APICAL MERISTEM FUNCTION

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A.thaliana mutation *na* causes premature termination of shoot apical meristem (SAM) function. SAM size in plants heterozygous for *na* is 2–3 fold smaller than in wild type plants, which indicates that na gene participates in the regulation of stem cell pool size or SAM cells proliferative activity. In order to understand whether na gene exerts the influence on these processes by affecting cell divisions we studied the effect of mutation on the expression level of transgene CycB1;1::GUS (reporter gene beta-glucuronidase *uidA*, fused to promoter of the cyclin gene *CycB1;1*), which is widely used as a cell division marker. Study of the CycB1;1::GUS expression in seedlings and juvenile plants revealed some differences in its expression pattern in *na* mutant in comparison to wild type. However, we found great variation in the activity of the reporter in different samples of mutant plants. Such variation may be due to the fact that the time of termination of the SAM proliferation in different plants may differ slightly, as seen from the variation of mutant phenotype. To avoid adverse effects on cell division associated with abnormalities of morphogenesis we continued our studies on callus culture that is characterized by a simple level of organization. We analyzed callus formation on Gamborg's B5 medium using wild type and *na* leaf explants. We found significant reduction in the proliferative activity of mutant cells compared to wild type. Already after 2 weeks of cultivation all wild type explants were transformed into callus and actively expressed the reporter gene. Explants from mutant formed only a few small foci of cell proliferation and much weaker expressed a reporter gene. The obtained data suggest a link between the breach of the functioning of SAM and anomalies in cell proliferative activity of the mutant. This work was supported by RFBR (project № 13-04-00122).

EVOLUTIONARY ASPECTS OF HORIZONTAL GENE TRANSFER FROM AGROBACTERIUM TO PLANTS

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Most genetic engineering of plants is based on *Agrobacterium* mediated transformation. In nature, insertion of *Agrobacterium* T-DNA in the plant genome and its subsequent transfer *via* sexual reproduction has been shown in several species in the genera *Nicotiana* and *Linaria*. There is a number of evidence suggesting that there were several independent T-DNA integration events into the genome of *Nicotiana*. In contrast, naturally transgenic Linaria plants form monophyletic group. Analysis of microorganisms in rhizosphere of *Nicotiana* has shown a number of differences between *N. tabacum* (containing T-DNA) and other *Nicotiana* species. Comparative analysis of sensitivity to fungal pathogens of *Linaria* plants containing rol-gene-like sequences and *Linaria* species without rol genes has shown that species composition of micromycetes on *L. vulgaris* and *L. genistifolia* is rather poor as compared to mycobiotes of other plants. During all survey, none dead or highly impaired by fungus infection *Linaria* plant was found. The evaluation of virulence against *L. vulgaris* and *L. maroccana* for 14 strains of 7 pathogenic micromycetes indicated that *L. vulgaris* plants are less amenable to pathogenic micromycetes than *L. maroccana*. We assume that regulation of plant disease resistance may happen at the level of regulation concentrations of Antirrhinoside and its derivatives. It seems likely that a possible function cT-DNA is to mediate how plants interact with their environment by secreting opines and/or by changing the amounts of secondary metabolites. This publication was prepared within the framework of the thematic plan of St. Petersburg State University ## 0.37.526.2013; 1.39.315.2014 and supported by a grant to Tatiana V. Matveeva from the Russian Foundation for Basic Research #14-04-01480

CONTROL OF FLOWERING TIME IN TRANSGENIC CHRYSANTHEMUM PLANTS

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Chrysanthemum is one of the most important commercial cut flowers in the world. Early-flowering cultivars are required to produce quality chrysanthemum flowers with a lower cost of production. To shorten the vegetative growth phase of chrysanthemum, three AP1-like genes from Asteraceae were constitutively overexpressed in 80 independent transgenic chrysanthemum lines. All lines were characterized by PCR and RT-PCR and demonstrated that overexpression of compositae AP1-homologs in transgenic chrysanthemum under long-day conditions had no effect on plant development compared to non-transgenic controls. Conversely, under short-day conditions, transgenic plants commenced bud initiation 2 wk earlier than non-transgenic chrysanthemum plants. Subsequently, transgenic chrysanthemum flowers showed color earlier and resulted in full opening of inflorescences 3 wk prior to non-transgenic control plants. These results open new possibilities for genetic improvement and breeding of chrysanthemum cultivars.

MONOSOMIC ANALYSIS OF LATENESS IN THE LINE 821 OF SARATOVSKAYA 29 CARRYING THE INTROGRESSIONS FROM *TRITICUM TIMOPHEEVII*

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The line 821 (L821) was obtained by E. Budashkina through crossing of cv. Saratovskaya 29 (S29) with *Triticum timopheevii*. According the data of I. Leonova introgressions are localized in 2A, 2B and 5A chromosomes. L821 and S29 should flower at the same dates as the introgression in 5A chromosomes does not involve *Vrn-A1* gene. Average flowering dates of S29 in a green-house is about 36 days and of L821 –about 39 days. The reason of lateness was studied through monosomic analysis for 5A and 5B chromosomes. According the data of A. Simonov (2010), flowering dates of monosomics for 5A and 5B chromosomes are moved away from parental cultivar on 20 and 10 days, correspondingly. The average period to flowering in F_1 hybrids mono 5A S29 x L821 was 53.8 days. This lateness may be explained by only one dose of the "strongest" gene *Vrn-A1* out of three homoeoallelic genes from *Vrn-1* locus. The average period to flowering in F1 hybrids mono 5B S29 x L821 consisted 44 days as the one dose the "weaker" gene *Vrn-B1* was not so influential on flowering date. The average meaning of the period till flowering in F_2 mono 5A S29 x L821 was about 57 days. All the plants in the population carried one dose of *Vrn-A1* gene and this extended flowering date as was indicated above. However, many plants delayed flowering to more than 100 days. In F_2 mono 5B S29 x L821 population the average date till flowering was about 44. But also here the plants were found with flowering dates which substantially exceeded the parental forms. Their occurrence may be explained by the effects of other genes determining the formation of reproductive organs. In the line 821 it may be the genes *Ppd-B1* in 2B chromosome and *Ppd-A1* in 2A chromosome carrying the introgressions from *Triticum timopheevii*.

POLYMORPHISM OF PROMOTER REGION OF THE PHOTOPERIOD *PPD-A1* GENE IN HEXAPLOID AND TETRAPLOID WHEAT

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The photoperiod response in wheat is one of the key of agronomically valuable traits and determined to a large extent by the homoeologous series of *Photoperiod 1* (*Ppd1*) genes. Recessive *ppd1* alleles confer sensitivity to day length, with flowering delayed under short days, while dominant *Ppd1* mutants are photoperiod insensitive, and result in rapid flowering under both short and long photoperiods. Photoperiod insensitive alleles in generally determined by mutations within *Ppd1* promoter region or by increasing gene copy number. The promoter region of *Ppd-A1* in 6 hexaploid and 6 tetraploid wheat species from 57 countries was investigated by PCR analysis. During investigation the polymorphism of PCR fragments was revealed. Multiple sequence alignment of the 452 bp promoter fragment identified 13 SNPs, forming 13 distinct combinations which are considered as individual haplotypes. According to data of proposed approach for inferring haplotypes phylogenies, the *Ppd-A1b* haplotypes form two haplogroups. These haplogroups

can be detected by anomalous migration of PCR fragments through polyacrylamide gel under certain conditions (low conformational dynamics of DNA molecules), while haplotypes identified using heteroduplex mobility assay. Mutations, which discriminate of the *Ppd-A1b* haplotypes, located within "critical promoter region", hence it cannot rule out they influence on regulation expression of *Ppd-A1* gene. Furthermore, some of these mutations accompanied by significant change of DNA bend that can also influence on the DNA-protein interactions. Nevertheless these assumptions require further experimental confirmation. Analysis of hexaploid wheat revealed a novel mutation within the "photoperiod critical" region in a subset of T. compactum accessions. This putative photoperiod insensitive allele (designated *Ppd-A1a.4*) includes a 684 bp deletion, which spans region in common with deletions previously identified in other photoperiod insensitive *Ppd1* alleles. New allele *Ppd-A1a.4* can be utilized as genetic source to breed early heading cultivars. Moreover, the contraction of the "critical region" it allows will help in the identification of the putative *Ppd1* promoter regulatory sequences, which currently remain undefined.

GENETICS OF SEED LONGEVITY – ARTIFICIAL SEED AGEING AT DIFFERENT STORAGE CONDITIONS REVEAL IMPORTANT LOCI IN BARLEY

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The ability of seeds to survive a certain period of time, termed seed longevity, is strongly dependent on the growth conditions of the mother plant, pre-storage and storage conditions and the genetic background. Under ambient storage conditions, seed survival of orthodox seeds can vary between few years (e.g. onion or lettuce) and several decades (e.g. pea). Scientific experiments on seed longevity are usually not designed to last decades. Therefore, methods which accelerate seed ageing rate and, finally, reduce seed vigour are widely used. The current study investigates seed vigour of 94 lines of the Oregon Wolfe Barley mapping population after a) three and b) five years of 'dry' cold storage at 18°C, c) controlled seed deterioration using 45°C and 18% seed moisture and modified storage atmospheres by d) increased nitrogen concentration and e) elevated partial pressure of oxygen, shortly EPPO. Highly significant differences in seed vigour were detected between 'dry' cold storage, controlled seed deterioration and the EPPO method. Interestingly, high-pressure oxygen triggers similar morphological ageing symptoms as dry storage. However, a low correlation coefficient (r = 0.14) between 'dry' cold storage and EPPO indicates different underlying biochemical mechanism. Composite interval mapping using Restriction Site Associated DNA (RAD) linkage revealed seven highly significant quantitative trait loci (QTL) whereas QTL for three years 'dry' storage could be only found on two and for EPPO and controlled deterioration on three chromosomes.

DEVELOPMENT AND PHYSICAL MAPPING OF SSR MARKERS ON CHROMOSOME 5BS OF THE HEXAPLOID WHEAT (*Triticum aestivum* L.)

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Despite numerous works on designing molecular markers for the hexaploid wheat, detection of polymorphic markers for individual chromosomal regions is currently a topical problem. Simple sequence repeats (SSR) are the markers widely used for genotyping of wheat cultivars as well as in marker assisted and genome selection. The advantages of these markers consist in a high level of polymorphism and stable results and the shortcomings, in a lower frequency as compared with SNP and ISBP markers. In this work, individual regions of the short arm of hexaploid wheat chromosome 5B have been saturated with the SSR markers designed using the sequencing data for BAC clones, namely, 134 BAC clones from 5BS BAC library sequenced on the IonTorrent platform assembled with MIRA. The periodicities have been detected using the algorithm utilizing the properties of complexity decompositions formed in a mode of sliding window. Analysis of 17 770 contigs with a total length of 25 879 921 bp has detected 253, 156, and 27 DNA sequences with a repeat period of 2, 3, and 4 bp, respectively, and a length of at least 20 nucleotides. Some of these SSRs are localized to the

ends of contigs, and some are located in the environment preventing the primer design. Thus, totally 113 primers for the SSRs with a periodicity of 2 bp, 79 primers for SSRs with a periodicity of 3 bp, and 23 for the SSRs with a periodicity of 4 bp have been chosen for further work. The efficiency of amplification, detection of polymorphism, and localization to chromosome have been tested for 67 markers with a periodicity of 3 bp. All tested markers mainly give one PCR fragment well detectable by agarose gel electrophoresis. Over half markers are able to distinguish between CS and CS-5Bdic. Eight markers have been localized to the distal region of the chromosome (bin 5BS6) with the help of a set of CS deletion lines for chromosome 5B; seven and three markers were localized to the interstitial region, bins 5BS5 and 5BS4, respectively. The utility of the developed markers for identification of the leaf rust resistance genes, *LrW*, in bin 5BS6 is discussed. The work was supported by the Federal Targeted Program of the Russian Federation (agreement no. 14.604.21.0106).

MATHEMATICAL MODELING OF AUXIN DISTRIBUTION IN A TRANSVERSE ROOT SECTION

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Plants differ in types of the root central cylinder: diarch, triarch, tetrarch, pentarch or polyarch. The type of the symmetry is the reflection of the relative positions of xylem and phloem bundles in a cross section of the root. The mechanisms forming different types of symmetries in the central cylinder remain poorly understood. It is assumed that vasculature differentiation is triggered and controlled by plant hormone auxin. We have developed a model that describes auxin flow through a cell layer, imitating a cross section of the vascular cylinder in a root. We have studied the stationary auxin distribution in the cell layer depending on the model parameters. A mathematical model was defined by a system of ODE. The processes of auxin diffusion, transporters synthesis and degradation, active transport described on the basis of the mass action law and in terms of generalized Hill functions. The model has been assembled using the MGSModeller software. We have proved that for any configuration of cell assemblies and any values of the parameters for uniform flow of auxin from the shoot to the root always there are both uniform minimum and non-uniform steady-state distribution of auxin. The simultaneous presence of uniform and non-uniform steady solutions shows that even if one of them has the target configuration, problem of it fixing can`t be solved due to internal mechanisms. It is concluded that at a certain stage of development must appear new formation factors necessary for morphogenetic field generation. One of such factors may be non-uniform flow of auxin from the shoot to the root. In numerical experiments by giving external flow corresponding configuration were obtained examples of stationary solutions in which patterns of auxin distribution and its transporters is well agreement with the experimentally-observed diarch type of the *Arabidopsis thaliana* central cylinder. The work is partially supported by the RFBR grants 13-01-00344, Integration project SB RAS 80, budget project (VI.61.1.2).

PRODUCTION OF TRANSGENIC TOMATO PLANTS EXPRESSING THE GENES OF PETUNIA TRANSCRIPTION FACTORS EOBI AND EOBII

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Fruit flavor is a mixture of molecules of various volatile substances that are synthesized during maturation, and is an essential feature of quality and ripeness of the fruit. Volatile organic compounds, forming a tomato flavor composition, are products of various plant metabolic pathways, and their synthesis is determined by a huge pool of different enzymes, whereby the aroma becomes complicated for the study and modification. Modern methods of genetic engineering can create such vectors, which will initiate a cascade transcription of the whole pool of gene in a certain ontogeny stage. The aim of our research was to obtain tomato plants with more attractive fruit aroma and improved consumer appeal. Recently a group of scientists led by Prof. A. Vainstein discovered genes of transcription factors EOBI and EOBII (Emission Of Benzenoids) recently found in petunia, they show that EOBII directly affects the level of transcription of several genes encoding enzymes of phenylpropanoid biosynthetic pathway of volatile organic compounds and genes of shikimate pathway. So we have decided to use these

gens for genetic transformation of tomato. We created two vectors that are equipped with the gene EOBI/EOBII under control of the fruit-specific promoter E8, which provides a high level of expression and tissue specificity. Also we chose 5 different varieties of tomatoes, including cherry, yellow and pink tomatoes, also tomatoes with increased lykopene. Currently we have more than 10 lines each variety with each of the vectors, insertion of the target gene was confirmed by PCR analysis, also gene expression were proved by RT-PCR. Changes in the content of several phenylpropanoid volatiles in the tomato fruit were identified by gas chromatography and mass spectrometry. Thus, the results demonstrate the effectiveness of the chosen approach to the modification of tomato fruit flavor that allows us to offer to use such a strategy in the future.

THE WHOLE *DE NOVO* GENOME SEQUENCING AND ASSEMBLY OF SIBERIAN LARCH (*LARIX SIBIRICA* LEDEB.) AND SIBERIAN PINE (*PINUS SIBIRICA* DU TOUR.)

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The whole genomes of Siberian larch (*Larix sibirica* Ledeb.) and Siberian pine (*Pinus sibirica* Du Tour.) were sequenced using Illumina HiSeq 2000 and MiSeq, and their first draft genome assemblies were generated. We searched for microsatellite loci in the assemblies and designed PCR primers to identify and develop highly polymorphic and informative SSR-markers for population genetic studies. For the first time the chloroplast genome of Siberian larch has been assembled and annotated. For Siberian pine we improved the chloroplast genome assembly published in Genbank (FJ899558.1) by closing gaps with the total gap length of 16085 bp. The draft assembly of mitochondrial genomes for these species has been done. The transcriptome assembly consisted of 43717 unigenes with a total length of ~26 Mbp. The longest unigene was 8512 bp; N50 = 1330 bp, and the number of unigenes longer than 1 Kbp was 6919. The obtained transcriptome assembly represented ~70% of the estimated total transcriptome in Siberian larch and was similar to other published conifer transcriptomes. This study was supported by Research Grant No. 14.Y26.31.0004 from the Government of the Russian Federation.

INTEGRATIVE ANALYSIS OF ANTISENSE TRANSCRIPTS IN PLANTS

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Integration of next generation sequencing data in specialized databases is challenging problem of computer genomics. Pairs of RNA molecules transcribed from partially or entirely complementary loci are called *cis*-natural antisense transcripts (*cis*-NATs), and they play key roles in gene expression regulation. Natural antisense transcripts (NATs) are capable of regulating the expression of target genes at different levels (transcription, mRNA stability, translation). The study presents integrative analysis of plant NATs aspects, including availability, conservation, coding capacity and functions. A promising experimental tool for profiling sense and antisense transcription is strand-specific RNA sequencing. Earlier, identification of chromatin signature of *cis*-NATs in *Arabidopsis* allowed to suggest a connection between *cis*-NAT transcription and chromatin modification in plants. An analysis of small-RNA sequencing data showed that ~4% of cis-NAT pairs produce putative *cis*-NAT-induced siRNAs. To meet issues of statistical analysis of sequencing data we developed set of computer programs to analyze *cis*-NAT pairs and miRNA genes. Text complexity as a measure of context dependencies was applied for nucleotide sequences containing mapped cis-NATs in plants, as previously we did it for monomer repeats analysis. Presence of low complexity regions was shown. We have analyzed data from PlantNATsDB (Plant Natural Antisense Transcripts DataBase) which is a platform

for annotating and discovering NATs by integrating various data sources (http://bis.zju.edu.cn/pnatdb/). It contains about 70 plant species. The database provides an integrative, interactive web graphical interface to display multidimensional data, and facilitate research and the discovery of functional NATs. We have compared gene structures containing NATs for wheat and related plant genomes. An alternative method for large-scale detection of sense-antisense transcript pairs involves the use of microarrays (Affymetrix Wheat GeneChip). About 100 sense-antisense transcript pairs were found. Analysis of the gene ontology terms showed a significant over-representation of transcripts involved in energy production. The work is supported in part by RFBR (15-04-05371) and ICG SB RAS Budget project VI.61.1.2.

THE INFLUENCE OF GENOME COMPOSITION IN WHEAT HYBRID LINES ON THEIR CYTOLOGICAL STABILITY

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The growing global demand for cereals dictates a need for development of elite wheat cultivars. It is possible to improve wheat by introgression of genetic material from cereals related species. Formation of wheat recombinant genome by hybridization with wild and closely related species is accompanied by a complex of structural and functional rearrangements. The previously conducted study of 14 hybrid lines - *T. aestivum/T. durum* and *T. aestivum/T. dicoccum* for genome composition has shown that the lines differ greatly in the data of SSR-analysis and C-banding from common wheat accessions used in crosses. The goal of this research was to study the patterns of chromosome behavior in meiosis of these lines and to estimate the influence of genome composition in these lines on their cytological stability. The study of the chromosome behavior in meiosis of these lines has shown that introgression of alien genetic material into wheat genome had no negative effect on its meiotic stability. Number of defective cells was small not only at the metaphase I stage, but also at the tetrad final stage. The range of variability in terms of the level of cytological stablity in the studied lines is the result of differences in the number and localization of genome introgression fragments of tetraploid wheat in hybrid genome. Comparative analysis of hybrid material has shown that the lines with the maximum number of univalents contained the highest number of alien fragments in their genome. So, the highest number of introgressed fragments – 12 was observed in the line 196-1 *T. durum* × Chinese Spring for which the lowest level of bivalent chromosome pairing was revealed. The influence of the cytoplasm on the formation of the karyotype of introgression lines of wheat was found. The research work was supported by the BRFFR, grant № B14R-013; the RFFR, grant № 14-04-90000.

STUDY OF ANDROGENESIS AND GAMETOCLONAL VARIATION DURING THE DEVELOPMENT OF DOUBLE HAPLOID INTROGRESSIVE LINES OF COMMON WHEAT

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Anther culture is the most widespread method to obtain double haploid (DH) lines which are homozygous organisms. In homozygous plants the activity of recessive genes is expressed together with dominant genes. Therefore, the selection of the target genotypes takes less time. Moreover, the production of homozygous lines is considered to be a method to obtain "ideal"genotypes that are characterized by durable resistance to biotic factors or fix heterosis. The aim of this work was to study androgenesis and gametoclonal variation during the development of DH lines of introgressive wheat genotypes. The forms and varieties of common wheat resulted from West Siberian breeding which carry genetic materials of other cereals (*S. cereale, T. turgidum, T. timopheevii, T. tauschii* (*Ae. squarrosa*), *Thynopyrum, T. dicoccum, H. marinum ssp. gussoneanum* etc). It was established that anther response to culture conditions, the frequency of embryo-like structures and the development of green plantlets are determined by alien genetic materials of studied wheat genotypes. Introgressive genotypes of common wheat with high anther culture ability were

obtained. Cytogenetic and molecular marker analyses have been used for selection of androgenic plants with gametoclonal variation and cytogenetic stable genotypes, followed by DH lines development. It was shown that the rate of gametoclonal variation depends on the origin of alien genetic materials introgressed to common wheat genome. The frequency of spontaneous generation of double haploid plants with restored fertility did not differ between different introgressive genotypes. DH lines obtained in this work are used on breeding programs of Siberian Research Institute of Agriculture, Omsk. This work was supported by the Russian Foundation for Basic Research (14-04-00674) and by the Russian Academy of Sciences ("Nature Biodiversity").

POLYMORPHISM OF AVENIN SPECIES *A.SATVA* L., *A.BYZANTINA* C. KOCH. AND *A.STRIGOSA* SCHREB.

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Electrophoretic separation of avenin was carried out by the method of polyacrylamide gel electrophoresis. Kernels of oat varieties used for the study belong to the species *A.sativa* L., *A.byzantina* C. Koch. and *A.strigosa* Schreb. Alleles of studied cultivars were identified by loci *AvnA*, *AvnB*, *AvnC* using the genetic nomenclature catalogue for common oat. In the spectra of common oat varieties the number of components was changed from 7 to 10. 49.5% of the samples were found monotype. Allele *A2* was most frequently met by the locus *AvnA* (56% of varieties). The rarest was the allele *A3* (2.7% of varieties). By the locus *AvnB* 44.9% of varieties had the allele *B1*. The greatest diversity had alleles of the locus *AvnC*, the most common were alleles *C1*, *C2*, *C3* and *C6**. The smallest number of components was met in the spectra of bristle oat - from 3 to 6. In electrophoregrams there were no protein components in the area of fast prolamins. Variety alleles of species *A.strigosa* did not correspond to the catalogue of genetic nomenclature. Variety spectra *A.byzantina* had from 6 to 14 components. In the identification of alleles at avenin-coding loci it has been established that some varieties of the red oat had in its spectrum alleles typical for common oat. Thus by the locus *AvnA* alleles *A2* and *A6* have been identified. By the loci *AvnB* and *AvnC* for varieties of this species almost all alleles except *B5* and *C4* have been found. New alleles by the loci *AvnA*, *AvnB* and *AvnC* have been found in red oats; 91.7%, 75% and 50% of varieties respectively. The presence of identical alleles in the spectra of common and red oat can be related to the origin of these species from a common ancestor - *A.sterilis* L. Differences in the frequency of occurrence of the identified alleles can be explained by the fact that *A.sativa* L. and *A.byzantina* C. Koch. have different centers of formation.

GEOGRAPHIC DISTRIBUTION AND DOMESTICATION OF WILD EMMER WHEAT (*TRITICUM DICOCCOIDES*)

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The transition from hunting and gathering to agriculture had revolutionary consequences for the development of human societies. Crops such as wheat, barley, lentil, pea and chickpea played a crucial role in the establishment of complex civilizations in south west Asia. Wild emmer wheat (Triticum dicoccoides) was one of the first cereals to be domesticated in the Fertile Crescent between c. 12,000 and c. 10,000 years ago. This step provided the key for subsequent bread wheat evolution. Wild emmer is found today in the western Fertile Crescent in Jordan, Syria and Israel, the central part of southeastern Turkey and mountain areas in eastern Iraq and western Iran. In this work, the geography and domestication of wild emmer wheat based on published molecular and archaeobotanical data and on our recent findings will be presented.

INFLUENCE OF INFECTING *CLAVIBACTER MICHIGANENSIS* SSP. *SEPEDONICUS* UPON THE DEVELOPMENT OF POTATO IN EAST SIBERIA

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Up to half the potato crops in North Europe countries and Canada is last as a result of such a disease as potato ring rot, which is caused by the gram-positive bacteria *Clavibacter michiganensis* ssp. *sepedonicus (Cms).* According to the data officially published by the European and Mediterranean Plant Protection Organization, ring rot infection is widely distributed in Russian European part regions, while *Cms* is not

the quarantine organism in Russia. It may be expected that, in connection with global warming, the areal of pathogen distribution shall extend, what may provoke distribution of the disease even in East Siberia. The objective of this paper implies the investigation of manifestation of the potato ring rot disease symptoms under the East Siberia conditions. Investigated were potato tubers (*Solanum tuberosum* L.), variety Lukyanovsky (susceptible to *Cms*) and the two *Cms* strains: strain Ac1405 and strain B66. The investigation is represented by one experiment with infecting the tubers with Cms of the two strains right before storing (autumn), analyses of influence the infecting upon growing the tubers (spring after storage), planting the tubers, analyses of vegetation and productivity. It has been revealed that infecting the tubers with two *Cms* strains did not influence the number of plantlets at the tubers, while it substantially shortens their length. Observation of the potato plants during the vegetation period has shown that *Cms* infecting increases the percentage of flowering potato plants with respect to the check samples. Infecting with strain Ac1405 plausibly increased the number of sprouts and their length. While infecting with strain B66 increased the number of sprouts and did not influence their length. This proves the fact of plants' stimulation and growth owing to bacterial infection. Infecting with strain Ac1405 caused some increase in productivity, while infecting with strain B66 reduced it. Furthermore, the scrutinized strains of *Cms* suppress the growing of potato tubers during the storage and do not influence the potato productivity. Meanwhile, infecting may stimulate the growth and development of plants, while increasing the number of sprouts and their length during the vegetative stage. Absence of the negative effect of polluting *Cms* upon the productivity is probably due to the disease latent character and the East Siberia climate conditions, which limit spread of the disease. The work has been conduct with the financial support via grant No.14–404107 of Russian Foundation for Basic Research, Siberia.

ALLOPLASMIC LINES OF WHEAT: GENETIC MODELS AND NEW GENOTYPES FOR BREEDING

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Alloplasmic lines (nuclear-cytoplasmic hybrids) combine the cytoplasm of one species together with the nucleus of a related species. These plants are an appropriate model to study nuclear-cytoplasmic interactions, as they have disrupted cross-talk between nucleus and organelles (mitochondria and chloroplasts). The aim of our work was the production and the study of common wheat alloplasmic lines with cytoplasm of barley species (H. vulgare and H. marinum ssp. gussoneanum) which exhibit different levels of nuclear-cytoplasmic compatibility/incompatibility. It was shown that a complex of in vitro culture methods is necessary for the development of barley-wheat hybrids, followed by substitution backcrossing with the aim to obtain alloplasmic recombinant and introgression lines. The role of certain common wheat varieties and chromosomes of related species for fertility restoration of alloplasmic lines was determined. Using molecular and phenotypic markers the alloplasmic wheat lines the barley cytoplasm were studied. It was shown that polyembryony is one of phenotypic trait of alloplasmic lines which emerges in certain hybrid combinations and resulted from anther culture. Fertility, genetic introgression and parental types of mitochondrial and chloroplast are determined by barley species used for the alloplasmic lines development. We revealed that the introgression of alien chromosomes to genome of fertile alloplasmic (Hordeum)-T.aestivum lines does not disrupt nuclear-cytoplasm compatibility. These alloplasmic wheat lines can be used to broaden the resources for wheat genetic improvement. Promising introgressive forms and lines derived from alloplasmic lines (Hordeum)-T.aestivum have been developed and included in breeding. One of these lines as a spring common wheat variety Sigma is being tested in State variety trial. This work was supported by the Russian Foundation for Basic Research (14-04-00674) and by Integration Project.

THE EFFECT OF ALIEN TRANSLOCATION ON QUANTITATIVE TRAITS AND RUST RESISTANCE IN COMMON WHEAT

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Wild relatives of wheat are useful sources of alien resistance genes for wheat breeding. Two the leaf rust resistant lines 73/00i and 81/00i with genetic material of *Aegilops speltoides* Toush (SS) was studied by fluorescence *in situ* hybridization (FISH) and SSR analysis. Was shown that line 73/00i carrying terminal translocations on long arms of chromosomes 5B (T5BS-5BL·5SL), 6B (T6BS-6BL·6SL) and in the short arm of chromosome 1B (T1SS·1BS-1BL). The line 81/00i is characterized the T1RS-1BL·1BS translocation from rye (*Secale cereale* L.), deletion on short arm of chromosome 6B and translocation on 7D chromosome (T7DS/7SS·7SL). To obtain the lines, carrying a single *Ae. speltoides* translocation, the lines 73/00i and 81/00i was crossed with four wheat cultivars, and the BC_1F_2 - BC_1F_3 populations were screening with FISH with probes Spelt1 and pSc119.2. The 15 lines with single translocations were obtained and were studied on resistance to leaf rust, powdery mildew and some quantitative traits such as: straw and spikelet length, spike size, fertility, kernel weight and other. The experiment was carrying out during three years at three locations of the Novosibirsk and it region. The wheat lines carrying the translocation T5BS-5BL·5SL were resistant to leaf rust, the lines carrying the T6BS-6BL·6SL translocation were displayed a moderate resistance. And the lines carrying the translocations T7DS/7SS·7SL were resistant to powdery mildew and leaf rust. The resistance gene to leaf rust from 5S chromosomes is a new gene and it was preliminarily designated as *LrAsp5*. No negative effects of the alien genetic material on yield and other quantitative traits were noted. That is why these lines can be recommended for using in breeding programs. At the present time the lines with translocations T5BS-5BL·5SL and T6BS-6BL·6SL is using in the some breeding programs as donors of resistance genes to leaf rust. This work was supported by Federal Targeted Program of the Russia Federation (agreement no. no. 14.604.21.0106)

SEQUENCE ANALYSIS OF MT-DNA FRAGMENT (NAD1INT2) IN SIBERIAN STONE PINE IN SOUTHERN YAKUTIA

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Siberian Stone pine is one of the most forest-forming species in boreal zone of Eurasia. At the eastern part of the species distribution hybridization of *P. sibirica* and *P. pumila* take place. *Pinus* species exhibit paternal chloroplast inheritance and maternal mitochondrial inheritance. It was shown that fragment mt-DNA (nad1int2) of two Stone pine species have a different size and nucleotide sequence - 2 530 bp length (specific for P. *sibirica*) and 2181 bp (specific for *P. pumila*), and it should be used to determined species input to genotype of hybrid trees. We study Siberian Stone pine population at the eastern distribution limit, Aldanskoe Nagorie (58°45 с.ш., 125°25 в.д.). There were two types of Siberian Stone pine trees. The one of these had atypical habitus, more rounded crown shape with curved upward lower branches. The second morphological trait of these trees was conelet morphology; seed scale shape was pumila-like. A cursory examination of the other Siberian Stone pines didn't reveal distinctive features and trees looked like typical individuals. DNA samples were extracted from fresh needles by CTAB method. We used follow PCR-primers: F 5'-gcattacgatctgcagctca-3'; R 5'-ggagctcgattagtttctgc-3'. Surprisingly, all studied Siberian Stone pine trees had nad1int2 sequences typical for *Pinus pumila*. *P. sibirica*-like trees results from repeated mating of *P. pumila* with P. sibirica and/or interspecific hybrids. Mentioned above some atypical phenotypic traits of studied trees confirm this assumption. We supposed that the unique phenomenon could be explained as the scar of ancient *Pinus sibirica* and *Pinus pumila* hybridization event.

ECOLOGICAL-GENETIC DIFFERENTIATION OF *PINUS SYLVESTRIS* L. POPULATIONS

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The study of structure features and horogenetic population differentiation depending on the gradients of environment is one of the cardinal problems of population biology. We determined almost complete phenological reproductive isolation of adjacent populations due to the sharp soil-termal gradients according to the phenophase dynamics of pollen

dispersal and reception of *Pinus sylvestris* L. in dryland and adjacent high bogs of Western Siberia and Russian Plain. But it is absent between the dryland or between the bog populations. Similar levels of isolation were revealed between pine and spruce settlements at different altitudes in the Carpathians. Clearly genetic border of populations in the continuous areal was found as a result of paleobotanic, factorial-ecological, phenological, phenotypical, and allozymic research between the pine settlements of dryland and adjacent high bogs. According to our genosystematic scale it was determined at the population level (Nei genetic distance, 1978; DN78 = 0.011). A gradient of this genetic distance DN78/D (where D – the distance between populations, km) is several times higher than between the adjacent dryland or between the bog populations.The reliable phenotypic differents are found between the natural populations and between their descendants at the levelled ecophone; 92% of 18 years old seedlings from the dryland had vertical roots, but 84% plants from the bogs had lateral roots only. Probably, genetically fixed adaptation of pine to the bog environment took place during the Holocene under the influence of mutations, disruptive selection and other factors of microevolution in two contrast environments by the almost complete reproductive isolation. The genetic differentiation at the level of DN78 0.017–0.015 was revealed also between reproductively isolated population of pine located at different altitudes in the Carpathians and in the Ural. This work was supported by the Program UrD RAS №15-12-4-13.

THE STUDY OF GENETIC CONTROL OF THE 1000 GRAIN WEIGHT IN SOFT SPRING WHEAT CULTIVARS IN DIALLEL CROSSINGS

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The purpose of the research was to determine the peculiarities of genetic control of the 1000 grain weight of soft spring wheat the varieties and the selection lines. The study was conducted in 2008-2009 at the experimental field of the laboratory of Genetics of SibRIPP&B. Mathematical treatment of value of the 1000 grain weight of the varieties and the hybrids F2 was carried out using the system of genetic analysis of quantitative traits Poligen A, developed by Merezhko A.F. Diallel analysis was carried out by the method of Zielke R.A. et al., 2006. The hybrids, resulted from the hybridization on full diallel scheme of the 6 varieties of soft spring wheat (Kantegirskaya 89, Novosibirskaya 67, Grekum 114 Sibirskaya 3, Sibirskaya 12, Novosibirskaya 89), and the recombinants (F_3), selected from F_2 the hybrid populations, were the material for the study. The 1000 grain weight is controlled by additive-dominant genetic system. The genes with additive effect bring more significant contribution to the trait expression. The growth of the 1000 grain weight depends on the accumulation of dominant alleles in the genotype. The variety Grekum 114 and the selection line Sibirskaya 3 include the greatest number of dominant alleles in their genotypes. The varieties Novosibirskaya 67 and Kantegirskaya 89 are characterized by the presence in their genotype of the genes with epistatic interaction or modifying genes that reduce of the 1000 grain weight in the hybrids F_1. The varieties Novosibirskaya 67 and Grekum 114 have 2 different genes (loci) which control the 1000 grain weight. There was observed dominant epistasis B>A (0,8) between the genes (loci). The recombinants selected from the hybrid populations with the variety Grekum 114, were characterized by the maximum value of the 1000 grain weight. Whereas recombinants with the varieties Novosibirskaya 67 and Kantegirskaya 89 were characterized by the minimum value of the 1000 grain weight.

MOLECULAR MARKERS FOR BARLEY BREEDING IN THE SOUTH OF RUSSIA

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Barley is one of the main cereal crops in the South of Russia. However, in the last 15 years the grain production decreased due to significant changes of climatic conditions (high summer temperatures and low amount of atmospheric precipitation). Barley breeders in the southern regions are looking for donors of early maturing alleles that allow the plants to pass the key stages of development in time before the beginning of drought. Recently, numerous candidate genes involved in the photoperiod and vernalization response were cloned along with *EPS*

(*earliness per se*) genes. The advanced barley cultivars and breeding lines developed in Kalinenko Institute of Grain Crops (Zernograd, Rostov area) were investigated using molecular markers for *Ppd-H1*, *Vrn-H1*, *Vrn-H2*, *sdw1/denso* (*Hv20ox2*) and *HvCEN* (*Mat-c*) alleles. Correspondence between genotyping data and barley phenotypes observed in the field is discussed.

INTRGRESSIONS FROM WILD CEREALS INTO BREAD WHEAT GENOME – A NEW SOURCE OF GENETIC VARIABILITY FOR TECHNOLOGICAL PROPERTIES OF GRAIN

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Diverse technological purposes of bread wheat grain requires the expanding the genetic base for selection for quality traits. The wild cereal species are widely used for improvement of wheat resistance in respect to fungus diseases through a wide hybridization. The resulted genotypes often carry the alien genetic material with known chromosomal location and rearrangements indicated with molecular markers. Such introgressions may affect technological properties of grain and flour and add to a genetic variability for technological properties of grain. The aim of this work was to investigate the influence of introgressions from wild cereal species, *Aegilops speltoides* and *Aegilops markgrafii*, and exotic tetraploid wheat *Triticum timopheevii* on milling properties, gluten content in grain and physical properties of dough. The novel gene for endosperm texture *Ha-Sp* was identified in winter and spring lines of wheat with introgression from *Ae. speltoides*. Introgression was detected as 5A/5S and was used to obtain the genotypes with two genes *Ha* and *Ha-Sp* for extra soft endosperm texture. Multiply introgressions from *Ae. markgrafii* into winter wheat chromosomes 2A, 2B, 3B, 4A and 6D resulted in increase of gluten content in grain and vitreousness and finally to a substantial improvement of physical properties of dough. Substantial increase of gluten content in grain was found in the line with introgression from *T. timopheevii* in 2A chromosome of spring cultivar. The effects of introgressions on technological properties of dough have been verified by introducing into another genetic background. The new substitution and isogenic lines with genotyped introgressions and improved technological properties of grain have been developed. They may be used in marker-assisted selection for quality traits.

CHALLENGES OF ASSEMBLING HUGE CONIFER GENOMES

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To assemble the Siberian pine (*Pinus sibirica* Du Tour.) genome we used the Illumina HiSeq2000 sequence reads obtained in the Laboratory of Forest Genomics at the Genome Research and Education Center of the Siberian Federal University and tested different assemblers that were used in the similar *Picea abies*, *Picea glauca* and *Pinus taeda* conifer genome projects: CLC Assembly Cell (trial version), ABySS and MaSuRCA, respectively. The input data size was 1.4 TB (~594 Gbp) that corresponds to 25-fold coverage of *Pinus sibirica* genome. The assembling was done using the IBM x3950 x6 supercomputer with 96 cores and 3 TB RAM. The best run (with the k-mer size = 39) of ABySS generated the total assembly length of 2 Gbp (10% of the expected complete genome length), with N50 for contig equalled 920 bp, and minimum and maximum length equaled 500 and 14020 bp, respectively. ABySS run took 145 hours using 30 cores and 634 GB of RAM. The CLC run quitted after completing 80% of the task and gave a memory allocation error warning. It took more than a month for the MaSuRCA pipeline on 64 cores with 2 TB of RAM to execute only the data preprocessing and creating of unitigs step. The next step, assembly was still in the progress by the time of the writing this thesis, with the expected running time of more than 2 months. Thus, our results demonstrated that ABySS was the most stable and fast running assembler. Low values of the basic assembly metrics for our data were most likely caused by the low genome coverage (25X). Our plan is to significantly increase the coverage to greatly improve the genome assembly.

INHERITANCE OF TRANSGENES IN *BRASSICA NAPUS* L., OBTAINED BY AGROBACTERIUM–MEDIATED GENETIC TRANSFORMATION

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Transgenic plants with a low copy number, obtained by agrobacterium-mediated transformation, usually have a progeny segregating in accordance to Mendelian 3:1, in some cases the progeny demonstrates non-Mendelian inheritance. The possible reason for non-Mendelian inheritance can be formation of chimeras, the organisms consisting of genetically heterogenous cells and producing generative organs from transgenic and untransformed cells. Finding the way to escape this phenomenon could allow to obtain much many transgenic plants. For this purpose we studied the inheritance of gfp and nptII genes in transgenic canola plants produced by different explants of two spring canola varieties. PCR analysis of cotyledon regenerants only revealed the simultaneous presence of gfp and nptII genes in a part of transgenic plants. They presented in 40% transgenic lines of v.Westar, and in 45% ones of v.Podmoskovny. However, only 70% of these plants fluoresced in UV expressing gfp gene. The transgenic plants were consecutively twice cut in vitro, and then the rooted plants were transplanted into the soil to produce seeds. Evaluation of the segregation results showed that although some Westar transgenic lines after the first cutting demonstrated the Mendelian inheritance of single feature characteristic of self-pollinated plants, the same lines after the second cutting demonstrated the non-Mendelian one. Only one transgenic line segregated according to the Mendelian after the second cutting, confirming its non-chimeric status. We only observed non-Mendelian segregation in Podmockovny transgenic plants. *Gfp* gene was not found out in the first generation of transgenic plants regenerated from leaf explants of both canola varieties. Studying the inheritance of this gene in T2 progeny showed that the most obtained regenerants demonstrated monogenic distribution of gfp gene. In the following generations the number of transgenic plants is increasing and there is a possibility to obtain some homozygous plants in the future.

PARASITIC PLANT MONOTROPA HYPOPITYS: GENOME STRUCTURE AND EVOLUTION

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Parasitic plants are a specific group of plants that receive nutrients directly from the tissues of other organisms – plants or fungi. The transition to parasitism is accompanied by different morphological changes, lose the ability to photosynthesize and the emergence of specific functions required for interaction with the host. *Monotropa hypopitys* L. (Monotropaceae), commonly known as pinesap or yellow bird’s nest, is a flowering perennial non-photosynthetic plant, that are mycoheterotrophic depending on carbon compounds obtained via fungus linkages to autotrophic host plants. We started the project that includes the Monotropa hypopitys whole-genome sequencing and further comparison with the genomes of related photosynthetic plants for estimation of genome changers that may be associated with the transition to mycoheterotrophy. The first result of this undertaking is the paired-end GS FLX pyrosequencing, the transcriptome sequencing via RNA-Seq (Illumina technology) and plastome sequencing and evolution. The obtained data will be discussed. This work was supported by RSF grant 14-24-00175

OBTAINING NEW FORMS OF WHEAT USING DISTANT HYBRIDIZATION AND LEAF-NURSE METHOD

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A protocol for raising new forms of wheat has been developed. It is based on the transfer of valuable traits from a wild wheat relative, intermediate wheatgrass (*Agropyron glaucum*) into wheat. The protocol includes raising wheatgrass double haploids *via* anther culture, the selection of wheatgrass genotypes that are able to produce green haploids in vitro, a proximate analysis of frost resistance, remote crosses of frost-resistant wheatgrass genotypes to wheat, and a new leaf-nurse method for transferring

some traits from wheatgrass to wheat. Numerous lines of spring and winter wheat with valuable economic traits were obtained and propagated during 2010-2014. In 2014 these lines were tested on an area of 4 hectares.

INTERSPECIFIC HYBRIDIZATION IN GRASS EVOLUTION: MOLECULAR PHYLOGENETIC RESEARCH

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Detection of the multiple interspecial and intergeneric events is of large methodological importance and a novel system of the grasses based on molecular phylogenetic data was proposed (Simon, 2007). We think that complicated system of intercrossing typical to the grass evolution makes this system unrealizable. Theoretically, taxa formation better agrees with the Genomic Concept of Genus suggested by Löve (each genus is a unique combination of genomes – Löve, 1984) in spite of the fact that attempts to apply this concept to real results of molecular phylogenetic analysis often disappoint the researchers. For evaluation of the relationships between hybrid grass species we sequenced and studied variability of the ITS1-5.8rRNA-ITS2 region (nuclear genome) and some genes and spacers of chloroplast genome (ndhF, matK, rbcL, trnL-trnF, trnK-rps16) of grasses from tribes Poeae, Triticeae, Meliceae, Phleeae. Comparing nuclear and plastid markers we determined some putative parental taxa of the hybrid species and genera. For example, we showed that the main part of the polyploid bluegrasses originated from intersectional crossings. Bluegrasses previously considered as subgenus Arctopoa (now – genus Arctopoa) and Andinae (now – genus Nicoraepoa) are intergeneric hybrids. Plastid genomes of Nicoraepoa and Arctopoa are relatives of Poa sect. Sylvestres (chloroplast genome Y), and North Eurasian genera Arctophila and Arctagrostis share the same genome. Thus we suppose that their common ancestor could live in northern latitudes. Nuclear genome of Arctopoa and Nicoraepoa is also close to that of Acrtophila and Arctagrostis but is distant from nuclear Y-genome of the sect. Sylvestres. Here we see an interesting phenomenon – close relationship between Arctic and South American (as well as Subantarctic) species. Earlier we showed this between North Pacific and Subantarctic bluegrasses (Poa s. str.). Research of nuclear and chloroplast sequences' variety allows us to detect cryptic species. In special issue (Shneyer, Kotseruba, 2014) we evaluated this phenomenon in grasses.

THE GENETICALLY-CAUSED REACTION OF DIFFERENT PLANT CULTIVARS ON ABIOTIC STRESS

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The attention of geneticists and breeders to beans, very important crop, increases at present time. The analysis of a complex of physiological, biochemical, anatomical and morphological features of the parental forms, defining of plant productivity and resistance to environmental stress is necessary for identification of the best genetic material sources, creation of new and improvement of already existing cultivars. Kaliningrad region is the territory with a complex of negatively-effected abiotic factors. Excess moistening, washing water mode of soil, snowless winter, low PAR, strong wind, pollution, and some others are among them. Therefore the purpose of this study was to carry out a comprehensive analysis of selected bean forms on the main genetically-determined quantitative characteristics, including plant productivity and resistance in order to find the best biological resources for selection and food value for conditions of the Northwest of the Russian Federation. 18 cultivars of beans (*Vicia faba* L.), entered in the annual "State Register of the Selection Achievements" in 2010-2014, were tested. Different morphometric, morphological, anatomical, phenological, and physiological parameters, characterizing growth, development and functioning of plants, crop formation, and biochemical mechanisms of stress resistance were analyzed. Our researches showed phenotype variability of genetically-determined parameters mentioned above in all bean cultivars. As a result, it was found out, that the best characteristics of productivity and resistance to abiotic stress under soil and climatic conditions of the Kaliningrad region had traditional bean cultivars Russkie Tcherny and Herts Frey. So these cultivars may be recommended for selection as

the best bean genetic material sources on the base of the complex of parameters.

MAJOR RESULTS AND CHALLENGES IN DURUM WHEAT BREEDING IN ALTAI TERRITORY (RUSSIA)

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Durum wheat appeared on Altai first half of XIX century and since that it has proved to be good yielder in the province. Area under the crop varies in a large scale – from 3000 ha till 400000 ha. At present Altai is the third largest durum producer among administrative regions of Russia with the sowing area about 50000 ha. For 1985–2014 durum productivity in competitive yield trial made up 3,25 t/ha (CV=29,7%). At this grain protein made up 15,4% with variation 13,2 – 21,3% (CV=9,7%), dough gluten – 33,0% with variation 28,0–44,0 (CV=11,0%). Weather characteristics determine more than 75% of general yield variation. Principle environmental constrains are short non-frost period and rain deficit combined with extremely high day temperatures especially at tillering, shooting and anthesis. Major challenges are: high and stable yield through adaptation to abiotic and biotic (loose smut, Septoria leaf blotch, common root rot, black point, ergot, cereal leaf beetle, sawfly and other) stresses; quality improvement – protein, gluten content and quality, vitreousness, semolina and pasta color, cooking strength, pasta firmness; resistance to lodging; ease of threshing and etc. Among biotic stresses problems of sawfly and Fusarium head blight are aggravating. For the development of original genetic diversity intra- and interspecies hybridization based on local genotypes is used. Sources of new germplasm are Vavilov's Institute as well as institutions of Russia (Omsk, Samara, Saratov, Voronezh institutes), Ukraine, Germany, USA, Canada and some others. To widen durum adaptability relative species *Triticum aestivum*, *T.dicoccum*; to a lesser extent *T.turgidum*, *T.persicum*, *T.timopheevii*, *T.bioticum*, *T.monococcum* were used. For 45 years of incessant breeding 7 durum wheat cultivars were developed and released and 2 are now in the State Variety Testing. For the period a breeding progress in yield made up 44% that is about 1% a year. Newly developed varieties Salyut Altaya, Solnechnaya 573 and Oasis have advantage over check cultivars in quality of grain and end products.

DOUBLED HAPLOID TECHNOLOGIES AS IMPORTANT TOOLS FOR EFFICIENT PLANT BREEDING

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Production of doubled haploid (DH) has become one of the key tools for plant breeding. Using DH techniques, the speed and efficiency of the plant improvement process is significantly enhanced. At present, several different technologies for the production of haploid or doubled haploid plants are available: haploids can be produced after pollination with distantly related species (wide hybridization). The female gametophyte is stimulated to develop into a haploid embryo, whereby the paternal chromosomes are eliminated after fertilization. Alternative methods have been developed that rely on the switching male or female gametophytes from their natural gametophytic pathway to an embryogenic (sporophytic) pathway. "Gynogenesis" is the induction of haploid egg cells to form a haploid plant, in "androgenesis" haploid plants are derived from *in vitro* cultured microspores. Now, technologies based on androgenesis facilitated a new strategy in the production of homozygous lines due to the large amount of microspores which are produced by a single plant. In addition to the potential for commercial mass production, an important advantage of anther and microspore culture is the occurrence of "spontaneous" (non-artificially induced) chromosome doubling during early cell divisions of the microspores. This process enables a one-step regeneration of fertile, double haploid plants without colchicine treatment. Doubled haploid technologies widely used for a variety of economically important crops, including wheat, barley, rye. DH methods are applied successfully as major tools for the accelerated production of homozygous lines from heterozygous parental genotypes without the necessity of time-consuming selfing. The rapid expansion of DH technologies for crop improvement is attributed to the recent achievements in tissue culture methods. However, the applied of DH plants is not limited to breeding programs: DHs can be used as material in several areas of genetics (mapping, gene discovery and identification) and to directly generate plants homozygous for the transgene(s).

A *THINOPYRUM INTERMEDIUM* CHROMOSOME IN BREAD WHEAT CULTIVARS AS A SOURCE OF GENES CONFERRING RESISTANCE TO FUNGAL DISEASES

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Wheatgrass *Thinopyrum intermedium* (Host), or *Agropyron intermedium* (Host) (2n = 42; genome formula, $EEE^{st}E^{st}StSt$) is one of the most valuable sources of highly effective resistance genes in wheat breeding. Bread wheat cultivars Tulaikovskaya 5, Tulaikovskaya 10, and Tulaikovskaya 100 derived from wheat—*Thinopyrum* crosses are highly resistant to leaf rust and powdery mildew, and moderately resistant to stem and yellow rust. C-banding, in situ hybridization, and assays with PLUG and SSR markers have demonstrated that wheat chromosome 6D in all three cultivars is substituted by the *Th. intermedium* homoeologous chromosome, 6Ai. This chromosome was designated 6Ai#2, because it differs from the earlier described homoeologous chromosome 6Ai#1. *In situ* hybridization with *Pseudoroegneria spicata* and *Dasypyrum villosum* genomic DNAs has allowed chromosome 6Ai#2 to be assigned to the E (=J) subgenome. Chromosome 6Ai#2 remained intact over long-term breeding efforts. Tests of leaf rust resistance of in F_2 and F_3 populations from a cross of a leaf rust–susceptible cultivar with Tulaikovskaya 10 has demonstrated that chromosome 6Ai#2 carries at least one gene locus for leaf rust resistance. This locus was designated *Lr6Ai#2*. It must be different from the known gene locus *Lr38*, located on the long arm of chromosome 7Ai#2 and now present in many translocation variants in bread wheat. The effect of a chromosome from the *Th. intermedium* subgenome E on resistance to powdery mildew, stem and yellow rust in Tulaikovskaya 5, Tulaikovskaya 10, and Tulaikovskaya 100 is discussed. This work was supported by Federal Targeted Program of the Russian Federation (agreement no. 14.604.21.0106).

SYNTENY AND PHYSICAL MAP OF THE SHORT ARM OF CHROMOSOME 5B OF COMMON WHEAT

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The IWGSC strategy for sequencing the bread wheat genome is based on first obtaining physical maps of the individual chromosomes. Our aim is to develop and use the physical map to study the organization and evolution of wheat chromosome 5B, which bears a number of agronomically important genes such as *Vrn*, *Skr*, *Ne1*, *Ph1* and genes conferring resistance to fungal diseases. The 5B chromosome-specific BAC-libraries were obtained from the Institute of Experimental Botany, Olomouc, Czech Republic with approximately 15-fold coverage for the short arm (290 Mbp) and the long arm (580 Mbp). BAC clones of 5BS were fingerprinted using the SNaPshotTM fluorescent restriction fragment labeling method and the fingerprint data were assembled with the LTC program. A minimal tiling path (MTP) consisting of 3161 overlapping BAC clones covered 258 Mbp (~89%) of chromosome 5BS. The 5BS BAC clones were assembled into 275 contigs, of which 90 with a size greater than 300 kb were designated as supercontigs. More than 40% of the supercontigs were anchored by using seventy-one markers mapped to chromosome 5BS using a F_2 population generated from a cross of CS and CS-5B dic. Further anchoring of BAC clones via SNP markers was achieved by genotyping the population of 116 Recombinant Inbred Chromosome Lines (RICLs) from a CS and CS-5Bdic cross using the Illumina Infinium 15k Wheat SNP array (TraitGenetics GmbH). 418 markers polymorphic between the parental lines were integrated to 5B map. Additionally, in order to link the non-anchored BAC-contigs to chromosome we applied the GenomeZipper approach that uses the collinearity relationship between grass genomes. The 134 BAC-clones from non-anchored contigs were sequenced on the IonTorrent platform, assembled with MIRA and compared with coding sequences from the Brachypodium distachyon genome. The 50 MIRA

contigs were assigned to 34 hypothetical genes located between the telomere and centromere of Brachypodium chromosome 4 that is collinear with wheat chromosome 5BS. Here, we demonstrate the approach of constructing physical maps of individual chromosomes of complex genomes such as wheat to generate resources that will be useful to accelerate map-based cloning, gain new insights into genome evolution, and provide a foundation for reference sequencing. The work was supported by the Russian Scientific Foundation (Project No. 14-14-00161).

USING SSR MARKERS FOR THE IDENTIFICATION OF THE COTTON MONOSOMICS

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Simple sequence repeats (SSR) have been applied as the most popular molecular markers for cotton genetics. In the last decade chromosome-deficient stocks of *Gossypium hirsutum* L. have been used for the development of chromosome substitution lines for *G. barbadense* L., *G. tomentosum* L. and *G. mustelinum* L. chromosomes or chromosomes segments. Several lint percentage trait associated SSR markers have been located to chromosomes 12, 18, 23 and 26 using deletion analysis in aneuploid chromosome substitution lines. We created new Cytogenetic collection of cotton in Uzbekistan after irradiation of seeds by thermal neutrons or pollen gamma-irradiation directly in M1, M2 and M3 generations. It includes a total 94 primary monosomics of *G. hirsutum* L. from the common genetic background of the highly inbred line L-458. We identified some of these monosomics using translocation lines of our Cytogenetic collection and a well-defined tester set of translocation lines of Cytogenetic collection of the USA. Considering a lot of DNA markers have already been assigned to the individual chromosomes of G. hirsutum L., we aimed to utilize chromosome specific simple sequence repeat (SSR) markers to identify and reconfirm the chromosome specificity of monosomic lines of our collection. We used of the monosomic substitution hybrids F_1 from crosses monosomic lines with doubled haploid Pima 3-79 line of *G. barbadense* L. for assignment of the SSR markers. Assignment of SSR markers was straightforward and in a manner described in previous reports that utilized a PCR amplification of chromosome specific markers in the genomic DNAs of hybrid plants. As results, we are identified 11 monosomic lines where three monosomes (Mo11, Mo16, Mo19) are chromosome 2, six monosome lines (Mo70, Mo71, Mo76, Mo81, Mo89, Mo90) – chromosome 4, two monosome lines (Mo13 and Mo67) – chromosome 6.

THE USE OF SOURCE MATERIAL OBTAINED BY BIOTECHNOLOGAL METHODS IN BREEDING OF THE SPRING BREAD WHEAT

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The success of breeding depends on availability of the genetically variable source material and application of modern biotechnological methods along with traditional breeding approaches. One of the approaches, allowing induction of resistance to abiotic and biotic stress factors, is a cellular breeding. Due to this approach, it is possible to get plant regenerants distinct from the original form by various characteristics, including resistance to fitopatogens. The purpose of this study was to investigate breeding value of wheat lines obtained from created by *in vitro* selection on the base of spring cultivars of bread wheat: Sibirskaya 59, Karagandinskaya 22, Karagandinskaya 70, Soltustyk, Karagandinskaya 31. The top productivity 18,6 c/h was achieved in line derived from Sibirskaya 59 using selection agent 20% culture filtrate of *Fusarium gramenium*. It was 3,1 c/h higher than grain productivity of standard Karagandinskaya 22. In addition, the line has high grain quality and resistance to root rot and septoria. The increased productivity was related with more intensive tillering, higher number of grains per spike and higher grain weight per spike. Drought tolerance of the material obtained was studied. The results demonstrate efficiency of *in vitro* selection for improving wheat productivity and resistance to biotic and abiotic stress factors. The most productive and resistant lines are included in further breeding programs.

OPTIMIZATION OF MICROPROPAGATION OF AQUATIC ORNAMENTAL PLANTS *IN VITRO*

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Ornamental aquatic plants (Staurogyne, Rotala, Hemianthus, Alternanthera) used for water gardens and phytoremediation. Therefore, a protocol for rapid and reproducible shoot organogenesis from apical shoot explants was developed. Optimum culture conditions for shoot proliferation were tested with MS medium containing different concentrations of 6-Benzylaminopurine (BAP), 1-Phenyl-3-1,2,3 thiadiazol –5-yl urea (TDZ) and gibberellic acid (GA3). Plant growth regulators significantly affected diameter, area and fresh weight of shoots clumps. With the exception, higher concentration of sucrose showed visible phenotypic growth inhibition and abnormalities observed *in vitro*. The regenerated shoots rooted efficiently on MS medium without growth regulators. The growth pattern of multiple shoots indicated their origin from the enlarged shoot base *via* proliferation of apical shoot. All *in vitro* regenerated plantlets acclimatized in aquarium successfully. Simplicity of the protocol and direct production of multiple shoots make this, a potential system that is highly amenable for true to type plant regeneration and maintain the genetic stability.

THE STRUCTURAL ORGANIZATION OF THE REGIONS SPANNING 5S rRNA GENES LOCATED ON 5BS CHROMOSOME OF *TRITICUM AESTIVUM* L.

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The aim of the study is obtaining the information on organization of the genomic regions (with length up to 100 kbp) containing the satellite clusters of 5S rRNA coding genes located on the short arm of chromosome 5B of the bread wheat *Triticum aestivum* (2n = 6x = 42, BBAADD). To date the data about the structure of these regions in the wheat genome is quite scarce. The availability of wheat chromosome-specific BAC-libraries and the high-throughput sequencing methods give us the opportunity to explore these regions. Using the PCR screening of 5BS-genome specific BAC library (43776 clones, 15-fold coverage) with primers specific to coding region of 5S rRNA gene we isolated two BAC-clones (010O13 and 025F09) containing 5S rDNA. The BAC-DNA of two clones was sequenced using Ion Torrent, then end-sequenced by Sanger method. Also we used the information about partial (6% or the arm length) 454-sequencing of 5BS. The 5,523,266 Ion Torrent reads (N50 = 244 bp) were *de novo* assembled by MIRA programme. The obtained 17770 MIRA contigs were annotated using BLAST with TREP and NCBI databases. The 689 of contigs with the length from 81 to 17042 bp had the regions of identity to 5S rDNA. We demonstrated that sequences flanking the 5S rDNA clusters represented the transposable elements, mainly the LTR-retrotransposons. Using PCR with primers designed specifically to 5S rDNA flanking sequences we partially determined the features of structure of 5S rDNA-containing BAC-clones 010O13 and 025F09. The information about the regions spanning 5S rDNA allows us to develop the new molecular markers for comparative studies in the genomes of diploid and polyploid wheat species that will give us the new view about the evolution of this group of genes in the wheat genome. The work was supported by grant of RFBR 14-04-32365.

STRATEGY OF SPRING BREAD WHEAT BREEDING IN THE CHANGING ENVIRONMENT CONDITIONS IN WESTERN SIBERIA

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Global climate changing, increase of air temperature increase and carbon dioxide concentration level in the atmosphere will make certain modification in national breeding programs in the nearest 50 years. Omsk data from 1971-2013 showed that each 10 years air temperature authentically increased on 0.3^0C and in general increased on 1.34^0C. On average for 43 years annual air temperature was 1.88^0C. The average annual sum of rainfalls for this period didn't change. The analysis of the hydrometeorological service on the vegetation period of wheat from sowing (May) to wax and full ripeness (August)

showed that not sufficient rainfalls (hydro-thermyc coefficient was 1,10–0,76) for analyzed period were noted in all months: in May – 23% of years, in June – 25%, July – 20%, in August – nearly 30% of years. Decrease of hydro-thermyc coefficient below 1,0 in May and June is evidence about getting dryness of climate in the initial vegetation period of wheat. The modern spring bread wheat varieties have yield productivity more than 6 t/h, however real average yield of wheat in the region of Western Siberia isn't higher than 1,5–2,0 t/h due to enormous losses of their potential under not favourable biotic and abiotic factors. Strategy of breeding for the nearest years should consider possible negative influence of weather conditions on yield productivity of spring bread wheat, especially in a consequence of climate getting warmer. Omsk State Agrarian University coordinates the KASIB program in Russia. Within this program the valuable initial material was received by use distant hybridization with wild species *Ae. squarrosa* and *T. dicoccon*, which have features of adaptability to biotic and abiotic stress. The spring bread wheat varieties which are characterized by complex resistance to diseases, high grain quality and high yield potential in the conditions of Western Siberia are selected.

SNP-CHARACTERIZATION OF GENETIC STRUCTURE AND DIVERSITY IN BELARUSIAN WHEAT (*Triticum aestivum* L.)

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Numerous wheat accessions are stored in Belarusian genebank with a little information about its genetic diversity and possible relationships. To characterize genetic structure in a collection of 58 winter and 27 spring accession including contemporary local Belarusian and foreign wheat varieties we used subset of 384 gene associated SNPs. A total 295 SNPs distributed on 21 chromosomes and 36 SNPs with not defined localization were genotyped, representing variability of 97.6% loci. The number of markers per chromosome ranged from 1 for 4D to 26 for 5B with following distribution through genome: A-genome – 129, B-genome – 138, D-genome – 28 SNPs. In total 653 alleles were detected at 331 marker loci. Polymorphic information content (PIC) values varied among chromosomes and depended on sub-group of wheat accessions (winter or spring). Average PIC values observed in our study ranged from 0.29 to 0.44 with high proportion of markers with PIC value above 0,35 (61,9%). Observed minor allele frequency (MAF) was shifted towards alleles with MAF>0.25. Of the 331 markers, only eight (2.4%) detected MAF≤0.05. The main loci with rare alleles are located on chromosomes 3D, 4B, 5A, 6D. Gene pools of winter and spring wheat significantly differ by frequencies of 248 variants of 174 SNP loci. Genetic diversity of spring wheat is 1.2 times lower both overall (π=0,37) and winter wheat (π=0,36) values. Higher proportion of low-frequency alleles and heterozygosity of a spring accessions versus winter accessions is confirmed by fixation index Fis (0.4 vs. 0.48 respectively). Genetic structure of Belarusian population of wheat is substantially similar to Russian and Ukrainian variety. Also we found a tendency of using a closely related accessions and even sibling lines, as evidenced by the presence of the subgroups of identity. Subsequently, our findings will contribute wheat improvement in Belarus through targeting selection for breeding.

COMPARISON OF SNP AND CAPS MARKERS APPLICATION IN GENETIC RESEARCH IN WHEAT AND BARLEY

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Barley and bread wheat show large differences in frequencies of Single nucleotide polymorphism (SNP) determined from genome-wide studies. These frequencies have been estimated as 2.3-3.1 times higher in barley than within each of diploid genomes of wheat (A, B or D). However, barley SNPs within individual genes are significantly more frequent than quoted. For example, one SNP is present per 7-64 bp in barley while per 335-613 bp in wheat. Differences between wheat and barley are based on the origin and evolutionary history of the species. Bread wheat contains rare SNPs due to the double genetic 'bottle-neck' created by natural hybridisation and spontaneous polyploidisation. Furthermore, wheat has the lowest level of useful SNP-derived markers while barley has been estimated with the highest level of polymorphism. As a result, different strategies are required for the development of most suitable molecular markers in the cereal species. For

example, Cleaved amplified polymorphic sequences (CAPS) are more popular and widely used in small scale experiments with highly polymorphic genetic regions containing multiple SNPs in barley. In contrast, SNP markers based on high-throughput technology Next generation sequencing are very effective and useful in bread wheat. However, in wheat, thousands of potential SNPs have to be assessed and several rounds of preliminary searches in different databases for potential SNPs have to be made to develop SNP markers. The presented results are based on the comparison of the example of two genes: (1) 2-oxoglutarate-Fe (II) oxygenage in barley, located in chromosome 6HS, and (2) Universal stress protein in bread wheat, located in the long arms of homeologous chromosome group 6. The presented results support the development of different strategies and the application of effective SNP and CAPS markers in wheat and barley.

STRUCTURAL VARIABILITY OF *HOX1* GENE IN POLYPLOID WHEATS AND THEIR DIPLOID PRECURSORS

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Data accumulated over the past decade provided the basis for a common scheme of genetic regulation of flowering in response to cold (vernalization) for hexaploid or common wheat *T. aestivum* (BAD). A key role in this scheme is given to the *VRN-1* gene encoding MADS domain containing transcription factor. The latter is actively accumulated in the apical meristem under the influence of vernalization conditions leading to the development of spike. However, which genes are targets for *VRN-1*, and what biochemical processes they regulate still remains unclear. Further perspectives in the study of flowering regulation were opened by the recent discovery of homeobox-containing gene *HOX1* in common wheat. The product of this gene forms a protein complex with the product of *VRN-1* gene and the stability of this complex affects the duration of vernalization period and time to heading. On the basis of primary structure analysis of the *HOX1* gene we developed specific primers for PCR amplification of homeologous copies of this gene from the three genomes of common wheat. These primers were used for PCR using genomic DNA of diploid species, precursors of A, B and D- genomes, as well as the wild tetraploid species *T. dicoccoides* (BA) as a template. Analysis of primary structure of the corresponding PCR products allowed us to establish the structural peculiarities of *HOX1* gene in diploid species comparing with their polyploid derivatives. Based on these data we presented a diagram of divergence of *HOX1* in diploid *Aegilops* and *Triticum* species, as well as in polyploids starting with the first (*T. dicoccoides*) up to the last (*T. aestivum*) stages of polyploidization. This study is supported by Russian Scientific Foundation (14-14-00161)

IDENTIFICATION OF NUCLEAR GENES CONTROLLING CHLOROPHYLL SYNTHESIS IN BARLEY BY RNAseq

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Chlorophyll synthesis is controlled by numerous genes localized in both nucleus and chloroplasts. Mutant phenotypes with full or partial chlorophyll deficiency have been described within various plant species. However, genes related with partial chlorophyll deficiency phenotypes formation remain unknown. Insight into structure of these genes and their involvement in gene pathways may elucidate organization and evolution of the complex regulatory machinery governing chlorophyll synthesis in plants, especially tissue specific part of this regulatory network. Current research aims to find individual genes responsible for formation of *albino lemma* (*Al*) phenotype in barley *Hordeum vulgare* and investigate their interactions with other genes and gene networks. Analysis of gene network of chlorophyll biosynthesis in *Arabidopsis thaliana* as well as lists of genes functionally connected with formation of albino and pale-green phenotypes in *A. thaliana* and several *Poaceae* species was carried out. Total RNA was extracted from developing spikelets of *Hordeum vulgare* near-isogenic lines differing by *Al* allelic state. Poly-A RNA was isolated, specific libraries for IonTorrent platform were produsedand size-selected. Sequencing of 3 biological repeat for each line was performed using IonTorrent platform and 318 chips. Reads were mapped to reference genome of *H. vulgare* and *de novo* transcriptome assembly was performed. Alignments and assemblies were compared in search of differential expression. We were able to identify 128 differentially expressed genes ($p<0.05$) from the set of genes annotated in *H.*

vulgare genome. Among differentially expressed genes those related with photosynthesis and chlorophyll biosynthesis turned out to be overrepresented. Additionally we identified many novel transcripts by de novo transcriptome assembling. Further study will be aimed on identification, analysis and verification of the candidate gene for *Al* among the differentially expressed genes, as well as on the reconstruction of gene network involved in tissue-specific regulation of chlorophyll biosynthesis in barley.

PURPLE AND BLACK PIGMENTATIONS OF BARLEY GRAIN ARE GENERATED FROM DISTINCT GENE NETWORKS

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Barley grain can have different pigmentation playing protective role under unfavorable environments. The most studied ones are purple and blue, which are caused by anthocyanin compounds synthesized in pericarp and aleurone layers, respectively. The genetic and biochemical basis of the anthocyanin biosynthesis is well studied: it has been revealed enzymes, participating in metabolic pathway, and structural genes encoding these enzymes as well as regulatory genes, predetermined temporal and spatial patterns of the anthocyanin biosynthesis structural genes expression. The less known is black pigmentation of barley grain, which is assumed to be caused by combination of phytomelanins with anthocyanins. Chemical nature of the barley phytomelanins is not clear yet. In the current study, to evaluate the role of anthocyanin or any other uncolored flavonoid compounds in black pigmentation formation, transcription analysis of anthocyanin biosynthesis genes was performed in barley grains of near-isogenic lines (NILs) having green, purple and black color. In NIL with purple colored grain, expression of the genes encoding chalcone synthase (CHS), chalcone-flavanone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavanol reductase (DFR), anthocyanidin synthase (ANS) and the regulatory gene *Ant2*, predetermined anthocyanin pigmentation in barley pericarp and lemma, was up-regulated in comparison with the green and black grains, whereas in black grained barley NIL, transcriptional activity none of the genes studied was differed from its expression in green control. These data demonstrated that anthocyanins as well as any others uncolored flavonoid precursor do not participate in black pigment synthesis in barley grain. Black and purple pigmentation of barley grain are generated from distinct gene networks. This study was partially supported by RFBR (grant no 14-04-31637).

DEVELOPMENT OF SUPERSOFT LINES OF BREAD WHEAT, THE CARRIERS OF TWO GENES *Ha* AND *Ha-Sp* FOR SOFT ENDOSPERM TEXTURE

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Endosperm texture parameters of bread wheat – vitreous/floury and hard/soft – are determined by only one gene *Ha* in 5D chromosome. Multiply allelism for this gene resulted in a continuous variation of endosperm structure from rather soft and floury to hard and vitreous as in durum wheat. Manifestation of the trait is modified by environment. The cultivar Chinese Spring may be an example of soft-grain wheat as its vitreousness varies in different conditions from 50 to 75% and flour particle size diameter (PS) from11 to 15 microns. The cultivar Rodina is hard-grained and has a vitreousness 85-90% and PS more than 23 microns. The introgression line 84/98^{w} obtained on the genetic basis of cv. Rodina carries the gene *Ha-Sp*, homoeological to *Ha* gene from *Aegilops speltoides* Tausch. in 5A chromosome. Therefore, the line has a soft grain and its vitreousness is about 50-70% and PS about 11-15 microns. The aim of the work was to combine two genes *Ha* and *Ha-Sp* for soft endosperm texture in one genotype. It was supposed that two genes will make the grain softness more expressed and less dependent from environment. To do this, the hybridization of Chinese Spring with the line 84/98^{w} was performed. Selection of genotypes was fulfilled in the number of generations accompanied with investigation of vitreousness and PS. From F_6-F_8 generations the lines were selected with very soft and floury endosperm with reduced vitreousness (from 15 to 50%) and PS 11-12 microns. These properties retained both in green-house and filed replications. Alveograph tests of the lines have showed that the

lines have a low dough strength (from 42 to 76 alveograph units) characteristic of "weak" gluten. Just this kind of flour is preferable for producing confectionary (cookies, cakes, pastries and biscuits) as it enables not apply chemical baking powder.

PLANT PROMOTERS FOR SPECIFIC TRANSGENE EXPRESSION IN TOMATO

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Tomato is one of the most important crop plants and has been a model plant for fruit research. A genetic modification of tomato has continuously expanded in recent years to include enhanced tolerance to biotic and abiotic stresses and improved nutrition and tastes of the fruit. Promoter is an important part of the genetic construct which largely determines the pattern of transcription of the transgene. Apart from a strong constitutive CaMV35S promoter, a toolbox of promoters with defined specificities is necessary for efficient expression of transgenes. The goal of our work was to find information about the promoters with tissue-specific and inducible activity in tomato plants and systemize it using the TGP database format (http://wwwmgs.bionet.nsc.ru/mgs/dbases/tgp/home.html). More than 60 promoters of different plant species have been investigated for their spatial and temporal control of transgene expression in tomato. A chimaeric construct of a pea *END1* promoter fused to a cytotoxic ribonuclease gene (*barnase*) was used to produce efficient male-sterility in tomato plants. This strategy may be used for the production of hybrid seeds. Pathogen attacks can cause serious losses in production. Taking care to negate transgene expression in fruits, *Arabidopsis thaliana* root-specific *NRT2.1* and *RB7* promoters were used to regulate the expression of the antifungal *NIC* and thionin *Thi2.1* genes, respectively. Resulting transgenic lines conferred enhanced resistance to *Fusarium oxysporum* and *Ralstonia solanacearum*. A *BADH* promoter of a halophyte *Suaeda liaotungensis* can drive increased expression of *BADH* gene in transgenic tomato under salt stress and increase salt tolerance without affecting plant growth. For tomato fruit improvement peach and apple *ACO* promoters, pineapple *MADS1* promoter may be used, while *GalUR* promoter from strawberry showed low activity in tomato fruit tissues. Wheat *Wcs120* promoter, cold-inducible in wheat, barley and cucumber, shows no cold inducibility in tomato. These examples indicate the presence of specific regulatory elements in *GalUR* and *Wcs120* promoters. TGP database allows selecting promoters with the desired stress-, tissue-, and stage-specific activities for proper transgene expression.

A DATABASE ON GENES PROVIDING RESISTANCE TO PATHOGENIC FUNGI

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Plants are exposed to a vast number of potentially pathogenic fungi. Their resistance to pathogens is based on a complex network of constitutive and inducible mechanisms of defense working at various levels. This process is controlled by dozens of genes and many of them are still unknown (especially in non-model plants, like cereals). We designed a special database, containing data on the cloned genes associated with resistance to fungal pathogens in bread wheat and the relative species. The database is manually curated and implemented on the SRS (Sequence Retrieval System) platform. It contains two cross-indexed sections with information on the related genes and their nucleotide sequences. The table of genes contains their formal descriptions with links to either resistant or susceptible plant varieties. The gene entry contains information about the gene name, localization of the gene on chromosome, the name of the corresponding protein, its function, reference to the UniProt database, gene activity, and the name of the disease caused by a specific pathogen. Links to the GenBank databases (AC) for corresponding nucleotide sequences are also provided. The nucleotide sequence of the gene is present in the sequence section and cross-linked with the gene entry. All the information is based on annotation of literature data with links to corresponding PubMed database entries. On demand, users may retrieve different information on the genes responsive to leaf rust, stem rust, powdery mildew, yellow stripe, septoriosis, etc. Database organization allows users to select individual defense genes with appropriate characteristics. The database can be used as a source of information for both a creation of crops resistant to parasitic fungi and annotation of genomic sequences to find new resistance genes. This work is supported by Federal Targeted Program of the Russian Federation (agreement no. 14.604.21.0107)

EFFECT OF ABIOTIC STRESS FACTORS ON GENETIC STABILITY OF *FRAGARIA VESCA* PLANTS

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Among the ever-changing components of the environment, increasing of solar irradiation and constantly rising ambient temperature are considered two of the most detrimental stresses. Stress disturbs the functions of regulation and repair systems of plant, causes alterations in expression of genes and structural reorganization of DNA and can indirectly leads to mutations. The diploid strawberry (*Fragaria vesca* L.) is a genetic model for the more complex octoploid commercial strawberry and *Rosaceae* family. The micropropagated plants of clone of *F. vesca*, cv. Reine des Vallee used in our study were maintained in vitro over 25 year. DNA of individual plants subjected light stress and heat stress were screened for genetic stability by PCR-methods (RAPD, ISSR, and REMAP). We compare the frequency of polymorphic bands in the plants after different stresses to that in plants of *F. vesca* cultivated *in vitro* without other stress. On preliminary data no additional polymorphic bands have been observed after light stress and heat stress. This work was supported by the Russian Foundation for Basic Research, project №14-04-31615.

THE CHLOROPLAST GENOME SEQUENCE OF THE FERN *DRYOPTERIS BLANFORDII* (C. HOPE) C. CHR.

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The fern genus *Dryopteris* is among the most common fern genera in temperate regions in the Northern Hemisphere and containing about 250 species worldwide. However this genus has been neglected in studies of genomics and chloroplast genome of any *Dryopteris* species has never been fully sequenced. In this study we report and characterize the chloroplast genomes nucleotide sequence of *Dryopteris blanfordii* (C. Hope) C. Chr. Alive young *D. blanfordii* was taken from the collection of the Moscow State University Botanical Garden. For rising of cost-effectivity of high-throughput sequencing we took chloroplast DNA (cpDNA) extracted form fresh leaves. For cpDNA purification the slightly modified sucrose gradient method proposed by Shi et al. (2012) was used. We sequenced the isolated cpDNA samples with Illumina (MiSeq) sequencing technology. Assembling of obtained paired-end reads was performed by Velvet and CLC software. Pteridium aquilinum 152362 b.p. chloroplas complete genome [HM535629] was used as reference. For *D. blanfordii* we achieved of 21 contigs with 42% GC content and 120298 b.p. summary contig length. This work was supported by grant from the Russian Scientific Foundation (14-04-01852).

ANALYSIS OF THE *Vrn-1* AND *Ppd-1* GENES IN SIBERIAN EARLY AND MEDIUM EARLY VARIETIES OF SPRING COMMON WHEAT

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The duration of the growing season is one of the most important traits that determine the value of varieties of crops and their suitability for cultivation in a particular climate area. Time to heading and, accordingly, the length of the growing season of spring wheat is largely affected by the *Vrn* genes which control the response of plants to vernalization. Besides, the *Ppd* genes controlling the sensitivity to photoperiod also affect the duration of vegetation. The objective of this work is to characterize the Siberian early and medium early varieties of spring wheat in relation to the genes that determine the length of the growing season, and comparing the results of molecular studies with actual field characteristics of genotypes. The length of the growing season in the studied cultivars was evaluated for 9 years (from 2005 to 2013) at the experimental field of the Siberian Research Institute of Plant Industry and Breeding. Allelic combinations of the *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-D1*, and *Ppd-B1* genes were analyzed in 48 spring wheat varieties from different breeding centers of Siberia using allele-specific primers. Six haplotypes were identified for *Vrn-1* genes, including the most abundant with two

dominant genes *Vrn-A1* and *Vrn-B1* and the recessive *Ppd-D1b* gene, which cause sensitivity to photoperiod. Only one variety (Tulun 15) was found containing the photoperiod-neutral *Ppd-D1a* allele in combination with the dominant *Vrn-A1* and *Vrn-B1* alleles. This variety showed the earliest ripening of all the studied accessions. A considerable variability was found within each haplotype in duration of vegetation, suggesting a strong influence of the "genetic background" on this trait. Nevertheless, the results can be used for marker-assisted selection of genotypes most appropriate for different growing conditions.

GENETIC CONTROL OF SPECIFICITY OF SYMBIOSIS BETWEEN "AFGHAN" VARIETIES OF PEA (*PISUM SATIVUM* L.) AND NODULE BACTERIA

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The specificity of legume-rhizobial symbiosis depends on the structure of the bacterial signal molecule (Nod-factor) being recognized by plant receptor kinases. Several genotypes within the pea (*Pisum sativum* L.) species originating from Middle East differ in their ability to perceive the Nod-factor structure, which results in their increased selectivity for bacterial symbionts. Plant gene controlling this trait was discovered more than 80 years ago and named *Sym2*. To date, *Sym2* was mapped in I linkage group of pea genome, but its nucleotide sequence, as well as the structure and function of its protein, remain unknown. In the present study we identified a previously unknown pea gene *LykX*, which has a number of characteristics of *Sym2*. *LykX* is localized in I linkage group and encodes a receptor kinase potentially capable of binding Nod-factor. There are two specific alleles of *Sym2* leading to amino acid substitutions in corresponding protein which correlate with the high selectivity in legume-rhizobial symbiosis. Thus, *LykX* is considered the most likely candidate for the *Sym2*. For a further description of the role of *LykX* in symbiosis we perform the TILLING analysis on pea mutant collection (in collaboration with INRA-URGV, France). 23 mutant families were identified, with 8 mutations presumably disrupting the function of *LykX* protein (according to the *in silico* prediction; SIFT program). We intend to conduct the allelism test between mutant *LykX* and *Sym2*. A positive test result will be the final confirmation of the hypothesis about *LykX* being *Sym2*. The work was supported by RFBR grants 14-04-32289 and 15-29-02737.

BIOTECHNOLOGICAL APPROACHES TO IMPROVEMENT OF ECONOMICALLY VALUABLE TRITICALE TRAITS

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Triticale is one of the most important cereals for Belarus and is currently grown on the area of about 500 thousand hectares. Modern triticale varieties are characterized by a high yield and grain feeding value, resistance to a number of diseases and adverse soil and climatic conditions. At the same time there are some significant problems in crop breeding which include, in particular, insufficient resistance of varieties to lodging and pre-harvest grain sprouting. These shortcomings are related to a certain extent with the lack of wheat D-genome in triticale in whose chromosomes a number of significant genes for manifestation of the above-mentioned traits are localized. Therefore, we have set a goal to evaluate the efficiency of using chromosome engineering technologies in combination with DNA-labeling of hybrid material for solving the stated problems. The secondary recombinant forms of triticale were developed by hybridization of chromosome-substituted forms of the 6x-triticale with modern crop varieties. Eight stable recombinant lines, of which four contained 1D(1A)-chromosome substitution, one – 2D(2B); one – 6D(6B); one – 1D(1A) and 2D(2B) and the latter – 1D(1A) and 6D(6B), were selected from hybrid populations F_4 by the C-banding method. The allelic composition of genes affecting formation of semidwarfness (Rht-B1, Rht8) and resistance to pre-harvest sprouting (Vp-1B) was studied by DNA-markers in these lines. Six recombinant lines were established to contain the mutant allele *Rht-B1b*, one line was characterized by the presence of the wild allele *Rht-B1a* and there are both wild and mutant alleles in another line. The allelic composition of the gene *Rht8* localized in the short arm of the chromosome 2D was studied in two lines with introgression of this chromosome. It was established that there was the wild-type allele *Rht8a* in the investigated forms. When analysing the gene *Vp-1B* in the secondary recombinant lines, two alleles – *Vp-*

1Ba and *Vp-1Bc* were identified. Four recombinant lines contained the mutant allele Vp-1Bc. The forms were selected for use in breeding.

SYSTEM CONTROL OF NODULATION

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Holistic principle in biology is one of the most fundamental principles, depending on how it appears you can judge about the integrity of multicellular organisms. M.E. Lobashev first formulated regulations about the control by the organism on the flow of genetic processes, in particular, the emergence of mutations. «Behavior as an active process of ontogenetic adaptation among animals is also a factor controlling genetic consequences caused by the influence of environmental conditions» (M.E. Lobaschew, Research on Genetics, 3. edition, publishing house of LSU, 1967, p. 15). However, a statement of the importance of the holistic principle of organization of the living system does not decide the issue of the mechanisms determining the very consistency, in particular its molecular genetic support. The better we understand, work of which genes causes that the congregation of cells "aware" itself as an organism, the more effective we will be able to manage the adaptation process. Existence of integrating systems by animals is also practically assured, but the laws of appearance and operation of such plants require special studies as it is possible to find a number of adaptations that are systemic in nature. One of the most well studied in this regard - the symbiosis of legumes and nodule bacteria. Being optional i.e. only essential in the absence of nitrogen in the environment, this adaptation, said more precisely the degree of its development, is under the control of the whole organism of plants, which was first demonstrated in studies of P.Greshoff and his colleagues. The appearance of nodule in the form of newly formed meristems is controlled by the plant, and mutations that lead to a breach of the system regulation, causes a decrease of the adaptive potential. Identification, cloning and sequencing of the corresponding genes lead to the assumption that the regulation system is based on controlling the emerging meristems on principles similar to those of regular meristems. The new, recently obtained data on the mechanisms of control of the system will be presented in the report. The present study was supported by the Russian Science Foundation, grant of RSF 14-24-00135.

CREATION OF MARKER-FREE TOMATO PLANTS WITH THE SUPERSWEET PROTEIN GENE UNDER THE CONTROL OF CIS-REGULATORY ELEMENTS

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Generation of transgenic plants without any foreign genetic material is capable to relieve some public concerns. In our work we used the pMF vector system containing of the recombinase R and a CodA-nptII bifunctional selectable gene for produce tomato plants carrying the super sweet thaumatinII gene from tropical plant under the control of tomato fruit-specific ELIP or E8 gene promoter and tomato RuBisCo terminator. After agrobacterium transformation, two strategies were followed for the selection of marker-free transgenic tomato plants. In the early negative selection approach, a total of 155 Km-resistant calluses were treated with dexamethasone to induce recombinase activity and after the selection on 5-fluorocitosine we obtained 116 plants from 40 lines. Eighty three shoots was non-transgenic escapes, 32 contained nptII gene fragment and only one marker-free plant with excised DNA was appear. In the alternative delayed strategy we have obtained a total of 170 transgenic tomato lines that have been analyzed by PCR for the presence of complete T-DNA and RS site sequences. About half of them contained a partial sequence of the T-DNA, but the majority of the checked by Southern blot had two or more inserts. Then we choose 35 transgenic tomato lines and after induction of recombinase activity in explants about half of them did not produce regenerants on medium with 5-FC. One hundred twenty one resistant sublines were obtained from 18 original lines. Most of them lost resistance to kanamycin in spite of the sequence of nptII gene were detected by PCR in 120 plants and only one fully marker-free transgenic plant was obtained. We suppose that an incomplete excision and chromosomal rearrangements due to the presence of multiple or partial T-DNA insertions occur in other cases. A total of two completely marker-free

transgenic tomato lines were obtained by two different selection strategies. The thaumatin II gene expression has been confirmed by RT-PCR, Western blotting and organoleptic analyses.

MUTANT RESOURCES OF VEGETABLE PEPPER AND THEIR USE IN THE PRACTICAL BREEDING

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The breeding of vegetable pepper in Prednistrovye is conducted first of all in the direction of the productive and diseases resistant varieties and hybrids F_1. Priority and urgent tasks are the creation of varieties and hybrids with the wide reaction norm to the biotic and abiotic factors of environment and in connection with this the various duration of phenological phases, forms thickness and colour of pericarp, with the improved biochemical characteristics of fruits. Mutagenesis and use of the mutant gene pool recommended themselves as one of the effective methods of the vegetable pepper breeding. Problem of today is the development of the scientifically substantiated recommendations regarding the use of mutant resources of *Capsicum* L into the productivity, the early ripeness and the quality breeding. For creating the fundamentally new breeding developments the mutants were used with the diverse types of sterility, resistant to the diseases, with the high content of bioactive compounds, with all possible pericarp colour patterns, with different types of branching and growth, and also mutants, with the various duration of vegetative period. The results of the mutant gene pool usage in the pepper breeding are as following: 1) the scientifically substantiated recommendations regarding the use of mutant resources in *Capsicum* breeding to the productivity, the early ripeness and the quality are developed; 2) the nursery of lines and also varieties and hybrids with high ß-carotene content and with the complex of the most important economically valuable characters are created; 3) Early-ripening varieties and hybrids with the complex of economically valuable characters are bred out; 4) the seed-growing of heterotic hybrids on the sterile basis is organized.

STUDY OF KARTALINSKAYA WHEAT IN THE NORTHERN TRANS-URALS AREA

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To preserve and restore the genetic variety it is necessary to use rare species of *Triticum* L. in selection. The tetraploid type *Triticum carthlicum* Nevski (=*Triticum persicum* Vav. ex Zhuk.) is of great importance for the forest-steppe zone of Northern Trans-Urals area. Studies of biological features and adaptable abilities of this species have begun in the Tyumen Region since 1992. Duration of the vegetative period for the years of research in kartalinskaya wheat variety samples was 81 days on the average, that is 3 days longer than in standard varieties of soft wheat. By seed efficiency not a single variety samples of kartalinskaya wheat exceeded the standards for certain. Grain quality analysis has shown that the protein content in variety samples K-13768, K-7882, K-32507, K-17581 varied from 17,3 to 18,3 per cent. Variety samples K-13768, K-7882, K-32507, K-17581 had high gluten content of second and third quality groups. Field and laboratory studies have allowed to single out kartalinskaya wheat variety samples resistant to *Septoria*, *Puccinia triticiana*, *Erysiphe graminis*. The nature of flowering has shown that the maximum and most vigorous flowering was on the first half of the day. There is a direct correlation between the number of blossom out flowers in kartalinskaya wheat and air temperature (r=0,698±0,09) and also relative air humidity (r=0,705±0,07). The analysis of storage protein component structure in kartalinskaya wheat variety samples has shown that all of them differed by electrophoretic mobility and component density. Intraspecific crossings have been carried out to study the inheritance nature of gliadin component structure.The parental forms and hybrids F_1, F_2 in hybrid combinations K-17581xK-7881 and K-17581xK-7887 have been analysed using the method of electrophoresis in polyacrylamide gel. The gliadin biochemical analysis in populations F_1 and F_2 has allowed to reveal the control of these proteins. Thus, allocated variety samples *Triticum carthlicum* can be recommended as sources of valuable signs and properties in selection of soft wheat.

SOMATIC POLYPLOIDYZATION IN THE CEREAL LEAF EPIDERMIS AND ITS RELATION TO CELL GROWTH

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Currently, the study of plant tissue and organ formation remains to be one of the important areas of science. With the use of new technologies it has become possible to trace the fate of individual cells, as well as the effects of different regulatory mechanisms on morphogenesis.Cell cycle is the important process associated with morphogenesis. In recent years, the evidence was obtained that somatic polyploidization plays an important role in plant tissues formation and leaf epidermis may be a useful object for the studies. The purpose of this work was to estimate the fraction of polyploid cells among the different types of epidermal cells in leaves of bread wheat and its wild relatives, differing in morphological characteristics of the epidermis. To visualize the geometry of cells and nuclei the classical fluorescence microscopy and laser scanning microscopy were used. Morphological characteristics of cells and nuclei were evaluated using ImageJ software.The results obtained evidenced for the prevalence of somatic polyploidy in the epidermisesof bread wheat and its wild relatives. Polyploid cells were found among the main cells of epidermis and among the trichome cells. A positive correlation was found between the size of the nucleus and the cells within each cell type. Character of the correlation differed in different cell types, indicating the differences in the regulation of cell growth. The abnormally large cells were found similar to the recently described in A. thaliana. The studied lines of bread wheat and its wild relatives are a perspective model for the genetic analysis of the mechanisms of cell cycle regulation during morphogenesis, particularly, during the transition of cells to somatic polyploidy.This work was supported by Russian Science Foundation (RSCF) grant № 14-14-00734.

SOMACLONAL VARIABILITY OF *LARIX SIBIRICA* AND *L. SUKACZEWII* CELL LINES *IN VITRO*

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Sixteen proliferation cell lines (CLs) were obtained from embryo cultures: four of *L. sibirica*, 11 CL of *L. sukaczewii* and one hybrid CL of *L. sibirica* x *L. sukaczewii* and fifteen CLs from megagamethophytes of *Larix sibirica* on AI medium (http://www.freepatent.ru/images/patents/5/2456344/patent-2456344.pdf). CLs differed in somatic embryo production: embryo quantity, size, and capability to maturate, to germinate, and to form viable plantlets. Embryo numbers ranged from 2040 to 11103 embryos per 1 gram of fresh weight of embryogenic callus (EC). The embryonal lines are capable of long-term (over five years) self-maintenance and mass production of somatic embryos and plantlets. In highly embryogenic CLs embryos were formed, which were normal in terms of morphogenesis. Cytological studies shown that embryos in proliferation cell mass contained diploid chromosomes and have high mitotic activity (mitotic index 5.6). In lines of low embryogenic productivity (hybrid CL) the small embryos do not matured on the medium AI with ABA. In this CL the agglutination of chromosoms were observed. In CLs number of healthy plantlets ranged from 11.1% to 82.4%. Plantlets planted in the greenhaus CLs derived from Siberian larch megagametophytes demonstrated the genetic instability of in vitro. From analysis of 11 nuclear microsatellite loci, none of the resulting CLs retained the original maternal haplotypes. Practically, all CLs were chimeric and contained mutational allelic variation in addition to the original maternal allele at one or more loci. Cytogenetic studies demonstrated that 73% CLs from megagamethophytes contained diploid chromosomes. Thus cytogenetic analysis revealed that Cls from embryo cultures characterized the high genetic stability but megagamethophytes cultures (cytogenetic and microsatellite analysis) has high frequency of somatic mutagenesis and genetic heterogeneity. This work was supported by RFBR grants № 15-04-01427 and by Research Grant No. 428 14.Y26.31.0004 from the Government of the Russian Federation

COMPLEMENTARY GENES OF HYBRID LETHALITY WERE REVEALED IN WHEAT-RYE CROSSES

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Ungerminated hybrid seeds with dead embryo and normally developed endosperm were obtained in crosses of bread wheat Chinese Spring (CS) and four inbred rye lines. It was shown that single rye gene is responsible for abnormal seed development. This gene has two alleles - compatible allele inherent in majority of tested rye lines and incompatibleone characteristic of two unrelated lines. Gene *Eml-R1* was localized on chromosome 6R with aid of molecular markers which have been genotyped in F_2 of corresponding rye hybrid and test for embryo lethality of wheat-rye hybrids conducted in F_5 of the same cross combination. Using additional molecular and isozyme markers, genotyped directly in F_5 and F_6 of rye hybrid, gene *Eml-R1* was included in the linkage group on chromosome 6R. The distance between *Eml-R1* and two co-segregated markers *Xgwm1103 (Xgwm732)* is 8.8±3.5 cM. Whereas the distance between isozyme locus *Est10 (EstR-5)* and *Eml-R1* is even 29.4±6.2 cM. It was shown that incompatible allele dominate over compatible allele in accordance to appearance of seeds with dead embryos in crosses of CS 6R addition line with rye line carrying incompatible allele of *Eml-R1* . The canonical interpretation of such manifestation is complementary gene interaction. To solve this question the set of nulli-tetra CS wheat lines was crossed to line carrying incompatible allele of *Eml-R1*. Germinated seeds were observed only in hybrids with CSN6A/T6B and CSNT6A/T6D lines, in all other crosses hybrid seeds have abnormal embryos inefficient to germinate. Thus, namely chromosome 6A is to carry incompatible wheat allele interacting with *Eml-R1* gene with 6B and 6D carry compatible or non-active homeoalleles. In accordance to genome location of wheat and rye *Eml* genes it is felt that embryo lethality of wheat-rye hybrids is result of interaction between wheat and rye homeoalleles.

GENETIC VARIATION OF SPRING WHEAT IN KAZAKHSTAN

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Ninety six cultivars of hexaploid spring wheat growing in Kazakhstan were genotyped by using the high-density wheat 90K Illumina SNP array. The analysis allowed to identify 30 288 polymorphic SNPs. A subset of 3541 high-quality SNPs was used for comparison of 690 wheat accessions representing landraces and varieties including Asia, Australia, Canada, Europe, Kazakhstan, USA and other parts of the world. Phylogenetic analysis showed a clear separation of wheat cultivars according to their geographic origin. In the phylogenetic tree accessions from Kazakhstan and USA formed two neighboring clusters with a common node and they were distinct from accessions of other regions of the world, including Europe. The results were used for association mapping of wheat agronomic traits and suggest new important insights in genetic relationships of the investigated wheat accessions.

IDENTIFICATION OF QTLS ASSOCIATED WITH SPOT BLOTCH RESISTANCE OF BARLEY

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Spot blotch caused by *Bipolaris sorokiniana* Shoem. is one of the most harmful diseases of barley in Kazakhstan. It is estimated that in certain years it downgrades grain yield up to 30%. Therefore, search for resistant forms of cultivated barley to *B. sorokiniana* is very important for local breeding programs. Seven hundred spring barley accessions,

including 96 cultivars and perspective lines from Kazakhstan, were grown in Karabalyk breeding organization (Northern Kazakhstan) and in controlled artificially infected environment in Dzhambul region (Southern Kazakhstan). The accessions were grown in triplicate randomized blocks in all testing sites and studied for grain yield, yield components and disease resistance to spot blotch. In controlled infected environment all accessions were inoculated at tillering stage and resistance was estimated at heading and seed maturation stages. As a result 7 QTLs associated with resistance to spot blotch were identified based on association mapping approach. Out of these 7 QTLs, 4 QTLs were mapped for juvenile stage of growth and 3 QTLs on adult stage. The research is further attempt to dissect QTL disease resistance to spot blotch of barley and results can be used for improvement of local barley disease resistance activities based on marker-associated selection.

STILBENE SYNTHASE GENES TRANSCRIPTIONAL REGULATION IN CULTURED CELLS OF *VITIS AMURENSIS* RUPR.

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Grape growing in Primorsky region of Russia, *Vitis amurensis* Rupr., was shown to posses the increased resistance to many diseases and to cold stress in conjunction with high level of resveratrol content in comparison to other grapevine cultivars. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a naturally occurring phenol that has been reported to exhibit a wide range of important biological and pharmacological properties. Stilbene synthase (STS, EC 2.3.1.95) directly catalyzes the reaction of resveratrol formation in vivo. It was shown, that STS represented as a multigene family in most stilbenoid producing species. Despite the fact that all enzymes involved in resveratrol biosynthesis were preciously described, mechanism regulating resveratrol biosynthesis are far to be characterized in detail. In course of our study, we investigated the role of cytosine DNA methylation, a powerful epigenetic factor, in regulation of resveratrol biosynthesis in vivo on cell cultures of V. amurensis. Interestingly, non-specific cytosine demethylation caused by addition of demethylation agent 5-azacytidine (5A) led to two-fold enhancement in resveratrol production in V. amurensis cell cultures. Further analysis revealed that some STS genes encoding key enzymes in resveratrol biosynthesis were significantly increased in their expression under DNA-demethylation effect of 5A. Furthermore, we observed negative manner of correlation between level of cytosine methylation within nucleotide sequences of different representatives of STS gene family and level of their expression at normal conditions and under 5A treatment. Moreover, analysis of salicylic acid (SA) and ultraviolet (UV-B) treatment of V. amurensis cell culture, revealed their up-regulating effect on resveratrol production and STS expression determined by selective cytosine demethylation of certain STS genes. According to the data obtained, the level of resveratrol production in vivo is regulated epigenetically in response to environmental stimuli. Hence, more detailed investigation of factors responsible for establishing cytosine methylation in particular loci could be useful in modulation of plant biosynthetic pathways.

PATTERN OF CYTOSIN METHYLATION IN SWEET PEPPER HYBRIDS AND ITS PARENTAL LINES

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Genomic DNA-strand contains epigenetic information that is predominantly caused by covalent modifications of nucleotide context by addition methyl groups. This specific feature, named DNA methylation, regulates different genetic pathways by altering of gene expression. Increasing evidence suggests that many aspects of heterosis manifestation also involve epigenetic regulation. To investigate extent and possible role of DNA methylation in heterosis we have used fluorescence labeled methylation sensitive AFLP (with *Hpa*II and *Msp*I esoschizomeres) for getting specific patterns heterotic and non- heterotic hybrids F_1 of a sweet pepper (*Capsicum annuum* L.) in three stage of vegetation (seedling, flowering, fruiting). To date, in total 203 loci from 4 AFLP markers were detected in plant seedlings P_1, P_2, F_1, from which 24 loci had variability in DNA-methylation. We found some differences between parental and maternal lines both in polymorphism of amplified loci and its *epi*-allelic variability. There were following cross combinations of allelic variants (P_1/P_2): Met/dMet; dMet/dMet;

Met/Met; Met/0; dMet/0. Noteworthy, heterotic hybrid characterized by presence allelic variants dMet (demethylated) independently of the methylation status of parental lines analyzed on seedling stage. Exceptions - *epi*-allele $TCTC_{240}$, which one was methylated in both parents (ver. Met/Met) and hybrids inherited the same status (Met).

TRANSLATIONAL GENOMICS FOR CHICKPEA IMPROVEMENT

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Chickpea (*Cicer arietinum*) is the second largest cultivated grain food legume globally. Terminal drought is one of the serious yield constraints that confer ca. 40% yield loss. A range of translational genomics approaches are being used to accelerate genetic gain in chickpea breeding and develop improved chickpea varieties for enhancing food and nutritional security in developing countries. In this direction, as a part of several initiatives and strategic collaborations with several partners from different countries, large-scale genomic resources including draft genome sequence, re-sequencing of >500 chickpea lines, comprehensive transcriptome assembly, high density genetic and BIN maps, QTL maps as well as physical maps have been developed. For trait mapping, by using linkage mapping approach, a "QTL-hotspot" harboring QTLs for several drought tolerance related traits was identified on linkage group 04 (CaLG04). The "QTL-hotspot" has been successfully introgressed in several elite chickpea cultivars using marker-assisted backcrossing (MABC) approach and many introgression lines have shown higher yield as compared to recurrent parent. Realizing the importance of this region, efforts are also underway to fine map this region. Similarly, MABC has also been used for introgressing resistance to Fusarium wilt (FW) and Ascochyta blight (AB) in elite chickpea cultivars. In parallel, by using genome wide association study (GWAS) approach, 335 significant marker-trait associations (MTAs) have also been identified. For a high-resolution GWAS and understanding the genome dynamics, re-sequencing of 3000 chickpea germplasm lines has also been initiated. In addition, ca. 1000 lines from multi-parent advanced generation intercross (MAGIC) population have also been sequenced at 2X-3X for mapping drought tolerance at high resolution. Furthermore, several functional genomics approaches such as RNA-seq, Massive Analysis of cDNA Ends (MACE) with parental genotypes of mapping populations as well NILs have provided some candidate genes for drought tolerance that are being validated through genetical genomics and/or TILLING approaches. Finally, genomic selection (GS) approach by phenotyping and genotyping a training population of 320 elite breeding lines is also being used. An overview on above results will be presented in the meeting.

PROTEIN INTERFERENCE FOR REGULATION OF GENE EXPRESSION IN PLANTS

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Regulation of gene expression in a cell can be on transcriptional, translational, and protein levels. Thus, regulation at the transcriptional and translational levels can be accomplished by RNA interference (RNAi). However, despite the blockage of transcript there are a number of transcription factors (TFs) that have been accumulated previously. At the protein level, regulation is usually occurred by controlled ubiquitin-dependent proteolysis. However, this pathway requires a certain time, which is critical in the presence of stress factors. Recently a new way of regulation of TF protein by small proteind was discovered. Discovered small proteins have been called "small interfering peptides/proteins" (siPEP/siPROT) because of the similarity with the molecular mechanism of action of small interfering RNA (siRNAs) in the RNA interference (RNAi) pathway. The mechanism of action of siPROT was named "peptide/protein interference" (PEPi/PROTi). To date, it is assumed the presence of small interfering peptides in all the major families of TFs such as: bHLH, homeobox proteins, MADS, MYB, NAC and WRKY. Analysis of the database revealed four candidate proteins - WRKY43, WRKY39, WRKY55, and WRKY15. The selected candidates have two isoforms of the protein obtained by an alternative splicing: a full-length isoform represents a functional TF and second, cut isoform has a natural deletion of various sizes. In the course of work *Arabidopsis thaliana* plants were treated by flagellin. Using RNAseq it was carried out a comparative analysis of gene expression in the infected and non-

infected plants. In was found that the treatment of plants by flagellin significantly alter expression of about 25 genes from the WRKY family, which include two candidate genes: *wrky55* and *wrky15*. Candidate genes *wrky39*, *wrky43* do not show significant changes in expression. Analysis of the literature reveals other stress factors which might cause a change in the expression level of the selected candidate genes. Among the many stress factors were selected: heat, cold, salt stress. According to the analysis using qPCR, only one gene - *wrky55* - is changing its expression in the presence of salt stress, and only the full-length splice variant of *wrky55*.

METABOLIC MODEL OF CENTRAL CARBON METABOLISM OF GROWING *ARABIDOPSIS THALIANA*

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The stoichiometric model describes central carbon metabolism of growing A.thaliana. The model includes three plant tissues, two growing tissues: autotrophic mesophyll, heterotrophic root and non-growing phloem, which is a connective tissue. The model describes biomass growth in autotrophic light-phase and heterotrophic growth on starch in dark-phase. The mesophyll has two inner sub-compartments: plastid and mitochondrion. The root has only one inner sub-compartment: mitochondrion. The phloem plays only transport role in the model. The model includes 334 transformers (194 – metabolic reactions; 126 – transporters, 14 – polymerization reactions), 346 balanced compounds, which all together are jointed into 67 pathways in all tissues. The metabolic flux analysis (MFA) has predicted flux distribution towards biomass formation. The results of MFA are used to compare with available experimental data and thus to validate the model. Additional validation of the model is experimental identification and relative quantification of metabolic enzymes (by shotgun MS-based proteomics), represented in the model. The long-term purpose of the stoichiometric model is to build a mathematical model suitable for further development into dynamic model that will have purpose to study roles of different sugar transporters in biomass A.thaliana growth.

PEA (*PISUM SATIVUM* L.) TRANSCRIPTOME DATABASE

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Next-generation sequencing (NGS) solutions open up new possibilities including transcriptomic investigations of species without the sequenced and annotated genome. For such species, these solutions allow to form the transcriptome assembly and perform sample transcriptome profiling. New transcriptome assemblies and services for their usage appear regularly now. Here we present the new combined database of transcribable elements of pea (*Pisum sativum* L.) called PEAtom. The PEAtom database incorporates data from different publicly available and personal resources: pea EST-sequences and pea transcriptomic studies carried out using different NGS-sequencing platforms and methods. The database comprises directly sequenced cDNA molecules and whole transcriptome assemblies obtained *via* NGS-solutions like RNA-seq (Illumina) or MACE (MAssive Analysis of cDNA Ends, GenXPro). MACE technology allows sequencing of regions nearest to 3’ or 5’-ends of a DNA molecule in contrast to RNA-seq methods cutting regions to be sequenced randomly along a molecule. Based on the available data the pea transcriptome meta-assembly was created. Sequences of different sources were reassembled in order to combine different versions and fragments of the same transcripts into continuous sequences (contigs), integrated into database. Obtained contigs were verified by the developed expert system which uses the information associated with parts of contigs such as source of sequences, sequence and/or assembly quality, homologues search in relative species (like Medicago truncatula Gaertn.). In addition, contigs were grouped into gene-families and annotated with GO-terms. The new incoming sequencing data will be used to create new versions of meta-assembly verifying and reclaiming previous ones. At present, we are working on a web-application which could make the use of the database convenient for ordinary users. It will be possible for researchers to use the services provided by the application through the search by keywords and GO-terms, BLAST-search, transcriptome analysis such as transcriptome profiling and comparison of transcriptomic profiles. Our research project has been supported by the grants of RSF (14-24-00135) and RFBR (14-04-32000).

NODULE TRANSCRIPTOME PATTERNS OF PEA (*PISUM SATIVUM* L.) MUTANTS WITH IMPAIRED NODULATION

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Nitrogen-fixing symbiosis of leguminous plants (*Fabaceae*) is the best-studied and at the same time the most specific and complex one. Legume plant develops special organs (symbiotic root nodules) to host symbiotic bacteria inside its tissues. The knowledge on genetic bases of nodule organogenesis is still not sufficient by now, despite its importance for breeding programs. Next-generation sequencing (NGS) technologies provide an opportunity to study total gene expression even in species with insufficient information on genome organization. The large and complicated genome of agriculturally important crop pea (*Pisum sativum* L.) is not discovered properly. Therefore use of NGS for studying pea gene expression by transcriptome sequencing appears to be a feasible approach aimed at discovering regulatory networks during development of nitrogen-fixing symbiosis. Transcription profiles of symbiotic nodules in several pea mutants with defects on different consequent stages of symbiosis development were examined in this work by transcriptome sequencing on Illumina HiSeq2000 using MACE (MAssive Analysis of cDNA Ends) technology. This approach, in contrast to conventional RNAseq, allows sequencing only a small part of each transcript and therefore appears to be much more sensitive to transcription changes. Annotation of short MACE contigs was performed by aligning those to nodule transcriptome of wild type pea line SGE (which was already sequenced and annotated by our workgroup). Differential expression profiles were analyzed in combinations "wild type *vs* mutant" and between different mutants, with attention paid to genes encoding transcription factors and genes known to be essential for symbiosis development. According to our data, some segments of gene expression networks can be drawn, which control consequent steps of symbiotic nodule development. Also, misexpression of several stress response genes was found to be associated with preliminary nodule senescence. In general, our results shed light upon the complexity of gene network governing the symbiotic nodule development in pea. The work is supported by the grants of RSF (14-24-00135) and RFBR (13-04-01702, 14-04-01442).

THE 1st IAEA REGIONAL PROJECT RER/5/013 EVALUATION OF NATURAL AND MUTANT GENETIC DIVERSITY IN CEREALS OF THE CENTRAL AND EASTERN EUROPE USING NUCLEAR AND MOLECULAR TECHNIQUES

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The IAEA since many years promote and funded a lot of projects basic, strategic, applied and adaptive related to peaceful utilization of atomic energy i.e. in the field of plant and animal genetics and breeding, application of novel molecular and cytogenetic approaches etc. Recent several years we observed increasing of interests to production and utilization of mutants of a number of agriculture crops in modern genetics and functional genomics research and application of TILLING, ecoTILLING, comet assay etc. Traditionally IAEA funded a number of projects with collaboration with African, Latin American and Asian countries by it Section Food and Agriculture which covered Plant Breeding, Animal Production and Health and Nuclear Science for Food Security. However, for more than 20 years the region of Central and Eastern Europe as well as post-Soviet countries did not cover by the interest of this Section of IAEA. Nevertheless in 2007 it was appeared the First IAEA Regional Project RER/5/013 for the Central and Eastern Europe which last 5 years and allow to more than 16 countries of region reveal their level in these investigations, study the possibilities of IAEA for supporting of the research and capacity building in their particular countries and promote the international collaboration. Under the funding of project 5 short training courses were organized: on mutant production and their analysis, molecular genetic techniques, molecular cytogenetics and *in vitro* techniques, TILLING, abiotic stresses research; scientific visits for senior scientists to the laboratories of Europe, attending the conferences, long-term trainings, expert visits, funding for their research and equipment for laboratories. In this presentation it will be explain how to get IAEA funding, benefits for institutions and scientific groups based on my experience as national coordinator of the IAEA Regional Project RER/5/013/

HOW SYMPLASTIC MODE OF GROWTH EFFECTS ON CELL MECHANICS IN UNIDIRECTIONAL GROWING PLANT TISSUE

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The feature of plants is symplastic growth where neighboring cell walls adhere and do not slide along each other. The epidermis of monocotyledon leaves consisting of parallel files of cells is convenient object to explore this one. We developed a simple mechanical cell-based model for symplastic growth of linear leaf blade. The challenge was to determine what restrictions on cell size symplastic growth creates compared to the free growing cells. We assumed a unidirectional growing cell ensemble starting from a meristem-like layer of generative cells and then generating parallel cell rows from every cell of the initial layer. Each cell was characterized by its growth function, and growth of the whole leaf blade was accompanied by mutual adjustment between all the cells. Cells divided asymmetrically once they had reached a threshold area. A mathematical model and its implementation were proposed for computational simulation of symplastic, unidirectional growth of plant tissues. The question analyzed was how a cell grows in a plant tissue if there is a mechanism for regulating the growth of an isolated growing cell and the behavior of the cell wall matter is elastoplastic. The results of the simulation of linear leaf blade growth were compared to those for a free-growing cell population considering different growth functions of individual cells. It was shown that in the model proposed symplastic growth causes a significant deviation of the actual cell length from its isosmotic length with regard to freely growing cells and that cells in the tissue undergo significant unsteady hypo- and hyperosmotic stress. This means that the control of symplastic cell growth should differ from that of free growth. This work was supported by RSF grant 14-14-00734 “Investigation of the molecular mechanisms of plant organ development using the systems biology approach”.

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