



# PlantGen2019

The Fifth International Scientific Conference

## Plant Genetics, Genomics, Bioinformatics, and Biotechnology



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**PLANT GENETICS, GENOMICS,  
BIOINFORMATICS, AND BIOTECHNOLOGY  
(PlantGen2019)**

The Fifth International Scientific Conference

Abstracts

June 24–29, 2019  
Novosibirsk, Russia

Editors:

Corr. Member of the RAS *Alexey V. Kochetov*  
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Novosibirsk  
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# **Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia**

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The Institute was founded in 1957, among the first institutions of the Siberian Branch of the Russian Academy of Sciences. It is situated in the Novosibirsk Akademgorodok. Presently, ICG SB RAS is an interdisciplinary biological center, which ranks among the leading biological institutions in Russia. The second step of the restructuring of the Federal Research Center Institute of Cytology and Genetics was completed in May 2017. Presently, ICG includes three affiliated branches: 1) Siberian Research Institute of Plant Production and Breeding (SibRIPPB). The institute is located in Krasnoobsk Village and the Novosibirsk rural area. It conducts academic, prospective, and applied studies including the collection, examination, preservation, and utilization of plant genetic resources for obtaining new biological knowledge; expansion and improvement of crop gene pools; 2) Research Institute of Clinical and Experimental Lymphology (RICEL); 3) Research Institute of Internal and Preventive Medicine (RIIPM). RICEL and RIIPM are situated in the Sovetskiy and Oktyabr'skiy districts of Novosibirsk. They conduct academic, prospective, and applied studies in molecular medicine and human genetics. They also provide medical care.

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*Strategic goal:* Integrated studies in plant genetics and breeding, animal genetics and breeding, human genetics, fundamental medicine, and biotechnology by applying methods of molecular genetics, cell biology, and computational biology from the generation of academic knowledge to the solution of top priority problems set by Russian agricultural, biotechnological, biomedical, and pharmaceutical industries.

*Staff:* As on January 1, 2019, ICG included 89 scientific units, which employed 1354 members; of them 487 researchers, 2 RAS Advisors, 8 Full Members of the RAS, 4 Corresponding Members of the RAS, 93 Doctors of Science, and 342 Candidates of Science.

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Institute of Cytology and Genetics is the founder of the following journals (mass media): *Vavilov Journal of Genetics and Breeding*, *Letters to Vavilov Journal of Genetics and Breeding*, *Atherosclerosis*, *Siberian Scientific Medical Journal*, and *Live Science*.

*The Federal Research Center Institute of Cytology and Genetics is looking to cooperate with scientific and commercial enterprises.*

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## Screening sugar beet samples for the presence of bolting gene

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Sugar beet (*Beta vulgaris*) is biennial plant used in sugar industry. It is an actual problem to increase the yield of sugar beet and reduce the cost of harvesting and sowing in the spring, and it's resistance to frost. However, overwintering sugar beets in the soil dramatically reduces the yield of plants and another important point – bolting. It was found that bolting depends on the effect of both environmental and genetic factors. Bolting dramatically reduces the yield of sugar beet and it's sugar content, and complicates the processes of harvesting. Thus, bolting is completely undesirable for agriculture (in the first year), although it is necessary for the production of seeds (the second year). Bolting is highly dependent on many factors. For this, the use of molecular markers closely related to the genes of bolting. A study was conducted on the collection of hybrids and sugar beet lines of the Kazakh Research Institute of Agriculture and Plant Growing with the use of CAU3903b marker, which is specific for the BR1 locus for the presence of bolting gene. As a result of the study, it was established that 39 out of 40 samples of sugar beet have resistant genes to bolting, and these samples are recommended for further breeding processes. An environmental test of 40 hybrids and sugar beet lines for adaptability, productivity, cold resistance and bolting in 3 climatic zones (Almaty, Pavlodar regions of Kazakhstan; Voronezh region of the Russian Federation) was conducted. The test showed that under the conditions of 2018, bolting of sugar beet in the first year was low in all three zones (0–0.7 %). 7 samples according to high adaptability and productivity and 10 samples according to high sugar content were identified in the Pavlodar region. Based on the preliminary testing results, 6 samples with high yield and 12 samples with high sugar content were selected in the Voronezh region. 3 samples by high yields, exceeding the standard Aisholpan hybrid in the Almaty region, were selected. Hybridization of sugar beet was carried out – 3 combinations of crossings. Hybrid seeds are obtained.

## Genetic resources in creating sustainable diseases of introgressive spring wheat forms

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Introgression spring wheat forms with *T. militinae*, *T. timopheevii*, *T. dicoccum*, *T. kiharae* and *T. zhykovskiyi* were screened for disease resistance (leaf, stem, yellow rust, septoria, mildew, smut) in: Almalybak, Karabalyk; OmSAU, Turkey; on the infectious background St. Petersburg, Otar; Moscow, Turkey; at the cytological and genetic level – KazRIAPG, ICG and ARRIPS, CIMMYT. On a natural infectious and background from 7 to 21 samples (23–50 %) were allocated for leaf rust resistance: 6583 × *T. timopheevii* (Lr34, Lr36, Lr68), Kazakhstanskaya 10 × *T. dicoccum* (Lr46, Lr68), 6569 × *T. militinae* (Lr34, Lr46), 6625 × *T. timopheevii* (Lr14, Lr46) and 6631 × *T. timopheevii* (Lr9, Lr14) and species *T. militinae* (Lr19, Lr68), *T. timopheevii* (Lr19, Lr68) and *T. kiharae* (Lr68). According to stem rust, advanced lines block was immune (Sr2) and highly resistant (5–10) relative to cvs-standard Kazakhstanskaya 10 (15–75) and to *T. militinae*, *T. timopheevii*, *T. kiharae* Sr36. 9 synthetic spring wheat lines were found (Kazakhstanskaya 10 × *T. dicoccum*, 6569 × *T. millitinae*-1, 6569 × *T. millitinae*-2, 6628 × *T. millitinae*, 6625 × *T. timopheevii*-1, 6625 × *T. timopheevii*-2, 6625 × *T. timopheevii*-3, 6628 × *T. timopheevii*-1 and 6628 × *T. timopheevii*-2), which show horizontal rust resistance. Genotypes 6628 × *T. militinae* and 6569 × *T. militinae*-1 (TIRS.1BL; estimated substitution 2B (2G) or translocation T2B-2G); 6569 × *T. militinae*-2 (TIRS.1BL; T3GS/3BL) (I.G. Adonina, unpublished data). According to maximum yield, genotypes with brown and stem rust resistance are 6569 × *T. militinae*-2 (3.2–5.2 t/ha); 6569 × *T. militinae*-1 (3.0–5.7 t/ha); 6628 × *T. timopheevii*-3 (3.0–5.7 t/ha). The following genotypes were distinguished by minimum powdery mildew infection: Kazakhstanskaya 10 × *T. timopheevii*; 6631 × *T. timopheevii* (0–5 %); 6625 × *T. timopheevii*-2 (10–15 %). Selected resistance forms were evaluated by high yield, quality, DUS-test, transferred to double haploid basis and for (Tim-biday, Gunticum, VEK).



## Wheat genetic resources as a raw material for healthy food

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23 introgressive winter investigated and 27 spring wheat forms for (1) macro- and microelement content, especially Zn, Fe, Cd; (2) protein and fractions content, including  $\alpha_6$  components; (3) content of amylose,  $\beta$ -glucan, arabinoxylane; (4) fatty acid composition. The source of high N, Mg, Mn and Fe, Zn contents can be considered *T. kiharae*; of N, P, S – *T. militinae*; Mn, Fe, Zn – *T. petropavloskyi*; of K and Zn – *T. compactum*. The protein content in the grain of various species was formed due to the predominance of different protein fractions: globulin in grain of *Ae. triaristata* (40.6 % to the total) and *T. militinae* (35.7 %); due to gliadin in *T. dicoccoides* (38.9 %), *T. dicoccum* (34.5 %) and *T. timopheevi* (33.7 %). By the ratio of protein fractions in the 70 % ground flour, winter introgressive forms were characterized by the predominance of albumin + globulin fraction from 40.4–44.0 %; 1721-9 and 2041-13 to 55.3 % (Bezostaya 1  $\times$  *Ae. triaristata*)  $\times$  Karlygash. The maximum content of  $\beta$ -glucan is characteristic of Aegilops (*Ae. triaristata* and *Ae. cylindrica*), then *T. dicoccoides* and *T. macha*. Consistently high content of  $\beta$ -glucan was observed in *T. shaerococcum* and *T. timopheevi*, content of arabinoxylane for Zhetysu  $\times$  *T. militinae*. Wild relatives were characterized by an amylose content in the range of: *T. timopheevi* (31.5 %) > > *T. dicoccoides* (29.3 %); *T. macha* (28.1 %); *T. persicum* (27.7 %) > *T. spelta* (27.0 %) > > *T. militinae*, *T. spharacoccum* (26.4 %). Amylose content in Aegilops ranged from 9.6 % (*Ae. triaristata*) to 13.3 % (*Ae. triuncialis*). Rising interest in natural and organic products led to the reopening of ancient wheat as a source of grain for healthy nutrition. Several wheat species are used and further adapted to cultivation in industrial scale, e. g., *Khorasan*, *T. spelta*, *T. compactum*. However, these wheat forms have drawbacks, which hinder their widespread use. In this regard, interspecific and intergeneric wheat hybrids are convenient and promising objects that ideally combine nutrient and technological properties with agronomic suitability.

## Triticale-wheat hybrid lines with the vaviloid type of spike branching

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The collection of wheat forms with the vaviloid type of spike branching consists of lines obtained from crossing a stable wheat-rye amphiploid (triticale) ABR ( $2n = 6x = 42$ ), used as the maternal form with the local variety of common wheat *Triticum aestivum* var. *velitimum* ( $2n = 6x = 42$ , BAD). Triticale was obtained by professor Aminov in 1975 from hybridization of synthetic wheat BAD (*T. durum* × *Ae. squarrosa* var. *meyeri*, catalog number in VIR – k-45918) with weed rye *Secale cereale* ssp. *segetale* ( $2n = 2x = 14$ , RR). Hybrid populations were studied for 10 years in an open field at the Absheron experimental base of the Genetic Resources Institute of ANAS. The forms with the vaviloid type of spike branching began to appear from the generation F4. Estimation of the morphological and quantitative traits of these lines was conducted. Significant differences were registered in such parameters as the number of grains per spike, the grain weight per spike. A molecular-cytological analysis of the lines was carried out using the methods of genomic *in situ* hybridization (GISH) and fluorescent *in situ* hybridization (FISH) with probes that allow the identification of wheat and rye chromosomes. It was shown that in all the studied lines, the chromosomes 2D was replaced by a pair of rye chromosomes, presumably 2R. In addition, a part of the lines contained a telocentric chromosomes, presumably corresponding to the short arm of a wheat chromosome 2D. Thus, the studied lines with the vaviloid type of spike branching can be divided into three groups: 1) lines with one telocentric chromosome; 2) lines with a pair of telocentric chromosomes; 3) lines without telocentric chromosomes.

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## Wheat–alien introgression breeding: current status and prospects in the 21st century

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Wheat–alien introgression breeding went through several stages. The first attempts to obtain hybrids between wheat and related species have been carried out at the end of the 19th century. Initially, the purpose of the experiments was to study the evolution and origin of species, the meiotic chromosome pairing. However, the main purpose of distant hybridization was to create wheat with exceptional properties. It seemed that this approach would provide much wider possibilities for enriching the wheat genome than traditional selection. Introgression breeding reached its peak in the second half of the 20th century. A significant progress has been made in developing strategies to produce hybrids of wheat with distant relatives, in the improvement of cytogenetic techniques, development of molecular markers to identify and characterize introgressed chromatin. These advances led to development of a large panel of introgression lines of various types and from a number of wild wheat relatives, carrying important traits. At present, based on the accumulated data, one can speak about certain regularities of distant hybridization of wheat. For example, in chromosomes 4A, 5A, the least alien introgression is observed. On the contrary, it is possible to distinguish “hot” sites on individual chromosomes and whole chromosomes, where introgression occurs more often. Nowadays, only a small number of commercially successful wheat cultivars have been created by distant hybridization. The potential of alien introgression breeding remains underused. What are the prospects for wheat–alien introgression breeding in the 21st century? Primarily, we need to develop theoretical knowledge. Almost nothing is known how the wheat genome interacts with introgressed genes and how it influences their function. The recent advances in genomics, transcriptomics, epigenomics, proteomics, in cytogenetics are promising to deliver the needed insights. The use of the new plant breeding techniques can be useful. In this respect, cis-genesis and genome editing, in particular based on CRISPR/Cas9, are promising.

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## Analysis of out of the reference transcripts from RNA-seq libraries in crops

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The analysis of crop gene expression based on RNA-seq experiments is one of the most effective ways to search for genes of biological significance. The results are important for geneticists and breeders in the breeding new lines and cultivars of improved stress response, the search for markers of new useful genes. However, most of the results of gene expression analysis published in articles and databases are based only on reference genomic sequences. For agricultural plants, there are more and more data on transcriptomes of varieties and lines, the genotype of which differs from the genotype of the reference organism. Most of these transcriptomes contain sequences that are not detected in the reference genome and can only be obtained by the *de novo* assembly method. In this work, a large-scale analysis of transcriptomes of 5 crops (maize, rice, tomato, potato and barley) taken from the available SRA archives of NCBI and EBI (over 1200 libraries in total) was carried out. We aimed at identification of “Out Of the Reference Transcripts” (OORT) in RNA-seq libraries and their annotation. For each of the libraries *de novo* transcript sequences were reconstructed and aligned to reference genome. Sequences of two types were identified: (1) transcripts aligned to the unannotated reference genome loci; (2) transcripts unaligned to reference genome. It is shown that the proportion of transcripts that are aligned to the reference unannotated loci varies from 20 to 25 %. Proportion of transcripts unaligned to the reference genome is up to 5 %. For sequences of “new” transcripts not aligned to the genome, the identification of ORFs and amino acid sequences was carried out and their annotation was performed. Some of such transcript were identified as non-coding RNAs, viral and pathogen sequences. We also identified candidate for plant resistance genes among OORT: 181 for unaligned transcripts and more than 1500 for unannotated. Transcripts that are homologous to genes of plant stress response to drought, oxidative stress, high temperatures and genes of plant resistance to pathogens were also identified.

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## Genome composition and divergence between Russian boreal species in the genus *Elymus* (Poaceae), as assessed by nuclear gene *GBSSI* sequencing

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Serial studies have been conducted previously by Dr. Mason-Gamer to confirm that the molecular phylogenetic analysis of sequences in the low copy gene granule-bound starch synthase 1 (*GBSSI*) significantly complements the cytogenetic data for the genomic constitution and evolutionary relationships both among North American and Asian species of the genus *Elymus*. We sequenced and compared *GBSSI* gene in 14 endemic *Elymus* species from Siberia and the Russian Far East in order to determine their genomic constitution and to assess the levels of divergence and phylogenetic differentiation. The species are: *E. charkeviczii*, *E. jacutensis*, *E. kamczadalarum*, *E. komarovii*, *E. kronokensis*, *E. lenensis*, *E. macrourus*, *E. margaritae*, *E. subfibrosus*, *E. sajanensis*, *E. transbaicalensis*, *E. peschkovae*, *E. uralensis*, *E. viridiglumis*. PCR-amplified gene fragments spanned from exon 9 to exon14 were cloned, and six clones per each species accession have been sequenced. All of them included St and H subgenomic variations of the gene. Most profound differences between St and H subgenomic fragments were located in the intron 13. This intron in the subgenome H contains a large deletion of 21 bp in all *Elymus* genotypes, likely obtained from a common ancestor of the H and P genomes. In place of this deletion all St and Y subgenomes have a relatively conservative sequence that is almost identical in nucleotide composition to the closely related genus *Pseudoroegneria*, whose ancestor is the donor of the modern St subgenome of all *Elymus* species. The phylogenetic cluster analysis revealed microevolutionary events and considerably added our previous biosystematic results in the group of boreal species from Siberia and the Russian Far East. New data obtained are needed for the construction of a phylogenetically oriented taxonomic system of the genus within Russia.

## Anatomo-morphological stem features of spring bread wheat varieties

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Using the anatomical method, the study of the main parameters of the internal stem structure of Siberian bread wheat varieties (internode diameter, mechanical layer thickness, number and diameter of vascular bundles of parenchyma) was performed in order to assess their impact on the resistance to lodging. The assessment focused on the study of the first two internodes located under the ear: EN1 and EN2, since these internodes reflect the genotypic features of the plant anatomy. An increase in the number of parenchyma vascular bundles from top to bottom was established. The number of bundles in EN1 varied from 14 to 24, while in EN2 from 16 to 33. On average, the number of bundles from EN1 to EN2 increased by 8.0 for the studied varieties. The decrease in the diameter of the vascular bundles is compensated by an increase in their number. This pattern was clearly observed in cultivars Novosibirskaya 18 (224  $\mu\text{m}$  and 18 bundles), Obskaya 2 (215 and 19), Trizo (228 and 18) and Bel (228 and 24) in the EN1; in Novosibirskaya 29 (268 and 26), Chernyava 13 (270 and 28), Novosibirskaya 18 (262 and 28) and Velut (239 and 29) in EN2. Bel and Novosibirsk 31 were found to differ from the other cultivars on the thickness of the mechanical layer. According to the combination of the stem internal structures (the number and diameter of vascular bundles and the stem diameter) the varieties Trizo, Velut, Novosibirskaya 29 and Novosibirskaya 31 were shown to overcome the others. The Obskaya 2 and Bel differed in a large number of vascular bundles of small diameter with a large stem diameter. Significant correlations ( $r = 0.91$ ) was established between the stem length and the length of the EN1. A positive correlation ( $r = 0.59\text{--}0.37$ ) was observed between the number of vascular bundles and resistance to lodging. The correlation coefficient between the stem diameter in EN1, EN2 and resistance to lodging does not exceed 0.21. The obtained results can be used in the evaluation of spring bread wheat for resistance to lodging.

## Efficient eradication of potato viruses by induction of posttranscriptional gene silencing in transgenic potato

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Existing technologies for obtaining virus-free seed potatoes using methods of apical meristem cultivation, cryopreservation, chemo- and thermotherapy do not eliminate the possibility of subsequent viral infection of plants in the soil. Potato varieties with genetically engineered resistance against viral diseases are of particular importance.

Potato virus S (PVS, genus Carlavirus) is most widely spread in Kazakhstan. It has positive-sense single-stranded genomic RNA of 8535 nucleotides encoding six ORFs. Fragments of cDNA with complete coding sequence of 25K-protein in sense and antisense orientations were inserted at the 5' and 3' flank of cat 1 gene intron from *Ricinus communis*. Two recombinant cassettes [35S-CaMV-25KSense-intron-25KAsense-nos] or [35S-CaMV-25KAsense-intron-25KSense-nos] were cloned in pCAMBIA2300 binary vector under the control of 35S-CaMV promoter and nos-terminator. Expression of each cassette *in planta* leads to production of double-stranded RNAs that induce of RNAi mechanism against PVS.

Six potato cultivars initially containing viruses PVS, PVM, and PVY were transformed by aforementioned constructs. Regenerated plants were analyzed for presence of recombinant DNA constructs, as well as for their RNA-transcripts. Transgenic plants were grown in soil and further tested for the presence of viruses using the DAS-ELISA. Laboratory tests were performed every 30 days during 6 months. Five promising transgenic lines (TL) were selected. TL-61, TL-67 of “Dunyasha” variety and TL-103 of “Kormilitsa” variety showed negative response for PVM, which was detected before transformation. The TL-119, TL-336 lines of “Zeren” variety were free from the complex PVM+PVS+PVY viral infection.

Micro-tubers of transgenic potatoes were planted in the field with elevated viral background for trials in 2017–2018. Samples from these plants were collected for ELISA tests three times during the vegetative season. These analyses confirmed the results of laboratory tests: no potato viruses were detected in the samples.



## Antioxidant enzyme activities of plants under conditions of combined temperature and viral stress

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Temperature is one of the important environmental factor influencing plant development in natural and diseased conditions. Reactive oxygen species (ROS) are produced as a result of environmental stresses such as temperature and viral infection. There are limited information about antioxidant enzyme activities upon combined abiotic and biotic stress factors. The aim of this work was to investigate the influence of combined temperature and viral infection on aldehyde oxidase (AO) and catalase (CAT) activities in *Nicotiana benthamiana* plants.

A 24-h experiment of cold and heat stress was performed with plants, 10 and 40 °C respectively. After that one part of *N. benthamiana* plants were infected with Tomato bushy stunt virus (TBSV), other part were treated with virus-free buffer. Mock-inoculated plants in room temperature treated with virus-free buffer were used as controls.

According to our results, under abiotic stress (room, cold and heat stresses) CAT activity was significantly higher in non-infected plants compared to infected plants. Imposition of heat stress had no significant impact on CAT activity in non-infected plants, whereas cold stress showed a decrement of this enzymatic activity. In response to individual temperature stress, AO activity did not change with respect to control values in leaves. Interestingly, under viral infection, CAT activity decreased, but AO activity increased in *N. benthamiana* plants compared to control levels. The combination of viral infection and temperature stresses negatively impacted on plants but the activation of the antioxidant machinery was associated to the ability to tolerate this stress combination.

As a result, in *Nicotiana benthamiana* plants the increment of AO activity along with the decline in CAT activity compared to control values could be partially responsible of its increased oxidative damage and sensitivity to the combination viral infection and high and low temperatures. Thus, *Nicotiana benthamiana* plants has the ability of efficiently coordinate AO and CAT activities involved in ROS detoxification.

## Short-stemmed hybrid lines derived from crosses of triticales with the synthetic wheat

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We present here the study results on plant height (PH) trait of triticales-like plants derived from triticales × synthetic wheat cross, in which the maternal plant is the locally produced hexaploid triticales ABR (genome AABBRR,  $2n = 42$ ) with PH =  $88 \pm 16.97$  cm and the paternal plant is the locally produced synthetic wheat ADS [*(T. beoticum* × *Ae. taushii*) × *Ae. speltoides*, genome AADDSS,  $2n = 42$ ] with PH =  $110 \pm 14.14$  cm. The maternal plant ABR is differentiated from other triticales by its obtaining in 1975 without any embryo rescue technique or hormone treatment procedure from sexual hybridization between Tanaka's (produced in Japan, Kyoto, AD 221-16a, in VIR- k-45918) synthetic wheat ABD (*T. durum* × *Ae. squarrosa* var. *meyeri*, genome AABBDD,  $2n = 42$ ), PH =  $950 \pm 7.87$  cm and local weed rye *Secale cereale* ssp. *segetale* (genome RR,  $2n = 14$ , collected from Lerik (Azerbaijan)), PH =  $1150 \pm 21.21$  cm.  $F_1$  plants from this cross with PH = 110 cm had 28 chromosomes and after selfing in field conditions without any colchicine treatment the segregated  $F_1$  plants with 56 chromosomes were obtained. The above-mentioned triticales ABR with the chromosome number 42 was selected from late selfed generations based on such desirable traits as short stem, large spike, early heading time, flag leaf width and etc. Starting from 1980 the triticales ABR was included in numerous crosses with many wheats (4x, 6x), triticales (4x, 6x, 8x) and wheat-alien amphiploids (6x, 8x) for the study its morphotype formation diapason and obtain morphotypes with novel traits. Selected for current study the 8 short-stemmed hybrid lines are the segregants obtained from the same selfed  $F_4$  plant that was derived from cross of triticales ABR with synthetic wheat ADS made in 2007. The range of PH for this sister plants was varied from 43 cm up to 66 cm. GISH analysis showed that the short stemmed line from this group has 42 chromosomes, 12 of which are rye chromosomes.

## Haploid biotechnology in the selection of *Triticum aestivum* L.

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Haploids are unique objects for cell selection and genetic engineering. Our work presents the results of the use of haploid biotechnology in the practical selection of *Triticum aestivum* L. for resistance to rust diseases. In the first series of experiments, the field resistance of the original wheat varieties and hybrids under conditions of an infectious nursery was determined. The greatest resistance to rust was shown by the isogenic line Lr 24: to yellow rust – 2/20, to brown – 2/10, and was immune to stem rust. Similar values of resistance to yellow and brown rust showed isogenic lines Lr 19, Lr 25. By resistance to stem rust, the isogenic line Lr 25 was weakly susceptible – 2/20, and Lr 19 showed an average susceptibility – 3/30, respectively. In the second series of experiments, the varieties of common wheat were crossed with donors of effective resistance genes – isogenic lines Lr 19, Lr 24. Valuable wheat hybrids were genetically stabilized by haploid biotechnology based on in vitro culture of isolated microspores. As a result of studies on the use of haploid biotechnology based on in vitro culture of isolated microspores in the selection of wheat *Triticum aestivum* L. for resistance to rust diseases, we obtained embryoids, morphogenic calli and regenerant plants from which DH lines were created. To determine the resistance of DH lines to rust diseases, they were studied in the conditions of an infectious nursery of South-East Kazakhstan. As a result of the studies conducted in an infectious nursery, DH wheat lines were selected, which showed a high level of resistance to rust diseases. Thus, it was shown that haploid biotechnology is an effective method for accelerating the selection process and rapid genetic stabilization of promising hybrids. In the early stages of the selection process, wheat DH lines carrying the genes for resistance to rust diseases were selected.

## Genetic resources of water caltrop *Trapa* L.

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Water caltrop (water chestnut) is a valuable aquatic plant. Its seeds are edible and rich in protein and starch. Antimicrobial compounds are found in the seed coat and can be used in medicine. Water caltrop is widely cultivated by men since Neolithic, however, on the territory of Europe and Russia its population decreased dramatically over the last century. The plant became very rare and now is included in the Red List of Threatened Species in 36 regions of Russia and protected in most of the EU countries. The extinction could happen due to the change in climate and water regime of the habitats, as well as human factor. Now northern border of its area lies near Moscow, however, several populations of water caltrop still remain in regions with even more severe and continental climate (near Ufa city in the Republic of Bashkortostan and in Novosibirsk and Altai mountains). It is not clear, why water caltrop is preserved on these territories. In spite of great morphological differences, using RAPD and ISSR primers, we did not detect any genetic polymorphism between water caltrops from different regions of Russia, except for several samples from Far East, distinguished as *T. maximowiczii*. Most probably, they belong to one species *T. natans*. To verify that, we performed sequencing of the ITS, trnH-psbA and several other regions. It is possible that water caltrop has a great potential to adapt to different climate conditions and can be reintroduced to its previous habitats from the south and be used as a food source again. Study of its genetic and morphological diversity is important for the survival and recovery of the unique populations.

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## Breeding value of partial waxy wheat samples in Tatarstan

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Non-functional alleles of *wx* genes affect the violation of synthesis and changes in the localization of amylose in cereal starch. Wheat samples carrying non-functional alleles of *wx* genes at one or two loci are called partial waxy wheat. From crossing the winter wheat variety Starshina (have non-functional allele *Wx-A1b*) and the line of spring wheat O-192-03-5 (have non-functional allele *Wx-B1b*) we obtained two promising lines of partial waxy wheat, which combines non-functional alleles *Wx-A1b* and *Wx-B1b*. These are the K-243-13Wx-2 and K-243-13Wx-6 lines. Test these lines was conducted in the Tatar Research Institute of Agriculture in 2017–2018. Tatar RIA is located in the northern part of the Middle Volga region of Russia. The average yield of the line K-243-13Wx-6 was 276 g/m<sup>2</sup>, which is much less than the standard variety Yoldyz – 550 g/m<sup>2</sup>. The line K-243-13Wx-2 has an average yield of 534 g/m<sup>2</sup>. The average weight of 1000 grains at the line K-243-13Wx-2 was 49.6 g. At the line K-243-13Wx-2 degree of lesion of leaf rust was 0–15 %, the degree of damage of stem rust was 15 %. This line is susceptible to powdery mildew, its resistance is 3 points (9 points – *maximum*). Line K-243-13Wx-6 is susceptible to leaf rust, the degree of damage was 15–50 %. Line K-243-13Wx-6 is susceptible to stem rust, the degree of damage was 30–70 %. Resistance to powdery mildew in this line in epiphytotic 2017 was 4 points. At the line K-243-13Wx-2 date of earing before on 1 day, than at the line O-192-03-5. At the line K-243-13Wx-6 date of earing occurred simultaneously with the line O-192-03-5. According to the analysis of the harvested grain in 2018 year, the lines K-243-13Wx-2 and K-243-13Wx-6 have a high protein content in the grain of 14.9 and 14.5 %, respectively, and have a high gluten content in the grain of 30.8 and 31.7 %, respectively. Thus, the evaluation of agronomically valuable properties of the obtained samples of partial Waxy wheat indicates the prospects of their use as a starting material for the production varieties of spring wheat with a modified composition of grain starch.

## Sources of high protein and gluten content in grain in some wheat species

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Despite the creation of a number of varieties of soft wheat combining high grain productivity and high grain quality, there are limits to increasing protein and gluten. The initial material plays a key role in the efficiency of selection for grain quality. Screening of the VIR wheat collection on the NIR analyzer, grown in the forest-steppe zone of the Republic of Tatarstan, revealed samples with the highest protein and gluten content. They can serve as a starting material for the selection of high-quality varieties of soft wheat. During three years (2016–2018) wheat samples including the following species were analyzed: *T. aestivum* L., *T. durum* Desf., *T. dicoccum* (Schrank) Schuebl., *T. polonicum* L. In high-protein samples *T. aestivum* L. protein content in grain ranged from 15 to 18.7 %, gluten in grain from 32.8 to 38.1 %. The maximum protein and gluten content was in varieties Long Fu 12 (K-65473, China), Long Fu 040671 (K-66200, China), Krasnoufimskaya 110 (K-65478, Russia, Sverdlovsk region), Ekaterina (K-65477, Russia, Sverdlovsk region), AC Tahoe (K-64977, Canada), Lillian (K-66203, Canada), Lovitt (K-66204, Canada), Molera (K-66033, Switzerland), Pamyati Maistrenko (K-65448, Russia, Omsk region), Polyushko (K-64856, Russia, Novosibirsk region), Manu (K-66029, Finland), Mayon 1 (K-65851, Syria). *T. durum* Desf. is widely used in hybridization with soft wheat and can be a source of high protein content. The maximum protein content was 16.4 % in the sample of Bezenchukskaya 182 (K-59890, Samara region), in other tested samples it did not exceed 15 %. Species *T. dicoccum* (Schrank) Schuebl, refers to high-protein, protein content in some samples reached 18.2 %, while the starch content is slightly lower than that of high-quality spring wheat. Protein content stand out K-7530 (Russia, Ulyanovsk region), K-10456 (Russia, Tatarstan), K-21961 (Germany). High protein content in *T. polonicum* L. – reached 17.8 %. The maximum protein and gluten content was in K-9277 (Israel) and Koko (K-62974, Syria).

## Development of sweet pepper F<sub>1</sub> hybrids based on MAS methods by fruit quality and resistance genes

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This work aims to assess the developed, using MAS methods, F<sub>1</sub> hybrids and parent forms of sweet pepper by fruit quality (*Ccs*, *cl*, *norc*) and pathogen resistant genes (*Me1*, *pm*) by a complex of biochemical (dry matter, carotene, vitamin C, soluble carbohydrates) and biometric (fruit mass, fruit wall thickness; early, commercial and gross yield) fruit characters and study the peculiarities of the characters' manifestation in F<sub>1</sub> hybrids. As an experimental material, 9 parental forms and 16 F<sub>1</sub> hybrids of sweet pepper were studied (crossing schemes 8×1 and 1×8). The features of the characters' manifestation in hybrids were evaluated during the period of three years (2016–2018) by the value of true heterosis and degree of dominance. As a standard, Troika pepper variety was used. Based on the three-year test results, valuable F<sub>1</sub> hybrids were identified (L45-11 × Shokoladnaya krasavitsa, L45-11 × Zhelty buket, L45-11 × L140/0, L140/0 × L45-11) characterized by the dry matter content at the level of 8.18–8.77 %; carotene – 19.95–32.73 mg/kg; vitamin C – 112.49–144.4 mg/kg; soluble carbohydrates – 4.36–4.77 %. Three best hybrid combinations with a complex of biometric features were selected: L45-11 × Shokoladnaya krasavitsa, L140/0 × L45-11 and L45-11 × L160-10 characterized by the fruit mass at the level of 136.6–175.4 g; fruit wall thickness – 6.8–7.5 mm; early yield – 0.68–0.77 kg/m<sup>2</sup>; commercial yield – 4.16–4.97 kg/m<sup>2</sup>; and gross yield – 4.27–5.01 kg/m<sup>2</sup>. Analysis of the true heterosis manifestation in hybrids revealed its multidirectionality depending on the conditions of the vegetation period by the majority of the studied characters. The most frequent manifestation of the values of yield, mass, and carbohydrate content was the dominance and overdominance in the direction of the increased characters. The thickness of the fruit pericarpium manifested by the intermediate inheritance type – overdominance in the direction of decreased characters.



## Evolution of the S-genome in *Triticum* and *Aegilops*

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Five diploid *Aegilops* species of the *Sitopsis* section: *Ae. speltoides*, *Ae. longissima*, *Ae. sharonensis*, *Ae. searsii*, and *Ae. bicornis*, two tetraploid species *Ae. peregrina* and *Ae. kotschy* (*Aegilops* section) and hexaploid *Ae. vavilovii* (*Vertebrata* section) carry different variants of the S-genome. The B- and G-genomes of polyploid wheats are also the derivatives of the S-genome. Evolution of the S-genome species was studied using C-banding and fluorescence in situ hybridization (FISH) with DNA probes representing 5S and 18S-5.8S-26S rRNA gene families and tandem repeats pSc119.2, pAesp\_SAT86, Spelt-1, Spelt-52, pAs1, pTa-535, and pTa-s53. To align the C- and FISH patterns we used the microsatellites (CTT)<sub>10</sub> and (GTT)<sub>9</sub>, which are major components of the C-heterochromatin in cereals. According to the results obtained, diploid species split into two groups corresponding to *Emarginata* and *Truncata* sub-sections, which differ in the C-banding patterns, distribution of rDNAs and other repeats. The B- and G-genomes of polyploid wheat are shown to be most similar to the S-genome of *Ae. speltoides*. The genomes of allopolyploid wheat evolved as a result of different species-specific chromosome translocations, sequence amplification, elimination and re-patterning of repetitive DNA sequences, which occurred independently in polyploidy wheat and in *Ae. speltoides*. The 5S rDNA locus on chromosome 1S was probably lost in ancient *Ae. speltoides* prior to formation of Timopheevii wheat, but after the emergence of ancient emmer. Evolution of *Emarginata* species was associated with an increase of C-banding and (CTT)<sub>10</sub>-positive heterochromatin, amplification of Spelt-52, re-patterning of the pAesp\_SAT86, and a gradual elimination of all D-genome-specific repeats. The emergence of *Ae. variabilis* and *Ae. kotschy* did not lead to significant changes of the parental S\*-genomes. However, partial elimination of 45S rDNA repeats from 5S\* and 6S\* chromosomes and alterations of C-banding and FISH-patterns were detected in both tetraploid species. Similarity of the S<sup>v</sup>-genome of *Ae. vavilovii* with the S<sup>s</sup> genome of diploid *Ae. searsii* confirmed the origin of this hexaploid. A model of the S-genome evolution is suggested.

## **Zinc finger A20/AN1 stress-associated genes, *HvSAP*, are differentially expressed under drought, salinity and dehydration in barley leaves**

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**Introduction and Aim:** A family of genes designated the Zinc finger A20/AN1 Transcription factors, encoding stress-associated proteins (*SAP*), represent a large group of genes in both plants and animals. The gene family, which includes 14*AtSAP* and 18 *OsSAP* genes, is well described in *Arabidopsis* and rice, where variable tolerance to multiple abiotic stresses were studied and a great diversity in structure and function of the *SAP* gene family was found in different plant species. The aim of this study was to identify all *HvSAP* genes in barley (*Hordeum vulgare* L.) and to carry out experiments determining gene expression in response to drought, salinity and dehydration in barley leaves.

**Methods:** Bioinformatic approaches were used to identify all *HvSAP* genes in barley and construct a molecular phylogenetic tree using publicly available databases and computer software. For gene expression, qPCR analysis was carried out on cDNA synthesized from mRNA extracted from control and treated barley plants.

**Results:** In our study with barley, 17 *HvSAP* genes were most commonly identified, which were strongly homologous to rice genes. Small rearrangements in the barley genome were found, where two *HvSAP* genes were duplicated, but three other genes were lost compared to the 18 *OsSAP* genes in rice. Multiple and quite variable responses in *HvSAP* gene expressions were found in treated barley plants compared to controls, where some but not all gene expression profiles were similar to those published in rice. Repeated experiments on *HvSAP* gene expression in response to drought, salinity and dehydration will verify the confidence of our results in barley in the nearest future.

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## Identification of the stem rust resistance genes in the introgression lines of spring bread wheat using molecular markers

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A total of the 58 introgression lines and 11 cultivars of spring bread wheat developed by Agricultural Research Institute of South-East Region and cultivated in the Volga Region were analyzed. The lines were obtained with the participation of CIMMYT synthetics, durum wheat cultivars, direct crossing with alien species such as *Agropyron elongatum*, *Ag. intermedium*, *Aegilops tauschii*, different species of the genus *Triticum* L., *Secale cereale* and triticale Satu. Cultivars and lines were evaluated for resistance to Lysogorsk and Omsk stem rust pathogen populations (*Puccinia graminis* f. sp. *tritici*) and to the Ug99 race group in Kenya (KARI) as well as analyzed for the presence of the known Sr resistance genes (*Sr22*, *Sr25*, *Sr26*, *Sr31*, *Sr35*, *Sr36*, *Sr38*, *Sr39*) using molecular markers. The gene *Sr31* remained effective to the local pathogen population. The 26 wheat lines out of 58 were resistant to all local pathogen populations taken into analysis and the 15 introgression lines were resistant to stem rust in Kenya. The genes *Sr31/Lr26*, *Sr25/Lr19*, *Sr22*, *Sr35* and *Sr38/Lr37* were identified in the introgression lines. The gene *Sr31/Lr26* was identified in 13 lines. All lines carrying 1RS.1BL translocation (*Sr31/Lr26*) were resistant to all local pathogen populations taken into analysis. The gene *Sr25/Lr19* was identified in 40 lines. The genes combination *Sr31/Lr26* + *Sr25/Lr19* was identified in 10 lines. The gene *Sr22* was identified in 2 lines, this fact will be checked in further work, gene *Sr35* – in one line and gene *Sr38* – in two lines. The genes combinations *Sr38/Lr37* + *Sr25/Lr19*, *Sr35* + *Sr25/Lr19* were identified. The lines with genes combinations *Sr38/Lr37* + *Sr25/Lr19* were resistant to local pathogen populations. The line with gene *Sr22* was resistant to local pathogen populations and to the Ug99 race group in Kenya. The genes *Sr26*, *Sr36* and *Sr39* were not detected in the analyzed wheat lines.

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## Genomic analysis in soybean breeding

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The patterns of heredity and genetic variability, which are peculiar to all organisms, are of great practical importance for agriculture. The role of the application of genotype-phenotype correlation analysis in the genomic selection of crops is currently growing. In the course of the work, foreign and Russian soybean breeding works were analyzed and the most significant SSR markers were identified. The most relevant for research are the genes with strongest impact on the traits of interest. To solve this problem, the following strategy was developed: 200 soybean varieties were selected from the VIR collection. Range of phenotypic traits of these grown varieties were analyzed. Obtained data have been recorded in journals with a view to further using it for GWAS analysis. The next stage of work, 50 soybean varieties were sequenced, having the best performance in the following ways: productivity, photoperiodism, drought resistance, protein content in beans, a short growing season. Sequencing has been carried out using Illumina technology. Primary data processing has been carried out using the FastQC utility, which allowed quality control to eliminate inconsistent results associated with the poor quality of the input data. Samtools and Hisat2 programs have been used for further data processing, which allow preparing the reference genome, align the reads, translate them from sam to bam format, sort them and index them. To prepare for the GWAS analysis, the GATK software package has been used:

- search for duplicate reads;
- local reorganization of readings;
- local recoding of readings around indels;
- synchronization of all information between each read and its paired pair;
- calibration of the quality assessment of the information base;
- verification of areas containing true SNP;
- hierarchical merging of bam files into a single gVCF.

As a result, we obtained gVCF files containing information about all 50K markers found in the samples. The next stage of our work will be GWAS analysis of 200 soybean varieties after NGS, which will allow genomic selection to create new appropriate soybean varieties.

## Plant VLP production system based on bacteriophage MS2 coat protein

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Some types of single-stranded RNAs regulate gene expression. To protect ssRNA from degradation and to deliver it to a target tissue precisely we can incorporate the molecule into a virus-like particle (VLP). Such VLP is a nanocarrier consisting of viral coat protein capsid and nucleic acid filling. Bacteriophage MS2's coat protein (CP) easily forms a stable capsid via interaction with MS2 operator – the 19-nucleotide sequence in the MS2 genomic RNA. Thus, target ssRNA, which mimics the MS2 genome, can be packed into a VLP. There are successful examples of bacteriophage coat protein application for VLP production. We aim to obtain *Nicotiana tabacum* plants steadily expressing MS2 CP and which can evolve into a VLPs producing system for ssRNA delivery. Bacteriophage MS2 (ATCC 15597-B1, United Kingdom) was produced in *E. coli* strain ER2738. Genomic RNA was purified by TRIzol Reagent and MS2 cDNA was synthesized by reverse transcription using iScript, BIO-RAD. CP gene sequence was amplified from MS2 cDNA by PCR with Phusion high-fidelity DNA polymerase (Thermo Scientific) and was transferred first in interim vector pJet1.2 (Thermo Scientific). Then the sequence was subcloned into another vector where the expression cassette harboring CP under control of Cauliflower Mosaic Virus double 35S promoter was assembled. The cassette was then embedded into destination binary vector for *Agrobacterium*-mediated stable plant transformation. The structure of every genetic construct was confirmed by restriction analysis and Sanger sequencing. *Agrobacterium* (strain AGL1)-mediated *N. tabacum* SR1 leaf explants transformation by created plasmid was conducted and primary regenerants T<sub>0</sub> plants were obtained after regeneration with hygromycin selection. PCR with genomic DNA template and RT-PCR with total RNA revealed 7 plants carrying CP gene which is being transcribed. We aim to investigate whether VLPs are able to form in the transgenic plants in the presence of RNA molecule containing MS2 operator.

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## **The influence of heavy metalions on proline accumulation and resistance of plants to saline stress**

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Various biotic and abiotic factors negatively affect plant growth as well as physiological and biochemical processes. It is known that salinity and the high concentration of heavy metals can be a cause of oxidative stress and metabolic malfunctions which in turn significantly reduce the yield of crops.

Molybdenum (Mo) is one of the required microelements for plants since it is included in the active center of various enzymes involved in redox reactions, as well as its low concentrations play a significant role in the growth and development of plants. The activity of Mo-enzymes can be inhibited by substitution of molybdenum by tungsten (W) in the active center of Mo-protein complexes, since they are analogues belonging to the VI group in the periodic table of elements.

We used molybdenum and tungsten-containing solutions to study the effect of heavy metals on the barley. We observed that the stems germination and the growth of the root system improved at the low concentration of Mo, whereas at the low concentration of W the growth of the root system significantly decreased with slight changes in the germination. Deterioration in the root system and the decline of stem growth were found at high concentrations of Mo. A completely negative effect was revealed when plants were grown at a similar concentration of W. Another type of stress was salinity, during which germination significantly decreased, compared with control plants. Interestingly, a combination of these stresses demonstrated positive effect on plant growth in general. We also studied an accumulation of proline, which is a well-known osmoprotector, during above mentioned combined stress. Proline is one of the most multifunctional stress metabolites of plants, performing chaperone, antioxidant and signal-regulatory role. It was found that under the influence of abiotic stresses on plants, the concentration of proline increased significantly.



## Expression of sheep pox viral A27L and L1R proteins in prokaryotic and eukaryotic systems

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Sheep pox is a severe illness causing mass death among small ruminants. Epizootics in different countries including Republic of Kazakhstan leads to great economic losses. Sheep pox virus (SPPV) belongs to *Capripoxvirus* genus of the *Poxviridae* family. Attenuated strains are used as vaccines for prophylactic means of the disease. Such vaccines are effective but has crucial disadvantage as virions could revert to virulent form by recombination. Genome sequencing and genetic engineering methods give opportunities to produce save vaccine with less expense. Nucleotide sequence of the SPPV “NISKHI” strain used in our work was analyzed to find orthologs of the immunogenic structural proteins of vaccinia virus. *sppv-niskhi-117* gene coding A27L ortholog (17.3 kDA) and *sppv-niskhi-060* gene coding L1R ortholog (20.3 kDA) were used for expression in various systems. Genes were cloned in bacterial pET-19b expression vector. Bacterially produced (*E. coli* BL21 strain) and purified proteins were used for rabbit immunization. Antibodies raised to recombinant proteins showed virus neutralizing activity. pACT2 vector was used to express proteins in *Saccharomyces cerevisiae* cells (CG 1945 strain). Only A27L protein successfully expressed in yeast cells. Transgenic *Nicotiana tabacum* and *Brassica napus* plants expressing viral proteins were obtained after transformation by pCambia 2300 vectors containing viral genes, translational enhancers and sequences for signal peptides to target recombinant proteins into chloroplast or endoplasmic reticulum. A higher recombinant protein yield was achieved when using transient expression (magnification) in *Nicotiana benthamiana* leaves. Maximal viral protein level was obtained in transplastomic *N. tabacum* plants carrying viral genes in the chloroplast genome. L1R protein tend to form di- and trimeric forms in *E. coli* and plants. Synthesized proteins may be used for development efficient recombinant subunit vaccine against sheep pox.

## Breeding of spring bread wheat for resistance to fungal pathogens in Western Siberia

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In connection with the mass spread of fungal pathogens, the task of breeders to produce highly productive and stress-resistant varieties. In our works, introgression lines of wheat, carriers of the genetic material of its relatives – *T. durum*, *T. dicoccum*, *T. dicoccoides*, *Agr. elongatum*, *Agr. intermedium*, *T. timopheevii*, *S. cereale*, are involved in hybridization to obtain initial material for breeding. Due to the inclusion of alien genetic material, the varieties that inhibit the development of leaf pathogens were produced: Omskaya 37, Omskaya 38, Omskaya 41, Sigma 2, Pamyaty Maystrenko, Uralosibirskaya. One of the direction of our work is the utilization of alloplasmic genotypes (*H. vulgare*)–*T. aestivum* and DH lines with a fixed combination of resistance genes of different origin. So, hybrid form 311/00-22 developed from the crossing of the alloplasmic DH(1)-17 line with line Com37 (CIMMYT), the source of the translocation *IRS.1BL*, proved to be successful for breeding. Lines L-311(1)–L-311(6) showed their advantage in comparison with the standard varieties for resistance to leaf and stem rust, yield, and grain quality. The breeding tests of alloplasmic lines L-311(5), L-311(4), L-311(6) resulted in varieties of spring bread wheat Sigma, Uralosibirskaya 2 and Ishimskaya 11, respectively. Line L-311(3) entered the pedigree of the new variety Karavai. These results confirm the fact that gene *Sr31* remains effective for protection against stem rust in the Omsk, Kurgan, Tyumen regions and Bashkortostan. DH lines that combine genes for resistance to powdery mildew, leaf and stem rust are studied. New lines are estimated for resistance to *Ug99* and yellow rust in Kenya (KARI).

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## Intraspecific variability and mechanisms of pea (*Pisum sativum* L.) tolerance to toxic metals

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Heavy metals are among the most common pollutants of agricultural lands. Remediation of such massive areas is difficult or impossible. Selection of resistant varieties with reduced accumulation of heavy metals can be a promising approach for obtaining environmentally friendly crop production on polluted soils. The aim of the present work was to study the intraspecific variability of pea (*Pisum sativum* L.) for resistance to toxic metals (Cd and Al) and for the ability to accumulate and transport various heavy metals (Cd, Co, Cu, Ni, Pb and Zn) from the root to the shoot and seed. A series of vegetation experiments were carried out in which the plants were grown under hydroponic conditions or in a soil supplemented with elevated concentrations of various metals. The content of heavy metals and nutrients in plants was determined using an ICPE-9000 spectrometer. The objects of research were more than 200 pea genotypes (primitive and modern cultivars, belonging to different subspecies and having various geographic origin) originated from the VIR collection (St. Petersburg). It was established that pea has a very large variability in resistance to cadmium and aluminum, as well as the content of heavy metals. Correlations were found between the content of various metals in the shoots and seeds of plants. It has been established that important mechanisms of pea resistance to Cd and Al are maintaining of nutrient homeostasis and modulating pH of the rhizosphere. An additional mechanism was related to formation of efficient symbiosis with microorganisms such as nodule bacteria, endomycorrhizal fungi and plant growth promoting rhizobacteria. The obtained results can be useful for successful selection and breeding programs aimed at creating plant cultivars with high productivity and quality products cultivating on contaminated and acid soils.

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## The study of state transitions in *phyA* and *phyB* mutants of *Arabidopsis thaliana*

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Photosynthetic apparatus of higher plants developed adaptation mechanisms to respond light changes. State transitions is one of these mechanisms. It presents redistribution of a moving part of external antenna of light-harvesting complex of photosystem II (LHCII). As a result the LHCII-LHCI-PSI supercomplex is formed. The supercomplex consists of LHCII mobile proteins associated with photosystem I.

It is known that state transitions are regulated by redox state of plastoquinone pool of thylakoid membranes. We assume that cytoplasmic photoreceptors may also be involved in regulation of state transitions. We exposed *phyA* and *phyB* mutant *Arabidopsis thaliana* plants to blue and red light during 2 hours. In this lines formation of the LHCII-LHCI-PSI supercomplex was detected under both blue and red light, while in the wild-type plants formation of the supercomplex occurred only at blue light. Therefore, there is disruption of state transitions in *phyA* and *phyB* mutants exposed to red light. Our data demonstrate for a first time a role of phytochromes A and B in regulation of state transitions.

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## Pollen parent transfer mitochondria to offspring

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In plant cells, DNA is located in three compartments: nucleus, plastids and mitochondria. Genes transmitted by chromosomes of the nucleus inherited by the offspring of Mendel. Plastids and mitochondria can be inherited from the mother, from the father, or from both parents. In most cases, the plastids are transmitted by the parent, since they are either not included in the cells of the generative sperm, or remain fertilized during fertilization. Only under experimental conditions was it possible to obtain plastid transmission from *Nicotiana* to the offspring with a frequency of  $10^{-4}$  to  $10^{-5}$ . Maternal inheritance of mitDNA in plants has several important consequences for its evolutionary dynamics. First of all, such a transfer determines the direct contribution of maternal mitDNA to fitness determined by the number and quality of the seeds produced. Transmission only on the maternal line is of particular importance for the formation of the population structure of plants, especially those in which pollen spreads over long distances. For a long time it was believed that the mitochondrial genomes of plants are transmitted to the offspring strictly from the mother parent. At the same time, such a method of their inheritance is based on the use of a number of special, finely organized mechanisms that ensure its rather strict functioning. However, there are cases of transmission of mitDNA from fathers, and it is possible that this phenomenon occurs quite often. In this communication, we analyzed the transfer of genetic information in hybrids of two *Zea mays* lines (B73 and Mo17) and *Tripsacum dactyloides* line by nuclear, chloroplast and mitochondrial genomes. To this end, partial nucleotide sequences of the nuclear *Pox3* gene, the chloroplast *trnL* gene and two mitochondrial genes (S-male sterility locus and *Nad4*), in the original parental species and F1 hybrids have been established. Based on the molecular phylogenetic analysis, it was found that the nuclear genome in hybrids comes from both parents, the chloroplast is transmitted exclusively on the maternal line, and the mitochondrial genome can be obtained both from the maternal and the paternal lines.

## Creation of early maturing productive forms of cereals using the cell biotechnology and physiologically active compounds

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Creation of early maturing genotypes of main cereal crops – spring wheat and barley, is an actual task for northern regions of Kazakhstan. We have distinguished the increasing of phenotypic variability by two approaches: use of long-term plant regeneration cell technology (wheat) and using physiologically active compounds (wheat, barley), both elaborated in our laboratory of cell biology (Institute of Plant Biology and Biotechnology). Then, we have selected during several years forms with inherited signs of precocity and high productivity. Both approaches are genotype independent and can be used for any commercially important variety or line. It's noteworthy, that these approaches allowed to obtain principally new forms, incorporating traits (early maturity, high productivity, drought resistance, grain quality, etc.), simultaneous combination of which is difficult or impossible to achieve using the methods of traditional classical breeding or genetic transformation. Most of these signs are under polygenic control. Obtained data are discussed from the point of possible epigenetic mechanisms underlying the arising of new forms. Intense breeding has eroded genetic diversity, and epigenetic diversity now emerge as a new source of phenotypic variations to improve adaptation to changing environments and ensure the yield and quality of crops. Approaches we propose for enhancing phenotypic diversity can be well used in breeding programs. Obtained forms are the good models for transcriptome search of new genes responsible for phenotype reprogramming.

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## Alterations of differential gene expression during the morphogenesis induction in wheat tissue culture (transcriptome analysis)

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Today, the most modern approach for study the gene expression is profiling of the entire transcriptome. The cell identity is defined by its transcriptome, i. e., by a complete set of expressed RNA transcripts. Profiling of the whole transcriptome is widely used to assess the relative gene expression in cells, tissues, organisms, or under different conditions. The main goal of this investigation is identification of differentially expressed genes by whole-transcriptome sequencing of wheat cell culture samples during the induction of long-term embryogenic cell lines. For this purpose the transcriptomes of 7-days wheat calli induced for long-term embryodogenesis and control uninduced tissues have been compared. Initial globular non morphogenic callus, subcultivated on MS nutrient media with 2,4-D have been used as a control uninduced sample. Control variants were compared with globular callus, transferred for the induction of long-term embryogenic cell lines on MS media with 2,4-D and high (stress) concentration of mineral salts. As a result of transcriptome RNAseq analysis, key genes involved in early stages of induction of long-term embryogenic potential have been identified. 974 genes with up-regulated expression have been established belonging to the categories of the protein proteolysis and cellular proteins involved in catabolism, 419 of them were recognized. 834 genes with down-regulated expression have been revealed belonging to the categories of response factors for signals, 403 of them were recognized.

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## Cytogenetic study of the hybrids $F_1BC_1$ with interspecific chromosome substitution of species *G. barbadense* L.

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As is known, the cytogenetic collection of cotton of the species *G. hirsutum* L., obtained at National University of Uzbekistan, was created by irradiation of pollen with gamma rays and irradiation seeds with thermal neutrons. To create aneuploid hybrids  $F_1BC_1$ , monosomic  $F_1$  hybrids with substitutions of individual chromosomes were crossed with the original 8 monosomic lines (recurrent parent), which acted as maternal parents. As a result of the study of meiosis at the stage of metaphase I in backcross  $F_1BC_1$  hybrids, 25 bivalents and one univalent of different sizes were found. Moreover, some cross family revealed several backcross hybrid monosomics. Backcross hybrid monosomics with the replacement of individual chromosomes were found in eight hybrids variants and in four families two hybrid monosomics ( $F_1BC_1Mo60 \times F_1694_5$ ,  $F_1BC_1Mo27 \times F_1687_4$ ,  $F_1BC_1Mo34 \times F_1688_9$ ,  $F_1BC_1Mo48 \times F_1529_{16}$ ) were identified, while in four families ( $F_1BC_1Mo58 \times F_1530_3$ ,  $F_1BC_1Mo59 \times F_1531_8$ ,  $F_1BC_1Mo75 \times F_1104_2$ ,  $F_1BC_1Mo92 \times F_1539_5$ ) – one hybrid monosome plant, at the stage of metaphase I meiosis 25 bivalent and univalent in a different size. Analysis monosome size in monosomic hybrid  $F_1BC_1$  plants with substitutions of individual chromosomes found average size univalents replacement of chromosome 4 ( $F_1BC_1Mo58 \times F_1530_3$ ,  $F_1BC_1Mo59 \times F_1531_8$ ,  $F_1BC_1Mo60 \times F_1694_5$ ,  $F_1BC_1Mo75 \times F_1104_2$ ) and chromosome 7 ( $F_1BC_1Mo27 \times F_1687_4$ ), whereas on chromosome 6 ( $F_1BC_1Mo34 \times F_1688_9$  and  $F_1BC_1Mo92 \times F_1539_5$ ), the large size of univalents was found, which confirmed the At-subgenomic identity of the above-mentioned monosomes and the absence of a change of univalents. Also in the variant of crossing  $F_1BC_1Mo48 \times F_1529_{16}$  with substitution on chromosome 18, the small size of the univalent was found, which confirmed the Dt-subgenomic identity of the above monosome. Thus, a comparative analysis of conjugation of chromosomes in 12 hybrid monosomics  $F_1BC_1$  with substitution of individual chromosomes 4, 6, 7 of At-subgenome and chromosome 18 Dt-subgenome revealed normal conjugation of chromosome for cotton monosomics and the presence of 25 bivalents and one univalent of different size at the stage of metaphase I appropriate to the size of the monosomes of the original cotton monosome lines.

## Mutagenic effect of phosphemide for induction of mutations of *Hordeum vulgare* L. and *Linum usitatissimum* L.

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Genetic erosion of plants leads to a reduction in the number of varieties, therefore, to a decrease in the productivity of agrocenoses at changing environmental conditions. The use of induced mutants in breeding programs led to the creation of 3222 varieties of 170 different plant species in more than 60 countries of the world. The significance of researches in this area increases significantly with the mutations induction at using a new or insufficiently studied mutagenic factor. The goal of our research is the development of the scientific basis for the application of the chemical mutagen phosphemide in the seed's treatment of barley (*Hordeum vulgare* L.) and flax (*Linum usitatissimum* L.). A prerequisite for the use of new methods is the presence of a mutant population. In experimental work should consider differences in the set of chromosomes: at *Hordeum vulgare*  $2n = 14$ ; and at *Linum usitatissimum*  $2n = 30$ . We have substantiated various concentrations for treating barley seeds (0.002, 0.01 %) and flax (0.005, 0.01, 0.1 %). The effectiveness of the mutagenic factor was determined by the sensitivity of barley and flax to the phosphemide in  $M_1$ , the frequency and spectrum of mutations in the  $M_2$  and  $M_3$  as in laboratory conditions so in field experience. The physiological status of seeds and the variability of plant morphometric parameters in ontogenesis are among the informative criteria. The express-diagnostics of chlorophyll content in leaves at different stages of ontogenesis was tested. Based on the readings of the SPAD 502 chlorophyll optical counter (Minolta Camera Co., Japan), significant differences were found in the accumulation and degradation of chlorophyll in the control and after phosphemide. The content of the mass fraction of starch and amylose in the barley grain was determined by the genotype and the concentration of mutagen. The mutagenic effect of phosphemide was confirmed by mutations in barley (10) and flax (10).

## The maintenance and exploitation of plant genetic resources – state of the art

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There are two main strategies for maintaining plant genetic resources: *ex situ* and *in situ* conservation. *Ex situ* conservation means the conservation of the biological diversity outside their natural habitats. *In situ* conservation means the conservation of ecosystems, natural habitats and viable populations of species in their natural surroundings. *Ex situ* conservation is the most significant and widespread mean of conserving plant genetic resources. Mainly, accessions are maintained in specialized facilities known as genebanks. World-wide 7.4 million accessions are stored in about 1,750 *ex situ* genebanks. Considering major crop groups about 45 percent of all the accessions in the world's genebanks are cereals, followed by legumes (15 percent), fruits and forage crops (each 6–9 percent) as well as roots and tubers, oil crops and fibre crops (each 2–3 percent). It is estimated that 90 % of all genebank holdings are stored as seeds whereas less than 10 % and less than 1 % are maintained *in vivo* (field genebanks) and *in vitro* (tissue culture and cryo preservation), respectively. Clearly, seed storage is the predominant mode of plant genetic resources conservation. With a total inventory of 150,000 accessions from 3,212 plant species and 776 genera, the 'Federal *ex situ* Genebank of Germany' in Gatersleben holds one of the most comprehensive collections worldwide. It comprises wild and primitive forms, landraces as well as old and more recent cultivars of mainly cereals but also other crops. Since the majority of genebank accessions globally are stored in the form of seed, seed longevity is of particular importance for crop germplasm preservation. At the IPK research was initiated for a range of crops stored in the genebank over decades. Variation between crop species was detected. However, there is also intraspecific variation within genebank collections. It was concluded that the differences in germination after long term storage are genetically based. Therefore, genetic analyses of seed longevity were initiated. Genetic mapping was performed for barley, wheat, oilseed rape and tobacco. In addition, mass spectrometry based untargeted metabolite profiling experiments were performed in order to detect biochemical changes coinciding with loss in seed germination. GC-MS analysis of the polar metabolome of wheat and barley identified glycerol and related intermediates as highly correlated to germination rate. Therefore, the lipidomic composition of a wheat panel was investigated using high-resolution liquid chromatography-mass spectrometry (LC-MS). A high proportion of tentative oxidized lipids was detected, suggesting lipid oxidation as the causal trigger for membrane degradation. Beside research on seed storability genebank accessions and genetic stocks have been extensively used for genetic and genomic studies. Data on mapping of loci/marker trait associations for a range of different traits will be presented.

## Study of the leaf rust resistance gene *Lr52* by targeted sequencing

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At present, more than 30 genes that control a number of morphological and quantitative traits, resistance to abiotic and biotic factors, have been mapped on 5B wheat chromosome (5BL = 580 Mb and 5BS = 290 Mb), but research and chromosomal localization of loci with agronomic character remains relevant. In that work we identified and annotated 5BS wheat chromosome sequences for previously unstudied leaf rust resistance gene *Lr52*. *Lr52* localization in the position from 6654000 bp up to 6956436 bp on 5BS pseudomolecule (IWGSC RefSeq v1.0 genome) was determined by mapping of markers linked to the gene *Lr52*. We selected 5 plants with *Lr52* and 5 plants without *Lr52* from mapping population F4 (line\_LrW (52) × hybrid215) according to the data of KASP and SSR genotyping together with screening for resistance. These plants were sequenced using the SeqCap EZ Target Enrichment System (Roche). After quality control (FASTX-toolkit) the obtained sequences were assembled using the BWA-MEM mapping program and the SPAdes genomic assembler. As a result of assembled sequences analysis using blastn and blastx algorithms in GrainGene, Ensembl Plants and PRGdb databases, more than 500 sequences with homology with potential coding sequences were identified, of which more than 40 are putative resistance genes with the NBS, CC, LRR, Tm and kinase domains.

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## Drought resistance in some *Prunus persica* (L.) Batsch cultivars damaged with *Plum Pox Virus*

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*Prunus persica* (L.) Batsch is one of the most valuable and widely cultured fruit crops, which is characterized by a rather high yield and resistance to abiotic stress factors. Nowadays, the great problem of this culture is its high degree of disease and pest damages. In this regard, a comparative analysis of peach cultivars, infected with *Plum pox virus* (PPV), resistance to air and soil drought on the Southern coast of the Crimea has been made. On the leaf blades of the damaged plants, narrow, indistinct stripes were observed in the form of a pattern, rarely-rings of light yellow or yellow color and leaf wrinkling. The degree of the tree crown damages was different: from 10 % ('Podarok Neveste') to 80 % ('Lakomyiy', 'Pushistiy Ranniy', 'Mechta'). The leaf blades in the damaged plants had a number of structural differences compared with asymptomatic ones: leaf thickness reduction, palisade index decrease (by 8–12 %, noted in 'Lakomyiy' and 'Krymskiy Shedevr'), formation of large intercellular spaces in spongy tissue, necrotic processes in palisade chlorenchyma, a greater amount of crystal inclusions in cell cytoplasm. Due to the cultivar differences, in optimal conditions of the growing season beginning, cultivars demonstrated the water regime changes because of the infection were noted: 'Mechta', 'Pushistiy Ranniy', 'Cardinal'. Their water retention capacity significantly reduced. The summer drought led to a decrease of the total water content in the leaf tissues. In asymptomatic plants water content was 59–70 %; in damaged plants it reduced to 55 % ('Karnavalniy', 'Pushistiy Ranniy', 'Ambergold'), water deficit was 12–26 % in healthy plants and 20–31 % – in damaged plants. High drought tolerance is generally characteristic of the cultivars: 'Krymskiy Shedevr', 'Dixired', 'Tulpan', 'Podarok Neveste'. PPV damages reduces the resistance to abiotic stress in the summer period in 'Karnavalniy', 'Pushistiy Ranniy', 'Demerdzhinskiy'.

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## ***Bacillus* bacteria in the resistance of potato plants to viruses**

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Plant viruses have a significant negative impact on major cultures of agronomic importance provoking a wide range of symptoms, representing a serious threat to global food security. Subsistence crops are often infected with viruses that cannot be controlled with pesticides. Biopreparations based on endophytic microorganisms may be an alternative. There are genes of ribonucleases (RNases) produced by many species of *Bacillus*. The synthesis of secreted degradation enzymes including RNases, this is one way to adapt bacteria to variable habitat conditions. The secreted RNase allows bacilli to have a broad spectrum of biological activities. In low concentrations, they stimulate the growth and physiological functions of plants and microorganisms. In high concentrations, they have antiviral activity. The microbial RNases are potential therapeutic agents, thus the assessment of plant protection against viruses by endophytic bacteria is becoming an important practical problem. In our laboratory there is a collection of bacteria produced various metabolites and have RNase activity. We compared growth and development indicators of potato treated with *B. subtilis* 26D and *B. thuringiensis*. The results showed that the inoculation increased the grain yield by 0.2–0.7 t·ha<sup>-1</sup>. In addition, the treatment of potato plants with bacteria of *Bacillus* significantly reduced the infection of potato plants with viruses. The prevalence of the disease in potato plants was significantly reduced from 75 % in the control to 18 % (*B. subtilis* 26D) and 33 % (*B. thuringiensis*) in the inoculated plants. Similarly, the index of viral infection development decreased from 5–10 % in the control to 1–5 % in the inoculated plants. The further study of molecular mechanisms related to bacterial induction of plant defense reactions in response to viral infections will lead to a better understanding of stress resistance problems. The endophytic microorganisms studied in this report may become basis for creation of biological agents for plant protection.

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## Phenotypic effects of the *Rht-17* dwarfing gene in spring wheat under two climatic conditions

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Such dwarfing genes as *Rht-B1b* (*Rht1*), *Rht-B1e* (*Rht11*) and *Rht-D1b* (*Rht2*) have been widely explored in wheat breeding to reduce plant height. The introduction of new variations of these genes into new wheat cultivars is relevant for sustainable agriculture. The *Rht-B1p* (*Rht17*) reduces plant height by 33 % and affects other economically valuable traits. Leaf elongation rate and coleoptile length are reduced by *Rht17* in early plant growth phases due to highly decreased sensibility to gibberellic acid that is similar to the effect of *Rht-B1b*, *Rht-D1b* and *Rht-B1e*. Our aim is to compare the effects of *Rht17* on nine valuable agronomic traits under conditions of Non-Black Earth Zone (Nechernozemye, Moscow) and Black Earth Region (Chernozemye, Krasnodar Territory). We studied the F<sub>3</sub> population of spring bread wheat [*‘Cltr17241’*(*Rht-B1p*) × *‘Novosibirskaya 67’* (*Rht-B1a*)] and F<sub>3</sub> population of spring durum wheat [*‘Chris Mutant’*(*Rht-B1p*) × *‘LD222’*(*Rht-B1a*)], designated M17 and T17, respectively. Seeds were sown by families in 0.4 m rows in 2018. The plants were genotyped using molecular markers. The plants homozygous for presence/absence of *Rht17* were compared and the significance of differences in measured traits were determined by Fisher’s analysis of variance. Here, we present some statistically significant results obtained in our work. In population M17 plant height is decreased by *Rht17* by 31 % in Black Earth Region and by 29 % in Non-Black Earth Zone, while in population T17 by 46 % and 41 %, respectively. Additionally, we revealed that *Rht17* decreases 1000 grain weight by 12 % in population M17 in Black Earth Region and by 15 % in Non-Black Earth Zone, while in population T17 by 27 % and 17 %, respectively.

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## Cultivated sunflower high-throughput genotyping and lipidomic profiling

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Cultivated sunflower is one of the key plants used by human. It is an important oilseed crop that was domesticated from the wild sunflower approximately 4000 years ago. Now sunflower is mainly planted for the seed oil. Selection of hybrids with changed oil properties is one of the basic directions in oilseed crops hybrid breeding. Full sunflower genome assembly released by Badouin et al. made good possibilities for large-scale genome wide association studies (GWAS) which result can be implemented in sunflower genomic selection and help to speed up breeding programs. In this study we perform high-throughput genotyping (GBS-sequencing) and lipidomic phenotyping on 600 inbred sunflower lines. For lipid profiling we use ultra high performance liquid chromatography coupled with mass-spectrometry (UPLC-MS). UPLC-MS is a very powerful tool for lipidomics, which allow simultaneous profiling of several hundreds different lipids extracted from a single plant sample. Here we perform comparative lipidomic study in sunflower and combine NGS based genotyping with high performance phenotyping technology and show advantages of this approach for agricultural proposes. We have identified 2360111 SNPs and 1000 lipid molecules. GWAS were performed. Significant associations between molecular phenotypes and SNPs were identified. Our results extend current knowledge of sunflower metabolism and give new insights on development of approaches in oil-seed crop genomic selection.

## The effect of various dominant *VRN* alleles and their combinations on the duration of development phases and productivity in common wheat lines

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Establishing the effect of different alleles of the *VRN* loci and their combinations on the duration of the developmental phases and the productivity of common wheat is of immediate practical importance for breeding. Since most varieties of Russia and Western Siberia carry the dominant alleles of *Vrn-A1* and *Vrn-B1* genes, were obtained two lines of winter cultivar Bezostaya 1 (Bez1) with the combination of alleles of the *VRN-I* locus: Bez1 *Vrn-A1a Vrn-B1a* and Bez1 *Vrn-A1a Vrn-B1c*. Homozygous plants were isolated in the F<sub>2</sub> generation using allele-specific primers for the *VRN-A1* and *VRN-B1* loci. Based on the genetic segregation of F<sub>2</sub> hybrids with tester isogenic lines, it was confirmed that the obtained lines carry two dominant genes: *Vrn-A1* and *Vrn-B1*. The presence of the *Ppd-D1a* allele in lines of cultivar Bez1 was shown with the use of PCR-marker. It was established that the lines with two dominant alleles headed on day 40, which was 2, 8 and 5 days shorter than in the isogenic lines i:Bez1 *Vrn-A1a*, i:Bez1 *Vrn-B1a* and i:Bez1 *Vrn-B1c*, respectively ( $p \leq 0.01-0.001$ ). Also, these lines have reduced the period of “tillering-first node” compared to the above lines by 2, 9, and 8 days, respectively ( $p < 0.001$ ). The study of the dynamics of the growth cone in the lines of the Bez1 and Sava cultivars showed that the differences began to appear from the “tillering-first node” stage. The lines with the dominant allele *Vrn-A1a* were ahead of other lines at the III–IV stages of organogenesis in the degree of differentiation and the size of the growth cone and the lines with the *Vrn-B1c* allele were ahead the lines with the *Vrn-B1a* allele. It was found that the lines with dominant allele *Vrn-B1c* were the most productive, and the Bez1 *Vrn-A1a Vrn-B1c* line was more productive than the Bez1 *Vrn-A1a Vrn-B1a*.

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## **Study of the lines of common wheat of breeding of National Center of Grain named after P.P. Lukyanenko on allele variants of *Waxy*-genes**

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The aim of this work was the molecular identification of promising genotypes common wheat by allele variants of *Waxy* genes (*Wx*) to create varieties with improved technological qualities of grain. 352 lines of common wheat were studied by allelic variants of genes *WxA1*, *WxB1*, *WxD1*. All lines were obtained in the department of breeding and seed production of wheat and triticale in the National Center of Grain named after P.P. Lukyanenko, by crossing with a mutant line carrying null alleles *WxA1b*, *WxB1b* and *WxD1b*. There were identified 205 lines carrying the wild type allele *WxA1*, 229 lines with the wild-type allele of the gene *WxB1* and 249 with the wild-type allele of the gene *WxD1*. 64 lines carried functional allele *WxB1e*, different from that wild type. The lines carrying zero alleles of genes *WxA1*, *WxB1* were selected (147 and 58 respectively). 13 lines, showing the heterozygous condition for the gene *WxD1*, and one line for the *WxB1* gene, were identified. Full technological evaluation was given to 57 promising lines and several standard varieties. The obtained results show that the lines carrying zero-allele *WxA1b* or *WxB1b*, as well as a combination of alleles *WxA1b*+*WxB1b*, are generally characterized by high protein content (15.3, 15.4, 15.5 % respectively), gluten (28.3, 28.6, 30.3 %). All three groups of lines are close to the “strong” wheat by the strength of flour (285 alveograph units, 270 a.u., 279 a.u.). The lines with the identified null alleles of the *Wx* genes are distinguished by a high water absorption capacity compared to the Tanya variety, which does not have these alleles in its genotype. The optimal combination of liquefaction indicators and high valorimetric evaluation probably contributes to the formation of high-volume bread with a high overall baking rating.

## Use of a synthetic form Avrodes for modification of the genome of common wheat

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The synthetic form Avrodes (BBAASS) was used for modification of the genome of common wheat. This form possesses the ability of *Aegilops speltoides* to suppress the activity of *Ph* gene(s) and stimulate homoeologous chromosome pairing. Synthetic recombinant forms (RS-forms) were obtained in which the first two genomes A and B originate from common wheat, and the third genome combining the chromosomes of the S genome from *Aegilops speltoides* with the chromosomes of other wild species. Introgression lines obtained on the basis of RS-forms can carry genetic material of two wild species, both separately and together. Currently a cytological study (C-banding and FISH) of lines derived from the synthetic recombinant form RS7 (BBAAUS) has been carried out. Lines are resistant to leaf rusts and may presumably have genetic material from both *Ae. speltoides* and *Ae. umbellulata*. Chromosomal changes affected 10 of the 12 studied lines. In most cases the lines carry translocations from *Ae. speltoides*. Translocations from this species were identified on chromosomes 1D, 2D, 3D, 2B, 4B, 5B and 7B. Lines with substituted chromosomes 1B(1S), 4D(4S), 5D(5S) and 7D(7S) were also identified. Lines with translocations only from *Ae. umbellulata* not identified. Two lines carry simultaneously genetic material from *Ae. speltoides* and *Ae. umbellulata*. In line 3379 translocations were detected in the short arm of chromosome 7D from *Ae. umbellulata* (T7DL.7DS-7US) and on chromosomes 5B, 1D, 2D from *Ae. speltoides*. Line 4626 presumably has a translocation on the long arm of chromosome 2D from *Ae. umbellulata* (T2DS.2DL-2UL) and translocation T7SS.7SL-7DL from *Ae. speltoides*. The translocations T1DS.1DL-1SL, T3DS.3DL-3SL from *Ae. speltoides* and T2DS.2DL-2UL, T7DL.7DS-7US from *Ae. umbellulata* obtained for the first time.

## **The collection of stone fruit cultures of the SBI SO SRI “Zhigulevskiye sady” – mobilization, studying, the prospects of use**

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The history of selection of stone cultures at the Samara experimental fruit and berry station originates since 1931 and proceeds so far (in the status of Science, State Budgetary Institution of Samara Region “Research Institute of Horticulture and Medicinal Plants “Zhigulevsk Gardens”). In the State Register of Breeding Achievements of the Russian Federation in 2018, 21 varieties of stone fruits from the breeding plant of the Research Institute “Zhigulevskiye Sady” were included. The main goal of our research is the creation of varieties of stone fruits of an intensive type, highly productive, having a compact low-growing crown, with high-quality fruits, dessert, universal and technical purposes, adapted to the conditions of the region. To achieve this goal, from 2011 to 2018 a genetic collection of stone fruit varieties was created: plums – 76, cherries – 60, sweet cherries – 24, and apricots – 22 varieties. The ecologo-geographical origin of the stone fruit varieties attracted to the collection covers many regions of the Russian Federation: Central, Central Black Earth, Middle Volga, Lower Volga, Ural, Northwestern, Far Eastern, Volgo-Vyatsky, North Caucasus. During the study, a preliminary assessment of the collection material in the climatic conditions of the past growing seasons was carried out. In 2015–2018, the harvest was obtained in most of the collection varieties. The following varieties of cherries were distinguished by the complex of economic and biological features: Saratovskaya baby, Volga dessert, Malinovka, Mayak, Molodezhnaya, Zhukovskaya, Kharitonovskaya, Victoria, Shakirovskaya, Rovesnitsa, Turgenevka, Azalia and others. In favorable conditions, growing seasons in 2016, 2018 were harvested sweet cherry varieties Tchernashnya, Fatezh, Tyutchevka, Bryansk pink. Plum is represented by varieties of home plum, Chinese plum and cherry plum. Since 2015, intensive flowering and annual fruiting have been noted in the Chinese plum sapings Nezhenka, Alyonushka, Krasa Orlovshiny, Orlovsky souvenir. The most fruitful were the varieties of homemade plum Etude, Start, Renklod Tambov, Record, Candy, Eurasia 21, Skoroplodnaya, Bolkhovchanka, Start. In 2016, 2018 there were favorable conditions for the culture of apricot. High yield, good quality of fruits are characterized by the varieties of apricot Round, Khabarovsky, Rattles, Zeus, Lel, Ulyanykhinsky.



## Clusters of transcription factor binding sites in plant genomes

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Analysis of transcription regulation in plants presents important problem in molecular biology challenging growing volume of sequencing data. The computer analysis of structure of transcripts and the genome organization of crop plants is important for biotechnology and agrobiology. ChIP-Seq technology allows to detect interactions between DNA and proteins and allow to analyze gene expression regulation in genome-wide scale for large genomes of eukaryotes including crop plants. In course of evolution of plants the specific transcription factors (TF) in plant genomes also have been changing with time as well as TF binding sites. Thus, comparison of genome-wide distribution of TF binding sites is important. It is critical to annotate clusters of binding sites of different transcription factors that may function as enhancers in complex fashion. We developed R scripts and computer tools for TFBS analysis, including following steps: Look for TFBS in genomes with or without the TF and test whether enrichment is detectable overall (plot TFBS density as threshold score); Refine analysis by checking the location of bound regions (intergenic/gene/promoter/intron); Refine by checking whether the enrichment increases with the size of the TF family; Search for best TFBS and look at density and arrangement in their vicinity (probability of detecting others, relative position). Then compare regions from a TF-containing genome and a relative plant genome depleted by such sites. We have developed set of computer programs and have integrated them to the program complex for transcription factor binding sites analysis. Construction of clusters of transcription factor binding sites allowed reconstruct gene regulatory networks. We continue work on integration of ChIP-seq transcription factor binding sites data in plant genomes using available data sources.

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## Preventive role of *Tomato bushy stunt virus* RNA-interference suppressor protein in plant immune response

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Complete resistance in plant to virus infection is referred to as immunity. Except protein defense factors, plants operate with RNA-interference defense system, which was firstly demonstrated for plant viruses. RNA-interference is a posttranscriptional RNA silencing process that is evolutionary conserved across eukaryotic kingdoms, which involved in development, differentiation, response to stress factors through regulation the levels of specific RNAs. As a countermeasure, viruses encode suppressor proteins, which disturb RNA-interference pathway. One of the best biochemically characterized suppressor is *Tomato bushy stunt virus* encoded P19 protein. During TBSV replication, abundantly accumulated single-stranded genomic RNAs and double-stranded RNAs performed as substrates for Dicer-mediated cleavage into duplex short interfering RNAs (siRNA). AGO-family proteins with siRNAs form RNA-induced silencing complex. This forms the catalytic entity for RNA degradation that uses the incorporated siRNA to specifically targeting complementary RNAs. The main function of P19 as suppressor protein is prevention the unwinding of viral siRNA and thus preventing the incorporation of single-stranded RNA into RISC complex, therefore – programming. The X-ray crystallographic structure of the P19 suppressor protein and specific siRNA complex revealed that caliper tryptophan residues on P19 dimers precisely measure the binding of 21-nucleotide siRNAs by P19 dimers. These siRNA duplexes have 2-nt 3' overhangs. P19 binds the duplex region of siRNAs. Usually, most suppressor proteins are multifunctional. P19 protein also appeared like pathogenicity factor. Inoculation of susceptible plant with TBSV P19-null mutant (TBSV $\Delta$ P19) leads to recovery. Cross-inoculation experiments with wild type TBSV, which was preliminary inoculated TBSV $\Delta$ P19 leads to recovery phenotypes instead systemic collapse.

## Quantitative real-time PCR as a supplementary tool for molecular cytogenetics

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Cytogenetics is one of the powerful tools of plant genetics that enables to explore structural and functional organization of the genome as well as evolutionary relationships between species. However, preparing the chromosome slides for analysis and the following procedures such as genomic and fluorescence *in situ* hybridization (FISH and GISH) are rather expensive, time and labor consuming. Tedious cytogenetics techniques have been highly facilitated by bioinformatic approaches on the basis of whole-genome sequencing data helping not only to reveal new DNA satellite sequences that can be applied as probes for chromosome labelling but also to predict its possible chromosomal localization. Quantitative real-time PCR (qPCR) can be applied in the selection for DNA repeated sequences that can be used as probes for FISH highly efficiently thus increasing the number of successful experiments. qPCR makes it possible to estimate the relative quantity of DNA repeated sequences (satellites or mobile elements) in the genome of the studied species and thus to carry out a preliminary selection of those that can be converted into chromosome- or genome-specific cytogenetic marker. The estimation can be performed even at a semi-quantitative level, therefore, the requirements for primer efficiency are not so strict as for quantitative assessment. We demonstrated the efficiency of qPCR as a supplementary tool for cytogenetics in several case studies, namely, with cytogenetic markers for sex chromosomes of buckthorn, satellite repeats localized to chromosomes of J, St and V genomes in diploid and polyploid grasses (including *Thinopyrum intermedium*), and tandem repeat-based cytogenetic markers in wheat. New application of qPCR approach has sufficiently reduced the costs and time required for the development of the novel markers that can be widely used in both fundamental and applied research in plant genetics.

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## Developmental pathways regulating wheat inflorescence architecture

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Wheat inflorescence architecture contributes to grain yield potential. The wheat inflorescence consists in a spike with a main axis carrying lateral sessile spikelets that are directly attached to the rachis and also a terminal spikelet. The spikelet constitutes the basal unit of the spike inflorescence. Wheat inflorescence architecture is determined by the activity of different meristem types and the timing of transitions between them. Although some of the genes controlling wheat meristem activities have been identified, understanding of the network of transcriptional regulators controlling this process is lacking. To address this, we used a set of classical and modern approaches of genetics and developmental biology, including high-throughput genotyping, light and electron microscopy and bioinformatics. We found the development of additional organs of the spikelet, glumes and flowers, in the *T. jakubzinerii* accession (*shr1*), which indicates disorders in spikelet meristem determinacy. The development of additional organs of the spikelet was not accompanied by a change in phyllotaxis. Thus, the function of the *Shr1* gene in the developing inflorescence of wheat is associated with determinacy of spikelet meristems. Genes *Shr1* and *Shr2*, *RS*, which determine the false-true branching of the ear and the false branching of the ear, are inherited independently. Thus, at least two different genetic pathways in wheat control the spikelet meristem determinacy.

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## The study of the localization, structure and expression of the genes regulating the development of the ligular region of the tribe Triticeae

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Leaves of Poaceae have a unique morphological feature: they consist of a proximal sheath and a distal blade separated by a ligular region. The sheath provides structural support and protects young developing leaves, whereas the main function of the blade is photosynthesis. The auricles allow the blade to tilt back for optimal photosynthesis and determine the angle of a leaf, whereas the ligule protects the stem from the entry of water, microorganisms, and pests. Research on liguleless mutants of maize and other cereals has led to identification of genes that are involved in leaf patterning and differentiation. A liguleless line of *Ae. tauschii* is an induced mutant (*Lgt*-mutant), whose phenotype is under control of the dominant gene *Lg<sup>t</sup>*. Using 3933 polymorphic DArTseq markers, a high-throughput genotyping of F2 population from the cross *Lgt*-mutant/KU-2126 was performed; highly saturated molecular-genetic maps of *Ae. tauschii* were constructed. The *Lg<sup>t</sup>* gene was placed on the short arm of chromosome 5D by molecular-genetic mapping. *In silico* mapping of the DArTseq markers on *Ae. tauschii* physical map allowed to establish the coordinates of *Lg<sup>t</sup>* on 5D pseudomolecule and to determine the list of the *Lg<sup>t</sup>* candidate genes. *Lg<sup>t</sup>* gene is not an orthologue of the previously studied *Lg4*, *Lgn1* and *Knox1* cereal genes, whose dominant mutations cause the liguleless phenotype, and presents a new cereal gene, involved in the genetic control of the development of the ligular region and the formation of the distal-proximal axis of differentiation. SNP and SSR markers help us to shorten the list of candidate genes for *Lg<sup>t</sup>*.

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## Evaluation of leek (*Allium porrum*) genomic polymorphism using the AFLP method

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Leek (*Allium porrum* L.) is an important green vegetable crop in Europe and Asia. In Russia, the popularity of leek increases every year due to its valuable taste and nutrition/dietary properties, which are a consequence of the high content of secondary metabolites. Despite the importance of this vegetable, data on the biochemical composition and phenotypic polymorphism of leek accessions, as well as molecular-genetic characteristics of *A. porrum* genome variability and individual genes polymorphism are extremely limited. In this study, the AFLP method was applied to determine the genomic variability of 65 leek accessions from the collection of the Federal Scientific Center of Vegetable Growing (FSCVG). As an outgroup, species *A. ampeloprasum*, *A. commutatum* and *A. pyrenaicum* were used, because together with the leek they are included into the *A. ampeloprasum* complex. Using two selected primer combinations (with six selective nucleotides at the 3'-end), for each sample analyzed, a unique AFLP spectrum was obtained. A total of 721 fragments were identified, of which 675 (93.6 %) were polymorphic for leek accessions, and 103 were accession-specific. The calculated genetic distances ranged from 0.32 to 0.75 (GDcp 0.58). According to PCO-analysis, the accessions of leek form two unequal groups (16 and 49 accessions), equally distanced from the outgroup. Four accessions, cultivars (Florena (Netherlands), Good fellow (Russia)) and breeding lines (2017, 2038), were distanced from the rest of the analyzed leek accessions, which reflects the difference in their genomes. Thus, an AFLP analysis revealed a high genomic polymorphism level of leek accessions from the FSCVG collection. *A. porrum* accessions formed an isolated sub-cluster that validates *A. porrum* as a separate species other than *A. ampeloprasum*.

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## New antimicrobial gene promoters from chickweed (*Stellaria media*) for biotechnology of cultivated plants

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Earlier we with colleagues found that hevein-like peptides genes from chickweed (*Stellaria media*) are the source of strong constitutive promoters for biotechnology of cultivated plants. E. g., in transient expression in *Nicotiana benthamiana* pro-SmAMP1 and pro-SmAMP2 promoters were 2–4 times more effective than the CaMV35S promoter and in rape (*Brassica napus*) and sugar beet (*Beta vulgaris*) plants were comparable to it. The functionality of the pro-SmAMP2 promoter was shown in the calluses of flax (*Linum usitatissimum*). In the homozygous lines of transgenic tobacco (*Nicotiana tabacum*), the pro-SmAMP1 and pro-SmAMP2 promoters are twice as strong as the CaMV35S promoter. The both promoters are at least as effective as the duplicated CaMV35S promoter for neomycin phosphotransferase II gene control in the selection of transgenic tobacco and *Arabidopsis* plants on media with kanamycin antibiotic at recommended concentrations. In present research we focused on the fact that pro-SmAMP1 and pro-SmAMP2 promoters are identical by 94 % differing by point mutations outside canonical cis-elements. Additional deletion analysis showed that in transient expression the minimal variant of pro-SmAMP1 (–119 b. p.) is twice stronger than minimal variant of pro-SmAMP2 (–121 b. p.). Along with this, pro-SmAMP2 is significantly more efficient than pro-SmAMP1 in control of selective gene *nptII* being comparable with duplicated 2xCaMV35S promoter. We employed the 9-nucleotide point polymorphism between sequences of two minimal promoters from chickweed for creation of new effective promoters featuring simultaneously high active and constitutive.

Significant similarity of nucleotide sequences in promoters of hevein-like genes precludes from using both of them within the same genetic construct so that recombination between the repeats be excluded. To create novel regulatory elements, we cloned the promoters of  $\alpha$ -harpinine gene (*pro-SmAMP-X*) and defensine gene (*pro-SmAMP-DI*) from the chickweed using genome walking method. These novel promoters do not have a homology with any other known promoters being comparable with promoter CaMV35S in efficiency of transient expression.

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## Biotechnological approaches in breeding and genetic research of soybean

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To obtain a new source material in soybean breeding in order to isolate valuable genotypes, we used the possibility to develop *in vitro* forms with the use of heavy metal ions as a mutagenic factor in nutrient media. The lines, which were obtained using Cd<sup>2+</sup> ions from the initial forms of soybean varieties Hodson and Primorskaya 301, were analyzed by the method of allozyme analysis and according to a complex of biochemical parameters. Soybean variety Primorskaya 4 was taken as a standard. As a result of the research there were identified samples with low and high peroxidase activity which ranged from 0.2 to 167.5 u/mg of protein. Seeds of varieties Primorskaya 4, Primorskaya 301 and regenerant R 1577 possessed low specific activity of peroxidase. The highest specific activity of peroxidase was observed in seeds of Hodson variety and regenerants R 1590, R 1585, R 1606 and R 1567. The specific activity of catalase in soybean seeds varied from 27.5 to  $249.9 \times 10^{-3}$  u/mg of protein. The highest activity of catalase was revealed in seeds of three samples: R 1567, R 1585, variety Hodson. The lowest catalase activity was noted in seeds of varieties Primorskaya 4, Primorskaya 301, regenerants R 1597 and R 1577. The remaining samples are characterized by average values of enzyme activity. According to the study of electrophoretic spectra of peroxidases and catalases in the seeds of the studied lines, a cluster analysis was carried out. As a result, two clusters were formed. The first included the lines where the original form of them was variety Primorskaya 301. In the other line there were forms obtained from the soybean variety Hodson. Maximum genetic distances were detected between soybean variety Primorskaya 4 and lines 1567 and 1585 (I.F. Hodson) and 1568 (Primorskaya 301). The distance between clusters was 0.8, inside the cluster which was formed by the lines P301 from 0.07 to 0.3, and between the lines I.F. Hodson from 0.1 to 0.5. The studied lines demonstrate different level of variability depending on the genotype of the initial form. Thus, there were defined 4 variants of the genotype with the maximum level of genetic differences of 0.3 from 6 lines which were developed from P301. While there were defined also 4 variants from 7 lines of variety Hodson, but with big level of genetic distances – 0.5. Line 1606 according to isozymes content of catalases and peroxidases were identical as their initial form (variety Hodson). Samples with high enzyme activity and the highest number of isoenzymes potentially have the greatest resistance to stress factors. As a result of the analysis of the biochemical composition of seeds there was defined variation in the values of individual indicators both upward and downward relative to the standard. There were defined three lines R 1609, R 1605, R 1584 having advantages on a complex of traits: the fat content, oleic, linoleic and linolenic acids, have average values of enzyme activity, which does not allow them to be considered as resistant to stress factors. In relation to the initial form (Primorskaya 301) the greatest genetic differences has R 1605 (0.3365). As for R 1609 and R 1584, they have the same level of distance (0.1542). The line R 1576 is defined as a source of high protein and oleic acid content, has high values of enzyme activity. In relation to the initial form of Hodson variety it is characterized by an average value of genetic distances 0.2412.

## Genetic effects of alien chromosome substitution or translocation in common wheat

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The polyploid nature of *Triticum aestivum* L. has opened up opportunities for the development of cytogenetic studies that allow targeted intervarietal and alien substitutions of chromosomes or their fragments and to study the effects of these chromosomes on the manifestation of a complex of characters. Genetic models, including substitution and translocation lines, introgressive lines and synthetic wheat with the participation of species of the Triticeae tribe contribute to the enrichment of the common wheat gene pool and play an important role in the selection for resistance to biotic and abiotic stresses. The aim of the work is to study the directed processes of transformation of the genome of common wheat as a result of intergenomic substitutions of chromosomes or their segments with chromosomes of taxonomically distant cereal species (*Ag. elongatum*, *Ag. intermedium*, *S. cereale*, *H. marinum*), to study the contribution of alien chromosomes to the formation of adaptive and agronomic traits. A set of ditelosomic wheat-barley substitution lines (*T. aestivum*–*H. marinum*) on chromosomes of the homeological group 7 was obtained. It has been shown that 7HIL<sup>mar</sup> chromosome of *H. marinum* was homoeologous to the group 7 chromosomes of common wheat. Wheat-rye 5R(5A) substitution lines for winter wheat varieties Ulyanovka and Filatovka were created. Field experiments conducted in the winter of 2017/2018 showed that these wheat-rye lines overwinter by 90–100 %, but at the same time they have facultative type of development and can grow out during spring sowing. Was isolated homozygous forms with a combination of genes controlling disease resistance (*Lr26/Pm8/Sr31* + *Lr19/Sr25* + *Lr6Ai Sr6Ai/Pm6Ai*), different color of grain (*Ba1* and *Pp-1/Pp3*) and winter type of development (*vrn-R1*) based on phenotypic markers and molecular analysis by PCR with specific primers. Phenotyping and genotyping of introgression lines for disease resistance in a field experiment was carried out and plants resistant to leaf rust were selected.

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## Decreasing wild potato toxicity by targeted modification of glycoalkaloid metabolism genes

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Wild relatives of crop plants carry a variety of genes controlling resistance to adverse biotic and abiotic environmental conditions and serve as donors of resistance genes. Many of wild potato species accumulate high amounts of steroidal glycoalkaloids (SGAs), which are toxic to humans. This fact impedes application of these wild species as donors for potato breeding. The targeted knockout of genes controlling SGA biosynthesis in wild potato could be a promising strategy to decrease SGA accumulation. The *GAME9* gene plays a key role in SGAs synthesis regulation in potato. It was shown that it is associated with domestication process and decreasing of potato toxicity. The steroidal alkaloid glycosyltransferase (SGT) genes are responsible for the final glycosylation steps in the biosynthesis of the SGAs. The *GAME9*, *SGT1*, -2, and -3 genes were selected as targets for gRNA/Cas9-mediated modification. The fragments of target genes were re-sequenced in few wild potato species and set of gRNAs was designed. The modification of wild potato genome is planned through transformation of protoplasts and subsequent regeneration of mutant plants. The both genetic constructs harboring Cas9 and gRNA genes and RNP Cas9/gRNA complexes will be used for protoplast genome modifications. The methods for gRNA efficiency evaluation and protoplast isolation and transformation have been established for potato. For evaluation of SGA content in plant tissues the HPLC-based protocol has been established.

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## Modern biotechnological approaches for improvement of nutritional value of grain sorghum

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One of the promising areas of biotechnology is the improvement of nutritional value of grain. This task is particularly important for sorghum – a high-yielding drought-tolerant cereal, which is a source of feed and food for millions of people in many countries around the world. However, compared with other cereals, majority of sorghum cultivars have a lower nutritional value, which is based on the resistance of sorghum prolamines (mainly, gamma-kafrin) to proteolytic digestion. Recently, in several laboratories in different countries, transgenic sorghum lines with genetic constructs capable of RNAi-silencing of kafrin genes were obtained. These lines were characterized by reduction of kafrin oligomers, an increase of *in vitro* digestibility of endosperm proteins, changes in the ultrastructure of endosperm protein bodies, which acquire an irregular shape with invaginations, and modification of endosperm texture, namely, reduction of the vitreous layer. Moreover, an important consequence of the silencing of the kafrin genes was the enhancement of synthesis of other proteins, including those that have a higher content of essential amino acids, lysine and threonine. Such a rebalancing of seed proteome is a common phenomenon for transgenic plants with genetic constructs for RNA-silencing of prolamine genes. In our experiments, in the offspring of plants with high protein digestibility, the introduced genetic construct functioned irregularly; in the course of plant ontogenesis, including the plants from the late generations (T<sub>4</sub>, T<sub>5</sub>), the elimination of the construct for RNA-silencing was observed. In this regard, the methods of genome editing have significant advantages, since they allow inducing genetic changes in the structure of kafrin genes. We started the work on creation of sorghum lines with a modified nucleotide sequence of the gamma-kafrin gene. These lines should have improved endosperm proteins digestibility and a higher level of essential amino acids. *Acknowledgements:* This work was partially supported by RFBR, grant No. 19-016-00117.

## The nature of bilateral symmetry

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According to the Genome Tree Theory, in terms of graph theory, the genome of a multicellular organism is structured in the form of an oriented binary tree, vertices of which correspond to the same type of logical elements “genome tree loop step”, and the arcs transfer the control between the “steps”. The step of the Genome Tree may be represented by three of non-coding regulatory genes with identical complex promoters. Each cell of a multicellular organism has its own step in its Genome Tree. It receives control from the step of the mother cell, initiates the execution of the “cell program” that determines its development and multiplication, regains control after it is completed, and transfers the control to the steps of the daughter cells. The classic description of the cell cycle leaves behind such important issues as the change in the orientation of the mitotic spindle of cell division, the “assembly” of membrane organelles and their spatial arrangement in the daughter cells taking into account the new cell orientation. Orientation of the mitotic spindle over any direction can be submitted in a spherical coordinate system by zenith and azimuthal angles. Cell cycle events are determined by the cell program, which is a multi-level nested Dijkstra loop organized mostly on non-coding regulatory genes. The cell program can be represented by one of the standard cell programs and some combination of subroutines. The change in the zenith and azimuth angles between the spindle apparatus of the mother cell and that of the daughter cell is presumably set by one of such subroutines. The progenitor cell of a symmetric organ or a group of cells divides into two daughter cells. The main feature of the bilateral symmetry of multicellular organisms (including plants) is presumably the use of the same branch of the Genome Tree by these two daughter cells in their subsequent cell cycles. Asymmetric organs and groups of cells of an organism develop from other cells under the control of other branches of the Genome Tree.

## Identification of DNA markers associated with starch granules morphology of *Solanum tuberosum* L.

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Polymers comprising raw starch granules are packed in a layered structure consisting of alternating crystalline and amorphous layers. Study of the morphological parameters of granules can provide a deeper understanding of the biochemical mechanisms of their formation and reactivity during (bio) chemical transformations. Morphology and size of starch granules are supposed to be primarily determined by starch biosynthesis genes, in particular, genes that encode SBEI and SBEII enzymes (Starch Branching Enzyme). However, it can be assumed that the set of genes affecting the morphology of the granules is wider than the structural genes of starch biosynthesis. The aim of this work is to search for genomic regions associated with parameters defining morphology of potato starch granules with genome-wide association studies (GWAS) using the Illumina 22K SNP potato array-genotyped collection (90 cultivars) from ICG “GenAgro” collection. Evaluation of starch granules was carried out on such parameters as the area, aspect ratio, circularity, roundness, feret, min. feret, solidity by the method described previously [1]. The data obtained as a result of phenotyping and genotyping were processed using Microsoft Excel, Tassel 5 and the R software. Currently, analysis for the granules circularity is completed. Parameter “circularity” characterizes how elongated the granules are. Granules with the same projection area may vary from round to elongated (from 0 to 1). For the studied potato starch samples parameter “circularity” varies in the range from 0.79 (cultivar Ladozhsky) to 0.87 (perspective line G.3-43-6). Data analysis with the use of GLM (Generalized Linear Model) + PCA (Principal Component Analysis) revealed a significant association with SNP located on chromosome 11. A detailed study of the identified genomic region is conducted in order to create proper diagnostic DNA-marker for further accelerated selection of plants with the required values of morphological starch granules parameters.

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## Molecular, cytogenetic, and morphological features of primary octoploid triticales

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Triticale ( $\times$  *Triticosecale* Wittmack) is artificial intergeneric hybrid that combine genomes of wheat (*Triticum* spp.) and rye (*Secale* spp.) Triticales are a convenient biologic model for study of genetic and epigenetic changes associated with allopolyploidization in Triticeae. Remote hybridization of plants induces genomic rearrangements, which may cause the elimination of chromosomes of one of the parents, chromosome number reduction owing to fusions and rearrangements, and gene expression modification. Faithful chromosome segregation in mitosis and meiosis depends on the absence of aberrations in centromere function. The centromere structure is determined by the centromeric histone H3 variant, CENH3. We studied the *CENH3* structure and expression in wheat-rye allopolyploids with various ploidies and their parental forms. The cytogenetic analysis included the comparison of in situ hybridization patterns with the highly repetitive pSc200 DNA probe and centromere measurement in the allopolyploids and parental forms. Allopolyploids (genome AABBDDRR) were synthesized by crossing isogenic line Triple Dirk D (*Triticum aestivum* L.) (AABBDD) with rye (*Secale cereale* L.) cv. Korotkostebel'naya 69 (RR). Hybrids of generations  $S_1$  through  $S_5$  were analyzed. The coding sequences of  $\alpha$ CENH3 N-terminal tail from hybrid  $S_2$ – $S_5$  plants with different chromosome numbers ( $2n = 42$ – $56$ ) and their parental forms were amplified with specific primers, and their analysis revealed nonsynonymous nucleotide substitutions characteristic of wheat and rye. The expression level of *CENH3* copies with SNPs characteristic of rye *CENH3* increased from generation  $S_2$  (11 %) to generation  $S_5$  (57 %). Fluorescent in situ hybridization analysis (FISH) with the highly repetitive DNA probe pSc200 showed identical hybridization patterns in hybrids having different numbers of chromosomes ( $2n = 42$ – $56$ ), which were also the same in the parental rye variety Korotkostebel'naya 69. Stable octoploid triticales ( $2n = 56$ ) preserved the rye chromosome set throughout generations  $S_2$ – $S_5$ , but the plants showed phenotypic differences.

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## Bioresource collections of vegetable plants as an initial material for breeding cultivars with high biochemical value and for obtaining functional foods

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The human habitat that has deteriorated in recent decades causes drastic changes in the assortment and variety of foodstuffs. It greatly contributes to the reduction of their biological value. Bioresource collections of vegetable plants created in Russia successfully used for breeding cultivars of cultivated plants and introduction of new crops. The breeding of vegetable crops – super producers of biologically active substances distinguished by the stability of this trait is an important task for the near future. The Central Siberian Botanical Garden SB RAS (CSBG) create a genebank of vegetable seeds and related wild species, numbering 10754 samples, including 133 species belonging to 44 genera and 13 families. Based on the registered collection UNU No. USU 440534 for the first time in Russia 5 varieties of new cultures have created and included in the Russian State Register of Breeding Achievements: cultivars of cowpea (*Vigna unguiculata*), bitter melon (*Momordica charantia*), kiwano (*Cucumis metuliferus*) and wax gourd (*Benincasa hispida*) distinguished by their high biochemical value. The content of ascorbic acid in the fruits of cowpea samples reaches 83.9 mg% (cultivar Yunnanskaya), whereas in common bean not exceed 22.9 mg%. Bitter melon fruits are distinguished by their high content of carotenoids (68.9–177.6 mg% in aryllus), 350.8–545.1 mg% in leaves, FW and ascorbic acid (72.5–127.5 mg%). Elevated concentrations of Zn are noted in the fruits of bitter melon, cowpea and kiwano (32.9–57.6 µg/g) whereas its content in the fruits of tomato is 18.5 µg/g. The fruits of cowpea accumulate an increased amount of Mo (5.47 µg/g). Coefficient of variation (Cv) of the macro-element content (K, Na, Ca, Mg, Fe) in the cowpea seeds, when grown in 2018 in the CSBG and in the Nikitsky Botanical Garden, Crimea was the smallest in cv. “Siberskiy razmer” and sample of *Vigna catjang* (14.3 %).

## **Influence of molybdenum and tungsten on the enzymatic activity of molybdenum enzymes**

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A serious problem in the study of the role of heavy metals is that many of them in plants perform the function of vital trace elements. Therefore, when considering the toxicity of heavy metals, it is necessary to carefully assess the dependence of the reaction of plants on the concentrations of each metal and to analyze the different effects of heavy metals on wild and cultivated plants. The aim of our work was to study the effect of heavy metals such as molybdenum ( $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ ) and tungsten ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ) in different concentrations (0.1 mM, 0.5 mM and 1 mM) on the Moco containing enzymes in barley *Hordeum vulgare* L. seedlings.

Morphometric parameters of plants showed that Mo has a positive effect on growth of shoot and root systems, while W inhibited the growth, especially in the root system compared to their controls. Further in gel activity of Moco containing – AO and XDH enzymes were examined.

The results showed three different isoforms of aldehyde oxidase – AO1, AO2, and AO3 in the 7 days old barley roots. While in the leaves, AO activity was mild, there was no obvious distribution among individual isoforms. At high concentrations (0.5 mM and 1 mM) of tungsten, AO activity was completely disabled compare to other treatments. Adding different concentrations (0.1 mM, 0.5 mM and 1 mM) of molybdenum salts increased AO activity of the AO1 isoform compared with the control samples.

Investigation of XDH activity showed higher intensity in Mo-treated (0.1 mM and 0.5 mM) seedlings, but high concentration (1 mM) of Mo inhibited of XDH activity. In addition, tungsten treated seedlings with 1 mM concentration had negative effect on activity of xanthine dehydrogenase compared to all other samples.

## Tissue-dependent transcription of the rye centromeric histone CENH3 variants

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The assembly site for the kinetochore complex of active centromeres is defined by the chromosomal location of the centromeric modification of histone H3 (CENH3). The loss of CENH3 from centromeres leads to improper chromosomal segregation during cell division. In most diploid genomes, including cereals (maize and rice), in which the structure and copy number of *CENH3* have been determined, CENH3 is encoded by a single gene. However, some diploid species in the tribe Triticeae have two variants of *CENH3* gene. Previously we have shown the presence of two main forms of protein,  $\alpha$ CENH3 and  $\beta$ CENH3 in rye species (the genus *Secale*, belonging to the tribe Triticeae). In rye the average nucleotide identity between  $\alpha$ CENH3 and  $\beta$ CENH3 is 81–83 %, with main amino acid sequence difference in NTT domain and in  $\alpha$ 1-helix and loop 1 of HFD domain (CATD). Due to the presence of two main forms of the histone CENH3 instead of one, it is of great interest to study their functions. We suppose that the comparative study of the expression levels of  $\alpha$ CENH3 and  $\beta$ CENH3 in different tissues can shed light on this problem. Here we determine the expression levels of  $\alpha$ CENH3 and  $\beta$ CENH3 in various tissues of rye, *Secale cereale* var. ‘Imperial’. The highest level of expression of both *CENH3* forms was found in reproductive tissues (anther and carpel). The expression ratio of  $\alpha$ CENH3 and  $\beta$ CENH3 varies depending on the type of tissue, for example, the average expression level of  $\alpha$ CENH3 was higher than the average expression level of  $\beta$ CENH3 in leaves but they were comparable in carpels. Our results differ from the results obtained on barley, where expression level of  $\beta$ CENH3 significantly exceeded expression level of  $\alpha$ CENH3 in all studied tissues.

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## Male sterility in potato – perspectives for developing hybrid seed breeding

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Common potato (*Solanum tuberosum* L.) is the most important non-grain crop. Until recently vegetative propagation by tubers has been the main method of potato cultivation. A shift of interest to sexual potato reproduction by true botanical seeds is due to the appearance of a new hybrid seed breeding strategy. The main expected advantages of this strategy is a decrease in the risk of plant contamination because most potato pathogens are not transmitted with pollen and seeds. Successful application of F<sub>1</sub> hybrid seed breeding for many other crops was supported by cytoplasmic male sterility and/or by nuclear(genic) male sterility which allow to avoid emasculation.

Eight cytoplasmic types can be distinguished in potato with DNA markers: A, M, P, W/beta, W/alpha, W/gamma, D, T; three last types are known as sterile which exhibit a different phenotypic appearance of male sterility. This investigation is focused on the study of cytoplasmic types in Russian potato gene pool and estimation the perspectives of their application for developing hybrid seed breeding.

Three major sterile cytoplasm types have been detected in the subset of ~220 Russian potato varieties: T (~43 %), D (~47 %), W/gamma (~10 %).

According to the results of pollen stainability test, many of varieties with T and D sterile cytoplasm types had a high pollen fertility level. Some of them were used as effective pollinators that indicating possible presence of functional alleles of nuclear fertility *Restorer* genes. Analysis of polymorphism in the sequences of *RFL-PPR* gene homologs in genotypes with T and D cytoplasm types demonstrate that the analyzed sequences belong to the *RFL-PPR* gene subfamily and may be considered as *Rf* gene candidates in potato.

In contrast, genotypes with W/gamma<sub>sto</sub> cytoplasm had always sterile pollen and were characterized by male tetrad sterility when tetrads fail to disintegrate in microspores. The data of metabolite profiling of the anthers in fertile and male sterile (W/gamma<sub>sto</sub>) genotypes support the nuclear-cytoplasmic male sterility postulated earlier for potato.

## Methods of computer vision to extract the quantitative characteristics of the wheat spike

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The shape and structure of the wheat spike is one of the most important characteristics of cereals associated with their economically valuable qualities such as productivity, the absence of ear fragility and ease of threshing. The study of the genes controlling these traits will allow us to purposefully create new varieties with improved characteristics in terms of yield, ease of thresh and resistance to environmental factors [1]. Evaluation of wheat spike characteristics in most modern studies is performed by an expert based on the visual analysis and measuring practices, which requires a significant investment of time, despite the fact that in modern experiments tens of thousands of plants are analyzed. Automation of this time-consuming process through the introduction of digital image analysis technologies is relevant for modern science. We propose the method of wheat spike morphometry based on the analysis of digital images. This method allowing extract a number of signs of the spike, such as the spike length, width, area projected on the image, color, awns volume, etc. The proposed approach allowing analyze the shape of the ear, which is an important characteristic that is closely related to the species of the plant, which in turn can be used to identify varieties. In this work, 1454 images of 382 plants were analyzed. The obtained morphometric data were loaded into the SpikeDroidDB database ([2], <http://spikedroid.biores.cytogen.ru>). The method showed high accuracy for determining the qualitative and quantitative characteristics of wheat spike.

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## Identification and characterization of a barley gene controlling cuticle wax formation

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The deposition of lipid-based polymers at the surface of plant epidermis affects a large set of diverse traits in crops. The group of *WAX INDUCER1/SHINE1 (WIN1/SHN1)*-like transcription factor genes belonging to the *APETALA2/ETHYLENE RESPONSIVE ELEMENT-BINDING PROTEIN (AP2/EREBP)* gene family has been shown to be involved in the regulation of lipid biosynthesis and cuticle formation in plants. Taking a reverse genetic approach, the present study aims to reveal functions of few *WIN1/SHN1*-like genes in barley. The site-directed knockout of four *WIN1/SHN1*-like genes in spring barley (cv. Golden Promise) was performed using Cas9 endonuclease targeted to a region conserved across all four related genes. An analysis of T0 and T1 mutants revealed different combinations of mutated target genes in individual plants. Various mutant phenotypes were identified in T1 families, which included a deficiency in cuticle wax deposition at the stem and leaf surface. Co-segregation analysis revealed this wax-deficient phenotype to be associated with mutations in one of target genes, namely *HvWin1*. The cuticle wax measurement and scanning electron microscopy showed that independent mutations in the *Win1* gene invariably lead to a significant reduction in total wax deposition at the leave blades, while stem and leaf sheaths of such mutants were almost entirely free of wax. These results suggest an organ-specific transcriptional regulation of cuticle wax accumulation and *Win1*-mediated activation of cuticle wax component biosynthesis or transport in barley. Cuticle wax-deficient barley mutants constitute a useful experimental model for further studies on the transcriptional regulation of cuticle organization and the role of cuticle composition in the context of biotic and abiotic stress resilience.

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## Study of genetic basis of the melanin biosynthesis in barley grain

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In barley (*Hordeum vulgare* L.), the black grain pigmentation trait caused by the formation of uncharacterized polyphenolic compounds melanins in hulls and pericarp was described. The trait is monogenically inherited by the *Blp* locus mapped on chromosome 1H. The locus contains 21 genes, nonetheless, the gene controlling black pigmentation has been not identified and isolated yet. The aim of the current study is to carry out comparative analysis of barley near-isogenic lines (NILs) differing by the *Blp* locus to reveal the most likely candidate gene for *Blp*. The uncolored cultivar Bowman and its sister line with black grain i:Bw*Blp* were chosen. A comparative morphometric characteristic of the NILs under abiotic stress conditions, as well as a cytological analysis of the developing grain and a transcriptomic analysis in the hulls and pericarp were carried out. Previously the increased resistance to biotic and abiotic stresses of crops with black grain was reported. However, our data demonstrated substantially reduction of the roots and coleoptiles length in the i:Bw*Blp* line under drought conditions, but similar response to salinity and heavy metal stress with Bowman. Comparative RNA-seq analysis in the hulls and pericarp of the NILs allowed establishing that the differences in allelic state of the *Blp* locus are associated with changes in the expression of more than a thousand genes. Among the genes with increased expression in i:Bw*Blp*, the genes of the phenylpropanoids and fatty acids biosynthesis pathways are mostly overrepresented, while the expression of cellulose biosynthesis genes is significantly reduced in this line compared to Bowman. Using light microscopy, the chloroplast localization of melanin pigments was determined.

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## Study of the role of *Arabidopsis thaliana* RNA-polymerase with dual-targeting RPOTmp in plant early development and stress response

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In a number of dicotyledonous plants, including *Arabidopsis*, transcription of organellar genes is performed by three nuclear-encoded RNA polymerases, RPOTm, RPOTmp, and RPOTp. RPOTmp is a dual targeting protein, which is presumably involved in gene expression control in both mitochondria and chloroplasts. A previous study of *Arabidopsis* insertion mutant *rpotmp* showed that it has retarded growth and development, altered leaf morphology, changed expression of mitochondrial and probably some plastid genes, and decreased activities of the mitochondrial respiratory complexes. To date, the particular importance of RPOTmp as a part of mitochondrial and plastid transcription machinery remains unclear. Despite of the obvious though not fully understood role of RPOTmp in mitochondrial transcription, the function of this NEP polymerase in chloroplasts of dicotyledonous plants is still under discussion. Transcription of plastid genome of lower plants including alga, with the probable exception of *Physcomitrella*, is performed by PEP-polymerase (Plastid Encoded Plastid) only. Contrastingly, benefits of angiosperms using phage-type NEP-polymerase (and eudicots using even two of these) remain unknown. The aim of this study is to elucidate what role RPOTmp could specifically play in organelles transcription regulation and possibly in retrograde regulation and cell stress-responses. Previously, plant lines overexpressing RPOTmp with either mitochondrial or plastid targeting based on the *A. thaliana* wild-type line as well as lines with RPOTmp complimentary functions based on the *rpotmp* mutant line were obtained and used for microarray analysis. The transcript level of selected genes isolated from these transgenic plants germinated in different conditions were used for real-time qPCR analysis. It was noticed that some lines with RPOTmp overexpression have increased germination rate under stress condition. We suppose that these lines might have an increased metabolism rate. Also, the bioinformatics analysis of the promoter region of the *Arabidopsis rpot2* gene encoding RPOTmp for different regulatory motifs is presented.

## Marker-based development of wheat near-isogenic and substitution lines with high anthocyanin content in grains

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Bread wheat (*Triticum aestivum* L.,  $2n = 6x = 42$ , BBAADD) is one of the most important cereal crop. Today there is increasing interest in production of wheat with high anthocyanin content in grain, as a source of functional foods. Anthocyanins can be produced in wheat grain either in pericarp (under control of genes *Pp*) or in aleurone (*Ba*). Previously, on the genetic background of Saratovskaya 29 cultivar we developed a set of near-isogenic lines having different combinations of anthocyanin biosynthesis regulatory genes *Pp-A1*, *Pp-D1* and *Pp3* as well as a blue-grained substitution line (BC<sub>7</sub> progeny) carrying the wheatgrass (*Agropyron elongatum* Host.) *Ba* genes. Chromosome C-banding, FISH and microsatellite analysis showed substitution of wheat chromosome 4D by *Ag. elongatum* chromosome 4Ag. The line was designed 'S29(*Ag. elongatum* 4Ag(4D))'. We constructed diagnostic markers for the dominant alleles of *Pp3* (*TaMyc1*) inherited from purple wheat and *Ba* (*ThMyc4E*) inherited from *Ag. elongatum*. The markers together with microsatellites linked to these genes were used for selection of plants with dominant alleles at the *Pp* and *Ba* loci. In the F<sub>2</sub> progeny, obtained after crossing the blue- with the purple-grained lines, in addition to deep purple-grained plants with the dominant alleles of the *Pp-1*, *Pp3* and *Ba* (4Ag/4D) genes, blue-grained plants with hairy leaves (*Hl* gene is localized in 4BL) were found. FISH and molecular marker analysis of these plants confirmed the substitution 4Ag/4B. The line was designed 'S29(*Ag. elongatum* 4Ag(4B))'. By crossing the new line with the purple-grained line, deep purple-grained plants with dominant alleles *Pp-1*, *Pp3* and *Ba* (4Ag/4B) in one genome were obtained. The substitution and isogenic lines and the allele-specific markers designed in the study can be applied for accelerated obtaining wheat with high anthocyanins content in grains.

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## Assessment of the genetic diversity of barley landraces maintained in the Vavilov Institute of Plant Genetic Resources (VIR) in the world scale

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The Vavilov Institute of Plant Genetic Resources (VIR) maintains a large barley (*Hordeum vulgare* L.) germplasm collection comprising more than 20,000 accessions from 24 different species. For the accessions “passport” data describing geographical origin, taxonomic status and some phenotypic characters are available. No attempt has yet been made to assess the genetic diversity of the collection with the large number of environmentally neutral, easily scorable molecular markers such as single nucleotide polymorphism (SNP). With the modern technology of Genotyping-by-Sequencing (GBS) available there is a good opportunity to evaluate the genetic diversity of the VIR barley collection for use in crop improvement programs. In the frame of the collaboration between VIR and Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) 501 barley landraces and local cultivars from the VIR collection were assessed using high throughput GBS technique. The 501 barley accessions originated from 46 countries and were randomly selected for the analysis based on their diverse phenotypic traits. Two individuals from each accessions were genotyped. Reference based variant discovery pipeline identified 76,501 SNPs out of which 23,733 SNPs with  $\leq 10$  % missing data were selected for downstream study. The yielded SNP data were compared with those of the ‘Bridge’ project combining genotyping data of 22,626 barley DNA samples from the National Crop Genebank of China (NCGC), the Institute of Crop Sciences of the Swiss National Genebank of Agroscope and the IPK barley germplasm collection. The results of GBS approach performed allowed to compare the genetic diversity of the barley landraces maintained at VIR with the barley germplasm diversity preserved at world gene banks.

## Association mapping of agronomically important traits in Russian collection of rapeseed

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Rapeseed (*Brassica napus*) is oilseed crop vastly used to produce vegetable oil for food, chemical, and biofuel industries. Despite the fact that rapeseed is quite popular in terms of trait-genotype association studies the Russian collections which are of high agricultural value remain poorly studied and described. Here we aim to map the agronomically important traits (lodging, plant height, glucosinolates content, time of flowering, seed mass, oil content) obtained for rapeseed collection from All-Russian Research Institute of Oil Crops (Krasnodar, Russia). In order to perform association mapping the information on abovementioned traits were obtained for 105 rapeseed lines. In order to perform genotyping, we applied restriction site DNA digestion procedure followed by Illumina high-throughput sequencing technique which allowed us to obtain around 8 million of paired-end reads for each plant sample in three replicates. Using GATK pipeline we have identified 761304 variants in total of which 221768 were presented by biallelic SNPs. After quality filtration procedure 8251 biallelic SNPs left which were used for downstream analysis. Population structure was revealed using admixture software which allowed to distinguish clear segregation of the rapeseed into two populations represented by winter and spring types of plant, respectively. The association mapping was performed in TASSEL software using mixed linear model approach. This allowed to reveal new potential genotype-phenotype associations. The results on loci significantly associated with glucosinolates content, plant height and flowering will be discussed in detail during the presentation.

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## Determination of loci associated with potato starch resistivity to hydrolysis by $\alpha$ -amylase

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Natural production of raw potato starch with pre-designed physical and chemical properties is a challenging task, which appears to be important for both its industrial biotechnological processing and developing diets for weight reduction as well as for maintenance/treatment and prevention of some diseases (diabetes, obesity, etc.). Resistant starch is considered as important dietary fiber for the nutrition of beneficial bacteria in the large intestine. For the development of potato cultivars with desired starch digestibility the next generation breeding techniques are required. Application of these techniques is based on the information about certain genetic mechanisms, underpinning desired properties. The purpose of this work was to identify the genomic SNP loci associated with resistance and digestibility of potato starch. 90 potato varieties and hybrids from the ICG “GenAgro” collection were genotyped on the Illumina 22K SNP potato array. Raw tuber starch from the same varieties and hybrids samples were isolated and tested for resistance to digestion by  $\alpha$ -amylase. Contrast forms with low (40–52 %) and high (up to 99 %) resistance were revealed. These data were used for further genome-wide analysis of genotype-phenotype associations (GWAS). Tassel 5 software and the R package were used for analysis. Using the GLM (Generalized Linear Model), a significant association of resistance of tuber starch to amylase digestion was found with the genomic region assigned to chromosome 5. Based on the results we plan to convert significant SNP to the convenient CAPs marker for further validation and application for marker-assisted selection of plants with different tuber starch digestibility.

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## Direct shoot organogenesis in vitro from mature embryos of maize

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Biotechnological and genetic engineering methods can significantly accelerate the selection process. An important step in these methods is the regeneration of plants in an in vitro culture. Currently, considerable experience has been gained in the regeneration of maize plants through somatic embryogenesis (Santos et al., 1984; Alatortseva, 1994; Huang, Wei, 2004; Obert et al., 2004; Tang et al., 2006; Ahmadabadi et al., 2007; Rakshit et al., 2010; Joshi et al., 2014; etc.). However, regeneration through callus cultures is often accompanied by somaclonal variability, which is undesirable when cloning unique genotypes and carrying out genetic engineering studies. Unwanted variability can be avoided by plant regeneration through direct organogenesis. Currently, there is information about single successful attempts to induce direct organogenesis for some maize genotypes (Ahmad et al., 2017; Ovchinnikova et al., 2018; Humud et al., 2018). The aim of our study was the clonal micropropagation through direct organogenesis without callusogenesis of the three maize lines: KM (Brown marker), AT-TM (bm, wx, y) and AT-TM (lg, y). The lines AT-TM (bm, wx, y) and AT-TM (lg, y) was developed at the Saratov State University. Mature embryos were used as the primary explants. The conditions for the sterilization of the kernels were detected. For optimal sterilization of grains the “Domestos”, 70 % ethyl alcohol and 0.1 % mercuric chloride were used. The different media for the initiation, micropropagation and rooting were tested. The best results were obtained on MS medium supplemented with 2.0 mg/l BAP. The 3–5 small axillary shoots developed in the basal parts of the explant after 3–4 weeks of cultivation. Microshoots were elongated on the MS medium supplemented with 0.2 mg/l BAP. Microshoots in the length 8–10 mm were rooted on the MS medium without hormones. *Acknowledgements:* This work was supported by the Ministry of Education and Science of the Russian Federation as part of the basic part of the state assignment in the field of scientific activity on assignment No. 6.8789.2017/BC.

## **Influence of viral suppressor expression on the activity of molybdoenzymes**

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The study of molecular interactions between plants and pathogens of paramount importance in creating methodological approaches to increase crop productivity. Currently, there are a large number of viruses that infect most plants. The TBSV virus is an effective and convenient model in studying the molecular interactions of plants and viruses. The P19 suppressor protein, encoded by the TBSV genome, protects the viral genomic RNA by binding small interfering RNA duplexes, thereby blocking RNA interference at the initial stage of infection. Systemic infection and the development of symptoms is inextricably linked to the level of P19 accumulation in the plant. The aim of our research is to study the effect of a suppressor protein expression on the activity of molybdoenzymes. These enzymes play an important function in plant metabolism and involved in resistance mechanisms to biotic and abiotic factors. Xanthin dehydrogenase plays a significant role in the metabolism of N-heterocyclic compounds. Plant aldehyde oxidase is a key enzyme in the synthesis of abscisic acid. Recent studies indicate the important role of this enzyme in the activation of an oxidative explosion in response to a viral pathogen. In the experiments were used the wild type TBSV virus and its suppressor-defective mutant  $\Delta$ P19 TBSV. Plants inoculated with  $\Delta$ P19 TBSV RNA mutant transcripts succumb to systemic necrosis and show moderate signs of viral infection. This phenomenon is explained by the inability of  $\Delta$ P19 TBSV to express a viral protein, a suppressor of RNA interference P19. P19 is able to isolate both siRNAs and miRNAs, causing morphological disturbances in plant growth. Using the suppressor-defective mutant of the TBSV virus, we have shown the key role of the P19 protein in the activation of aldehyde oxidase isoforms. The multifunctional role of the viral suppressor in the activation of plant defense systems is discussed.



## **Study of *Sorghum bicolor* L. for bioethanol production in the conditions of the South-East of Kazakhstan**

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Sorghum is the fifth most important cereal crop after wheat, corn, rice, and barley. Food sorghum is used as food in 30 countries for more than 500 million people living in tropical Africa and South Asia. Forage sorghum is the main ingredient for the preparation of feed for divorce. Sugar sorghum is grown on an industrial scale for the production of syrup, malt, starch, and protein is also a promising raw material for the production of bioethanol. We have conducted studies on the factors affecting the frequency of formation of morphogenic calluses in a culture of somatic sorghum cells grown in the conditions of the South-East of Kazakhstan. During the cultivation of somatic sorghum cells, it was noted that the frequency of callus cell formation and their morphology was significantly influenced by the original genotype of the donor plant. It should be noted that during the cultivation of somatic sorghum cells, the main problem for most genotypes is the phenolic compounds, which are secreted by somatic cells on the seventh to the tenth day of in vitro cultivation. Phenolic compounds are substances of an aromatic nature containing one or more hydroxyl groups of an aromatic ring. In the process of the research it was found that the frequency of callus formation during the cultivation of somatic sorghum cells in vitro grown in the conditions of the South-East of Kazakhstan depends on the original genotype. Also, sorghum genotypes have been found that are capable of forming morphogenic calli that can be used in cell selection for resistance to biotic and abiotic environmental stress factors. Sorghum genotypes with maximum sugar content in cell sap were selected. The maximum sugar content was found in the Kazakhstani 20, Hybrid 3 and Ac-64 genotypes, where the sugar content was 21.4 %, 20.7 % and 20.3 %, respectively.

## WildPetotaDB – a database for genotype and phenotype of wild tuber-bearing species of the genus *Solanum*

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The development of high throughput phenotyping platforms is necessary to satisfy the increasing demand in fast and precise introduction of desired traits into elite crop material. In ICG, a range of high-performance phenotyping technologies is being developed. These methods allow one to characterize a large number of plants in a short period and avoid the subjective description of plants by man. Most of these methods are based on image processing and allow one to reveal stable phenotypic characteristics and quantitatively describe all phenotypic diversity. The WildPetotaDB database is being created for digital phenotypic data obtained from different samples of wild potato species from VIR collection. The purpose of creating this database is to evaluate and apply the developed digital phenotyping methods for wild potato material and provide a useful tool of optimal experimental sample selection for potato breeders and biotechnologists. The data deposited in the WildPetotaDB include the following fields for each sample: general description with photo, growing conditions, general tuberization ability, set of tuber morphological characteristics, pubescence characteristics, tuber starch content and morphology of starch granules, steroidal glycoalkaloid content and microsatellite markers of each sample. The database is being developed in a way to collect the most relevant information about the accessions, necessary for their further involvement to potato next-generation breeding programs.

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## Creation and characterization of the soft wheat line with centric translocation T2R.2D

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Alien chromatin introgression into the genome of common wheat *Triticum aestivum* L. is the most efficient way to enrich the gene pool of this crop. To enhance the breeding value of wheat, rye *Secale cereale* L. is used as a source of traits. Today, 1RS.1BL translocation is widely used throughout the world, transmitted to the genome of commercial wheat varieties. However, other chromosomes of the rye genome also carry valuable traits such as resistance to biotic and abiotic factors. This work is devoted to the creation and analysis of new forms of soft wheat with the rye chromosome 2R introgression. Backcrossing of the wheat-rye substituted line 2R(2D) with the varieties of soft wheat Saratovskaya 29 (S29) and Novosibirskaya 67 (N67) was carried out. The 2R(2D) line is characterized by high yield, as well as the quality of grain and bread. The plant karyotypes were analyzed with C-banding, GISH and FISH methods. Wheat-rye centric translocations of T2R.2D and 2R chromosomes with long arm terminal deletions were found in the progeny of backcrosses with N67 and S29 respectively. Changes in the chromosome structure influenced the morphological characteristics of plants. Plants with T2R.2D translocations were analyzed according to eight quantitative characteristics: plant height, spike length, number spikelets per spike, number grains per spike, number grains per plant, weight of grains per spike and plant, weight of 1000 grains. A positive effect on the decrease in height in plants with translocation T2R.2D was revealed. The lines with T2R.2D centric translocation were lower (from  $83.32 \pm 1.79$  to  $116.22 \pm 4.71$  cm) than the parental S29 ( $126.3 \pm 2.33$  cm) and N67 ( $121.37 \pm 1.16$  cm) varieties. Since the creation of varieties with a short stem is preferable in breeding for resistance to lodging, these lines may have practical value in breeding programs. Almost all lines with translocations were lower or corresponded to the level of varieties S29 and N67 for the rest of the indicators of yield structure.

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## miRNA and genes of the MYB plant family involved in the response to stress

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MYB transcription factors (TFs) family play a great role in the development, metabolism and plant responses to biotic and abiotic stress. The free energy of miRNA binding, the value of free energy of interaction, the position and schemes of potential binding sites (BS) were calculated using the MirTarget program. To identify miRNAs whose targets are genes of the TF MYB family, a search of 738 miRNAs BS in mRNAs of 124 MYB family genes of *O. sativa* was performed. 56 genes were identified as targets for 40 miRNAs. The BS of osa-miR529a-3p, osa-miR414-5p, osa-miR2919, osa-miR2868-5p, osa-miR2097-5p, osa-miR156a-5p, osa-miR1442-5p in the mRNAs of the *O. sativa* MYB family genes were located in 5'UTR, while osa-miR818a-3p BS in the mRNA of MYB family genes of the *O. sativa* were located in 3'UTR. The osa-miR5075-3p and osa-miR159c-3p BS were located in 5'UTR and CDS mRNA of the *O. sativa* MYB family genes. osa-miR2102 binds to mRNAs of 21 MYB genes. The free energy of interaction of osa-miR2102-5p with the mRNA of these genes varied. osa-miR5809-3p had BS in the mRNA of nine target genes. osa-miR5075-3p and osa-miR5833-5p had four and six target genes, respectively. The remaining miRNAs had only one or two target genes. Studying the binding of 125 miRNAs to the mRNAs of 258 genes of the *T. aestivum* MYB family resulted in a finding that 48 genes were targets for 28 miRNAs. miRNA BS in the mRNA of MYB family genes were located only in the CDS. tae-miR159b-3p, tae-miR164-5p and tae-miR444b-3p had BS in the mRNA of seven genes. tae-miR10518-5p had five target genes. For tae-miR5084-3p, tae-miR171a-3p, and tae-miR10517-5p, targets were mRNA of three genes. tae-miR9676-5p, tae-miR9666a-3p, tae-miR9662b-3p, tae-miR5384-3p, tae-miR398-3p, tae-miR319-3p, tae-miR1127b-3p had two BS in the mRNA MYB genes. The remaining miRNAs had only one target gene. The target genes from MYB family of *O. sativa* and *T. aestivum* for miRNAs have been identified, the associations of which were identical for both plant species.

## Redesign of starch biosynthetic pathway in rice by CRISPR/Cas9-mediated genome editing toward human diets

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CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated Cas9 endonuclease)-mediated genome editing has revolutionized biological research and crop improvement because of its specificity, simplicity, and versatility. Editing a gene by CRISPR/Cas9 only has three requirements: (1) expression of the nuclear localized Cas9 protein; (2) production of a guide RNA (gRNA) molecule, whose first 20 nucleotides are complementary to the target gene; (3) the NGG PAM site that is located immediately adjacent to the 30 end of the target sequence. The majority of the reported CRISPR/Cas9-mediated gene editing in plants belongs to this category. CRISPR/Cas9-mediated gene editing technology has the potential to greatly facilitate plant breeding. However, so far only a very few examples of improvement of agronomic important traits and creation of novel germplasm in crop plants have been reported. Here, we succeeded in constructing individual target gene editing objects for 22 genes related to starch biosynthesis in rice via CRISPR/Cas9. A total of 1685 T0 plants (60 sgRNAs per each) were analyzed by NGS for genetic modification, resulting in a mutation in the target gene of 965 individuals. From these mutants, T1 seeds were grown through single copy and homo-, hetero-, di-allelic studies and used for selection null plants. We defined the roles of some genes example SBEI and SBEIIb etc, in determining the amylose content, fine structure of amylopectin, and physiochemical properties of starch. This work enables the improvement of nutritional properties of starch in rice grain, thus potentially providing health benefits to many people.

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## Reduced ethylene production in tomato fruits upon CRISPR/Cas9-mediated LeMADS-RIN mutagenesis

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Recent progress in genome editing methods has opened new opportunities for reverse genetics-based studies in plants. The clustered regularly interspaced short palindromic repeat (CRISPR) system is a novel strategy used to induce mutations in a specific genomic region of a variety of organisms, including plants. Here, we describe a high-frequency targeted mutagenesis utilizing *Agrobacterium*-delivered CRISPR/Cas9 in tomato. This system consists of an *Agrobacterium* binary vector and three guide RNAs for single gene targeting. We evaluated the system for its mutagenesis frequency and heritability using LeMADS-RIN gene of tomato. T<sub>0</sub> transgenic events carrying mutations in the LeMADS-RIN gene occurred at rates over 10.6 % mutants per transgenic event in both ‘Mamirio’ and ‘Golden bell’ tomato genotypes. Three independent T<sub>1</sub> transgenic lines and wild-type (WT) tomato plants were used for ethylene analysis. Compared with WT plants, edited mutants exhibited more incompletely-ripening fruits and lower ethylene contents. Following genetic combination through segregation, null segregants carrying only the desired mutant alleles without the CRISPR transgene could be retrieved among the T<sub>1</sub> progeny. These Cas9/gRNA transgenic lines, therefore, can be used to convey the CRISPR-based mutagenesis by genetic cross to tomato lines that are not amenable to genetic transformation.

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## Study of the introduction collection of the *Miscanthus*

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Currently, the most accurate methods for establishing variety identity are DNA polymorphism analysis methods. One of such methods widely used for certification is microsatellite analysis. Microsatellite markers developed for *Miscanthus* have not yet been introduced into practical application for the certification of varieties. The aim of the current study was to develop a method of genetic certification of samples of the *Miscanthus* collection based on microsatellite analysis. A total of 47 *Miscanthus* samples were studied: 23 accessions from the introduction collection (21 accessions of *M. sacchariflorus*, 1 hybrid of *M. giganteus* and 1 hybrid sample, which taxonomic assignment is still to be clarified) and 24 plant samples of *M. sacchariflorus* cv. Soranovsky, having different morphotypes. The latter was included to study intravarietal polymorphism of the cv. Soranovsky to check whether there are genetic differences between different ecotypes. When selecting plants cv. Soranovsky, attention was paid to such signs as the presence (plants no. 1–7) and absence (plants no. 9–16) of a full panicle, as well as a tendency to lodging (plants no. 17–24). A total of 10 primer pairs for amplification of highly polymorphic microsatellite loci were selected from the literature. Based on the cross-assessment of these primers for the formation of dimers, the optimal combinations for multiplex PCR were selected. The PCR testing was carried out on individual primer pairs on the extracted DNA. Three sets of fluorescently labeled markers were designed for further multiplex PCR and fragment analysis. Identification of various samples of *Miscanthus*, as well as evaluation of intraspecific polymorphism of the cv. Soranovsky, is carried out.



## Expression in potato plants of phosphomimetically mutated gene *AteIF2 $\alpha$* , coding for alpha subunit of translation initiation factor 2 from *Arabidopsis thaliana*, provides resistance to drought

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The cDNA-gene *AteIF2 $\alpha$*  from *A. thaliana* was amplified by RT-PCR and cloned in pUC19 vector. *In vitro* mutagenesis was carried out replacing the 56th serine codon with triplet encoding phosphomimetic aspartic acid. *AteIF2 $\alpha$ (S56D)*-gene was cloned in binary agrobacterial vector pCambia2300 under the control of either constitutive (35SCaMV) or stress-inducible (*rd29A*) promoter. Translational enhancer  $\Omega$  (5'UTR of TMV) was inserted downstream of *rd29A* promoter.

The resulting DNA constructs [*35SCaMV*-(*His-tag*)-*AteIF2 $\alpha$ (S56D)*-*nos*\_pCambia] and [*rd29A*- $\Omega$ -(*His-tag*)-*AteIF2 $\alpha$ (S56D)*-*nos*\_pCambia] were transfected into agrobacteria cells, which were then used for vacuum infiltration of tobacco leaves for transient expression. Using RT-PCR, the transcription of (*His-tag*)-*AteIF2 $\alpha$ (S56D)*-transgene is shown in leaves on the third (from 35S) and fifth day (from *rd29A*). On the same days, the synthesis of (*His-tag*)-*AteIF2 $\alpha$ (S56D)*-protein was confirmed by immunoblotting using anti(*His-tag*) antibodies.

Stable transformation of virus-free potato plants of 'Milena' variety was carried out. Regenerated plants were tested by PCR for presence of transgenic inserts. Total RNA preparations were isolated from transgenic plants and analyzed by RT-PCR to assess the levels of transgene mRNA synthesis. Synthesis of mRNA was confirmed only in plant lines that were genetically modified (GM) by DNA-construct [*rd29A*- $\Omega$ -(*His-tag*)-*AteIF2 $\alpha$ (S56D)*-*nos*\_pCambia]. These lines were propagated and tested for resistance to elevated temperatures and to water deficiency.

In particular, the tested GM-potato line No. 91 expressing the *AteIF2 $\alpha$ (S56D)* transgene showed significant resistance to drought. Similarly, all control potato plants of 'Milena' variety died after 14 days without watering, whereas all GM-plants of No. 91-line survived after 21 days of drought.

This technology for improving plant resistance to abiotic stresses can apply not only to potatoes, but also to other economically important crops.

## MIGREW database: typical use cases

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Recently we have released the MIGREW database (<https://migrew.sysbio.cytogen.ru/migrew>). It stores information on wheat immunity to rusts and powdery mildew that shared between the following objects: diseases, plants, chromosomes, genes, markers, protocols, pathogens and papers. The data focuses on effectiveness of wheat resistance genes in different regions of the Russian Federation. For each object of the database, one can obtain all levels of objects it is related with. Some typical use cases with the database were implemented in the MIGREW web application. On the other hand, these use cases are hardcoded through the application web forms. It is a challenge to get information that is not presented in the predefined author's logic. To take into account possible users requirements we have also developed REST API for the MIGREW database ([https://migrew.sysbio.cytogen.ru/migrew\\_api](https://migrew.sysbio.cytogen.ru/migrew_api)). It is the direct way to the data acquisition that could be used by any programming/modeling tool that support REST service calls (Python, R or Matlab, for instance). If one uses Python in a daily work, he/she is familiar with the wide set of available tools and libraries for data analysis and data representation. Using these tools, one can write a script for data processing and results visualization. Here we demonstrate several use cases of the MIGREW data access and their visualization on global map with Python through the REST API: first – visualization of effectiveness of the wheat resistance gene or group of co-segregated resistance genes in the regions of the Russian Federation; second – occurrence rate of the virulence gene in the pathogen populations of the Russian Federation. Using these scenarios as a basis, one can develop his/her own scripts for MIGREW data accessing/processing and visualization in Python.

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## Assessment of genetic diversity among Siberian stem rust isolates using SSR markers

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The genetic diversity of the fungus *Puccinia graminis* is extremely high. Within the species, there are several special forms (f. sp) virulent to different cereals, and there is a differentiation of *P. graminis* f. sp. *tritici* (Pgt) determined by the resistance of wheat varieties. The objective of this study was to develop the panel of SSR markers to estimate the level of genetic diversity among the Siberian stem rust isolates. The effectiveness of 20 SSR markers used in the international rust laboratories for Pgt differentiation (Zhong 2009, Berlin 2012, 2017) was tested on a sample of 14 plants infected with stem rust: wheat varieties and wild grasses with urediniospores from Omsk, Novosibirsk, and Altay regions, together with the sexual progeny on barberry leaves (aeciospores) in Novosibirsk. Thirteen markers were polymorphic and only five of them gave amplicons with the sizes expected from published data: 227AAGR/F, 24R/F, CAA53F1/R1, PgCAA8F1/R, CAA49F1/R1. Therefore, this suggests the large genetic distance between Western Siberian and European populations of stem rust. Pgt samples from the same wheat variety – Chernyava 13, collected in Omsk and Novosibirsk regions, showed differentiation on the SSR-profiles of five markers: 109AGGF/R, 227AAGR/F, 293F/R, PgCAA8F1/R1, CAA49F1/R1. These results indicate that two distant Pgt populations exist in these regions of Western Siberia. Four SSR markers (109AGGF/R, CAA98F1/R1, CAA53F1/R1, CAA49F1/R1) were able to differentiate the single pustule isolates obtained from the Novosibirsk population. This set of markers may be useful to inspect the extended sample of Pgt isolates. Most of the tested markers gave the same SSR-profiles for samples of aeciospores and urediniospores from wild grasses, but not from wheat. These results tell us that another special form, not Pgt, could be segregated in sexual progeny on barberries in Novosibirsk.

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## **New breakthrough CRISPR/Cas9 biotechnology of genome editing is a powerful tool for improvement of agricultural crops**

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The technology, a genome-editing tool called CRISPR/Cas9, revolutionized the life sciences when it appeared on the market in 2013. It is now proving useful in the plant science community as a powerful tool for the improvement of agricultural crops. CRISPR/Cas9 system is a simple, inexpensive and versatile tool for gene/genome editing, resulting in which has become known as the ‘CRISPR craze’. The multitude of functions that can be performed with CRISPR/Cas9 make it a molecular tool that will open new opportunities in the complicated world of plant-pathogen interactions and help design durable crop resistance to pathogens. Our goal is to create elite barley cultivars, resistant to viral diseases, in Kazakhstan. Using the CRISPR/Cas9 tool editing of eIF4E gene that conferred resistance to multiple viruses has been successfully engineered in 5 commercial Kazakhstan barley cultivars. Elite crops cultivars will benefit farmers and the local economy. Generally, the CRISPR/Cas9 system for plant genome editing is a breakthrough technology in breeding – prospect for creation of elite high yielding crops that could be exempt from GM classification.

## Genetic diversity of genes involved in fatty acid biosynthesis in a collection of flax cultivars

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Flax (*Linum usitatissimum* L.) is one of the major sources of omega-3 fatty acids (FAs), which provide health benefits for humans. Flaxseed oil is composed of palmitic (PAL, C16:0), stearic (STE, C18:0), oleic (OLE, C18:1), linoleic (LIO, C18:2), and linolenic (LIN or ALA, C18:3) acids, with high levels of LIN and moderate levels of LIO, the essential FAs, being attributed the nutraceutical properties of flaxseed. Genetic control of FA biosynthesis in flax has been studied and genes encoding the enzymes that perform FA synthesis have been identified, however, there is still little information regarding the relationship between the genetic diversity of these genes and fatty acid composition in flax. In the present study, our goal was to analyze the genetic variability for *SAD* (stearoyl-ACP desaturase) and *FAD* (fatty acid desaturase) genes in flax by sequencing these genes in 288 flax accessions with different proportion of FAs, obtained from the Institute for Flax (Torzhok, Russia). For genetic variation analysis, we used generated DNA sequences with an average coverage of 100x for an individual sample from the Illumina platform. Genetic variation data were correlated with FA composition data, in order to determinate the key polymorphisms leading to different proportion of FAs that will provide further information in order to understand the genetic factors controlling FA composition in flax.

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## Expression analysis of intracellular vesicle trafficking superfamily genes, CaRab-GTP, in response to drought, dehydration and salinity in leaves of chickpea (*Cicer arietinum* L.)

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**Introduction and Aim:** Environmental stresses such as drought and salinity inhibit plant growth and productivity. Chickpea is an important legume, moderately tolerant to high temperatures, drought and salinity stress during the growing season. *CaRab*-GTP, intracellular vesicle trafficking superfamily genes, play essential role in response to these stresses. *CaRabC*, belonging to the family of *Rab*-GTP genes, was identified from an SNP database using bioinformatic and molecular genetic analyses. The aim of this study was to identify and analyse the role of *CaRabC* in tolerance to drought, salinity and rapid dehydration in chickpea.

**Methods:** Bioinformatics and systems biology methods were applied in this study to confirm the potentially important role of the target gene in tolerance to abiotic stresses in chickpea. Three experiments applying abiotic stress treatments (salinity, slow drought and rapid dehydration) were carried out. For gene expression, RNA was extracted from control and stressed plants with subsequent cDNA synthesis and qPCR analysis. For SNP identification, Amplifluor SNP analysis, sequencing and bioinformatics were used.

**Results:** Eight sub-families with 54 isoforms of *CaRab* genes were identified and clearly distinguished in the phylogenetic tree based on protein sequences. Levels of *CaRabC* expression were very high in plants subjected to salinity and rapid dehydration, but down-regulated under slowly developing drought. Five isoforms of *CaRabC* were strongly stress- and genotype-dependent, showing differential expressions.

**Conclusion:** *CaRabC* is part of the large *Rab*-GTP gene family. All five isoforms were expressed differently in response to salinity, rapid dehydration and drought. This confirms the important role of this gene in the tolerance of chickpea plants to abiotic stresses.

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## DNA barcodes from four loci provides poor resolution on phylogenetic relationships between the *Triticum* species

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DNA barcoding relatively a novel approach, which was developed to provide rapid, accurate and automatable species identification by using standardized DNA regions. The Consortium for the Barcode of Life (CBOL) plant-working group recommended the 2-locus combination as the standard plant barcode. The evolutions of the chloroplast regions combine with nuclear gens are sufficiently rapid to allow discrimination between closely related species. In this study, we tested the phylogenetic utility of the DNA barcoding loci (ITS2, *matK*, *psbA-trnH* and *rbcL*) for efficient discrimination of *Triticum* species. To assess the barcoding efficiency to resolve the species discrimination, a total of 109 accessions representing 16 recognized genotypes in the *Triticum* genus have been sampled. Topologies of the phylogenetic trees based on combination of DNA barcode analyses were similar, but a few accessions were placed into distant phylogenetic groups. The 109 accessions analyzed in this study were placed into three groups supported by high bootstrap values. However, as expected the barcoding analyses were not able to discriminate some closely related *Triticum* species. Thus, we have proposed, molecular studies with more diverse markers and species will be required to clarify the ambiguities surrounding the phylogeny of these important genera.



## Identification of grain and flour quality determinants in common wheat using GWAS

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In this study we investigated common wheat varieties adapted to the environment of Western Siberia and the Ural. The qualitative characteristics of flour and grain determine the technological properties of the product and its nutritional quality. The decrease of grain baking parameters result in the inability to produce a sufficient amount of good quality bread. Thus, it is necessary to identify new resources to improve these traits. We analyzed grain quality traits, such as nitrogen, protein, thousand grain weight, grain bulk density, vitrescence, falling number, gluten; and flour quality traits: flour particle diameter, flour surface, flour strength, dough resilience, dough strength and extensibility. The population of 92 common wheat varieties was genotyped using the 15K Illumina Infinium SNP array (TraitGenetics GmbH). We used Mixed Linear Model implemented in EMMAX algorithm of R package GENESIS to perform association analysis. The results revealed SNP markers significantly associated with every trait. Some of the detected loci corresponded to known loci, but the others were firstly described. The results obtained will be tested in repeated study. Using the SNP sequences associated with the traits, markers will be developed to use them in further selection process.

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## High-throughput genotyping and transcriptome analysis reveals candidate genes associated with wheat heading time

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Variability of heading time may affect wheat adaptation to different environments. Thus, the detection of new heading time determinants is important for improving cereals. In this work, we used common wheat cultivar Chinese Spring (CS) and the substitution line of CS with 5B chromosome from *T. dicoccoides* (CS-5Bdic), different in their flowering time by two weeks, to detect determinants of heading time on 5B chromosome. Using the RICL population from a cross of CS x CS-5Bdic, we detected QTL in pericentromeric region of chromosome 5B, that was significantly associated with heading time. To determine candidate genes, that affect the trait of our interest, we analyzed transcriptomes of CS and CS-5Bdic using the RNA-seq. Three replicate samples from each genotype were harvested at four time points over 24 hours since the beginning of the light period (0, 3, 9 and 16 hours). The genes showed differential expression between the substituted line and CS were identified in each time point. GO analysis revealed that the DEGs were mainly involved in nitrogen assimilation and metabolism, photosynthesis, regulation of transcription, ATP metabolism. Among the genes, differentially expressed between CS and CS-5Bdic, one of the most interesting is TraesCS5B01G075300, which is higher expressed in CS at all time-points. This gene is localized in the region of the heading time QTL, detected previously. qPCR confirmed the revealed differences in the expression level. The *TraesCS5B01G075300* gene encodes Myb transcription factor. Best hit resulted from blastp analysis against *A. thaliana* is EFM (EARLY FLOWERING MYB PROTEIN) protein, involved in regulation of flowering.

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## Evaluation of genetic diversity of *Fagopyrum esculentum* Moench variety using method of ISSR-analysis

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Species of the genus *Fagopyrum* Mill. are prospective sources of flavonoids, the main among which is the 3-O-rutinoside quercetin. The most important task of selection is to develop new varieties ones with a high content of flavonoids. The study of intraspecific genetic diversity of *F. esculentum* is extremely relevant, due to the presence of a large number of the variety, which differ in the content of flavonoids. In this regard, the aim of this study was to evaluate the genetic polymorphism of the buckwheat variety of different origin with a high content of flavonoids using the molecular marking. Using method of ISSR-analysis there were investigated 5 varieties of *F. esculentum*: Izumrud (Primorsky Krai, Russia), Kitawase 1 (Japan), Kitawase 2 (Japan), Cheremshanka (Tatarstan, Russia), Bashkirskaya with red stem (Bashkortostan, Russia), using four primers. As a result of the PCR there were revealed 106 amplicons, 105 of which were polymorphic. The polymorphism (P) in the joined sample was 99.6 %. However, the variability within the varieties significantly differs from the minimum  $P = 50$  and 50.94 % in Izumrud and Bashkirskaya with red stem accordingly, to the maximum  $P = 75.47$  % in Cheremshanka. Varieties Kitawase 1 and Kitawase 2 have close values of polymorphism  $P = 65.09$  and 64.15 %, respectively. Based on the analysis of the distribution pattern of the revealed fragments, there were calculated the indices of differences – the genetic distances ( $D_N$ ). The highest value of  $D_N = 0.2296$  were defined between the varieties Izumrud and Bashkirskaya with red stem, the smallest  $D_N = 0.0284$  was found between Kitawase 1 and Kitawase 2. Varieties Izumrud, Bashkirskaya with red-stem and Kitawase 1, which have the greatest genetic differences, present the practical interest for the development of new genotypes with a high content of flavonoids.

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## Molecular screening of wheat entries for resistance to tan spot toxins Ptr ToxA and Ptr ToxB *Pyrenophora tritici-repentis*

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Tan spot, caused by *Pyrenophora tritici-repentis*, is a serious foliar disease of wheat in Kazakhstan. The aim of this study was the identification of wheat genotypes resistant to *P. tritici-repentis* against Ptr race 1 and race 5 and their host-selective effectors Ptr ToxA and Ptr ToxB. The common wheat collection of 41 accessions were characterized using the molecular markers Xfcp623 and XBE4444541, diagnostic for the Tsn1 and Tsc2 genes conferring the sensitivity to fungal toxins. The accuracy of marker XBE4444541 with race 5 was 92.11 %, and to Ptr ToxB – 97.37 %. Genotyping results using the Xfcp623 marker confirmed the expected response to Ptr ToxA; the presence/absence of the Xfcp623 marker completely (100 %) coincided with the sensitivity/resistance to race 1 and Ptr ToxA. It demonstrates the reliability of a diagnostic marker of Xfcp623 for identifying wheat genotypes with resistance the fungus and insensitivity to toxin Ptr ToxA. The study of the reaction of wheat germplasm to the fungal inoculation and toxins infiltration showed that out of 38 analyzed 30 genotypes (78 %) exhibited resistance to both race 1 and race 5, and insensitivity to toxins Ptr ToxA and ToxB. Of the most significant interest are eight wheat genotypes that showed resistance/insensitivity both to the two races and two toxins. The results of phenotyping were reconfirmed by the molecular markers used in this study. Sensitivity to Ptr ToxB is not always correlated with susceptibility to race 5 and is dependent on host's the genetic background of the wheat genotype, i.e. from a specific wheat genotype. The results of the study are of interest for increasing the efficiency of breeding based on the elimination of the genotypes with dominant alleles Tsn1 and Tsc2, sensitive to the toxins Ptr ToxA and ToxB.

## Cereal signs analysis associated with color on digital images

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The color and textural characteristics of the ear and grains are associated with a number of phenotypic traits that are important for selection. The color of the ear can be associated with the stage of plant development, the content of various metabolites in the plant tissues, or the stressful effects of the environment. Textural signs can be used to detect pathogens or anatomical parts of the spikelet. We propose the results of an evaluation of the method for classifying cereal grains based on color and textural characteristics using machine learning approaches. As part of this work, a data set was created in which images of grains of different genotypes are presented: parental forms of the ITMI mapping population that carry nonallelic genes of red grain; the Opata-85 sort carries the R3 gene (chromosome 3BL), and the synthetics R-93 carries the R1 gene (3DL chromosome). Among 110 recombinant lines, there are genotypes – carriers of one or two genes of the color, or white grain (recessive alleles of both genes), on which the nature and intensity of the color depends. We calibrated the color of images by method used in epilluminous microscopy using the target ColorChecker Mini Classic, which was placed in the area of each frame. Such a correction made it possible to eliminate color distortions in the image arising due to different lighting conditions. The grains in the image were recognized by the method proposed by us earlier and implemented in the SeedCounter mobile application. As a result, a set of metrics that are suitable for describing the texture and color of wheat grains was determined. We evaluated the importance of features in the task of classifying grains according to color characteristics and implemented the calculation of these features in the SeedCounter mobile application. *Acknowledgements:* This work was supported by the Russian Science Foundation, the project 17-74-10148. Authors are grateful to Pshenichnikova T.A. for plant seed material.

## Metabolic factors of resistance of bread wheat *Triticum aestivum* L. to fungal infections

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Protective reactions of plants from fungal infections are based on structural features of plants tissue and contents of any metabolites having protective effect.

In some of researches participation in protective mechanisms of products of a fenilpropanoid way of metabolism – lignin, lignan and aromatic glycosides is noted. Also resistance of plants can be connected with degree of a mineralization of tissues of a stalk and a leaf, in particular, with silicon content. Terminal reaction of a fenilpropanoid way – formation of monolignol (aromatic alcohols) – is controlled by family of CAD enzymes (cinnamil alcohol dehydrogenase; EC 1.1.1.195). The of F3 and F4 progenies of spring bread wheat differing on CAD genotypes, received from crossing of a cultivar Novosibirskaya 9 with nulli-tetrasomic lines of a cultivar Chinese Spring. The progenies were landed on the infectious field and showed various degree of susceptibility to brown rust. These plants were used for the analysis of leaves tissues on micromorphological and chemical characters. Large plaques and spot consisting of mineral compounds were observed on the leaf surface of the more resistant plant. On a surface of leaves of a sample, susceptible to brown rust, the high content of salts of calcium whereas on a surface of leaves of steady genotypes silicon oxide prevails is revealed. In steady against defeat by brown rust samples the content of lignin is higher concerning carbohydrates. Leaves of plants unstable to a fungal infection contain less chlorophyll b that can cause an arrest of development and flowering of plants. In leaves of steady genotypes the high content of aromatic acids is revealed. It is possible that the observed differences lead to afflict the plants with leaf rust to such different degrees. In that case these characteristics can be used for diagnostics of potential resistance of cultivars to fungal infection.

## Differences in the genetic mechanism of the response to stress between wheat varieties

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**Motivation:** *Triticum aestivum* is one of the most important agricultural plants. Abiotic stresses such as drought and cold are a common cause of reduced productivity of this culture. Resistance to abiotic factors is a complex (multigenic) traits. The study of the stress response mechanisms of *T. aestivum* is an actual goal of basic research and has practical significance. To identify a complete set of genes an analysis of full transcriptome data is necessary. Transcriptome sequencing was performed using MACE method under control and stress conditions.

**Method and algorithm:** In this work we used plants of bread wheat (*Triticum aestivum* L.) varieties Saratovskaya 29 and Yanetskis Probat with different abiotic stress tolerance. The plants were planted in mobile hydroponic pots and grown under controlled day/night cycle (16/8 hour). Cold stress treatment were simulated in a climatic chamber at +4 °C for 6 and 24 hours with the same lighting conditions. Drought simulated by discontinuation of watering. After treatments tissue samples were sampled, freezed in liquid nitrogen and stored at –80 °C. Sequencing was performed on Illumina NexSeq 500 platform. The FastQC program was used to analyze the quality of the libraries. Trimmomatic was used to filter the libraries. STAR was used to mapping reads to reference genome. Search for the differentially expressed genes was performed by featureCounts from package subread-1.6.3-source and EdgeR.

**Results:** Analysis of *T. aestivum* transcriptomic data revealed 1292 genes differentially expressed under stress conditions. Most of the genes are differentially expressed in cold. The gene lists of cold-response between the studied varieties are intersect more strongly. Differences in the mechanisms of reaction to stress between the studied varieties were revealed. Identified genes can be used in subsequent genome selection.

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## Identification of the molecular markers linked to the chosen genes in cereals

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Nowadays, plant breeding worldwide is routinely supported by the application of the solutions developed by biotechnology. One of the most important milestones, which allowed for significant improvement of the selection process was the application of the molecular markers for genotyping of the plant material (marker assisted selection, MAS). The major advantage of this approach is fast and reliable identification of the materials carrying desirable genes in the genome. However, the limitation of the MAS is often the insufficient availability of the molecular markers useful for application. Because of that fact development of the new molecular markers linked to important genes (e. g. dwarfing or resistance genes) is of great importance.

Research performed in the Institute of Plant Genetics, Breeding and Biotechnology allowed for the development of the novel sequence specific molecular markers, which has a potential for application in plant breeding. The most important findings include identification and development of molecular detection methods for the novel genetic sources of dwarfism in triticale as well as two genetic sources of powdery mildew resistance in oat. These new genes were introduced into triticale and oat genomes from wild relatives. Subsequently, on the basis of obtained plant material, reliable methods of their molecular detection, based on sequence specific markers, have been designed. Moreover, as a result of our studies, we have adapted molecular markers developed for detection of the several wheat leaf rust (*Lr*) and powdery mildew (*Pm*) resistance genes to an application in triticale in both singleplex as well as multiplex format. Developed molecular markers may provide a valuable tool for detection of the presence in cereals genomes selected genes important for their breeding programs.

## A transcriptome-base analysys of lilac apical complexes *in vivo* and *in vitro*

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Comparative transcriptome analysis of vegetative apices of *Syringa vulgaris* L. during physiological rest or in phase of active growth *in vivo* and *in vitro* were carried out. For transcriptome analysis the two type of lilac's shoots were used: (1) the aseptical culture of *Syringa vulgaris* L. shoots cultivated in the lab since 2008, (2) the adult plants growing in the open ground of MSU Botanical Garden. For experiment the aseptical microshoots were precultivated under slow-growth culture condition during 270 days after then apices of one half of shoots were cutted and fixed in RNA-later. The remaining shoots were transferred to normal condition and growing for 28 days. Likewise the vegetative apices of adult *S. vulgaris* shrubs were collected under physiological rest (in winter) and from active growth (in springtime). The total RNA was purified with RNeasy Plant Mini Kit (Qiagen) from 20 mg of each fixed samples. The libraries were prepared using NEBNext® Poly(A) mRNA Magnetic Isolation Module and NEBNext®Ultra RNA Library Prep Kit for Illumina (NEB) and sequenced using HiSeq 2500 System. Reference transcriptome was assembled by Trinity, transcript quantification were made by Salmon and differential expression analysis were conducted using edgeR. A pairwise comparison of samples showed different level transcripts of two  $\beta$ -glucosidase isoforms, five superoxide dismutase isoforms, four peroxidase isoforms and three xyloglucan xylo glucosyltransferase isoforms in slow-growth condition *in vitro* vs. physiological rest lilac's plant apices *in vivo*. And different level transcripts of seven superoxide dismutase isoforms, three  $\beta$ -glucosidase isoforms and cytochrome p450 isoforms in lilac's shoot apices of active growth plants *in vivo* vs. *in vitro*.

## **Postgenomic technologies in practical forestry: development of DNA markers and population genetic databases for timber origin identification, genetic monitoring, breeding and other applications**

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The forest genetics, tree improvement and protection can greatly benefit from complete genome sequence data made recently available for several major conifer species. They allow to identify and annotate genes, other functional elements (sRNA, transcription factors, regulatory elements, etc.), and genetic networks that control adaptation and disease resistance. They can be used to develop highly informative genetic markers that can be applied in population genetic studies to create database of barcodes for individual populations to fight illegal timber harvest and trade. They are very much needed for development of genome-wide genetic markers for association studies for linking genetic variation (SNPs, alleles, haplotypes, and genotypes) with environmental factors, adaptive traits and phenotypes for better understanding genetic control of agronomically and economically important traits. They can be also used to develop genome-wide genetic markers for genomic-assisted selection to breed for better adapted, stress resistant and climate change resilient trees with desirable quality ecological and economic traits. Finally, whole genome sequences allow to integrate proteomics, transcriptomics, and metabolomics and provide reference genomes for resequencing. One of the most important practical applications of genomics in forestry, which will be presented in detail is development of highly polymorphic and informative DNA markers for several very important boreal forest species in Eurasia, Siberian larch (*Larix sibirica* Ledeb.), Siberian stone pine (*Pinus sibirica* Du Tour), and Scots pine (*Pinus sylvestris* L.), based on the whole genome data obtained in the “Genomics of the Key Boreal Forest Conifer Species and their Major Phytopathogens in the Russian Federation” project funded by the Government of the Russian Federation (grant No. 14.Y26.31.0004).

## Perspectives of using Illumina MiSeq for identifying obligate symbionts of plants – arbuscular mycorrhiza fungi

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Arbuscular mycorrhiza fungi (AM) form one of the most common symbiosis with majority of land plants. AM fungi supply the plant with various mineral elements, primarily phosphorus, and improve the water supply. Search of the most symbiotic effective AM strains and the creation of microbial preparations on their basis is an important task for modern biology. The identification of AM is very difficult. This is primarily due to the high genetic AM polymorphism, as well as the difficulties of their cultivation without a host plant. The morphological identification of AM is often unreliable due to high number of cryptic species among AM. In recent years increases the number of AM biodiversity studies performed by modern NGS-based methods, in particular Illumina MiSeq. Using the Illumina MiSeq eliminates the need for a sequencing of large number of clones. Currently, there are still many questions in the identification of AM fungi. The most important of them are the choice of a genetic marker for the barcoding of AM fungi – conservative or variable sequences, as well as the choice of primers – specific for AM or universal. Another significant problem for molecular genetic identification of AM is DNA isolation. In our work, we successfully use universal primers ITS3 and ITS4 for the sequencing in Illumina MiSeq the 5.8SrRNA–ITS2 region, which contains both a conservative and variable regions. The effectiveness of identification of AM isolated from the roots of a host plant varies around 50 percent. When DNA is isolated from a spore, efficiency dropped to 10 percent.

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## Marker-assisted selection of new barley genotypes accumulating anthocyanins in grain

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Barley (*Hordeum vulgare* L.) is an agronomically important crop. In the structure of world grain production, it ranks fourth after corn, wheat and rice. Barley grain contains a lot of fiber and little starch, which makes this culture a promising dietary food. To increase the nutritional value of grain crops, there is a steady world trend of saturation of grain with biologically active compounds anthocyanins. In barley, these compounds can be accumulated in pericarp (where their biosynthesis is controlled by the complementarily genes *Ant1* and *Ant2*), in the aleurone layer (*HvMyc2*), or in two tissues simultaneously, giving the grain a purple, blue and dark purple color, respectively. However, to date, barley varieties with colored grain have not been created and are not cultivated in our country. The purpose of this work was a marker-assisted breeding of new barley genotypes, accumulating anthocyanins in aleurone layer and pericarp based on cultivated Siberian varieties. The varieties Vorsinsky 2, Aley and Tanay were chosen as maternal forms. The near-isogenic lines of cultivar Bowman “Intence blue aleurone” and “Purple lemma and pericarp” (obtained from the Nordic Gene Bank), which are donors of the *HvMyc2* and *Ant1/Ant2* genes, respectively, were chosen as paternal forms. The F<sub>1</sub> hybrids were self-pollinated to get F<sub>2</sub> plants. Among F<sub>2</sub> offsprings, homozygous plants with blue and purple grain color were selected using diagnostic PCR markers (AFLP, CAPS and STS) for the *HvMyc2* and *Ant1/Ant2* genes. These plants were sown in the field, where they were backcrossed with the original Siberian varieties. During the year, 350 BC<sub>1</sub>F<sub>2</sub> plants (backcross of the first generation) were obtained, which will be subjected to a further 5–6 fold backcrossing and a marker-selection selection of homozygous plants.

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## The role of expansin and xyloglucan endotransglycosylase genes in the regulation of plant growth under changing environmental conditions

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The discrepancy of cellulose microfibrils during cell expansion is achieved by three basic mechanisms: hydrolysis of a part of binding glycans with endoglycanases, cutting and new crosslinking of glycans with xyloglucan endo-transglycosylases/hydrolases (XTHs), and the violation of hydrogen bonds between the microfibrils of cellulose and glycan chains, which is carried out by expansins. We have created transgenic tobacco plants with increased expression of the genes encoding the tobacco expansins: *NtEXPA1*, *NtEXPA5* and tomato xyloglucan endo-transglycosylase – *tXET-B2*. Increased expression of *NtEXPA1* and *NtEXPA5* expansin genes led to an increase in the growth rate and root length both under normal plant growth conditions and at 12 °C and 50 mM NaCl. Increased expression of expansin genes influenced the changes in the fresh and dry mass of a shoot, leading to an increase in their exposure to hypothermia. Overexpression of the *tXET-B2* gene promoted tobacco root growth in a medium containing 50 mM NaCl. Under drought conditions, overexpression of *tXET-B2* gene resulted in a considerable increase in fresh and dry weight in many of the studied transgenic lines. The totality of the obtained data may indicate the involvement of *NtEXPA1*, *NtEXPA5*, and *tXET-B2* genes in the regulation of growth under hypothermia, drought, and salinity. We will be using these target genes for the genetic transformation of cultivated plants.

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## Effect of combined temperature-drought stresses on antioxidant activity of plants

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Altering climatic conditions and drought stress drastically affects the crop yield in agriculture. Plants respond and adapt to these abiotic stresses invariably by complex mechanisms inducing various morphological, biochemical, physiological, and molecular aspects. Additionally, it is known, that abiotic stresses results in the excessive production of Reactive Oxygen Species (ROS), leading to oxidative stress. Plants have evolved a wide range of enzymatic and non-enzymatic mechanisms to scavenge ROS and protect their cells against oxygen toxicity. However, the role of the antioxidant defense involving ROS-scavenging enzymes in the tolerance of barley plants to combined drought and temperature stresses is currently unknown.

The aim of the present work was to determine the importance of the modulation of the antioxidant system in barley to the combination of drought with high (+40 °C) and low (+10 °C) temperatures. To achieve this, plants were exposed to combined stresses for 5 days. Indeed, morphological parameters of the barley plants showed less root and shoot biomass accumulation and higher chlorophyll degradation compare to their controls. Further we checked the activity of the CAT and aldehyde oxidases (AOs) activities, where catalase (CAT) directly converts  $H_2O_2$  into  $H_2O$  and AO may participate in stress responses, because it catalyzes the oxidation of abscisic aldehyde to ABA, in the last step of ABA synthesis.



## WOX and KNOX transcription factors in symbiotic nodule development

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WOX and KNOX transcription factors (TFs) regulate different aspects of plant development, including meristem formation and maintenance. Their involvement in diverse developmental programs allows unraveling the evolution of regulatory mechanism that accompanies the formation of new organs in plant. We studied the role of WOX and KNOX TFs in symbiotic nodule formation in *Medicago truncatula*. WOX TFs are known as key regulators of plant meristems working together with CLV1-like receptor and CLE peptides. We have analyzed their role in nodulation and found that *WOX5* gene is involved in nodule development. Moreover, using *sun* supernodulating mutant we found that *WOX5* expression is dependent on CLV1-like kinase SUNN that regulates nodule number in *M. truncatula*. The role of other members of WOX family in nodulation has been studied as well.

Together with WOX family, KNOX TFs regulate diverse developmental programs in plants. Specifically, in the shoot apical meristem KNOX TFs activate the expression of isopentenyl transferase (IPT) genes involved in cytokinin biosynthesis. We found that nodule development is regulated by a member of KNOX TF family KNOX3. We found that in developing nodules KNOX3 activates the expression of genes involved in cytokinin biosynthesis, in particular, genes of *IPT* and *LOG* families, by direct binding to their regulatory sequences.

To summarize, studying the role of WOX and KNOX TFs in nodule development suggests that different developmental programs in plants are regulated by common regulatory modules involved WOX-CLV system and KNOX TFs with their target genes.

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## Knockout of abscisic acid (ABA)-dependent transcription factor gene OsVP1 using CRISPR/Cas9 system improves germination velocity and pre-harvest sprouting in rice (*Oryza sativa* L.)

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Seed dormancy is a condition that has not germinated during a specific period, even in environmental conditions that are prone to sprouting. These phenomena vary in proportion to the dry storage (after ripening) of the seeds and are genetically controlled by the genotypes of both the mother plant and embryo. The dormancy imposed by the coat is enhanced by the tissue that covers the seed, ie, glue and pale (or crust), pericarp and testis, and optionally endosperm (Bewley et al., 2013). Embryonic dormancy of the endosperm is finely controlled during development (Sugimoto et al., 2010). In cultivated rice, seed dormancy is commonly removed with dry after-ripening to achieve rapid and uniform germination on seed sowing. In this report, Pre-harvest sprouting is a phenomenon that seeds germinate while still attached onto the maternal plants in the condition of cloudy and rainy weather, and is also a restrictive factor of rice production and seed propagation. The phenotype of rice pre-harvest sprouting is very similar to that of maize seed-specific vp1 mutant. VP1 gene is essential for seeds maturation and dormancy, and is also a key transcription factor of ABA signal transduction pathway. Thus, it is of great significance to effectively control the occurrence and hazard of rice pre-harvest sprouting. The aim of the current investigation is to dissect the biological function of homologous gene OsVP1 by using CRISPR/Cas9 system in rice. Germination experiment showed that the percentage of germinated seeds from T1 knockout lines was higher than that of wild-type plants. Under the different concentrations of abscisic acid (ABA) treatment, the inhibition of germination ratio of *osvp1* gene knockout seeds was not significantly different when comparing with wild-type plants. Therefore knockout lines of OsVP1 gene using CRISPR/Cas9 system can increase germination velocity of seeds and also lead to pre-harvest sprouting.

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## Fine mapping of rice bacterial leaf blight resistance loci to major Korean races of Xoo (*Xanthomonas oryzae*) and development markers

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Bacterial leaf blight (BLB), caused by *X. oryzae* pv. *oryzae* (Xoo), is one of the most destructive diseases of rice due to its high epidemic potential. Understanding BLB resistance at a genetic level is important to further improve the rice breeding that provides one of the best approaches to control BLB disease. In the present investigation, a collection of 192 accessions was used in the genome-wide association study (GWAS) for BLB resistance loci against four Korean races of Xoo that were represented by the prevailing BLB isolates under Xoo differential system. A total of 192 accessions of rice germplasm were selected on the basis of the bioassay using four isolated races of Xoo such as K1 and K2. The selected accessions was used to prepare 384-plex genotyping by sequencing (GBS) libraries and Illumina HiSeq 2000 paired-end read was used for GBS sequencing. GWAS was conducted using TASSEL 5.0. The TASSEL program uses a mixed linear model (MLM). The results of the bioassay using a selected set of 192 accessions showed that a large number of accessions (93.75 %) were resistant to K1 race and K2 resistant germplasm proportion remained between 66.67. The genotypic data produced SNP matrix for a total of 293,379 SNPs. After imputation the missing data was removed, which exhibited 34,724 SNPs for association analysis. GWAS results showed strong signals of association at a threshold of  $[-\log_{10}(P\text{-value})]$  more than 5 (K1 and K2) for nine of the 39 SNPs, which are plausible candidate loci of resistance genes. These SNP loci were positioned on rice chromosome 2, 9, and 11 for K1 and K2 races. The significant loci detected have also been illustrated and make the CPAS markers for *NBS-LRR* type disease resistance protein, *SNARE* domain containing protein, *Histone deacetylase 19*, *NADP-dependent oxidoreductase*, and other expressed and unknown proteins. Our results provide a better understanding of the distribution of genetic variation of BLB resistance to Korean pathogen races and breeding of resistant rice.

## **Polyphenolics compound variation in foxtail millet (*Setaria italica*) germplasm and establish a core collection**

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*Setaria italica* (L.) P. Beauv. is one of the most widely cultivated species of millet in the Korea and have considerable attention due to its nutritional quality related to high content of dietary fiber, protein, starch patterns and high level of minerals. This research was aimed to study variation of polyphenols compound in foxtail millet germplasm and combined it with basic agronomic traits and molecular markers to establish a core collection. Total phenolic content ranged from 11 to 87 mg gallic acid equiv (GAE)/100g and antioxidant activity was showed from 3.3 50 51 % by DPPH scavenging activity. Here we assessed the genetic diversity and population structure in a large germplasm collection of 785 accessions by employing EST-SSR markers, morphological traits he phenolic content and antioxidant activity. The germplasm collection was separated into three groups based on population structure analysis, whereas principal coordinate analysis (PCoA) could not cluster accessions according to their geographic origin. Subsequently, a core collection with a total of 170 accessions (21.66 %) was selected from the whole set of germplasm by combining allelic variations of 22 EST-SSR markers and their traits. The core collection optimally represented the whole germplasm collection and displayed a similar level of genetic diversity, population structure, and phenotypic variations based on various genetic analyses such as Shannon-Weaver and Nei's diversity indices and PCoA, while phenotypic traits were analyzed by mean, range, and principal component analysis. This core collection of foxtail millet will be a primary resource for further genetic analysis and development of appropriate.

## Genome-wide association study of powdery mildew resistance in collection of common wheat varieties (*T. aestivum* L.)

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Powdery mildew, caused by the fungal pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the economically important disease of common wheat *T. aestivum* L. One of the most effective and environmentally important ways of wheat protection against *Bgt* is cultivation of the varieties with genetic resistance. The aim of this work was to study the genetic diversity of collection of common wheat varieties and breeding line on resistance to *Bgt*. Wheat panel consists of 100 Russian wheat cultivars and 60 breeding lines containing introgressions from Triticeae tribe species. The results of the evaluation of the resistance level of wheat cultivars showed that no more than 10 % of the varieties have low level of susceptibility to the population of *Bgt*, specific to the Western Siberian region. Among introgression lines, more than 30 % showed a moderate to highly resistant infection types. Association mapping, performed on the basis of SNP genotyping and phytopathological evaluation during three environmental seasons identified ten loci in chromosomes 1AL, 1DS, 2AL, 2BL, 5AS, 5DS, 6AL, 6DL, 7AL, and 7BL. A high impact to the phenotypic manifestation of the trait was established for genetic factors localized in chromosomes 5AS, 6AL and 6DL. In the long arm of chromosome 6D, two loci were mapped which provide effective protection from powdery mildew pathogen. One of them was introduced from the wheatgrass *Th. intermedium*, another – from tetraploid wheat *T. timopheevii*. Based on comparative analysis of the chromosomal localization of the known *Pm* resistance genes and loci mapped in this work, an assumption was made that the QTLs on chromosomes 1DS, 2BL, 5AS, 6DL are new, not previously described resistance loci. The obtained results can be used in breeding programs for selection of target loci and for development of molecular markers specific for *Bgt* resistance loci.

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## Application of biotechnological approaches in genetic and pre-breeding studies of bread wheat

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The development of new forms of common wheat using conventional breeding takes a long period and does not allow to utilize the genetic potential of sources of agronomically important traits. The paper presents the results of the use of biotechnological approaches for screening of wheat varieties on the genetic loci determining valuable traits. To identify QTLs responsible for resistance to fungal pathogens and for yield components, two methodological approaches were used: genome-wide association study (GWAS) and marker-assisted selection. QTL identification was done with help of collection of wheat varieties and mapping populations obtained from hybridization of wheat was cultivars from different groups of ripeness. Loci associated with resistance to fungal diseases were found on chromosomes 5A, 6D, 1D, and 1B. The results of GWAS was confirmed by screening a collection of wheat varieties using STS and SSR markers recommended for marker-assisted selection. Association mapping based on the results of long-term evaluations of wheat varieties on productivity traits revealed 34 loci. For the trait “ear grain number” most SNP are located in the 6th homoeologous group. Loci in chromosome 6A have a positive effect on the trait, whereas QTLs in chromosomes 6B and 6D are negative. Ten significant SNP were found for “ear grain weight”. Chromosome 5B has two loci with positive and negative effects. For “1000 grain weight”, 14 informative markers were identified in chromosomes 2D, 3A, 4A, 5A, 5B, and 1B. Genetic mapping of the loci for the “ear grain number” and “ear grain weight” was carried out based on the results of trait evaluation in F3 mapping populations. Loci, associated with “ear grain number” were identified in chromosomes 2D, 4A, 5A, 5D and 7B, with “ear grain weight” – in chromosomes 2D, 3A, 6D and 7A. The obtained results will be used for development of KASP markers associated with yield traits.

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## Polyphenol oxidase gene family in barley (*Hordeum vulgare* L.): structural organization and functional activity of the genes in respect to black grain pigment formation

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Polyphenol oxidase (PPO) is an enzyme of the class of oxidoreductases. PPO plays an important role in response to abiotic stress and wounding. The enzyme catalase the enzymatic browning reaction in damaged plant tissues by oxidizing *o*-diphenols to highly reactive *o*-quinones. However, its functions in intact tissues have been not well understood. The aim of the current study is to characterize the polyphenol oxidase gene family and to establish their role in formation of the black pigmentation of barley grain. The uncolored cultivar Bowman and its near-isogenic line with black grain i:BwBlp were chosen. Based on the known barley *Ppo* genes sequences mapped on chromosome 2H (*Ppo1*, *Ppo2*), we identified two more copies: *Ppo3* and *Ppo4* localized on chromosomes 3H and 4H, respectively. All copies contain a conservative tyrosinase domain and have an intact tertiary protein structure. Nevertheless, the exon-intron structure of each copy differs as well as their promoter structure. Using the PLACE *cis*-regulatory elements database, we predicted light-, cold- and drought-responding elements, MYB- and MYC-recognizing elements as well as elements involved in abscisic acid-mediated abiotic stress response (ABRE). We found DRE elements only in the promoters of the *Ppo2* and *Ppo4* genes and the cold-responding elements only in the *Ppo2* promoter. We analyzed the *Ppo* gene expression in intact tissues of near-isogenic lines (coleoptile, root, leaf, stem, pericarp and hulls) and in roots and coleoptile of plants exposed to salt stress. We showed that all the *Ppo* genes in barley have different patterns of expression, while expression of the *Ppo2* gene increases in the pericarp and hulls of the i:BwBlp line as the black pigment appears. The *Ppo4* gene is not transcriptionally active in any of the intact tissues of both lines.

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## Genomics of non-photosynthetic plants

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The capability to photosynthesis is one of the most prominent characteristics of plants. However, several species have lost this ability and have adapted to obtain energy from organic compounds derived from other organisms: either from other plants (parasitism) or from fungi (mycoheterotrophy). Heterotrophy is not confined to any specific lineage of plants; it has occurred repeatedly in the course of evolution. The switch to heterotrophy leads to profound changes at the phenotypic level (reduction of leaves, loss of green colour, reduction of the vegetation period) that are highly parallel in different lineages. Heterotrophic plants are difficult to cultivate in experimental conditions; this hampers classic genetic and physiological studies. Advances in DNA sequencing technologies permit the application of a genomic approach for elucidation the genetic changes associated with heterotrophy. I will summarize recent discoveries on the genomics of non-photosynthetic plants, with focus on the following aspects: 1) structure and evolution of organellar genomes, 2) loss and expansion of specific gene families, 3) horizontal gene transfer, 4) mycoheterotrophy and parasitism – differences and similarities at genomic level.

## 3D-microscopy of prophase nucleus in the meiosis I of wheat-rye amphihaploids

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In wheat-rye hybrids there is no homologous chromosome pairing. In this regard, hybrids can be used as a model to study disorders of meiotic prophase I. The goal of the present study was to understand the structural and functional organization of prophase I nuclei in amphihaploids with different genetic background (Silkova and Loginova, 2016). Combination of immunostaining with antibodies against ASY1, CENH3, and ZYP1 with confocal and high-resolution microscopy (3D-SIM) enable to understand the centromeres and synaptonemal complex (SC) dynamics and organization. CENH3 signals differed in number, size and shape during prophase I propagation. Differences between bivalent and univalent centromere organization were seen more clearly at pachytene stage when we used 3D-SIM. The dynamic of SC component loading mostly were studied with confocal microscopy, but structure organization at some cases we analyzed with 3D-SIM. SC dynamics at diplotene until diakinesis in wheat is similar to the SC dynamics described for rye, but differs from that of rye during pachytene. In the wheat-rye hybrids, despite the lack of homologues, the loading of ZYP1 occurred. Multiple long extended (linear) signals of ZYP1 appeared at zygotene. Disappearance of anti-ASY1 after full loading of ZYP1 at zygotene and pachytene was observed. As a result, at diakinesis almost all chromosomes are univalent, which indicates desynapsis and the normal functioning of *Ph*-locus.

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## Features of the effect of winter wheat selection on grain quality in the conditions of the South-Eastern region

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Improving the quality of winter wheat grain is one of the priority areas of selection, whose efficiency is largely determined by source material. Evaluation of the quality of wheat grain may be different, depending on the field of its use. Grain quality is a set of technological and biochemical, baking and food properties, which determines the economic value of the variety. From the breeder's viewpoint, grain quality consists of many components which best correspond to the genetic basis of the varietal material which the breeding work is carried out with. Quality features, which are quantitative in nature, are very strongly influenced by environmental conditions. As a result of modifications, there may be situations when individuals with identical (by this or that sign) genotypes will be completely dissimilar relative to its phenotypic manifestation. When choosing the raw material for selection, one needs to take into account not only the genotypic potential of grain yield and quality, but also the degree of their preservation in adverse environmental conditions, the frequency of high-quality grain formation, and the reaction to changes in conditions during the formation and loading of grains. It is believed that in the arid conditions of the Volga region, the main limiting factor in the formation of high-quality grain is the amount of precipitation during the growing season of wheat and the uniformity of their distribution. More than 20 indicators of winter wheat quality are evaluated annually. With an ever-increasing amount of work on studying wheat quality in the selection process, the expression of many indicators through a smaller number is very important. Our study of the quantitative expression of the rheological properties of the dough on a Mixolab device makes it possible to study both the protein-proteinase complex and the carbohydrate-amylase one in one sample during one experiment. The data obtained indicate sufficient information content for selection (varietal diversity manifestation) of such indicators as water absorption capacity (WAC); the energy absorbed by dough during kneading (RA), dough stability, dough dilution (C2), and starch retrogradation index (C5). Dough formation time is the least informative. In 100 % of cases, genotype-environmental interactions by this indicator were observed. The variation limits of the meal whiteness index was 5.9–14.6 units for the red wheat group and 14.3–28.1 for the white wheat group. The variation limits of the falling number in the white wheat group were 92–428 s; the average value of this index was 248 s. The variation limits of the falling number for the red wheat group were 290–382 s; the average value of this index was 341 s. No relationship between the falling number and the meal whiteness index was proven: the correlation coefficient was 0.3482 only.

## The study of the genetic diversity of oat varieties cultivated in the Tyumen region, by avenin-coding loci

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Oats – one of the main cultivated crops in the Tyumen region. Due to the active breeding work, currently in the region grow oats varieties only local selection. An important condition for increasing yields and crop resistance to adverse factors is the preservation of the high genetic diversity of the species. To evaluate this indicator, alleles of prolamin-coding loci are very convenient. We analyzed the electrophoretic spectra of prolamins of 18 varieties of oats, included in the State Register of breeding achievements in the Tyumen region from the 1930s to the present. The alleles of avenin-coding loci (ACL) *AvnA*, *AvnB*, *AvnC* are identified. In total, 12 alleles were identified at the *AvnA* locus, 10 at the *AvnB* locus, and 8 at the *AvnC* locus. Among the studied varieties, 11 were homogeneous in component composition of avenin. The remaining varieties had two biotypes. On the basis of data on the frequency of occurrence the alleles of ACL, the average gene diversity (H) was calculated. In order to evaluate the genetic diversity of varieties in different periods of time, all the studied samples were combined into groups. One group included varieties cultivated in the same ten-year period. It was established that the minimum values of genetic diversity (0.33) were characteristic of varieties cultivated in 1930–1950. With the advent of new varieties, including domestic breeding, the H value increased and reached its maximum (0.78) in 1970–1980. By 2000, this figure fell to 0.70, which is associated with the processes of variety changing and substitution of foreign varieties with local breeding varieties. As a result, the frequency of occurrence the alleles of ACL characteristic of these varieties has changed. To date, the genetic diversity of oat varieties cultivated in the region is 0.75. The high value of H of oats indicates a well-conducted breeding work with this crop in the Tyumen region.

## Molecular-genetic analysis of DNA plasmotype of rye-wheat secalotriticum amphidiploids (RRAABB, $2n = 42$ )

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In order to achieve a balanced expression of the original species genomes and enhancement of the rye genomes expression in triticales, we carried out research on the creation of a new type of triticales with rye-type cytoplasm – hexaploid secalotriticum (RRAABB,  $2n = 42$ ), by hybridization of tetraploid rye (RRRR,  $2n = 28$ ) with hexaploid triticales (AABBRR,  $2n = 42$ ) and a single backcross of rye-triticales  $F_1$  hybrids (pentaploids) on the initial triticales. Restriction analysis of species-specific DNA sequences of chloroplasts (*ndhH* locus) and mitochondria (8S/5S-repeat) showed that for rye-type cytoplasm (S-cytotype) the absence of restriction was detected by the *MspI* endonuclease recognition site (fragment of 750 bp) and the presence of restriction by the recognition site of the endonuclease *Sall* (restriction fragments of about 250 bp in length); for wheat (T-cytotype) – restriction with the *MspI* endonuclease (restriction fragments 500 and 250 bp long) and the lack of restriction with the *Sall* endonuclease (500 bp fragment). It has been established that rye-triticales  $F_1$  hybrids (pentaploids, RRABR,  $5x = 35$ ), rye-wheat amphiploids  $F_1BC_1$  ( $5-7x = 35-49$ ) and hexaploid amphidiploids of secalotriticum  $F_{1-15}$  had a stable inheritance of DNA markers of rye cytoplasmic organelles. However, in contrast to original rye cultivars, the analysis of restriction results of the mitochondrial DNA *tMet-18S/5S* region by the *Sall* endonuclease detected the presence of restriction fragments for about 250 bp in length, which is characteristic for rye, and a 500-bp non-restriction fragment. The presence of this fragment may indicate a partial transfer of the paternal cytoplasm (two-parent inheritance of mitochondria) during the hybridization of rye with triticales. In support of this, a comparative analysis of sequencing the mitochondrial DNA locus *tMet-18S/5S* of the secalotriticum lines as well as the initial rye and triticales cultivars will be carried out.

## Genetic resources of *Durum* wheat in Russia on the content of yellow pigment in grain

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The content of yellow pigments in the grain, along with the quantity and quality of gluten, is one of the important components of grain quality in world markets. According to experts, this trait determines the general quality level of durum wheat grains by 20 %. In the process of breedings exists unceasing increase the yellow pigment content in grain, straight and pasta from durum wheat. In Russia, this direction of breeding has been developing most intensively since the creation of the Saratovskaya zolotistaya cultivar. Obviously, the accumulation of transgressions is associated with changes in the composition and activity of the system of genes of complex and multistage biosynthesis pigment content and oxidative enzymes. In process of the breedings for this trait based on the genetic system of Saratovskaya zolotistaya in Agricultural scientific institute of Samara region are received transgressions, exceeding level of the initial cultivars on 12.5 % (Pamayati Chehovicha), 14.2 % (Bezenchukskaya krepost), 20.6 % (1429d-10), 28.4 % (Bezenchukskaya zolotistaya). Some commercial cultivars (Bezenchukskaya steppe, Bezenchukskaya 210, Bezenchukskaya 205, Bezenchukskaya niva, Elizavetinskaya) has contents of the yellow pigment at a rate of Saratovskaya zolotistaya. The cultivar Bezenchukskaya zolotistaya in the conditions of the Middle Volga region is the absolute record among the studied genotypes of Russian and foreign breeding (Australia, Austria, Germany, Italy, Kazakhstan, Canada, USA, CIMMYT). The Accumulation of the pigment in grain of this cultivar reaches 9 mg/kg. In the process of studying cultivars and breeding lines, genotypes were identified that exceed the Saratovskaya zolotistaya in value trait: Gordeiforme 677, Gordeiforme 878, Gordeiforme 879 (Altai Agricultural scientific institute), 2012d-6, 1981d-12 (Samara Agricultural scientific institute), Duroflaus (Austria), Hyperno, Tjikuri (Australia). The Coefficient heritability trait in broad sense of the word ( $H^2$ ) has amounted to 0.52, genotype – environment interactions were of low. The Defining influence genotype in variation of the trait suggests high efficiency to breedings on base of the genetic systems studied cultivars.

## Occurrence and variability of polyembryonic seedlings in triticale-wheat hybrid line

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Polyembryony is suggested as an important feature due to producing more plants per unit area by using the same number of seeds. However, yet little is known about this reproductive phenomenon to increase its frequency up to 100 percent for involving in the breeding process. In our work the constant polyembryonic line #908 ( $2n = 42$ ) was spontaneously derived from the crosses of triticale (genome AABBRR (*T. durum*/*Ae. squarrosa*//*Secale cereale* ssp. *segetale*),  $2n = 42$ )) with common wheat *T. aestivum* var. *velutinum* (genome AABBDD,  $2n = 42$ ). Correlated positively with the spike fertility the polyembryonic seed frequency of this line is varying from 0.26 % to 11.98 % per plant. The highest frequency distribution of polyembryonic seeds was observed in central spikelets and basal (closest to the rachis) florets of spike, i. e. in the positions favoring seed formation. Polyembryonic seedlings morphologically can be divided into following types: i) with common coleoptile; ii) with half or completely conjoined coleoptiles, and iii) with splitted coleoptiles. With low frequency the conjoining was observed also for leaves and roots either in mono- and polyembryonic seedlings of this line. The highest frequency of appearance is for twin plants with splitted coleoptiles, followed by triple, quadruple, quintuple and sextuple. Seeds with more than double seedlings can combine its splitted with common or conjoined coleoptile(s). Seedlings from the same polyembryonic seed can develop either equally or unequally determining a wide variation of plant height and spike fertility traits. The trait exhibits a recessive mode of inheritance in the crosses with other poly- and non-polyembryonic lines. Compared with the monoembryoid the polyembryoid seeds were more sensitive to high doses of gamma radiation. GISH analysis showed that line #908 has 14 rye chromosomes, i. e. it is hexaploid triticale. It can be assumed that the increased polyembryony frequency is associated with a specific combination of the genes of the A- and B-genomes of *T. durum* and *T. aestivum* resulting from crosses.



## Testing safety of genetically modified products of rice: Case study on Sprague Dawley rats

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Rice is the staple food of more than half the world's population. However, rice is one of the products that are severely damaged by pests. In order to stand against this damaged, genetic engineering is considered as one of the best ways by adding new genes inside the grain named genetically modified (GM) foods. It is important to find any additional genes in tissues after consumption of GM foods. Therefore, in this study, the remaining of *cryIA(b)* gene and P35 were assessed in the liver of Sprague Dawley rats fed with GM rice. Overall, 20 male and 20 female SD rats were fed by pellets made by GM rice in 50 % of needed carbohydrate for 90 days. Then, sampling was done from liver. DNA extraction was done based on the protocol. The quality and quantity of the extracted DNA was done by agarose gel electrophoresis and spectrophotometry, respectively. Detection of GM genes residues, including *CryIA(b)*, P35, and T35 was done by Polymerase Chain Reaction using specific primer pairs. The results were analyzed by agarose gel electrophoresis alongside with 50 bp DNA ladder. The results were compared with the ones in control groups with feeding by standard pellet. All tests were done in triplicates. Analysis of the amplification of P35, *CryIA(b)* and T35 showed no residues inside the liver tissue. The results showed no significant difference in the presence of transgenic gene in the liver tissue between the control and experiment groups. Therefore, this study rejects the possibility of gene settle of GM rice gene residues in liver tissue.

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## Genetic diversity of rare iris species in the Southern Urals

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*Iris scariosa* and *I. pumila* are two rare species of iris found in the steppes of the Southern Urals. Both of them are included in the Red Data Book of the Russian Federation. *I. pumila* is an ancestor of many modern cultivars, and *I. scariosa* is known for its drought and salt tolerance and can also be used in breeding. The Republic of Bashkortostan and Orenburg oblast are situated on the eastern border of *I. pumila* habitat and western border of *I. scariosa* habitat, so Southern Urals is the area where these two species coexist. Therefore, it is important to study these unique populations, their morphological and genetic diversity and possibilities of hybridization. We studied DNA samples from 29 populations of iris discovered in Southern Urals using different RAPD and ISSR primers. We observed not only phenotypical, but also genetic interspecific and interpopulation variability. Moreover, several samples distinguished as *I. scariosa* on the basis of external features, genetically showed higher similarity to *I. pumila* and might be hybrids.

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## DNA methylation as a sensitive biomarker of environmental abiotic factor exposure

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The great number of biotic and abiotic factors affect plant under natural growing conditions growth. DNA sequence does not carry the complete information necessary to determine the phenotype of the organism. DNA methylation controls genomic integration, regulates genome expression and cell differentiation, as well as plant response to biotic and abiotic stresses. Epigenetic regulation involves various reversible chemical modifications occurring on both the DNA itself and the proteins interacting with it, which as a result affects the chromatin structure and function, without, however, altering the sequence of nucleic residues in the DNA.

We have previously shown on wheat seedlings *Tr. aestivum* (v. Nairi) the importance of the role of DNA methylation in the formation of a response to abiotic stress, as well as the transformation of these changes into the next generation. The results of our research have proved once again that DNA methylation is a sensitive biomarker for the environmental impact of environmental factors. However, in order to understand the role of epigenetic changes in the adaptation and evolution of plants, require further studies of DNA methylation on the model-based *Tr. aestivum*, *A. thaliana*, *O. sativa*, *Z. mays*, and on non-model plants.

Now special importance is attached to the study of the population of pasture plants, since environmental pollution through animal food can be transmitted to human. Due to these circumstances, it is imperative to regularly monitor pasture plants, both for relocating animals to more environmentally friendly meadows, and environmental protection measures aimed at improving damaged pastures. Among pasture plants *Bromus inermis*, *Medicago sativa*, *Onobrychis arenaria*, *Agropyron pectinoforme*, etc. are widely distributed. Currently we have studied the DNA methylation of esparcet – *Onobrychis arenaria* and *Agropyron pectinoforme*. The obtained data on epigenetic changes on the studied plants will improve the methods of pasture monitoring, taking into account the ecological, climatic conditions of the regions and the agricultural sector of the countries' economies.

## Features of the interaction of the effector genes *ToxA* and *ToxB* with the susceptibility genes *Tsn1* and *Tsc2* in different species of wheat

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The *Triticum aestivum*–*Pyrenophora tritici-repentis* pathosystem is well studied in durum and common wheat. The purpose of our study is to assess the distribution of the sensitivity genes *Tsn1* (5BL) and *Tsc2* (2BL) in species of the genus *Triticum* L. from the VIR collection and the response of wheat accessions containing these genes to infection by isolates with complementary genes effectors *ToxA* and *ToxB*. All 72 accessions of 16 wheat species were evaluated for resistance to two isolates ToxA<sup>+</sup> originating from Kazakhstan and Russia, and one ToxB<sup>+</sup> from Greece. Using gene-specific primers, *Tsn1* and *Tsc2* were not detected in the diploid species *T. urartu*, *T. boeoticum*, and *T. monococcum*. In the wild tetraploid wheats *T. dicoccoides* and *T. araraticum* and six cultivated tetraploid species, the *Tsc2* gene and the polymorphism of the *Tsn1* were detected. In the *T. timopheevii*, only the *Tsc2* gene was identified. Polymorphism for both genes was observed in all hexaploid species (genome *BBAADD*). The manifestation of necrosis and/or chlorosis on wheat leaves is observed when the plant and the pathogen have both of the dominant genes *Tsn/ToxA* and/or *Tsc2/ToxB*, respectively. All diploid wheat species had no susceptibility reactions. The gene-on-gene *Tsc2/ToxB* gene interaction was observed in accessions of *T. aethiopicum* and *T. turgidum*, as well as for most accessions of hexaploid species. All accessions of *T. dicoccoides* and *T. dicoccum*, despite the presence of the *Tsc2* gene, were resistant to the ToxB<sup>+</sup> isolate. The reasons for this resistance are being studied. When evaluating the interaction of the *Tsn1/ToxA*, 11 accessions of different tetra- and hexaploid species of wheat aroused particular interest. The *Tsn1* was not detected in these accessions, but a strong necrosis was observed when infected with ToxA<sup>+</sup> isolate, which is possibly due to the presence of other unknown susceptibility and effector genes.

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## Modern biotechnologies for the targeted modification of wheat genome

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The application of innovative research tools for the targeted genome modification open new opportunities for understanding wheat genomics and developing improved cultivars. At the same time, the application of various modern technologies such as tissue and time-specific gene expression, RNAi-based gene silencing, chloroplast transformation, T-DNA insertion mutagenesis, and genome editing is available only for the restricted number of wheat genotypes. Most of the actual procedures involve the introduction of foreign DNA/RNA/RNP complexes into plant tissue and then regenerating the plants containing the modified genome. The focus of the present work is genetic sequences transfer methods for efficient production of fertile genetically modified plants of di-, tetra- and hexaploid wheat germplasm. We present a routine procedure for a transfer of heterologous sequences by biolistic delivery method. Hundreds of independent transgenic plants, including einkorn, emmer wheat, timopheevii wheat, and bread wheat have been already producing using the optimized parameters. Various details concerning the in vitro tissue culture productivity, DNA delivery in the target tissue, an appropriate method to select the transformed plants, genetic transformation efficiency, and stability of transgene expression will be discussed. We also will speculate future trends in genetic engineering as a tool for targeted genome editing to manipulate of wheat genome in order to increase yield and enhance stress tolerance.

## Increasing the protein and gluten content in the grain of bread wheat using marker-assisted selection

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Yeast bread-baking is possible due to the gluten, which creates a three-dimensional frame that holds gas bubbles inside the raw dough. The grade of grain and its use for technological purposes are determined according to the amount of gluten in the grain and parameters of dough elasticity and extensibility. Since the amount of gluten closely correlates with the amount of protein in the grain, the percentage of gluten determines the nutritional value of bakery products. In our work, we studied the collection of cultivars of domestic selection for the raw gluten content in grain (RGCG). A correlation analysis was carried out between the alleles of 24 microsatellite (*Xgwm*) markers located on different chromosomes and RGCG percentage. One of the highest values of the RGCG was associated with the *Xgwm261* marker allele with a length of 185 base pairs. The marker is located on chromosome 2 of the D-genome of wheat, in the short arm. To verify the role of this chromosome region in the formation of a high RGCG, a chromosome with the above-mentioned allele was introduced into the well-studied cultivar Saratovskaya 29 (S29) by backcrossing to the monosomic line for 2D chromosome. The donors of the allele associated with a high content of gluten were the old cultivars Sibirka 1818 and Cesium 111 bred in Siberia. By this way the single chromosome inter-varietal substitution lines S29 (Sibirka 1818 2D) and S29 (Cesium 111 2D) were obtained. The interim control of the gluten content during the backcrosses showed that the lines exceed the recipient cultivar for this trait.

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## The gene expression level of enzymatic and non-enzymatic antioxidant system of potato plants under chloride salinity

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The growing season duration for potato plants is one of the key criteria for their regionalization in a certain climate zone. For the Siberian region, characterized by a short summer season, early-maturing varieties are most preferred. However, the increase of saline areas sets priority on salt tolerance varieties. Salinization negatively affects the implementation of many physiological processes in plants, largely due to the generation of reactive oxygen species and the development of oxidative stress, which leads to disruption of the photosynthetic apparatus, a decrease in the intensity of photosynthesis and, as a consequence, a decrease in the productivity of plants; induction of aging or premature death of the plant. In response to the oxidative stress progress and metabolic disturbances, the plant responds by activating the cellular antioxidant system, which includes antioxidant enzymes (catalases, peroxidases, superoxide dismutases, etc.) and low-molecular-weight organic compounds with antioxidant properties (proline, phenolic compounds, carotenoids). The gene expression of antioxidant system will compare the functioning of the protective system for two varieties (early and mid-season) the plant *Solanum tuberosum* in response to chloride salinity. We estimated the effect of salinity on growth (length of axial organs, area of assimilating surface) and physiological (osmotic potential of cell exudate, lipid peroxidation degree, content of photosynthetic pigments, flavonoids, proline, activity of antioxidant enzymes) for early and mid-season varieties *Solanum tuberosum* (Zhukovsky early and Lugovskoy). The gene expression levels of enzymatic (APX1, APX3) and non-enzymatic (P5CS1, P5CR, PDH) plant protection were detected by real time PCR. For the first time, the causes of potato plant resistance to NaCl action for analyzed varieties were identified and a detailed comparison of the functioning of the protective systems of potato plants depending on the maturation period was made.

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## Duplication of the dominant *Vrn-A1b.2* allele in *Triticum dicoccum* lineage

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The *Triticum dicoccum* lineage includes domesticated hulled tetraploid wheat of *T. dicoccum* and hexaploid wheat species of *T. spelta*, *T. macha* and *T. vavilovii*, which were derived from hybridisation events between the *T. dicoccum* and free-threshing hexaploid wheat of *T. aestivum*. The dominant *VERNALIZATION-A1* alleles determine spring growth habit (without vernalization requirement) and early flowering of wheat. The *Vrn-A1b.2* allele is frequent in tetraploid wheat of *T. dicoccoides* and *T. dicoccum* as well as in hexaploid wheat of *T. spelta*. In *T. spelta* this allele as well as *Vrn-B1c* are major determinants of the spring phenotype, while *T. macha* and *T. vavilovii*, as a rule, are characterized the strong vernalization requirement (winter type) and carry the recessive *VRN1* genes. The *VRN1*-ratio test, based on end-point qPCR was optimized to estimate the copy number variation (CNV) of *VRN1* in wheat. Applied to the analysed accessions this test showed a two-fold increase in signal for the *VRN-A1* fragments in two accessions of *T. dicoccum* from Israel and Palestine carrying *vrn-A1b.3* and numerous accessions of *T. spelta* from Europe carrying *Vrn-A1b.2* and *vrn-A1b.3*. This difference in amplification of the *VRN-A1* fragments was preserved at different level of the genomic DNA fragmentation, excluding the genomic environment effect, and was confirmed during TaqMan real-time PCR assay with the different endo- and exogenous controls. Duplication of *Vrn-A1b.2* in accessions of *T. spelta* was strongly associated with the *Vrn-A1b.2/Vrn-B1c* genotype and awned spikes, indicating the bottleneck and founder effects. It is known that gene dosage of the dominant *VRN1* alleles positively correlates with early flowering of polyploid wheat. On the other hand, multiplication of the recessive *vrn-A1* is associated with the later flowering. In any case the effect of the *Vrn-A1b.2* duplication on phenotype provides additional advantages in manipulation of the flowering time of wheat.

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## Production of *T. aestivum* L. hybrids with *Ae. neglecta* under conditions of Azerbaijan

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*Ae. neglecta* [syn. *triaristata* Willd.; ( $2n = 4x = 28$ , genome *UUMM*)] is an important gene source for favourite alleles and genes introducing via intergeneric hybridization in wheat improvement. The common wheat line 171ACS {*Aegilotriticale* [(*T. durum* Desf.  $\times$  *Ae. tauschii* Coss.)  $\times$  *Secale cereale* L. ssp. *segetale* Zhuk.]  $\times$  *T. aestivum* L. 'Chinese Spring'} crossed reciprocally with 3 genotypes of *Ae. neglecta* originated and collected from different populations of Azerbaijan. 1 genotype of *Aegilops* collected from Siyazan (#1), 2 [black (#2) and white (#3) accessions] collected from Girdimanchay. Field works were carried out at the Absheron Research Station of Genetic Resources Institute of ANAS. No embryo rescue or hormone treatment was applied for the production of  $F_0$  seeds. Seed setting and germination ability were different depend on the genotypes. The highest seed setting observed in combination 171ACS  $\times$  #3 (51.72 %). The second place taken by common wheat hybrid with #2 (23.58 %). It is followed by 171ACS  $\times$  #1 that demonstrated 20.00 % seed-production rate. The lowest seed setting observed reciprocal hybrids of last combination (15.62 %). They gave 27, 19, 7 and 1 seedlings, respectively, that were transplanted into the experimental field for further investigations. Morphologically,  $F_1$  hybrids between bread wheat and #1 resembled goatgrass, but others remain common wheat plant. However, phenotype of their reciprocal hybrids doesn't differ from each other. Pollen mother cells (PMCs) for studies of meiotic chromosome behavior were prepared by means of the standard Carnoy fixative and acetocarmine squash method and observed 35 chromosomes in  $F_1$  plants, as expected. Significant differences in chromosome conjugation of reciprocal hybrids had not been observed, thus, they had approximatedly same amount of bi- and univalents.

## Genetic diversity of hexaploid wheat accessions conserved ex situ at the Japanese gene bank NBRP-Wheat

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The National BioResource Project (NBRP)-Wheat, the Japanese gene bank of wheat and its relatives, is aimed to promote wheat sciences by providing high quality genetic materials. Currently, the NBRP-Wheat stores more than 12,000 wild species, landraces, and experimental strains of wheat. The main body of collections of wild species and landraces derived from several expeditions Kyoto University have made to the heart of wheat cultivation areas. Some of them are hardly available at the collection sites today. In addition to the primary tasks to maintain the genetic resources, we are surveying genetic diversity among wheat accessions. We established the core collections representing 3,500 hexaploid (AABBDD), 1,900 tetraploid (AABB and AAGG), and 300 diploid (AA) wheat accessions conserved ex situ at NBRP-Wheat. The hexaploid core collection, consisting of 188 accessions of *Triticum aestivum*, *T. spelta*, *T. compactum*, *T. sphaerococcum*, *T. macha*, and *T. vavilovii*, was intensively genotyped by DArTseq markers. Overall, the core collection was divided into seven clusters. Non-admixture accessions in each cluster indicated that the clusters reflect the geographic distribution of the accessions. We analyzed genetic factors controlling seed morphology in hexaploid wheat by utilizing the core collection. The analysis indicated that the hexaploid core collection is a useful genetic tool to dissect complex traits in wheat such as grain morphology analyzed here. Currently we are developing a nested-association mapping (NAM) population representing genetic diversity of East Asian wheat accessions. Twenty four parental accessions, selected from the core collection based on genetic diversity, were crossed with Norin 61 wheat whose genome is de novo sequenced by the International Wheat 10+ Genome Project. The most advanced individual of the NAM population is currently in the F<sub>4</sub> generation. Our goal is to prepare and release the East Asian NAM population with more than 3,000 individuals collectively in the F<sub>6</sub> or more advanced generations.

## Ecological strain testing of breeding lines of soft spring wheat in Bagan created on the basis of distant hybridization

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The article presents long-term results of studying under the conditions of risky agriculture Kulunda steppe the ecological homeostasis of spring soft wheat breeding lines, created using remote hybridization. The studies were carried out at the breeding and seed sector of grain and feed crops of the SFNTSA RAS Siberian Research Institute of Feed North-Kulunda Department. One of the important indicators characterizing the resistance of plants to adverse environmental factors is homeostasis – a universal property in the system of the relationship between the genotype and the environment. Homeostasis is the ability of the genotype to minimize the effects of adverse external conditions. Statistical processing was performed using a package of Snedecor applications. There were carried on an analysis of the homeostasis of the selection lines according to the algorithms developed by Martynov S.P., an yield variance analysis of multiyear experience according to Tomilov, and multidimensional ranking of varieties on the main breeding traits, which was designed by I.A. Uzhakov. A breeding line (1459-E-06) of spring soft wheat with high responsiveness to the environmental conditions and stable high yield was selected. This line was made by crossing a spelt with a durum wheat and further backcrossing with soft wheat. It gave a significant increase in yield, in relation to the Omsk standard 36 0.3 t/ha, due to the plants survival. This line is advisable to use as a source of high productivity of plants in the selection process, to obtain new genotypes with high adaptive properties and is recommended for transfer to the state strain testing.

## Phylogenetic analysis of high-throughput sequencing data for a non-transcribed spacer 5S rDNA of *Triticum aestivum* relatives

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The 5S rRNA is one of the most important structural and functional component of the large subunit of the ribosomal. 5S rDNA has long been a favored target for cytological and phylogenetic studies due to their high interspecific divergence and the tandem arrays repetitive units. The repeating unit of 5S rDNA contains a 120 bp coding region and a non-transcribed spacer sequence (NTS). The former is highly conserved in structure, whereas the NTS is polymorphic in both length and nucleotide sequence. Thus, sequencing of tandem repeats is difficult task. To multiplexing sequencing NTS of the 53 accessions of relatives of *T. aestivum* (3 acc. *T. monococcum*, 3 acc. *T. baeoticum*, 6 acc. *T. urartu*, 16 acc. *T. dicoccoides*, 6 acc. *T. araraticum*, 3 acc. *T. timopheevii*, 16 acc. *Ae. speltoides*) by high-throughput method, we have developed six pair primers to NTS for inner barcoding. Obtained results were transformed by FastX Tool Kit ([www.galaxy.org](http://www.galaxy.org)). The molecular phylogenetic analysis of common sequences allowed to reveal two major evolutionary branches of NTS: Short type and Long type. Short type branch consisted of three minor types: ShortA1, ShortA2 and ShortG1 (in agreement with the classification of Baum and Bailey, 2001), and Long type branch consisted of two minor types: LongS1 (Baum and Bailey, 2001) and LongA1 (Baum and Bailey, 2004). Thus, the multiple sequence alignment problem, one of the most difficult problems in computational molecular biology, can be solve.

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## Functional characterization of papain-like cysteine proteases genes in rice

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Papain-like cysteine proteases (PLCP) are key enzymes involved in cell death as response to biotic stress. Functional genetic investigation of cysteine protease family members has been performed in a fragmentary scale to understand its specific role in plants. Highlights of research milestone for these proteases provide strong evidence on their diverse and overlapping roles in basal immunity and effector-triggered immunity. The objective of this study was to provide useful insights into biological function of three cysteine protease genes, *OsCP2*, *OsCP3*, and *OsCP5*, in rice. Overexpression of rice cysteine protease attenuated the virulence of *Xanthomonas oryzae* pv. *oryzae* race K3a in all transgenic lines which displayed moderate resistance as indicated by shorter lesion lengths (*OsCP2ox*, 6.82 cm; *OsCP3ox*, 5.55 cm; and *OsCP5ox*, 5.40 cm) than wild type Dongjin (16.07 cm) whereas RNAi-mediated knockdown of *OsCP3* resulted in severe bacterial leaf blight symptoms (17.1 cm). Abiotic screening revealed the biological significance of these three cysteine protease genes, especially of *OsCP3*, against salinity stress for which rice exhibited moderate tolerance (salinity score = 5.0 to 5.2). This study provides experimental evidence for roles of papain-like cysteine protease in improving resistance of rice against *Xanthomonas oryzae* pv. *oryzae* and tolerance against salinity stress, suggesting that these genes could be used as a valuable resource to be employed in rice breeding program to improve its ability to withstand biotic and abiotic stresses.

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## Reactivation of *VaSTS1* expression in transgenic *Arabidopsis thaliana* plants by retransformation with *2b* from *Cucumber mosaic virus*, isolate NK

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Since transgene silencing in genetically transformed plants is a serious limitation for a wide application of genetic engineering techniques, studying mechanisms ensuring stability of transgene expression is vital. Molecular mechanism underlying transgene silencing is RNA interference (RNAi). RNAi protects plant cells from the expression of viral and foreign DNA. Small interfering RNAs (siRNAs) are key components in RNAi. The siRNA-protein complexes inhibit transgene expression at the post-transcriptional and transcriptional levels by degrading target mRNA transcripts and establishing DNA methylation within transgene nucleotide sequences, respectively. Multiple investigations concerning viral suppressors of gene silencing revealed that 2b protein from *Cucumovirus* (CMV) effectively represses assembly and targeting of the RNA-induced silencing complex. Current study presents unique data on using the *2b* gene from CMV-NK isolate for transgene silencing reduction in *A. thaliana* plants earlier transformed with *VaSTS1*. In our study, two *VaSTS1*-transgenic lines were retransformed with *2b* and derived plants were analyzed. Our data demonstrated that *A. thaliana* plants with decreased expression of *VaSTS1* transgene increased transgene expression in up to 3.0-fold upon retransformation with *2b* from CMV NK. Interestingly, the more pronounced effect of *2b* retransformation regarding increase in transgene expression was shown for *nptII* used as selective marker for transformants selection upon transformation with *VaSTS1* of *A. thaliana* plants. The *nptII* gene expression increased in more than 10.0-fold in lines retransformed with *2b* being compared to initial plants transformed with *VaSTS1*. Moreover, the decrease in the level of *VaSTS1* expression in transgenic *A. thaliana* plants was associated with enhancement of the cytosine DNA methylation level within *VaSTS1* sequence. The mentioned fact implies that *VaSTS1* expression was repressed at transcriptional level and our data demonstrates that *2b* from CMV NK can reactivate a silenced transgene.



## **Association mapping for physio-biochemical traits under salt stress in wheat RILs population developed from cross between Frontana × Pasban90**

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In present study a population of recombinant in hybrid lines develop from cross between Frontana and Pasban90 parents was evaluated for (5) morphological, (7) physiological and (6) biochemical traits under normal and salt stress environment in hydroponic culture for two cropping season 2014–15 and 2015–16. Parents and their RILs showed considerable variation in studied traits. Total 202 polymorphic microsatellite markers (SSR) and composite interval mapping approach was used. Present study identified ninety-two significant QTLs out of which 27 were major and 65 were minor and most were concentrated on chromosome 2A, 2B and 7A (7 each). Twelve major QTLs were reported on B genome followed by D and A while chromosome 4B had maximum number of major QTLs. QTLs for sodium and potassium content were concentrated on D genome due to presence of Kna1 gene on D genome. Furthermore, thirty-two pleiotropic regions were identified and chromosome 2A contained pleiotropic region associated with five different traits. The identified MTAs may be helpful in improving wheat tolerance to salt and can be employed in marker-assisted selection.

## Polymorphism of flax pathogens assessed using deep sequencing

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Pathogens decrease the crop yield and reduce the quality of products obtained from flax (*Linum usitatissimum* L.). The knowledge on the genetic diversity of flax pathogens is necessary for the development of molecular marker system for fungus identification and subsequent application of proper defense actions. In the present work, the polymorphism of fungal pathogens of flax was assessed using deep sequencing. Twenty-four strains of *Fusarium oxysporum*, 8 strains of *Fusarium moniliforme*, 5 strains of *Fusarium solani*, 5 strains of *Fusarium culmorum*, 4 strains of *Fusarium gibbosum*, 3 strains of *Fusarium sporotrichiella*, 3 strains of *Fusarium avenaceum*, 2 strains of *Fusarium semitectum*, 20 strains of *Colletotrichum lini*, 9 strains of *Melampsora lini*, 9 strains of *Aureobasidium pullulans*, and 8 strains of *Septoria linicola* were obtained from Institute for Flax (Torzhok, Russia). Internal transcribed spacer (ITS) region of the rRNA genes and regions of genes encoding translation elongation factor 1-alpha (tef1), beta-tubulin (tub2), and RNA polymerase II subunits (RPB1 and RPB2) were chosen for assessment of genetic diversity of flax fungal pathogens. For DNA library preparation, the method proposed by Illumina for 16S metagenomic sequencing library with some modifications was applied. Primers with overhang adapters were used at the first stage of DNA library preparation followed by the second stage of amplification using Nextera XT index primers. DNA libraries were obtained for 100 fungal pathogen strains and sequenced on MiSeq Illumina with 300+300 nucleotide read length. About 4000 reads were generated for each sample. The pipeline was developed for the analysis of the sequencing data. The results of our work allow to characterize the genetic diversity of flax fungal pathogens and to determine the DNA sequences that are the most suitable for identification of fungus that cause *L. usitatissimum* diseases using molecular markers.

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## Flax (*Linum usitatissimum* L.) response to *Fusarium oxysporum* infection on transcriptome level

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Flax (*Linum usitatissimum* L.) is used for production of textile, vegetable oil, composite materials, and pharmaceuticals. Fusarium wilt caused by the fungus *Fusarium oxysporum* sp. lini is the most harmful pathogen that reduces flax production. For obtaining high yields of flax, cultivation of cultivars that are resistant to *F. oxysporum* is necessary. Methods of classical genetics revealed different genes for resistance to *F. oxysporum*, however, sequences and products of these genes are still unknown. In the present study, susceptible and resistant to Fusarium wilt flax cultivars and lines, including those that according to classical genetic analysis carry different resistance genes, were studied. Transcriptome sequencing was performed for susceptible and resistant genotypes grown under control conditions and 48 hours after inoculation with *F. oxysporum* spores. About 250 million reads were generated on NextSeq Illumina. After trimming of the reads using Trimmomatic and filtering against the *F. oxysporum* genome, the flax transcriptome assembly and annotation were performed. Then reads were mapped to the assembled transcripts and quantified using bowtie2 and rsem. The scoring was developed to identify genes with diverse expression alterations in resistant and susceptible to *F. oxysporum* genotypes, and resistance gene candidates were identified. Our study contributes to the understanding of the key mechanisms of flax response to *F. oxysporum*, identification of resistance genes, and development of molecular markers for breeding of flax cultivars carrying several resistance genes simultaneously.

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## Molecular mechanisms of the drought tolerance in common wheat – a transcriptomic approach

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Water deficits represent a major global abiotic stress that limits plant productivity by inhibiting their growth and development. The defense mechanism against drought stress in plants contains several elements including enzymatic antioxidant system activation to protect cells against toxic reactive oxygen species (ROS) and the accumulation of osmolytes such as proline. Proline is involved in direct ROS scavenging, stabilize proteins and antioxidant enzymes, and promote cellular signaling. The major signaling system activated in response to stress in plants is based on mitogen activated protein kinases (MAPK) cascades. The objective of presented study was to examine wheat responses to short-term drought, as measured by the expression level of genes involved in signal transduction (MAPK3 and MAPK6), the activity of the antioxidant system and the proline biosynthesis in common wheat seedlings. Furthermore, using intervarietal single chromosome substitution lines (ISCSLs), we identified chromosomes associated with the initial response to short-term drought stress in wheat.

The results of our study revealed that the first reaction of the tested wheat lines was characterized by changes in the catalase transcript level. Furthermore, examined stress induced the expression of genes involved in proline biosynthesis and the *MAPK6*-mediated signaling pathway. In the present study, we identified chromosomes associated with the initial wheat response to short-term stress using a set of common wheat ISCSLs of variety ‘Janetzkis Probat’ (JP) in the genetic background of ‘Saratovskaya 29’ (S29) with varying drought tolerance. The data indicated that the substitution of chromosomes 3B, 5A, 7B, and 7D had the largest impact on the expression level of all tested genes and could play a critical function in controlling tolerance to water deficits in the wheat genome.

## Nanocomposite selenium – containing substances and effect on ring rot of potatoes

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Potato plants are susceptible to ring rot disease caused by the bacterium *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*). In the fight with disease use of aggressive and environmentally safe reagents. The study uses nanocomposite substances containing selenium (NC Se) in the natural matrix membranes of starch, karaginan and arabino-galactan as environmentally safe means of combating the disease. Synthesis of NC Se oxidation was carried out organil desalineamiento of sodium by hydrogen peroxide with the subsequent formation of selenium nanoparticles and their simultaneous stabilization of natural matrix. All NC Se were synthesized in Irkutsk Institute of Chemistry SB RAS. They are easily soluble in water and convenient to use their aqueous solutions. The synthesized NC is a well water-soluble powder, possessing bacteriostatic properties. Selenium concentration, which was 0.000625 %, was used for the experiments. Potato plants grown *in vitro* of the susceptible variety Lukyanovskii were incubated with the bacterium *Cms* of the strain Ac-1405 for 18–21 days and monitored the change in biometric parameters of plant growth and development. Studies have shown that NC Se does not have a negative effect on plants, but when infected, its effect decreases. In the study of peroxidase activity, it was found NC Se/AG reduces the index, which indicates the presence of stress in plants. In studies on the *Cms* during incubation with solutions of NC expressed negative effect of NC was not revealed. But in vain consideration of the growth rate of bacterial biomass was found NC Se/AG inhibits bacterial growth. Se accumulation in plant tissues was investigated by X-ray energy dispersive microanalysis. The data obtained did not reveal Se accumulation. The results indicate the prospects of the study of NC Se as an environmentally safe means for the improvement of potatoes with bacteriostatic and bactericidal properties.

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## Sequencing and iterative assembly of *Ixiolirion tataricum* plastome from total DNA using 2nd and 3rd generation HTS platforms

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The *Ixiolirion* genus is a systematically isolated group, the exact position of which within the order Asparagales is not completely clear. Chloroplast genomes are commonly ~150 kb long and widely used for phylogenetic reconstruction. We've collected the tiny specimen of *I. tataricum* in the spurs of Kyrgyz Alatau ridge, Republic of Kyrgyzstan in 2018 with the aim of plastome sequencing. The amount and condition of sample was not sufficient for laboratory manipulations to extract chloroplast fraction, and we couldn't repeat the sampling. Therefore, we had to extract total DNA and perform WGS using Illumina MiSeq. The total numbers of reads were theoretically enough for complete plastome assembling, but in fact only up to ~25 kb long contigs were assembling (using whole library with Spades 3.13.0 assembler). To improve assembling we've performed additional sequencing by Ion Torrent and Oxford Nanopore HTS platforms. We have tested different approaches to extract chloroplast reads and assemble. The best approach we've found includes filtering by k-mer frequency and combined iterative filtering and assembly with enrichment of chloroplast reads and depletion of nuclear and bacterial contamination reads by separation of chloroplast and non-chloroplast nodes and clusters in de Bruijn graphs of Spades' assemblies using Bandage software, Blastn and BBmap. Assembly has much improved containing longer contigs and covering most of the LSC, SSC and IRs regions. We have found 5,527 bp inversion in SSC region, similar to the inversion we have observed in *A. paradoxum*; and that *I. tataricum* draft plastome sequence showed the highest similarity to complete plastome of *Astelia pumila*, compared to all other complete plastomes from GenBank.

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## Databases and computer resources on plant miRNA to study its role in abiotic stress response

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Non-coding RNA (ncRNAs) play crucial roles in transcriptional and post-transcriptional regulation of gene expression in plants. New data indicates that ncRNAs, including miRNA and long ncRNA (lncRNAs), have emerged as key regulatory molecules in plant stress responses. Plants are exposed to set of stress factors, such as viral infection, salt, drought, cold, and heat, which limit plant growth and productivity. To adapt and survive plants have utilized multiple gene regulatory mechanisms to restore cellular homeostasis. ncRNAs play a critical role in the regulation of gene expression in response to stress conditions. We review contemporary set of tools and resources to study its role in plant abiotic stress response. Functional RNAs with low protein-coding potential (ncRNAs) are classified as small ncRNA (<30 nt), medium-sized ncRNA, and long ncRNAs (lncRNAs) (>200 nt). miRNA expression is up-regulated or down-regulated in response to stress similar to changes in expression of protein-coding genes. For example, in *Arabidopsis*, in response to nitrogen (N) deficiency, expression of miR-160 is upregulated whereas that of miR-169 is downregulated.

Bioinformatics analysis of available sequencing data is important. Several computational tools have been established to identify and predict miRNAs in plants. According to sRNA sequencing (sRNA-seq) data, miRPlant, miRanalyzer, miRA and miRDeep-P could be used to predict new miRNAs. Several databases have been established for archiving miRNAs and their annotation: miRBase collects miRNAs from experimental or computational identification of various species, while Rfam provides miRNA sequences based on homology relationship. Plant miRNA database (PMRD) and Plant microRNA Knowledge Base (PmiRKB) are two well-known plant-specific miRNA annotation databases. Although remarkable progress in studying of miRNAs and lncRNAs role in plant adaption to stress, details are still have to be discovered using new sequencing technologies.

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## **The virulence of isolates of *Ustilago tritici* (Pers.) Jens. collected in Western Siberia**

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Knowledge about the racial composition of the causative agent of loos smut and its ongoing inside-population changes, a necessary condition in developing of determined genetic protection of created varieties. The virulence of isolates, which have been collected mainly in the fields of the Novosibirsk region as well as Omsk and Altai Krai in the period 2015–2018 years evaluated by infecting varieties-differentiators. Two sets used for inoculation. The first – Russian, developed by V.I. Krivchenko (1987) to identify the races of loos smut. It consists of nine varieties, three are durum wheat and other are soft wheat. Another – Canadian, created by Nielson, Thomas (1996), used of foreign investigation, consists of 19 samples, of which TD-1, TD-11, TD-19 – durum wheat. In all on the virulence have been estimated 15 isolates, it assembled from different varieties of spring soft wheat. The isolate was one ear with loos smut. Isolate considered virulent to the differentiator, if more than 10 % of plants affected. The key proposed by V.I. Krivchenko and J. Nielsen, P. Thomas carried out identification of races. 66 race dominated in the Novosibirsk region by identified in the Russian set. This race met in the Altai region. In the Canadian set, it is registering as T8. Also in these regions revealed race 23, according to the reaction of differentiators Canada, race is identifying as T18. In the Novosibirsk and Omsk egions registered 12 race. In addition, 78 races identified in the Novosibirsk region, 58 – in the Altai region, and 1 race – in the Omsk region. All races specialized to varieties *T. aestivum* and did not able to infect hard wheat varieties.

## Molecular-cytogenetic analysis of common wheat lines with *T. kiharae* genetic material

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*T. kiharae* (A<sup>t</sup>A<sup>t</sup>GGDD, 2n = 42) is of interest for the improvement of common wheat varieties as a source of high protein and gluten content and resistance to diseases. The aim of the study was to analyze the genomic structure and cytological stability of the common wheat lines containing genetic material of *T. kiharae*. To achieve this goal, the C-banding method and SSR- and SNP-analyses were used. Using the C-banding, the presence in karyotypes of hybrid lines whole chromosomes of *T. kiharae* (2A<sup>t</sup>, 3A<sup>t</sup>, 2G), replacing the homoeologous chromosomes of wheat A- and B-genomes, and chromosome arms (1A<sup>t</sup>L, 2A<sup>t</sup>S, 5A<sup>t</sup>L, 6A<sup>t</sup>L, 5GL, 6GL) among aberrant chromosomes composed by the *centricbreak-fusion* type. The change in the C-banding pattern observed in some chromosome regions may be a consequence of introgression of small fragments of *T. kiharae* chromatin. This was confirmed by the results of SSR-analysis showing the presence from 2 to 7 *T. kiharae* fragments in the chromosomes of all three wheat subgenomes. The highest frequency of introgressions is shown for chromosomes 1A, 1B, 2A, 2B, 5A and 5B, while in chromosomes 4A, 4B and 7B no alien chromatin was detected. The data obtained indicate that recombination events with the participation of *T. kiharae* chromosomes or their fragments occurred in all studied introgression lines. It was shown that introgression lines obtained on the base of the Saratovskaya 29 cultivar are characterized by a higher number of alien introgressions compared to cv. Festivalnaya and Rassvet. Analysis of microsporogenesis indicated a high level of bivalent chromosome pairing at the stage of metaphase I (95.87–99.76 %) in all introgression lines. Most lines are also characterized by a high percentage of normal tetrad (88.18–93.0 %). The meiotic stability of hybrid lines creates prerequisites for the preservation of alien introgressions in a number of subsequent generations, which makes it possible to use this material to expand the gene pool of wheat.

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## To live in genetic diversity: wild emmer in Fertile Crescent and its use for plant breeding

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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 southwest Asia. Wild emmer wheat (*Triticum dicoccoides*,  $2n=28$ , BBAA) was one of the first cereal to be domesticated in the Fertile Crescent between c. 12,000 and c. 10,000 years ago. The wild emmer wheat is the progenitor of the domesticated durum wheat (AABB), and fully fertile in cross with it. This step provided the key for subsequent bread wheat evolution. Therefore, it is called as “Mother of wheat”. Wild emmer is found today in the western Fertile Crescent in Jordan, Syria, Israel, and the central part of southeastern Turkey. Wild emmer harbors a wide spectrum of genes and alleles lost during domestication and breeding, nevertheless, a number of genes and alleles positively contributing to biotic and abiotic stress tolerance, yield components and quality traits have been found in wild emmer and transferred to cultivated wheats by crossing experiment. Recently, a wide collection of wild emmer accessions has been established to recover useful genetic diversity for cultivated wheats. This collection is being used for genome-wide association (GWAS) for a number of traits. In this presentation, we will discuss its domestication progress and its use for a wheat breeding program.

## Gene pool of common beans in Western Siberia

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The problem of import substitution in the seed market is primarily the problem of creating competitive varieties. To solve this problem, it is necessary to ensure the genetic potential of the crop varieties. Functional products are of particular importance for the implementation of the food task. Grain and leguminous crops are of interest. Common beans (*Phaseolus vulgaris*) has a unique balanced composition of seeds and green beans with a high content of protein, vitamins and microelements. Importance for the creation of varieties is the use of collection material of different ecological and geographical origin. There is a need to study the gene pool to identify sources and donors of valuable traits. From 1997 to the present time in the Novosibirsk State Agrarian University has studied more than 150 breeding samples of different geographical origin, including recombinant forms. The studied varieties of beans are divided by origin: most 51 % – introduced, including 47 % – of European and 4 % – of Asian origin, the samples of hybrid origin. Breeding samples were studied on the main features: the duration of the growing season, the nature of growth, plant height and attachment of the lower beans, shape and length, color of beans. The variability of these features with the establishment of correlations, evaluation of the nature of the inheritance of individual features. According to the duration of the growing season varieties were divided into 4 groups of ripeness (early maturing – 7 %, of which can be identified very early: Nika, Secunda, Maxi; mid-season: Sunray, G135 – 43 %, middle: Viola, G171 – 37 %, and late – 13 %). Indicators of high productivity and quality of green beans were found in 44 % of samples: G135, Peak, Rocqentcant, Sunray, Nika with a yield of more than 3.5 kg/m<sup>2</sup>. The creation of varieties with a complex of certain selected characteristics on the basis of available breeding samples of the Siberian gene pool for specific soil and climatic conditions will ensure guaranteed high productivity and product quality.

## Analysis of the evolution of gene expression patterns in flowering plants

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Polyploidization with subsequent functional divergence (neo- and subfunctionalization) or loss of duplicated genes is one of the major mechanisms of plant genome evolution. In particular, whole genome duplications are associated with the emergence of such large clades as monocots and core eudicots. However, the patterns of these processes remain unclear in many aspects. The way to gain insight into them is the study of the evolution of gene expression profiles as soon as the expression reflects the function. Using the method of gradient busting we performed comparison of detailed expression maps for five plants, representing large groups of angiosperms: rosids (the model object *Arabidopsis thaliana*), asterids (*Solanum lycopersicum*), caryophyllids (*Fagopyrum esculentum*), monocots (*Zea mays*) and basal angiosperm *Amborella trichopoda*. This allowed identification of groups of genes with conserved expression profiles, present in all five species. These profiles are presumably inherited from a common ancestor of angiosperms and can be called the core pan-transcriptome of flowering plants. Based on these data it is possible to trace the genes that changed their function after duplication and ones that underwent neo- and subfunctionalization. Also, the analysis of the coexpressed groups of non-homologous genes allows for the search of conserved regulatory modules, in particular, via identification of transcription factor binding sites in promoters. Besides this the data on evolutionary conservation of expression profiles will contribute to the translation of knowledge gained through studies of model species to non-model plants.

## Alloplasmic introgression and DH-lines of (*H. vulgare*)–*T. aestivum* and (*H. marinum* ssp. *gussoneanum*)–*T. aestivum*: research models and initial material for breeding

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Introgressive hybridization is a basic method for increasing the genetic diversity of bread wheat. In our studies, genotypes of bread wheat with new intergenomic interactions by replacing the cytoplasm of wheat with an alien one are creating. These genotypes (alloplasmic lines) are using for production of introgression and homozygous introgression (DH) lines with pyramids of genes controlling resistant to fungal pathogens. Developed a set of methods aimed at restoring fertility and overcoming nuclear-cytoplasmic conflict when crossing between individual species of barley and wheat, as well as the subsequent reconstruction of nuclear genes as a result of the integration of alien genetic material into the nuclear genome of alloplasmic lines (*H. vulgare*)–*T. aestivum* and (*H. marinum* ssp. *gussoneanum*)–*T. aestivum*. It was shown that the peculiarities of restoring the fertility of alloplasmic lines and integration of chromosomes of barley in genome of alloplasmic lines depending on the species of barley. It was discovered that the patterns of fertility restoration of alloplasmic lines associated with the variability of nuclear and cytoplasmic genomes. It has been established that the conditions of anther culture for production of DH lines do not negatively affect nuclear-cytoplasmic compatibility in alloplasmic genotypes (*H. vulgare*)–*T. aestivum*. In our work, alloplasmic and DH lines are used as a new genotypes in breeding. DH lines serve to accelerate the selection of the desired genotypes with target genes and are included in the hybridization to obtain the initial material for breeding. Alloplasmic wheat-barley substitution and addition lines (*H. marinum* ssp. *gussoneanum*)–*T. aestivum* with chromosomes of *H. marinum* are used to obtain euplasmic wheat-barley introgression lines.

The positive effect of individual chromosomes of this wild barley on the content of protein in the grain was shown.

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## Bioinformatics analysis of the structures of CRISPR/Cas-systems in the genomes of phytopathogenic bacteria

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The study of phytopathogenic bacteria is a topical issue in most discussion. Since that time, when microorganisms disrupt the carbohydrate and protein exchanges of a plant. The object of the study is full genome sequence of the *Agrobacterium fabrum* str. C58 chromosome circular, downloaded from the RefSeq database (NC\_003062.2.). Pathogenic strains of *Agrobacterium* spp. carry at least one Ti or Ri – plasmid. To date, to avoid mass death of plants, targeted genetically modified approaches are being developed, for example, CRISPR/Cas. To find CRISPR/Cas-systems MacSyFinder were used. The research for the structural and functional characteristics of the Cas-genes was carried out using the HMMER and makeblastdb software. CRISPI, CRISPRFinder, CRISPRDetect were used to select CRISPR-arrays in the genome of the bacterium. To looking for phages through decoded spacer sequences was taken the BLASTn. The results of the bioinformatics study have showed the following: 1 CRISPR-array was found, having 3 spacer sections separated by repeat. Spacers are 18 b.p., repeat – 5 b.p. The total length of the CRISPR-array was 510 b.p. with repeat (C-GGCGGCTGTTCGGCAGG). The obtained spacer sites were analyzed in the CRISPR-array for phage identification in such software: Phages, CRISPRTarget. It was possible to identify the belonging of the spacers to the phages of Mycobacterium Phage Shipwreck (13 repeats), Gordonia phage GMA2 (15 repeats). Also, using the BLASTn, we have found that *Agrobacterium fabrum* has full identity with *Plantactinospora* sp. BC1 chromosome, *Streptomyces* sp. IH-SSA4 genome, *Xanthomonas perforans* 91-118 etc. These results help us to suggest that, in addition to virulent plasmids, the total repeat plays a role in pathogenicity trough the CRISPR-array. Thus, the bioinformatics analysis of the CRISPR/Cas-system of the phytopathogenic bacterium *Agrobacterium fabrum* str. C58 chromosome circular made it possible to find out which part of the DNA in the bacterium in order to change its pathogenic properties.



## Developing of scientific resources for marker-assisted selection of a new legume crop – *Cyamopsis tetragonoloba* (L.) Taub. as a base for guar gum industry in Russia

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Since Sept. 2017 Vavilov Institute has been leading the national project focusing on the development of new varieties of guar using marker-assisted selection for import substitution of guar gum for the oil, gas and food industries in Russia. Guar, an herbaceous legume plant, is widely cultivated in India, Pakistan, Afghanistan, Kenya, Australia, and semi-desert regions of the United States. Guar gum, extracted from the seeds of guar, is in demand in the oil and gas industry. Until now, guar gum has been fully exported from abroad. Nowadays, there is an evident need to develop new guar varieties, adapted to the conditions of cultivation in the territory of the Russian Federation. In frame of the project various approaches of modern breeding were employed. Among them are ecological testing of a number of genotypes in different climatic conditions, field evaluation of VIR guar germplasm collection in Kuban experimental station (Krasnodar area), high throughput genotyping of the diversity panel of ~200 genotypes by RADseq method using Illumina HiSeq2500 and Genome-Wide Association Study. Since guar is a short-day plant, a special effort was made to discover the photoperiod insensitive genotypes among the guar genetic diversity tested. Valuable genotypes with the shortened vegetation period were discovered. Original laboratory methods have been developed for estimating the percentage and viscosity of gum in guar seeds. Root nodule bacteria were isolated from guar, their 16S rRNA sequences were deposited in NCBI. As the result, five most promising breeding lines were suggested as the new guar varieties adopted to the climate conditions of Krasnodar region. Astrakhan and Volgograd regions are suggested as the regions where the new crop can be successfully propagated under irrigation.

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## Polymorphism of *CAD* and *CESA* genes in flax (*Linum usitatissimum* L.)

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Flax (*Linum usitatissimum* L.) is used for production of fiber and seed. The content of lignin and cellulose is an essential characteristic that determines the suitability of fiber for textile and composite productions. It has been shown that cinnamyl alcohol dehydrogenases (CADs) are involved in the processes of lignin formation, while cellulose synthases (CESAs) play a key role in cellulose biosynthesis. In the present work, polymorphism of *CAD* and *CESA* genes was estimated in 288 flax cultivars and lines with lignin content varied from 2 % to 7.5 % and cellulose content varied from 82 % to 88 %. DNA was extracted from pools of seedlings for each sample using CTAB protocol. Primers were designed for amplification of *CAD1* and *CESA4* genes and their putative promoter regions with 450–500 bp DNA fragments. DNA libraries were obtained using two successive PCRs which allowed us to add sequences that are necessary for sequencing on the Illumina platform. Quality and concentration of DNA libraries were evaluated by Agilent 2100 Bioanalyzer (Agilent Technologies) and Qubit 2.0 fluorometer (Life Technologies). Deep sequencing was performed on MiSeq Illumina with 300+300 read length. The average coverage for studied *CAD* and *CESA* genes in an individual sample was over 100x. The pipeline was developed for analysis of the sequencing data, and genetic polymorphism of *CAD1* and *CESA4* genes was assessed within 288 flax samples. Obtained data contribute to the evaluation of genetic diversity of flax, determination of association between *CAD* and *CESA* allelic variants and fiber qualities, and could be used in breeding of improved cultivars.

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## Mapping of loci associated with drought tolerance in chromosomes 2A and 2D of bread wheat and the search for responsible candidate genes

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The study of the genetic mechanisms of wheat adaptation to environmental conditions is necessary for successful selection of cultivars tolerant to abiotic stress factors, drought being the most harmful. The study of the genetic architecture of traits that affect drought tolerance is of great, if not crucial, importance for overcoming the negative impact of drought on crop yields. All of them have a polygenic control and provide coordination of many physiological and biochemical processes. Analyzing the associations between the alleles of molecular markers and target traits in bi-parental mapping populations or by association mapping is the most effective strategy in studying the genetic control of polygenic traits. In our work, the both approaches were used to identify the regions associated with the tolerance of photosynthetic apparatus to water deficiency in chromosomes 2A and 2D. Using the introgressed lines Chinese Spring (Synthetic 6x) for D-genome the two regions in chromosome 2D were identified associated with photosynthetic parameters and the activity of antioxidant enzymes under stress conditions. Regions were marked within the *Xgwm455-Xgwm261* and *Xgwm1419-Xgwm539* microsatellite markers, respectively. Using the substitution recombinant lines Saratovskaya 29 (Yanetskis Probat 2A) the two regions were identified in the long arm of the target chromosome associated with the regulation of stomatal conduction, the rate of photosynthesis, the activity of antioxidant enzymes and the yield components under water deficit conditions. Bioinformatic methods of analysis of these regions have revealed the candidate genes that are homologous to the known Arabidopsis genes involved in the signaling pathways of the hormonal regulation of stress response.

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## The role of E-box-, G-box- and RY-motif-binding proteins in regulation of ethylene response in *Arabidopsis thaliana*

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Plant hormone ethylene is a major regulator of growth and development that plays an important role in various processes such as seed germination, response to biotic and abiotic stresses, senescence etc. Ethylene signaling proceeds via a linear pathway that activates EIN3 and EIL1 transcription factors (TF) – the key regulators of ethylene response. Besides EIN3 binding to gene promoters, co-factors are often required to trigger gene expression upon ethylene treatment. In this work, we perform a genome-wide analysis of EIN3 binding to investigate complex mechanisms of EIN3-mediated gene expression upon ethylene treatment. *De novo* motif search in EIN3 ChIP-seq peaks (publicly available data) with Homer tool found a variety of enriched motifs. Besides EBS-like motif (well-known EIN3-binding site) ( $p < 1e-109$ ), E-box-like motif ( $p < 1e-125$ ), G-box-like motif ( $p < 1e-47$ ), and RY-like motif ( $p < 1e-52$ ) were in the top. Intriguingly, E-box-like motif rather than EBS-like motif was the first ranked. Using Tomtom tool, E-box-, G-box- and RY-like motifs were annotated as the binding sites for bZIP family group I TFs, PIF family TFs and FUS3 binding sites, correspondingly. Enrichment of the motifs annotated as the binding sites of EIN3-unrelated TFs supports indirectly a possible role of these TFs as EIN3 co-factors. To further investigate this possibility, we performed an analysis of EIN3 and bZIP29/PIF4/FUS3 binding co-occurrence in gene promoters and found it statistically significant ( $p < 2.2e-16$ ). It is noteworthy that FUS3, PIF family and bZIP family group I TFs are known to take part in several ethylene-regulated processes. Therefore, according to our results we suggest that FUS3, PIF family and bZIP family group I TFs could influence EIN3 functioning to regulate gene expression on the genome-wide level. These TFs are promising candidates for future investigations of EIN3 functional partners.

## Sex-associated genome region of poplar

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Poplars (genus *Populus*) are dioecious plants, and sexual dimorphism in males and females is actively studied. Recent investigations on the determination of poplar sex revealed sex-associated single nucleotide polymorphisms (SNPs) and genome regions of their localization. However, the improvement of the genome assembly is necessary for accurate mapping of the sex locus. The key genes that are involved in poplar sex determination are still unknown. The goal of our work was the obtaining of high-quality genome assembly of *Populus* × *sibirica*, which is the most common poplar in Moscow, and identification of sex-specific DNA polymorphism. Twenty male and twenty female plants of *P.* × *sibirica* were collected in different regions of the Moscow city. DNA was extracted and amplicon libraries for sequencing on the Illumina platform were prepared. Deep sequencing of genes encoding MET1 and homolog of ARR17, which contain a number of sex-associated SNPs, was performed for the 40 samples on MiSeq (Illumina), and about 4000x coverage was obtained for each sample. We identified 17 (11 in MET1 and 6 in ARR17) sex-specific SNPs. All females were homozygous, while all males were heterozygous for identified sex-specific SNP sites. Moreover, in one allelic variant, males had the same nucleotides as females, while in the other one – sex-specific SNPs. Thus, the colocation of sex-specific SNPs was revealed. Two males and two females of *P.* × *sibirica* were selected for the whole genome sequencing. The Nanopore platform with long reads and the Illumina one with high-precision short reads were used. Combination of these sequencing platforms enabled high-quality genome assembly and accurate identification of the sex-specific locus in *P.* × *sibirica* genome. Obtained results contribute to the understanding of the mechanisms of poplar sex determination.

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## Assembling of the Siberian larch mitochondrial genome using long nucleotide sequence reads, the largest currently known mitogenome

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Plants have very large mitochondrial genomes (mitogenomes) varying from 200 Kbp to 11.3 Mbp. They have also very complex structure in plants. So far, only a few mitochondrial genomes have been sequenced and published in conifers. We succeeded a high-quality assembly of the Siberian larch (*Larix sibirica* Ledeb.) mitogenome using both short and long nucleotide sequencing reads generated by the Illumina HiSeq2000 and MinION (Oxford Nanopore Technologies) sequencers, respectively, and a hybrid approach based on the MaSuRCa pipeline. To select mitochondrial sequences from nuclear and plastid sequences, assembled contigs were mapped to the nucleotide database of complete and partial plant mitogenomes. The final assembly of the Siberian larch mitogenome consists of 9 contigs with a total length of 11,662,539 bp (N50 = 3,031,766 bp). The longest contig is 4,008,762 bp, the shortest – of 24,767 bp. Finally, 40 protein-coding, 34 tRNA, and 3 rRNA genes were annotated. This mitogenome is currently the largest one.

## Introducing CGMS genes to the commercial and hopeful cotton cultivars of Iran

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This study was carried out in order to investigation of possible cytoplasmic-genetic male sterile lines production of commercial and hopeful cotton cultivars of Iran through transferring of these traits from male sterile lines. In this kind of male sterility, the cytoplasm of male sterile at the presence of dominance fertility restorer alleles, lose their effects which cause to return the ability of anthers to produce fertile pollen. This investigation was performed during four years in three regions of Varamin, Gorgan and Kashmar. Annually selfing crosses were done among male sterile and male fertility restorer lines and all cultivars as well as selfing in order to multiplication of lines. In order to transferring the male sterility, crosses were done between cultivars and A line. Harvested seeds from these crosses were cultivated along with their parents in the farm for back-crossing purpose during next years. Also crosses were done between cultivars and R line in order to transferring the male fertility gene. In order to retrieve the cultivars along with male fertility gene, back-crossing between offspring and maternal parent was continued during next years. Because of male fertility gene, the offspring from these crosses were male fertile. Assessment of the male sterile flowers showed that, the filament of flags were short and the anthers were thin and unable to burst. The sterility situation of the anthers was stable and in higher temperature no pollens were seen.



## Development of new methods for obtaining hybrid forms of spring and winter wheat with the involvement of the gene pool of wheatgrass and soybean and confirmation of the applicability of these methods in practical breeding

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To increase biodiversity and improve the quality of wheat, breeders widely use the method of intergeneric hybridization of existing wheat varieties with its wild relatives, in particular, with different types of wheatgrass. Wheatgrass has the following characteristics that it is desirable to pass on to the cultivated wheat: winter hardiness, salt and drought resistance, high content of protein and gluten in the grain, resistance to diseases, low demands on soil fertility, non-lodging. As the source material for producing of new forms of winter WWHs, winter wheat cv. Filatovka and androgenic plants of wheatgrass *Elytrigia intermedium* were taken in our work. An original leaf nurse method of transmitting traits between different species of plants without hybridization has been developed. To produce LN-lines (lines produced by the leaf-nurse method), a winter wheat cv. Bagrationionka, and wheatgrass leaves, are taken. As a result of the application of the leaf nurse method, the appearance of plants with awned spike and elongated grains was noted. In some experiments, soybean leaves were used as a nurse. The disappearance of the awnes of the spike and the decrease in the height of plants are noted in this case. In 2017, 52 genotypes of winter WWHs and LN-lines were tested. Thirty genotypes had a significantly higher yield and quality of grain than standard cv. Novosibirskaya 40. In the competitive test 2017–2018, 68 genotypes of WWHs and LN-lines were taken. Most of them showed high yields and resistance to lodging. No marked disease was observed. There was no damage to kernel smut. The root system of many genotypes was significantly more developed than the standard. This sign is clearly transmitted from the wheatgrass, which has a powerful root system. Analysis of the technological qualities of the grain showed that in many respects the lines created are significantly superior to the standard grade. Spring forms of WWHs were obtained by backcrossing the produced winter WWHs by spring wheat. As a result of selections in 4 generations, 32 promising non-lodging spring forms of WWHs were obtained. The yield, the size of the spike and the weight of 1000 grains of most of the accessions exceed the standard commercial cultivars. In order to expand genetic diversity and improve grain quality, the best accessions of spring WWHs were crossed between themselves and with two cvs of spring wheat Novosibirskaya 31 and Omskaya 37. Further selection was carried out in the greenhouse and in the field. In 2019, the best 129 genotypes from 419 were selected for further investigations. Thus, the obtained results prove the great promise of applying the used selection methods to obtain new valuable forms of spring and winter wheat.

## Intragenomic polymorphism of internal transcribed spacer ITS1 in the locus 35S rRNA of polyploid *Avena* species

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Using sequence-tagged Roche 454 platform, we studied intragenomic polymorphism of ITS1, a spacer region of the multiple 35SrRNA genes in four polyploid *Avena* species. Comparation of ITS1 sequences from the diploid species, earlier sequenced by Sanger approach, allowed to detect two indels specific for all ITS1 of C-genome diploid species. It enabled easily identifying rare C-subgenome-specific ITSs among hundreds of ITS1 reads characteristic for the A-subgenomes. Instead of expected 50 % C-variant reads of 35S rDNA in *A. insularis* (karyotype AACC or CCDD) and 33 % C-variant reads in hexaploids *A. fatua*, *A. ludoviciana* and *A. sterilis* (all AACDD), the actual rate consisted only about 4 % in *A. insularis* and 2–3 % C-variant reads in hexaploid genomes. The C-genome-originated 18S (fragment), ITS1 and 5.8S (small fragment) were 10 times more variable than the same sequences originated from to A-genome. Some of the sequences of C-subgenomes contained deletions, including deletions in the 18S rRNA region. As for the origin of C-subgenome ITS1s in the polyploid species, some sequences revealed similarity to the ITS1 variants of either *A. macrostachya* (Cm) or *A. ventricosa* (Cv), but the core variant of C-subgenome ITS1 on the genetic tree is approximately equidistant from all the present-day C-genome diploid species. The A-type ITS1 of *A. insularis* is represented by few families, one of which is close to the A-genomes of *A. longiglumis*, *A. hirtula* and *A. wiestii*. As for hexaploids, there are two more frequent families of A-type rDNA reads in their genomes. We believe that one of them, represented by a higher number of reads, is located in NORs of D- and another one in NORs of A-subgenomes.

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## The association mapping of quantitative resistance loci to net blotch and spot blotch in barley

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Spot blotch, caused by *Cochliobolus sativus*, and net blotch, caused by *Pyrenophora teres* f. *teres* are two of the most widespread and harmful diseases in barley. The identification of genetic loci associated with resistance to both *C. sativus* and *P. teres* is the important task for future marker-assisted selection. The goal of the current study was to identify loci conferring seedling resistance to different pathotypes of *C. sativus* and *P. teres* in the Siberian spring barley core collection. The collection included 96 spring barley cultivars and lines was created. All of them were phenotyped at the seedling stage with two *C. sativus* isolates (Kr2 and Ch3) and four *P. teres* isolates (S10.2, K5.1, P3.4.0, A2.6.0). About 42–47 % and 15–40 % genotypes were resistant to spot blotch and net blotch, respectively. A total of 94 genotypes were analyzed with the barley 50K Illumina Infinium iSELECT assay. 27,319 SNPs from total 44,040 SNPs passed filtering threshold and were used for association mapping. The GLM analysis revealed 3 and 27 SNPs for spot blotch isolates (Kr2 and Ch3) and 6, 3, 29 and 3 SNPs for net blotch isolates (S10.2, K5.1, P3.4.0, A2.6.0), respectively. In total three genomic regions on chromosomes 1H, 2H and 3H were assisted with resistance to spot blotch and seven genomic regions on chromosomes 1H, 2H, 3H and 6H were assisted with resistance to net blotch. The data were assessed using PASS, Tassel 5 and R. Information of SNPs related can be used further for development of DNA-markers convenient for diagnostics of resistance-associated alleles in barley breeding programs.

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## The introgression peculiarities of the wheatgrass 6Ai chromosome in various varieties of common wheat

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Wheatgrass *Thinopyrum intermedium* (Host) is a source of agronomically valuable traits in wheat breeding. Using wheat-wheatgrass substituted line *Agis* 1 varieties of spring wheat Tulaykovskaya 5 and Tulaykovskaya 10 were produced. In the genome of the cultivar Tulaikovskaya 10, chromosome of wheat 6D is replaced by the wheatgrass 6Ai chromosome, which carries the genes for resistance to fungal diseases, providing immunity to plants in various ecological-geographical zones. In this work the hybridization of wheat varieties Saratovskaya 29 (C29), Novosibirskaya 15 (H15), Novosibirskaya 31 (H31) and Novosibirskaya 67 (H67) with the variety Tulaikovskaya 10 (T10) was carried out with the aim of obtaining plants with centromere breaks of the wheatgrass chromosome in  $F_2$ , and in the longer term – plants with centric wheat-wheatgrass translocations. The meiosis of  $F_1$  hybrids was analyzed. In meiosis, centromere breaks of chromosomes 6Ai and 6D occurred, on the basis of which a prediction was made about the presence of 6Ai telocentrics in the  $F_2$  generation.  $F_2$  plants were analyzed using MF2/MR1r2 primers to the long arm of chromosome 6AiL *Th. intermedium*, Te6HS476 to the short arm of the chromosome 6AiS *Th. intermedium* and MF2/MR4 to the long arm of chromosome 6DL. According to PCR analysis of genomic DNA, the frequencies and transmission patterns of chromosome 6Ai are influenced by both the genotype of the recipient variety and the use of T10 as the maternal form. Among the studied plants, those (66) were selected for further analysis, in which primers were only amplified from the long or short arms of the wheatgrass chromosome. Plants  $F_3$  T10  $\times$  C29 with 6AiS telocentric were grown under field conditions, they were characterized by resistance to powdery mildew and leaf rust.

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## Patterns of durum wheat response to favorable environments

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Responsiveness is an important part of durum wheat adaptation taking that the crop is usually sown after good preceding crop supplied with major nutrients and moisture. For the period 1985–2018 the yield in competitive yield trails of spring durum wheat in Ob' forest-steppe of Altai territory sown after fallow field made up 0.1...5.1 t/ha. In 2018 durum wheat produced the highest yield for the time being – 5.6 t/ha. In previous four years the yield of the same set of genotypes made up 3.3 t/ha. The rise of yield was accompanied with the increase of the number of spikes per plant (+55 %), above-ground plant weight (+85 %), grain weight of main spike (+52 %), grain weight of side spikes (+135.3 %), grain weight per plant (+120 %). Number of kernels per spike and 1000 kernel weight increased less +17.6 % and +27.2 % respectively. Parameters of crop density either were similar (spikes/1 m<sup>2</sup>) or lower (plants/1 m<sup>2</sup>) than in previous years. A mid-ripening line Hordeiforme 881 and a mid-late line Hordeiforme 748 were top-yielders with grain yield 6.90 and 6.47 t/ha that was 1.22 and 0.81 t/ha higher than corresponding checks had. They have advantage in above-ground plant weight, grain weight of main and side spikes and plant as a whole and a number of kernels per spike. Hordeiforme 881 produced kernels 4.5 g larger than check variety. Low-yielding genotypes in favorable environments did not reach mean yield because of the lower level of a complex of related traits with largest impact of plant weight and grain weight of side spikes. Old local varieties made the majority of the group.

## Major approaches in improving wheat resistance to the crucially dangerous diseases in Kazakhstan

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In Kazakhstan, rusts (*Puccinia graminis*, *P. triticiana* and *P. striiformis*) and leaf spots (*Zymoseptoria tritici*, *Pyrenophora tritici-repentis*, *Parastagonospora nodorum*) are most common among the crucially dangerous diseases of wheat. Since 2018 science and technology program “Development of the innovative systems for increasing the resistance of wheat varieties to especially dangerous diseases in the Republic of Kazakhstan” is realized in the country under financial support of the Ministry of Agriculture (program number BR06249329). The specialists in plant pathology, mycology, breeding, genetics and molecular biology from various scientific institutions take part in implementation of the program. As a result in 2018 sources of wheat resistance to the above mentioned diseases have been ascertained. Among the tested spring wheat cultivars 44 genotypes appeared to be resistant to rust and leaf spot species. New breeding lines (91) of spring wheat were created with use of disease resistance donors. Fifty two perspective cultivars of spring soft wheat from the breeding institutions of Kazakhstan and Russia were studied and the cultivars with effective combinations of Lr- and Sr-genes were revealed among them. The data of genotyping the collection of common wheat using new genomic technologies (Illumina 20K SNP array technology) were obtained. On the basis of a genome-wide association analysis of the soft wheat resistance to the most dangerous diseases at the adult plant stage, 11 QTLs associated with resistance to 4 fungal pathogens were identified. Alignment and analysis of nucleotide sequences of *Z. tritici*, *P. tritici-repentis* and *P. nodorum* genes resulted in identification of polymorphic sites that were used to develop species-specific primers ensuring highly sensitive diagnosing of all studied fungi by PCR.

## The resistance of different wheat species to greenbug aphid *Schizaphis graminum* Rond.

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In our work, we studied the expression of genes encoding PR proteins and redox enzymes of wheat species *Triticum aestivum* L., *T. monococcum* L. and *T. timopheevii* Zhuk., infected by *Schizaphis graminum* Rond. It was detected that hydrogen peroxide performs an important signaling function in triggering defense reaction in the short-term response of plants to insect feeding. In the long-term response of plants to pest infection, the processes of detoxification of reactive oxygen species, in which peroxidase and catalase involve, were important. The processes of reactive oxygen species detoxification were important in the long-term response of plants to pest infestation. Analysis of antibiosis and tolerance (persistence) showed that *T. aestivum* Omskaya 35, *T. timopheevii* k-58666 and *T. monococcum* k-39471 were tolerant to *S. graminum*. *T. monococcum* k-39471 was the most resistant species. The most tolerant to *S. graminum* species *T. monococcum* k-39471, showed the highest peroxidase activity throughout the experiment, and the catalase activity was regulated depending on the stage of infection. It is known that infection by aphids forms jasmonate/ethylene and salicylate-dependent defense in plants. In our work, the expression of genes encoding PR proteins, markers and regulators of salicylate (*TaRboh*, *TaPAL*, *Tapr1*, *TaPrx*) and jasmonate signaling pathways (*TaPI*, *TaLOX*, *TaPrx*) was studied. In the early stage of infection in susceptible *T. aestivum* varieties, expression of only jasmonate-dependent genes was activated, which reflected the response of plants to damage. In the resistant accession k-58666, expression of only salicylate-dependent genes was activated, while the aphid practically did not reproduce. In the tolerant accession k-39471, expression of both salicylate-dependent and jasmonate-dependent genes was activated, while the aphid mortality rate was the highest among all studied samples (39 %). Aphids attack set off jasmonate-dependent activation of proteinase inhibitors and lipoxygenases in tolerant samples. Thus, salicylate signaling pathway is probably the joint mechanism of antibiosis and repelling aphids in resistant plant forms.



## What we know about vernalization process in wheat

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A transition from vegetative to reproductive stage is a crucial step in the plant development. Some plants have to undergo a few weeks of cold treatment – vernalization. Bread wheat (*Triticum aestivum* L.,  $2n = 6x = 42$ ), is one of the most cultivated crops worldwide and occurs in two growth habits: a spring type and winter type. Winter wheat varieties are characteristic for their vernalization requirement for transition from vegetative to reproductive phase. To date, four genes controlling this process are known, denominated as *VRN1*, *VRN2*, *VRN3* and *VRN4*. An allelic variability in the *VRN-1* gene is associated with large differences in flowering time and thus fast adaptability of wheat to diverse locations. These changes are linked with variation at the DNA sequence level (rearrangements in the promoter region and the first intron) and also with copy number variation (CNV) of the genes. In addition, epigenetic modifications like DNA methylation and histone modifications are associated with the gene expression and triggering of flowering. We focused on the role Polycomb repressive complex 2 (PRC2) in vernalization process. Here, we describe individual components of these complexes in bread wheat for the first time. *In silico* identification of PRC1 and PRC2 subunits in hexaploid wheat was supported by gene expression data from RNAseq. Moreover, we are studying influence of different sequence motives of *VRN-1* gene and promoter to determine the most critical region for vernalization requirement. New molecular tools could contribute to better understanding of molecular mechanism of this important biological process.

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## **Towards genome-based and environment-informed breeding intensification**

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Chickpea is the second most widely grown food legume, providing a vital source of nutritional nitrogen for ~15 % of the world's population. With success in chickpea domestication came an incremental loss in adaptive variation and currently genetic and adaptive variation has nearly disappeared from elite high-yielding chickpea cultivars. Given this perspective there is a compelling need to re-introduce genetic variation into chickpea lines. The Vavilov seed bank contains numerous landraces collected nearly hundred years ago, before intensive breeding of most crops, and thus is a potential reservoir of “genomic gems” that might offer solutions for improving elite varieties. Here we analyze the genomes and ancestral environments of ~500 of Vavilov's original landraces, sampled from major historic centers of chickpea cultivation and secondary diversification. We dissect important phenotypes to their underlying genetic basis, including large-effect genes and whole-genome smaller-effect contributions, enumerate beneficial alleles, identify polymorphisms that assort by environment and that are candidates for local adaptation and figure how to this standing variation can be combined to produce improved cultivars. This knowledge could then be used to optimize chickpea breeding.

## Identification and numeration of the univalent chromosomes for cotton monosomic lines by means the tester translocations

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We have continued to identify cotton monosomic lines of the cytogenetic collection of Uzbekistan using a well-defined tester set of translocation lines of the USA, kindly provided by Prof. D.M. Stelly, Texas A&M University, USA. Monosomes were identified by analyzing meiotic metaphase I configurations of monosomic translocation heterozygous  $F_1$  hybrid plants. The tests with translocation lines showed, that the monosomes Mo76 and Mo89 are chromosome 4 At – subgenome of cotton, because the monosomic  $F_1$  hybrids from crosses with two translocation lines TT4L-19R and TT4R-15L had the MI pairing configurations  $24^{II} + 1^{III}$ . Also the study detected, that the monosomes Mo34 and Mo95 are chromosome 6 At – subgenome of cotton, because the monosomic  $F_1$  hybrid plants of Mo34xTT3L-6L, Mo34xTT6L-14L and Mo95xTT3L-6L, Mo95xTT6L-10R had the MI pairing configurations  $24^{II} + 1^{III}$ . The tests indicated, that the monosome Mo93 is chromosome 2 At-subgenome, because the MI pairing configurations in the monosomic  $F_1$  hybrid plants of Mo93xTT2R-8Ra, Mo93xTT2R-8Rb and Mo93xTT2R-14R were  $24^{II} + 1^{III}$  and common chromosome 2 had in all translocations. The test showed, that the monosome Mo48 could be from chromosome 7 or 18, because in the monosomic  $F_1$  hybrid of Mo48xTT7L-18R the MI pairing configuration was  $24^{II} + 1^{III}$ . Molecular marker data suggested that the monosome Mo48 is chromosome 18 Dt - subgenome. The test with translocation line TT15R-16Ra pointed out, that the monosome Mo82 could be from chromosome 15 or 16 Dt – subgenome, because in the monosomic  $F_1$  hybrid of Mo82xTT15R-16Ra the MI pairing configuration was  $24^{II} + 1^{III}$ . The utilization of the translocation lines in our investigation provided the unified numeration of the monosomes for 10 monosomic lines of the Uzbek cytogenetic collection: one is chromosome 2 At- subgenome, two are chromosome 4 At- subgenome, two are chromosome 6 At- subgenome, one is chromosome 18 Dt- subgenome and the monosome Mo82 could be from chromosome 15 or 16 Dt-subgenome of cotton.

## Systems biology study on the WOX5 role in the distal part of the root meristem in *Arabidopsis thaliana*

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Root stem cell niche functioning requires formation and maintenance of specific “auxin-rich domain” governed by directional auxin transport and local auxin production. Auxin maximum co-localizes with WOX5 expression domain in mitotically inactive quiescent center (QC) that separates mitotically active proximal and distal root meristems. Columella stem cells prematurely differentiate in loss-of-function mutant *wox5-1*. An opposite effect was observed in 35S::WOX5-GR overexpression transgene upon DEX treatment where columella stem cell daughters (CSCDs) do not undergo normal differentiation. We showed that WOX5 modulates TAA1-mediated auxin synthesis and PIN-mediated auxin transport in root apical meristem. We hypothesized that modulation TAA1-mediated auxin synthesis resulted in observed changes in anatomical structure of columella under knockout and overexpression of WOX5 genes.

We developed 1D computational model of auxin distribution in columella with growth and division of cells. Using the mathematical modeling approach we showed that WOX5-dependent changes in TAA1-mediated auxin synthesis are sufficient to reproduce all other changes observed in WOX5 knockout and overexpression lines. We can conclude that the main role of WOX5 gene for auxin distribution is modulation of TAA1-mediated auxin synthesis. In addition, we predicted that additional division occurred only in the distal part of columella while the cells in the proximal part of columella remain quiescent in 35S::WOX5-GR transgenic line. That was confirmed by experiment with kynurenine treatment.

## Genome-wide association and epistatic scan for unravelling the genetic architecture of complex traits and their practical applications in a breeding program

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Genome wide association mapping in conjunction with comprehensive epistatic scans of the genome can help unravelling the genetic architecture of complex traits such as grain yield and yield stability. Important genomic regions identified this way can propel common genomics-assisted breeding strategies implemented in a breeding program such as marker-assisted selection (MAS) and genomic selection (GS). We conducted in-depth investigation on the above on a large set of seven cohorts of advanced bread wheat lines ( $n = 6,518$ ) within CIMMYT elite yield trials, which were genotyped with genotyping-by-sequencing markers and phenotyped under contrasting (irrigated and stress) environments. Haplotype-based genome wide association mapping identified a repertoire of beneficial alleles for GY and yield stability (superiority index  $P_i$ ) in CIMMYT germplasm on chromosomes 1A, 1B, 2B, 3B, 4A, 4B, 5B, 6B and 7B with allelic effects ranging from 2 to 10 %. Epistatic interactions contributed to an additional 5 to 9 % variation on average. Stepwise regression of these genomic regions unraveled the best combination of alleles that can be used for MAS. We also explored whether integrating a subset of the consistent associations as fixed variables in prediction models improves prediction accuracy for GY and yield stability. For GY, the model led to up to 10 % increase in prediction accuracy in cross-validation. Lines have been identified with different combinations of beneficial alleles to be included in crossing and line development schemes.

## Assessment genetic structure of Azerbaijan wild and cultivated barley genotypes by biochemical marker

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A serious problem agriculture currently faces is the decreasing variability in genetic resources, which has resulted from the number of economically important crops being reduced and in addition, fewer varieties of these being used in place of many traditional varieties. Diversity in plant genetic resources provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits and breeders preferred traits.

Extreme diversity of the soil and climatic conditions of Azerbaijan Republic support a very rich diversity of plant genetic resources. To preserve and use efficiently in the future the current genetic pool, it is necessary to evaluate the extent of this diversity by identification and distinction of Azerbaijan barley accessions, as well as the determination of genetic relationship between cultivated and wild relatives.

In order to investigate polymorphism resulting from monomeric prolamine and hordein, 49 wild and cultivated barley genotypes obtained from National Genbank of Azerbaijan Republic were analyzed. Monomeric prolamins and hordeins, which are part of storage proteins were identified by Acid-PAGE technique, using the Poperelya and Mujarinko's method. The difference in bands pattern can appear as an efficient tool in polymorphism. By analyzing of the hordeins, in each region 6 different patterns were observed separately. The average genetic diversity index for these proteins was calculated as  $PIC = 0.96$ . In the analysis of the monomeric prolamins, 9, 7, 7, and 8 various patterns were observed for  $\omega$ ,  $\gamma$ ,  $\beta$  and  $\alpha$  areas, respectively. Also, the calculated average of the genetic diversity index for these proteins was the same of hordein seed storage  $H = 0.95$ .

Monomeric prolamines and hordein analyses were applied for the first time to obtain preliminary information on genetic profile of wild type barley samples in Azerbaijan. Our data suggests both markers are effective and reliable genetic markers for accurate assessment of genetic variation. Our analyses revealed high diversity between *H. spontaneum* and *H. vulgare*. Therefore, this information could be very useful for further management of Azerbaijan barley germplasm.

## The role of *Eutrema salsugineum* cold shock domain protein EsCSDP3 in the cold-acclimation

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Plant cold shock domain proteins (CSDPs) are DNA/RNA-binding proteins. CSDPs contain the conserved cold shock domain (CSD) in the N-terminal part and glycine-rich regions with varying number of the retroviral-like zinc fingers in the C-terminus. At present, the number of studies suggest an involvement of CSDPs in adaptation to the abiotic stress and the role in the plant growth and development.

The primary goal of this study is to understand the role of RNA-binding protein EsCSDP3 from *Eutrema salsugineum* in the cold-acclimation.

Our previous study have indicated that overexpression of EsCSDP3 increase tolerance to freezing in transgenic *Arabidopsis thaliana*.

RNA pull-down assay was conducted on *A. thaliana* with stable expression of EsCSDP3-Halotag or Halotag only. Initial analysis determined that chimeric protein EsCSDP3-Halotag saves ability to bind RNA with high affinity.

Rosette leaves from plants cultivated at 23 °C and then transferred to 4 °C for 3 weeks were ground in liquid nitrogen. Whole cell extracts containing the Halo tagged bait protein were incubated with affinity magnetic beads for 2 h at 4 °C. The beads were washed with lysis buffer. Bound RNA-protein complexes were eluted by incubating the beads with buffer containing TEV protease. Total RNA was prepared from either whole cell extract or from the Halo purified samples using ExtractRNA reagent.

Comparative RNA profile were analyzed by Bioanalyzer 2100 using reagents for total and small/microRNA. RNA was reverse transcribed and the resulting cDNA was analyzed by PCR followed by agarose gel electrophoresis.



## Effect of root exudates and rhizobacteria on colonization of barley roots by phytopathogenic fungi *Fusarium culmorum*

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The studies were aimed at clarifying the question: why *Fusarium culmorum* in 1–4 days from seeds germination more actively colonized roots that were colonized by *Pseudomonas fluorescens*, but not sterile roots. Daily and four-day exudates of sterile barley and colonized, separately and jointly, by fungi and bacteria were obtained. The quantitative composition of root exudates and nutrition requirements of the fungi and bacteria was established using an ultra-efficient liquid chromatography. The effect of barley root exudates on the ability of the fungi to colonize barley roots and the nature of the interaction between the facultative phytopathogenic fungi *F. culmorum* and the antagonistic bacteria *P. fluorescens* has been evaluated. It was established that glucose is the main component of the root exudates used both by the fungi and bacteria. The bacterial strain, more active than the fungi, utilized of organic acids and amino acids. Similar nutritional needs of the fungi and bacteria can lead to competitive relationships between these microorganisms. It has been established that root exudates of barley that were colonized by *P. fluorescens* stimulate the growth of the macroconidium growth tubes of *F. culmorum*. A comparative analysis of low molecular weight components of root exudates showed that exudates of barley roots that were colonized by bacteria contain a significantly higher amount of glucose and lower amounts of aromatic acids (plant defense compounds having antimicrobial activity) than root exudates of sterile barley. Stimulating the growth of the fungi could be the reason for more active colonization by the fungi of the roots colonized by bacteria. It has been established that both fungi and bacteria that colonizing the roots of barley mainly influenced on the amount of low molecular weight exometabolites, and not the qualitative composition of root exudates. *Acknowledgements:* These studies were funded by RFBR (project 18-016-00111).

## Application of Amplifluor-like SNP markers in plant genotyping

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Single nucleotide polymorphisms (SNP) represent a very useful tool, used successfully in a wide array of plant genotyping projects. There are various methods for SNP analyses, most of which have been commercialised. The Amplifluor (Amplification with Fluorescence) SNP method is based on competitive allele-specific PCR, similar to that applied in KASP markers. Two steps are required to carry out Amplifluor SNP analyses: PCR using Universal probes (UPs) and then with Gene-specific primers (GSPs), which are developed independently. Each of the two UPs contains a fluorophore and a quencher with a 'hair-pin' fragment in-between. During PCR with a DNA template containing one of two alleles at the SNP position, the amplification with GSPs will result in the release of fluorescence from one of the UPs. The two UPs are relatively expensive, but their 'universality' allows for their purchase as a 'one-off' order that provides a stock for all further SNP analyses. This makes the application of the UP mixture much cheaper, since GSPs for each SNP cost the same as ordinary oligos. The significantly lower cost of Amplifluor-like SNP markers is accompanied by a high degree of freedom, completely 'in the minds' and 'in the hands' of researchers. Various SNP genotyping studies using self-designed Amplifluor-like markers were presented for analyses of many genes in different plant species. Examples include: Transcription factors *DREB5* and *NFYC-A7*, and transcriptional repressor *Dr1* in wheat; intracellular vesicle trafficking genes, *RabC-GTP* in chickpea; phytochrome C and *SAP* genes encoding stress-associated proteins in barley; chitinase genes in sugar beet; as well as a genetic polymorphism in different species of crested wheatgrass. All these cases provide useful 'fuel' for further candidate gene studies and Marker-assisted selection.

## Structural peculiarities and polymorphism of the SQS-gene controlling the synthesis of squalene in amaranth

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Amaranth (genus *Amaranthus*) is a unique dicotyledonous plant with great potential as a grain, vegetable and fodder crop. Oil from amaranth grain is enriched with valuable lipid compounds, in particular, squalene, the content of which (2.2–10 %) significantly exceeds the content of squalene in other plant oils [1]. Squalene is widely used in medicine: as an adjuvant in vaccines, immunomodulator and antioxidant in the complex therapy of a such diseases as diabetes, ischemic disease, etc., as part of cosmetics [2]. The key gene in squalene biosynthesis is the gene encoding the enzyme squalene-synthase (SQS), which catalyzes the final stage of the formation of a squalene molecule. The aim of our work is to study the polymorphism of SQS-gene in different species of *Amaranthus*, as well as to search for those features in its structure that may determine the variation of squalene concentration in amaranth tissues. On the basis of the reference genomic sequence of *A. hypochondriacus*, available in the GoGe database (id40120; <https://genomevolution.org/coge/>) we have established a nucleotide sequence of the SQS-gene and designed specific primers for the promoter (~1 kbp upstream ATG-codon) and coding regions of this gene. As the latter, a region between exons 5 and 9 was taken, containing 3 functionally conservative domains. Using these primers, isolation and analysis of primary structure of the SQS-gene in various species, the representatives of the “grain” amaranth (*A. hypochondriacus*, *A. cruentus*, *A. caudatus*) and their wild predecessors was carried out. The association of revealed structural polymorphism of SQS-gene with squalene concentration in amaranth grain tissue was analyzed.

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## Phenotypic and genotypic evaluation of bread wheat line with introgression from *T. timopheevii* into 2B chromosome

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The gene pool of bread wheat wild relatives is used to transfer genes for resistance to biotic and abiotic stresses. It can serve also as a source of genes for improving the wheat grain quality. Functional alleles of *Gpc-B1* gene (chromosome 6BS) of the wild tetraploid *T. dicoccoides* were introduced into commercial cultivars of tetra- and hexaploid wheats, resulting in the development of new high-protein and high-yielding genotypes. Earlier, we studied the line 821 of bread wheat with introgressions from *T. timopheevii* into the short arms of chromosome 2A, 2B and long arm of chromosome 5A. The aim of this work was to obtain a line of bread wheat with a single introgression on 2B chromosome from *T. timopheevii* and its assessment in terms of grain quality parameters and yield components. The transfer of the desired chromosome into the genetic background of Saratovskaya 29 (S29) cultivar was done using the backcrossing to the corresponding monosomic line of this cultivar. Chromosome substitution correctness was monitored using cytological analysis of hybrids, as well as using the microsatellite markers. In preliminary studies, in greenhouse conditions, the S29 (821 2B) line increased the amount of gluten in grain by more than ten percent compared to the control cultivar. Yield components were evaluated in the line and control under normal and restricted water supply in greenhouse conditions, as well as under natural field growing. Gluten content in grain and milling grain parameters were determined. Additionally, the field grain material was evaluated for the physical properties of dough on alveograph. The line retains a high gluten content and physical property of the dough corresponding to the strong-flour parent S29. Breeders can use the line as a material for improvement of grain quality in newly created wheat cultivars.

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## Genome-Wide Association Mapping of diverse set of spring wheat germplasm in Western Siberia

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The research object was the set of 150 accessions, which comprises: CIMMYT synthetic wheat lines with *Ae. tauschii* genome (44 accessions); synthetic lines from Kyoto University (14); spring bread wheat varieties from the USA and Canada (15); varieties and lines of Omsk State Agrarian University (18), varieties and lines of Omsk ARI (17); wheat varieties of network KASIB-18 from Russia and Kazakhstan (42). The SNPs with MAF less than 5 % and missing data more than 20 % were removed from the analysis. After filtering, 46.268 SNP markers of 143 accessions were used for genetic diversity analysis. The GBS derived SNPs were well distributed across the 21 chromosomes. The B genome had the highest number of SNPs (17.309, ~37 %), followed by the A genome (15.776, ~34 %), and the D genome (13.183, ~29 %). There were 1.258 SNPs located in scaffolds that are not anchored to any of the chromosomes. The number of SNPs per chromosome ranged from 808 (4D) to 3.148 (2B). A total 143 accessions were evaluated on 35 economically valuable traits under field conditions in Omsk in 2017 and 2018 for genome-wide association study. GWAS detected 243 MTAs distributed across 21 chromosomes for traits with phenotypic variance explained (PVE) ranging from 0.3 % to 25.0 %. The highest number of MTAs was detected in the A genome (72), the B genome (65), and the D genome (62). Thus, for the first time in the Russian Federation genome-wide association study under conditions of Western Siberia was carried out. Were identified 243 MTAs associated with 35 economically valuable traits. Some of identified MTAs were reliable in both research years, and had a pleiotropic effect on 2–4 traits at the same time. The markers associated with these traits were identified.

## Radial plant growth – Cellular coordination during growth in two dimensions

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Body shaping in multicellular organisms depends on the activity of distinct stem cell niches coordinated over long distances. Radial growth of plant shoots and roots is a stem cell-driven process fundamental for the mechanical and physiological support of enlarging plant bodies. In most dicotyledonous species, the underlying stem cell niche, the cambium, displays a strictly bifacial character generating xylem (wood) inwards and phloem (bast) outwards. Despite its importance and intriguing dynamics, the functional characterization of cambium stem cells was hampered by the lack of experimental tools for accessing distinct cambium sub-domains. Investigating the hypocotyl of *Arabidopsis thaliana*, we mapped stem cell activity in the proliferating cambium. Through pulse-labelling and genetically encoded lineage tracing, we established different transgenic markers defining a proximal, a central and a distal cambium domain. We further demonstrated that the proximal domain represents a site of xylem formation and the distal cambium domain contains cells determined for phloem development. Moreover, using tissue-specific transcriptomics, chromatin analysis and local genetic perturbation, we unravelled regulatory circuits specifically regulating the transition of common stem cells to xylem or phloem tissues. Thereby, our analysis provides a cellular fate map of a strictly organized plant meristem and reveals determinants instructive for a bifacial tissue production.

## Assessment of genetic diversity in the wheat genetic resources based on agricultural traits

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Wheat is one of the most widely consumed food crops in the world, in which many studies have been conducted on quantitative and qualitative traits, to improve its productivity. In this study, we have evaluated the genetic diversity of agricultural traits in the wheat genetic resources, which was collected and preserved at the National Agrobiodiversity Center to improve their utilization. A total of 450 wheat genetic resources, which was collected from Afghanistan, China and India were used in the study. The wheat genetic resources were planted and investigated with agricultural traits such as number of days to maturity, culm length, and ear length in between 2017–2018. Among the 450 wheat accessions, 16 accessions had the shortest number of days to maturity (209 days); the remaining 15 accessions except India K215537 were from China. In the culm length, China K141112 showed the shortest with 49.7 cm, while India K199831 was the longest with 152 cm. Similarly, 10 Chinese accessions, in which China K250987 showed the longest ear length with 15 cm, 4 Afghanistan accessions and an Indian accessions showed longest ear length. The mean number of days to maturity according to the collection area was  $217.9 \pm 5.6$  days in Afghanistan,  $214.4 \pm 6.6$  days in China, and  $216.3 \pm 6.3$  days in India. The mean culm length was  $131.2 \pm 30.4$  cm in Afghanistan,  $95.2 \pm 52.0$  cm in China,  $111.4 \pm 24.0$  cm in India. Similarly, the ear length was  $10.1 \pm 3.0$  cm in Afghanistan,  $9.8 \pm 3.5$  cm in China and  $9.3 \pm 2.2$  cm in India. The distribution of agricultural traits in the wheat genetic resources showed wide genetic diversity in Chinese resources, which is probably due to the China's various climatic zones. The results of this experiment could provide basic information for the development of wheat varieties, such as lodging tolerance including early-maturity varieties.



## Unified automated information system for the formation of highly structured plant genomes and proteomes

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Formation of new genomes requires creation of effective ways of extraction, storage, analysis and interpretation of omics data. Currently existing file systems with scripting languages require a fairly deep knowledge of bioinformatics, which makes it difficult to use such systems are not bioinformatics. The report considers the unified automated information system for the formation of highly structured representations of plant genomes and proteomes based on the technology of databases and knowledge bases. The developed system is equipped with an intelligent interface to work with omics data on the basis of semantically understandable terms (DNA, genes, transcripts, exons, introns, mRNA, proteins, metabolites, reactions, metabolic pathways, etc.), forming ontological knowledge of the subject area, and does not require programming knowledge. The system is based on the restructuring of the primary omics data of the world portals (NCBI, EnsemblPlants, etc.), represented by text formats (FASTA, TXT, XML), indexed highly structured database format with storage on a local server or personal computer. The system can also work with experimental data obtained during genome sequencing and/or proteome mass spectrometry. The restructuring and local storage of data, in turn, ensure the system's autonomous operation and high performance. Periodically, the data is updated in an automated mode with connection to the world portal. The system has extensive functionality of working with omics data: access DNA, genes, transcripts, proteins up to nucleotide or amino acids with their coordinates; search and transformation omics data; modeling of signaling and metabolic pathways; the search for motifs of transcription factors; modeling of mutations at the level of genes, transcripts, proteins. Setting up the system for a new genome is carried out in an automated mode and includes the following stages: loading file data from ftp-portals; restructuring and indexing the loaded omics data format of databases; coding data; automatic translation of genes into proteins. Minimum memory requirements: RAM from 4 GB, disk memory of 500 GB. The system can be used for both research and education.

## Transcriptomic changes underlying partial albinism in barley nearly isogenic line

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Chlorophyll is a plant pigment involved in photosynthesis. Abnormalities in chlorophyll synthesis lead to albinism and premature death of the plant. However, cases of partial albinism are known. Such plants are a perfect model to investigate specific details of chlorophyll biosynthesis, plastid development, and interaction of plastid and nuclear genomes. An example of plant with partial albinism is a *Hordeum vulgare* nearly isogenic line i:Bw*Alm* which contains a mutant allele of gene *Alm* located in the short arm of chromosome 3H. However, neither structure of the gene nor its functions are currently known. Microscopic analysis was implemented for phenotypic characterization of i:Bw*Alm* line. Total mRNA of the i:Bw*Alm* line and control Bowman isogenic line was sequenced using IonTorrent platform, and computational analysis was performed in order to identify genes involved in the process. Most of the differentially expressed genes are down-regulated in i:Bw*Alm* line. Among these are genes that take part in chlorophyll biosynthesis, photosynthesis and nitrogen utilization. Down-regulation of genes located in plastid genome was observed. Additionally, *de novo* transcriptome assembly was performed, and putative new barley genes were predicted.

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## Anthocyanin pigmentation in wheat and barley: identification of genes controlling the trait and their allelic diversity

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Anthocyanins play important role in plant-environment interactions including protection of plant against stress. Due to convincing data on their health benefit, there is steady tendency to increase anthocyanin content in food. Particular attention in this aspect is paid to breeding new varieties of cereals with high anthocyanins content in grain that is not possible without knowledge of genetic control of its biosynthesis, diversity of the pigmentation patterns and sources of valuable genes. In wheat and barley, the pigments can be accumulated in pericarp and aleurone, imparting, respectively, purple and blue color to grain, as well as in vegetative parts of plant. Here we identified the full sets of genes encoding flavonoid biosynthesis MBW regulatory complexes that activate anthocyanin synthesis in tissue-specific fashion in wheat and barley. Using sets of near-isogenic lines differing by pigmentation we revealed features of anthocyanin biosynthesis regulation in the two related species. Although the metabolic pathway is universal, there are differences among species in regulation of structural genes encoding enzymes of the pathway. In addition, we noted that wheat and barley genotypes differ by the presence of pigmentation and its intensity that we assume to be caused by allelic diversity of regulatory genes. Studying accessions of different Triticeae tribe species from VIR collection, we found that dominant alleles of some genes determining anthocyanin pigmentation are widely distributed among the accessions investigated (eg. *TaCI* and *HvAnt1*, controlling red color of coleoptile and leaf sheath, respectively), whereas some of them are rare and have local origin (eg. *TaMyc1*, determining in conjunction with *TaCI* purple color of grain).

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## The protective functions of progesterone system of hormonal regulation in higher plants

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We have established that one of the most important functions of the ancient progesterone system of steroid hormonal regulation preserved in plants along with the brassinosteroids is protection from biotic and abiotic stresses. It is shown that the expression of the mammalian *CYP11A1* cDNA of cytochrome P450<sub>sc</sub> in transgenic tomato plants increases their resistance to a wide range of biotic stress factors: phytopathogens *Botrytis cinerea*, *Alternaria* spp., *Oidium neolycopersici* and *Cladosporium fulvum*. Plants of line No. 7 in the generations T1 to T3 showed almost no signs of infections by all the above mentioned pathogens: vegetative organs of plants remained intensely green before the end of the vegetation period, which ceased only with the onset of frost. Elevated synthesis of endogenous progesterone increases resistance of Solanaceae plants (tomato, tobacco) also to abiotic stresses (drought, salinization). We even have observed the phenomenon of targeted protection of mitochondria of mesophyll cells in *CYP11A1* transgenic tobacco plant leaves after NaCl-induced stress damage (Proc. Latv. Acad. Sci. Section B., 2018;72(6):334-340). Taken together, our data indicate that progesterone system of hormonal regulation in higher plants (BMC Plant Biol., 2017;17(Suppl 1):189) is important for nonspecific, general protection against biotic and abiotic stresses. Mitochondrial cytochrome CYP11A1 is able to provide direct protection of these cell organelles in the leaves of plants under salinity treatment.

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## Analysis of chromosome structure in *Musaceae* using oligo painting

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The family *Musaceae* comprises genera *Musa*, *Ensete* and *Musella*. While *Ensete* ( $2n = 18$ ) and *Musella* ( $2n = 18$ ) are represented by a few and one endemic species, respectively, genus *Musa* contains about 70 different species. Based on plant morphology and basic chromosome number, *Musa* species are classified into four sections. The largest of them, Eumusa ( $x = 11$ ) comprises most of edible banana cultivars. They originated by intra- and inter-specific crosses between wild diploids *M. acuminata* and *M. balbisiana*. The section Rhodochlamys ( $x = 11$ ) contains ornamental species, which are closely related to those of Eumusa. The section Australimusa ( $x = 10$ ) is represented by species growing in Southeast Asia and contains a peculiar group of edible banana clones known as Fe'i. The section Callimusa is the most diverse and contains species differing in basic chromosome number ( $x = 9, 10$ ) and species which seem to be closely related to *Ensete* and *Musella*. In order to analyze chromosome structure and karyotype evolution in *Musaceae*, we used the recently developed method of oligo painting FISH. Pools of chromosome arm-specific oligomers were designed using the reference genome sequence of double haploid *M. acuminata* 'DH Pahang'. The oligomers were labeled using reverse transcription either by hapten- or fluorescently-labeled primers and used for FISH in a set of accessions representing different subspecies of *M. acuminata*, in *M. balbisiana*, and their intra- and inter-specific hybrids. The oligo painting probes were also tested for their suitability to detect chromosome rearrangements in more distant species of the family *Musaceae*. The chromosome oligo painting enabled anchoring pseudomolecules to individual chromosomes, creation of molecular karyotypes and identification of large structural chromosome rearrangements. The method of oligo painting FISH thus opens avenues for comparative analysis of structural chromosome changes to shed light on karyotype evolution in *Musaceae*.

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## Development of the substitution lines of bread wheat with introgressed pubescence from *T. timopheevii* and their study in contrasting irrigation conditions

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The leaf pubescence serves as a mechanism of adaptation to adverse environmental conditions by forming a near-surface protective layer that delays air currents and scatters solar radiation. In bread wheat, the trait is controlled by dominant genes in chromosomes 4B and 7B. *T. timopheevii* species has rare but very long pubescence. The introgressive line 821 carries this new type of pubescence and contains the fragment from *T. timopheevii* on 5A chromosome marked with the *Xgwm179* microsatellite marker. This pubescence was introduced into the single chromosome substitution lines for 5A chromosome of two cultivars, Saratovskaya 29 (S29) and Diamant 2 (Dm2), contrasting for leaf pubescence and drought tolerance. The effect of this introgression on a number of physiological parameters has been established. In the line Dm2(821 5A), the stomatal conductance and transpiration rate decreased by 2.5–4 times under normal irrigation and drought, the photosynthetic activity decreased by about a quarter, and the water use efficiency (WUE) doubled in comparison with the poorly haired recipient. This introgression practically did not affect the physiology of S29 under normal watering. However, under drought conditions, S29(821 5A) more than doubled the stomatal conductance and the rate of transpiration. The WUE more than doubled, while the photosynthetic activity remained at the same level. The productivity parameters of the line S29(821 5A) did not differ significantly from the recipient under normal watering. The line Dm2(821 5A) surpassed the recipient by one and a half times for the weight of grain and the number of grains from the plant. Under drought, the plants of both substitution lines switched to a moisture saving mode, and reduced the productivity more than the recipients did. Chromosome substitution increased the raw gluten content in grain by a quarter in S29 and to some extent in Dm2.

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## Plant genetic resources in India: management and utilization

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Plant genetic resources (PGR) are the foundation of agriculture as well as food and nutritional security. The ICAR-NBPGR is the nodal institution at national level for management of PGR in India under the umbrella of Indian Council of Agricultural Research (ICAR), New Delhi. India being one of the gene-rich countries faces a unique challenge of protecting its natural heritage while evolving mutually beneficial strategies for germplasm exchange with other countries. The Bureaus activities include PGR exploration, collection, exchange, characterization, evaluation, conservation and documentation. It also has the responsibility to carry out quarantine of all imported PGR including transgenics meant for research purposes. The multifarious activities are carried out from ICAR-NBPGR headquarters and its 10 regional stations located in different agro-climatic zones of India. It has linkages with international organizations of the Consultative Group on International Agricultural Research (CGIAR) and national crop-based institutes to accomplish its mandated activities. NBPGR collects and acquires germplasm from various sources, conserves it in the Genebank, characterizes and evaluates it for different traits and provides ready material for breeders to develop varieties for farmers. ICAR-NBPGR encompasses the National Genebank Network and at present, the National Genebank conserves more than 0.40 million accessions. NBPGR works in service-mode for effective utilization of PGR in crop improvement programmes which depends mainly on its systematic characterization and evaluation, and identification of potentially useful germplasm. NBPGR is responsible for identifying trait-specific pre-adapted climate resilient genotypes, promising material with disease resistance and quality traits which the breeders use for various crop improvement programmes. The system has contributed immensely towards safeguarding the indigenous and introducing useful exotic PGR for enhancing the agricultural production. Presently, our focus is on characterization of *ex situ* conserved germplasm and detailed evaluation of prioritized crops for enhanced utilization; assessment of impact of on-farm conservation practices on genetic diversity; genome-wide association mapping for identification of novel genes and alleles for enhanced utilization of PGR; identification and deployment of germplasm/landraces using climate analog data; validation of trait-specific introduced germplasm for enhanced utilization. Gene banks have often, through necessity, focused mainly on the immediate but long-term conservation of plant genetic resource activities. There is currently a major gulf between the operations of PGR collections and modern plant breeding. The conservation of genetic resources must be linked to their increased and sustainable use if they are to play a key role in climate change adaptation. This could be achieved through active engagement with all stakeholders in order to assure the functionality of the entire “Genetic resource-chain”. The recent progress in genomics has opened up enormous possibilities, both for introgression of specific traits and for base broadening in pre-breeding. Development of molecular markers since 1980s has seen a striding development from RFLPs to SNPs because of progress in high throughput genomics at very low costs. A few case studies on alien introgression in rice, wheat, lentil, and Brassica involving use of genomics tools for rapid introgression of desirable variability from un-adapted germplasm to high yielding varieties in India and their commercialization will be presented.



## Barley alloplasmic lines – the spectra of peculiar plasmon types

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The coordination of nuclear and organelle genomes is substantial for functioning of green plants, especially for coadaptation of plants to different environmental conditions. Intensive use of next generation sequencing technologies for nuclear genomes exploration allowed to accumulate the tremendous amount of data about various organisms. Organellar genomes are studied not so intensively. Alloplasmic lines are the suitable model for evaluation of functioning the same nucleus on different cytoplasms, revealing the effects of nuclear-cytoplasmic interactions. The collection of alloplasmic lines of barley *Hordeum vulgare* with cytoplasm of *Hordeum spontaneum* was created and studied in our laboratory in the pre-NGS time. At present we performed the isolation of chloroplast and mitochondrial DNA of 12 samples from this collection, and sequenced them on Illumina MiSeq. Quite novel data were obtained concerning the variability of *Hordeum* chloroplast and mitochondrial genomes. Totally 6 indels, 58 SNP, 15 SSR in cpDNA were detected between studied set of samples. The most important data were verified by Sanger sequencing. Concerning the plastid DNA, the alloplasmic lines with W8 cytoplasm and different nucleus grouped in one subcluster. The lines with W3 and W4 cytoplasms demonstrated dissimilarities in clusterization depending on the definite nucleus genomes, varying cpDNA types were detected. Mitochondrial genome sequencing revealed similar trend, but the level of variability was much lower. The cause of observed differences in the organelle genomes of alloplasmic lines with the cytoplasm from the same source and different nucleus is not clear. Probably it is the initial heterogeneity of cytoplasms in the samples of wild barley taken for creation of alloplasmic lines or it alternatively may be the outcome of some organelle genome modifications during the coexistence of a new combination of nucleus and cytoplasm. So, next generation sequencing of organellar DNAs gives new breakthrough for analysis of the chloroplast and mitochondrial genomes. Revealing the organellar variability facilitates further development of our knowledge about nuclear-cytoplasmic interactions.

## Systems analysis of chilling stress induced transcriptomes in *Arabidopsis thaliana*

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Cold or chilling stresses are severe environmental factors affecting plant growth and development, limiting crop productivity and influencing geographical distribution of plant species and varieties. The evolution of plants from temperate regions has resulted in the emergence of several mechanisms contributing to their cold resistance. Among them cold acclimation, when plant adaptation to chilling temperature makes them tolerant to freezing. Molecular and physiological processes underlying cold response and tolerance are widely studied. However, current knowledge is incomplete and contradictory. The aim of this study is to overview the data on chilling-stress induced gene expression with the help of systems biology methods. For this, we perform meta-analysis of all available chilling-stress-induced transcriptomes in *Arabidopsis thaliana* from different studies and compare them with the data on 370 TF binding regions. Totally, from 30 microarray and 10 RNA-seq experiments we found half of Arabidopsis genome to be differentially expressed in response to cold at least in one experiment. Among them, the most robust genes were selected to reconstruct the gene network. TFs which binding regions were overrepresented in the differentially expressed genes provide a new dimension to the network. Functional annotation of chilling-stress induced genes and their comparison with the phytohormones metabolism and signalling pathways provided us a clue for the molecular mechanism of cold acclimation.

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## Polymorphism of the stem rust population on avirulence genes in Western Siberia

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Effective breeding rust resistant wheat varieties relies on monitoring efforts that include field screening of pathogen gene structure and seedling tests conducted in quarantine-equipped laboratories. Since 2009, stem rust has appeared among the pathogenic complex in Western Siberia, causing significant damage to wheat crops. A list of avirulence genes identified in the rust population is desirable in order to focus on complementary *Sr*-resistant genes that can be used in a breeding program. Disease incidence and severity were assessed in Novosibirsk region during 2016–2018. Stem rust responses of wheat varieties with known *Sr*-genes (more than 64 entries) were recorded at post-flowering stage using the Roelfs' scale (1992). Infection types (ITs) on seedlings were recorded in 14 days after inoculation addressed to Stakman's scale (1962). Based on field screenings, stem rust population local in Novosibirsk region is polymorphic for most of known avirulence genes. No polymorphism was revealed for genes avirulent to wheat lines with *Sr9e*, *Sr20*, *Sr28*, *Sr29*, *Sr32*, *Sr33*, *Sr39*, and *Sr2* complex. After the seedling test of 20 differential lines, there were polymorphic genes avirulent to *Sr7b*, *Sr6*, *Sr8a*, *Sr9b*, *Sr9e*, *Sr21*, *Sr30*, *Sr36* and non-polymorphic genes avirulent to *Sr11*, *Sr24*, *Sr31*, *SrTmp* revealed in the local stem rust population. The twelve wheat resistant genes *Sr9e*, *Sr11*, *Sr24*, *Sr20*, *Sr28*, *Sr29*, *Sr31*, *Sr32*, *Sr33*, *Sr39*, *Sr2 complex*, and *SrTmp* are considered effective against stem rust pathogen in Novosibirsk region.

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## Application of genetic resources and markers in breeding of potato resistant to late blight

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Potato (*Solanum tuberosum* L.) is the fourth most important crop plant worldwide and its economically most important disease, late blight, is caused by an Oomycete, *Phytophthora infestans* (Mont.) de Bary. Breeding potatoes resistant to this disease is a valid and environment-friendly alternative to the currently applied intensive chemical control. The pathogen is fast-evolving and can quickly adapt and infect new resistant cultivars of the host. Therefore new strategies of using late blight resistance (*Rpi*) genes in improving durability of the resistance are developed. They are all based on the access to multiple broad-spectrum *Rpi* genes. In Plant Breeding and Acclimatization Institute – National Research Institute, Młochów Research Centre, four *Rpi* genes were identified and introduced into potato pre-breeding program: *Rpi-phul* from *S. phureja* from International Potato Center (CIP), *Rpi-rzc1* from *S. ruiz-ceballosii* and *Rpi-mch1* from *S. michoacanum* from Vavilov Collection (St. Petersburg, Russia) as well as the *Rpi-Smiral* gene from the cultivar Sárpo Mira. The genes were mapped on a potato genetic map and markers are used for marker-assisted pyramiding of the genes. The *Rpi-phul* has been cloned and sequenced and we use gene-derived marker for selection, while for other genes we use closely linked markers. Spectrum and durability of provided resistance is monitored in the Polish population of *P. infestans* in virulence detached leaflet tests. The effect of pyramiding is tested both in the laboratory and in the field tests. Using qPCR we test the expression of the *Rpi-phul* gene and the corresponding effector during the host-pathogen interaction for better understanding of virulence/avirulence. The first cultivar with the *Rpi-phul* gene has been registered in Poland by the Zamarte Potato Breeding Ltd. Group IHAR in 2018, ca 48 years after arrival of gene donor from CIP to Poland which demonstrates that introgression breeding is a long, laborious and challenging process.

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## Biochemical, molecular and genetic aspects of fruit ripening in green-fruited and red-fruited tomato species

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Tomato fruit ripening is a complex process characterized by dramatic physiological and biochemical changes. It determines crop yield, and fruit color, flavor, dry matter content and shelf life. RIPENING INHIBITOR (RIN) is one of the main ripening adjusters controlling ethylene biosynthesis, cell-wall metabolism, sugar and secondary metabolite accumulation, and transcription factors expression. Wild and cultivated tomato species (*Solanum* sect. *Lycopersicon*) are known to be the most suitable model system to understand the mechanism of fleshy fruit ripening. The section *Lycopersicon* includes cultivated tomato *S. lycopersicum* and 12 wild relatives that differ significantly in fruit physiology. In this work, biochemical and expression analysis of red-fruited (RF) and green-fruited (GF) tomato species during ripening was performed. HPLC analysis showed that RF fruits accumulate glucose and fructose and change color from green to red as a result of carotenoid synthesis. Ripe fruits of GF tomatoes accumulate sucrose and are green due to the absence of carotenoids. The expression pattern of genes encoding RIN, sucrose hydrolyzing TAI (vacuolar invertase) and carotenoid-related PSY (phytoene synthase) was analyzed at four fruit developmental stages in GF and RF species. It was shown that the absence of sucrose degradation and carotenoid accumulation in GF species is due to the loss of *TAI* and *PSY* expression, at the same time the expression level of their activator *RIN* was high. Structural analysis revealed that in RF species, each of *TAI* and *PSY* promoters contain two RIN-binding sites, while in GF species, only one of two possible. Thus, the polymorphism of *TAI* and *PSY* promoter regions in RF and GF species may determine the differences in fruit morphophysiology.

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## Stress-inducible and tissue-specific promoters in transgenic tomatoes

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Tomato is one of the most important crop plants. 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. More than 170 gene promoters of different plant species have been investigated for their stress-inducible, developmental- and tissue-specific expression in tomatoes. Pollen-specific promoters may be used for a hybrid seed production. RNAi silencing of *S*-adenosylmethionine decarboxylase genes under the control of tapetal-specific AtA9 promoter results in male sterility of tomato plants. PsEND1 and LeFRK4 promoters fused to a cytotoxic ribonuclease gene also produced efficient male-sterility in tomatoes. For fruit quality improvement, MdACO, PpACO, and AcMADS1 promoters may be used. Le2A11 promoter was used for hepatitis B virus large surface antigen and other target genes expression in tomato fruits. Over-expression of mouse ornithine decarboxylase gene under the control of Le2A11 fruit-specific promoter enhances fruit quality in tomato. Root-specific AtNRT2.1 and AtRB7 promoters were used to drive the expression of the antifungal *NIC* and *Thi2.1* genes. Resulting tomato plants conferred enhanced resistance to *Fusarium oxysporum* and *Ralstonia solanacearum*. CYP97A29, DFR, FLS, NIK and PME1 promoters displayed different activity patterns in nematode-infected and uninfected transgenic hairy roots. Expression of *LeBADH* gene under control of its own promoter can increase salt tolerance without affecting plant growth. TGP database gathers information on constitutive, tissue-specific, and inducible promoters, the activity of which has been characterized in transgenic plants. The TGP database interface enables searching for promoters with certain characteristics. The TGP database allows to select promoters with the desired stress- and tissue-specific activities for transgene expression in tomatoes.

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## Population genomics and analysis of agronomic traits of green gram (*Vigna radiata*) and black gram (*Vigna mungo*)

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*Vigna radiata* and *Vigna mungo* are two important Asian pulse crops, important as dried seeds, sprouts, and in the green form as vegetables. Among the merits of these crops is the presence in seeds of at least 25 % protein and essential aminoacids such as lysine, histidine and tryptophan, which are not produced by the human body, many vitamins and minerals. With good heat and drought tolerance, these crops are often grown as summer pulses in contrast to winter pulses such as chickpea and lentil, making them important for food security and nutritional diversity in many parts of Asia. The World Vegetable Gene Bank, in Taiwan, houses a unique genebank of green gram (*Vigna radiata*). Phenotyping of 293 accessions of the mini-core collection of green gram (*Vigna radiata*) from the World Vegetable Gene Bank (Taiwan) at Kuban experimental station of VIR in 2018 revealed a wide range of inter-varietal variability. Genotyping by sequencing of these accessions from different countries identified 8,466 segregating single nucleotide polymorphisms (SNP). We performed Genome-wide association study (GWAS) to find associations between SNPs and phenotypic data obtained at Kuban experimental station. GWAS analysis identified a large number of genome intervals and potential gene candidates that may affect important agronomic traits.



## Zetri – the cereal of future?

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The most common cereal in the 20th century was maize (*Zea mays* ssp. *mays*,  $2n = 2x = 20$ ). In the 21st century, its production in the world reached 1 billion tons and continues to increase annually. First of all, this is due to the high adaptability of its cultivation and the fact that being a  $C_4$  plant of the photosynthesis pathway, it most effectively transforms solar energy into plastic substances. One of the problems associated with its cultivation, is the need for annual production of F1 hybrids, because they are more productive. For a long time, researchers tried to consolidate heterosis by transferring the corn to a cornless way of reproduction from a wild relative, the gamagrass (*Tripsacum dactyloides*,  $2n = 4x = 72$ ). However, contrary to expectations, this trait was under complex genetic control and its expression requires the presence of 9 chromosomes of the wild relative, therefore, the resulting plants are far from maize.

In this regard, we propose to create apomictic 56-chromosomal maize hybrids with gammagrass ( $2n = 4x = 56 = 20 Zm + 36 Td$ ). As the source of the genomes from maize, the maize lines used in hybrid selection for heterosis in F1 are taken. At present, such hybrids have been obtained, but they are male-sterile, and forced pollination of corn is required to obtain seeds. Nevertheless, we believe that these hybrids have so many beneficial economic traits that the work of identifying pollen-producing plants among them is justified and should be expanded and continued. First of all, these hybrids are salt tolerant and do not suffer from short-term waterlogging, and are also resistant to many infectious diseases and other biotic pests. Since the maize genome is unusually dynamic, the successful breeding improvement of such hybrids is fully justified.

## The comparative plastome analysis of twelve *Allium* species: adaptation to shaded environments could be accompanied by the complete loss function of the NDH genes

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We have analysed plastome sequences of twelve wild and cultivated *Allium* species (ten of them were sequenced, assembled and annotated in our lab). We have found some plastome features that are characteristic for species of three evolutionary lines of the genus, i. e. reflect evolution processes. In general the number and position of genes were similar, but *A. paradoxum* plastome sequence differed markedly from other *Allium* species. It had a large 4,825 bp long inversion in the SSC region and showed loss of pseudogenization of all genes encoding the NDH complex. The functional role of *ndh* genes in plants is presumably related to adaptation to different habitats, because this complex is required to optimize photosynthesis. In *ndh* defective plants the photosynthesis rate decreases under excess light stress conditions. Naturally *A. paradoxum* occurs in shady forests and does not grow in open areas or at forest edges. We assume that the shade-loving nature of *A. paradoxum* is caused by *ndh* genes impairment (as a result of NDH complex interruption). No one of other analyzed *Allium* species demonstrated the same features, including phylogenetically related shade-loving *A. ursinum*.

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## **Biogenesis of siRNA and miRNA upon infection of *Nicotiana benthamiana* plants with a virus and its mutants**

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As a result of morphogenesis features and evolutionary development pathway, among eukaryotic organisms, epigenetic variability is most effectively employed by plants. Double stranded RNA directed gene silencing (also referred as RNA-interference) is a biological mechanism present in all eukaryotes and known as one of the central anti-viral defence mechanisms in plants. In this system produced by viral RdRp double stranded RNA molecules are recognized by PTGS system and cleaved into small interfering RNA by RNase like III enzyme DICER. Following then, these siRNA molecules are loaded into RISC complex, which leads to the post-transcriptional gene degradation or “silencing arrest”. Viral protein p19 encoded by Tombusviridae family member *Tomato bushy stunt virus* suppresses silencing system by binding with short interfering RNA. This protein widely used in RNA-interference studies, because it binds with high affinity to 21–24 nt dsRNA in sequence-independent manner. We used mechanic inoculation of model plants *Nicotiana benthamiana* by *Tomato bushy stunt virus* and its p19 deficient mutant to study the levels of siRNA circulation during infection. After infection of plants we isolated total RNA from tissues and polyacrylamide gel electrophoresis with urea was done to detect and segregate siRNA. Then we transferred RNA molecules to the membrane and incubated them with DIG. The purpose of this work is to study the effect of viral infection on the generation of a pool of siRNA molecules in plant tissues compared to uninfected plants.

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## Development of spring wheat lines with a reduced period from germination to heading using the marker-assisted selection

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The time of heading of wheat is an important factor that determine the adaptation of plants to environmental conditions. The heading time of spring wheat is determined by genes that control the plant reaction to vernalizing temperatures (*VRN* – vernalization genes), and photoperiod sensitivity (*PPD1* – photoperiod response genes). The different combinations of alleles of these genes result in different heading time. Marker-assisted selection enables to accelerate the development of spring wheat forms, differing in heading time. Using the results of the analysis of the allelic variation of the *VRN* and *PPD1* genes in Siberian spring wheat varieties, we selected the early Tulun 15 variety, with photoperiod insensitive *Ppd-D1a* and rare *Vrn-B3a* allele, associated with early flowering. The cultivar Tulun 15 was crossed with the middle early cultivar Obskaya 2. Using allele-specific primers  $F_2$  generation plants with different combinations of *VRN* and *PPD1* alleles were detected. These plants were divided into four haplotypes. The  $F_3$  generation plants resulted from self-pollination of these plants were sown in the field to study the effect of the haplotypes on the heading time. The time from germination to heading of the plants with different haplotypes was as follows: haplotype 1 (*Vrn-A1a Vrn-B1c vrn-D1 Vrn-B3a Ppd-D1a*) – 34.5 days; haplotype 2 (*Vrn-A1a Vrn-B1c vrn-D1 vrn-B3 Ppd-D1a*) – 37.3 days; haplotype 3 (*Vrn-A1a Vrn-B1c vrn-D1 Vrn-B3a Ppd-D1b*) – 38.0 days; haplotype 4 (*Vrn-A1a Vrn-B1c vrn-D1 vrn-B3 Ppd-D1b*) – 40.3 days; Tulun 15 (*Vrn-A1a Vrn-B1c vrn-D1 Vrn-B3a Ppd-D1a*) – 37.5 days; Obskaya 2 (*Vrn-A1a Vrn-B1c vrn-D1 vrn-B3 Ppd-D1b*) – 42.9. The results obtained demonstrated that *Vrn-B3a* and *Ppd-D1a* alleles accelerate heading of plants with the same allelic composition of *Vrn-A1a*, *Vrn-B1c*, and *vrn-D1* genes in the long day conditions of Novosibirsk region. **Acknowledgements:** The study has been supported by the Budget project 0324-2019-0039.

## Study of the interphase period “shoots–earring” of 8x and 6x triticale with different dominant *Vrn* genes

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Duration of the interphase period “shoots–earring” of two groups of octaploid (8x) and a group of hexaploid (6x) triticale with different dominant *Vrn* genes affecting the length of the plant period of vegetation was studied in 2014 and 2016–2018. Four 8x triticale forms were produced in the Siberian Research Institute of Plant Production and Breeding – Branch of the Institute of Cytology and Genetics, SB RAS by crossing between a winter diploid rye variety Korotkostebel'naya 69 and nearly isogenic lines of soft wheat Triple Dirk D, Triple Dirk B, Triple Dirk E and Triple Dirk F (obtained from N.P. Goncharov), bearing respectively dominant genes *VrnA1*, *VrnB1*, *VrnD1* and *VrnD4*, and by subsequent doubling of the wheat-rye hybrids' chromosome number. By selection of the most early maturing plants in the progeny of hybrids made by crossing on 4 combinations: 8x*VrnA1* × 8x*VrnD1*, 8x*VrnB1* × 8x*VrnD1*, 8x*VrnA1* × 8x*VrnD4*, 8x*VrnD1* × 8x*VrnD4*, new 8x triticale genotypes, bearing pairs of dominant *Vrn* genes were obtained. Spring hexaploid triticale forms were developed by selection of the most early maturing plants in the progeny of the hybrids F<sub>3</sub>–F<sub>4</sub> between the 8x forms and a winter 6x triticale variety Sears 57, bearing recessive *vrn* genes. It was found that triticale plants of both ploidy levels had a short interphase period “shoots–earring” if they contain one of the dominant genes *VrnA1* or *VrnD1*, in contrast to those triticale plants that contained one of the genes *VrnB1* or *VrnD4*. In 2018 the interphase period “seedling–heading” lasted 49.5 ± 2.6 days (genotype 8x*VrnD1*), 51.0 ± 1.8 days (genotype 8x*VrnA1*), 71.2 ± 4.3 days (genotype 8x*VrnB1*) and 74.3 ± 1.7 days (genotype 8x*VrnD4*) in the group of 8x triticale. Hexaploid triticale plants have the same dominant *Vrn* genes as their initial 8x forms. The *VrnA1* and *VrnD1* genes of hexaploid triticale plants conditioned short “shoots–earring” interphase period in comparison with the *VrnB1* and *VrnD4* ones. In 2018 the 8x triticale forms, selected from the combinations of crosses 8x*VrnA1* × 8x*VrnD1* and 8x*VrnA1* × 8x*VrnD4* had the shortest interphase period “shoots–earring” – 42.8 ± 4.0 days and 43.0 ± 1.8 respectively. Their plants have two dominant genes. The gene *VrnA1* is likely to have the strongest effect. On the basis of this research work, a collection of spring octaploid and hexaploid triticale forms bearing identified dominant *Vrn* genes responsible for both the spring type of plant development and the duration of the vegetative period is being made. Some of spring hexaploid triticale forms are used in breeding programs.

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## Regulation and evolution of flavonoid biosynthesis pathway in polyploid plants

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Higher plants, including cereals, synthesize secondary metabolites flavonoids. The regulation of the expression of flavonoid biosynthesis genes is under control of genetic and epigenetic mechanisms. Genetic regulation occurs with the MBW complex, which is formed due to the action of the Myb, bHLH and WD40 transcription factors (TF), while DNA methylation is important for the binding of TF with *cis*-regulatory genes regions. The aim of this work was the characterisation of *Myb*, *bHLH* and *WD40* gene copies in the Triticeae tribe, on the one hand, and the studying of the methylation patterns of promoters of flavonoid biosynthesis genes in wheat genome, on the other hand. In this work, we identified and characterized in the genomes of the Triticeae tribe *bHLH* gene copies in 2 and 4 groups of chromosomes, *Myb* gene copies in 4 and 7 groups of chromosomes and *WD40* gene copies in 6 chromosomes. A study of the structure organisation and transcriptional activity revealed the full range of regulatory MBW genes controlling the synthesis of anthocyanins in the pericarp and aleurone layer of wheat and barley. We demonstrated that bHLH-coding gene *HvMyc2* is the main regulator of the appearance of blue colour in barley grain. The bHLH-coding candidate gene *TaMyc-B1* determining the colour of the wheat coleoptile was also detected. In addition, analysis of methylation patterns of promoters of flavonoid biosynthesis genes in wheat showed that the methylation does not make a significant contribution in the specific expression pattern of the studied genes. In general, the results of the comparison of the flavonoid biosynthesis genes copies demonstrate that the maintenance of their functional state is the cause of their tissue-specific activity in members of the Triticeae tribe.

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## Differential gene expression in roots of yellow lupin sprouts under *Fusarium* treatment

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**Motivation and Aim:** Yellow lupin (*Lupinus luteus* L.) is a valuable high-protein legume plant. Nevertheless, its implementation as field crop is restricted by heavy damage caused by fungal pathogens. One of the most devastating disease for yellow lupin is fusariosis. The aim of this work was to assess the feasibility of using SRAP (sequence-related amplified polymorphism) for transcriptome profiling analysis of yellow lupine samples under *Fusarium* treatment.

**Methods and Algorithms:** RNA was isolated from the roots of ten-day seedlings of yellow lupine varieties Tremosilla, BSHA 13, BSHA 19, Nadezhny. There were four groups of seedlings: intact and exposed to three *Fusarium* isolates. cDNA was synthesized on the RNA matrix and was used in a series of PCR reactions with 12 combinations of 3 forward (Me8, f12, f16) and 4 reverse (Em5, Em12, r14, r9) SRAP primers. SRAP fragments associated with resistant seedlings were isolated and sequenced. Their putative function was assigned based on alignment to known sequences.

**Results:** The results showed that 12 primer combinations produced a total of 107 clear bands. The proportion of polymorphic bands varied in the range from 81.3 % to 100 %. Three SRAP fragments were associated with longer roots (f12-Em5-120, f16-r9-500) and hypocotyl (f12-Em5-200) in seedlings treated with *Fusarium* isolates. Sequencing and subsequent analysis of these fragments allowed to associate fragment f12-Em5-120 with fumarylacetoacetase mRNA, f16-r9-500 with BURP domain protein USPL1-like mRNA, f12-Em5-200 with hydroxyphenylpyruvate dioxygenase mRNA.

**Conclusion:** The results obtained indicate that the SRAP method is suitable for the analysis of yellow lupine cDNA and can be used to study the differential gene expression of this culture. Three SRAP fragments associated with mRNA expressed in *Fusarium* treated seedlings were found.



## DNA import into plant mitochondria: studying of the translocation pathways *in organello* and *in vivo*

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The active participation of plant mitochondria in horizontal gene transfer, which has recently received more and more evidence, one can consider as an important fundamental feature of the mitochondrial genetic system. We assume that these processes involving mitochondria are possible, as well, due to a phenomenon of the plant mitochondria natural competence to uptake (“import”) DNA. Although the mechanism of DNA import into plant mitochondria is obviously of ancient evolutionary origin, it is still insufficiently studied due to the obvious complexity of mechanisms providing DNA transfer through two membranes. The working model of DNA import into the plant mitochondria includes VDAC and a precursor protein of  $\beta$ -subunit of ATP synthase localized in the outer membrane, CuBPP subunit of complex I in intermembrane space, and ADP/ATP carrier in the inner membrane. The results obtained to date suggest the existence of more than one mechanism of mitochondrial DNA import. To study the features of DNA import into plant mitochondria under conditions close to *in vivo*, we have developed and tested a new approach based on use in parallel of an (i) *in organello* and (ii) isolated protoplast system. In an advanced *in organello* system, the detection of imported DNA in the mitochondrial matrix is based on the use of fluorescently labeled substrates and on the assessment by quantitative PCR. The *in vivo* system is used to investigate the DNA transport from the cytoplasm to the mitochondrial matrix in *Arabidopsis* protoplasts. This approach allows to establish whether the regularities of the mitochondrial DNA import detected *in organello* are preserved *in vivo*. We demonstrated that DNA of various lengths is actively imported into mitochondria of *Arabidopsis* protoplasts. We showed that mitochondria lacking one porin isoforms, VDAC1, VDAC2 or VDAC4, transport DNA more efficiently compared to the wild-type mitochondria.

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## Prediction of time to flowering in soybean with artificial neural network

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A number of days from emergence to flowering is an important trait in soybean that strongly depends on temperature and day length. We investigate this dependence using mathematical modeling with Artificial Neural Networks (ANN). Experimental data was obtained in 1999–2013 in Leningradskaya oblast (Pushkin) and Krasnodarskii krai for nine early maturing soybean accessions with low photosensitivity. We adapted ANN to predict time to flowering in this dataset in two steps. Firstly, we added scaling constants for network inputs, optimized high and low temperature thresholds and base day length. Training of 121 model parameters resulted in the MSE value of 0.026. Investigated accessions were characterized by decreased high temperature threshold in comparison to literature data ( $23\text{ °C} < 30\text{ °C}$ ) and increased low temperature threshold ( $12\text{ °C} > 5\text{ °C}$ ). The increase of day length from 12 h to 13 h confirmed the adaptation to a longer day. The average error was ~2.4 days. ANN has to be trained for each group of plants from the same accession. Secondly, we modified the network topology to include the nine input nodes that represent the membership of a plant to an accession group. Thus the same ANN is used for any plant. An average error for the second model was ~3.1 days. The average errors for ANN models from both steps are less than the error of 5.2 days of the previous model based on temperature thresholds. We applied the first model to the generated weather for different future greenhouse gas emission scenarios and predicted flowering time for nine accessions in changing climate for years 2019–2030. Mean time to flowering decreases from measured 39.21 days to 36.33 days predicted for 2030 significantly with Mann–Witney–Wilcoxon criterion ( $P = 0.0097 < 0.01$ ) but may stay constant or fluctuate in several cases.

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## Delayed flowering of guar plants (*Cyamopsis tetragonoloba* L. (Taub.)) in terms of metabolome

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Guar is an economically valuable crop used as a source to produce guar gum that is demanded by many industries. Guar is a short-day plant that performs best with shortened photoperiod (12–13 h). The date of flowering of guar is an important agro biological trait since it usually correlates with productivity of the crop. Identification of key molecules associated with the transition to generative phase of guar will help to detect genes controlling their biosynthesis and, accordingly, contributing to early flowering. Metabolomic profiling being a new approach of the system biology allows to reveal the internal factors determining the transition to the generative stage of guar. In our experiments 96 guar genotypes with different sensitivity to photoperiod from the VIR world collection were grown under long day conditions. For the photoperiod-sensitive genotypes, the prolonged day light caused obstruction for switching the flowering program, which results in a strong delay of flowering. We detected differences in the metabolomic response between two guar groups: the first group of plants that started to flower without delay (“early flowering plants”) and the second group of plants that delayed flowering significantly “delayed flowering plants”. Metabolomic analysis showed that the metabolome of the “early flowering plants” and “delayed flowering plants” differs each from another by concentration of 7 molecules. We suggest to consider those as the key metabolites involved in the transition to flowering in guar.

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## Alloplasmic wheat lines, their photosynthetic activity and drought-tolerance

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They are investigated morphophysiological and photosynthetic indicators of response to drought-stress of nine wheat alloplasmic lines carrying the cytoplasm of tetraploid species *T. dicoccum* Shuebl. They were noted and significant differences in the regulation of growth and photosynthetic activity of alloplasmic lines between themselves under drought conditions and differences from euplasmic parental forms were shown. The most tolerant to drought alloplasmic lines D-d-05 b, D-b-05, D-41-05 and the most sensitive D-f-05 have been identified.

The use of 21 SSR (simple sequences repeats) markers for evaluation of genomic polymorphism of the wheat alloplasmic lines and the cv. Mironovskaya-808, used as the paternal form in crossing, showed that level of polymorphism for 7 lines and Mironovskaya-808 does not exceed 15 %. The lowest level of polymorphism (less than 5 %) was observed between the lines D-b-05 and D-a-05.

The D-n-05 line is distinguished because it differs from the studied lines in 9 SSR fragments out of 21. Of interest is the D-f-05 line, since it, unlike other lines, identifies *T. dicoccum* genetic material marked with *Xgwm357* (1AL chromosome), *Xgwm192c* (4BS) and *Xgwm155* (3AL).

Obviously, selection on the basis of productivity and grain quality for 7 years by Dr. Khailenko, also led to the selection of wheat alloplasmic lines in which the presence of *T. dicoccum* nuclear genes is necessary to maintain high productivity based on nuclear-cytoplasmic interactions, which compensates for its most sensitive to drought on the studied parameters, relative to other lines.

Thus, it was showed that the combination the nucleus and cytoplasm originating from parental forms belonging to different species can both improve and worsen the stress-resistance and photosynthetic activity of wheat alloplasmic lines.

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## The role of suppressor protein in acquired resistance to viral infection

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Plants are constantly exposed to abiotic and biotic stresses. One of the main types of biotic stress is viral agents. The viruses cause tremendous damage; therefore, plant viral diseases are an important problem in phytopathology, as they cause great damage to agriculture. We have been studying TBSV, which has a broad host range under experimental conditions and has been reported to infect over 120 plant species spanning 20 families. Tomato bushy stunt virus (TBSV) is the type species of the *Tombusvirus* family. TBSV has a single +ssRNA genome with length of about 4800 nt, which is wrapped by 180 subunits of capsid protein. The virus contain 5 open reading frames: p33 and p92 responsible for replicase, which translated from genomic RNA, capsid protein p41 is translated from subgenomic RNA1, whereas p22 and p19 from subgenomic RNA2. The p22 protein is a movement protein that is required for the virus to spread from cell to cell. P19 is a major viral pathogenicity determinant and functions as a suppressor of defensive mechanism RNAi. This protein binds siRNAs and prevent their incorporation into RISC, thereby allowing viral propagation in the host. Therefore, manipulations with this protein can impart resistance to viral pathogen. *Nicotiana benthamiana* plants, which initially inoculated with mutant of TBSV-157 survived, due to absence p19, then these plants were grown. After that, the seeds from these plants (157 s *N.b.*) were collected. Collected seeds were planted, and after 30 days growth plants were inoculated with wild type TBSV. For experiments were taken *N. benthamina*, 157 s *N.b.* plants, which were infected by TBSV and showed the same symptoms. But only 157 s *N. benthamiana* plants survived after 14 days post inoculation. Taken together, it is possible to assume that the infection with suppressor mutant, may effectively trigger systemic resistance of plants in the next generation.

## Genetic resources of the genus *Triticum* L. for breeding in the conditions of the Tyumen region

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Wheat is the most important crop. Further progress in breeding work with this culture will be based on the involvement in the hybridization of the entire diversity of species of the genus *Triticum* L. In this regard, starting from 1992, in the Tyumen region, in field conditions, the varieties and variety samples of the most promising species of wheat for the local region were studied: a single-grain cultural (*T. monococcum* L.) with the A<sup>b</sup> genome, durum (*T. durum* Desf.) with the A<sup>u</sup>B genome, kartalinskaya (*T. carthlicum* Nevski.) with A<sup>u</sup>B genome, abyssinskaya (*T. aethiopicum* Jakubz.) with A<sup>u</sup>B genome, wheat sharozernaya (*T. sphaerococcum* Perciv.) with A<sup>u</sup>BD genome. Varieties of soft wheat of different groups of ripeness Scala, Tyumenskaya 80, Rang, Novosibirskaya 15 were used as standards. The weather conditions that have developed over the years of research allowed us to give the varieties and variety samples of the studied wheat species the most complete assessment of the length of the growing season and seed productivity. The late-ripening varieties turned out to be round-grain wheat. The length of the growing season they ranged from 84 to 87 days. For 3–5 days faster than them ripened varieties of durum and then kartalinskaya wheat. According to the results of the research, the variety samples of kartalinskaya wheat (K-19764, K-17581) and cultivated single-grain crops (K-17534) ripening at the level of ripening standard varieties were identified. Durum wheat varieties surpassed other species in seed production. They significantly exceeded the standards by 58.6–71.3 g/m<sup>2</sup>. The number of single-grain grains (K-17534; 376.3 g/m<sup>2</sup>, CV = 35.9 %), kartalinskaya wheat (K-18772; 282.1 g/m<sup>2</sup>, CV = 37.7 %), and abyssinskaya wheat (K-19611; 266.5 g/m<sup>2</sup>, CV = 45.2 %) yielded some to Novosibirskaya 15. Thus, as a result of studies conducted from the collection of rare wheat species, promising variety samples, varieties and lines were identified using a set of economically valuable traits. The source material is currently used in wheat breeding programs in the Tyumen region.

## The *Septoria* blight on the spring wheat varieties in the Western Siberia

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The aim of the work was to clarify the *Septoria* causative agents species composition on leaves and spike and to evaluate the spring wheat varieties effectiveness in the disease controlling in the Western Siberia forest-steppe zone. Studies were carried out in 2016–2018 according to generally accepted methods. Spring wheat leaves and ears *Septoria* blight is widespread in Western Siberia, causing a decrease in yield by up to 50 % or more with the deterioration in the grain quality. The *Septoria* blight causative agents' specific composition is represented by *Parastagonospora nodorum*, *Septoria tritici* and *S. avenae* f. sp. *tritici*, and the species ratio varied by region, variety and within plant organs: in the Novosibirsk region the strongest *P. nodorum* domination was revealed, in the Tyumen region *P. nodorum* dominance was not absolute and was disturbed in some geographical points by *S. tritici* and *S. avenae* f. sp. *tritici*. In the Altai Territory, the *P. nodorum* dominance was revealed at all points, but it was less significant compared to the Novosibirsk Region and was accompanied by the widespread occurrence of *S. tritici*. The immunological assessment of spring wheat 23 varieties collection from different origin did not allow identification of samples immune to *Septoria*. A differentiated manifestation of resistance signs to leaves and ear *Septoria* disease has been established. The complex revealed resistance some varieties (Orenburg 23, Vyatchanka, also Long Chun 7 Hao from China) have shown, they combined reduced susceptibility to *Septoria* disease of the leaves and ear. The varieties collections study from three regions of Siberia in the epiphytotic year made it possible to identify the following trend: compared with the Omsk and Kurgan regions, the *Septoria* blotch causative agent transmission was most active with seeds of the Novosibirsk breeding varieties.



## Association mapping of tuber eye depth and golden cyst nematode resistance traits using ICG collection of *Solanum tuberosum* L.

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Potato is one of the most important crops in the world. It's yield and quality depend on many genetic factors. The genomic regions associated with tuber eye depth and the resistance to the golden potato cyst nematode (GPCN) were found in this study by analyzing *Solanum tuberosum* potato varieties from the ICG "GenAgro" collection, genotyped using an Illumina 22K SNP potato array DNA chip. Data processing was carried out using programs: Tassel 5, package R, Microsoft Excel. Four statistical models were used for data analysis: GLM (general linear model) without or with (GLM+Q, GLM+PCA, GLM+Q+PCA) taking into account the population structure. Data on resistance to the GPCN was taken from the database of the State Register of breeding achievements approved for use. The study showed the presence of 18 significant SNPs on chromosomes 1, 5 and 11. Association with three SNPs was highly significant when using each of the four statistical models. The tuber eye depth was determined on a three-point scale: 1 – small (less than 1.0–1.3 mm), 2 – medium (1.4–1.6 mm), 3 – deep (more than 1.7 mm). 27 significant SNPs associated with the tuber eye depth were found on chromosomes 4, 5, 6 and 10. Association with five SNPs was highly significant when using each of the four statistical models.

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## The genetic variability of proliferative cell lines of *Larix sibirica*

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The quality of 23 proliferative embryogenic cultures (EC) and the genetic changes associated with somaclonal variations in the cell lines (CLs) and cloned plants of *Larix sibirica* were studied. The age of CLs was from 6 month to 9 years. CLs were obtained from explants (zygotic embryos) of Siberian larch trees as a result of open and controlled pollination. The frequency of EC formation was 4.5–23 % on the nutrient medium AI, supplemented by plant growth regulators: (2,4-D:6-BAP, 2:0,5). All CLs actively formed embryonal-suspensor mass (ESM), in which globular embryos propagated through cleavage, budding formation and proliferation of embryonic tubes of the suspension. Cytogenetic studies of proliferating CLs of Siberian larch showed that the cells of young cell lines (age 1–2 years) contained mainly cells with a normal number of chromosomes for this species ( $2n = 24$ ). Analysis of long-cultivated (7–9 years) CLs showed that majority of them were genetically unstable and only one (CL6) was characterized by stability ( $2n = 24$ ). The genetic stability of this line was confirmed of the microsatellite analysis of nine microsatellite loci. Molecular genetic studies of proliferating CLs, conducted using RAPD analysis allowed us to obtain a diversified line-specific PCR spectra that can be used as markers of ECs. Somatic embryos matured on a nutrient medium AI with ABA (32 mg/l). The number of mature somatic embryos in different cell lines varied from 9 (CL16.19) up to 1220 (CL 4) per 1 g of fresh ESM. Somatic embryos germinated (83 % CL4) on the medium AI without hormones and rooted (5–15 %). Stable maturation and germination of embryos was observed in CL6. For 7 years cloned trees grow to the station “Pogorelsky Bor” IF SB RAS successfully. Microsatellite analysis clones showed their full compliance with this cell line.

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## Study of transferability of *H. vulgare* EST markers for characterization of introgression bread wheat – *H. marinum* subsp. *gussoneanum* lines

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*H. marinum* subsp. *gussoneanum* ( $2n = 28$ ) is a valuable source of genes that determine resistance to abiotic stresses (salinization, flooding, waterlogging, temperature severe changes). In addition, accessions of this barley with a high content of protein in seeds have been isolated. These traits may be transferred to wheat, since *H. marinum* subsp. *gussoneanum* is able to cross with bread wheat. To efficiently obtain introgression genotypes of wheat with the genetic material of barley, it is necessary to use methods for reliable and quick identification of its chromosomes in the *T. aestivum* background. For this purpose, we evaluated the possibility of using *H. vulgare* EST markers for studying wheat – *H. marinum* subsp. *gussoneanum* substitution and addition lines of bread wheat. The work included the developed lines of bread wheat, carrying the chromosomes of *H. marinum* subsp. *gussoneanum*. The presence of wild barley chromosomes was detected using GISH and C-banding. The applicability of 78 EST markers localized in different chromosomes of *H. vulgare* barley for analysis of *H. marinum* ssp. *gussoneanum*. Of all the markers studied, 35 were suitable for the analysis of lines carrying the chromosomes of *H. marinum* ssp. *gussoneanum*. At the same time, for 20 EST markers out of these 35, localization in the short or long arm of *H. vulgare* chromosomes is known. The identified EST markers specific for *H. marinum* ssp. *gussoneanum* were amplified in the lines obtained in our work with the chromosomes of wild barley. It was shown that the results of the identification of chromosomes of barley using EST markers confirmed the C-banding data. Thus, it has been established that the *H. vulgare* EST markers can be successfully used to identify the chromosomes of the *H. marinum* subsp. *gussoneanum*. **Acknowledgements:** This work were supported by project No. 0324-2019-0039 and the RFBR grant No. 17-04-01738.

## Phylogenetic relationships between FMO classes and the origin of YUCCA

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YUCCA proteins, a flavin monooxygenases (FMO), are important plant enzymes involved in the biosynthesis of one of the key hormones, auxin [1]. It is believed that these genes originated as a result of horizontal transfer from bacteria to plant genome [2]; though it is not clear at what stage of plant evolution it occurred: in multicellular algae or land plants. To clarify the early evolution of YUCCA genes, we reconstructed phylogenetic relationships between sequences of flavin monooxygenases, the protein superfamily to which YUCCA belongs. The analysis of active sites, domain composition and similarity of spatial structures of different FMO groups was performed. The analysis made it possible to distinguish three clades on the tree of FMO and YUCCA proteins: Yucca proteins, classic FMOs and a new group called “type II FMO-B”, which, apparently, have a unique ability to catalyze both oxidizing heteroatom-containing compounds (characteristics of FMO) and Baeyer–Villiger oxidations of ketones (characteristics of Baeyer–Villiger monooxygenases) [3]. We demonstrated that *Klebsormidium nitens* kfl00109\_0340 protein, which previously was a candidate for the function of YUCCA enzymes in Charophyta [4], belongs to this new class of enzymes. The results support the hypothesis of horizontal transfer of the ancestral YUCCA genes from of bacterial genomes to the genomes of the ancestor of land plants. In addition, according to our analysis using Riebel et al. data [3], the “type II FMO-B” group of proteins is probably a new family of proteins, interesting for biocatalysis and includes proteins of bacteria, fungi, algae, higher plants. Therefore, further research for experimental verification of the functional properties of type II FMO-B is required.

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## Strategy of genetic protection of common spring wheat from leaf rust in Southern Ural due to changes pathogen population structures

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Breeding of spring common wheat in the Chelyabinsk Scientific Research Institute of Agriculture (ChRIA) has a traditional focus on resistance to a combination of stress abiotic and biotic stress factors. The varieties created by the institute play a significant role not only in increasing gross grain yield in the Chelyabinsk region, but also in improving the phytopathological situation. Leaf rust caused by *Puccinia triticina* Erikss. is a widespread and devastating disease in the South Ural. The use of the intraspecific genetic potential of common wheat is not able to provide a sufficient protection against the pathogen, therefore, since 1990, in ChRIA has been conducting targeted selection for leaf rust resistance with the involvement of donors with alien translocations carrying effective resistance genes. Firstly donors with translocation from *Aegilops umbellulata*, carrying gene *Lr9* (= *LrTr*) were used in 1990 and new varieties Duet, Chelyaba 2, Pamyati Ryuba, Chelyaba jubileinaya, Chelyaba stepnaya, Chelyaba rannyaya were created. But in 2007 the effectiveness of *Lr9* was lost and the actual task of breeding has become the expansion of the genetic diversity of common wheat for leaf rust resistance. The new resistance donors with alien gene translocations from *Secale cereale*, *Aegilops speltoides*, *Agropyron elongatum*, *Agropyron intermedium*, *Aegilops tauschii*, *Triticum ventricosum* are involved. A new set of varieties such as Chelyaba 75, Chelyaba 80, Pamyati Odintsova, Ilmenskaya 2 was created. In whose genomes a new resistance gene *LrSp* from *Aegilops speltoides* was introduced. Presently the high effective gene *Lr24* may be recommended for breeding in Southern Ural. Also for stabilization of phytosanitary situation in the Ural' region, the effective combination of genes *Lr9* or *Lr19* with *Lr26* and other *Lr*-genes may use for developing of new resistant wheat varieties. Based on this principle the spring wheat variety Silach was created. The high resistance to leaf rust in this variety due to a combination of the *Lr9* with *Lr26* genes, each of which in separately is not effective to leaf rust population in Southern Ural. Permanent monitoring of virulence of *Puccinia triticina* population in Southern Ural will permit to identify the effective genes of combinations of genes providing reliable protection.

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## Prediction and verification of auxin-ethylene crosstalk gene networks

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Plant hormones auxin and ethylene are the key regulators of plant growth and development, which are widely used in agriculture. Crosstalk between these hormones is essential for the regulation of various physiological processes. Morphogenetic effects of ethylene are often provided by modulation of auxin biosynthesis and transport that cause changes in auxin distribution. The crosstalk is reciprocal since auxin induces ethylene biosynthesis. However, the molecular mechanisms underlying this crosstalk are still poorly understood. Here, we perform a genome-wide prediction of the molecular events of the auxin-ethylene crosstalk and their associations with certain biological processes, as well as investigate the underlying molecular mechanisms. We suggest an algorithm to predict the genes potentially involved in the crosstalk between two signaling pathways – based on their functional annotation followed by transcription factor (TF) binding and expression pattern analyses. We applied the algorithm to identify auxin-sensitive genes that are associated with ethylene biosynthesis, signaling or ethylene response. As a result, we predicted over a hundred potential mediators for auxin-ethylene crosstalk, most of them were not reported to mediate auxin-ethylene crosstalk previously. Recruiting DAP-seq data, for some candidates we predicted potential mechanisms that could be utilized to regulate auxin-ethylene crosstalk. By analysis of expression patterns of predicted mediators and their putative regulators we made assumptions about the biological processes targeted by auxin-ethylene crosstalk. Some of the predictions were verified by qRT-PCR.

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## Integration of molecular marker and doubled haploid technologies for wheat breeding in the North Africa region

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Wheat is the major staple food crops of Morocco, Algeria and Tunisia and grown mainly under rainfed conditions. Biotic stresses such as yellow rust, leaf rust and the Hessian fly and abiotic stresses mainly drought constrains for wheat production, resulting in insufficiency in wheat production and import wheat most of the year for its domestic consumption. Developing stress tolerant varieties is one of the most efficient and economical approach to manage these stresses. However, traditional breeding approaches are in-efficient and take long time (10–25 years). As a first step and baseline information, we evaluated phenotypic and genotypic diversity of a set of improved and local landraces of bread wheat cultivars from Morocco, Algeria and Tunisia. The results revealed that many of the wheat cultivars were susceptible to prevailing biotypes of the Hessian fly and yellow rust. Therefore, the exotic wheat cultivars having various useful known genes were deployed and being used to make crosses with the North African cultivars. Integration of molecular marker and doubled haploid technologies within traditional breeding systems had enabled to select superior genotypes for traits that are difficult to select based solely on phenotype or to pyramid desirable combinations of genes into a single genetic background. The targeted crosses were made and subsequent generations were carried forward through traditional breeding systems and also in some cases through doubled haploids (DH) to speed up development of homozygous plants. Though marker-assisted breeding (MAB) can be applied to all segregating generations, we most commonly applied to early generations, including haploids,  $F_2$ ,  $BC_1F_1$ ,  $BC_1F_2$  and the  $F_1$  of complex crosses to enrich populations with favourable genes and their combinations. MAB also offered the opportunity to hasten transfer of desirable alleles from un-adapted exotic genetic backgrounds into a desirable germplasm through cross-breeding. Once, the selected homozygous genotypes were validated for the selected traits under field conditions, the selected lines were seed increased for subsequent preliminary yield trials and multilocation trials. In Morocco, three bread wheat varieties namely 'Kharoba', 'Khadija' and 'Malika' were released using these tools. In Algeria and Tunisia, integration of these tools in breeding is in progress. In conclusion, application of MAB and DH technologies greatly enhanced efficiency and effectiveness of utilization of the germplasm and enhanced genetic gains in the breeding programs.

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## Breeding for high sugar content, plant stalk juice and plant height characters in sweet sorghum

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Sweet sorghum (*Sorghum bicolor* L. Moench) with high biomass and high sugar content in its stalk is one of the most important energy crops. The high correlation between plant height and biomass and fermentable sugar in stem make sweet sorghum an important plant as a renewable bioenergy source. The potential of higher biomass and bioethanol with lower input in sweet sorghum comparing to maize make this crop valuable in large areas worldwide. The important characters for obtaining high biomass and sugar are mainly sugar and stem juice content per plant and plant height. 551 accessions were screened and several superior accessions were determined for these bioenergy related characters. Pyramiding different QTLs in a single line would obviously result in higher plant height, sugar content and biomass as the characters studied were under additive genetic behavior. QTL mapping is crucial to find out different QTLs for each character and thus “Erdurmus” line with high sugar content, plant height and stem juice was crossed with Ogretmenoglu which has contrasting values for the characters. The  $F_1$ s were selfed and the segregating  $F_2$  population were generated. 376  $F_2$  individuals were phenotyped for the bioenergy related characters. Of those, 200 individual DNAs were extracted and subjected to genotyping by sequencing (GBS) analyses. The corresponding  $F_3$  lines will be phenotyped in two different environments in this summer. PCR-based molecular markers developed from the SNP regions related to the characters will be tested in other advance lines which have high sugar content, plant height and stem juice. If the marker produces no information about any advance line, it will be assumed that this line may contain different QTL(s) and it will be used for pyramiding. This study proposes an authentic pipeline to find out different QTLs.

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## **Study of the genetic diversity of maize samples with dark colored grains from the gene pool of Azerbaijan**

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In the corn grain (*Zea mays* L.), flavonoid grouping and possessing many health properties (antioxidant, antimicrobial, anti-carcinogenic) anthocyanin pigments can accumulate. Numerous studies have found that regular consumption of food rich in anthocyanins leads to a significant decrease in the level of diabetes, obesity, cardiovascular and oncological diseases. The beneficial effect is manifested depending on the type and amount of anthocyanins in the ration component. The combination of nutritional and therapeutic value of the rich content of useful anthocyanins of corn kernels characterizes it as a functional product. The accumulation of anthocyanins in corn grain is controlled by the coordinated expression of structural and regulatory genes, the combination of different allelic variants of which determines the quantity and quality of the anthocyanins synthesized. Out of more than 400 maize samples from the National Gene Bank of Azerbaijan, forms with dark-colored grains were selected to study and use their genetic potential in breeding. The purpose of this work was to study the genetic diversity of maize samples with dark colored grains adapted to local conditions for further selection on grain quality. The paper presents the results of genotyping 38 maize samples using intermicrosatellite ISSR primers selected from the literature (inter simple sequence repeats), the most widely used and effective classes of DNA markers for genotyping, certification and classification of plant varieties. 6 primers generated polymorphic, well reproducible PCR fragments. For each sample, individual ISSR spectra were obtained, differing in the number of amplicons. A total of 65 fragments were synthesized, of which 63 are polymorphic. Thus, the average level of polymorphism in the 6 primers used was 96.9 %, which indicates a wide range of genetic variability in the sample under study, the samples of which can serve as the starting material for the selection of corn to improve the qualitative and quantitative composition of anthocyanins in the grain.

## Non-brittle rachis 1 (*Btr1*) gene in genera *Triticum* L. and *Aegilops* L.

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The non-fragility of the spike rachis trait was a key factor in the process of wheat domestication, determined the harvest effectiveness. However, the genetic control of this trait is not well understood in some cultivated and artificial wheat species. It is known that the *Q* and *Tg* genes are the main regulators of the wheat fragility vs. non-fragility of the spike rachis. Nevertheless, recently it was shown that rachis fragility of the second type (brittle rachis) in diploid wheat is determined by the *Btr1* and *Btr2* genes which are the homologous to those of barley. These genes have been studied only for einkorn wheat species (*Triticum monococcum* L. and *T. boeoticum* Boiss.). The study, conducted by Pourkheirandish et al., 2018, was allowed for the conclusion that a single non-synonymous amino acid substitution at 119 position (alanine to threonine) in *Btr1* gene is responsible for the non-brittle rachis trait in domesticated einkorn wheat. In the present study we investigated variability of *Btr1-A* gene in di-, tetra- and hexaploid wheat species, *Btr1-B* and *Btr1-D* genes from *Aegilops speltoides* Tausch and *Aegilops tauschii* Coss., respectively. We used a combination of bioinformatical tools and molecular biological methods to determine the full-length sequences of *Btr1* gene. *Btr1-A* gene in tetra- and hexaploid wheat species was contained 2 bp deletion at 292 nucleotide position, leading to formation of premature stop-codon.

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## Production of transgenic tomato plants to increase the efficiency of phytoremediation of soils contaminated with heavy metals

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Phytoremediation is the elimination, neutralization or conversion of pollutants to a less toxic form with the help of plants. This method is often used in cases of soil contamination with heavy metals (HM), using plants – hyperaccumulators of HM to restore the biological productivity of ecosystems. For effective phytoremediation, the search for soil bacteria that increase the availability of HM for plants is extremely important. The most promising in this regard are bacteria of the genus *Pseudomonas*, which are widespread in the rhizosphere of plants. Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in agriculture. In recent years, studies on the accumulation of HM in tomato plants, protection of this culture from HM, and the potential use of tomatoes for phytoremediation, including in conjunction with bacteria-microsymbionts, have become popular. The strain *Pseudomonas* sp. 102 can significantly increase the growth parameters and biomass of tomato plants, including under the toxic effects of cadmium. The greatest positive effect was observed in plants transformed with the bacterial adhesin gene *rapA1*, the product of which is important for colonization of plant roots by bacteria. It was also shown that shoots of transgenic tomato plants accumulated the greatest amount of cadmium during inoculation of *Pseudomonas* sp. 102. The ability to extract high concentrations of cadmium and accumulate a large biomass under stress opens up prospects for the further use of associative interactions between tomato and *Pseudomonas* for phytoremediation.

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## Effect of the *Stagonospora nodorum* effector SnTox3 on regulation of plant redox metabolism

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The most important virulence factors of the *Stagonospora nodorum* are multiple fungal necrotrophic effectors (NEs) that cause necrosis and/or chlorosis on wheat lines possessing dominant susceptibility genes (*Snn*). One such NE is SnTox3, which evokes programmed cell death and leads to disease when recognized by the wheat *Snn3-B1* susceptibility gene. In this study, combinations of different genotypes of wheat variety and fungal isolate ( $Snn3^+/Tox3^+$ ,  $snn3^-/Tox3^+$ ,  $Snn3^+/tox3^-$ ,  $snn3^-/tox3^-$ ) were studied. Some *S. nodorum* isolates, characterized by the presence or absence of the Tox3 ( $Tox3^+$  and  $Tox3^-$ ) effector gene and genetically characterized wheat samples were used. Full compatibility reaction in combination of genotype  $Snn3^+/Tox3^+$  was shown. The suppression of hydrogen peroxide generation in a compatible  $Snn3^+/Tox3^+$  interaction was most likely due to high activity of catalase, low activity of peroxidase and reduce of expression of genes encoding NADPH-oxidase (*TaRboh*), anionic peroxidase (*TaPrx*) and superoxide dismutase (*TaSod*) at the early stage of infection (24 hours), which further led to the formation of extensive lesions. The increase expression of ethylene biosynthesis genes *ACS* (ACC synthase), *ACO* (ACC oxidase) and ethylene signaling pathway genes *EIN3*, *ERF1* after 24 hours of infection in this genotype was shown. Incompatibility reaction in combinations  $snn3^-/Tox3^+$ ,  $Snn3^+/tox3^-$ ,  $snn3^-/tox3^-$  was shown. The increase of hydrogen peroxide generation due to alterations in redox enzymes activity and increasing expression of *TaRboh* and *TaSod* genes, as well as the absence of activation of biosynthesis and signaling pathway genes of ethylene at early stage of infection, which led to the development of hypersensitivity reactions and inhibition of the pathogen mycelium growth was found in incompatible interactions. Thus, the pathogen effector SnTox3 influenced the biosynthesis and the signaling pathway of ethylene with a view to regulate the redox metabolism of infected wheat plants for successful colonization of the host.

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## The transcriptomic analysis of Scots pine trees from the Chernobyl zone reveals pattern of adaptation to chronic radiation exposure

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Radioactive contamination of the natural areas is one of the most long-lasting anthropogenic impacts on the environment. Scots pine (*Pinus sylvestris* L.) is an important species for radiation protection of biota due to its high radiosensitivity, although the genome size of Pinacea species has imposed obstacles for high-throughput radiation-related studies so far. We conducted the analysis of the *de novo* assembled transcriptome of Scots pine populations growing in the Chernobyl-affected zone, which is still today contaminated with radionuclides. The transcriptional response indicates a continuous modulation of the cellular redox system, enhanced expression of chaperones and histones, along with the control of ions balance. Our data suggest that the modulation of ROS level occurs mainly through the control of glutathione- and thioredoxin-related responses and most likely involves a fine-tuning of ROS-generating processes. The SNPs analysis indicated enrichment of polymorphisms occurring on transcripts related to the antioxidant system and oxidation-reduction processes. While ROS-generating processes are being repressed, several pieces of evidence suggest the occurrence of control of gas exchanges via stomata, what would directly impact on the photosynthesis rate, a major ROS source. It is interesting to note that the control of stomata movement, although relying on several ABA target genes, apparently does not involve the modulation of ABA levels. A perspective for future work is the confirmation of ABA attenuation and of the control of photosynthesis rate on chronically irradiated plants. These adaptive responses are triggered by radiation doses 30 times lower than the one accepted as a safe for biota species by international regulations. This suggests that the environmental management in radiation protection should be reviewed.

## Elements of technology of adaptive seed production of vegetable beans in Western Siberia

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Today adaptive seed production of crops is a current direction. First of all seed breeders face the problem of adapting varieties to different soil and climatic conditions and reducing the quality of seed production of crops. To introduce vegetable beans into production crops, it is necessary to establish its seed production due to the plasticity of the varieties and the development of elements of the varietal cultivation technology, which will ensure the production of high-quality seed material of the crop. The purpose of the study is to improve the elements of adaptive seed technology for vegetable beans for the conditions of Western Siberia. The object of study is two varieties of common beans Nika and Solnyshko. With the observance of the optimal timing and sowing scheme, it becomes possible to establish seed-growing of vegetable bean in the conditions of the Siberian region. Sowing dates have a significant impact on the formation of the vegetative and generative parts of the plant. With the presence of amicable shoots, the productivity of the crop is significantly increased due to active flowering and bean formation, which creates the conditions for the uniform formation of the crop. Sowing qualities are determined by the term and scheme of sowing culture, taking into account the seed germination energy. When sowing in the third decade of May–the first decade of June, the whole complex of natural factors develops favorably for the growth and development of common bean plants, and it is possible to predict a high seed productivity of the crop. It was established that with an increased seeding rate, a rapid transition to the stage of biological ripeness and, accordingly, increased seed productivity with high quality seed culture is observed. Obtaining a assured yield of high-quality varietal seeds is possible subject to the developed methods of adaptive varietal seed production, recommended for specific growing conditions.



## Physiological tests in assessing of winter wheat gene pool for adaptability and productivity

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The predicted global climate change in the world is of particular importance for Kazakhstan, the main areas of agricultural production of which are characterized by high variability of environmental factors. According to the Second National Report on Climate Change (UNFCCC, 2009), the temperature regime in Kazakhstan changes mainly in the direction of warming. High temperature can be one of the main factors limiting the productivity of all agricultural crops, including wheat. In this regard, the understanding of physiological problems associated with stress caused by high temperature is of great importance. As an indirect selection criterion for heat tolerance, the CTD (canopy temperature depression) feature, calculated as the difference between the canopy temperature and the environment was used. According to the CTD value, the accessions were ranged from 11.4 °C to 14.2 °C. The tendency to increase in productivity with an increase in CTD ( $r = 0.69$ ) was revealed. The most productive was the variety Kharkovskaya-106 (9.1 t/ha, CTD = 13.6 °C). Monitoring of winter wheat gene pool using the “Green Seeker” (optical sensor) showed a change in the plant biomass index (NDVI) in more than 70 % of the accessions, depending on: 1) the cultivation conditions (irrigation, rain fed); 2) year of study; 3) growth stage. A high NDVI value was observed on irrigation in the heading stage (0.50–0.82) and a decrease (0.34–0.73) during the grain filling period. In 25 % of the accessions the high NDVI remained at the same level during the 2 measurement phases, which affected the yield. In rain fed conditions the NDVI value ranged from 0.20 to 0.39. According to the indicators of photosynthetic activity, the accessions of winter wheat were divided into 3 groups: high, medium and low productivity. In relatively high-yielding varieties (5.0–7.7 t/ha), the assimilation surface ranged from 50.4 to 72.9 thousand m<sup>2</sup>/ha.

## **$\beta$ -glucan elicitor from *Schizophyllum commune* induces expression of defense genes and protective effect against *Phytophthora* blight disease of pepper**

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Mushroom, *Schizophyllum commune* is a white wood-rotting fungi and produce a large of  $\beta$ -glucan on culturing. The  $\beta$ -glucan has industrially been used as cosmetics and medicines for enhancing immunity. This study was to investigate the availability of culture filtrate of *S. commune* (Sc-cf) on controlling *Phytophthora* blight disease of pepper. Sc-cf was precipitated by absolute ethanol and the precipitant and supernatant fractions inhibited mycelial growth of different phytopathogenic fungi including *Phytophthora capsici*, *Rhizoctonia Solani*, *Pythium ultimum*, *Botrytis cinerea* and *Colletotrichum acutatum*. The high content (16.88 g/100g) of  $\beta$ -glucan was detected in the precipitant, polysaccharide. The Sc-polysaccharide suppressed *Phytophthora* blight disease of pepper seedlings more than 60 %. In quantitative real-time PCR, the gene expression of *CaBPR1* (PR protein 1), *CaBGLU* ( $\beta$ -1,3-glucanase), *CaPR-4* (PR protein 4), and *CaPR-10* (PR protein 10) were significantly induced on the Sc-polysaccharide and DL- $\beta$ -aminobutyric acid (BABA) treated pepper leaves. In addition, the salicylic acid (SA) content was also increased in the Sc-polysaccharide treated pepper samples. These results suggest that  $\beta$ -glucan from *S. commune* can be used as an elicitor for the control of *Phytophthora* blight disease of pepper.

# Gene expression of phosphorus transport and sugar metabolism in *Medicago lupulina* plants with inoculation by *Rhizophagus irregularis* under conditions of low phosphorus levels in the substrate

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The aim of this study is to assess the expression level of the main phosphorus transport genes and carbohydrate metabolism genes in highly mycotrophic line MIS-1 *Medicago lupulina* inoculated with highly efficient arbuscular mycorrhizal fungus *Rhizophagus irregularis* under conditions of low level of phosphorus available for plant nutrition in the substrate. In our work, we used the original method for estimating the expression of genes, the sequences of which were selected by homology with the known sequences for *M. truncatula* and according to the data of the transcriptome analysis for *M. lupulina*. In particular, carbohydrate metabolism genes (*MIHKK1*, *MISUS*, *MISUS2*, *MISUC4*, *MlrbcS*, *MISTP13*) and phosphorus transport (*MIPT1*, *MIPT2*, *MIPT4*, *MIATP1*) were detected. For each genes, three pairs of primers were selected, of which the most effective ones were left to assess relative expression using the generally accepted 2- $\Delta\Delta$ CT method (reference gene – actin). Accounting for the parameters of productivity, efficiency of arbuscular mycorrhiza (AM), mycorrhization, microelements content, and expression of genes of interest were carried out in the key stages of *M. lupulina* plant development (6 terms up to flowering stage). Pronounced dynamics were shown for all genes of interest except *MIHKK1* and *MIRUB*. The study showed that two genes – *MIPT4*, *MIATP1* are specific for the development of effective AM-symbiosis of *M. lupulina* with the fungus *R. irregularis*. *MISUS2* showed significantly higher expression in plant roots with AM against control. Since 2019, it is planned to add several new genes to the analysis, such as invertase genes, as well as the *MST* or *SWEET* genes, among the latter screening for the presence of plant mycorrhiza-specific sugar transporters can be performed.

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## Marker-trait associations for agronomic traits in soybean harvested in Kazakhstan

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Soybean is becoming one of the most important oilseed crops in Kazakhstan. In last ten years the area under soybean is expanded from 45 thousand hectares (ha) in 2006 to 120 thousand ha in 2016. The general trend of soybean expansion is from south-eastern to eastern and northern regions of the country, where average temperatures are lower and growing seasons are shorter. These new soybean growing territories were poorly examined in terms of general effects on productivity level among the diverse sample of soybean accessions. Phenotypic data were collected in three separate regions of Kazakhstan and entire soybean sample was genotyped for identification of marker-trait associations (MTA). In this study, the collection of 113 accessions representing five different regions of the World was planted in 2015–2016 in northern, eastern, and south-eastern regions of Kazakhstan. North American accessions showed the highest yield in four out of six trials. The collection was genotyped with 6K SNP Illumina array. 4,442 SNPs found to be polymorphic and were used for whole genome genotyping purposes. Genotyping and field data were used for GWAS (genome-wide association study). Thirty SNPs appear to be significant in 42 MTAs in six studied environments. Overall thirty SNP markers associated with time to flowering and maturation, plant height, number of fertile nodes, seeds per plant and yield were identified. Physical locations of 32 identified out of 42 total MTAs coincide well with positions of known analogous QTLs. This result indicates importance of revealed MTAs for soybean growing regions in Kazakhstan. Obtained results would serve for forming and realization of specific breeding programs towards effective adaptation and increased productivity of soybean in three different regions of Kazakhstan.

## Phosphomimetically mutated and thus constitutively active kinase of ribosomal protein S6 from *Arabidopsis thaliana* (AtRPS6K2) does phosphorylate TaRPS6 in wheat (*Triticum aestivum*) 40S ribosomal subunit

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Coordination of growth and division in eukaryotic cells depending on availability of nutrients, energy, and in response to internal and external stimuli, is adjusted by multilevel cascade of serine-threonine protein kinases that transmit various signals to the protein-synthesizing apparatus causing its activation or inhibition. Phosphorylation of RPS6 by RPS6-kinase stimulates production of new ribosomes via preferential translation of 5'TOP-mRNAs that encode most proteins of translational apparatus (ribosomal proteins, elongation factors and many of initiation factors, poly(A)-binding proteins, etc.) and proteins of proliferation control. The mechanism of preferential translation of 5'TOP-mRNAs is unknown. Most studies of RPS6-kinase regulation in plants performed on *A. thaliana* that contains AtRPS6K2, which phosphorylates AtRPS6 in 40S ribosomal subunit (40S RS). For full activation, AtRPS6K2 requires phosphorylation by upper-level kinases: pPDK1 (at Ser296) and pTOR (Thr455, Ser437). To investigate the role of RPS6-phosphorylation in preferential translation of some viral and cellular 5'TOP-mRNAs it is important to obtain constitutively active AtRPS6K2. For this purpose we cloned *AtRPS6K2* cDNA-gene and carried out *in vitro*-mutagenesis, replacing codons of Ser(S)296, S437 and Thr(T)455 by triplets that encode phosphomimetic amino acid Glu(E). After expression in *E. coli*, two recombinant proteins were isolated: original AtRPS6K2 and phosphomimetic AtRPS6K2(S296E;S437E;T455E). These kinases were tested *in vitro* for their ability to phosphorylate either purified recombinant AtRPS6 (~30-kDa) or its homolog TaRPS6 in composition of 40S RS isolated from wheat germ (*T. aestivum*). Neither original nor phosphomimetic kinases were able to phosphorylate purified recombinant AtRPS6. Phosphomimetic kinase did phosphorylate TaRPS6 in composition of isolated 40S RS as was evident from SDS-PAGE-electrophoresis and subsequent radioautography by incorporation of radioactivity from [ $\gamma$ -<sup>33</sup>P]ATP into 30-kDa polypeptide. Besides *in vitro* studies, such an approach can find biotechnological applications.

## Chromatin and cytoskeleton reorganization in meiosis of wheat-rye substitution line (3R3B)

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Meiosis is a type of cell division that halves the chromosome number. This phenomenon shows a considerable degree of diversity among species. Unraveling molecular mechanisms of the meiotic machinery has been mainly based on meiotic mutants, where the effects of a change were assessed on chromosomes of the particular species. An alternative approach is to study the meiotic behavior of the chromosomes introgressed into different genetic backgrounds. The effect of rye chromosomes in meiosis of wheat-rye substitution lines is studied actively. Previously various groups of scientists studied the rye chromosome effect in substitution lines. It is known that chromosomes 1R, 3R, and 7R showed a regular meiotic behavior, and, based on the finding, polyembryony was regarded as a phenotypic expression of nuclear-cytoplasmic interactions where an important role is played by rye chromosomes 1R and 3R. Moreover, asynapsis was observed in 3R3B substitution line. The behavior of individual pair of rye homologues added to wheat (3R3B) has been monitored in first and second meiotic division. Using Navashin's modified fixative and immunostaining with  $\alpha$ -tubulin and CENH3 antibodies we observed such abnormalities as curved spindle, prolonged prometaphase in the first meiotic division. Moreover 20–50 % of meiocytes have an abnormal shape of the cell in late meiotic prophase. In the second telophase 50–20 % of the cells show such abnormalities as unequal division, absence of spindles and chromosomes in the second cell from a pair. In the stage of tetrads we observed monads, dyads, tetrads and unequal tetrads as a result. Thus there are some differences that we found by this study.

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## Comparison of spring oats varieties in response to the effects of root rot toxins in an *in vitro* culture

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The effect of the *Fusarium poae* and *Fusarium equiseti* toxic metabolites was studied in vitro in oats immature embryos. The Murashige–Skoog medium (MS) and the classical 3-stage cultivation were used: induction, proliferation of calli and plant regeneration. Toxic metabolites obtained on the basis of isolates of the author's collection of regional pathogens on the nutrient medium Chapeka. Filtrates of toxic metabolites of the species *F. poae* and *F. eqiseti* were used as selective agents on the proliferation medium of calli in concentrations of 30 %, 40 % and 50 %. The volume of the material was 2578 immature embryos of eight varieties: Tubinsky, Sayan, Kazyr, Selma, Talisman, Zolotoi pochatok, Golets, Tyumen holozerny. Callus induction took place on MS with the addition of 2,4-D – 3 mg/l and IAA – 2 mg/l, proliferation – from 2,4-D – 1.5 mg/l, regeneration – with kinetin – 1 mg/l and IAA – 0.5 mg/l. Inhibition of proliferation of calli by metabolites is highly significant even for lower concentrations of 30–40 % (from 49.4 to 71.0 % for *F. poae* and 37.6–66.1 % for *F. eqiseti*). Under these conditions the Tyumen holozerny and Kazyr varieties responded less to stress. Organogenesis and the formation of full-fledged regenerants in the presence of metabolites was above average for these same forms. Some dependence of the varietal reaction on the type of micromycetes was observed. The varieties Selma and Talisman compared with others showed greater resistance to the metabolites of *F. eqiseti*, and not of *F. poae*. In general, the metabolites of *F. poae* caused a stronger inhibitory effect on the stages of morphogenesis. Less resistant to the inhibitory effects of two types of micromycetes p. *Fusarium* varieties Golets, Tubinsky and Zolotoi pochatok. In this way, *in vitro*, oat varieties were tested for resistance to toxic metabolites of two types of micromycetes *F. eqiseti* and *F. poae*.



## Expression analysis and regulation of general transcription repressor, *TaDr1*, in bread wheat under drought

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**Introduction and Aim:** The key factor in achieving increased yields of spring wheat is the enrichment of the gene-pool using germplasm collections from foreign countries. The study and introgression of wheat germplasm accessions from various ecological and geographical origins is important for the wheat breeding process. These genetic materials can be used to study candidate genes responsive to abiotic stress and associated with agronomically important traits. The study of genetic polymorphism and expression of the transcriptional repressor gene, *TaDr1*, together with the *TaVrn1* and *TaFT1* genes which control the transition to the reproductive stage and flowering, can help to better understand the mechanism of drought tolerance and improve wheat yields in Northern Kazakhstan.

**Results:** High- and low-yielding wheat accessions were identified through screening of the world spring wheat germplasm collection via environmental tests in the local environment of Northern Kazakhstan with its harsh continental climate. As a result of molecular studies, the Amplifluor-like SNP marker KATU-W62 was developed based on *TaDr1* gene polymorphism, for accurate wheat genotyping. Increased expression of *TaDr1* was observed in response to drought, while the level of expression was higher in high-yielding wheat samples than in low-yielding accessions. Additionally, it was revealed that *TaDr1* is associated with two genes controlling wheat plant development, *TaVrn1* and *TaFT1*. All three studied genes, *TaDr1*, *TaVrn1*, and *TaFT1*, had similar changes in expression in response to drought, which is of particular interest for further research.

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## The wheat leaf epidermal pattern as a model for studying the effect of stress conditions on morphogenesis

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**Motivation and Aim:** The leaf epidermis of cereals is a widely used model system for studying the mechanisms of pattern formation for plant tissues, as it contains readily observable specialized cells. In this work we used a growing wheat leaf to study stress-induced dynamic changes in morphogenesis of specialized epidermal cells. In the process of formation, the leaf of wheat remains in the stationary growth phase for long time period. This fact permits us to observe a series of successive morphogenetic events recorded in the cellular structure of the mature leaf.

**Methods and Algorithms:** High-resolution 3D LSM-images allow extracting quantitative characteristics describing the cellular structure of leaf epidermis. However, to obtain a large amount of statistical data methods of high throughput computer based image segmentation should be used. We developed a workflow for detection of structural properties of leaf epidermis from 3D images obtained from confocal laser scanning microscopy. The workflow includes the protocol of sample preparation, image processing ImageJ-plugin [1] and data extraction algorithms.

**Results:** We showed significant aberrations of stomatal morphogenesis in the epidermis of boot leaves of wheat varieties Saratovskaya 29 and Yanetskis Probat in response to cold stress. We found that nonfunctional stomata predominated in the zone of maximum manifestation of stress, whereas in the zones formed before and after the stress impact, the developmental anomalies come to the disturbance in the morphogenesis of subsidiary cells [2]. In Saratovskaya 29, a significant amount of ectopic trichomes formed in rows predetermined to stoma formation. The proposed approach can provide standardized qualitative and quantitative data on stressinduced morphogenesis aberrations in wheat leaf epidermis. Subsequently, these data can be used for verification of computer models of leaf morphogenesis. Further study of the mechanisms of the effect of cold stress on morphogenesis will add to the search for additional opportunities to increase wheat yields in areas of risky agriculture.

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## Исходный материал мягкой яровой пшеницы на устойчивость к полеганию и продуктивность

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Повышение урожайности неразрывно связано с устойчивостью к полеганию, которое обусловлено анатомо-морфологическими признаками стебля. При этом длина стебля отрицательно коррелирует с устойчивостью к полеганию –  $r = -0.570 \dots -0.670$  (при  $R \geq 0.273$ ), что характерно и длине второго нижнего междоузлия –  $r = -0.528 \dots -0.781$ . В то же время устойчивость к полеганию в большей степени зависит от диаметра второго нижнего, опорного междоузлия, что подтверждается положительной сопряженностью –  $r = 0.476 \dots 0.586$ . Положительно выраженные различной степенью сопряженности анатомических признаков: число и диаметр СВП, толщины склеренхимного кольца и стенок междоузлий с основными элементами морфологии стебля позволяют использовать последние в качестве маркерных признаков при оценке сортов на устойчивость к полеганию. При этом следует учитывать, что длина стебля до значительной степени ( $r = 0.655 \dots 0.756$ ) оказывает влияние на продуктивность, а полегание отрицательно ( $r = -0.305 \dots -0.438$ ) коррелирует с качеством зерна: белок, клейковина, ИДК. Низкостебельные сорта, несмотря на хорошо выраженные анатомо-морфологические признаки стебля, слабо адаптированы к местным условиям, поэтому используются в селекционной работе как промежуточные формы. В то же время среднерослые, устойчивые к полеганию генотипы Скандинавских стран и Северной Европы довольно хорошо адаптированы к местным агроклиматическим условиям, где показывают хорошую продуктивность и широко используются в селекционной работе. Из изученных 126 сортообразцов коллекции ВИР – 12 устойчивые к полеганию, с высотой растений 76–93 см, при высоте стандарта Тюменская 29 – 86–95 см и полегании 3–4 балла. Среди них шесть сортообразцов: *Aletch* (к-65011), *Remus* (к-66025), *Trappe* (к-66027), *Lona* (к-66030), *Greina* (к-66031), *Toronit* (к-66032) и *Molera* (к-66033), в благоприятных условиях выделились довольно высокой средней урожайностью – от 497 до 567 г/м<sup>2</sup> при осредненном стандарте Тюменская 29 – 447 г/м<sup>2</sup>. Сортообразцы: *Aletch* (к-65011), *Toronit* (к-66032), *India 288* (к-65116), Мажор (к-65271), Гренада и АВИАДа устойчивы к предуборочному прорастанию зерна в колосе. Повышенная белковость (17.3–17.7–18.0 %) наблюдалась у Бисерти, Жигулевской, Тюменской 29 и Боевчанки. Высокой продуктивностью выделяются *Jin Mai 71* (к-65813) (559 г/м<sup>2</sup>), Арка (561), Гренада (592) и Алабуга (633 г/м<sup>2</sup>). Выделенные сортообразцы рекомендуются для системных скрещиваний, ступенчатой гибридизации и беккроссной селекции.

## Селекция интенсивных сортов гороха (*Pisum sativum* L.) зернового направления

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Все возрастающие требования производства к урожайности и технологичности сортов гороха предполагают создание в первую очередь интенсивных сортов. Этим требованиям отвечают созданные и зарегистрированные в последнее десятилетие сорта Русь, Кумир и Томас. Сорта короткостебельные, среднеспелые, устойчивы к полеганию и по качеству семян отнесены к ценным. Потенциальная продуктивность сортов равна 5–6 т/га. Реальная урожайность, полученная за 2010–2018 гг., при изучении в КСИ составила: 3.53 т/га – у Руси, 3.96 – у Кумира, 4.00 – у Томаса. Сорта отличаются относительно высокой стабильностью урожайности. Даже в острозасушливый 2012 г. они превышали по этому показателю стандартный сорт Ямалский на 7.3–21.0 %. Реальная урожайность сортов в производстве была близка к потенциальной. В 2010 г. в КХ «Пчела» с 20 га было намолочено по 6.0 т гороха Русь, а в КХ Шабалина за 2015–2017 гг. получено по 3.8–4.2 т/га гороха Кумир. Сорта созданы методом гибридизации. Отборы в питомниках проводились согласно выявленным закономерностям формирования урожайности сортов зернового гороха в условиях лесостепи Тюменской области. Итоги изучения 36–45 селекционных линий гороха в КСИ за 2014–2018 гг. показали более тесную взаимосвязь урожайности с массой семян с одного растения ( $r = 0.769–0.909$  за 2014–2017 гг. при пороге 5 % достоверности 0.294–0.320). И только в 2018 г. связь была слабая, но достоверная ( $r = 0.370–0.329$ ). Взаимосвязь урожайности с густотой стояния продуктивных растений была значительно слабее (от 0.167 до 0.597) и чаще недостоверной. Это связано с тем, что до конкурсного сортоиспытания «доходят» линии, устойчивые к стрессовым ситуациям резко континентального климата зоны. Густоту стояния растений в питомниках поддерживаем согласно рекомендациям для зоны, высевая 120–130 зерен на 1 м<sup>2</sup>. Взаимосвязь массы семян одного растения с элементами, ее слагающими, также не однозначна. Доказана наиболее тесная связь массы семян с количеством бобов на растении за 2015–2018 гг. ( $r = 0.509–0.818$ ), и только в 2014 г. связь отсутствовала. Связь с количеством зерен в бобе была значительно слабее ( $r = 0.063–0.519$ ) и часто недостоверной. Взаимосвязь в анализируемом наборе линий гороха массы семян одного растения и урожайности с массой 1000 семян отсутствует.

## Создание и оценка гибридного материала для селекции нейтральнотроневной крупноплодной земляники (*Fragaria* × *ananassa* Duch.) в Западной Сибири

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Крупноплодная земляника (*Fragaria* × *ananassa* Duch.,  $2n = 8x = 56$ ), благодаря ценному микроэлементному составу ягод и их диетическим свойствам, занимает лидирующие позиции в мире по занимаемым площадям и объему продукции. В настоящее время селекция этой культуры наполнилась новыми направлениями. Появились сорта с розовыми и ярко-красными цветками, развивается направление создания нейтральнотроневных F1 гибридов, репродуцируемых семенами, создаются сорта под выращивание в закрытом грунте и т.д. Одним из активно развиваемых направлений современной селекции культуры является создание нейтральнотроневных сортов, которые могут непрерывно плодоносить независимо от длины светового дня. Такие сорта хорошо себя зарекомендовали для выращивания в закрытом грунте. Однако нейтральнотроневные сорта отечественного происхождения, пригодные для выращивания в Сибири, практически отсутствуют. Цель данного исследования – расширение генофонда нейтральнотроневной крупноплодной земляники для повышения эффективности селекционной работы. В работе использовали внутривидовую гибридизацию (сортолинейные и межлинейные скрещивания, инбридинг) и межвидовую гибридизацию. В качестве материала для отбора исследования использовали потомство от скрещиваний, используя в качестве материнских форм нейтральнотроневные гибриды № 08/15-4-5, 08/17-4-5 и 3-6, полученные в результате последовательных скрещиваний и отборов по нейтральнотроневному типу цветения, а в качестве опылителя – гибрид ОФ-33-2011 однократного типа цветения, отобранный за позднеспелость, компактный габитус куста, плотность и хороший вкус ягоды, темноокрашенную мякоть, низкую побегообразовательную способность и относительную устойчивость к болезням. Основное внимание было уделено оценке селекционного материала на адаптивность, устойчивости на естественном инфекционном фоне, элементам продуктивности. В результате анализа семенных потомств в течение двух лет отобрано 26 нейтральнотроневных гибридов и 12 с однократным типом цветения. Отобранные в полевых условиях растения отличаются крупные, среднеплотные ягоды с хорошим вкусом (4–5 баллов), устойчивость к грибным заболеваниям и засухе, низкая побегообразовательная способность. В настоящее время материал размножается вегетативно для дальнейшего скрининга в экологических испытаниях и последующего выделения элит.

## Эпигенетические механизмы кариогеномной системы зрелых зародышей пшеницы, выведенной в условиях холодового стресса

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Пониманием базовой основы закономерностей морфогенетического развития, особенно в условиях гибридизации растений, занимается биоинформационная наука, которая с этой целью разрабатывает эффективные информационно-компьютерные технологии, исходя из того, что в основе любого признака лежит генная сеть – функциональная группа координированно экспрессирующихся генов. В ряде таких работ виртуально выделен их блочно-модульный характер, где блоки генных сетей образуют иерархическую структуру, в которой включение порядка и времени соответствуют формированию морфофизиологических компартментов. Связь между блоками генных сетей осуществляют сигнальные молекулы. По сравнению с биохимическими признаками молекулярные механизмы адаптивной эволюции морфогенетических признаков изучены слабо. В настоящее время методический прогресс сильно продвинул понимание молекулярно-генетической организации интерфазного ядра. Становится очевидным, что функциональная динамика доменной топологии интерфазного хроматина вовлечена в контроль регуляции различных взаимосвязанных базовых процессов в определенных областях ядра. Мы предположили, что один из механизмов в супрадоменной реорганизации хроматиновой матрицы может выполнять *Arg-X* протеазо-процессинг. Это предположение основывается на том, что хроматин ядра богат аргинином, и из всех аминокислот только он способен связываться с определенными пуриновыми и пиримидиновыми основаниями ДНК. Целью данной работы было рассмотрение кариогеномного анализа локализации *Arg-X* процессинга в топологически ассоциированных супраблоках гексаплоидной системы интерфазного хроматина в зрелых зародышах пшениц, адаптированных к холодовому стрессу. Объектом исследования служили семена суперэлиты пшениц (*Triticum aestivum* L.) сорта Артемовка (яровая) и выведенного из нее сорта Мироновская 808 (озимая), полученных из коллекции Всероссийского института растениеводства им. Н.И. Вавилова. Экспериментальная работа была проведена на основе собственных патентов: (1) по оценке морфофизиологического состояния проклюнувшихся зародышей; (2) выделенных из них клеточных ядер и (3) их супраструктурных ансамблей, а также (4) негистоновых и гистоновых белков, в которых (5) выявлена локализация *Arg-X* протеазо-процессинга. Впервые экспериментальные данные по эпигенетическим механизмам кариогеномного интерфазного хроматина гексаплоидной пшеницы представлены методами эписиохимии с применением терминологии кариогеномики. Выявлены зоны локализации *Arg-X* протеазо-процессинга в негистоновых и коровых гистонах, топологически ассоциированных кариогеномных доменах, в клеточных ядрах мезокотилей вегетативного периода ростового морфогенеза зрелых зародышей пшеницы, адаптированной к холодовому стрессу. Приведенные данные необходимы для разработки логико-математических схем теории и практики биологической специфичности и могут войти в базу данных онтологии стадий роста и развития кариогеномных растений.



## Перспективы создания голозерных сортов овса в зоне северной лесостепи Тюменской области

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Создание голозерных сортов овса – одно из перспективных направлений селекции в мире. Овес – культура универсальная и широко используется как на кормовые, так и продовольственные цели. Для создания новых сортов голозерного овса необходимо учитывать основные направления: скороспелость, иммунитет, высокое качество зерна, урожайность, технологические и морфологические показатели. В связи с этим проведена оценка 213 голозерных образцов овса различного эколого-географического происхождения. Для включения в селекционный процесс могут быть рекомендованы перспективные образцы, сочетающие высокую продуктивность и скороспелость: к-15014 Левша (Кемеровская область), к-15136 Avenuda (Jakub) (Чехия), к-15137 Detvan (Словакия), к-1926 HUU-LESS (Китай). Выделены источники комплексной устойчивости к пыльной головне, корончатой ржавчине и красно-бурой пятнистости: к-14365 Белорусский голозерный (Белоруссия), к-11663 Caesar (Германия), к-15094 MF9521-247 (США), к-15091 MF9224-336 (США). По биохимическим показателям качества зерна выделен перспективный исходный материал для использования в селекционной практике. В этом плане большой интерес представляют: к-15132 Местный (Франция), к-14944 Местный (Нидерланды), к-2353 Местный (США). Особо следует отметить образец к-2299 Polard (Канада), который наряду с высокими показателями качества формировал достаточно высокий урожай зерна. Выделены источники хозяйственно ценных признаков, которые могут быть рекомендованы для использования в селекции овса голозерного на продовольственные цели: без опушения зерновки – к-2122 Avoine nue grosse (Франция), к-14602 Krypton (Великобритания), к-15305 Gehl (Канада); с низким содержанием пленчатых зерен – к-5321 Местный (Пермский край), к-7439 Местный (Красноярский край), к-14719 Вандроуник (Беларусь), к-15120 Гоша (Беларусь); крупнозерные с высоким содержанием эндосперма – к-8427 Местный (Приморский край), к-8739 Голозерный (Мордовия), к-14717 Пушкинский (Ленинградская область), к-14960 Вятский голозерный (Кировская область), к-14227 Бег 2 (Беларусь), к-14182 Hja 76037 N (Финляндия), к-15299 Gkzalon (Монголия).



## Селекция мягкой яровой пшеницы в условиях изменяющегося климата

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Полное генеалогическое древо сортов-родителей позволяет установить географию их происхождения и определить динамику лим-факторов среды в данной географической точке. Это дает информацию о генетико-физиологической системе адаптивности родителей, которая может быть передана гибридному потомству. Потепление климата требует наследственного повышения засухоустойчивости, которая обусловлена эколого-генетической организацией количественных признаков, оперирующей семью генетико-физиологическими системами (ГФС), определяющими урожай: аттракции, микрораспределений аттрагированных пластических веществ, адаптивности, горизонтального иммунитета, оплаты сухой биомассы лим-фактора почвенного питания, толерантности к загущению и продолжительностью фаз онтогенеза. Одной из определяющих является ГФС адаптивности к разным лим-факторам в зоне. Показатель адаптивности зависит от взаимодействия «генотип–среда» (ВГС), меняющегося при смене рангов продуктивности сортов. В Северном Зауралье из 25 % генетической составляющей в формировании урожайности, около 20 % обусловлены ВГС. Проявление урожайности резко снижает засуха, которая обусловлена 22 компонентами физиологического и морфологического состояния растений, входящими в семь ГФС. Из них важным компонентом является корневая система, изменяющаяся в онтогенезе. Крупный зародыш имеет хорошо дифференцированные элементы, что позволяет отбирать генотипы с 5–6 активно растущими зародышевыми корнями. К кущению корни проникают в почву на 50–70 см, колошению – 130–150 см и к полной спелости – 170–185 см. Повышение засухоустойчивости должно быть направлено на использование диких видов. Примером этого служит сорт Серебряна – соматическая гибридизация интенсивной Казахстанской 10 и неэкстрифугированного ядерного материала пырея сизого, отличающаяся сочетанием засухоустойчивости с выносливостью к патогенам и вредителям. Засухоустойчивость и адаптивность пшеницы повышаются с помощью трансгенеза – введением генов амфидиплоидов, путем встраивания чужеродной ДНК в геном реципиента. Получены селекционные формы с участием тритикале и *Sphaerococcum*. ГФС полигенного иммунитета (горизонтальной устойчивости) – ИММ обусловлена сильным кутикулярным восковым налетом листьев, стебля и колоса, препятствующим прорастанию спор – септариоза, ржавчины, мучнистой росы, и негативному влиянию засухи. Густое опушение листьев и листовых влагалищ предохраняет от поражения скрытностебельными и как альбедо предохраняет от нагрева. Повышение урожайности должно идти за счет оптимального соотношения озерненности колоса (30–34 шт.), абсолютной массы зерна (38–41 г) и его продуктивности (1.1–1.2 г). При хорошей толерантности к загущению – 530–600 колосьев/м<sup>2</sup>, формируется урожайность 5.8–7.2 т/га.

## Исходный материал для создания сортов овса универсального использования

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В условиях Северного Зауралья в 2016–2018 гг. была проведена комплексная оценка 80 коллекционных образцов (из коллекции ФГБНУ Федеральный исследовательский центр «Всероссийский институт генетических ресурсов растений им. Н.И. Вавилова») и 22 селекционных номеров, созданных в НИИСХ Северного Зауралья. Результаты изучения показали достаточно высокую изменчивость по урожайности зерна, зеленой массы и сбору сухого вещества. Свыше 30 % изученных образцов формировали высокий урожай зерна, но уступали стандарту (Талисман) по урожаю зеленой массы и сбору сухого вещества. Большая часть образцов (52 %) превосходила стандарт (Талисман) по урожаю зеленой массы и сбору сухого вещества, но уступала ему по урожаю зерна. Перспективный исходный материал для создания сортов овса универсального использования (на зерно и зеленый корм) составил 16 %. Большой интерес в этом плане представляют номера: К-14376, Avalanche, (Франция); К-15279, 50h2035 (Московская область), ТМ 08-123-5, ТМ 04-22-2, ТМ 07-84-8, ТМ 08-140-2, Тобояк (Тюменская область). Выделенные образцы формировали урожай зерна от 544.3 до 615.9 г/м<sup>2</sup> (при урожае стандартного сорта Талисман 524.5 г/м<sup>2</sup>). Сбор сухого вещества у них варьировал от 1367.8 до 1490.5 г/м<sup>2</sup>, у стандартного сорта Талисман – 1263.7 г/м<sup>2</sup>.

## Гетерозис у 56-хромосомных апомиктичных кукурузно-трипсакумных гибридов

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Апомиксис обладает потенциальной возможностью революционизировать растениеводство и значительно повысить рентабельность и урожайность в этой весьма затратной отрасли сельского хозяйства. Все действующие в настоящее время селекционные технологии по использованию гетерозиса основаны на работе с сексуально размножающимися растениями. В этой связи они несут свойственный им недостаток – сегрегацию по хозяйственно ценным признакам в ряду поколений после  $F_1$ , что требует проведения ежегодного и очень затратного воспроизводства гибридов. Эта проблема может быть решена с привлечением бесполосеменного размножения. Один из путей практического использования апомиксиса для закрепления гетерозиса связан с заимствованием его у диких сородичей культурных растений. В этой связи необходимо оценить, как сильно генетический материал дикого родителя будет влиять на экспрессию гибридной мощности. Исследования, проведенные на растениях разных родов, показывают, что межвидовые гибриды, как правило, проявляют более высокий гетерозис, чем внутривидовые гибриды, если генетическая разница между видами или родами не мешает им формировать совместимые скрещивания. С целью закрепления гетерозиса у кукурузы и получения перспективного селекционного материала был предложен путь гибридизации между *Zea mays* и *Tripsacum dactyloides* ( $2n = 4x = 72$ ) – донора признака апомиктического способа репродукции. Создание 56-хромосомных кукурузно-трипсакумных гибридов ( $2n = 56 = 20Zm + 36Td$ ) строилось по схеме последовательной гибридизации линий кукурузы (линия 573 и линия 611), используемых для получения коммерческих гибридов  $F_1$ , с гамма-грассом. Из полученных форм 56-хромосомных гибридов три формы с родительскими  $2n = 56 = (20Zm \text{ (линия 573)} + 36Td)$ ;  $2n = 56 = (20Zm \text{ (линия 611)} + 36Td)$  и гетерозисной ( $2n = 56 = [(10Zm \text{ (линия 573)} + 36Td) + 10Zm \text{ (линия 611)}]$ ) комбинациями кукурузных геномов экспрессируют признак апомиктического воспроизводства. Исследование данных форм показало, что 56-хромосомные апомиктичные гибриды, у которых присутствуют геномы линий 573 и 611, превосходят контроли, где оба генома только от одной из этих линий: по массе зерновки, скорости роста, биохимическим показателям и урожайности зеленой массы, что говорит о том, что у данной формы гибрида экспрессируется гетерозис.

## **Фитосанитарная обстановка и влияние пестицидов на формирование урожая в агроценозах новых сортов яровой пшеницы**

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Для реализации потенциала новых сортов пшеницы в сельскохозяйственном производстве, важно иметь информацию об их групповой или комплексной устойчивости к вредным организмам и их отзывчивости на внесение пестицидов в различных условиях выращивания. Такая информация позволит оптимизировать элементы системы защиты для каждого отдельно взятого сорта пшеницы, сократить затраты и повысить рентабельность производства, улучшить экологическую обстановку окружающей среды. Сравнительную оценку фитосанитарной ситуации в посевах сортов мягкой яровой пшеницы (раннеспелый – Новосибирская 16, среднеранние – Новосибирская 41 и Сибирская 21) проводили в 2018 г. на стационаре СибНИИРС – филиал ИЦиГ (в ОПХ «Элитное»). Наши исследования показали некоторые различия пораженности посевов пшеницы болезнями и заселенности их вредителями. Сорт Новосибирская 16 сильнее других поражался обыкновенной корневой гнилью (6.8 %), бурой листовой ржавчиной (5.2 %) и септориозом (3.5 %), его посевы интенсивнее повреждались внутрискосовыми вредителями, однако меньше их заселяли хлебная полосатая блошка (164 шт./м<sup>2</sup>) и пшеничный трипс (59 личинок/колос). Растения пшеницы Новосибирская 41 слабее других поражались обыкновенной корневой гнилью (2.1 %) и повреждались внутрискосовыми вредителями, в большей степени поражались мучнистой росой (10.1 %) и заселялись хлебной полосатой блошкой (252 шт./м<sup>2</sup>). Сорт Сибирская 21 проявил самую высокую устойчивость к листостебельным болезням (индекс развития болезней составил 0.4–1.2 %) и был менее привлекательным для пшеничного трипса (18 личинок/колос). Также он показал самую высокую урожайность, как без применения фунгицидов и инсектицидов (5.13 т/га), так и с их использованием в различных сочетаниях (от 5.27 до 6.19 т/га). Наименьшую урожайность отмечали у сорта Новосибирская 16, в первом случае она составила 2.87 т/га, во втором – изменялась от 3.26 до 4.06 т/га. Продуктивность сорта Новосибирская 41 варьировала в зависимости от варианта от 3.91 до 5.77 т/га. Таким образом, изучаемые сорта определенным образом влияют на формирование фитосанитарной ситуации в отношении болезней и вредителей, по-разному отзываются на внесение пестицидов и дают различную прибавку урожая при их внесении.

## Состояние и перспективы селекции зернофуражных культур в условиях Северного Зауралья

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Работы по созданию сортов ярового овса и ячменя в условиях Северного Зауралья до конца 1970-х гг. прошлого столетия практически не велись. Первые сорта овса (Тюменский 82, Вагай), созданные в данном регионе, были получены на опорном пункте СибНИИСХ при НИИСХ Северного Зауралья. В 1981 г. в НИИСХ Северного Зауралья была создана лаборатория селекции зерновых и кормовых культур. Самостоятельная лаборатория по селекции зернофуражных культур была организована в 1995 г. За истекший период времени было создано и передано в государственное сортоиспытание 15 сортов овса и 9 сортов ячменя. В настоящее время в Государственный реестр селекционных достижений включено 5 сортов овса (Мегион, Талисман, Отрада, Фома, Тюменский голозерный) и 2 сорта ячменя (Абалак, Зенит). Потенциальная урожайность пленчатых сортов овса 6.0–8.0 т/га, голозерного – 4.0 т/га, ячменя – 6.5–7.5 т/га. Сорта высокопластичны и рекомендованы для использования в ряде регионов РФ (Мегион – 4, 10; Талисман, Фома – 10, 11, 12; Отрада – 9, 10, 12; Тюменский голозерный – 4, 7, 9, 10, 11; Абалак – 4, 10, 11; Зенит – 4, 12). Селекция овса в Северном Зауралье ведется в двух направлениях: создание сортов зернового типа и зерноукосного. Решается также задача по созданию сортов универсального использования (на зерно и зеленый корм). Таким сортом, внесенным в Государственный реестр, является Талисман. С 2018 г. государственное сортоиспытание проходит новый сорт универсального использования – Тоболяк. За годы изучения в условиях Северного Зауралья (питомник конкурсного сортоиспытания 2014–2017 гг.) он превзошел стандартный сорт Талисман по урожаю зеленой массы на 5.3 т/га, по сбору сухого вещества на 1.3 т/га, по урожаю зерна на 1.0 т/га. Селекционные работы по ячменю направлены главным образом на получение новых сортов кормового и пищевого использования, также ведется отбор низкобелковых форм, перспективных для пивоваренной промышленности. В государственное сортоиспытание передан высокопродуктивный сорт ячменя Кудесник, у которого содержание белка за годы испытания на заключительном этапе селекционного процесса (2015–2018 гг.) составило 9.58–11.68 %.

## Основные направления селекции и семеноводства люцерны в Европейской России

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Люцерна – одна из важнейших широко распространенных древних мировых культур, которая является спутником интенсивного товарного животноводства и скотоводства, требующих для своего развития прочной, стабильной и качественной кормовой базы. На современном этапе наиболее интенсивная селекционная работа ведется с несколькими видами рода *Medicago*: *Medicago sativa* L. и *Medicago varia* Mart. В скрещивания для создания специализированных сортов для особых условий возделывания, например луговых почв, затопляемых пойм, участков с кислыми почвами, меловыми обнажениями и др., включаются менее распространенные виды: *M. falcata* auct., *M. falcata* subsp. *romanica* (Prodan) Schwarz et Klinkovski, *M. borealis* Grossh., характеризующиеся высокой зимостойкостью, способностью к возделыванию на затопляемых участках, корнеотпрысковостью и др. В настоящее время работа по селекции люцерны активно ведется в Белгородской области в Белгородском государственном национальном исследовательском университете на базе природно-ландшафтного комплекса «Ботанический сад» и лаборатории биологических ресурсов и селекции растений кафедры биологии, в тесном сотрудничестве с аграрными предприятиями региона. Созданы и районированы сорта: Белгородская 7, Краснояружская 1, Краснояружская 2. В Государственном сортоиспытании находятся сорта Алексеевская 1 и Глория. Сорта дифференцированно рекомендованы к возделыванию как в условиях интенсивных севооборотов, так и на низкопродуктивных почвах. Селекционная работа с люцерной как кормовой культурой направлена на достижение нескольких основных целей: повышение продуктивности кормовой массы в конкретных почвенно-климатических условиях, увеличение сбора белка с единицы площади и повышение переваримости кормовой массы. Параллельно ведется работа на повышение семенной продуктивности как основы эффективного семеноводства. Успешная селекционная работа немыслима без создания системы устойчивого семеноводства как экономической основы эффективного внедрения достижений науки. Тесное сотрудничество группы по семеноводству многолетних трав Белгородского университета с одним из крупнейших предприятий региона ЗАО «Приосколье» позволило создать систему первичного и элитного семеноводства люцерны на базе собственных сортов. Площади семенников люцерны в отдельные годы достигают 1.5 тыс. га, а производство семян высокими репродукциями – 300 т/год. Вложение ренты от использования и реализации семян позволяет финансировать селекционные программы сортов с использованием метода рекуррентной селекции, метода поликросса, гибридизации с использованием *mf*-мутаций, микроклонального размножения. Коллекционный фонд люцерны формировался преимущественно на базе генетического материала, полученного в природных условиях региона. Основой для него стали коллекции, собранные, начиная с 60-х годов прошлого века, на Полтавской опытной станции по многолетним травам, во Всероссийском институте растениеводства, генетики и селекции им. В.Я. Юрьева, Белгородском сельскохозяйственном институте. Затем коллекции пополнялись за счет местных образцов, отобранных в овражно-балочных комплексах с меловыми обнажениями, современного гибридного материала, полученного в Белгородском государственном национальном исследовательском университете. Коллекция люцерны в настоящее время составляет около 3000 тыс. образцов, сохраняемых в виде семян и живых растений на территории Ботанического сада университета. Отработанные технологии микроклонального размножения позволяют ускорить размножение наиболее ценных форм. Таким образом, созданная система селекции и семеноводства люцерны позволяет поддерживать стабильное производство качественных кормов на основе селекционных сортов люцерны, адаптированных к различным условиям возделывания.



## Генетический потенциал сибирского генофонда мягкой яровой пшеницы

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В музее изучалось 67 сортов сибирской селекции: раннеспелых – 20, среднеспелых – 35 и позднеспелых – 12, с выраженной засухоустойчивостью к ранне-летней засухе и ограниченной (1.3–1.7) продуктивной кустистостью. У позднеспелых сортов колос длинный и многозерный, что подтверждается сопряженностью между ними и периодом кущения ( $r = 0.420 \dots 0.654$ ) (при  $R \geq 0.325$ ). Урожайность в основном формируется за счет продуктивности колоса ( $r = 0.663 \dots 0.756$ ). Разнонаправленная сопряженность массы 1000 зерен с озерненностью колоса ( $r = -0.164 \dots +0.426$ ) позволяет создавать крупнозерные формы с многозерным колосом. Крупное зерно отличается утонченным перикарпием, что благоприятствует проникновению молекул воды и фитофагов. Оно отрицательно коррелирует с содержанием белка, клейковины, ИДК ( $r = -0.395 \dots -0.598$ ). Масса зерна с колоса на одном уровне, значимо коррелирует с его озерненностью ( $r = 0.616 \dots 0.787$ ) и абсолютной массой зерна ( $r = 0.679 \dots 0.715$ ), поэтому у новых сортов должно быть оптимальное их соотношение: озерненность колоса – 29–31 шт., масса 1000 зерен – 38–41 г. Высокая озерненность ведет к щуплости зерна и снижению абсолютной массы. Предуборочное прорастание зерна в колосе снижает его продуктивность ( $r = -0.540$ ) и урожайность ( $r = -0.602$ ). На урожайность отрицательное влияние патогены: бурая ржавчина, септариоз, пыльная головня ( $r = -0.334 \dots -0.441$ ). Многозерностью колоса (31–33 шт.) выделяются сорта Тюменская 80, Омская 20, Лютесценс 70, Омская 35, Серебрина, Ильинская, Ария, АВИАДа и раннеспелые сорта Омская 26, Новосибирская 29, СУРЭНТа-6; 34–38 зерен в колосе у Ранга, Чернявы 13, СКЭНТа-3, Икара, Омской 18. Крупная масса 1000 зерен (43–47 г) у Стрелы, Тюменской 80, Чернявы 13, Ильинской, АВИАДы, Веры, Казахстанской 10, Омской 35, Nadine. Мелкозерность (32–34 г) отмечена у Тулунской 12 и Новосибирской 15. Устойчивые к прорастанию зерна в колосе у сортов Лютесценс 70, СУРЭНТа-7, Сибирская 14, Лавруша. Высокой белковостью выделяются: Цезиум 111 (17.0–17.2 %), Хитон (16.0–16.6) и Новосибирская 29 (15.4–17.7 %). Горизонтальная устойчивость к септариозу и бурой ржавчине у сортов Гренада и Лютесценс 368. Выделенные сорта рекомендуются для включения в селекционные программы.



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ООО «Максим Медикал» – научно-производственная фирма, основанная в 2016 г. Компания осуществляет поставку, обслуживание научного и медицинского оборудования и разрабатывает системы анализа и инспекции на базе машинного зрения и микросенсоров.

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**KASP™** – экономичная и точная аллель-специфическая ПЦР, генотипирование для поиска и валидации селекционно-значимых маркеров до 1000 SNP.

**RPA** – рекомбиназная полимеразная амплификация (RPA) является экспресс-альтернативой ПЦР, позволяет диагностировать фитопатогены/ГМО в течение 10–15 мин без специального оборудования.

**BHQ** – зонды **Black Hole Quencher** с улучшенными спектральными параметрами, где требуется высокая специфичность real-time ПЦР.

**Sbeadex** – наборы для выделения ДНК из растений, тканей животных, крови, бактерий (плазмидная ДНК) и микропроб.

Расходные материалы для ПЦР: мастер-миксы **KlearTaq**, «пластик» **Sarstedt** для ПЦР и микробиологии.

**Intelliqube** – высокопроизводительный автоматизированный комплекс для генотипирования, включающий модуль жидкостного дозирования сборки ПЦР, амплификации и детекции. Вместо планшетов запаиваемая 768-луночная лента (Array Tape), что значительно экономит реагенты.

- Производительность до 24 000 SNP / 8 часов;
- Модуль детекции для широкого спектра красителей 480–620 нм (15 фильтров);
- Приложения: ПЦР по конечной точке, real-time ПЦР, мультиплекс, изотермическая ПЦР.



**HiPoint** – модульные камеры роста растений и растительных культур, теплицы.

- Широкий выбор источников LED-освещения, включая искусственный солнечный свет;
- Модульная и масштабируемая конструкция;
- Автоматический контроль параметров: освещение, температура, влажность, CO<sub>2</sub>.



**Beckman Coulter** – цитометры, центрифуги, счетчики клеток, сортеры клеток.

**Phenospex** – роботизированные системы фенотипирования растений от 10 см до 1.1 м на основе лазерных 3D сканеров Planteye F500.

Автоматический анализ параметров: NDVI, EVI, цифровая биомасса, высота растения, наклон листа, площадь листовой поверхности, степень озелененности и др.



### Услуги генетического анализа

ООО «Максим Медикал» – авторизованный провайдер услуг генетического анализа LGC Genomics (Англия).

Вам требуется лишь передать образцы нашим специалистам, мы возьмем на себя всю рутину пробоподготовки.

- **Генотипирование «Все включено»:** выделение ДНК, синтез праймеров, генотипирование, выдача результатов. В основе патентованные технологии SeqSNP™ и KASP™. Масштаб проекта от 1 до 10 000 SNP.
- **SeqSNP** – уникальная технология целевого NGS на анализаторах Illumina. Идеально для ускорения селекционных программ: масштаб от 100 до 100 000 SNP.

Сроки выполнения работ от 1.5 до 3 месяцев в зависимости от масштаба эксперимента и вводных данных.

Наши контакты: [www.maxmedikal.com](http://www.maxmedikal.com), [www.kasp.ru](http://www.kasp.ru),  
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# ThermoFisher SCIENTIFIC

Компания Thermo Fisher Scientific – крупная международная корпорация с годовым оборотом свыше \$ 20 млрд, насчитывающая 65 000 сотрудников в разных странах мира. Помимо продукции Life Sciences, компания производит аналитическое, диагностическое и общелабораторное оборудование и расходные материалы.

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**СИНТОЛ** – научно-производственная компания, специализирующаяся в сфере технологий синтеза и анализа нуклеиновых кислот.

Мы производим наборы реагентов для выделения ДНК и РНК, ПЦР-диагностики туберкулеза, опасных и особо опасных инфекций, для анализа SNP, ГМО, анализа ДНК в криминалистике, идентификации сырьевого состава мясной и рыбной продукции, выявления патогенов растений, генетической паспортизации картофеля, выявления ДНК-маркеров устойчивости к возбудителям заболеваний картофеля. В сотрудничестве с ИАП РАН мы производим генетические анализаторы «Нанофор-05» и амплификаторы «АНК». Кроме того, мы производим вспомогательное оборудование для ПЦР-лабораторий. Компания «Синтол» проводит курсы обучения секвенированию по Сэнгеру, фрагментному анализу, анализу ГМО методом ПЦР в реальном времени.

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Компания **«Аксиома БИО»** предлагает реактивы и расходные материалы научно-исследовательским лабораториям, специализирующимся в области биохимии, молекулярной и клеточной биологии, и научно-производственным биотехнологическим фирмам.

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**Takara BIO** – инновационные реагенты для молекулярной биологии – ферменты, наборы реагентов для создания клонотек для мультиплексного секвенирования, реагенты для клонирования, протеомики, популярные наборы PicoPLEX WGA для преимплантационного скрининга и многое другое;

**Zymo Research** – высококачественные наборы для выделения и очистки нуклеиновых кислот, эпигенетики, работы с дрожжевыми культурами – достойная альтернатива популярным наборам по демократичной цене;

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**Electron Microscopy Sciences** – крупнейший производитель расходных материалов и реагентов для электронной микроскопии. В большом ассортименте предлагается также продукция Diatome (ножи для ультрамикротомов), Dumont (ультратонкие инструменты), Ideal-Tek, Rubis, Summers Optical;

**USBiological** – американская компания, производитель биохимических реагентов, антител и питательных сред, реагентов для молекулярной биологии, ИФА наборов самого высокого качества. В каталоге представлено более 1 млн наименований;

**BIOplastics** – высококлассный пластик для ПЦР из Нидерландов. Широчайший выбор микропробирок для всех известных на рынке амплификаторов, разнообразные наконечники для всех типов пипеток и многое другое.



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The Fifth International Scientific Conference

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- Plant biotechnology in the post-genome era.
- Plant systems biology and digital technologies.

The working languages of the Conference are English and Russian.

The organizing Committee provides an opportunity to arrange workshops on topical areas of plant breeding, genetics, and biotechnology within the Conference.

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