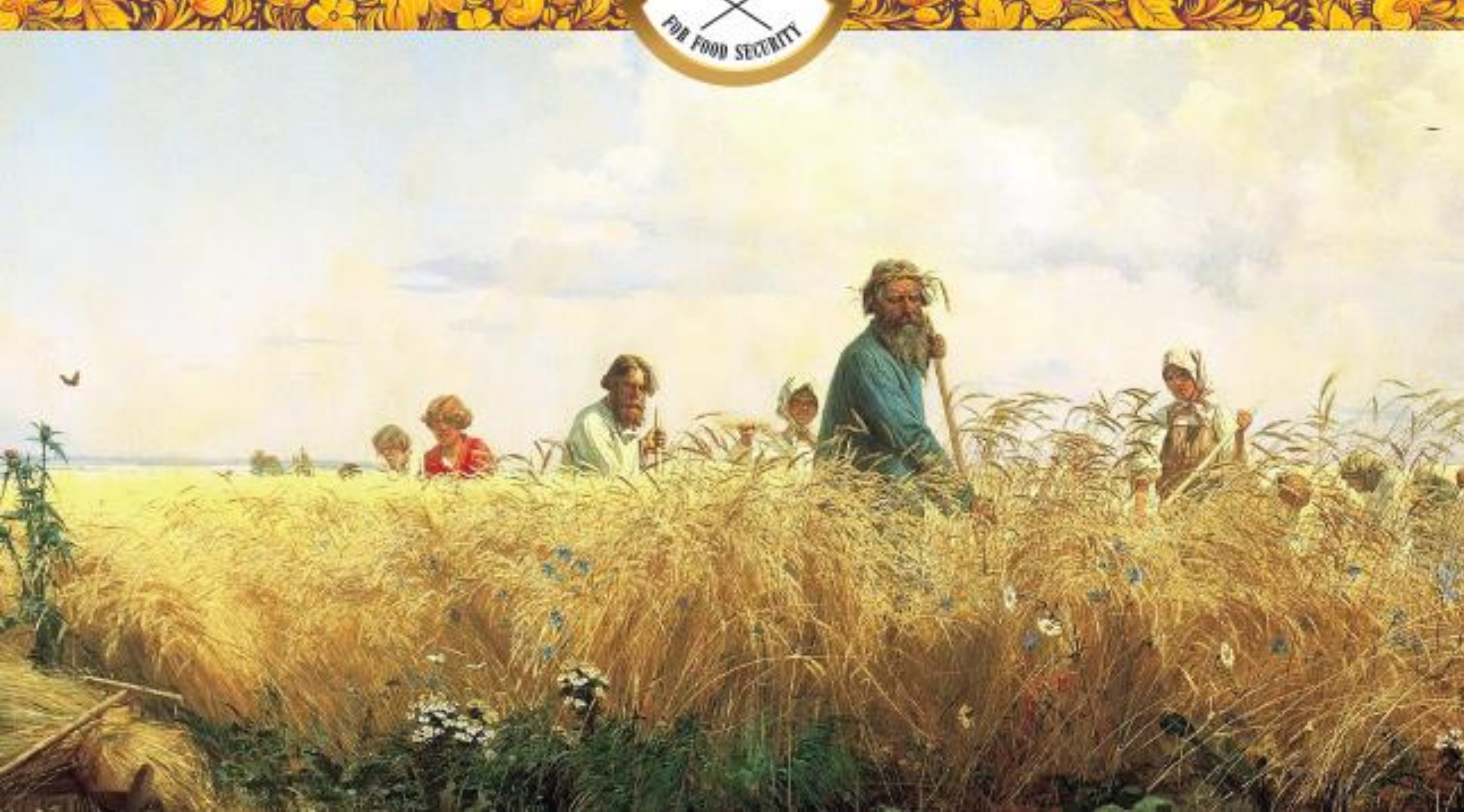
THE 1ST INTERNATIONAL WORKSHOP “PLANT GENETICS AND GENOMICS FOR FOOD SECURITY”

PGGFS-2016
Novosibirsk, Russia
August 26–28, 2016



Federal Research Center Institute of Cytology and Genetics
of the Siberian Branch of the Russian Academy of Sciences

Novosibirsk State University

Federal Agency for Scientific Organizations

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Abstract Book

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ORGANIZERS

Federal Research Center Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk State University, Federal Agency for Scientific Organizations, Siberian Branch of the Russian Academy of Sciences, Vavilov Society of Geneticists and Breeders, Faculty of Natural Sciences of the Novosibirsk State University, Food Security Research Center of the Novosibirsk State University, Council of Young Scientists of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk Branch of the Vavilov Society of Geneticists and Breeders, Vavilov Journal of Genetics and Breeding

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GENOTYPE-ENVIRONMENT INTERACTION PATTERNS OF SOYBEAN GROWING IN KAZAKHSTAN

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As part of diversification policy in agriculture, the harvesting area under soybean is constantly growing and increased from 45K hectares in 2006 to 114K hectares in 2014. This suggests that in addition to basic agronomy knowledge there is a necessity to have a detailed study for plant adaptation to new expanded environments. Therefore, it is necessary to determine the important genotype-environment patterns in order to find optimal cultivars for major soybean growing regions in the country. In this study 120 soybean accessions from different parts of the world, including 18 cultivars and prospective lines from Kazakhstan, were tested in 2015 in field conditions of South-eastern, Eastern, and Northern regions of the country. Results allowed the grouping of studied accessions into different maturity groups and the assess of the yield components. The highest yield in the entire collection among three regions was recorded in Northern Kazakhstan where the most productive samples were characterized by *E1e4* genotype, which was not present in local breeding lines. Application of AMMI and BiPlot methods allowed determining that East and South-East regions as one mega-environment and North Kazakhstan as an additional one. The results provide important insights into the relationship between genetic and phenotypic patterns in new soybean growing territories in Kazakhstan.

GENETIC DIVERSITY OF BARLEY RESISTANCE TO LEAF BLIGHTS AND DEVELOPMENT OF INITIAL MATERIAL FOR BREEDING

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Net blotch caused by *Pyrenophora teres f. teres (Ptt)* and spot blotch caused by *Cochliobolus sativus (CS)* are two most harmful fungal pathogens of barley around the world. To increase the diversity for net blotch and spot blotch resistance in cultivated barley we studied resistance of more than 10000 barley accessions from the collection of N.I. Vavilov Institute of Plant Genetic Resources. Four double haploid (DH) populations from crossing of resistant to *Ptt* and CS accessions with susceptible cultivars were developed by use of barley anther culture. Phenotyping of seedling resistance to Ptt and CS and genotyping by SNP and DArT markers allowed us to determine three major genes, controlling resistance to *Ptt* isolates mapped to chromosome 6H (11_11067, position 58cM) and 3H at the interval 37,46 – 44,24 cM and 48.32cM – 51.35cM. Also four novel isolate specific QTLs controlled resistance to *Ptt* were determined on chromosomes 1H, 4Hи 5H, and five novel QTLs associated with resistance to *CS* were found on chromosomes 2H, 3H, 5H and 6H. The stock of resistance genes is necessary for successful barley breeding on durable resistance to pathogens by combining major and minor QTLs. The sources of barley resistance to Ptt were included in prebreeding program. From 3 till 6 ackcrosses with commercial susceptible cultivars, and evaluation of seedling resistance and using MAS allowed us to develop resistant barley lines, which can be used for barley improvement on resistance to pathogens. This work was supported by the RFBR № 14-04-00400 and by № 15-54-12365.

WHEAT LEAF PUBESCENCE PHENOTYPING AND ANALYSIS

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Leaves of many angiosperm species develop trichomes. These epidermal outgrowths have been exploited to study the determination of cell fate, plant cell differentiation mechanisms and cell morphogenesis in the model plant species. It was found that even simple shape trichomes (leaf hairs) offer protection against both biotic and abiotic stress factors. Recently we developed the high-throughput approach to wheat leaf pubescence phenotyping in wheat based on the folded leaf image analysis. This method estimates the trichome number and trichome length distribution. Using our technique we estimated the phenotypic effect of the known bread wheat leaf pubescence genes. Our results demonstrated that these genes differed in their effect on trichome growth, initiation and patterning. A model of the action and interaction of these genes has been proposed to explain the genetic basis of trichome length and number. We analyzed the diversity of the adaptive morphological trait, leaf pubescence among the relatives and the ancestors of hexaploid wheats, to establish the variability of its phenotypic manifestation as the result of evolution and domestication. This was achieved through the study of quantitative characteristics of leaf pubescence among 47 representatives of di -, tetra- and hexaploid species of genera *Triticum* and *Aegilops* - the donors of the individual genomes of the allopolyploid *Triticum* species. As a result, the main morphological types of pubescence were described inherited by the cultural polyploid wheat from wild ancestors, and the types of pubescence which are observed only in diploid species. To determine the effects of the drought stress conditions on the change in the leaf pubescence characteristics in a number of wheat genotypes were carried out the experimental study with four isogenic and single chromosome substitution lines of bread wheat with different combinations of allelic states of genes controlling leaf pubescence. The results of this experiment demonstrated that wheat loci associates with leaf pubescence might be involved in plant physiological response to drought conditions. The work supported by RSF grant 14-14-00734.

GERMPLASM COLLECTIONS – THE SOURCE FOR FUTURE BREEDING AND FOOD SUSTAINABILITY

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Plant genetic resources play a major role for global food security. The most significant and widespread mean of preserving plant genetic resources is *ex situ* conservation. World-wide 7.4 million accessions are stored in about 1,750 *ex situ* genebanks. The largest numbers of accessions stored are of wheat (855,000), rice (775,000), barley (465,000) and maize (325,000). Other large germplasm holdings include bean, sorghum, soybean, oat, groundnut and cotton. One of the ten largest *ex situ* collections of our globe is located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, conserving 150,000 accessions from 3,200 plant species and 780 genera. Since the majority of genebank holdings globally are stored as seed, seed storability is of exceptional importance for germplasm conservation. Seed storage is managed in large cold chambers at -18°C. Seeds are kept in glass jars, covered with bags containing silica gel. The maintenance of the collection requires regeneration. Each year between 8 and 10% of the collection is grown either in the field or in glasshouses. Regeneration is carried out locally to ensure genetic integrity and to minimize genetic erosion. Special attention has to be given to out-pollinating species, which are either multiplied in small glasshouses or in isolation plots in the field. A pre-requisite for any further exploitation activities is to maintain a high seed quality during storage. The lifespan, or longevity, of seeds is crop specific. However, there are also strong hints of an intraspecific variability which is genetically determined. Consequentially, studies were initiated determining genetic loci responsible for seed storability. At IPK investigations were performed on barley, wheat, oilseed rape and tobacco. An efficient exploitation of the germplasm collections is often hampered by the huge numbers of accessions stored in the seedbanks. Therefore, core collections representing the genetic variation of the whole set must be created. The utilisation of such an approach for unlocking the genetic diversity 'sleeping' in genebanks will be discussed. Examples are given for an efficient exploitation of the germplasm collections for genetic studies of traits important in plant breeding.

BASIC APPROACH FOR GAPLOID AND APOMICTIC MAIZE FORM DEVELOPMENT

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Apomixis (asexual reproduction through seeds) is characteristic for wild-growing and rare in the cultural forms of agriculture plants. The introduction of apomixes technologies into crop plants is revolution for agriculture, since it would provide a mechanism to maintain agriculturally important phenotypes and fix complex, important traits of hybrids. We observed for the first time the gamete-fusion genes in the maize genome. The sequenced PCR products from the ZM_*hap2* (1467 bp) and ZM_*gex*2 (737 bp) genes of the haploid-inducing (SPEM) and a control (GPL-1) maize lines were identical and also were identical to the reference maize (B73) sequences from the GenBank (excluding two mismatched nucleotides for ZM_*gex*2). The PCR products (656 bp region of ZM_*hap2*) for the ZM_BFb0162K03 (1925 bp) and for the 737-bp region of the ZM_*gex*2 (XM_008671984.1, 2904 bp) maize genes were observed for the cDNA of pollen grains, ovary, roots and leaves of the haploid-inducing and control maize lines. The development of parthenogenetic proembryo (autonomous parthenogenesis) from unpollinated ovules was observed for AT-3 maize line at 3-12 days after the delay of the pollination. We observed for the first time the DNA methylation genes expression in AT-3 maize line with autonomous parthenogenesis. In particular, the data on the expression of DNA methylation genes (dmt102, dmt103, hdt104, hon101, chr106), associated with switching from a sexual to apomictic way of development in maize, in the generative tissue (before and after fertilization), was obtained. It was found that expression of dmt102, dmt103, hdt104, hon101, chr106 genes was observed in ovule sac before pollination but surprisingly suppressed in germ 3-7 days after pollination for AT-3 and control (S-32) maize lines. The principal approaches for preparing of technologies for apomictic plant reproduction will be discussed. This work was supported in part by RFBR grant 15-04-08413.

INTROGRESSION OF LEAF RUST RESINTANCE FROM *AEGILOPS SPELTOIDES* INTO BREAD WHEAT (*TRITICIM AESTIVUM* L.)

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Leaf rust of wheat (*Triticum aestivum* L.) caused by the fungus *Puccinia triticina* Erikss. is one of the important foliar diseases of this crop. The use of resistant varieties is the most efficient way to protect wheat crops from this disease. *Aegilops speltoides* is a diploid wild relative of wheat, exhibits a high frequency of leaf rust resistance. The synthetic form Avrodes (BBAASS) was used as a bridge to transfer leaf rust resistance genes from *Ae. speltoides* to common wheat. Introgression lines obtained from the crosses of Avrodes and susceptible common wheat cultivars were evaluated in a field leaf rust nursery. Resistance levels varied from high to moderate. Avrodes and 27 Avrodes */T. aestivum* introgression lines were tested by molecular markers for the presence of already transferred from *Ae. speltoides* leaf rust resistance genes *Lr28, Lr35, Lr47* and. *Lr51*.Gene *Lr28* was present in the synthetic form Avrodes. Gene *Lr35* has been identified in the synthetic form Avrodes and in two lines- 771 and 2506. Genes *Lr47* and *Lr51* were not identified in the Avrodes and introgression lines. Thus, many lines appeared to have genes different from *Lr28, Lr35, Lr47* and *Lr51*. Cytological analyses established that the majority of the lines have 21 chromosome pairs. Transfer of a genetic material from Avrodes into common wheat basically occurs through translocations. Lines with translocation on chromosomes 2D and 5D were identified by C-banding.

USE OF MOLECULAR MARKERS FOR IDENTIFICATION OF THE LEAF RUST RESISTANCE GENES *LR21, LR22A, LR32, LR39* IN *AEGILOPS TAUSCHII*, SYNTHETIC FORMS AND INTROGRESSIVE LINES OF WHEAT

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One valuable source of genes for resistance to diseases is the diploid form of *Aegilops tauschii Coss*. Relatively easy crossability with the D genome can significantly facilitate the transfer of the necessary traits of this species to common wheat. With the use of molecular markers 22 accessions of *Ae. tauschii*, synthetic forms carrying the *Ae. tauschii* D genome (*Triticum miguschovae* (GGAADD), *Triticum palmovae* (AbAbDD), *M.it./ Ae.tauschii* (BBAADD)) and 84 introgressive lines of common wheat derived from them were screened for the presence of genes conferring resistance to leaf rust (*Lr21, Lr22a, Lr32, Lr39*). The GDM35 molecular markers linked with a highly efficient gene *Lr39* was detected in 17 samples of *Ae. tauschii* k-2423; k-3186; k-3187 – k-3199; k-1354; k-1356 as well as in lines 1731, 1755 derived from *T. miguschovae*, in lines 3261, 3265 derived from *T. palmovaei* and in the line 4141 *M.it./Ae.tauschii*. Marker BARC135 linked to *Lr32* gene was identified in 8 samples of *Ae. tauschii*: k-2464, k-2603, k-2605, k-2607, k-2609, k-2610, k-2642, k-2613. However, it has not been found in synthetic forms and lines. Gene of adult resistance *Lr22a* was identified with the help of the GWM455 marker. This marker was identified in 14 samples of *Ae. tauschii* – k-2423, k-2603, k-2823, k-3180, k-3181 – k-3183, k-3191 – k-3194, k-570810, k-865, k-1361, a synthetic form of *T. miguschovae., T. palmovae and M.it./Ae. tauschii* and line 2425 with the genetic material from *T. miguschovae*. The marker Lr21F/R linked to the *Lr21* gene was not detected in any of the samples.

DEVELOPING CLIMATE RESILIENT CROPS VIA MANIPULATING NOVEL STRESS-ASSOCIATED PROTEINS

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Abiotic stresses including drought, heat, salinity, and heavy metal toxicity as a result of climate change have far-reaching implications for global food security and already substantially impact agricultural production worldwide. To increase crop productivity for meeting the global food demand, it is imperative to understand the underlying molecular and biochemical mechanisms for developing crops resistant to multiple abiotic stresses. We have characterized the members of Stress-Associated Proteins (SAP) gene family in plants for their roles in providing tolerance to multiple abiotic stresses. The SAPs contain either A20 or AN1 or both A20/AN1 with extra Cys2-His2 RING zinc finger domains at the N- or C-terminal. Overexpression of SAP10 and SAP13 in Arabidopsis provided strong tolerance to multiple abiotic stresses such as salt, drought, heat, and various toxic metals including zinc, cadmium, arsenic, nickel and manganese, without causing a significant difference in metals accumulation except Zn. These plants attained significantly higher biomass and longer roots as compared to wild type plants under the stress condition in greenhouse conditions. However, the mode of action of this SAP member in providing tolerance to multiple abiotic stresses is largely unknown. We hypothesized that the expression of SAP genes might be regulated through the interaction of *cis*-elements present in the SAP gene promoters with abiotic stress related *trans* factors via protein-DNA interactions under different abiotic stresses. Through yeast one hybrid assay, we have proved this hypothesis and identified several transcription factors such as DREB, ERE, ZIP, HSE etc. that are interacting with the AtSAP13 promoter. Results from Arabidopsis were translated into *Brassica juncea* and when grown in greenhouse, transgenic brassica plants showed strong tolerance to multiple abiotic stresses including drought, salt and heavy metals. These results showed that members of the SAP family have the potential to be used for developing ‘climate resilient crops’ to ensure global food security.

INTEGRATED BIOINFORMATICS ANALYSIS OF FREEZING AND DROUGHT RESISTANT PLANTS

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The computer analysis of structure of transcripts and the genome organization of crop plants on the basis of integration of high-performance sequencing data is important for the solution of fundamental molecular and biological problems and applied agricultural tasks. The analysis of structure of the genes connected with architecture of an ear in crop plants, morphological parameters of drought- and cold resistance for wheat can be added with the bioinformatics methods and data for model organisms. We present here joint research project devoted to development of an integrative computer platform for the analysis of high-performance sequencing data and publicly available computer genomics data. In genetic sense, the mechanisms of drought resistance can be grouped into three categories, drought escape, drought avoidance and drought tolerance. However, crop plants use more than one mechanism at a time to resist drought. Drought escape is defined as the ability of a plant to complete its life cycle before serious soil and plant water deficits develop. This mechanism involves rapid phenological development (early flowering and early maturity), developmental plasticity. Such studies need molecular-genetics approaches and integration of available data. For example, a drought resistance gene, *Drt1* in rice, which is linked with genes for plant height, pigmentation, hull color and awn, and has pleiotropic effect on the root system was described. The technical purpose of the works is the analysis of structure of transcripts in plants genomes, search and the description transcripts structures and its location to the protein coding genes. The expected results include development and adaptation of the computer database of potential genes and antis-sense transcripts in model organisms of plants – *Arabidopsis*, rice, bread wheat. Integration of data in the project will include data of sequencing technologies RNA-seq and Hi-C (for *Arabidopsis*). With use of the developed programs and algorithms the general platform of the bioinformatics analysis in plant genomes using high-performance sequencing data will be developed.

PEPTIDE CLE41 IS A REGULATOR OF SECONDARY ROOT GROWTH AND DEVELOPMENT OF ABNORMAL MERISTEMS

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Peptide CLE41 is a representative of the CLE (CLV3 / ESR) family of peptide phytohormones, whose members play a role in controlling the activity of different types of meristems, regulating the expression of *WOX* genes. In particular, CLE41 have been identified as a regulator of the cambium activity: it stimulates cell proliferation in this lateral meristem and inhibits differentiation of xylem vessels. The target of the action of the signal path induced by CLE41 is *WOX4* gene encoding a homeodomain-containing transcription factor, which is necessary for initiation and maintenance of the cambium. Cambium is a meristem making the major contribution to the secondary growth of the axial organs in which the ring formed from the cambium gives the concentric layers of the secondary conductive elements - xylem and phloem. At the present time, despite the great economic importance of this trait, genetic control of the secondary growth rather poorly studied. The main object of our research - radish *Raphanus sativus* - is related to *Arabidopsis* - model object of genetics, but, unlike the latter, it is able to form the storage root at the expense of the process of secondary growth. Furthermore, some of the inbred lines from radish genetic collection form spontaneous tumors anatomically similar to tumors induced by *Agrobacterium tumefaciens*, on the root. We have found that during the development of storage root of radish activation of the expression of certain genes CLE occurs - CLE41 in the cambium and phloem, CLE19 in differentiating xylem. At the same time, in *Raphanus raphanistrum* – the wild ancestor of radish, which does not form the storage root, enhancing of the expression of these genes is not observed. In experiments on the overexpression of individual genes CLE in *R. sativus* and *R. raphanistrum*, it was shown that overexpression of CLE41 in *R. sativus* increases the number of layers of cambium cells, disturbs the orientation of their division, inhibits vascular lignification and xylem parenchyma formation, as well as causes a meristematic foci from the xylem parenchyma, which give the additional xylem and phloem (anomalous secondary growth). In *R. raphanistrum* overexpression of this gene leads to a significant increase in the number of layers of the cambium and impaired orientation of cell division that leads to proliferation of vascular bundles and the appearance of neoplasms, similar to spontaneous tumors of inbred lines of *R. sativus* in the root. Activation of CLE41 expression in radish also is found in spontaneous tumors and tumors induced by *Agrobacterium*. Thus, we revealed the central role of CLE41 in the regulation of secondary growth and formation of the storage root, as well as its participation in the development of abnormal areas of meristematic activity - tumors. The research is supported by the RFBR grants 14-04-00591, 15-29-02737 and RSF grant 16-16-10011.

NOVEL REGULATORS OF COLD-INDUCED PHENYLPROPANOID METABOLISM IN THE MODEL PLANT *ARABIDOPSIS THALIANA*

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Our main research interest is the biosynthesis and regulation of secondary metabolism in plants. In particular, we are interested in different branches of the phenylpropanoid pathway. Important aspects are the protective functions of secondary compounds against abiotic and biotic stresses *in planta*, but also to their potential health effects as a part of the human diet. Some fruits, vegetables and especially berries contain high amounts of anthocyanins, a class of polyphenols known for their antioxidative properties. A diet rich in anthocyanins is considered beneficial for health and may protect from cardiovascular diseases, rheumatoid arthritis and chronic inflammation. Phenylpropanoids comprise several branches including cinnamic acids, cinnamic acid conjugates, coumarins and flavonoids such as anthocyanins. In addition, lignins are derived from this pathway. The phenylpropanoid pattern of plants is rather complex, tissue-specific and dependent on developmental programs, but also shows a strong responsiveness towards environmental stimuli, e.g. nutrient supply and abiotic as well as biotic stress factors. Major goals of our studies are to gain further insights into regulatory programs and mechanism of resource allocation into different branches of secondary metabolism and to unravel tissue specific metabolite functions. Integrative approaches combining proteomics, metabolomics and transcriptome analysis are applied to study the integration of secondary metabolism into overall cellular defence mechanisms. The functional characterization of regulatory factors of coumarin biosynthesis was performed using knock-out mutants and wild type plants grown under conditions favouring coumarin accumulation; in particular cold stress regulation was analysed. One of the factors was shown to be a component of the RNA silencing machinery. Stress experiments and subcellular localization of metabolites were performed to further characterize individual candidate genes. Two transcription factors were shown to be involved in regulation of anthocyan accumulation indicating competition with coumarin synthesis. The phenylpropanoid metabolism has also been investigated in barley populations grown either in the field or under different conditions (cold stress, elevated CO_2) in growth cabinets and in the field. Major aims are to correlate metabolic patterns with hyperspectral data of field grown plants and to identify novel regulatory genes of phenylpropanoid metabolism in barley. I will briefly mention the hyperspectral imaging performed for sugar beet genotypes as a non-invasive method to monitor plant performance. Specific metabolites associated with a higher pathogen tolerance were identified, and we have evidence that phenypropanoids contribute to the hyperspectral signatures of leaves. Funding of our research by different grants is gratefully acknowledged (BMBF IPAS FKZ 031A352B; BLE Hyperspectral, FKZ 22010512; EU grant ATHENA, 245121; BMBF ANTHOPLUS, grant 031A006A).

THE MANIFESTATION AND PHYTOHORMONE RESPONSE OF LEAF PUBESCENCE GENES IN BREAD WHEAT

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The leaves of many angiosperm species develop trichomes. This trait is known to make a significant contribution to the protection from pests and adaptation to environmental factors in bread wheat. However the genetic basis of wheat trichome formation is poorly understood although a wide variation was found among *Triticeae* species with different ploidy level. Currently Catalogue of Gene Symbols for wheat contains only two loci associated with this trait: the gene *Hl1* in 4B chromosome and the gene $Hl2^{aesp}$ in 7B chromosome. Molecular function and regulation of these genes are currently not known. The present research was sought to establish the individual and joint effect on trichome patterning and growth of each of three known wheat leaf pubescence genes (*Hl1, Hl3* and $Hl2^{aesp}$) and effect of phytohormone treatment on their phenotypic expression. Various lines carrying *Hl1, Hl3* and $Hl2^{aesp}$ and specially created nearly isogenic lines were used to quantitatively compare leaf pubescence using a high throughput phenotyping method (wheatdb.org/lhdetect2). This method allows to obtain rapidly quantitative characteristics of leaf pubescence (length of individual trichomes and their number) among many plants. These genes differed in their effect on trichome formation. *Hl1* and *Hl3* more affected trichome initiation and growth, while $Hl2^{aesp}$ modified trichome length. Their action was independent to a large extent. A model of the action and interaction of *Hl1, Hl3* and $Hl2^{aesp}$ has been proposed to explain the genetic basis of trichome length and number. The effects of phytohormones on trichome cell growth and initiation while *Hl1, Hl3* and $Hl2^{aesp}$ genes manifestation were explored. The effects of auxin (IAA), gibberellic acid (GA3), cytokinins (6-BAP, Kinetin), metyl jasmonate (MeJa), ethylene (ACC) have been investigated and described. Our data revealed a key role of cytokinin signaling pathway in *Hl1* and *Hl2* gene manifestation. This work was supported by Russian Science Foundation (RSCF) grant № 14-14-00734.

PHYLOTRANSCRIPTOMIC HOURGLASS PATTERNS OF EMBRYONIC AND POST-EMBRYONIC PLANT DEVELOPMENT

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The historic developmental hourglass concept depicts the convergence of animal embryos to a common form during the phylotypic period. Recently, it has been shown that a transcriptomic hourglass is associated with this morphological pattern, consistent with the idea of underlying selective constraints due to intense molecular interactions during body plan establishment. Although plants do not exhibit a morphological hourglass during embryogenesis, a transcriptomic hourglass has nevertheless been identified in the model plant Arabidopsis thaliana. We investigated whether plant hourglass patterns are also found post embryonically, and we found that the two main phase changes during the life cycle of Arabidopsis, from embryonic to vegetative and from vegetative to reproductive development, are associated with transcriptomic hourglass patterns. In contrast, we found that flower development, a process dominated by organ formation, is not associated with transcriptomic hourglass patterns. These findings suggest that plant hourglass patterns (i) are decoupled from organogenesis and body plan establishment and (ii) might reflect general transitions through organizational checkpoints.

INTRASPECIFIC POLYMORPHISM OF *SUS1* GENE IN *PISUM SATIVUM*

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Symbiosis with nitrogen-fixing bacteria of the genus *Rhizobium* is one of the most important characteristics of *Pisum* species, as well as other Fabaceae species. Sucrose plays an important role in symbiotic nitrogen fixation and sucrose metabolism is partly controlled by sucrose synthase. Sucrose synthase in plants is encoded by *Sus* gene family. Despite the great economic importance of legumes the data on the coding sequences of sucrose synthase genes in Fabaceae is limited. In this study, for the first time the full *P. sativum* sucrose synthase gene sequences were determined. *Sus1* gene intraspecific variability of eight *P.sativum* accessions, including five accessions characterized by the level of symbiotic nitrogen fixation was analyzed. The obtained sucrose synthase gene sequences included 13 exons and 12 introns and their length varied from 3568 bp to 3585 bp. According to their exon-intron structure the studied sequences were attributed to SUS1 dicot group of sucrose synthase gene family. The studied sequences contained SNPs and indels and their total polymorphism level was 3.7%. In introns both SNPs and insertions and deletions were detected while exons were more conservative (1.9%). The most variable was exon VIII. *Sus1* nucleotide sequences were translated and the length of the corresponding proteins was 806 amino acids. Five radical and seven conservative amino acid substitutions were detected. The analysis revealed no nucleotide or amino acid substitutions associated with the level of symbiotic activity. The work was supported by RFBR project № 16-34-00981.

GENETICALLY RECONSTRUCTED SOYBEAN SOMACLONES DEVELOPED USING BIOTECHNOLOGY AND GENETIC ENGINEERING

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Soybean (*Glycine max* (L.) Merrill) is a unique and significant legume in the world. Exclusivity of it among all the other field crops is due to the rich chemical composition of seeds. The research objects were the regenerant lines of soybean of 10 original forms, which were developed using chemical and physical mutagenesis, agrobacterial transformation. As a result of research the genetically modified somaclones of soybean were obtained which significantly exceeded the original forms for a number of economically valuable traits. According to the results of the study in the control nursery nine lines exceeded the standard on productivity by 3,0 - 36,9%. According to biochemical parameters the three lines performed high content of oil and histidine in the seeds having a low index of linolenic acid (R 1485, R 1357, R 1490). Relative electrophoretic mobility of peroxidase with a zone of 0,36-0,40 Rf was revealed in forms obtained in the medium with Cu2+ ions. The more mobile electrophoretic spectra were defined in R 1496 (0,43-0,45 Rf), R 1490 (0,48-0,50 Rf), and R 1518 (0,42 Rf; 0,47 Rf). Applying laser and gamma irradiation of seeds, for the first time in our practice, the lines were obtained resistant to septoria (R646) and cercosporosis (R623). Analysis of the transgenic somaclones of soybean lines containing in its genome a gene AMP I, which allowed to extend the limits of plant resistance to pathogens, showed that the leaves of plant transformants were damaged by septoria (*Septoria glycines* Hemmi) 25.7% less than the standard variety. As for cercosporosis (*Cercospora sojina* Hara) and downy mildew peronosporosis (*Peronospora manshurica* (Naum.) Syd.) the leaf damage was 20.0% less than the standard. These results show the effectiveness of using these directions in biotechnology and genetic engineering in soybean breeding in order to develop initial material.

THE TRANSGENIC SORGHUM WITH IMPROVED DIGESTIBILITY OF STORAGE PROTEINS OBTAINED USING RNAi TECHNOLOGY

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Development of approaches to the management of gene expression, affecting the quality of agricultural products is one of the important areas of cereal genomics. RNAi technology down-regulating expression of genes, which reduce the grain quality, is an efficient technique for these purposes. This approach is especially important for sorghum – the unique drought tolerant cereal crop that is characterized, however, by a relatively poor nutritive value in comparison with other cereals. It is considered that one of the reasons of the low nutritive value of the sorghum grain is the resistance of one of its seed storage proteins, gamma-kafirin, located in the outer layer of endosperm protein bodies, to protease digestion. Using *Agrobacterium*-mediated genetic transformation, we obtained transgenic sorghum plants harboring a genetic construct for RNAi silencing of the gamma-kafirin gene. *In vitro* protein digestibility (IVPD) analysis showed that the amount of undigested protein in transgenic plants from the T_3 generation was reduced by 2.9-3.2 times, in comparison with the original non-transgenic line and the digestibility index reached 85-88% (in comparison with 59% in the original line). A high IVPD value was observed both in plants having kernels with floury endosperm, and kernels with modified or vitreous endosperm types. In the electrophoretic spectra of endosperm proteins of transgenic plants with increased digestibility, the proportion of 20 kDa protein that is encoded by the gamma-kafirin gene, was significantly reduced, in comparison with the original non-transgenic line. HPLC analysis showed total amino acid content in two out of the three studied transgenic plants from the T_2 generation was reduced in comparison with the original non-transgenic line, while the lysine proportion increased by 1.6-1.7 times. The results of experiments demonstrate that it is feasible to develop transgenic sorghum lines with improved protein digestibility.

THE STUDY OF THE DEVELOPMENTAL PHASES OF SPRING OCTOPLOID AND HEXAPLOID TRITICALES UNDER CONDITIONS OF NEAR OB REGION OF WESTERN SIBERIA

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Octoploid (8x) triticales with dominant *Vrn* genes and 78 spring cultivars and samples of hexaploid (6x) triticales were studied under natural growth conditions (Krasnoobsk township). 8x triticales with dominant genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Vrn-D4* were obtained by cross of common wheat Triple Dirk NILs with winter diploid rye Korotkostebel'naya 69, following chromosome doubling of F_1 plants (Stepochkin, 2009). In each cross the combination of several numbers, or families, were obtained. The number of days before heading of 8x triticale families with dominant *Vrn-A1* gene varied within the interval 60-77 days, 8x*Vrn-B1* families – 72-94 days, 8x*Vrn-D1* families – 70-97 days, 8x*Vrn-D4* families – 68-103 days. In some triticale families with dominant genes *Vrn-D1* and *Vrn-D4*, among spring plants, facultative and winter plants appeared. These differences on heading time between families with one dominant *Vrn* gene may be explained by probable gamete heterogeneity of parental rye form, due to its tendency to cross-pollination, or by aneuploidy, typical for 8x triticales. We have also created 8x triticales with two dominant *Vrn* genes: *Vrn-A1 Vrn-B1*, *Vrn-A1 Vrn-D1*, *Vrn-A1 Vrn-D4*, *Vrn-B1 Vrn-D1*, *Vrn-B1 Vrn-D4*, *Vrn-D1 Vrn-D4*.

Number of days before heading of 78 6x triticale samples at the I sowing term was 31-49 days, at the II sowing term – 30-52 days. These triticales headed earlier than 8x triticales with dominant *Vrn* genes which confirms that the vegetation period lengthens after increasing of the ploidy level (Kaminskaya *et al.*, 2005; Stepochkin, 2009). The majority of 6x triticales (82%) headed 1-9 days earlier at the II sowing term, than at the I term, which happened, probably, due to shortening at the II sowing term of the period “shoots – the first node” in most triticales (87%). Hexaploid triticales had shorter periods “shoots – the first node” and “stem elongationg – heading” compared to 8x triticales, which, probably, caused earlier heading of 6x triticales. This work was supported by IC&G budget project №0324-2015-0005.

PLASTID GENOME POLYMORPHISM IN *ALLIUM SATIVUM* ACCESSIONS OF DIFFERENT ECOLOGICAL AND GEOGRAPHICAL ORIGIN

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Garlic (*Allium sativum*) is cultivated for more than 7000 years and still is one of the most important vegetable crops. The cultivated garlic does not produce fertile seeds, and as a result, it is propagated only vegetatively. The center of origin of garlic is Central Asia - Uzbekistan, Kyrgyzstan, Tajikistan, Turkmenistan and South Kazakhstan, whence it spread through the trade routes to every continent except Antarctica. Genetic diversity of garlic is poorly studied, in comparison to other cultivated species, which may be due to the huge genome size. In this study six chloroplast genome fragments (*rpl32-trnL*, *ndhJ-TabE*, *psbM-ycf6*, *trnC-ycf6*, *trnT-trnL*, *rps16* gene intron) were analyzed in *A. sativum* accessions from wild populations of the primary center of origin, cultivated accessions and related species of section *Allium*, provided by the Vavilov Research Institute of Plant Industry (VIR) (St. Petersburg, Russia) and Dr. N. Friesen (the Botanical garden of Osnabruck University, Germany). The total size of the six analyzed *A. sativum* chloroplast genome fragments varied from 5191 bp to 5246 bp. For each of the analyzed chloroplast genome fragments haplotypes were identified and polymorphism rates were calculated. *TrnC-ycf6* spacer turned out to be the most polymorphic of the studied fragments. Also, sample specific SNPs were found in a number of the studied garlic accessions. Thus, variability of the six chloroplast genome fragments was for the first time studied in wild and cultivated *A. sativum* accessions. As a result of the cluster analysis, based on the chloroplast genome polymorphism data, the wild section *Allium* species that may be the likely ancestors of the cultivated *A. sativum* were identified. The work is supported by 0104-2014-0210.

THE SUCCESS OF RUSSIAN RICE BREEDING ACHIEVED WITH THE USE OF MAS

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All-Russian Rice Research Institute (ARRI, Krasnodar, Russia) actively conducts the programs of marker-assisted selection for resistance to rice blast disease, improving the quality of grain, resistance to cold and other agronomically important traits. Breeding and seed production schemes are accompanied by marker control of the target genes (genes of resistance to the fungal pathogens: *Pib*, *Pita*, *Piz*, *Pi9*, *Pi40*, etc.; genes determining cooking quality of grain: *Waxy*, etc.; genes of red coloring of caryopsis: *Rc*, etc.; OTLs of resistance to cold) on all stages of development of breeding samples with specified traits. In the course of these programs the valuable breeding resources of rice are developed.

In particular, the breeding nursery of the Institute currently evaluates hundreds of lines with 3-5 introduced - including pyramided ones - genes of resistance to blast. Some of them are already passing State trials for being registered as varieties. In the report we observe breeding material obtained with MAS method. In the primary seed production the molecular genetic approach is also used for quality control of produced rice seeds. Thus, control of genetic purity of reproduced varieties and of absence of red-grain dockage is carried out using methods of DNA diagnostics. For diagnostics of the traits mentioned above we use the well-known markers from the literature as well as our own ones, developed by ARRRI’s biotechnology and molecular biology laboratory. The institute is also using cell engineering methods (experimental haploidy) for many years to accelerate breeding schemes. Each year we develop 1500-2000 double haploid lines of rice as well as cabbage and pass them to the breeders.

NUD AND *VRS* GENES IN BARLEY- TARGETS FOR KNOCKOUT USING CRISPR/CAS SYSTEM

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Cereals are of outstanding value among crop species. Genome editing of monocot plants with large genomes, especially wheat and barley is an important challenge for biotechnology and plant breeding. CRISPR/Cas system allows performing different genome modifications, such as gene knockout, gene insertions and trait stacking, regulation of gene expression, etc. The system consist of Cas9 endonuclease and chimeric single guide RNA (sgRNA). A 20-nt guide sequence within the sgRNA directs Cas9 to the desired target site via Watson-Crick base pairing. Cas9 cleaves the DNA resulting in a double strand brake (DSB). DSB reparation via non-homologous end-joining is often imprecise and frequently introduces small deletions or insertions at the target site. The protocol of targeted gene mutagenesis contains the following steps: sgRNA design, vector construction, plant transformation, identifying of CRISPR/Cas-induced mutations, off-target activity evaluation, obtaining transgene-free mutant plants. The aim of the present work is targeted mutagenesis of barley *Nud* and *Vrs* genes using CRISPR/Cas system. *Nud* gene controls covered *vs* naked caryopsis. Expected knockout phenotype has naked caryopsis. *Vrs* gene controls two *vs* six-row spike, knockout phenotype has six-row spike. With the help of online target-design tools the few sgRNAs have been designed to pair with diverse sites of the barley *Nud* and *Vrs* genes. The potential off-target sites with few nucleotide mismatches have been identified. The vector construction and plant transformation are in progress. The work is supported by Russian Science Foundation (project 16-14-00086).

BREAD WHEAT LINES HAVING DIFFERENT COMBINATIONS OF ANTHOCYANIN BIOSYNTHESIS REGULATORY GENES: MARKER-ASSISTED DEVELOPMENT AND PRACTICAL VALUE

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There is a growing interest in cereals having grains producing anthocyanins – natural antioxidants beneficial for human health. Anthocyanins can be produced in pericarp (under control of *Pp* genes) and aleurone layer (under control of *Ba*) of bread wheat (*Triticum aestivum* L.). A current set of the common wheat cultivar Saratovskaya 29 near isogenic lines having different combinations of anthocyanin biosynthesis regulatory genes consists of 10 NILs with combination of dominant and recessive *Pp-A1*, *Pp-D1* and *Pp3* alleles as well as a blue-grained substitution line (BC_7 progeny) carrying the *Agropyron elongatum* Host. *Ba* gene in chromosome 4D. The 4D/4Ag substitution was established by chromosome C-banding, GISH and microsatellite analysis. Pyramiding of *Pp* and *Ba* genes in order to obtain the wheat with higher anthocyanin content is in progress. The comparative assays of the antioxidant status of grains and bread obtained from the NILs is performed in order to find out if antioxidant activity is retained after baking. In addition, it was shown that *Pp* genes have the practical value for long seed storage and viability after low-dose irradiation treatment. Purple-grained and control lines grains are introduced into the diet of laboratory mice to study potential health benefits of anthocyanins produced in wheat grain. The study was supported by the Russian Science Foundation (Project No 16-14-00086).

APPLICATIONS OF METABOLOMICS FOR PLANT RESEARCH

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Metabolomics is a relatively new field to comprehensively study of all metabolites of organisms. It has been proven to be instrumental to unravel important metabolites as well as molecular pathways governing genotypes and phenotypes of an organism. Numerous metabolomics applications in plant genomics research focus on identification of genes and biomarkers at particular traits to rationally develop new varieties with better yields and desirable nutritional contents. At the National Center for Genetic Engineering and Biotechnology (BIOTEC, Thailand), we have established metabolomics capacity to address various research problems related to agriculture. Two examples of metabolomics applications will be presented. First, we employed ^{1}H NMR spectroscopy, GC-MS and HR-LC/MS to decipher important metabolites and relevant pathways of the brown planthopper (BPH) resistance in Thai rice in order to facilitate a selective breeding program. The finding from multi-platform metabolomics analyses provided comprehensive insight into biochemical processes involved in the amino acid metabolism, shikimate pathway, nucleotide biosynthesis, phenylpropanoid and octadecanoid pathways for the BPH resistance mechanism leading to help identifying BPH resistance genes underlying these traits to improve precision breeding program. Second, we attempted to understand the mechanism how a biocontrol *Streptomyces* bacterium inhibit growth of anthracnose-causing *Colletotrichum* fungi by using untargeted metabolomics approach. Upon the understanding of the mechanism, we will be able to enhance the stability of antagonistic effect as well as increase the production of inhibitory secondary metabolites. In summary, metabolomics have been successfully proved as a high-throughput screening method in functional genomics to generate more fundamental knowledge of the biochemical process underlying these crucial traits.

ANTHOCYANINS IN WHEAT: GENETIC STUDIES FOR FUTURE MARKER-ASSISTED BREEDING FOR NUTRITION VALUE

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Natural antioxidants anthocyanins can be produced either in aleurone layer or in pericarp of wheat grain. Therefore whole-grain products obtained from colored wheat can benefit human health. Anthocyanins are known for their protection of DNA from cleavage, membrane strengthening, lipid peroxidation. These compounds can regulate immune responses and alter development of hormone-dependent disease symptoms. They possess anti-inflammatory activity, decrease capillary permeability and fragility, prevent cancer and neurodegenerative diseases. The genetic bases of anthocyanin synthesis in wheat grain have been studied. The regulatory network and the target genes are described and their peculiarities in wheat in comparison with other species are described in the current report. Allelic variants of the regulatory genes, their expression at the phenotyping, biochemical and genetic levels as well as suitable markers for marker-assisted breeding are presented. The study was partially supported by the joint projects of the Belarusian Republican Foundation for Fundamental Research, National Academy of Sciences (B-15 SB 051) and the Siberian Branch of the Russian Academy of Sciences for 2015-2017 (project No. 22).

TRANSGENIC PLANTS: GENETIC MODELS FOR INVESTIGATION OF STRESS TOLERANCE MECHANISMS AND DESIGN OF NEW RESISTANT GENOTYPES FOR AGRICULTURE

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Transgenic plants became an invaluable instrument for investigation of genetic control of different traits. Plant gene engineering provides a broad variety of unique tools including (a) overexpression of gene of interest; (b) suppression of target gene *via* antisense RNA, dsRNA, or artificial microRNA production. Virus-induced gene silencing (VIGS) technique opened the way to investigate the effects of target gene suppression in adult plants. Recent progress in gene engineering technologies (genome editing) makes possible the precise modifications of target nucleotide sequences in plant genomes.

Here we consider some genetic models developed for investigation of plant stress tolerance mechanisms. They include the transgenic lines of *Nicotiana tabacum* and *Solanum tuberosum* with either up- or downregulated expression of extracellular ribonucleases, and *Nicotiana tabacum* lines with suppressed proline dehydrogenase gene. Novel functions of above mentioned genes in biotic and abiotic stress tolerance mechanisms as well as the development of new breeding strategies are discussed. This work was supported by the Russian Science Foundation 16-16-04073.

ELEMENTAL METABOLOMICS: REFERENCE VALUES AND CLASSIFICATION BY ELEMENTAL SIGNATURES IN RICE

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Elemental profiles in plants are variable - they depend on multiple factors, most important are genetic origin, geographic location, year, maturation stage at harvest, and variations of production system. For food crops it is important to identify reference values (reference intervals) for elements because they give informative ranges for concentration of elements expected for plants grown under regular conditions and the nutritional ranges in food. Values that deviate from reference values indicate the possibility, among others, of error, systematic measurement shift, contamination, departure from standard agricultural practices, crop stress, biofortification, parboiling, or adulteration of food. In this study we defined the reference values for 17 elements in rice. We also defined elemental signatures and explored their use for prediction of rice production system, year of production, and variety (indica, japonica, aromatic, and aus). Reported minimum and maximum values for 51 elements were taken from a systematic review of the literature and used as a comparison baseline. We analyzed more than 24,000 elemental profiles of rice samples from the ionomics database and more than 3400 profiles from an ionomic study and calculated reference intervals for 17 elements using Reference Value Advisor program. Elemental signatures that correlate to rice production system, year of production, and specific location were defined and analyzed. We defined reference limits for 17 elements in rice variety from 24000 samples representing rice from all over the world. The most pronounced differences are due to flooding as production system affecting As, Cd, Cu, and Ni. Some effects of variety to elemental concentration were observed for Ca, As, Cd, Co, Rb, and Sr. We estimate that genetic variability is responsible for concentration variation of up to one order of magnitude. Elemental metabolomics provides a new methodology and techniques suitable for biomonitoring of rice and other crops. It can be used for classification of samples by their origin (geographic, production system, genetic origin), and nutritional or toxicity status.

SeedCounter APPLICATION AND WHEAT EAR RECOGNIZING ALGORITHM FOR HIGH THROUGHPUT WHEAT PHENOTYPING

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Qualitative and quantitative analysis of morphological characteristics of wheat yield is an important step in developing new high-yielding varieties. This includes spike and grain phenotyping. Traditionally, spike analysis performed manually. The following characteristics identified visually: the type of spike, number of grains, dimensions, color, etc. Grain characteristics (length, width, shape, roundness) are also important but difficult to measure. Therefore, it is important to develop computational tools for fast and efficient morphometry of these traits. In this work, we present the methods for grain and spike phenotyping using image processing. For phenotyping of wheat spike we developed an algorithm for recognizing spike on the image and estimation of its shape characteristics. The algorithm detects the spike on a A4 white paper sheet, determines its contour and performs its morphometry. The problem of mass phenotyping using computer image analysis can be divided into the following steps: 1) Identification of the measured object on the image; 2) Digitization of data of identified object; 3) Create and populate the database by collected information; 4) Comparative data analysis methods. Wheat spike recognition algorithm is implemented on the Java programming language. The OpenCV library for image processing was used for algorithms implementation. Input program data is image of wheat spike located on a white sheet of paper. We have implemented an Android mobile application for high-throughput wheat grain phenotyping that recognizes wheat grains on a sheet of paper and measures their quantitative characteristics. We used Android platform as a widespread system of open source software for mobile applications. Developed wheat spike recognition algorithm and SeedCounter application can be used for high-throughput phenotyping of wheat in field conditions when special equipment is unavailable as well as in the laboratory. The SeedCounter application is available on GooglePlay: https://play.google.com/store/apps/details?id=org.wheatdb.seedcounter. The work supported by RFBR grant 16-37-00304 and budget project № 0324-2015-0003.

INFLUENCE OF A LIGNIFICATION AND MINERALIZATION OF LEAF TISSUES ON RESISTANCE OF BREAD WHEAT PLANTS TO A BROWN RUST

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Aromatic substances in plant tissues can have protective effect against fungal diseases. When studying a collection of cultivars of bread wheat *Triticum aestivum* L. on lignification enzymes the distinctions between them on extent of susceptibility to brown rust are found (infecting agent *Puccinia recondita* f. sp. *tritici*). On a phytopathologic plot, against an artificial infection with spores of brown rust, two samples of spring bread wheat 15-4 and 14-3 were affected for 1-5% and for 30%, respectively. The analysis of various substances content in the leaf tissue at the samples was conducted. Significant differences in the content of mineral compounds were detected. Large plaques and spot consisting of mineral compounds were observed on the leaf surface of the more resistant plant. In ashes of leaves and ashes of a lignin differences on the content of a number of mineral elements are also found. Cross-sections of the leaves were differed in the intensity of autofluorescence. Tissues of the more resistant sample have stronger fluorescence than tissues of the susceptible sample. These differences persisted after extraction of the low molecular weight components from the leaf tissues with alcohol and toluene. After extraction the leaves with the polar and non-polar solvents the cell wall consists exceptionally of polymers, and among them only lignin is capable of autofluorescence. Thus, differences found between the samples are apparently due to the differences in lignin content and structure. Lignin content in the dry mass of a leaf differed slightly, respectively 14,2% and 12,3%, but the IR spectroscopy data showed the differences in its chemical composition. Probably, the discovered differences lead to afflict the plants with leaf rust in such different degree. In that case these characters can be used for diagnostics of potential resistance of cultivars to fungal infection.

IDENTIFICATION AND TAGGING OF ALLELIC VARIANTS OF THE *NUD* GENE IN THE SIBERIAN BARLEY COLLECTION

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Hulled/ naked barley (*Hordeum vulgare* L.) trait is determined by a single gene *Nud* located on the long arm of chromosome 7H. Hulled barley has a dominant gene *Nud*, while naked barley has the recessive one – *nud*. The gene consists of two exons and one intron. The dominant *Nud* allele controls a formation of lipids which are involved in the bonding of the lemmas and a formation of hulled grain. The recessive *nud* allele does not provide the formation of epidermal lipids on grains and hence there are no bonding lemmas. The naked barley's lemmas are easily separated from seeds by the threshing.

The collection of cultivars of spring barley (about 100 samples) is generated for high-throughput genotyping and phenotyping. Development of diagnostic DNA-markers for a marker-assisted selection is performed. Using primers amplifying the *Nud* gene with adjacent regions a screening of hulled and naked cultivars of the collection has been performed. It was shown that the naked Siberian cultivars have a deletion in the *Nud* gene. The pairs of primers have been selected as dominant diagnostic markers for hulled/ naked phenotype, co-dominant markers' development is in progress. In addition, potential target genes for genome editing using CRISPR/Cas9 system are analyzed in the study. Recent works demonstrate the possibility of transgene-free plants generation using the CRISPR/Cas9 system. The *Nud* gene was chosen as the most convenient first target for editing. Knockout of the *Nud* gene in hulled barley cultivars will provide to obtain naked phenotype. The cultivars "Biom", "Talan", "Aley", "Acha" and "Krasnoyarskiy 91" have been chosen as candidates for genome editing. The *Nud* gene of the chosen cultivars has been sequenced to clarify the design of the sgRNA structure for the further editing. All chosen cultivars were brought into the *in vitro* culture, the efficiency of callus induction and regeneration has been tested.

The study was supported by the Russian Science Foundation (Project No 16-14-00086).

PURPLE-GRAINED BARLEY: MARKER ASSISTED DEVLOPMENT OF NILs AND GENETIC DISSECTION

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Purple lemma and pericarp (PLP) trait is due to accumulation of anthocyanins in these tissues. The *Ant2* (chromosome 2H) gene encoding bHLH-transcriptional factor was previously shown to control PLP trait in barley. In this study we have split the Bowmans' purple-grained NIL carrying donor's fragments in chromosomes 2H (with the gene *Ant2*) and 7H (with the gene *Ant1* determining leaf sheath color and encoding MYB-transcriptional factor) into 2 lines (one with donor's fragment in 2H and another one with the fragment in 7H) using morphological, *Ant*-genes-specific and microsatellite markers. The two lines have uncolored lemma and pericarp. Furthermore, qRT-PCR with primers to *Ant2* in pericarp of the novel and initial lines showed this gene to be activated in the presence of dominant *Ant1* only. We suggested that (1) the *Ant1*-encoded MYB-factor is needed for transcriptional activation of the *Ant2* gene, (2) the *Ant2*-encoded bHLH-factor may require interaction with the Ant1 product to activate anthocyanin biosynthesis structural genes. Since anthocyanins are implicated in plant resistance to a number of abiotic and biotic stress factors and are known for their health benefits, the additional studies (i.e. the comparative assays of the antioxidant activity in grains of the developed and initial barley NILs) is performed in order to find out if *Ant1* and *Ant2* genes can become the target genes in certain barley breeding programs. The study was supported by the Russian Science Foundation (Project No 16-14-00086).

PEA (*PISUM SATIVUM* L.) TRANSCRIPTOMICS AS A MODERN APPROACH IN AGRICULTURAL SCIENCE

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Plant genetics sufficiently contributes to the agriculture development and, subsequently, to a global food security. Garden pea (*Pisum sativum* L.) is among the valuable pulse crops all over the world. The large size and complexity of the pea genome hamper its sequencing as well as the discovery of this crop plant's genetic resources, both of which are desperately needed for molecular and genomics-assisted breeding. Over the last few years the development of next generation sequencing technology made a considerable progress in pea transcriptome sequencing. Comparative transcriptome analysis of different pea lines, carried out by separate scientific groups, has allowed developing a large number of molecular markers. We integrated a large number of pea genic markers into a single database with simple intuitive interface. This online tool – Pea Marker Database (PMD) (www.peamarker.ru) – comprises information about 2484 pea markers including the chromosome location of the marker, the sequence of corresponding pea transcript and the name of the relevant gene in *M. truncatula* genome. Using PMD, we found appropriate markers and performed fine mapping of 10 symbiosis-related genes in pea. Another trend of transcriptomics research is study of expression profiles of comprehensive collections of pea mutants, representing genes associated with valuable traits. This analysis can help to better understand the mechanisms underlying the plant-microbe interactions, organogenesis, stress response etc. In our work, we characterized the transcriptome of unique pea mutant SGECd^t (tolerant to cadmium) and initial line SGE (sensitive to cadmium). Transcriptional responses to cadmium stress appeared to be remarkably different in these lines. Genes associated with nutrient deficiency, stress reactions and transmembrane transport were affected in SGE, and genes related to ion binding and DNA reparation processes, were affected in SGECd^t. Such studies demonstrate that transcriptomics is a good tool for studying the bases of agriculture valuable traits. This work was supported by Russian Foundation for Basic Research (grant 16-34-60132) and Russian Science Foundation (grant 14-24-00135).

NEW BREEDING TOOLS AND EFFECT OF CHANGED AGRONOMY ON PLANT ATTRIBUTES IN CENTRAL AND NORTH EAST PLAINS ZONE OF INDIA

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Although, wheat is considered to be a highly adapted crop across the world ranging from the tropics to close to the Arctic Circle, but one variety itself may not perform well or even survive in this range of environments. The wheat varieties are adapted to this by the interaction between the genetics and the environment. The key environmental factors interacting with wheat variety are temperature and photoperiod and these determine the lifecycle of the crop so as to provide the best fit to the environment. Due to advancement in the wheat agronomical practices in last few years, emphasis is being given to breed genotypes best suited the changed agronomy. To save the time and natural resources, resource conservation technologies (RCTs) are being promoted and adapted by large number of farmers in South Asia and other parts of the world. The RCTs serve several advantages over conventional method in several ways including water saving, improving soil health, reduced cost of cultivation, reduced lodging in the end of wheat season and most important is saving time after rice crop. Since Rice-Wheat is a major cropping system in South Asia, planting of wheat is delayed due late harvesting of rice. Further, planting of wheat is delayed by 2-3 weeks while preparing the field. The late planting of wheat coincide with terminal heat stress resulting reduced yield and loss to the farmers. Considering the issue of farmers particularly in South Asia, the zero tillage is proposed and one of the solution to late planting and reduce the cost of cultivation. Generally, the wheat breeding programs across the world are breeding wheat under conventional tillage while not giving much emphasis on breeding under different agronomic management conditions. In other words, the genotype × environment × management (G×E×M) has not been explored. However, the Genotype × Environment have been studied in details by several researchers. Due to lack of this component, the variety developed under one management i.e., conventional tillage may not perform well under zero tillage. In the area where drip irrigation system is being adopted due to limited availability of water, it become more important to develop varieties responsive to particular management. Therefore, to realize the maximum yield potential, breeding under changed agronomy need to be considered. To prove the concept of breeding variety under particular management, as set of 53 most potential genotypes developed using conventional tillage method with 7 international checks were tested under two management conditions (conventional and zero tillage) in the first year and another set of 21 genotypes including selected lines from previous year under four different management conditions, e.g., subsurface drip with sensor based application of nitrogen, subsurface drip irrigation, zero tillage with flood irrigation and conventional method in second year. The trial was conducted at two locations viz., Ludhiana, Punjab and Pusa Bihar representing two different wheat growing zones North West Plains Zone (NWPZ) and North East Plains Zone (NEPZ) respectively. Data was recorded on 8 agronomic traits and yield per plot. The analysis suggested significant genotype × management (G×E) as well as genotype × environment × management (G×E×M) interactions for plot yield. The other agronomic traits e.g., spike length showed significant interaction with the management. The lines produced maximum yield under particular management were selected and will be tested at larger plot to realize the maximum potential under given set of management.

MAPPING OF SPOT BLOTCH DISEASE RESISTANCE USING NDVI AS SUBSTITUTE TO VISUAL OBSERVATION IN WHEAT

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Evaluation of wheat for spot blotch disease resistance relies on various visual observation methods. The person who evaluate lines need to be experienced to score disease severity. To facilitate high-throughput phenotyping, hand-held green seeker NDVI sensor was used to map spot blotch disease resistance QTL. A total of 108 germplasm lines along with 335 SSD derived lines (F_4 and F_5 generations) originating from the cross ‘YS116 × Sonalika’ were used. The population was evaluated at BISA, Pusa Bihar; a hot spot for spot blotch, for two consecutive years. Data was recorded using NDVI as well as by visual observation as % disease severity. Correlation coefficient was calculated between two scoring methods (NDVI and DS) recorded at different growth stages. High negative correlation was observed between NDVI and % DS at GS69 and GS77 on Zadoks scale. With both methods, the QTL was mapped in the same chromosomal region on 5BL. Using NDVI value, the detected QTL explained up to 54.9% of phenotypic variation while it explained up to 56.1% using DS. The *Sb2* gene was mapped between the markers *Xgwm639* and *Xgwm1043* with an interval of 0.62 cM. Markers linked to *Tsn1* gene is found to be 1.15 cM apart from the *sb2* gene. It is concluded that NDVI may be used as an alternative to visual scoring of spot blotch disease in wheat and create new avenue for high-throughput phenotyping.

RYE CHROMATIN INTROGRESSION INTO THE BREAD WHEAT GENOME. CREATION OF THE PREBRIDING FORMS

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Bread wheat (*Triticum aestivum* L.), the main cereal crop, is constantly exposed to breeding programs. Today, wheat selection for high yield potential led to a reduction in plasticity and adaptability of varieties to adverse environmental factors. To increase the genetic diversity of varieties, hybridization with wild relatives of the tribe Triticeae used. Rye, *Secale cereale* L is an important genetic resource for the breeding of wheat because of its outstanding biotic and abiotic stress tolerance. However, F_1 hybrids are often sterile. Unreduced gamete formation is the main mechanism of hybrid fertility restoration. Complex molecular cytogenetic analysis of the chromosome behavior in meiosis of amphihaploids *T. aestivum* L. x *S. cereale* L., in which wheat 1A, and 6A chromosomes are substituted by rye homoeologs, revealed two mechanisms of unreduced gamete formation. The first one is a mitotic-like division that is characterized by mitotic centromere architecture, robust bipolar spindle, one-step loss of arm and centromere cohesion, and sister chromatid separation in the first and only meiotic division. The second type involves a monopolar spindle formation. It prevents chromosome segregation in meiosis I, with a bipolar spindle distributing sister chromatids to the poles in meiosis II. Both mechanisms were observed in 1Rv(1A)xR, and 6R(6A)xR, but mitotic-like division was primary. To determine the connection of unreduced gamete formation with the hybrid fertility, the seed set of F_1 and F_2 offspring, obtained by amphihaploid 1Rv(1A)xR, 6R(6A)xR and C29xR self-pollination, was characterized. The F_3 hybrid formation was accompanied by a change in chromosome set of 1Rv(1A)xR, 6R(6A)xR and C29xR genomes: rye chromosomes substituted wheat chromosomes; 1Rv/1A substitution affect the rye chromosome elimination and 6R/6A substitution tend to maintain almost complete set of rye chromosomes. Individual contribution of chromosome substitutions to the F_3 plant morphotype and productivity was observed. Cytologically stable hybrids with high productivity selected as prebreeding forms and their chromosomal constitution was characterized. The work was supported by the Russian Science Foundation (grant no. 16-16-00011)

GENETIC DIVERSITY OF SPRING BARLEY VARIETIES FROM RUSSIA ASSESSED BY ALLELES OF HORDEIN-CODING LOCI

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To confer food security of country it is necessary to preserve the national genetic resources - animal breeds and plant varieties. Barley is one of the most important crops. It is widely used for food and industrial purposes, for feeding animals and birds, as well as being the main malting material for brewing industry. During 20th century intravarietal heterogeneity and genetic diversity of spring barley varieties grown in Russia have been decreasing over the course of breeding. By now assortment of varieties grown in Russia has changed considerably. Therefore, barley genetic diversity monitoring using different approaches is essential. Starch gel electrophoresis was used to identify alleles of hordein-coding (*Hrd*) loci for 211 spring barley varieties. We assessed allelic diversity and frequencies of *Hrd* alleles determined a sample of 160 spring barley varieties, approved for use in Russian Federation in 2014, and comparison with a sample of 101 spring barley varieties, approved in 1999; 49 varieties being common to them. It is shown that intravarietal heterogeneity tended to decrease mainly due to the introduction of foreign varieties homogeneous for *Hrd* loci. For investigated period the number of foreign varieties increased more than in 5 times. Polymorphism by hordein-coding loci in modern spring barley varieties slightly increase. The number of *Hrd A* alleles increased by five, and for *Hrd B* locus - by nine alleles. Along with the previously recorded alleles, we identified three novel alleles of *Hrd A* and four of *Hrd B*. We have not found any new alleles of *Hrd F* locus, and the frequencies of four already known alleles changed insignificantly. Statistically significant changes have been observed in the frequencies of some *Hrd A* and *Hrd B* alleles. All novel *Hrd A* and *Hrd B* alleles were found with extremely low frequencies, therefore their emergence did not cause the statistically significant increase of genetic diversity deduced from μ and *PIC* values, despite the expanded number of alleles. This work was supported by Russian Foundation for Basic Research, grant no. 14-04-00247.

SPECIFIC FEATURES OF ORGANIZATION OF CELLULAR T-DNA IN NATURALY TRANSGENIC PLANTS

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Most genetic engineering of plants uses *Agrobacterium* mediated transformation to introduce novel gene content. In nature, insertion of T-DNA in the plant genome and its subsequent transfer via sexual reproduction has been shown in several species in the genera *Nicotiana*, *Ipomea* and *Linaria*. In our study T-DNA-like sequences has been detected and characterized in different *Linaria* species, belonging to two sections *Linaria* and *Speciosae*. In all characterized *Linaria* species the cT-DNA is present in two copies and organized as a tandem imperfect direct repeat and contained the same T-DNA oncogenes and the *mis* gene, however there are different mutations inside the T-DNA in the investigated forms. In *Nicotiana* there are several types of cT-DNA. Molecular markers, based on different types of cT-DNA are developed in our laboratory for evolutionary studies of Nicotiana. Characterization of structure of T-DNA in close species of naturally transgenic plants is important, since they could be good model system for study of delayed environmental risks of GMO

ADAPTATION OF *GLOBODERA ROSTOCHIENSIS* TO MODERATE RESISTANT POTATO HYBRID CLONES

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Globodera rostochiensis is considered one of the most harmful quarantine potato pathogen in Russia and worldwide. Resistance to *G. rostochiensis* was studied 40 inter-species hybrids of potatoes were screened for resistance to *G. rostochiensis* pathotype Ro1. New sources of resistance were found and could be used in breeding. For study the possibility of nematode adaptation to potato genotypes with moderate resistance four interspecies hybrid clones obtained from crosses of double haploid susceptible cultivated potato with accessions of wild species *Solanum incamayoense*, *S. doddsii* and *S. alandiae* were used. DNA was isolated from cysts, reproduced after the second or third passage. Each population was represented by a DNA pool from 31–50 cysts. DNA pools were genotyped by RAPD method. The findings suggest that changes in genotypic composition of the parasite population selected after 2–3 passages on potato accessions depends on the host plant genotype. The experimental data seem to confirm the hypothesis of adaptive selection of virulent *G. rostochiensis* genotypes with the "bottleneck" mechanism for 2–3 parasite generations. The genetic nature of nematode resistance of hybrid clones developed with using of *S. incamayoense* is different from hybrids with genomes of *S. doddsii* and *S. alandiae*.

FINGERPRINTING FOR DROUGHT AND HEAT TOLERANCE IN SOME EGYPTIAN WHEAT CULTIVARS

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We discuss here the joint project considering an important problem of bread wheat varieties and crop production, as well as their tolerance to abiotic stress and climatic conditions in Egypt and Russia. Drought and heat stresses are most significant stresses which limits wheat productivity. Egyptian wheat genotypes from Egyptian Agricultural Research Center department are planned to be used as the experimental materials for this investigation. The genotypes differ from each other in morphological as well as genetically characters. Dehydrins are a group of intrinsically disordered proteins that respond to abiotic and biotic stresses. The importance of DHNs has been demonstrated by genetic and protein evidence *in vitro*; however, *in vivo* protective mechanism has not been elucidated. The wheat DHNWZY2 is induced by drought, low temperature, and other stresses. The data indicate that WZY2 exerts its function as a protectant. The K-segment is a major functional component of this DHN. The proteins with two K-segments (WZY2 and YS) had the most significant impact on improving cold and heat stress tolerance. Dehydrins (DHNs), group of late embryo genes is abundant (LEA) proteins, are up regulated in most plants during cold, drought, heat, or salinity stress. Physiological parameters and expression levels of drought related genes were analyzed in early vegetative stage of two bread wheat cultivars (Sids and Gmiza) differ in drought tolerance capacity. Both cultivars were imposed to gradual water depletion started on day 17 till day 32 after sowing. Sids, the more tolerant cultivar to drought showed higher fresh and dry weights than the drought sensitive genotype, Gmiza. Under water stress, Sids had higher membrane stability index (MSI), lower accumulated H_2O_2 and higher activity of the antioxidant enzymes. We will conduct study of gene crops, associated with tolerance to drought, to salt content in the soil cover and other abiotic stress factors.

PLANT DEFENSE PEPTIDES AS VALUABLE ANTIMICROBIALS FOR CROP PROTECTION

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Plant diseases cause considerable losses in cultivated crops by reducing yields and deteriorating nutritional quality of plant-derived products. The main currently used strategy to control infection includes treatment with fungicides and pesticides which dramatically affects ecological safety. A promising, environmentally friendly approach should be based on the employment of plant's innate immune mechanisms. Antimicrobial peptides (AMPs) constitute an important part of plant defense machinery. The aim of our work is to study AMPs and their genes in highly resistant and wild plant species. From the wheat species *Triticum kiharae* Dorof. et Migusch., 24 novel AMPs were isolated and structurally characterized, among them, the hevein-like peptides WAMPs and hairpinins. The biological activity of WAMPs and hairpinins was assayed against a wide range of plant pathogens; their genes were cloned and expression studied under biotic and abiotic stress conditions. The amino acid sequences and three-dimensional structures of these novel AMPs were determined. Both types of AMPs were shown to be produced from precursor proteins. The modular structure of hairpinin genes encoding several AMPs was disclosed. Genes closely related to WAMPs and hairpinins were discovered in related cereal species. Testing biological activity of WAMPs showed that they are active against phytopathogenic fungi including *Fusarium* species. Analysis of their mode of action demonstrated that WAMPs are inhibitors of fungal metalloproteinases that target plant defense chitinases. The model for plant-microbe interactions involving wheat plants and *Fusarium* fungi was suggested. AMP biodiversity in wheat was further explored by high- through-put transcriptome sequencing, and dozens of putative AMP transcripts belonging to different AMP families were identified. From a cosmopolitan weed species *Stellaria media* L., a number of novel AMPs inhibiting growth of phytopathogens at micromolar concentrations were isolated. Even more putative AMP genes were revealed by transcriptome sequencing. The results obtained demonstrate the abundance of candidate AMP genes in plants for the improvement of disease resistance in crops. The work was partly supported by Russian Foundation for Basic Research (grants no. 15-04-04680 and no. 15-29-02480).

PROBLEM OF MODERN BREAD MARKET IN RUSSIA IN THE CONTEXT OF FOOD SECURITY

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The bread market in Russia was dramatically changed. It is shown that the commodity offer of bakery products is characterized by illusiveness of assortment whose line items differ by names more, than by their nutritional and biological value, the percentage of products, made using chemical additives and fat, increases. Bread producers face a problem of low baking properties of bread flour, first of all the insufficient flour strength (gluten strength), and also the presence in it of "potato" illness spores, etc. in industrial production. Now the low-quality products with such characteristics as raised friability, thick crusts, lack of a typical "grain" smell and taste, the increased mouldiness dominate in the Russian bread market. The statistics on the volume and structure of grain quality in Russia in 2011–2014 is analyzed. An essential deformation of the quality structure of the produced wheat grain in favor of less valuable classes is observed. Search for the issue solutions of increasing grain technological properties is carried out in various spheres and it is necessary to concentrate on multidisciplinary projects and to combine not only agricultural science, but also modern economic and genetic approaches, combined with traditional breeding methods. It is noted that the modern way of the increased chemicalization does not comply with the food security principles and does not provide the population with baking products of necessary quality and nutritional value.

APPLICATION OF GENETIC MARKERS IN THE LABORATORY SEED CONTROL OF BARLEY

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Electroforesis of alcohol-soluble storage proteins - hordeins is a basic method of the Laboratory seed control on the assessment of varietal authenticity and seed purity in cultivars of malting barley. Protein components of hordeins detected by starch gel electrophoresis are inherited in groups (blocks) and are encoded by seven linked loci: *Hrd A, Hrd B, Hrd C, Hrd D, Hrd E, Hrd F* and *Hrd G*. Three loci - *Hrd A*, *Hrd B* and *Hrd F* is highly polymorphic. As a result of analysis of 1600 barley cultivars from different germplasm collections worldwide, 157 alleles of *Hrd A*, 272 of *Hrd B* and five alleles of *Hrd F* have been identified. In theory, this allows distinguishing of more than 370 000 barley varieties. However, in fact, hordeins electrophoresis can discriminate not more than 80% of modern barley varieties from the Russian Register. The number of foreign and domestic breeding varieties, identical by hordeins is growing. The same formula of hordeins has been determined in some brewing and forage varieties, as well as in Null-Lox and conventional varieties of malting barley. This may cause errors in the identification of barley varieties and estimating varietal purity of barley seeds. Thus, there is an urgent need in additional genetic markers for reliable varietal identification. For discrimination of barley varieties identical by hordeins, the electrophoretic analysis of TBE-PAGE soluble seed proteins can be successfully used.

ADVANCES IN GENETICS AND GENOMICS OF FOXTAIL MILLET (*SETARIA ITALICA* L.) FOR CROP IMPROVEMENT OF MILLETS, CEREALS AND BIOENERGY GRASSES

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Foxtail millet (*Setaria italica* L.) has recently been regarded as a tractable experimental C_4 model crop for studying functional genomics of millets, cereals and bioenergy grasses. Further its potential abiotic stress tolerance has invited much research attention and we attempted to generate genomic resources and to decipher the molecular mechanism of stress tolerance. Though our efforts have generated considerable knowledge on structural and functional genomics of foxtail millet, the release of genome sequence significantly accelerated the genomic research to a new dimension, as evidenced by large-scale development of genomic resources, development of web-based database, genome-wide association studies, genome-wide functional genomics and standardization of molecular protocols specific to foxtail millet. Our research contributions in both the functional and structural genomic aspects of foxtail millet are remarkable and our efforts have now resulted in the transformation of this orphan crop into a nutri-crop with rich genomic and genetic resources. These resources would promisingly assist in crop improvement programs involving millets, non-millets and bioenergy grass species, thus ultimately addressing global food security in the scenario of changing climate.

ASSOCIATION OF INTROGRESSIONS IN 2A, 2B AND 5A CHROMOSOMES OF BREAD WHEAT FROM *TRITICUM TIMOFEEVII* TAUSCH. WITH PARAMETERS OF GAS EXCHANGE, CHLOROPHYLL FLUORESCENCE AND ACTIVITY OF ANTIOXIDANT ENZYMES UNDER NORMAL AND WATER DEFICIT CONDITIONS

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Alien hybridization in cereals is used for comparative investigations of genome structure and evolution as well as for extracting the useful genes from a wild gene pool. The tetraploid species *Triticum timopheevii* has long been used as a source of genes for resistance to fungi diseases. The line 821 was developed by E. Budashkina on the genetic background of drought resistant but very susceptible for diseases cultivar Saratovskaya 29 (S29), and resistant to leaf rust. According the genotyping data, the line carries big introgressions in 2A and 2B chromosomes and a small introgression in subtelomeric region of 5A chromosome. The line was studied for a number of physiological and biochemical parameters under conditions of normal and restricted water supply. It was found that the line substantially differed from the initial drought tolerant cultivar for gas exchange, chlorophyll fluorescence and activity of antioxidant enzymes under both conditions. Under normal watering the parental cultivar differed by decreased parameters of gas exchange and photosynthesis comparing to the line 821 but had higher water use efficiency (WUE). Under drought, the line noticeably lowered these parameters in contrast to S29 in which these parameters increased and WUE was also substantially higher. In addition, the activity of antioxidant enzymes, dehydroascorbate reductase and catalase, inactivating an excess of reactive oxygen species considerably elevated and WUE was higher. Thus, it may be supposed that 2A, 2B and 5A chromosomes carry the important genetic complexes responsible for reaction of living systems of wheat plant on changeable environment.

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THE USE OF CYTOGENETIC COLLECTIONS OF BREAD WHEAT AND THEIR MOLECULAR-MARKED DERIVATIVES FOR THE SEARCH OF A NEW GENETIC VARIABILITY FOR DROUGHT TOLERANCE AND GRAIN QUALITY

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The dynamic development of plant breeding based on the achievements of genomic investigations is taking place in 21 century. The quick progress on this direction is especially important for ensuring a food security under the conditions of unfavorable climate changes on the Earth. In wheat, the genotyped collections of single chromosome substitution lines and the lines with introgressions from the wild relatives may be the sources of various genetic variability as well as the good models for genetic investigations of physiological basis of stress tolerance and end-use quality. In the report, the data will be presented about genetic investigations of drought tolerance components and grain quality in wheat. Substitution and molecular marked recombinant substitution lines on the genetic basis of cultivars Chinese Spring and Saratovskaya 29 were used for QTL-mapping of different physiological and grain quality traits. The set of recombinant introgression lines for D genome of *Aegilops tauschii* was used to map QTL for manifestation of many physiological and biochemical traits under drought. The two main regions were detected in 2D and 7D chromosomes carrying loci responsible for gas exchange and chlorophyll fluorescence traits and antioxidant enzyme activity. Chromosomes 2A and 4D substitutions in the genetic background of S29 were found to lead to a critical decrease of water-deficit tolerance of drought tolerant recipient. The initial bread wheat lines with introgressions from *Triticum timopheevii* and *Ae. speltoides* were used for obtaining the derivatives – the new substitution and isogenic lines carrying the genes for high protein content in grain. The development and involvement of this material in the genomic investigations will promote further researches in the field of food security and sustainable agriculture. The work was supported by RFBR grant #15-04-02762/15 and ICG project (No 0324-2015-0005).

FUNCTIONAL GENOMICS TO IMPROVE WHEAT DISEASE RESISTANCE

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Recent innovations in sequencing technologies and computational genomics now allow the sequencing of many wheat varieties. In our group, we are advancing functional wheat genomics to overcome major barriers to wheat crop improvement. We are integrating massive data sets including wheat POPSEQ genetic bin maps with a w^{2}rap wheat assembly constructed recently and exome-capture data. In addition we are resolving gene cluster regions by targeting specific gene families with an exome-capture approach and PacBio sequencing. As a result, we are generating a platform for detailed comparative genomics of wheat genotypes that are currently being sequenced in-house. Broad scale integration of wheat genomic data is crucial to expedite isolation of genetic variants underlining the phenotype of interest. Currently, our specific effort is focused on disease resistance pathways in tetraploid durum wheat. Previously, mutagenized *Triticum turgidum* cv 'Kronos' lines were phenotyped in the field and mutant lines with increased resistance to yellow rust were identified. Using an exome-capture approach and a mapping-by-sequencing approach we aim to isolate the underlying causative mutations for this phenotype. We have already sequenced the M_4 segregating progeny of a mutant with increased yellow rust resistance and identify the chromosomal region that contains the causative mutation. We are currently screening the F_2 segregating population derived from the cross of the mutant and wild type 'Kronos' with markers to confirm the region of interest. The homozygous resistant and homozygous susceptible F_2 plants will then be sequenced and analyzed to identify the causative mutation responsible for increased resistance.

THE USE OF GENOME TECHNOLOGIES FOR PLANT GENETIC PROTECTION

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Recently, the approaches utilizing genome sequencing data for solving the topical problems of plant genetic protection have been actively developed. Currently, the most efficient in this area are the methods of marker-assisted selection and genome editing. The Laboratory of Plant Molecular Genetics and Cytogenetics utilizes the marker-assisted selection for over last 10 years to breed the bread wheat lines resistant to fungal pathogens. The markers were selected for the genes/loci that determine the bread t wheat resistance to leaf rust, stem rust, and powdery mildew. The spring lines donated the resistance genes were selected with the help of microsatellite markers from the populations produced by crossing the hybrids *T. aestivum* × *T. timopheevii* (collection of E.B. Budashkina) and *T. aestivum* × *Ae. speltoides* (collection “Arsenal” of I.F. Lapochkina) with the cultivars Saratovskaya 29 and Novosibirskaya 29. The winter donor lines were selected using the designed gene-specific markers from the population produced by crossing the spring donor lines and cultivar Tulaikovskaya 10 (carrying a set of resistance genes from couch grass) with the winter cultivars Biiskaya ozimaya, Filatovka, Novosibirskaya 3, and Novosibirskaya 40. The selected winter and spring donor lines carrying the target resistance genes were tested for resistance to fungal pathogens under field conditions. The following soft wheat lines were chosen for our further work: (i) Lines 21-4, 17-7, and 11-8, carrying the genes from *Ae. speltoides* rendering resistance to fungal pathogens and localized to chromosomes 5B, 6B, and 7D, respectively; (ii) Lines 5366-180 and 3862-5, carrying the leaf and stem rust resistance genes from *T. timopheevii* and localized to chromosomes 5B and 2B, respectively. The spring bread wheat lines selected using the markers and carrying the *Lr* gene of line 21-4 and rye translocation (1RS.1BL) were involved in breeding, since they displayed resistances to powdery mildew and leaf rust along with a higher yield in Western Siberia and Ural. The remaining wheat lines developed using the produced donor lines and markers are in the process of testing at breeding centers.

SLEEP OF REASON IN THE ANALYSIS OF THE RESULTS OF RESEARCH ON MATERIALS «PROTEOMIC INFORMATION OF SPRING WHEAT VARIETIES DIFFERING IN RESISTANCE TO INFECTION AFTER *PUCCINIA RECONDITA* INOCULATION»

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Systemic acquired resistance (SAR) to *Puccinia recondite* (pathotype TKT/Y) in wheat cultivars was studied by using a proteomic approach. The effect of leaf rust on the regionalized in the Akmola region of Kazakhstan spring wheat cultivars was studied in pot experiments. The peptides were analyzed by using nano-HPLC (Agilent Technologies 1200), which is directly related to the ion-trap mass spectrometer (Bruker 6300 series), equipped with nano-electrospray source. The gradient of acetonitrile from 5% to 90%, duration 25 minutes. Voltage of fragmentation 1.3 V. A total of 104 proteins were identified using a combination of peptide mass fingerprinting (PMF) and MSMS fragmentation. Only detected in the control plants: Fructan 1-exohydrolase w1;w2; w3; Histone H1; NAD(P)H-quinoneoxidoreductase subunit 1,chloroplastic; NAD(P)H-quinoneoxidoreductase subunit H, chloroplastic; NADP-dependent glyceraldehyde-3-phosphate dehydrogenase; Mitochondrial outer membrane porin; Cytochrome c oxidase subunit 2; Arf-GAP with Rho-GAP domain; Elongation factor 1-beta; 30S ribosomal protein S8, chloroplastic; 30S ribosomal protein S7, chloroplastic; Eukaryotic initiation factor iso-4F subunit p82-34; Retinoblastoma-related protein 1; Ubiquitin-activating enzyme E1 1;Cysteine synthase. Only detected in the infected plants: peroxidase; ATP synthase subunit 9, mitochondrial; 50S ribosomal protein L23, chloroplastic; cytochrome b6-f complex subunit 4; Ubiquitin; 50S ribosomal protein L16, chloroplastic; 30S ribosomal protein S3, chloroplastic; DNA-directed RNA polymerase subunit beta; DNA-directed RNApolymerase subunit alpha; glutathione S-transferase 1; Small heat shock protein, chloroplastic; probable non-specific lipid-transfer protein 3. Multiple changes in the activity of enzymes were observed, in spite of that the intracellular pool of substrates has been found to not allow such a significant change. The results suggest the impossibility of plant life without the above proteins and errors in the identification of compounds by chromatograph. Interpretation for the identification of proteins and determining the direction of changes activity of enzymes should be based on common sense.

PHENOTYPING OF SYNTHETIC WHEAT WITH *AE.SQUARROSA* GENOME IN THE CONDITIONS OF SOUTHERN FOREST-STEPPE OF WESTERN SIBERIA

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Wheat synthetics created on the base of the wild relatives' genetic potential are widely used in breeding programs for creating of the initial material for the breeding of wheat adaptive high-yielding varieties in the world and also in Russia, which contributes to a significant extension of genetic diversity of initial material for breeding. Selection estimation of the material received according to KASIB and shuttle breeding program of CIMMYT, created by the distant hybridization with wild species *Ae. squarrosa* and *T. dicoccon* was performed. For period 2010–2015 in Omsk State agrarian university 2734 populations, included 350 "synthetic-populations" were studied. Comparative select results of hybrid populations in nurseries of breeding process showed that the selecting percent of "synthetic-populations" in general is high and ranged from 11 to 30%. Their selection value was determined, above all, by complex resistance to diseases. The results of evaluation of the selected spring bread wheat varieties received with participation of wild species in Preliminary and Yield Variety Trials are presented. Resistance genes to stem and leaf rust were identified in varieties Lutescens 24-12 – *Lr34*, Lutescens 27-12 – *Sr35*, Lutescens 34-12 – *Lr9/ Lr26 / Sr21* using molecular markers. Above mentioned varieties as well as Lutescens 106-11, Lutescens 15-12, Lutescens 96-12 which are characterized by complex resistance to diseases, high grain quality and high potential yield in the conditions of Western Siberia were selected. As a result of team-work of Japan Kyoto University and International Center of maize and wheat improvement CIMMYT were obtained synthetic hexaploid wheat lines by crossing varieties of *Triticum durum* with 12 different forms of *Ae. squarrosa*. In 2016 collection of 126 synthetic lines was transferred to Omsk SAU for phenotypic evaluation in Western Siberia in order to select the sources of valuable agronomic traits and disease resistance for their inclusion to breeding process. Work is performing under financial support of Russian Foundation for Basic Research (grant № 16-16-10005).

GENE CASCADE CONROLS EARLY FLOWERING TIME IN UNIQUE SPRING BREAD WHEAT SST843 FROM SOUTH AFRICA

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The spring bread wheat cultivar SST843 originated from South Africa has a unique genotype that differs from other wheat germplasms. Plants of SST843 have a significantly shorter period until flowering and maturation, regardless of day length and temperature; with flowering initiating 15-16 days earlier compared to various wheat genotypes used in the current study. Genetic regulation between the Transcription factor *TaHDZipI-2,* Vernalisation-responsive gene *TaVrn1*, and Flowering time gene *TaFT1*, were identified as the genetic components most up-regulated during the growth of SST843 plants and its derivatives, which result in the earlier flowering habit of the plants. Other Flowering time genes are expressed co-ordinately, but their roles are less important for the early development of SST843 plants. A significant level of genetic polymorphism has been found in sequences of the promoter regions of the *TaVrn1* gene in homoeologous chromosomes 5A and 5D of SST843. In the promoter of the *TaVrn-A1* gene, a unique insertion of 232 bp was identified, while 21 SNPs and four small insertions were found in the *TaVrn-D1* promoter of SST843. Alterations in both promoters can significantly change the regulation of *TaVrn1* due to the presence of more binding motifs for the *TaHDZipI-2* TF regulating the expression of *TaVrn1*. The identified regulation of gene cascade, *TaHDZipI-2 - TaVrn1 - TaFT1*, is the most possible mechanism explaining the early flowering phenomenon in SST843 plants.

IDENTIFICATION OF CANDIDATE GENES FOR DROUGHT TOLERANCE IN SPRING BARLEY BASED ON SNP-KASP TECHNOLOGY

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The high throughput technology of Kompetitive Allele Specific Polymorphism (KASP) markers allows for the rapid and accurate identification of SNPs for their application in various plant species. In the drought environment of Northern Kazakhstan, 35 prospective domestic spring feed barley cultivars were genotyped and their seed yield was measured in field trials. From seven preliminary selected KASP markers four showed strong associations with seed yield production under drought. The four KASP markers were developed based on SNPs found in sequences of the following genes: *HvGI*, Gigante; *HvFT5*, Flowering time 5; *HvPhyC*, Phytochome C; and *HvTFL1*, Terminal flower 1. These four genes are involved in plant development, transition to flowering and flowering time. Majority of high- and low yielding barley germplasms shared different alleles for these four genes. The associations revealed in this work indicate the importance of these developmental factors and their encoding genes for barley seed production in the drought conditions of Northern Kazakhstan. The genes are located in chromosomes 3HS, 4HL and 5HL, with known contig identifications in the Morex genome. Marker Assisted Selection (MAS) using these and other novel KASP markers is now in planning, in order to improve and accelerate the breeding of spring feed barley for better seed production under conditions of strong drought stress in Northern Kazakhstan.

MAPPING OF QTLS ASSOCIATED WITH THE TECHNOLOGICAL PROPERTIES OF GRAIN AND FLOUR USING INTERVARIETAL SINGLE CHROMOSOME SUBSTITUTION LINES AND SUBSTITUTION RECOMBINANT DOUBLE HAPLOID LINES SARATOVSKAYA 29 (YANETSZKIS PROBAT 4D*7A)

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The unique spring Russian cultivar Saratovskaya 29 (S29) produces such kind of grain which retains the high quality under various environmental conditions. The aim of this work was to reveal and map in the bread wheat genome the locus (loci) responsible for this valuable technological trait. At the first stage, a full set of single chromosome substitution lines S29(YP) was studied in order to identify the chromosome carrying the locus under study. The donor of single chromosomes, spring cultivar Yanetzkis Probat (YP) had a low flour strength and dough tenacity comparing to the recipient cultivar S29. Milling properties of grain were studied as well as physical properties of dough using Chopin alveograph. The only substitution line which showed low physical properties of dough compared to the donor was the line S29(YP 4D*7A). The new set of substitution recombinant double haploid lines was developed on the basis of this line to map the locus responsible for variability in technological properties of grain. The lines were genotyped with 16 and 14 microsatellite loci on 4D and 7A chromosomes, correspondingly. The grain of SRDH lines was studied for physical properties of flour and for grain vitreousness. Two major QTLs for two traits - dough strength and tenacity- were mapped to the one and the same positon on 4D chromosome near the marker *Xgwm0165*. The donor of both loci was the high quality cultivar S29. This finding may be used in wheat breeding for improving the physical properties of dough. Additionally, one major QTL was mapped to the region of the marker *Xgwm0870* in 7A chromosome associated with a high vitreousness. The donor of higher meanings of the trait was the donor cultivar YP.

IMPACT OF DIFFERENTIAL DNA POLYMORPHISM OF TOMATO (*SOLANUM LYCOPERSICUM* L.) INTO F_1 HYBRIDS PERFORMANCE

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Classical theory considers F_1 hybrids heterozygosity as the main factors contributing to heterosis and therefore presuppose a linear relationship between value of genetic polymorphisms of parental lines and heterotic response of its F_1 offspring. To investigate the impact of differential DNA-polymorphisms to the superior F_1 performance, we carried out a screening of 12th SSR loci and performed a genetic analysis of parental lines and its hybrids in testcross (4×6) design. In the result we found out a significant positive link between the total number polymorphic loci in pairwise cross-combinations, high-parents and mid-parents heterosis (r=0.43; 0.47), but its value is insufficient to predict best hybrid performance. But when we divided F_1 in non-heterotic and heterotic groups we revealed a close relationship of differential polymorphism and heterotic F_1 hybrids superiority – the coefficient of determination (r^2) reached 75% and 84% for mid- and high-parents heterosis correspondingly. There was a significant impact of differential polymorphism into specific combining ability (SCA). The number of non-polymorphic loci was negatively associated to heterosis. According our result, total number of polymorphic loci and ratio between polymorphic and non-polymorphic loci have high predictive value and could be good prognostic criteria for the selection potentially heterotic cross-combinations. Therefore, overall and locus-specific heterozygosity are important for F_1 heterotic response and its evaluation may be useful for tomato breeding.

MOLECULAR-GENETIC DIVERGENCE AS A CRITERION FOR THE SELECTION PROMISING CROSS-COMBINATIONS IN CROP

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Heterosis as the increasing performance of hybrid progeny compared to their inbred parent is one of the intriguing phenomena of genetics. First attempts to find underlying mechanisms were based on theoretical models, which were useful, but couldn’t characterize this phenomenon as a whole. With a developing of molecular markers the big efforts were made to identify genomic regions causing heterotic response and clarify prospects of using information about molecular divergence as criterion for F_1 performance prediction. The genetic basis of heterosis was studied at Institute of Genetics and Cytology for many years with using of classical and contemporary molecular genetic tools. We evaluated different molecular-genetics models for the selection of potentially heterotic cross-combinations in crop. Promising results were obtained in vegetables. We found out that hybrid performance of sweet pepper caused by ISSR genetic divergence (GD) on 86% and RAPD GD - on 69%. With microsatellite markers (SSR) we got a close relationship ($r = 0.71$) SSR GD and heterosis for fruit length of sweet pepper. Similar work using SSR markers for tomato revealed that SSR evaluation could be useful, but it has limitations, since only part of heterozygous advantage F_1 can be explained by GD of their parents. When we selected "positive" markers, total score GDs changed and correlations (r) were increased to level 0,51-0,9 for high-parent and 0,61-0,81 for mid-parent heterosis, depending on the trait. Good prospects of using GDs have been shown for cabbage and triticale - all selected this way cross-combinations gave F_1 superiority against mid- and better parent. However, in genome-wide SNP study of wheat we found that evaluation of overall GD could not be predictive for particular traits. Despite the lack of a linear relationship between the level of genetic distances and F_1 heterosis, our data support the conclusion of promising prospects of using GDs in plant breeding, provided its detalization and selection “positive” or key markers.

ALBINO LEMMA AND PERICARP MUTATION IN BARLEY: CHANGING EXPRESSION OF TARGET GENES, PLEIOTRPIC EFFECT ON PHENOTYPE AND PRODUCTIVITY

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Chlorophyll deficiency in plants can result in photosynthesis impairment, abnormal plant development and premature death. These abnormalities are frequently encountered in interspecific crosses and tissue culture experiments. Analysis of albino mutant phenotypes with partial chlorophyll deficiency can shed light on genetic determinants and mechanisms of albinism. Barley (*Hordeum vulgare*) i:Bw*Alm* (*Albino lemma and pericarp mutation*) line with tissue-specific albinism is a promising object for such study. Comparison of TGW (1000 grain weight) in the mutant line and control (cultivar Bowman) revealed 1,2 fold reduction of this value in the mutant line, suggesting contribution of chlorophyll synthesis in lemma and pericarp to grain weight. Pleitropic effect of *Alm* was observed on auricles (and adjacent leaf blade regions), stem node (and adjacent stem regions) and sheath of the first leaf of the i:Bw*Alm* line. Chlorophyll distribution was examined in different parts of i:Bw*Alm* and Bowman plants using chlorophyll fluorescence microscopy. Difference revealed between i:Bw*Alm* and Bowman in the cell shape and arrangement demonstrated that *Alm* gene, in addition to effects on chloroplasts, results in modification of cell morphogenesis. Results of RNAseq analysis demonstrated that the i:Bw*Alm* line has decreased expression level of plastid genes. Statistically significant differential expression was observed for several plastid operons containing protein coding genes, rRNA and tRNA-coding genes. We identified nuclear genes with differential expression in two barley lines. Functional differentiation between genes with higher and lower levels of expression in the i:Bw*Alm* line was detected. Genes with lower level of expression in the i:Bw*Alm* line are mostly associated with photosynthesis and chlorophyll synthesis, while genes with higher expression level are functionally associated with vesicle transport. Differentially expressed genes are shown to be involved in several metabolic pathways, most represented being Calvin-Benson-Bassham cycle. Finally, *de novo* assembly of transcriptome contains several transcripts, not annotated in current *H. vulgare* genome version. The study was supported by the Russian Science Foundation (Project No 14-14-00734).

DEVELOPMENT OF SUPERSOFT LINES OF BREAD WHEAT, THE CARRIES OF TWO GENES *HA* AND *HA-SP* FOR SOFT ENDOSPERM TEXTURE

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Endosperm texture parameters of bread wheat – vitreous/floury and hard/soft – are determined by one gene *Ha* in 5D chromosome. Multiply allelism for this gene resulted in a continuous variation of endosperm structure from rather soft and floury to hard and vitreous. Manifestation of the trait is modified by environment. The cultivar Chinese Spring may be an example of soft-grain wheat as its vitreousness varies in different conditions from 50 to 75% and flour particle size diameter (PS) from 11 to 15 microns. The cultivar Rodina is hard-grained and has a vitreousness 85-90% and PS more than 23 microns. The introgression line 84/98w obtained on the genetic basis of cv. Rodina carries the gene *Ha-Sp*, in 5A chromosome homoeological to *Ha* gene from *Aegilops speltoides* Tausch.. Therefore, the line has a soft grain and its vitreousness is about 50-70% and PS about 11-15 microns. The aim of the work was to combine two genes *Ha* and *Ha-Sp* for soft endosperm texture in one genotype. It was supposed that two genes will make the grain softness more expressed and less dependent from environment. To do this, the hybridization of Chinese Spring with the line 84/98w was performed. A significant transgression for tow traits was found in the second generation. Selection of genotypes in the number of generations was fulfilled accompanied with investigation of vitreousness and PS. From F_6-F_8 generations the lines were selected with very soft and floury endosperm with reduced vitreousness (from 15 to 50%) and PS 11-12 microns. These properties retained both in green-house and filed replications. Alveograph tests of the lines have showed that the lines have a low dough strength (from 42 to 76 alveograph units) characteristic of “weak” gluten. Just this kind of flour is preferable for producing confectionary (cookies, cakes, pastries and biscuits) as it enables not apply chemical baking powder.

BREEDING FOR STEM RUST RESISTANCE FOR SUSTAINABLE IMPROVEMENT OF FOOD SECURITY IN RUSSIA

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The stem rust population of central Russia is flexible after sexual process on barberry and potentially open to airborne infection. An international set of differentials (courtesy of Dr. McCallum, Agriculture and Agri-food Canada) were used to obtain the rust field survey data. The virulence to *Sr6, Sr8a, Sr9b, Sr9e, Sr9d, Sr10, Sr21, Sr30 Sr36* were identified in Central Russia which was a basic subset of Ug99 complex and a new Digalue race apart from *Sr5, Sr7b, Sr9a, Sr17, Sr21,Sr24, Sr31, Sr38, SrTmp* and *SrMcN*. Siberian spring wheat varieties are competitive with global germplasm but greatly affected by fungal leaf infections in favorable years. Among them the most devastating is stem rust which recently spread through the western Siberia and caused the local outbreak in the Omsk region in 2015. Although fungicide application may be necessary but preventive approaches of using resistant cultivars are the most environmentally effective and consistent with the current programs for spring wheat improvement of the Kazakhstan-Siberian Network on Wheat Improvement (KASIB). After the germplasm screening a majority of varieties were highly susceptible but a set of 148 locally-cultivated were resistant to stem rust. Genes *Sr25, Sr31, Sr36, Sr6Ai, Sr6Ai#2* were postulated for them using 16 pathotypes and 16 molecular markers. Complex resistance to both stem and leaf rusts were provided with gene linkage *Sr25* to *Lr19* and *Sr6Ai#2* to *Lr6Ai#2*. Adult plant resistance was revealed in 26 genotypes, including eight with *Sr57*. The limiting genetic basis of resistance to stem rust together with the high risk of outbreaks in Western Siberia revealed the requirement of the spring wheat breeding strategy for developing stem rust resistant varieties for Russia. Acknowledgements to the Ministry of Education and Science of the Russian Federation (agreement No 14.604.21.0106 from 07.07.2014; identification number RFMEFI 60414X0106).

RECONSTRUCTION OF THE GENE NETWORK OF THE WHEAT RESPONSE TO FUNGAL PATHOGENS

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A vast variety of pathogens is able to infect wheat tissues and cause diseases. Rust and mildew cause severe crop losses. Numerous plant genes control a complex network of defense mechanisms based on both constitutive and inducible processes. Several dozens of resistance genes were identified in wheat but only few of them have been sequenced. The gene network of the wheat response to fungal pathogens was created with the use of GenNet technology, analysis of literature data and manual curation. The gene network can be found at http://wwwmgs.bionet.nsc.ru/mgs/gnw/genenet//viewer/Plant%20fungus%20pathogen.html. Effector molecules produced by pathogens are the major inducers of the gene network. Receptor proteins encoded by resistance genes can identify pathogen effectors and switch on signal transduction pathways that contribute to the formation of the defense response. Reactive oxygen species (ROS) and hypersensitive response are involved in a formation of wheat resistance to rust pathogens. The transcriptional activity of the genes involved in the defense mechanisms usually increases in response to the infection with the exception of the MDHAR, which increases ROS scavenging. miRNA participates in regulating the resistance of wheat plants to stripe rust by targeting a NAC21/22 transcription factor, which plays a negative role in defense response. The levels of some hormones that regulate the synthesis of defense proteins change in response to infection. A directed change in the content of such hormones is considered as a variant of the strategy of increasing the resistance. Salicylic acid plays an important role in the defense response. The effect of salicylic acid results in an increase expression of pathogenesis-related genes, a receptor protein RLP1.1, a transcription factor MYB4, and ADF7. RAR1/SGT1/HSP90 complex required for correct folding and stability of some NBS-LRR defense proteins. The reconstruction of gene networks that are responsible for wheat defense against pathogenic fungi is required for the elucidation of the underlying molecular mechanisms as well as for the development of new approaches to crop improvement.

DEVELOPING OF THE GENETIC CLASSIFICATION OF *AEGILOPS COLUMNARIS* ZHUK

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Aegilops columnaris Zhuk. is a potential source of new traits for wheat improvement. However, so far this species has not been used in practical breeding. In the present work we reported for the first time the molecular-cytogenetic characterization of the newly developed 20 introgressive *T. aestivum* x *Ae. columnaris* lines by means of C-banding and fluorescent *in-situ* of hybridization (FISH). Fifteen lines carried from 1 to 3 pairs of *Aegilops* chromosomes as addition(s) or substitution(s) to wheat chromosomes. Based on substation spectra these chromosomes were identified as $3Ae^1$, $3Ae^2$, $5Ae^2$, $6Ae^1$ and $6Ae^2$. Another *Aegilops* chromosome has been found in the line 2305/1 as a monosomic addition; due to the lack of group-specific markers genome or genetic group affinity of this chromosome have not been determined; this chromosome was temporary designated Ae-*a*. In several lines acrocentric and telocentric chromosomes were also revealed (Ae-*b* and Ae-*c*). Different alleles of the gliadin loci have been identified using PAAG electrophoresis in introgressive lines L-2310/1 and L-2304/1 with substitutions of the chromosome 6D by two different chromosomes of *Ae. columnaris*. This observation confirmed that lines L-2310/1 and L-2304/1 contained non-identical 6Ae chromosomes. Taking into consideration our previous results of FISH analyses, the three other *Ae. columnaris* chromosomes can be assigned to homoeologous groups 1, 5 and 7 of the U-genome based on the location of 5S and 45S rDNA loci (1U and 5U) or pSc119.2 probe distribution (7U). Thus, based on our current data as well as on the results of earlier work, we can identify eight out of the 14 chromosomes of *Aegilops columnaris*. The authors thank Drs. S.N. Sibikeev and Dr. A.E. Druzhin (Agricultural Research Institute for South–East Region, Saratov) for providing the material. Work was supported by the Russian Foundation for Basic Research (grant № 15-04-00296).

COPIES OF THE *TaMyc1* GENE DETERMINING ANTHOCYANIN PIGMENTATION OF WHEAT GRAIN: IDENTIFICATION AND ANALYSIS OF THE EVOLUTIONARY FEATURES

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Bread wheat producing grain in which the pericarp is purple is a useful source of dietary anthocyanins. The trait is under the control of the gene *TaMyc1*. *TaMyc1* was strongly transcribed in the pericarp and, although at a lower level, in the coleoptile, culm and leaf. It encodes a MYC-like transcription factor, which is an essential part of the regulatory complex «MYB + MYC + WD40», which is necessary for gene activation in flavonoid biosynthesis. In the current study, we identified, characterized and compared full-length sequences of duplicated homoeologous and paralogous copies of the *TaMyc1* gene. With the search of homologous sequences in databases for not annotated wheat sequences using BLAST we identified 10 copies of the gene *TaMyc1* in a common wheat genome. The exon-intron organization of these genes is the same to the *TaMyc1* structure. All available sequences have a conserved bHLH domain. None significant change in the motive, which could lead to changes in gene function, has been identified among the annotated *Myc* genes of *Triticum aestivum* and related species. The nucleotide substitutions rate (k) for *Myc* was calculated by the formula $Ks/2T$. The obtained value ($k=10.04\times10^9$) was used for the calculation of divergence time of duplicated copies. Analysis of genetic similarity showed that the first duplication of *Myc* gene was in the diploid common ancestor of the tribe Triticeae. The duplication has undergone from two to four further acts of duplication in *Triticum* and *Aegilops* genomes. Maintaining functional duplicated genes is likely due to their specialization. It is assumed that *TaMyc1* copies may be involved in the synthesis of various flavonoid compounds in different parts of the plant. Also it is possible that the maintenance of the functional duplicated copies of genes in wheat is one of the factors that ensure its high ecological plasticity. Acknowledgements: This study was partially supported by the RAS MCB Programme (0324-2015-0016) and by RFBR (16-04-00912).

ANALYSIS OF *DDM1* AND *DRM2* ORTHOLOGS REVEALS SPECIFIC FEATURES OF RICE DNA METHYLATION PATHWAYS

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Plant DNA methylation that occurs at CG, CHG, and CHH sites (H = A, C, or T) is a hallmark of the repression of repetitive sequences and transposable elements (TEs). The rice (*Oryza sativa*) genome contains about 40% repetitive sequence and TEs and displays specific patterns of genome-wide DNA methylation. The mechanism responsible for the specific methylation patterns is unclear. Here, we analyzed the function of *OsDDM1* and *OsDRM2* in genome-wide DNA methylation, TE repression, small RNA accumulation, and gene expression. We show that *OsDDM1* is essential for high levels of methylation at CHG and, to a lesser extent, CG sites in heterochromatic regions and also is required for CHH methylation that mainly locates in the genic regions of the genome. In addition to a large member of TEs, loss of *OsDDM1* leads to hypomethylation and up-regulation of many protein-coding genes, producing very severe growth phenotypes at the initial generation. Importantly, we show that *OsDRM2* mutation results in a nearly complete loss of CHH methylation and derepression of mainly small TE-associated genes and that *OsDDM1* is involved in facilitating *OsDRM2*-mediated CHH methylation. Thus, the function of *OsDDM1* and *OsDRM2* defines distinct DNA methylation pathways in the bulk of DNA methylation of the genome, which is possibly related to the dispersed heterochromatin across chromosomes in rice and suggests that DNA methylation mechanisms may vary among different plant species.

BREEDWHEAT: BREEDING FOR SUSTAINABLE WHEAT VARIETIES, AN INTEGRATED APPROACH FROM GENOMICS TO SELECTION

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The challenge for wheat breeding is to produce safe, high-quality, and health-promoting food and feed in a sustainable manner across environments affected by global change. The BreedWheat project is conceived to help support the breeding sector to achieve this goal. To ensure that the knowledge, resources, and methods are translated rapidly into products and varieties the project gathers 15 public research laboratories, 10 private companies, 1 technical institute, 1 competitiveness cluster and 1 company specialized in project management and technology transfer. BreedWheat not only participates in new technological developments of markers and phenotyping methods, but also integrates high throughput genotyping, phenotyping, physiological and modeling to decipher the molecular and ecophysiological basis of major traits of interest for Northern Europe. This long-term project (2011-2020) includes the sequencing of chromosome 1B, the detection of new structural genetic polymorphisms, large scale SNP production, genetic and physical mapping of those SNP, and the generation of more than 2 billion genotyping data points. A large field experimental network was developed to phenotype association panels for nitrogen use efficiency, grain proteins composition, drought tolerance, *Septoria* leaf blotch and *Fusarium* head blight resistance. Phenotyping includes both the development of a wheat crop model and the use of high throughput phenotyping platforms developed within the PHENOME project (www.phenome-fppn.fr). Moreover, 5 000 wheat lines from the INRA Biological Resources Centre (BRC) were characterized and used to construct a new association panel representing the worldwide genetic diversity. Innovative statistical tools, including genomic selection models, are developed within a real size selection program, in order to address both technical and socio-economic aspects of the implementation of novel breeding strategies in seed companies. Finally, a robust bioinformatics platform enabling efficient data storage and breeder friendly access is established. As the BreedWheat project reaches mid-term, several relevant results were already delivered to the breeders.

RFBR-SUPPORTED RUSSIAN-VIETNAMESE SCIENTIFIC COOPERATION FOR RESEARCH MUSHROOMS AS FOOD AND IN VIEW OF BIOTECHNOLOGICAL BENEFITS

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Macrobasidiomycetes from the family Ganodermataceae are known to be extremely important mushrooms in fundamental biological, medicinal, socio-economic aspects. There are numerous Ganodermataceae species that are native to Vietnam. Within a framework of the joint research RFBR-VAST project. the field experiments in Vietnam were realized for collecting a number of species from *Ganoderma* and *Tomophagus* genera, including rare *G. colossus*, *G. neojaponicum*, *T. cattienensis* along with the strains of *G. lucidum, G. applanatum* intrinsic for this area, aimed at the subsequent comparative studies with the Russian-sourced herbarium strains of *G. lucidum, G. applanatum,* and *G. valesiacum*. Cultural characterization on solid and liquid fermentation, scanning electron microscopy of morphology along with chemical analysis served as the supporting identification and comparison factors. The studied micromorphological, cultural and selected biochemical characteristics provided a basis for identifying the most promising strains as biotechnological subjects. The selenium-enriched preparations of higher-fungal origin are of especial interest owing to their food availability, biocompatibility, and the proved biological activity. The organoselenium compound (kindly gift from Prof. B.I. Drevko) with the severely decreased toxicity served as the exogenous Se-source. The physicochemical techniques-assisted research on selenium level and zero oxidation state in the fungal extracellular specimen enabled us to judge on the *Ganoderma* and *Tomophagus* submerged cultures' capability to form the Se particles and Se-composites. The approach developed allows the bioproduction of submicrostructured selenium using edible mushrooms cultures to be put into practice. The peculiarities of lipidic pool of low-molecular-weight chemical constituents of mycelium, which could likely be significant for taxonomic differentiation, were elucidated. Valuable substances detected in pigmented mycelia and submerged cultures are of promising biotechnological applications including the food supplement, lipid-based drug delivery systems, and biodiesel-related items.

USE OF A TRANSCRIPTION FACTORS FOR ENHANCING CROPS TOLERANCE TO THE ABIOTIC STRESSES BY TRANSGENIC TECHNOLOGY

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Drought, salinity, heat and cold are the abiotic stresses that affect crop plants productivity, thereby causing large economic loss of agriculture. It is possible and very important create crops tolerant to the multiple environmental stresses with high yields (Wang et al, 2016). Instead the manipulation of single functional gene, the way of engineering certain regulatory genes has emerged as an effective strategy for controlling the expression of many stress-responsive genes. Transcription factors (TFs) are the best candidates for genetic engineering creation of stress-tolerant crop because of their role as master regulators of hundreds stress-responsive genes. Transcriptome analysis has indicated that distinct environmental stresses induce similar responses. Tolerance to drought, salinity, heat and cold are quantitative traits determined by polygenic genes quantitative trait loci (QTL) (Bita and Gerats, 2013). TFs are proteins with a DNA domain that binds to the cis-acting elements present in the promoter of a target gene. They induce (activators) or repress (repressors) the activity of the RNA polymerase, thus regulating gene expression. The presence of transcription factors regulating transcription of target genes often involves a whole cascade of signaling events determined environmental condition (Wyrick & Young, 2002). Transcription factors have become incredibly powerful and efficient components for precise regulation of targeted plant transgene expression (Liu and Stewart, 2015). Introducing only one gene TF of DREB1A in the wheat genome allowed to induce expression of more than 40 genes, involved in the drought response, and obtained drought tolerant transgenic wheat (Pellegrineschi et al, 2004). There are plenty of work that has been shown success usage of TF genes for obtaining barley, rice and wheat tolerant to the stresses (Kovalchuk et al 2013; Sahoo et al, 2012). We have found a few dozens of international patents which describe the way of obtaining wheat tolerant to the abiotic stresses and more than 80% of patents have used the genes of transcription factors. The work was supported by the Ministry of education and science of the Russian Federation (The Agreement №14.613.21.0052).

RESULTS AND THE PROSPECTS FOR THE QUALITY BREEDING OF VEGETABLE PEPPER

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Quality genes perform the dual purpose: the control of pericarp colour and the biological activity of nutrients. One of such candidates is mutant gene *bc*, obtained by S.Daskalov and colleagues as a result of the gene *Crtz-1* mutation. We refined the nature of the *bc* gene expression and its localization on the genetic map and developed the early testing of the gene product by the method of HPLC in leaves which makes it possible to accelerate the breeding in the selective nursery. However, this testing does not differentiate genotypes on the earliest phases. Therefore, the method of marker assistant breeding was used. For its realization the working collection of primers was selected and the PCR was worked out. In connection with the development of hybrids on the sterile basis with gene *bc* the same reliable system of the nuclear male sterility early genes identification is necessary. Labor-consuming work on the removal of the fertile analogs of *ms* maternal form during its seed-growing is a drawback in the classical method of operation with the nuclear genes. Innovation approach with the molecular markers will make it possible to save the occupied areas and to optimize very process of seed-growing. The proposed by American researchers and tested by us the genes identification system of *cos II* – markers did not operate and did not reveal reliable markers for the present. Not solved, but promising is also a problem of the early genotype differentiation with the high content of flavonoids in the fruits. Preliminary results showed that this character is inherited as polygene; its expression depends on the work of a number of modifiers, which is manifested in the diverse colour of pericarp. As a result different hybrids with the improved biochemical composition of fruits were developed.

TECHNOLOGICAL ASPECTS OF QUALITY OF RICE VARIETIES OF SPECIAL PURPOSE

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Rice is the oldest cultivated cereal and staple food for more than half of humanity, which serves as an excellent source of complex carbohydrates, minerals, vitamins and antioxidants. Domestic rice-growing industry is now an integral part of the grain agro-industrial complex, which has important place in its socio-economic sphere. Varieties of special and functional purposes occupy a special place in rice assortment and the products made from them often have the unique, more high nutritional qualities. The objective of the present work was to study technological parameters of domestic varieties of special purposes. In 2014-2015 we have conducted the research of technological quality traits and nutritional qualities of rice varieties with colored pericarp of ARRRI breeding: Mars, Mavr, Yuzhnaya Noch, Gagat, Ryzhik. Thousand weight of dry grain of studied forms was from 21,5 g for variety Yuzhnaya Noch in 2015 to 27,6 g in 2014 for variety Ryzhik; hull content - from 18,0% for Mars in 2014 to 22,0% for Gagat in 2015; vitreousness - from 70% for Rubin in 2015 to 91% for Mars in 2015; fracturing - from 12 % for Mars and Gagat in 2015 to 21% for Mavr in 2015. All varieties have shown tendency for lowering of 1000 dry grain weight in 2015. Mars and Mavr belong to the long-grain varieties, Rubin and Mavr – to the medium-grain, Ryzhik and Yuzhnaya Noch – to the short-grain. The smallest outcome of brown rice was marked in variety Gagat in 2015– 77,9%, maximum – in short-grain variety Ryzhik - 81,9 % in 2015. Amylose content in rice grain starch is the most important biochemical trait of the variety. In 2015 increase of the trait «amylose content un the grain» was typical for all the varieties. Yuzhnaya Noch is non-amylose glutinous variety, which doesn’t have amylose in grain starch. The smallest accumulation of amylose in grain starch was shown in variety Mars: in 2014 - 18,0%, in 2015 – 19,9%. In 2014 maximum amylose content was in variety Mavr – 22,7% , in 2015 – in Rubin – 20,8%. By amylose content varieties Rubin, Mavr, Gagat belong to group of medium amylose varieties, Mars – to group of low-amylose. Rice varieties of domestic breeding with colored pericarp are characterized by different rates of technological traits. Being glutinous, low- and medium-amylose they can be used in functional nutrition for cooking both sticky and crumbly dishes.

ADAPTABILITY OF SPRING BARLEY COLLECTION GROWN IN KAZAKHSTAN

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Barley is important crop worldwide and in Kazakhstan, and valuable source for animal feeding, malting industry, and dietary food. In Kazakhstan there is united barley genetic and breeding community working on understanding of plant adaptation to various environmental regions of the country. In this study we assessed yield performance of spring barley accessions from Kazakhstan and the USA. The collection consisted from 92 samples from Kazakhstan and 574 accessions from the USA and grown in key barley growing regions of Kazakhstan during 2009-2015 The analyses allowed the identification of a large number of accessions from the USA with high agronomic performances in several regions of Kazakhstan. The AMMI and Biplot methods were used for the assessment of genotype-environment interaction patterns. The relationship among plant growth parameters and yield components were established. In addition, the collection tested for several key grain quality traits. Ninety two accessions from Kazakhstan were genotyped by using 9K SNP Illumina array. The genetic variation studied using selected 5K polymorphic SNPs for Kazakh samples and 3K SNP data for the US barley accessions. Genetic and phenotypic data used for GWAS mapping of QTL associated with agronomic traits, grain quality parameters and resistance to stem rust and spot blotch. The results can be used in developing DNA markers associated with agronomic traits of barley for enhancement of breeding activities in Kazakhstan.

PROBLEM OF MANAGEMENT OF TECHNOLOGICAL WHEAT GRAIN PROPERTIES WITHIN THE FOOD CHAIN "GRAIN-FLOUR-BREAD"

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The main criteria of food security include: physical and economic availability, quality and safety of food products. Providing physical and economic availability, quality of bread and bakery products is determined by availability of sufficient production volumes of grain with proper baking properties for providing the population with finished goods according to demand and rational consumption rates. Now the low-quality products with such characteristics as raised friability, thick crusts, lack of a typical "grain" smell and taste, the increased mouldiness dominate in the Russian bread market. Search for the issue solutions of increasing grain technological properties is carried out in various spheres, including the scope of molecular and genetic approaches. Plants genomes sequencing and modern biotechnological approaches open new prospective of receiving the grain, having the necessary baking quality. However, the problem of finished goods production from low-quality grain even more often is resolved due to usage of chemical additives and improvers in practice. It is shown that the molecular and genetic approaches, combined with traditional selection methods, create opportunities for the accelerated creation of the new grades, adapted for conditions and requirements of the baking industry due to usage of natural genetic potential of wheat. Besides, obtaining the new types of products with the increased nutritional and biological value is possible. For example, trial usage of the grain, possessing the increased antioxidant activity at bread production are given. This example is an illustration of feasibility of focusing in the course of molecular and genetic research on the need of coordination of initial and final stages of the food chain "grain-flour-bread". Thus, the use of molecular and genetic approaches for management of technological properties of wheat grain will allow not only preserving bread as a traditional Russian product, but also having a new opportunity of creation of functional food in the market of bread and bakery products.

FINE ANALYSIS OF ChIP-SEQ DATA FOR EIN3 BINDING IN *A. THALIANA* L. REVEALS DIFFERENT LAYERS OF EIN3 REGULATION IN ETHYLENE SIGNALING

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Plant hormone ethylene regulates a wide range of physiological processes during plant development and coordinates different stress responses. Among others, ethylene controls such practically significant characteristics of agricultural crops as fruit ripening rates and plant tolerance to stress conditions. For this reason, understanding molecular mechanisms underlying ethylene action is one of the basic questions in plant biology. Transcription factor (TF) EIN3 is a master regulator of ethylene signaling pathway, but the mechanisms implied by EIN3 for regulation of a majority of transcriptional responses are still largely unknown. We used chromatin immunoprecipitation followed by genome-wide sequencing (ChIP-Seq) data analysis and the whole-genome maps of chromatin types in *Arabidopsis thaliana* to investigate EIN3-DNA interactions and reveal details of EIN3 action. We found the heterogeneity of peaks distribution in gene promoters at various distances from transcription start sites (TSS), within the gene bodies, introns and intergenic spacers. EIN3 peaks were enriched within 300 bp upstream TSS and in intergenic spacers. To finer characterize EIN3-DNA interactions, we distinguished EIN3 peaks located in chromatin domains of states 2 and 4 according to the whole-genome map of chromatin types in *A. thaliana*. These domains were referred to as proximal and distant 5’regulatory regions of genes, correspondingly. The nucleotide context of EIN3 binding sites was more conserved in distant chromatin domains than in proximal ones. *De novo* motif search in close proximity to EIN3 motifs, conducted with Homer tool, revealed several distinct motifs, specific for either proximal or distant chromatin domains. We believe that they represent binding sites for EIN3 partner TFs. Distinct functional roles of these motifs was supported by gene ontology (GO) annotation results. Based on the results we consider that EIN3 regulation of gene expression is mediated by at least “proximal” and “distant” types of EIN3-DNA interactions. The work was supported by RFBR 15-34-20870.

POPULATION OF EASTERN EUROPEAN WINTER COMMON WHEAT VARIETIES AS THE MODEL OBJECT AND SOURCE FOR FURTHER UNDERSTANDING OF MOLECULAR MECHANISMS OF END-USE QUALITY FORMATION IN WHEAT

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Bread-making quality is one of the main characteristics of common wheat. It extensive investigation since 60th-80th of XX century allowed to make hypothesis about three main genetic systems: gliadins (*Gli*), glutenins (*Glu*) and grain hardness (*Ha*), which predominantly can predict end-use quality in common wheat. However a number of recent investigations demonstrated that a lot of QTL from different chromosomes of wheat which genetic nature still is unclear as well made their input in final expression of end-use quality traits. In Ukraine since 60th XX the main aim of breeding programs was to develop the common winter wheat varieties with strong and extra strong characteristics without decrease in yield. The most breeding institutions applied MAS of accessions with favorite gliadins and glutenins alleles for which the positive influence on bread-making was reported. Such approach increased the quality of registered varieties, but in state register still there are varieties with strong, filler and valuable bread-making characteristics. In present research we studied 4 main trait of wheat quality (grain protein content (GPC), gluten content (GC), bread loaf volume (BLV) and dough strength (DS)) and allelic characteristics of *Gli*, *Glu* and *Ha* loci of more than 129 winter common wheat varieties allowed to dissemination in Ukraine in 2007-2009. The low level of polymorphism was observed in all of those three genetic systems: *Ha* (2 allels), *Glu-A1* (a, b), *Glu-B 1*(b, c, al), *Glu-D1* (d) and the same tendency observed in *Gli* loci. Despite the wide range of variability of investigated varieties by GPC, GC, BLV and DS the allele variations of *Gli*, *Glu* and *Ha* loci did not explain their high and low end-use characteristics with exception of *Glu-B1*al (DS) and several *Gli-A1* (DS) and *Gli-B1* (BLV) alleles. Therefore selected varieties are very promising sources for further investigation of genetic and epigenetic mechanisms of end-use quality formation in wheat due to their high variability by investigated bread-making traits and low polymorphism in *Gli*, *Glu* and *Ha* loci.

SPONTANEOUS RYE MUTATIONS OF ANTHOCYANIN BIOSYNTHESIS GENES AS A SOURCE FOR DEVELOPMENT OF WHOLE GRAIN FUNCTIONAL FOODS

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Rye grain is reach in numerous phytochemicals with certain pharmacological properties. Anthocyanins are shown to reduce the risk of cardiovascular, neurodegenerative, ophthalmological diseases and some types of cancer. Recessive mutations in any of six genes *vi1-vi6* lead to absence of anthocyanins in all parts of plant including grain. Two dominant genes control anthocyanin biosynthesis in aleurone (*C*) or pericarp (*Vs*), resulting in green or violet grain color, correspondingly (Smirnov, Sosnikhina, 1984). The recessive homozygotes for each of these genes have yellow grain color. The anthocyanin composition of rye grains with different genotypes is not well studied. Meanwhile, qualitative and quantitative content of anthocyanin and their precursors may differ to a great extent between genotypes, and so health-improving effect may also differ. Three rye lines were used for HPLC analysis of anthocyanin in grains. One line carries dominant *Vs* gene and two other were homozygous for anthocyaninless mutation *vi1*. No anthocyanins at detectable level were found in the grains of these two lines. At the same time the violet seeds contain a set of 10 anthocyanidin glycosides, which were identified as three derivatives of cyanidin, one of delphinidin, four of peonidin and two of petunidin. Delphinidin 3-o-rutinoside which is predominant in blue-green-seeded rye lines (Dedio et al. 1969) was not identified. The reason may be the homozygosity of violet-seeded line for recessive allele for green aleurone gene (c). Violet color of pericarp conceals the yellow or green color of aleurone, and chromatography is needed to find lines or segregants with dominant alleles (C and Vs) to produce genotype enriched with anthocyanins. Another promising mean to combine mutations in different genotypes is development of corresponding molecular markers and it is now being devised. The work is supported by RFBR grant № 16-04-00411 and Program of Presidium RAS “Bioderversity of Natural System” grant № 0112-2015-0012.

AUTHOR INDEX

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