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«ИСПОЛЬЗОВАНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ ПШЕНИЦЫ В СЕЛЕКЦИИ»

MAINTENANCE AND UTILISATION OF WHEAT GENETIC RESOURCES FOR FUTURE BREEDING

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Key words: *genetic integrity, genetic diversity, molecular tools, seed longevity, seed dormancy, association mapping*

As estimated by FAO world-wide existing germplasm collections contain about 7.4 million accessions of plant genetic resources. Wheat (*Triticum* and *Aegilops*) represents the biggest group with about 900,000 accessions. One of the eight largest *ex situ* genebanks of our globe is located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben. As on the global scale wheat is the largest group having almost 30,000 accessions. Beside the long term storage and frequent regeneration of the material molecular tools are used for characterization and utilization of the germplasm. Here data are presented on: (I) The genetic integrity of long term stored genebank accessions. For the self-pollinating crop wheat a high degree of identity was revealed, which underlines the efficiency of the precautions taken by the IPK genebank to preserve the genetic integrity. (II) The genetic diversity of wheat germplasm collected at intervals of 40 to 50 years in comparable geographical regions. Here a qualitative, rather than a quantitative shift in diversity was detected. (III) The inter- and intraspecific variation of seed longevity and its relationship to seed dormancy. Genetic studies were initiated. Numerous QTLs were detected indicating the complex and quantitative nature of the trait seed longevity. Some of the loci identified are in genomic regions which co-localize with genes determining agronomic traits as biotic and abiotic stress response. (IV) The exploitation of genebank collections using a genome wide association mapping analysis of a core collection of wheat. The collection was evaluated for agronomic important traits. In order to investigate trait-marker associations the wheat lines were genotyped using diversity array technology (DArT) markers. Many of the associated and mapped markers were detected in genomic regions where major genes or QTLs have been described earlier. However, in addition new loci appeared, providing new opportunities to monitor genetic variation for crop improvement in plant breeding programs.

SMALL GRAINS CEREALS GENETIC RESOURCES NETWORK IN FRANCE

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Key words: *cereals, genetic resources, data management, genotyping, phenotyping*

Genetics resources (GR) are used by breeders since many decades in their plant breeding programs to produce modern varieties by introducing genes of interest (i.e. resistance genes). These resources appear nevertheless under estimated if we focus on abiotic stress tolerances or new agricultural techniques which combine productivity and respect of environment. More recently, new users, such as scientists and farmers, discovered diverse and new sources of interest in screening and exploiting natural diversity conserved in GR collections.
Presently, the major question is how better promoting genetic resource present in collection to answer both to socio-economic evolution of the agriculture and to the development of basic scientific knowledge in genetic and genomic?
In the case of the French cereals GR Network, a share of responsibility based on knowledge and ability of its members have been decided: phenotyping activities are carried out by private breeders who describe genetic resources for specific descriptors through multilocal evaluation network, while the Genetic Resources Centre (GRC) at INRA Clermont-Ferrand (http://www.clermont.inra.fr/umr1095/) produces molecular data in relationship with its high-throughput genotyping platform and manages collections of small grains cereals (multiplication, conservation and distribution) under quality process. Main species of *Triticum* (wheat), *Hordeum* (barley), *Secale* (rye), *Triticosecale* (triticale), *Avena* (oat) genus and their wild relatives are hold in the collection. The main part of the preserved accessions are patrimonial genetic resources (landraces, breeding lines, registrated cultivars) which represent about 11 800 hexaploid wheats, 2 800 tetraploid wheats and wild relatives, 6 550 barleys, 1 200 triticales, 1 200 oats, 450 *Aegilops* sp. and 85 ryes accessions. All these genetic resources form a valuable toolbox as much for fundamental genomic approaches as for basic breeding processes.
Combining phenotyping and genotyping data, the whole genetic resource collection have been structured in smaller functional groups of accessions (i.e. soft wheat FAO collection, INRA core collection,...) in order to facilitate the reply to an increasing number of different requirements by the distribution of adapted samples of accessions.
New panels are under process to give to breeders and scientists new useful tools to study for instance stresses resistance or to develop association studies. All these evaluation data obtained from the French small grains cereals Network will be progressively available through INRA Genetic Resource Website (http://urgi.versailles.inra.fr/siregal/siregal/welcome.do).

RUSSIAN WHEAT GENETIC RESOURCES: CURRENT STATUS AND UTILIZATION

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Key words: *molecular markers, pre-breeding, wheat breeding, wheat genetic resources*

Wheat genetic resources form the basis for breeding and sustainable agriculture development in Russia. The N.I. Vavilov All Russian Institute of Plant Industry (VIR) is responsible for their collecting, conservation, study and utilization. The VIR wheat collection contains more than 38000 accessions and is characterized by a wide botanical, geographical and agro-ecological diversity. The uniqueness of the VIR wheat collection is determined by: (1) a more than 100-year historical period of the formation; (2) comprehensive performance of wheat, collected in Russian Empire before 1917, the USSR from 1922 to 1991 and Russian Federation from 1991; (3) presence of wild and primitive wheats, landraces, collected by the VIR expeditions, in different countries of the world; (4) great intraspecific variability for different traits. Introduction of new material into the wheat collection, multiplication and regeneration of accessions, characterization and documentation of them continue to be the main objectives of the collection management. But all these activities are subordinated to solving of the most important task: screening of collection and revealing of sources with the required genetic variability for wheat improvement. For the successful wheat improvement it is necessary to have genetically diverse initial material. VIR has a rich historical experience in pre-breeding researches. In 1931 C. Flyaksberger and M. Yakubtciner offered to use the global diversity of the wheat collection as starting material for crosses. Providing a support to this approach N.I. Vavilov (1935) insisted on creation of a broad genetic base for breeding, that will include a variability of the whole species and, first of all, the variability related to physiological and agronomic traits. By the late 1980s it is became obvious that effective utilization of wheat resources requires comprehensive knowledge of their genetics. A. F. Merezhko (1994) made an attempt to solve this problem through revealing of an essential part of intraspecific variability for important economic traits and development of donors for breeding. Significant progress in identification of wheat variability was connected with the study of enzymes and seed storage proteins as markers. A celebrated school of proteins research and their use for wheat germplasm evaluation was developed by V. Konarev (Konarev et al., 1996) in VIR. Seed storage proteins, first among all gliadins, were used in estimation of wheat genome constitution, cultivars identification, assessment of the genetic structure of various sets of accessions, identification of duplicates, and testing authenticity of accessions. Research works on these directions are continued up to the present. In recent years, great advances have been made in the world towards the development and application of molecular markers for characterization of plant genetic resources. We used different types of DNA markers in order to investigate genetic differentiation of hexaploid wheats (2n=*6x*=42, *AABBDD*) which is associated with their adaptation to local environments. More close integration of efforts and collaboration among geneticists, molecular biologists and wheat breeders is required for the further progress in pre-breeding researches.

CHARACTERIZATION OF VARIETIES AND BREEDING LINES OF SPRING BREAD WHEAT CARRYING ALIEN GENETIC MATERIAL

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Key words: *spring bread wheat, alien genes, resistance to leaf rust, resistance to powdery mildew, yield*

Transfer of alien genes to wheat from its relatives is the effective way to improve its genetic variability. The first approach to solve this problem is the production of new forms of wheat resulted from a remote hybridization. The study of these forms makes it possible to select the most promising ones which can be used in breeding as donors of new genes determining resistance to biotic and abiotic stresses. The second approach is the development of recombinant wheat genotypes as new forms for breeding using previously established varieties with alien genes that are effectively expressed in the background of common wheat. Both approaches are used in joint research of Siberian Agricultural Research Institute (Omsk) and Institute of Cytology and Genetics (Novosibirsk) for the production of new varieties and promising lines of spring bread wheat. Immune lines of 'Saratovskaya 29' (S29) resulted from the cross between bread wheat variety 'S29' and Dr. P. Savov's (Bulgaria) synthetic amphiploid *T.timopheevii* x *Ae. tauschii*. The immune lines of 'S29' are characterized by a complex resistance to fungal pathogens. A commercial variety of spring wheat 'Pamyati Maystrenko' has been developed on the basis of one of the immune lines. The utilization of wheat varieties carrying wheat-rye translocation 1RS.1BL and genetic material of *Thinopyrum elongatum* (=*Agropyron elongatum*) led to the effective combination of genes controlling resistance to biotic and abiotic stresses in the new varieties of spring wheat 'Omskaya 37', 'Omskaya 38', 'Omskaya 41'. A set of promising lines possessing resistance to stresses, high yield and grain quality were developed using hybridization of alloplasmic lines of common wheat *(Hordeum vulgare*)-*T.aestivum* with wheat lines carrying wheat-rye translocation 1RS.1BL or genetic materials of *T. durum*.

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«ИСПОЛЬЗОВАНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ ПШЕНИЦЫ В СЕЛЕКЦИИ»

IMPLEMENTATION OF GENOME-WIDE SELECTION IN WHEAT

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Key words: *genomic selection, Breeding value, BLUP, LASSO*

With the expected development of thousands of molecular markers in most crops, the marker-assisted selection theory has recently shifted from the use of a few markers targeted in QTL regions (or derived from candidate or validated genes) to the use of many more markers covering the whole genome [1, 2]. Such genome-wide scans are already used for association analysis between polymorphisms at anonymous markers and qualitative or quantitative traits. The condition for success is that a sufficient level of linkage disequilibrium does exist between the markers used for genotyping and the true genes or QTL. This LD is known to show huge variation among species and type of genetic material considered. In selfing species, and particularly among breeding lines, it is expected to range up to 1 cM or more. In such conditions, anonymous markers can be used, for example in mixed model equations, to predict the breeding value of a trait, without reference to actual QTL. We present a case study using DArT markers on the INRA wheat breeding programme, in an attempt to implement whole genome selection as an alternative to phenotypic selection [3]. This investigation assesses different models – Pedigree BLUP, Ridge Regression BLUP, Bayesian Ridge Regression and Lasso – ability to predict yield of genotypes grown during one year (Target Population) with marker and yield data from genotypes grown during 9 other years (Training Population). The prediction coefficients obtained when the target population is a random sample (i.e. cross-validation) of the data are of the same magnitude of those reported by Crossa et al. [4]. However, when the target population is a subset of genotypes studied in a given year (out of 10), the prediction quality is much lower. Implication of results for practical implementation of genomic prediction in breeding programmes is discussed.

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SELECTION AND GENETIC ESTIMATION OF SPRING BREAD WHEAT POPULATIONS OF SIBERIAN SHUTTLE BREEDING NURSERY OF CIMMYT

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Key words: *spring wheat, shuttle breeding, varieties, hybrid populations, initial material, breeding estimation, resistance, leaf and stem rust.*

Western Siberia is one of the leading country regions for production of high quality wheat. However, the regional yield significantly varies by years due to frequent droughts and epidemics of fungal diseases in wet years. The numerous data suggest spring wheat yield losses of up 20–30% during leaf rust epidemics. The stem rust has being observed in Western Siberia for the last 3–4 years. In 2009, the wheat yield losses were 25-30% in the fields of Omsk region. Shuttle breeding effectively solves the matters of adoptive and ecological trends in wheat breeding with the help of involvement new sources of valuable Biological and Economical features as initial material from the world's gene pool. Annually 200–1000 lines and hybrid populations of spring bread wheat in Siberian shuttle breeding nursery (SSBN), created according to the shuttle breeding program between scientific institutions of Western Siberia, Kazakhstan and the International Maize and Wheat Improvement Centre CIMMYT, are studied. The local varieties adapted to region conditions were crossed with sources of resistance to leaf and stem rust from CIMMYT collection, and at their basis a resistant initial material for wheat breeding in conditions of Western Siberia was created. The breeding evaluation of the initial material and isogenic lines for resistance to leaf and stem rust in Mexico, Kenya and Western Siberia was performed. The most competitive hybrid populations of spring bread wheat that are resistant to a wide range of races of stem and leaf rust, including a virulent race Ug99, which can be used in different regions in the case of the global spreading of stem rust, were selected.

SPORE GERMINATION OF *TILLETIA CONTOVERSA* KÜHN (DWARF BUNT OF WHEAT) USED IN DISEASE SELECTION NURSERIES

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Key words: *Dwarf Bunt, TCK*

Tilletia controversa Kühn (TCK) is the causative organism for dwarf bunt, and has been studied due to the potential for yield losses and quality degradation from contaminated grain. The teliospores are slow to germinate and have been characterized as having a long viability [1]. Resistance to dwarf bunt in the US has largely been from germplasm collected from Turkey, and a limited number of resistance genes are known. Since new races occur infrequently, it is important to know viability for teliospores in the soil.

The Utah State Agricultural Experiment Station has collected spores from field infection nurseries over sixty years. Spores had been stored as sori in intact wheat heads in ambient conditions at Logan, UT, USA, and this collection of spores was utilized to evaluate germination in soil extract agar at 4 C under low lights [2] over an eight week period.

During the germination period, spore germination from younger collections was more rapid with initial germination at two weeks. Some germination from spores older than twenty five years was observed. The pattern of germination and differences in conidial morphology of different collections are discussed.

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PROSPECTS FOR THE USE OF CYTOGENETIC COLLECTIONS AND THEIR DERIVATIVE FORMS IN BREAD WHEAT FOR IDENTIFICATION AND MAPPING OF NEW GENES

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One of the staple world crops, bread wheat, has a complex polyploid genome (AABBDD, *2n=6x*=42). Specially developed cytogenetic collections including aneuploid and single chromosome substitution lines are used for the genetic investigations of the species for many years. They enable to determine chromosome carrying the gene for any character. Elaboration of the molecular chromosome maps in wheat gives the possibility to involve into genetic investigations new recombinant material obtained on the basis of substitution lines. It comes from crossing the substitution line with the recipient cultivar and after molecular genotyping the recombinant lines may be used for a precise mapping of genes or loci earlier attributed to the certain chromosome. Three examples of the use of the substitution lines and their derivative forms, recombinant and introgression lines, for search and mapping of new genes and loci will be presented.

(1) In the set of spring wheat substitution lines Saratovskaya 29/Janetzkis Probat (S29/JP) the flowering date was investigated in West Siberia, Bulgaria and Germany. The line for 4D substitution was found to substantially delay flowering. Molecular genotyping of the line showed that it carries in addition a donor fragment of chromosome 7A. A set of recombinant substitution double haploid (RSD) lines was derived and genotyped using both 4D and 7A microsatellite markers. After field tests of the RSD lines two QTLs on 4D (interval *Xwmc0720-Xgwm3000*) and 7A (interval *Xgwm974-Xgwm0060*) chromosomes for flowering date were identified in Bulgaria and Germany and only one on 7A chromosome in Siberia. This may be explained by the shortest growing period in Siberia falling at the longest summer days suggesting the QTL on 4D to be photoperiod responsive.

(2) Activity of the enzyme lipoxygenase participating in many cell physiological processes was studied in the set of Chinese Spring/Synthetic 6x substitution lines. The low level was found in the donor and in the line with 4D substitution. Using the set of introgression lines for this chromosome a new gene for lipoxygenase activity was localized on chromosome 4DS near the marker *Xgdm129*. This position is comparable with that of homoeoallelic lipoxygenase gene on 4B chromosome in durum wheat.

(3) Activity of the enzyme glutathione reductase (GR) supporting a pool of reduced glutathione participating in many metabolic pathways including detoxification of xenobiotics was studied in S29/JP set of lines. The low level of activity was characteristic of the donor and the lines with substitution of 2A, 2B and 2D chromosomes. Therefore, chromosome location of the genes responsible for GR activity was determined for the first time in wheat.

GENE POOL OF WHEAT AND TRITICALE DEVELOPMENT AND USE IN BREADING

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Key words: *accessions, collection, gene pool*

The registered Siberian gene pool of spring wheat in the Institute of Plant Growing and Selection contains more than 2.5 thousand accessions of different origin. Predominantly they are varieties and lines of bread wheat (*Triticum aestivum* L.). There are also 211 registered accessions of winter and 186 of spring triticale.

Apart from them there are about 300 wheat and 200 triticale forms which are in fact heterogenic populations. They are still segregated wheat interspecies hybrids and unstable triticale forms. From these populations we select the typical for the accession plants and deviating ones to form a working collection. If the selected plants give a constant offspring, we register them and include in the Siberian gene pool.

Each accession of the Siberian gene pool has its own catalogues number, VIR catalogues number (if it is registered in VIR, S.Petersburg), the place of origin (country, city), the subspecies name and the name of the variety or line. For example: C-02825, K-65024, the Ukraine, *v.erythrospermum,* Suita.

The sources of the collections of wheat and triticale are: the world collection of VIR, the local old and new varieties, breeders' lines and heterogenic populations of hybrids, spontaneous mutants. VIR sends 50-100 accessions of wheat every year and 30-50 accessions of winter and spring triticale once in three years. Breeders usually transfer 5-15 forms of their competitive trials annually.

We also include in the Siberian gene pool spontaneous mutant spring forms of wheat and triticale, selected from populations of winter varieties, sown in spring. We use spring sowing of winter crops as a selective background for spontaneous mutant plants. These forms are able to transit from vegetative to the generative development in summer sown in spring without vernalization like spring varieties as well as sown in autumn like winter ones. Their winter hardiness does not differ from one of the initial winter populations.

On the ground of the unique collection of the Siberian gene pool, adapted to the local conditions, the breeders of the Siberian Institute of Plant Growing and Selection of Russian Academy of Agricultural Sciences created 17 spring wheat varieties. Such varieties as 'Novosibirskaya 67' (made in collaboration with the Institute Cytology and Genetics of Russian Academy of Sciences), 'Lutescens 25', 'Novosibirskaya 22', 'Novosibirskaya 89', 'Novosibirskaya 29' occupied several hundred thousand ha. Now 'Novosibirskaya 29' is sown on the area about 1 million ha. The total area of growing the spring wheat varieties created by the breeders of the Siberian Institute of Plant Growing and Selection in the Siberian region is more than 1.5 million ha. Winter triticale varieties 'Tsecad 90' and 'Sirs 57' are grown in this region on the area about 10 thousand ha.

COMPARISON OF METHODS OF OBTAINING THE GENETIC DIVERSITY FOR THE SELECTION OF WHEAT TO WINTER HARDINESS IN SIBERIA

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Studies in our laboratory showed that the winter-hardiness of wheat depends on its ability to maintain a minimum level of metabolism (inner rest) during the winter and the ability to develop frost resistance in excess of the minimum temperatures in the soil. Plants of 11 varieties of the intensive type were treated by gibberellic acid (GA3) in the fall in two consecutive generations. Plants wintered in the soil under the temperature not below -10 ° C. As a result, 225 samples were isolated, overwintering at 85-100%. Survival of the original samples did not exceed 10%. Seventy two samples of 225 were preserved after overwintering under the soil temperature 0°C. Selection on the ability to maintain inner rest took place in these experiments. More rigorous selection for this indicator has increased the proportion highly frost-resistant genotypes. This showed 3-year test of survival in the steppe (the soil temperature dropped below -30°C). Seventeen genotypes of the first group and 40 genotypes from the second survived. Therefore, the low level of metabolism (inner rest) during the winter is crucial for selection for frost resistance. This is confirmed by the results of similar tests of wheat hybrids. One of their parents were the genotypes selected by GA3 (long inner rest) and the other were the lines with high frost resistance, isolated from the variety 'Ul'yanovka' (long deep rest and a high frost resistance). Four hundred and forty eight specimens (72%) of 662 hybrids survived in the steppe. In our view, the presence of one or both components of hardiness contributed to this efficiency.

In the case of wheat-wheatgrass hybrids (WWH) the survival was lower (57%). But in this case, the donors of both components of hardiness were inbred clones of wheatgrass (3-5 generations of selfing). Wheat parents of WWH are varieties from European part of Russia that are not wintering in Siberia. In addition, the proportion of WWH survived after exposure to temperature -24°C for 3 days was almost 9 times higher than among the genotypes of wheat origin. Therefore, a wheatgrass was a strong contributor for creating the winter hardiness genotypes, overwintering in Siberia. This is particularly important because only 12 of approximately 15000 wheat sample collection of VIR (S-Petersburg) survived in the steppe.

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«ЭВОЛЮЦИЯ И ТАКСОНОМИЯ ПШЕНИЦЫ»

EVOLUTION OF WHEAT - CYTOGENETIC POINT OF VIEW

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Key words: *wheat, genome, chromosome, C-banding, polymorphism, translocations, intraspecific divergence, evolution*

Evolution of wheat at the karyotype level was studied using C-banding technique and *in situ* hybridization with highly repeated DNA sequences. Comparison of polyploid wheats with their putative diploid progenitors *Triticum urartu* (A-genome), *Aegilops speltoides* (B/G-genome), and *Aegilops tauschii* (D-genome) showed that formation of tetraploid species has led to significant alterations of parental genomes due to chromosomal rearrangements; heterochromatin re-patterning as a result of gain or loss of particular C-bands or changes in their sizes; elimination or amplification of repetitive DNA families; activation or suppression of rDNA loci. On the contrary, formation of hexaploid wheat did not induce any significant chromosome modifications relative to the parental species.

Wild tetraploid wheat showed the highest level of chromosome diversity; many geographic populations were characterized by the specific spectra of chromosomal rearrangements, and the "region-specific" peculiarities of the C-banding patterns were also observed. Comparison of *T. dicoccoides* with cultivated tetraploid species revealed that domestication of wheat caused the significant decrease of chromosome diversity. Evolution of wheat under domestication has probably promoted accumulation of several types of chromosomal rearrangements in wheat. In particular, translocation between 5B and 7A chromosomes dominated among European accessions of *T. dicoccum,* whereas translocation T5B:7B was highly abundant among common wheat cultivars from Western Europe.

THE IMPACT OF TRANSPOSABLE ELEMENTS ON WHEAT GENOMIC DIFFERENTIATION

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Keywords: *LTR-retrotransposon, DNA-transposon, Fatima, Caspar, Lila, genomic differentiation*

Transposable elements constitute a considerable part of the wheat genome, and are distributed over all the chromosome length; several families are accumulated in particular chromosomal regions such as centromeres and subtelomeres. The amplification of transposable elements is the main source of interspecific genomic divergence, which is essential for formation of allopolyploid genome.

We demonstrated the impact of transposable elements amplification on wheat genomic differentiation for the three families – two *gypsy* LTR-retrotransposons *Fatima* and *Lila*, and CACTA DNA transposon *Caspar*. FISH with the probes derived from the elements sequences and with the BAC-clones containing *Fatima*, *Lila* and *Caspar* demonstrated the different distributions in individual hexaploid wheat genomes (A/B and D). The phylogenetic analysis found a clear distinction in the elements sequences between the different wheat genomes. The estimation of the timing of insertion of LTR-retrotransposons showed that proliferation of specific elements took the place during the formation of diploid progenitors 0.5-2.5 MYA of wheat genomes and before the hybridisation event that led to formation of the wild tetraploid wheat *T. dicoccoides.*

The data supposed the common scenario of emergence and proliferation of genome-specific variants of transposable elements, mainly in the diploid species, is characteristic of the evolution of all three genomes in hexaploid wheat.

August 29

ORAL PRESENTATIONS

SECTION

«GENETICS AND BREEDING IN A CHANGING ENVIRONMENT»

29 августа

УСТНЫЕ СООБЩЕНИЯ

СЕКЦИЯ

«ГЕНЕТИКА И СЕЛЕКЦИЯ В ИЗМЕНЯЮЩИХСЯ УСЛОВИЯХ ОКРУЖАЮЩЕЙ СРЕДЫ»

PROSPECTIVES FOR HIGH THROUGHPUT PHENOTYPING IN THE FRAME OF CLIMATE CHANGE

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Key words: *Abiotic constraints, Biotic constraints, FACE, Modelling, Sensors*

Phenotypic analyses are challenged (1) by the probable increased frequency of unfavourable environmental scenarios involving abiotic and biotic stresses and (2) the rapid progress of genotyping. DNA marker assays and sequencing technologies now allow dense genotyping of several thousands of plants at a relatively low cost. The objective of high throughput phenotyping is to provide the agronomic research community (academic and industrial) with infrastructures able to fully characterize panels of genotypes under different environments. This is to be done in the context of rapid development of association genetics and genomic selection for a better plant resilience to environmental cues associated with climate changes. This should involve building highly instrumented platforms in controlled conditions, in field conditions, and at the "Omics" level. They should be able to (i) cope with throughputs of 200-300 genotypes with the necessary number of repetitions, (ii) manipulate and control environmental conditions in order to impose well-characterised scenarios, (iii) perform at a common throughput innovative phenotyping measurements based on functional imaging, innovative sensors and "Omics" measurements. It also involves developing new applications linked to the platforms with technological jumps, in particular concerning new sensors, phenotyping methods, data analysis and databases able to cope with millions of datapoints. Finally, it involves the development of networking and training activities in close interaction between partners, especially in interaction with the scientific community in the field of phenotypic analysis. It is expected that these platforms will participate to the development of sustainable agricultural strategies involving both new cropping systems and cultivars adapted to climate change and utilizing natural resources more efficiently.

GENETIC POLYMORPHISM IN LOCI DETERMINING GRAIN QUALITY OF UKRAINIAN WHEAT VARIETIES

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Key words: *bread wheat, molecular markers, quality of grain*

Eighty-five winter bread wheat varieties and lines that have been mostly developed in Ukraine were analyzed by NIR for hardness and compared with data obtained by PCR-analysis of alleles of puroindoline genes. The 98% of modern wheat cultivars of Plant Breeding and Genetics Institute (PBGI) are characterized by the *Pina-D1a* and *Pinb-D1b* alleles and have hard structure of endosperm. The variation of hardness parameters that were revealed in case of low polymorphism of puroindoline genes indicates the presence additional genes that have influence on the parameters of hardness. By using introgressions from wild relatives breeders increase the variability of milling characteristics and create varieties with special technological qualities. For example, the modern cultivar 'Oksana' that has been developed from hybridization with *Triticum palmovae* is "super soft".

PCR-analysis of modern wheat varieties with primers to the *Wx* loci revealed that mainly Ukrainian varieties have not null-alleles of the *Wx* genes in their genotypes. Molecular breeding were applied for development of new wheat genotypes with low content of amylose in the starch from crossing cv. 'Kuyalnik' × Wx-12 (99ID524) by using set of PCR-markers for the *Wx-A1* (7AS), *Wx-B1* (4AL), *Wx-D1* (7DS) loci.

Alleles of the *Gli* and *Glu* genes make either positive or negative contribution to the baking and technological qualities of flour. For the majority of the best varieties of PBGI a gliadin formula is closed to a formula of 'Albatros odessky'. In general, polymorphism among Ukrainian wheat varieties by high-molecular-weight (HMW) glutenin subunits is low. The three alleles are found at each the *Glu-A1* and *Glu-B1* loci. At the same time, 98-99% if these varieties have no difference in the *Glu-D1* locus (they have subunits 5+10). We have applied PCR-markers recommended by Zhang et al. (2003, 2004) for analysis of polymorphism in the *Gli-1* and *Glu-A3* loci of 14 varieties and 6 near-isogenic lines. We revealed a lower level of genetic polymorphism by allele-specific PCR compared to the whole complex of genetic polymorphisms determined by electrophoresis of storage proteins, but showed that PCR method possesses a capacity to differentiate wheat genotypes that belong to different groups by the allelic variants of the blocks of gliadin components.

ADULT RESISTANCE GENES EFFECTIVE AGAINST LEAF RUST IN WESTERN SIBERIA AND MECHANISMS OF THEIR ACTION

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Key words: *common wheat leaf rust, adult resistance genes, the resistance mechanisms*

For the stable protection of crops against diseases the genes ensuring a durable resistance as well as the mechanisms of their action are of great interest. In the International center CYMMIT, durable resistant wheat varieties with the effect of slow rusting were created on the basis of adult resistance genes [1]. The effectiveness of adult resistance genes to leaf rust varies in the regions of the world. The genes *Lr13* and *Lr22b* protected wheat in Canada, Europe and the European part of Russia, but were ineffective in India [2]. In addition, it is well known that the expression of some genes is influenced by the environmental conditions. Investigations of adult resistance genes effectiveness were made on the model of cv. 'Thatcher' (*Lr22b*) and its near-isogenic lines with the *Lr12*, *Lr13*, *Lr18*, *Lr22a*, *Lr22b*, *Lr34*, *Lr35* or *Lr37* genes in Western Siberia (Omsk, Russia). The *Lr22b* gene of 'Thatcher' was not effective in Western Siberia. *Lr34* had a slow effect at daily average temperature above 18-20^{o}C while at 10-14^{o}C the plants were middle resistant. Under epidemic conditions leaf rust developed at a lesser degree in the lines with the *Lr12, Lr13, Lr18, Lr22a, Lr35* or *Lr37* genes regardless of the temperature. Mechanisms of adult resistance genes action are far from understanding. Cytophysiological aspects of the interaction between the fungus *Puccinia triticina* Erikss. and the lines with the *Lr13*, *Lr34*, *Lr37* genes were investigated. At heading phase, 65-73% of the inoculum was inhibited on flag leaves. Resistance mechanisms appeared on different stages of pathogenesis: appressorium formation was extensively suppressed at leave surface; more than one third of inoculum died at the stage of invasion into stomata; haustorium formation in the colonies was reduced; some of the colonies were aborted before the sporogenesis; spore production was decreased. First active plant reaction was oxidative burst at some of stomatal guard cells which led to a partial death of the appressoria and substomal vesicles. The hypersensitive reaction did not developed, plant cells in the zones of abortive colonies and pustules slowly died at the late stage of pathogenesis. At the same time appositions consisted of polysaccharide callose and lignin accumulated at cell walls. Lignin autofluorescence in colony zones and conducting bundles was similar, but differed from yellow autofluorescence in hypersensitive zones. It is proposed that the adult resistance genes have pleiotropic effect on the pathogenesis. Active mechanisms are connected with oxidative burst at the part of stomata and, probably, induced resistance. Induced resistance may be stimulated both by oxidative burst at the stomata and elicitors from necrotic cells of abortive colonies. Complex of defense mechanisms provide basis for the durable resistance of the crops protected with adult resistance genes.

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RESISTANCE SOURCES OF SOFT WHEAT, ITS RARE TYPES AND *AEGILOPS TAUSCHII* TO NORTH CAUCASIAN RUST AND BLOTCH DISEASES PATHOGENS

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Key words: *wheat, Aegilops tauschii, cultivars, collection samples, resistance sources, pathogens*

North Caucasian region is one of the main areas of winter wheat cultivation; its gross yield of grain is up to 48 % of the total crop yield in Russia. However, the high concentration of spiked crops and favourable weather conditions of the region predispose to frequent and injurious outbreaks. The sources of winter wheat pathogen complex are brown (*Puccinia triticina* Rob. ex Desm. *f.sp. trititci* Erikss. et Henn.) and yellow (*Puccinia striiformis* West. *f.sp.tritici* Erikss. et Henn.) rust pathogens, *Pyrenophora tritici–repentis (*Died.) Drechsler, *Septoria tritici* Rob. et Desm and others. In such situation resistant cultivars occupy an important place in the system of the host plant biological protection. The constant search of reliable sources with wheat group resistance, of its main species and wild varieties is necessary to widen its genetic diversity - that was the aim of the conducted research.
The immunologic assessment of the winter wheat cultivars of P.Lukyanenko Krasnodar Agricultural Research Institute and I.Kalinenko All-Russian Research Institute of Grain Crops (Zernograd, Rostov Region) selection included in RF State Directory or passing state tests (from 37 to 53 depending on a pathogen) was conducted under condition of artificial infectious background. In addition, 393 collection wheat and *Aegilops* samples of N.Vavilov All-Russian Research Institute of Plant Growing were estimated regarding brown (*Puccinia triticina*) and yellow (*Puccinia striiformis)* rust pathogens, as well as *Pyrenophora tritici–repentis* and *Septoria tritici*. Twenty-nine winter wheat cultivars were singled out (55.8 % out of studied ones), resistant to the brown rust pathogen, 12 (25.0 %) – to yellow rust, 5 (13,5 %) – to *Pyrenophora tritici–repentis*; but the cultivars resistant to *Septoria tritici* were not registered. Nine cultivars have group resistance to the pathogens and it should be taken into account by territorial distribution. One hundred fifty-eight sources resistant to *P. triticina,* 117 – to *P. striiformis*, 37 – to *P. tritici–repentis*, 127 – to *S. tritici* were registered among the collection cultivar samples. One hundred twenty samples have group resistance and they are of great interest for selection not only in southern but in other regions of Russia as well.

CHARACTERIZATION OF GENES THAT ARE INVOLVED IN CONTROL OF SPIKE DEVELOPMENT IN WHEAT AND ITS RELATIVES

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Detection and analysis of genes that are involved in the control of spike development in common wheat and related species and affect spike morphology add to the understanding of the genetic regulation of inflorescence development in Poaceae. The goal of this project is investigation of genetic regulation of the cereal inflorescence development in bread wheat (*T. aestivum* L.) and its close relatives. Molecular-genetic studies were conducted on cereal mutants with altered spike morphology that characterized by development of additional spikelets at rachis nodes. It was found that the multirow spike (MRS) character in common wheat and the monstrosum spike in rye had the monogenic recessive mode of inheritance. The *mrs1* gene was mapped on wheat chromosome 2DS and localized in chromosome deletion bin 2DS-5. The *mo1* gene was mapped on rye chromosome 2RS. Location of the *Mrs1* and *Mo1* genes in regions of conservative synteny and similarity between the phenotypical manifestation of *mrs1* and *mo1* mutants may suggest that *Mrs1* and *Mo1* are orthologs. A region of conservative synteny was identifed in the long arm of rice chromosome 7 using COS-SSCP approach and *in silico* mapping of the RFLPs fanking *Mrs1* in the consensus map of chromosome 2DS in the genome of rice (*Oryza sativa* L.). This region hosts the *FRIZZY PANICLE* (*FZP*) gene. A mutation in this gene disrupts the transition from spikelet meristem identity to foral meristem identity. Plants mutant for *fzp* phenotypically resemble *mrs1* and *mo1*. Homologs of the *FZP* gene from rice were identifed in common wheat, rye, cultivated barley, and diploid donors of common wheat genomes A, B, and D. The *FZP* homologs from rye, barley, and Aegilops species of the Sitopsis section were localized on chromosomes of homeologous group 2. In addition to *Mrs1* and *Mo1*, other genes of wheat and related species are known whose mutations are associated with the presence of supernumerary spikelets in rachis nodes and/or rachis branching. For example, the *bh* gene determines multiple spikelets in two common wheat lines, 3492 and 1611, independently obtained by chemically induced mutagenesis. Our study showed that the *bh* gene also locates on chromosome 2D.

QTL ANALYSIS FOR THOUSAND-GRAIN WEIGHT UNDER TERMINAL DROUGHT STRESS IN BREAD WHEAT (*TRITICUM AESTIVUM* L.)

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Key words: *chemical desiccation, drought stress, quantitative trait loci, T. aestivum L., thousand-grain weight, translocation*

Grain yield under post-anthesis drought stress is one of the most complex traits, which is inherited quantitatively. The present study was conducted to identify genes determining post-anthesis drought stress tolerance in bread wheat through Quantitative Trait Loci (QTLs) analysis. Two cultivated bread wheat accessions were selected as parental lines. Population phenotyping was carried out on 133 $F_{2:3}$ families. Two field experiments and two experiments in the greenhouse were conducted at IPK-Gatersleben (Germany) with control and post-anthesis stress conditions in each experiment. Thousand-grain weight was recorded as the main wheat yield component, which is reduced by post-anthesis drought stress. Chemical desiccation was applied in three experiments as simulator of post-anthesis drought stress whereas water stress was applied in one greenhouse experiment. Analysis of variance showed significant differences among the $F_{2:3}$ families. The molecular genetic linkage map including 293 marker loci associated to 19 wheat chromosomes was applied for QTL analysis. The present study revealed four and six QTLs for thousand-grain weight under control and stress conditions, respectively. Only one QTL on chromosome 4BL was common for both conditions. Five QTLs on chromosomes 1AL, 4AL, 7AS, and 7DS were found to be specific to the stress condition. Both parents contributed alleles for drought tolerance. Taking the known reciprocal translocation of chromosomes 4AL/7BS into account, the importance of the short arms of homoeologous group 7 is confirmed for drought stress.

ABOUT POSSIBLE APPLICATION OF THE WHEAT-GRASS HOMOLOGS *VP-1* (VIVIPAROUS) GENES IN IMPROVEMENT OF SOFT WHEAT

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Key words: *bread wheat, Thinopyrum intermedium, wheatgrass, preharvest sprouting, Viviparous gene, wheat-wheatgrass amphiploids, wide hybridization, polymerase chain reaction, molecular markers, marker-assisted selection*

Preharvest sprouting (PHS) results from an early break of seed dormancy and leads to the activation of physiological processes and the beginning of embryo growth. An important stage in studying seed germination (and its special case – preharvest sprouting) is the discovery of *Viviparous* genes. The *Viviparous1* gene (*Vp-1*) is a regulator of late embryogenesis in cereals. Since PHS leads to great financial losses to wheat growers it is necessary to develop resistant cultivars. One of the ways to increase the PHS resistance is to introduce *Vp-1* homologues into the genome of bread wheat from its wild relatives by wide hybridization. Various species of wheatgrass (*Thinopyrum sp.*) are of great interest in such studies. Partial wheat-wheatgrass amphiploids are very useful as an intermediate step in the breeding programs to transfer alien genes into wheat. The aim of our study was to identify the lines of octoploid wheat-wheatgrass amphiploids (*2n* = 56, The Department of Distant Hybridization, Tsitsin Main Botanical Garden) carrying *Vp-1* homologues in their wheatgrass genome and to estimate their resistance to PHS.

The sequencing of intron-exon site of the *Vp-1* homologue of *Th. intermeduim* was performed. On the basis of its sequence we have developed the CAPS marker that allows us to distinguish the *Vp-1* gene of wheatgrass from wheat. The identification of the *Vp-1* wheatgrass homologue in the genome of twelve wheat-wheatgrass amphiploids was carried out using our CAPS marker. As a result, line 209 has appeared to carry the *Vp-1* homologue of wheatgrass origin. The resistance to PHS was estimated by the calculation of weighted germination index (GI). Among the other lines, line 209 has shown the highest level of PHS resistance (GI=0,4). Therefore, line 209 was classified as relatively resistant, while the other lines as susceptible.

To sum up, we have developed the CAPS marker which allows us to identify the *Vp-1* homologue of wheatgrass in the presence of *Vp-1* of wheat. Wheat-wheatgrass amphiploid 209 has been found to carry the *Vp-1* homologue of wheatgrass. It has been demonstrated that line 209 is characterized by the higher resistance to PHS, than the other studied samples. The given line can be useful as a donor of *Vp-1* of wheatgrass origin for further introgression of it into bread wheat genome using the CAPS marker developed by us.

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ORAL PRESENTATIONS

SECTION

«BIOTECHNOLOGY AND MOLECULAR-GENETIC METHODS FOR NEW GENOTYPES DEVELOPMENT»

30 августа

УСТНЫЕ СООБЩЕНИЯ

СЕКЦИЯ

«БИОТЕХНОЛОГИЯ И МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ МЕТОДЫ ДЛЯ ПОЛУЧЕНИЯ НОВЫХ ГЕНОТИПОВ»

PARENTAL TYPES OF MITOCHONDRIAL AND CHLOROPLAST DNA IN THE PROGENIES OF HYBRIDS OF COMMON WHEAT DEPEND ON CROSS DIRECTION AND CYTOPLASM ORIGIN

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Key words: *barley-wheat hybrids, rye-wheat and wheat-rye hybrids, alloplasmic lines, euplasmic lines, mitochondrial DNA, chloroplast DNA*

This work dealt with the study of mitochondrial (mt) and chloroplast (cp) genomes of alloplasmic and euplasmic wheat lines obtained on the basis of barley-wheat, rye-wheat and wheat-rye hybrids. Alloplasmic line with $7H^{m}L(7D)$ chromosome substitution was obtained by repeated backcrossing of *Hordeum marinum* subsp. *gussoneanum* x *Triticum aestivum* hybrids with wheat variety Pyrotrix 28 and self-pollination. Euplasmic line with the same type of chromosome substitution was obtained by crossing of monosomic 7D line (maternal parent) with alloplasmic line with $7H^{m}L$ chromosome addition (paternal parent). Thus, alloplasmic and euplasmic lines with the same type of chromosomes substitution were obtained.

We found out the relationship between the parental type of mitochondrial (*18S/5S*) and chloroplast (*ndH, infA, rpo, psaA*) examined loci and the level of plant fertility in alloplasmic $7H^{m}L(7D)$ substituted lines. Mitochondrial DNA heteroplasmy (simultaneous presence of barley and wheat copies) was detected in partly fertile lines and homoplasmy (barley copies) was detected in sterile lines. All examined lines contained maternal barley copies of cpDNA. Comparative analysis of euplasmic lines, containing wheat cytoplasm and wheat genome with the same type of barley-wheat chromosome substitution as in alloplasmic lines, revealed that they possessed wheat mitochondrial and chloroplast copies. Thus, it enabled us to conclude that in case of alloplasmic lines the detection of barley cytoplasmic copies wasn't the result of barley chromosomes DNA amplification.

Another type of alloplasmic and euplasmic lines was obtained by backcrossing and self-pollination of rye-wheat and wheat-rye hybrids. The degree of fertility differs in reciprocal crossing: most of the progenies of wheat-rye hybrids were fertile whereas the progenies of rye-wheat hybrids in most cases were sterile. Study of 18S/5S mtDNA and *ndH, infA, rpo, psaA* chloroplast loci in reciprocal F_1 hybrids, triticale and wheat-rye additional lines revealed different parental types of mt- and cpDNA copies. The progenies of *S. cereale* x *T. aestivum* hybrids contained heteroplasmy (rye and wheat copies) of 18S/5S mtDNA and homoplasmy (rye copies) of cpDNA. All progenies of *T. aestivum* x *S. cereale* hybrids contained wheat copies of mt- and cpDNA loci examined.

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DEVELOPMENT OF A MODEL SYSTEM TO STUDY GENE INTERACTIONS OF PARENTAL FORMS IN WHEAT-RYE HYBRIDS AND PRIMARY TRITICALE

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Key words: *wheat-rye hybrids, genetic analysis, embryo lethality, hybrid incompatibility*

A special genetic model system to study gene interaction of parental forms in wheat-rye hybrids and primary triticale was developed. This model permits to reveal and map the wheat and rye genes which are specifically expressed in new complex triticale genome. Genetic model includes the well-characterized common wheat cultivar 'Chinese Spring' ('CS'), the sets of nulli-tetrasomic (CSNT), wheat-rye additional (WRA) and deletion 'CS' lines as maternal forms and the big set (100 lines and more) self-fertile inbred lines from genetic collection, interline F_1 rye hybrids and the set of RILs developed from F_2 population of such hybrids as paternal forms. 'CS' and its derivates also carry genes for high crossability with rye that allow to take in big set of offspring from each cross. Using this model system the genetic analysis of embryo lethality arises as the result of interaction of wheat and rye speciation genes was performed. Rye gene, causing embryo lethality in crosses with common wheat was revealed using interline F_1 rye hybrids and named *Eml* (Embryo lethality). Therefore, it was concluded that the *Eml* gene has two alleles: compatible (*Eml-R1a*) and incompatible (*Eml-R1b*) with wheat genome. Molecular markers associated with F_2 genotypes derived from a contrasting rye inbred progeny (L7xL2) were used for a linkage study. Recombinant inbred lines of F_5 population served as testers. *Eml-R1b* maps to chromosome arm 6RL, along with two co-segregating microsatellite loci, *Xgwm1103* and *Xgwm732*. Inheritance mode of *EmlR1* was tested in crosses with 'CS' wheat-rye addition lines (WRA6R, WRA6RL). It was shown, that the mutant allele *Eml-R1b* causing embryo lethality in crosses of common wheat with rye is dominant, and the wild type allele *Eml-R1a* is recessive. The wheat gene complemented to rye *Eml-R1b* was revealed in crosses of CSNT lines with rye line carrying the incompatible *Eml-R1b* allele. Only in crosses of lines N6A/T6B and N6A/T6D with such rye line the embryos have shown the normal embryogenesis so it follows that wheat chromosome 6A carries a gene complementing to rye gene *Eml-R1b*. This wheat gene was named *Eml-A1*. Analysis of hybrid embryos revealed morphological differences at the age of 16 days after pollination. It was found that interaction of wheat and rye incompatible alleles arrest the formation of shoot meristem (SAM), but had no influence on root meristem (RAM) formation. Method for overcoming such kind of hybrid embryo lethality between common wheat and rye was developed and plantlets with "lethal" genotyped were successfully grown. Now the model for studying the differences in interaction and expression of incompatible wheat and rye alleles causing embryo lethality is developed: 1) after double fertilization both incompatible alleles (*Eml-A1* and *Eml-R1b*) are present in hybrid genotype – SAM is arrested – the interaction is present - conflict between incompatible alleles; 2) after double fertilization both incompatible alleles are present in hybrid genotype – callus induction from abnormal embryos – regeneration from callus culture – SAM is present – the interaction is present (?) – no conflict between incompatible alleles; 3) embryogenesis in triticale with "lethal" genotype (not studied yet); 4) The crosses of N6AT6B or N6AT6D with L2 – the hybrid embryos carries only rye incompatible allele *Eml-R1b* - SAM is present – the interaction is absent.

WHEAT ANTIMICROBIAL PEPTIDES

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Key words: *plant immune system, antimicrobial peptides, amino acid sequence, cDNA and gene structure, regulation of gene expression, biotic and abiotic stress*

Antimicrobial peptides (AMPs) are natural antibiotics produced by all living organisms to resist infection by pathogens. They are important effector molecules of the innate immune system both in animals and plants. AMPs display broad-spectrum antimicrobial activity, and thus show promise for engineering pathogen-resistance in crops and development of novel pharmaceuticals.

AMPs from the wheat *Triticum kiharae* Dorof. et Migusch., which is highly resistant to pathogens were studied. Using multidimensional chromatography in combination with MALDI TOF mass spectrometry and Edman sequencing 24 novel AMPs were isolated from wheat seeds, which were partially or completely sequenced. The results showed that wheat seeds contain nearly all known families of plant AMPs. Novel structural types were also discovered. Among them, the 10-Cys hevein-like AMPs named WAMPs and a family of 4-Cys peptides with a characteristic cysteine motif: XnCXXXC(11-14X)CXXXCXn and potent antimicrobial activity. The cDNA and genomic DNA sequences encoding WAMPs and 4-Cys peptides Tk-AMP-X1 and Tk-AMP-X2 were determined. The deduced precursor proteins of WAMPs consist of a signal peptide, a mature peptide domain and a C-terminal prodomain. The Tk-AMP-X1 and Tk-AMP-X2 peptides are derived from complex multimodular precursors consisting of 5 or 7 4-Cys peptides with a characteristic cysteine pattern, a signal peptide region and a C-terminal prodomain. No introns were found in the protein-coding regions of the genes studied. The distribution of WAMP and 4-Cys peptide genes in related *Triticum* and *Aegilops* species was analyzed, and highly homologous sequences were discovered in a number of species tested. Analysis of *wamp* mRNA accumulation revealed constitutive expression of *wamp* mRNA in 3-5-day-old seedlings. The reaction of seedlings to pathogens depended on the fungus tested. Infection with *Fusarium* species caused a considerable increase in *wamp* mRNA level. Quantification of mRNA level in response to *A. niger* revealed no upregulation of *wamp* genes. However, sequencing of cloned PCR products revealed several transcripts: instead of transcripts detected in healthy seedlings, a novel predominant transcript was identified in diseased seedlings. Thus, infection of wheat with *A. niger* induced transcription reprogramming and post-transcriptional alternative polyadenylation. Analysis of *wamp* mRNA accumulation in response to elevated NaCl concentrations revealed a considerable increase in *wamp* mRNA levels. The 4-Cys AMP genes were similarly upregulated by biotic and abiotic stress highlighting specific roles of these genes in wheat stress response mechanisms.

The work was supported in part by the RFBR grant no. 09-04-00250 and grant of Ministry of education and science GC no. 16.512.11.2156.

DEVELOPMENT AND UTILIZATION OF LEAF RUST RESISTANCE LINES WITH MULTIPLE AND SINGLE INTROGRESSIONS OF THE *T. TIMOPHEEVII* GENOME

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Key words: *introgression line, pathogen resistance, microsatellite markers, T. timopheevii*

Broadening of genetic diversity of modern wheat cultivars by identification and introgression of useful genes from wild wheat relatives is important goal of plant breeding. A collection of 80 introgression lines (ILs) was obtained from crosses of tetraploid wheat *T. timopheevii* ssp. *viticulosum* with six common wheat (*T. aestivum* L.) cultivars: 'Saratovskaya 29', 'Skala', 'Novosibirskaya 67', 'Pirotrix 28', 'Irtishanka', and 'Tcelinnaya 20'. Pathogen resistance of introgression line collection to leaf and stem rusts, powdery mildew, spot blotch and loose smut was evaluated in the field tests in Central and West Siberian Regions of Russian Federation, Caucasia and Kazakhstan. All the lines proved to have a higher resistance to leaf rust than the recipient wheat cultivar, however, the resistance varied in different lines from immunity to moderate resistance. In addition, three lines with high immunity to stem rust, 20 lines resistant to powdery mildew, 6 lines resistant to spot blotch, 15 lines with loose smut resistance and 6 lines possessing complex resistance to various fungal diseases were found among the introgression lines.

Chromosomal localization of the *T. timopheevii* genome fragments analyzed by means of microsatellite (SSR) markers showed high frequency of substitutions and translocations in chromosomes 1A, 2A, 2B, 5B, and 6B. Comparison of ILs obtained by different cross combinations demonstrated that the genotype of parental wheat cultivar determined the level of introgression of the *T. timopheevii* genetic material. Molecular mapping revealed tree loci *QLr.icg-5B*, *QLr.icg-2A*, and *QLr.icg-1A* on chromosomes 5B, 2A and 1A, respectively, controlling resistance to leaf rust.

To create a set of introgression lines carrying single introgressions or combinations of *QLr.icg-5B*, *QLr.icg-2A*, and *QLr.icg-1A* loci, ILs with multiple introgressions were backcrossed three times to initial common wheat cultivar. Selection of plants containing loci for resistance was performed in BC_2F_1 and BC_3F_1 progenies with microsatellites specific for *T. aestivum* (BBAADD) and *T. timopheevii* (GGA^tA^t) genomes. As a result, eighteen ILs carrying different combinations of leaf rust resistance loci were developed. It was shown that all the lines containing *QLr.icg-5B* loci were resistant or moderately resistant to leaf rust pathogen. Occurrence only minor loci *QLr.icg-2A* and *QLr.icg-1A* in genome of introgression line decelerated disease development. The obtained ILs can be used as a source of resistance genes in breeding programs for development of new common wheat cultivars.

ALLELIC DIVERSITY OF *VRN* AND *PPD* GENES OF THE RUSSIAN LOCAL WHEAT CULTIVARS AND THEIR APPLICATION FOR MARKER ASSISTED SELECTION

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Key words: *wheat, photoperiodic sensitivity, vernalization, Ppd, Vrn, allele-specific markers, marker-assisted breeding*

In wheat, flowering time is a highly variable phenotypic trait with major implications for adaptation to geographic regions. The major genetic determinants of this physiological trait are the *Ppd* genes that control photoperiod response and the *Vrn* genes that determine requirement for a period of low temperature for a plant to make the transition from a vegetative to a reproductive state. Acting together the two groups of genetic factors determine flowering time and hence the basic adaptation of a genotype for a particular environmental condition. There are various wheat ecotypes propagated across regions of the Russian Federation. It is expected that each of the ecotypes can be described by the certain allele combination of the *Ppd and Vrn* genes that match most perfectly to the specific local climatic condition. To develop productive genotypes with optional parameters of vegetative period breeders might need information about *Ppd* and *Vrn* alleles existing in local wheat cultivars as well as in breeding lines under selection. The recent cloning of vernalization and photoperiod response genes in wheat has facilitated the development of gene-specific markers. These markers provide a unique opportunity to screen large collections of wheat germplasm for allelic diversity at the *Vrn* and *Ppd* genes.

A research project was initiated in the spring of 2011 to assess *Vrn/Ppd* allele combinations among 320 wheat cultivars registered in the Russian Federation and the Republic of Belarus. As a first step of the project we examined 60 advanced hexaploid bread wheat varieties that currently are propagated in the North Caucasus (Krasnodar) and the South Ural (Chelyabinsk) using the diagnostic *Vrn* and *Ppd* gene-specific molecular markers. For all of the wheat varieties alleles of the *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-D1* genes were identified. The relationships between *Vrn/Ppd* allele combinations and heading date in the presence/absence of vernalization were investigated under long and short day conditions. The wide-ranging distribution of the photoperiodic insensitive *Ppd-D1a* allele among Krasnodar wheat cultivars was observed whereas only the sensitive *Ppd-D1b* allele was detected among wheat cultivars grown in the South Ural region. The *Vrn/Ppd* allele combination was found to significantly affect both earliness and productivity of the wheat cultivars. Marker assisted selection of wheat genotypes with determined *Vrn/Ppd* allele pattern might be employed as a tool for establishing of sustainable wheat production throughout the diverse agro-ecological zones of Russia.

SYNTHETIC FORMS: A BASIS FOR PRESERVATION AND USING OF A GENE POOL OF WILD RELATIVES OF COMMON WHEAT

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The gene pool of numerous wild relatives represents an inexhaustible reserve of a genetic variability for wheat improvement. One of the important approaches to achieve a successful gene transfer is production and use of synthetic forms instead of wild relatives.

The synthetic genome substitution forms 'Avrodes', 'Avrosis', 'Avrolata', 'Avroale', and 'Avrocum', where the D genome of common wheat, *Triticum aestivum*, was substituted for genomes of *Ae. speltoides*, *Ae. sharonensis*, *Ae. umbellulata*, *S. cereale*, and *Ag. glaucum*, respectively, were obtained. In addition, the genome-added forms, in which the genome D from *Ae. squarrosa* was added to the *T. militinae* AG and durum wheat *Mutico italicum* AB genomes, were developed. These forms represent a qualitatively new product, where genetic material of the wild relatives has become more accessible. The above forms have the same ploidy level with common wheat and are easily crossed to it. The progeny of such crosses have a good viability. Neither monosomic series nor alien chromosome addition lines are necessary for the transfer of alien genetic material to wheat genome.

As a result of regular observation and the cytological control it was determined that the synthetic forms remain constant in respect to the morphological and biological attributes and maintain a high cytological stability during many years. The synthetic forms show 21 bivalent or close configuration in MI of PMC. In the synthetic form 'Avrodes', 18.4 bivalents, 2.3 univalents and 0.57 multivalents were observed on the average. The highest number of multivalents in the form 'Avrodes' is a result of its ability to suppress the activity of wheat *Ph* system and to promote pairing between homoeologous chromosomes due to the presence of the S genome from *Ae. speltoides.*

Synthetic forms were used for producing secondary recombination synthetic forms (RS forms), in which the first two genomes A and B are derived from common wheat, and the third genome is represented by chromosomes of different alien species. Fertile RS forms with genome formulae AABBSR and AABBSS^{sh} were obtained.

Through the use of the synthetic forms a large set of cytologically stable lines with resistance to diseases was produced. Some of them display a high protein content. In most cases, introgression of genetic material from *T. miguschovae* and *Ae. speltoides* to the common wheat genome was realized *via* chromosome substitutions and translocations. Genetic material of *Ae. umbellulata* and *Ag. glaucum* in the lines is presented by one pair of substituted chromosomes. The hybridological and PCR analysis indicated that leaf rust resistance genes of the lines differ from each other and from the known effective genes *Lr9, Lr19* and *Lr24*.

The synthetic forms represent a unique genetic basis for preservation and use of a gene pool of wild relatives in breeding of wheat and open new opportunities in cytogenetic and phylogenetic studies. Five common winter wheat cultivars were developed with the use of the synthetic forms.

August 31

ORAL PRESENTATIONS

SECTION

«BIOINFORMATICS AND GENOMICS»

31 августа

УСТНЫЕ СООБЩЕНИЯ

СЕКЦИЯ

«БИОИНФОРМАТИКА И ГЕНОМИКА»

STRUCTURAL ORGANIZATION AND TRANSCRIPTION OF A NEW *VRN-B1* ALLELE OF WHEAT *T. AESTIVUM* L.

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Key words: *vernalization, near-isogenic line, gene, allele, earing time, transcription, wheat*

Effects of various alleles of the dominant *Vrn-B1* gene on earing time have been prviously studied in two near-isogenic lines (NILs) of winter wheat 'Bezostaya 1' having genotype *vrn-A1*, *Vrn-B1*, *vrn-D1*. It has been found that the earing time difference between the lines grown in different settings is related to two differently expressed alleles: $Vrn\text{-}B1^{S} > Vrn\text{-}B1^{Dm}$. The earing time in the lines with the $Vrn\text{-}B1^{S}$ allele inherited from 'Saratovskaya 29' begins 7–10 days earlier than in the lines with weak $Vrn\text{-}B1^{Dm}$ of 'Diamant 2' [1].

We found significant differences in the structure of the first intron of the $Vrn\text{-}B1^{S}$ allele when compared to $Vrn\text{-}B1^{Dm}$ (*Vrn-B1a*), specifically, the deletion of 0.8 kb coupled with the duplication of 0.4 kb. We suggested that these changes in intron 1 of $Vrn\text{-}B1^{S}$ caused earlier ear emergence in the corresponding NIL. The unusual structure of intron1 within the $Vrn\text{-}B1^{S}$ allele was described for the first time in this study. We designated the new $Vrn\text{-}B1^{S}$ allele as *Vrn-B1c* to distinguish it from the previously studied *Vrn-B1a* allele [2]. We showed that transcription of the *Vrn-B1c* allele starts earlier than transcription of the *Vrn-B1a* allele, leading to earlier initiation of reproductive phase in corresponding NIL. We also observed that the dominant *Vrn-1* allele induces the transcription of the recessive alleles of homoeologous genes, so the coordinated transcription of dominant and recessive alleles may contribute to an attainment of the *Vrn-1* transcript level required to trigger flowering initiation in polyploid wheat.

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HOMOEOLOGOUS CHALCONE FLAVANONE ISOMERASE GENES IN ALLOHEXAPLOID WHEAT

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Key words: *chalcone flavanone isomerase, flavonoid biosynthesis, common wheat, homoeologous genes, gene mapping*

Biosynthesis of flavonoids is the best characterized pathway among plant species at the biochemical and genetic level. However, despite known essential roles of these compounds not only in plant defense against visible and UV-B radiation, drought and low temperature but also in human diets, because of their antioxidant, antibacterial and anti-cancer activity, genetic bases of flavonoid biosynthesis in different organs in the main agricultural crop - common wheat – are not adequately explored. In particular, only a few structural genes coding flavonoid biosynthesis enzymes were isolated and characterized in common wheat.
In the current study, we isolated full-length sequences of three wheat genes encoding chalcone flavanone isomerase (CHI, E.C. 5.5.1.6) catalyzing cyclization of chalcone into (2S)-naringenin – precursor of all flavonoid compounds. Comparison of nucleotide and deduced amino acid sequences revealed high level homology (96-97%) among these three genes. Structure analysis showed that they have identical exon - intron structure and consist of three exons and two introns. Interestingly, the others cereals related to wheat have different number of exons and introns in *Chi* gene. For instance, *Chi* genes of maize and rice have four exons and three introns, whereas barley *Chi* gene has the same exon-intron structure as wheat *Chi* genes. Physical and/or genetic mapping of the three *Chi* copies in wheat showed their location in the distal region of the long arm of each of 5A, 5B and 5D chromosomes. It was concluded that these three genes are homoeologues, and therefore they were designated *Chi-A1*, *Chi-B1* and *Chi-D1*, respectively. RT-PCR-based and *in silico* analyses of *Chi* transcripts both showed transcription of these gene in different plant organs (root, coleoptile, leaf, stem, glume, and grain pericarp). Among the three homoeologous copies, *Chi-A1* is characterized by scant transcription activity. This may be related with some differences observed in promoter regions of the homoeologous copies. The 3D-structure analysis of the *Chi-A1*, *Chi-B1* and *Chi-D1* products suggested that all these three genes encode functionally active enzymes.

STRUCTURE, ORIGIN AND SPECIALIZATION OF THE *F3H* GENE DUPLICATION IN WHEAT

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Key words*: wheat, gene duplication and specialization, flavonoid biosynthesis, stress response*

The *F3h* gene is a single-copy gene in plant genomes and encodes flavanone 3-hydroxylase – a key enzyme of flavonoid biosynthesis. Duplications of the *F3h* gene have been described in none of the plant species. Recently, we have found the *F3h* gene duplication in the B-genome of hexaploid *Triticum aestivum* L. (BBAADD) [1]. Two *F3h* copies have been mapped on the long arm of chromosome 2B. The *F3h-B1* gene has been mapped in position highly comparable with those of the *F3h* genes in the wheat genomes A and D, that in the rye R-genome, and that in the barley H-genome. The duplication designated *F3h-B2* has been found 40 cM distal to the *F3h-B1* gene position [2].

In the current study, we isolated the full-length *F3h-B2* sequence from *T. aestivum* 'Chinese Spring' and partial sequences of *F3h-B2* homologues from *T. timopheevii* (GGAA) and *Aegilops speltoides* (SS). The *F3h-S2* and *F3h-G2* genes of *Ae. speltoides* and *T. timopheevii*, respectively, were genetically mapped on chromosomes 2S and 2G in positions highly comparable to that of *T. aestivum F3h-B2*. The finding of the *F3h-B2* gene homologues in *T. timopheevii* and in the putative diploid progenitor of the B- and G-genomes, *Ae. speltoides*, but not in the A- and D-genomes or their diploid progenitors, led us to a conclusion that the *F3h* duplication appeared in the B/G/S-genome ancestor.

Like *F3h-1* genes, *F3h-B2* has three exons and two introns. At the same time, significant nucleotide sequence divergence between the *F3h-B2* gene and the *F3h-1* genes was observed, and the *F3h-B2* predicted amino acid sequence was at the C-terminus 30 residues shorter than those of the *F3h-1* genes. These 30 residues belong to the C-terminal signal peptide, whereas the amino acid sequence corresponding to the enzyme had the same length and about 95% homology between *F3h-B2* and *F3h-1*. Furthermore, amino acid residues important for correct 3D-structure and functional activity of the F3H enzyme were conserved in the *F3h-B2* gene. From RT-PCR-based and *in silico* analyses of *F3h* transcripts in wheat it was clear that the *F3h-B2* gene is not cotranscribed with the *F3h-1* genes. It has different specialization, and its transcription is likely to be stress-induced.

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DE-NOVO SEQUENCE ASSEMBLY OF WHEAT CHROMOSOME 7B

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The genome of bread wheat (*Triticum aestivum*) is about 17Gb which is five times larger than the *Arabidopsis* genome and consists of large amount of highly-conserved, high-copy retrotransposons and other repetitive sequences. This, as well as its polyploidy nature makes sequencing and assembly extremely challenging. Illumina sequencing has been applied to flow-sorted arms of wheat chromosome 7B. Paired-end reads were assembled by different *de-novo* sequence softwares, including CLC, SOAPdenovo and Abyss. Abyss yielded the best assembly, achieving maximum contig sizes of 30964 bp and 50938 bp for the long and short arm, respectively. Scaffolding of contigs by using low coverage mate pair data reduced total number of contigs and produced a significant increase of the N50. Comparative genomic analyses identified ~1000 scaffolds containing full length and near full length genes.

NUCLEOSOME ORGANIZATION IN PLANT SATELLITES

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Key words: *satellite repeat, nucleosome positioning, DNA curvature profile, Fourier transform*

We performed analysis on the data of plant satellite monomers retrieved from PlantSat database [1]. The preference for the monomer sizes around 140-170 and 330-350 was observed on satellite sequences. Therefore, it was speculated that this length preference may be influenced by nucleosome DNA wrapping size of 140-180bp, whilst more than a half of the monomers dropped out of these length intervals. We targeted our research on elucidating the nature of length variance and its implication on the nucleosome organization.
We worked out an appropriate statistic linking *in silico* predicted DNA curvature profile and monomer length to specify nucleosome positioning preference in satellite repeats. A statistically significant deviation was found in satellites compared with random sequences considering their DNA curvature profiles. By means of Fourier transform we elucidated that as many as 50% of the monomers maintain the period in the vicinity of 170bp corresponding to single nucleosome occupation within top two harmonics of DNA curvature profile. We observed that subtelomeric satellites nucleosome positioning scheme differs significantly from that of centromeric ones in plants.
For the monomers with significant maximum amplitude value of DNA curvature profile we observed mostly U-shaped binding affinity profiles. As long as the regular period of nucleosome binding profile usually equals to monomer length except for the cases with minor peaks with a period equaling to 140-160bp, the smooth junction of monomers implies the affinity score at the beginning of monomer should be approximately equal to the affinity score at the end. This feature impacts DNA sequence content of the significant number of monomers. There are some cases of reverse U-shaped monomers, but their frequency is significantly less than U-shaped ones. The cases of non-symmetric monomers with the DNA curvature profile values either monotonously increasing or decreasing are exceedingly rare. The cases of monomers with irregular or non-significant nucleosome affinity trend usually results in quite low value of maximum amplitude. These monomers probably don't contain any nucleosome positioning preferences.
Our results support the hypothesis that the satellite repeats might exemplify several assembling schemes, including one and two stage assembling modes. It's also plausible that nucleosome spacing in satellite repeats depends on the chromosome location.
The work is partly supported by RFBR grant (11-04-01206-a) and Integration Grant SB RAS.
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HIGHT THROUGHPUT PHENOTYPING APPROACH TO ANALYSIS OF THE LEAF HAIRINESS IN WHEAT

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Key words: *Triticum aestivum, leaf hairiness, trichome, high-throughput phenotyping*

Leaf hairiness in wheat is of great importance for protection from pests and for adaptation to environmental factors. For example, this trait is characteristic of a number of drought resistant wheat cultivars referred to the steppe ecological group. Study of the features of leaf hairiness morphology and identification the corresponding genes will allow to obtain varieties resistant to hard climatic conditions and certain pests. To identify the genes responsible for the leaf hairiness, mass analysis of a great number of plants belonging to different hybrid populations is needed, accompanying with a laborious manual job.

Here we provide a description of a novel protocol for photomicroscopy of the transverse folds of wheat leaves and a novel algorithm, which allows such images to be used for rapid quantitative evaluation of pubescence. This algorithm is implemented as the LHDetect2 software program, which comes in two flavors: as a console application and as a Web service. A comparison of LHDetect2 and its previous version LHDetect [1] performance demonstrated that the error in trichome number identification with LHDetect2 is 1.2 times as lower as that with LHDetect.

To integrate information of the wheat phenotype, genotype and environment we developed WheatPGE computer system. It is designed to solve the problem of integration of genotypic and phenotypic data and parameters of environment as well as to analyze the relationships between genotype and phenotype in wheat. The system is used to integrate heterogeneous data about plant, for storing and processing of various morphological characteristics and genotypes of the wheat plants as well as of various environmental factors.

The developed system was successfully used for analysis of leaf hairiness in plants of different genotypes at different environmental conditions [2].

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August 28-29

POSTER PRESENTATIONS

28-29 августа

СТЕНДОВЫЕ СООБЩЕНИЯ

MOLECULAR-GENETIC BASIS OF GRAIN TEXTURE IN KAZAKHSTAN WHEAT CULTIVARS

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Key words*: wheat, hardness, allele, Pinb-D1 gene*

Spring wheat area occupies about 12-13 million ha in the North of Kazakhstan, and 1-1.5 million ha in the South of the Republic is occupied by winter wheat. Due to environmental conditions and breeding Kazakhstani wheat cultivars have high protein content. But the end-use quality and marketing classification of wheat are determined by grain hardness too. Wheat grain hardness is controlled by the hardness (*Ha*) locus and tightly linked with the genes *Pina-D1*, *Pinb-D1* and *Gsp-D1* on the short arm chromosome 5D. Kernel hardness change in bread wheat is a result of seven single nucleotide mutations in *Pinb* and a *Pina* null mutation, i.e. only one of the two known *Pina* and *Pinb* mutant alleles may differ in grain hardness. The objectives of the current study were to analyze the allelic diversity at the *Pinb-D1* gene in Kazakhstani wheat cultivars, to study distribution of these alleles in Kazakhstan and to identify hard grain cultivars. Pyrosequencing-based identification of *Pinb-D1* alleles was performed for 40 spring and 48 winter wheat including as elite cultivars released in Kazakhstan in different years as some advanced breeding lines, and 70 spring wheat accessions from the 10th KazSib (Kazakhstan and Siberia) Network. Within the South Kazakhstan winter wheat, two types of alleles at the *Pinb-D1* locus were observed. The prevailing allele was *Pinb-D1b* (58.7%), the *Pinb-D1a* allele showed frequency 26.1%, the rest 15.2% were cultivars with mixed *Pinb-D1a* and *Pinb-D1b* alleles. Within the North Kazakhstan spring wheat, the allelic diversity at the *Pinb-D1* locus was higher than that in the South Kazakhstan. In the North, the *Pinb-D1b* allele was also prevalent (30%), the *Pinb-D1a* allele was characteristic of 5% cultivars, 40% cultivars had mixed *Pinb-D1a* and *Pinb-D1b* alleles. Cultivars with *Pinb-D1f* and *Pinb-D1c* alleles were seldom, and they appeared mainly in combination with *Pinb-D1a* allele. In the 10th KazSib spring wheat cultivars, the alleles *Pinb-D1b, Pinb-D1a, Pinb-D1f, Pinb-D1c* occur with frequencies 41.5%, 29.2%, 4.6%, and 3.1%, respectively. Different alleles of the *Pinb-D1* locus and their frequency in various regions of Kazakhstan showed us genetic relationships between cultivars.

CYTOGENETIC AND MOLECULAR ANALYSIS OF LEAF RUST RESISTANT WHEAT CULTIVARS WITH GENETIC MATERIAL OF *AGROPYRON INTERMEDIUM*

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Key words: *T. aestivum, Agropyron, leaf rust, in situ hybridization, SSR-analysis*

Brown leaf rust (pathogen *Puccinia triticina* Eriks.) still remains the most devastating disease of wheat, despite the progress in studying the mechanisms of resistance and successes in breeding. Only few leaf rust resistance genes: *Lr9*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr36*, *Lr38* and *LrTR* are effective against the *P. triticina* population in the Samara region of European Russia; the others lost their efficiency and did not use in breeding practice any more. At the same time, efforts to introduce leaf rust resistance genes from wild relatives into common wheat genome are continued.

Wheat - *Ag. intermedium* substitution line, Agis 1, was used for introgression of *Ag. intermedium* genetic material to the gene pool of *T. aestivum*. Wheat cultivars Tulaykovskaya 5 (Erythrospermum 865 × Agis 1), Tulaykovskaya 10 and Tulaykovskaya 100 (Tulaykovskaya 5 × Albidum 653) created on the basis of line Agis 1 are characterized by a high immunity to leaf rust. It was shown that the resistance of Tulaykovskaya 5 cultivar is determined by one dominant gene *LrAg*, which was introgressed to Tulaykovskaya 10 and Tulaykovskaya 100 cultivars without changing of the chromosomal location.

Genotyping of Tulaykovskaya 5, Tulaykovskaya 10 and Tulaykovskaya 100 by using of genomic *in situ* hybridization (GISH) in combination with probes pSc119.2 and pAs1 demonstrated that in all three cultivars chromosome 6D of *T. aestivum* was replaced by unidentified chromosome of *Ag. intermedium*. Full chromosome substitution was confirmed by molecular analysis with the help of SSR-markers specific for 6D chromosome of bread wheat.

Genetic analysis showed that *LrAg* gene is not identical to the *LrAgi-1* gene, which was also introduced from *Ag. intermedium* genome into 6D chromosome of spring wheat. Segregation analysis for resistance to leaf rust of F2 and F3 generations derived from the crosses of Tulaykovskaya 5, 10 and 100 cultivars with the lines and varieties carrying the genes *Lr9*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr36*, *Lr38* and *LrTR* indicated that *LrAg* gene is not allelic, not identical and not tightly linked to the any of *Lr* genes used in breeding practice in the Samara region.

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APPLICATION OF MOLECULAR MARKERS IN WINTER WHEAT AND TRITICALE BREEDING IN KRASNODAR LUKYANENKO RESEARCH INSTITUTE OF AGRICULTURE

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The Wheat and Triticale Breeding Department of the Krasnodar Lukyanenko Research Institute of Agriculture (KNIISH) in collaboration with a range of Russian scientific and educational centers is running a program aimed at application of molecular markers in creating new winter wheat and triticale varieties. Researches are being conducted simultaneously in several directions.

1. Screening the presence of effective genes of resistance to leaf (*Lr*), stripe (*Yr*) and stem (*Sr*) rusts, genes controlling the plant height (*Rht*), vernalization requirement (*Vrn*), response to photoperiod (*Ppd*), resistance to pre-harvest sprouting (*Vp*) in commercial varieties and breeding material. Ninety-nine commercial varieties and more than 200 breeding lines obtained in KNIISH were analyzed. The genes *Lr19-Sr25* were detected in 2 varieties and in a range of breeding lines, obtained with the participation of those varieties. The gene *Lr9* was identified in a range of advanced breeding lines. The rye translocation (1BL.1RS), carrying the genes *Lr26*, *Sr31*, *Pm8* and *Yr9*, was detected in 37 of 99 varieties. The genes *Lr24-Sr24*, *Lr37-Yr17-Sr38*, *Lr39(41)*, *Yr10*, *Sr36* were not identified.

The presence of plant height reduction genes was studied. The gene *RhtB1b* (*Rht1*) was detected in 41, and the gene *RhtD1b* (*Rht2*) - in 13 of 99 examined varieties. The different alleles of the gene *Rht8* were discovered in all varieties. The allele *Rht8a* was found in 2 varieties, and the alleles *Rht8b* – in 9, *Rht8c* – in 75, *Rht8g* – in 3, *Rht8?* (unidentified allele) in 8 varieties. The collection of 24 facultative wheat varieties and lines was examined in order to find the genes controlling vernalization requirement and response to photoperiod. The dominant alleles of the gene *Vrn-A1* were indicated in 10 samples, *Vrn-B1* – in 12, *Vrn-D1* – in 6, *Ppd-D1* – in 15 samples of the collection.

2. Application of marker assistant selection in transferring the complex of leaf (*Lr*), stripe (*Yr*), stem (*Sr*) rusts resistance genes from source varieties to the material which is adapted to local environmental conditions. Formation of pyramids of these genes.

A range of KNIISH commercial varieties was crossed to carriers of the genes *Lr9*, *Lr19-Sr25*, *Lr24-Sr24*, *Lr28*, *Lr37-Yr17-Sr38*, *Lr39(41)*. In the year 2010, backcrosses to the adapted parent were conducted. The plants carrying the target gene were selected by the help of molecular markers. This year we are planning further recurrent crossings and hybridizations to form the pyramids of different effective genes.

3. Searching for the most effective ways of application of molecular markers in traditional breeding scheme. Three schemes of application of molecular markers in traditional breeding process were evaluated at different breeding stages of individual selection from the population at the level of breeding and check nurseries.

COMPARISON OF LOW TEMPERATURE-RESPONSIVE GENES, *TAIRI* AND *TACBF*, IN WINTER AND SPRING CULTIVARS OF *TRITICUM AESTIVUM* L.

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Key words: *freezing tolerance, Triticum aestivum, wheat, antifreeze protein, AFP, ice recrystallization inhibition, IRI, CBF, C-repeat binding factor*

Cold acclimation induces the expression of cold-regulated genes needed to protect plants against freezing stress. The CBF (C-repeat binding factor) transcription factor family is an important part of this process. CBF genes may form clusters, in Triticeae there are 11 different *CBF* gene orthologues at the *FR-H2* locus of barley and the *FR-A^{m}2* locus of einkorn wheat, *T. monococcum* [1, 2]. It was shown that the structure of the *CBF* genes at *FR-2H* differs between winter and spring barley [3].

Among the genes induced by low temperature in plants there are several genes encoding antifreeze proteins (AFPs) These proteins have an affinity for ice and possess the properties of recrystallization inhibition and thermal hysteresis. The binding of AFPs restricts the ice crystal growth in the apoplast, enabling plants to survive under freezing conditions. Among these there are the *TaIRI* (*Triticum aestivum* ice recrystallization inhibition) genes up-regulated during cold acclimation in freezing-tolerant species. The accumulation of *TaIRI* transcripts is associated with a freezing tolerance level of different wheat cultivars. In the current study, we used PCR primers to five *CBF* genes (*TaCBFIIIc-B10*, *TaCBFIIId-B12*, *TaCBFIVc-B14*, *TaCBFIVd-B4*, and *TaCBFIVd-B22*) that were chosen from wheat CBFs by their high capacity to cold response [4]. Two full length (AK252915, AY968589) and two partial (FJ594448, FJ594449) *TaIRI* cDNAs from the EST database (http://www.ncbi.nlm.nih.gov/Database/) were carefully analyzed for primers design. Winter wheat cultivars 'Ulyanovka' and 'Bezostaya 1' and spring cultivar 'Chinese Spring' were genotyped using specific primers to the *TaIRI* and *TaCbf* genes. The results of the genotyping are discussed.

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3. Knox et al. (2010) CBF gene copy number variation at Frost Resistance-2 is associated with levels of freezing tolerance in temperate-climate cereals. Theor Appl Genet, 121:21-35.
4. Badawi et al. (2007) The CBF gene family in hexaploid wheat and its relationship to the phylogenetic complexity of cereal CBFs. Mol Genet Genomics, 277:533–554.

MANAGEMENT OF PLANT GENETIC RESOURCES – EXAMPLES ON SEED LONGEVITY

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Key words: *ex situ conservation, seed storability, artificial ageing, association mapping, marker-trait associations*

Plant genetic resources for food and agriculture (PGRFA) play a major role for global food security. The most significant and widespread mean of conserving PGRFA is *ex situ* conservation. The majority of accessions is conserved as seed in specially designed cold stores. The storability/longevity of the seeds however is limited. It depends on the environmental conditions during regeneration and storage but also on the individual genotype. Bi-parental genetic studies in Arabidopsis, rice and barley revealed the presence of quantitative trait loci determining seed longevity. In the present study we applied an association genetics approach originated from human genetics. We used barley as a model for wheat. The population under investigation comprised 163 cultivars/landraces originated from 30 countries of the four continents Europe, Asia, Africa and Australia. Seeds of the 163 accessions were artificially aged (43±0,5°C, 72h, 4 replicates of 50 seeds) following the rules of the International Seed Testing Association (ISTA). Germination tests were performed using the artificial aged seeds together with untreated controls. Significant marker-trait associations were detected on five out of the seven barley chromosomes. It was concluded that association mapping is a feasible approach to detect loci responsible for the storability of seeds in other crops as e.g. wheat.

HAPLOTYPE *RHT8C PPD-D1A* IN UKRAINIAN WINTER BREAD WHEAT VARIETIES

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Key words: *genetic distance, Rht8, Ppd-D1, bread wheat*

The gene *Rht8c* is one of the most distributed dwarfing genes (90%) among Ukrainian winter bread wheat varieties (Chebotar et al., 2006, 2008, 2011). The gene *Ppd-D1* is one of the most potent genes affecting the photoperiod response of wheat (*Triticum aestivum* L.) (Worland et al., 1998; Beales et al., 2007; Guo et al., 2010). Of the six known alleles of *Ppd-D1* gene only one (*Ppd-D1a*) results in photoperiod insensitivity (Guo et al., 2010). The alleles *Rht8c* and *Ppd-D1a* are used in breeding programs all over the world, because of their agronomic advantages, such as height reduction and photoperiod insensitivity.

The aim of this work is to estimate the distance between the genes *Rht8* and *Ppd-D1* because the haplotype *Rht8c Ppd-D1a* on 2D chromosome is presented in the majority of wheat cultivars of Plant Breeding and Genetic Institute (Odessa, South of Ukraine). We assume that this haplotype have been transferred to Ukrainian wheat germplasm by crossing bread wheat cultivars from the South region of Ukraine with cv. 'Bezostaya 1'. According to Worland et al. (1998) the allele *Rht8c* have been transferred to cv. 'Bezostaya 1' from cv. 'Akkakomugi' through Strampelli's cv. 'Ardito' and then through cultivars 'Klien33', 'Skorospelka 2' and 'Bezostaya 4'.

The population F_2 from crossing 'Kooperatorka' (*Rht8a Ppd-D1b*) × 'Kooperatorka K-90' (*Rht8c Ppd-D1a*) has been tested with the help of molecular markers, namely, the microsatellite marker *Xgwm261* that is linked to *Rht8* on the distance of 0,6 cM (Korzun et al., 1998) and the allele-specific markers to *Ppd-D1* (Beales et al., 2007). The genetic distance between *Rht8c* and *Ppd-D1a* was calculating by using the program GENE2 (Corol et al., 1990) and JoinMap (Stam, 1993) applying the Kosambi map-unit function. According to the data of molecular genetic analysis the genes *Rht8* and *Ppd-D1* are linked on the distance 23.3±0.05 cM.

During more then 50 years of breeding programs in the South of Ukraine haplotype *Rht8c Ppd-D1a* was selected as one of the most important adaptive complex for the plants of this region. It can be expected that the segment of 2D chromosome with size 23.3±0.05 cM that is located between alleles *Rht8c* and *Ppd-D1a* is saturated by the numbers of other genes according to Gasperini et al. (2009). This segment have been co-selected with *Rht8c* and *Ppd-D1a* alleles and saved in the genotypes of a number of Ukrainian wheat varieties. For example, haplotype *Rht8c Ppd-D1a* was presented in 27 modern wheat varieties (2006-2010 years of registration) from different breeding centers of Ukraine except of cv. 'Dolya' (Dnepropetrovsk Agrarian University).

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ALLELE VARIATION IN LOCI CONTROLLING HEIGHT, GROWTH AND SENSITIVITY TO PHOTOPERIOD IN UKRAINIAN AND RUSSIAN BREAD WHEAT VARIETIES

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Key words: *bread wheat, plant height, adaptive response*

The adaptation of bread wheat (*T. aestivum* L.) to diverse environmental conditions is greatly under the control of genes involved in determination of vernalization response (the major genes - *Vrn-A1*, *Vrn-B1*, *Vrn-D1*), photoperiod response (the major genes – *Ppd-A1*, *Ppd-B1*, *Ppd-D1*), and *earliness per se* (there are about twenty-five *Eps* loci according to Snape et al. (2001)). The genes of these three systems have significant influence on variation of plant height and yield components (Stelmach 1993, 1998; Worland, Sayers, 1985; Low, Worland, 1997; Shah et al., 1999, Pankova et al., 2007). In addition to these genes, the *Rht* (reduced height or dwarfing) genes have significant effect on the agronomically important traits.
The distribution of allelic combinations of the *Vrn*, *Ppd* and *Rht* genes in Ukrainian and Russian varieties cultivated in different regions at different historically periods is under investigation. The current results are discussed in this report.

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IDENTIFICATION OF LEAF RUST RESISTANCE GENES IN WILD RELATIVES, SYNTHETIC FORMS AND INTROGRESSION LINES OF COMMMON WHEAT (*TRITICUM AESTIVUM* L.)

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Leaf rust of wheat (*Triticum aestivum* L.) caused by the fungus *Puccinia triticina* (formerly *P. recondita* f. sp. *tritici*), is one of the most important foliar diseases of this crop. Breeding of wheat cultivars with resistance to leaf rust is the most effective, economically and environmentally friendly method of disease control and was used in numerous wheat breeding programs worldwide (Kolmer, 1996). Most of the efficient genes for leaf rust resistance were derived from wild relatives of common wheat.

The samples of *Aegilops umbellulata*, *Ae. speltoides* and *Ae. ventricosa*, synthetic forms 'Avrolata', 'Avrodes' and introgressive lines of common wheat with genetic material from *Agropyron glaucum* and *Ae. umbellulata* were screened for the leaf rust resistance genes *Lr9*, *Lr19*, *Lr24*, *Lr28* and *Lr37* with the use of molecular sequence tagged site (STS) markers. The markers for *Lr9* were detected in all fourteen samples of *Ae. umbellulata* (U) from geographically remote groups and the synthetic form 'Avrolata' (genome AADDUD). Screening of the gene pool of eight samples of *Ae. ventricosa* (DDUU) showed the presence of the resistance gene *Lr37*.

Amplification of an STS marker linked to the *Lr28* gene was not specific, because the amplification product (378bp) was found in the susceptible cultivar 'Avrora'. Forty two introgressive lines with the genetic material of *Ag. glaucum* were analyzed for the presence of the leaf rust resistance genes *Lr19* and *Lr24*. Only in one of them **(**3/1) the gene *Lr19* was detected. PCR analysis of eighty nine lines with genetic material of *Ae. umbellulata* did not show the presence of the leaf rust resistance gene *Lr9*. On the basis of the results obtained we can assume that the investigated introgressive lines contained leaf rust resistance genes differed from the known effective *Lr9*, *Lr19* and *Lr24* genes.

ANALYSIS OF THE ORIGIN AND DISTRIBUTION OF CULTIVATED EMMER *TRITICUM DICOCCUM* (SCHRANK.) SCHUEBL. USING C-BANDING METHOD

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Key words: *emmer, C-banding patterns, C-banding polymorphism*

Hulled tetraploid emmer *Triticum dicoccum* (Schrank) Schuebl. is considered to be a primitive form of wheat of the Emmer group. Emmer was among the eight "Founder crops" of ancient agriculture (Zohary, 2004) that was domesticated by Neolithic man in the Middle East approximately 12000-10000 PB. Although domestication of emmer is one of the key events of the beginning of agriculture, it is still not completely clear where this important wheat species was first taken in cultivation. To solve this task we study intraspecific diversity of *T. dicoccum* using C-banding technique. In total, 223 accessions of *T. dicoccum* maintained in the genbanks of the State Scientific Center of Russian Federation, Vavilov All-Russia Research Institute of Plant Industry (VIR, Russia) – 111 and INRA (Clermont-Ferrand, France) - 71, and ICARDA – 41 are examined. Our sample covers all areas of emmer wheat cultivation (23 countries from the Middle East, Europe, Asia and North Africa), which allow us not only to estimate polymorphism levels in particular populations, but also to trace historical ways of emmer wheat dispersion in the past.

Chromosome analysis reveals differences between geographical populations in the level of C-banding polymorphism. It is the highest in Moroccan and Algerian populations, which can be caused by multiple and repeated introduction of wheat in these countries due to their specific geographic locations and also to historical reasons. Transcaucasian, Indian, and North-European groups of Emmer prove to be the least polymorphic which can reflect an important role of "originator effect" in their evolution. Some populations are found to possess the unique C-banding patterns of particular chromosomes which are the "markers" of these geographical regions. For example, the abundance of translocation T7A-5B is characteristic of North-European populations of emmer. This translocation is also found in few accessions from Turkey, Iran and Algeria which can be an indicative of ancient origin of this rearrangement. Many emmer wheat accessions from Volga basin, Ural region and from Balkan countries are characterized by a small C-band in subtelomeric region of the chromosome 4A long arm, which discriminates them from North-European emmer accessions carrying large C-band in the same position. Comparison of the C-banding patterns of emmer wheat cultivated in Volga basin or in the Ural foothills allows to suggest two possible roots of wheat expansion to these regions: directly from the center of origin through the Caucasus and indirectly, from the Balkan countries though the southern Russia and Ukraine. There are no indications of links between emmer wheat from Volga basin and the Ural foothills, from one side, and those from Northern Europe, from another side. It is interesting that the translocation T7A-5B marker for emmer wheat populations from Northern Europe is also found in few accessions of wild emmer growing close to each other in the territories of Syria, Lebanon and Israel. This fact points to an important role of wild emmer germplasm from this region in the evolution of cultivated form.

ANALYSIS OF LEAF HAIRINESS MORPHOLOGY WHEAT: COMPUTATIONAL APPROACHES USING IMAGE PROCESSING TECHNIQUE

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Keywords: *leaf hairiness, trichome, drought stress, bread wheat, high throughput phenotyping*

Leaf hairiness in wheat is of great importance for adaptation to environmental factors including protection from pests. For example, this trait is the characteristic of a number of Russian drought resistant wheat cultivars referred to the steppe ecological group. Study of leaf hairiness morphology and identification of the corresponding genes will allow obtaining the varieties which are resistant to hard climatic conditions and certain pests. To identify the genes responsible for the leaf hairiness, mass analysis of a great number of plants belonging to different hybrid populations is needed, accompanying with a laborious manual job. Furthermore, the more accurate description of the morphological properties of the trait for correct determination of phenotypic classes is timely. We developed a computer-based technology for descriptions of quantitative traits of leaf hairiness. It contains the LHDetect program with the feature of image processing. Using the LHDetect program one can count the trichome number, the mean length of the trichomes, and evaluate the trichome length distribution vector for each leaf sample. In the investigation, we used the LHDetect program for determining the morphological properties of leaf hairiness on a number of wheat genotypes. The technology appeared to be effective for a large scale analysis of leaf hairiness morphology in individual plants. This approach along with genotyping will be useful for mapping of genes responsible for leaf hairiness. In this study, we carried out the detailed morphology analysis of leaf hairiness among eight wheat cultivars and lines representing a wide range of leaf hairiness morphology: the trichome density, length and distribution pattern greatly varied. Positive correlation between trichome number and the mean trichome length was found: leaves with a higher trichome density usually had longer trichomes. One of the cultivars was grown in contrast conditions. It was shown that drought stressed plants form more trichomes on the leaf surface, but they are significantly shorter than those from plants grown in a favourable conditions. There are at least two possible explanations of the observations. First, much more trichomes are needed to form the microclimat in the drought conditions. Second, plant cells cannot produce enough turgor pressure to form a long trichomes while the drougt stress.

EVOLUTIONARY HISTORY OF *AEGILOPS TAUSCHII* COSS. AS REVEALED BY CHLOROPLAST DNA NON-CODING SEQUENCES POLYMORPHISM

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Key words: *Aegilops tauschii; chloroplast DNA; evolutionary history; intraspecies phylogeny*

Aegilops tauschii Coss. (*2n* = 14, genome DD) is a diploid goat-grass species, the D-genome progenitor of common wheat, *Triticum aestivum* L. *Ae. tauschii* genetic variability is an important natural resource, since this species is the most important donor of agriculturally important genes for improvement of common wheat. Therefore, an understanding of how this variability was formed in the course of *Ae. tauschii* evolutionary history and how it is presented throughout the species area is of particular importance.

In order to reveal the peculiarities of *Ae. tauschii* origin, intraspecies divergence and geographic expansion, the sequencing of the four cpDNA fragments from the *trnC-rpoB*, *trnF-ndhJ*, *ndhF-rpl32* and *atpI-atpH* intergenic regions, about 3000 b.p. totally, was carried out according to [1]. One hundred and twelve *Aegilops tauschii* accessions, 56 of ssp. *tauschii* and 56 of ssp. *strangulata* representing all the species range were analysed. One inversion, 8 insertions/deletions, 18 base pair substitutions and 5 microsatellite loci were found. The data obtained revealed that: (1) *Ae. tauschii* originated in Caucasia. (2) Neither of the two *Ae. tauschii* subspecies was an ancestor one to another. *Aegilops tauschii* divided into ssp. *tauschii* and ssp. *strangulata* at the very beginning of its existence as a species. (3) Subspecies *tauschii* was the first to start geographic expansion and relatively rapidly occupied a vast area from Caucasia -eastward up to central Tien Shan and western Himalayas. (4) In contrast to ssp. *tauschii*, a geographic spread of ssp. *strangulata* was a complicated, multi-stage and slow process. At the beginning of ssp. *strangulata* evolutionary history its major phylogenetic lineage had existed as a small isolated population for a long time span. Several forms of ssp. *strangulata* had originated, better adapted to a relatively more humid and cool habitats. Each of these forms has gradually forced out ssp. *tauschii* from some part of its area in the west, up to central Kopet-Dag.

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PRODUCTION OF WHEAT-RYE SUBSTITUTION LINES OF COMMON WHEAT RESISTANT TO BIOTIC AND ABIOTIC STRESSES

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Key words: *common wheat, rye, alien substitution line, heading time, vernalization genes Vrn, cold/freezing tolerance, translocation 1BL.1RS*

The success of selection for tolerance against biotic and abiotic stresses is determined by many factors, the most important of which are genetic resources. Using of different sources of resistance makes it possible to produce new forms of plants with enriched gene pool. We have studied frost tolerance of winter wheat-rye 5R(5A) substitution lines (‘Rang’ and ‘Mironovskaya krupnozernaya’ cultivars) during several years. They have a winter type of development which is controlled by recessive alleles of *vrn* genes from spring wheat cultivars ‘Rang’ and ‘Mironovskaya krupnozernaya’ and rye cultivar ‘Onokhoiskaya’. In favorable conditions of winter 2007/2008, the substitution lines displayed tolerance to low temperature and capability to stand hard frost, but in a less degree than standard frost-hardy cultivars ‘Mironovskaya 808’ and ‘Ul'yanovka’, 30-40% against 60-70% of hibernate plants, respectively. However, the frost tolerance of cultivars and lines of common wheat was significantly below the frost tolerance of winter rye cultivars, which had 99% hibernate plants. The conditions of winter 2009/2010 conduced to plant death and only 15% plants of wheat-rye lines survived, whereas in the control winter cultivars, 30% plants of ‘Ul'yanovka’ and 20% plants of ‘Mironovskaya 808’ survived. The results obtained allow us to conclude that frost tolerance might be due to recessive *vrn* alleles of spring wheat and rye cultivars, which can enhance winter traits and probably frost tolerance of winter wheats.

Wheat-rye lines with substitutions of homeologous group 5 chromosomes carrying 1BL.1RS translocation were obtained. For this purpose two lines of ‘Saratovskaya 29’ cultivar with alien chromosome substitution 5R(5A) and 5R(5D) were crossed with Line L2075 carrying 1BL.1RS rye translocation. L2025 was kindly provided by Dr. S.N. Sibikeev. It is known that the short arm of rye chromosome 1R carries different disease resistance genes (*Lr26*, *Pm8*, *Yr9*, *Sr31*). Consequently, in F_5 and F_6 populations, homozygous forms with complex resistance to brown leaf, stem rust and powdery mildew, containing alien chromosome substitution 5R(5A) and 5R(5D), were isolated.

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THE STUDY OF MULTIPLE ALLELISM OF THE DOMINANT *VRN-B1* GENE IN COMMON WHEAT *T. AESTIVUM* L.

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Key words: *common wheat, Vrn genes, heading time, molecular markers*

Wheat can be spring, winter and facultative according to the growth habit. Several genes responsible for growth habit in common wheat, namely *Vrn-A1*, *Vrn-B1* and *Vrn-D1* on chromosomes 5AL, 5BL and 5DL, respectively, and *Vrn-D4* on chromosome 5DL. Winter growth habit is determined by recessive *vrn* alleles at all loci, and spring – by one or more dominant *Vrn* genes. Heading time in wheat, in addition to the *Vrn* genes, is influenced by the *Ppd* and *Eps* genes. Multiple allelism at the *Vrn-B1* locus was found by Maystrenko (1992) in substitution lines for chromosome 5A of cultivars 'Saratovskaya 29' and 'Diamant 2'. The lines with the *Vrn-2a*^{S} (*Vrn-B1c*) allele from 'Saratovskaya 29' headed 10-12 days earlier, than those with *Vrn-2b*^{D} (*Vrn-B1a*) from 'Diamant 2'. In our work, in addition to these lines we studied substitution lines of winter wheat 'Sava' with 5B chromosomes from 'Saratovskaya 29' or 'Diamant 2', and near-isogenic lines of winter wheat 'Bezostaya 1' with the dominant *Vrn-B1* alleles from these cultivars (Efremova et al., 2011). In these lines, the studied alleles are introduced separately into the same genotype, so they can be more precisely compared with each other.

When we studied the heading time of these lines, we found, that without vernalization the lines with the *Vrn-B1c* allele headed 2-15 days earlier, than the lines with *Vrn-B1a*. 'Saratovskaya 29' and 'Diamant 2' did not differ from each other on heading time, due to the dominant *Vrn-A1* gene. After 30-days vernalization the difference in the period from shoots to heading has shortened from 15 to 10 days in some lines, in the other lines, plants with the *Vrn-B1c* allele headed 1-6 days later than plants with *Vrn-B1a*. It can mean, that without vernalization the *Vrn-B1c* allele is stronger, than *Vrn-B1a*, but this weak *Vrn-B1a* allele responds to vernalization stronger, than *Vrn-B1c*.

Duration of the period from shoots to heading in F_1 plants from the cross between two near-isogenic lines carrying the *Vrn-B1c* and *Vrn-B1a* alleles was similar to this of late-ripening plants from line with the *Vrn-B1a* allele (58 and 57 days respectively, in contrast to 53 days in *Vrn-B1c*). We suggest that in F_1 generation, the late ripeness determined by the weak *Vrn-B1a* allele dominates. Frequencies of the alleles of the dominant *Vrn-A1*, *Vrn-B1* and *Vrn-D1* genes among 52 cultivars from Russia and adjacent regions were defined using PCR-analysis. Sixty percent of cultivars had the *Vrn-A1a* allele, and only 3.8% (2 cultivars) had *Vrn-A1b*. The *Vrn-B1a* allele was a little more widespread among the cultivars, than *Vrn-B1c* (47 and 38%, respectively). Dominant *Vrn-D1a* was presented only in 2 cultivars, in all other it was recessive. Substitution and near-isogenic lines confirmed the existence of two alleles of the *Vrn-B1* gene, which are determining the difference in heading time in wheat.

TECHNOLOGICAL PROPERTIES OF GRAIN AND FLOUR IN BREAD WHEAT LINES WITH INTROGRESSIONS FROM *AEGILOPS SPELTOIDES* AND *AEGILOPS MARKGRAFII*

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The wild cereal species are widely used in crosses with bread wheat for improvement of resistance to fungus diseases. Such collections may contain the introgressions that affect technological properties of grain and flour. The aim of this work was to investigate the influence of introgressions from *Aegilops speltoides* and *Aegilops markgrafii* on milling properties, gluten content in grain and physical properties of dough.

Spring and winter bread wheat lines of 'Arsenal' collection (Lapochkina, 2001) with introgressions from *Ae. speltoides* into cv. 'Rodina' and two winter lines of cv. 'Alcedo' with introgressions from *Ae. markgrafii* (Weidner, 2004) were used as the material for investigation. The spring lines from 'Arsenal' collection and the winter lines of cv. 'Alcedo' were grown under field conditions in several replicates in different seasons. The winter lines from 'Arsenal' collection were grown under green-house conditions according their vernalization requirements. Technological properties of grain and flour were determined following the procedures accepted in Russia.

Previous studies of the lines from 'Arsenal' collection have shown that they carry the genes for resistance to powdery mildew and leaf rust (Lapochkina et al. 2003) and several morphological and physiological traits from *Ae. speltoides* (Pshenichnikova et al. 2007). The lines with introgressions from *Ae. markgrafii* carry the insertions in three chromosomes identified using molecular markers (Iqbal et al. 2007).

Maternal cultivar 'Rodina' has high milling properties of grain and low physical properties of dough. Three spring and two winter lines showed a significant increase of gluten content in grain comparing to 'Rodina'. A pronounced effect of introgression on grain vitreousness and hardness was observed. One spring line and one winter line showed a significant decrease of flour particle size and had a soft endosperm texture. It was shown that the winter line carries substitution of 5A chromosome for homoeologue 5S (Pshenichnikova et al. 2010). Two spring lines resistant to powdery mildew and leaf rust improved physical properties of dough. They had higher flour strength and an increased dough tenacity comparing to 'Rodina'.

Introgression from *Ae. markgrafii* into cv. 'Alcedo' was associated with increase of weight of 1000 grains and vitreousness. Gluten content also showed the tendency to increase.

Introgressions from wild species in bread wheat genome enlarge the genetic diversity not only for disease resistance but for technological properties of grain as well. Novel genes may be useful for breeding purposes.

RESISTANCE TO POWDERY MILDEW IN AN INTROGRESSIVE WHEAT LINE DERIVED FROM *TRITICUM MILITINAE*

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Keywords: *Triticum aestivum, Triticum militinae, powdery mildew, QTL, doubled haploids*

Wheats of the timopheevii group (A^tA^tGG) are considered to be a useful reservoir of disease resistance genes. Introgressive line 8.1 (*2n* = 42) derived from the *Triticum aestivum* × *Triticum militinae* cross exhibited effective adult plant resistance (APR) to powdery mildew in field tests, and showed also resistance to population of the pathogen in the seedling stage of plant growth.

A doubled haploid (DH) mapping population was generated from the cross of *T. militinae*-derived introgressive wheat line 8.1 and the susceptible cv. 'Tähti', and tested for adult plant resistance (APR) and for seedling resistance to 11 different *Blumeria graminis* sp. *tritici* (Bgt) isolates and 5 different mixtures of Bgt. Five introgressive chromosome regions (on chromosomes 1A, 4A, 5A, 5B, and 7A) were found to carry *T. militinae*-origin QTLs for seedling resistance, three of them (on chromosomes 4A, 5A and 7A) were also detected in all APR assessments.

The main QTL for APR and seedling resistance to an artificial Bgt mixture that had been detected earlier on chromosome 4A (*QPm.tut-4A*) was confirmed and was found to be effective on all Bgt races tested in the seedling stage. With the exception of the minor QTL on chromosome 5B, none of the detected minor QTLs showed race-specifity. *QPm.tut-4A* was found to account on average for 49% of seedling resistance variance and for 33% of APR variance, while each of the detected minor QTLs was responsible for 10-17% of the resistance variance. It was shown that *QPm.tut-4A* does not require any other QTL for its activity.

An additional F_2 mapping population from a cross between susceptible cv. 'Chinese Spring' and resistant line 8.1 was constructed. In the novel mapping population, recombination was observed in the translocation region carrying *QPm.tut-4A* locus and *QPm.tut-4A* for powdery mildew resistance was mapped in a proximal 2.5 cM region of *T. militinae* translocation on the distal end of the wheat chromosome arm 4AL close to the 4AL.7BS translocation breakpoint. Possible origin of *QPm.tut-4A* from the chromosome 7GS of *T. militinae* is discussed.

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POLYMORPHISM OF THE EXON 11 OF THE *LR34* LOCUS IN UKRAINIAN WHEAT CULTIVARS

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Key words: *polymorphism, leaf rust, genetic markers, haplotype, SNP*

Leaf rust caused by the fungus *Puccinia triticina* Erikss. is one of the most frequent and pathogenic diseases of wheat (*Triticum aestivum* L.). Crop losses from it may reach 10-15 c/ha and more (http://agroua.net/plant/chemicaldefence/sickness/s-3/). Resistant allelic state of the *Lr34* locus confers nonspecific slow-rusting adult plant resistance in first 7-10 days of infection, decreasing quantity of uredinia per leaf area [1, 2]. Wheat haplotypes on *Lr34* locus differ by insertion-deletion in exon 11 (which made possible the development of co-dominant STS marker *cssfr5* [3]), insertion-deletion in exon 10, and SNPs in intron 4 and exon 12. A total of 5 haplotypes were identified, and only one of them confers leaf rust resistance [4].

Bread winter wheat cultivars developed in the Plant Breeding and Genetics Institute (PBGI) of the Natl. Academy of Agrarian Sciences (NAS) of Ukraine in Odessa, the Mironovka Institute of Wheat (MIP) of the NAS of Ukraine and the Institute of Plant Physiology and Genetics (IPPG) of the NAS of Ukraine (in total 80 cultivars) were studied for the exon 11 polymorphism using the co-dominant STS-marker *cssfr5* [3]. Our purpose was to make preliminary analysis of wheat cultivars with one of the most specific molecular marker of the *Lr34* resistance allelic state [4] and to use the derived data for further discrimination of other possible haplotypes. We detected the frequency of the *Lr34* haplotype with deletion (associated with the resistance allelic state) to be about 61% among the cultivars of PBGI. The frequency of this haplotype among the cultivars of MIP and IPPG was lower (about 13%). The average frequency of the *Lr34* resistant haplotype in the total sample of Ukrainian wheat cultivars was about 44% being higher than that among European ones.

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DISTRIBUTION OF THREE DWARFING GENES (*RHT*) IN WINTER WHEAT DEVELOPED BY KRASNODAR LUKYANENKO AGRICULTURAL RESEARCH INSTITUTE

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Key words: *winter wheat, dwarfing genes, Rht genes, polymerase chain reaction, molecular markers, allelic structure, marker-assisted selection*

One of the basic strategic directions in modern breeding of bread wheat is the application of *Rht* dwarfing genes (reduced plant height). *Rht* genes are used to reduce plant height, increase resistance to lodging and, thus, productivity of wheat. By now about 21 wheat *Rht* genes with significant effect on plant height have been identified. Currently, many dwarf and semi-dwarf cultivars of bread wheat developed at Krasnodar Lukyanenko Agricultural Research Institute are widely cultivated in Russia and abroad. However, the complete information on the distribution of *Rht* genes among them is not available. The purpose of this study was to investigate the prevalence of the three dwarfing genes (*Rht-B1b*, *Rht-D1b* and *Rht8*) in the pool of cultivars developed at Krasnodar Lukyanenko Agricultural Research Institute. The given information can be useful both in breeding programs and genetic research of winter wheat.
The allelic state of the dwarfing genes *Rht-B1*, *Rht-D1* and *Rht8* in 94 winter wheat cultivars developed at Krasnodar Lukyanenko Agricultural Research Institute was analyzed using diagnostic molecular markers *STS-Rht-B1*, *STS-Rht-D1* and *Xgwm261*, respectively. The alleles *Rht-B1b* and *Rht-D1b* have been revealed in 10 and 37 cultivars, respectively. However, cultivars with both alleles have been not found. Seventy-three cultivars have appeared to carry the *Xgwm261* 192bp allele known to be associated with the *Rht8c* allele. In addition, the following alleles have been revealed in this marker locus: 174bp (nine cultivars), 165bp (two cultivars), 194bp (two cultivars), 196bp (three cultivars), 202bp (one cultivar), 204bp (one cultivar), 211bp (two cultivars), 213bp (one cultivar). All cultivars with the exception of 'Lastochka' carry *Rht8c* along with *Rht-D1b*. Twenty-six cultivars carry *Rht8c* along with *Rht-B1b*, and 11 cultivars have either *Rht-B1b* or *Rht8c*.
The absence of the combination "*Rht-B1b+Rht-D1b*" may be due to a very strong additive effect of the genes. At the same time, the combinations "*Rht8c+Rht-B1b*" and "*Rht8c+Rht-D1b*" stands for 28% and 9% of the studied collection, respectively. Apparently, such allelic distribution might be caused, first, by multidirectional effect of the given genes and, second, by the weaker influence of *Rht8c* on plant height in comparison with *Rht-B1b* and *Rht-D1b*. Therefore, the simultaneous presence of the given alleles in genotype does not have negative effect on various traits of winter wheat cultivars.

THE STUDY OF WAXY-GENES ALLELES IN THE COLLECTION OF SOFT WHEAT

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Granule-Bound Starch Synthase I (GBSSI) is a key enzyme providing biosynthesis of a linear polysaccharide – amylose which together with amylopectin forms the endosperm's starch of wheat caryopsis. The genome of soft wheat *Triticum aestivum* L. has three homologous genes coding isoforms of the GBSSI enzyme. These genes designated *Wx* are located on chromosomes 7A (*Wx-A1*), 4A (*Wx-B1*) and 7D (*Wx-D1*). Mutations in the *Wx* genes affect the amount of amylose and, accordingly, the physical-chemical and functional properties of starch. It has been shown that the *Wx-B1* gene has the greatest influence on the amylose content and the end-product quality, followed by the *Wx-D1* and *Wx-A1* genes. Various functional allelic variants of the *Wx* genes of soft wheat have been revealed along with the null-alleles. However, the effect of the functional alleles on the amylose content is not very well studied because of difficulties associated with their revealing and creation of wheat lines with various allelic variants. The aim of our work was to study the allelic variants of the *Wx* genes in the collection of soft wheat. In this study, a collection of 128 varieties and lines of soft wheat was used. The larger part of the collection (99 varieties) included species of KLRAI selection. By this time, several molecular markers have been developed to each of the null-alleles of the *Wx* genes. By means of three various molecular markers to the *Wx-A1* gene it was found that varieties 'Starshina' and 'Sila' have in their genomes *Wx-A1* null-allele. For detection of various allelic variants of the *Wx-B1* gene it is necessary to use a combination of molecular markers because some markers may fail to distinguish the *Wx-B1e* allele from the null-allele (*Wx-B1b*), giving false-positive results of presence of the null-allele in genome. On the basis of amplification of four various systems of molecular markers we established that varieties 'Nota', 'Korotishka' and 'Lastochka' have the *Wx-B1e* allele in their genomes. The varieties having null-alleles of the *Wx-B1* gene were not found. In the *Wx-D1* gene, only wild-type alleles were revealed. Thus, among 128 varieties of the collection, only two varieties with the null-allele (*Wx-A1b*) of the gene *Wx-A1* and three varieties with the *Wx-B1e* allele of the Wx-B1 gene were found. The *Wx-B1e* allele has been poorly studied. Thereby, we cloned and sequenced an 804 bp fragment of the *Wx-B1e* allele. This allele was found to have a 34 bp insertion, an 8 bp deletion and 23 nucleotide substitutions in comparison with the *Wx-B1a* allele. The BLAST analysis showed the highest homology of the *Wx-B1e* allele with the sequences of the *Wx* genes of *T. spelta* and *T. durum,* suggesting the *Wx-B1e* allele to be introduced into the soft wheat genome from either dinkel or durum wheat as a result of spontaneous or selective crossings.

DEVELOPMENT OF STEM RUST RESISTANCE GERMPLASM UNDER APPLICATION OF MOLECULAR MARKERS

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Key words: *wheat, rust, breeding, resistance genes, molecular marker*

The region of Central Asia is one of the most important wheat areas in the world. As there was practically no breeding work for the rust in the past, most of the varieties released for commercial production are susceptible to the disease. The development of donors and potential breeding lines resistant to rust is a very important task. The stem rust of wheat is one of the most damaging diseases of wheat throughout the world. Ug99, which has the designation of TTKSK, is a race of stem rust *(Puccinia graminis Pers.f.sp.tritici)* that is virulent to the great majority of wheat varieties. Unlike other rusts, which only partially affect crop yields, stem rust can bring 100% crop loss. Up to 80 percent of all Asian and African wheat varieties are susceptible to the fungus and major wheat-producing nations to Iran's east – such as Afghanistan, India, Pakistan, Turkmenistan, Uzbekistan and Kazakhstan – should be on high alert as FAO (2008) warned. The aim of the present work was to find sources of stem rust resistance and to develop disease-free germplasm. The objectives of the study are 1) to find the new rust donors with identified stem rust resistance genes; 2) development of wheat collection from advanced lines resistant to stem rust. We have evaluated a wide range of wheat lines from various parts of the world against number races of *Puccinia graminis* collected in different parts of Kazakhstan. The wheat lines with moderate to good levels of resistance were identified. The subject of the study was 42 lines of wheat including advanced lines and hybrids F_4-F_5 were used. For the identification of the *Sr* gene sources PCR with microsatellite primers associated with the effective *Sr* genes was performed. The most attention was paid to the genes effective against Ug99 - *Sr22*, *Sr24* and *Sr46*. Identification of wheat lines resistant to stem rust using molecular markers associated to the *Sr22* gene localized in the short arm of chromosome 7A was done for 42 breeding lines using co-dominant SSR markers cfa2019 and cfa2123 flanking *Sr22* at the distance 5,9cM and 6.0 cM, respectively (Khan et al., 2005). Results of the PCR analysis showed that the carriers of *Sr*22 were 8 out of 42 studied entries: 185-6 (More/Currawong/LawsonC90-1(Marquis*8/*Sr39Lr35*)**,** *Sr22* TB, KS1 (Zhenis/PPG), KS5 (Zhenis/*T. dicoccum*), KS9 (Saratovskaya 29 /*T. macha*), KS12 (Zhenis/*T. compactum*), KS15 (Raminal) and KS16 (Zhenis/96Argus). The presence of the genes *Sr24-Lr24* was observed in 7 wheat lines resistant to stem rust, among them: 19W-207 Progress/94*Sr36*, 29W-306-94-*Sr36*/Progress, MV10-2000/4/AGRI/NAC//KAUZ, KALYOZ-18//8229/OK81306/4 /AGRI/NAC//KAUZ/3/1D13.1/MLT, SILVERSTAR/4/338-K1-1//ANB/BUC/3/GS50A/5/ TAM200/KAUZ, 338-K1-1//ANB/BUC/3/GS50A/4/4_22/5/BAYRAKTAR and TREGO/BTYSIB//ZARGANA-3/3/TAM200/KAUZ. The resistance gene *Sr46* was identified only in one line - 338-K1-1//ANB/BUC/3/GS50A/4/4_22/5/BAYRAKTAR. These lines now are being tested in the next step of breeding process – for the yield potential. These results can be used in the programs for Marker Assisted Selection. So, molecular markers accelerated the development of wheat cultivars with superior resistance by rapid identification of related genes and their transfer into modern cultivars.

PATTERNS OF RYE CHROMOSOME TRANSMISSION TO COMMON WHEAT GERMPLASM

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Key words: *T. aestivum, wheat-rye substitution lines, C-banding, GISH, introgression, chromosome translocations*

Introgressions of rye chromatin into wheat through wheat-rye chromosomal substitutions and translocations have increased the genetic diversity of bread wheat cultivars for various characters (Friebe et al., 1996; Rabinovich, 1998). Through wheat-rye translocations, 1BL.1RS and 1AL.1RS, a number of useful genes of rye (*Secale cereale* L.) were transferred to wheat (Zeller and Hsam, 1984; Lukaszewski, 1990; Villareal et al., 1998). The presence of resistance genes, promising quality traits and good agronomic performance associated with 2RL make this chromosome arm an attractive target for wheat improvement (Knacksted et al., 1994; McIntosh et al., 1995; Merker, Forsstrom, 2000; Hysing et al., 2007). Rye chromosome 5R is source of agronomic traits, too (Schlegel et al., 1993; Efremova et al., 2006). We have identified 1Rv(1A), $2R(2D)_1$, $2R(2D)_3$ and 5R(5D) as promising lines for transmission of desirable agronomic traits of wheat(Silkova et al., 2006). A detailed analysis of the introgressed rye chromatin is essential for applied breeding. Our objective was to analyze transmission of 1Rv, 2R and 5R chromosomes to common wheat backgrounds. Rates of wheat/rye substitutions and types of rearrangements for each rye chromosome were different. We obtained 45.35% plants 5R chromosome among F_2 hybrids of double monosomics 5R5D (5R(5D) × 'Saratovskaya 29' ('S29')); 10.37% plants had aberrant 5R chromosome. These were t5RS, isochromosomes i5RS and chromosomes with terminal deletion T5RS.5RL-del. Absence of amplification of SSR-markers mapped on 5RS and identification of PCR products for a number of 5RL markers allowed us to identify 9 plants containing 5RL only. Since cytological analysis did not detect t5RL, the results suggest the presence of small interstitial 5RL segments in 5D or other wheat chromosomes. We obtained 44.44% plants with 1Rv chromosome among F_2 hybrids of double monosomics 1Rv1A (1Rv(1A)xS29); 11.11% plants had a short arm of 1R and 2.8% translocation T1RS.1A. We detected 58.33% plants with 2R chromosome and 2.63% plants with telocentric t2R among F_2 hybrids of double monosomics 2R2D ($2R(2D)_1$ × 'S29'). In BC_1F_2 $2R(2D)_1$ × 'S29', $2R(2D)_1$ × 'Novosibirskaya 67' ('N67') and $2R(2D)_3$ × 'S29', $2R(2D)_3$ × 'N67', both the genotype of the wheat-rye substitution line and of the cultivar involved in the cross affected the frequency and transmission pattern of 2R chromosome. In backcrosses to cultivar 'N67', chromosomes 2R more frequently substituted 2D chromosomes than in cross combinations involving 'S29', so that 26% of hybrid offspring formed telocentric t2R and translocations T2R.2DL. The recent results would be of interest in development of more effective tools for applied wheat breeding.

MOLECULAR CHARACTERIZATION OF *T. AESTIVUM* × *T. DURUM* AND *T. AESTIVUM* × *T. DICOCCUM* HYBRID LINES RESISTANT TO BIOTIC STRESSES

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Key words: *leaf rust, stem rust, microsatellite markers, T. durum, T. dicoccum*

Rust diseases are the most important fungal pathogens of common wheat worldwide. One of the best approaches to overcome the threat from new races of pathogens is the development of new wheat varieties or intermediate lines resistant to the diseases. Involvement of new genes conferring resistance to various fungal diseases from relatives and progenitors of common wheat is a topical problem. It is known that the gene pool of tetraploid wheats *T. durum* and *T. dicoccum* contains many economically important alleles both for resistance and for yield and other agronomic important traits. A number of hybrid lines (F_6 generation) obtained through crosses between common wheat cultivars *T. aestivum* (BBAADD) with tetraploid wheats *T. durum* and *T. dicoccum* (BBAA) were evaluated on resistance to fungal diseases at adult plant stage. Among sixteen lines there were found eight lines resistant to leaf rust (*Puccinia triticina)*, six lines resistant to stem rust (*Puccinia graminis*), and five lines with moderate level of resistance to powdery mildew (*Blumeria graminis*). Comparative analysis of hybrid lines on agronomic valuable traits showed that a number of lines exceeded parental wheat cultivars on ear length, grain per spike, thousand grain weight and protein content.

For molecular characterization of hybrid lines and identification of *T. durum* and *T. dicoccum* introgression fragments, genomic wheat SSR markers (GWM, GDM) with known chromosome localization were used. Of the 145 microsatellite markers tested on parental forms (*T. durum*, *T. dicoccum*, wheat cultivars 'Chinese Spring', 'Pitic S62', 'Belorusskaya 80', and 'Skala'), 130 (90%) were found to be polymorphic. Microsatellite analysis by means of 111 polymorphic SSRs demonstrated that hybrid lines contain from 5 to 13 introgressive fragments. The obtained results revealed chromosomes with high and low levels of introgression of *T. durum* and *T. dicoccum* genomes in chromosomes of common wheat. Thus, all hybrid lines were found to have introgressions in chromosomes 1A, 3B, and 7A. More than 80% of the lines contained introgressions in chromosomes 2A, 5B, 6B, and 7B. Lower level of introgression (< 20%) was detected for chromosome 4B. Introgressions in chromosomes 5A and 7B were detected only in the long chromosome arms. Intervarietal differences were observed both for chromosomal localization of introgressive fragments and a number of the fragments. This study provides the intermediate hybrid lines that may be used in breeding programs for increasing resistance of modern wheat cultivars to biotic stresses and improving grain quality.

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A NEW APPROACH IN BREEDING ENLARGING THE RANGE OF VARIABILITY OF COMMON WHEAT (*TRITICUM AESTIVUM* L.)

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Keywords: *common wheat, Triton X-100, epigenetic*

Earlier a treatment of series of cultivars of common wheat with plant niacin acid and its derivatives resulted into development of the changed genotroph-plants having sets of economically valuable and adaptive traits which are retained throughout more than 65 generations [1]. We have attributed the obtained variability to epigenetic variability. At the present time, for induction of similar alterations the synthetic niacin acid possessing low biological activity is used. However, for deducing of an organism from an equilibrium state and induction of epigenetic variability the exposure of substances possessing a high biological activity is necessary [2]. That is why a search for such inductors is relevant. It is known that the majority of biochemical processes in a cell and in its nucleus occurs with a participation of biological membranes. Thereby, it is a great interest to study the effect of the substances influencing membrane state and membrane-related proteins on plant phenotype. Since 2005, we have been investigating such effects induced by a surface-active substance Triton X-100 (TX-100). The presowing treatment of seeds of spring common wheat 'Alem' with 1% TX-100 solution caused full destruction of plants. After the treatment with, respectively, 0.1% and 0.01% TX-100, 22% and 44% plants survived. Among the survived plants some alterations interesting from the breeding point of view were revealed, such as: a square head ear with doubled spikelets, a long friable ear with doubled spikelets and increased number of grains per spikelet. The changed forms have become late-ripening for 7-10 days on average in comparison with the initial cv. 'Alem', and the obtained traits alterations are retained throughout 6 generations. The revealed alterations possess a theoretical and practical meaning. To increase wheat productivity genotypes with non-standard ear morphology may be used: floral knobs formation reinforces ear attractive ability and leads to maximum use of assimilates increasing number of grains per spikelet. The inherited alterations induced by TX-100 treatment were also shown at a molecular level. For example in sugar beet (*Beta vulgaris* L.), PCR-profiles of enzyme locus *Adh1* differed between the control initial plants and those treated with TX-100, suggesting TX-100 to influence DNA of vegetative genome and nuclear membrane to play a role in structural and functional organization of plant genome.

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GENERATION OF TRANSGENIC WHEAT THROUGH CALLUS INITIATION FROM MATURE EMBRYOS

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Keywords: *somatic embryogenesis, plant regeneration, transgenic wheat*

Genetic transformation is a powerful tool for plant molecular genetics and breeding. For practical use in breeding programs, a large number of independently transformed lines are desirable. Tissue culture and plant regeneration systems play a critical role in successful production of transgenic plants. In wheat, immature embryos are the most widely used explant to regenerate transgenic plants, but they are inconvenient due to their temporal availability and production requirements. Tissue culture derived from mature seeds/embryos is an alternative approach that can be used to save time and space, and reduce greenhouse costs associated with growing plants for the collection of immature embryos. A method for producing transgenic wheat plants from mature seeds using the endosperm-supported callus induction technique has been developed. En effective regeneration frequency (up to 65%) sufficient for transformation experiments was achieved by the optimal tissue culture medium and screening of a diverse set of spring and winter Russian wheat genotypes. A reproducible transformation system for spring wheat has been developed based on the combination of the *gfp* and *bar* genes for transgene recovery. The biolistic approach was used to produce transgenic plants with average frequency of 0.7%. The influence of plant hormone composition of medium on the efficiency of mature embryos tissues transformation was shown. The inheritance of the transgenes in the wheat T1 and T2 generation as well as the resistance to herbicide treatment of transgenic plants were demonstrated. This alternative wheat transformation protocol may facilitate production of multiple transgenic plants and significantly reduce the time, costs and labor.

BREAD WHEAT GERMPLASM RESISTANCE TO STEM RUST RACE UG99

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Western Siberia has met Siberian races of stem rust back in 2007 as they broke the resistance of wheat cultivars putting the wheat production under a threat. Years of disease outbreaks in the region may reduce the wheat yield by as much as 50%. During "the Cold War" stem rust was considered as a biological weapon that could wipe out the yield of cereal crops. An aggressive virulent race of stem rust (Ug99) was first reported in Uganda in 1999. The race may reach the Central Asian region in the nearest 3-5 years from where this pathogen may be carried away to the Western Siberia. Therefore, finding sources of resistance are increasingly important for wheat breeding.

Goal of the research is to identify the sources of resistance to the aggressive race Ug99 and race populations of stem rust present in the Western Siberia. Resolving this goal will involve using shuttle breeding conducted jointly with the International Wheat and Maize Improvement Center (CIMMYT headquartered in Mexico). Assessments and observations for the germplasm complied with VIR methodological guidelines for wheat collection study (M, 1979). The stem rust research helped identify and map genes resistant to Ug99. (1). Major genes: a number of well-known genes - *Sr25*/*Lr19*, *Sr1A.1R*, *SrACCadillac* (6DS, close to *Bt10*); a number of recently mapped genes –*SrSha7* and *SrSharp*; non-mapped genes - *SrHUW234*. (2). Minor genes - polygenic resistance. (3). Unknown genes found in the following varieties from Kazakhstan, Omsk and Chelyabinsk: 'Stepnaya 62', 'Omskaya 37', 'Chelyaba 75'. Analysis of Western Siberian Germplasm of Spring Bread Wheat identified 9 sources of resistance (BC1E.59/L.20639(22889); NS888 Lr19/Kormovaya 12; Om.20/Irt.10//L.444/3/Akt; NS888 Lr19/Lut.45-95; Eritr.23334; Eritr.23442; BC2E.59/L.20639 (22918); Lut.23419; 'Chelyaba 75') to the Siberian collection of stem rust races and in particular, to Ug99.

MOLECULAR-GENETIC EVIDENCE OF (1B)1R SUBSTITUTION AND 1BL.1RS TRANSLOCATION IN WHEAT INTROGRESSION STOCKS

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Keywords**:** *genetic linkage, hairy leaf, STS-markers, rust resistance, Triticum aestivum*

For breeding purposes a number of introgression stocks with high resistance to leaf and stem rust, frost tolerance, high protein content and some morphological characters were developed as a result of wide crosses (Motsnyy et al., 2000). The aim of this work was a marker assisted identification of 1B-1R wheat-rye chromosome substitution and translocation, analysis of their meiotic behavior, as well as plant pathological analysis and phenotypical evaluations of the introgression stocks. The investigation was carried out within a program for the development of a genetic collection of bread wheat lines with qualitative characters.

By using of molecular-genetic and cytological analysis the 1B-1R wheat-rye chromosome substitution and translocation have been identified in the original introgression stocks. The pairing between short arms of the $1B_L.1R_S$ translocation and of bread wheat chromosome 1B was observed with very low frequency. Tree and two genes for resistance, respectively, to leaf and stem rust were revealed, and *Lr26* and *Sr31* among them have been recognized and determined to be effective. No recombination between the *Lr26* and *Sr31* has been found. The association of these genes with STS-markers (REMS1303, SR1R003, ω-sec-P3 and ω-sec-P4) on the short arm of $1B_L.1R_S$ translocation chromosome was detected. For the *Hg1* gene determining hairiness of glumes, Mendelian mode of inheritance was observed. Three major linked genes determining hairiness of the leaf upper surface (Hl^{up}), lower surface (Hl_{low}) and leaf margin (*Hlm*) were revealed with location, supposedly, on the long arm of chromosome 4D. The rust resistance genes were contributed by cv. 'Avrora' and originated from rye 'Petkus'. The glume hairiness gene was contributed by old cv. 'Hostianum 237'. The leaf pubescence genes were contributed by a synthetic (*T. timopheevii* Zhuk./*Aegilops tauschii* Coss) and, therefore, originated from *Ae. tauschii*. The Hl^{up}, Hl_{low} and *Hlm* loci seem to be non-allelic to *Hl1* gene.

DEVELOPMENT OF BIOTECHNOLOGICAL METHODS FOR PRODUCTION OF FROST-RESISTANT WHEATGRASS LINES AND THEIR USE FOR CREATION OF *TRITICUM-AGROPYRON* HYBRIDS

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Key words: *Triticum-Agropyron* hybrids, frost resistance, anther culture, leaf nurse

The problem of obtaining stable yields of winter wheat has not yet been resolved. Winterkill of a winter wheat occurs periodically during the harsh winters. The own genetic potential of winter wheat is not enough for the desired increase in cold resistance. One way of obtaining high frost-resistant varieties of winter wheat is a distant hybridization with wild relatives, in particular with certain species of wheatgrass, among which the most promising is *Agropyron glaucum* (Desf.). Breeders of various countries carried out the hybridization of wheat with wheatgrass. The works of Academician N.V. Tsitsin are the best known. The drawback in these studies is the small amount of used genotypes of wheatgrass, no clear definition of frost-resistance of donor wheatgrass plants, and the lack of methods for determining the morphogenic properties of wheatgrass pollen. To overcome these difficulties we have done a lot of long-term work, resulting in the following: 1) A massive collection of genotypes of wheatgrass *Agropyron glaucum* was produced from the original material collected in Eastern Kazakhstan, on the elevated site with little snow which suggests that they have high frost resistance [1]. 2) The efficient method for rapid analysis of frost resistance of winter wheat, wheatgrass and other crops was developed [1]. 3) Biotechnology for obtaining pure wheatgrass androgenetic lines *via* anther culture was developed [2]. 4) A method for testing of wheatgrass genotypes in their ability to produce green haploids was developed. 5) A method for identifying wheat genotypes possessing good crossability with wheatgrass and responsiveness to biostimulants was developed. 6) A large-scale breeding of cold-resistant winter wheat varieties and more frost-resistant lines of wheatgrass was made. As a result of the hybridization and subsiquent backrossing the extensive material of wheat-wheatgrass hybrids was obtained. This material is used by us in further work towards a creation of frost-resistant varieties of winter wheat. 7) An alternative method of transfer of frost resistance character from wheatgrass to wheat by means of "leaf nurse" is being developed [3].

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DEVELOPMENT OF MOLECULAR AND GENETIC STOCKS FOR A PHYSICAL MAP OF 5B CHROMOSOME OF COMMON WHEAT

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Chromosome 5B is nearly 870 Mbp (5BL = 580 Mbp and 5BS = 290 Mbp) and is known to carry more than 30 genes that control a number of morphological and quantitative traits resistance to abiotic and biotic stress and gene, such as *Ph1* involved in nonhomoeologous pairing.

During the work the data of 188 SSR and EST-SSR markers of 5B chromosome were collected. The structures of primers, PCR condition, and the length of PCR fragments for seven lines and cultivars of bread wheat were included in the 5B markers Databank. The cytological markers for specific 5B chromosome regions, including telomeric regions, were developed and checked.

Three genetic maps of 5B chromosome were developed by using F_2 population from crossing 'Chinese Spring' and 'Renan', 'Skala' and Line 842, 'Skala' and Line 832. The basic consensus genetic map of 5B chromosome of common wheat was constructed based on current and previously obtained data.

Twenty eight radiation hybrids from the cross of 'Saratovskaya 29' with nulli-tetrasomic lines of 'Chinese Spring' were developed and analyzed. Seven plants have the deletions of one or two markers. It was shown that the 20 krad radiation doses not influence plant growing and we were able to obtain the chromosomal microdeletions.

The physical mapping of 40 SSR markers has been done by using 14 deletions lines obtained from Kansas State University.

These results are an important step for future work on physical mapping and localization of BAC clones on 5B chromosome of common wheat.

CHROMOSOMAL LOCATION OF THE GENE Q^S INTROGRESSED IN BREAD WHEAT FROM *AEGILOPS SPELTOIDES* TAUSCH, AND IT'S INTERACTION WITH THE GENE *Q* FROM *TRITICUM SPELTA* L.

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The gene *Q* of hexaploid wheats determining a spike shape is considered to be an analogue of APETALA-2 gene of *Arabidopsis* responsible for floral initiation (De Faris et al. 2003). As the allelic state of the gene determines the attribution of the hexaploid to bread or spelta wheat, it has a domestication importance. The gene *Q* pleiotropically influences threshing ability, plant height and flowering date. It has been mapped on chromosome 5AL; the functional homoeoallelic genes on wheat chromosomes 5B and 5D have not been found yet.

We studied the bread wheat line $84/98^w$ from 'Arsenal' collection (Lapochkina et al. 2003) developed on the basis of cultivar 'Rodina' by crossing with *Aegilops speltoides* Tausch., the wild diploid progenitor of the B genome of wheat. In contrast to 'Rodina', Line $84/98^w$ has a speltoid spike. Earlier it was shown that the trait is controlled with a dominant gene designated as Q^s (Simonov et al. 2009).

The first aim of the work was to determine a location of the gene Q^s, introgressed from *Ae. speltoides* into Line $84/98^w$ within the genome of bread wheat. The second aim consisted in study of the mode of interaction of this gene with the dominant allele of the gene *Q* of *T. spelta*.

Monosomic lines for the fifth homoeological group of the cultivars 'Saratovskaya 29' and 'Diamant 2' were used for chromosomal location of the gene Q^s. Through monosomic analysis it was shown that the gene inherited from *Ae. speltoides* is localized on chromosome 5A. Therefore, non-homoeological introgression 5S/5A took place.

To study the interaction between the two genes, two *T. spelta* accessions and a substitution line 'Chinese Spring'(*T. spelta* 5A) were used. F_1 hybrids of Line $84/98^w$ with these genotypes significantly overcame the parental forms for spike length and spikelets number and were named as 'superspeltoids'. They appeared constantly in F_2-F_5 segregating generations. Because of the homoeological introgression into chromosome 5A this phenotype was characteristic only for heterozygotes. Therefore, the gene Q^s of *Ae. speltoides* and the gene *Q* of *T. spelta* interact additively.

GENETIC DETERMINATION OF PLANT WATER REGIME OF BREAD SPRING WHEAT *TRITICUM AESTIVUM* L.

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Key words: *Water retention, drought, water content*

Water-retaining capacity largely reflects an adaptive metabolism and determines the resistance of plants to dehydration, that is, with increasing water stress prevents from decreasing water content in tissues. Structural state of protein molecules, the physical properties of water permeability of cell membranes regulate this process at the molecular level. Mechanisms exist to counter dehydration in order to maintain a high plant water status under water deficit. The volume of total water content is used as an integrated indicator of ecological and physiological characteristics of the water treatment plants and their adaptation to environmental conditions. In our study, an attempt was made to identify and to map QTL responsible for features that form the water regime: water-holding capacity, hydration, water scarcity and dry matter content. Measurements of these traits were conducted on leaves of spring wheat mapping population ITMI (International Triticeae Mapping Initiative). Detected QTL (LOD $\geq$ 2,5) were distributed throughout the genome. Thus, QTLs for water-holding capacity after three hours were located on chromosomes 1AL, 2BL, 6DS, QTL for water-holding capacity after 24 hours - on chromosome 6BS. QTLs for water content were found on chromosomes 2DL (LOD 2.3) and 7AL. QTL determining dry matter content was detected on chromosomes 2BL, 6BS, and QTLs for responding to water deficit were located on chromosome 1BS, 1DL, 2BS, 6DS. Moreover, it should be noted the almost complete coincidence of the QTL positions on chromosome 6B for traits "dry matter content" and "water-retaining capacity in 24 hours" and on chromosome 6D for traits "water-retaining capacity in 3 hours" and "water scarcity" was found. The coincidence of QTLs for traits "water content" and "water shortage" on chromosome 1B and 2D was also observed. The traits "water scarcity" and "solids content" have the common QTL position on chromosome 2A, but their value was less than the threshold value of LOD. The detected QTLs had dominant and additive effects. The significance of such studies is evident, both in terms of identifying the role of different genomes in the evolutionary formation of the mechanisms of drought resistance in modern wheat species, and for the creation of highly drought-resistant wheat varieties.

WHEAT PROMOTER SEQUENCES FOR TRANSGENE EXPRESSION

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A toolbox of promoters with defined specificities is a valuable resource in controlling the expression of transgenes in desired tissues for both wheat improvement and molecular farming. However there is limited information regarding the gene expression profile for wheat-specific promoters. A number of wheat promoters have been isolated and investigated for their spatial and temporal control of transgene expression. These promoters have been tested in the homologous cereal (wheat, barley, rice) systems as well as in the heterologous (tobacco, *Arabidopsis*) systems. It is important that the expression patterns are predetermined in the specific genotype targeted for transformation because the same promoter-transgene construct can produce different expression patterns in different host species. Information on the promoter size, nucleotide sequence, and transcription patterns, as well as regulators influencing the wheat promoter activities are represented in TGP database format (http://wwwmgs.bionet.nsc.ru/mgs/dbases/tgp/). All promoters in the TGP database are accompanied by links to the relevant publications. TGP also includes accession numbers of nucleotide sequences from GenBank database, promoter positions according to the transcription start site or translation initiation site, and target species name. Each annotated wheat promoter is supplied with a corresponding gene description. TGP includes constitutive, targeted, or inducible wheat promoters that have been characterized in specific plant species. The wheat germ promoter is regulated by biotic and abiotic stress, including induction by heavy metals, wounding, and tobacco mosaic virus. The wheat defensin promoters are the potential tools for engineering disease resistance in cereal grains. The wheat AIDFa promoter drives expression of the reporter gene in wheat calli under drought, salinity, low-temperature stress conditions, and is also activated by exogenous abscisic acid. The wheat chloroplast-targeted protein *cor15* gene and *Wcs120* gene promoters are induced under low-temperature conditions. The wheat HMW-glutenin, LMW-glutenin, gliadin storage protein promoters, small cysteine-rich protein (PR60) promoter, puroindoline PinA and PinB gene promoters maintain seed-specific expression in wheat, barley or rice. The PinA promoter is also induced by wounding and by *Magnaporthe grisea*. The wheat early-maturing (Em) promoter is up-regulated by abscisic acid and can be used as a strong promoter to direct transgenes in specific tissues of barley and wheat grain. The wheat phosphate transporter PHT1.2 and PT2 promoters are specifically induced by phosphate-starvation and predominantly expressed in the roots. The wheat GstA1 promoter in combination with the WIR1a intron is found to drive strong and constitutive expression in wheat epidermis. The wheat CKX2.1 promoter is mainly expressed in the reproductive organs and the vasculature in the vegetative organs. The wheat *Cab-1* gene promoter is responsible for the circadian clock-regulated and phytochrome-regulated gene expression. The representation of wheat promoters in the TGP database provides a possibility to select a promoter with the desired properties including appropriate stress-, tissue-, and stage-specific activities for different experimental tasks. These data may be used for design of genetic constructs to conduct experiments for both fundamental and biotechnological investigations.

A GENE REGULATORY NETWORK MODEL FOR VERNALIZATION AND SEASONAL FLOWERING RESPONSE IN WINTER WHEAT

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Key words: *systems biology, VRN1, etpigenetic, vernalization, VRN1, day length*

The transition from vegetative to reproductive development in wheat is regulated by seasonal cues including vernalization and photoperiod. Vernalization accelerates flowering in winter cereals and mainly regulated by the *VRN1* (*Arabidopsis* AP1 homologue), *VRN2*, and *VRN3* (*FT* homologue) MADS box genes. Flowering during autumn is prevented by the VRN2 downregulation of *VRN3* and low *VRN1* transcription. After vernalization MADS-box transcription factor VRN1 is induced and promoted of flowering. Vernalization releases *VRN1* chromatin from an inactive state and results in a gradual increase in its transcript levels.
During the long days of spring, *VRN1* is upregulated by a RAF kinase inhibitor–like protein. VRN3 interacts with the bZIP protein FDL2, which binds to the *VRN1* promoter. Vernalization induces *VRN1*, which is followed by the down-regulation of *VRN2*, thereby increasing VRN3 level. The expression of VRN3 promotes further increases in *VRN1* transcription level, generating a positive feedback loop that enhances *VRN1* transcripts until a threshold level required to initiate flowering.
The interactions between CCT and NF-Y proteins play an important role in the integration of the vernalization and photoperiod seasonal signals. The PPD1 and CO2, that code photoperiod genes, increase VRN3 expression under the long days. *VRN3* transcription is modulated through interactions involving CCT-domain proteins and NF-Y transcription factors. The CCT domains present in VRN2 and CO2 proteins interact with the same subset of NF-Y proteins. The regulation of flowering is reflected in the competition of flower repressor VRN2 and activator CO2 proteins for interaction with NF-Y complex.
Seasonal changes in day length are perceived by plant photoreceptors and transmitted to the circadian clock to modulate developmental responses, such as flowering time. Blue light-sensing cryptochromes, the E3 ubiquitin-ligase COP1, and clock-associated proteins ELF3 and GI, regulate this process. The COP1 acts with ELF3 to mediate day length signaling from CRY2 to GI within the photoperiod flowering pathway. ELF3 acts as a substrate adaptor, enabling COP1 to modulate light input signal to the circadian clock through targeted destabilization of GI.
Here we integrate data on vernalization and photoperiod genes in a gene network. We have developed a simple logic model of the wheat development gene network. Using a synchronous boolean model we have simulated the network dynamics. The found fixed points correspond to the wheat development patterns of year seasons. This model can be useful to test the coherence of experimental data and to hypothesize gene interactions that remain to be discovered.

THE ROLE OF THE GENES FOR VERNALIZATION REQUIREMENT (*VRN*), ADAPTATION TO PHOTOPERIOD (*PPD*) AND EARLINESS *PER SE* (*EPS*) IN ADAPTATION OF WHEAT VARIETIES TO CONDITIONS OF SOUTH - EAST KAZAKHSTAN

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Key words: *wheat varieties adaptation, speed of development up to heading, Vrn, Ppd, Eps genes.*

The role of various combinations of the main genes of vernalization requirement (*Vrn*), adaptation to photoperiod (*Ppd*) and earliness *per se* (*Eps*) in adaptation of wheat varieties to conditions of South-East Kazakhstan has been studied on a material of the international nursery GAWYT – Global Adaptation Wheat Yield Trial (CIMMYT). The nursery included the key combinations of *Vrn*, *Ppd*, and *Eps* genes in 5 groups of genotypes which differed on reaction to temperature and photoperiod changes. Because 75% of a hereditary variation for vegetative period length is caused by the system of vernalization requirement (*Vrn*) genes, the genetic system of the *Vrn* genes was taken for a basic analysis. The genetic system of the *Ppd* genes providing 30% of bread wheat variability for rate of development and the third system, *Eps* genes, which determines 5% of the remained differences, were considered as combining. Isogenic lines 'Triple Dirk' (TD): TDD (*Vrn1*), TDB (*Vrn2*) and TDE (*Vrn3*) with neutral reaction to the photoperiod and not accelerating development at rise of temperature were used as standard lines. In the conditions of South-East Kazakhstan (elevated temperatures and more short photoperiod in comparison with zones of middle latitudes), the genetic variability created by combination of the *Vrn* genes with genetic system of sensitivity to photoperiod and earliness *per se* has led to a considerable phenotypic variability. Effects of dominant loci of standard lines TDD and TDE were identical and higher than of TDB line. The length of the period "number days to heading" of the earliest genotype of a set, 'Sonora 64' (*Vrn1Vrn3*), was the same as of the standard lines TDE and TDD. Polymorphism in terms of the "number days before heading" found between the varieties having the same *Vrn* genotype can be attributed to the flexibility of the genetic system supervising speed and type of development (Halloran 1967; Koval, Goncharov 1998). The lines with *Eps* genes (participating in regulation of development rates, but genetically insufficiently studied) considerably accelerated the development up to heading at rise of temperature. The early maturing line CLT/H471.71A//3*CLT/4/-CLT/H471.71A//2*CLT/3/-PVN (*Vrn1Vrn3*) is selected. The analysis of three groups of genotypes - spring photosensitive (V1HB, V3CS), spring photo neutral (V3P1TDE, V3P2CS) and facultative type wheat (V2TDB) obtained on the base of winter photosensitive variety 'Stephens' showed the possibility to manipulate with the specified genes for obtaining of genotypes with various rate of development up to heading.
The following range of genotypes with elevating period "days to heading" (from early maturity to late maturity) has been received: *Vrn1Vrn3 > Vrn1Vrn2 > Vrn2Vrn3 > Vrn1 > Vrn3 > Vrn2* (dominant alleles are specified). Positive correlation ($r \geq 0.5$) between number of days before heading and the length of vegetative period was established, i.e. acceleration of development up to heading clearly defined the overall acceleration of development.

ANALYSIS OF EVOLUTION OF THE TRITICEAE SPEACES USING FAT SEQUENCE

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Evolution of the Triticeae species was studied using FISH with the *Fat* element that was isolated from BAC-end sequences of wheat chromosome 3B. Fifty one species representing eight genera of Poaceae (*Aegilops, Triticum, Agropyron, Pseudoroegneria, Elymus, Secale, Hordeum, Avena*, and Triticale) were examined using fluorescence *in situ* hybridisation (FISH). The *Fat* sequence was not found in oats and in two barley species, *Hordeum vulgare* and *H. spontaneum.* Only very low amounts of the *Fat* element were detected on the chromosomes of two other barley species, *H. geniculatum* and *H. chilense*, with different genome compositions. The chromosomes of other cereal species exhibited distinct hybridisation patterns with the *Fat* probe, and labelling intensity varied significantly depending on the species or genome. The highest amount of hybridisation was detected on chromosomes of the D-genome of *Aegilops* and *Triticum* and on chromosomes of the S-genome of *Agropyron*. The *Fat* element is tandemly organised, however, hybridisation with *Fat* probe produces uneven, diffuse signals in the proximal regions of chromosomes. In some of the genomes we investigated it also forms distinct, sharp clusters in chromosome-specific positions, and the brightest fluorescence was always observed on group 4 chromosomes. Thus, the *Fat* element represents a new family of Triticeae-specific, repeated DNA elements with a clustered-dispersed distribution pattern. These elements may have first emerged in cereal genomes prior or at the time of divergence of the genus *Hordeum* from the last common ancestor. During subsequent evolution the amount and chromosomal distribution of the *Fat* element changed due to amplification, elimination and re-distribution of this sequence. Because the labelling patterns that we detected were highly specific, the *Fat* element can be used as an accessory probe in FISH analysis for chromosome identification and investigation of evolutionary processes at the chromosomal level.

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