The International Conference

Plant Genetics, Genomics, and Biotechnology

PlantGen 2010

PlantGen
Novosibirsk, Russia
June 07–10, 2010

Institute of Cytology and Genetics SB RAS

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General genetics. The ecological, population-genetic and evolutionary bases of biodiversity. Development of novel methods applied in animal and plant genetics and breeding for efficient use of gene pools.

Scientific publications in 2007-2009

Total – 1735
Including:
monographies in Russian – 11;
articles in reviewed Russian journals – 610;
monographies in English – 2;
articles in peer-reviewed
international journals – 344.

The research grants in 2007-2009

Grants of the Russian Foundation for Basic Research – 301
Projects of the Federal Target Scientific Technological Program - 30
Grants of the Russian Academy of Sciences - 86
Grants of Siberian Branch of the Russian Academy of Sciences – 100
Grants of the President - 18
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Total – 802

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Abstract book

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June 7

ORAL PRESENTATIONS

SECTION

«PLANT GENOME SEQUENCING IN THE 21ST CENTURY»

7 июня

УСТНЫЕ СООБЩЕНИЯ

СЕКЦИЯ

«СЕКВЕНИРОВАНИЕ ГЕНОМА РАСТЕНИЙ В XXI ВЕКЕ»

THE INTERNATIONAL WHEAT GENOME SEQUENCING CONSORTIUM (IWGSC): BUILDING THE FOUNDATION FOR A PARADIGM SHIFT IN WHEAT BREEDING

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As the staple food for 35 % of the world's population, wheat is one of the most important crop species. Wheat and maize, alone, provide almost two-thirds of the world's food energy intake and more than 60 % of the total calories and proteins for daily life is derived from wheat, rice, and maize. With the world population estimated to reach almost 7 billion this year with more than 1 billion facing food insecurity, it is critically important to accelerate cereal crop improvement. Genomics offers powerful tools for understanding the molecular basis of phenotypic variation, accelerating gene cloning and marker assisted selection, and for improving the efficiency of exploiting genetic diversity. All of these combined allow breeders to reduce substantially the time from discovery to commercialization. While rice and maize improvement is profiting already from genome sequences as well as whole genome-based tools and resources, genome sequence-based tools to accelerate wheat improvement remain significantly behind. In 2005, a group of wheat growers, breeders, and plant scientists launched the International Wheat Genome Sequencing Consortium (IWGSC, www.wheatgenome.org) with the goal of sequencing the bread wheat genome as bread wheat is grown on more than 95 % of the wheat growing area and on more area than any other crop. The IWGSC utilizes a milestone-based strategy designed to provide breeders access to an increasing array of tools and resources without having to wait for the completed physical maps or sequence. Whole genome physical mapping and sequencing of the bread wheat genome is a significant challenge, however, because it is allohexaploid ($2n = 6x = 42$), highly repetitive (~80 %), and 40 times the size of the rice genome with 17 billion base pairs. To approach the wheat genome, the IWGSC is following a chromosome-specific approach based on the ability to sort individual chromosomes or chromosome arms at high speed using laser flow cytometry and aneuploid lines. The development of the chromosome-based physical maps provides breeders immediate access to resources while also building the substrate for sequencing. In 2008, the physical map of the largest wheat chromosome (3B, ~1Gb) was developed, confirming the feasibility of this approach for physical mapping the 21 bread wheat chromosomes. Funding is in place for physical maps of more than half of the chromosomes and projects for the remaining are underway. In 2010, the Genoscope in France began the process of sequencing the bread wheat genome by starting the sequencing of chromosome 3B. An overview of the IWGSC strategies and projects will be presented.

CHROMOSOME-BASED APPROACHES FOR GENOMICS OF TRITICEAE

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Key words: *Flow cytometry; Chromosome sorting; BAC libraries; Physical mapping; Shotgun sequencing;*

Barley, rye and wheat, the major crops of the tribe Triticeae, are characterized by huge genomes, which consist mainly of various types of dispersed repetitive DNA sequences. In wheat, the evolution of cultivated tetraploid and hexaploid species was accompanied by polyploidization events, which further increased the sequence redundancy and resulted in the presence of homoeologous loci. These genome features hamper development of markers associated with traits of interest, positional gene cloning and genome sequencing. With the aim to facilitate genomics in barley, rye and wheat, we have been developing chromosome-based approaches, which considerably reduce the genome complexity. This is achieved by dissecting the genomes into individual chromosomes and chromosome arms, which represent only a few per cent of the whole genome. Individual chromosomes can be purified by flow cytometric sorting. However, as flow cytometry classifies chromosomes according to relative DNA content, chromosomes that do not differ enough in size cannot be resolved. Consequently, only a few chromosomes can be sorted from stocks with wild-type karyotypes. This obstacle has been overcome by means of chromosome deletion and alien addition lines from which particular chromosomes and chromosome arms can be sorted. DNA of sorted chromosomes is intact and the chromosomes are suitable for a range of applications. One of them — the construction of chromosome-specific BAC libraries — has been adopted by the International Wheat Genome Sequencing Consortium to develop physical map of hexaploid wheat. This strategy avoids problems in contig assembly due to the presence of homoeologs, facilitates division of labor and international collaboration. To date, BAC libraries from 12 wheat chromosomes have been constructed. Other significant uses of sorted chromosomes include molecular cytogenetic mapping, physical mapping using PCR, high-throughput mapping on DNA arrays, and targeted isolation of molecular markers for saturation of genetic maps for specific chromosomes. The advent of the next generation sequencing technology expanded the application of chromosome sorting as large amounts of sequence data can be obtained from target genome regions in a short time and at affordable cost. For most of the applications, fractions of sorted chromosomes and/or their DNA can be prepared and distributed to other laboratories so that they do not have to develop their own infrastructure for sorting. Thus, the chromosome-based approach can be employed in different laboratories across the world. Our work has been supported by the Czech Science Foundation (grant awards 521/08/1629, 501/10/1740 and 501/10/1778) and Czech Republic Ministry of Education, Youth and Sports (grant award LC06004).

B-GENOME EVOLUTION AND 5B CHROMOSOME

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Three key periods that were accompanied by considerable rearrangements in the B genome of wheat and its progenitor can be considered. The first period covers the period from the divergence of diploid *Triticum* and *Aegilops* species from their common progenitor (2.5–6 million years ago) to formation of the tetraploid *T. diccocoides* (about 500 thousand years ago). Significant genomic rearrangements in the diploid progenitor of the B genome, *Ae. speltoides* (SS genome), involved a considerable amplification of repeated DNA sequences, which led to an increase in the number of heterochromatin blocks on chromosomes relative to other diploid *Aegilops* and *Triticum* species. Our analysis has demonstrated that during this period Spelt1 repeats intensively amplified as well as several mobile elements proliferated, in particular, the genome-specific *gypsy* LTR-retrotransposon *Fatima* and CACTA DNA-transposon *Caspar*. The second period in the B-genome evolution was associated with the emergence of tetraploid (BBAA genome) and its subsequent evolution. The third most important event leading to the next rearrangement of the B genome took place relatively recently, 7000–9500 years ago, being associated with the emergence of hexaploid wheat with the genomic formula BBAADD. The evolution of the B/S genome involved intergenomic and intragenomic translocations and chromosome inversions. So far, five rearrangements in the B-genome chromosomes of polyploid wheats has been observed and described; the majority of them took place during the formation and evolution of tetraploid species. Our mapping of the S-genome chromosomes and comparison with the B-genome chromosome maps have demonstrated that individual rearrangements pre-existed in *Ae. speltoides;* moreover, *Ae. speltoides* is polymorphic for these rearrangements.
The translocations and inversions of chromosome 5B/5S, which could have taken place in the evolution of *Ae. speltoides* and allopolyploid wheats, yet has not been detected so far. On the other hand, the changes in chromosome 5B that had brought forth the locus *Ph1*, critical for correct mitosis and meiosis in the allopolyploid nucleus, took place due to certain yet unknown mechanisms. Chromosome 5B is nearly 870 Mbp (5BL = 580 Mbp and 5BS = 290 Mbp) and is known to carry important genes controlling the key aspects of wheat biology, in particular, *Kr1,* controlling interspecific incompatibility; the genes controlling hybrid necrosis and response to vernalization, *Ne1* and *Vrn-B1* loci; and genes controlling resistance to various pathogens and bread-making quality. Construction of the physical map for chromosome 5B and determination of its primary structure are the goals for further research

A PHYSICAL MAP OF CHROMOSOME 3B AS A FOUNDATION FOR SEQUENCING AND MARKER DEVELOPMENT IN HEXAPLOID WHEAT (*TRITICUM AESTIVUM* L.)

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Key words: *Wheat, Physical map, Genetic map, Molecular markers, BAC*

Physical maps anchored to genetic maps are the substrate for genome sequencing and they provide efficient tools for marker development, map based cloning, QTL mapping, as well as for structural, functional, and comparative genomics studies. In the framework of the International Wheat Genome Sequencing Consortium, we have developed a physical map of the hexaploid wheat chromosome 3B (995 Mb) and established a proof of concept for physical mapping of the 21 bread wheat chromosome through a chromosome-based approach. The 3B physical map consists of 1.036 contigs with an average size of 783 kb that cover 811 Mb i.e. 82 % of the chromosome. To date, the 10x physical map is anchored to cytogenetic and genetic maps with 1.443 markers thereby providing a framework for efficient map-based cloning and marker development through BAC end and contig sequencing [1]. Fourteen contigs of 500 kb to 3.2 Mb representing different regions of the chromosome have been sequenced and annotated providing valuable information about the genome composition and organisation and supporting strategic decisions for sequencing the entire chromosome in the near future. An update about the status of the physical map and the efforts in sequencing chromosome 3B as well as the application of these resources to genomic studies and wheat improvement will be presented.

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ORAL PRESENTATIONS

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«CHROMOSOME AND CELL BIOTECHNOLOGY»

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«ХРОМОСОМНАЯ И КЛЕТОЧНАЯ БИОТЕХНОЛОГИЯ»

DEVELOPMENT AND CHARACTERIZATION OF ALIEN CHROMOSOME TRANSLOCATION IN WHEAT

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Wheat relatives have proved useful to wheat improvement as sources of valuable traits, particularly pest and disease resistance, and tolerance to abiotic stress. However, the introduction of alien useful genes is difficult because pairing and recombination between wheat and alien chromosomes rarely occurs. The development of alien addition lines, substitution lines and translocation lines, especially small segment translocation lines, is an efficient strategy for alien useful genes transfer. In Cytogenetics Institute, Nanjing Agricultural University, translocation lines between common wheat and *Haynaldia villosa, Leymus reacemosus, Roegneria kamoji* and *Secale cereale* etc. have been developed by irradiation, gametocyte effect and chromosome pairing homoeologous system (*ph1b*, Ph^I), and characterized using chromosome C-banding, genomic *in situ* hybridization (GISH), molecular marker analysis, telosomic test analysis as well as target gene tracing. *T. aestivum-H. villosa* translocation lines T6VS·6AL with powdery mildew resistance, T4VS·4DL with wheat spindle streak music virus resistance, and the *T. aestivum-L. racemosus* translocation lines T4BS·4BL-7Lr#1S and T6AL-7Lr#1S with *FHB* resistance have been used as parents in wheat breeding, and more than 10 new varieties have been developed and released.

To increase the induce frequency and transmission rate of translocation chromosomes, mature male or female gametes before flowering on the spikes were ^{60}Coγ-rays irradiated at doses ranging from 800 to 2240 Rad, and then crossed with untreated common wheat. GISH was used to identify the translocations in M_1, this allows us to identify more translocations in a relatively small population. The M_1 plants with translocations were pollinated with normal fresh pollens from common wheat, and this enhanced the transmission rate of various chromosome structural changes in the next generation. A series of small segment translocations have been obtained by irradiating the female gametes of T6VS·6AL. The Chinese spring *ph1b* mutant and the Ph^I were also used to induce compensate translocation. *T. durum-H. villosa* amphiploid was irradiated for the mass production of various translocation lines. Translocations involving different fragments and regions of chromosome 1V to 7V have been identified. They will be further used to construct chromosome translocation pool, which will be useful genetic resources for physical mapping, the introgression and further utilization of alien useful genes.

This research was supported by the grants from the Hi-Tech Research and Development (863) Program of China, the National Natural Science Foundation of China and McKnight Foundation, USA.

HETERO- AND HOMOPLASMY OF 18S/5S MITOCHONDRIAL DNA REPEAT ASSOCIATES WITH PLANT FERTILITY IN THE PROCESS OF ALLOPLASMIC RECOMBINATION LINES FORMATION BASED ON THE PROGENIES OF BARLEY-WHEAT HYBRIDS

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Key words: *mitochondrial DNA (mtDNA) heteroplasmy, nuclear-cytoplasmic interactions, barley-wheat hybrids, alloplasmic lines*

Wide hybridization is the factor of angiosperms speciation and is used for creation the new forms of cultivated plants. The efficiency of viable and fertile hybrid plants formation is determined by the influence of nuclear-cytoplasmic interactions. Thereby the study of nuclear and cytoplasmic genomes variability in process of morphogenesis of wide hybrids is of great importance.

This work dealt with simultaneous study of nuclear and mitochondrial genomes in process of alloplasmic lines formation based on backcrossed and self-pollinated progenies of barley-wheat hybrids *H. marinum* subsp. *gussoneanum* Hudson (2*n* = 28) x *T. aestivum* L. (2*n* = 42) and *H. vulgare* L. (2*n* = 14) x *T. aestivum* L. (2*n* = 42)

By using molecular and cytogenetic methods it was found out that the process of morphogenesis during backcrossing and self-pollination of *H. marinum* subsp. *gussoneanum* x *T. aestivum* hybrids involved either integration of wild barley chromosomes in wheat genome, which leads to formation of substituted and additional lines, or elimination of all barley chromosome from wheat genome. The genomes of backcrossed and self-pollinated progenies of *H. vulgare* x *T. aestivum* hybrids contained only wheat chromosomes.

We found out the relationship between the parental type of mitochondrial 18S/5S repeat, nuclear genome organization and fertility of plants. However, both similarities and differences in these features were detected between lines (*H. marinum* subsp. *gussoneanum*)–*T. aestivum* and (*H. vulgare)–T. aestivum*. All examined alloplasmic lines with restored fertility contained a single wheat type of 18S/5S repeat (wheat homoplasmy). Homoplasmy of barley type was revealed in sterile (*H. marinum* subsp. *gussoneanum*)–*T. aestivum* lines irrespective of barley chromosomes presence in the nuclear genome. MtDNA heteroplasmy (simultaneous presence of barley and wheat copies) in (*H. marinum* subsp. *gussoneanum*)–*T. aestivum* lines associated with barley chromosomes in wheat genome. As for (*H. vulgare)–T. aestivum* lines, which didn't contain barley chromosomes in nuclear genome, heteroplasmy was detected in partly fertile and sterile plants.

SOMATIC EMBRYOGENESIS OF CONIFEROUS SPECIES IN SIBERIA

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Despite the active studying somatic embryogenesis in gymnosperms this technique still remains problematic for coniferous species growing in Siberia. The aim of this study is the development of efficient biotechnogical protocols of *Pinus sibirica, Pinus pumila, Larix sibirica, Larix dauruca, Picea obovata, Picea ajanensis* involved in somatic embryogenesis and optimization them using cytoembryological control.

Experiments of culturing the immature isolated embryos of Siberian coniferous species were carried out on modified media MS, MSG, LV, DCR and MA with different hormone concentrations and their different proportions. For induction of embryogenic callus every species needs the optimized medium supplemented with L-glutamine, casein hydrolysate, ascorbute acid and hormones. The active proliferation of embryonal mass (EM) is observed on the same medium with reduced concentration of cytokinins. The proliferation of EM was significantly improved when they were subcultured after dispersing in liquid medium. The somatic embryos from embryonal mass mature on basal medium with ABA (60–120 mM) and PEG.

In spite of species specificity the morphogenesis of embryogenic structures had the same scheme: elongation of somatic cells, formation of initial cells and embryonal tubes, development of globular, torpedo and bipolar somatic embryos. The first step of formation of an embryogenic callus is elongating somatic cells and their asymmetric division. The second step of formation of embryogenic callus is an active medium components, hormonal regulation and tree genotypes. Using the effective biotechnology of somatic embryogenesis in co formation of embryogenic mass: the elongated cells are divided and produce globular embryos and embryonal tubes surrounding them. The third step of somatic embryogenesis is an formation of bipolar embryos and their maturing at basal medium with ABA and PEG. Processes of somatic embryogenesis in *Larix* and *Picea* proceeded 4–6 months, in *Pinus* — 7–10 months.

However, not all donor-plants of coniferous species can form morphogenic callus and somatic embryos. As a rule, heterosis genotypes and hybrids formed somatic embryo intensively. The active development of embryonic callus and formation of somatic embryos is observed in hybrid seeds of *Pinus sibirica* and *Larix sibirica*. Embryogenic callus was produced from the unique genotypes of *P. sibirica* with annual development cycle of female cones and their hybrids. We have found 5 selective cell lines in *Larix sibirica* and one line in *Picea ajanensis.*

The success of the somatic embryogenesis is due to the stage of explant development, mbination with selective programs (hybridization works — control pollination with selection of parents, early selection, testing of improved genotypes, mass propagation) is one perspective task of forest genetic and selection.

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COMMON WHEAT LINES RESISTANT TO TAKE-ALL (*GAEUMANNOMYCES GRAMINIS* var. *TRITICI*) PRODUSING BY CELL SELECTION APPROACH

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Key words: *Triticum aestivum* L., *Gaeumannomyces graminis* var. *tritici*. (Sacc.) Arx & D. Olivier var. *tritici* J. Walker, *cell cultures*, *cultural filtrate*, *cell selection*, *ISSR*.

Take-all, caused by the fungus *G. graminis*, is one of the most damaging root diseases of cereals, which is widely distributed worldwide [1]. Among the small grains the wheat is appeared to be the most susceptible. Only the different levels of succeptibility were revealed among wheat accessions, and there are no resistant ones [2]. For wheat improvement, the resistance of its relatives *S. cereale*, *H. vulgare*, *Ae. tauschii, H. chilense* [1], *Haynaldia villosa* [1, 3], whose genetic material can be transferred into wheat gene pool, has been screened. However, only in one accession of *H. villosa* [3] the promising gene on chromosome 3V was identified. In all other cases, the transferring of alien chromosomes did not improve this trait, which may be caused by its polygenic nature in wheat relatives otherwise by the masking of the genetic effect of resistance in the wheat genome [1]. So, it was considered that only one of the possible approaches to improve the resistance should be GM technologies [4]. In our work, we applied wheat cell selection approach on the cultivated medium possessed up to 50 % of cultural filtrate of fungus *G. graminis*. After 7th passage on such cultural medium resistant calluses were selected. Two of 34 ISSR (inter simple sequence repeats) primer pairs used for genotyping revealed polymorphism between resistant and susceptible calluses, suggesting genetic changes in callus DNA occur during cultivation *in vitro*. Regenerated plants from resistant callus formed 4 wheat lines with resistant grade 0–0.5 according to 0–3 scale, 6 lines — with grade 0.6 — 1, 11 lines — with 1.1 — 1.5 and 14 lines — with 1.6 — 2.0, while the initial parental variety has grade 2.5. After obtaining the grains from these lines the same resistant reaction was confirmed in two generations, demonstrating the possibility of development of resistant and improved lines of wheat. The next step, which will be performed, is genetic analysis of lines in order to understand the genetic mechanism of resistance to the take-all in wheat.

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«GENETICS AND BREEDING IN A CHANGING ENVIRONMENT»

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«ГЕНЕТИКА И СЕЛЕКЦИЯ В ИЗМЕНЯЮЩИХСЯ УСЛОВИЯХ ОКРУЖАЮЩЕЙ СРЕДЫ»

HERBIVORY INDUCED TRANSCRIPTOME CHANGES IN TEA

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Key words: *SSH,Camellia sinensis,herbivory,transcriptome,Helopeltis theivora*

Assam tea, *Camellia sinensis* sub sp. *assamica* is one of the most important cash crops in India whose cultivation and production is challenged by many biotic and abiotic stresses. Among the biotic stresses, infestation by *Helopeltis theivora*, the tea mosquito bug is the most economically important (15 % production loss). Using PCR based Suppression subtractive hybridization (SSH) and Real time quantitative PCR, we report the up regulation of a broad repertoire of defense related transcripts in response to *H. theivora* infestation.
Infested cultivars showed increased defense related gene up regulation, both in terms of variety and quantity. Out of a total of 7891 ESTs generated, more than 25 % of them represented defense related genes in contrast to control, while 10 % of these showed more than 4 times increase in their intensity of expression with respect to their controls. BLAST analysis of the ESTs revealed genes related to both general and specific antiherbivory strategies undertaken by the plant, notable among the latter being 8-Hydroxymethyl glutathione dehydrogenases, Thaumatin like proteins, Hypersensitive induced reaction protein, 12-Oxophytodienoate reductases, asmonate-0-methyltransferases, Salicylic acid methyl transferases, Calmodulin like proteins, Importins, Transmembrane super family proteins, Epithio specifier proteins (ESPs) and Leucine rich repeat (LRRs) defense genes. Moreover, up regulation of genes encoding transcription factors were also encountered, like ethylene response transcriptional activators, zinc finger domains etc. Time course analysis of infestation between 30 min and 8 hrs gave valuable insight into the early and late gene expression patterns indicating that early gene expressions are more dynamic and diverse than their late counterparts. We hypothesize that wound inducible defense gene expression is very rapid, with marked differences in their expression patterns with time and there is a greater possibility of cell wall/membrane reorganization around the infested area to prevent necrosis effects in the adjoining tissue from the site of infestation, where differentially expressed transmembrane and ion transporter genes play a substantial role. Many of the unknown ESTs generated, need to be investigated for their novel possible roles in antiherbivory strategies. The infestation induced gene expression types have significant overlap over those induced by drought and other biotic stresses, and that infestation by *H. theivora* not only induces expression of defense related genes but also sets off a cascade of biochemical changes that reshapes the metabolome of the plant with far reaching economic consequences.

THE PHYSIOLOGICAL ROLE OF δOAT GENE IN PLANT DEVELOPMENT AND STRESS RESPONSE

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Key words: *ornithine-δ-aminotransferase, transgenic plants, salt stress.*

Ornithine-δ-aminotransferase (δOAT) is a mitochondrial enzyme containing pyridoxal-5'-phosphate as a cofactor, which catalyzes the conversion of L-ornithine to L-glutamate γ-semialdehyde using 2-oxoglutarate as a terminal amino group acceptor. There are some experimental data on its participation in proline biosynthesis, stress response and nitrogen recycling. However, the exact physiological role of this enzyme in plants remains unclear. It may be hypothesized that this enzyme plays an important role in junction between stress response and nitrogen metabolism.

In order to compare the effects of δOAT gene over- and under-expression, we made the transgenic *Nicotiana tabacum* plants bearing genetic constructs for overexpression and for suppression of δOAT. Also, to investigate the pattern of transcriptional control of δOAT gene the set of reporter genetic constructs has been developed. Including the translation initiation codon, the 5'-upstream segments of 1850 bp, 1300 bp and 450 bp of *Arabidopsis thaliana* δOAT (corresponding to promoter region) gene were fused to beta-glucuronidase reporter. The localization of GUS expression in transgenic tobacco plants carrying the fusion constructs was analyzed.

It was shown that:

1) δOAT overexpression in transgenic tobacco plants did not yield any increase in proline levels under both normal and salt stress conditions (comparing to control plants). However measurements of shoot and root biomass showed that the constitutive overexpression of the OAT enzyme increases the growth performance of these transgenic plants under 300 mM NaCl.
2) Transgenic plants bearing antisense suppressor against δOAT exhibited a strong suppression of root growth under 300 mM NaCl. However, under normal conditions these plants were similar to nontransgenic control.
3) Analysis of transcriptional control of δOAT gene promoter segments showed that its activity was strongly associated with proliferating cells (terminal and axillary buds, young leafs, developing inflorescence)

These results allow us to assume that δOAT does not contribute to proline biosynthesis but possibly involved in salt stress response and could be important for proliferating cells.

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CHARACTERIZATION OF A NOVEL PLANT DEFENSIN FROM *STELLARIA MEDIA* L. SEEDS

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Antimicrobial peptides are known to be an important component of the innate immunity in mammals, insects, amphibians and plants. Plant defensins are small (about 5 kDa), basic and cysteine-rich antimicrobial proteins. These proteins were found in seeds, stems and roots, and were located both inside and outside the cell. Defensins demonstrate anti-bacterial and/or antifungal activity as a result of pathogen membrane permeabilisation. In high concentrations, plant defensins can potentially be dangerous for insects due to their ability to inhibit animal α-amylases and proteinases.

We isolated new plant defensin Sm-D1 from the seeds of *Stellaria media L.*, a cool-season annual plant grown in a moist soil. Sm-D1 amino acid sequence was determined by N-terminal sequencing carried out by automated stepwise Edman degradation. Similarly to most plant defensins, Sm-D1 consists of 50 amino acids residues and has eight cysteine residues. Sm-D1 exhibit a broad spectrum of antifungal activity since it is active against all 8 tested plant pathogenic fungi. Concentrations required for 50 % inhibition of fungal growth were 0.35 μM for *Fusarium oxysporum*, 0.52 μM for *F. gramineanum*, 0.52 μM for *F. avenaceum*, 0.5 μM for *Helminthosporium sativum*, 1.0 μM for *Botrytis cinerea*, 0.52 μM for *Phoma betae* and 1.0 μM for *Phythium debaryanum*. Such data demonstrated high antifungal activity of Sm-D1 in micromolar concentration.

Primary nucleotide sequence of Sm-D1 cDNA was determined by 5'- and 3'-rapid amplification of cDNA ends with degenerate primers which were synthesized to match possible nucleotide sequences which can code the amino acid sequence of the mature peptide. cDNA of Sm-D1 included 580 bp and consisted of 5'- and 3'-nontranslated regions (42 and 292 bp, respectively) and a coding region that encoded endoplasmic reticulum signal domain (96 bp) and a mature defensin (150 bp). cDNA structure of Sm-D1 was similar to the one of many plant defensins that are assumed to be included in the secretory pathway and to have no obvious signals for post-translational modification or subcellular targeting.

In summary, the amino acid and the nucleotide sequences of a new defensin Sm-D1 from *Stellaria media* seeds were determined. As Sm-D1 showed high antifungal activity, it could be used as plant protection product against some fungal diseases. The advantage of this kind of crop protection lies in the use of natural plant protein which is a part of natural plant immunity, not a chemical pesticide. Moreover, determination of nucleotide sequence of Sm-D1 cDNA gives an opportunity to produce various transgenic plants expressing this peptide and thus becoming resistant to fungal diseases.

BREEDING CHANGES IN WHEAT AND THEIR RELATION TO SPIKE SINK CAPACITY AND MORPHOLOGY

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Key words: *wheat, yield potential, genetic gain, spike morphology*

Wheat yield potential improvement is associated with increases in above-ground biomass (improving radiation use efficiency — RUE) and dry matter partitioning to grain. In production areas with stable growing conditions in high-input farming systems where the sources are not limited, it is possible to reach yields more than 10 t ha^{-1} in the large field areas. It is evident that sink strength is a critical limiting factor of yield in the high-input farming systems and the balance between source and sink is the most promising approach for yield increasing. The genetic way of increasing the biomass production per unit area is not easy. It could be possible to manipulate with rubisco photosynthetic enzyme or potentially with CO_2 efficiency (incorporation of the C4 biosynthetic system via genetic transformation or wide hybridisation). An easy way could be to continue increasing the harvest index (HI) via decreasing the stem weight (shortening the stem length) or increasing the grain weight per spike (improving the spike productivity). Breeding changes are usually evaluated by comparing differences between sets of old and new cultivars and are characterised by a genetic gain parameter (usually expressed in per cent per year). We attempted to generalise breeding changes in important traits that were associated with physiological and morphological changes of plant. The main result of breeding process is substantial shortening the stem and increasing grain weight per spike. Modern cultivars with normal spikes (with one spikelet per one rachis node) have usually a higher kernel number per spikelet than spikelet number per spike. The higher number of spikelets in normal spike usually leads to prolongation of the grain filling period and to late ripening. Further increase in harvest index through shortening the stem length will obviously result in the development of cultivars with lower yield stability. Further increasing of the yield potential will be also accompanied by lowering protein content and increasing starch content in grain (since less metabolic energy is needed for starch biosynthesis than protein biosynthesis). An alternative way of increasing spike productivity could be the breeding use of the donor with supernumerary spikelets (SS), where more than one fertile spikelet arise from one spike rachis node, and also the three pistils (TP), which are able to produce three kernels in one floret. An overview of the genetic differences in wheat spike morphology is presented. We suppose that SS or TP will be advantageous for realisation of yield potential in high-input farming. They could be a good model for the study of the sink-source relations in wheat. (MEYS, Czech Republic: ME10063)

A MAP-BASED CLONING APPROACH FOR IDENTIFICATION AND MAPPING OF A NOVEL Na^+ EXCLUSION LOCUS IN BARLEY

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Key words: *map-based cloning, Na^+ exclusion, salinity tolerance, co-linearity, CAPS markers, vacuolar H^+-inorganic pyrophosphatase, flowering time / vernalization genes, barley, Hordeum spontaneum*

In a barley mapping population (Barque, *Hordeum vulgar*e and CPI-71284, *H. spontaneum*), 75 F_1-derived DH lines were screened for sodium exclusion in supported hydroponics. A single, highly significant QTL originating from the paternal parent CPI-71284 was identified on the short arm of chromosome 7H, with nearest molecular markers bPb-9269 and GBM-1519. Analysis of the QTL attributed the control of the Na^+ exclusion trait to a single locus named *HvNax3*. Co-linearity of SSR and DArT markers across the entire QTL region was compared with the rice genome and 14 suitable sites in rice genes (non-retrotransposon) were identified. Based on the sequence of genes in the corresponding rice interval, primers were designed and PCR products were sequenced for identification of SNPs and suitable restriction enzymes which could cut PCR products specific to one of the two parents. Suitable combinations of PCR primer pairs and restriction enzymes were identified; these markers are known as *c*leaved *a*mplified *p*olymorphic *s*equence (CAPS) markers. Finally, twelve CAPS markers were identified and used to genotype lines from an advanced backcross QTL population (AB-QTL) and F_2 segregating populations to verify linear order and to identify recombination events under the QTL. This enabled *HvNax3* to be mapped precisely to a 1.3 cM interval between two CAPS markers, HYI and NOD. This chromosome fragment contains 33 genes and one relatively large repeat in homoeologous rice chromosome 6. Comparative analysis of the rice and *Brachypodium* intervals identified 16 different classes of proteins within the interval and a single likely candidate gene: Vacuolar H^+-inorganic pyrophosphatase (*HVP* or *V-PPase*). This gene is known to be involved in responses to salinity stress, but it is the first time it has been identified from hybrid populations using map-based cloning. Expression of the gene was 2.2-fold higher in shoots of the paternal parent CPI-71284 compared to Barque, on the 3rd day of salt treatment. Two flowering time/vernalization genes, *HvFT* and *VRT-2*, also occurred within the interval containing *HvNax3*. However, *HvFT* has now been shown to be 6.3 cM distal to *HvNax3*, while *VRT-2* is 2.4 cM from the Na^+ exclusion locus on the proximal side. These findings suggest that Na^+ exclusion is not a consequence of developmental effects controlled by either *HvFT* or *VRT-2*. The role of the candidate gene *HVP* in both sodium exclusion and salinity tolerance, and its co-segregation with *HvNax3*, is under further investigation.

OBTAINING OF NEW STRESS-RESISTANT TOMATO FORMS IN CHANGING ENVIRONMENT WITH HELP OF MOLECULAR ANALYSIS

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Key words: *molecular analysis, stress-resistance, tomato forms*

Tomato is the leading vegetable crop in the world and one of the main vegetable cultures in Russia. Low temperature, *Phytophthora infestance* DB and Tobacco mosaic virus (TMV) are the most injury stressors for tomato at the Middle part of Russia. Using 3 primers designed by Ohmori et al. [1], we could support the presence of 3 RAPD-markers of *Tm-2/Tm-2a* genes (resistance to TMV) in 10 tomato forms selected earlier at the infection background of the TMV-wild strain [2]. However, the *Tm-2a* gene lost its protective effect under low temperature stress at the stage of seeds germination. So, we decided to study consequences of low temperature stress at the early stages of plant development at the molecular level. cDNA-AFLP analysis of seedlings after low temperature stress showed that a cold-resistant genotype (*Lycopersicon esculentum var. racemigerum*) has 8 fragments with stress-induced increase of the level of expression, whereas a cold-susceptible genotype has only 3 fragments with increased expression. Further sequencing of the fragments showed that among them there is a sequence highly homologous to the part of the known gene for jasmonate and ethylene-responsive factor (*JERF-3*). Jasmonates are well-known signal molecules responsible for the resistance to mechanical damage and a number of abiotic stresses. Phytohormone ethylene induces protective reactions against viruses. Thus, we observed simultaneous expression of unspecific resistance genes and virus-specific resistance genes in cold-resistant genotypes under low temperature stress. Taking that innovation into account, we developed a new technology for breeding of stress-resistant tomato forms, actual for ecological agriculture, i.e. for (1) monitoring of stress situation and evaluation the injury of main stresses; (2) study of the genetic (with help of molecular techniques) and other mechanisms of the stress-resistance; (3) estimation of genetic resources (with help of molecular techniques) for the breeding of stress-resistant forms; (4) analysis of effectiveness of resistance genes and techniques for increasing resistance under controlled conditions and selection of stress-resistant forms and the best technique; (5) evaluation of stress-resistant forms and techniques of increasing resistance under natural stresses; (6) selection of basic material with effective gene-resistance and the most effective technology for improvement of plant resistance to stresses.

Five new forms with complex resistance to abiotic (cold) and biotic (TMV, *Alternaria solani* Sor., *Botrytis cinerea* Pers.) stresses were obtained using this technology. These forms can maintain to stresses under low temperature action at the stage of seeds germination. Fruits of these forms had orange color and showed good quality.

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SYMBIOTIC INTERACTIONS OF LEGUME PLANTS WITH SOIL MICROORGANISMS: SEARCHING THE MECHANISMS OF EFFICIENT SYMBIOSIS IN THE PRESENCE OF ENVIRONMENTAL STRESSES

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Key words: *abiotic stress, mycorrhiza, plant mutants, rhizobia, symbiosis*

Symbioses are one of the main forms of life on the Earth. Development of effective symbiotic interactions of plants with soil microorganisms leads to the mutual adaptation to different abiotic stress-factors. The aim of our study is to investigate the mechanisms underlying resistance of plant-microbe systems to stresses using two types of effective legume symbioses: nitrogen-fixing legume-Rhizobium symbiosis and symbiosis with arbuscular mycorrhizal fungi (AM fungi). The research was performed with recently obtained plant models: (1) cadmium tolerant pea mutant SGECd^t [1]; (2) obligate-mycotrophic black medic line (*Medicago lupulina* L.) having fast response to mycorrhization, and different mutants of this plant, which were not able to establish effective AM symbiosis [2]. It was shown that the pea mutant SGECd^t formed efficient symbiosis with nodule bacteria *Rhizobium leguminosarum* bv. *viciae* in the presence of toxic Cd concentrations, however there was no differences in the growth response to water deficit between the mutant and wild type plants. A knock-out mutants of *R. leguminosarum* bv. *viciae* defective in ACC deaminase gene *(acdS)*, playing important role in symbiosis development under stressful conditions, were obtained. An obligate-symbiotrophic line of black medic (*Medicago lupulina* L.), which was not able to growth properly in the absence of AMF and showed dwarf phenotype under deficit of phosphorus in soil, was selected. Formation of symbiosis with AM fungus *Glomus intraradices* resulted in restoration of a normal phenotype. A chemical mutagenesis was successfully applied to obtain black medic mutants characterized by defective AMF symbiosis. The obtained plant-microbe systems are unique models for the study of molecular-genetic and biochemical mechanisms of efficient symbiosis between legume plants and nodule bacteria or AM fungi under single and combined environmental stresses, such as metal toxicity, drought and nutrient deficiency.

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GENETIC TRANSFORMATION OF MITOCHONDRIA IN TOBACCO USING VECTOR CONSTRUCTS WITH INTEGRATIVE PROPERTIES

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Key words: *mitochondrial genome, Nicotiana tabacum, biolistic DNA delivery, leaf disks, cell culture, gfp gene, modified cob gene, homologous recombination*

Up till now the expression of foreign genes in mitochondria of higher plant species could not be addressed, despite its fundamental and biotechnological interest. This is mainly due to the impossibility to transform mitochondria in high eukaryotes with conventional methods and to select cells with transgenic mitochondria. Failure to transfect mitochondria in plant cells can actually be considered as a paradox, as these organelles are able to take up both DNA and RNA. We previously demonstrated that isolated plant mitochondria can actively import and retain double-stranded DNA [1]. It is important that the natural competence of isolated mitochondria to uptake foreign DNA is characteristic not only for plants but for animals and yeasts also [2]. We are building on these data to develop mitochondrial transformation method for *in vivo* conditions. For this we have created two variants of genetic constructs with the integrative vector's properties. To study the efficiency of genetic construct transfer into mitochondria under transfer of DNA to leaf disks by the method developed in the Laboratory of Plant Genome Expression (Head Prof Dr. Kusnetsov V.V.) of Timiryazev Institute of Plant Physiology the genetic construct with integrative properties and containing the GFP (green fluorescent protein) reporter gene in polycistronic unit from tobacco mitochondrial genome has been used. The possibility to integrate a sequence of interest into the organelle genomic DNA following import into mitochondria *in vivo* has thus been explored for the first time with the genetic construct containing GFP gene flanked by sequences identical to relevant regions of the tobacco mitochondrial DNA. PCR analysis of DNA from cell cultures derived from transformed leaf disks showed in some cases the integration of GFP gene sequences in tobacco mitochondrial genome. Using RT-PCR we revealed also the GFP gene transcripts appearance in transformed leaf disks. Another evidence of GFP gene expression in transformed plant material was the appearance of fluorescence zones in these leaf disks only. The more detailed analysis of DNA and RNA from tobacco mitochondria in plants regenerated from transformed leaf disks is under the way now. Second variant of genetic construct is involved (i) GFP reporter gene and (ii) modified tobacco apocytochrome *b* gene encoding resistance to the respiratory inhibitor antimycin A. Under using of this construct in genetic transformation of suspension and calli cultures in *Nicotiana tabacum* by biolistic method the existence of GFP reporter gene sequences in mitochondria of transformed cells was shown.

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AGROBACTERIUM-MEDIATED TRANSFORMATION OF MAIZE GERM CELLS

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Key words: *Agrobacterium, corn, transformation, T-DNA, transfer*

Most methods of *Agrobacterium*-mediated transformation are based on the coincubation of plant vegetative organs and tissues (leaves, roots, stems, or meristems) and a bacterial cell suspension [1]. This study was focused on investigating of the possibility of *Agrobacterium*-mediated T-DNA transfer to male and female gametophyte cells of maize plants. A transfer DNA (T-DNA) carrying the marker gene *npt*II was detected in the genomes of diploid and first time of haploid maize plants obtained after the treatment of pistil filaments with a suspension of *Agrobacterium* during artificial pollination [2]. Integration of T-DNA into the maize female gametophyte cells was confirmed by PCR (the *npt*II and *gus* reporter genes), by hystochemical staining of the seedling tissues (GUS) obtained from the transformed seeds, and by fluorescence microscopy of GFP expression observation. PCR analysis of total DNA isolated from 155 canamycin_resistant diploid F1 seedlings revealed T-DNA insertions in the genomes of 111 plants (32.7 % of the total number of analyzed seeds). Using microscopy and PCR analysis, we found evidence for agrobacterial T-DNA delivery into the egg cell after treatment of female-based haploid plants. The example of matroclinal haploids was used to demonstrate that T-DNA may be transported to the egg cell by the growing pollen tube (PT). Twelve out of 16 analyzed haploid plants contained the T-DNA insertion. The possible mechanism of the transfer of the *Agrobacterium* T-DNA to the maize genome during pollination is discussed.

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SEQUENCES HOMOLOGOUS TO T-DNA OF AGROBACTERIUM IN PLANT GENOMES

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Key words: *Agrobacterium, horizontal gene transfer, plant genomes*

Agrobacterium mediated transformation is the traditional way of genetically engineering plants. This method is based on natural vector system, Agrobacterium tumefaciens, or A. rhizogenes. These bacteria transfer fragments (T-DNA) from a large tumor-inducing (Ti) plasmid or root-inducing (Ri) plasmid respectively to their plant hosts. The evaluation of plant transformation has raised in our modern society a question of the safety of transgenic foods. At the same time, a number of "untransformed" plants, such as some Nicotiana species, contain DNA sequences, homologous to the T-DNA from A. rhizogenes. Thus A. rhizogenes must have transferred T-DNA to Nicotiana species, and these genes have played a role in the evolution of the genus.

No one has reviewed a large number of plants to determine how wide the transfer of T-DNA from Agrobacterium to plants is spread. For this purpose we applied a TaqMan modification of Real-time PCR. It combined positive features of PCR and Southern hybridization in a single reaction. Real-time PCR was done with degenerate primers and probes to screen 127 plant species, belonging to 38 families of Dicotyledones for the presence of oncogenes, homologous to ones from Agrobacterium tumefaciens and Agrobacterium rhizogenes. We found that only Linaria vulgaris contains genes, homologous to rolB, rolC, ORF13, ORF14 of A. rhizogenes.

Our results demonstrate that horizontal gene transfer of oncogenes from Agrobacrerium to plants is not a unique feature of Nicotiana. The data presented in this report indicate that horizontal gene transfer took place in the evolution of plant species L. vulgaris belonging to Scrophulariaceae family. The absence of T-DNA homologs in the other 126 analyzed species lead us to a conclusion, that horizontal gene transfer from Agrobacterium is a rare event in the evolution of plants.

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THE ROLE OF dsRNA BINDING AND HYDROLYTIC ACTIVITIES IN THE VIRUS-RESISTANCE OF HIGHER PLANTS

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Key words: *plant antiviral immune function, RNA silencing, silencing suppression*

Viral diseases are responsible for substantial agronomic losses of crop plants worldwide. Therefore, a detailed understanding of plant antiviral immune function will underpin crop improvement for food, fibre and biofuel production. In plants, RNA silencing is an efficient antiviral system, and therefore successful virus infection requires suppression of silencing. Although many viral silencing suppressors have been identified, the common molecular basis of silencing suppression is poorly understood. It was proposed that dsRNA binding is a general silencing suppression strategy. In the case any additional dsRNA binding activity would reduce plant resistance to viral pathogens. Nevertheless, a mutant bacterial RNAse III gene lacking hydrolase activity but capable of binding to dsRNA was used to produce transgenic wheat plants and proved the high resistance of the plants against the barley stripe mosaic virus.

In our study we wanted to resolve the apparent contradiction and to determine the role of dsRNA binding activity, dsRNA hydrolytic activity and their localization in the resistance of higher plants to phytopathogenic viruses. For this aim we have created several vectors bearing a native, chimeric and mutant *S. marcescens* nuclease gene. This enzyme strongly prefers dsRNA and was used as antiviral agent for plant and insect protections.

We have got the mutant *S. marcescens* nuclease gene, lacking of hydrolytic activity, by means of PCR site-directed mutagenesis. The mutant protein contains substitution in codon 110 that leads to the amino acid replacement of histidine to alanine. The chimeric nuclease gene consists of (a) nucleotide sequence of leader peptide of *Zinnia elegans* extracellular ribonuclease gene (ZRNaseII) and (b) leaderless sequence of *S. marcescens* nuclease gene. Besides we constructed mutant chimeric gene that consists of (a) nucleotide sequence of leader peptide of ZRNaseII and (b) leaderless sequence of mutant *S. marcescens* nuclease gene, lacking of hydrolytic activity. Finally, we constructed vector that contains leaderless sequence of mutant *S. marcescens* nuclease gene, lacking of hydrolytic activity. All these sequences were placed under the control of strong constitutive promoter of CaMV 35S RNA at the vector pEarleyGate 101 for Gateway Cloning System (Invitrogen).

We have transformed tobacco plants with all these vectors and started the assays of the resulted transformants for the level of nuclease activity, presence of transgenic mRNA/DNA and resistance to phytopathogenic viruses. The ongoing research would clarify one of the molecular mechanisms underlying suppression of RNA silencing in the plant-virus interplay and could provide new approaches to attempt a difficult task protecting of transgenic plants against multiple viruses.

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ORAL PRESENTATIONS

SECTION

«GENOME ORGANIZATION AND EVOLUTION»

9 июня

УСТНЫЕ СООБЩЕНИЯ

СЕКЦИЯ

«ОРГАНИЗАЦИЯ И ЭВОЛЮЦИЯ ГЕНОМА»

ORIGIN AND EVOLUTION OF THE Y GENOME IN ELYMUS AND ITS RELATIONSHIPS TO OTHER GENOME IN TRITICEAE

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Key words: *Genome evolution, phylogeny, polyploidy, Triticeae.*

Elymus L. is a large and exclusively polyploid genus in the tribe Triticeae. Cytogenetic analyses show that all its members include the **St** genome in combination with one or more of four other genomes, the **H**, **Y**, **P**, and **W** genomes. Diploid species with the **H**, **P**, and **W** genomes are known, but there is no known diploid with the **Y** genome. The ITS sequence data suggested that the **Y** was derived by gradual differentiation from the **St** genome. This hypothesis is also supported by RAPD based STS-PCR markers in which one accession of *Pseudoroegneria spicata* (Pursh) A. Löve was found to have markers closed related to those of the **StY** *Elymus longearistatus* (Boiss.) Tzvelev. Sequence dada from single copy nuclear genes β-amylase gene and DNA polymerase subunit II (*RPB*2) sequence support the Dewey's hypothesis that the **Y** genome had an independent origin from a **Y** diploid species, all of which are now extinct or undiscovered. The purpose of our study was to try and clarify the origin of the **Y** genome by examining differentiation of nucleotide sequences (elongation factor G) closely linked to the *vrs*1 locus in wider range of species, both diploid and polyploid, than has been included in previous studies. We analyzed the single copy nuclear gene coding for elongation factor G (*EF-G*) from 28 accessions of polyploid *Elymus* species and 45 accessions of diploid Triticeae species in order to investigate the relationship of the **Y** genome to other genomes in the tribe *Triticeae*. Sequence comparisons among the **St**, **H**, **Y**, **P**, **W** and **E** genomes detected genome-specific polymorphisms at 66 nucleotide positions. The **St** and **Y** genomes are relatively dissimilar. The phylogeny of the **Y** genome sequences was investigated for the first time. They were most similar to the **W** genome sequences. The **Y** genome sequences were placed in two different groups. These two groups were included in an unresolved clade that included the **W** and **E** sequences as well as sequences from many annual species such as *Aegilops* species, *Triticum monococcum*, *Crithopsis* and *Taeniatherum*. The **H** genomes sequences were in a clade with the **F**, **P**, and **Ns** genome sequences as sister groups. Theses two clades were more closely related to each other and to the **L** and **Xp** genomes than they were to the **St** genome sequences. These data support the hypothesis that the **Y** genome was originated from a diploid species that is different from the **St** genome species.

EVOLUTIONARY CHANGES OF THE S-GENOMES IN *TRITICUM* AND *AEGILOPS* ANALYZED USING C-BANDING AND FISH WITH DIFFERENT TYPES OF REPEATED DNA SEQUENCES

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Key words: *evolution, Triticum, Aegilops, S-(B/G)-genome, chromosomes, C-bands, FISH, highly repeated DNA sequences*

Alterations in the amount and chromosomal distribution of highly repeated DNA sequences during radiation of the S-genome diploid *Aegilops* species (*Ae. speltoides, Ae. longissima, Ae. sharonensis, Ae. searsii, Ae. bicornis*) and in the course of formation of polyploid *Aegilops* (*Ae. variabilis, Ae. kotschyi*) and *Triticum* were studied using C-banding technique and *in situ* hybridization with the *pSc119.2, Spelt-1, Spelt-52,* and *Fat* probes. The S-genomes split into two major groups: (1) *Ae. speltoides* and polyploid wheat species and (2) the diploid *Aegilops* species of Emarginata section and polyploid *Ae. peregrina* and *Ae. kotschyi*. *Ae. speltoides* chromosomes were characterized by large pericentromeric and subtelomeric heterochromatin complexes, predominantly interstitial location of the *pSc119.2* sites, very poor hybridization with *Fat* sequence and by the presence of both *Spelt-1* and *Spelt-52* sequences. The chromosomes of polyploid wheat species were similar with the chromosomes of *Ae. speltoides* in respect of distribution of *pSc119.2* sequence and in the presence of large pericentromeric heterochromatin blocks; however, the number and sizes of *Spelt-1* and *Spelt-52* loci on the B- and G-genome chromosomes was much smaller and they lost prominent subtelomeric C-bands. Diploid *Aegilops* species of Emarginata section possessed predominantly intercalary C-bands although they differed significantly in heterochromatin content. Their chromosomes contained large subtelomeric *pSc119.2* sites, lack *Spelt-1* sequence, and hybridized with *Fat* probe; labeling intensity of *Fat* decreased in the order *Ae. bicornis* →*Ae. searsii* →*Ae. sharonensis* →*Ae. longissima.* The *Spelt-52* repeat was found in two of four species of this group, *Ae. sharonensis* and *Ae. longissima.* Hybridization patterns of this probe were highly polymorphic and individual plants may contain up to 15 signals located predominantly in subtelomeric chromosome region and rarely in distal parts of group 1 and 7 chromosomes. The S-genome chromosomes of an artificial amphiploid of *Ae. umbellulata x Ae. sharonensis* did not differ from the parental line of *Ae. sharonensis* in the distribution of all types of repetitive sequences, however, in natural polyploids *Ae. peregrina* and *Ae. kotschyi* we observed some changes in the number and location of *pSc119.2* sites as well as the significant decrease in the number and size of *Spelt-52* loci.

PHYLOGEOGRAPHY OF WILD REPRESENTATIVES OF *Pisum Sativum* L.

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The genus *Pisum* L. (pea) has rather a confusing taxonomy. It contains: 1) a clear-cut wild species *P. fulvum* Sibth. et Smith. endemic to the Near East; 2) a cultivated (and wild?) *P. abyssinicum* A. Br., the specific status of which is uncertain since its being a clear-cut lineage but not so strongly differing from 3) the huge species *P. sativum* L. which embraces the overwhelming majority of cultivated peas and quite diverse wild representatives ranging in the Mediterranean, from Portugal to Iran and from Crimea and the Caucasus to Israel (and Ethiopia?). Among wild representatives of *P. sativum* from Israel and Turkey, Ben-Ze'ev & Zohary (1979) isolated two karyologic groups, one of which, including 2 'northern' accessions, also includes cultivated peas. We analysed 108 accessions of different pea taxa (12 *P. fulvum*, 7 *P. abyssinicum*, 45 wild and 44 cultivated *P. sativum* L.) for three functionally unrelated dimorphic molecular markers residing in three different cellular genomes: *rbcL* (plastid genome), *CoxI* (mitochondrial genome) and *SCA* (nuclear genome). *RbcL* either contains or not a recognition site for restriction endonuclease *Asp*LEI (*rbcL*+ vs. *rbcL*–); *cox1* either contains or not recognition site for *Psi*I (*cox1*+ vs. *cox1*–); SCA may be represented either by slow (SCA^s) or fast (SCA^f) electromorph. It turned out that most accessions had two combinations: 32 had SCA^f *cox1*+ *rbcL*+ (combination A) and 50 had SCA^s *cox1*– *rbcL*– (combination B). All accessions of *P. fulvum* and *P. abyssinicum* had combination A, an overwhelming majority of cultivated *P. sativum* had combinatoin B, while wild representatives of *P. sativum* had both combinations A, B as well as less frequent ones. Hence, combination A is ancestral for the genus as inherited by two species as a plesiomorphic character, further mutations took place within *P. sativum* in the wild, while cultivated forms diverged from some wild population(s) already having combination B. Based on geographical distribution of the allele combinations analysed we suggest a putative history of wild representatives of *P. sativum*. The ancestor of this species belonged to lineage A and appeared in East Mediterranean, then spread westward most probably during one of the Pleistocene coolings when the sea was smaller, so that representatives of lineage A remained in the Eastern Mediterranean and on the islands of Sicily and Menorca. Mutation leading to the loss of the restriction site for *Psi*I in *coxI*-, gave rise to combination C (SCA^f, *rbcL*+, *coxI*-) which spread widely in the Mediterranean and is now found in France, Greece and Ethiopia. Mutation leading to *rbcL* - gave rise to combination D (SCA^f, *rbcL*-, *coxI*-), now found in Egypt (cultivated) and Spain (wild?). Mutational transition of SCA^f to SCA^s most probably took place in North-Eastern Mediterranean since the resulting combination B now occupies the Tauro-Caucasian area. In Asia Minor and North Israel, combination B met the ancestral combination A so that both lineages coexist there presently, corresponding to the karyologic classes by Ben-Ze'ev & Zohary. The lineage B gave rise to the cultivated *P. sativum* subsp. *sativum*. Our phylogenetic analysis of nucleotide sequences of the histone H1 subtype 5 gene quite well corresponds to the above data. It resolves divergence between *P. fulvum*, *P. abyssinicum* and *P. sativum* with combinations A and C versus wild and cultivated *P. sativum* mostly with combination D and B. *P. fulvum* and *P. abyssinicum* tend to form separate branches inside the former group. A histone H1 gene appeared good for plylogenetic analysis on an intra-species level.

FALL AND RISE OF SATELLITE REPEATS IN ALLOPOLYPLOIDS OF *NICOTIANA* OVER *C.* 5 MILLION YEARS

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Rationale: Allopolyploids represent natural experiments in which DNA sequences from different species are combined into a single nucleus and then coevolve together, enabling us to follow the fate of parental DNA sequences over time. Here we examine the fate of satellite DNA over 5 million years of divergence in plant genus *Nicotiana* (family Solanaceae).
Methods: Using molecular-clock analyses, we estimated the likely age of *Nicotiana* allopolyploids. We isolated subtelomeric, tandemly repeated satellite DNA from *Nicotiana* diploid and allopolyploid species and analysed patterns of inheritance and divergence by sequence analysis, Southern blot hybridization and fluorescent *in situ* hybridization (FISH). We used genomic *in situ* hybridization (GISH) to determine how the chromosome compartments evolve over time.
Results: We observed that parental satellite sequence redistribute around the genome in allopolyploids of *Nicotiana* section *Polydicliae*, formed *c.* 1 million years ago (mya), and that new satellite evolved and amplified in section *Repandae*, formed *c.* 5 mya. In some cases that process involved the complete replacement of parental satellite sequences. Typically, novel variants of subtelomeric satellites arise at preexisting loci probably by amplification of low copy monomers. While the parental chromosomes can be efficiently visualized in relatively recent alloplyploids by GISH, after *c.* five million years of divergence GISH fails.
Conclusions: The rate of satellite repeat replacement is faster than theoretical predictions suggest assuming the mechanism involved is unequal recombination and cross-overs. Instead we propose that this mechanism occurs with the deletion of large chromatin blocks (perhaps fundamental chromatin loops) and reamplification, perhaps *via* e.g. rolling circle replication. The changes in satellite structure correlated with the age of an allopolyploid and with the GISH signal changes. Genome downsizing, commonly observed in allopolyploids over long time frames (millions of years) may be reversed occasionally through the amplification of satellite repeats and other repeat families. The ongoing satellite homogenization must increase the homology at chromosomes potentially influencing hoemologous pairing. Thus satellite repeats significantly contribute to chromosome evolution in allopolyploid species.

GENE EXPRESSION UPON GENOME COMPLEXIFICATION: STUDIES ON FLAVONOID BIOSYNTHESIS GENES IN WHEAT AND WHEAT-ALIEN HYBRIDS

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Key words: *wheat, wheat-alien hybrids, gene expression, flavonoid biosynthesis*

The joining of evolutionary divergent plant genomes in allopolyploids creates regulatory incompatibilities between genomes that must be reconciled. Analysis of newly formed allotetraploids showed that wide hybridization and chromosome doubling affect gene expression via genetic and epigenetic alterations immediately upon allopolyploid formation [1, 2]. Nonadditive expression and transcriptional dominance are observed in allopolyploids [3]. Exploiting flavonoid biosynthesis (FB) model system, which provides a colorful tool for studies in genetics and biotechnology, we studied for the first time relationships between homoeologous structural genes and their regulatory homoeologous genes in allopolyploid genome and investigated gene transcription in a foreign background in wide hybrids. We performed genetic and/or physical mapping of the 26 FB regulatory and 11 structural (target) gene loci in wheat and its relatives. From these genes, *Rc* and *F3h* represented the most convenient regulatory-target gene model. *Rc* (red coleoptile) controls pigmentation of coleoptile. In wheat (*Triticum aestivum*, AABBDD, $2n = 6x = 42$), 3 homoeologous *Rc* genes (*Rc-A1*, *Rc-B1*, *Rc-D1*) were mapped to chromosomes 7AS, 7BS and 7DS, respectively. In diploid relatives of wheat, the *Rc* genes were localized on chromosome 4R of rye (the gene *Rc-R1*), 7H of barley (*Rc-H1*), 7S of *Aegilops speltoides* (*Rc-S1*) and 7D of *Ae. tauschii* (*Rc-D1*). The presence of a dominant *Rc* allele induced expression of *F3h* (flavanone-3-hydroxylase) in coleoptiles. Quantitative examination of *F3h* expression using real-time PCR on cDNA from coleoptiles of the genotypes carrying different combinations of the *Rc* and *F3h* genes led us to the following conclusions: (i) the FB gene regulation cuts across genomes of allopolyploid wheat; (ii) the regulatory FB genes contribute more to the functional divergence between the diploid genomes of allopolyploid wheat than do the structural genes; (iii) phenomenon of transcriptional dominance takes place in the wheat-rye chromosome substitution line 2R(2D); (iv) the regulatory *Rc* genes of different Triticeae species are able to activate the wheat target genes *F3h*, demonstrating good cooperation of the wheat and alien FB gene systems within the hybrid genomes; (v) however, the bigger genetic distance between wheat and a donor species, the lower transcriptional level of wheat *F3h* genes, suggesting that successful insertion of an alien gene into recipient genome still does not guarantee the desirable level of its transcription, because of donor-recipient gene expression networks divergence; (vi) overall, the FB genes provide an effective tool for studies into gene regulation in plants having complex genomes.
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NUCLEAR ENVELOPE AND NUCLEAR PORE STRUCTURE IN HIGH PLANT

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Key words: *nucleus, nuclear envelope, nuclear pore complex, high plant*

Motivation and Aim: The nuclear envelope (NE) of all eukaryotic cells is perforated by nuclear pore complexes (NPCs) that facilitate controlled communication between nucleus and cytoplasm. The basic architecture of the NPC is conserved among metazoans and yeasts. In eukaryotes with an open mitosis, NPCs assemble during two stages of the cell cycle. Despite recent progress of the plant nuclear transport field, our understanding the plant machinery is far behind its animal counterpart. Fine microscopic data on NPC structure in plants apart from early electron microscopy investigations (1967–1970) that provided a rough overview of the NPC in plants. We aimed to fill in this gap and analysed the detailed ultrastructure of plant NPCs.
Methods and Algorithms: We have used FESEM (Field Emission in lenz Scanning Electron Microscope) to investigate the structure of NPCs in plant BY-2 cells and onion root cells.
Results: We have shown that NPCs in the NE from high plants resembled to some extent the NPCs previously described in *Xenopus* oocytes. The NPCs in the onion roots resembled NPCs from tobacco BY-2 cells in both size and conformation. In some NPCs we observed cytoplasmic filaments arranged in various manners. We detected structural differences between NPCs of dividing and quiescent nuclei. The conformations of the NPCs as observed from the cytoplasmic side of the nucleus varied considerably between 3- and 10-day-old cells. Widely open NPCs with thinner cytoplasmic ring were predominant in 3-day-old cells while more closed conformation and thicker ring were typical features of the NPC in 10-day-old cells. We found that NPCs in cells in different phases of the life cycle harboured different proportions and NPC intermediates. Importantly, we also traced the organisational pattern of the NPCs and observed non-random NPC distribution over the nuclear surface. We observed also an organised filamentous protein structure that underlies the inner nuclear membrane and interconnects NPCs [1].
Conclusion: For the first time, we describe the NPC from both sides of the NE in rapidly growing and quiescent cells. Differences in NPC conformation that were related to the different stages of the life cycle have been demonstrated. Using feSEM, we have also observed for the first time a complex and highly organised filamentous lattice underlying the inner nuclear membrane of the tobacco BY-2 cells. The respective structure spread among and was connected to the nucleoplasmic rings of the NPCs. This network of filaments has an organisation and structural details resembling to some extent the animal nuclear lamina.
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INFERRING EVOLUTIONARY RELATIONSHIPS IN WILD AND CULTIVATED WHEAT SPECIES BASED ON POLYMORPHIC RETROTRANSPOSON INSERTIONS

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Key words*: retrotransposons, molecular evolution, genetic markers, wheat*

Solid evolutionary studies in wheat are possible only if a complex approach is utilized, employing morphological analysis, cytogenetics, resequencing of important genomic and chloroplast genes, and DNA fingerprinting. When it comes to the latter, several problems can arise in wheat: insufficient polymorphism, difficulties in obtaining reliable fingerprints from a large and complex chromosomes, and inability to distinguish between polymorphic loci located in different wheat genomes (A, B or G, and D). In addition, the nature of cultivated wheat genepools requires analyzing lots of accessions, so the fingerprinting methods should be high-throughput, efficient and reproducible, enabling accumulation of data. In our hands, the modified SSAP (Sequence-Specific Amplification Polymorphism) protocol revealing polymorphism at multiple *BARE-1* and *Jeli* retrotransposon insertion sites allowed to address most of the abovementioned issues. Using this approach, we studied the genetic diversity in 92 diploid and 81 tetraploid wheat accessions representing wild and cultivated species, mainly *Triticum boeoticum, T. urartu, T. monococcum, T. dicoccoides, T. dicoccum, T. araraticum* and *T. timopheevii*. About 30–50 DNA fragments amplified in a single reaction were separated by high-resolution capillary electrophoresis and scored automatically; in total, over 500 polymorphic markers were obtained for each ploidy level. The marker data clearly demonstrate the significant distance between A^u and A^b and also between BBAA and GGAA genome species. Within the major genomic groups, several clusters with high support values were discovered, which correspond to either botanical subspecies or geographical groups. In general, we conclude that a retrotransposon-based genetic fingerprinting in wheat is established as a high-throughput method providing relevant information to address the questions of evolution.

VRN1 PROMOTER REGION ANALYSIS: EVOLUTIONARY DINAMICS AND *CIS*-REGULATORY ELEMENTS PREDICTION

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Key words: *vernalization, cold adaptability, wheat, transcription factors, regulatory cascade*

In crops such as common wheat (*Triticum aestivum* L.), a vernalization requirement (a long exposure to low temperatures) distinguishes winter varieties from spring ones. Besides being an important trait for adaptation to environments, the requirement of vernalization is also of a great agronomical importance. *VRN1* gene is dominant for spring growth habit and it is the main initiating factor of the flowering regulatory cascade. It has been demonstrated that damages in either intron or promoter regions are sufficient to accelerate flowering under LD (long day) condition. However less is known about functional significance of regulatory sites and consequently genetic network control vernalization response in wheat.
Molecular biology methods used in the present study included *total DNA isolation, primer design, PCR amplification, cloning, DNA sequencing. For bioinformatic research* UniPro Ugene software together with the integrated plugins: Sitecon for TF site recognition, MUSCLE 3,4 and KAlign algorithms for alignment analysis, HMM profiles for transposable elements search was used (http://ugene.unipro.ru). For transcription factors analysis tools of NCBI (http://www.ncbi.nlm.nih.gov), EMBL (http://www.ebi.ac.uk/embl), DBD (http://www.transcriptionfactor.org), Jaspar (http://jaspar.cgb.ki.se) and other databases were used.
Variability of the *VRN1* promoter region of the unique collection of spring polyploid and wild diploid wheat species was investigated. Sequence analysis indicated great variability in the region from -62 to -221 nucleotide positions of the *VRN1* promoter region. Different indels were found within this region in spring wheats. In the present work, we carried out searching and analysis of the *cis*-regulatory elements (recognition sites for transcription factors, TF) inside of the *VRN1* promoter and its homologs in crops and *Arabidopsis*. Such information helps to investigate the possible consequences of the observed damages in the promoter of the main initiation factor of flowering, and possibly is related to the mechanism of growth habit changing. Some transcription factor recognition sites including hybrid C/G-box for TaFDL2 protein known as the *VRN1* gene upregulator were predicted inside the variable region. Information available in model plants helped to simulate more detailed genetic network responsible for growth habit (spring vs. winter).
It was also shown that deletions leading to promoter damage occurred in diploid and polyploid species independently. Therefore the spring polyploids are not related in their origin to spring diploids. DNA transposon insertions first occurred in polyploid species. At the same time, the amplification of the promoter region was observed in the A genome of polyploid species. We can conclude that supposed molecular mechanism of the *VRN1* gene activating in cultivated diploid wheat species *T. monococcum* is common also for wild *T. boeoticum* and was inherited by *T. monococcum*. All obtained data are useful for deeper insight into regulatory network underlined the origin of spring wheat forms in evolution and domestication process.

MOLECULAR-GENETIC AND PHYSICAL MAPPING OF MUTANT GENES INVOLVED IN THE INFLORESCENCE DEVELOPMENT IN BREAD WHEAT AND ITS CLOSE RELATIVES

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Key words: *inflorescence morphology, spike branching, Triticum aestivum*, *Secale cereale, microsatellite mapping, COS-SSCP approach*

The inflorescence morphology is influential in the processes of pollination and seed formation, and thus plays a role in the determination of grain yield. Identification and characterization of genes involved in these processes will add to our understanding of how inflorescence development and architecture is regulated. The inflorescence of wheat and its close relatives, barley and rye, is referred to as a spike, and variation in spike morphology is one of the most widely used criteria used in grass taxonomy. Although spike length and density can vary intraspecifically, the number of spikelets at each rachis node is usually constant. Wheat and rye spikes normally bear one spikelet per rachis node, and the formation of supernumerary spikelets (SS) is rare. The term SS includes sessile additional spikelets at a rachis node, additional spikelets on an extended rachilla, and the ramified spike seen in some tetraploid and hexaploid wheats. A synonym of RS is "branched spike". A strongly branched spike in rye is referred to as a "monstrosum ear". The locus responsible for the 'multirow spike' or MRS trait in wheat was mapped by genotyping F_2 populations with microsatellite markers. The MRS trait is under the control of a recessive allele at a single locus. The *Mrs1* locus is located on chromosome 2DS, co-segregating with the microsatellite locus *Xwmc453*. The placement of flanking microsatellite loci into chromosome deletion bin 2DS-5 (FL 0.47–1.0) delimited the physical location of *Mrs1* to the distal half of chromosome arm 2DS, within the gene rich region 2S0.8. The use of the COS - SSCP approach and *in silico* mapping of sequenced RFLPs linked to the mutant locus allowed us to identify the syntenic chromosomal region on the rice chromosome 7. The region hosts the *Fzp* (*FRIZZY PANICLE*) gene, whose mutation altered the identity of the spikelet meristem, causing inflorescence branching. The wheat orthologues of the gene were isolated. Allelic relationships of *mrs* and other genes determined the SS/branched spike trait were tested using bread wheat material of different origin. The practical importance of the MRS spike is that it produces more spikelets per spike, and thereby enhances the sink capacity of wheat, which is believed to limit the yield potential of the crop. Along with the mutant allele that determines SS trait in bread wheat, the gene for the 'monstrosum' trait in rye, *mo1*, was mapped by genotyping F_2 population with microsatellite markers. The *Mo1* locus maps about 10 cM from the centromere on chromosome arm 2RS. The similar effect on phenotype of *mo1* and *mrs1*, together with their presence in regions of conserved synteny, suggest that they may well be members of an orthologous set of *Triticeae* genes governing spike branching.

SEQUENCING AND ANALYSIS OF BUCKWHEAT FLORAL TRANSCRIPTOMES

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Genetic control of the flower development is one of the most thrilling and the most complicated topics in plant developmental genetics. Until recently experimental studies in this field were confined to several model species with well characterized genomes, primarily *Arabidopsis thaliana*. The advent of next generation sequencing technologies allowed rapid identification of thousands of genes expressed in any structure or developmental stage that facilitates the genetic studies on non-model species.
We applied this approach to buckwheat (*Fagopyrum*). Plants of the genus *Fagopyrum* are interesting not only for practical but also for fundamental reason as they possess the floral structure that is not characteristic for most dicotyledonous plants. Continuing our research on the genetic control of flower development in buckwheat we identified the genes expressed in flower and inflorescence in two species of buckwheat — *Fagopyrum esculentum* (common buckwheat) and *F. tataricum*. These species are closely related but differ in their flower morphology. For this purpose normalized floral/inflorescence cDNAs were subjected to high-throughput sequencing using 454 (Roche) technology. Sequencing resulted in about 250 thousands of reads for each species with average length of 350 bp. These reads were assembled using MIRA program into 29689 contigs with N50 equal to 655 for *F. esculentum* and 28873 contigs with N50 equal to 626 for *F. tataricum*. 65452 reads for *F. esculentum* and 62221 reads for *F. tataricum* were retained as singletons. Based on blastx search more than 60 % of contigs had significant matches in NCBI protein sequence database. In both transcriptomes the orthologs of *Arabidopsis* genes that are known to play a key role in flower and inflorescence development were found. These genes include MADS box transcription factor genes many of which are known to control transition to flowering and floral organ identity, homeobox genes that control meristematic activity of the cells, MYB transcription factor genes and many others. Phylogenetic analysis of these gene families provided further insight into their evolutionary history, specifically, revealed some taxon-specific duplication in MADS-box gene lineage. Further qualitative and quantitative analysis of these two transcriptomes is expected to reveal the genes that are responsible for the morphological difference between species.
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ORAL PRESENTATIONS

SECTION

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УСТНЫЕ СООБЩЕНИЯ

СЕКЦИЯ

«ГЕНОМИКА И СИСТЕМНАЯ БИОЛОГИЯ»

USE OF THE REFERENCE GENES FOR QUANTITATIVE GENE EXPRESSION ANALYSIS IN DIFFERENT SPECIES

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Key words: *Quantitative Real-Time RT-PCR, reference genes validation.*

Quantitative Real-Time RT-PCR is a powerful tool for the study of gene expression widely used in many fields of plant research. It is accepted that accurate comparisons of any samples by this method require procedure called normalization that is necessary to correct for variability not caused by the differences inherent to control and experimental sample. There are several strategies of normalization, but the most popular is the use of reference gene. In an ideal case this is a gene whose expression is stable in control and experimental conditions. In plants, the stability of gene expression was investigated on a model species *Arabidopsis thaliana* in a genome-wide survey and a set of potential reference genes was suggested [1]. However there is increasing evidence that for any new experimental system the validation of potential reference genes is required [2]. There is a number of articles reporting the validation of reference genes in different plant species (mostly model and/or agriculturally important) such as *Populus*, rice, wheat, tomato, potato. But all these studies were focused on a single species; the question whether it is possible to find the reference gene (or several genes) suitable for interspecific comparison of gene expression level has never been raised. The comparison of gene expression level between different species is an integral part of many studies on plant developmental genetics thus the identification of reference genes suitable for this purpose can greatly improve its accuracy and reliability. In the present work the stability of expression of 8 potential reference genes was tested in five species of Brassicaceae: *Arabidopsis thaliana, Lepidium sativum, Matthiola longipetala, Alyssum tortuosum, Iberis amara* in three structures — leaves, inflorescences and flowers. These genes represent orthologs of *Arabidopsis thaliana* genes At1g27450, At5g08290, At5g60390, At5g25760, At3g11150, At4g33380, At5g46630 and At4g34270 — genes that were found to be stably expressed in *Arabidopsis thaliana* in a genome-wide survey of gene expression stability [1]. PCR efficiency was accessed using Miner 2.2, for the estimation of gene expression stability two programs — geNorm_win_3.5 and NormFinder. It was shown that for accurate normalization in interspecific comparisons of identical types of organs three reference genes should be used. For all types of organs two most stably expressed genes are At4g34270 and At5g46630. The third potential reference gene is different for different types of organs: in leaves it is At4g33380, in flowers and inflorescences — At5g60390.

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CHARACTERIZATION OF ARABIDOPSIS MUTANT WITH INACTIVATED GENE ENCODING FOR Fe-S SUBUNIT OF RESPIRATORY CHAIN COMPLEX I

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Key words: *Arabidopsis thaliana, respiratory complex I, insertion mutagenesis, mitochondrial-nuclear interactions*

The respiratory chain of plants, compared to the electron transport chain of animals, has a number of distinctive features, the most important of which is the presence of alternative electron transport pathways diverting electron flow around the main respiratory complexes. According to that, mutations affecting respiratory complexes in plants are not always lethal. The plants carrying such mutations can be a useful tool for clarifying the role of each respiratory chain component.

The mutant lines of *Arabidopsis thaliana* obtained by insertion mutagenesis of the *fro* gene (at5g67590) encoding the 18 kD Fe-S subunit of mitochondrial respiratory chain complex I were characterized. The homozygous plants carrying the insert were selected by PCR genotyping. The absence in these plants of transcripts corresponding in size to the *fro* gene was confirmed by gene expression analysis. The obtained homozygous plants were characterized by several phenotypic features, such as late seed germination, retarded growth and development, the increased number and deep-green color of leaves. At variance with earlier data obtained for splicing-defective mutants of the Arabidopsis *fro* gene, the mutant plants obtained in this study did not exhibit a lower resistance to abiotic stresses. Despite the complete inactivation of the *fro* gene, the mutant plants were similar to the wild-type plants in terms of biomass growth and productivity. Among all respiratory mutants studied so far, the characterized Fro^- line is only the one which displays high productivity, forms normal seeds and have no signs of cytoplasmic male sterility. Because of their unaffected productivity, the plants of Fro^- line can be a suitable model system for studying the role of complex I in mitochondrial functions and mitochondrial-nuclear interactions.

Study of intracellular reactive oxygen species (ROS) level in suspension cell cultures which we obtained from plants of wild-type and Fro^- lines revealed clear differences between these lines. The decreased level of superoxide radical formed by mutant cells was shown. We have also found differences in rate of increase of the hydrogen peroxide level upon the stress treatments. Hydrogen peroxide level in the cells treated with prooxidants raised a few times in wild-type cells but remained almost unchanged in the cells of Fro^- line. This fact indicates the elevated ability of the latter line to scavenge ROS. Additionally, we have shown that plants of the wild-type line accumulate several times more anthocyanins upon the combined drought and light stress if compared with plants of the mutant line. Apparently, plants with the modified respiratory complex I appeared to be more resistant to this stress. On the basis of the obtained data, we suggest that the absence of the Fe-S-subunit (and probably of the complex I function) leads to numerous readjustments of the signal and metabolic pathways which result in defect compensation and increased stress resistance of the mutant plants.

UNUSUAL APPLICATIONS OF PCR WITH FLUORESCENT PROBES

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Key words: *PCR, plant genomes, transgenic plants*.

Plant genomes are difficult for investigation because of many reasons, such as polyploidy, big number of different kinds of repetitive sequences etc. Sometimes application of classical variants of PCR is not sufficient for certain tasks of investigation of plant genomes, especially for search for sequences with unknown level of similarity to ones described earlier, or for the characterization of sites of different kinds of insertions. The main difficulty in such studies is big number of DNA fragments to be analysed. To solve this problem we adopted procedures, based on Real-time PCR with fluorescent DNA probe.

Real-time PCR with fluorescent probes combines positive features of PCR and Southern hybridization in a single reaction. Traditionally, it is used to characterize a DNA sample quantitatively. In this case, primers similar to the template DNA, anneal to the sequence of interest. DNA probe 5'-labelled with fluorescent dye and 3'-labeled with quencher anneals to sequences between the primers. During the elongation stage Taq polymerase reaches the probe and cleaves it from the 5' to 3' end. The detection of fluorescence reports the successful amplification of a specific fragment. This general feature of TaqMan Real-time PCR was used for our purposes.

In the presentation we will discuss PCR with degenerate primers and probes, corresponding to the most conserved regions of the genes of interest. This method allowed us to detect sequences, homologous to those, described earlier. The method is quick, and data obtained by this method conform data obtained by the traditional methods for the same species.

Several modifications of PCR based approaches for highly effective identification of the integration site of insertion will be discussed. We will focus on modifications of Tail PCR with fluorescent probes and different types of adapter oligonucleotides and semi-specific primers.

Approaches for quick Real-time PCR-based screening of genomic libraries will be also mentioned.

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COMPUTATIONAL GENOMICS: SEARCHING FOR NEW PROTEOME COMPONENTS

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Key words: *genome annotation, alternative translation starts, protein isoforms*

Modern methods of experimental analyses generate large volumes of raw data on mRNA and protein profiles in different cells, tissues and stages of development. These data provide a valuable source for reconstruction of biological pathways and regulatory circuits. However, such a reconstruction demands not only well-developed techniques of computational analyses but also a reliable background (i.e., genome structure, transcriptome and proteome contents, etc.). Currently, genome annotation procedures are based on certain premises limiting accuracy of gene structure predictions. In particular, it is commonly considered that a typical eukaryotic mRNA can encode only one protein. However, it was recently demonstrated that a considerable part of mRNAs could contain several alternative start codons from which translation of functionally different protein isoforms (or unrelated peptides) can be initiated. A contribution of alternative translation to eukaryotic proteome complexity is discussed.

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DEVELOPMENT OF SHOOT APICAL MERISTEM *IN SILICO* UNDER PHYTOHORMONES REGULATION

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Key words: *shoot apical meristem, mathematical modelling, molecular-genetic systems, auxin*

Motivation and Aim: Auxin participates in regulation of cell differentiation in development of embryo, leaves, vascular tissue, fruit, primary and lateral root and in controlling apical dominance and tropisms. Indole-3-acetic acid (IAA) is physiologically active in the form of the free acid, but can also be found in conjugated forms in plant tissues. The regulation of the IAA metabolism is enough complex and may explain in some aspects how this simple substance is able to influence such diverse processes. Mathematical modeling of IAA metabolic gene network can help reveal the main factors governing this complex process.
Methods and Algorithms: To reach this aim, we first reconstructed a gene network of auxin metabolism by annotating experimental data from 107 published papers into GeneNet computer system. This gene network after reduction was input into converter [1] to generate the mathematical model of auxin metabolism.
Results: We have reconstructed the gene network and developed the mathematical model of auxin metabolism in arabidopsis shoots. The model allows to reproduce some phenomenological and molecular-genetic aspects of the auxin role in the plant development. The obtained results confirm adequacy of the developed model [2]. *In silico* experiments testify to qualitatively rapid processes of the molecular genetic regulation of the systems homeostasis.
Conclusion: Earlier we've developed the cellular automaton model that imitates morphodynamics of embryo development by means of regulation of signals produced by different embryonic cells is a first step in modelling the process of development in general and in modelling the gene network for morphogenesis in particular [3]. The next step in mathematical modeling application to studying of the plant development rules is integration of the spatial distributed hierarchical model with model of the intracellular auxin metabolism.
This work has been partially supported by the RFBR grant № 08-04-01008-a, Grant NSh-2447.2008.4, and by the SB RAS Interdisciplinary project №№ 107, 119, and also by the RAS projects 21.26, 22.8, 23.29.
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TARGETS PREDICTION FOR AtARF FAMILY TRANSCRIPTION FACTORS, MEDIATING PRIMARY RESPONSE TO AUXIN

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Background: Auxin regulates many plant developmental and physiological processes. Auxin response transcription factors (ARFs) mediate auxin-induced gene expression. ARFs specifically bind to TGTCnC-containing auxin response elements (AuxREs) [1].
Aims and Motivation: It is suspected that presence of TGTCnC motif in a gene promoter is the evidence for auxin regulated gene expression. Since TGTCnC motifs are widely spread throughout the *A. thaliana* genome, almost all genes could be regulated by ARFs factors. A more precise approach for prediction of ARF-targets is required.
Methods: A sample (1) of 11 experimentally proven AuxREs from arabidopsis, rice, pea and soybean and a sample (2) with promoters of 53 auxin-regulated genes were created using published data. The optimized PWM method [2] was used for prediction of potential AuxRE sites. For the estimation of predicted targets significance, we used the microarray data on gene expression after plant treatment with 1 μM IAA for 1 hour [3]. First, we obtained the ratios reflecting the auxin-induced changes in gene expression. A group of genes showing the smallest changes were used as the control group. We compared the enrichment/depletion of AuxRE sites in the upstream regions of *A. thaliana* genes that respected to either repression or activation. χ^2 test was used to verify reliability of observed difference.
Results: First, the set of *A. thaliana* promoters with proved TSS was created. For that purpose, we superposed the data on the upstream regions of *A. thaliana* genes [3] to the experimentally proved TSS distribution [4], by this way we obtained 8688 promoters. Analysis of the sample (2) by the PWM method using th
e sample (1) showed, that the density of predicted AuxREs in the regions (-300; +1) relative to an annotated TSS nearly twofold exceeded that for the (-2000; -300) regions. With the threshold estimated for the sample (2) we predicted 1242 AuxREs in (-300; +1) regions of 1154 genes out of 8688. The analysis of the presence of AuxRE sites in the promoter regions of 4321 from 8688 genes (the genes which have a unique reference to the microarray data), showed a significant AuxREs enrichment for the genes displaying auxin-induced repression or activation of expression.
Conclusion: A novel effective approach for AuxRE sites recognition was developed. We first demonstrated that (-300; +1) regions of auxin-regulated genes are enriched by AuxREs. The list of genes was created consisting the most probable targets of AtARFs.
Acknowledgements: This research was supported in part by the RFBR (№ 08-04-01214-a), Nsh-2447.2008.4, the SB RAS Integration Project No. 119 and RAS Programs №№ 22(8), 23(29).
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POSTER PRESENTATIONS

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СТЕНДОВЫЕ СООБЩЕНИЯ

INTROGRESSION OF *AEGILOPS* GENETIC MATERIAL INTO THE GENOME OF HEXAPLOID TRITICALE AND INFLUENCE OF GENOME STRUCTURE ON FORMATION OF ECONOMIC-VALUABLE TRAITS

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Key words: *triticale, Aegilops, genotyping, chromosome substitution, translocation*

The present work is focused on studying of formation of valuable properties in hexaploid triticale (the synthetic hybrid of wheat and rye) depending on its genome structure. For this purpose we have performed genotyping of triticale lines with introgressions from wild *Aegilops* species and have investigated their productivity, resistance to diseases, winter hardiness. Two approaches have been used for genotyping of ten triticale lines: 1) molecular-cytological which consisted in the comparative analysis of chromosome structure by GISH and FISH; 2) molecular-genetic, connected with studying genomic DNA of a hybrids and parental forms (with using microsatellite and chromosome-specific markers). According to GISH with DNA of *S. cereale* 8 lines have 14 chromosomes of rye, translocations and recombination between wheat and rye chromosomes were not detected. There were 12 chromosomes of rye and one pair chromosomes of *Ae. umbellulata* in two lines. It was shown (data of microsatellite analysis and analysis by using chromosome-specific STS markers) that rye chromosome 1R was replaced by chromosome 1U *Ae. umbellulata* in one line, and there was a replacement 2R(2U) in another line. FISH with species-specific probe Spelt1 and a probe pSc119.2 (to identify individual chromosomes of wheat and *Aegilops*) was used to detect genetic material of *Ae. speltoides* in the investigated lines. The chromosome substitution 1B(1S) was revealed in one line. Translocation T1SS/1BS·1BL was observed in five lines, translocation T4AS·4AL/4SL was detected in one line and another line has translocation T7BS·7SL. Productivity standards of lines, such as plant height, tilling capacity, length of the main ear, weight of grains of the main ear etc were investigated. It was established that chromosome 2U of *Ae. umbellulata* made negative impact on all production standards. On the contrary, the line with chromosome substitution 1B(1S) was characterized by increasing of basic elements of productivity. The line with replacement 1R(1U) can represent potential interest to improve bread-making quality of triticale [1]. Degree of winter hardiness of all lines was estimated in the conditions of Belarus and was close to indicators of parental forms or exceeded them. The most essential increase of winter hardiness is observed in the lines with translocations T4AS·4AL/4SL and T7BS·7SL. All lines have shown resistance to powdery mildew and to leaf rust in the conditions of West Siberia. This work was supported by the Federal Targeted Program of the Russian Federation (state contract P409) and SB RAS (Integration project No. 129) for financial support.

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APPROACHES FOR PHYTOSTEROL METABOLISM MODIFYING IN POTATO PLANTS

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Key words: *phytophthora, sterol composition, silencing*

Late blight disease is one of the most devastating diseases of potato plants leading to great losses in yield, so, searching for the new sources of resistance is very important for agriculture effectiveness. The idea of the research based on the fact of Phytophthora dependence on phytosterols which are important for growth and propagation of Phytophthora spp. [1, 2], and sterol composition of host plants is an essential moment [3]. So, potato plants with altered sterol composition will be less "attractive" for pathogen and more resistant to pathogen attack.
For changing of sterol composition of potato plants the strategy of silencing of some potato genes (*smt1, smt2, dwf1*) was chosen because of their great influence on end products of sterol pathway, especially, on sitosterol (most suitable phytosterol for Phytophthora). Agrobacterial strains carrying modified vectors pKannibal [4] for inducing of silencing with cloned parts of potato genes were produced. *In vitro* transformation of susceptible potato cultivar Zhukovskiy ranniy and tobacco plants allowed producing of transgenic plants. Molecular characterization (by real-time PCR) revealed reducing of target *dwf1*-gene mRNA level in transgenic tobacco plants. This result indicates of possible using of this approach for sterol metabolism modifying in plants.
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THE RADIOBIOLOGICAL INVESTIGATIONS OF THE TERRESTIAL ECOSYSTEMS IN THE EAST-URAL RADIOACTIVE TRACE

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Key words: *radionuclides, plants, populations, seed progeny, viability, mutability, radioresistance, allozyme analysis*

The Kyshtym accident at the Mayak Production Association (Chalyabinsk region, Russia) in 1957 resulted in radionuclide discharge that formed the Eastern Ural Radioactive Trace (EURT). Nine years later, the Eastern Ural State Reserve was established in the frontal part of this trace and actually became the test ground for experiments in nature, which had no analogs in the world.

In the EURT, concentrations of Sr-90, the main contaminant, in the upper topsoil was estimated to be 40–17000 times over the global level. The density of contamination decreased with the distance from the accident plot according to exponential law. The resulting doze loads shown an excess over the background level of about 1–3 orders of magnitude. Vegetation in the head part of the EURT is represented with synanthropic and seminatural communities undergoing different phases of degradations and recovery successions. Phytocenosis degradation is caused by the failures during the accident, subsequent reinstatement and restoration activities and also by the initial anthropogenic load. The ecological and genetic effects of permanent ionizing radiation on plants are evident from a wider spectrum of variability for all indicators of the living ability of seed posterity and their increased mutability. The effect of radioadaptation, i.e. increased the resistance to the additional irradiation, in the EURT plants was not found.

DIVERSITY OF SIBERIAN GRASSES (POACEAE) AND PROSPECTS OF SYSTEMS AND APPLIED RESEARCH

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Key words: *grasses of Siberia, phylogenetic diversity, endemism*.

Grasses (*Poaceae* Barnh.) are one the largest and thoroughly studied family of flower plants. In Siberian flora, it is represented by 483 species and subspecies belonging to 73 genera [1]. Species grow everywhere in Siberia and are of great economic importance. However, data on their diversity in Siberia are very limited. Necessity of study of the diversity of Siberian grasses is caused, first of all, by revealing the main trends in genesis of the family under increasing influence of anthropogenic factors on the environment. Study of endemism and peculiarities of distribution of endemic taxa in Siberia is of great interest. The aim of our work was to study phylogenetic diversity and endemism using the example of Siberian grasses to reveal history of characters. Diversity, endemism and distribution of taxa have been determined with the use of published data [1–3] and herbarium collections (NSK, NS, TK). Complexes of tribes at the rank of subfamilies represent 5 phylogenetic lines in the family. The complex *Pooideae* Benth. comprises 11 tribes: *Stipeae* Dum., *Triticeae* Dum., *Aveneae* Dum., *Phleeae* Dum., *Bromeae* Dum., *Phalarideae* Benth., *Poeae* R. Br., *Brachypodieae* (Hack.) Hayek., *Meliceae* Endl., *Nardeae* Anderss., *Scolochloeae* Tzvel. and 56 genera. The line *Ehrhartoideae* Link. is represented by the tribe *Oryzeae* Dum. with 2 genera. The tribes *Aeluropodeae* Nevski ex Bor., *Pappophoreae* Woods. and *Cynodonteae* Dum. in the line *Chloridoideae* Burmeist. comprise 6 genera. The complex *Panicoideae* Link. includes the tribes: *Arundinelleae* Stapf., *Paniceae* R. Br., *Andropogoneae* Dum. and 7 genera. The tribes *Molinieae* Jiras and *Arundineae* Dum. in the line *Arundinoideae* Burmeist. comprise 2 genera. The number of species and subspecies in the genera of the family varies from 1 to 69. Genera with 1 species amount to 41 %. The family has no endemic genera. Endemic taxa account for 10 % and belong to 13 genera: *Poa* L. — 11 species; *Festuca* L. — 10; *Koeleria* Pers. — 8; *Puccinellia* Parl. — 7; *Agrostis* L. — 3; *Helictotrichon* Besser. — 2; *Hyalopoa* (Tzvel.) Tzvel. — 2; *Calamagrostis* Adanson — 1; *Ptilagrostis* Griseb. — 1; *Setaria* Beauv. — 1; *Stipa* L. — 1; *Deschampsia* Beauv. — 1; *Alopecurus* L. — 1. Diversity of species increases from peripheral to intracontinental floristic provinces. Interrelationship between a general number of species and the same number of endemic taxa in genera was revealed (r = 0.78). The largest genera diversity among 5 lines of the family is in line *Pooideae* (77 %). The family contains 38 endemic species and 11 subspecies. Endemic taxa are represented in 2 evolution lines of the family: *Panicoideae* (2 %) and *Pooideae* (98 %). Established diversity permits studying the history of plant characters and genes which determine them and use them as a new source of material for breeding.

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GENETIC ANALYSIS OF NUCLEAR-CYTOPLASM INCOMPATIBILITY IN *PISUM*

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In pea, we found a nuclear-cytoplasmatic conflict in crosses of some wild forms with cultivated peas. In the cross VIR320 x WL1238, where VIR320 represents the wild subspecies *Pisum sativum* subsp. *elatius* and WL1238 is a testerline, at least two unlinked nuclear genes, designated as *scs1* and *scs2*, were shown to participate in the conflict, which is manifested as mosaic chlorosis and underdeveloped leaflets and stipulae if alleles of both genes in heterozygote are present on the background of plastids inherited from VIR320. Their location was determined based on linkage analysis of a number of molecular and visible markers available in a population of recombinant inbred lines (RIL) derived from the reciprocal cross WL1238 x VIR320. For a fine location of these genes on the pea linkage map we utilized synteny of the pea and barrel medic (*Medicago truncatula*) genomes and worked out molecular markers using publicly available [1] nucleotide sequences of the latter genome. The closest to *scs1* known marker appeared *PhlC* (linkage group III). For a next close marker we chose the gene coding for a protein of the GRAS family, *nsp2* in the barrel medic genome, which corresponds to the pea gene encoded in AJ832139 (*Pisum sativum sym7* gene for GRAS family protein), further we designate this gene as *sym7*. For mapping, we used two F_2 populations derived from F_1 plants heterozygous for *scs1, PhlC* and *sym7*. Segregation in F_2 deviated from the Mendelian 1: 2: 1 since allele *scs1_1238* appeared to be sporophytic and/or gametophytic lethal in the background of the cytoplasm from VIR320. We tested 103 plants for genotypes of *PhlC* and *sym7*. Genotype for *scs1* was detected by mycroscopic analysis of pollen: we found that heterozygotes for the LG III markers studied had high (~ 50 %) sterility of pollen, so we considered plants with semisterile pollen as heterozygotes scs1_1238/scs1_320 and the ones with fertile pollen as homozygotes scs1_320/scs1_320, while homozygotes scs1_1238/scs1_1238 were regarded as non-viable in the VIR320 cytoplasm. Of the 206 gametes involved, 3 were cross-overs between *PhlC* and *scs1* (~ 1.46 cM), and 2 were cross-overs between *scs1* and *sym7* (~ 0,97 cM); total distance between *PhlC* and *sym7* thus comprised 2.43 cM and gene *scs1* was mapped between *PhlC* and *sym7*. For a more precise mapping of gene *scs2* on LGV we tried genes residing in the barrel medic gemone assembly near the *cri* gene ortholog. We selected the gene coding for polynucleotidephosphorylase, corresponding to the pea sequence AF010578 *(Pisum sativum* polynucleotide phosphorylase (*pnp*) mRNA). Segregation data in the mapping RIL population suggest *scs2* to reside between *gp* and *pnp*; Haldane formula applied for the data provides an estimate of 15.5 cM between the latter markers.

1. http://www.medicago.org.

INVESTIGATION OF INTROGRESSIVE (*TRITICUM AESTIVUM* X *TRITICUM TIMOPHEEVII*) LINES OF COMMON WHEAT — THE DONORS RESISTANCE GENES TO BIOTIC STRESS

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Key words: *common wheat, introgressive lines, biotic resistance*

It is well known that the genetic diversity of common wheat (*Triticum aestivum* L.) genes providing its resistance to pathogens is very limited. It can be significantly enhanced by moving into its genome of genes from species that have shaped their gene pool in the centers of the joint evolution of the host plant and pathogens. The aim of our long-term work was to enrich the gene pool of common wheat with genes of tetraploid species *Triticum timopheevii*, which is characterized by a complex resistance to diseases, and to create a pool of resistance genes for breeding. To realize this goal we have created a collection of introgressive lines of common wheat. These lines ($2n = 42$) represent the secondary gene pool, which can be used in breeding as donors of resistance genes to powdery mildew, leaf and stem rust. The purpose of this communication is to evaluate the possibility of introgressive lines as donors in the breeding process. This is necessary to evaluate cytological stability as well as to identify and locate the fragments of the *T. timopheevii* genome and loci that ensure the sustainability of these lines to pathogens. We have previously shown that the resistance of some lines is controlled by 2 genes with different types of interaction between them. These genes are effective non-allelic resistance genes used in plant breeding. The investigated lines are characterized by multiple sites of introgression fragments of the *T. timopheevii* genome into different chromosomes.

A detailed cytogenetic analysis of introgressive lines showed that some of them are characterized by cytological instability: a significant percentage of cells with disruptions in the chromosome pairing during meiosis. Determination of chromosomal localization of the *T. timopheevii* genome fragments in unstable lines showed that these lines are characterized by a larger number of introgressions in comparison with the stable ones. Part of the introgressive lines collection was transferred to Omsk. In 2005–2006, these lines were included in the crossing with promising varieties of wheat breeding laboratory.

In the study of meiosis of hybrids F_1 (Omsk varieties x introgressive lines), it was shown that the genotype of the recipient variety influences on the chromosome pairing in PMCs. The smallest number of abnormalities of chromosome pairing observed in combination with cytologically stable lines. These lines were mainly characterized by a small number of genomic introgressions from *T. timopheevii* and by none *T. timopheevii* introgression into 5BL chromosome. Hybrids F_1 (Omsk varieties x line 140) had no irregularities in the chromosome pairing. This is probably due to the fact that in its genome only two chromosomes (2AL and 6BL) were found to carry fragments of the *T. timopheevii* genome. This line is used extensively in the breeding process. In assessing the F_1 and F_2 hybrids, dominance of resistance in almost all studied hybrid combinations was shown.

GENETIC DIVERSITY OF SOUTH-EAST KAZAKHSTAN WILD SPECIES *AEGILOPS CYLINDRICA* HOST. AND *AEGILOPS TAUSCHII* ON THE GRAIN STORAGE PROTEIN COMPOSITION

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Key words: *Aegilops cylindrica, Aegilops tauschii, gliadin, HMW-GS, electrophoresis*

Aegilops genera is the richest source of valuable biological properties and economic traits for wheat breeding [1]. The molecular characterization of wheat wild relatives ecotypes reveals a huge variability of intra- and inter-populations, that allows clear identification of material, enables to preserve germinal plasma and to select forms valuable for selection. *In situ* and *ex situ* conservation of wild relatives populations including *Aegilops* species and investigation of them are necessary to save biological variability of plants.

Fifty samples of *Aegilops cylindrica* populations and 14 accessions of *Aegilops tauschii* from various growth places in Almaty, Zhambyl and the South-Kazakhstan regions were collected during the expeditions to Southeast Kazakhstan carried out by the Crops Genofond Department of Kazakh Scientific Research Institute of Farming and Plant Growing in 2003–2009.

With the purpose of identification and screening of the samples maintained in the gene bank, spectra of wheat wild relatives seeds storage proteins, detected by SDS and acid polyacrylamide gel electrophoresis, were studied. In gliadin spectra of *Ae. cylindrica* and *Ae. tauschii*, absence of the α zone components along with saturation by components of the γ and β zones was typical for the first species, whereas ω and β saturated zones of the protein spectrum were characteristic for the second one. Analysis of gliadin structure of *Ae. cylindrica* accessions has revealed significant inter- and intra-population polymorphism at the gliadin coding loci. As a whole, in all populations, 5 variants of the protein electrophoretic spectra were identified. The number of prolamin biotypes varied in separate samples from 2 to 4. Structure of high-molecular glutenins was identical in all samples and included 3 subunits corresponding to the zone of the spectrum characteristic for high-molecular glutenin subunits of bread wheat, designated 1Cx, 1Cy and 1Dy by Wan et al. [2]. Inter- and intra-population polymorphism of *Ae. tauschii* at the gliadin loci was considerably higher than that of *Ae. cylindrica*. Thirteen variants of the protein spectrum were revealed. So, genotypes with a specific protein spectrum were prevailed in practically each sample. In the zone of high-molecular weight glutenin subunits (HMW-GS) of the spectrum of *Ae. tauschii*, 2 subunits were found out. The level of polymorphism among *Ae. tauschii* accessions analyzed in the current study at the glutenin coding locus was not significant.

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THE EFFECTS OF ALLELES OF DWARFING GENES AND *Ppd-D1* GENE ON THE AGRONOMICAL TRAITS OF WHEAT

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Key words: *dwarfing genes, Ppd-D1, agronomical traits, winter wheat*

The short arm of chromosome 2D of bread wheat carries genes which are important for determining the adaptation of wheat varieties. The dwarfing gene *Rht8* and the photoperiodic insensitivity gene *Ppd-D1* are linked on the chromosome 2D [Worland et al., 1998] at the distance 20.9 cM [Pestsova et al., 2002]. The *Rht8c* and *Ppd-D1a* genes were actively involved in breeding programs of PBGI.

Lines-analogues, that are genetically different in plant height, were created by V.V. Khangildin on the genetic background of historically well known varieties such as: Kooperatorka, Odesskaya 3, Odesskaya 51, Stepnyak. The allelic composition of the lines-analogues, recurrent and parental forms regard to dwarfing genes (*Rht8, Rht-B1, Rht-D1*) has been tested by PCR-analysis and the test of sensitivity to gibberellic acid [Chebotar et al., 2008]. PCR-analysis of alleles of *Ppd-D1* gene had been done according to Beales et al. (2007) and had shown that the varieties which were selected before 1960, such as Kooperatorka (1929), Gostianum 237 (1929), Odesskaya 3 (1938) and Odesskaya 16 (1958) were sensitive to photoperiod and did not carry any dwarfing gene. Lines-analogues that have been created on the basis of these varieties carry dwarfing genes from the parental forms Odesskaya semidwarf and Karlik 1 and are insensitive to photoperiod.

To investigate the effects of the dwarfing and photoperiod insensitivity alleles complex the lines were grown up in the field using wide rows in 2004–2009 year conditions. The data for estimation of correlation characteristics were measured in 2009 and calculated by Statistica 8. For the correlation analysis plants were divided into 3 groups (morphotypes) depending on their height: high, medium (one dwarfing allele) and dwarf (two dwarfing alleles).

The agronomic characteristics of the lines-analogues and tall parental varieties of bread wheat installed significant differences by height, weight of thousand kernels (WTK), the rate of development and yield structure elements. The lines-analogues with *Ppd-D1a* gene were earlier in earring and flowering. The WTK parameter of line Kooperatorka K-90 was significantly higher in comparing with dwarf photoperiod insensitive line-analogue Kooperatorka K-70 and high photoperiod sensitive line-analogue Kooperatorka. The productivity of the main ear has had the significant impact in the yield of the tall varieties. The influence of the secondary ears is increased in the yield structure of the dwarf or semidwarf lines, particularly, the ears of the second level (0.8 height). Comparison of phenotypic and genotypic correlations between the agronomical traits showed their ambiguous, but phenotypic correlations between height and productivity of the plants were positive independently from the morphotype. Moderate positive correlation between the grain number and the WTK has been revealed in the dwarf morphotypes.

ANALYSIS OF LEAF HAIRINESS IN WHEAT *TRITICUM AESTIVUM* L. USING THE HIGH-THROUGHPUT PHENOTYPING APPROACH

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Key words: *Triticum aestivum, leaf hairiness, trichome*

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.

Furthermore, a more accurate description of the morphological properties of the trait for correct determination of phenotypic classes is timely. Using of new computer–based technologies for descriptions of quantitative characteristics of leaf hairiness is the important step in this direction. In the course of the work, we have developed the LHDetect program for determining the degree of leaf hairiness and its morphological properties on the basis of its microscope image processing [1].

The suggested method appeared to be the effective approach for a large scale analysis of leaf hairiness morphological peculiarities in individual plants. For example in according with genotyping this approach can be useful to quantitative trait loci (QTL) mapping.

In this study we detailed analysis of hairiness in wheat as a complex feature. For two different cultivars with similar leaf hairiness differences were shown. The disjoining of hairiness trait in F_2 generation hybrids was studied for several combinations of parents. This allowed us to estimate qualitatively the possible number of genes that may control the hairiness trait in different cultivars. It was shown that this trait for several cultivars is polygenic. Also we show correspondence between trichome middle length and number of trichomes on several cultivates.

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1. A.V. Doroshkov et al. (2009) Using the computer-based image processing technique in genetic analysis of leaf hairiness in wheat *Triticum aestivum* L., VOGiS Herald, 13: 1: 218–226.

STUDY ON EFFECTS OF CHROMOSOME RECONSTRUCTION IN HEXAPLOID TRITICALE GENOME ON EXPRESSION OF SPIKE PRODUCTIVITY TRAITS

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Key words*: triticale, intergenomic chromosome substitutions, spike productivity traits, differential staining of chromosomes (C-banding)*

Nowadays chromosome technologies are the most promising approach to solving such problems in triticale breeding as low bread-making quality and insufficient resistance to phytodiseases. These disadvantages can be eliminated by introgressing chromosomes of the wheat genome D into karyotype of hexaploid wheat-rye amphidiploids. We have initiated research on genetic effects of introgression of different chromosomes from the wheat genome D into the karyotype of hexaploid wheat-rye hybrids for working out an optimum strategy of developing triticale recombinant forms of selection value.

Development of the line collection of hexaploid wheat-rye amphidiploids with different types of D(A)- and D(B)-chromosome substitutions was a necessary stage preceding the given research. The line material was selected from hybrid populations of 6x-triticale chromosome-substitution forms, produced in four crossing combinations of 8x- x 4x-triticale, and karyotyped by the method of Giemsa differential staining of chromosomes. As a result, the collection was made from 24 lines of hexaploid wheat-rye amphidiploids characterized by different qualitative and quantitative composition of intergenomic chromosome substitutions.

Effects of different types of D(A)- and D(B)-chromosome substitutions on expression of spike productivity traits of hexaploid triticale were studied in the line material. Such parameters as spike length, number of spikelets and flowers per spike, number of set seeds, 1000-grain weight were analysed. The presence of 6D(6B)-chromosome substitution in the karyotype of hexaploid triticale was revealed to exert a negative effect on the studied traits. The observed effect is not related to introgression of chromosome 6D into genome but is a consequence of chromosome 6B pair deficiency in their karyotype. Hence, it can be concluded that introgression of chromosome 6D into karyotype of hexaploid triticale should be more preferably performed as 6D(6A)-substitution. It was also shown that introgression of chromosome 4D into the karyotype of 6x-triticale resulted in a considerable increase of spike length, however, a positive correlation between this trait and spike productivity was not observed in our material. An addition, an increase in plant height was noted in the studied forms due to their susceptibility to lodging.

The data obtained, on the one hand, demonstrate visually the opportunity to use the developed line material for studying effects of chromosome introgression of the wheat genome D into the karyotype of 6x-triticale, but, on the other hand, indicate that intergenomic substitutions can have a significant effect on manifestation of not only qualitative but also polygenically inherited quantitative traits.

ALLELE VARIATION OF FOUR ENZYME-ENCODING GENES IN *AEGILOPS TAUSCHII*: SPATIAL PATTERNS THROUGH THE SPECIES AREA

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Key words: *Aegilops tauschii, allozymes, genetic variation, natural selection*

Aegilops tauschii Coss. genetic variation is an important natural resource for the improvement of common wheat. Therefore it is important to investigate how this variation is presented through the vast species area and among its subspecies, *tauschii* and *strangulata*. Investigations of enzyme-encoding genes allelic variation are of particular interest since it is known to be directly involved in plant adaptation to environment peculiarities. An investigation of spatial patterns of adenylate kinase (AK, EC 2.7.4.3), catalase (CAT, EC 1.11.1.6), endopeptidase (EP, EC 3.4.21-24.-) and fructose-1,6-diphosphatase (FDP, EC 3.1.3.11) encoding genes allelic variation in *Ae. tauschii* was carried out through this study. About 300 accessions, representing all the species range were taken for the investigation. *Cat2* and *Fdp* loci are completely monomorphic in ssp. *strangulata* and in the western part of ssp. *tauschii* range as well. Both *Cat2* and *Fdp* are highly polymorphic in the eastern part of ssp. *tauschii* range, with the patterns of this polymorphism being discordant in these two loci. Ak^{108}, a rare allele with a sporadical spatial occurrence, was found in ssp. *tauschii* only. On the contrary, Ak^{92} is absent in ssp. *tauschii*, while it is the most common *Ak* allele in ssp. *strangulata* in Precaspian Iran, the most moist part of the area, and is very rare in other parts of ssp. *strangulata* area. *Ep* is a highly polymorphic locus with the highest level of variation in the west of *Ae. tauschii* area, where this species had originated. *Ep* allele variation patterns are rather similar in ssp. *tauschii* and ssp. *strangulata*. The data reveal an adaptive nature of *Ak, Cat2,* and *Fdp* allele variation, while *Ep* allele polymorphism seems to be mostly neutral. It can be seen than enzyme-encoding genes allelic variation patterns in *Ae. tauschii* were to a large extent formed by natural selection. As a rule, these spatial patterns are irrespective of the history of *Ae. tauschii* geographic expansion through the species area and reflect adaptation of the species local populations to environmental conditions.

EFFECTS OF CHROMOSOMES OF HOMEOLOGICAL GROUP 5 BEARING VARIOUS *Vrn-1* LOCI ON EARING TIME AND GROWTH HABIT IN WHEAT

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The three major loci for vernalization requirement in common wheat, *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, are located on homologous chromosomes 5A, 5B, and 5D, respectively [Law et al., 1976; Worland et al., 1996]. *Vrn-1* loci are all linked with the *Fr-1* (frost resistance) loci, i.e. *Vrn-A1* with *Fr-A1*, *Vrn-B1* with *Fr-B1*, and *Vrn-D1* with *Fr-D1* [Galiba et al., 1995; Snape et al., 1997; Iwaki et al., 2002; Tóth et al., 2003]. In rye, only one gene, *Vrn-R1*, is known to determine the spring/winter growth habit. It is located on chromosome 5R [Plaschke et al., 1993]. Vernalization genes determine flowering time and hence the basic adaptation of a genotype to a particular environmental condition [Worland et al., 1998].

Effects of various alleles of the dominant *Vrn-B1* gene on earing time were studied in near-isogenic lines (NILs) of winter cv. Bezostaya 1 and intervarietal substitution lines of winter cv. Sava for chromosome 5B. It was found that the earing time difference between lines grown in different settings was related to two differently expressed alleles: $Vrn\text{-}B1^{S} > Vrn\text{-}B1^{Dm}$. The earing time in lines with the $Vrn\text{-}B1^{S}$ allele inherited from cv. Saratovskaya 29 began 7–10 days earlier than in lines with weak $Vrn\text{-}B1^{Dm}$ of cv. Diamant 2. The NILs and substitution lines are late-ripening, and grain fails to complete its ripening in the Novosibirsk climate. On the base of the information we gathered, we conclude that ear emergence time is polymorphic in wheats at least because of the allelism of the dominant *Vrn* genes.

We developed 5R(5A) substitution lines harboring chromosomes of spring rye cv. Onokhoiskaya [Efremova et al., 2006]. As a result of this substitution, two lines descending from spring varieties Rang and Mironovskaya krupnozernaya acquired the winter habit. These lines fail to ear in the summer when sown in the spring in fields near Novosibirsk, whereas part of the plants sown in the autumn hibernate successfully. The winter habit of the substitution lines is determined by the recessive wheat genes *vrn-B1* (chromosome 5B) and *vrn-D1* (5D) and the lack of expression of the rye *Vrn-R1* gene for the spring habit. Molecular analysis indicated that Onokhoiskaya rye had the same gene on chromosome 5R, but its expression was low [Malyshev et al., 2001]. It can be conjectured that spring rye plants were heterozygous for spring habit genes, and chromosomes 5R of the substitution lines had the recessive vrn-*R1* allele. Hibernating plants constituted 25 to 27 % of the 5R(5A) lines for Rang and Mironovskaya krupnozernaya; about the same as in many winter varieties (Mironovskaya 808, Kavkaz, Avrora, Odesskaya 51, etc.) but less than in Ul'yanovka (30 %), which is the most frost-tolerant variety in Siberia. Both our results and data from the literature show that chromosome 5R bears important frost tolerance genes [Fowler et al., 1999; Snape et al., 2001]. The most appropriate models for further winter hardiness studies are lines with wheat–rye 5R–5A, 5R–5B, and 5R–5D translocations in the vicinity of *Vrn-1* genes obtained on the base of frost-tolerant varieties of winter wheats and rye.

DEVELOPMENT AND CHARACTERIZATION OF *TRITICUM AESTIVUM* x *T. TIMOPHEEVII* NEAR-ISOGENIC INTROGRESSION LINES BY USING MARKER-ASSISTED SELECTION

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Key words: *introgression, leaf rust, marker-assisted selection, T. timopheevii*

Introgression lines of common wheat *Triticum aestivum L*. are characterized by effective resistance to leaf rust and powdery mildew as a result of transferring resistance genes from tetraploid species *T. timopheevii*. Molecular-genetic analysis by means of genome-specific microsatellite markers showed that *T. timopheevii* genome fragments was localized mainly in the first, second and fifth chromosomes of the A genome and in the second, fifth and sixth chromosomes of the genome B. Molecular-genetic mapping of the line 832 containing multiple *T. timopheevii* introgression fragments detected that leaf rust resistance is determined by three loci in chromosomes 1A (*QLr.icg-1A*), 2A (*QLr.icg-2A*) and 5B (*QLr.icg-5B*). These loci are responsible for 10, 11 and 54% of the phenotypic variance of the trait, respectively. To investigate interaction of the genes providing the leaf rust resistance in the line 832, the introgression lines with single introgressions containing the *QLr.icg-1A*, *QLr.icg-2A* and *QLr.icg-5B* loci or combination of introgressions were created. For this purpose, the line 832 was backcrossed three times with the initial common wheat cultivar. Selection of plants carrying the loci *QLr.icg-1A*, *QLr.icg-2A* and *QLr.icg-5B* was performed in the progenies of the second and third backcrosses by means of SSR (simple sequences repeats) markers specific for *T. aestivum* (BBAADD genome) and *T. timopheevii* (GGA^tA^t genome). The selected lines were additionally analyzed using *in situ* hybridization with the probes pSc119.2 and Spelt1 specific for the B and G genomes to discriminate additional introgression fragments from chromosomes 3G and 4G, which have not been revealed by molecular analysis. As a result, six introgression lines carrying the loci *QLr.icg-5B*, nine lines with *QLr.icg-2A*, one line with *QLr.icg-1A*, four lines carrying both *QLr.icg-1A* and *QLr.icg-5B* loci and one line carrying both *QLr.icg-2A* and *QLr.icg-5B* were developed. The resistance of the lines to the population of the leaf rust pathogen prevalent in West Siberian region of Russia was evaluated at the seedling and adult plant stages. It was found that all near-isogenic introgression lines carrying the major locus *QLr.icg-5B* expressed high (R) or moderate (MR) level of resistance to the natural population of *Puccinia triticina* at the adult plant stage (score 0-1 and 2 according to the Mains–Jackson immunity scale). It was found that the lines containing only *QLr.icg-1A, QLr.icg-2A* or *QLr.icg-5B* were less resistant than lines containing two or three loci. The data assumed that the presence of the minor resistance loci *QLr.icg-1A* and *QLr.icg-2A* in combination with *QLr.icg-5B* increase the resistance of introgression lines to a wider set of leaf rust races. The obtained data evidenced that application of SSR-analysis allows to select the genotypes carrying target loci or genes already at the stage of the second and third backcrosses and significantly reduces the time of development of *T. aestivum* lines, containing only one introgression fragment from wild species. This work was supported by the Federal Targeted Program of the Russian Federation (state contract P409).

IDENTIFICATION OF THE NEW GENE *Ha2*, DETERMINING ENDOSPERM TEXTURE OF BREAD WHEAT GRAIN, INTROGRESSED FROM *AEGILOPS SPELTOIDES* TAUSCH

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Endosperm texture is the most important trait of wheat grain determining its end-use purposes. It reflects strength of linking between the two main grain components — starch granules and protein inclusions. The bread-making flour is manufactured from the grain of cultivars with hard texture of endosperm. The main genetic determinant of this trait is a locus situated in 5DS chromosome which responsible for biosynthesis of two proteins — puroindolines (PinA и PinB) and grain softness protein (Gsp-1). Mutations in the two first proteins result in hard grain texture. No other such genes have been found in wheat genome until now.

In this work, the new homoeologous gene determining the soft endosperm structure is described introgressed from *Aegilops speltoides* Tausch., a wild diploid cereal which is considered to be a possible progenitor of B genome of bread wheat. The gene was discovered in the winter line $84/98^{w}$ from "Arsenal" collection developed by Dr. I. Lapochkina with co-workers in the Institute of Agriculture of Non-Chernozem Regions of Russia (Nemchinovka, Moscow region). The collection was obtained by direct crossing of spring wheat cultivar "Rodina" with a specimen of *Ae. speltoides*. The maternal cultivar has a hard type of endosperm texture and the winter line with introgression — a soft type.

The winter habit and speltoid awned spike of the line proved the introgression to be non-homoeologous; 5S substituted for 5A chromosome of bread wheat. With the use of monosomic analysis the introgressed chromosome from the line $84/98^{w}$ was introduced into genotypes of two additional bread wheat cultivars Saratovskaya 29 and Diamant 2 with hard type of endosperm. This also resulted in the softness of their endosperm texture. The association of grain hardness with virtuousness — another endosperm trait characterizing milling properties of grain was also investigated.

Therefore, the species *Aegilops speltoides* is a carrier of the dominant allele of the locus determining endosperm texture which is homoeologous to the locus situated in 5DS chromosome of bread wheat. It is suggested the designation *Ha-Sp* to this gene.

FROST TOLERANCE AND MICROSATELLITE ANALYSIS OF RECOMBINANT-INBRED LINES

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Key words*: winter bread wheat, RILs, frost tolerance, microsatellite analysis*

Bread wheat (*Triticum aestivum L.*) is the most widely distributed cereal in the world. Freezing temperatures are often become the cause of losses in agricultural wheat productivity. The only insignificant part of varieties that are growing up in conditions of South Ukraine possesses sufficient frost and winter hardiness.

Inheritance of frost resistance in winter wheat is regarded as a quantitative trait controlled by many loci at least on 15 of the 21 pairs of chromosomes in hexaploid wheat. The major genes affecting frost resistance have been mapped on the long arms of chromosomes of homeologous group 5: *Fr-A1* and *Fr-A2* (chromosome 5A), *Fr-B1* (5B) and *Fr-D1* (5D) [1–3].

The analysis of frost tolerance according to [4] and microsatellite analysis of 5B chromosome were performed on 113 recombinant-inbred lines (RILs) derived from a cross between winter wheat varieties Luzanovka odesskaya (tolerant to frost) and Odesskaya krasnokolosaya (susceptible to frost). Parental forms analyzed by using of microsatellite markers located on the long arm of chromosome 5B (*Xcfd7*-5BL, *Xbarc89*-5BL, *Xbarc88*-5BL, *Xbarc*4-5BL, *Xgwm371*-5BL, *Xbarc1061*-5BL, *Xcfa2070*-5BL and *Xbarc74*-5BL). Polymorphism between parental varieties was observed for 75 % of markers. The association between allelic differences at microsatellite loci and frost resistance was studied. At the freezing test of the 113 RILs the population average frost resistance was 84 %, and the frost resistance of the separate lines varied from 0 % to 100 %. The level of the frost resistance of 56 lines that have allele 204bp of *Xcfd7* locus from the parent Odesskaya krasnokolosaya was 77 %, and the frost resistance of 57 lines with allele 194bp of Luzanovka odesskaya was 90 %. Therefore 13 % difference in the resistance of RILs F_7 Luzanovka odesskaya/Odesskaya krasnokolosaya were associated with alleles of *Xcfd7*-5BL locus from Luzanovka odesskaya.

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METABOLIC ENGINEERING OF PLANTS FOR STRESS RESISTANCE

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Key words: *environmental stress, proline metabolism, metabolic and gene regulation, transgenesis*

In many plants, free proline accumulates under stress conditions. The induction of proline accumulation in response to environmental stress suggests that genetic engineering overproduction in agriculturally important crops might increase their overall environmental tolerance and enhance productivity. The cellular concentration of proline can be regulated by increasing biosynthesis, decreasing degradation, and/or modifying rates of uptake or release. Thanks to knowledge of a metabolic pathway, signal transduction pathway and gene regulation whose products involved in a pathway, approach to understanding and predicting metabolic flux of proline can become more clarified. Using the GeneNet technology, the metabolic and gene network that control proline metabolism in plants are described. By accumulating the relevant literature data the metabolic pathway and gene network regulation are reconstructed. In higher plants, proline is synthesized from either glutamate or ornithine, and glutamate pathway is the primary route for proline biosynthesis. Proline is synthesized from glutamate via two intermediates, namely GSA (glutamic-semialdehide) and P5C (delta-pyrroline-5-carboxylate). Two enzymes catalyze this pathway: P5C synthase (P5CS) and P5C reductase (P5CR) in final step. Genes encoding two enzymes p5cs and proc1 in proline biosynthesis have been isolated from various plants. The level of proline and activity of P5CS is controlled via a regulatory circuit with a feedback regulation. P5cs gene was induced by high salt treatment, dehydration, whereas there was nothing induced the proc1 gene to a significant extent by any of stress. P5CS is the rate limiting enzyme in proline metabolism. Specific CaM isoform CaM4 enhances the DNA binding activity of transcription factor AtMYB2 and enhance transcription of p5cs gene and mediates salt-induced Ca^{2+} signaling, resulting in salt tolerance in plants. Proline is metabolized to glutamate by two enzymes: proline dehydrogenase (ProDH) and P5C dehydrogenase (P5CDH) in the final step. The key enzyme in proline degradation is ProDH. Expression of pox gene encoding of ProDH is provoked by both the application of exogenous proline and rehydration. In hypoosmotic conditions, activation of pox gene is induced by ATB2 subgroup transcriptional factor through ACTCAT sequence on promoter region of gene. Proline synthesized in the cytosol while metabolized in mitochondria. Accumulation proline in mitochondria involves proline transport between cell compartments, and its distribution through the whole plant.

Genes encoding proline transporters were isolated from a number of plant species, but knowledge of these genes properties is rather poor. Considering the results obtained through reconstruction metabolic and gene network on improving the pool of free proline has made possible to increase proline accumulation by transgenic technology using key enzymes: P5CS for proline biosynthesis and ProDH antisense for proline degradation, and it may initiate to enhance stress resistance in plants.

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SUPRAMOLECULAR STRUCTURES OF CELLULAR NUCLEUS OF PLANTS IN THE CHANGING ENVIRONMENTAL CONDITIONS

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Keywords: *a cell nucleus, nucleoplasm, chromatin, nuclear matrix, wheat*

Scientists have been interested for a long time, how whole genomic DNA upon the accessibility of its certain sequences for regulatory factors and transcriptional enzymes is accommodated in the cellular nucleus with a diameter of only ca. 10 microns. This challenge is solved at the level of packaging of DNA into chromatin, which proceeds in several stages: nucleosome formation, DNA loops formation and loop anchorage on the protein skeletal structure of a nucleus — nuclear matrix. At present the mechanism underlying work of the factors of chromatin remodeling is not clear enough. Our attention to this problem is focused on proteins with arginine content. It is known that arginine-enriched histones are evolutionary stable in plants and animals, indicating that they may play an important role in processes of chromatin remodeling. Arginine possesses on the surface of nucleosomes, therefore it is exposed to different modifications, including possible relaxation of protein globule owing to *Arg-X* specific proteolysis. The proteolysis it considered to be a kind of the biological control giving fast physiological answer to the changing environmental conditions. The aim of the current study was detection of *Arg-X* protease sensitive zones, as one of the supramolecular-genetic mechanisms of the large-scale spatiotemporal chromatin organization during the biochemical adaptation to new living conditions. The object of research was the elite seeds of wheat from the VIR collection: spring cultivar Artemovka and winter cultivar Mironovskaya 808 originating from Artemovka. Cellular nuclei and their supramolecular structures (nucleoplasm, chromatin, nuclear matrix) were isolated from air-dry wheat embryos (0ч), and also during their further germination: 3 → 6 → 9 → 12 → 15 → 18 → 21 h of the G_1 phase of a cellular cycle. *Arg-X* proteolysis activity was assessed by cleavage of *Arg-X* bounds in the arginine-enriched protein protamine-*Salmine-A-I* («*Merk*»), which consists of 33 amino acid residues (22 Arg, 4 Ser, 3 Pro, 2 Glu, and 2 Val). Activity was measured in nmoles of arginine per second and milligram of protein. It was shown, that the initial cultivar Artemovka has high mass of protein on the nucleus during the introduction in S phase of the cell cycle (18 → 21 h). The winter cultivar has delay of transition G_1 phase into S phase of the cell cycle (18 → 21 h). Probably, this is related with changing of protein-synthetic processes necessary for formation of newly-formed nucleoprotein systems of nucleus. Furthermore, it was shown that molecular mechanisms underlying ontogenetic adaptation to cold stress are at the level nuclear matrix, responsible for assembling of multifermentative complexes of replication and transcription. The study was supported by the presidential grant for young scientists of Republic Bashkortostan.

ANALYSIS OF MICROSATELLITE LOCI POLYMORPHISM IN CULTIVATED BUCKWHEAT

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SSR analysis of 11 microsatellite loci variability in 32 cultivars and wild accessions of two cultivated buckwheat species *Fagopyrum esculentum, F. tataricum* was performed. Polymorphisms of the analyzed SSR loci have been evaluated. Four SSR markers showed high variability (PIC = 0.71–0.86) signifying that microsatellites can be used as powerful tools in buckwheat genetic studies. The average PIC for the four markers was 0.80 and was higher than the average PIC values reported for SSR markers for other cereal crops.

Predominate and rare allele phenotypes have been determined for buckwheat cultivars and wild accessions. A total of 43 allele phenotypes were identified for *F. esculentum* and 15 for *F. tataricum*. As a whole SSR loci allele variability in *F. esculentum* is higher than in *F. tataricum* that can be explained by self-incompatiblity of *F. esculentum*. To estimate within-cultivar diversity SSR analysis of 29 individuals each for 2 cultivars and 1 wild accession of *Fagopyrum esculentum* have been performed and high intracultivar variability shown.

Based on SSR analysis for each cultivar specific allele patterns of analyzed microsatellite loci have been described and unique allele formulas were established. The latter can be used for cultivar molecular genotyping. Diagnostic sets of most informative SSR markers enabling identification of the genotypes of all examined buckwheat cultivars of Russian breeding were determined.

STRUCTURE AND FUNCTION OF A NOVEL GENE CODING FOR UNIQUE HEVEIN-TYPE ANTIMICROBIAL PEPTIDE OF WHEAT *TRITICUM KIHARAE* DOROF. ET MIGUSCH

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Antimicrobial peptides (AMPs) are the key components of the first line of defense used by plants to combat pathogenic microorganisms and environmental stress. AMPs display broad-spectrum antimicrobial activity, therefore comprehensive analysis of their structure and biological properties supplemented by gene isolation and regulatory control studies present both theoretical and practical interest. Earlier we showed that a hexaploid wheat species *Triticum kiharae* is a valuable source of numerous AMPs. About 50 AMPs belonging to virtually all families found in plants were isolated from this species.

In addition to known structural types, a new antimicrobial peptide named WAMP-1a with a unique 10-Cys motif was isolated from *T. kiharae* seeds and sequenced. Using 3'- и 5'-RACE the nucleotide sequence of its full-length mRNA was subsequently determined. The transcript contained a short 5'-untranslated region, the peptide precursor region (a signal peptide, the mature peptide domain and the C-terminal domain) and the 3'-untranslated region. The genomic sequence coding for WAMP-1a was also cloned and sequenced. It was shown that the ORF encoding WAMP-1a has no introns.

Regulation of WAMP-1a gene expression by biotic and abiotic stress and organ specificity of expression were also studied. mRNA expression levels were evaluated at three temperature regimes: +4°C, +37°C, and +22°C (control). Salt stress was induced by 100 and 200 mM. NaCl concentrations. Biotic stress was stimulated by incubation of seedlings in the presence of fungal pathogens *Fusarium graminearum, F. oxysporum, Helminthosporium sativum* and *Aspergillus niger*. Organ specificity of *wamp-1a* expression was studied in 3- and 14-day seedlings. To quantify the amount of RNA, RT-PCR was used.

We showed that expression of WAMP-1a was induced by salt stress, elevated temperatures and infection with *F. oxysporum*, which causes wilting, but was independent of the plant organ and developmental stage. The results obtained point to diverse functions of WAMP-1 peptides, which are involved both in plant defense against pathogens and abiotic stress. Since expression of *wamp-1a* is inducible, its promoter is of considerable scientific interest, and the relevant research is now in progress.

BREEDING PINK-FLOWERING EVERBEARING STRAWBERRY ADAPTED TO THE VARIABLE CONDITIONS OF WEST SIBERIA

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Keywords: *pink-flowering strawberry, everbearing capacity, ecological plasticity*

At recent time the assortment of abroad and domestic origin pink-flowering strawberries (Fragaria × ananassa Duch., $2n = 8x = 56$) numbers no more than 30 cultivars, hybrids and varieties. It is recommended for everbearing strawberries to cultivate them in southern parts of Russia due to their low winter-hardiness in the conditions of outdoor growing. In addition, pink-flowering strawberry is a "raw" breeding material and therefore it usually produces nubbin fruits (irregular-shaped berries) due to poor seed set. That is why the productivity of pink-flowering strawberry is much lower than the one of ordinary everbearing cultivars, so pink-flowering strawberry cultivars are grown mainly in ornamental purpose [1]. Another problem of these strawberry cultivars is mediocre fruit flavor. These disadvantages actuated in the Institute of Cytology and Genetics a special breeding program on creation high-yielding everbearing pink-flowering cultivars adapted to the conditions of West Siberia. Inbreeding, outbreeding, inbreeding lines crossing were used to produce seeds. As a source of petal pink color initially ornamental cultivar 'Pink Panda' and later, F_1 hybrid C141 of ABZ Seeds (Holland) [2] were involved. During the hybridization work, donors with desirable characters such as winter-hardiness, everbearing capacity and good fruit flavor were used. These donors were obtained as a result of long-term selection from the gene pool of hybrids and lines of the Institute of Cytology and Genetics SB RAS.

Taking into consideration extreme climatic conditions of Siberia for plant existing, particular attention is given to winter-hardiness of promising hybrids. The gene pool of ornamental strawberries including 5 cultivars of foreign origin and more than 800 different winter-hardy hybrids adapted to the conditions of West Siberia was established. 36 promising hybrids with optimal combination of ornamental and fruit traits were selected as candidates to create a new cultivar. All these plants are capable of perpetual bearing during the whole growing season and have great ecological plasticity.

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SEARCHING FOR NUCLEOTIDE SEQUENCES LIKE AGROBACTERIAL GENE FRAGMENTS IN PLANT GENOMES

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Key words: *genome evolution, bioinformatics, BLAST, Clustal X 1.81*

Searching for nucleotide sequences similar to agrobacterial T-DNA, *rol*C fragments in plant-genome data banks for agrobacterial genes spreading in plant genomes was conducted. T-DNA borders, *rol*C and *npt*II (as a negative control) gene sequences was evaluated in GenBank and DNA Data Bank of Japan using the BLAST and Clustal X 1.81 programs. A set nucleotide sequences (13–17 bp with E = 5.8 for 17 bp) similar to the T-DNA right border-like fragments (TRBLF) was found mostly in corn and arabidobsis genomes. The 18-nucleotide-long *npt*II (0.02 % of full-length *npt*II) fragment was also found in the *Arabidobsis* and *Zea* genome. Full-length *npt*II gene (with an identity of 99.9 % and E = 0) was found only in the *eIF-4A1* gene for the translation initiation factor eIF-4A1 (exons 1–5 from the *Arabidopsis thaliana* plant) and within the *gus* gene for β-glucuronidase protein, as a result of transfer of binary vector pBI121 to the *Arabidobsis* genome. Within the *Nicotiana tabacum* genome, we found 542-nucleotide-long fragments similar with the gene *rol*C (89–100 % identity for five top results). Thus, we found a set of short (up to 17 bp or 40–60 % of full-length T-DNA) T-DNA right border-like fragments, and short (0.01–0.02 % of full-length *npt*II) *npt*II-like fragments within different plant genomes; and 542 nucleotides fragments similar with gene *rol*C in the *Tobacco* genomes. We hypothesize that agrobacterial T-DNA insertion into the plant genome could exist and is possible involved in plant evolution.

SEARCH FOR MOLECULAR MARKERS OF APOMIXIS IN *ZEA MAYS* × *TRIPSACUM DACTYLOIDES* L. 39-CHROMOSOME HYBRIDS

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The eastern gamagrass (*Tripsacum dactyloides* L.), a distant relative of maize (*Zea mays* L.), reproduces apomictically and can donate this reproduction method to maize by hybridization. Unique 39-chromosome hybrids of gamagrass with maize reproducing via agamospermy (asexual reproduction through seeds) have been bred at the Laboratory of Cytology and Apomixis. These hybrids carry 30 maize chromosomes and 9 gamagrass chromosomes. Note that the loss of one of the nine *T. dactyloides* chromosomes switches the reproduction to a normal sexual manner. Search for the genes that determine an apomictic embryo development in these hybrids requires a set of marker traits indicating the involvement of both whole gamagrass chromosomes and their fragments in this process. For this purpose, the primary sequences of *dek1* and *Agpsem* gene fragments of *T. dactyloides* were determined. Comparison of a gamagrass *dek1* gene fragment with the corresponding maize sequence demonstrated a homology level of 99 %. The found differences comprise several single-letter substitutions and one three-letter deletion/insertion. Taking into account this three-letter deletion/insertion, we constructed a primer pair allowing the *dek1* L gene fragment of *T. dactyloides* to be amplified, whereas the corresponding fragment of maize gene did not amplify. Using this primer pair, we have demonstrated that the 39-chromosome hybrids carry this *T. dactyloides* gene fragment. The *Agpsem* gene fragments of gamagrass and maize display 87.4 % homology. The differences consist in one- and two-letter substitutions and deletions/insertions of various lengths. The studied fragment of this gamagrass gene is shorter by several tens of nucleotides, which is evident from the electrophoresis of PCR fragments. Analysis of the 39-chromosome hybrids demonstrated that they carry the *Agpsem* gene fragment characteristic of gamagrass.

EFFECTS OF DROUGHT STRESS AND SELENIUM ON ANTIOXIDANT ENZYMES ACTIVITY IN CHICKPEA VARIETIES

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Key words: *Drought Stress, Irrigation, Antioxidant Enzymes, Chickpea Varieties*

In order to evaluate the effects of drought stress on activity levels of antioxidant enzymes in three chickpea cultivars, this experiment was conducted. At first we test the cultivars in mannitol solutions at germination stage. We used factorial experiment basis on RCB (Randomized Complete Block) design with 2 factors, cultivars in 3 levels and mannitol in 4 levels (-12 bar, -8 bar, -4 bar and 0 bar) with 4 replications. The results showed significant differences between studied cultivars in cases of radicale length, pedicle length and viability. At the first stage we used spilt-split plot design on the basis of RCBD with 4 replications. Main factor was in 2 levels (normal irrigation and drought stress), and sub factor was in 2 levels selenium (0 and 20 gr/ha) and sub-sub factor was three chickpea cultivars. The results showed that activity rate of Malon dealdehyde (MDA), Catalase (CAT), Glutation peroxidase (GPX) and super oxide dismutase (SOD) enzymes had significant differences. A logical relationship was observed in 2 experiments, and we showed that "Bivanij" was a cultivar that had longer radical length in comparison with the other cultivars. The results of biochemical experiments showed that in stress conditions and with using selenium, the activity levels of antioxidant enzymes increased (exept CAT with using selenium). Finally, we concluded that there is a correlation between stress condition and increased levels of antioxidant enzymes, and these enzymes have important roles in plant drought stress tolerance.

DEVELOPMENT OF BIOLOGICALLY ACTIVE SUBSTANCES WITH ANTIBACTERIAL ACTIVITY FROM YAKUTIAN PLANTS

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Key words: *Yakutia, wild plant species, essential oils, antibacterial activity*

The research on antibacterial activity of biologically active substances such as essential oils from Yakutian plants biomass was conducted for the first time in permafrost zone. Searching and screening of biologically active herbal substances such as essential oils is one of urgent approaches to development of biological preparations for the practical needs of agriculture, medical science, food industry etc.
Goal: research of Yakutian wild plants essential oils biological activity
Objectives: picking, fixing and transportation of herbal materials; extracting and storage of herbal essential oils; assessment of subject essential oils antibacterial activity.
Climate conditions in Yakutia. Sakha Republic (Yakutia) is the region that has not only the largest territory among Russian regions, but also has the most sever climate conditions. In Yakutia with it acutely continental climate temperature range is about 100°C. In summer temperature goes up to + 40°C and during winter it falls down to - 60°C. Yakutian Oimyakon region is so called "Pole of Cold"; in absolute figures of lowest temperature and coldest days duration Sakha Republic is on the first place in the world. Weather with temperature below zero lasts for 6.5–9 months in a year.
Research Objects: *Ribes alpinum* L., *Pinus pumila* (Pall.) Regel, *Ledum palustre* L., *Artemisia jacutica* Drob., *Thymus bitominosus* Klok., *Schizonepeta multifida* (L.) Brig.
Test-cultures: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus.*
Research Methods: geobotanical description of vegetation; collection, transportation and fixation of research objects; allocation of essential oils by vapor-phase distillation; storage and use of essential oils for screening; screening of antibacterial activity through the disco-diffusion method with modifications; mass-spectrometric analysis.
Conclusions: Studied essential oils are often selective with respect to various test cultures. In relation to the growth and development of Staphylococcus aureus the essential oils from phytomass *Ribes alpinum* L., *Ledum palustre* L., *Artemisia jacutica* Drob. are active. Essential oil of *Ledum palustre* L. phytomass has high activity with respect to the growth of *Escherichia coli* and *Proteus vulgaris*. Essential oil of *Pinus pumila* (Pall.) Regel is an active suppression of growth in respect of all the used test-cultures, except for *Staphylococcus aureus*. Qualitative analysis shows the presence of major groups of compounds that is common for appropriate types of essential oils.
The data could form the basis for the development of alternative means for prevention of certain human diseases on the basis of biologically active substances such as essential oils from wild plant species of Yakutia.

LEAF DEHYDROASCORBATE REDUCTASE AND CATALASE ACTIVITY IS ASSOCIATED WITH DROUGHT TOLERANCE IN BREAD WHEAT

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Levels of activity of the enzymes dehydroascorbate reductase (DHAR) and catalase (CAT) were correlated with flag leaf relative water content and two indices of grain yield components across a set of bread wheat whole chromosome substitution lines Chinese Spring / Synthetic 6x (CS/Syn). The lines carrying a synthetic hexaploid homologous pair of chromosomes 1B, 1D, 2D, 3D or 4D all expressed a low constitutive level of DHAR and CAT, but were able to increase this level (by four fold for DHAR and by 1.5 fold for CAT) in response to drought stress. When challenged by drought stress, these lines tended to be the most effective in retaining the water status of their leaves, and preventing their grain yield components to be compromised. Therefore, it was shown that DHAR and CAT activity in wheat plays a role in determining its drought tolerance. The best performing CS/Syn lines were derived from substitutions from the D genome. This suggests that *A. tauschii*, the natural range of which is characterized by arid soils, may serve as a donor of tolerance o water deficit conditions. It is suggested that DHAR activity, and its regulation under drought stress, could be explored as a useful indirect selection criterion for drought tolerance.

SIBERIAN STONE PINE (*PINUS SIBIRICA* DU TOUR) AND SIBERIAN DWARF PINE (*PINUS PUMILA* PALLAS REGEL) NATURAL HYBRIDISATION: ALLOZYME DIVERSITY AND MITOCHONDRIAL DNA POLYMORPHISM

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Key words: *natural hybridization, Pinus sibirica, Pinus pumila, allozymes, mtDNA, climate change.*

We studied genetic variation and mating structure in Siberian Stone Pine, Siberian Dwarf Pine and natural hybrids in Baikal region. Two sites in Baikal lakeside were examined: Barguzin Biosphere Natural Reserve, Davsha bight (BR) (*P. sibirica* forest stand with scattered *P. pumila*), and delta of Upper Angara (DUA) (swamped area, *P. pumila* dominated and *P. sibirica* occurred rarely). In both studied sites there was natural hybridization between the Stone Pine species, the number of hybrids was approximately 5 plants per hectare. Genetic structure of *P. pumila*, *P. sibirica* and hybrids in the mixed stands was analyzed by means of 29 allozyme loci controlling 16 enzyme systems. For most loci the hybrids had intermediate allele frequencies relative to *P. pumila* and *P. sibirica* samples that confirmed hybrid nature of these trees. All hybrids were heterozygous at diagnostic *Skdh-2* locus. In the site where *P. sibirica* dominated 27 % of *P. pumila* seeds were sired by *P. sibirica* but no *P. pumila* specific alleles were found in Siberian Stone Pine seeds embryos genotypes. In the site with alternative ratio of pure species only 1.4 % of *P. sibirica* seeds had *P. pumila* paternal contribution, all *P. pumila* seeds were sired by the same species pollen. Studying of hybrids mating structure demonstrates that main portion of cross-pollinated embryos had paternal contribution from parental species dominated in the site and second species sired 13–14 % of embryos. Portion of hybrid pollen in effective pollen pool of hybrid seeds in DUA was approximately 12 % that is more than threefold compare to BR. It was shown that hybrids took part in pollination of pure species rarely occurred in a site.

It is well known, that *Pinus* species exhibit paternal chloroplast inheritance and maternal mitochondrial inheritance. We used length variation of PCR products of nad1 intron2 (mtDNA) to determine paternal and maternal input of parental species into genotypes of adult hybrid trees. It was shown that in 15 of 16 hybrids from BR the PCR product was 2 530 bp length (specific for *P. sibirica*), and in 21 of 22 hybrids from DUA the mtDNA fragment length was 2181 bp (specific *for P. pumila*).

Our results confirm the possibility of back-crosses and F_2 hybrids existence and demonstrate that at least initial stages of introgressive hybridization take place in the mixed stands with contrasting partitioning of the two stone pine species. Possible evolutionary perspectives of natural hybrids especially under climate change conditions are discussed.

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THE MUTATION PRODUCING THREE PISTILS IN A FLORET IN WHEAT (*TRITICUM AESTIVUM* L.)

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Key words: *Triticum aestivum, three-pistils, Pis1, floret, kernel, sink capacity, yield*

The spontaneous mutation of bread wheat (*Triticum aestivum* L.) producing three pistils (TP) in a floret is presented. The TP mutant was found in a spring Chinese landrace and can form up to three kernels in a floret and thus to increase grain number per spike. Restricted space in florets causes kernel flattening. Doublets or triplets of kernels form clusters where the basal parts arise from the receptacle and ventral groove of kernels is outside oriented. In some cases, these groups of kernels are visible after threshing. The TP mutant is determined by the dominant *Pis1* gene located on the long arm of chromosome 2D [1, 2] between the markers *Xgwm539* and *Xgwm349* [1]. The original Chinese landrace was grown in field tests at Kromeriz (Czech Republic) in 2007. Its yield was only 44 % in comparison with mean yield of check registered cultivars of spring wheat Vanek, Granny and SW Kadrilj (6.46 t ha^{-1}). TP wheat exhibited low resistance to fungal pathogens, low 1000-kernel weight (TKW) (27.9 g), low volume weight (75.4 kg hl^{-1}), lower germination vigour, high protein content (17.7 %) and was 10 and 7 days earlier at heading and maturity, respectively. The TP mutant was crossed to significant cultivars of winter wheat aiming to transfer the gene *Pis1* to the genetic background of currently grown cultivars. TKW of the harvested F_1 plants was around the average of parents. The TP can be used as a potential gene resource for increasing reproductive spike capacity (a kernel number per spike) and spike sink capacity. At present, there is some progress in developing winter TP lines with a medium level of frost hardiness. These lines will be compared with common European cultivars as standards in trials with complex fungicidal protection and gradually increasing nutrient doses. We will try to find out whether the TP lines will have yield responses to available nutrients better than common cultivars. For exact evaluation of the importance of the gene *Pis1* it will be necessary to develop near-isogenic lines distinguishing in TP trait and with identical genetic background. (MEYS, Czech Republic: ME10063).

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TRANSFER OF THE CHARACTER OF FROST RESISTANCE FROM THE WHEATGRASS TO WHEAT BY THE METHOD OF LEAF NURSE

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Key words: *wheat, wheatgrass, frost resistance, transfer*

With the help of the developed method [1] of express train — analysis of frost resistance of plants — frost resistance of various cultivars of spring and winter wheat, and also of androgenic haploids and doubled haploids of wheatgrass *Agropyron glaucum* [2] and wheat-wheatgrass hybrids has been studied. It is shown, that plants of wheatgrass and wheat-wheatgrass hybrids possess wide polymorphism on frost resistance. Most of the plants possess very high frost resistance, and during cold treatment before irreversible leaves damage they surpass many times the most cold-resistant cultivars of winter wheat. Leaves of high frost resistant plants of wheatgrass and wheat-wheatgrass hybrids have been used as a nurse for growing of winter and spring wheat seeds in specially designed plates. The analysis of frost resistance has shown that up to 20 % of wheat plantlets growing with leaf nurse possessed higher frost resistance than control plants. The obtained high frost resistant wheat plantlets were grown in a greenhouse and in a field. By this time seeds of cold-resistant plants of the 3-rd generation of two cultivars of winter wheat Bagrationovka and Filatovka and the 4-th generation of the spring wheat Novosibirskaya 89 have been obtained. High frost resistance level of the obtained samples of the spring wheat proves their winter hardiness and ability to give seeds next year similarly to winter wheat. The percent of survived plants reached 14.5 %, whereas in the control represented by the standard cultivar none of the plants have survived. This gives evidence of the transfer of frost resistance from wheatgrass to wheat by the method of leaf nurse.

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INFLUENCE OF MEDIUM, PHYSICAL, AND GENETIC FACTORS ON HAPLOMORPHOGENESIS IN ANTHER CULTURE OF THE WHEATGRASS *AGROPYRON GLAUCUM* (Desf)

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Key words: *wheatgrass, anther culture*

Wheatgrass *Agropyron glaucum* (Desf.) possesses many valuable characters, and it is widely used by breeders for wheat improvement. Wheatgrass is a crossbreed culture possessing high heterozygosity which complicates allocation of the forms combining the necessary attributes. Thereby it is necessary to obtain homozygous lines of wheatgrass. Availability of such lines will enable controlled introduction of the alien genetic material to the cultural cereals genomes. One of the fast methods of the development of homozygous lines of plants is cultivation of isolated anthers on nutrient mediums. The first viable green haploids of wheatgrass *A. glaucum* have been obtained in 1998 [1]. Low ability to androgenesis and development of albinous impractical haploids are disadvantages of this approach, demonstrated for a plenty of wheatgrass genotypes [2]. The current study was aimed on the further improvement of the method of androgenesis and was carried out on the basis of a comprehensive wheatgrass collection of the Institute of Cytology and Genetics. Influence of the following factors on processes of induction and regeneration of wheatgrass androgenic haploids in isolated anthers culture was investigated:

a) various phitohormones and others biologically active substances in the composition of nutrient mediums;
b) microwave, ultra-violet, x-ray radiation and cold treatment;
c) self-fertility level of researched plants.

Positive effect of phitohormones such as abscisic and hibberelic acids, vitamins ribophlavine and nicotinic acid, biopreparation Novosil, and of wheatgrass leaf extract on androgenic haploids yield was revealed. For each of these substances effective concentration was picked up. It was shown that in known Chinese nutrient medium Potato II for androgenesis induction the potato extract can be replaced with maltose without loss of efficiency. Direct correlation between androgenic structures yield and level of self-fertility of researched wheatgrass plants was shown. Threshold dozes of microwave, ultra-violet and x-ray radiation, inhibiting development of androgenic structures, were determined. The results showed that the method of androgenesis *in vitro* can be used successfully for development of pure androgenic lines of wheatgrass, and also as the sensitive test of damage effects caused by various agents. High sensitivity of this test is due to haploid status of developing androgenic structures.

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THE PROLIFERATION OF TRANSPOSABLE ELEMENT FAMILIES DURING GENOME DIVERGENCE OF DIPLOID AND POLYPLOID WHEATS

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Key words: *transposable element, Caspar, Fatima, polyploid wheat*

Transposable elements (TEs) are the most rapidly evolving fraction of eukaryotic genome that contributes to interspecific genomic divergence. TEs are likely to influence considerably the differentiation of genomes in the allopolyploid nucleus. The repetitive sequences, especially different families of TEs, contribute for more than 80 % of the wheat genome. The investigation of chromosomal distribution and phylogenetic relationships of different families of TE enable us to make some conclusions about evolution of genomes in the polyploid wheat and its diploid relatives and proliferation of TE families during evolution. We analyzed the two families of TEs abundant in wheat genome which belong to the TE classes with differing mechanisms of transposition: *gypsy* LTR-retrotransposon *Fatima* and CACTA DNA-transposon *Caspar*. Since the complete wheat genomic sequence is still unknown, we can perform phylogenetic analysis only for a part of TE sequences available in public nucleotide databases, and use fluorescent *in situ* hybridization (FISH) to investigate chromosomal distribution of TEs over *Triticum* and *Aegilops* chromosomes. Using FISH we found the DNA-transposon *Caspar* being localized predominantly in the subtelomeric chromosomal regions of the bread wheat and its diploid relatives. The LTR-retrotransposon *Fatima* was localized mainly on the chromosomes belonging to the B-genome of the polyploid wheats and on those of *Aegilops speltoides* (putative donor of the genome B). The phylogenetic analysis demonstrated that both the *Caspar* and *Fatima* families formed distinct genome- and species-specific groups and that the major part of amplification events in genomes appeared during the divergence of the diploid *Triticum* and *Aegilops* species and before the *T. dicoccoides* allotetraploid formation. We demonstrated the impact of the DNA-transposon *Caspar* to the formation and differentiation of the subtelomeric chromosomal regions of different genomes in the polyploid wheat and the impact of the LTR-retrotransposon *Fatima* to the divergence of the B-genome of the polyploid wheats and *Ae. speltoides*. Our results point the important role of the TE families in structuring and evolution of grass genomes.

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ESTIMATES OF PARENTAL DIVERSITY FOR PREDICTING HETEROSIS IN VEGETABLE PLANTS

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Key words: *DNA-heterogeneity, heterosis, sweet pepper, cabbage*

Efficiency of using DNA markers in Breeding for heterosis of vegetable crops was studied. RAPD and SSR loci were analysed in 30 selection accession of white cabbage and 23 accessions of sweet pepper.
Based on the data obtained, cluster analysis UPGMA was made, with accessions being divided into polymorphic groups. Intra- and intergroup crosses were performed according to complete diallel scheme. In white cabbage high-heterotic progeny (high parents heterosis HPH > 30 %) was produced by intrapool hybridization. Significant effects of heterosis by interpool hybridization were observed only in some combinations.
In sweet pepper, intrapool hybridization has not taken significant heterosis effect in F_1. However, use of the most divergent genotypes in crosses proved successful-significantly high HPH for the majority of productivity traits was obtained in F_1.
The results allow conclusion that it is necessary to use intrapool hybridization at selecting parental pairs in collections with a high level of DNA heterogeneity, whereas in collections with a low level of divergence, preference should be given to interpool hybridization.
Based on the data obtained, the search for loci — candidates for key markers in heterotic breeding of white cabbage is under way.

SYMBIOTIC MUTANTS IN PEA BREEDING TO ENHANCE NODULATION AND NITROGEN FIXATION

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Key words: *Pisum Sativum, cultivar, supernodulating mutant, nitrogen fixation, recurrent selection, productivity*

Supernodulating pea mutants have been studied phenogenetically with a view to using them in breeding practices. Positive results have been obtained when a supernodulating mutant, K301(*nod4*), was crossed with two forage pea cultivars, Druzhnaya and Novosibirskaya 1, marked with a symbiotic gene, *Nod5*, controlling hypernodulation. Recurrent selection for productivity and symbiotic traits allowed a series of constant lines F_7 with high levels of nodulation, high nitrogen fixation rates and high productivity to be developed. Based on the results of analysis of recurrent F_7 lines developed by crossing the supernodulating mutant K301 to cv. Druzhnaya, we identified the seven best for productivity and nodulation. As was found, the plants in the recurrent lines were basically as high as Druzhnaya plants. Seed production was considerably higher in the recurrent lines than in Druzhnaya. Importantly, in addition to being highly productive, any plant in all the lines had many more nodules than the supernodulating mutant plant. By comparing the reciprocal hybrids, we found that a larger number of productive lines (five) resulted from crosses with cv. Druzhnaya as the maternal form. When it was the supernodulating mutant that was used as the maternal form, only two productive lines were developed. In three recurrent lines generated using cv. Novosibirskaya 1, a reduction in plant height was observed, especially when the maternal form was the supernodulating mutant. Plants in two lines developed using cv. Novosibirskaya 1 as the maternal form were taller. Only three recurrent lines had higher seed production than cv. Novosibirskaya 1. All the recurrent lines generated using cv. Novosibirskaya 1 had abundant nodulation. Nitrogen fixation activity inferred from the activity of the enzyme nitrogenase was considerably higher in all the recurrent lines than in the cultivars. The highest values were obtained for the recurrent lines generated using Druzhnaya and Novosibirskaya 1 as the maternal plants.

Nitrogenase activity in the pea cultivars and recurrent lines, nmolC_2H_4/plant/h

Mutant, cultivar		Recurrent lines	
Supernodulating mutant K301	1242	K301 x Druzhnaya	1123 to 1226
		Druzhnaya x K301	2312 to 2380
cv. Druzhnaya	424	K301 x Novosibirskaya 1	1235 to 2107
cv. Novosibirskaya 1	463	Novosibirskaya 1 x K301	2399 to 2702

The presence of two symbiotic genes, *nod4* and *Nod5*, in a single genotype is favorable for symbiotic properties and productivity.

MEIOSIS IN ABDR (*TRITICUM AESTIVUM* L. × *SECALE CEREALE* L.) AMPHIHAPLOIDS

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Key words: *Triticum aestivum* L., *Secale cereale* L., *disomic wheat-rye substitution lines, amphihaploids, unreduced gametes, type division of univalents, meiotic restitution*

The correct chromosome segregation in meiosis requires the presence of a pair of homologs, whereas a haploid plant, capable of developing vegetatively, displays the meiosis with numerous abnormalities, which in the majority of cases lead to sterility. An analogous situation is observed in the meiotic division of distant hybrids whose genome is formed of several haploid genomes. The genome of wheat hybrids with its wild relatives contains only homeologous chromosomes, which form bivalents with one another in single cases. Despite this fact, meiotic division is launched in the sporogenic cells of amphihaploids (interspecies or intergeneric hybrid carrying half genome of each parent), and seeds are sometimes set by selfing, giving new polyploid forms. The nature of this phenomenon is in formation of the gametes with nonreduced number of chromosomes. Such gametes, providing a partial fertility of polyhaploid hybrids, enhance their survival and stabilization of the genomes. The male gametogenesis in sterile and partially fertile ABDR amphihaploids produced using *Triticum aestivum* L. cultivar Saratovskaya 29 (S29) ($2n = 42$, BBAADD) and *Secale cereale* L. ($2n = 14$, RR) as well as disomic wheat–rye substitution lines (*T. aestivum* L./*S. cereale* L.) 1R(1A), 1Rv(1A), $2R(2D)_1$, $2R(2D)_2$, $2R(2D)_3$, 5R(5D), 5R(5A), and 6R(6A) ($2n = 42$) and *S. cereale* L. was studied. Numerous abnormalities in the first and second divisions and a high variation of the abnormalities interfered with a correct estimation of the meiotic patterns. Therefore, the male gametogenesis was studied in microsporocytes of individual anthers from individual hybrids. This allowed us to detect the hybrid combinations displaying uniform cytological patterns, which enhanced detection of three microsporocyte types with characteristic (1) reductional, (2) equational, and (3) reductional + equational divisions of univalent chromosomes. In the majority of microsporocytes, all hybrids $2R(2D)_1 \times R$ and $2R(2D)_2 \times R$ in anaphase I displayed a random segregation of univalent chromosomes between the poles; chromosomes never accumulated at the equational plane and did not separate into sister chromatids (reductional division). Meiosis ended in tetrad formation; the plants were always sterile. Individual plants of partially fertile hybrids in anaphase I displayed a predominant chromosome accumulation at the equator and separation of chromosomes into chromatids, which segregated to the poles (equational division); on completion of meiosis, the hybrids formed dyads and set seeds by selfing. All the studied hybrids contained microsporocytes where some chromosomes divided in an equational manner and the other did not (equational + reductional division). These results suggest that the chromosome division type in anaphase I choose between a switch-on/blocking of the second division. Thus, the analysis of meiosis based on assessment of the type of chromosome division makes it possible to take into account the function of the centromeric region in univalent chromosomes as a key mechanism of the meiotic regulation in amphihaploids.

A DATABASE OF PLANT PROMOTERS FOR TRANSGENESIS

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Key words: *promoter, transgenesis, database, gene engineering, plant*

A detailed planning of genetic construct design is a necessary pre-condition for successful transgene expression. In many cases, expression of a foreign gene in plants should follow a certain pattern to fit the specific demands of experiment (e.g., tissue- or stage-specific expression). Thus, choosing of an adequate promoter is considered to be an important stage in planning of gene engineering experiment. In each particular case, this selection may principally be based on manual systematic analysis of the relevant published experimental data; however, this approach is not efficient. Probably, this is the reason why a very limited number of promoters are used in the vast majority of experiments with transgenic plants.

We designed a special database, named TGP (TransGene Promoters), containing data on the transcriptional activities of plant promoter regions verified in experiments with transgenic plants. TGP is manually curated and implemented on the SRS (Sequence Retrieval System) platform. It contains three cross-indexed tables with information on the related genes, promoters and their nucleotide sequences.

Each gene in the TGP database is accompanied by information on a promoter and its deletion mutants with different activities. Current release of the database comprises 446 entries including 94 genes, 176 promoters, and 176 experimentally verified annotated nucleotide sequences. Genes and promoters of 26 plant species are represented. The TGP contains information on promoter size, promoter nucleotide sequence, patterns of transcriptional activity of different promoter segments, as well as on external factors, influencing the promoter activity. Host species name is also presented because the same promoter-transgene constructs can produce different tissue-specificity and activity in different species transformed. TGP also contains description of a promoter host native gene, including gene expression patterns. TGP is based on annotation of experimental literature data. Each promoter and gene is hyperlinked to PubMed database. EMBL nucleotide sequence databases accession numbers are also included.

On demand, users may retrieve the promoters sensitive to one of 36 exogenous and endogenous stimuli where heavy metals, elicitor, cold, drought, salt, dehydration and infections are of practical interest.

Database organization and a simple interface allow users to select individual promoters with appropriate transcriptional characteristics. TGP can be used as the source of information for both plant biotechnological and basic investigations.

The TGP is available at http://wwwmgs.bionet.nsc.ru/mgs/dbases/tgp/

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CHARACTERIZATION OF MICROALGA *BOTRYOCOCCUS BRAUNII* STRAIN UTEX 2441 AS A FEEDSTOCK FOR SECOND GENERATION BIOFUELS PRODUCTION

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Key words: *microalgae*, *biofuel, photobioreactor*

There are currently intensive global research efforts aimed at developing methods for intensive biomass production as a feedstock for commercial biofuels, especially from rapeseed and palm oil. But now it is obvious that above mentioned cultures if used for mass biofuel production will compete with traditional farming, and its effectiveness will depend on crop capacity. It is considered that microalgae offer novel aquatic biomass systems with higher fuel yield potential than traditional cultures and lower water demand than terrestrial biomass, thus will become a promising resource for biofuel production.

We studied properties of microalga *Botryococcus braunii* UTEX 2441 to define its applicability for biofuel production. Biomass production process using 110 L flat-plate airlift photobioreactor, allowed to produce 0.55 g/L *B. braunii* UTEX 2441 biomass on modified Bold's basal medium with 1.5 % CO_2 feeding, under optimized culturing conditions. To define fatty acid composition GC-MS analysis was performed. The main fatty acids were found to be palmitic (23.7 %) and linoleic (27.09 %).

The main point for developing of technology of biofuel production from micralgae biomass are the intensive studies on physiology of candidate strains and definition of factors influencing their growth and productivity. Due to this we performed a proteome analysis of *B. braunii* UTEX 2441 proteins with 2D gel electrophoresis, the proteomic maps were made.

MOLECULAR DIVERSITY OF TIBETAN WILD AND WORLDWIDE ORIGINATED BARLEY GERMPLASM AND ASSOCIATION MAPPING OF QUANTITATIVE TRAITS

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Key words: *Barley germplasm; Tibetan wild barley; Microsatellite; Molecular Diversity; Association mapping*

Molecular diversity of 40 accessions of Tibetan wild barley (TB), 10 Syrian (SY), 72 North American (NA), 36 European (EU), 9 South American and 8 Australian varieties were characterized using multiple microsatellite loci. The 42 SSR primers amplified 123 SSR loci across the 175 barley accessions tested in the present study. The average gene diversity for the whole sample was 0.3387 whereas the mean value for the each population was as follows: TB = 0.3286, SY = 0.2474, EU = 0.299, AU = 0.2867, NA = 0.3138, SA = 0.2536. Clustering analysis based on Nei's original genetic distance showed that the EU and NA barley populations were grouped together. The TB population was well separated from the other 5 barley populations. Associations between microsatellite markers and 14 quantitative traits (leaf area, stem diameter, grains per plant, filled grains per plant, grain weight per plant, plant height, spikelets on main spike, grains on main spike, grain weight on main spike, length of main spike, density of main spike, length of the 1st internode, length of spike neck, and awn length) were also investigated. Significant associations were found for 64 microsatellite marker loci. The number of marker loci associated with each trait ranged from one (stem diameter, filled grains per plant, grain weight per plant, and awn length) to nine (plant height and grain weight on main spike). The percentage of the total variation explained by each marker ranged from 4.59 % (*HVM2-2* associated with plant height) to 17.48 % (*Bmac90-1* associated with density of main spike). This study provides candidate markers for further QTL mapping of these traits and for marker-assisted selection.

BIOSYNTHESIS OF FLAVONOIDS UNDER SALINITY STRESS IN *TRITICUM AESTIVUM* L.

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Key words: *anthocyanin pigmentation, salinity stress, bread wheat*

It is known that the scavenging of reactive oxygen under stressed environmental conditions is one of the suggested roles of flavonoids plant tissues. The level of the flavonoids, particularly anthocyanins, tends to increase under different stresses. For example, drought, cold, toxic metals in soils, UV-B irradiation, and pathogens attack induce biosynthesis of anthocyanins in different plants species [1]. In bread wheat (*T. aestivum* L.), anthocyanins pigments are found in culm, leaf, auricle, pericarp, coleoptile and anther [2]. However, protective role of anthocyanin pigmentation of wheat plant organs has not been widely studied yet.
In the current study, the relationships between salt stress and anthocyanin content in the coleoptiles of cultivar 'Saratovskaya 29' (S29) carrying *Rc* (red coleoptile) gene determinig weak pigmentation and near-isogenic line i:S29Pp1Pp2 having additional *Rc* gene conferring strong coleoptile pigmentation, along with 2 complementary *Pp* genes for anthocyanin pigmentation of pericarp [3], have been investigated. The germinated one-day old seedlings, growing on filter paper at 20°C under 12 hours daily cycle, have been exposed to 0 (control), 100 mM and 200 mM NaCl in three replicates for each concentration. Relative anthocyanin content in the coleoptiles was evaluated by spectrophotometry at 530 nm wavelength. Under salinity stress, the growth of wheat seedlings was suppressed significantly, whereas anthocyanin content increased 1.4-fold and 1.6-fold in S29 under 100 mM and 200 mM NaCl, respectively, and 1.5-fold (100 mM) and 1.9-fold (200 mM) in i:S29Pp1Pp2. The correlation between salt concentration and relative anthocyanins content was significant ($P < 0.05$).
Thus, using different genetic models we were able to show significant intensification of anthocyanin biosynthesis in wheat coleoptiles under salinity stress. Further comparisons between wheat cultivars having pigmented and nonpigmented coleoptiles (and/or pericarp), are needed to estimate contribution of the anthocyanin pigmentation into salinity stress tolerance of wheat seedling.
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GENETIC CHARACTERISTIC OF CARROT FORMS ON RATE OF MATURATION AND PHOTOPERIODIC RESPONSE

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The research on the detection of genetic characteristics of plants from varietal populations that differ in rate of maturation and photoperiodic response is the one of the important goal of breeding.

The plant genotypes of varietal populations "Nantskaya 4" and "Moskovskaya zimnaya A-515", their inbred lines and hybrids F_1, F_2, and BC_1 were used in this study. The evaluation of plant genotypes on rate of maturation and photoperiodic response was carried out under various photoperiodic conditions: long day (14–17 h, V–VII months), short day (7–10 h in the greenhouse, XII–II months, dark cage in the field, 10 h, V–VII months). The analysis was done at the different stages of plant ontogenesis: the root-plants were analyzed in the field at long day; the seed-plants were analyzed in the field at long day and short day, as well as in the greenhouse at short day.

The result of research showed that the seed-plants determined under short day as the early ripening forms in the condition of long day were late ripening, and *vice versa* — the genotypes determined under short day as the late ripening forms in the condition of long day showed the early ripeness. The high inverse correlation (r = -0.83) between the growing season length of the root-plants and the seed-plants under different photoperiods (long and short day) was revealed. At the same time, this correlation was positive (r = 0.82) under the condition of equal long-term photoperiod (14–17 h). In the condition of long day, the segregation in the inbred progenies was different. In part of the inbred progenies the ratio of the late ripening neutral plants and the early ripening sensitive plants was 3:1, and other part of the inbred progenies showed the prevalence of early ripening sensitive plants (1:3). Taking into account the different segregation in the progenies, we can speculate that the late ripening neutral genotypes posses dominant genes *Lt* (Late) and *Ppd* (photoperiod), but the early ripening sensitive genotypes have recessive genes *lt* and *ppd*. Moreover, there are suppressor genes interacting with *Lt* and *Ppd* genes by way of recessive epistasis (i-l > lt and i-p > ppd). As a result of research, the new source of early ripeness and neutral photoperiodic response in carrot were identified.

INDUCTION AND PLANT REGENERATION OF CALLUS FROM IMMATURE EMBRYOS OF KAZAKH RICE CULTIVARS (*ORYZA SATIVA* L.)

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Key words: *Kazakhstan rice cultivars, in vitro, embryogenic callus, 2, 4-D, immature embryos.*

PIn this study, we have demonstrated that *Oryza sativa* (Kazakh rice cultivars) callus induced from immature seeds can produce of plant regeneration and somatic embryogenesis, even following a prolonged period of subculturing.
Embryos were on then excised and transferred onto agar medium placed with rounded scutellar surface up. The basal culture medium consisted of Murashige and Skoog (MS) medium, supplemented with 2, 4-D, 2.5 mg/l exhibited better performance of callus induction. Callus induction frequency was equal to 95 % in Bakanassky, as against 84 % in Madina and 83 % in Marzhan. For induction of morphogenesis, callus was placed on MS medium, supplemented with kinetin 2.0 mg/l and NAA 0.2 mg/l. Four weeks after transferring callus to differentiation medium, some portion of callus turned to green and from where roots regenerated. By 14 days, shoots started to emerge and all plantlets regenerated were green.
Therefore, the conclusion was made that application of medium MS is advantageous to obtain overall efficiency of callus induction and plant regeneration from immature embryos of various Kazakhstan rice genotypes.

ANALYSIS OF THE DEGENERATE MOTIFS IN PROMOTERS OF AUXIN RESPONSIVE GENES

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Motivation and Aim: The plant hormone auxin plays a key role in plant development. In promoters of early auxin responsive genes the Auxin Response Elements (AuxREs) were found that are specifically bound by transcription factors of ARF (Auxin Response Factor) family. Experiments display that AuxRE often operates as a part of composite element (CE) [1]. We developed the ARGO_CEL [2] program for recognition of potential CEs in regulatory regions of auxin responsive genes.
Methods and Algorithms: Two sets of sequences were created using published data: (1) [-100;100] regions relative to the experimentally proved AuxREs; (2) [-2000;1] promoter regions of auxin responsive genes [3]. The ARGO system [4] based on the Field-Programmable Gate Array and Graphic Processor Unit technologies is used for detection of degenerate region-specific oligonucleotide motifs.
Results: About dozen of high-significant degenerate motifs are obtained per every set. The most significant motif **TGTCNC**, found in all sites, corresponds to the well known TGTCTC-like AuxRE sequence. A few other motifs, overlapping with it describe alternative variants of the flanking regions. Other significant motifs, revealed in set (1) don't relate to TGTCTC-like context, and are located in regions surrounding **TGTCNC.** Some of them co-present with alternative motifs of **TGTCNC** flanks, significantly anti-correlate with other alternative motifs of **TGTCNC** flanks forming potential CEs (like AuxRE/MYBcore). The most significant motifs revealed in the set (2) locate in core promoters and correspond to transcription factor binding sites, like TATA-box *etc*. Groups of co-presenting motifs form potential CEs. We demonstrate that the CEs found in the set (1) are significantly over-represented in core promoters of early auxin response genes.
Conclusion: We revealed potential CEs covering experimentally proved AuxREs, and in promoters of early auxin regulated genes. These CEs consist of ARFs binding site and of motifs, corresponding to binding sites of another transcription factors. The data can be used for the experimental analysis of ARF targets and for recognition of auxin regulated genes.
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DEVELOPMENT OF INITIAL MATERIAL FOR BREEDING OF NAKED BARLEY (*HORDEUM VULGARE* L.) VARIETIES

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> A new variety raised by man will be a far more important and interesting subject for study than one more species added to the infinitude of already recorded species.
>
> Ch. Darwin, *The Origin of Species*.

The general trend in the climate is an increase in annual air temperature and precipitation. Therefore, it is not improbable that quite different crop varieties will be grown in the future. In particular, naked barley varieties can be at least as promising as hulled ones. At present, little attention is given in Russia to naked barley breeding. They have two grave disadvantages: (1) poorer yield than in hulled barleys and (2) protrusion of the central embryo root outside the grain surface, which causes its damage during thresh. We started our work with investigation of a collection of 50 naked barley accessions. The collection included 26 foreign varieties, 20 lines obtained by crossing and mutagenesis, and 4 varieties released in Russia. The following traits were recorded: growing season, yield per 1 m^2, weight of 1000 grains, total and productive tilling capacity, ear density, ear length, and protein content.

Families promising for further breeding were recognized on the base of field and structural analysis according to the following indices:

- early ripening, growth season 70 days;
- high yield, 360 g/m^2;
- 1000 grain weight 54 g;
- productive tillering 14–20 ears;
- ear density 17 grains for two-rowed barleys and 21 grains for six-rowed ones;
- ear length 11 cm;
- protein content 17.7 %;
- lysine content 647 mg/100 g

The promising accessions selected by us were tested in the field in the Altai Institute of Agriculture. They outperformed standard naked barley variety Omskii 2 in grain yield by 16–28 %. Their grain yield was close to values characteristic of hulled varieties. Thus, our work gave rise to breeding material for developing highly productive naked barley varieties fit to the changing ambient conditions. This material can also be used for choosing parents for crossing.

Our further work is aimed at the breeding of naked awnless barley varieties without central embryonic root protrusion (wheat-type grain) that would contain much protein and lysine and be resistant to fungal and viral diseases.

A STUDY OF THE GENETIC VARIABILITY IN SELECTED TRITICALE GENOTYPES USING SSR MARKERS

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Key words: *triticale, microsatellites, SSRs*

The genetic variability in 16 genotypes of triticale (*2n = 6x* = 42, BBAARR) was studied using microsatellite markers. Five cultivars originating from Poland (Gutek, Kitaro, Lamberto, Presto and Tornado), three from Germany (Lupus, Ticino and Triamant), one from Russia (Valentin-90) and seven lines with different 1R.1D translocations of chromosome 1R carrying segments from 1D (1R.1D$_{5+10}$-1, 1R.1D$_{5+10}$-2, Valdy), and from 1B (MA1) derived from cv. Presto were analysed. The translocations were developed with the aim to improve bread-making quality by A.J. Lukaszewski. The selected SSR markers were linked with different chromosomes of the A, B, D, and R genomes. The dendrogram significantly differentiated cv. Valentin-90 from all of the other 15 samples which were split into three subclusters. The first one includes cvs. Gutek, Tornado, Presto, and also all lines with translocations of 1R chromosome. The second subcluster consists of cvs. Kitaro, Lamberto, Ticino, and Triamant. The third subcluster contains cv. Lupus only. We detected 184 alleles from 48 markers with an average of 3.83 alleles per locus (ranging from 1 to 9 per locus). The calculated average polymorphic information content was 0.48 and ranged between 0.00 and 0.85. Six different microsatellite markers were detected which were linked with more than one chromosome. Two chromosomes were linked with four SSR markers and three chromosomes with two SSR markers. Subsequently two or three zones with different size were detected in two cases only using one microsatellite. It was caused by the similarity of the homoelogous chromosomes from closely related genomes. In cv. Tornado six SSR markers were found that detected two alleles simultaneously in one DNA isolation. This cultivar is composed of two sister lines and this was confirmed by polymorphism of prolamin gel electrophoresis. As the DNA was isolated from a mixed sample, it was not possible to distinguish sister lines or a heterozygote constitution. This distinguishing would be possible on the basis of higher number of separately analysed plants. In two lines with translocation of chromosome 1R (Presto MA1 /line 2/ and Presto Valdy LH /line 67/) two alleles were detected using one SSR marker. It confirmed their heterogeneity. A certain degree of genetic variability was found among the triticale lines derived from cv. Presto with specific type of translocation. This variability could be explained by the heterogeneity of initial samples which were used for the development of analysed lines. The presented methods can be used for the study of the level of variability and purity of the analysed samples in breeding programs. (MEYS, Czech Republic: MSM2532885901)

ALLELE COMPOSITION OF *PINa* AND *PINb* LOCI OF EASTERN EUROPEAN COMMON WHEAT VARIETIES AND THEIR IMPACT ON BREAD-MAKING QUALITY CHARACTERISTICS

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Key words: *Triticum aestivum* L., *grain hardness, Ha, puroindolines, Pina, Pinb*

Grain hardness is one of the most important quality characteristics of common wheat which determine the processing properties of wheat, affecting both the milling and bread-making. Generally hard wheat is suitable for bread and other yeast-leavened foods, whereas soft wheat is used for cookies, cakes and pastries. It is known that grain texture is controlled by Hardness (*Ha*) locus, located on the chromosome 5DS [1] and consisted of the *Puroindoline a* and *b* genes (*Pina* and *Pinb*) [2]. Soft texture (*Ha*) is the result of the wild-type allelic composition (*Pina-D1a*, *Pinb-D1a*) while hard texture (*ha*) is the result of mutation in either *Pina* and *Pinb* [2]. Up to date the most mutations were identified in *Pinb* gene, whereas in *Pina* only 2 alleles Pina-D1b [2] and Pina-D1c were found out. Those results were mainly obtained by investigation of Western European, American and Asian wheat germplasm, whereas Eastern European collections have not been intensively studied for their allele composition of *Pina* and *Pinb* genes. Traditionally, breeding institutions of Ukraine and Southern Russia focused their attentions mainly on increasing of bread-making quality in winter wheat, therefore it was suggested that varieties may possess mutations in *Pina* or *Pinb* genes. To confirm this suggestion the molecular studies of *Pina* and *Pinb* of 51 common winter wheat varieties suitable for dissemination in Ukraine in 2008, belonging to different groups of quality (strong, valuable, fillers) and possessing of hard and semi hard consistence of endosperm, was performed. It has been revealed that the most of them possess allele combination *Pina-D1a Pinb-D1b* and only two varieties Zymoyarka and Tsyganka have *Pina-D1a Pinb-D1c,* which suggested that the grain hardness in this group of varieties is strongly affected by modification effect. Despite expected low characteristics of some bread-making traits i.e. dough strength (W) of Zymoyarka and Tsyganka based on studies of allele combination of their storage proteins (gliadins and glutenins), indeed they stably performed W around 316–364 and average bread loaf volume 900–1100 ml, which suggested strong positive influence of *Pinb-D1c* allele on those traits mainly by increasing of water adsorption of dough.

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