Intergenomic substitutions and translocations in wheat-alien lines and their use in studying the characteristics that determine adaptation and stress resistance

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Abstract: The authors have created new genetic models of common wheat with different types of intergenomic chromosome substitution or translocation that include genetic materials of taxonomically unrelated species (S. cereale, Ag. elongatum, Ag. intermedium, H. marinum) for the introgression of genes that control adaptation and resistance to biotic and abiotic stress factors, along with the studying of homology between chromosomes. For the identification of rye, barley and wheatgrass chromosomes in the genome of common wheat, the authors used the molecular-cytological analysis of lines based on the genomic in situ hybridization and PCR analysis with the use of specific primers. The genetic relations have been studied in terms of the degree of substitution and compensation ability of particular chromosomes. To study the homology and homeology of chromosomes and control the peculiar features of alien chromosome transmission and elimination via gametes, the authors performed an analysis on chromosomes' synapsis and assortment in the offspring of wheat-alien substitution lines. Newly created models were examined to identify and localize the genes or gene alleles in chromosomes of different genomes and homeologous groups, which control such traits as the type of development and heading stage, and resistance to the impact of unfavorable weather conditions (winter- cold tolerance, stem and leaf rust and powdery mildew tolerance).

Key words: common wheat; rye; wild barley; precise genetic stocks; chromosome substitution; isogenic lines; introgression; *in situ* hybridization; PCR analysis; adaptation; biotic and abiotic stress.

1. Introduction

Polyploidy of *Triticum aestivum* L. provides new opportunities for cytogenetic research, which allows for targeted intravarietal and alien substitution of chromosomes or their parts and examination of the effects of these chromosomes on the manifestation of a complex of traits (Sears, 1952, 1972). Diploid and polyploid species of Triticeae relative to common wheat contain the genomes that are not homologous to the A, B or D genomes of wheat. The examples of such gene pools are diploid and polyploid species of *Aegilops, Thinopyrum, Secale* and *Hordeum*. The genomes of these species are homologous to the genomes of common wheat. An alien chromosome belonging to the same homeologous group can compensate for the corresponding chromosome of wheat as a result of a substitution or a translocation.

Chromosome engineering describes the technologies that allow for manipulating specific chromosomes or their segments and obtain new wheat genetic lines (Qi et al., 2007; Chan, 2010). A successful transfer of alien genes based on the homeologous recombination depends on the *Ph* system. There are two known base loci of the *Ph* system: *Ph1* is located on the long arm of chromosome 5B, and *Ph2* is located on chromosome 3DS (Sears, 1972). The *Ph1* gene of common wheat to a significant extent prevents homeologous pairing, thus, only homologous chromosomes pair up and recombine, which results in the formation of bivalents in Metaphase I of common wheat meiosis. Homeologous pairing of chromosomes may occur either due to the nullisomy or deletion of *Ph1* or the recessive mutation of ph1b. Researchers have described several methods that allow for chromosome or chromosome segment transferring from related species to wheat: 1) the use of ionizing radiation (Sears, 1956); 2) the use of homeologous recombination between chromosomes based on chromosome 5B with *Ph1* gene elimination or use of *ph1b* mutation (Sears, 1972); 3) the use of the univalent chromosomes trend to misdivision (Sears, 1952); 4) the use of gametocidal chromosomes (Gc) (Endo, Gill, 1996).

The genetic models obtained and applied for the studying of common wheat can be divided into several types. First, there are various types of aneuploid lines, which were first obtained by E.R. Sears (1952, 1966) in the cv. 'Chinese Spring'. They include full sets of monosomic, nullisomic-tetrasomic, tetrasomic, ditelosomic and monotelosomic lines. He developed new cytogenetic methods of common wheat analysis with the use of different types of aneuploids.

Another group of genetic models is represented by the wheat-alien substitution and addition lines, as well as the lines with alien translocations. The cytogenetic research based on the common wheat aneuploids has shown that in most cases an alien chromosome replaces wheat chromosomes of only one homeologous group. In some cases the alien chromosome is capable of replacing chromosomes from different homeologous groups, which indicates the presence of chromosome structural changes of different character. At the same time, the higher the compensation effect in the intergenome substitution, the closer the genetic affinity of genomes. Those genetic models that include lines with chromosome substitution and translocation, introgressive lines and synthetic wheats with the participation of Triticeae species, provide for the enrichment of the common wheat gene pool and play an important role in selection concerning the biotic and abiotic stress resistance (Jiang et al., 1994); with their help, scientists study the homeology of wheat chromosomes and genomes with other species of grasses (Qi et al., 2007; Chan, 2010) and specificities of chromosome behavior during the meiosis in the process of selection. Therefore, the researchers' interest in obtaining new wheat-alien lines is still intense, and new species are used as a source of new genes (Jiang et al., 1994; Friebe et al., 2001; Qi et al., 2011).

We have created new genetic models represented by near isogenic, introgressive and substitution lines, which possess a set of unique combinations of alien genetic material of different origin, to identify the influence imposed by donor chromosomes on stress resistance and adaptation to new environmental conditions. The results of this study are presented in the article.

2. Alien substitution lines

For the first time we obtained wheat-barley substitution lines (T. aestivum-H. marinum ssp. gussoneanum Hudson 4x) for the chromosomes of homeologous group 7, in which common wheat chromosomes 7A, 7B and 7D are substituted by barley H. marinum telocentric chromosome 7HL^{mar} (Efremova et al., 2013, 2018). The analysis of the meiosis Metaphase I (MI) has shown that the ditelosomic (DT) lines obtained are characterized by cytologically stable meiosis, and most plants in each line have the following chromosome configuration: $20^{"}+t^{"}$. The GISH-analysis of substitution lines has shown that they bear a pair of barley telocentric chromosomes. The ditelosomic analysis allowed us to define the lines as DT7HL^{mar}(7A) and $DT7H^{mar}(7B)$. On the basis of the compensation test and studying of the substitution lines' meiotic stability, we determined that H. marinum chromosome 7HLmar is homeologous to the common wheat chromosomes of the seventh group (Efremova et al., 2018).

In order to increase the adaptive potential of plants, we created the wheat-rye 5R(5A) substitution lines based on the winter wheat cv. 'Filatovka' and cv. 'Ul'ynovka' to identify the role of rye chromosome 5R in controlling the winter-cold-resistance feature. It is important to point out that as of today there are almost no research papers referring to the influence of rye chromosome 5R on the plants' winter resistance. Field tests performed in winter of 2017/2018 have shown that 90–100 % of wheat-rye 5R(5A) substitution lines survive the winter in the conditions of the Novosibirsk Region.

3. Introgression lines

Modern biotechnological methods are aimed at reducing the share of alien material in the cultivated grasses' genomes; thus, the studying of methods of inducing the chromosome homeologous recombination for acquiring translocations with the genes responsible for valuable traits of wild species has great practical and theoretical value (Qi et al., 2011). In order to find the combinations of crossing that induce a high frequency of chromosomes with translocations, we studied monosomic wheat-rye 5R(5A) and 5R(5D) substitution lines and their

hybrids with line L2075. The cytologic analysis has shown that rye chromosome 5R rather frequently undergoes misdivision both at the self-fertilization of monosomic alien lines and in $F_{1,4}$ hybrid offspring [5R(5A)×L2075] and [5R(5D)× L2075] at the self-fertilization of dimonosomics with the chromosome configuration of 20"+1'+1'. In the MI meiosis, as a rule, chromosomes in the wheat-rye dimonosomics 5R-5A and 5R-5D are represented as univalents. However, within each hybrid combination from 15 to 18 % of plants with a telocentric chromosome were selected. We have also found that univalent chromosomes 5A and 5D differ in the frequency of telocentric chromosome formation. Univalent chromosome 5A undergoes misdivision with high frequency (19 %) in comparison with chromosome 5D (0.46 %). As a result, based on *in situ* hybridization, among F_4 [5R(5A)×L2075] hybrid plants we selected plants with the 5AS.5RL translocation, and among $[5R(5D) \times L2075]$ hybrids we detected no such wheatrye translocation. This translocation might have appeared in the case of the simultaneous presence, in the hybrids, of two univalents and a spontaneous misdivision process in the rye and wheat chromosomes followed by telocentric fusion. Thus, the mechanism of Robertsonian translocation formation in the offspring of dimonosomics can be used for transferring an alien chromosome arm to the wheat genome.

We obtained two wheat-rye lines identified as T5AS.5RL+ T1RS.1BL and 5R(5D) + T1RS.1BL. The rye chromosomes are of different origin: 5R originates from the spring rye cv. 'Onohoskaya', and 1RS from the winter rye cv. 'Saratovskaya 5'. Based on in situ hybridization and C-banding, we found that one line had a Robertsonian translocation, so that the short arm of wheat chromosome 5AS had been transferred to the long arm of rye chromosome 5RL. We found no such translocation in the second line: the obtained lines have the 5R(5D) substitution. To identify chromosomes 1RS and 5RL, we used rye-specific markers. The field tests of adult plants and sprouts tolerance analysis have shown that the T5AS.5RL + T1RS.1BL and 5R(5D) + T1RS.1BL lines possess leaf rust and powdery mildew tolerance, as well as moderate stem rust tolerance in conditions of Western Siberia (Efremova et al., 2014).

We created the introgressive lines of common wheat that possess a complex of useful traits that were transferred from phylogenetically distant species (Ag. elongatum, Ag. inter*medium*, S. cereale) united in a single genotype. These new genetic models include several alien genes which control diseases tolerance (a pyramid of rye and wheatgrass genes: Lr26/Pm8/Sr31+Lr19/Sr25+Lr6Ai/Sr6Ai/Pm6Ai), various grain colors (a combination of wheatgrass and common wheat genes, Bal and Pp-1/Pp3), and winter development type (the rye gene vrn-R1), for studying the peculiarities of development of traits under examination depending on the combination of alien chromosomes or their segments. A combination of two/ three complex genetic systems responsible for stability, adaptation and morphobiological traits in one genotype is a rather complicated issue. One of the most promising approaches to its solution is to combine classic cytogenetic methods and MAS selection methods. The selection of a homozygous form with the combination of genes that controls disease tolerance, grain color and winter type of development has been performed based on phenotypic markers and molecular analysis with the help of PCR with specific primers. We performed phenotypic tests on the introgressive lines for disease tolerance in a field test and selected the plants that are tolerant to leaf rust.

Among the genes under examination we also studied the *Ba1* gene which controls blue grain color. Using the genetic models with colored grain, we create new lines with the combination of genes that control the blue color of the aleuron layer and complex disease tolerance. In parallel, we are working on obtaining lines with a combination of genes that control purple pericarp and blue aleuron layer color in the genotype of the cv. 'Saratovskaya 29' ('S29') to increase the amount of anthocyanin in grains.

As a result of kernel section analysis and visual estimation of grain color in F_3 hybrids, we managed to select individual plants with brown grain, purple pericarp and blue aleuron layer. Among F_4 -hybrids we selected, as a result, homozygous S29 lines $Pp-1Pp3^{PF}/Ba1$, S29 $Ba1/Pp-1Pp3^{PF}$ and S29 $Ba1/Pp1Pp3^{PF}$ which have shown no grain color splitting. Grains are dark-brown. Among F_{3-4} -hybrids of S29 $Pp-1Pp3^{P}/Ba1$ we continued to select homozygous forms. It was shown that the strength of kernel color may be different, which probably depends on the presence of genes in the plant genotype and their status. We can assume that the combinations of different alleles of the Pp-1/Pp3 and Ba1 loci are expressed in different ways.

We tested three samples of flour for the anthocyanin content. The estimation of total content of anthocyanins in hydrolisates of three flour samples has shown that original isogenic lines differ in content of anthocyanins; for the line with blue grain (S29 4Ag(4B)), this parameter exceeds the parameter of the purple-grain line (i:S29*Pp-1Pp3^{PF}*), 135.2 mg/100 g and 81.1 mg/100 g correspondingly. The content of anthocyanins in the new line, S29 *Pp1Pp3^{PF}/Ba1*, exceeds that in the isogenic lines (166.2 mg/100 g).

Thus, the 'S29' lines obtained possess the complementary genes *Pp-1/Pp3* which control the purple color of the grain pericarp (the donors are the common wheat cv. 'Purple Feed' and cv. 'Purple') and the *Ba1* gene, which is responsible for the blue color of the aleuron layer (the donor is the wheatgrass *Ag. elongatum*). These lines contain larger amounts of anthocyanins than red-grained and blue-grained samples.

4. Near-isogenic lines

The existing allele differences of *Vrn* genes are basic for the wide area of wheatgrass cultivation and the high level of adaptation to environmental conditions. Noteworthy, all studies of structural-functional regulation of the duration of the wheat vegetation period are connected with the determination and description of the new allelic variants of the *VRN-1* gene, but do not refer to the influence of structural changes in the regulatory parts of the *VRN-1* gene on earliness. We have created the near-isogenic lines of the common winter wheat cv. 'Bezostaya 1' with different alleles of the *VRN-1* gene and with their help performed an experimental study of the role that *VRN-1* allele polymorphism has in the regulation of the duration of the vegetation period and the duration of particular phases of development for the first time (Efremova et al., 2011; Emtseva et al., 2013). We found that the isogenic line with the Vrn-A1a allele has a shorter "tillering – first node" phase in comparison with the lines with the Vrn-B1c and Vrn-B1a alleles. This line approached the heading stage earlier than the other isogenic lines. The same result was obtained earlier during the study of these lines in greenhouse conditions. This fact testifies one more time that gene Vrn-A1 has the strongest effect on the duration of the tillering stage and controls the heading stage in plants. Under the conditions of the naturally long day in the Novosibirsk Region, we found no difference between the isogenic lines with the Vrn-B1c and Vrn-B1a alleles in the duration of the "tillering - first node" phase, and the difference in the duration of the heading stage was 2 days. However, under the conditions of a short day in a greenhouse, the difference in the duration of the tillering phase between the Vrn-B1c and Vrn-B1a alleles appeared to be 12 days, and the difference in heading stage duration was 35 days (Emtseva et al., 2013). Based on these results, we can assume that the winter wheat cv. 'Bezostava 1' has a weak response to the photoperiod. It is known that not only the effects of Vrn genes, but also the *Ppd* genes that control photoperiod response, impose a significant influence on the developmental phases. Thus, under short day conditions, the effect of the Vrn-B1c and Vrn-B1a gene alleles in the genetic background of cv. 'Bezostaya 1' manifests in the increase of vegetation period duration, especially the "tillering - first node" phase between the lines. Under ong day conditions, we observed no differences between the Vrn-B1c and Vrn-B1a alleles.

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