# Development and study of new spring wheat lines containing alien genetic material from *Th. intermedium* and *Ae. speltoides*

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**Abstract:** This paper presents the results of the introduction of leaf rust resistance genes inherited from *Th. intermedium* and *Ae. speltoides* into the genome of spring wheat cultivars differing in the length of the vegetation period. A total of 409 $F_4$ families from cross combinations with the early ripening cultivar Novosibirskaya 15, mid-early 'Novosibirskaya 31', mid-ripening 'Udacha' and mid-late 'Sibirskaya 17' and the donors of *Lr* resistance genes 'Tulaikovskaya 10' and 'Chelyaba 75' were studied for leaf rust resistance and the duration of vegetation period. A significant reduction in heading date was revealed in the $F_4$ lines obtained on the base of the mid-late variety Sibirskaya 17. In contrast, the lines originating from of the 'Udacha' × 'Chelyaba 75' and 'Novosibirskaya 15' × 'Tulaikovskaya 10' crosses were characterized by an elongated period from germination to ear emergence. According to the results of molecular and phytopathological testing, breeding lines were selected that differ in pathogen resistance, length of vegetation period and productivity.

**Key words:** leaf rust; *Lr* gene; wheat varieties; heading date; *Th. intermedium*; *Ae. speltoides*.

## 1. Introduction

One of the important problems of modern breeding is the creation of new wheat cultivars carrying a complex of agronomically important traits. Wild wheat relatives such as wheatgrass (*Thinopyrum* ssp.) and goatgrass (*Aegilops* ssp.) are an inexhaustible source of widening the genetic diversity of bread wheat by genetic loci that determine resistance to biotic stress factors and adaptability. However, the alien genetic material of these species is transferred to the genome of common wheat in the form of extended translocations, or substitutions of whole chromosomes or their arms (Friebe et al., 1996; Petrash et al., 2016). Introgression of a large amount of foreign chromatin can lead to disruption of the functioning of the gene networks responsible for the formation of the traits. Therefore, when introducing disease resistance genes into commercial wheat cultivars, it is important to take into account the effects of alien translocations on such characters as heading date and yield. The purpose of this work was to create new resistant lines of bread wheat, differing in heading date and ripening. The selection of genotypes was carried out with the help of molecular markers that detect alien genetic material.

## 2. Materials and methods

For hybridization, we used four spring wheat varieties, Udacha, Novosibirskaya 15 (N15), Novosibirskaya 31 (N31) and Sibirskaya 17, highly susceptible to leaf rust and belonging to different groups of ripeness (Piskarev et al., 2017). The wheat cultivars Tulaikovskaya 10 (T10, the *Lr6Ai* = 2 gene from *Th. intermedium*) and Chelyaba 75 (the *LrSp2* gene from *Ae. speltoides*) were used as donors of leaf rust resistance genes. $F_0$ and $F_1$ hybrids were multiplicated under greenhouse conditions to produce $F_2$ plants; $F_{2\text{-}4}$ progeny were estimated in field condition on the experimental plots of the Institute of Cytology and Genetics, SB RAS, in 2016–2018. The evaluation of resistance to leaf rust was carried out at the adult plant stage under local population of pathogen. The degree of susceptibility was assessed using a modified Cobb's scale (Peterson et al., 1948). Screening of genotypes for the presence of the *Lr6Ai* = 2 and *LrSp2* genes was performed using the *Xicg6Ai* = 2 and *Xksum73* markers as previously described (Leonova et al., 2017; Adonina et al., 2018). Separation of PCR products was performed in 2 % agarose gel or on an ABI3130XL automatic sequencer in a 6 % denaturing polyacrylamide gel.

## 3. Results and discussion

A total of 409 recombinant $F_2$ plants was studied, 110 of them from cross combinations with the early ripening cv. N15, 102 plants with the mid-early cv. N31, 99 with the mid-ripening cv. Udacha and 98 with the mid-late cv. Sibirskaya 17. According to the results of the genotyping of the $F_2$ progeny with primers to the *Lr6Ai* = 2 and *LrSp2* genes, samples containing and not containing alien genetic material were selected.

The evaluation of $F_4$ families for the response to the leaf rust pathogen showed that the donors of resistance genes, 'T10' and 'Chelyaba 75', exhibited from immune to medium-resistant (0R-10MR) reaction type, respectively. The parental varieties of bread wheat were highly susceptible to the pathogen (70–90S). Among the $F_4$ offspring, 312 lines were resistant to leaf rust (0R-20MR), including 116 obtained on the base of 'T10', 196 lines with 'Chelyaba 75'. The susceptible response was shown by 75 families from 'T10' cross combinations. It should be noted that among the offspring originating from 'Chelyaba 75', plants susceptible to leaf rust were not detected. As was shown previously, this effect may be associated with the gametocidal action of the *Ae. speltoides* translocation containing the *LrSp2* gene (Adonina et al., 2018).

Genotyping of $F_4$ offspring using the *Xksum73* marker, showed that in all samples obtained with the participation of 'Chelyaba 75', a fragment of 194 bp, specific to *Ae. speltoides*, was amplified (Figure 1). When using the *Xicg6Ai* = 2 marker developed for the 6DL/6Ai translocation in 'T10', a fragment

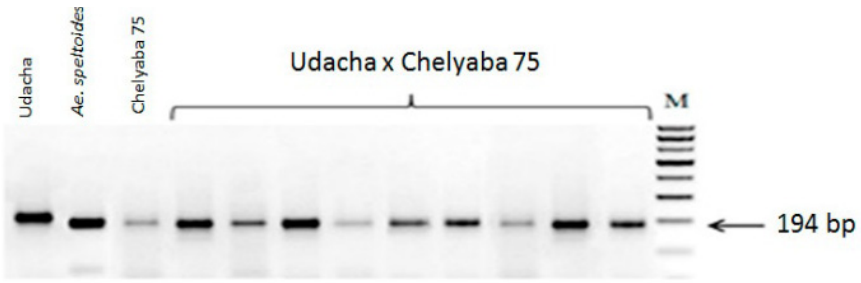


**Figure 1.** PCR products obtained by amplification of genomic DNA of $F_4$ progeny and the parental forms Chelyaba 75 and Udacha with ksud73 primers. Lane M is a DNA ladder; the arrow indicates the diagnostic fragment.

specific to *Th. intermedium* chromatin was amplified in all resistant plants. The results indicate the effectiveness of the primers for screening plant material for the presence of translocations inherited from the varieties Chelyaba 75 and T10.

A significant reduction was revealed in the length of heading date of the $F_4$ lines obtained on the base of the mid-late variety Sibirskaya 17 compared with all the paternal donor cultivars. Resistant and susceptible lines based on the early cultivar N15 moved to flowering 2.0 ('N15' × 'T10') and 4.1 days ('N15' × 'Chelyaba 75') later than the maternal wheat cultivars. The heading date of the lines from the 'N31' × 'T10' cross combination was overall shorter than in 'N15', and a similar reaction was observed with the lines from the 'Udacha' × 'T10' cross. In contrast, the lines of the 'Udacha' × 'Chelyaba 75' cross combination were characterized by an elongated period from germination to ear emergence in comparison with the maternal form.

A significant increase in the period from germination to maturation' from 3.6 days (resistant lines Sibirskaya 17 × Chelyaba 75) to 16.7 days (resistant lines N31 × T10) was found. In general, the length of the vegetation period of resistant lines selected from combinations involving cultivars of the early and mid-early groups increased by 5.6 days ('N31' × 'Chelyaba 75') and 15.8 days ('N31' × 'T10') in comparison with the maternal wheat cultivars. The results also indicate that the susceptible $F_4$ lines obtained in cross combinations with 'T10' are characterized by shortened periods 'germination – ear emergence' and 'germination – maturation' compared with the resistant ones.

We have previously shown in cross combinations of winter wheats with spring donors of resistance genes that the use of the cv. T10 as a donor of the *Lr6Ai* = 2 gene increases heading date of spring progenies (Stasyuk et al., 2017). However, this effect was not associated with the presence of fragments introgressed from an alien genome.

ANOVA analysis of heading date in resistant and susceptible wheat lines obtained on the base of 'T10' indicates that the variance of the factors «genotypes of maternal wheat cultivar» (A), «resistance to leaf rust» (B) and «environments» (C) is reliable at the 1 % significance level. The effect of the environmental factor was 43.9 % in comparison with the factors «genotypes of maternal wheat cultivar» (31.4 %) and «resistance to leaf rust» (14.6 %) (Figure 2). Analysis of factors affecting the period from germination to ripening indicates that the main contribution is made by the genotype of the maternal wheat (46.1 %).

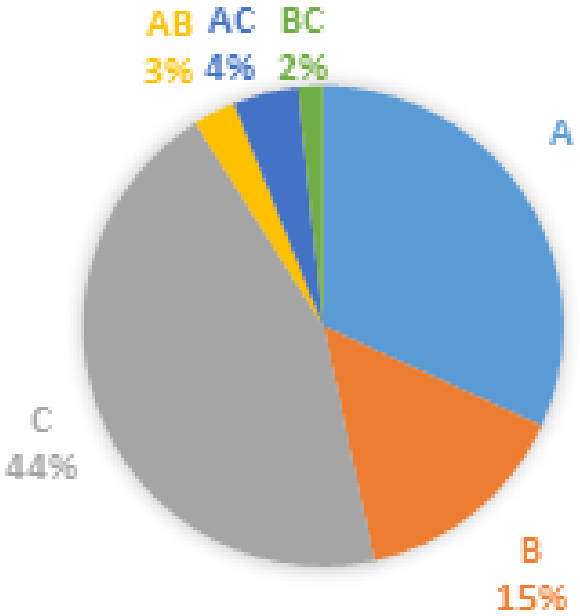


**Figure 2.** Effects of the genotypes of the maternal wheat cultivars (A), resistance to leaf rust (B) and environments (C), and their interaction on heading date in wheat lines derived from crosses between the varieties N15, N31, Udacha and Sibirskaya 17 with T10.

## 4. Conclusions

Thus, our study have shown that the use of donors of leaf rust resistance genes, originating from *Th. intermedium* and *Ae. speltoides*, in most cases leads to a prolongation of the developmental stages. It has been established that changes in the heading date is not always associated with the presence of alien translocations. The genotype of the maternal wheat variety makes a major contribution to the phenotypic manifestation of the trait "time from germination to ripening". The results also indicated the effectiveness of the use of molecular markers specific for alien translocations to select genotypes for target loci at the early stages of breeding.

## References

Adonina I.G., Bukatich E.Yu., Piskarev V.V., Tyunin V.A., Shreider E.R., Salina E.A. Inheritance of the translocation in chromosome 2D of common wheat from *Aegilops speltoides* Tausch with leaf rust resistance genes. *Rus. J. Genet*. 2018;54:989–993. DOI 10.1134/S1022795418080021.

Friebe B., Jiang J., Raupp W.J., VcIntosh R.A., Gill B.S. Characterization of wheat-alien translocation conferring resistance to diseases and pests: current status. *Euphytica*. 1996;91:59–87. DOI 10.1007/BF00035277.

Leonova I.N., Stasyuk A.I., Skolotneva E.S., Salina E.A. Enhancement of leaf rust resistance of Siberian winter wheat varieties by marker-assested selection. *Cereal research Commun*. 2017;45:621–632. DOI 10.1556/0806.45.2017.048.

Peterson R.F., Campbell A.B., Hannah A.E. A diagrammatic scale for estimating rust intensity of leabes and stems of cerials. *Can. Res. Sec. C*. 1948;26:496–500.

Petrash N.V., Leonova I.N., Adonina I.G., Salina E.A. Effect of translocation from *Aegilops speltoides*. Tausch on resistance to fungal diseases and productivity in common wheat. 2016;52:1253–1262. DOI 1134/S1022795416120097.

Piskarev V.V., Boylo N.I., Kondratieva I.V. Sources of agronomically important traits for breeding soft spring wheat (*Triticum aestivum* L.) in the Forest steppe of Novosibirsk region. *Rus. J. Genet.: Applied Research*. 2017;7:281–289. DOI 10.1134/S2079057717030133.

Stasyuk A.I., Leonova I.N., Salina E.A. Variability of agronomically important traits in spring wheat hybrids obtained by marker-assisted selection from crosses of winter wheat with spring wheat donors of resisyance genes. *Agricultural Biol*. 2017;52:526–534. DOI 10.15389/agrobiology.2017.3.526.eng.

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**Conflict of interest.** The authors declare no conflict of interest.