# Production and characterization of the bread wheat lines with introgression of chromosome 6Agi2 *Thynopirum intermedium*

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## INTRODUCTION

*Thinopyrum intermedium* is a source of agronomically valuable traits for common wheat. In breeding programs, partial wheat-wheatgrass amphidiploids and lines with wheatgrass chromosome substitution are widely used. The line Agis 1 (6Agi2/6D) is included in the cultivar Tulaykovskaya 10 pedigree. The wheatgrass chromosome 6Agi2 carries complex resistance to fungal diseases in various ecological-geographical zones and does not reduce the yield and grain quality. Varieties with 6Agi2/6D substitution are used in breeding programs.

## AIM

The aim of the work was to study the peculiarities of the transfer of the *Th. intermedium* 6Agi2 chromosome to wheat varieties, to obtain the breeding material with introgression of the 6Agi2 chromosome and to study it by economically valuable traits.

## MATERIALS AND METHODS

We used the varieties of spring bread wheat Saratovskaya 29 (S29), Novosibirskaya 31 (N31) and Tulaykovskaya 10 (T10), as well as hybrids S29×T10 $F_2$, $F_3$ generations, T10×S29 $F_2$-$F_6$ generations and N31×T10 $F_3$ generations, obtained from self-pollination of $F_1$ hybrids.

To identify chromosome 6Agi2, we used DNA markers specific to the long and short arms of this chromosome (PCR analysis), as well as genomic *in situ* hybridization (GISH).

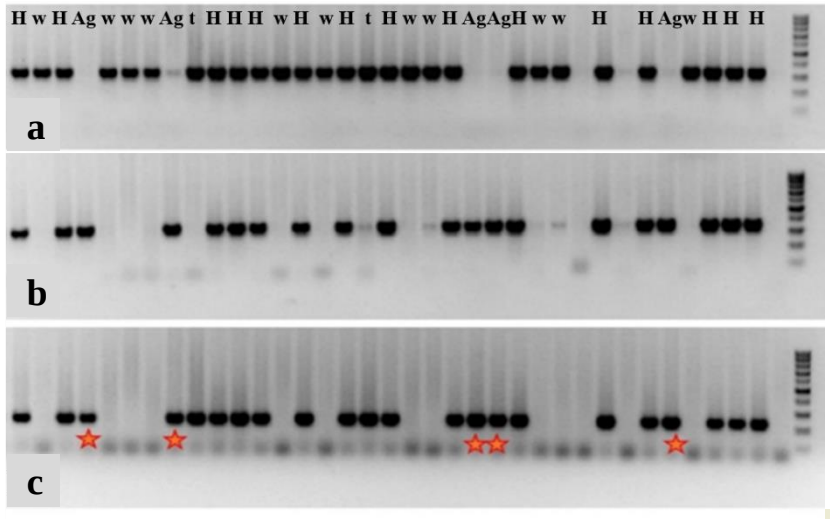
The degree of plant damage to fungal diseases was assessed using the CIMMYT scale (Koishybaev et al., 2014). The content of protein and gluten in grain was determined on an infrared express analyzer of grain OmegAnalyzer G.

# Isolation of plants with 6Agi2/6D substitution

The presence of chromosomes or individual chromosome arms in the $F_{2-3}$ generations of hybrids S29xT10, T10xS29 and N31xT10 according to PCR data.

| Generation of hybrids | Studied DNA samples | Only 6AgiL (number/%) (t - type) | Only 6AgiS (number/%) (t - type) | Present 6Agi (number/%) (Ag - type or H - type) | Absent 6Agi (number/%) (w - type) |
|---|---|---|---|---|---|
| $F_2$ S29 x T10 | 116 | 9/7,56 | 7/5,88 | 50/41,8 | 48/40,34 |
| $F_3$ S29 x T10 | 20 | 2/10 | 4/20 | 3/15 | 11/55 |
| $F_2$ T10 x S29 | 45 | 0 | 14/31.1 | 12/26.7 | 19/42.2 |
| $F_3$ T10 x S29 | 35 | 1/2.86 | 4/11.43 | 14/40 | 16/45.71 |
| $F_3$ N31 x T10 | 45 | 6/13.3 | 6/13.3 | 24/53.4 | 9/20 |

According to molecular analysis, 6Agi2 was transmitted to almost half of the studied plants in the $F_2$ and $F_3$ generations.




The presence of amplification fragments using two markers to the short and long arms indicated the presence of a whole chromosome 6Agi2, and depending on the presence of chromosome 6D, one can speak of either a complete substitution of 6Agi2/6D (Ag-type) or a heterozygous state of the chromosome in these samples ( H-type).

**Fig. 1.** Electrophoregram of marker amplification to the long arm of chromosome 6DL(a) to the short(b) and long (c) arms of the 6Agi2 chromosome in $F_2$ generation plants of the S29xT10 crossing. Plants with 6Agi2/6D substitution are marked with an asterisk.

Plants with amplification of markers to the short and long arms of the wheatgrass chromosome were selected for GISH analysis.

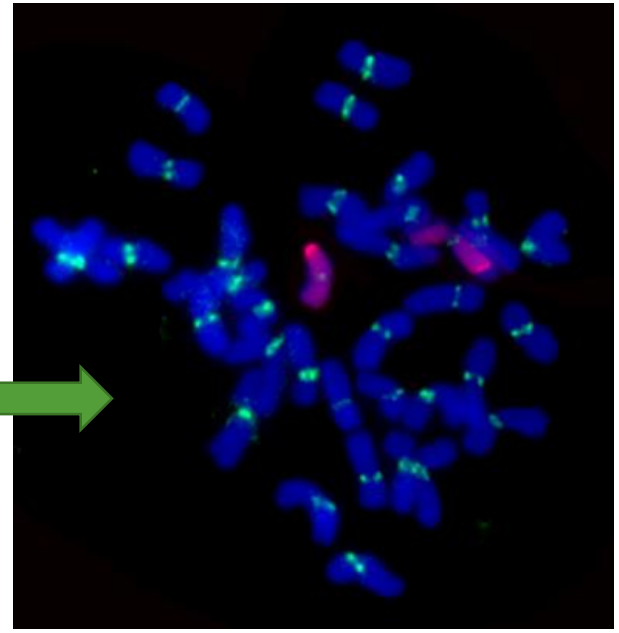


**Fig. 2.** Karyotype of the $F_5$ hybrid T10xS29. GISH. Two chromosomes of wheatgrass are red, centromeric regions of chromosomes (pAet6-09) are green. DNA repeat pAet6-09 kindly provided by Dr. A. Lukaszewcki (Riverside University, California, USA).

Analysis of the karyotypes of plants carrying substitutions according to PCR analysis revealed 42 chromosomes, among which two whole wheatgrass chromosomes were found (Fig. 2), on the long arm of which there is a large subtelomeric heterochromatin block, which corresponds to the distribution of Gimsa C-bands on chromosome 6Agi2 in the karyotype of cultivar Tulaykovskaya 10 (Sibikeev et al., 2017).

# Productivity traits of lines with 6Agi2/6D substitution

Elements of productivity and grain quality of offspring of line 49-14 ($F_5$ T10xS29) and varieties T10, C29 (summer 2020)

| Indicators | S29 | Line 49-14 | T10 |
|---|---|---|---|
| Plant height, cm | 108.33±1.63 | 106.25±1.44 | 98.54±1.32### |
| Productive tillering, pcs | 6.63±0.43* | 5.27±0.42 | 5.04±0.4# |
| Main spike length, cm | 11.08±0.31 | 10.75±0.26 | 10.35±0.19 |
| The number of spikelets in the main spike, pcs | 17.25±0.27 | 16.86±0.25 | 18.02±0.29## |
| The number of grains in the main spike, pcs | 51.75±1.62*** | 63.36±2.19 | 56.66±1.77# |
| The number of grains per spikelet in the main spike, pcs | 3.01±0.09*** | 3.77±0.1 | 3.4±0.08# |
| The mass of grains in the main spike, g | 2.51±0.09 | 2.66±0.13 | 2.42±0.09 |
| The number of grains per plant, pcs | 249.38±19.14 | 259.36±22.49 | 216.68±19.18 |
| The mass of grains per plant , g | 10.62±0.89 | 10.04±0.93 | 8.49±0.83 |
| The mass of 1000 grains, g | 42.53±0.73*** | 38.44±0.59 | 37.45±0.91 |
| Protein content,% | 15.88±1.02 | 17.91±1.23 | 18.81±0.73 |
| Gluten content,% | 35.56±1.63 | 40.55±2.47 | 40±0.88 |

* significant differences in the values of the variety S29 and 49-14. (*at p≤0.05, ***at p≤0.001)
# significant differences in the values of the variety T10 and 49-14. (# at p≤0.05, ##at p≤0.01, ###at p≤0.001)

According to the results of the field experiment in 2020, the samples N31xT10 and T10xS29 was characterized by high productivity traits. The mass of grains per plant and the number of grains per plant did not significantly differ from the parent varieties and the N31 variety. Plants of line 49-14 were characterized by the ability to tie 3.77±0.1 grains per spike, the range of variability of the trait was from 2.93 to 4.62 in individuals. The protein content in the grain was 17.91%, the gluten content was 40.55%.


The main ears of plants with the highest values of the indicator "number of grains per spike in the main spike" of cultivar S29 (3.44) (a); lines 49-14 (4.35) (b); spike of line 49-14 in the stage of waxy ripeness (c), on the leader - spikelets of the central part of the spike (ci); T10 (3.88) (d).

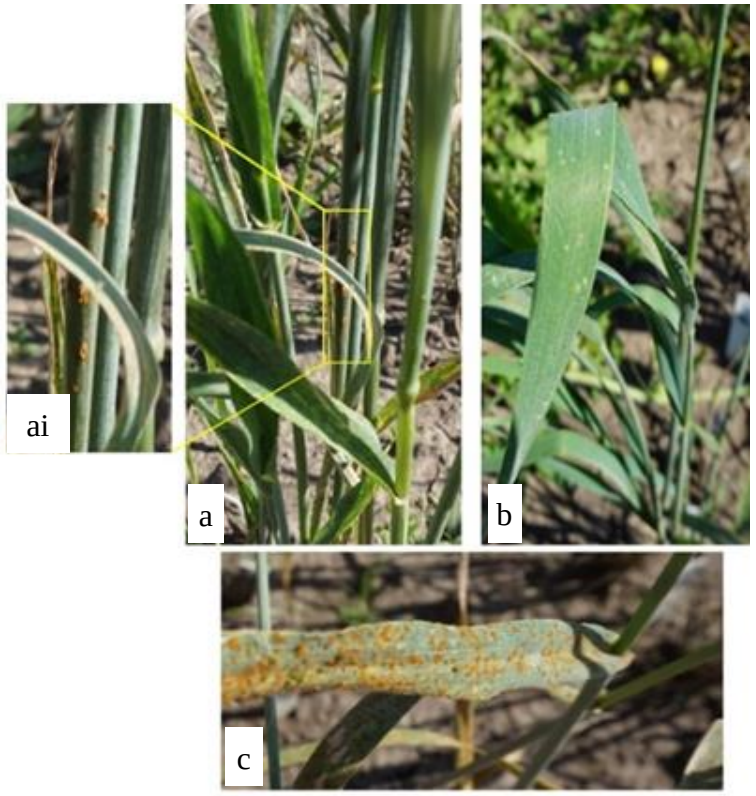
Elements of productivity and grain quality of hybrids $F_3$ N31xT10 and varieties N31, T10 (summer 2020)

| Indicators | N31 | $F_3$ N31xT10 | T10 |
|---|---|---|---|
| Plant height, cm | 110.69±1.14 | 112.67±1.27 | 98.54±1.32### |
| Productive tillering, pcs | 6.23±0.77 | 5.50±0.46 | 5.04±0.4 |
| Main spike length, cm | 12.17±0.45 | 11.45±0.29 | 10.35±0.19### |
| The number of spikelets in the main spike, pcs | 20.46±0.35 | 19.14±0.23** | 18.02±0.29 |
| The number of grains in the main spike, pcs | 59.69±3.29 | 53.75±1.77 | 56.66±1.77 |
| Density of the main spike | 1.68±0,4 | 1,67±0,26 | 1.74±0.24 |
| The mass of grains in the main spike, g | 2.79±0.17 | 2.29±0.09** | 2.42±0.09 |
| The number of grains per plant, pcs | 312.92±35.27 | 250.89±24.34 | 216.68±19.18 |
| The mass of grains per plant , g | 13.24±1.55 | 9.67±0.96 | 8.49±0.83 |
| The mass of 1000 grains, g | 42.17±0.79 | 38.44±0.45*** | 37.45±0.91 |

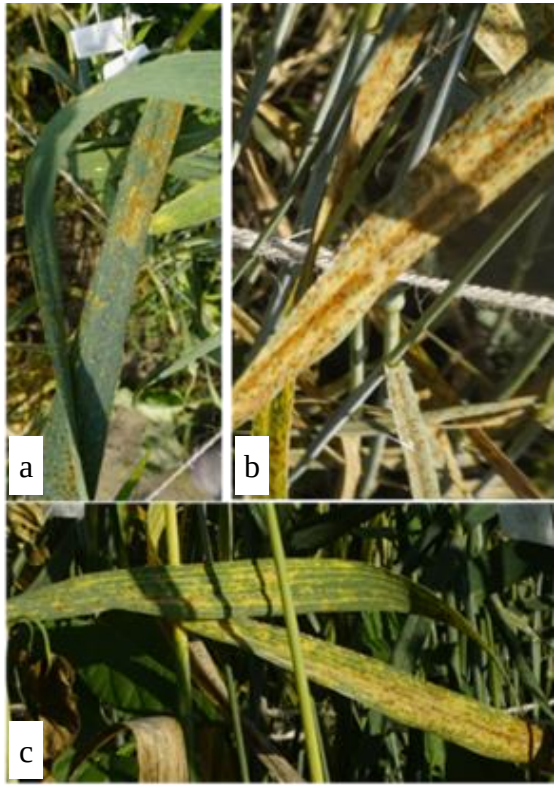
* significant differences in the values of the variety N67 and hybrids $F_3$ N31xT10. ** at p≤0.01, ***at p≤0.001
# significant differences in the values of the variety T10 hybrids $F_3$ N31xT10. ###at p≤0.001

# Screening for fungal diseases resistance

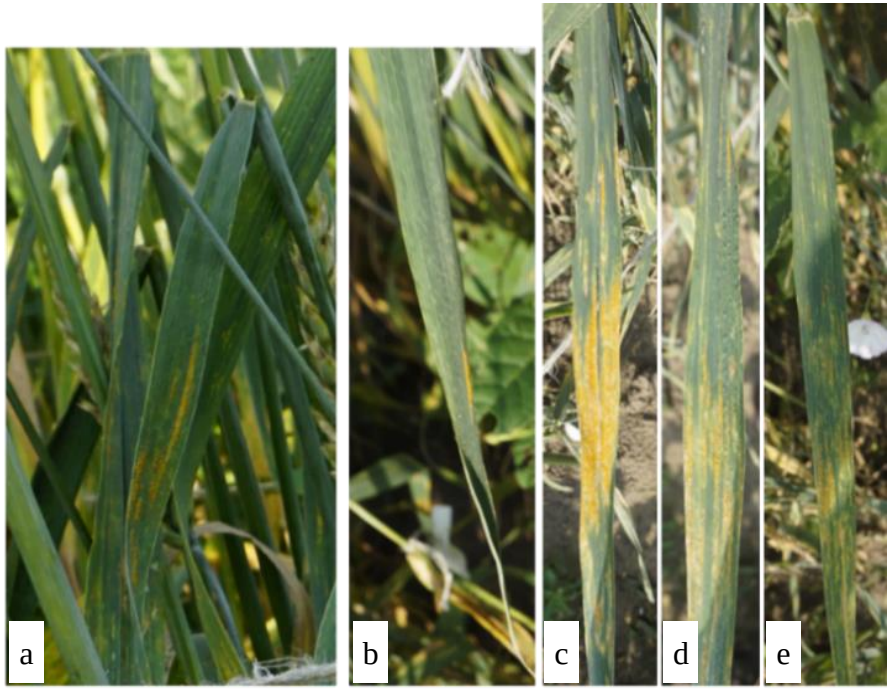
A selection line 49-14 was immune to West Siberian populations of leaf rust pathogens. The type of reaction to the pathogen of yellow rust was characterized by medium resistance and medium susceptibility, probably due to the presence of races of different aggressiveness in the population. Damage to stem rust, noted in only one plant, was assessed as immunity to the dominant races of stem rust.




Absence of leaf rust damage in hybrids $F_4$ T10xS29 (a, b), leaf rust damage of S29 variety (c). On the callout, the straw is affected by stem rust (ai) (August 18, 2018).




Damage to plant leaves with yellow and brown rust in varieties N31 (a) and S29 (b, c) (August 2, 2020).




Resistance of plants of line 49-14 to leaf rust (a-e) and various degrees of damage to plant leaves by yellow rust (a-e) (August 5, 2020).

# CONCLUSION

The use of chromosome-specific primers and genomic *in situ* hybridization made it possible to accurately identify 6Agi2/6D substitution.

New line 49-14 was characterized by high productivity traits.

Possibly chromosome 6Agi2 *Th. intermedium* carries the gene/genes that control the synchronicity of metameric growth of spikelets in cultivar T10, and in line 49-14 (T10xS29), an additive character of the "number of grains per spikelet" trait is observed, which provides normal development of up to 6 flowers per spikelet.

According to the screening for fungal diseases resistance carried out in the field of 2018 and 2020, chromosome 6Agi2 preserves immunity in plants to the West Siberian population of brown rust and to dominant races of stem rust, also provides medium resistant and medium susceptible types of reaction to yellow rust.

Lines/varieties of bread wheat with wheatgrass chromosomes 6Agi2 can be used in breeding for increasing the protein content in the grain, for resistance to leaf-stem diseases, and for creating multi-flowered forms.

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