

Karyotyping of triticale-wheat hybrid lines with the sham ramification of spike

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DOI 10.18699/ICG-PlantGen2019-80

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Abstract: Triticale-wheat hybrid lines from collection of the Genetic Resources Institute of ANAS (Azerbaijan) significantly differ in the ear morphology (manifestation of the vaviloid type of spike branching), in some quantitative traits, and in karyotypes. Karyotypic differences are mainly associated with chromosomes of the second homeologous group. The chromosomes 2D are replaced by a pair of rye chromosomes, presumably 2R in all the studied lines. Some lines have telocentric chromosomes, presumably corresponding to the short arm of a wheat chromosome 2D. We also observed rye chromosomes variability in different lines: loss of one arm or the deletion of subtelomeric heterochromatin accompanied by the loss of the pSc119.2 signal.

Key words: triticale-wheat hybrid lines; vaviloid type of spike branching; karyotype; FISH.

1. Introduction

The wheat forms with the spike branching are studying already about a hundred years. However, there is still a lot of unexplained. Sham ramification (Martinek, 1998) or vaviloid type of branching is trait in species *Triticum vavilovii* (Thum.) Jakubz. (BBAADD). In this case, additional spikelets on the rachis node do not develop, but lengthening of the spike axis, on which many flowers form, is observed. Sham ramification was also found in tetraploid wheat *T. jakubzineri* Udacz. et Schachm. ($2n = 4x = 28$). Aliyeva and Aminov (2013) showed that forms with a vaviloid type of spike branching can occur as a result of distant hybridization. This article is dedicated to the study of the triticale-wheat hybrid lines with the vaviloid type of spike branching.

2. Materials and methods

The collection of wheat forms with the vaviloid type of spike branching consists of lines obtained from crossing a stable wheat-rye amphiploid (triticale) BBAARR ($2n = 6x = 42$), used as the maternal form with the local variety of common wheat *T. aestivum* var. *velutinum* ($2n = 6x = 42$, BBAADD). Triticale was obtained by Professor Aminov in 1975 from hybridization of synthetic wheat BBAADD (*T. durum* × *Ae. tauschii* var. *meyeri*, catalog number in VIR – k-45918) with weed rye *Secale cereale* ssp. *segetale* ($2n = 2x = 14$, RR). Hybrid populations were studied for 10 years in an open field at the Absheron experimental base of the Genetic Resources Institute of ANAS. The forms with the vaviloid type of spike branching began to appear from the generation F4. Estimation of the morphological and quantitative traits of these lines was conducted.

A molecular-cytological analysis of the lines was carried out using the methods of genomic *in situ* hybridization (GISH) with *S. cereale* genomic DNA and fluorescent *in situ* hybridization (FISH) with probes that allow the identification of wheat and rye chromosomes:

pAs1 – a 1-kb fragment derived from *Ae. tauschii*, which allows the identification of the D-genome chromosomes (Rayburn and Gill, 1986b);

pSc119.2 – a 120-bp-long sequence isolated from rye (Bedbrook et al., 1980), which hybridizes mainly to the B-genome chromosomes (Mukai et al., 1993).

3. Results and discussion

Aliyeva and Aminov (2011) showed that sham ramification or vaviloid type of branching controlled by one recessive gene. Subsequently, Amagai et al. (2014) localized the *sham ramification 1* (*shr1*) gene, which controls the false branching of the *T. jakubzineri* spike, on the long arm of chromosome 5A. In the same work, it is shown that the recessive gene, *shr2*, localized on the long arm of chromosome 2A, controls the false branching of *T. turgidum* spike. However, differences are found between the phenotypes of *T. turgidum* and *T. jakubzineri* after a detailed analysis of the spike structure. The trait controlled by the *shr2* gene is isolated into a separate morphotype – false-true ramification (Amagai et al., 2017).

The rye has forms with the spike branching too. One of the genes associated with the emergence of such forms, *monstrosus ear 1* (*mo1*), was mapped in the short arm of the 2R chromosome (Benito et al., 1991).

Dobrovolskaya et al. identified key regulators of cereal inflorescence development – the *Wheat FRIZZY PANICLE* homeologous genes (*WFZP-A*, *WFZP-B* and *WFZP-D*) in the genome of common wheat (Dobrovolskaya et al., 2015). They found that mutations in the functional domain of the *WFZP-D* and *WFZP-A* genes result to the formation of additional supernumerary spikelets and the branched spike. It was detected that the contribution of the *WFZP-A*, *WFZP-D* and *WFZP-B*, localized on 2AS/2BS/2DS, in the genetic control of the trait “supernumerary spikelets” of common wheat is not the same. The most functionally active copy of the gene, *WFZP-D*, belongs to the subgenome D. Research of different “supernumerary spikelets” wheat lines done by Dobrovolskaya and co-authors showed that replacement of the 2D chromosome and deletions in the short arm of this chromosome can affect the spike morphology. The appearance of wheat forms with supernumerary spikelets or branched spike in result of distant

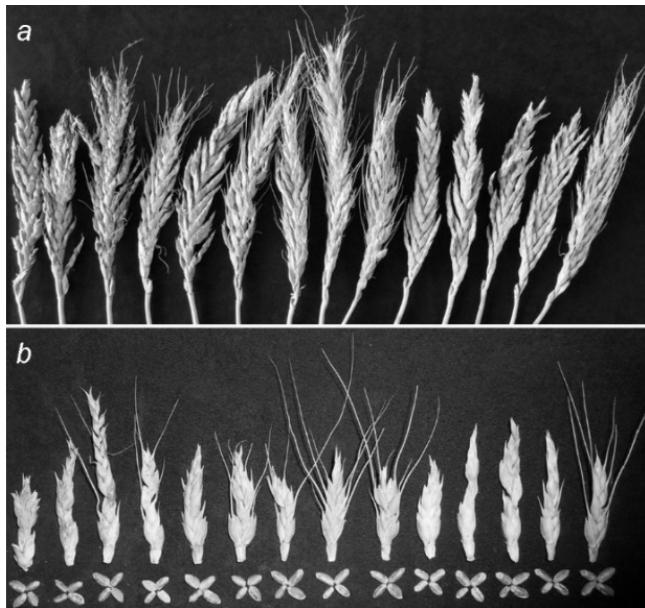


Figure 1. Manifestation of the vaviloid type of spike branching trait in different tritcale-wheat hybrid lines. Spikes (a) and spikelets (b).

Figure 2. Examples of karyotypes of different hybrid lines: $2n=39W+2R\text{-del}+2tW$ (a); $2n=40W+2R\text{-del}+1tW$ (b); $2n=41W+2R\text{-del}+1*$ (c); $2n=40W+1R+1tR$ (d). The probes: pAs1 – red; pSc119.2 – green (a, b, c); rye DNA – green (d).

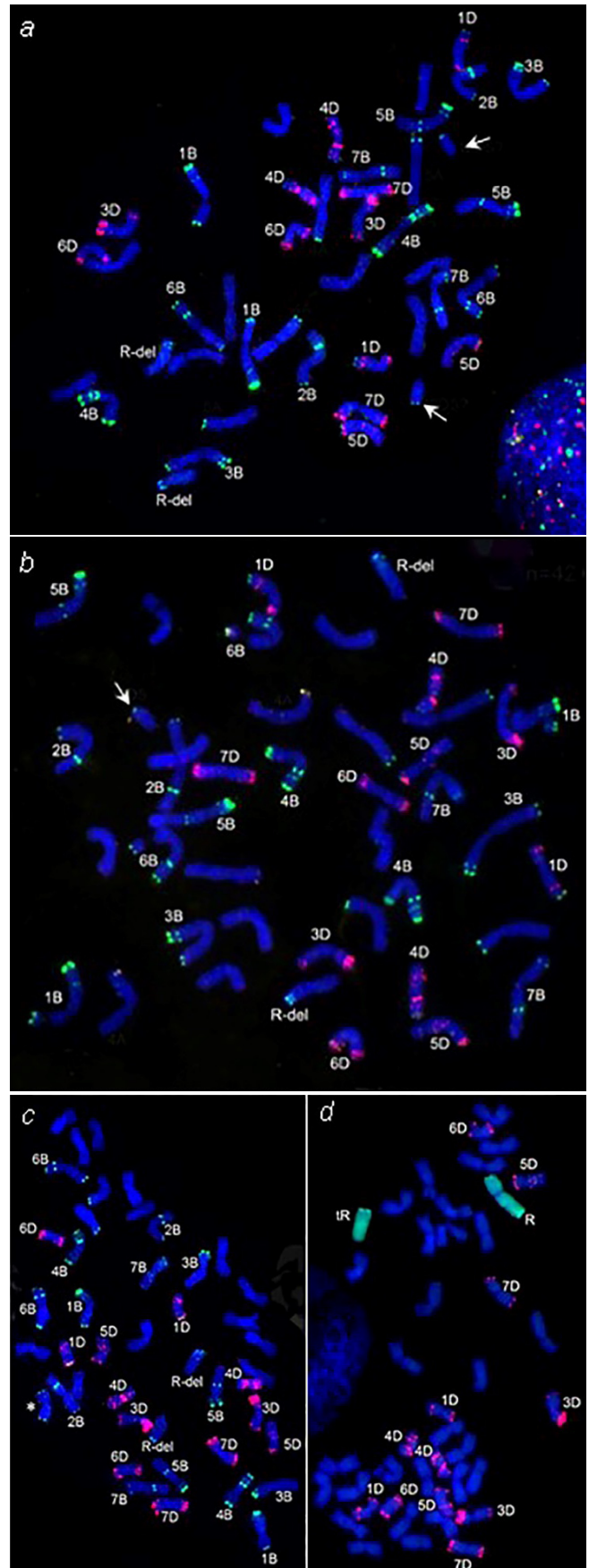
Note: W – wheat, R – rye, t – telocentric chromosome, R-del – rye chromosome with deletion of subtelomeric heterochromatin; the arrows indicate wheat telocentrics; the asterisk indicates chromosome, which was apparently formed because the centric fusion of two wheat telocentrics.

hybridization can serve as a marker of rearrangements of the second homeologous group of chromosomes.

Tritcale-wheat hybrid lines with the vaviloid type of spike branching obtained in the Genetic Resources Institute of ANAS (Azerbaijan) are significantly different in morphological characteristics of the ear (Figure 1) and in some quantitative traits, such as the number of grains in main spike, the grain weight in main spike. The number of grains in main spike varies between 27–123 in different lines, the grain weight in main spike ranges from 1–4.5 grams.

The lines karyotype analysis revealed a chromosomal instability, the number of chromosomes varies from 42 to 44 (Figure 2). Molecular-cytological analysis showed that the chromosomes of the B-genome are not changed. The chromosomes 2D are replaced by a pair of rye chromosomes, presumably 2R in all the studied lines. The number of A-genome chromosomes varies from 12 to 14. In addition, some lines have telocentric chromosomes, with the subtelomeric hybridization signals of pSc119.2 (see Figure 2, a, b). Presumably, these telocentrics correspond to the short arm of wheat chromosome 2D. In one of the lines, we found a chromosome without pair, which was apparently formed because the centric fusion of two telocentrics (see Figure 2, c).

We also observed rye chromosomes variability. We detected the loss of one arm (see Figure 2, d) or the deletion of subtelomeric heterochromatin accompanied by the loss of the pSc119.2 signal (see Figure 2, a–c) in different lines. Earlier,



Alkhimova with co-authors (Alkhimova et al., 1999) observed similar changes when studying rye chromosome variability in wheat-rye addition and substitution lines.

4. Conclusions

Thus, at this stage of the study, we found that the tritcale-wheat hybrid lines from collection of the Genetic Resources Institute of ANAS (Azerbaijan) significantly differ in the ear morphology (manifestation of the spike branching trait), in some quantitative traits, and in karyotypes. Karyotypic differences are mainly associated with chromosomes of the second homeologous group. We also found that the lines themselves are heterogeneous in karyotypic characteristics (the number of chromosomes, the presence of telocentrics). Therefore, we plan to choose contrasting lines in terms of the manifestation of the spike branching trait and characterize the individual plants at the next stage of the work. Molecular-genetic analysis is also required for accurate identification the rye chromosomes and wheat telocentric chromosome.

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Acknowledgements. We are grateful to the Common Use Center of Microscopy of Biologic Objects SB RAS for the provided equipment (<http://www.bionet.nsc.ru/microscopy/>).

The work was supported by grant No. 0324-2019-0039.

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