Chromosome constitution and activity of the centromeric histone H3 (*CENH3*) variants in octoploid triticale lines

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Abstract: Hybrids between wheat and rye (Triticale) are a promising biologic model for studying genetic and epigenetic changes associated with remote crossing in Triticeae. Newly synthesized wheat-rye allopolyploids were obtained from *Triticum aestivum* 'Triple Dirk D' × *S. cereale* 'Korotkostebel'naya 69' and investigated by fluorescent in situ hybridization (FISH). FISH with the highly repetitive DNA probe pSc200 allows identification of all rye chromosomes and shows identical hybridization patterns in hybrids with different numbers of chromosomes (2*n* = 42–56). In hybrids, including plants that have lost entire genomes, we revealed that only wheat chromosomes were eliminated. Yet the nucleotide structure of the centromeric histone H3 (*CENH3*) was studied in allopolyploids and their parental forms. The synthesis of *CENH3* copies with the rye-characteristic SNPs increases in hybrids continuously from F_3 to F_5, regardless of the chromosome number.

Key words: wheat-rye allopolyploids; remote hybridization; centromeric histone H3 (*CENH3*).

1. Introduction

Triticale derived from crossing wheat and rye is the first synthetic allopolyploid cereal. The main impetus for the development of triticale was the idea of obtaining a wheat-rye hybrid that combines a high yield and grain quality as in wheat with adaptation and tolerance to abiotic and biotic factors as in rye. Yet triticale is a promising model for studying the rapid changes in the hybrid genomes associated with severe rearrangements in the genomes of the parental forms, which is the most vivid manifestation of the "genomic shock" which occurs when the parents' genomes are combined in a hybrid cell (McClintock, 1984) and is accompanied by various chromosomal abnormalities, including those affecting the centromere structure. Incompatibility of centromeres of different species seems to be the main reason for the chromosome elimination of one of the parental genomes in the hybrids (Sanei et al., 2011; Ishii et al., 2015). In the present study we carry on a karyotypic analysis of octoploid triticale derived from common wheat 'Triple Dirk D' × rye 'Korotkostebel'naya 69'. Then, we performed a comparative analysis of the nucleotide sequences of the N-terminal tail of the *CENH3* genes in wheat–rye allopolyploids of various ploidy as well as their parental forms.

2. Materials and methods

Allopolyploids (genome AABBDDRR) were synthesized by crossing isogenic line Triple Dirk D (*Triticum aestivum* L.) (AABBDD) with the rye (*Secale cereale* L.) cv. Korotkostebelnaya 69 (RR). Young F_1 seedlings were treated with 0.05 % colchicine solution, and allopolyploids were obtained after chromosome doubling. Although most of the F_1 plants were sterile, a few F_2 seeds were obtained and F_2 seedlings carrying more than 5-7 seeds were chosen for further work. Primary octoploid triticale from the 'Triple Dirk D' × 'Korotkostebel'naya 69' cross was designated as TDK. The lines were propagated for several generations by strict self-pollination. Three lines of F_3-F_6 plant generations were analyzed: TDK 92, 94 and 96. The chromosome number was determined by staining chromosomes after Feulgen (De Tomasi, 1936). The DNA probes for FISH-assisted identification of rye chromosomes were pSc 200, pTa71. The tandem repetitive sequence, pSc200 and the clone pTa71 were labeled by biotin-16-dUTP (Roche) and digoxigenin-11-dUTP (Roche for FISH analysis. These probes were hybridized to mitotic metaphase chromosomes of the parental rye and allopolyploids. Total RNA was isolated from young leaves of rye, wheat and allopolyploids using TriReagent (MRC); cDNA was created and used in a PCR series as a template with primers synthesized specially for amplification of the N-terminal tail (NTT) of rye α*CENH3*(αSc*CENH3*). The product was 268 bp in size. PCR products were cloned into plasmid pTZ57R/T and Sanger-sequenced.

3. Results and discussion

We performed a karyotypic analysis of 30 plants of wheat-rye allopolyploids, F_3 generation. The chromosome number in the somatic cells of these plants was different, ranging from 52 to 56 chromosomes. Then two F_3 plants were chosen for further analysis and designated as TDK 94 (55 chromosomes) and TDK 96 (56 chromosomes). The progenies of these plants were analyzed in F_4, F_5 and F_6 generations. The total number of chromosomes and the number of rye chromosomes were determined in the lines in each generation. Two plants of TDK 96 (TDK 96.1 and TDK 96.2) preserve the full sets of 56 chromosomes in F_4-F_6, while the progenitors of the third plant (TDK 96.3) lose individual chromosomes in subsequent generations with the minimum chromosome number 41 in F_6. DNA probe pSc200 gives a chromosome-specific FISH pattern allowing identification of all rye chromosomes. FISH signals of probe pSc200 are localized at both arms of all 14 chromosomes of the parental rye 'Korotkostebel'naya 69' (Figure 1, *a*). Additionally, pTa71 was used for identification of chromosome

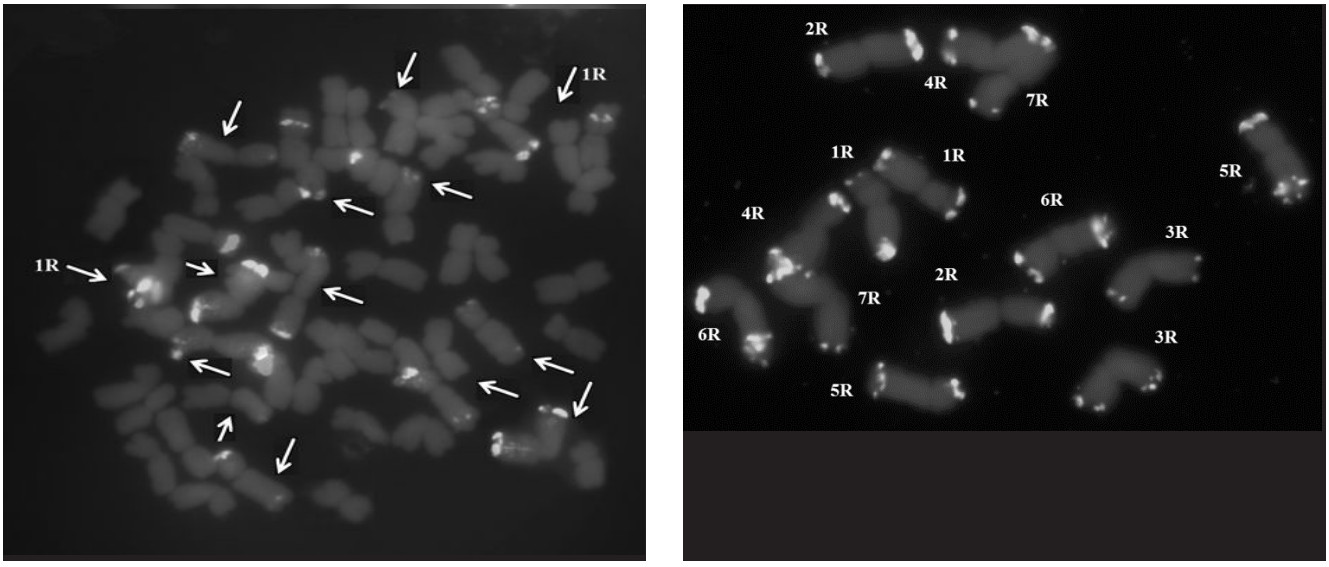

Figure 1. Fluorescent in situ hybridization (FISH) of pSc200 and pTa71 on the spreads of metaphase chromosomes of a root tip of (*a*) *Secale cereale* cultivar Korotkostebel'naya 69 (*b*) octoploid triticale (2*n* = 56, line TDK 96F3). Note: White arrows point to rye chromosomes.

Table 1
Distribution of specific non-synonymous SNPs across the N-tail of *CENH3* of wheat, diploid rye and octoploid triticale

Plants	Number of clones	Number of forms with SNPs at positions of ORFs, %										
		28	32	50	56	73	82	84	99	122	130	145
T. aestivum Triple Dirk D (*AABBDD*), 2*n* = 42	18		11.1		5.6		55.6	55.6	27.8			
Korotkostebel'naya 69 (*RR*) diploid rye, 2*n* = 14	23					8.7	7.4	7.4	30.4			21.7
Octoploid triticale F_3:												
plant 1, (2*n* = 56), TDK 96_1	18	5.6		5.6		16.7	5.6	5.6	11.1	5.6		5.6
plant 2 (2*n* = 52), TDK 92	6	17		16.7					16.7	33.3		
plant 3 (2*n* = 54), TDK 92	10	30	10				10					
Octoploid triticale F_4 (derived from F_3 plant 1):												
plant 1 (2*n* = 56), TDK 96_1	20	5		5		35	10	10	10	15		5
plant 2 (2*n* = 56), TDK 96_2	20	10			5	30	10	10		10		
plant 3 (2*n* = 49), TDK 96_3	15		6.7			33.3	6.7	6.7	6.7		6.7	6.7
Octoploid triticale F_5 (derived from F_4 plant 2):												
plant 1 (2*n* = 56), TDK 96_1	14		7.1			50					7.1	
plant 2 (2*n* = 43), TDK 96	15					57.1		7.1		14.3		14.3

1R in the allopolyploids. Stable octoploid triticales (2*n* = 56) preserved the R genome chromosomes throughout generations F_3-F_5 (Figure 1, *b*). Unstable triticales produced by lines 92, 94 and plant 96.3 have lost chromosome arms, individual chromosomes and sets of seven chromosomes.

An interesting fact is that only wheat chromosomes are eliminated, while the whole set of the rye chromosomes is present in all analyzed plants including those that have lost entire wheat genomes (2*n* = 41, 42, 49). FISH with pTa71 demonstrated that rye chromosome 1R carrying the genes coding for centromeric histone CENH3 is present in all studied lines (see Figure 1, *b*).

To uncover possible relationships between chromosome elimination and *CENH3* activity in triticale, the coding sequences of the α*CENH3* NTT were analyzed in 8 F_3-F_5 hybrid plants with different chromosome numbers (2*n* = 43–56) and in their parental forms. The sequencing of randomly selected clones from wheat and diploid rye revealed 99 % identity between parental *CENH3*s at a nucleotide level. However, *CENH3* rye and wheat variants have specific positions (Table 1). The top number of SNPs in wheat is mapped to nucleotide positions 82 and 84 (55.6 % of wheat clones). These substitutions in rye *CENH3* are very rare. SNPs at four rye-specific positions (28, 73, 122, and 145 bp) were identified within *CENH3* ORFs. These substitutions are non-synonymous. We revealed that the wheat-rye allopolyploids contain all nucleotide substitutions that are specific for the parental rye form. This implies that rye-specific *CENH3* forms are successfully synthesized in the wheat cytoplasm. The distribution of rye-specific and wheat-specific SNPs

in *CENH3* sequencesfrom the triticale hybrids suggests a preferred synthesis of rye *CENH3*. This conclusion is in a good agreement with the fact that *CENH3* genes are located on chromosome 1R (Lipikhina et al., 2017). A special case is the rye-specific substitution at position 73 of the α*CENH3* nucleotide sequence, which is not present in the parental wheat cultivar. F_3-F_5 triticale plants increase the synthesis of *CENH3* copies with SNPs at position 73 bp, typical of rye *CENH3* (16–57 % in the clones) (see Table 1).

With this substitution, *CENH3* loses the only serine phosphorylation site predicted for the rye N-tail. It is possible that the lack of posttranslational *CENH3* modifications reduces the diversity of *CENH3* forms and assists the meiosis setting of a complex hybrid genome.

4. Conclusions

Fluorescence in situ hybridization (FISH) karyotyping of primary octoploid triticale revealed that individual chromosomes and whole genome chromosomes were eliminated, but the chromosomes of R genome were retained in triticale lines through generations F_3-F_6. Both rye and wheat variants of *CENH3* were expressed in the wheat-rye allopolyploids, regardless of how many chromosomes were ultimately lost. However, it is not yet clear whether all the expressed *CENH3* variants are integrated in the centromeres of the hybrids' chromosomes. Taken together, our results provide evidence for the involvement of the *CENH3* N-tail structure in the formation of new hybrid genomes.

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