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ORIGINAL RESEARCH

Alteration of Terminal Heterochromatin and Chromosome Rearrangements in Derivatives of Wheat-Rye Hybrids

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ABSTRACT

Wheat-rye addition and substitution lines and their self progenies revealed variations in telomeric heterochromatin and centromeres. Furthermore, a mitotically unstable dicentric chromosome and stable multicentric chromosomes were observed in the progeny of a Chinese Spring-Imperial rye 3R addition line. An unstable multicentric chromosome was found in the progeny of a 6R/6D substitution line. Drastic variation of terminal heterochromatin including movement and disappearance of terminal heterochromatin occurred in the progeny of wheat-rye addition line 3R, and the 5RS ditelosomic addition line. Highly stable minichromosomes were observed in the progeny of a monosomic 4R addition line, a ditelosomic 5RS addition line and a 6R/6D substitution line. Minichromosomes, with and without the FISH signals for telomeric DNA $(TTTAGGG)_n$, derived from a monosomic 4R addition line are stable and transmissible to the next generation. The results indicated that centromeres and terminal heterochromatin can be profoundly altered in wheat-rye hybrid derivatives.

KEYWORDS: Wheat-Rye addition lines; Chromosome rearrangements; Multiple centromeres; Minichromosomes; Heterochromatin

INTRODUCTION

Heterochromatin plays an important role in maintaining the structure and function of centromeres and telomeres. Both terminal and centromere regions contain satellite repeats and retrotransposons that are usually packaged into heterochromatin (Pearce et al., 1996; Miller et al., 1998; Henikoff et al., 2001; Houben and Schubert, 2003; Bühler and Gasser, 2009). The dynamics of centromere and telomere evolution in plants has been reviewed (Nagaki et al., 2003; Fajkus et al., 2005; Ma et al., 2007). It has been reported that the activation of heterochromatic transcription can be affected by environmental and genetic stress conditions (Tittel-Elmer et al., 2010). Interspecific hybridization is among the stresses that trigger reorganization of the parental genomes (McClintock, 1978). Alteration of rye (*Secale cereale* L.) terminal heterochromatin in triticale (*Triticosecale* Wittmack) and in progeny derived from triticale × wheat (*Triticum* ssp.) has been investigated (Appels et al., 1982; Gustafson et al., 1983; Lukaszewski and Gustafson 1983; Lapitan et al., 1984). Subsequently, fluorescence *in situ* hybridization (FISH) was used to detect the variation of terminal/subterminal heterochromatin of rye chromosomes in wheat-rye addition and substitution lines (Alkhimova et al., 1999). These previous studies focused on the variations of rye terminal/subterminal heterochromatin and the main variation patterns were deletion and amplification. Several studies on centromeres have focused on centromeric DNA sequences and proteins, epigenetic regulation and centromeric activity (Henikoff et al., 2001; Heit et al., 2006; Dalal et al., 2007). Although centromeres are usually maintained as unique loci on chromosomes, alteration in centromeres such as neocentromeres, holocentromeres, dicentric chromosomes, multiple centromeres and stable chromosomes without

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centromeric repeats have been reported (Kynast et al., 2000; Hiatt et al., 2002; Nasuda et al., 2005; Guerra et al., 2010; Gao et al., 2011; Fu et al., 2012; Neumann et al., 2012; Masonbrink et al., 2013; Fu et al., 2013). Holocentric chromosomes contain a kinetochore that spans the whole length of a chromosome. Holocentromeres have been reported in organisms such as insects (Heteroptera), worms (*Parascaris univalens*, *Caenorhabditis elegans*) and plants (Guerra et al., 2010). Dicentric chromosomes were formed through asymmetric chromosome translocation. In maize, dicentric chromosomes that involved A-B chromosomes and A-A chromosomes were described (Han et al., 2006, 2009; Gao et al., 2011). A transmissible dicentric chromosome was mentioned in wheat by Sears (1946). Sears and Camara (1952) further observed that this chromosome was derived from an isochromosome involving the short arm of chromosome 7, which was later specified to be chromosome 7B. This chromosome has three functional centromeres (Zhang et al., 2010). Multicentric chromosomes were reported for animals (Paweletz et al., 1989; Hadlaczky et al., 1991), wheat (where gametocidal genes could lead to the formation of multicentric chromosomes) (Kynast et al., 2000) and pea (*Pisum sativum*) (Neumann et al., 2012).

In this study, we investigated the alterations of terminal and centromeric heterochromatin in wheat-rye alien addition, substitution and translocation lines. Some interesting phenomena including change of terminal heterochromatin position and formation of multi-centromeres, dicentric chromosomes and minichromosomes were uncovered.

RESULTS

Centromere variation in the progeny of the 3R^{CI} disomic addition line

In the self progeny of a 3R^{CI} disomic addition line, we obtained a dicentric chromosome, which was a wheat-rye translocation chromosome (Fig. 1A). This chromosome is unstable because its two kinetochores may bind to spindle fibers from opposite poles, resulting in chromosome breakage during mitotic divisions, as evidenced by FISH screening in the root tip cells two months after planting (Fig. 1B). Simultaneously, a wheat chromosome with multiple centromeres was observed in the progeny of the 3R^{CI} disomic addition line. This chromosome probably descended from chromosome 1B based on the arm ration and presence of a satellite (Fig. 1C). Immunolocalization of H2AThr133ph can detect multiple active centromeres (Dong and Han, 2012) that may in some cases behave as a single centromere during mitosis (Fig. 1D). In the next generation, this multi-centric chromosome was lost, suggesting its meiotic instability.

Variations of rye terminal heterochromatin

In the 3RS ditelosomic line, the rye terminal heterochromatic blocks can be distinguished by strong genomic *in situ* hybridization (GISH) signals at one end of the 3RS telosome (Fig. 2A). Subsequent FISH with the rye-specific subtelomeric tandem repeat pSc200 and the highly repetitive DNA sequence pSc119.2 labeled the terminal region of 3RS (Fig. 2B) which coincided with the rye terminal heterochromatin. In another 3RS ditelosomic addition line 3RS-1, the terminal heterochromatin of the 3RS has moved to a subterminal position of 3RS (Fig. 2C) as did the signals of repetitive sequences, pSc119.2 and pSc200 (Fig. 2D). Among the selfed progeny of 3RS-1, in some plants the rye terminal heterochromatin and the highly repetitive DNA sequences pSc119.2 and pSc200 were lost (Fig. 2E and F).

In a line of the selfed progeny of the 5RS ditelosomic addition line (MK5RS^{dite}), all mitotic root cells possessed a chromosome fragment containing the terminal heterochromatin of rye chromosome arm 5RS (Fig. 3). However, the terminal heterochromatin was moved into a subterminal position (Fig. 3A and C). Subsequent FISH using pSc119.2 as probe confirmed this presumption (Fig. 3B and D). Another kind of minichromosomes derived from 5RS, which lost the terminal heterochromatin, but retained the centromeric retrotransposon of wheat signal, were also observed in some mitotic cells (Fig. 3C and D).

Minichromosomes in progeny of 4R addition lines

One particular wheat line (MK4R$^{mon\text{-}mini}$) was detected among the self progeny of the 4R monosomic addition line MK4R^{mon}. GISH and FISH using rye genomic DNA (green) and CRW (red) as probes indicated that the two minichromosomes were rye chromosomes (Fig. 4A). However, only one minichromosome contained telomere signals. The other minichromosome is either a ring or a telomere-depleted linear chromosome (Fig. 4B). Some self progeny of MK4R$^{mon\text{-}mini}$ still contained minichromosomes, and four types of minichromosomes were observed. The first one was the same as that in MK4R$^{mon\text{-}mini}$. The second one contained two minichromosomes with telomere signals indicating a linear structure (Fig. 4C). The last two types contained only one minichromosome with or without telomere signals (Fig. 4D and E). Immunolocalization revealed CENH3 on minichromosomes with and without the telomere signals (Fig. 4F). The minichromosomes with telomere signals could be transmitted to the next generation at a frequency of about 55%, while the transmission frequency of minichromosomes without telomere oligonucleotide signals was only about 10%.

Variation of centromere in 6R/6D substitution line

In one of the self progeny of the 6R/6D substitution line, several kinds of special chromosomes were discovered. These special chromosomes included a minichromosome that was almost totally composed of rye centromere sequence (Fig. 5A), a small ring chromosome having two rye centromeres (Fig. 5B), a large ring chromosome possessing four rye centromeres (Fig. 5C) and a multicentromeric chromosome containing four rye centromeres (Fig. 5D). In this multicentromeric chromosome, the four centromeres were similar in size and appeared as individual units interspersed by segments of noncentromeric