Creating wheat-rye translocation lines by monosomic addition lines

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ABSTRACT

An elite wheat line R149 was obtained from the selfed progeny of the monosomic addition lines between common wheat cultivar 'Mianyang 11' and a rye inbred line 'baili', with obvious phenotypic characters of the parental rye inbred line 'baili', such as disease resistance and the yield advantage. Biochemical assay of high molecular weight (HMW) glutenin subunits showed that R149 had the same subunit pair 5+10 as the parental wheat cv. Mianyang 11, which contributes the most to the baking quality of wheat flour. Sequence analysis and genomic *in situ* hybridization (GISH) further revealed that a small segment of rye chromosome containing the 2085-3265bp (about 1.1kb) segment of pAWRC.1, centromere-specific repetitive sequence of rye were translocated to the terminal regions of wheat chromosomes in line R149. Furthermore, the fragment of pSc119.1 cloned from R149 had only 86% homology with its original sequence. These results indicated that the reconstruction of translocation chromosomes was concerned not only with simple exchange of chromosomal segments but also with the rearrangement of DNA sequences. Learning about the mechanism of reconstruction of this kind of translocation is helpful to study the organization of chromosome and the control of gene expression. Besides, the use of monosomic addition line is an effective approach to transfer the small segments of alien chromosomes into wheat.

INTRODUCTION

Rye has some important traits beneficial for wheat's yield and resistance improvement. So far, many disease resistance genes have been transferred to wheat (Friebe et al. 1996). Plant breeders using alien genetic introgression frequently wish for very small segments of chromosomes to be introgressed, to minimise the transfer of deleterious genes (Ribeiro-Carvalho et al. 2001). Although several attempts have been made to transfer alien segments that are smaller than complete chromosome arms (Sears 1993; Miller et al.1994; Hu et al.1999; Wang et al.2004), only a few examples of small-segment-translocation (SS translocation) lines which had superior agronomic performance have hitherto been reported (Masoudi-Nejad, 2002).

Ren and Zhang (1997) found that the monosomic chromosome of rye added to wheat genome was instable in heredity. The high frequency of rye chromosome breakage could induce fragmentation and elimination of wheat chromosomes, which led to wheat-rye translocation lines with small rye chromosome segments formation. We have reported that the small rye chromosome segments with powdery mildew-resistant gene(s) were transferred to wheat through this method (Fu et al, 2006). In present study, we charactered another wheat line containing small rye chromosome segment.

MATERIALS AND METHODS

Monosomic addition of R chromosome of rye inbred line 'baili' in common wheat 'Mianyang11', were developed according to the method described by Ren and Zhang (1997). From the selfed progenies of the monosomic addition lines (BC_2F_7), wheat line R149 was selected because of its excellent agronomic traits.

Two sets of primer pairs, Pr119.1 (5'TTGGC CCTCA TGCCT TTAGT CCTTG C3'; 5'CTTGG CCCTC TCCGC TTGAC CGTTG CTC3') and PrAWRC (5'AAGAT GCCGA GGCTA ACCGC3'; 5'GAAGG ACTT G TGTCC ACGGC3'), were designed from pSc119.1 sequence (McIntyre et al, 1990) and from nucleotides 2085-3265 of pAWRC.1 sequence (Francki et al., 2001), respectively.

Genomic DNAs were extracted according to Zhang et al. (1995). PCR amplification and sequence cloning were according to Tang et al. (2008). Sequence alignment was carried out with the software DNAMAN Version 4.0.

Rye genomic DNA was labelled with digoxigenin-11-dUTP according to the manufacturer's instruction (Roche). GISH protocol was as described in Tang et al. (2008).

The identification of various HMW glutenin subunits was according to Yang et al. (2001). 'Chinese Spring'('CS') and 'Mianyang11' were used as the control.

RESULTS

The wheat line R149 possesses superior agronomic traits including high yield and high resistance to stripe rust and powdery mildew. It has also good quality because high molecular weight (HMW) glutenin subunit pair

5+10 was detected by SDS-PAGE (Figure 1), which contributes the most to the baking quality of wheat flour.

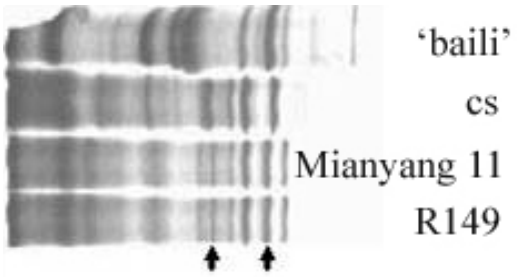

Figure 1. SDS-PAGE patterns of endosperm protein extracts from rye inbred line 'baili'; 'CS'; 'Mianyang11' and R149. Arrows showed high molecular weight (HMW) 5+10 glutenin subunits.

The target products of Pr119.1 and PrAWRC were produced only from the genomic DNAs of rye 'baili' and wheat line R149, however, no products were amplified from the genomic DNAs of 'Mianyang11' and 'CS'. This indicates that the two primer pairs were rye-specific. The target products of the two primers from R149 were cloned and sequenced. The fragment amplified by PrAWRC from R149 had 97% similarity to pAWRC.1 sequence.. In addition, the fragment of pSc119.1 cloned from R149 had 86% similarity to the original sequence. This result indicates that R149 contains rye chromatin.

Further GISH analysis was used to characterize the size and translocation breakpoint of rye segments. As shown in Figure 2, small chromosome segments of rye origin translocated to the terminal region of the short arm of one pair of wheat chromosomes.

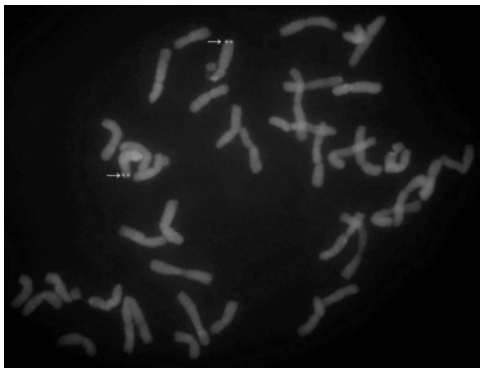

Figure 2. GISH to the mitotic metaphase chromosomes of R149. Arrows indicate two chromosomes with terminal regions stained in brighter colour.

DISCUSSION

PCR amplification, sequence alignment and GISH analysis showed a small rye chromosome segment, containing the nucleotides 2085-3265 of of pAWRC.1, were translocated to the terminal regions of wheat chromosomes in line R149. Because pAWRC.1 is rye centromere-specific repetitive sequence (Francki et al., 2001), the small rye chromosome segment in R149 maybe came from rye centromere. This urged us to think about the mechanism of small-segment translocation induced by monosomic addition of rye chromosome in wheat. In the selfed generations of monosomic addition lines, not only the single added rye chromosome in wheat was eliminated rapidly, but also the wheat chromosomes existing in pairs exhibited a tendency toward cytological instability (Ren et al., 1991). This kind of instability caused high frequency of breakage and disruption between rye and wheat chromosomes during meiosis. Then the small segments of rye chromosomes incorporated into wheat chromosomes by fusion. Because of the randomicity of the breakage and disruption of chromosome, it is possible for the centromeric segments of rye chromosomes transferring to terminal regions of wheat chromosomes. Perhaps, there was another mechanism to explain this kind of translocation. Sequence of pAWRC.1 is retrotransposon-like element (Francki et al., 2001). When monosomic rye chromosome was introduced into wheat, the retrotransposon-like element was provoked to transpose because of the change of genetic background.

The sequence of pSc119.1 cloned from R149 was only 86% similarity to the original one. This indicated that the repetitive DNA sequence rearranged during reconstruction of translocation chromosomes. So the reconstruction of translocation chromosomes was concerned not only with simple exchange of chromosomal segments but also with the rearrangement of DNA sequences.

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