

Variation in repetitive elements in sibling wheat cultivars containing 1BL.1RS translocation

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ABSTRACT

Three kinds of rye-specific repetitive DNA sequence pSc119.1, pSc20H and pAW161, from three sibling wheat cultivars containing the 1BL.1RS translocation, were investigated by sequencing and alignment analysis. pSc20H and pAW161 are more conserved than pSc119.1. Non-random nucleotide substitution throughout the sequence pSc119.1 was found. Furthermore, the three cultivars were derived from F₄ single wheat plants. PCR analysis using 210 wheat SSR markers indicates that the wheat A, B and D genomes have high genetic identity among the three cultivars. We presume that the variation in repetitive DNA may be related to the variability for some agricultural traits among the three cultivars. Different repetitive elements may play different roles in genomic systems. The results in this study have given a better understanding of genetic variation of the 1BL.1RS translocation and imply that the variation in different repetitive DNA sequences may be used as a new kind of molecular marker to select elite genotype in wheat breeding programs.

INTRODUCTION

Some papers have indicated that repetitive elements play roles in genomic/epigenetic systems (Sternberg, 2002; Zuckerandl, 2002). Wheat (*Triticum aestivum* L.) is one of the best models for the study of repetitive elements, because species in the tribe Triticeae have large genomes and the majority of their genomes is made up of repetitive DNA sequences (Flavell, 1986). The introduction of alien chromosomes from related species into wheat has been widely used in wheat breeding programs and has proven to be useful for increasing the genetic diversity available to wheat breeders. However, some sibling wheat lines derived from the same combination of wide hybridization demonstrated great variability for some agronomic traits (Warburton et al, 2002). From the genomic system architecture perspective (Shapiro, 2005), the variability for agronomic traits between sibling wheat lines may be related to the variation of repetitive elements. In this study, variation in several repetitive DNA sequences in three sibling wheat cultivars was investigated.

MATERIALS AND METHODS

A monosomic addition line, monosomic addition of 1R chromosome of rye inbred line L155 in common wheat 'Mianyang11' ('MY11'), was developed according to the method described by Ren and Zhang (1997). Wheat line 91S-23 was derived from the monosomic addition line. Three cultivars 'CN12', 'CN17' and 'CN18' were derived from a F₄ single plant derived from 91S-23×'MY11'. The C-banding technique was used according to Ren and Zhang (1995). The total genomic DNA of rye Jingzhou-heimai was used as a probe to hybridize to metaphase chromosomes of the three cultivars. The probe was labelled with digoxigenin-11-dUTP according to the manufacturer's instructions (Roche). A thin layer of antifade solution containing 1µg/ml propidium iodide (PI) was added to each slide, for detection of *Secale cereale* DNA. A total of 210 Xgwm microsatellite markers (Röder et al, 1998) were used. One primer pair (Pr161) designed for pAW161, which is a rye subtelomeric sequence, was described by Zhang et al. (2005). The other two sets of primer pairs, Pr119.1 (5'TTGGC CCTCA TGCTT TTAGT CCTTG C3'; 5'CTTGG CCCTC TCCGC TTGAC CGTTG CTC3') and Pr20H(5'GTTGG AAGGG AGCTC GAGC3'; 5'GTTGG GCAGA AAGGT CGACA TC3'), were designed according to the original pSc119.1 sequence (McIntyre et al, 1990) and pSc20H sequence (Ko et al, 2002), respectively. Powdery mildew isolates (No.9 and No.3) collected from Ya'an, Sichuan, were used to inoculate seedlings of the three cultivars and 'MY11'. PCR amplification and sequence cloning were according to Tang et al. (2007). Common wheat 'Chinese Spring' ('CS') was used as the control.

RESULTS

Both C-banding and GISH analyses indicate that the three cultivars are 1BL.1RS translocation (Fig. 1).

'MY11' was highly susceptible to powdery mildew. 'CN18' was immune to powdery mildew, however, 'CN17' and 'CN12' displayed a high level of resistance to powdery mildew. In addition, 'CN18' matured earlier than the other two cultivars.

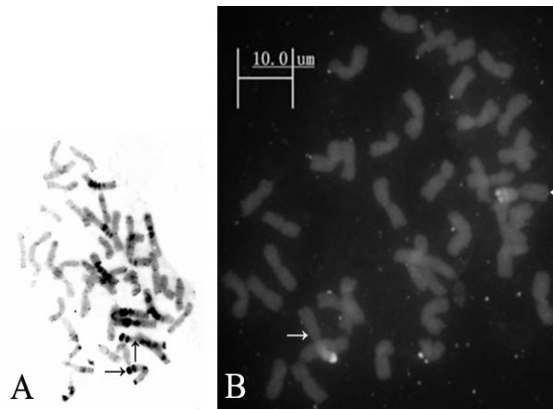


Fig. 1 C-banding (A) and GISH analysis (B) of 'CN17'. Arrows indicate IBL.1RS translocation.

Of the 210 *Xgwm* microsatellite markers, 203 produced the same band pattern among the three cultivars. Additionally, there were 26 that exhibited polymorphism between 'MY11' and the three cultivars. The remaining 184 SSR markers produced the same marker pattern among 'CN12', 'CN17', 'CN18' and 'MY11'.

The target products of Pr119.1 and Pr20H were produced only from the genomic DNA of rye L155, and no products were amplified from the genomic DNAs of 'MY11' and 'CS'. This indicates that the two primer pairs were rye-specific. Furthermore, the primer pair Pr161 has already been proved to be rye-specific by Zhang et al. (2005).

The amplified products of Pr119.1, Pr20H and Pr161 from L155 were cloned and five inserts were selected randomly from each product of the three primers for sequencing. All of the five sequences from L155 produced by Pr119.1 have 97%-99% similarity to the original pSc119.1. All of the five sequences from L155 produced by Pr20H have 95%-96% similarity to the original pSc20H. Finally, all of the five sequences from L155 produced by Pr161 have 98% similarity to the original pAW161.

The PCR-amplified fragments of Pr119.1, Pr20H and Pr161 from the three cultivars were also cloned. For each cultivar, 10 inserts produced by Pr119.1, five inserts produced by Pr20H and five inserts produced by Pr161 were selected randomly for sequencing. The 30 sequences produced by Pr119.1, the 15 sequences produced by Pr20H and the 15 sequences produced by Pr161 were analysed by sequence alignment with the original pSc119.1, pSc20H and pAW161, respectively. All of the 15 sequences produced by Pr20H from 'CN12', 'CN17' and 'CN18' have 94%-96% similarity to the original pSc20H. Of the 15 sequences produced

by Pr161 from the three cultivars, two sequences from 'CN12', one sequence from 'CN17' and three sequences from 'CN18' have 97% similarity to the original pAW161, and all the other sequences have 98% similarity to the original pAW161. However, the 30 sequences produced by Pr119.1 showed high levels of sequence. The sequences produced by Pr119.1 from 'CN12' have 91%-95% similarity to the original pSc119.1. The sequences produced by Pr119.1 from 'CN17' have 93%-96% similarity to the original pSc119.1. The sequences produced by Pr119.1 from 'CN18' have 91%-94% similarity to the original pSc119.1. Of the 30 pSc119.1-like sequences, 15 which have 93%-96% similarity to the original pSc119.1 were analysed by software CLUSTALX. Throughout the pSc119.1 sequence, there were 172 positions which were involved in base substitutions. Of the 172 changed positions, 70 positions were involved in more than one sequence. Interestingly, multiple alignment analysis indicated that the same base substitution occurred at the same changed position in 63 of the 70 positions.

DISCUSSION

The PCR analysis using SSR markers indicates that the wheat A, B and D genomes have high genetic identity among the three cultivars. This case leads us to think that the variability for some agronomic traits among the three cultivars may be attributable to variation in the 1RS arm. Although the sequences homologous to the pSc119.1 and pSc20H were searched in NCBI GenBank database, sequence comparisons indicated that our successful rye-specific amplification was ensured by sequence diversity near the 3' termini of primers of Pr119.1 and Pr20H. The PCR products of Pr119.1 and Pr20H were derived from the rye chromosome. So the changes of rye-specific repetitive DNA sequence pSc119.1 can indicate variation in the 1RS arms among the three cultivars. Investigating the variation of repetitive elements might give a better understanding of genetic variation in the 1RS arm. The variation of repetitive elements might be used as a new kind of molecular marker to select elite genotypes in wheat breeding programs.

Of the three kinds of rye-specific repetitive DNA sequence investigated in this study, pSc20H and pAW161 are more conserved than pSc119.1. This result suggests that different repetitive elements may play different roles in genomic systems. Variation in the pSc119.1 sequence may be important for the genetic diversity of the 1RS arm. However, the repetitive elements such as pSc20H and pAW161 were conserved for maintaining the stability of 1RS arm. It is interesting that the nucleotide substitution throughout the sequence of pSc119.1 was non-random.

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