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Full Length Research Paper

Hereditary variety of 1RS arm between Siblinng wheat lines containing 1BL.1RS translocation

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3 sets of sibling 1BL.1RS translocation wheat lines (each set containing 2 sibling lines) derived from 3 different F_4 single plant. Between 2 sibling wheat lines, different resistance to powdery mildew was observed. In addition, different bands in -gliadin mobility zone between 2 sibling wheat lines were also significantly observed by acid polyacrylamide gel electrophoresis (A-PAGE). However, the nucleotide sequence of / -gliadin precursor gene cloned from 2 sibling wheat lines were 100% similarity.
Polymerase chain reaction (PCR) analysis using 180 wheat microsatellite markers dispersed on 7 homeologous groups of wheat indicated that little genetic variation occurred in wheat A, B and D genomes of these sibling wheat lines. Structural variation of 1BL.1RS chromosome arm was detected by PCR analysis. Different rye-specific repetitive DNA exhibited different variation. The different structure of 1RS chromosome arms may be related to the genetic variation between the 2 sibling wheat lines. The results lead us to think that the variation of 1RS arm within a species should be utilized in wheat breeding program. To detect variation of repetitive DNA can provide better understanding of the genetic variation of 1BL.1RS translocation and will be useful for utilization of 1RS arm in wheat breeding program.

Key words: 1BL.1RS translocation, repetitive DNA, sibling wheat lines, gentic variation.

INTRODUCTION

The introduction of alien chromosomes from related species into wheat (*Triticum aestivum* L.) has proven to be useful for increasing the genetic diversity available to wheat breeders. Rye (*Secale cereale* L.) has been used extensively as a source of elite genes introduced into wheat genome. Translocation 1BL.1RS involving the short arm of rye chromosome 1R and the long arm of wheat chromosome 1B is widely used in wheat breeding program. The 1BL.1RS translocation has been introduced into hundreds of wheat cultivars worldwide through the Russian wheat cultivars 'Kavkaz', 'Aurora' and their derivaties (Raiaram et al., 1990; Rabinovich, 1998). Because the 1RS arm carries genes for resistance to diseases and positively affects agronomic traits including yield performance, yield stability and wide adaption (Schlegel and yield stability and wide adaptation (Schlegel and Meinel, 1994; Moreno-Sevilla et al., 1995; McKendry et al., 1996; Kim et al., 2004), wheat-rye 1BL.1RS translo-cation lines will be still useful in wheat breeding program. It has already reported that the effect of source of rye chromatin is greater than its position effect in wheat genome and selection of 1RS source is important in pro-ducing constantly higher grain yield in 1RS translocation lines (Kim et al., 2004) . Approaches have been made to the production of new 1BL.1RS translocation line from different origins of cultivated rye, with aim to improve the genetic polymorphism of the 1RS chromosomes for better diseases resistance (Lukaszewski et al., 2001; Ko et al., 2002a). With the diversified 1BL.1RS translocation lines developed, the cytogenetic and biochemical techno-

Figure 1. Steps for obtaining wheat lines.

logies were exploited to characterize the genetic variation of 1BL.1RS translocation in wheat background (Li et al., 2002; Yan et al., 2005). To better understand the genetic variation of 1BL.1RS translocation will be useful for utilization of 1RS arm in wheat breeding program.

However, besides cytogenetic and biochemical technologies, DNA sequences can also be used to investigate the genetic diversity of 1BL.1RS translocation. It has reported that the major determinants of genome system architecture are the repetitive elements in the genome, such as tandem repeats and dispersed repeats (Shapiro, 2005). Species in the tribe *Triticeae* hold large genomes and the majority of their genomes consist of repetitive DNA sequences (Flavell and Smith, 1976; Flavell, 1986). Taken these reports together, it is possible and reasonnable to use repetitive DNA sequences to detect genetic diversity induced by 1BL.1RS translocation. Furthermore, some sibling wheat lines/cultivars which were developed in wheat breeding program exhibit diversity in some traits. These sibling wheat lines are good materials for studying the mechanism by which repetitive DNA sequences induce genetic variation.

In this study, we selected 3 sets of sibling 1BL.1RS translocation lines from 76 wheat lines. Each set of sibling 1BL. 1RS translocation line was derived from a F_4 single plant. However, some different traits were observed between 2 sibling translocation lines. We mainly investigated the structure of 1BL.1RS translocation chromosome using repetitive DNA to discover the probable molecular mechanisms which in-duce the genetic variation in sibling 1BL.1RS translocation lines.

MATERIALS AND METHODS

Plant materials and pedigree

Common wheat (*T. aestivum* L.) 'Mianyang11' ('MY11') was used as recipient and rye (*Secale cereale* L.) inbred lines R3, R12 and Baili were used as donors. 'MY11' was released in 1981 and was a widespread high-yielding wheat cultivar in Southwest China. It has already been susceptible to several diseases such as stripe rust, powdery mildew and *Fusarium* head blight for many years. Wheat lines 96-132-1 and 96-132-2 were obtained from the cross between 'MY11'×R3. Wheat lines 96-137-2 and 96-137-3 were derived from 'MY11'×R12. Wheat lines 96-212-1 and 96-212-2 were derived from 'MY11'×Baili. These wheat lines were obtained by following steps (Figure 1).

The same single plant selected from each wheat line was used for each analysis in this study, such as disease resistance observation, cytological analysis, seed storage protein electrophoresis, southern blot analysis and PCR analysis. Wheat cultivars Chinese spring ('CS') and rye inbred line L155 were also used in this study.

Disease resistance observation

The 3 sets of sibling wheat lines were evaluated for resistance to powdery mildew. Seedlings were inoculated by the epidemic predominant race of powdery mildew in southwest China. 6 infection types were recorded on a scale of 0, 0;,1, 2, 3 and 4. 0 is no visible symptoms, 0; is necrotic flecks, 1 is highly resistant, 2 is moderately resistant, 3 is moderately susceptible and 4 is highly susceptible.

Cytological analysis and seed storage protein electrophoresis

A-PAGE and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) were used to separate endosperm gliadin proteins and glutenin subunits, respectively. The procedures were described by Yang et al. (2001). For the identification of the chromosomes, the C-banding techniques described by Ren and Zhang (1995) were applied. Meiotic behavior of the 6 wheat lines was investigated according to Li et al. (2002).

Selection and design of primer

3 sets of primer pairs were designed according to original pSc119.1 sequence (McIntyre et al., 1990), pSc20H sequence (GenBank accession No.AF305943) and alpha-/beta- gliadin storage protein precursor gene (GeneBank Accession NO. DQ166376). This gene was selected randomly. The primer pairs of sequences pSc119.1, pSc20H and the gene are named as Pr119.1 (5'TTGGC CCTCA TGCCT TTAGT CCTTG C3'; 5'CTTGG CCCTC TCCGC TTGAC CGTTG CTC3'), Pr20H (5' GTTGG AAGGG AGCTC GAGCT G 3'; 5'GTTGG GCAGA AAGGT CGACA TC3') and Pr -gli (5'CATCC TTGCC CTCCTT GCTA3'; 5'TGGTA CCGAA GATGC CAAAT3'), respectively. Sequences pSc119.1 and pSc20H are all rye-specific interspersed repetitive DNA (McIntyre et al., 1990; Ko et al., 2002b). Additionally, one microsatellite marker *SCM* 9 mapped on chromosomal arm 1RS (Saal and Wricke, 1999) and 180 microsatellite markers dispersed on 7 homeologous groups of wheat (Röder et al., 1998) were also used in this study.

PCR analysis, cloning and sequencing

Genomic DNAs were extracted from each single plant according to the method described by Zhang et al. (1995). Each PCR reaction (25 L) using simple sequence repeat (SSR) primers, Pr119.1 and Pr20H contained 50 mM KCL and 10 mM Tris-HCL (pH 8.8), 1.5 mM $MgCL_2$, 200 M of dNTP, 200 M of each primer, 1.0 unit of *Taq* polymerase (Promega), and 40-60 ng of genomic DNA. The annealing temperature of SSR markers was according to Röder et al. (1998) and Saal and Wricke (1999). The annealing temperature

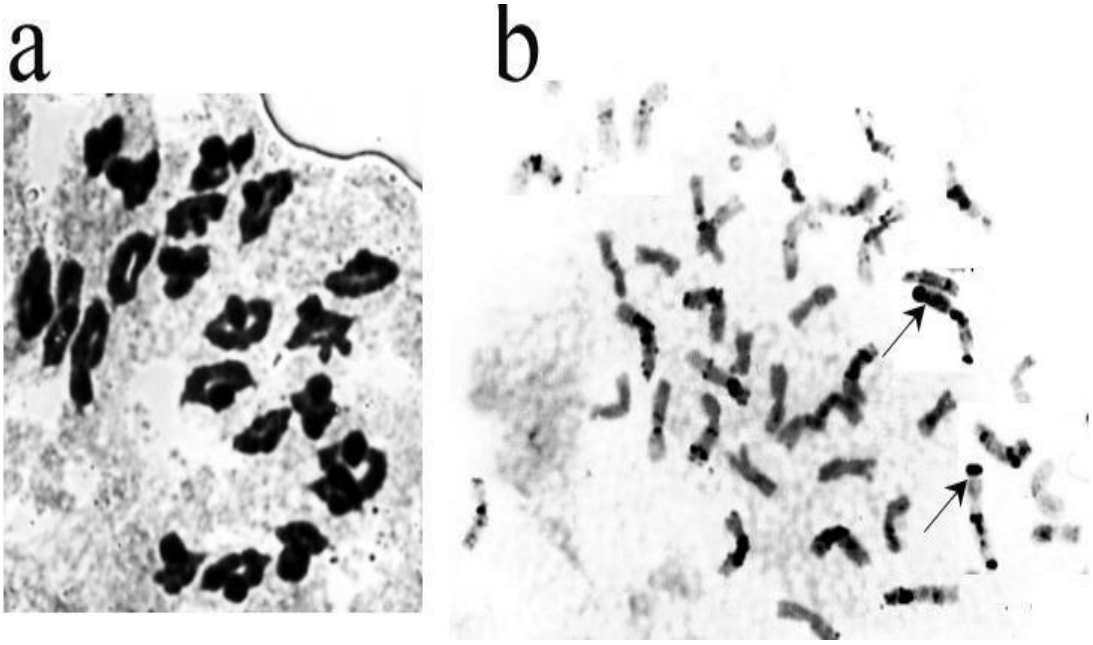

Figure 2. Cytological analysis of 6 wheat lines. (a) 21 cycle bivalents of 6 wheat lines at meiotic metaphase I (Only 96-132-1 is shown); (b) C-banding of 6 wheat lines (Only 96-132-1 is shown). Arrows show the 1BL.1RS chromosomes.

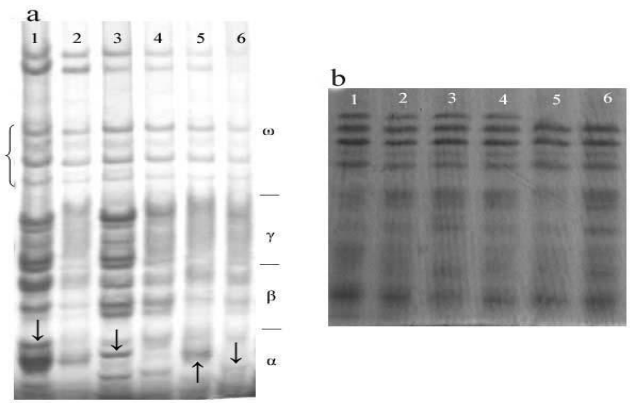

Figure 3. Storage protein electrophoresis of 6 wheat lines. (a) Gliadin storage protein separated by A-PAGE. (b) Glutenin subunits separated by SDS-PAGE. Lane 1, 96-132-1; Lane 2, 96-132-2; Lane 3, 96-137-2; Lane 4, 96-137-3; Lane 5, 96-212-1; Lane 6, 96-212-2. Bracket indicates bands of rye secalins. Arrows indicate different bands in -gliadin mobility zone between two sibling wheat lines.

of Pr119.1 and Pr20H was 60°C. Each PCR reaction (25 L) using Pr -gli contained 80 ng of template, 2.5 L 10×PCR buffer, 1.5 mM $MgCL_2$, 200 M of dNTP, 200 M of each primer, 1.25 unit of Ex *Taq* polymerase (TaKara, Japan), the annealing temperature was 60°C. PCR amplifications were carried out on an MJ research PTC-200 thermocycler, using a program that consisted of initial denaturation for 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 30 s at annealing temperature, 2 min at 72°C and final exten-sion for 10 min at 72°C. The amplified products were analyzed on on 2% agarose gels (FMC brand, Spain). Using 0.5 Tris-borate-EDTA buffer with constant power 120 v (4 v/cm). The PCR fragments were visualized using ethidium bromide staining methods.

Target PCR product of Pr -gli was recovered using gel extrac-tion kit (Omega E. Z. N.A., USA) and products were cloned into pMD18-T simple vector (Takara, Japan). Clones were sequenced by the commercial company Invirtrogene biotechnology (Shanghai) Co., Ltd. Sequence analysis was performed with the software DNAMAN Version 4.0.

Southern blot analysis

The procedure of southern blot was described as Tang et al. (2006). Genomic DNAs of wheat lines were digested by *Bam*H . The plasmid pSc119.1 (gifted by Dr. Gustafson, University of Missouri Columbia, USA) was used as probe. The probe was labeled with [^{32}P] dCTP using the random primed DNA labeling kit (TaKaRa Biotechnology Co., Ltd, Dalian).

RESULTS

Characteristics of the 3 sets of sibling wheat lines

The meiotic behavior of the 6 wheat lines was investigated at metaphase I and 21 cycle bivalents were observed (Figure 2a). The regular meiotic behavior reveals their cytological stability. C-banding analysis indicates that all the 6 wheat lines are 1BL.1RS translocations (Figure 2b) . The resistance to powdery mildew is different between 2 sibling wheat lines of each set. Wheat lines 96-132-1, 96-137-2 and 96- 212-1 are highly susceptible to powdery mildew, however, wheat lines 96-132-2, 96-137- 3 and 96-212-2 showed highly resistant to powdery mildew. The seed gliadin A-PAGE patterns of the 6 wheat lines are shown in Figure 3a, where one can clearly observe that bands in -gliadin mobility zone between 2 sibling wheat lines are significantly different. However, the glutenin composition between 2 sibling wheat lines is same (Figure 3b). Furthermore, the nucleotide sequence of / -gliadin

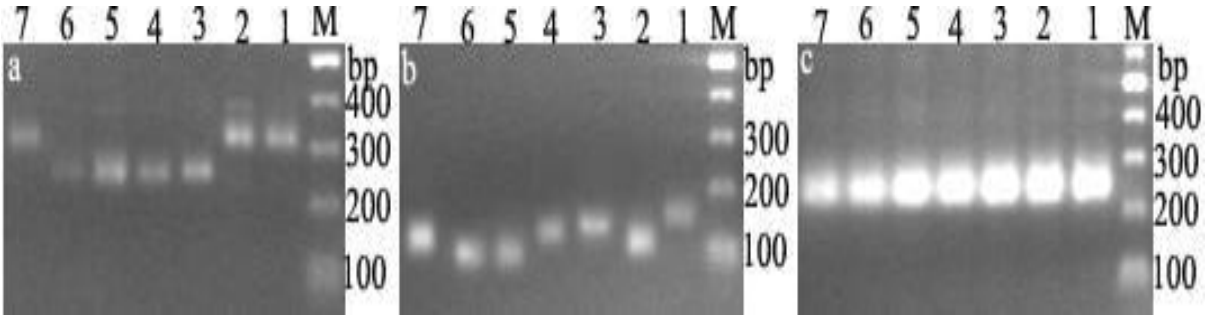

Figure 4. PCR amplification with wheat SSR markers. (a) Amplification products of X*gwm*140; (b) Amplification products of X*gwm*359; (c) Amplification products of X*gwm*4. Lane 1, 96-132-1; Lane 2, 96-132-2; Lane 3, 96-137-2; Lane 4, 96-137-3; Lane 5, 96-212-1; Lane 6, 96-212-2. Lane 7, 'MY11'; M, DNA size marker.

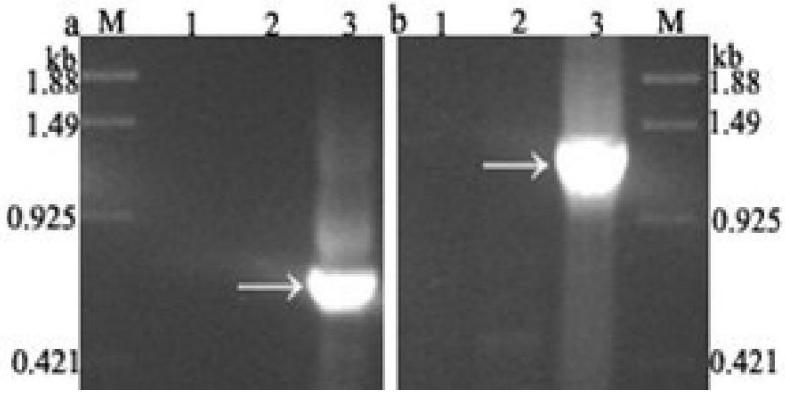

Figure 5. Specialty testing of primer pairs. (a) Amplification product of primer Pr119.1. (b) Amplification product of primer Pr20H. Arrows indicate the target fragment of each primer pair. Lane 1, 'CS'; Lane 2, 'MY11'; Lane 3, rye L155; M, DNA size marker.

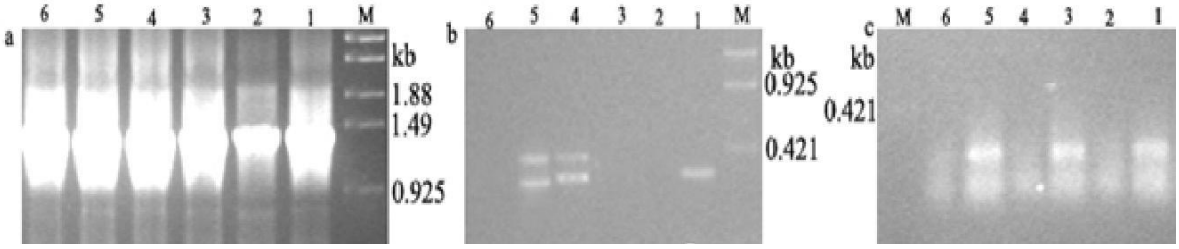

Figure 6. PCR amplification of 6 wheat lines using primers Pr20H (a), Pr119.1 (b) and *SCM*9 (c). Lane 1, 96-132-1; Lane 2, 96-132-2; Lane 3, 96-137-2; Lane 4, 96-137-3; Lane 5, 96-212-1; Lane 6, 96-212-2. M, DNA size marker;

precursor gene cloned from 2 sibling wheat lines from each set showed 100% similarity among them. The gene sequences cloned from the three sets of sibling wheat lines were submitted to the public database of NCBI GenBank. Sequence with Genbank Accession NO. EF165554 was cloned from 96-132-1 and 96-132-2, sequence with Genbank Accession NO. EF165555 was cloned from 96-137-2 and 96-137-3 and sequence with Genbank Accession NO. EF165556 was cloned from 96-212-1 and 96-212-2. Out of the 180 wheat SSR markers, only 5 markers displayed polymorphism among 'MY11' and the 6 wheat lines (Figures 4a, 4b). These SSR markers are X*gwm*140, X*gwm*408, X*gwm*469, X*gwm*497 and X*gwm*539. The other wheat SSR markers amplified the same band pattern in 'MY11' and the analyzed 6 wheat lines (Figure 4c).

Rye-specificity of primers Pr119.1 and Pr20H

The genomic DNA extracted from rye L155, 'MY11' and 'CS' were amplified with the two sets of primer pairs Pr119.1 and Pr20H. All the primer pairs amplified the target products only from the genomic DNA of L155, however, no products were amplified from the genomic DNA of 'MY11' and 'CS' (Figure 5), thus showing that the 2 primer pairs are rye-specific.

Structural variation of 1BL.1RS translocation chromosomes

Primer pairs Pr20H amplified the target fragments from the genomic DNA of all the 6 wheat lines (Figure 6a). However, primer pairs

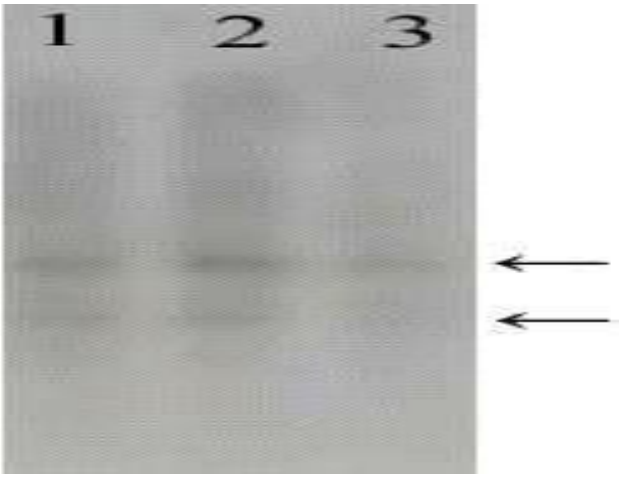

Figure 7. Southern blot of the genomic DNA extracted from 96-132-2, 96-137-2 and 96-212-2 using pSc119.1 as probes. Lane 1, 96-132-2; Lane2, 96-137-2; Lane 3, 96-212-2. Arrows indicate the hybridization signals.

Pr119.1 amplified only the target fragments from the genomic DNA of wheat lines 96-132-1, 96-137-3 and 96-212-1, but not from the wheat lines 96-132-2, 96-137-2 and 96-212-2 (Figure 6b). Furthermore, a non-target fragment was also amplified from the genomic DNA of wheat lines 96-137-3 and 96-212-1 using primer pair Pr119.1 (Figure 6b) . The PCR-amplified bands using rye micro-satellite markers *SCM*9 were different between 2 sibling wheat lines (Figure 6c). Southern-blot analysis using pSc119.1 as probe indicated that the sequence pSc119.1 are not eliminated from wheat lines 96-132-2, 96-137-2 and 96-212-2 (Figure 7).

DISCUSSION

Structure variation of 1BL.1RS chromosomes

Rye-specific dispersed repetitive DNA sequence pSc119.1 is located throughout the 14 rye chromosome arms (McIntyre et al., 1990). The difference of PCR amplification using Pr119.1 between 2 sibling wheat lines discovers the variation of pSc119.1 and indicates the structural variation of 1RS arm at the same time. Furthermore, the different amplification products of *SCM*9 between 2 sibling wheat lines detects the variation of flanking regions of microsatellite and also suggests the structural variation of 1RS arm. It has already been reported that variation of repetitive elements will occur immediately following wide hybridization (Ma et al., 2004; Salina et al., 2004; Han et al., 2005; Ma and Gustafson, 2006). In this study, the 6 wheat lines were also derived from wheat-rye wide hybridization. The variation of repetitive DNA sequences has still been observed in these wheat lines although they are in F_5 generation, indicating that variation of repetitive element may be continual in progeny derived from wide hybridization. PCR analysis did not discover the variation of repetitive sequence pSc20H, indicating that different interspersed repetitive elements play different roles in genomic variation.

Mechanism inducing genetic divergence between 2 sibling wheat lines

Although the 3 sets of sibling wheat lines were all derived from a single F_4 plant, some differences of traits such as resistance to powdery mildew and seed gliadin composition was observed between 2 sibling wheat lines. Whether did the mutation of genes induce the diver-gences of traits? The cases that wheat lines are sibling and / -gliadin precursor gene cloned from 2 sibling wheat lines were 100% similarity lead us to think diver-gences of traits probably did not result from mutation of genes. Shapiro (2005) indicated that it is possible for 2 genomes in different species to have identical coding sequences but distinct signals and genome system archi-tectures and the different patterns of coding sequence expression will lead to phenotypic and ecological diver-sity. Madlung et al. (2002) have described the mecha-nisms of epigenetic gene regulation resulting in phenol-type instability among sibling allotetraploids of Arabido-psis. Furthermore, the PCR analysis using 180 wheat SSR markers does indicate that the wheat A, B and D genomes are high genetic similarity between 2 sibling wheat lines. The case that the glutenin composition between 2 sibling wheat lines is same also indicates that 2 sibling wheat lines are genetic identity. Taken together, it is reasonable to presume that the divergences of traits between 2 sibling wheat lines probably were induced by the variation of 1RS arm.

As well known, in wheat breeding process, heterozy-gosity still exists in wheat lines even though they are in high generation. The results in this study lead us to think that using repetitive DNA sequences may elucidate the reasons why the heterozygosity exists in wheat lines when they are in high generation.

Conclusions

In conclusion, the genetic variation of 1RS could occurred within a species and repetitive DNA sequences are useful for investigating the genetic variation

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