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A simple approach based on intron length polymorphism (ILP) for chromosomal localization of Gene *WAG-2*

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Abstract

Intron polymorphism can be used to develop potential intron polymorphism (PIP) markers. Intron length polymorphism (ILP) is one of the most easily identified PIP markers. *WAG-2*, a class C MADS-box gene, had been isolated from the wheat expressed sequence tag (EST) database. Rich polymorphisms, such as intron allele variation, and length polymorphism occur in *WAG-2* gene introns. Especially, a significant length polymorphism is shared by intron IV. In present study, based on intron IV length polymorphism, using wheat nullisomic-tetrasomic (NT) and ditelosomic (DT) lines, a pair of ILP primers were designed in exons flanking introns IV and the *WAG-2* gene was located on the short arm of homologous group 3 chromosome in the wheat genome. The gene location approach by using ILP markers combining with aneuploidy is convenient, reliable, specific, codominant, and stable. It is expected that ILP markers will play important roles in the genetic studies and breeding of wheat.

Key words: *WAG-2*, intron length polymorphism, chromosomal localization, nullisomic-tetrasomic, ditelosomic

Introduction

Most protein-coding genes consist of exons and introns. Introns belong to a non-coding sequence in genes (Hawkins 1988). The smaller selection pressure that introns bear causes more variation (Gaut 1988; Batzoglou et al. 2000). Intron variation may be classified as loci variation and length variation. Intron polymorphism can be used to develop potential intron polymorphism (PIP) markers (Choi et al.2000; Yang et al. 2007). Intron length polymorphism (ILP) is one of the most easily identified PIP markers. First, the introns in a gene must be identified. Then, ILP primers must be designed in exons flanking the target introns with length polymorphisms and detection must be performed via PCR amplification. This method is called exon-primed intron-crossing PCR (EPIC-PCR) and has been applied to fields of rice, *Arabidopsis,* fern, foxtail millet (Bierne et al. 2000; Wang et al. 2005; Seoighe et al. 2005; Galasso et al. 2011; Muthamilarasan et al. 2014).

Given ILP primers are designed in conservative exons, which have more advantages compared with those designed in non-coding regions and hold broader application prospects, including population genetic structure analysis, genetic map construction, molecular marker-assisted selection, and quantitative trait locus (QTL) location (Lessa 1992; Corte-Real et al. 1994; Wydner et al. 1994; Daguin et al. 1999).

WAG-2, a class C MADS-box gene, had been isolated from the wheat expressed sequence tag (EST) database (Ogihara et al. 2003). The genetic diversity and evolution of the *WAG-2* gene based on new *WAG-2* alleles isolated from wheat and its relatives had been examined (Wei et al. 2011). During the evolution of polyploid wheat, only single nucleotide polymorphisms (SNPs) and no insertions and deletions (indels) were found in exon sequences of *WAG-2* gene from different species. Rich polymorphisms, such as intron allele variation, and length polymorphism occured in *WAG-2* gene introns. Length polymorphisms usually originated from random indels within *WAG-2* gene introns. Intron length polymorphisms of the *WAG-2* gene were largely manifested by A, S and D genomes of diploid wheat, A and B genomes of tetraploid wheat, and A, B and D

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genomes of hexaploid wheat. Especially, a significant length polymorphism was shared by intron IV. In the present study, based on intron IV length polymorphism, using wheat nullisomic-tetraploid (NT) and ditelosomic (DT) lines, a pair of ILP primers were designed in exons flanking introns IV and the *WAG-2* gene was located.

Materials and methods

Plant materials

One accession each of *Triticum urartu* (2n = 2X = 14, AA), *Aegilops speltoides* (2n = 2X = 14, SS), *Ae. tauschii* (2n = 2x = 14, DD), *T. dicoccoides* (2n = 2x = 28, AABB), *T. aestivum* cv. Chinese Spring (CS, 2n = 6x = 42, AABBDD) and its nullisomic-tetrasomic (NT) lines, ditelosomic (DT) lines were used in this study. NT and DT lines were provided by the American National Genebank. All materials were cultivated in the field located at China West Normal University in Nanchong, China. Genome DNA was extracted from fresh leaves using the modified CTAB procedure.

Primer design

Based on the length polymorphisms within intron IV of WAG-2 gene, we developed ILP primers in exons flanking the intron IV that can be used to isolate intron IV in genomes A, B and D. The forward primer was WIN4-3F (5'-CAAAGGCATAGCC AAGATAAGAG-3') and the reverse primer was WIN4-3R (5'-ACGTCTCTGAGTTGAAGTCGTATGTAG-3'). Fig. 1 schematically showed the intron IV sequences of the WAG-2 gene for genomes A, B, and D as well as the locations of the primers.

PCR amplification and polyacrylamide gel electrophoresis detection

PCR amplification was performed in a volume of 10 μL containing 5.0 μL 2×Taq PCR Master Mix (TIANGEN), 0.5 μL of DNA, 0.4 μL of each primer (10 μmol/L) and 3.7 μL of ddH_2O. The PCR cycling included pre-denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 59°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min.

The PCR product was first detected with 1.0% agarose gel electrophoresis. If targeted bands were amplified, 8% polyacrylamide gel electrophoresis was performed at 200V. After 6h, the gel slug was washed with ionized water, treated with 0.5% silver staining solution, and subjected to gentle shaking for 15 min. After staining, the gel slug was washed with running water to remove redundant silver ions, treated with 500 mL of cooling developer, and gently shaken until bands appeared. Finally, the developing reaction was terminated using deionized water.

Results and discussion

The gene location approach used in the present study is novel that employs an intron length polymorphism as a genetic marker. Obviously, the key point of use and detection of an ILP is to identify suitable introns (Yang et al. 2007). ILP is detected by PCR requiring some specific knowledge of DNA or cDNA sequences (Wydner et al. 1994). Previous studies had revealed that the exon-intron structure were largely conserved among homologous *WAG-2* genes from different species (Wei et al. 2011). In addition, the intron lengths of *WAG-2* gene differ in genome A, S or B, and D. For example, the length of intron IV is 151 bp in diploid, tetraploid and hexaploid A genome, 193 bp or 206 bp in S/B genome and 231 bp in D genome (Wei et al. 2011). These information are high enough to allow developing PIP markers in wheat and its relatives by designing primers in exons flanking the target intron.

In the present study, the chromosomal location of *WAG-2* gene was mapped using seven NT lines (N1B-T1D, N2B-T2D, N3B-T3D, N4B-T4D, N5B-T5D, N6B-T6D and N7B-T7D), one accession each of *T. urartu*, *Ae. speltoides*, *Ae. tauschii*, *T. dicoccoides* and CS (*T. aestivum*). Chromosome location of the *WAG-2* should be thus determined by a lack of amplifying bands for NT lines. Fig. 2 showed that single band was obtained from *T. urartu*, *Ae. speltoides* and *Ae. tauschii*. Two bands were achieved from wild *T. dicoccoides* corresponded to those amplified from *T. urartu* and *Ae. speltoides*. The three bands amplified from CS were similar to the bands from three diploid species in size and position. In the line N3B-T3D, two amplified bands corresponding to the *WAG-2* gene located on chromosome 3A and 3D were obtained, but the second (middle) amplified band corresponding to chromosome 3B was missing. On the other hand, three bands corresponding to the *WAG-2* gene located on chromosome 3A, 3B and 3D were achieved in other six NT lines. These results demonstrated that the first band corresponded to the *WAG-2* gene located on chromosome 3D, the second to one on chromosome 3B, and the third to one on chromosome 3A. Given that N3B-T3D lacked a pair of 3B chromosomes, consequently *WAG-2* gene was located on homologous group 3 chromosome in the wheat genome. Our findings also demonstrated that the *WAG-2* gene had one copy in diploid genome species *T. urartu, Ae.*

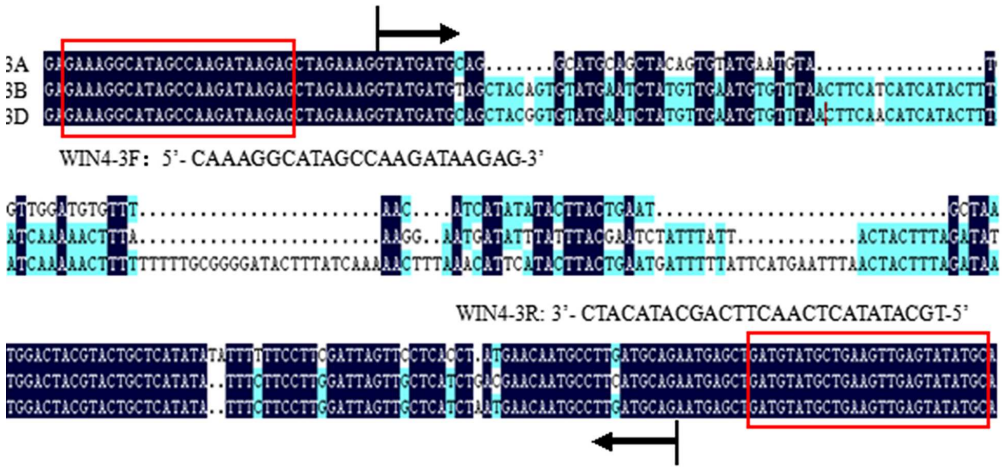

Fig. 1. Sequences and location of amplifying intron IV of *WAG-2* gene. The primers WIN4-3F and WINF-4R were indicated as red box. The intron IV was indicated as blank arrow

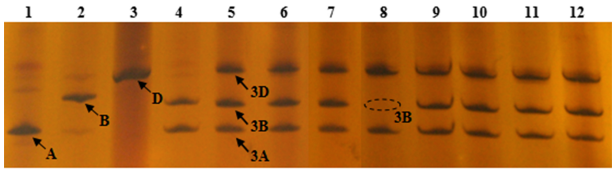

Fig. 2. Polyacrylamide gel electrophoresis of PCR product amplified by primers WIN4-3F and WIN4-3R from 12 accessions of wheat. Lane 1 = *T. urartu*; 2 = *Ae. speltoides*; 3 = *Ae. tauschii*; 4 = *T. dicoccoides*; 5 = *T. aestivum* cv. Chinese spring; 6 = N1B-T1D; 7 = N2B-T2D; 8 = N3B-T3D; 9 = N4B-T4D; 10 = N5B-T5D; 11 = N6B-T6D; 12 = N7B-T7D. The lower, middle and upper bands respectively showed in lane 1, 2 and 3 are the ones corresponding to the *WAG-2* genes located on genome AA, SS and DD. Three bands corresponding to the *WAG-2* gene located on chromosome 3A, 3B and 3D were indicated as blank arrow in lane 5. The second amplified band indicated as elliptical bulkhead corresponding to chromosome 3B was missing (lane 8)

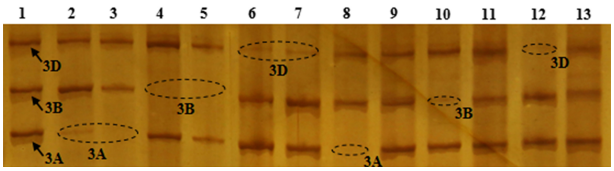

Fig. 3. Polyacrylamide gel electrophoresis of PCR product amplified by primers WIN4-3F and WIN4-3R from six nullisomic-tetrasomic and six ditelosomic lines. Lane 1 = Chinese Spring; 2 = N3A-T3B; 3 = N3A-T3D; 4 = N3B-T3A; 5 = N3B-T3D; 6 = N3D-T3A; 7 = N3D-T3B; 8 = Dt3AL; 9 = Dt3AS; 10 = Dt3BL; 11 = Dt3BS; 12 = Dt3DL; 13 = Dt3DS. Three bands corresponding to the *WAG-2* gene located on chromosome 3A, 3B and 3D were indicated as blank arrow in lane 1. The missing bands corresponding to chromosome 3A, 3B and 3D were indicated as elliptical bulkhead (lane 2,3 lane 4,5 and lane 6,7). Also the missing bands corresponding to chromosome 3AS, 3BS and 3DS were indicated as elliptical bulkhead (lane 8,10 and 12)

speltoides and *Ae. tauschii* respectively, and two copies in the tetraploid wheat genomes.

To verify whether or not the *WAG-2* gene was located on homologous group 3 chromosome and determine if it was in the long or short arm of the chromosome, six NT lines (N3A-T3B, N3A-T3D, N3B-T3A, N3D-T3B, N3D-T3A and N3D-T3B) and six DT lines (Dt3AS, Dt3BS, Dt3DS, Dt3AL, Dt3BL and Dt3DL) were used for PCR amplification. As seen from Fig. 3, only two bands were amplified from the six NT lines. The N3A-T3B and N3A-T3D lines lacked the bands corresponding to the *WAG-2* gene located on chromosome 3A, the N3B-T3A and N3B-T3D lines lacked the bands corresponding to one on chromosome 3B, and the N3D-T3A and N3D-T3B lines lacked the bands corresponding to one on chromosome 3D. These results showed that the *WAG-2* gene was surely located on homologous group 3 chromosome in the wheat genome. Three bands were amplified in Dt3AS, Dt3BS and Dt3DS, while only two bands were amplified in Dt3AL, Dt3BL and Dt3DL. The missing bands corresponded to the *WAG-2* gene located on chromosome 3AS, 3BS and 3DS, which demonstrated in the wheat genome there were three homologous *WAG-2* genes located on the short arm of homologous group 3 chromosomes.

The copy number of many MADS-box genes in wheat were conducted by Southern blot analysis (Meguro et al. 2005; Paolacci et al. 2007). We have known previously that there were a high number of MADS-box genes dispersed throughout the whole wheat genome (Murai 1997). To avoid cross-hybridization of the *WAG-2* probe with other MADS-box genes due to highly conserved MADS-box region, the probe should be designed in 3'-region that is specific for each MADS-box gene. However, the southern blot analysis is tedious and time consuming which requires the physical availability of a suitable probe, probe-labeling reactions. In the present study, the gene location approach by using ILP markers combining with aneuploidy can overcome these limitations. It is possible to determine a molecular genotype directly by PCR amplification and gel electrophoresis (Wydner et al. 1994). ILPs markers have many desirable characteristics, including convenient, reliable, specific, codominant, neutral and stable. However, ILPs has not been widely utilized. To date, ILP markers only in a few species have been exploited (Choi et al. 2004; Wang et al. 2005; Wei et al. 2005; Seoighe et al. 2005; Ishikawa et al. 2002; Muthamilarasan et al. 2014). Nowadays, common wheat have got a large number of cDNA/EST sequences available in public databases. Therefore, large-scale exploitation of ILP markers in wheat becomes possible. We expect that ILP markers will play important roles in the genetic studies and breeding of wheat.

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