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Full Length Article

Developing Specific Molecular Marker for Wheat Q Gene

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Abstract

Wheat *Q* gene located in the homoeologous group 5 chromosomes is extraordinarily important for the widespread cultivation of wheat, because of its effects on many domestication-related morphological traits. The full length of *Q* gene from each locus of 5A, 5B and 5D is 3.2-3.4 kb, which makes it difficult to verify them from each other by PCR and electrophoresis. This study focused on developing molecular makers to verify the three different wheat *Q* gene copies from homoeologous chromosomes 5A, 5B and 5D. The locus-specific markers based on the INDELs in the 9th intron region of *Q* gene were designed to verify the three homoeologous loci from each other. Three gene segments of Gene *Q* were cloned from different wheat materials as molecular markers. These three makers of 536 bp, 665 bp and 578 bp were identified from each homoeologous loci of 5A, 5B and 5D. These markers are useful for tracing different loci of *Q* gene and can also be used in the identification of genetic materials involved in homoeologous group 5 chromosomes. © 2015 Friends Science Publishers

Keywords: *APETALA2* (*AP2*) gene; *Q* gene; Molecular marker

Introduction

Bread wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD) is one of the major cereal crop consumed by human. It appears to be an allohexaploid with three homoeologous genomes A, B and D. It was derived originally from twice allopolyploidization. The first allopolyploidization is hybridization between *T. urartu* (2n=2x=14, AA) and *Aegilops speltoides* (2n=2x=14, SS) or analogs, which produced teraploid *T. turgidum* L. (2n=4x=28, AABB) (Petersen *et al*., 2006; Kilian *et al*., 2007; Forster *et al*., 2012). The second is hybridization of *T. turgidum* L. with diploid goatgrass *Ae. tauschii* coss.(2n=2x=14, DD), which produced bread wheat (Feldman, 2001; Salamini *et al*., 2002).

The *Q* gene located in the long arm of chromosome 5A is extraordinarily important for the widespread cultivation of wheat, because of its influences on many domestication-related traits such as free-threshing character of seeds, rachis fragility, spike length and emergence time, glume shape and tenacity, plant height, etc. (Faris and Gill, 2002; Faris *et al*., 2003; Forster *et al*., 2013). Wild and primitive wheat with *q* allele characterized by spear-shaped spike, elongated rachis and difficulty of seed threshing. The mutation of gene *Q* leads to free-threshing character, which is essential to mass production and mechanical harvesting, accordingly having significant impact on agriculture and the history of human civilization. Gene *Q* is one of the most important crop genes in human history and has been highly concerned by researchers for a long time since it was revealed at the beginning of the 19th century (Simons *et al*., 2006).

Previous researches in molecular level have suggested that gene *Q* is a member of transcription factor *AP2* (*APETALA2*) family, which is also called *WAP2* gene (Simons *et al*., 2006). In *Arabidopsis*, AP2 consists of two typical AP2 domain and plays an important role in development (Theissen and Saedler, 1999). It can also affect seed mass and yield (Jofuku *et al*., 2005; Ohto *et al*., 2005) that brings great value in crop genetics and breeding research.

Southern hybridizing analysis by Simons *et al*. (2006) has given evidence to the presence of a single copy of gene *Q* in each of the homeologous group 5 chromosomes, 5A, 5B and 5D. These three gene *Q* copies from each locus have been all cloned and sequenced (Simons *et al*., 2006; Ning *et al*., 2009). Due to the nearly same molecular weight, these three copies from different locus can't be distinguished from each other by a simply PCR process. Based on these known gene sequences, we developed specific molecular maker to verify gene *Q* from different chromosomes in this study. This knowledge will be of fundamental importance for further wheat breeding.

Materials and Methods

Plant materials: Chinese Spring; Chuanmai 36; Chuanmai 28; Chuanyu 12; 87429; 3854; Yiyuan 2; AS1510; AS332; AS908; *A. tauschii* AS60; Langdon(LDN) and its disomic substitution line with chromosome D: LDN 1D(1A), LDN 2D(2A), LDN 3D(3A), LDN 4D(4A), LDN 5D(5A)+5AL, LDN 5D(5B), LDN 6D(6A), LDN 7D(7A).

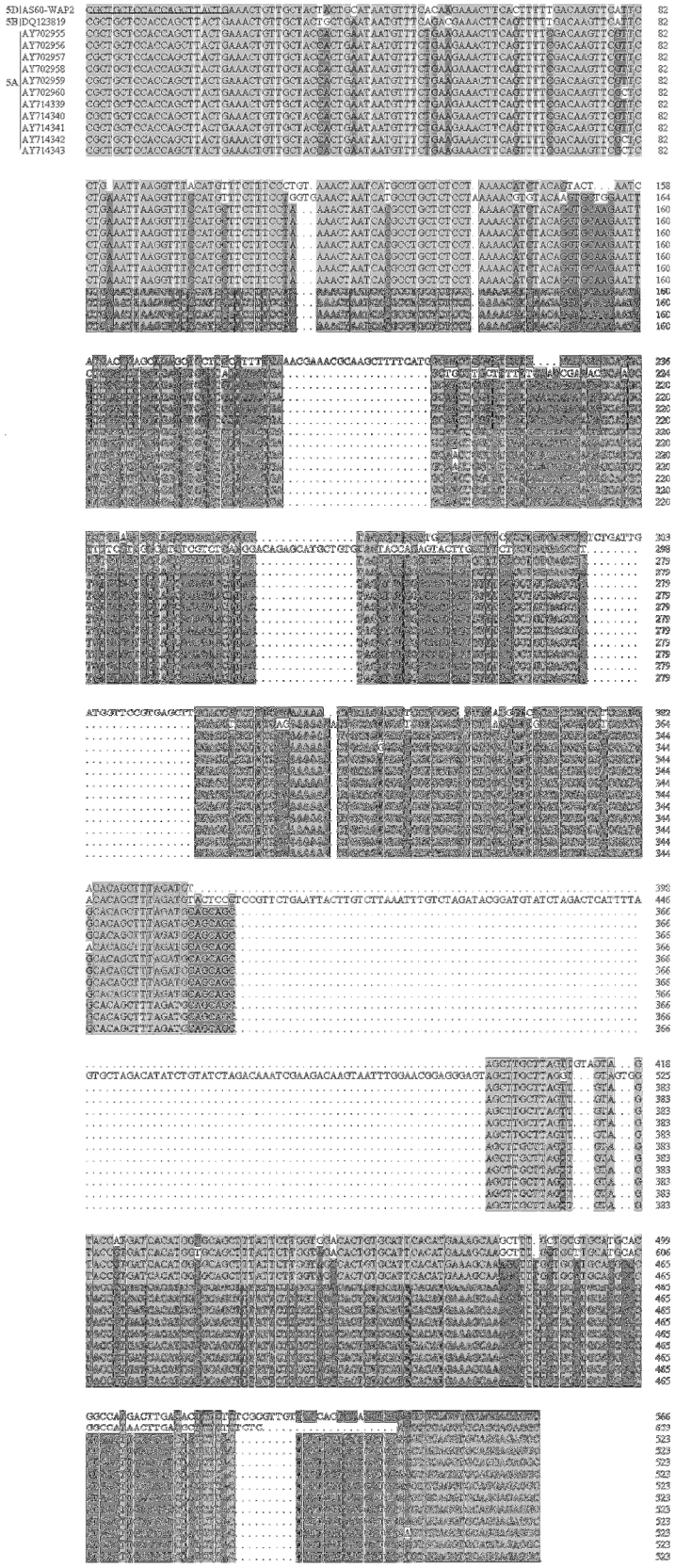

Fig. 1: Sequence alignment of the region of the ninth intron. Dots indicate deletions. The sequences of primers designed in this study were underlined

These aneuploid lines each have a pair of A or B homoeologues replaced by D-genome chromosomes (Joppa and Williams, 1988). Due to lack of 5A chromosome leads to infertility, disomic substitution line LDN 5D(5A) retained the long arm of 5A[LDN 5D(5A)+5AL].

PCR amplification: Young leaves of selected plants were used to DNA extraction according to Saghai-Maroof *et al.* (1984) and Zhang *et al.* (2004). Based on the alignment of *Q/q* sequences from chromosomes 5A, 5B and 5D suitable primers for PCR amplification were designed for three different locus (F: 5'-CGCTGCTCCACCAGCTTACTG-3'; R: 5'-GCCTCTCCTGCACCTGCAC-3'). PCR reaction mix included the following reagents in a total volume of 25 µL: 100 ng of template DNA, 10×Ex*Taq* PCR buffer 2.5 µL, 25 mmol/L $MgCl_2$ 1.5 µL, 2.5 mmol/L dNTPs 1.5 µL, 10 µmol/L primer 1 µL, 5U/µL Ex*Taq* DNA polymerase (TaKaRa) 0.2 µL, ddH_2O. The following PCR conditions were used on PTC-200 Thermocycler (MJ Research Inc., Watertown, MA, USA): initial denaturation (94°C, 4 min), 30 cycles of denaturation (94°C, 40 s), annealing (60°C, 1 min), synthesis (72°C, 2 min), followed by a final extension (72°C, 10 min). Amplicon size was characterized in a 2% agarose gel.

Results

Q/q gene consists of 10 exons and 9 introns (Simons *et al*., 2006). Sequence data have been submitted to the EMBL/GenBank Data Libraries under accession no. AY702958 from *T. urartu*; AY702955, AY702957, AY702959, AY714339 and AY714343 from *T. turgidum*; AY702956, AY702960, AY714340, AY714341 and AY714342 from *T. aestivum* locate on chromosome 5A; DQ123819 from *T. turgidum* located on 5B; AS60-*WAP2* from *A. Tauschii* located on 5D (Ning *et al*., 2009). Compared with these sequences, we found a large number of DNA deletion/duplication within the region of the ninth intron, causing length variation of 5A, 5B and 5D loci (Fig. 1). We designed a pair of primers according to comparing results (F: 5'-CGCTGCTCCACCAGCTTACTG-3'; R: 5'-GCCTCTCCTGCACCTGCAC-3'), which supposed to amplify three segments of 536 bp, 665 bp and 578 bp from each locus of 5A, 5B and 5D.

By using the designed primers, three different fragments were amplified from each DNA template of ten common wheat and the results showed that there were only two fragments of teraploid wheat LDN (Fig. 2). The disomic substitution line of LDN can be used easily to determine the chromosomal location of genes. Band of 665 bp didn't exist in LDN 5D (5B) indicating that it was not from locus of 5B (Fig. 3, Line 8). Segment of 578 bp was only found within *A. Tauschii* (Fig. 3, Line 1), LDN 5D(5A)+5AL(Fig. 3, Line 7), LDN 5D(5B) (Fig. 3, Line 8) indicating that they were from locus of 5D.

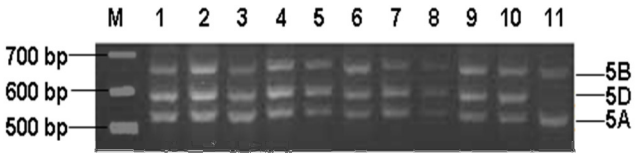

Fig. 2: The PCR products of ten common wheats (line 1-10) and *T. turgidum* Langdon (line 11). M, Marker; 1, Chinese Spring; 2, Chuanmai 36; 3, Chuanmai 28; 4, Chuanyu 12; 5, 87429; 6, 3854; 7, Yiyuan 2; 8, AS1510; 9, AS332; 10, AS908; 11, Langdon

Fig. 3: The PCR products of *Ae. tauschii*, Langdon (LDN) and its D-genome disomic substitution lines. M, Marker; 1, *Ae. tauschii*; 2, LDN; 3, LDN 1D(1A); 4, LDN 2D(2A); 5, LDN 3D(3A); 6, LDN 4D(4A); 7, LDN 5D(5A)+5AL; 8, LDN 5D(5B); 9, LDN 6D(6A); 10, LDN 7D(7A)

The band of 536 bp could be found within all the materials containing chromosome 5A (Fig. 3, Line 2-10). All the results revealed that the primers can efficiently distinguish *Q/q* genes from different homeologous chromosomes.

Discussion

The *Q* gene has been extensively studied as one of the most important gene of agriculture history. But most of the researches focus on the locus of chromosome 5A. Recently, the *Q* gene was proved also locating in the other two homeologous chromosomes 5B and 5D by southern hybridization (Simons *et al.,* 2006), which was further confirmed by our study. Follow-up studies on *Q* gene from these three homoeologous loci will be needed for further research of wheat, but the full length of *Q* gene from each locus is 3.2-3.4 kb, which makes it difficult to verify them from each other by PCR and electrophoresis. The specific molecular markers developed by our work could verify these three loci, then it can be applied on intensive research of *Q* gene as well as identify genetic materials involved the group 5 homoeologous chromosomes. *A. tauschii*, LDN and its disomic substitution line with chromosome 5D could be used on further research of *Q* gene from different locus and their interaction on phenotypic characteristics of wheat.

Conclusion

Molecular makers of three different wheat *Q* gene copies from homoeologous chromosomes 5A, 5B and 5D were developed in this study. These markers are useful for tracing different locus of *Q* gene and can also be used in the

identification of genetic materials involved in homoeologous group 5 chromosomes. And this knowledge will further facilitate the wheat breeding.

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