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Isolation and Characterization of A Novel *Glu-Bx* HMW-GS Allele from Tibet Bread Wheat Landrace

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Abstract: A novel HMW-GS of Bx6** , with slightly slower migration rate than that of Bx7 presented in wheat cultivar Chinese Spring, was found in a Tibet bread wheat landrace using SDS-PAGE. The gene for this subunit was isolated and its sequence was obtained. This gene was very similar to Bx7 both in nucleotide and deduced amino acid sequence. At the nucleotide sequence level, Bx6** different from Bx7 by the deletion of an 18 bp fragment and three nucleotides replacement at position 455 A/G, 2046 G/A and 2208C/G, respectively. At the deduced amino acid sequence level, the only difference is that Bx6** shorter than Bx7 by the deletion of a hexaploid peptide unit (PGQGKQ). These results suggested that Bx6** was a derivation of Bx7 and was formed by replication slippage.

Key words: Bread wheat, *Glu-B1*, x-type HMW-GS, sequence analysis, evolution relationship

INTRODUCTION

There are two types of important storage proteins in wheat seed endosperms: glutenins and gliadins. The glutenin components can be subdivided into high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS) and the gliadins can be classified into α, β, γ and ω components by their relatively molecular size and biochemical properties (Lawrence and Shepherd, 1981; Shewry *et al*., 1995). Among them, HMW-GSs account for approximately 10% of the total storage proteins in wheat endosperm, but their roles in determining the end-use quality of wheat flours could not be ignored (Shewry *et al*., 1995; Shewry and Halford, 2002).

In hexaploid wheat, HMW-GSs are encoded by the *Glu-1* complex loci, which situated on the long arms of homologous group one chromosomes (1A, 1B and 1D) and each locus encode two tightly linked genes (Lawrence and Shepherd, 1981). Due to the gene silencing and allelic variations, there are usually 3 to 5 HMW-GSs expressed in most of the hexaploid wheat cultivars (Lawrence and Shepherd, 1981; Payne, 1987). The numbers and the quality of superior functional HMW-GSs have the profound influence on the baking quality of wheat flours (Luo *et al*., 2001; Payne, 1987). To date, orthologous genes encoding for at least 40 HMW-GS alleles have been isolated and characterized from wheat and wild relative species (Anderson *et al*., 1989; De Bustos and Jouve, 2003; Forde *et al*., 1985; Guo *et al*., 2005; Halford *et al*., 1992; Li *et al*., 2004; Liu *et al*., 2003; Reddy and Appels, 1993; Shewry *et al*., 2003; Wan *et al*., 2005; Wang *et al*., 2004, 2006; Yan *et al*., 2002, 2006; Yang *et al*., 2006).

In hexaploid wheat, the *Glu-B1* locus encoded more HMW-GS alleles than *Glu-A1* and *Glu-D1* did (Shewry *et al*., 1995). Previous study showed that the *Glu-B1* locus encoded x-type genes can be

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divided into two subgroups: one subgroup with Bx7 and Bx17 and the other with Bx14 and Bx20 as representatives, respectively (Li *et al*., 2004; Robin *et al*., 1999). The former subgroup, like other typical x-type HMW-GS genes, had 3 conserved cysteine residue, while the latter had only one conserved cysteine residue in their N-terminal domains (Anderson and Greene, 1989; Li *et al*., 2004; Reddy and Appels, 1993; Shewry *et al*., 2003). Based on this character, the HMW-GS allele *Glu-B1x23* can be classified into the former subgroup (Yang *et al*., 2006). There are so many *Glu-Bx* alleles present in hexaploid and tetraploid wheats as revealed by SDS-PAGE (Shewry *et al*., 1995). However, the sequence information about these genes was still limited and thus impeded us for understanding their biochemical performances, quality potentials as well as evolution relationships.

In present previous study, a novel HMW-GS allele with abnormal migration rate, its protein designated as Bx6**, has been found in Tibet bread wheat landrace using SDS-PAGE (Yan *et al*., 2007). In the present study, we isolated and characterized the gene sequence of this allele. The results would help us understanding the evolution relationship between HMW-GS genes *Glu-B*x7 and *Glu-Bx6***.

MATERIALS AND METHODS

Plant Materials

This study was conducted in September 2006 at Dujiangyan City, Triticeae Research Institute of Sichuan Agricultural University. Tibet wheat landrace As1510 was used in this study. Two wheats, As1513 (HMW-GS: Dx2+Dy12, Bx6+By8) and Chinese Spring (CS) (HMW-GS: Dx2+Dy12, Bx7+By8), were use as controls.

SDS-PAGE Analysis

One half of wheat endosperm was crushed into powder and added the HMW-GS extraction buffer (4 mg sample/100 μL extraction buffer) extracted at room temperature for three hours. The extraction sample was heated in boiling water for 5 min to denature and then centrifuge at 10000 rpm for 5 min and 5 μL of the supernatant was used for separated HMW-GS on vertical SDS-PAGE gel (Yan *et al*., 2002).

DNA Extraction

Genomic DNA was extracted from the young leaves of As1510 by CTAB method (Yan *et al*., 2002).

Isolation and Sequencing of HMW-GS Gene Glu-Bx6**

A pair of oligonucleotide primers were used to amplify *Glu-Bx6***. The sequences were: BxF, 5'-ATG, GCT, AAG, CGC, CTG, GTC, CT-3'; BxR, 5'-AGC,TGC, AGA, GAG, TTC, TAT, CA-3'. The PCR conditions and parameters for amplification of HMW-GS genes of *Glu-Bx6*** were the same as described by our previous study (Yan *et al*., 2002). The PCR products were separated on 0.8% agrose gel and then the targeted DNA fragments were recovered and ligated into pMD18-T vector (China, Dalian, Takara company). The full coding sequences were acquired by sequencing three different resulting plasmids and a set of overlapping subclones, which were made by the nested deletion methods. The sequences were translated into amino acid sequences using the ORF (Open Reading Frame) finder program (http://www.ncbi.nlm.nih.gov/gorf/gorf.html).

Phylogenetic Analysis of Amino Acid Sequences

The phylogenetic tree was constructed using the signal and the N terminal amino acid sequences by the MEGA program using the Neighbor-Joining (NJ) method (Kumar *et al*., 2001). During the

analysis, the complete deletion option was used with respect to gaps in the aligned sequence and the evolutionary distances were measured by calculating *p*-distances for each pair of aligned sequences. The bootstrap value was estimated based on 2000 replications using the same software.

RESULTS AND DISCUSSION

The HMW-GS allelic variations encoded by the genes presented in the *Glu-D1*, *Glu-B1* and *Glu-A1* loci have been found in wheat (Shewry *et al*., 1995). However, In the *Glu-B1* locus, there are more HMW-GS variations have been documented (Shewry *et al*., 1995). In SDS-PAGE analysis, it was showed that a novel *Glu-Bx* allele presented in Tibet bread wheat landrace As1510. The electrophoresis migration rate of this *Glu-Bx* HMW-GS lain between Bx7 and Bx6, slightly slower than Bx7, we designated its protein as Bx6** (Fig. 1).

In the PCR amplification experiment, two DNA bands, with approximately 2.3 and 1.8 Kb, respectively, were amplified by using the total genomic DNA of As1510 (Fig. 2). The larger DNA fragment of 2.3Kb, with approximately the same size of Bx6**, was recovered and the 1.8Kb non-specific amplification DNA fragment was omitted. The complete gene sequence of Bx6** was obtained by sequencing three different resulting clones and a series of subclones, which were made by nested deletion methods (Sambrook *et al*., 1989).

The complete gene sequence of *Glu-Bx6*** was 2355 bp in nucleotide length and 783 amino acid residues in deduced protein sequence and deposited in GenBank under accession number EU287439. *Glu-Bx6*** had the similar primary structure with that of the published HMW-GSs by containing a 21 amino acid leader peptide and a conservative N and C terminal flanking by repetitive domains. The N and C terminal domain and the repetitive domains of HMW-GS *Glu-Bx6*** consist of 81, 21 and 639 amino acid residues, respectively.

After retrieve *Glu-Bx6*** gene sequence in the NCBI net service (http://www.ncbi.nlm.nih.gov/blast), it was indicated that *Glu-Bx6*** was a Bx gene. The protein sequence of *Glu-Bx6*** was very similar to that of Bx7 in hexaploid wheat. Therefore, the sequences of both nucleotide and amino acid sequence were further compared between *Glu-Bx6*** and Glu-Bx7, respectively.

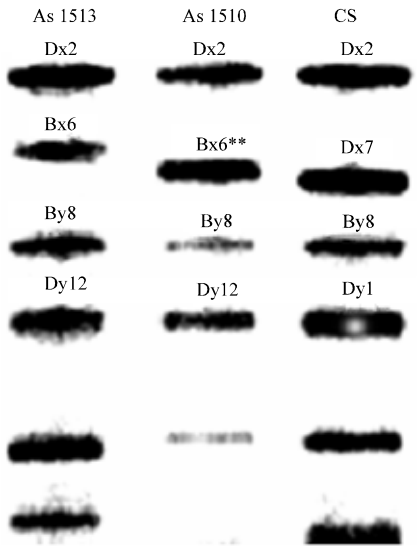

Fig. 1: SDS-PAGE analysis of the novel HMW-GS Bx6** in As1510

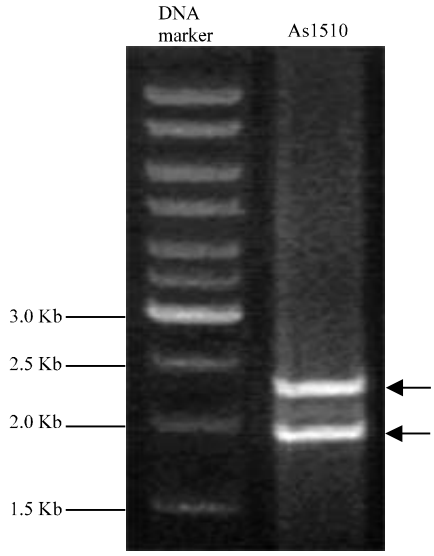

Fig. 2: PCR amplification of the HMW-GS gene Bx6** (long arrowhead) in As1510, the short arrowhead indicated the non-specific amplification band

In bread wheat, there were so many HMW-GS allelic variations presented in it (Shewry *et al*., 1995). Some of genes corresponding to these allelic variations have been sequenced and characterized (Anderson and Greene, 1989; Anderson *et al*., 1989; De Bustos and Jouve, 2003; Forde *et al*., 1985; Halford *et al*., 1987; Yang *et al*., 2006). Sequence alignments among the nucleotide and the deduced amino acid sequences, respectively, have revealed the general high degree of identity in their sequences and the presence of the short deletions and/or insertions within their repetitive domains (Anderson and Greene, 1989; Anderson *et al*., 1989; De Bustos and Jouve, 2003; Forde *et al*., 1985; Halford *et al*., 1987; Yang *et al*., 2006). The comparison data showed that the nucleotide sequence of *Glu-Bx6*** shorter than that of *Glu-Bx7* by the deletion of an eighteen nucleotides DNA fragment with the sequence of AAACAACCAGGACAAGGA in its repeptitive domain. Besides, there were three single base nucleotide replacements at position 455 A/G, 2046 G/A and 2208C/G, respectively (Fig. 3a). In the deduced protein sequence, the only difference was that Bx6** shorter than Bx7 by the deletion of a hexpeptide unit (PGQGKQ) in its repeptitive domain (Fig. 3b).

Several mechanisms, such as replication slippage, transposition, gene conversion, or unequal crossing over, can cause the gene length modification of the wheat HMW-GS genes (D'Ovidio *et al*., 1996; Shewry *et al*., 2003). In the *Glu-D1* locus, the complete sequences of the genes of HMW-GS Dx5, Dx2, Dx2.2 and Dx2.2*, etc, have been obtained and characterized (Anderson *et al*., 1989; Sugiyama *et al*., 1985; Thomson *et al*., 1985; Wan *et al*., 2005). It is suggested that the Dx2.2 and Dx2.2* genes were the derivation of Dx2. The comparative analysis among these gene sequences have indicated that the large size of Dx2.2 and Dx2.2* is due to the duplication event within the repetitive domain of Dx2 (Wan *et al*., 2005). Based on present study, it is suggested that the replication slippage may responsible for the shorter sequence of Bx6** in comparison with Bx7.

The high similarity between Bx6** and Bx7 both in nucleotide and deduced amino acid residues suggested that the novel Bx6** allele was probably derived from Bx7 and could be then added to the subgroup of Bx7 and Bx17 but not the subgroup of Bx14 and Bx20 (Fig. 4).

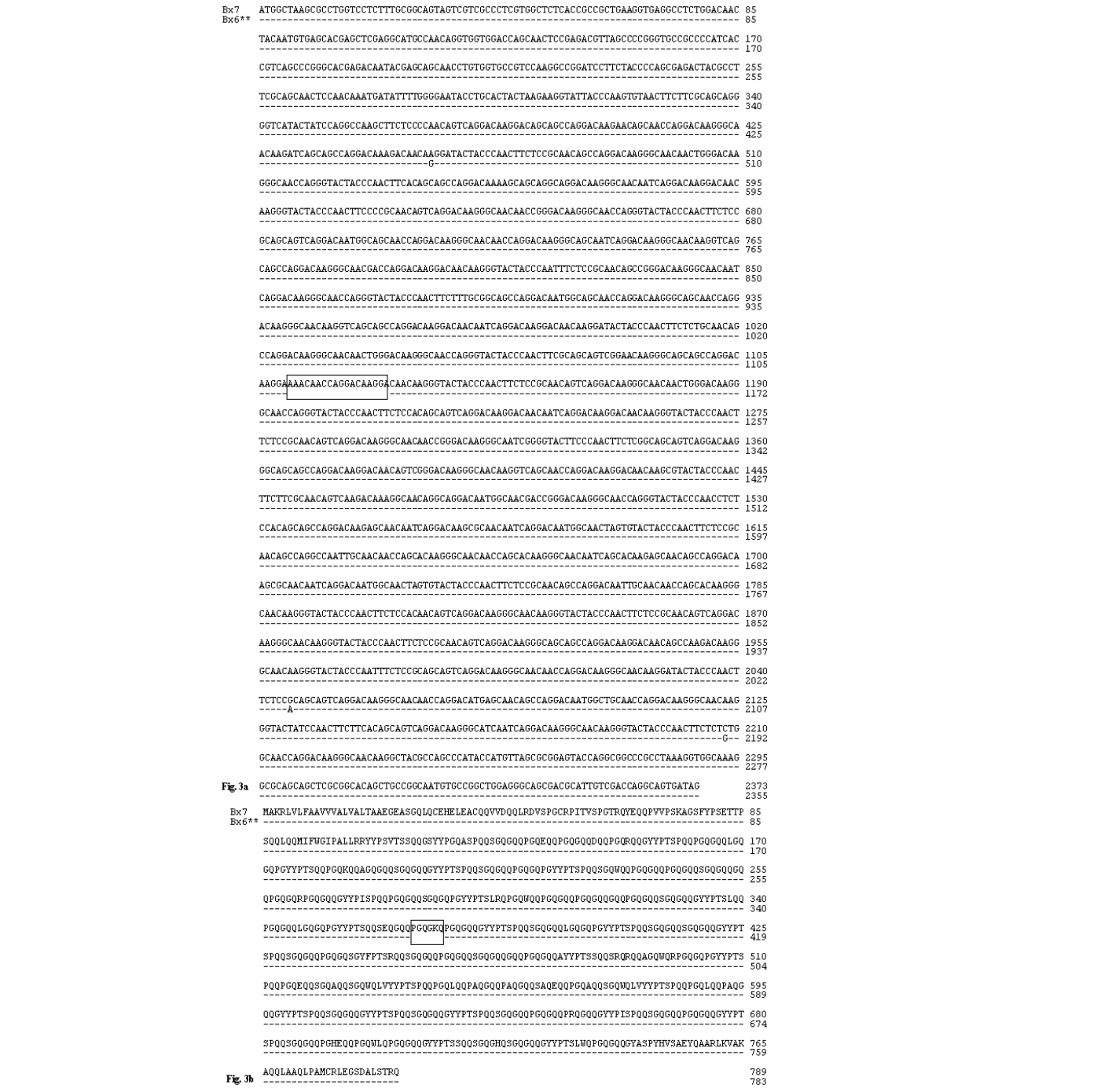

Fig. 3: Comparison of nucleotide (a) and deduced amino acid sequence (b) of HMW-GS gene between Bx7 and Bx6***. The Genbank accession number for Bx7 and Bx6*** were X13927 and EU287439, respectively. The Short bars indicated the amino acids or nucleotide were the same for two genes, while boxes indicate the deletions in nucleotide or amino acid

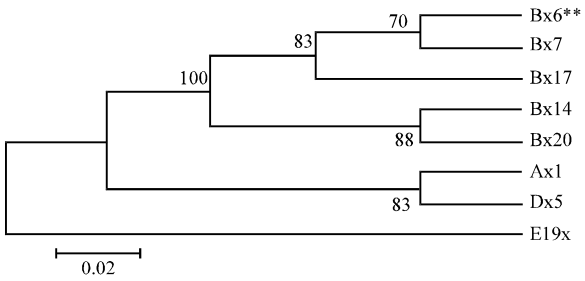

Fig. 4: Phylogenetic relationship of deduced amino acid sequence of HMW-GS gene Bx6** with previously characterized genes representatives, The genes are *Glu-B1-1* (Bx7, Bx17, Bx14 and Bx20), *Glu-A1-1* (represented by 1Ax1) and *Glu-D1-1* (represented by 1Dx5) alleles. The *Glu-E1-1* of *E. elongata* E19x (as an outgroup). The Genbank accession numbers for Bx6**, Bx7, Bx17, Bx14, Bx20, Ax1, Dx5, E19x were EU287439, X13927, JC2099, AY367771, AJ437000, X61009, X12928 and AY299520, respectively. The bootstrap values were calculated based on 2000 replications

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