ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2014) **12**(2); 191–198
doi:10.1017/S1479262113000476

Characterization of HMW glutenin subunit $Bx7^{OE}$ and its distribution in common wheat and related species

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Received 7 August 2013; Accepted 4 October 2013 – First published online 29 October 2013

Abstract

The overexpression of wheat Bx7 subunit ($Bx7^{OE}$) encoded by the *Glu-B1al* allele is originated from a duplication event of the *Bx7* gene, and has a positive effect on gluten strength. Thus, it is an important genetic resource for wheat quality improvement. In this study, the $Bx7^{OE}$ subunit from a large number of bread wheat and related species was characterized by sodium dodecyl sulphate–polyacrylamide gel electrophoresis, reversed-phase high-performance liquid chromatography (RP-HPLC) and Sequence-Tagged sites (STS) markers. Only 31 bread wheat varieties were found to carry $Bx7^{OE}$. RP-HPLC quantification analysis revealed that the mean proportion of the $Bx7^{OE}$ subunit to the total amount of high-molecular-weight glutenin subunits among the 31 bread wheat varieties was 41.8%, which is much higher than that of varieties with the normal Bx7 subunit (generally at 30%). Flour quality analysis of seven representative varieties with $Bx7^{OE}$ and three with the normal Bx7 subunit showed that the varieties with $Bx7^{OE}$ generally displayed better gluten strength than those with the normal Bx7 subunit. STS markers demonstrated that, in addition to the 31 bread wheat varieties with $Bx7^{OE}$, no PCR products were obtained from the related *Triticum* and *Aegilops* species. This suggests that the retroelement-mediated recombination event at the *Glu-B1* locus could have occurred more recently, later than the formation of hexaploid wheat. The $Bx7^{OE}$ subunit is mainly distributed in some bread wheat varieties from American countries with a low frequency, which is of particular importance for the quality improvement of wheat gluten.

Keywords: quality improvement; RP-HPLC; SDS–PAGE; STS markers; *Triticum aestivum* L

Introduction

Wheat storage proteins, mainly composed of polymeric glutenins and monomeric gliadins, primarily determine the processing quality of wheat flour with its unique viscoelastic properties for the production of bread and other food products (Shewry *et al.*, 1992). High-molecular-weight glutenin subunits (HMW-GS), which are the important components of glutenins, play a key role in governing bread-making ability to form large polymeric structures through disulphide bonds (Wrigley, 1996).

HMW-GS are encoded by tightly linked x-type and y-type genes at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci on the long arms of chromosomes 1A, 1B and 1D, respectively (Payne, 1987). Three encoding loci, especially for *Glu-B1*, showed extensive allelic variations, and at least 20 HMW-GS alleles were identified and catalogued in bread wheat (Payne and Lawrence, 1983). Considerable work has shown that allelic variations at the *Glu-1* loci are strongly related to the processing quality of wheat flour, even though HMW-GS account for only 8–10% of

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the total protein in wheat grains (Branlard and Dardevet, 1985; Payne *et al.*, 1988; Lukow *et al.*, 1992; He *et al.*, 2005). In addition, the high expression of some HMW-GS also has important effects on grain quality (Lukow *et al.*, 1992; Wang *et al.*, 2013). Particularly, the overexpression of the HMW Bx7 subunit ($Bx7^{OE}$) in wheat cultivars and landraces, designated as *Glu-B1al*, has shown a strong correlation with improved dough strength (Lukow *et al.*, 1992; Marchylo *et al.*, 1992; D'Ovidio *et al.*, 1997; Lerner *et al.*, 2003).

In terms of the overexpression mechanisms of the HMW Bx7 subunit, Lukow *et al.* (1992) found two functional copies presented in the gene encoding the Bx7 subunit by Southern blot analysis. The simultaneous expression of the two copies leads to the overproduction of the Bx7 subunit, which was also supported by D'Ovidio *et al.* (1997). Afterwards, a Bacterial Artificial Chromosome (BAC) clone encompassing the *Glu-B1* locus from the wheat cultivar Glenlea was sequenced, and a 10.3 kb duplication including the gene encoding the Bx7 subunit was identified (Cloutier *et al.*, 2005). Two STS markers, designed based on the structural organization of the locus, could specifically amplify the right and left junction sequences of a long-terminal-repeat (LTR) retroelement that lies between the duplicated areas (Ragupathy *et al.*, 2008; Ren *et al.*, 2009).

In this study, the overexpression feature of the Bx7 subunit from a large number of wheat collections was characterized by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE), reversed-phase high-performance liquid chromatography (RP-HPLC) and STS markers, and its effects on flour quality were further investigated. Based on these analyses, the distribution and the evolutionary origin of *Glu-B1al* in *Triticum* and related species were investigated. Our results provide useful information for further exploitation and utilization of $Bx7^{OE}$ genetic resource for wheat quality improvement.

Materials and methods

Plant materials

The materials used in this study included the diploid, tetraploid and hexaploid *Triticum* and *Aegilops* species that were mainly collected from the Chinese Academy of Agricultural Sciences (CAAS), the national gene pools of the USA and Germany and the International Wheat and Maize Improvement Centre (CIMMYT). The diploid accessions contained 32 *Aegilops speltoides* (SS), 30 *Aegilops searsii* (S^sS^s), 26 *Aegilops longissima* (S^lS^l), 28 *Aegilops markgrafii* (CC), 24 *Aegilops comosa* (MM), 22 *Aegilops uniaristata* (NN), 20 *Aegilops umbellulata* (UU), 52 *Aegilops tauschii* (D^tD^t), 35 *Triticum monococcum* (A^mA^m) and 27 *Triticum urartu* (A^uA^u). Four tetraploid *Triticum* species included 205 *Triticum dicoccum* (AABB), 120 *Triticum dicoccoides* (AABB), 45 *Triticum durum* (AABB) and *Triticum timopheevii* (AAGG) accessions. The hexaploid common wheat varieties and landraces (AABBDD) included 130 club wheat (*Triticum compactum*), 270 spelt wheat (*Triticum spelta*), 23 *Triticum macha*, 21 *Triticum sphaerococcum*, 135 synthetic wheat and 325 bread wheat mainly from China, Europe, North and South America and Australia (Table 1).

SDS–PAGE

HMW-GS were extracted from wheat grains using a modified method proposed by Gao *et al.* (2010). Identification of $Bx7^{OE}$ by SDS–PAGE was carried out as described previously by Yan *et al.* (2003a).

RP-HPLC

RP-HPLC analysis of HMW-GS was as described by Dong *et al.* (2009) and performed on an Agilent 1100 using a Zorbax 300SB-C18 column (300 Å pore size and 5 μm particle size). The solvents (A) water and (B) acetonitrile both contained 0.06% (v/v) trifluoroacetic acid and filtered (0.45 μm) and degassed before use. For the analyses, 5 μl for each sample were injected. Proteins were eluted at 1 ml/min using a gradient from 21 to 48% B over 65 min, running for 20 column volumes. The column was maintained at 50°C and the proteins were monitored at 210 nm.

STS marker analysis

Genomic DNA extraction from dry seeds and PCR amplifications were as described previously by An *et al.* (2006). Two pairs of allele-specific PCR primers were used to specifically detect $Bx7^{OE}$ (Ragupathy *et al.*, 2008). Two STS markers flanking the LTR retrotransposon borders and the duplicated region were designed at the left and right junctions of the retroelement. The left-junction primers were 5′-ACGTGTCCAAGCTTTGGTTC-3′ and 5′-GATTGGTGGGTGGATACAGG-3′, and the right-junction primers were 5′-CCACTTCCAAGGTGGGACTA-3′ and 5′-TGCCAACACAAAAGAAGCTG-3′. The PCR conditions for the right and left junction markers of the retroelement were according to Ragupathy *et al.* (2008). The PCR products were analysed by 1% agarose gel electrophoresis in Tris–acetic acid–EDTA buffer, and stained with ethidium bromide and visualized under ultraviolet light.

https://doi.org/10.1017/S1479262113000476 Published online by Cambridge University Press

Table 1. Identification of HMW-GS from bread wheat and related species by SDS–PAGE, RP-HPLC and STS markers

Species	Genome	No. of accessions					Proportion of Bx7 subunit to total HMW-GS (%)
		Surveyed	Overexpressed HMW-GS	Bx7	Bx7^{OE}	Left and right LTR junction	
Aegilops speltoides	SS	32	0	0	0	0	–
Aegilops searsii	S^sS^s	30	0	0	0	0	–
Aegilops longissima	S^lS^l	26	0	0	0	0	–
Aegilops markgrafii	CC	28	0	0	0	0	–
Aegilops comosa	MM	24	0	0	0	0	–
Aegilops uniaristata	NN	22	0	0	0	0	–
Aegilops umbellulata	UU	20	0	0	0	0	–
Aegilops tauschii	D^tD^t	52	0	0	0	0	–
Triticum monococcum	A^mA^m	35	0	0	0	0	–
Triticum urartu	A^uA^u	27	0	0	0	0	–
Triticum dicoccum	AABB	205	0	27	0	0	56.2 ± 3.6
Triticum dicoccoides	AABB	120	0	46	0	0	57.4 ± 3.1
Triticum durum	AABB	45	0	24	0	0	56.8 ± 2.8
Triticum timopheevii	AAGG	20	0	0	0	0	–
Triticum compactum	AABBDD	130	0	67	0	0	31.6 ± 3.6
Triticum spelta	AABBDD	270	0	47	0	0	30.4 ± 4.3
Triticum macha	AABBDD	23	0	14	0	0	29.7 ± 4.7
Triticum sphaerococcum	AABBDD	21	0	12	0	0	30.3 ± 4.3
Synthetic hexaploid wheat	AABBDD	135	0	42	0	0	31.4 ± 3.9
Triticum aestivum	AABBDD	325	31	120	25	25	41.8 ± 4.6

Gluten quality test

Gluten quality parameters were tested at Wheat Quality Laboratory, CAAS. Cleaned grain samples were pulverized using a Buhler experimental mill. A 10 g Mixograph (National Manufacturing) was performed to evaluate the quality properties of dough based on the procedure described by Yan *et al.* (2009). The Mixograph assays were performed by the 54-40A American Association of the Cereal Chemists method (AACC, 2000), and the results were automatically processed, mapped and displayed by Mixsmart software.

Results

Characterization of the overexpressed Bx7 subunit in bread wheat cultivars

A large number of bread wheat and related species collections listed in Table 1 were initially analysed by SDS–PAGE. The materials that were confirmed to carry the Bx7 subunit were further identified by RP-HPLC and STS markers. According to the relative proportion of the Bx7 subunit to total HMW-GS and the presence of the left and right LTR junctions, only 31 varieties among the 325 bread wheat varieties (120 with the Bx7 subunit) were found to have the Bx7^{OE} subunit, and most of them originated from the American countries. Of the 31 varieties with the Bx7^{OE} subunit, ten were from Canada (AC Vista, Bigger, Blue Sky, Burnside, Glenavon, Glenlea, Laura, Roblin, Oslo and Wild Cat), eight from Argentina (Buck Pucara, Calidad, El Gaucho, Klein Atlas, Klein Sendero, Pampa INTA, Retacon INTA and Victoria INTA), three from the USA (Red River 68, Nordic and Prospur), three from Australia (CD87, Kukri and Chara), two from Mexico (Bajio and Kanchan), two from Uruguay (Klein Credito and Olaeta Calandria), one from Brazil (Toropi), one from Portugal (Branco) and one from China (Demai 3), respectively.

The mean proportion of the Bx7 subunit to the total amount of HMW-GS among the 31 overexpressed Bx7 varieties was 41.8% (Table 1), which was much higher than that of the normal Bx7 subunit (generally about 30%). In addition to the Bx7^{OE} subunit present in the 31 bread wheat varieties, no other overexpressed HMW-GS among the three encoding loci were found. This suggests that *Glu-B1al* is likely to be originated from a rare duplication event that occurred only in bread wheat varieties.

The HMW-GS of seven representative bread wheat varieties (Olaeta Calandria, Demai 3, Bajio, Laura, Victoria INTA, Wild Cat and Calidad) with Bx7^{OE} as well as three varieties with the normal Bx7 subunit identified by SDS–PAGE are shown in Fig. 1. Although the sensitivity of SDS–PAGE was relatively low, the differences in expression between the normal and overexpressed Bx7 subunits could be easily differentiated on the gel. The HMW-GS compositions and the relative quantification proportion of the Bx7 subunit to the total

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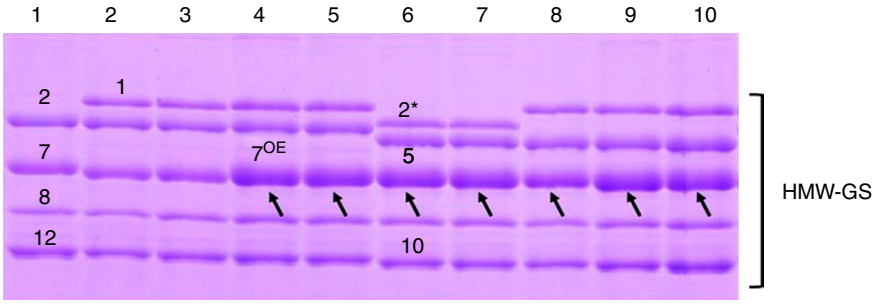

Fig. 1. SDS–PAGE of HMW-GS from the ten common wheat varieties. The arrows indicate the Bx7^{OE} subunit. 1. CS, 2. Sinvalocho, 3. Xinchun 15, 4. Olaeta Calandria, 5. Demai 3, 6. Bajio, 7. Laura, 8. Victoria INTA, 9. Wild Cat and 10. Calidad.

amount of HMW-GS in ten bread wheat varieties were determined by RP-HPLC, as shown in Fig. 2 and Table 2. According to the results summarized by Ragupathy *et al.* (2008), a variety could be defined as Bx7^{OE} if the proportion of this subunit to the total amount of HMW-GS was more than 36%. The proportion of Bx7^{OE} from the seven cultivars ranged from 38.8 to 42.3%, while that from the three normal cultivars (Chinese Spring (CS), Sinvalocho and Xinchun 15) were 26.9–32.8%, which was well corresponding to the results identified by SDS–PAGE (Fig. 1).

The *Glu-B1* locus of the wheat cultivar Glenlea was sequenced (Cloutier *et al.*, 2005). The tandem duplication of 10.3 kb comprised two copies of the *Bx7* gene and

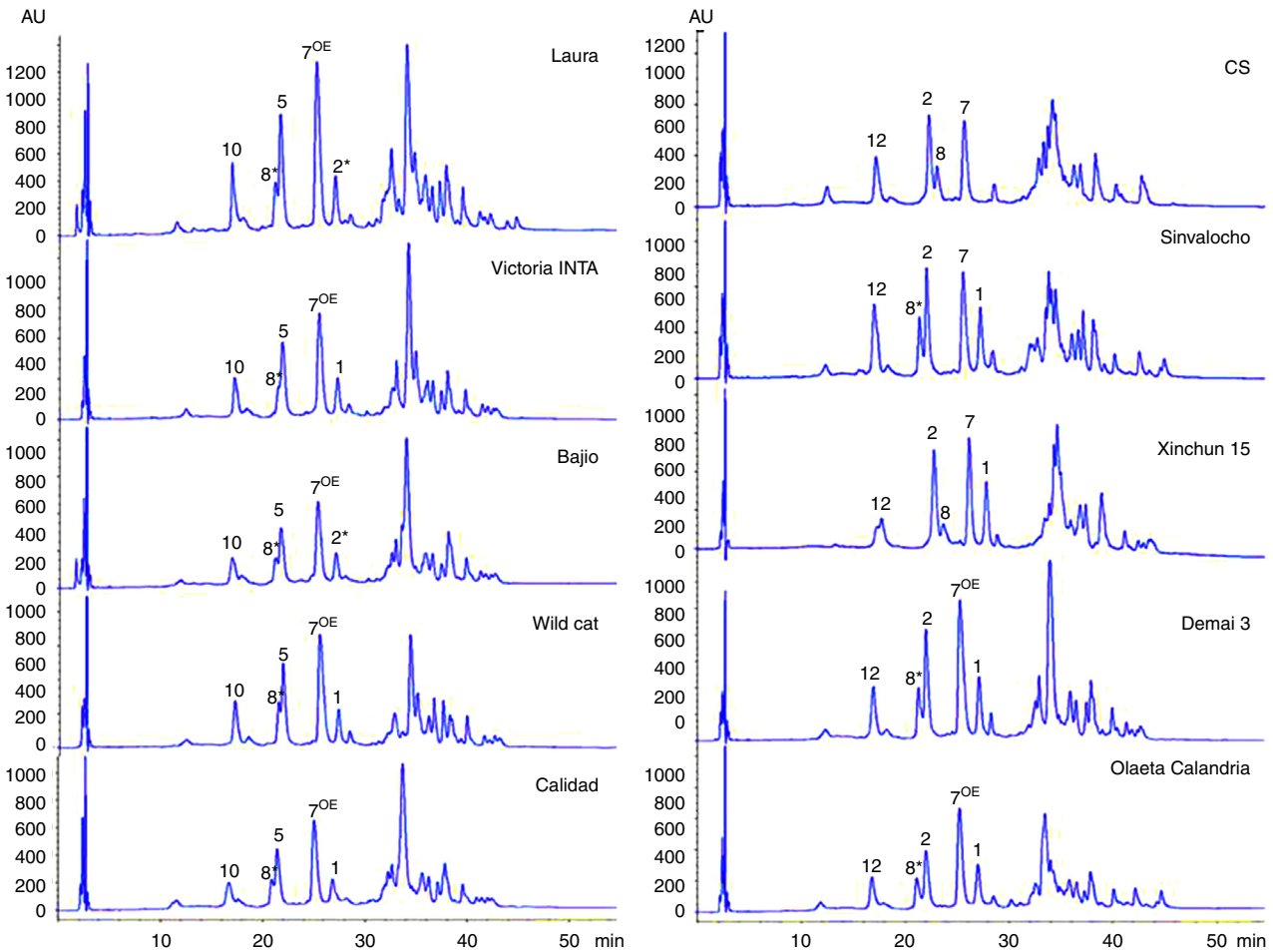

Fig. 2. RP-HPLC profiles of HMW-GS from the ten common wheat varieties. The composition of HMW-GS in each variety is indicated.

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Table 2. HMW-GS compositions and relative proportion of Bx7 subunit to total HMW-GS in the ten common wheat cultivars determined by RP-HPLC

Cultivars	Origin	*Glu-A1*	*Glu-B1*	*Glu-D1*	Proportion of Bx7 subunit to total HMW-GS (%)
CS	China	N	7 + 8	2 + 12	32.8 ± 1.2
Sinvalocho	Argentina	1	7 + 8	2 + 12	26.9 ± 1.3
Xinchun 15	China	1	7 + 8	2 + 12	27.4 ± 1.8
Olaeta Calandria	Uruguay	1	7^{OE} + 8	2 + 12	38.8 ± 1.4
Demai 3	China	1	7^{OE} + 8	2 + 12	39.1 ± 0.9
Bajio	Mexico	2*	7^{OE} + 8	5 + 10	39.3 ± 1.1
Calidad Victoria	Argentina	1	7^{OE} + 8	5 + 10	40.0 ± 1.7
INTA	Argentina	1	7^{OE} + 8	5 + 10	40.0 ± 1.6
Wild Cat	Canada	1	7^{OE} + 8	5 + 10	41.9 ± 1.1
Laura	Canada	2*	7^{OE} + 8	5 + 10	42.3 ± 1.3

flanked a complete and a partial LTR retroelement. According to this structural organization, Ragupathy *et al.* (2008) designed two pairs of STS primers that can specifically detect the *Bx7*^{OE} gene. In this study, both STS markers were used to further detect the presence of the Bx7^{OE} subunit identified by SDS–PAGE and RP-HPLC. As shown in Fig. 3, two fragments of 447 and 844 bp specific for the *Bx7*^{OE} gene were amplified in all the seven typical varieties containing Bx7^{OE}, confirming that these varieties contain two duplication events of the *Bx7* gene.

Effect of the Bx7^{OE} *subunit on flour quality parameters*

As shown in Table 2, the seven representative varieties with Bx7^{OE} mainly originated from the American countries and three with normal Bx7 had similar HMW-GS compositions. The Mixograph quality parameters of these ten varieties were tested, and the results are shown in Table 3. The peak time (min) was used to test the time when dough reached maximum resistance, and the midline peak integral (%torque × min), also called area at 8 min, referred to the energy input in 8 min and indicated a synthetic indicator according to dough strength and rubbing resistance. The width at 8 min (mm) could determine the rubbing resistance of the dough. The cultivars with a larger value of peak time, peak integral and width at 8 min could have strong gluten and better flour quality. According to the results as well as gluten types estimated by the values of these Mixograph parameters, all varieties with Bx7^{OE} except for Olaeta Calandria displayed strong or medium strong gluten quality (Table 3), generally better than those with the normal Bx7 subunit. This further supports previous reports that wheat cultivars with an over-expressed Bx7 subunit generally have superior gluten quality (Radovanovic *et al.*, 2002; Butow *et al.*, 2003).

Distribution of the Bx7^{OE} *subunit in* Triticum *and related* Aegilops *species as revealed by STS markers*

Both Bx7^{OE} STS markers were used to detect whether the LTR retroelement-mediated duplication at the *Glu-B1* locus was present in *Triticum* and related species, as listed in Table 1 and Fig. S1 (available online). The results demonstrated that there were no accessions containing the left and right junction markers except for the

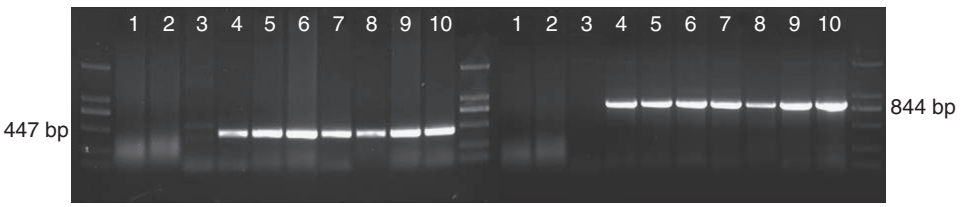

Fig. 3. PCR amplification of the ten common wheat varieties with two STS markers. A 447 bp amplicon yielded by the amplified primers of the left junction of the retroelement and an 844 bp amplicon by the amplified primers of the right junction are shown. 1. CS, 2. Sinvalocho, 3. Xinchun 15, 4. Olaeta Calandria (Bx7^{OE}), 5. Demai 3 (Bx7^{OE}), 6. Bajio (Bx7^{OE}), 7. Laura (Bx7^{OE}), 8. Victoria INTA (Bx7^{OE}), 9. Wild Cat (Bx7^{OE}), 10. Calidad (Bx7^{OE}).

https://doi.org/10.1017/S1479262113000476 Published online by Cambridge University Press

Table 3. Quality parameters of the ten common wheat varieties

Parameters	Protein content (%)	Peak time (min)	Midline peak integral (%torque × min)	Midline right slope (%/min)	Width at 8 min (mm)	Gluten type
CS	15.30 ± 0.21	2.25 ± 0.05	72.04 ± 1.12	−2.34 ± 0.06	6.10 ± 0.08	Weak gluten
Sinvalocho	15.80 ± 0.23	1.76 ± 0.03	69.91 ± 0.91	−2.68 ± 0.08	6.76 ± 0.15	Weak gluten
Xinchun 15	14.40 ± 0.17	2.42 ± 0.04	136.30 ± 1.82	−2.55 ± 0.05	16.83 ± .051	Medium gluten
Bajio	14.10 ± 0.15	4.93 ± 0.09	166.75 ± 2.53	−2.61 ± 0.06	18.23 ± 0.44	Strong gluten
Laura	15.8 ± 0.24	3.72 ± 0.06	166.35 ± 2.66	−2.85 ± 0.07	15.92 ± 0.23	Medium strong gluten
Victoria INTA	14.30 ± 0.18	5.96 ± 0.11	237.66 ± 3.77	−0.76 ± 0.01	26.92 ± 0.88	Strong gluten
Wild Cat	16.30 ± 0.19	3.30 ± 0.06	161.40 ± 2.43	−2.05 ± 0.06	15.96 ± 0.45	Medium strong gluten
Calidad	15.7 ± 0.14	4.01 ± 0.11	171.18 ± 2.56	−1.36 ± 0.04	22.33 ± 0.75	Strong gluten
Demai 3	15.80 ± 0.11	3.10 ± 0.05	131.91 ± 1.76	−2.44 ± 0.07	18.39 ± 0.32	Medium strong gluten
Olaeta Calandria	14.50 ± 0.13	2.82 ± 0.07	94.42 ± 1.12	−2.06 ± 0.04	12.20 ± 0.36	Medium gluten

31 bread wheat varieties, which was well consistent with the identification results by SDS–PAGE and RP-HPLC. The S, S^s and S^l genomes of *Aegilops*, as potential ancestors of the B genome and the B genome of related tetraploid *Triticum* species (*T. durum*, *T. dicoccoides* and *T. dicoccum*) and synthetic hexaploid wheat varieties from durum wheat × *Aegilops tauschii*, did not possess the structure of the LTR retroelement-mediated duplication. These results suggest that the $Bx7^{OE}$ variant occurred only in bread wheat varieties with a lower frequency, which is mainly present in a few bread wheat varieties from the American countries.

Discussion

Association of allelic variations and expression at the Glu-1 *loci with gluten quality*

SDS–PAGE is a traditional method for the separation and identification of HMW-GS in wheat varieties. An overexpressed Bx7 subunit can be visualized by a relatively higher-intensity staining (Lukow *et al.*, 1989). However, this method is not precise and sensitive in the quantitative aspect. Allelic variation at the locus encoding the Bx7 subunit among different varieties can be quantitatively detected by RP-HPLC (Marchylo *et al.*, 1992; Butow *et al.*, 2004). Molecular markers can also detect the corresponding alleles with a lower cost, providing a much more convenient tool for rapid genetic analyses (Pagnotta *et al.*, 1995).

Some investigations of the effects of Bx7 and $Bx7^{OE}$ at the *Glu-B1* locus on dough strength have been reported. Marchylo *et al.* (1992) suggested that the Bx7 subunit might be associated with greater dough strength and decreased extractability of gluten proteins. Some genetic variability for gluten strength was accounted for the overexpression of the Bx7 subunit originating from the cultivar Glenlea-derived line 87E03-S2B1 (Radovanovic *et al.*, 2002). A strong association between the overexpression of Bx7 and high dough strength was present (Butow *et al.*, 2003). In addition, there was an additional impact of Glu-D1 alleles on dough properties, with lines containing both the overexpressed Bx7 subunit and Glu-D1 5 + 10 having the highest levels of dough strength. Cornish *et al.* (2005) also found that wheat varieties with both the overexpressed Bx7 subunit and the 5 + 10 subunits produced extra-strong dough properties. However, there was no statistically significant epistatic interaction between the Glu-B1 and Glu-D1 loci. The cultivars with $Bx7^{OE}$ possessed a significantly higher ($P < 0.001$) proportion of HMW-GS (56.80 ± 3.25%) encoded by the B genome, suggesting that the proportion of *Glu-B1* subunits relative to the total amount of the expressed HMW-GS had a major effect on dough strength (Vawser and Cornish, 2004). A recent report has also found that two novel HMW-GS from the S^l genome of *Aegilops longissima* had a higher expression level in the CS substitution line and led to significantly improved gluten strength (Wang *et al.*, 2013). In this study, six varieties with $Bx7^{OE}$ displayed a strong or medium strong gluten property (Table 3), demonstrating the positive effect of HMW-GS overexpression on flour quality in addition to allelic variations of the *Glu-1* loci.

Evolutionary origin of the Glu-B1al *allele*

Among the three *Glu-1* loci, the *Glu-B1* locus displayed the most extensive allelic variations in bread and spelt wheat varieties (Gianibelli *et al.*, 2001; Yan *et al.*, 2003b). The extensive allelic variations at the *Glu-1* loci mainly resulted from single nucleotide polymorphisms and insertions/deletions (InDels), probably through unequal crossing over, slip mismatching, point mutations and illegitimate recombination (Zhang *et al.*, 2008).

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These allelic variations in HMW-GS provide rich genetic resources for wheat quality improvement. The modern hexaploid wheat is an allohexaploid species with genomes A, B and D and an extremely large and complex genome up to 17,000 Mb. Genetic and phylogenetic studies have revealed that *T. urartu* and *Ae. tauschii* are A and D genome donors of hexaploid wheat, respectively, while the B genome is probably originated from the S genome of *Ae. speltoides* (Dvořák *et al.*, 1988, 1990; Petersen *et al.*, 2006). Hexaploid wheat is the product of the hybridization between tetraploid wheat (AABB) and diploid goat grass (DD), which took place about 10,000 years ago (Zhang *et al.*, 2008).

According to our results, the *Glu-B1al* allele (Bx7^{OE}) was not found in wheat-related species through the analysis from a large number of collections, including spelt, club (*Triticun compactum*), dot (*Triticum sphaerococcum*) and durum wheat varieties, synthetic hexaploid wheat varieties from crossing between durum and *Ae. tauschii* as well as *T. dicoccum*, *T. dicoccoides* and several diploid *Aegilops* species carrying the S genome (Fig. S1, available online). The *Glu-B1al* allele appears to be present only in a few bread wheat varieties with a much low frequency (Table 1). This suggests that the *Glu-B1al* allele is originated from a rare mutation event. The retroelement-mediated recombination event at the *Glu-B1* locus could have occurred more recently, later than the formation of hexaploid wheat. This result also provides the evidence for the role of retroelements on the evolution of agriculturally important loci.

The recent report on the analysis of the bread wheat genome has revealed that the gene family size is generally decreased in concurrent with domestication, whereas the gene family size for certain genes associated with crop productivity (defence response, energy metabolism, growth) and grain quality such as nutritional content and storage proteins is increased (Brenchley *et al.*, 2012). Presumably these increases have been unconsciously selected during cultivation over the thousands of years since the species was formed to constitute a part of the co-evolutionary history of cereals and humans (Gill *et al.*, 2004). The *Glu-B1al* allele that has originated very recently with a lower frequency may provide an example to support this evolutionary trend. This suggests that the retroelement-mediated recombination may play important roles in the increase of gene family size in bread wheat during the evolutionary process.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262113000476

Acknowledgements

We are grateful to Dr Xianchun Xia and Dr Yan Zhang from the Institute of Crop Science, CAAS for constructive suggestions in reviewing the manuscript and flour quality analysis, respectively. This research was financially supported by grants from the National Natural Science Foundation of China (31271703 and 31101145), the China–Australia Cooperation Project from the Chinese Ministry of Science and Technology (2013DFG30530) and the National Key Projects for Transgenic Crops of China (2011ZX08009-003-004).

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