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Distribution of low-molecular-weight glutenin subunit Glu-B3 alleles in mini core collections of Chinese wheat germplasms

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Abstract

Low-molecular-weight glutenin subunit (LMW-GS) Glu-B3 plays an important role in the processing quality of the wheat used to make bread and noodles. A total of 214 accessions of common wheat (*Triticum aestivum* L.) comprising both landraces and improved cultivars collected from all the three growing zones in China were tested for ten alleles using 10 allele-specific PCR markers at the Glu-B3 locus. The frequency of the 'i' allele (20.6%) was greater than those of the other alleles. The 'i' allele was found in 18.7% of landraces and 23.8% of improved cultivars. The alleles, from most to least common, were 'i'>'a'>'d'>'g'>'f'>'b'>'e'>'c'>'j'>'h.' In the spring wheat zone, the Glu-B3 alleles were mainly 'g' and 'i,' but in the winter wheat zone, they were mainly 'a' and 'i.' The 'h' allele was only found in the spring wheat zone, but the 'a' and 'c' alleles were found in small proportions in the spring wheat and spring-winter wheat zones. The frequency of the 'i' allele was more than twice that of the b allele (9.3%). Because the 'b' allele has a more pronounced effect on gluten strength, varieties with the 'b' allele can be considered suitable for the breeding of new, high-quality cultivars.

Keywords: Wheat (*Triticum aestivum* L.); low-molecular-weight glutenin subunit; Glu-B3; PCR; mini core collections.
Abbreviations: PCR – polymerase chain reaction; LMW-GS – low molecular weight glutenin subunit; HMW-GS – high molecular weight glutenin subunit; SDS-PAGE –sodium dodecyl sulfate polyacrylamide gel electrophoresis; RP-HPLC – reversed phase high performance liquid chromatography; MALDI-TOF – matrix-assisted laser desorption/ionization time-of-flight; DE – two dimensional gel electrophoresis; MS – mass spectrometry; STS - sequence-tagged-site.

Introduction

The end use quality of bread wheat depends on the plant's seed storage proteins. These proteins determine the strength and unique viscoelastic properties of the dough by adjusting the quantity and quality of the gluten formed. The gluten fraction is composed of gliadins and glutenins, which can be distinguished from each other by their different levels of solubility in alcohol-water mixtures. Glutenins have been recognized as the main determinants of the quality of bread, noodles, and other foods. Glutenins include two types of subunits: high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS). These are linked by disulfide bonds. They are divided by their mobility during sodium dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE). HMW-GS is very important in determining wheat dough elasticity, and LMW-GS is related to dough extensibility and gluten strength (Cornish et al., 2001; Maruyama-Funatsuki et al., 2004; Tanaka et al., 2005; Ma et al., 2005). The characterization of the specific LMW-GS allele genes that encode these glutenin subunits has become the basis for the successful improvement of wheat quality in breeding programs. Generally, the LMW-GS genes are encoded by gene families at the Glu-A3, Glu-B3, and Glu-D3 loci. These are on the short arms of chromosomes 1A, 1B, and 1D. These loci are tightly linked to the Gli-1 loci (D'Ovidio and Masci, 2004). There are three subclasses of LMW-GSs: LMW-m, LMW-s, and LMW-i, that are named for the first amino acid residue of their mature proteins, methionine, serine, and isoleucine, respectively (D'Ovidio and Masci, 2004). Because cysteine residues form intramolecular and intermolecular disulfide bonds in the gluten macropolymer, previous researchers have divided the LMW-GSs into six classes on the basis of the locations of cysteine residues (Ikeda et al., 2002). A number of LMW-GS genes from bread wheat and its relatives had been cloned, however, because of the lack of efficient ways of distinguishing members of this complex, heterogeneic, co-migrating multigene family, the exact copy numbers of the LMW-GS genes remain unknown (Appelbee et al., 2009). Researchers have used SDS-PAGE, reversed-phase high-performance liquid chromatography (RP-HPLC), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), two-dimensional gel electrophoresis (2-DE), and mass spectrometry (MS) to investigate the polymorphic LMW-GS complex in bread wheat (An et al., 2006; Balmer et al., 2006; Ikeda et al., 2006; Dong et al., 2010; Liu et al., 2010). However, the complex band/peak patterns of LMW-GSs and the overlapping mobility between LMW-GSs and gliadins pose a problem, particularly when testing allelic variations of LMW-GSs in various wheat varieties (Mamone et al., 2009). High financial and labor costs are also an issue. Molecular markers are a convenient aid to rapid genetic analyses, allowing researchers to distinguish HMW-GS alleles from

LMW-GS alleles. Many functional markers have been developed for glutenin loci (Ma et al., 2003; Lei et al., 2006; Butow et al., 2003). These include a set of PCR markers designed to distinguish allelic variations at the Glu-A3 locus (Zhang et al., 2004). Zhao et al. developed several functional markers to discriminate certain Glu-D3 and Glu-B3 haplotypes (2007a, 2007b). Recently, STS markers had been developed for the Glu-B3 protein alleles ('a,' 'b,' 'c,' 'd,' 'e,' 'f,' 'g,' 'h,' and 'i') (Wang et al., 2009) and used to detect the alleles distribution in Chinese wheat (Gao et al., 2011).

Mini core collections of crop germplasms contain the minimum number of germplasm resources that can represent the total diversity of the genetic resources of a single species. In this paper, we used a set of STS markers specific to the Glu-B3 locus to screen mini core collections of Chinese wheat germplasms to determine the distribution of Glu-B3 protein alleles of low-molecular-weight glutenin in Chinese wheat zones. This may provide a basis for further research.

Results

Detection of Glu-B3 alleles using specific PCR primers

A total of 214 accessions of Chinese wheat germplasms were screened using the 10 pairs of primers and corresponding PCR conditions described by Wang et al. (2009). The results showed that the primers related to Glu-B3 alleles were very specific, as indicated by their ability to amplify a specific or unique product respectively (Fig. 1, see supplementary data for specifications). The 'i' allele was found to be the most common, appearing 20.6% of the time. It was followed by the 'a' allele (15.9%) and d allele (13.6%). The 'h' allele was the rarest (1.4%). The frequencies of alleles 'b,' 'c,' 'e,' 'f,' and 'g' were 9.3%, 6.1%, 7.0%, 10.7%, and 12.1%, respectively. Only 7 cultivars carrying Glu-B3j were detected in the 214 germplasms, the frequency was 3.3% (Table 1).

Distribution of Glu-B3 alleles in materials from various wheat zones

We evaluated the distribution of the Glu-B3 alleles of 214 germplasms from three wheat zones (Table 2, Fig. 2). Alleles 'g' and 'i' were found to be the most common alleles in the spring wheat zone. Alleles 'a' and 'i' were found to be the most common in the winter wheat zone. Allele 'h' was only present in the spring wheat zone and 'a' and 'c' were rare in the spring wheat and spring-winter wheat zones. Alleles 'b,' 'd,' 'e,' and 'f' were mainly distributed in the winter wheat zone. Among the 13 winter zone cultivars found to carry the 'b' allele, 10 cultivars were from the Huang-Huai valley winter wheat region. These included 7 improved cultivars and 3 landraces. This shows that the wheat quality in China has been improved significantly.

Comparison of Glu-B3 alleles in landraces and improved cultivars across different growing zones

The distribution of the Glu-B3 alleles among the 134 landraces was very different across the three growing zones (Table 2). The landraces carrying either of 'a' or 'c' only presented in the winter wheat zone. The frequencies of these two alleles were 21.6% and 8.2%, respectively. Neither 'a' nor 'c' was found in the spring wheat zone or spring-winter wheat zone. Only one landrace carrying the 'h' allele was found in the spring zone, it was not found in the winter zone or spring-winter zone. The disparity between the frequencies of the 'b,' 'd,' and 'g' alleles was never more than 5.0% and remained relatively stable across the three zones. Alleles of 'e,' 'f,' and 'i' were mainly distributed in the landraces of winter wheat zone. Among the landraces from the spring wheat zone, the frequencies of the 'i,' 'g,' and 'd' alleles were higher than those of other alleles. In the winter wheat zone, 'a' was the most common allele. In the spring-winter wheat zone, higher frequencies were found for 'd' and 'g' alleles than for other alleles.

Among the 80 improved cultivars, the most common allele was 'g' in spring wheat zone, 'd' in the winter wheat zone, and 'g' in the spring-winter wheat zone (Table 2). Alleles 'd' and 'e' were not detected among the 15 improved cultivars in spring wheat zone. Alleles 'e' and 'h' were not detected among the 58 improved cultivars in the winter wheat zone. Only 'f', 'g' and 'i' were present in the 7 improved cultivars of the spring-winter wheat zone. Among the improved cultivars of winter wheat zone, alleles of 'd,' 'i,' 'b,' and 'f' were more common than 'a,' 'c,' or 'g.' In the 16 improved cultivars carrying allele of 'd,' there were 10 cultivars from Huang-Huai valley winter wheat region, 3 from the northern region, 2 from the southwestern region, and 1 from the southern winter wheat region. Wheat from the Huang-Huai valley winter region showed good quality with respect to dough extensibility.

Discussion

The LMW-GS and HMW-GS proteins are important parts of the gluten complex in wheat. They are encoded by a highly variable gene family. Because of their importance in wheat flour quality and the difficulties involved in distinguishing them using traditional SDS-PAGE techniques, it was necessary to develop allele-specific markers to identify different LMW-GS alleles (Gale, 2005; Bagge et al., 2007). This is a simple and accurate way of detecting these alleles at every stage of wheat growth. The coding regions of LMW-GS alleles contain no introns and their N-terminal regions and C-terminal regions are more conserved than their middle portions at the nucleotide level. These characteristics make it possible to amplify low-molecular-weight gluten in genes directly from the genome using PCR. Studies of the mini core collections of wheat germplasms may provide information regarding their genetic diversity and allow researchers to select materials suitable for breeding programs. This study screened the mini core collections of Chinese wheat germplasms using the 10 Glu-B3 locus-specific primers (*glu-B3a*, *glu-B3b*, *glu-B3c*, *glu-B3d*, *glu-B3e*, *glu-B3fg*, *glu-B3g*, *glu-B3h*, *glu-B3i*, *glu-B3bef*) developed by Wang et al., (2009). Results suggest that the 10 specific primer pairs can be used to identify the majority of species reliably. They allowed us to confirm that each Glu-B3 allele was detected in the mini core collections. The highest percentage was observed for the 'i' allele at 20.6%. This was followed by 'a' (15.9%) and 'd' (13.6%). The lowest percentage was observed for 'h' (1.4%). In the present study, the frequency of the 'b' allele (9.3%), which makes a large contribution to gluten strength and dough development time (He et al., 2005), was only half that of the 'i' allele. The frequency of the 'd' allele, which makes a large contribution to dough extensibility (He et al., 2005), was slightly lower in landraces than that in improved varieties. This indicates that the 'b' and 'd' alleles could be gradually enhanced during the selection process. The 'h' allele appeared only in the spring wheat zone. The 'a' allele was rare in the spring wheat zone. At present, the distribution of the Glu-A3 and Glu-B3 loci alleles in the mini core collections of Chinese wheat germplasms is well understood. If Glu-D3 locus allele-specific markers can be

Table 1. Distribution of Glu-B3 alleles among landraces and improved cultivars in mini core collections of Chinese bread wheat germplasms.

alleles	No. of cultivars with allele			Frequency (%)		
	improved	landrace	subtotal	improved	landrace	subtotal
'h'	2	1	3	2.5	0.7	1.4
'c'	2	11	13	2.5	8.2	6.1
'e'	0	15	15	0.0	11.2	7.0
'b'	10	10	20	12.5	7.5	9.3
'f'	10	13	23	12.5	9.7	10.7
'g'	11	15	26	13.8	11.2	12.1
'd'	16	13	29	20.0	9.7	13.6
'a'	5	29	34	6.3	21.6	15.9
'i'	19	25	44	23.8	18.7	20.6
'j'	5	2	7	6.3	1.5	3.3
subtotal	80	134	214	100	100	100

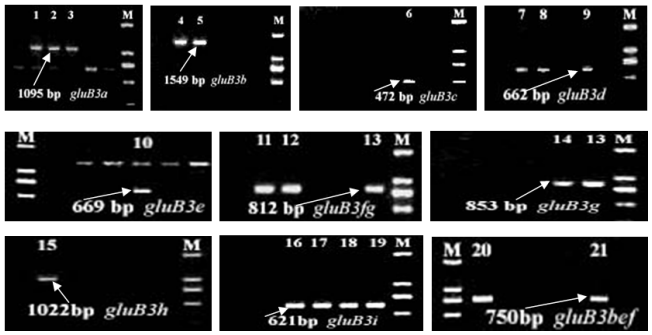

Fig 1. Agarose gel electrophoresis of PCR products amplified from some varieties using 10 allele-specific markers: gluB3a, gluB3b, gluB3c, gluB3d, gluB3e, gluB3fg, gluB3g, gluB3h, gluB3i and gluB3bef. The cultivars were as follows: 1 mahuaban, 2 jiahongmai, 3 hongjinmai, 4 xiaobaibang, 5 zhongyou9507, 6 niuzhijia, 7 taishan1, 8 jinan2, 9 bainong3217, 10 yizhihua, 11 liying5, 12 sumai3, 13 neimai11, 14 dabaipi, 15 dabaimai, 16 zaoxiaomai, 17 lanxizaoxiaomai, 18 wangshuibai, 19 wuyuanmai, 20 fuzhuang30, 21 pingyang27. M, DNA ladder 2000 (2000, 1000, 750, 500, 250, 100 bp).

Table 2. Distribution of 214 cultivars with different Glu-B3 alleles in the three wheat-growing zones

Alleles	spring zone			winter zone			spring-winter zone		
	improved	landrace	subtotal	improved	landrace	subtotal	improved	landrace	subtotal
'a'	1	0	1	4	29	33	0	0	0
'b'	1	3	4	9	4	13	0	3	3
'c'	1	0	1	1	11	12	0	0	0
'd'	0	5	5	16	4	20	0	4	4
'e'	0	3	3	0	9	9	0	3	3
'f'	1	1	2	7	10	17	2	2	4
'g'	6	5	11	2	6	8	3	4	7
'h'	2	1	3	0	0	0	0	0	0
'i'	3	6	9	14	16	30	2	3	5
'j'	0	1	1	5	1	6	0	0	0
subtotal	15	25	40	58	90	148	7	19	26

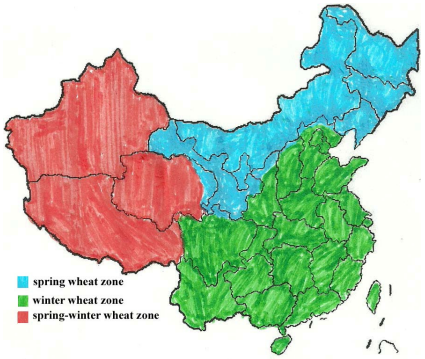

Fig 2. Three wheat zones in China.

developed, they will promote comprehensive understanding of the distribution of LMW-GSs in Chinese wheat germplasms and provide important references for the breeding of new varieties.In conclusion, the markers developed by Wang et al., were here found to be suitable for the detection of Glu-B3 alleles. Although the frequency of 'b' is increased in the improved cultivars, it still remains half that of 'i,' which is the most common allele in the 214 wheat germplasms. Therefore, the selection of 'b' allele should be stressed in the breeding program.

Materials and methods

Plant materials

A total of 214 wheat materials including 134 landraces and 80 improved cultivars were obtained from mini core collections of Chinese wheat germplasms. They came from three major growing zones (Fig. 2), i.e., the spring wheat zone, the winter wheat zone, and the spring-winter wheat zone, all three of which comprise Chinese wheat growing region. All the materials were provided by the Crop Genetic Resources and Improvement, Institute of Crop Science, CAAS, China.

DNA extraction and PCR amplification

Genomic DNA was extracted from seeds using the CTAB procedure as reported by Gale (2005). PCR was performed in a 10 µl volume containing 20 ng of genomic DNA, 100 µM of each dNTPs, 0.3 µM of each primer (*glu-B3a*, *glu-B3b*, *glu-B3c*, *glu-B3d*, *glu-B3e*, *glu-B3fg*, *glu-B3g*, *glu-B3h*, *glu-B3i*, *glu-B3bef*; Wang et al., 2009), 0.1 U of Taq DNA polymerase (Trans), and 1 × PCR buffer (containing 2.5 mM $MgCl_2$). PCR cycling conditions for gene-specific primers (Wang et al., 2009), were 5 min at 94°C followed by 38 cycles of 45 s at 94°C, 45 s at 56–61°C, 90 s at 72°C, and a final extension step of 8 min at 72°C. The allele-specific primers used in this study were adopted from Wang et al. (2009). Amplified PCR products were separated on a 1.2% agarose gel.

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