特约综述 **Invited Review**

Genetic Variation of High Molecular Weight Glutenin Subunits Associated with Processing Quality Improvements in Wheat

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Abstract: High-molecular-weight glutenin subunits (HMW-GS) are polymeric protein components of wheat endosperm which could form the glutenin polymers through the inter-molecular disulphide bonds with each other and/or with low-molecular-weight glutenin subunits (LMW-GS) in dough. Due to their particular importance for dough viscosity and elasticity in wheat processing, a majority of studies on the gluten proteins associated with wheat processing quality have focused on the HMW-GS in the past few decades. Abundant research results have been achieved in recent years on the characterization of HMW-GS and their encoding genes, the variation of these genes and their roles in wheat processing quality. This review will concern the recent research results on the HMW-GS and focus on the variation of HMW-GS proteins and their potential role in wheat quality breeding.

Key words: wheat; genetic variation; HMW-GS; processing quality

小麦高分子量麦谷蛋白亚基的遗传变异与小麦加工品质改良

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摘要: 高分子量麦谷蛋白亚基(HMW-GS)是小麦胚乳中一种具有多态性的蛋白质组分, 在面团中它们可以通过相互之间或与低分子量麦谷蛋白亚基(LMW-GS)之间形成二硫键来组成麦谷蛋白多聚体。由于其在小麦面粉加工所需的粘性和弹力方面具有极其重要的作用, 过去几十年间在小麦加工品质相关蛋白研究方面的工作大多数集中在高分子量麦谷蛋白亚基上。近几年在高分子量麦谷蛋白亚基及其编码基因的鉴定、基因的遗传变异以及不同变异在小麦加工品质中的作用方面进行了大量研究。本文对近几年在HMW-GS领域的研究进展进行综述并且重点讨论HMW-GS的变异及其对小麦品质育种的重要意义。

关键词: 小麦; 遗传变异; 高分子量麦谷蛋白亚基; 加工品质

Wheat is one of the most important crops in the world due to its high production (678 million tons in 2009, FAO), wide geographical range and high proportion of human consumption (Shewry et al. 2003a). Unlike rice and maize, wheat can be processed into a wide range of foods like bread, cake, cookies, noodles, pasta, and other products, which is determined by a group of wheat specific proteins, the gluten proteins. Gluten is commonly produced by washing dough with water and it is mainly comprised of alcohol-soluble gliadins and insoluble glutenins. The gliadins consist of monomeric proteins, which are separated into α, β, γ, and ω groups by polyacrylamide gel electrophoresis at low pH. The glutenins are polymeric proteins consisting of two groups of glutenin subunits called high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS), which are stabilized through

Received 2011-04-14 **Accepted** 2011-04-29

This work was supported by the Natural Science Foundation of China (31000568 and 30871320), the National Transgenic Project (2009ZX08002-014B) and the Doctoral Foundation of Shandong Province (BS2010NY013).

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the inter-chain disulphide bonds. The LMW-GS could be further classified into B-, C- and D-types, while the HMW-GS comprised x- and y-type subunits based on their different mobilities during electrophoresis.

Within the context of improving protein quality (e.g. high extensibility and appropriate dough strength) by wheat breeding, research has conclusively shown the importance of glutenin, particularly with emphasis on those subunits with high molecular weights (Payne et al. 1981a, 1987; Branlard and Dardevet 1985; Gupta and MacRitchie 1994; Popineau et al. 1994). In the past few decades, a majority of studies on the gluten proteins associated with wheat processing quality have focused on the HMW-GS, not only due to their accessibility for analysis (They appear at the top of the electrophoresis gel pattern and are well separated from all the other gluten protein subunits.), but also due to their particular importance for dough viscosity and elasticity in wheat processing (Shewry et al. 1992, 1997). The HMW-GS only account for about 12% of the total protein in the endosperm of common wheat (*Triticum aestivum* L.), while their allelic variation explain about 45% to 70% of the variation in bread making performance within European wheat cultivars (Branlard and Dardevet 1985; Payne et al. 1987, 1988). There have been some excellent reviews on their genetics, structure and relationship with wheat processing properties (Shewry et al. 1995, 2003a, b). Abundant research results have been achieved in recent years on the characterization of HMW-GS and their encoding genes, the variation of these genes and their roles in wheat processing quality, thus, this review will primarily build on recent research results and focus on the variation of HMW-GS proteins and their potential role in wheat quality breeding.

1 Characterization of HMW-GS proteins

Genetic studies indicate that the HMW-GS protein genes exhibit simple co-dominant Mendelian inheritance (Payne et al. 1981b; Payne 1987), and the genes encoding the HMW subunits are located on the long arms of the homoeologous group 1 chromosomes of hexaploid bread wheat (1AL, 1BL, 1DL) at the loci designated *Glu-1* (Lawrence and Shepherd 1980; Payne et al. 1980). Each locus contains two tightly linked genes encoding two types of subunits. The greater one is named x-type and the smaller one y-type (Harberd et al. 1986). Bread wheat cultivars contain a large number of allelic forms of the subunits encoded by each locus. SDS-PAGE electrophoretic analyses of about 300 wheat varieties identified a total of 3 alleles at the *Glu-A1* locus, 11 at the *Glu-B1* locus and 5 at the *Glu-D1* locus. A numbering system to designate the different alleles was proposed based on the relative mobilities of the subunits in SDS-PAGE (Payne and Lawrence 1983). Subsequent analyses of the different wheat cultivars and tetraploid or diploid wheat have resulted in continuing increases in the number of alleles detected at each of the three loci.

Because electrophoresis is simple and easy to perform, SDS-PAGE has become a widely used method for screening HMW-glutenin subunits around the world. However, it still has obvious disadvantages because the identification of the HMW-GS is based on their relative mobilities during SDS-PAGE. Sometimes it is difficult to correctly identify subunits that have very similar physical and chemical properties such as 1Ax2* and 1Dx2 (Gao et al. 2010). In recent years, some new techniques, such as acid polyacrylamide gel electrophoresis (A-PAGE), capillary electrophoresis (CE), reversed phase-high performance liquid chromatography (RP-HPLC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), were used to better characterize HMW-GS with the improvement of resolution and accuracy (Marchylo et al. 1989; Morel 1994; Werner et al. 1994; Sutton and Bietz 1997; Dworschak et al. 1998; Garozzo et al. 1999; Foti et al. 2000), which led to the characterization of many

novel HMW-GS.

For example, several bread wheat HMW-GS with similar mobilities on SDS-PAGE, such as 1Bx7 and 1Bx7*, 1By8 and 1By8*, 1Dx2 and 1Ax2*, 1Bx6 and 1Bx6.1, were found to be well separated with RP-HPLC (Dong et al. 2009). Through one and two-dimensional polyacrylamide gel electrophoresis combined with capillary electrophoresis, the allelic compositions of HMW-GS among European spelt (*Triticum spelta* L.) and related hexaploid and tetraploid *Triticum* species were investigated and some novel alleles encoding subunits 1Ax2.1*, 1Bx6.1, 1Bx13*, 1By19*, 1By22*, 1By22.1 were detected, which had not been found in previous investigations on common bread wheat (Yan et al. 2003a, 2004). In another study, the HMW-GS compositions of 205 accessions of cultivated emmer wheat (*Triticum turgidum* ssp. *dicoccum* Schrank) were examined using the same techniques, and two novel alleles at *Glu-A1* encoding 1Ax1.1 and 1Ax2.1′ were found while seven new subunits (1Bx17*, 1Bx6′, 1Bx13′, 1Bx20*, 1By9*, 1By14.1, and 1By8.1) at the *Glu-B1* locus were detected (Li et al. 2006).

As the genetic base of modern wheat cultivars is generally narrow, the wild progenitors and relatives of wheat are potentially rich resources of useful genes for improvements in wheat quality due to their high variability in the composition of their seed storage proteins (Nevo and Payne 1987; Ciaffi et al. 1993). Through SDS-PAGE analysis, more than 14 allelic variations at the *Glu-D^{t}1* locus of *Triticum tauschii* were detected (Lagudah and Halloran 1988; William et al. 1993). Through analyzing a large collection of accessions of *T. tauschii*, a large range of allelic variation was found and some novel subunits were observed in both the x- and y-type glutenin subunits, including x- or y-type null forms (Gianibelli et al. 2001). Recently the use of high resolution methods such as CE, RP-HPLC and MALDI-TOF-MS contributed to the identification of some novel HMW-GS in *Aegilops tauschii,* which had slightly different mobilities compared to those of bread wheat such as 1Dy10.4^t, 1Dy10.3*^{t}, 1Dy12.1*^{t}, 1Dy12.2*^{t}, 1Dy12.4*^{t}, 1Dy12.5^t, 1Dy12.1^t, 1Dy10.1^t, 1Dy12.2^t, 1Dx5*^{t} and 1Dx5.1*^{t} (Yan et al. 2003b, 2004; Zhang et al. 2006, 2008, 2009). The HMW-GS have also been characterized for some other wild relatives such as rye, *Ae. umbellulata*, *Ae. caudate*, *Ae. searsii, Ae. cylindrical*, *Agropyron elongatum* (*Thinopyrum elongatum*), *Lophopyrum elongatum*, *Pseudoroegneria stipifolia* and *Australopyrum retrofractum* (De Bustos et al. 2001; Liu et al. 2002, 2003, 2008a, b, 2010; Wan et al. 2002; Sun et al. 2006; Li et al. 2008).

2 The exploration of HMW-GS encoding genes

Due to the particular importance of HMW-GS to the processing quality of wheat, exploring the genes encoding these subunits will not only enable the identification of specific residues that are associated with good or poor breadmaking quality but will also improve wheat quality through genetic manipulation. The first full length HMW glutenin subunit gene sequences were isolated from the genomic library of common wheat in 1985 which encoded silent Glu-Aly, Glu-1Dx2 and Glu-1Dy12 (Forde et al. 1985; Sugiyama et al. 1985; Thompson et al. 1985). The gene sequences for Glu-1By9, Glu-1Ax2*, Glu-1-Bx7, Glu-1Dx5, Glu-1Dy10, Glu-1Ax1 and Glu-1-Bx17 were subsequently isolated and sequenced (Halford et al. 1987; Anderson and Greene 1989; Anderson et al. 1989; Halford et al. 1992; Reddy and Appels 1993).

The huge size of the wheat genome makes it laborious to perform library construction and screening, while the length of the HMW subunit genes (typically greater than 2 kb) and repetitive structure makes it difficult to amplify the sequences with the polymerase chain reaction (Shewry et al. 2003a). However, these difficulties have been overcome due to the availability of more reliable and robust polymerases (D'Ovidio et al. 1995; Wan et al. 2002), which, in turn, makes PCR a more efficient method than library

screening for the characterization of HMW-GS genes. The employment of PCR has led to the identification of abundant HMW-GS genes from wheat cultivars, the wild progenitors and relatives of wheat in recent years, including HMW-GS genes from the A, B, D, R, Ee, St, K, Ta and W genomes (D'Ovidio et al. 1996; Mackie et al. 1996a, b; De Bustos et al. 2001; Liu et al. 2002, 2003, 2008a, b, 2010; Wan et al. 2002, 2005; Shewry et al. 2003c; Bai et al. 2004; Li et al. 2004, 2008; Guo et al. 2005; Sun et al. 2006; Yan et al. 2006; Yang et al. 2006; Pang and Zhang 2008; Jiang et al. 2009).

The availability of a large number of HMW-GS genes from cultivated wheat, their wild progenitors and their relatives enabled a detailed comparison of their amino acid sequences. All of these proteins possess highly conserved structures, with each subunit containing a long repetitive region flanking two highly conserved terminal nonrepetitive domains, N and C. The N-terminal domain of the x-type subunits contains 81–89 amino acid residues while that of the y-type subunits contains 104 or 105 residues. The central repetitive domains of both x- and y-type subunits comprise hexapeptide and nonapeptide motifs while the x-type subunits also contain tripeptide motifs. The similarity in structures of different HMW glutenin subunits indicates that they likely evolved from the same ancestor (Shewry and Tatham 1990; Shewry et al. 1995). The first step in the evolutionary process of HMW subunits was the duplication of a single ancestral gene into two closely linked copies and these copies diverged to be distinguishable (x- and y-type) before the speciation of wheat and wheat-related species (Wan et al. 2002; Shewry et al. 2003b). The *Glu-1* gene duplication event was tentatively dated at 7.2–10.0 million years ago (MYA) while the origin of the A, B, D and G genomes at 5.0–6.9 MYA (Allaby et al. 1999)

The recently characterized HMW-GS from *A. retrofractum* comprised a novel type of repeat motif containing 12 residues in this subunit, which was the fourth type of repeat motif presented in HMW-GS (Liu et al. 2010). This subunit also contains some other special modifications when compared with known subunit from other species and it separated from both published x- and y-type subunit genes with phylogenetic analysis, which indicated that the W genome underwent a special evolutionary process different from other Triticeae species (Liu et al. 2010). Moreover, some y-type HMW-GS from the E and St genomes had more similarity with the x-type subunits when the phylogenetic tree was constructed based on the C-terminal sequences, which indicated they were an intermediate state in the divergence between the x- and y-type subunits.

3 Induced variation in HMW-GS

Even though a large number of HMW-GS and their encoding genes have been characterized from wheat and related species, most studies to improve wheat quality by wheat breeding focus on those HMW-GS controlled by the D genome, particularly 1Dx5+1Dy10. One of the reasons for this might due to the lack of more superior subunits than 1Dx5+1Dy10 in cultivated wheat varieties, which could be more easily used for plant breeding through hybridization. Searching for other high quality subunits in wheat still remains a huge challenge due to the narrow genetic range of cultivated wheat varieties. However, inducing novel variations in HMW-GS might be a convenient and feasible route for exploring high quality subunits and some progress has been made at this area in recent years.

It has been proposed that the plant cell culture itself could generate heritable somaclonal variation, which provides a novel source of variability for plant improvement (Larkin and Scowcroft 1981). Extensive variations including the variation of gliadin proteins have been observed among 142 regenerants of a Mexican breeding line Yaqui 50E and their progeny (Larkin et al. 1984). Similar variations have also been detected in regenerated plants of the winter wheat

line ND7532, except that gliadin changes occurred less frequently in ND7532 than in Yaqui 50E (Cooper et al. 1986). Significant changes in bands of gliadins and glutenins in seeds of wheat regenerated from immature embryo cultures included the appearance of new bands, the loss of specific protein bands and changes of band intensity relative to the parent (Hu et al. 1998). SDS-PAGE analysis revealed the HMW-GS composition of some seeds from wheat plants regenerated from tissue culture variation from the 1Ax1, 1Bx7+1By8, 1Dx2+1Dy12 to 1Bx7+1By8, 1Dx2+1Dy12 and 1Bx7, 1Dx2+1Dy12 (Zhang et al. 1997). Similar phenomenon was also observed in the progeny of regenerated plants derived from a single cell culture in spring wheat NE7742 (HMW-GS composition 1Ax1, 1Bx7+1By8, 1Dx2+1Dy12) where three kinds of novel HMW-GS combinations were detected and 8 lines expressed 1Bx7+1By8, 1Dx2+1Dy12, one line expressed 1Bx7, 1Dx2+1Dy12 and 5 lines expressed 1Bx7+1By9, 1Dx2+1Dy12 (Hu et al. 2002).

In addition to somaclonal variation, changes in HMW-GS have also been induced by other approaches such as chemical and/or physical mutagenesis and introducing exogenous DNA into the wheat. Chemical mutagenesis of wheat cv. Viginta using nitrosoethylurea provided a mutant line with altered composition of HMW-GS where the 7+9 subunits encoded by *Glu-B1* locus changed to 6 (Svec et al. 1999). Four somaclones were derived from gamma ray irradiated immature embryos of wheat and lost the subunits encoded by *Glu-A1*, *Glu-B1*, and/or *Glu-1Dy* (Zhang et al. 2002). Introducing the total DNA from sorghum into spring wheat Ganmai No. 8 also induced variation in the HMW-GS in one of the progeny where subunits 2+12 of Ganmai No. 8 were substituted by 5+10 in progeny 89144 (Wang et al. 2000). Chemical mutagenesis of an elite Chinese wheat variety Xiaoyan 54 led to a mutant where the expression of the 1Bx14 subunit was specifically blocked and the silencing of this subunit might due to a base substitution that resulted in a premature stop codon in the mutant ORF (Zhu et al. 2005).

4 Genetic variation in HMW-GS induced by somatic hybridization

For the purpose of quality improvement, the generation of novel HMW-GS was more significant compared to the loss of subunits. However, all of the above-mentioned studies indicated less frequent generation of novel variation of HMW-GS than those lost and no explanations were presented to describe how the novel HMW-GS were created. Fortunately, high frequency generation of novel HMW-GS was reported in the introgression lines of somatic hybrids between wheat and tall wheatgrass. Moreover, the mechanisms of novel HMW-GS formation were discussed (Zhao et al. 2003; Feng et al. 2004; Liu et al. 2006, 2007, 2009; Gao et al. 2010).

Asymmetric somatic hybridization between the protoplast of common wheat (*T. aestivum* L cv. Jinan 177) and the UV-irradiated protoplast of *A. elongatum* generated fertile introgression lines with superior agronomic traits. In particular the bread making quality was improved (Xia et al. 2003; Zhao et al. 2003; Liu et al. 2006). SDS-PAGE analysis of the seeds of 175 somatic hybrid lines indicated that about 35% of the hybrid lines express novel high quality HMW-GS not present in Jinan177 or either parents. Six novel HMW-GS 1Ax1, 1Ax2*, 1Bx13, 1By16, 1By8, 1Dx5, and three allelic HMW-GS combinations 1Bx13+1By16, 1Bx7+1By8, and 1Dx5+1Dy12 occurred in the hybrids (Zhao et al. 2003). More importantly, most of these novel HMW-GS or combinations correlated with higher flour processing quality than the parent wheat (Liu et al. 2006) and they could be stably inherited (Liu et al. 2009). Characterization of the coding sequences of the novel subunits indicated that additional cysteine residues were present in at least three novel subunits, which could partially explain the higher flour processing quality of some novel subunits (Feng et al. 2004; Liu et al. 2009).

Through sequence alignments of the novel subunits with those of both parents, the mechanisms of how these new subunits were created were elucidated (Liu et al. 2007, 2008, 2009). Except for two genes directly introgressed from the donor parent *A. elongatum*, most of the novel hybrid HMW-GS genes were derived from point mutations and replication slippage of the parental genes. Six subunit genes were produced by point mutations and 10 subunit genes resulted from unequal crossover or slippage of the parental wheat genes (Liu et al. 2007, 2009). In addition to the above mechanisms, reactivation of transposons and shuffling of parent genes to produce novel chimeric HMW-GS genes was also found to be responsible for the generation of novel subunits (Liu et al. 2007, 2009). Novel HMW-GS were also present in the fertile regenerants of symmetric somatic hybridization between the protoplasts of common wheat (*T. aestivum* L cv. Jinan 177) and *A. elongatum,* which are morphologically similar to tall wheatgrass, but contain some introgression segments from wheat (Cui et al. 2009; Gao et al. 2010). We would expect there to be similar mechanisms for the generation of these novel subunits in symmetric somatic hybrids with those that occured in asymmetric somatic hybrids, and this similarity between different hybrids indicates that the response to genomic shock triggered by the merger and interaction of biparent genomes might be mainly responsible for the sequence variation of HMW-GS genes in the introgression lines (Gao et al. 2010).

The formation of these novel hybrid genes has similarity to the evolutionary mechanisms of natural HMW-GS genes mentioned by Anderson and Greene (1989), which include: (a) single base changes, (b) deletions or additions within a repeat, (c) single repeat changes, and (d) deletions or duplications of blocks of repeats. The results suggest that asymmetric somatic hybridization is a unique pathway to rapidly produce novel HMW-GS alleles that can be used for wheat quality improvement and this technique may represent a valuable means of extending the variation at these functionally important genes.

5 Conclusions

The importance of wheat in food processing and the tight correlation between HMW-GS composition and flour processing quality has stimulated a massive volume of studies on the HMW-GS and their encoding genes. Transformation of wheat with HMW-GS genes has been widely used over the past decade to modify the functional properties of dough. However, the choice of target subunits has also almost certainly been restricted to 1Ax1, 1Dx5 and 1Dy10 due to the limited availability of high quality subunits (Altpeter et al. 1996; Blechl and Anderson 1996; Barro et al. 1997; He et al. 1999, 2005; Pastori et al. 2000; Alvarez et al. 2001). The exploration of novel high quality subunits from cultivated wheat, the wild progenitors, and relatives of wheat together with chemical, physical and/or biological inducement of novel subunits has significantly expanded the gene pool that could be used for wheat quality improvement by genetic engineering technology.

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