

EFFECTS OF *Glu-1* AND *Glu-3* ALLELIC VARIATIONS ON WHEAT GLUTENIN MACROPOLYMER (GMP) CONTENT AS REVEALED BY SIZE-EXCLUSION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (SE-HPLC)

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Gluten macropolymers (GMPs), formed by seed storage proteins glutenins and gliadins through intermolecular disulfide bond, confer dough viscoelasticity and wheat processing quality. Glutenins consist of high and low molecular weight glutenin subunits (HMW-GS, LMW-GS) encoded by *Glu-1* and *Glu-3* loci, respectively. The allelic variations at both loci have important effects on GMP content and breadmaking quality. In this study, GMP extraction and size-exclusion high performance liquid chromatography (SE-HPLC) separation were optimized, and then applied to investigate the effects of *Glu-1* and *Glu-3* allelic variations on GMP content using different wheat genotypes, chromosome

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substitution lines and near-isogenic lines (NILs). The results showed that the optimized GMP extraction and SE-HPLC protocol could obtain a reproducible separation and reliable quantitation of GMP content with small samples. The allelic variations at *Glu-1* and *Glu-3* were closely related to GMP content. Particularly, *Glu-D1d* encoded 1Dx5+1Dy10 subunits, *Glu-A3a* and *Glu-B3h* encoded an abundant LMW-B subunit respectively had positive effects on GMP content and breadmaking quality. The introgress of HMW-GS and LMW-GS into bread wheat from related genomes could significantly increase GMP content, indicating that wheat related species has potential gene resources for breadmaking quality improvement. Our results demonstrated that SE-HPLC could serve as an effective tool for rapid separation and quantitation of GMPs and had potential application for gluten quality screening in the early generations during wheat quality improvement program.

Keywords: bread wheat, HMW-GS, LMW-GS, glutenin macropolymer, SE-HPLC

INTRODUCTION

Wheat (*Triticum aestivum* L., AABBDD, $2n=6x=42$), as one of the most important grain crops in the world is an important protein source for human consumption. It is also used as raw materials for making bread, fodder, medicine, spices and other products (SHEWRY *et al.*, 2002). It is generally accepted that the quality of protein and the level of wheat production are the most important factors determining quality of manufacture high-quality of food (HE *et al.*, 2003). In wheat endosperm, the major storage proteins, glutenins and gliadins, are synthesized and accumulated during seed development. Glutenins and gliadins are the major determinant factors of the viscoelasticity and extensibility of dough, respectively (PAYNE, 1987). According to their different molecular weights, glutenins are divided into two groups: high molecular weight (70000–90000 Da) glutenin subunits (HMW-GS) and low molecular weight (20000–45000Da) glutenin subunits (LMW-GS), which mainly determine the unique elasticity and viscosity of wheat dough, respectively. Although HMW-GS account for less proportion of total protein (~10%), they play important roles in breadmaking quality. In the course of storage protein accumulation, HMW-GS and LMW-GS as well as gliadins are linked into polymers by intermolecular disulfide bonds and form the largest protein molecule glutenin macropolymers (GMPs) that significantly affect the rheological properties of wheat dough (WRIGLEY, 1996).

HMW-GS are encoded by genes at the orthologous *Glu-1* loci on the long arms of chromosomes 1A, 1B and 1D (*Glu-A1*, *Glu-B1* and *Glu-D1*) (PAYNE, 1987). Each locus possesses two paralogous genes encoding one larger x- and one smaller y-type subunit (SHEWRY, 1995). LMW-GS are encoded by *Glu-A3*, *Glu-B3* and *Glu-D3* loci on the short arms of group 1 chromosomes (GUPTA and SHEPHERD, 1990), which are further divided into B-, C- and D-group subunits according to their mobilities in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (D'OVIDIO and MASCI, 2004). HMW-GS contain 4-7 cysteine residues and LMW-GS have six conserved cysteine residues and one or more additional cysteine residues (KOH *et al.*, 2010). Most of these residues form intrachain disulfide bonds, whereas some of these residues form interchain disulfide bonds to form GMPs (ORSI, 2001). Both *Glu-1* and *Glu-3* loci showed extensive allelic variations that are closely related to dough quality (PAYNE and LAWRENCE, 1983; GUPTA and SHEPHERD, 1990; GUPTA *et al.*, 1994). Particularly, some HMW-GS alleles such as *Glu-D1a* (1Dx5+1Dy10), *Glu-A1a* (1Ax1), *Glu-A3a*, *Glu-B3h* are highly related to

superior breadmaking quality (SHEWRY *et al.*, 2002; ZHEN *et al.*, 2014; WANG *et al.*, 2016). HMW-GS are considered to be the key factor to form GMPs, and their relative quantity and subunit compositions are closely related to the number and particle size of gluten polymers (DON *et al.*, 2006; LIANG *et al.*, 2008). Recent study has showed that the deletion of *Glu-B3h* led to significant decrease of GMP content and breadmaking quality (WANG *et al.*, 2016).

According to protein classification system by the Osborne method, only partial proteins can be soluble in sodium dodecyl sulfate (SDS) extract and their protein molecular weight is small, named as extractable polymeric protein (EPP). The glutenins insoluble in SDS extract can be dissolved using ultrasonic treating, such unextractable polymeric protein (UPP) is named as glutenin macropolymer (GMP). Studies showed that the content and proportion of storage proteins is the main influential factor of the wheat quality, especially the content of glutenin subunits and GMPs (SAPIRSTEIN *et al.*, 1998). GMPs play an important role in the dough forming (DON *et al.*, 2003), and high-quality flour has more glutenins and GMPs in adulterated flour (HUANG *et al.*, 1997). There are correlation between the changes of total sulfhydryl and disulfide content of GMP and changes of GMP content (CHEN *et al.*, 2005). The higher content of GMP, the larger the elastic properties of dough it acts on (SI *et al.*, 2005). Meanwhile, by virtue of the results observed, GMPs have great effects on the formation of dough and GMP content is significantly correlated with rheological properties such as gluten index, flour sedimentation value, formation of dough and loaf volume (HU *et al.*, 2004; YAN *et al.*, 2010).

Size exclusion-high performance liquid chromatography (SE-HPLC) is a chromatographic method in which molecules in solution is separated by their size, and in some cases molecular weight. It has been widely used for the separation of large molecules or macromolecular complexes such as proteins and industrial polymers (YANG *et al.*, 2015). In wheat, SE-HPLC has been successfully used to separate and characterize seed proteins, including water-soluble proteins (BEAN *et al.*, 2003; WANG *et al.*, 2008). In this work, we optimized GMP extraction and SE-HPLC method for GMP separation and quantitation, and then used to investigate the effects of glutenin allelic variations on GMP content. Our results provided new evidence to confirm the close relationships between allelic variations at *Glu-1* and *Glu-3* and GMP content.

MATERIALS AND METHODS

Plant materials

Wheat materials used in this study included 14 bread wheat cultivars with different glutenin compositions: 11 from China (Jingdong 8, Jing 411, Jimai 19, Jimai 20, Neixiang 188, Xiaoyan 6, Xiaoyan 54, Zhongyou 9507, Zhengmai 366, Zhongmai 175, and Chinese Spring), two from Germany (Hanno and Imbros), and one from Australia (Hortag); 5 glutenin near-isogenic lines (NILs): CS and its *Glu-A3a* deletion line CS-n, CB037A (1Ax1, 1Bx17+1By18, 1Dx2+1Dy12; *Glu-A3a*, *Glu-B3h*, *Glu-D3d*), CB037B (1Ax1, 1Bx17+1By18, 1Dx5+1Dy10; *Glu-A3a*, *Glu-B3h*, *Glu-D3d*) and CB037C (1Ax1, 1Bx17+1By18, 1Dx5+1Dy10; *Glu-A3a*, Null, *Glu-D3d*) developed in our laboratory (ZHEN *et al.*, 2014; WANG *et al.*, 2016; DONG *et al.*, 2016); 3 Chinese Spring (CS) chromosome substitution lines CS-1C/1A (1C from *Aegilops caudata* replaced 1A from CS), CS-1S¹/1B (1S¹ from *Aegilops longissima* replaced 1B from CS) and CS-1U/1B (1U from *Aegilops umbellulata* replaced 1B from CS), kindly provided by Dr. S. Hsam, Plant Breeding Department of Technical University of Munich; 4 durum wheat Lira 42, Lira 45, Creso and Simeto-1; and one cultivation einkorn. The detailed information of all materials used were listed in Table 1.

Table 1a. Wheat materials used in this study

Materials	Origin	Species	HMW-GS compositions	Materials	Origin	Species	Glutenin compositions
Jingdong 8	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	Null, 7+8, 2+12	Chinese Spring (CS)	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	Null, 7+8, 2+12; <i>Glu-A3a</i> , <i>Glu-B3a</i> , <i>Glu-D3a</i>
Jing 411	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	Null, 7+8, 2+12	CS-n	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	Null, 7+8, 2+12; Null, <i>Glu-B3a</i> , <i>Glu-D3a</i>
Jimai 19	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 7+8, 2+12	CB037A	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1,17+18,2+12; <i>Glu-A3c</i> , <i>Glu-B3h</i> , <i>Glu-D3a</i>
Neixiang 188	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 7+9, 5+10	CB037B	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1,17+18,5+10; <i>Glu-A3c</i> , <i>Glu-B3h</i> , <i>Glu-D3a</i>
Hanno	Germany	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 14+15, 5+10	CB037C	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1,17+18,5+10; <i>Glu-A3c</i> , Null, <i>Glu-D3a</i>

Table 1b. Wheat materials used in this study

Materials	Origin	Species	HMW-GS compositions	Materials	Origin	Species	Glutenin compositions
Imbros	Germany	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 14+15, 2+12;	CS-1C(1A)	Germany	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	Null, 7+2**, 3*+12 <i>Glu-A3</i> is replaced by <i>Glu-C3</i>
Jimai 20	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 13+16, 5+10	CS-1S'(1B)	Germany	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1S' ¹ x2.3*+1S' ¹ y16*, 2-12 <i>Glu-B3</i> is replaced by <i>Glu-S'3</i>
Xiaoyan 6	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 20x+20y, 2+12	CS-1U(1B)	Germany	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	Null, 7+8, 2+12; <i>Glu-B3</i> is replaced by <i>Glu-U3</i>
Xiaoyan 54	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 20x+20y, 2+12	Lira 42	Italy	AABB, 2n=4x=28	Null, 7+8; LMW-1

Table 1c. Wheat materials used in this study

Materials	Origin	Species	HMW-GS compositions	Materials	Origin	Species	Glutenin compositions
Hortag	Australia	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 17+18, 5+10	Lira 45	Italy	AABB, 2n=4x=28	Null, 7+8; LMW-2
Zhengmai 366	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 7+8, 5+10	Creso	Italy	<i>Triticum durum</i> , AABB, 2n=4x=28	N, 6+8
Zhongmai 175	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	Null, 7+9, 2+12	Simeto-1	Italy	<i>Triticum durum</i> , AABB, 2n=4x=28	Null, 13+16
Zhongyou 9507	China	<i>Triticum aestivum</i> L., AABBDD 2n=6x=42	1, 7+9, 5+10	Einkorn	Iran	<i>Triticum monococcum</i> L., A ^m A ^m 2n=2x=14	1A ^m x+1A ^m y

Field planting and sampling

For analyzing the synthesis and accumulation patterns of GMP during grain development, three NILs CB037A, CB037B and CB037C were planted in the experimental field of CAAS, Beijing during 2015-2016 growing season under same field cultivation conditions. Each material was planted with three replications and each replication had 10 lines (500 plants). Developing grains were collected for five different grain developmental stages (5, 11, 14, 20, and 29 day after flowering, DAF). All collected materials were stored in -80°C prior to use.

GMP extraction

Different grain samples were grinded into flour, and grinded grain flours from 50 mg, 40 mg, 30 mg and 20 mg were used for optimizing GMP extraction based on WEGELS *et al* (1996). Flour samples were oscillated for about 20 min in 1800 µL extraction buffer (0.05 M PBS with 2% SDS). After centrifugation for 15 min at 12000 g, the supernatant was thrown away. The residues were put into ultrasonic instrument, following by oscillating for about 20 min in 1800 µL extraction buffer again with ultrasonic power 5 W and last 30 s at every turn. Every sample was undertaken three times, and then kept on oscillating for about 1 h. After centrifugation for 15 min at 12000 g, the supernatant was filtrated into another centrifugal tube and then centrifuged for 10 min at 13000 g and used for HPLC analysis.

SE-HPLC

On the basis of previous reports (LAGRAIN *et al.*, 2005), SE-HPLC for the separation and characterization of GMPs was optimized from different flow rates (0.35 ml/min and 0.5 ml/min by using agilent bio sec-5 column and diameter 5 µm with column pressure around 80 bar on Agilent 1100 (Agilent Technologies). The mobile phase was 0.05 M PBS with 0.1% SDS. Through this method, we can effectively separate wheat samples. After ultrasonic treatment with 70 W for 10 min, the peak area (1000 AU/S) was used as the measurement of GMP content in different wheat materials.

RESULTS

Optimization of GMP extraction and SE-HPLC separation

We firstly improved GMP extraction on the basis of WEGELS *et al.* (1996), and tried to reduce the seed sample amount. The results showed that GMPs extracted from 20 mg flour (about half grain) were enough for SE-HPLC analysis, significantly lower than traditional 50 mg. This would dramatically decrease the amount of grain samples and benefit rapid screening of GMP content in the early generations during wheat quality improvement program. To optimize SE-HPLC separation, two different flow rates (0.35 and 0.5 mL/min) were tested and compared. The results indicated that the latter should be the optimal flow for the GMP separation (Figure 1a). Rapid separation of GMPs from one sample could also been achieved under this condition, generally less than 10 min. The main components of GMPs were eluted from 3.8 min to 6.5 min (Figure 1b). To analyze the reproducibility of SE-HPLC, 10 consecutive runs of GMPs from Chinese Spring were performed under the optimized condition (Figure 1c). The relative standard deviations (RSD%) of migration time (< 0.1%), peak height (< 5%) and peak area (< 5%) of 9 main GMP components from consecutive 10 runs were good (Table 2), demonstrating that high reproducible separation with small sample amount can be obtained by the optimized condition.

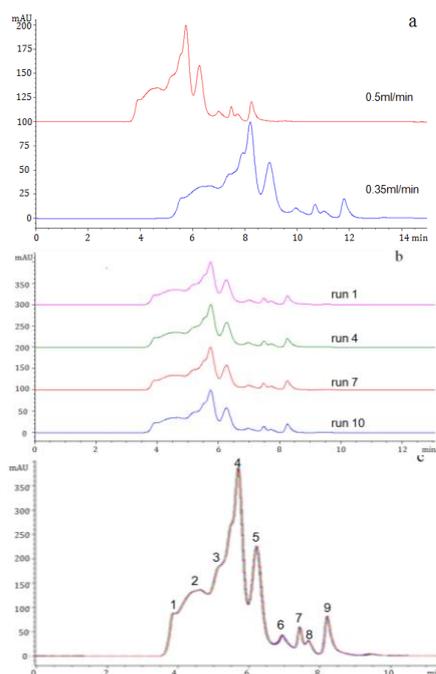


Fig. 1 Optimization (a), reproducibility (b) and characterization (c) of GMPs in Chinese Spring by SE-HPLC

Table 2. Repeatability of GMPs in Chinese Spring separated by SE-HPLC

GMP peak*	Migration time (min)	RSD%	Peak height (1000 AU)	RSD%	Peak area (1000AU/S)	RSD%
1	3.873±0.005	0.072	84.40±4.20	4.024	944.700±46.90	3.929
2	4.620±0.004	0.052	129.70±5.95	3.051	6250.500±166.90	4.239
3	5.700±0.003	0.038	358.60±6.60	1.068	14232.400±363.95	4.252
4	6.215±0.004	0.037	213.30±5.10	1.711	4721.900±152.20	4.741
5	6.923±0.006	0.061	40.00±2.00	4.391	913.150±62.95	4.241
6	7.434±0.003	0.026	56.50±2.60	4.177	614.800±30.00	4.335
7	7.676±0.004	0.034	29.35±1.75	4.786	399.150±23.25	4.905
8	8.208±0.004	0.026	76.55±4.05	4.138	1117.900±82.50	4.865
9	9.700±0.006	0.093	3.20±0.20	4.979	60.300±6.50	4.741

*Nine main GMP peaks are indicated in Fig.1c.

GMP separation and content determination from wheat genotypes with different glutenin compositions by the optimized SE-HPLC

The optimized SE-HPLC method was used to separate and quantify GMPs from diploid, tetraploid and hexaploid *Triticum* species (Table 3, Figure 2) as well as different glutenin NILs and chromosome substitution lines (Table 4, Figure 3). The peak area (1000 AU/S) was used as GPM

quantitation. The results showed that all wheat genotypes with different glutenin compositions displayed significant variations in GMP content. The rank of GMP content (peak area) was hexaploid bread wheats (from 10438.44 to 18056.60) > tetraploid from 7938.59 to 8806.57) > diploid species with 6908.70 (Table 3), suggesting that GMP content was significantly increased along with the *Triticum* genome expansion. Apparently, modern cultivated bread wheat cultivars had much higher GMP content than tetraploid and diploid species, indicating that GMP content and glutenin quality were significantly improved in the modern wheat breeding program. In addition, high GMP content in hexaploid bread wheat may reflect the important contribution of the D genome to dough strength and breadmaking quality.

Table 3. GMP content from diploid, tetraploid and hexaploid wheat species with different glutenin compositions and quality properties determined by SE-HPLC

Materials	Peak area (1000 AU/S)	Materials	Peak area (1000 AU/S)
Einkorn	6908.70±23.43	Imbros	12529.55±127.65
Lira 42	7938.59±34.58	Jimai 20	15307.23±87.36
Lira 45	8512.59±38.26	Xiaoyan 6	13140.61±90.64
Creso	8806.57±56.74	Xiaoyan 54	16093.29±112.39
Simeto-1	8650.01±41.35	Hortag	16261.36±70.58
Jing 411	10438.44±85.36	Zhengmai 366	17232.50±78.37
Jimai 19	17342.60±53.68	Jingdong 8	10525.91±67.38
Neixiang 188	16688.52±59.25	Zhongmai 175	10556.60±68.83
Hanno	17986.39±64.54	Zhongyou 9507	18056.60±148.83

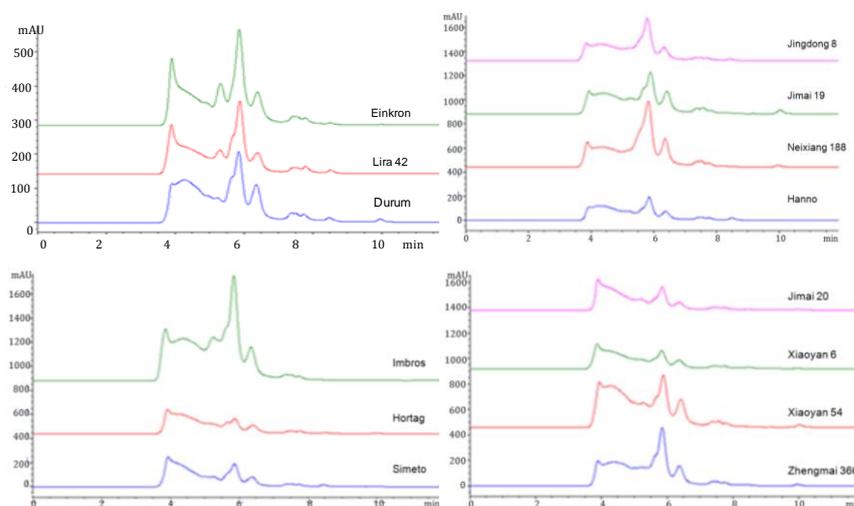


Fig. 2. Separation and characterization of GMPs from diploid, tetraploid and hexaploid wheat species with different glutenin compositions and quality properties by SE-HPLC

Table 4. GMP content from wheat near-isogenic lines and substitution lines determined by SE-HPLC

NILs	Peak area (1000AU/S)	RSD%	NILs	Peak area (1000AU/S)	RSD%
CS	11773.08	20.14	CB037C	10831.12	15.35
CS-n	10707.67	14.09	CS-1C(1A)	12869.25	18.58
CB037A	9009.69	10.61	CS-1S ¹ (1B)	15854.87	19.71
CB037B	11906.72	16.18	CS-1U(1B)	14625.20	16.65

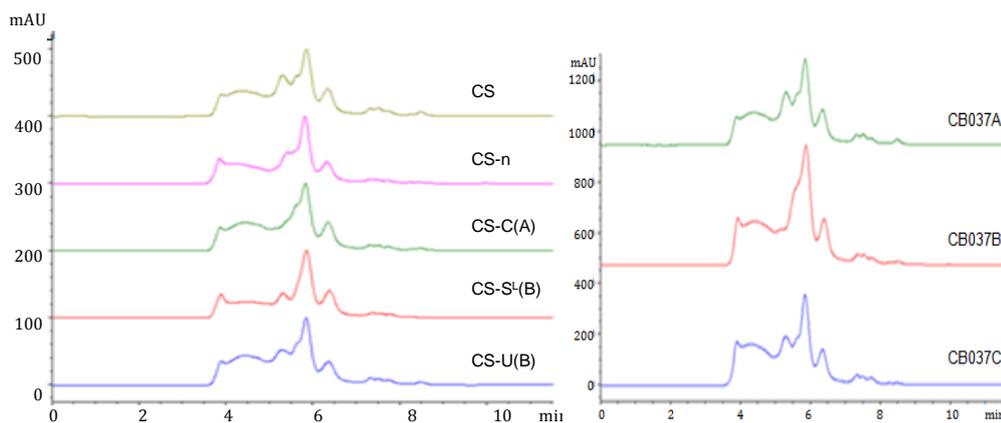


Fig. 3 Separation and characterization of GMPs from wheat substitution lines and near-isogenic lines by SE-HPLC.

As shown in Table 3, although hexaploid bread wheat cultivars have higher GMP content than diploid and tetraploid wheats, they also showed greater GMP content differences. In particular, 10 elite Chinese bread wheat cultivars that were widely cultivated in the main wheat production areas of China in the past several years also showed significant variations in GMP content, ranging from 10438.44 to 18056.60. Among them, seven cultivars (Zhongyou 9507, Xiaoyan 6, Xiaoyan 54, Jimai 20, Zhengmai 366, Neixiang 188, and Jimai 19) with superior gluten quality had higher GMP content (13140.61-18056.60) while three with poor gluten quality (Jing 411, Zhongmai 175 and Jingdong 8) possessed lower GMP content (10438.44-10556.60). In addition, three foreign superior quality cultivars (Hanno and Imbros from Germany and Hortag from Australia) also had higher GMP content (12529.55-17986.39). Generally, these bread wheat cultivars have different HMW-GS compositions, indicating the important effects of *Glu-1* loci on GMP content and gluten quality.

The results from different glutenin NILs and chromosome substitution lines showed that HMW-GS and LMW-GS as well as particular chromosome substitution had important effects on

GMP content (Table 4). Particularly, variations at *Glu-1* or individual *Glu-3* locus deletion could result in significant changes of GMP content. For example, when 1Dx2+1Dy12 subunits encoded by *Glu-D1a* in CB027A was replaced by 1Dx5+1Dy10 subunits encoded by *Glu-D1d* in CB037B, GMP content was significantly increased from 9009.69 to 11906.72. Meanwhile, the deletion of *Glu-A3a* in Chinese Spring and *Glu-B3h* in CB037C led to significant reduction from 11773.08 to 10707.67 and 11906.72 to 10831.12, respectively. In addition, the durum NIL Lira 42 with LMW-1 and Lira 45 with LMW-2 from Italy also showed significant difference in GMP content that was increased from 7938.59 to 8512.59 (Table 4).

Certain particular chromosome substitutions from wheat related species can result in significant increase of GMP content due to the introgress of HMW-GS or LMW-GS from related genomes. As shown in Table 4, all three Chinese Spring chromosome substitutions CS-1C(1A), 1S^l(1B) and CS-1U(1B) showed higher GMP content compared to CS, which was significantly increased from 11773.08 to 12869.25, 15854.87 and 14625.20, respectively.

Relationships between glutenin allelic variations as well as particular chromosome substitution and GMP content

Analysis from diploid, tetraploid and hexaploid species with different *Glu-1* and *Glu-3* allelic variations indicated that GMP content is closely related with glutenin compositions. Generally, the cultivars with 1Dx5+1Dy10 (*Glu-D1d*), 1Ax1 (*Glu-A1a*), 1Bx14+1By15 (*Glu-B1h*), and 1Bx17+1By18 (*Glu-B1i*) had higher GMP content than those with 1Dx2+1Dy12 (*Glu-D1a*) and Null (*Glu-A1c*). Particularly, the mean GMP content of 6 cultivars with 1Dx5+1Dy10 subunits was 16922.10, much higher than the other 7 cultivars with 1Dx2+1Dy12 subunits (12946.71) (Table 3). When 1Dx2+1Dy12 subunits in CB037A were replaced by 1Dx5+1Dy10 in CB037B, GMP content was significantly promoted (Table 4). These results demonstrated that 1Dx5+1Dy10 subunits have positive effects on GMP content and gluten quality. At *Glu-3* loci, both deletions of *Glu-A3a* and *Glu-B3h* encoded an abundant LMW-B subunit resulted in significant decrease of GMP content, indicating their important roles in GMP formation.

Wheat related species *Aegilops caudate*, *Aegilops longissima* and *Aegilops umbellulata* contain homologous glutenin loci. Thus, when 1A or 1B in CS was substituted by 1C, 1S^l or 1U, respectively, GMP content was significantly promoted. This demonstrates that the related genomes contain potential glutenin gene resources that can improve GMP formation and gluten quality. Particularly, the introgressing of several new HMW-GS with higher molecular weight or LMW-GS from related genomes into three substitution lines such as 1S^lx2.3*+1S^ly16* (Table 1), could lead to significant improvement of GMP content (Table 4).

Synthesis and accumulation changes of GMPs during grain development in three near-isogenic lines of CB037

The developing grains from six different stages (5, 11, 14, 20, and 29 DAF) in three near-isogenic lines of CB037 (A, B and C) were collected and then used for investigating the synthesis and accumulation patterns of GMPs (Table 5, Figure 4). The results demonstrated that the synthesis and accumulation of gluten polymers could be easily detected at 5 DAF and then GMP content increased rapidly until grain maturity. The accumulation of GMPs generally had a sharply rising from 5-11 DPA and 20-29 DPA, respectively (Figure 5). Comparative analysis found that the amount of GMP in CB037A at 5 day is slightly higher than those in CB038B and CB037C. But GMP accumulation at 29 DPA in CB037B and CB037C was significantly higher than that in

CB037A while CB037B was significantly higher than CB037C at 11, 14 and 20 DPA.

Table 5. Synthesis and accumulation patterns of GMPs during grain development in three near-isogenic lines (NILs) of spring wheat cultivar CB037

DPA	CB037A		CB037B		CB037C	
	Peak area (1000 AU/S)	RSD%	Peak area (1000 AU/S)	RSD%	Peak area (1000 AU/S)	RSD%
5	528.35	2.041	508.85	4.377	425.70	5.778
11	829.90	4.613	1244.70	3.008	836.25	6.049
14	1048.90	6.761	1285.50	6.887	1117.70	9.398
20	1339.10	5.471	1384.60	8.699	1214.85	8.622
29	1392.10	8.009	1885.90	6.149	1849.40	13.09

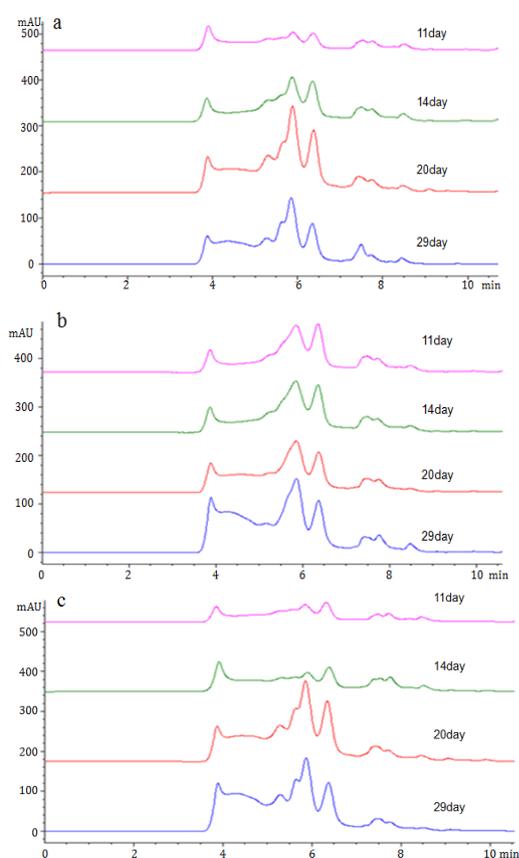


Fig. 4 Separation and accumulation patterns of GMPs from four grain developmental stages of CB037A (a), CB037B (b) and CB037C (c) by SE-HPLC.

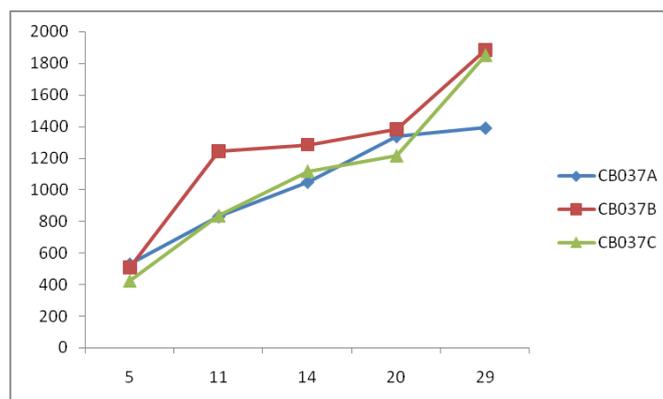


Fig. 5 The accumulation patterns of GMPs during grain development of three near-isogenic lines of

DISCUSSION

The fast identification of GMPs is highly important for wheat gluten quality improvement. Since GMPs have higher molecule weight, up to even millions of Dalton, they can not be separated effectively by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). At present, multistacking (MS)-SDS-PAGE is often used to separate GMP. Its advantage is capable of designing different gradient concentrations and contract GMP particle size distribution in different concentration SDS-PAGE, but it is time-consuming and high cost. Although biuret method is simple to operate, it has lower reliability and separation capability. Compared to above methods, SE-HPLC has advantages for fast, reproducible and high-resolution separation of GMPs. The present work reported an optimized GMP extraction and SE-HPLC separation method that can rapidly and accurately measure GMP content as well as its accumulation pattern during grain development with small sample amount. Therefore, SE-HPLC has potential application for rapid detection and screening of GMP content in the early generations of wheat quality improvement programs.

Considerable work revealed that high GMP content is closely related with the characteristic of greater dough strength, bigger volume of bread and good quality (SAPIRSTEIN and FU, 1998; SUN *et al.*, 1998). The results from 24 locations of elite Chinese bread wheat cultivar Jimai 20 by reversed-phase (RP) HPLC and SE-HPLC indicated that GMP content was significantly and positively correlated with loaf structure and score (TANG *et al.*, 2007). In 2008, analysis of 43 wheat genotypes further indicated that GMP content had significant and positive effects on bread appearance, loaf structure and score ($p < 0.001$).

GMPs consist of HMW-GS and LMW-GS as well as gliadins connecting by disulfide bond. Thus, the allelic variations at *Glu-1* and *Glu-3* have important effects on GMP content and breadmaking quality. In particular, HMW-GS have the characteristic of forming higher GMP content compared to LMW-GS, possibly due to longer glutamine (Q) repeats and richer cysteine residues forming disulfide bond present in HMW-GS. A large number of studies confirmed that the subunit pair 1Dx5+1Dy10 has positive influence on breadmaking quality while its allelic pair 1Dx2+1Dy12 (PAYNE, 1987). Similar to previous report (WEEGELS *et al.*, 1997), we found that the wheat cultivars with 1Dx5+1Dy10 subunits had higher GMP content than those with 1Dx2+1Dy12

(Table 3). The introgress of larger HMW-GS from related genomes also resulted in significant increase of GMP content (Table 4) and improvement of dough strength and breadmaking quality (WANG *et al.*, 2013). This may be attributed to an extra cysteine present in 1Dx5 subunit and longer repeat sequences including more Q repeats in HMW-GS. In addition, we also found that LMW-GS alleles *Glu-A3a* and *Glu-B3h* as well as the introgress of LMW-GS from related genomes had important effects on GMP content (Table 4). Recent reports have showed that the deletion of both alleles respectively encoding an abundant LMW-B subunit resulted in significant decrease of dough strength and breadmaking quality (ZHEN *et al.*, 2014; WANG *et al.*, 2016). These results demonstrate that the expression amount of LMW-GS as well as the *Glu-3* loci in the related species has important roles in GMP accumulation and breadmaking quality.

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UTICAJ *GLU-1* I *GLU-3* ALELNIH VARIJACIJA NA SADRŽAJ GLUTENINSKOG MAKROPOLIMERA PŠENICE (GMP) PRIMENOM EKSKLUZIONE TEČNE HROMATOGRAFIJE VISOKE PERFORMANSE

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Izvod

Makropolimeri glutena (GMP), koje formiraju rezervni proteini semena glutenini i gliadini kroz intermolekulske disulfidne veze, doprinose viskoelastičnosti testa i tehnološkom kvalitetu pšenice. Glutenini se sastoje od gluteninskih subjedinića visoke i niske molekulske mase (HMW-GS, LMW-GS) koje su kodirane sa *Glu-1*, odnosno *Glu-3* lokusa. Alelne varijacije na oba lokusa imaju značajan uticaj na sadržaj GMP i kvalitet hleba. U ovom istraživanju, GMP ekstrakcija i separacija ekskluzionom tečnom hromatografijom visoke performanse (SE-HPLC) su optimizovane, a zatim primenjene u cilju istraživanja uticaja *Glu-1* i *Glu-3* alelnih varijacija na sadržaj GMP korišćenjem različitih genotipova pšenice, linija sa hromozomskim substitucijama i blisko izogenih linija (NIL). Rezultati su pokazali da optimizovana ekstrakcija GMP i SE-HPLC protokol mogu da obezbede ponovljivo razdvajanje i pouzdanu kvantifikaciju sadržaja GMP sa malim uzorcima. Alelna varijacija na *Glu-1* i *Glu-3* bila je blisko povezana sa sadržajem GMP. Posebno, *Glu-D1d* koji kodira subjediniću 1Dx5+1Dy10, *Glu-A3a* i *Glu-B3h* koji kodiraju obilnu podjediniću LMW-B imaju pozitivni uticaj na sadržaj GMP, odnosno kvalitet hleba. Introgresija HMW-GS i LMW-GS u hlebu pšenice iz srodnih genoma može značajno da poveća sadržaj GMP, što ukazuje na to da vrste srodne pšenici imaju potencijalne genske izvore za popravku kvaliteta hleba. Naši rezultati su pokazali da SE-HPLC može da služi kao efikasno sredstvo za brzo razdvajanje i kvantifikaciju GMP-ova i da ima potencijalnu primenu za proveravanje kvaliteta glutena u ranim generacijama tokom programa unapređenja kvaliteta pšenice.

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