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Effects of sulphur fertilizer on glutenin macropolymer content and particle size distribution in wheat grain

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ABSTRACT

Two wheat cultivars (Gaocheng8901 and Yumai50) grown were used to investigate the effect of sulphur fertilizer on the glutenin macropolymer (GMP) size distribution and the contents of glutenin subunits in wheat. The results showed that the contents of GMP, high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS) were improved by sulphur fertilizer under lower nitrogen (N) condition in both cultivars. Under normal N (240 kg N/ha) conditions, sulphur application improved the contents of HMW-GS, LMW-GS and GMP within sulphur rates from 30–60 kg/ha, while decreased when sulphur rate of 90 kg/ha. The volume percentage of GMP particles < 60 μm decreased within sulphur rates from 30–90 kg/ha under lower N treatments. Under normal N condition, the volume percentage of GMP particles > 60 μm increased within the sulphur rates from 30–60 kg/ha, while decreased when excessive sulphur of 90 kg/ha was applied. It is suggested that appropriate sulphur fertilizer was favourable for the formation of large GMP particles, but too much of it was unfavourable under normal nitrogen condition. Sulphur fertilizer did not significantly affect the number distribution of GMP particles in both cultivars. The volume percentage of GMP particles > 60 μm was positively correlated with H/LMW-GS (the ratio of HMW-GS and LMW-GS) and GMP content. It indicated that larger GMP particles had more the ratio of HMW-GS and LMW-GS. And the higher the proportion of larger particles, the higher the content of GMP in wheat grain.

Keywords: winter wheat (*Triticum aestivum* L.); glutenin macropolymer; nitrogen and sulphur interaction; size distribution

It has been generally accepted that the quality of wheat-based food is related to the characteristics of glutenin aggregates which are widely accepted as glutenin macropolymer (GMP) fraction (Weegels et al. 1997). GMP consists of very large structures of both high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS) linked by disulphide bonds (S-S) (Don et al. 2003, Goesaert et al. 2005). The quantity and composition of HMW-GS and LMW-GS determine most of the dough characteristics and end-use qualities (Wieser and Kieffer 2001, Zhang et al. 2007). HMW-GS plays an important role in determining the gluten network structure (Don et al. 2006), and LMW-GS are major components of the glutenin aggregations with about 5–6 times more abundant than HMW-GS (D'Ovidio and Masci 2004).

Variation in total protein content alone could not result in difference in dough characteristic and processing quality, however, both quantity and quality of gluten protein were very important for end-use quality of wheat (Zhu and Khan 2001, Zhang et al. 2007). Gluten is mainly composed of gliadin and glu-

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tenin in wheat grains. Significant variation in particle size distribution of glutenin macropolymer occurred across environment and resulted in variation in dough properties and breadmaking quality (Ciaffi et al. 1996, Jia et al. 1996, Don et al. 2006). GMP was reported to be more sensitive to changes in environmental conditions, compared with total protein content in wheat grains (Don et al. 2005, Spiertz et al. 2006).

Many investigators have demonstrated the effects of some cultivation measures on accumulation of HMW-GS and GMP in wheat (DuPont et al. 2006, Jiang et al. 2009, Ni et al. 2014). Starch content was not influenced by the sulphur fertilizer applied (Dostalová et al. 2015). However, sulphur slightly improved the protein content and Zeleny sedimentation values in wheat (Hřivna et al. 2015). To our knowledge, a few studies are available on the effects of sulphur fertilizer on the contents of GMP and glutenin subunits and GMP particle size distribution. The objectives of the present study were to determine the effects of sulphur fertilizer on GMP content, glutenin subunits content and GMP size distribution in wheat grains. The insights may give guidance to improve the grain quality through both wheat breeding and field cultivation.

MATERIAL AND METHODS

Experimental design. The field experiments were carried out in growing season from November 2012 to May 2013 and from November 2013 to May 2014 at the Fengyang experimental station of Anhui Science and Technology University, Fengyang, China (32°51'N, 117°33'E). The soil was sandy loam, and the 0–20 cm soil layer contained 11.2 g/kg organic matter, 76.2 mg/kg available N and 6.62 mg/kg available S, respectively. Maize (*Zea mays* L.) was the previous crop. There were two nitrogen rates of 120 kg N/ha (N_L) and 240 kg N/ha (N_N). There were three sulphur rates of 30 kg S/ha (S_3), 60 kg S/ha (S_6) and 90 kg S/ha (S_9). Nitrogen and sulphur fertilizer were used in urea and sulphur. First part of nitrogen (50%) was applied as the basal fertilizer and another 50% was top-dressed at returning green of wheat in the spring. The sulphur was as the basal fertilizer. In addition, the basal fertilizer was applied at the rate of 16.4 kg P/ha and 49.8 kg K/ha before planting. The plot size was 2.5 × 6.0 m with 8 rows (20 cm between rows). Plants were sown on 15 November, 2012 and 18 November, 2013 with the density of 375 seedling/m^2.

Two high-yielding winter wheat (*Triticum aestivum* L.), Gaocheng8901 and Yumai50 were used in the present research. At maturity, wheat kernels were dried at 70°C for the analysis of GMP particle size distribution and determination of HMW-GS and LMW-GS content.

GMP determination. GMP content was measured by the method of Weegels et al. (1996) with some adaptations. Grain samples (50 mg) were suspended in 1 mL of 1.5% sodium dodecyl sulfonate (SDS) solution and centrifuged at 15 000 × *g* for 30 min at 20°C. The N content of sediment was measured as the GMP content.

Isolation of GMP gel. GMP gel was extracted from wheat grain was performed to the methods of Graveland et al. (1985) and Don et al. (2003) with some adaptations. GMP gel was isolated by dispersing 1.5 g flour into 30 mL of 0.05 mol/L SDS and then centrifuged at 75 000 × *g* for 30 min at 20°C. The supernatant was decanted, and the gel-layer was collected as GMP gel.

Particles size analysis. GMP particles size distribution analysis was carried out following by Don et al. (2003) with some modifications. About 100 mg of GMP gel was weighed into the 10 mL tube and suspended with 5 mL of 1.5% (w/v) SDS solution. And its size was measured with a Coulter Laser LS13320 (Beckman Coulter Instruments, Brea, USA).

Determination of HMW-GS and LMW-GS content. A Waters 1525 Binary HPLC pump + 2998 diode-array detector with Nucleosil 300-5 C8 column (4.6 mm × 250 mm) was used according to Wieser et al. (1998).

Statistical analysis. Analysis of variance (three-way ANOVA) was performed with the SPSS statistical analysis package (SPSS Inc., Chicago, USA). When significant treatment effects were detected, multiple comparisons among the treatments were conducted with the Tukey's range test. Pearson's correlation coefficients were calculated to determine the relationship between GMP volume distribution and H/LMW-GS and GMP content. The experimental data from 2012–2013 were reported in this paper.

RESULTS

HMW-GS and LMW-GS content. Sulphur fertilizer could improve the contents of HMW-GS

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Table 1. Effects of sulphur fertilizer on HMW-GS (high molecular weight glutenin subunit) and LMW-GS (low molecular weight glutenin subunit) content (mg/g) in grain

Cultivar	Treatment	HMW-GS	LMW-GS	H/LMW-GS
Gaocheng 8901	N_LS_3	9.6^{cd}	26.8^{bc}	0.359
	N_LS_6	10.0^{c}	26.6^{bc}	0.376
	N_LS_9	11.5^{bc}	29.1^{ab}	0.395
	N_NS_3	13.0^{ab}	29.5^{ab}	0.439
	N_NS_6	15.6^{a}	31.1^{a}	0.502
	N_NS_9	10.5^{bc}	30.4^{ab}	0.346
Yumai50	N_LS_3	5.6^{e}	17.1^{e}	0.327
	N_LS_6	6.2^{e}	19.3^{de}	0.321
	N_LS_9	6.4^{e}	19.9^{de}	0.322
	N_NS_3	6.8^{e}	22.1^{e}	0.306
	N_NS_6	7.2^{de}	22.5^{cd}	0.321
	N_NS_9	6.1^{e}	20.8^{e}	0.294
F-value	N	35.3^{**}	78.4^{**}	
	S	6.5^{*}	4.9^{*}	
	N × S	18.3^{**}	7.6^{**}	

Means within column followed by different letters are significantly different at $P < 0.05$. $^{*}P < 0.05$; $^{**}P < 0.01$. H/LMW-GS – ratio of HMW-GS and LMW-GS. N_L – 120, N_N – 240 kg N/ha; S_3 – 30, S_6 – 60, S_9 – 90 kg S/ha

and LMW-GS (Table 1). The contents of HMW-GS and LMW-GS all increased with increasing sulphur rate from 30–90 kg/ha under N_L treatment in both cultivars. However, the contents of HMW-GS and LMW-GS increased in response to sulphur rate from 30–60 kg/ha and decreased at a sulphur rate of 90 kg/ha under N_N treatment for both cultivars. It indicated that more sulphur fertilizer is beneficial for the HMW-GS and LMW-GS content under low nitrogen condition, and appropriate sulphur fertilizer could improve the contents of HMW-GS and LMW-GS under normal nitrogen condition. The ratios of HMW-GS and LMW-GS (H/LMW-GS) increased with increasing sulphur rate from 30–90 kg/ha under N_L treatment for cv. Gaocheng8901. For cv. Yumai50, the H/LMW-GS showed no significant difference among sulphur rates under N_L treatment. Under N_N conditions in both cultivars, the H/LMW-GS showed the order of $S_6 > S_9 > S_3$. It is suggested that the response of HMW-GS and LMW-GS to sulphur fertilizer differed between nitrogen rates. Nitrogen rate, sulphur rate and N × S interaction had a significant effect on the HMW-GS and LMW-GS contents.

GMP content. The GMP content in both cultivars under the two N conditions followed a similar pattern with different sulphur rates. Sulphur significantly affected the GMP content at maturity under two N conditions for both cultivars (Figure 1). The GMP content in grains of both cultivars shows an increase in sulphur application under N_L treatments, and it also shows that S_6 is the best level for the GMP content under N_N treatment in both cultivars. It is indicated that the appropriate sulphur fertilizer could improve the GMP contents, but too much of it was unfavourable under normal nitrogen condition.

Volume distribution of GMP particles. The GMP particles sizes were in the range of 0.375–324 μm for cv. Gaocheng8901 and 0.375–223 μm for cv. Yumai50, respectively (Figure 2). Contribution from the GMP particle with diameter smaller than 60 μm to the total volume in grains of cv. Gaocheng8901 and cv. Yumai50 were 50.7–61.3%

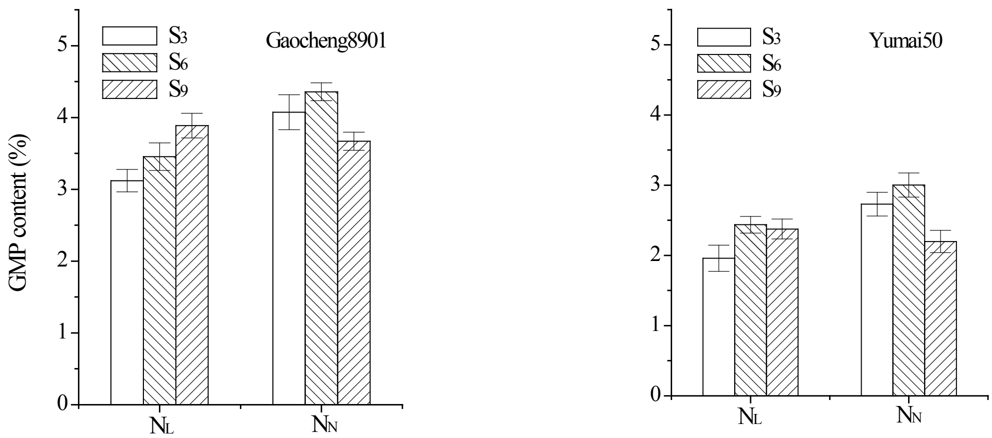

Figure 1. Effects of sulphur fertilizer on GMP content in wheat grain. GMP – glutein macropolymer; N_L – 120, N_N – 240 kg N/ha; S_3 – 30, S_6 – 60, S_9 – 90 kg S/ha

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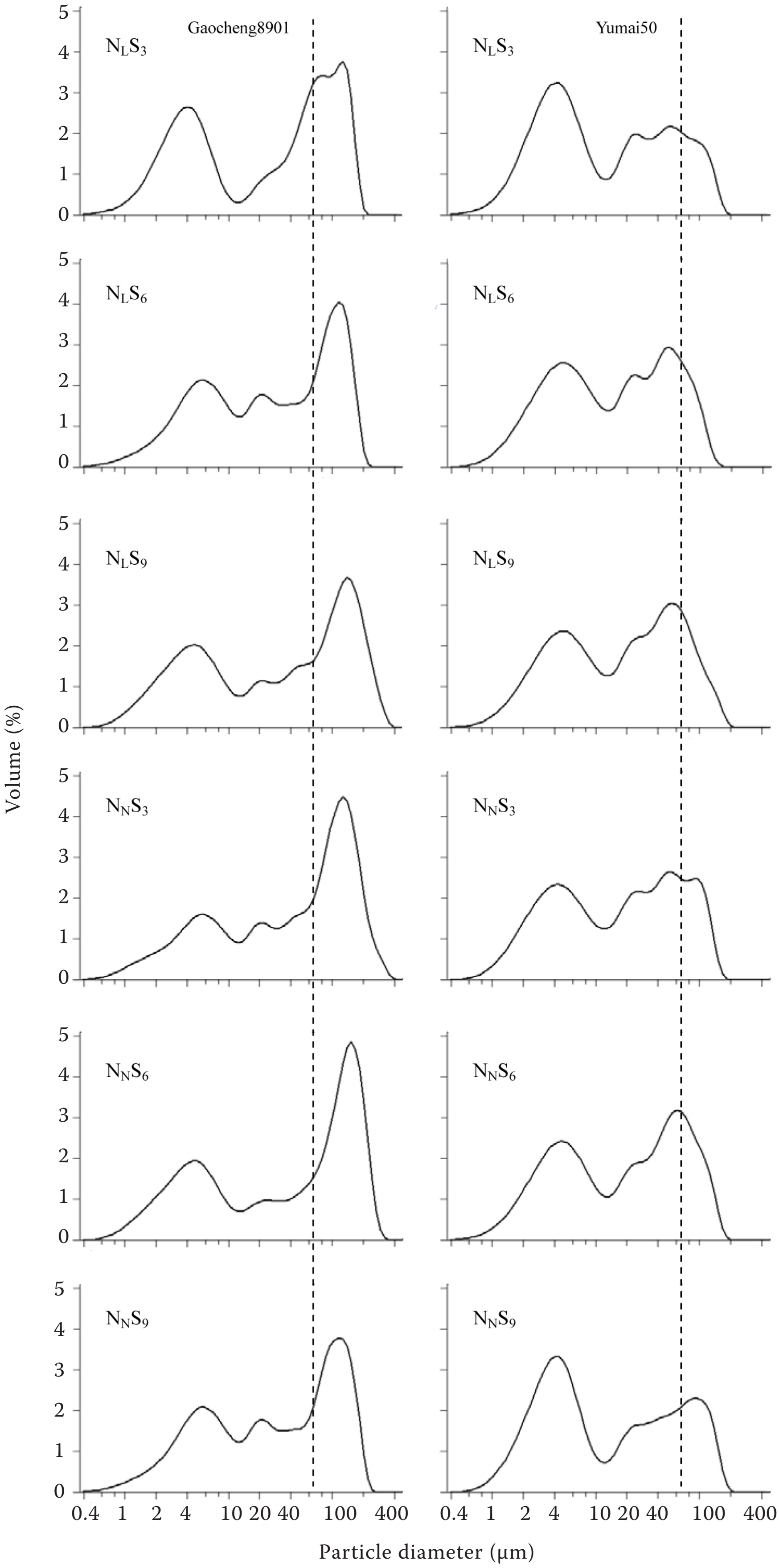

Figure 2. Effects of sulphur fertilizer on volume distribution of GMP particle in wheat grain. N_L – 120, N_N – 240 kg N/ha; S_3 – 30, S_6 – 60, S_9 – 90 kg S/ha

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Table 2. Effect of sulphur fertilizer on volume distribution of GMP (glutein macropolymer, μm) particle in grain (%)

Cultivar	Treatment	Particle diameter of GMP < 60	Particle diameter of GMP > 60	D(4,3) (μm)
Gaocheng 8901	N_LS_3	61.3[c]	38.7[b]	51.3[b]
	N_LS_6	61.1[c]	38.9[b]	52.9[b]
	N_LS_9	56.7[cd]	43.3[ab]	70.9[a]
	N_NS_3	51.3[d]	48.7[a]	75.1[a]
	N_NS_6	50.7[d]	49.3[a]	77.7[a]
	N_NS_9	60.8[c]	39.2[b]	55.8[b]
Yumai50	N_LS_3	84.5[a]	15.5[d]	27.8[d]
	N_LS_6	82.5[a]	17.5[cd]	28.8[cd]
	N_LS_9	80.0[ab]	20.0[cd]	33.2[cd]
	N_NS_3	78.7[ab]	21.3[cd]	33.5[cd]
	N_NS_6	76.1[b]	23.9[c]	35.9[c]
	N_NS_9	77.9[ab]	22.1[cd]	33[cd]
F-value	N	40.7**	40.5**	108.6**
	S	1.2	1.2	2.3
	N × S	14.6**	14.5**	108.7**

Means within column followed by different letters are significantly different at $P < 0.05$. *$P < 0.05$; **$P < 0.01$; D(4, 3) – weighted average volume; N_L – 120, N_N – 240 kg N/ha; S_3 – 30, S_6 – 60, S_9 – 90 kg S/ha

and 76.1–84.5%, respectively (Table 2). Volume percentage of GMP particle with diameter higher than 60 μm was in the range of 38.7–49.3% in cv. Gaocheng8901 and 15.5–23.9% in cv. Yumai50. This indicates cv. Gaocheng8901 had greater particles > 60 μm and fewer particles < 60 μm compared with cv. Yumai50. The D(4,3) of GMP showed an increase in sulphur application under N_L treatments, and it showed that S_6 is the best level for the D(4,3) of GMP under N_N treatment in both cultivars. The volume percentage of GMP particle < 60 μm decreased within sulphur rates from 30–90 kg/ha in N_L treatments. Under N_N treatments, the volume percentage of GMP particle > 60 μm increased within the sulphur rates from 30–60 kg/ha, while it decreased when excessive sulphur of 90 kg/ha was applied. It is suggested that appropriate sulphur fertilizer was favourable for the formation of large GMP particle, but too much of it was unfavourable under normal nitrogen condition. Nitrogen rate and N × S interaction had a significant effect on volume distribution of GMP, however, sulphur rate alone had no effect on GMP volume distribution. This suggested that sulphur fertilizer affects volume distribution of GMP by nitrogen and sulphur interaction.

Number distribution of GMP particles. Number distribution of the GMP particles in grains is given in Table 3. In this study, 99.96–99.99% of the total number came from particle with diameter smaller than 15 μm. Only 0.01–0.04% of the number was distributed by particle with diameter higher than 15 μm. Sulphur fertilizer did not significantly affect the number distribution of both cultivars under two N conditions.

As shown in Table 4, the D(4, 3) (weighted average volume of the particles) was positively correlated with the H/LMW-GS (0.9093**) and the GMP content (0.9579**), respectively. The H/LMW-GS was positively correlated with volume percentage of GMP particles > 60 μm (0.8606**), and negatively correlated with volume percentage of particles < 60 μm (–0.8606**). The content of GMP was positively related to volume percentage of GMP particles > 60 μm (0.9549**), and negatively related to volume percentage of particles < 60 μm (–0.9549**). It indi-

Table 3. Effect of sulphur fertilizer on number distribution of GMP (GMP – glutein macropolymer) particle in grain (%)

Cultivar	Treatment	Particle diameter of GMP (μm) < 15	Particle diameter of GMP (μm) > 15
Gaocheng8901	N_LS_3	99.98[a]	0.02[a]
	N_LS_6	99.96[a]	0.04[a]
	N_LS_9	99.98[a]	0.02[a]
	N_NS_3	99.97[a]	0.03[a]
	N_NS_6	99.98[a]	0.02[a]
	N_NS_9	99.96[a]	0.04[a]
Yumai50	N_LS_3	99.96[a]	0.04[a]
	N_LS_6	99.97[a]	0.03[a]
	N_LS_9	99.96[a]	0.04[a]
	N_NS_3	99.96[a]	0.04[a]
	N_NS_6	99.96[a]	0.04[a]
	N_NS_9	99.97[a]	0.03[a]
F-value	N	0.1	0.1
	S	0	0
	N × S	0.3	0.3

Means within column followed by different letters are significantly different at $P < 0.05$. N_L – 120, N_N – 240 kg N/ha; S_3 – 30, S_6 – 60, S_9 – 90 kg S/ha

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Table 4. Correlation coefficients between GMP (glutein macropolymer) volume distribution and H/LMW-GS (ratio of HMW-GS and LMW-GS) and GMP content

Item	Diameter of GMP particle (μm)		
	< 60	> 60	D(4,3)
H/LMW-GS	–0.8606**	0.8606**	0.9093**
GMP	–0.9549**	0.9549**	0.9579**

*$P < 0.05$; **$P < 0.01$. D(4, 3) – weighted average volume

cated that larger GMP particles had more the ratio of HMW-GS and LMW-GS in wheat grains.

DISCUSSION

It was reported that cultivation measures, such as nitrogen, phosphorus and sulphur, significantly affect the accumulation of GMP and HMW-GS (Jiang et al. 2009, Ni et al. 2014, Hřivna et al. 2015). However, little was known about the influence of the sulphur fertilizer on the content of glutenin subunit. In this study, the amounts of the GMP and the content of HMW-GS and LMW-GS were improved by sulphur rate at maturity under lower N treatment. Under normal N condition, sulphur application improved the accumulation of HMW-GS, LMW-GS and GMP, but too much of it was unfavourable. The increase in GMP content was found to be correlated to the increase of HMW-GS and LMW-GS under sulphur rates. This is consistent with the other findings (Jiang et al. 2009). It is suggested that sulphur application improved the accumulation of HMW-GS, LMW-GS and GMP at maturity and also favoured the quality of the wheat grain, but too much of it was unfavourable under normal nitrogen condition.

A few studies reported that the diameter of GMP particles was influenced by genotypes and environment, diameter of GMP particle was in the range of 1–300 μm (Don et al. 2006, Wang et al. 2011, Li et al. 2013). In the present study, under the N_L and N_N treatments, diameter of GMP particles was 0.375–324 μm for cv. Gaocheng8901 and 0.375–223 μm for cv. Yumai50. The different records on the particle diameter were most likely due to different cultivars growing conditions. The volume percentage of GMP particles < 60 μm decreased within sulphur rates from 30–90 kg/ha under lower N treatments. Under normal N conditions, the volume percentage of GMP particles > 60 μm increased within the sulphur rates from 30–60 kg/ha, while decreased when excessive sulphur rate of 90 kg/ha was applied. It is suggested that appropriate sulphur fertilizer was favourable for the formation of large GMP particle, but too much of it was unfavourable under normal nitrogen conditions.

This research showed no significant impact on the percentage of GMP particles < 15 and > 15 μm; small GMP particles (< 15 μm) amounted for 99.96–99.99% of the total number of particles. Then the number distribution of GMP particles was rarely regulated by the sulphur fertilizer.

The proportion of HMW-GS and LMW-GS played an important role in determining the size distributions of glutenin aggregates (Zhu and Khan 2002, Wang et al. 2004). SDS-insoluble glutenin polymers which have higher content of HMW-GS and LMW-GS, were larger in size compared with SDS-soluble polymers (Lindsay and Skerritt 1999). Liang et al. (2008) reported that the HMW-GS contents were negatively correlated with volume percentage of GMP particles < 100 μm, but positively correlated with that of particles > 100 μm. In this study, the parameter H/LMW-GS was positively correlated with volume percentage of GMP particles > 60 μm, and negatively correlated with volume percentage of particles < 60 μm. The GMP content was positively related to volume percentage of particles > 60 μm, and negatively related to volume percentage of particles < 60 μm. It indicated that larger GMP particles had higher ratio of HMW-GS and LMW-GS in wheat grain. And the higher the proportion of larger GMP particles, the higher the GMP content in wheat grain.

The results indicated that sulphur had a significant effect on the formation of GMP particles in wheat grains. The appropriate sulphur (60 kg/ha) fertilizer improved the accumulation of HMW-GS and LMW-GS, and hence improved the formation of large GMP particle.

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