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Effect of different water supply on accumulation of high molecular weight glutenin subunits and glutenin macropolymers in near-isogenic wheat lines

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ABSTRACT

Accumulations of high molecular weight glutenin subunits (HMW-GS) and glutenin macropolymer (GMP) in wheat grains are important indicators of grain quality. Two near-isogenic wheat lines, Line 11 (HMW-GS null, 7 + 9, 5 + 10) and Line 12 (HMW-GS null, 17 + 18, 5 + 10), were used to evaluate the impacts of different water supply on HMW-GS and GMP accumulation, and the GMP particle distributions in the grains. Three irrigation levels were implemented in a field and a pot experiment, respectively. Results indicated that drought is beneficial for grain desiccation in the two wheat lines at late filling stage. Compared to mild and excess watering, the total HMW-GS concentration at maturity was much lower when subjected to soil water deficit. Both drought and excess watering led to a reduced glutenin particle size and GMP content at maturity, indicating that a mild water supply could promote the accumulation of GMP and formation of lager glutenin particles. As opposed to Line 12, Line 11 showed an increase in accumulation of GMP and larger glutenin polymers.

Keywords: *Triticum aestivum*; quality of wheat; flour; synthesis of glutenin; polymerization

Wheat (*Triticum aestivum* L.) is the most widely consumed food crop in the world, being processed to give a range of breads, other baked goods, pasta, and noodles. In wheat, glutenin macropolymers (GMP), consisting of spherical glutenin particles, are a major component of the grain and an important factor affecting the processing quality of wheat (Don et al. 2005). Previous studies demonstrated that the amount of GMP in wheat flour correlates closely with baking quality (Weegels et al. 1996). Besides GMP content, GMP particle size and distribution are important in wheat bread-making quality. Evidence indicates that GMP particle size strongly correlates with dough development time (Don et al. 2006).

GMP consists of high molecular weight glutenin subunits (HMW-GS) linked with low molecular weight glutenin subunits (LMW-GS) through disulfide bonds. HMW-GS are encoded by polymorphic genes at the *Glu-1* loci on the long arms of group 1 chromosomes. Hexaploid wheat usually contains 3–5 subunits, zero or one encoded by *Glu-A1*, one or two by *Glu-B1* and two by *Glu-D1* (Lawrence and Payne 1983). Of the glutenin subunits, composition of HMW-GS has been assured to contribute greatly to bread-making quality. Meanwhile, HMW-GS concentration is also considered a very important grain quality trait in wheat (Yue et al. 2007).

Accumulations of HMW-GS and GMP in wheat grains are both genetically and environmentally controlled (Irmak et al. 2008). Of the different environmental effects, one of the greatest in certain regions (e.g. in northern China) is thought to be that of soil water stress (Ma et al. 2007). Water stress is known to influence dry matter production as well as quality of wheat (Yang et al. 2011). Drought promotes HMW-GS accumulation in the early grain filling stage, whereas the opposite effect occurs at late grain filling (Jiang et al. 2009). Both

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dough development time and dough stability time were longest with a single post-anthesis irrigation, whereas a second irrigation led to shortened dough development and dough stability times and weakened gluten strength, as well as a decreased glutenin polymerization index and average sized GMP (Jia et al. 2012).

Earlier studies with near-isogenic wheat were carried out on synthesis of glutenin. It was reported that the wheat biotype with HMW-GS 5 + 10 (*Glu-D1d* line) accumulated larger polymers more quickly than the biotype with allelic subunits 2 + 12 (*Glu-D1a* line) (Gupta et al. 1996). The line with the *Glu-D1d* allele showed an earlier polymerization of glutenin than its allelic counterpart and a higher molecular weight of glutenin at maturity (Irmak et al. 2008). The HMW/LMW ratio of GMP is lowered by heat stress, but glutenin particles become larger (Don et al. 2005).

Although numerous studies have been conducted on size distribution and properties of GMP particles in wheat grains, there is limited information about the synthesis of GMP in near-isogenic wheat under water stress conditions. In the present study, two wheat near-isogenic lines were used to minimize the genetic background. The aims were to compare the differential responses of HMW-GS and GMP accumulation to different water supply at the reproductive stage.

MATERIAL AND METHODS

Experiment description. Two near-isogenic wheat lines, Line 11 (HMW-GS null, 7 + 9, 5 + 10) and Line 12 (HMW-GS null, 17 + 18, 5 + 10), were used in this experiment. This experiment (included a field and a pot experiment) was carried out on the experimental farm of the Research Institute of Agricultural Science (37°N, 116°E), Dezhou, China in 2013–2014 wheat growing season. In field experiment, three irrigation levels during the growing season were used: irrigating at before-wintering, jointing, anthesis, and grain filling (W2), irrigating at jointing, and anthesis (W1), and no irrigation (W0). The water amount applied to every stage was 750 m^2/ha. The experiment was a 2 × 3 (two lines and three levels of irrigation) factorial design with six treatments. Each of the treatments had three plots as repetitions in a complete randomized block design. The moisture content in soil after anthesis is shown in Figure 1.

In pot experiment, pots with depth and diameter of 40 × 30 cm were filled with 13 kg mixture of peat substrate and loamy soil (1:4). The two near-isogenic wheat lines were sown and 10 plants were maintained in each pot after thinning. From 5 days after anthesis (DDA) until maturity, three levels of soil water potencial (ψ_{soil}) were imposed on the plants by controlling water application. The well-watered treatment (WW) was maintained at –0.02 MPa, the mild water-deficit treatment (WD) was maintained at –0.05 MPa, and the severe water-deficit treatment (WS) was maintained at –0.08 MPa. A tension meter was installed in each pot to monitor. When the reading dropped to the designed value, a specific amount of tap water was added to the plants. A rain shelter was used to protect the pot during rains.

After full heading, spikes flowering on the same date were labelled with thread. From 7 DAA, thirty

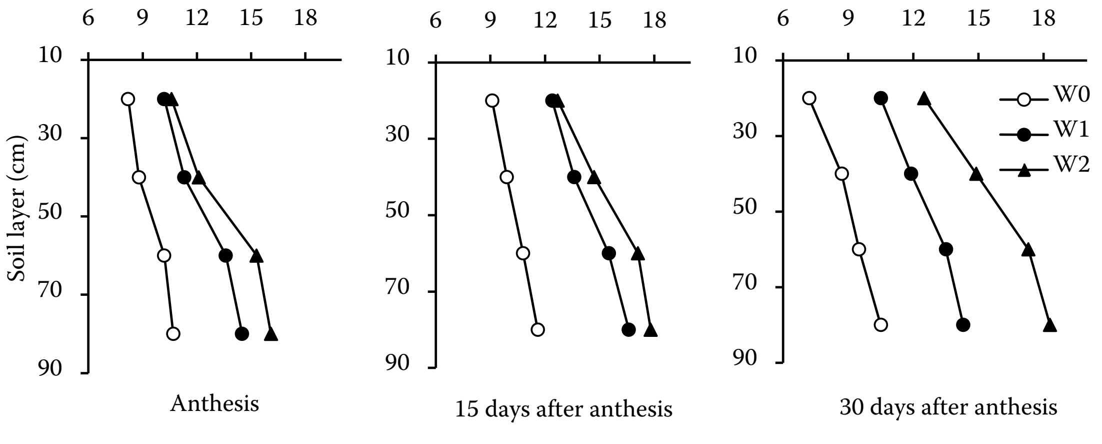

Figure 1. The soil water content (%) after anthesis in field experiment. Irrigation levels: W2 – before-wintering, jointing, anthesis, and grain filling; W1 – at jointing, and anthesis; W0 – no irrigation

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spikes were sampled at 7-day intervals till maturity (35 DAA). The grains from these spikes were weighted to get the fresh mass and then oven-dried at 70°C for 72 h to get the dry mass. The dried grains were then ground into flour for the measurement of GMP and HMW-GS content, and GMP particle size distribution.

Determination of GMP and HMW-GS content. GMP concentration was measured by the method of Weegels et al. (1996). HMW-glutenin subunits were separated by SDS-PAGE, and quantification of each individual subunit was performed according to the method of Yue et al. (2007), and total HMW-GS concentration was the sum of each individual subunit.

Determination of GMP particle size distribution. Particle size characteristics of GMP were determined according to the method of Jia et al. (2012).

Statistical analysis. Analysis of variance was performed with the SPSS statistical analysis package (Chicago, USA). Data from each sampling date were analysed separately. Duncan's new multiple range test was employed to assess differences between the treatment means at P = 0.05.

RESULTS

Changes in grain water content. The grain water content in both near-isogenic lines followed a similar pattern under different irrigation levels (Figure 2). With the process of grain filling, water content in grains increased before 21 DAA and then declined after reaching a maximum in all treatments. In field experiment (Figures 2a,b), the grain water content for treatment W0 was higher than that for treatment W1 and W2 at 7–21 DAA, while declined rapidly at 21–28 DAA to values lower than those in treatment W1 and W2. In pot experiment (Figures 2c,d), the water content in WS grains were markedly increased during 7–14 DAA,

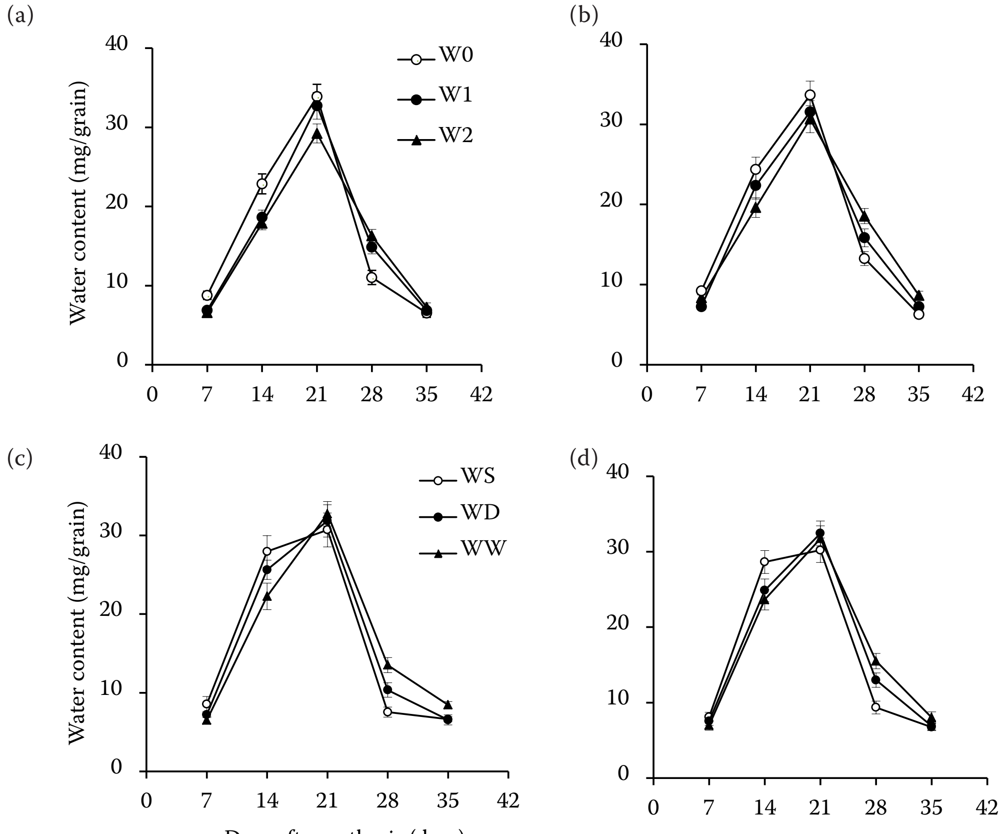

Figure 2. Changes of water content in grains of Line 11 (a, c) and Line 12 (b, d) in field (a, b) and pot (c, d) experiments. Irrigation levels: W2 – before-wintering, jointing, anthesis, and grain filling; W1 – at jointing, and anthesis; W0 – no irrigation; WW – well-watered (–0.02 MPa); WD – mild water-deficit (–0.05 MPa); WS – severe water-deficit (–0.08 MPa)

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gradually reached its peak 21 DAA and then sharply decreased thereafter when compared with that in WW and WD grains. This suggests that water limitation is beneficial for the increase of grain water content at early filling stage and grain desiccation at late filling stage.

HMW-GS accumulation. Changes in HMW-GS accumulation of all treatments in the two experiments showed a similar trend, which was initially formed at 14 DAA and increased gradually during grain filling (Figure 3). In treatment W0 (field experiment) and WS (pot experiment), the total HMW-GS concentration increased rapidly from 14–28 DAA, and then slowed down after 28 DAA. As for other treatments, the HMW-GS accumulated continuously during the whole grain filling stage. As a result, the total HMW-GS concentration at maturity was much lower in W0 and WS than in the other treatments. The results suggested that soil water deficit were disadvantageous for HMW-GS accumulation.

GMP accumulation. In the two experiments, GMP content in both near-isogenic lines increased at 7–14 DAA, and declined at 14–21 DAA or 14–28 DAA, and then increased during late grain filling (Figure 4). The decline may be related to the rapid accumulation of starch at this phase. At maturity, the GMP content in field experiment (Figures 4a,b) was ordered as follows: W1 > W0 > W2 in Line 12, and W1 > W2 > W0 in Line 11. In pot experiment (Figures 4c,d), the GMP content was the highest in WD, followed by WW, and the lowest in WS. The results indicated that mild watering could promote the accumulation of GMP.

At maturity, the GMP contents in Line 11 were increased by 8.0–18.0% and 11.1–12.3% when compared with Line 12, respectively, in field and pot experiment. This suggested that more glutenin was accumulated in Line 11.

Glutenin particle size distribution. The percent volumes of GMP particles with diameters < 10, 10–100 and > 100 μm in all test samples made up 19.7–35.5%, 45.9–54.3% and 15.0–27.7% of the total GMP particles, respectively (Table 1). Under different irrigation levels, the percent volumes

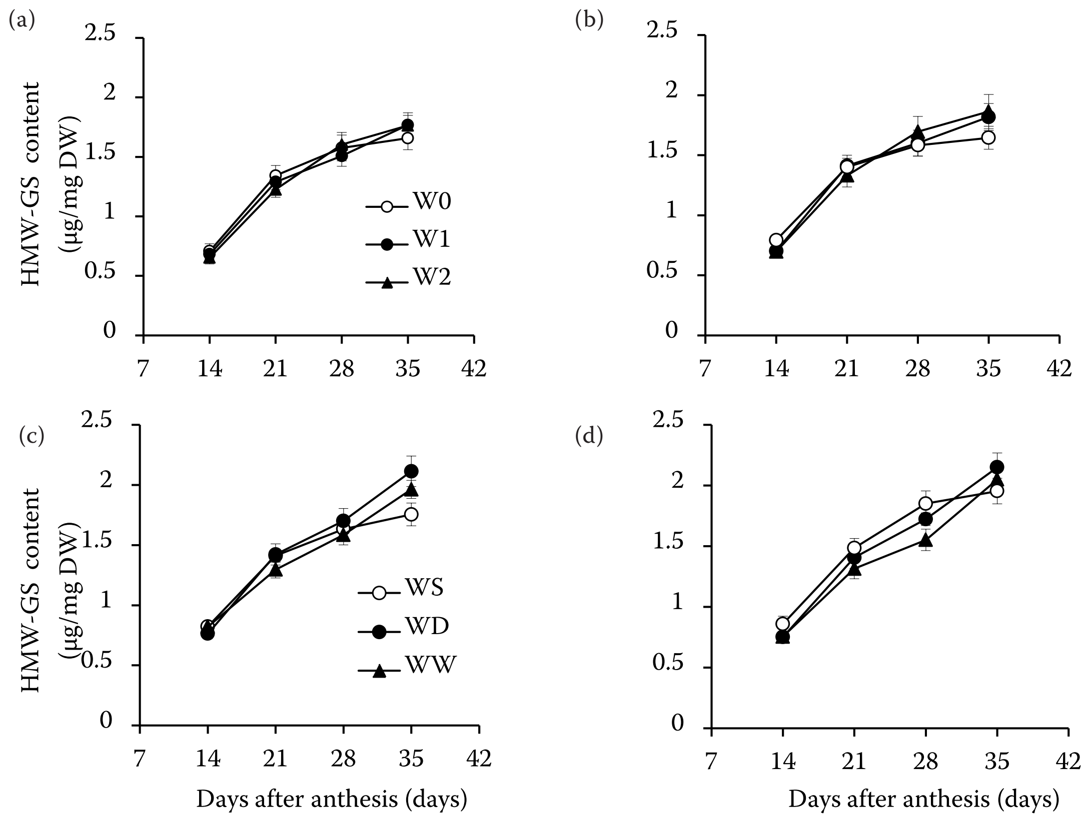

Figure 3. High molecular weight glutenin subunits (HMW-GS) content in grains of Line 11 (a, c) and Line 12 (b, d) in field (a, b) and pot (c, d) experiments. DW – dry weight. Irrigation levels: W2 – before-wintering, jointing, anthesis, and grain filling; W1 – at jointing, and anthesis; W0 – no irrigation; WW – well-watered (–0.02 MPa); WD – mild water-deficit (–0.05 MPa); WS – severe water-deficit (–0.08 MPa)

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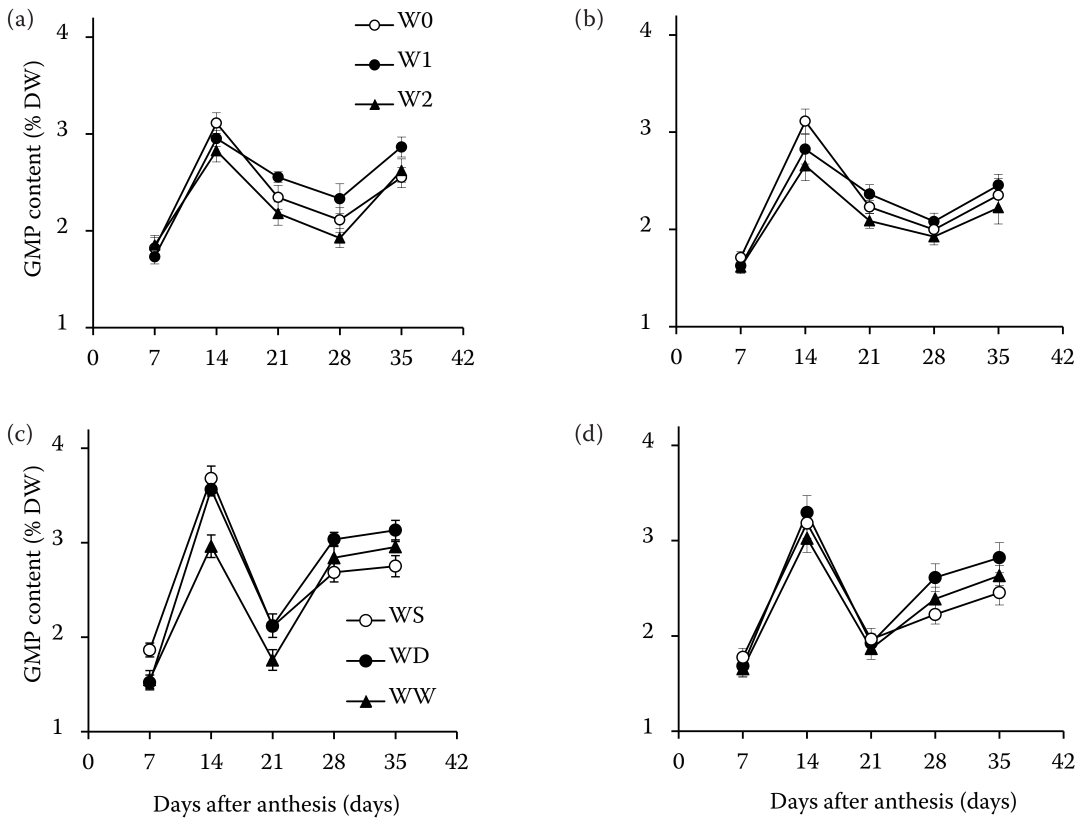

Figure 4. Glutenin macropolymer (GMP) content in grains of Line 11 (a, c) and Line 12 (b, d) in field (a, b) and pot (c, d) experiments. DW – dry weight. Irrigation levels: W2 – before-wintering, jointing, anthesis, and grain filling; W1 – at jointing, and anthesis; W0 – no irrigation; WW – well-watered (–0.02 MPa); WD – mild water-deficit (–0.05 MPa); WS – severe water-deficit (–0.08 MPa)

of > 100 μm in three treatments were ordered as follows: W1 > W0 > W2 in field experiment and WD > WW > WS in pot experiment, respectively, whereas those of < 10 μm were opposite. The results showed that both water deficits and excess watering led to an increase in volume percentage of smaller particles (< 10 μm) and a decrease in that of lager particles (> 100 μm), respectively.

Compared with Line 12, the percent volumes of > 100 μm in Line 11 were significantly increased, whereas those of < 10 μm were markedly decreased in the two experiments. This indicated that Line 11 had higher volume percentages of larger particles when compared with Line 12.

DISCUSSION

At maturity, the GMP contents and larger glutenin particles in Line 11 (HMW-GS null, 7 + 9, 5 + 10) were significantly increased when compared with Line 12 (HMW-GS null, 17 + 18, 5 + 10) in field and pot experiment. This suggests that the subunit 7 + 9 play an important role in the formation of GMP, which could be attributed to the structure of *Glu-B1*. Previous studies have showed that glutenins from wheat near-isogenic lines with HMW-GS 5 + 10 at *Glu-D1* and 7 + 9 at *Glu-B1* start to polymerize earlier and reach higher MWs than lines with HMW-GS 2 + 12 at *Glu-D1* and 20x + 20y at *Glu-B1* (Gupta et al. 1996). The faster polymerization may be explained by a higher concentration of S-H groups as reactants in the formation of S-S bonds in the polymerization reaction. The HMW-GS 7 (*1Bx7*) as the individual subunit of the 7 + 9 pair that is the major contributor to dough strength. This subunit contains two more cysteine residue than subunit 26 and 20x (Naeem et al. 2012). Butow et al. (2003) proposed that the 643 bp insertion in the DNA matrix attachment region of *1Bx7* alleles increased transcriptional efficiency.

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Table 1. Distribution of glutenin macropolymer (GMP) percent volume in Line 11 and Line 12 under different irrigation treatments

Experiment	Cultivar	Treatment	Diameter of GMP particles (μm)		
			< 10	10–100	> 100
Field	Line 11	W2	26.4^{b}	49.8^{d}	23.8^{c}
		W1	19.7^{d}	52.6^{bc}	27.7^{a}
		W0	22.5^{c}	51.9^{c}	25.6^{b}
	Line 12	W2	34.5^{a}	45.9^{e}	19.6^{d}
		W1	22.0^{c}	54.3^{a}	23.7^{c}
		W0	24.2^{bc}	53.0^{ab}	22.8^{c}
Pot	Line 11	WW	26.0^{d}	52.4^{a}	21.6^{b}
		WD	23.6^{e}	51.5^{ab}	24.9^{a}
		WS	33.0^{b}	49.6^{c}	17.4^{c}
	Line 12	WW	30.9^{c}	51.0^{ab}	18.1^{c}
		WD	26.3^{d}	52.8^{a}	20.9^{b}
		WS	35.5^{a}	49.5^{c}	15.0^{d}

Data in the table are the mean of 3 replicates. Values followed by a different letter within columns are significantly different at $P < 0.05$. Irrigation levels: W2 – before-wintering, jointing, anthesis, and grain filling; W1 – at jointing, and anthesis; W0 – no irrigation; WW – well-watered (–0.02 MPa); WD – mild water-deficit (–0.05 MPa); WS – severe water-deficit (–0.08 MPa)

Previous studies demonstrated that both composition and concentration of HMW-GS in wheat grains are closely related to the formation of GMP (Don et al. 2006). In the present study, there have been no significant differences in HMW-GS concentration between Line 11 and Line 12 under the same treatment, whereas the GMP contents in Line 11 significantly increased when compared with Line 12. This indicates that the composition of HMW-GS plays an important role in accumulation of GMP. However, the results are inconsistent with previous findings showing that GMP concentration is closely correlated to the individual and total HMW-GS concentrations (Li et al. 2012). It is due to the fact that both quantities and composition of HMW-GS play a role in determining the quantity and size of insoluble glutenin (León et al. 2009). In addition to quantity, the number or type of HMW-GS helps control the HMW/LMW ratio which in turn controls the apparent size of the glutenin particles isolated from wheat flour (Don et al. 2006).

Wheat kernel development can be divided into three phases i.e. cell division, cell enlargement and dehydration. During the dehydration phase, the polymerization of glutenin subunits occurs to form very large glutenin polymers. A close correlation has been found between the accumulation of GMP and the rapid loss of water during desiccation (Stone and Nicolas 1996). In this study, the grain water content for treatment W0 and WS were rapidly decreased after 21 DAA when compared with that in other treatments, indicating that drought promoted the grain desiccation. The desiccation rate during late grain filling was ordered as follows: W0 > W1 > W2 and WS > WD > WW in field and pot experiment, respectively. This is consistent with previous studies that multiple heat and drought stresses modified the early dough stage and maturity, and shortened the kernel desiccation period (Zhang et al. 2013). Jia et al. (2012) also reported that the time of grain rapid dehydration was postponed with the increase of irrigation amounts.

In parallel with grain desiccation, the accumulation of GMP for treatment W0 and WS was mainly at early grain filling; however in treatment W2 and WW it was at late grain filling. As a result, the GMP content and large glutenin particles (> 100 μm) for treatment W1 and WD were significantly higher than those for other treatments. It indicates that water availability may influence the accumulation of GMP and the size distribution of the glutenins through the modification of grain dehydration. This confirms the results of Jia et al. (2012), who found that drought or excess water led to less and smaller GMP formed, indicating irrigation levels influenced both glutenin biosyn-

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thesis and glutenin particle formation. These data have shown that water availability and genotype are important factors that influence the formation of yield and flour quality, which is consistent with the results of Madani et al. (2012). While managing for wheat yield and quality, growers should consider both the water and genotype factors to improve the end-use quality of wheat.

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