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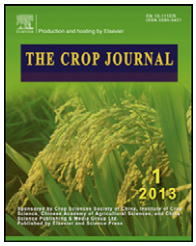

Variation of high-molecular-weight glutenin subunits and glutenin macropolymer particle distribution in wheat grains produced under different water regimes

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ABSTRACT

The components and contents of high-molecular-weight glutenin subunits (HMW-GS) in wheat grains affect glutenin macropolymer (GMP) size, which is considered an important flour quality trait in wheat. Four wheat cultivars (Shiluan 02-1, Yannong 24, Jinan 17 and Lumai 21) with different end-use qualities were used to investigate the HMW-GS and GMP contents, and the GMP particle distributions in grain produced under irrigated and rainfed conditions. The percent volume of GMP particles and the contents of HMW-GS and GMP were affected by genotype and soil water. Genotype × soil water interaction was significant only for GMP particles <12 μm and >100 μm in the growing season of 2010–2011. Irrigated and rainfed conditions had different influences on the GMP particle distribution in the four cultivars. Compared to irrigated treatment, the rainfed treatment had higher accumulations of HMW-GS and GMP, especially in cultivars Yannong 24, Jinan 17 and Lumai 21. Rainfed conditions also increased the proportion of large size particles of GMP, indicating that different water regimes had an effect on grain quality. According to correlation coefficients (*r*), the contents of HMW-GS and GMP in grains were negatively correlated with the volume of <12 μm GMP particles, but positively correlated with GMP particles >100 μm.

1. Introduction

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) are a major component of the grain and an important factor affecting the processing quality of wheat [1]. Previous studies demonstrated that the amount of GMP in wheat flour correlates closely with baking quality [2,3]. Besides GMP content, GMP particle size and distribution are important in wheat bread-making quality [4]. Evidence indicates that GMP particle size strongly correlates with dough development time [5].

GMP consist of high molecular weight glutenin subunits (HMW-GS) linked with low molecular weight glutenin subunits

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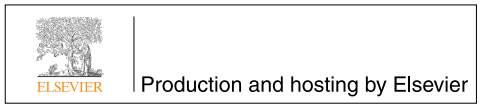

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(LMW-GS) through disulfide bonds [6]. HMW-GS play an important role in determining the glutenin protein network structure [5], and LMW-GS may also have a specific effect on glutenin aggregation [4]. GMP consisting of a higher ratio of HMW-GS to LMW-GS is correlated with improved wheat flour quality [7]. Therefore, subunit composition and GMP characteristics determine the rheological properties of wheat dough, and a close correlation between GMP characteristics and end-use quality has been shown.

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* [8].

The content and size distribution of GMP in wheat grains are both genetically and environmentally controlled. Drought promotes HMW-GS accumulation in the early grain filling stage, whereas the opposite effect occurs at late grain filling [9]. Increasing N fertilizer increases the proportion of GMP in wheat flour [10]. Clay soil results in the accumulation of HMW-GS and GMP when compared to loam soil and sandy soil [11]. When under high temperature stress during the kernel filling period, the contents of particular Glu-D1 HMW-GS in weak-gluten wheat are much more sensitive than that in strong-gluten wheat [12].

In recent years, frequent soil water stress in northern China has influenced both dry matter production and quality of wheat [13]. Increased N levels promoted the accumulation of HMW and LMW-GS, GMP content and proportion of larger GMP particles under irrigated conditions. Under rainfed conditions, increased N fertilizer also increased protein content [14]. Both 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 [15]. However, information about the impact of different irrigation patterns on accumulations of GMP in wheat grain is still limited.

Although numerous studies have been conducted on size distribution and properties of GMP particles in wheat grains, there is limited information about the size distribution of different quality types of wheat under irrigated and rainfed conditions. The objective of the present study was to investigate differences that may occur in GMP accumulation in field-grown wheat cultivars under irrigated and rainfed regimes. HMW-GS and GMP contents and GMP particle distributions in four wheat cultivars were therefore investigated.

2. Materials and methods

The experiment was conducted on the experimental farm of the Research Institute of Agricultural Science (37°N, 116°E), Dezhou, China. Four recently released winter wheat cultivars with different end-use qualities were used. They were Shiluan 02-1 (HMW-GS 1Ax1, 1Bx7 + 1By9, 1Dx5 + 1Dy10) and Jinan 17 (1Ax1, 1Bx7 + 1By8, 1Dx4 + 1Dy12) with strong gluten strength, Yannong 24 (1Ax1, 1Bx7 + 1By8, 1Dx5 + 1Dy10) with medium gluten strength, Lumai 21 (1Ax1, 1Bx7 + 1By8, 1Dx5 + 1Dy10) with weak gluten strength. Shiluan 02-1, Yannong 24, and Lumai 21, were used in the growing season of 2010–2011. The 0–20 cm soil layer contained 83.6 mg kg^{-1} of available nitrogen, 18.2 mg kg^{-1} of available phosphate and 95.2 mg kg^{-1} of available potassium. Wheat cultivars Jinan 17 and Lumai 21 were used in the 2009–2010 growing season when the soil contained available nitrogen-phosphate-potassium at 81.5, 17.6 and 93.6 mg kg^{-1}, respectively. Two contrasting water regimes (irrigated and rainfed) were used. The irrigated treatment was two irrigations with the total water amount of 1500 m^3 ha^{-1} over the whole growth period (750 m^3 ha^{-1} each at jointing and booting stages, respectively), whereas the rainfed treatment had no irrigation. The moisture content in soil after anthesis is shown in Fig. 1. The experiment was a complete randomized block design with three replicates. Plot dimension was 3 m × 3 m. Plants were sown on 12 October 2010 and 15 October 2009, respectively, at a density of 180 seeds m^{-2}. Normal crop farming practices were implemented to minimize pest, disease and weed incidence. After full heading, spikes flowering on the same date were labeled with thread. At maturity (14 June 2011 and 15 June 2010, respectively), the labeled heads were sampled and used to determine the GMP particle distributions. GMP and HMW-GS contents were also determined.

The content of GMP was analyzed as follows: 0.05 g of flour was dispersed into and mixed with 1 mL of SDS and then centrifuged at 15,500 ×*g* for 15 min using an Allegra X-64R centrifuge (Beckman, San Francisco, CA, USA) and the supernatant was retained. Glutenin macropolymer content was measured using TU-1901 dual-wavelength spectrophotometer (Persee Instruments, Beijing, China). Glutenin macropolymer content was calculated using a set of Kjeldahl protein values.

Glutenin macropolymer-gel was isolated by dispersing 1.4 g of defatted flour in 0.05 mol L^{-1} SDS (pasteurized, 28 mL) and then centrifuged at 80,000 ×*g* for 30 min at 20 °C using a Beckman L-60 ultracentrifuge (Beckman, San Francisco, CA, USA) as described [16]. The GMP gel-layer was collected from the top of the supernatant.

For Coulter laser particle size analysis, 1 g of GMP-gel was added to 8 mL of 0.05 mol L^{-1} SDS solvent. The tube was sealed and placed on a roller-bank for 3 h at room temperature and analyzed with a Coulter Laser LS13320 (Beckman Coulter Instruments, San Francisco, CA, USA). The GMP surface area distribution and volume distribution were measured and calculated from the resulting pattern.

Quantification of HMW-GS was performed according to the following method [17]. In brief, HMW-GS were first separated by SDS-polyacrylate gel electrophoresis (SDS-PAGE) according to Khan et al. [18]. A 40 mg grain sample was defatted with chloroform and then mixed with 1 mL of extraction buffer containing 62.5 mmol L^{-1} Tris–HCl (pH 6.8), 50% isopropyl alcohol, 5% SDS and 1% DTT. The mixture was incubated at room temperature for 30 min with continuous shaking, and then at 60 °C for 1 h, followed by centrifugation at 10,000 ×*g* for 15 min. The supernatant was used for SDS-PAGE.

The SDS-PAGE gel was 16 cm × 16 cm and 1 mm thick. The acrylamide concentration in the resolving gel was 10% and 4% in the stacking gel. Glutenin extract (20 μL) was loaded in each lane. After electrophoresis, the gel was stained with 0.05% Coomassie Brilliant Blue B250 for 24 h, and then destained in distilled water for 48 h. Thereafter, each band was separately cut from the gel, placed in an Eppendorf tube and depending on the intensity of each band, 1 mL of 50% isopropyl alcohol