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Nitrogen topdressing timing influences the spatial distribution patterns of protein components and quality traits of flours from different pearling fractions of wheat (*Triticum aestivum* L.) grains

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ABSTRACT

The major components determining wheat flour quality such as protein and protein fractions are unevenly distributed in different fractions of grains, which result in the spatial variations in processing quality of the corresponding flours. In this study, wheat grains were pearled into nine fractions from the outermost to the innermost layers. The effect of nitrogen topdressing timing basing on leaf age on the spatial distribution of bread-baking quality and contents of proteins within flour from different layers of grains in two wheat cultivars were analyzed. The contents of gliadin, glutenin and gluten presented a unimodel curve and peaked at the 2nd (P2) or the 3rd (P3) layer from the outmost of grains. Baking quality was the best with the breads made of flour from the P3 and P4 fractions. Delaying application of topdressed nitrogen increased contents of gluten proteins in each fraction, especially for layers of the aleurone and the outer endosperm. In addition, topdressing at the emergence of the topmost leaf of the main stem (the flag leaf, TL1) resulted in the highest bread-baking quality traits of volume, sensory score and texture profile analysis indexes with flour from each pearling fraction. The results indicated that better bread-baking quality could be achieved by selecting flour from the optimal pearling fractions of grains, and by delaying application of topdressed nitrogen.

1. Introduction

Wheat is a worldwide crucial food source for humans, contributing to over 45% of the global calories supply (kcal capita^{-1} day^{-1}) and over 40% of the global protein supply (g capita^{-1} day^{-1}) over the past decades (http://faostat.fao.org). It is reported that wheat products feed nearly half of Chinese people and more than 85% of wheat grains is consumed as various flour-derived products such as bread, noodles, cookies (Li, 2006).

Bread is the most important wheat product in the world. The baking quality of bread is highly relied on the content and quality of storage proteins in wheat grains. In general, the minimum protein content of 12% is required for bread-making according to the AACC protocols (Pomeranz, 1988). In terms of function, wheat grain protein can be classified into two groups: the non-gluten proteins of albumin and globulin with very limited role in bread-making, and the gluten proteins playing a crucial role in bread-making (Goesaert et al., 2005). The gluten proteins can be further divided into two functionally distinct groups, the monomeric gliadins and the polymeric (extractable and unextractable) glutenins. Of the glutenins, the sodium dodecyl sulfate (SDS) insoluble glutenin macropolymers (GMP) are very crucial in determining wheat dough quality (Don et al., 2003). GMP are glutenin complexes with high molecular weight glutenin subunits (HMW-GS) linked with low molecular weight glutenin subunits (LMW-GS) by disulfide bonds. Both HMW-GS and LMW-GS play an important role in determining the structure of the glutenin protein network as well as glutenin aggregation (Don et al., 2006; Dupont and Altenbach, 2003).

The contents of minerals and other chemical compounds vary largely within different pearling fractions of wheat grains (Liu et al., 2007; Xue et al., 2014; Guttieri et al., 2015). Xue et al. (2014) reported a progressive decrease in the contents of phosphorus, Fe, and Zn from the outer to the inner layers of grain. In contrast, a greater proportion of cadmium is observed in the endosperm, the inner part of grain (Guttieri et al., 2015). It is very interesting that proteins also non-homogeneously distribute in grains. Protein content per unit fresh volume in the outer region of developing endosperm was nearly as twice as that in the

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region adjacent to the endosperm cavity (Ugalde and Jenner, 1990). Ando et al. (2002) also reported that the protein content decreased continuously from the surface to the center of the eight milling fractions of wheat grains. Similarly, spatial gradients exist in the proportions of different types of gluten protein. The LMW-GS, ω- and α-gliadins in the subaleurone are more abundant than in the inner endosperm layers (Tosi et al., 2011). In addition, the outermost endosperm layers were rich in protein, especially for gliadins, while the inner layers were low in total protein content but were rich in HMW-GS (Tosi et al., 2011; He et al., 2013). However, knowledge is still limited on the spatial distribution of contents of the gluten proteins in wheat grains and its relationship with bread-making quality.

Nitrogen fertilization (N) is one of the most important management factors affecting grain yield and quality in wheat (Triboi et al., 2000). N increased contents of GMP and HMW-GS in wheat within a rate range between 0–300 kg ha^{-1}(Yue et al., 2007). N topdressing timing also significantly affects grain yield and quality in wheat. Weber et al. (2008) reported that delaying topdressing N from Zadoks 49 (late-boot stage) to Zadoks 59 (emergency of inflorescence completed) resulted in a significant or slight decrease in grain yield. In Ottman et al. (2000) study, N application at near anthesis stage could increase grain protein yield and kernel size as compared with earlier N application treatments. And there was a consensus that split applications of N with the final application at boot or later growth stages usually reach much higher protein (Ayoub et al., 1994; Brown et al., 2005). Furthermore, N also affected the distribution and quantity of glutenin (namely, the HMW-GS and LMW-GS). N is reported to improve HMW subunits accumulation in the outer layers than the inner layers of endosperm, whilst reversed for LMW-GS subunits and gliadins (He et al., 2013). However, it is unknown what the effects of N topdressing timing on spatial distributions of gluten proteins are.

In this study, two wheat cultivars with medium gluten content were grown in the field. Different timing of nitrogen topdressing was applied as referred to the leaf age. Mature grains were pearled into nine fractions. The distribution of total protein and protein components and their responses to nitrogen topdressing timing were evaluated. In addition, the quality of bread made of flour from different pearling fractions was also compared. We aimed to reveal the effects of nitrogen topdressing timing on the spatial distribution of gluten proteins and on bread-making quality. The results should help to provide a novel view for improving quality of bread product by pearling grains into different fractions and by nitrogen fertilization.

2. Material and methods

2.1. Experimental design

The experiment was conducted at the Tangquan Experimental Station of Nanjing Agricultural University, Nanjing (32°08′ N and 118°51′ E), and Tongshan Experimental Station of Xuzhou Academy of Agriculture Science, Xuzhou (34°26′ N and 117°20′ E), Jiangsu Province, P. R. China in wheat growth seasons of 2013–2014 and 2014–2015. The soils were clay, and their compositions are shown in Table S1-1. The average temperature and precipitation in the growing season are shown in Fig. S1. The amount of basal fertilizer and timing and the amount of topdressing nitrogen are shown in Table S1–2. Briefly, three nitrogen topdressing timings were included as at the emergence of the top-fifth-leaf (TL5), the top-third-leaf (TL3, in accordance to the jointing stage, which is the recommendation timing of nitrogen topdressing in practice), and the top-first-leaf (the flag leaf, TL1) of the main stem, respectively. Two winter wheat (*Triticum aestivum* L.) cultivars of Yangmai 16 (YM16, widely-grown in the downstream of Yangtze River wheat eco-region) and Zhongmai 895 (ZM 895, widely-grown in the Huang-Huai-Hai Plain) were planted in Tangquan and Tongshan, respectively. The plot size was 3 m × 3.2 m with a sowing density of 180 seedlings m^{-2} and row space of 0.25 m. The experiment was a randomized complete block design, with three biological replicates for each treatment (topdressing timing).

At maturity, heads of four m^2 were harvested to get grain samples. Grains were dried and kept for at least two months before milling.

2.2. Grain pearling

After hand-cleaning and adjusting the moisture to 14.0% on dry base, eight pearling fractions were sequentially prepared using the Foodstuff Machine (Streckel & Schrader, Germany). The grains were pearled into nine fractions from the surface to the center following previous reports with a minor modification (Liu et al., 2007; Hemery et al., 2009). Briefly, the pearling fractions were collected as flour enriched in husk (P1), aleurone layer (P2), outer endosperm (P3-P4), middle endosperm (P5-P6), and inner endosperm (P7-P9), and the innermost fraction (P9) accounting for ca. 20% of the grain. The P9 was milled with a universal high-speed grinding machine. Each pearling fractions were sieved at 80-mesh.

2.3. Contents of ash, protein and protein components

Ash content was determined according to an Approved Method 08-01 (AACC, 2000). The contents of protein and protein fractions (albumin, globulin, glutenin and gliadin fraction) were determined by the micro-Kjeldahl method of AACC 46-13.01 (AACC, 2004).

2.4. GMP content

Fifty mg flour from each layer were suspended in 1 ml of SDS (1.5%) solution and then centrifuged at 15 500*g* at 20 °C for 30 min. The sediment was washed twice with SDS solution (1.5%). Then the sediment was dissolved in 2 ml NaOH (0.2%) for 30 min. Afterwards, 3 ml Biuret reagent was added to the solution to evaluate the N content for further calculation of GMP content (Weegels et al., 1996; Zhang et al., 2013)

2.5. Quantifications of HMW-GS and LMW-GS

Total HMW-GS and LMW-GS were separated by our previous method (Yue et al., 2007). In general, 80 mg flour was dissolved in 1 ml isopropyl alcohol (50%), then water bathed at 65 °C for 20 min, following by centrifuging at 9 200*g* for 5 min. The sediment was kept and the supernatant containing gliadins was discarded. Then, 100 μl extraction buffer A containing 2% Dithiothreitol, 40 mM Tris-HCl (pH 8.0), SDS (2%), isopropyl alcohol (25%) was added to sediment, and the mixture was water bathed at 65 °C for 30 min to reduce disulfide bonds of proteins. Afterwards, 100 μl extraction buffer B containing 4-Vinylpyridine (1.4%), 40 mM Tris-HCl (pH 8.0), SDS (2%), isopropyl alcohol (25%) was added to the mixture, and water bathed at 65 °C for 15 min to allow for better separation of proteins by alkylating proteins. After centrifuging at 9 200*g* for 5 min, the supernatant containing proteins was collected and then put in a new centrifuge tube, to which 100 μl glutenin extraction buffer containing 62.5 mM Tris-HCl (pH 6.8), SDS (0.2%), β-mercaptoethanol (5%), sucrose (40%), bromophenol blue (0.5%) was added. The mixture was water bathed at 100 °C for 5 min followed by centrifugation at 9 200*g* for 5 min. The supernatant was used for SDS-PAGE.

The SDS-PAGE was carried out with our previous methods (Yue et al., 2007; Jiang et al., 2009). Glutenin extract (10 μl) was loaded in each lane. Quantifications of HMW-GS and LMW-GS were conducted by analysis software QUANTITY ONE. During the quantification procedure, a standard protein (Cat NO. 1610373, Bio-Rad, USA) with given concentration was separately loaded in 5, 10 and 15 μl volumes in three lanes on the same gel. The standard proteins were used to give a standard curve of known concentration. The content of each HMW-GS and LMW-GS in each lane was then quantified. A representative SDS-PAGE image was shown in Fig. S2.