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Zinc fertilization alters flour protein composition of winter wheat genotypes varying in gluten content

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ABSTRACT

Wheat flour protein components affect the processing quality of wheat. While it is known that zinc (Zn) fertilization can change flour protein content, there is little knowledge about Zn influence on flour protein composition. A pot experiment was conducted with five Zn fertilization treatments and three wheat genotypes differing in protein concentration and gluten composition. Zn fertilization up to 10 mg Zn/kg soil increased activity of nitrate reductase and glutamine synthetase in flag leaves after flowering, but 40 mg Zn/kg soil caused a genotype-dependent decline in these activities. Similarly, an increase in Zn fertilization was associated with a genotype-dependent increase in grain protein content and concentrations of gliadins, glutenins, albumins and globulin in flour, followed by a decrease of all three protein types at 40 mg Zn/kg soil. These results demonstrate that Zn nutrition can alter flour protein content and composition in wheat that has differential end uses, influencing flour quality.

Keywords: *Triticum aestivum*; plant-available Zn; seed bread; nutritional value; nutrient

Flour quality is determined by both genetic and environmental factors (Johansson et al. 2003). Many studies have reported that environmental conditions have a stronger effect on flour quality traits than genotype. In addition, plant nutritional status may also influence wheat grain quality (Naeem et al. 2012). Much of the published work has focused on the responses to the macronutrients, especially nitrogen and sulphur (Ercoli et al. 2011). Micronutrients also have an impact on nutrition and quality of wheat flour. For example, copper deficiency affected the extensibility of dough (Flynn et al. 1987). Zinc (Zn) plays an important role in the formation of protein and nitrogen assimilation process in grains of winter wheat (Li et al. 2011); Zn nutrition can affect flour quality by influencing the proportion of monomeric gliadins vs. polymeric glutenins. However, the specific mechanisms were not clear (Peck et al. 2008).

Soils with low Zn availability occur in many wheat-growing regions all over the world, including Turkey, India, Pakistan, China and Australia (Ozturk et al. 2006). Increasing the concentrations of plant-available Zn in soils can improve flour protein content (Hemantaranjan and Garg 1988). Starks and Johnson (1985) found that most of Zn applied to wheat was found in the flour protein, particularly in glutenins. Nitrate reductase (NR) and glutamine synthetase (GS) activity in the flag leaves affects the total protein content in wheat flour (Zhao et al. 2013), but may also influence the content of various protein components of flour. Zn fertilization can increase the activity of NR and GS in the flag leaves (Crawford 1995). Furthermore, Zn nutrition can alter the proportion of cysteine residues (Peck et al. 2008) and thus can affect interlinking of glutenin chains, influencing the structural and rheological properties of gluten (Tamás et al. 2002).

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The aims of this work were to characterize the effects of Zn nutrition on three wheat genotypes differing in protein content and composition and thus the end use in order to provide a scientific basis for improving the commercial value of wheat.

MATERIAL AND METHODS

Plant growth description. A pot experiment was conducted from October 13th, 2012 to June 1st, 2013 in a greenhouse at the farm of the Henan Agricultural University, Zhengzhou city, China (34°52'11''N, 113°35'26''E). The experimental soil was alluvial, and was collected from the farm.

The soil was air-dried, ground and sieved through a 2-mm sieve. The soil properties were: pH (v_{soil}:v_{water} = 1:5) 8.11, organic matter 12 g/kg soil, alkaline N (1 mol/L NaOH) 73 mg/kg soil, Olsen-P 7.2 mg/kg soil, available K 170 mg/kg soil, DTPA-Zn 0.75 mg/kg soil.

Experimental design. Each plastic pot (diameter 25 cm, height 30 cm) contained 10 kg of soil. Urea $CO(NH_2)_2$, $Ca(H_2PO_4)$ and KCl were applied in each treatment at the rate of 90 mg N, 90 mg P and 75 mg K per kg soil before sowing. In addition, 0.2 g urea/kg was added to each treatment at the jointing stage. $ZnSO_4 \cdot 7\ H_2O$ was used as Zn fertilizer. All chemical reagents were analytical grade; deionized water was used throughout.

Five Zn treatments (0, 5, 10, 20 or 40 mg Zn/kg) and three wheat cultivars were tested in a fully-factorial arrangement. Each treatment was replicated 16 times, with 12 replicates used to determine the nitrate reductase and glutamine synthetase in flag leaves at 0, 7 and 21 days after flowering (four replicates each, sampled in the morning on a sunny day). The remaining four replicates were grown to grain maturity (June 1st, 2013), and grain protein compositions were determined.

The seed of bread wheat cultivar (high-gluten wheat, Zhengmai9023), noodle wheat cultivar (moderate-gluten wheat, Aikang58 (AK58)) and biscuit wheat cultivar (weak-gluten wheat, Zhengmai004) was obtained from the Henan Provincial Academy of Agricultural Sciences. Ten seeds were sown, and the pots were thinned to five seedlings per pot.

Plant and grain analyses. The activity of NR and GS in flag leaf was determined according to the methods of Yu and Zhang (2012). Grain protein composition was measured by the sequential extraction method. Albumin, globulin, gliadin and glutenin were extracted by distilled water, 2% w/v NaCl, 70% v/v ethanol and 0.5% w/v KOH in succession. The extracts were read at 278 nm.

Statistical analyses. All data were statistically analyzed with a two-way ANOVA procedure using the SPSS 19.0 software (Chicago, USA). The main effects and the interaction were analyzed using *F*-value test. The mean values were subjected to multiple comparisons using the Duncan's-test at the $P = 0.05$ level.

RESULTS

Effects of Zn on grain yields of three wheat cultivars. Grain yield was significantly influenced by cultivar, Zn application and the cultivar × Zn interaction ($P < 0.01$) (Figure 1).

Zn application significantly increased ($P < 0.05$) grain yield of three wheat cultivars, with the highest grain yield in the treatments of Zn10 (Zhengmai 9023 and AK58) and Zn5 (Zhengmai 004). Compared with the treatment of Zn0, the highest grain yields of three wheat cultivars were increased by 20.14% (Zhengmai 9023), 5.18% (AK58) and 14.62% (Zhengmai 004), respectively.

Effects of Zn on the activity of nitrate reductase in flag leaves of three wheat cultivars after the flowering stage. NR activity in flag leaves was not significantly influenced by the cultivar × Zn

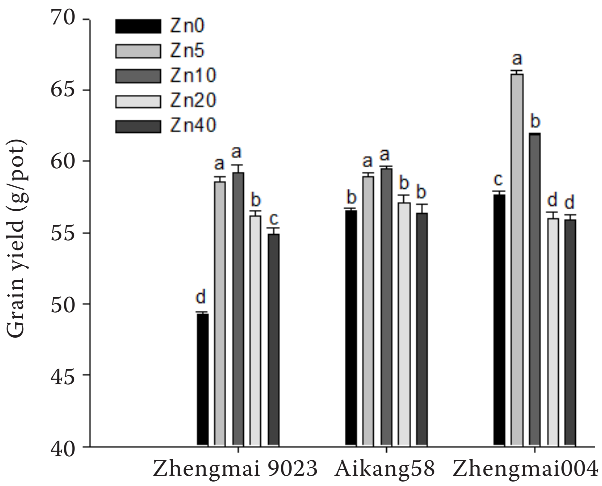

Figure 1. Effects of zinc (Zn) on grain yield in three wheat cultivars. The lowercase letters indicate significant differences among Zn fertilization rates by the Duncan's-test ($P < 0.05$). *F*-test: $F_{(cultivars)} = 132.26^{**}$; $F_{(Zn)} = 180.67^{**}$; $F_{(cultivars \times Zn)} = 40.45^{**}$. $^{*}P < 0.05$; $^{**}P < 0.01$; Zn treatments: 0, 5, 10, 20 or 40 mg Zn/kg

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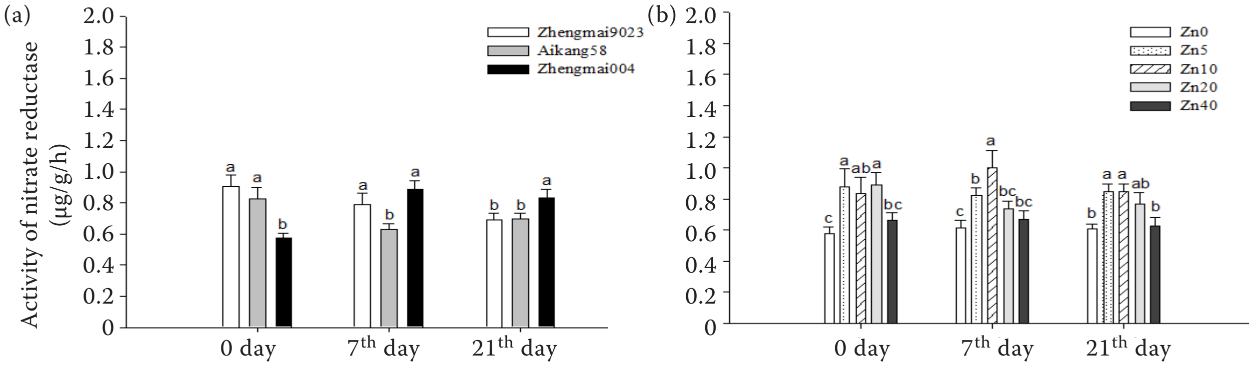


Figure 2. The main effects of (a) cultivars and (b) zinc (Zn) fertilizer on the activity of nitrate reductace of flag leaves after flowering stage. The lowercase letters indicate significant differences among cultivars (a) or Zn fertilization rates (b) by the Duncan's-test ($P < 0.05$). F-test: 0 day: $F_{(cultivars)} = 10.44^{**}$; $F_{(Zn)} = 4.08^{**}$; $F_{(cultivars \times Zn)} = 1.97$; 7th day: $F_{(cultivars)} = 9.19^{**}$; $F_{(Zn)} = 7.39^{**}$; $F_{(cultivars \times Zn)} = 2.11$; 21th day: $F_{(cultivars)} = 3.56^{*}$; $F_{(Zn)} = 4.63^{**}$; $F_{(cultivars \times Zn)} = 0.94$. $^{*}P < 0.05$; $^{**}P < 0.01$; Zn treatments: 0, 5, 10, 20 or 40 mg Zn/kg

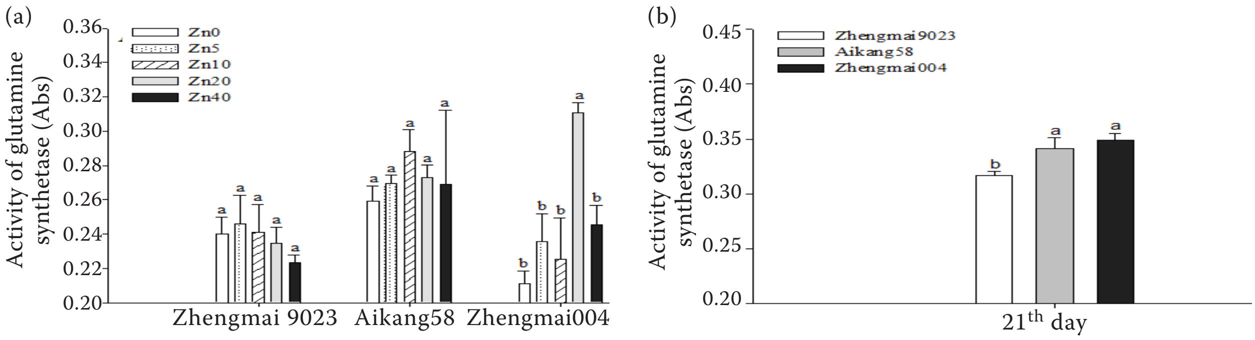
interaction, but the main effects were significant ($P < 0.01$ or $P < 0.05$) (Figure 2).

On day 0, Zhengmai9023 and Zhengmai004 had the highest and the lowest NR activity, and there was no significant difference between Zhengmai9023 and AK58 (Figure 2a). On the 7th day, Zhengmai004 and Zhengmai9023 had the similar NR activity that was significantly higher than that of AK58. On the 21th day, Zhengmai004 had higher NR activity than Zhengmai9023 and AK58.

The NR activity significantly increased ($P < 0.05$) and then decreased ($P < 0.05$) with the increase in Zn fertilization from 0 to 40 mg Zn/kg (Figure 2b). On days 0 and 21, the extreme Zn fertilization rates (0 or 40 mg Zn/kg) were associated with lower NR activity than the most of the other Zn fertilization treatments.

Effects of Zn on the activity of glutamine synthetase in flag leaves of three wheat cultivars after the flowering stage. The activity of GS was affected by the significant interaction cultivar × Zn at anthesis (day 0) and after 7 days. However, 21 days after anthesis, the interaction was non-significant (Figure 3a).

At days 0 and 7 after anthesis, there was a general trend of GS activity being lower at 0 and 40 mg Zn/kg



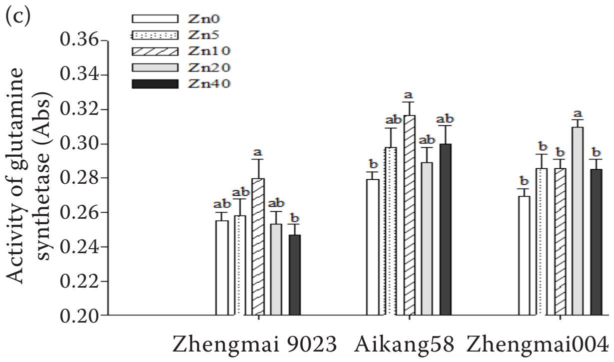


Figure 3. Effects of cultivars and zinc (Zn) fertilizer on glutamine synthetase in wheat flag leaves after flowering, including (a) 0, (b) 7 and (c) 21 days after anthesis. The lowercase letters indicate significant differences among cultivars and Zn fertilization rates (a,b) or cultivars (c) by the Duncan's-test ($P < 0.05$). F-test: 0 day: $F_{(cultivars)} = 7.15^{**}$; $F_{(Zn)} = 1.93$; $F_{(cultivars \times Zn)} = 2.35^{*}$; 7th day: $F_{(cultivars)} = 31.32^{**}$; $F_{(Zn)} = 4.40^{**}$; $F_{(cultivars \times Zn)} = 2.29^{*}$; 21th day: $F_{(cultivars)} = 6.22^{**}$; $F_{(Zn)} = 2.04$, $F_{(cultivars \times Zn)} = 1.21$. $^{*}P < 0.05$; $^{**}P < 0.01$. Zn treatments: 0, 5, 10, 20 or 40 mg Zn/kg

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than at other Zn fertilization levels (Figure 3b). Some of these differences were significant, and most of them were not. However, all these differences were relatively small and unlikely to have strong biological significance.

At day 21 after anthesis, neither the interaction nor the Zn fertilization effect was significant (Figure 3c). The activity of glutamine synthetase was higher in AK58 and Zhengmai004 than Zhengmai9023 ($P < 0.05$).

Effects of Zn fertilizer on grain quality in three winter wheat cultivars. Grain protein content was significantly influenced by cultivar, Zn application and the cultivar × Zn interaction ($P < 0.01$) (Figure 4). Zn application significantly increased ($P < 0.05$) grain protein content of three wheat cultivars, the highest grain protein content all occurred in the treatments of Zn10. Compared with the treatment of Zn0, the highest grain protein content of three wheat cultivars were increased by 13.63% (Zhengmai 9023), 3.71% (AK58) and 7.47% (Zhengmai 004), respectively.

For both gliadins and glutenins, lower concentrations were obtained under 0 or 40 mg Zn/kg than at other Zn rates, except for the Zhengmai004-glutenin combination (Figures 5a,b). The Zn rates associated with greater gliadin concentration than at extremes (0 and 40 mg Zn/kg) were: for Zhengmai9023 Zn5 and Zn10, for AK58 Zn10 and Zn20 and for Zhengmai004 Zn5 and Zn10 (Figure 5a). For glutenins these Zn rates were: Zn10 for Zhengmai9023 and Zn5 and Zn10 for AK58 Zn5, whereas for Zhengmai004 higher grain concentration was associated with Zn rates from 10 to 40 than from 0 to 5 mg Zn/kg (Figure 5b). Particularly significant increase with an increase in Zn fertilization from 0 to 5 mg Zn/kg were noted for gliadins in Zhengmai004 (Figure 5a) and for glutenins in AK58 (Figure 5b).

A similar trend, generally, was also observed in albumin concentration (Figure 5c). There was an increase in Zhengmai9023 and AK58 with increasing Zn application rates from Zn0 to Zn10 followed by a significant decrease when Zn rates were increased from 10 to 40 mg Zn/kg (Figure 5c). In Zhengmai004 higher albumin concentration was observed in treatments with 5 to 40 mg Zn/kg compared with Zn0 (Figure 5c).

With an increase in Zn fertilization globulin content decreased in Zhengmai9023 and AK58, but increased in Zhengmai004 (Figure 5d).

Bread wheat cultivar (Zhengmai9023) with high gluten had significantly higher ($P < 0.05$) glutenin content than the cultivar with relatively low gluten (Zhengmai004) (Figure 5b). In contrast, albumin content was higher ($P < 0.05$) in Zhengmai004 than the other cultivars (Figure 5d).

DISCUSSIONS

NR is very important for absorption and utilization of nitrogen and influenced yield and quality of crops (Raun and Johnson 1999). GS is a multifunctional enzyme in nitrogen metabolism involved in the regulation of many metabolism processes, and its activity may reflect the strength of nitrogen assimilation capacity (Foyer et al. 2003). In previous studies, many researchers observed that NR and GS activity were different between cultivars and soils available Zn concentration influenced the activities of NR and GS (Ghosh and Srivastava 1994). In our study, 5–20 mg/kg Zn application resulted in the increases in the activity of NR and GS, and three winter wheat genotypes indicated different influences on NR and GS. Therefore, the level of Zn nutrition can influence the activity of NR and GS in flag leaves, but the mechanism of these changes needs to be deeply studied.

Gluten is the main component of flour protein, and gluten proteins include gliadins (monomeric proteins) and glutenins (polymeric proteins), which

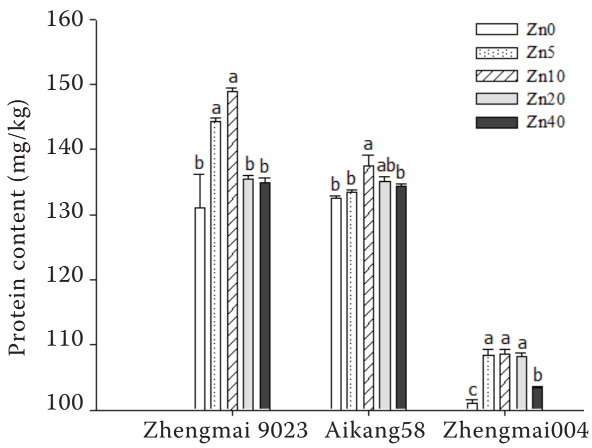

Figure 4. Effects of zinc (Zn) on grain protein content in three wheat cultivars. The lowercase letters indicate significant differences among cultivars and Zn fertilization rates by the Duncan's-test ($P < 0.05$). F-test: $F_{(cultivars)} = 684.14^{**}$; $F_{(Zn)} = 19.30^{**}$; $F_{(cultivars \times Zn)} = 5.26^{**}$. $^{**}P < 0.01$; Zn treatments: 0, 5, 10, 20 or 40 mg Zn/kg

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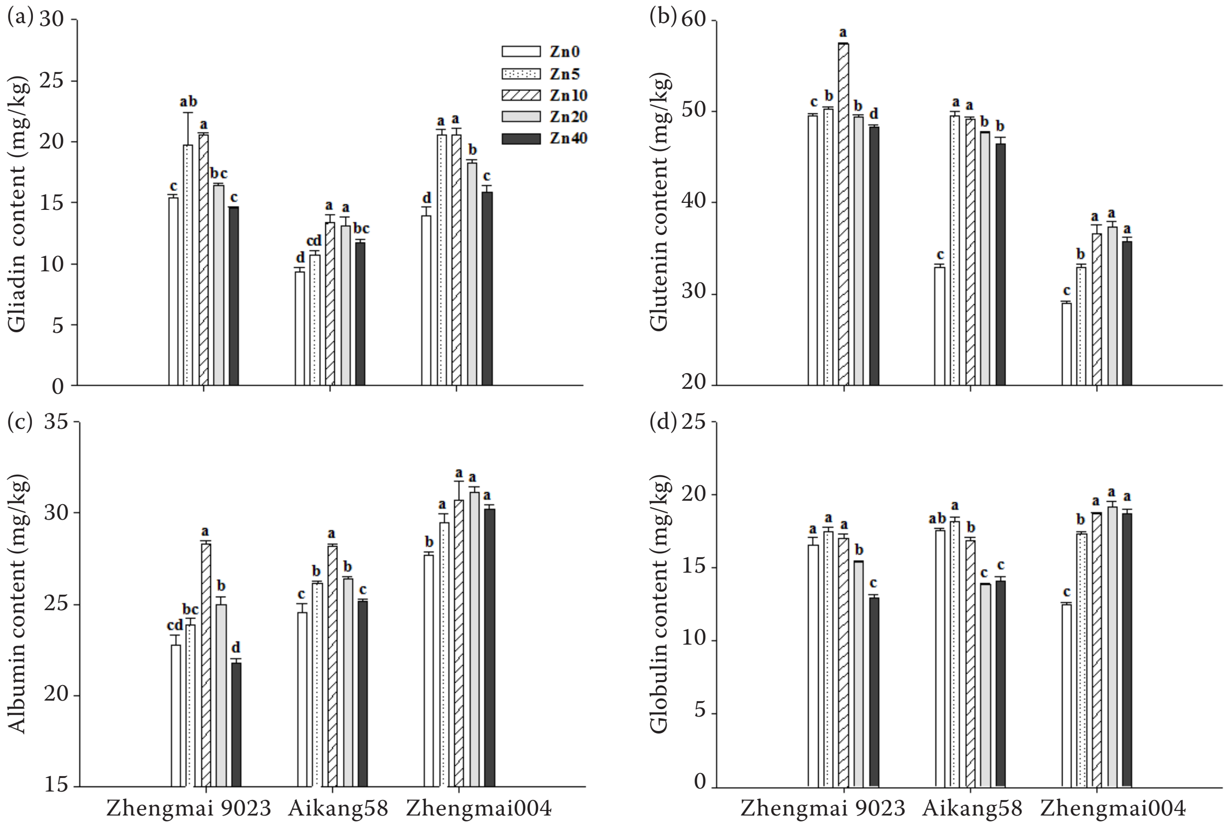

Figure 5. Effects of zinc (Zn) on grain protein composition, including (a) gliadin, (b) glutenin, (c) albumin and (d) globulin, in three wheat cultivars. The lowercase letters indicate significant differences among cultivars and Zn fertilization rates (a,b,c) by the Duncan's-test ($P < 0.05$). F-test: gliadin content: $F_{(cultivars)} = 229.42^{**}$; $F_{(Zn)} = 43.54^{**}$; $F_{(cultivars \times Zn)} = 7.35^{**}$; glutenin content: $F_{(cultivars)} = 39.43^{**}$; $F_{(Zn)} = 52.12^{**}$; $F_{(cultivars \times Zn)} = 76.70^{**}$; albumin content: $F_{(cultivars)} = 89.58^{**}$; $F_{(Zn)} = 21.21^{**}$; $F_{(cultivars \times Zn)} = 3.52^{**}$; globulin content: $F_{(cultivars)} = 1935.29^{**}$; $F_{(Zn)} = 246.67^{**}$, $F_{(cultivars \times Zn)} = 76.51^{**}$. $^{**}P < 0.01$; Zn treatments: 0, 5, 10, 20 or 40 mg Zn/kg

are predominant storage proteins and could directly affect the processing quality of wheat flour (Wang 1996, Goesaert et al. 2005). In contrast, non-gluten proteins are albumins and globulins, which are mainly structural proteins with high nutritional value and rich in the most limiting amino acid lysine (Wieser and Seilmier 1998). Not only flour protein content, but also the proportions of each protein type are closely related to flour quality (Deng et al. 2006) and thus the end use. Based on the end use, there are various wheats: bread wheat (high protein concentration and strong gluten strength), noodle wheat (moderate protein contents and gluten strength) and biscuit wheat (low protein contents and wet gluten content) (Shewry et al. 1997). It has been reported that N, S, Cu and Zn nutrition had impacts on flour protein components, especially glutenins (Zhao et al. 1999, Peck et al. 2008). The increase grain of albumin, globulin, gliadin and glutenin contents which were involved in increasing Zn fertilization could enhance the enzymes activity in nitrogen assimilation courses (Shi et al. 2011). In our study, Zn application had the significant effects on grain protein content and flour protein composition. The content of gliadin, glutenin, albumin and globumin all increased as the dose of Zn fertilizer in the proper level increased (5–20 mg Zn/kg). Gliadin, glutenin, albumin and globumin contents in flour changed with Zn fertilizer applied, which may be the results of Zn as disulfide bonds. Zn is closely related to cysteine, cysteine Zn proteome of Zn binding ligand ratio is 28%, the highest percentage for all amino acids (Zhang et al. 2012). However, because our study was conducted in a pot experiment, more work needs to be done in the field.

In conclusion, Zn application increased grain yield and influenced NR and GS activity, affecting the

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synthesis of grain protein content and components, thus impacting on processing quality and nutritional quality of different wheat genotypes. Especially for processing quality of flour, the role of Zn nutrition due to cultivars is different. For this study, the optimum amount of Zn application for Zhengmai9023 and AK58 is 10 mg/kg, for Zhengmai9023 the suitable Zn application amount is 20 mg/kg.

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