Effects of Nitrogen Application and Post-Anthesis Soil Relative Water Content on Nitrogen Uptake, Translocation, and Distribution During Grain Filling in Black-Grain Wheat (Postprint)

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Abstract

Using black-grained wheat 'Luozhen No. 1' as the experimental material, a pot experiment under shelter was conducted to investigate the effects of different nitrogen application rates and post-anthesis soil relative water content on nitrogen absorption, translocation, and distribution in 'Luozhen No. 1' plants, as well as on grain protein and its component contents. The results showed that under the same nitrogen application rate, grain nitrogen content and protein accumulation in black wheat decreased with intensifying water stress. Changes in various protein component contents differed among nitrogen application rates; under low nitrogen [N1, 150 kg(N) · hm−2] conditions, albumin, globulin, and gliadin contents increased with intensifying water stress, whereas under high nitrogen [N3,300 kg(N) · hm−2] conditions, albumin and globulin contents increased while gliadin content decreased. Under the same water stress conditions (soil relative water content of 55%~65%, W2; soil relative water content of 35%~45%, W3), grain nitrogen content and protein accumulation increased with increasing nitrogen application rate, while the proportion of grain nitrogen content to total nitrogen content at maturity decreased. However, under sufficient water supply (soil relative water content of 75%~85%, W1), the medium nitrogen treatment [N2, 240 kg(N) · hm−2] achieved the highest grain protein accumulation, with the translocation amount and translocation rate of stored nitrogen from vegetative organs to grains reaching maximum values and a relatively high contribution rate to grains. Under W1 treatment, albumin, globulin, and gliadin contents increased with increasing nitrogen application rate, with glutenin reaching its maximum under N2 treatment; whereas under W2 and W3 treatments, the N2 treatment resulted in the highest contents of various protein

components in wheat. In summary, under the experimental conditions, nitrogen application rate and post-anthesis soil relative water content significantly affected nitrogen metabolism in black-grained wheat; excessively high or low nitrogen application rates and water stress were all detrimental to the effective progression of nitrogen metabolism processes in black-grained wheat. Overall, the combination of sufficient post-anthesis water supply (W1) and medium nitrogen level (N2) exerted favorable regulatory effects on nitrogen absorption, translocation, and distribution in black-grained wheat.

Full Text

Introduction

Nitrogen metabolism is one of the most fundamental metabolic processes in plants, and this holds true for wheat (*Triticum aestivum*) as well. Nitrogen metabolism exerts important influences on both yield formation and quality improvement in wheat. In addition to the genetic characteristics of varieties themselves, cultivation practices represent a crucial factor affecting wheat nitrogen metabolism [**?**]. Xu et al. [**?**] demonstrated that improving soil moisture conditions can promote post-anthesis nitrogen translocation from vegetative organs to grains, thereby increasing both total nitrogen yield and biomass production while enhancing the proportion of fertilizer nitrogen absorbed by various organs. Ma et al. [**?**] reported that under the same nitrogen application level, either excessively high or low post-anthesis soil relative water content is detrimental to free amino acid synthesis in leaves, affecting their translocation to grains and consequently hindering grain protein accumulation. Under identical soil moisture conditions, grain protein content and accumulation increase with nitrogen application rate, though excessive nitrogen reduces the magnitude of this increase.

Ma et al. [**?**] investigated water consumption characteristics and nitrogen distribution in wheat under different nitrogen rates, concluding that appropriate nitrogen application or adequate irrigation under nitrogen-fertilized conditions both facilitate nitrogen translocation from vegetative organs to grains. Meanwhile, nitrogen application rate and soil relative water content also influence grain protein components to some extent [**?**]. Black wheat 'Luozhen 1' , a type of colored wheat, has attracted considerable research attention due to its high protein and amino acid content, as well as its richness in essential trace elements such as Fe, Zn, and Se [**?**], offering high nutritional value and development potential [**?**]. Although colored wheat possesses high nutritional value, its yield remains relatively low, primarily attributable to poor photosynthetic capacity and inefficient matter translocation during critical yield formation stages [**?**]. However, previous studies on the effects of nitrogen application rate and post-anthesis soil relative water content on nitrogen metabolism processes and grain protein composition in colored wheat are scarce. Therefore, this experiment selected black wheat 'Luozhen 1' as the test material to investigate the effects

of nitrogen application and post-anthesis soil relative water content on nitrogen absorption, translocation, and distribution, aiming to provide theoretical and technical references for improving matter translocation capacity during critical yield formation stages and achieving high-yield, high-quality cultivation of black wheat.

1.1 Experimental Site Overview

The experiment was conducted during the 2014-2015 growing season under a rainproof shelter at the Institute of Crop Science, Chinese Academy of Agricultural Sciences. The test soil was loam with the following nutrient contents: organic matter 9.84 $g \cdot kg^{1}$, total nitrogen 0.58 $g \cdot kg^{1}$, alkaline hydrolysis nitrogen 91.91 $mg \cdot kg^{1}$, available phosphorus 21.55 $mg \cdot kg^{1}$, available potassium 146 $mg \cdot kg^{1}$, and pH 7.86.

1.2 Experimental Design and Treatments

The test material was black wheat 'Luozhen 1' . A two-factor randomized block design was employed, with factors being nitrogen application rate (N) and post-anthesis water control (W). Three water control gradients were established: 1) Adequate water supply throughout the growth period (W1), with soil relative water content at 75%-85% of field capacity; 2) Moderate water stress treatment (W2), with water management identical to W1 before anthesis, but soil relative water content maintained at 55%-65% of field capacity from anthesis to harvest; and 3) Severe water stress treatment (W3), with water management identical to W1 before anthesis, but soil relative water content maintained at 35%-45% of field capacity from anthesis to harvest. During the water treatment period, pots were weighed daily and water was supplemented according to the control standards [**?**]. Three nitrogen application rates were established: low nitrogen treatment (N1) at 150 $kg(N) \cdot hm^{2}$, medium nitrogen treatment (N2) at 240 $kg(N) \cdot hm^{2}$, and high nitrogen treatment (N3) at 330 $kg(N) \cdot hm^{2}$. Urea (46% N) was applied as the nitrogen source at a base fertilizer to jointing fertilizer ratio of 5:5.

A pot experiment was conducted under an artificial rainproof shelter using soil as the substrate. The pots had a diameter of 26 cm, a surface area of 0.053 m^2, and contained 21 kg of dry soil per pot. Fifteen plants were grown per pot and thinned to the desired density at the five-leaf stage.

1.3.1 Determination of Nitrogen Content in Various Organs

Sampling began at anthesis (the day water treatment commenced and before water application) and continued every 7 days thereafter. Three replicates per treatment (nine plants total) were collected and separated into stems, leaves, and spikes. At maturity, samples were separated into stems, leaves, glumes + rachises, and grains. Samples were immediately brought indoors, placed in an oven at 105 °C for 30 minutes to deactivate enzymes, then dried at 80 °C to

constant weight before weighing and recording dry matter. Grains were ground using an FS-II laboratory cyclone mill, while other plant parts were ground using a high-speed grinder. Ground samples were weighed [grains (0.100±0.005) g, other parts (0.200±0.005) g], mixed with catalyst (a 10:1 mixture of potassium sulfate and copper sulfate by mass), and 6 mL of concentrated sulfuric acid was added. Samples were digested on a heating block at 420 °C until completely digested, and nitrogen content was determined using a K1302 Kjeldahl nitrogen analyzer from Shanghai Sheng Sheng Company.

Grain protein content (%) = grain nitrogen content (%) × 5.7 (1)

1.3.2 Determination of Grain Protein Components

Mature grains were collected from each treatment with three replicates (one pot per replicate). Grains were stripped, placed in an oven at 105 °C for 30 minutes, then dried at 80 °C to constant weight. After drying, grains were ground using an FS-II laboratory cyclone mill for protein component extraction and determination.

Protein components were extracted using a sequential extraction method. Ground grain samples (0.5 g, weighed on a 0.001 g precision balance) were placed in 10 mL centrifuge tubes. Following the extraction sequence of albumin, globulin, gliadin, and glutenin, the extraction solutions used were distilled water, 2% sodium chloride solution, 70% ethanol solution, and 0.5% potassium hydroxide solution, respectively. The extraction procedure was as follows: 5 mL of extraction solution was added to the sample, stirred continuously with a glass rod until completely broken down, then shaken on a shaker (30 minutes for the first extraction, 20 minutes for subsequent extractions). Samples were then centrifuged at 4,000 rpm for 5 minutes, and the supernatant was transferred to a stoppered glass tube. This process was repeated three additional times, and the four extracts were combined and mixed thoroughly for analysis. For determination, 5 mL of each component extract was taken and analyzed using the method described in Section 1.3.1.

1.3.3 Calculation Methods for Nitrogen-Related Indices

Nitrogen accumulation in each organ (mg · plant ^{1}) = nitrogen content (mg · g ^{1}) × dry matter weight of each organ (mg · plant ^{1}) (2)

Nitrogen accumulation in vegetative organs at anthesis (mg · plant ^{1}) = sum of nitrogen accumulation in stems, leaves, and spikes at anthesis (mg · plant ^{1}) (3)

Pre-anthesis stored nitrogen translocation amount (mg · plant ^{1}) = nitrogen accumulation per plant at anthesis - nitrogen accumulation in non-grain parts (stems, leaves, rachis + glume) per plant at maturity (4)

Pre-anthesis stored nitrogen translocation efficiency = (pre-anthesis stored nitrogen translocation amount / nitrogen accumulation per plant at anthesis) ×

100% (5)

Contribution rate of pre-anthesis accumulated nitrogen to grains = (pre-anthesis stored nitrogen translocation amount / total nitrogen content in grains at maturity) × 100% (6)

Nitrogen accumulation in grains at maturity (mg · plant ^{1}) = nitrogen content in grains at maturity × grain weight at maturity (mg · plant ^{1}) (7)

Nitrogen accumulation in vegetative organs at maturity (mg · plant ^{1}) = sum of nitrogen accumulation in stems, leaves, and spikes at maturity (mg · plant ^{1}) (8)

1.4 Data Processing

Data processing and analysis were performed using Microsoft Excel 2013 and DPS 15.10 statistical analysis software. Multiple comparisons were conducted using the LSR method.

2.1 Effects of Nitrogen Rate and Post-Anthesis Soil Relative Water Content on Grain Protein Accumulation in Black Wheat

As shown in Table 1 , grain protein accumulation in all treatments increased continuously with growth progression, reaching maximum values at 35 days post-anthesis, though differences existed among treatments. Under the same nitrogen rate, grain protein accumulation increased with intensifying water stress during 7–21 days post-anthesis; however, after 28 days post-anthesis, it decreased significantly with increasing water stress. Under the same irrigation level during 7–14 days post-anthesis, grain protein accumulation in N2 and N3 treatments was greater than in N1, though differences were not significant. At 21 days post-anthesis, under W1 and W2 irrigation, protein accumulation in N1 and N3 treatments was higher than in N2, while under W3, protein accumulation increased with nitrogen rate but differences remained non-significant. At 28 days post-anthesis, under W1 irrigation, protein accumulation followed the pattern N2 > N1 > N3, whereas under W2 and W3, it followed N1 N3 > N2, with no significant differences among treatments. At 35 days post-anthesis, under W1 irrigation, protein accumulation still followed N2 > N1 > N3, while under W2 and W3, it increased significantly with nitrogen rate. These results indicate that during the late grain-filling stage, increasing nitrogen rate can significantly enhance grain protein accumulation under moderate (W2) and severe (W3) water stress conditions, whereas adequate water supply (W1) combined with medium nitrogen rate (N2) effectively improves protein accumulation, with both insufficient and excessive nitrogen being unfavorable for grain protein accumulation.

2.2 Effects of Nitrogen Rate and Post-Anthesis Soil Relative Water Content on Nitrogen Accumulation in Various Organs at Maturity

Nitrogen rate and post-anthesis soil relative water content significantly affected nitrogen accumulation in grains and vegetative organs of black wheat at maturity (Table 2). Regarding grains, post-anthesis soil relative water content significantly influenced nitrogen accumulation under the same nitrogen level, which decreased with intensifying water stress, following the pattern W1 > W2 > W3. The proportion of grain nitrogen to total plant nitrogen showed the same trend. Under the same soil relative water content, grain nitrogen accumulation increased with nitrogen rate, while its proportion of total plant nitrogen decreased, indicating that increased nitrogen application enhances grain nitrogen accumulation but excessive nitrogen is detrimental to nitrogen translocation to grains. Regarding vegetative organs at maturity, under the same nitrogen rate, nitrogen accumulation in stems and sheaths decreased with intensifying water stress, while accumulation in leaves and rachises + glumes increased, as did their proportions of total nitrogen, suggesting that water stress hinders nitrogen translocation from vegetative organs to grains. Under the same soil relative water content, nitrogen accumulation in all vegetative organs increased with nitrogen rate, as did their proportions of total nitrogen, indicating that excessive nitrogen application increases nitrogen accumulation in vegetative organs at the expense of translocation to grains.

2.3 Effects of Nitrogen Rate and Post-Anthesis Soil Relative Water Content on Pre-Anthesis Stored Nitrogen Translocation to Grains

As shown in Table 3 , both nitrogen rate and post-anthesis soil relative water content significantly regulated nitrogen translocation from vegetative organs to grains in black wheat. Between 68.6% and 89.3% of grain nitrogen originated from translocation of pre-anthesis stored nitrogen in vegetative organs. The translocation amount and efficiency of stored nitrogen from vegetative organs to grains reached maximum values under the W1 irrigation and N2 nitrogen treatments, with a relatively high contribution rate to grains, indicating that the W1N2 water-nitrogen combination was most favorable for nitrogen translocation. The contribution rate of translocated nitrogen to grains reached its maximum under the W3N3 combination, suggesting that under severe water stress, high nitrogen application ensures greater translocation of vegetative nitrogen to grains; however, due to relatively low total nitrogen accumulation in vegetative organs under severe water stress, both translocation amount and efficiency were lower than under adequate water supply and moderate water stress. Comparing within the same nitrogen treatment, when nitrogen rate was N1, the translocation amount and efficiency of stored nitrogen from vegetative organs initially increased then decreased with intensifying water stress, following W2 > W1 > W3, indicating that moderate water stress favors nitrogen translocation when nitrogen application is low. When nitrogen rates were N2 and N3, both translocation amount and efficiency decreased continuously with intensifying

water stress, following W1 > W2 > W3. Under the same soil relative water content, the translocation amount, efficiency, and contribution rate to grains of stored nitrogen in vegetative organs all showed a trend of initially increasing then decreasing with nitrogen rate, with W2 > W3 > W1, demonstrating that increased nitrogen application significantly enhances nitrogen translocation capacity to grains, but excessive nitrogen is similarly detrimental to this process.

2.4 Effects of Nitrogen Rate and Post-Anthesis Soil Relative Water Content on Grain Protein and Component Contents and Proportions

As shown in Table 4 , nitrogen rate and post-anthesis soil relative water content significantly affected total protein content in black wheat grains. Under the same water treatment, total protein content increased significantly with nitrogen rate. Under low nitrogen conditions, water stress treatments showed significantly higher total protein content than adequate water supply; under medium nitrogen conditions, water stress treatments resulted in lower total protein content than adequate water supply, though differences were not significant; under high nitrogen conditions, total protein content initially increased then decreased with intensifying water stress.

Nitrogen rate and post-anthesis soil relative water content significantly affected albumin and globulin contents but not gliadin or glutenin contents. Nitrogen rate significantly affected all protein component contents except gliadin. Under the same nitrogen level, post-anthesis soil relative water content significantly influenced albumin, globulin, and gliadin contents, which increased with water stress intensification at N1 and N2 rates. At N3 rate, albumin and globulin contents increased with water stress while gliadin content decreased. Post-anthesis soil relative water content had some influence on glutenin content, but differences were not significant. Under the same water control condition, different nitrogen rates significantly affected all protein component contents. At W1 water content, albumin, globulin, and gliadin contents increased with nitrogen rate, while glutenin reached its maximum at N2. At W2 and W3 water contents, all protein component contents peaked at medium nitrogen rate (N2), indicating that under water stress conditions, both excessively high and low nitrogen rates are unfavorable for protein component accumulation.

Analysis of Table 5 reveals that the interaction of nitrogen and post-anthesis water control affected the proportions of all protein components in total protein. Post-anthesis soil relative water content significantly influenced the proportions of globulin, glutenin, and storage proteins, while nitrogen rate significantly affected the proportions of globulin, gliadin, and glutenin.

3.1 Effects of Nitrogen Rate and Post-Anthesis Soil Relative Water Content on Nitrogen Accumulation, Translocation, and Distribution in Black Wheat

Nitrogen rate and post-anthesis soil relative water content regulate nitrogen accumulation and distribution in wheat, with significant interactive effects between the two factors. Previous studies [**?**] have shown that increased grain protein content primarily depends on enhanced nitrogen accumulation capacity in grains and increased contribution of vegetative organ nitrogen to grains. Zheng et al. [**?**] reported that nitrogen rate and irrigation amount significantly affect wheat grain protein content, while drought stress influences fertilizer efficiency, which can only be realized under proper water supply conditions [**?**]. Duan et al. [**?**] found that within the nitrogen application range of 0–150 kg·hm ^{2}, increasing nitrogen rate significantly improved plant nitrogen accumulation at various growth stages, grain nitrogen accumulation at maturity, post-anthesis nitrogen absorption rate, and pre-anthesis vegetative nitrogen translocation amount; however, when nitrogen rate exceeded 150 kg·hm ^{2}, further increases showed no promoting effects and instead reduced grain nitrogen accumulation and distribution proportion at maturity. Ma et al. [**?**] noted that without nitrogen application, the contribution rate of vegetative nitrogen translocation to grains showed a trend of initially increasing then decreasing with irrigation amount, whereas under nitrogen application conditions, the contribution rate increased with irrigation amount, and the contribution rate of stored vegetative nitrogen to grains also increased with nitrogen rate across all water treatments, demonstrating that rational water and fertilizer conditions can enhance the contribution rate of stored vegetative nitrogen to grains. Our results indicate that during the late grain-filling stage of black wheat, increasing nitrogen rate significantly improved grain protein accumulation under moderate (W2) and severe (W3) water stress, while adequate water supply (W1) combined with medium nitrogen rate (N2) effectively enhanced protein accumulation, with both insufficient and excessive nitrogen being unfavorable. Under the same nitrogen treatment, water stress reduced grain protein accumulation, likely because water stress impaired nitrogen translocation from vegetative organs to grains, thereby reducing the grain' s own nitrogen accumulation capacity [**?**].

After anthesis, nitrogen in wheat vegetative organs undergoes continuous translocation and distribution, primarily transported to grains. Over 60% of grain nitrogen originates from remobilization of pre-anthesis stored nitrogen in vegetative organs [**?**]. Under water stress conditions, the translocation amount and efficiency of pre-anthesis stored nitrogen in vegetative organs decrease, reducing grain nitrogen accumulation and yield [**?**]; improved soil moisture conditions promote nitrogen transfer from vegetative organs to grains and increase total nitrogen yield [**?**]. Our results demonstrate that increased nitrogen application enhances grain nitrogen accumulation in black wheat, but excessive nitrogen is detrimental to nitrogen translocation to grains. Under the same nitrogen level, nitrogen accumulation in stems and sheaths at maturity

decreased with intensifying water stress, while accumulation in leaves and rachises + glumes increased, as did their proportions of total nitrogen. This occurs because water stress causes premature senescence in black wheat, reducing nitrogen translocation amount and efficiency from vegetative organs and resulting in nitrogen loss. Under the same water control condition, nitrogen accumulation in all vegetative organs increased with nitrogen rate, as did their proportions of total nitrogen. In summary, water stress impedes translocation of stored nitrogen from vegetative organs to grains, while increased nitrogen application significantly enhances this translocation capacity, though excessive nitrogen is similarly unfavorable.

3.2 Effects of Nitrogen Rate and Post-Anthesis Soil Relative Water Content on Grain Protein and Component Contents in Black Wheat

The effects of nitrogen rate and soil relative water content on wheat grain protein and its component contents have been extensively studied, though findings remain inconsistent. Zhao et al. [**?**] reported that nitrogen application significantly increased grain protein and component contents, with all components increasing with nitrogen rate. Shi et al. [**?**] suggested that responses of protein component contents to nitrogen rate differ among varieties, showing that strong-gluten wheat 'Jimai 20' and medium-gluten wheat 'Taishan 23' exhibited initial increases then decreases in grain protein and component contents with nitrogen rate, while weak-gluten wheat 'Ningmai 9' showed significant increases in all protein components with nitrogen rate. Zhao et al. [**?**] argued that different protein components respond inconsistently to nitrogen rate changes. Numerous studies have examined the effects of different water treatments and water-nitrogen interactions on grain protein and component contents [**?**, **?**, **?**], with varying results, indicating that grain protein and component contents exhibit different sensitivities to water and nitrogen, with variations among varieties. Our results show that total protein content in black wheat grains increased with nitrogen rate under the same water treatment, but decreased with intensifying water stress at high nitrogen rates. Under the same nitrogen level, water control significantly affected albumin, globulin, and gliadin contents but not glutenin. Under the same water control condition, nitrogen rate significantly affected all component contents. Under normal water supply (W1), albumin, globulin, and gliadin contents increased with nitrogen rate, while glutenin peaked at N2. Under water stress conditions (W2, W3), all protein component contents were highest at medium nitrogen rate (N2), indicating that under water stress, both excessively high and low nitrogen rates are unfavorable for protein component improvement.

The interaction of nitrogen and post-anthesis water control had no significant effect on the proportions of protein components in total protein. Similar to common wheat, nitrogen rate, post-anthesis soil relative water content, and their interaction all influence nitrogen accumulation, translocation, distribution, and protein component contents in black wheat 'Luozhen 1' . At low nitro-

gen rates, water stress can appropriately increase total grain protein content, while at medium or high nitrogen rates, water stress hinders total protein content increase. Under our experimental conditions, adequate water supply (W1) combined with medium nitrogen rate (N2) effectively regulated the nitrogen metabolism process in black wheat. Therefore, considering all factors, the optimal water-nitrogen combination was W1N2. Since this experiment used pot cultivation, root absorption and utilization of deep soil moisture differ from field conditions; thus, further validation through field experiments is required before broader application of these results.

References

[1] Zhao G C, Chang X H, Chen X M, et al. Effects of different treatment of nitrogen and irrigation on grain yield and quality in different wheat varieties[J]. Journal of Plant Genetic Resources, 2007, 8(4): 447–450 [2] Wang D M, Yu Z W, Zhang Y L, et al. Changes in nitrogen accumulation, distribution, translocation and nitrogen use efficiency in different wheat cultivars under different irrigation conditions[J]. Plant Nutrition and Fertilizer Science, 2010, 16(5): 1041–1048 [3] Flowers M, Weisz R, Heiniger R, et al. In-season optimization and site-specific nitrogen management for soft red winter wheat[J]. Agronomy Journal, 2002, 96(1): 124–134 [4] Xu Z Z, Yu Z W, Wang D, et al. Nitrogen accumulation and translocation for winter wheat under different irrigation regimes[J]. Journal of Agronomy and Corp Science, 2005, 191(6): 439–449 [5] Wang X C, Wang X H, Xiong S P, et al. Differences in nitrogen efficiency and nitrogen metabolism of wheat varieties under different nitrogen levels[J]. Scientia Agricultura Sinica, 2015, 48(13): 2569–2579 [6] Xu Z Z, Yu Z W, Wang D, et al. Effects of irrigation amount on absorbability and translocation of nitrogen in winter wheat[J]. Acta Agronomica Sinica, 2004, 30(10): 1002–1007 [7] Ma D H, Wang Y F, Zhao C X, et al. Effects of nitrogen fertilizer rate and post-anthesis soil water content on characteristics of nitrogen metabolism and grain protein content in wheat[J]. Plant Nutrition and Fertilizer Science, 2008, 14(6): 1035–1041 [8] Ma X H, Wang D, Yu Z W, et al. Effect of irrigation regimes on water consumption characteristics and nitrogen distribution in wheat at different nitrogen applications[J]. Acta Ecologica Sinica, 2010, 30(8): 1955–1965 [9] Zhao G C, Wan F S, Chang X H, et al. Effects of irrigation on yield and protein content of grains and their stability in strong-gluten wheat[J]. Acta Agronomica Sinica, 2008, 34(7): [10] Ma S K, Zhao G C, Chang X H, et al. Effects of water and nitrogen treatment on yield and protein quality in strong gluten wheat Jimai 20[J]. Acta Agriculturae Nucleatae Sinica, 2010, 24(4): 815–820 [11] Wang M, Zhao G C, Shi S B, et al. Effect of nitrogen rate on photosynthesis after anthesis, nitrogen distribution and content of protein and its component in maturity stage in wheat with different seed color[J]. Journal of Triticeae Crops, 2015, 35(6): 829–835 [12] Feng M, Zhao G C, Chang X H, et al. Effect of irrigation on grain yield and quality of wheat cultivars with different grain colors[J]. Journal of Triticeae Crops, 2009, 29(3): 460–463 [13] Chen Z C. New resources and distinctive nutrition food of wheat[J]. Grain Processing, 2008, 33(4): 15–18 [14] Sun Q, Sun B

Q, WANG J H. Characters of seed pigment of black kernel wheat[J]. Seed, 2004, 23(6): 18-20 [15] Su D M, Qi B J, Zhao R Y, et al. Preliminary evaluation for nutritional compositions of black wheat Luozhen No.1[J]. Cereal & Feed Industry, 2000(8): 1-2 [16] Lü Q, Xiong Y, Ma C, et al. Studies on differences and physiological basis of yield formation between color wheat and general wheat[J]. Corps, 2008(1): 41-43 [17] Xu Y. Post-anthesis dry matter and nitrogen dynamics in winter wheat as affected by nitrogen supply and water deficit[D]. Beijing: University of Chinese Academy of Sciences, 2013 [18] Triboi E, Triboi-Blondel A M. Productivity and grain or seed composition: A new approach to an old problem-invited paper[J]. European Journal of Agronomy, 2002, 16(3): 163-175 [19] Zheng Z S, Wang C Y, Niu J Y, et al. Effects of irrigation and fertilization coupling on protein and amino acids contents in grains of winter wheat[J]. Chinese Journal of Eco-Agriculture, 2011, 19(4): 788-793 [20] Zhai B N, Li S X. Effect of water and nitrogen cooperation on winter wheat yield and quality[J]. Plant Nutrition and Fertilizer Science, 2003, 9(1): 26-32 [21] Duan W X, Yu Z W, Zhang Y L, et al. Effects of nitrogen fertilizer application rate on nitrogen absorption, translocation and nitrate nitrogen content in soil of dryland wheat[J]. Scientia Agricultura Sinica, 2012, 45(15): 3040-3048 [22] Yang X Y, Yu Z W, Xu Z Z. Effects of irrigation regimes on water consumption characteristics and nitrogen accumulation and allocation in wheat[J]. Acta Ecologica Sinica, 2009, 29(2): 1955-1965 [23] Zhang Y L, Yu Z W. Effects of irrigation amount on nitrogen uptake, distribution, use, and grain yield and quality in wheat[J]. Acta Agronomica Sinica, 2008, 34(5): 870-878 [24] Fan X M, Dai T B, Jiang D, et al. Effects of nitrogen rates on carbon and nitrogen assimilate translocation in wheat grown under drought and waterlogging from anthesis to maturity[J]. Journal of Soil and Water Conservation, 2004, 18(6): 63-67 [25] Zhao G C, He Z H, Liu L H, et al. Study on the co-enhancing regulating effect of fertilization and watering on the main quality and yield in Zhongyou 9507 high gluten wheat[J]. Scientia Agriculture Sinica, 2004, 37(3): 351-356 [26] Zhao J Y, Yu Z W. Effects of nitrogen fertilizer rate on nitrogen metabolism and protein synthesis of superior and inferior wheat kernel[J]. Scientia Agriculture Sinica, 2005, 38(8): 1547-1554 [27] Guo M M, Zhao G C, Guo W S, et al. Effects of nitrogen rate and row spacing on winter wheat grain quality[J]. Chinese Journal of Eco-Agriculture, 2015, 23(6): 668-675 [28] Shi Y, Zhang Y L, Yu Z W. Effects of nitrogen fertilization on protein components contents and processing quality of different wheat genotypes[J]. Plant Nutrition Fertilizer Science, 2010, 16(1): 33-40 [29] Zhao G C, Chang X H, Yang Y S, et al. Grain yield and protein components responding to the amount and rate of nitrogen application in winter wheat[J]. Journal of Triticeae Crops, 2009, 29(2): 294-298 [30] Chi Z Z, Zhao G C, Zheng J G, et al. Effects of nitrogen application amount on protein components in grain of different varieties wheat[J]. Southwest China Journal of Agricultural Sciences, 2010, 23(2): 427-431 [31] Wang Y F, Yu Z W, Li S X, et al. Effects of nitrogen application amount on content of protein components and processing quality of wheat grain[J]. Scientia Agricultura Sinica, 2002, 35(9): 1071-1078 [32] Zhao G C, Chang X H, Liu L H, et al. Grain yield and protein components responses to irrigation in strong gluten wheat[J]. Acta Agronomica Sinica, 2007, 33(11):

1828–1833 [33] Li Q X, Wang C Y, Ma D Y, et al. Effects of irrigation and nitrogen application on grain yield, protein content and quality traits of winter wheat in high-yielding area[J]. Journal of Triticeae Crops, 2014, 34(1): 102–107 [34] Wang X Y, Yu Z W. Effect of interactions between water management and nitrogen fertilizer on wheat processing quality and protein fractions[J]. Journal of Triticeae Crops, 2009, 29(4): 632–638

Note: Figure translations are in progress. See original paper for figures.