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# Coupling effects of irrigation and nitrogen fertilization on grain protein and starch quality of strong-gluten winter wheat

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**Abstract** Effects of irrigation and nitrogen fertilization on the grain yield, protein composition, protein quality, starch composition and starch pasting properties of a strong-gluten winter wheat were investigated in a high fertility field. Compared with non-irrigation treatment, grain yields under irrigation treatments were significantly increased, but the content of grain protein, monomeric protein and flour wet gluten was reduced. There were no significant differences in the above parameters between the irrigation treatments. Nitrogen application could significantly increase grain yield under low irrigation frequency ( $W_0$  and  $W_1$ ), while the neglected effect on yield was observed with high irrigation frequency ( $W_2$  and  $W_3$ ). With the increase of irrigation frequency, the glutenin content leveled off, but the changes of glutenin composition were not uniform, in which the soluble glutenin content was increased, while the insoluble glutenin content and polymerization index (the ratio of insoluble glutenin to total glutenin) were reduced. Both dough development time and stability time became shorter with the increased irrigation frequency. Nitrogen application improved the content of all grain protein fractions and grain quality, in which the increased degree in non-gluten protein (albumin and globulin) was higher than gluten protein (gliadin and glutenin), and the increased degree in soluble glutenin was found higher than that of insoluble glutenin. The interactive effects of irrigation and nitrogen on starch composition were significant. Starch content and amylopectin content was increased as irrigation frequency added in non-nitrogen treatment. Compared to non-irrigation treatment, irrigation significantly increased the starch

content and the amylopectin content in nitrogen application treatment, but the starch and amylopectin content had no significant difference between irrigation treatments. Amylose content and the ratio of amylose to amylopectin were reduced while RVA indexes (peak viscosity, breakdown, final viscosity and setback) were increased as irrigation frequency was increased. Nitrogen application significantly improved the amylopectin content and decreased the amylose content in lower frequency irrigation, while the amylopectin content was decreased and the amylose content was enhanced by nitrogen application in higher frequency irrigations.

**Keywords** strong-gluten wheat, coupling of irrigation and nitrogen, protein, starch

## 1 Introduction

Irrigation and nitrogen fertilization have been found to be the major factors influencing protein composition, starch composition, and grain quality of wheat (Cao et al., 2005; Fan et al., 2004; Jia et al., 1996; Johansson et al., 2001; Lan et al., 2004; Pechanek et al., 1997; Wang et al., 2004a, 2004b; Wang et al., 1997; Xu et al., 2003a, 2003b). It is generally accepted that high soil moisture or increased irrigation frequency, especially irrigation after flowering, may result in worsening protein quality (Xu et al., 2003b; Wang et al., 1997). In many studies, nitrogen fertilization improved grain protein quality and applications of N later in the season were more effective than earlier applications in improving the protein quality (Yu et al., 2002; Dai et al., 2005; He et al., 2005). Several studies have documented the effects of irrigation and nitrogen fertilization on starch composition but the results are inconsistent. Cao et al. (2005) observed that nitrogen fertilization decreased the amylose content and the ratio of amylose to amylopectin which improved the noodle quality. Wang et al. (2004b) reported that adding irrigation frequency increased the content of total starch and amylopectin and decreased the ratio of amylose to amylopectin. Xu

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et al. (2003a) found that severe water deficit significantly decreased the content of total starch and all starch fractions as well as the ratio of amylose to amylopectin. There is a strong cultivar impact in which the starch properties of some cultivars have more intense responses to nitrogen fertilization and irrigation than others (Jiang et al., 2004). Until now, there are many studies that examined the integrative influence of irrigation and nitrogen fertilization on grain protein characteristics under medium and low grain yield conditions. However, fewer studies addressed the interactive effects of irrigation and nitrogen fertilization on starch properties of winter wheat and no information about these effects is available under high yield conditions.

The objectives of this study were: (1) to investigate the interactive effects of irrigation and N fertilization on the composition of protein and starch as well as grain quality under high yield conditions; (2) to find out the fraction of grain protein which is the most responsible for worsening grain quality when over-irrigation is encountered; (3) to identify the management practices which can maximize both the grain yield and the grain quality.

## 2 Materials and methods

### 2.1 Experimental design

A field experiment was carried out on loamy soil at Qianzhuliu Village, Longkou County, Shandong Province, China, during the 2003–2004 season. The content of soil nutrients before sowing (Table 1) indicated that the experimental field was of high fertility soil. The rainfall in this season was 228.5 mm and 11.3 mm more than the average precipitation in a common year, including 82.9 mm from sowing to over-wintering, 33.3 mm from over-wintering to jointing, 28 mm from jointing to anthesis and 84.3 mm from anthesis to maturity. Jimai-20 (a strong gluten winter wheat cultivar), which is commonly cultivated in this region, was sown on October 4, 2003 and harvested on June 18, 2004.

A split-plot design was used in our experiment, with four frequencies of irrigation being in the main plot and two rates of nitrogen in the subplot. The four irrigation frequencies were arranged as none ( $W_0$ ), once ( $W_1$ : at jointing stage), twice ( $W_2$ : before winter and at jointing stage), thrice ( $W_3$ : before winter, at jointing stage and anthesis stage), and the water amount of each irrigation was 60 mm. The two rates of nitrogen were 0 kg·hm<sup>-2</sup> ( $N_0$ ), 84 kg·hm<sup>-2</sup> applied at preplanting+84 kg·hm<sup>-2</sup>

topdressed at jointing stage ( $N_1$ ). The basal nitrogen was applied together with 105 kg·hm<sup>-2</sup> of P<sub>2</sub>O<sub>5</sub> and 105 kg·hm<sup>-2</sup> of K<sub>2</sub>O before sowing. Each treatment had 3 replicates, with a plot area of 4.5 m × 30 m.

### 2.2 Quality test

Grain meal and flour was milled in Perten 3100 Mill and Brabender Quadrumat Junior, respectively. Quality analyses were performed according to the following procedures: grain protein content (GB2905–1982), wet gluten content (GB131506–8), Zeleny sedimentation volume (AACC56–61), dough rheology parameters (AACC 54–21). The contents of amylose and amylopectin were measured by using the UV absorption method. The pasting properties of starch were determined using Rapid Viscosity Analyzer (Newport Scientific, Australia).

### 2.3 Protein fraction test

Proteins were fractionated according to a modified procedure of Wang and Kovacs (2002a, 2002b, 2002c). This procedure classified the proteins into three fractions: monomeric protein, soluble glutenin, and insoluble glutenin. The monomeric protein was extracted with 3 × 1.0 mL of 0.3 mol·L<sup>-1</sup> NaI-7.5% (v/v) 1-propanol at 30°C for 30 min from 100 mg of grain meal and three supernatants were pooled after a centrifugation at 15000 × g for 5 min. Similarly, the soluble glutenin was extracted with 3 × 1.0 mL of 40% (v/v) 1-propanol at 30°C for 30 min and three supernatants were pooled after a centrifugation at 15000 × g for 5 min. The insoluble glutenin was extracted with 3 × 1.0 mL of 40% (v/v) 1-propanol containing 0.2% (w/v) dithiothreitol (DTT) at 60°C for 30 min and three supernatants were pooled after a centrifugation at 15000 × g for 5 min. Subsamples (0.4 mL) of supernatant for all protein fractions determinations were mixed with 4 mL of 40% (w/v) trichloroacetic acid. After laying up for 35 min, the turbidity was determined at 590 nm. Calibration curves for all protein fractions were prepared based on standard wheat flour (cultivar Genesis) extracts obtained according to the same procedure as described above. The protein content of each protein fractions obtained from Genesis was determined by Approved Method 46–13 (N × 5.7) (AACC 2000). The calibration curves were developed by diluting the known protein content fractions with 40% 1-propanol. Absorption values at 590 nm for protein fractions were converted to protein concentration expressed in terms of the percentages of grain.

**Table 1** The content of soil nutrients

soil depth/cm	organic matter/g·kg <sup>-1</sup>	total N/g·kg <sup>-1</sup>	alkali-hydrolyzed N/mg·kg <sup>-1</sup>	available P/mg·kg <sup>-1</sup>	available K/mg·kg <sup>-1</sup>
0–20	23.8	1.2	102.4	20.5	134
20–40	4.9	0.9	71.6	7.9	87

### 2.4 Data analysis

All data processing and statistical analysis were performed using the following software: Excel, Originpro 7.5 and DPS 7.05 for Windows.

## 3 Results

### 3.1 Effects of irrigation and nitrogen fertilization on wheat grain yield and yield components

Whether nitrogen fertilizer was applied or not, grain yields of irrigation treatments ( $W_1, W_2, W_3$ ) were significantly higher than those of non-irrigation treatments ( $W_0$ ). However, no significant differences of grain yield were found between our irrigation treatments (Table 2). The response of the grain yield to nitrogen fertilization depended on the irrigation frequency. Under low irrigation frequency ( $W_0$  and  $W_1$ ), nitrogen application ( $N_1$ ) significantly increased the grain yield compared with non-nitrogen treatment ( $N_0$ ), but the grain yield had no significant response to nitrogen application under high irrigation frequency ( $W_2$  and  $W_3$ ) which suggested significant interactive effect between irrigation and nitrogen fertilization on the grain yield.

### 3.2 Effects of irrigation and nitrogen fertilization on grain protein, protein composition and quality

The content of grain protein, monomeric protein (containing albumin, globulin and gliadin) and wet gluten of irrigation treatments ( $W_1, W_2, W_3$ ) were significantly

lower than those of non-irrigation treatment ( $W_0$ ). Whereas, there were no significant differences among irrigation treatments (Table 3).

In the non-irrigation treatments ( $W_0$ ), the content of grain protein and wet gluten of nitrogen application treatments ( $N_1$ ) were significantly higher than those of non-nitrogen treatments ( $N_0$ ). In the irrigation treatments ( $W_1, W_2, W_3$ ), the grain protein content of nitrogen application treatments ( $N_1$ ) was also significantly higher than that of non-nitrogen treatments ( $N_0$ ), but there was no significant difference in wet gluten content between nitrogen application treatments ( $N_1$ ) and non-nitrogen treatments ( $N_0$ ), which indicated that the increase degree of non-gluten protein (albumin and goblin) was higher than that of gluten protein (gliadin and glutenin) as the grain protein content was increased by nitrogen fertilization under irrigation condition.

With the increase of irrigation frequency, the soluble glutenin content increased and total glutenin content leveled off while insoluble glutenin content, glutenin polymerization index, dough stability and development time all decreased. On the other hand, nitrogen application ( $N_1$ ) increased the content of all grain protein fractions in different degrees and improved grain quality. However, the glutenin polymerization index was decreased (Table 3). Correlation analysis shows that soluble glutenin content was significantly and negatively correlated with the insoluble glutenin content, glutenin polymerization index, dough development and stability time. However, the insoluble glutenin content and glutenin polymerization index were significantly positively correlated with dough stability and development time (Table 4).

**Table 2** Response of the wheat grain yield and yield components to irrigation and nitrogen fertilization

treatment	spike No./ $\times 10^4 \cdot \text{hm}^{-2}$	grains per ear/ $N_0$ .	1000-grain weight/g	grain yield/ $\text{kg} \cdot \text{hm}^{-2}$
$W_0N_0$	682.2 f	23.0d	47.2 a	6250.0 d
$W_0N_1$	705.8 e	25.2 c	46.1 b	7152.3 bc
$W_1N_0$	733.4 d	27.7 b	42.4 c	7571.6 b
$W_1N_1$	758.7 bc	29.0 ab	41.8 cd	8149.6 a
$W_2N_0$	765.3 b	28.0 b	41.9 de	7830.5 ab
$W_2N_1$	808.1 a	29.2 a	41.0 f	8331.0 a
$W_3N_0$	739.6 cd	29.0 ab	41.4 ef	7713.6 ab
$W_3N_1$	760.1 b	29.4 a	40.2 g	7870.6 ab

Note: Different letters denote significances at  $P < 0.05$  level (the same below).

**Table 3** Protein composition content and quality parameters of different irrigation and nitrogen fertilization treatments

treatment	grain protein/%	monomeric protein/%	soluble glutenin/%	insoluble glutenin/%	glutenin/ $\%$	glutenin polymerization index/ $\%$	zeleny sedimentation volume/mL	flour wet gluten/ $\%$	dough development time/min	dough stability time/min
$W_0N_0$	13.543 b	6.904 b	1.152 f	5.487 cd	6.639 b	82.6	36.25 c	30.76 b	9.73 a	18.75 a
$W_0N_1$	14.757 a	7.631 a	1.281 e	5.845 a	7.126 a	82.0	40.50 a	33.53 a	9.85 a	18.80 a
$W_1N_0$	12.820 c	6.109 c	1.230 e	5.481 cd	6.711 b	81.7	38.00 b	28.33 c	8.13 d	15.70 c
$W_1N_1$	13.768 b	6.634 b	1.434 d	5.701 b	7.135 a	79.9	38.50 b	28.35 c	8.80 b	17.33 b
$W_2N_0$	13.001 c	6.191 c	1.398 d	5.412 d	6.810 b	79.5	38.00 b	28.40 c	8.06 d	13.76 d
$W_2N_1$	14.088 b	6.898 b	1.680 c	5.510 c	7.190 a	76.6	39.00 b	28.55 c	8.43 c	15.16 c
$W_3N_0$	12.790 c	6.138 c	1.823 b	4.729 e	6.652 b	71.1	38.33 b	29.00 c	7.36 e	11.70 e
$W_3N_1$	14.018 b	6.749 b	2.168 a	5.101 d	7.269 a	70.2	39.33 b	29.13 c	7.43 e	12.23 e

### 3.3 Effects of irrigation and nitrogen fertilization on starch fractions content and starch pasting properties

As shown in Fig. 1, the interactive effects of irrigation and nitrogen fertilization on total starch content and starch composition were significant. In low irrigation frequency treatments ( $W_0$  and  $W_1$ ), amylopectin content was increased while both amylose content and the ratio of amylose to amylopectin were decreased when nitrogen fertilizer was applied ( $N_1$ ) compared with the non-nitrogen treatment ( $N_0$ ). However, in high irrigation frequency treatments ( $W_2$  and  $W_3$ ), the synthesis of amylose was promoted but the accumulation of amylopectin was reduced and the ratio of amylose to amylopectin in grain was increased after nitrogen fertilization.

In non-nitrogen treatments ( $N_0$ ), the content of total starch and amylopectin increased when irrigation frequency was increased. In the nitrogen application treatment ( $N_1$ ), the content of total starch and amylopectin of irrigation treatments ( $W_1$ ,  $W_2$ ,  $W_3$ ) were significantly higher than those of non-irrigation treatment ( $W_0$ ), but there were no significant differences among irrigation treatments. Whether nitrogen was applied or not, amylose content and the ratio of amylose to amylopectin decreased as irrigation frequency added.

Starch pasting properties are important indices to evaluate the noodle processing quality of wheat and correlate with noodle streaming time, stability and rate of water absorption (Yang et al., 2004). In our experiment, paste traits such as peak viscosity, through viscosity, break down, final viscosity and setback all increased as irrigation frequency increased. Paste traits were lightly influenced by nitrogen application (Table 5). Amylopectin content was significantly and positively correlated with peak viscosity, break down and setback, and the correlation coefficients between amylopectin content and peak viscosity, break down, setback were 0.874, 0.897 and 0.772, respectively. Amylose content and the ratio of amylose to amylopectin were significantly and negatively correlated with peak viscosity, break down and setback (Table 6).

## 4 Discussion

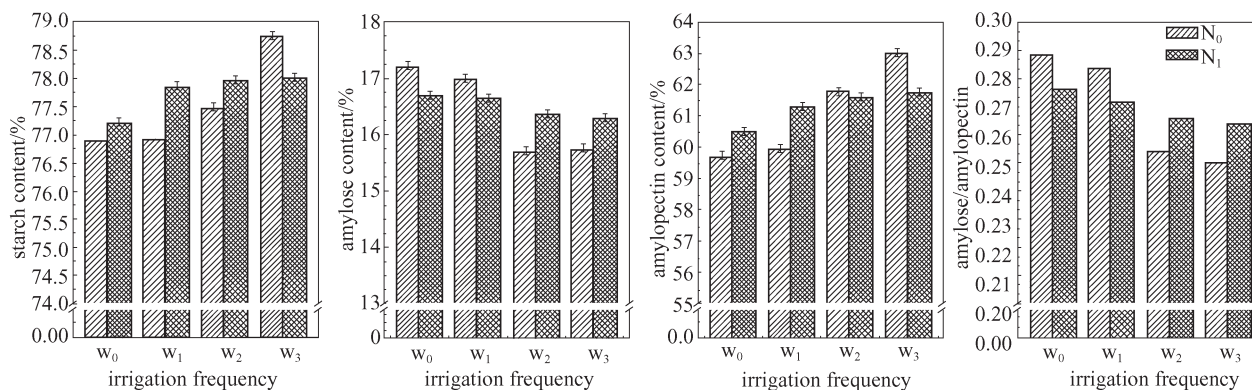
### 4.1 Regulatory effects of irrigation and nitrogen fertilization on wheat grain protein composition and quality

Protein content and quality are crucial factors influencing the wheat bread-making quality. Both the bread and dry

**Table 4** Simple relationship of quality characteristics related to protein

	monomeric protein	soluble glutenin	insoluble glutenin	glutenin	glutenin polymerization index	zeleny sedimentation volume	flour wet gluten	dough development time	dough stability time
grain protein	0.948**	0.076	0.351	0.500	0.019	0.657	0.931**	0.475	0.338
monomeric protein		-0.028	0.529	0.544	0.123	0.594	0.840**	0.587	0.491
soluble glutenin			-0.734*	0.660	-0.988**	0.346	-0.039	-0.783*	-0.835**
insoluble glutenin				0.025	0.765*	0.247	0.381	0.812*	0.867**
glutenin					-0.608	0.782	0.363	-0.254	-0.270
glutenin polymerization index						-0.266	0.135	0.805*	0.843**
zeleny sedimentation volume							0.684	-0.076	-0.109
flour wet gluten								0.445	0.333
dough development time									0.967**

Note: \*and \*\* denote significant correlation and very significant correlation, respectively (the same below).



**Fig. 1** Effect of irrigation and nitrogen fertilization on grain starch components content and ratio

**Table 5** Flour paste traits of different irrigation and nitrogen fertilization treatments

treatment	peak viscosity/RVU	through viscosity/RVU	break down/RVU	final viscosity/RVU	setback/RVU	peak time/min	pasting temperature/°C
W <sub>0</sub> N <sub>0</sub>	240.88 c	184.88 b	56.00 c	281.8 c	96.92 c	6.56 a	87.23 a
W <sub>0</sub> N <sub>1</sub>	242.13 c	185.96 b	56.17 c	287.8 b	101.84 b	6.64 a	87.18 a
W <sub>1</sub> N <sub>0</sub>	242.28 c	185.28 b	57.00 c	288.1 b	102.82 ab	6.60 a	87.18 a
W <sub>1</sub> N <sub>1</sub>	245.79 b	186.51 b	59.28 b	289.5 b	102.99 ab	6.60 a	87.20 a
W <sub>2</sub> N <sub>0</sub>	246.17 b	185.75 b	60.42 b	290.3 b	104.55 a	6.60 a	87.23 a
W <sub>2</sub> N <sub>1</sub>	245.99 b	186.55 b	59.44 b	290.3 b	103.75 a	6.64 a	87.20 a
W <sub>3</sub> N <sub>0</sub>	253.96 a	191.17 a	62.79 a	295.8 a	104.63 a	6.60 a	86.35 b
W <sub>3</sub> N <sub>1</sub>	253.50 a	190.46 a	63.04 a	296.4 a	103.94 a	6.56 a	86.35 b

**Table 6** Simple relationship of quality characteristics related to starch

	amylose/%	amylose/amylopectin	peak viscosity/RVU	through viscosity/RVU	break down/RVU	final viscosity/RVU	setback/RVU	peak time/min	pasting temperature/°C
amylopectin	-0.904**	-0.947**	0.875**	0.788*	0.897**	0.852**	0.772*	0.118	-0.645
amylose		0.987**	-0.727*	-0.591	-0.801*	-0.749*	-0.788*	-0.151	0.480
amylose/amylopectin			-0.806*	-0.687	-0.861**	-0.812*	-0.793*	-0.136	0.572
peak viscosity				0.963**	0.972**	0.938**	0.657	-0.237	-0.911**
through viscosity					0.873**	0.890**	0.534	-0.224	-0.971**
break down						0.924**	0.723*	-0.235	-0.805*
final viscosity							0.846**	-0.004	-0.819*
setback								0.356	-0.408
peak time									0.382

white Chinese noodle (DWCN) qualities were affected by flour quality parameters of the protein content, SDS sedimentation value, dough development and stability time. Loaf score and volume performed a significantly positive linear relationship with the flour quality parameters mentioned above, while the association between DWCN score and flour quality parameters significantly fitted the quadratic regression model (Yang et al., 2004). Glutenin is a major factor influencing wheat grain quality (Wang et al., 2002). The content of soluble glutenin and insoluble glutenin is significantly correlated with part of the rheology parameters and protein quality index, and the percentage of monomeric protein, soluble glutenin and insoluble glutenin in the grain may be the main difference between noodle wheat and bread wheat (Hu et al, 2004). Wang et al. (1997) reported that irrigation ranging between 0 and 225 mm had a diluting effect on grain quality and this effect can be lessened by nitrogen application.

In this study, the grain protein and wet gluten content of irrigation treatments (W<sub>1</sub>, W<sub>2</sub>, W<sub>3</sub>) were lower than those of the non-irrigation treatment (W<sub>0</sub>), but the content of grain protein and wet gluten of irrigation treatments (W<sub>1</sub>, W<sub>2</sub>, W<sub>3</sub>) did not decrease as irrigation frequency increased and there was no significant difference in the content of grain protein and wet gluten between irrigation treatments. Dough development and stability time decreased (Table 3), which could not be explained by the dilution effect. Further analysis of glutenin composition show that the glutenin content leveled off

as irrigation frequency was increased, but the soluble glutenin content increased, while the insoluble glutenin content and glutenin polymerization index (ratio of insoluble glutenin to total glutenin) was reduced. Variations in both dough development time and stability time show the similar tendency for insoluble glutenin and polymerization index. Correlation analyses indicated that the insoluble glutenin content and glutenin polymerization index were strongly and positively correlated with dough development and stability time, while soluble glutenin content was negatively correlated with insoluble glutenin content, glutenin polymerization index, dough development and stability time (Table 4). These results suggested that the retarded synthesis and accumulation of insoluble glutenin were the major reason for worsening grain quality as irrigation frequency increased.

Nitrogen application increased the content of all grain protein fractions (including soluble and insoluble glutenin), but the increase degree in soluble glutenin was higher than that in insoluble glutenin, resulting in a decrease of the glutenin polymerization index. Glutenin polymerization index declined as irrigation frequency increased because irrigation decreased the insoluble glutenin content and increased the soluble glutenin content. Therefore, nitrogen application could only lessen the adverse effects of irrigation on protein quality to some extent, but could not completely prevent protein quality from worsening. The above results suggested that the mechanism of nitrogen application and irrigation influencing protein quality was different and the mechanism

is still a matter of speculation. Furthermore, the results could be reaffirmed in common years because the rainfall in this season was a little more than that in common years.

#### 4.2 Regulatory effects of irrigation and nitrogen fertilization on wheat grain starch composition and pasting properties

Starch has less impact on the wheat bread-making quality (Yang et al., 2004), but the ratio of amylose to amylopectin and starch paste traits have a strong impact on noodle appearance and edibility characteristics. Slight fluctuation of the amylose content can lead to a remarkable variation in noodle processing quality. Higher amylopectin content in flour can make noodle smooth and tasty daintily and the higher peak viscosity can make noodle smooth, elastic and tenacious. Paste breakdown is strongly and positively correlated with noodle smoothness but negatively correlated with noodle elasticity, tenacity and softness (Wang et al., 2005). It was shown that irrigation ranging between 0 and 225 mm could increase the amylopectin and starch contents, but decrease the ratio of amylose to amylopectin. Nitrogen application decreased the amylose content and the ratio of amylose to amylopectin (Wang et al., 2004a, 2004b). However, Wang et al. (2003) proposed that nitrogen application ranging between 0 and 240 kg·hm<sup>-2</sup> increased the content of all starch fractions but decreased the ratio of amylose to amylopectin.

In our study, nitrogen application increased the amylopectin and total starch contents, and decreased the amylose content and the ratio of amylose to amylopectin when the plot was irrigated with 0–60 mm of water, which is in agreement with previous reports. However, nitrogen application decreased the amylopectin content and increased the amylose content and the ratio of amylose to amylopectin when the plot was irrigated with 120–180 mm of water, which led to worsened noodle quality. Obviously, irrigation and nitrogen fertilization regimes had significant interactive effects on starch composition. The determination of the optimal irrigation and nitrogen fertilization regimes with excellent starch quality needs further researches.

In conclusion, grain protein quality can be significantly worsened, while starch quality is improved as irrigation frequency increased. Nitrogen application can improve the grain protein quality parameters to different extents. Under low irrigation frequency treatments (0–60 mm), nitrogen application contributed to promoting the synthesis of amylopectin and decreased the accumulation of amylose. Therefore, the ratio of amylose to amylopectin was decreased accordingly. However, the reverse was true in the high irrigation frequency treatments (120–180 mm). In practice, the integrative effects of irrigation and nitrogen fertilization on both protein and starch quality should

be taken into account in the course of establishing management strategies for ideal end-use suitability of wheat.

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