Effect of irrigation and nitrogen application on grain amino acid composition and protein quality in winter wheat

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Abstract

Water management and nitrogen application are critical factors in wheat grain yield and protein quality. This study aimed to evaluate the effect of irrigation and nitrogen application on the grain yield, protein content and amino acid composition of winter wheat. Field experiments were conducted in a split-plot design with three replications in high-yielding land on the North China Plain in 2012/2013, 2013/2014 and 2014/2015. Three irrigation treatments were examined in main plots: no irrigation, irrigation at jointing, and irrigation at jointing plus anthesis, while subplots were assigned to nitrogen treatment at four different rates: 0, 180, 240, 300 kg N ha⁻¹, respectively. The results indicated that irrigation at jointing and at jointing plus anthesis improved grain yield by an average of 12.79 and 18.65% across three cropping seasons, respectively, compared with no irrigation. However, different irrigation treatments had no significant effect on grain protein content in any cropping season. Compared with no N treatment, 180, 240, and 300 kg N ha⁻¹ N application significantly increased grain yield, by 58.66, 61.26 and 63.42% respectively, averaged over three cropping seasons. Grain protein and the total, essential and non-essential amino acid content significantly increased with increasing nitrogen application. Irrigation significantly improved the essential amino acid index (EAAI) and protein-digestibility-corrected amino acid score (PDCAAS) compared with no irrigation; however, N application decreased them by an average of 7.68 and 11.18% across three cropping seasons, respectively. EAAI and PDCAAS were positively correlated, however, they were highly negatively correlated with yield and grain protein content.

Introduction

Owing to its ability to adapt to various environmental conditions and its utilization in a wide variety of food products, wheat is the most widely cultivated food crop in the world [1,2]. Achieving both high yield and grain quality is therefore a major goal in wheat production [3,4]. Wheat grain quality is determined by genetic and environmental factors: cultivar
selection, climate conditions and management practices [5–7], mainly through their effects on protein content and composition [8,9].

Protein content and the balance of amino acids largely determine the nutrient quality of wheat grains. The essential amino acid (EAA) content in protein is lower than the non-essential amino acid (NAA) content, with glutamic acid (Glu) accounting for the majority of wheat grain protein [10,11]. Among EAA, lysine (Lys), tryptophan (Trp) and methionine (Met) are the most limiting in wheat grains, and as a result, have received much attention [12]. The content of Lys in wheat grains was found to average only 3.85, 3.37 and 3.15% in six emmer wheat, four old bread wheat and two modern bread wheat varieties, respectively [13]. Enhancing protein quality, especially the balance of amino acids in wheat grains, is therefore a critical issue in wheat production. Evaluation standards of the protein nutritive quality of amino acids have been set using the chemical score (CS) and protein-digestibility-corrected amino acid score (PDCAAS) [14,15], while the biological value is expressed by the essential amino acid index (EAAI). However, to the best of our knowledge, little has been done to determine the effect of crop management practices on EAAI and PDCAAS.

Irrigation is a key measure in improving grain yield in wheat production, especially in arid and semi-arid areas. With an increase in irrigation level, wheat grain yield is significantly improved [7]. Irrigation at critical stages of wheat growth such as early tillering, jointing, heading and flowering was found to result in higher grain yield through an increase in spike number, fertile florets and heavier single grain weight [16–18]. However, supplemental irrigation was also found to decrease the protein content of wheat grains [19]. Wang et al. [20] found that irrigation performed two or four times at the grain filling stage resulted in a significant decrease in the grain protein content. On the other hand, moderate water deficits during the grain filling stage were found to increase grain protein content, although a slight decrease in grain yield was also observed [21]. Despite these findings, few studies have evaluated the effect of irrigation on the amino acid composition of wheat grains.

Among the management practices employed in wheat production, nitrogen application is often found to be the most limiting factor in terms of yield and grain quality [22]. Increased nitrogen application, as well as optimization of the fertilizer type and timing of fertilization, is a common strategy aimed at increasing the spike or grain number per spike and improving the nitrogen content, and thereby yield and quality [23]. Moreover, the economic N application rate, which is based on the average yield potential and soil N test, matching nitrogen demand in time and space, is also important in terms of economical yield and quality [24]. Although nitrogen application has little effect on the ratio of EAA/total amino acid (TAA), it can significantly increase the content of TAA in wheat grains [25]. With increasing nitrogen, the percentage of Glu + glutamine (Gln) in the TAA was found to increase, while that of Lys and the ratio of cysteine (Cys) to Met decreased [26]. However, little is known about the effect of nitrogen rates on protein quality parameters in wheat grains.

Interactions between nitrogen application and irrigation have also been observed in wheat. Nitrogen uptake was greater under irrigation treatment compared with rain-sheltering conditions [27], and water use efficiency was significantly improved when nitrogen application was increased [28]. Bandyopadhyay et al. [29] also found that irrigation increased both water and nitrogen use efficiencies, resulting in an improvement in wheat grain yield. A factorial experiment in wheat showed that a higher grain yield but a lower protein content was observed with increasing irrigation across four nitrogen treatments, while under each individual irrigation treatment, grain protein content was increased with increasing nitrogen application [30]. The dilution effect of irrigation on grain protein content at higher nitrogen levels was greater than that at lower nitrogen rates [30,31]. These findings suggest that understanding the optimum
regime of irrigation and nitrogen application is an important strategy in improving both grain yield and protein quality in winter wheat.

In China, wheat is a staple food, ranking second next to rice in terms of area and production [32]. Improving the protein quality of wheat grains through increased protein content and a better combination of amino acids is therefore urgently required. Effects of irrigation management, nitrogen fertilization application and the interaction between the two on wheat yield and protein content have already been researched in China [1,28]; however, little is known about the effects on grain amino acid composition and protein quality under field conditions. The objectives of this study, therefore, were to evaluate the effect of irrigation and nitrogen application management on wheat grain yield, amino acid composition and protein quality in winter wheat growing on the North China Plain. It was hypothesized that an appropriate increase in irrigation and nitrogen application rate would enhance the protein quality of the wheat grains.

Materials and methods

Field experiments

Long-term field experiments were commenced in 2010 at Wenxian (34°92′N, 112°99′E), Henan province, North China; a semi-arid area in the Huanghuai region for crop production of wheat-summer maize rotation. The land was owned by Pingan Seed Company Limited, and leased by Henan Agricultural University. We confirm that the field studies did not involve endangered or protected species.

Experiments were laid out in a split-plot design, with three irrigation treatments in the main plots (no irrigation, I0; irrigation at jointing, I1; irrigation at jointing plus anthesis, I2; irrigation of 750 m³ ha⁻¹ each time) and four nitrogen rates in the sub-plots (0, 180, 240 and 300 kg N ha⁻¹; N0, N180, N240 and N300, respectively). Three replicates were performed for each treatment. All sub-plot treatments were randomized in each main plot treatment. Individual sub-plots were 6.1 m in length and 2.5 m wide, and consisted of 12 rows. Irrigation treatment involved uniform watering using movable pipelines, with the amount of water calculated using a water meter. Nitrogen fertilizer was applied as urea (46%), and phosphorus (P) and potassium (K) fertilizer as calcium superphosphate (15%) and potassium chloride fertilizer (60%) at rates of 150 (P₂O₅) and 120 (K₂O) kg ha⁻¹, respectively. At 1/2 urea, calcium superphosphate and potassium chloride fertilizer was sprayed onto the soil prior to soil preparation. Residual N fertilizer was then applied at the jointing stage in selected plots.

Yumai 49–198, a widely produced winter wheat cultivar in Huanghuai area, was used in this experiment. The sowing rate was 135 kg ha⁻¹ with thinning to the recommended plant density in all subplots (approximately 55 plants per meter within a row) when most plants had 3–4 leaves. Protective management against pests and disease was carried out in all treatment plots to ensure healthy growth.

Wheat and soil samples were collected in the cropping seasons of 2012/2013, 2013/2014 and 2014/2015. Daily weather data for the three cropping seasons were obtained from a meteorological station located in the experimental field. The trends in temperature and rainfall relative to wheat growth are shown in Fig 1. Rainfall was limited and distributed mostly during the late stage of growth. The lowest daily maximum temperature (Tmax) was approximated to be 30°C and the average minimum temperature (Tmin) as 12.3°C during the wheat grain filling stage (May). Since soil nutrition could be affected by the different nitrogen rates, soil samples were collected before sowing at 0–30 cm from each plot under nitrogen treatment. Soil total N was measured by a semi-micro-Kjeldahl procedure. Available N was analyzed by the alkaline hydrolysis diffusion method, available P by the Olsen method, and available K using an atomic
absorption spectrophotometer. Organic matter was determined by the K2Cr2O7-H2SO4 oxidation method, and pH was measured using an Orion Ionalyzer Model 901 pH meter in a 1:2.5 soil: water solution [33,34]. Soil chemical characteristics listed in Table 1 showed that, although significantly lower available N was observed in the N0 treatment in each cropping season, and lower available K content in 2014/2015, there were no significant differences in organic matter, total N, available P or pH among the different nitrogen treatments in the three cropping seasons.

Sample preparation and analysis of grain yield and protein quality

At maturity, wheat was hand-harvested in a 6 m² area (2.4 m in length for 12 rows) in the middle of each plot. Grains were then threshed with a thresher and dried at 75°C until a constant weight was reached. Grain yield at corresponding moisture contents were then recorded and expressed against a standard moisture content of 13%.

Grain protein was calculated from the nitrogen content by multiplying by 5.7, and nitrogen content was measured using a nitrogen analyzer (Kjeltec 2300, FOSS, Sweden) according to

Table 1. Initial chemical characteristics of the soil during the three cropping seasons.

| Cropping Seasons | Treatments | Total N (g kg⁻¹) | Available N (mg kg⁻¹) | Available P (mg kg⁻¹) | Available K (mg kg⁻¹) | Organic matter (%) | pH   |
|------------------|------------|------------------|----------------------|----------------------|----------------------|-------------------|------|
| 2012/2013        | N0         | 0.91a            | 73.43c               | 22.07a               | 157.07a              | 16.98a            | 8.31a |
|                  | N180       | 0.95a            | 85.23b               | 23.60a               | 163.77a              | 17.12a            | 8.29a |
|                  | N240       | 1.04a            | 84.45b               | 24.18a               | 166.69a              | 17.55a            | 8.24a |
|                  | N300       | 0.97a            | 93.96a               | 24.23a               | 163.74a              | 17.85a            | 8.19a |
|                  | mean       | 0.97             | 84.27                | 23.52                | 162.82               | 17.37             | 8.26  |
| 2013/2014        | N0         | 0.89a            | 70.28c               | 17.90a               | 102.69a              | 16.10a            | 8.28a |
|                  | N180       | 0.99a            | 89.64ab              | 17.64a               | 103.10a              | 16.21a            | 8.19a |
|                  | N240       | 1.03a            | 85.29b               | 17.95a               | 112.30a              | 16.35a            | 8.20a |
|                  | N300       | 0.95a            | 90.36a               | 17.41a               | 112.81a              | 16.60a            | 8.23a |
|                  | mean       | 0.97             | 83.89                | 17.73                | 107.73               | 16.32             | 8.23  |
| 2014/2015        | N0         | 0.89a            | 72.59c               | 13.09a               | 116.77b              | 15.08a            | 8.32a |
|                  | N180       | 0.90a            | 79.28b               | 12.25a               | 120.25b              | 16.67a            | 8.20a |
|                  | N240       | 1.01a            | 84.54a               | 11.92a               | 146.97a              | 16.82a            | 8.26a |
|                  | N300       | 0.85a            | 89.56a               | 13.10a               | 158.36a              | 16.01a            | 8.21a |
|                  | mean       | 0.92             | 81.49                | 12.59                | 135.59               | 16.15             | 8.25  |

Note: Data represent the average value; values with different letters in the same column indicate a significant difference at the 5% level.
the ICC Standard Method 105/2. The proportions of particular amino acids were determined using an L-8800 and L-8900 amino acid analysis meter (Hitachi High-Technologies Corporation) according to the ISO 13903–2005 method. EAA included threonine (Thr), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His), Met and Lys. Other amino acids comprised the NAA. EAAI was calculated by the EAA of protein in each sample and the EAA of reference egg protein [35], and PDCAAS was determined using the method described by Schaafsma et al. [36]:

\[
EAAI = \left( \frac{(EAA_1 \times EAA_2 \cdots EAA_n)}{(EAA_{\text{sample}})} \right)^{1/n} \\
PDCAAS(\%) = \text{amino acid score (AAS)} \times \text{true nitrogen digestibility (TD)} (\%) \\
AAS = \frac{\text{Content of the first limiting amino acid in the test protein (mg kg}^{-1})}{\text{Content of the corresponding amino acid in the reference protein (mg kg}^{-1})}
\]

Here, the standard amino acid content in the protein of an adult (WHO/FAO/UNU, 2007) was used as the reference, and TD was 86% according to Tome [37].

Statistical analysis

For all investigated parameters, analysis of variance (ANOVA) was performed using the SPSS statistical package based on a split-plot design. Irrigation and nitrogen application were taken as fixed factors, while cropping season was considered as a random factor due to unpredictable weather conditions. The F-test was used and when significant, differences were compared using the least significant difference (LSD) test at the 0.05 probability level. Correlation analysis was performed to determine the relationship among yield, protein content and quality across all treatments.

Results

Grain yield and amino acid composition

The results showed that the grain yield of winter wheat was significantly affected by irrigation and nitrogen application (Table 2). Grain yield was improved by irrigation and followed the trend I0 < I1 < I2. Compared with I0, I1 and I2 treatments increased the grain yield of winter wheat by 7.61 and 29.26% in 2012/2013, 16.72 and 25.65% in 2013/2014, and 12.86 and 15.61% in 2014/2015, which averaged 12.79 and 18.65% across all three cropping seasons. Nitrogen application significantly improved the grain yield of winter wheat. Compared with N0, N180, N240 and N300 increased the grain yield by an average of 58.66, 61.26 and 63.42%, respectively, across the three cropping seasons (Table 2). This result revealed a clear increase between N0 and N180 treatments, but additional nitrogen application did not increase the yield any further. However, the two-way interaction between irrigation and nitrogen application was not significant among the three cropping seasons. N240 under I2 in 2014/2015 and N0 under I0 in 2012/2013 exhibited the highest and lowest grain yields (9.11 and 3.71 t ha\(^{-1}\)), respectively.

Compared with N0, nitrogen application significantly increased grain protein content in each cropping season. In contrast, no significant protein content response to irrigation was observed (Table 2). However, a two-way interaction between nitrogen application and irrigation on grain protein content was significant in 2012/2013 and 2014/2015. For example, in the cropping season of 2014/2015, no difference in grain protein content was observed among the
three nitrogen treatments under I0, while under I1 and I2, the protein content of N180 was significantly lower than those of N240 and N300 respectively.

As shown in Table 3, TAA, EAA and NAA levels were not significantly affected by different irrigation regimes in 2012/2013 and 2014/2015, but all were significantly decreased by irrigation in 2013/2014. Furthermore, N application significantly increased all three parameters in the three cropping seasons. The highest values were observed in treatment N2, but no significant differences were observed between N2 and N3. The interaction of irrigation and nitrogen application had a significant effect on TAA, EAA and NAA content, and a similar trend was observed for grain protein content. The highest TAA, EAA and NAA levels were obtained at N300 under I2 in 2014/2015 (144.80, 43.90 and 100.90 mg g\(^{-1}\), respectively). Leu and Phe accounted for a large proportion of the EAA content. Lys levels ranged from 2.57 to 3.83 mg g\(^{-1}\), 2.90 to 3.77 mg g\(^{-1}\) and 2.77 to 4.13 mg g\(^{-1}\) in the three cropping seasons, respectively.

### Table 2. Effects of irrigation and nitrogen application on grain yield and protein content in winter wheat in 2012/2013, 2013/2014 and 2014/2015, and interactions between irrigation and nitrogen application; summary of F significance from analysis of variance of the effects of main factors and interactions.

| Treatment | Yield (t ha\(^{-1}\)) | Protein (%) |
|-----------|----------------------|-------------|
|           | 2012/2013  | 2013/2014  | 2014/2015  | 2012/2013  | 2013/2014  | 2014/2015  |
| I0        | 4.99b      | 6.16b      | 6.92c      | 14.76      | 15.33      | 14.33      |
| I1        | 5.37b      | 7.19a      | 7.81b      | 13.34      | 14.91      | 14.33      |
| I2        | 6.45a      | 7.74a      | 8.00a      | 14.22      | 14.89      | 14.35      |
| F-test    | **         | **         | **         | ns         | ns         | ns         |
| N0        | 4.58b      | 4.47b      | 4.80b      | 11.57c     | 14.20b     | 10.86c     |
| N180      | 5.73a      | 7.84a      | 8.41a      | 14.32b     | 15.22a     | 15.17b     |
| N240      | 6.03a      | 7.76a      | 8.55a      | 14.91ab    | 15.40a     | 15.54a     |
| N300      | 6.08a      | 8.05a      | 8.54a      | 15.62a     | 15.34a     | 15.77a     |
| F-test    | **         | **         | **         | **         | **         | **         |
| I0×N0     | 3.71b      | 3.57b      | 4.32b      | 12.57b     | 14.37b     | 11.50b     |
| I0×N180   | 4.94ab     | 6.67a      | 7.73a      | 15.33a     | 15.53a     | 15.10a     |
| I0×N240   | 5.62a      | 7.34a      | 7.81a      | 15.37a     | 15.83a     | 15.10a     |
| I0×N300   | 5.69a      | 7.07a      | 7.81a      | 15.77a     | 15.57a     | 15.60a     |
| F-test    | *          | **         | **         | **         | **         | **         |
| I1×N0     | 4.46b      | 4.60b      | 5.11b      | 11.50c     | 13.83b     | 10.67c     |
| I1×N180   | 5.58a      | 8.39a      | 8.51a      | 12.40b     | 15.13a     | 15.03b     |
| I1×N240   | 5.76a      | 7.63a      | 8.73a      | 13.93ab    | 15.30a     | 15.67a     |
| I1×N300   | 5.70a      | 8.13a      | 8.87a      | 15.53a     | 15.37a     | 15.93a     |
| F-test    | **         | **         | **         | **         | **         | **         |
| I2×N0     | 5.56b      | 5.25b      | 4.98b      | 10.63b     | 14.40b     | 10.40c     |
| I2×N180   | 6.65a      | 8.48a      | 8.99a      | 15.23a     | 15.00a     | 15.37b     |
| I2×N240   | 6.72a      | 8.31a      | 9.11a      | 15.43a     | 15.07a     | 15.87a     |
| I2×N300   | 6.86a      | 8.93a      | 8.93a      | 15.57a     | 15.10a     | 15.77a     |
| F-test    | *          | **         | **         | **         | **         | **         |
| Grand mean| 5.60       | 7.03       | 7.57       | 14.11      | 15.04      | 14.33      |
| I×N (F-test) | ns        | ns        | ns        | **        | ns        | **         |
| CV (%)    | 4.32       | 6.73       | 0.57       | 9.02       | 0.74       | 0.55       |

Note: ns, not significant at P < 0.05; * Significant at P < 0.05; ** Significant at P < 0.01; Data in the same column with different letters indicate a significant difference at P < 0.05.

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significant increase in EAA content was observed following higher nitrogen application in 2012/2013 and 2014/2015 (Fig 2). The ratio of EAA to TAA was affected by irrigation only in 2013/2014, but it was significantly decreased with increasing nitrogen application in the three cropping seasons. The interaction of irrigation and nitrogen application had a significant effect on the EAA/TAA ratio in all three cropping seasons, which varied from 30.11 to 32.58% (Table 4).

### Protein quality evaluation

A significant increase in EAAI following irrigation was observed in 2012/2013 and 2014/2015, whereas increasing nitrogen application significantly decreased EAAI in 2013/2014 and 2014/2015 (Table 4). The interaction between irrigation and nitrogen application significantly

### Table 3. Effects of irrigation and nitrogen application on TAA, EAA and NAA content in wheat grain in 2012/2013, 2013/2014 and 2014/2015, and interactions between irrigation and nitrogen application; summary of F significance from analysis of variance of the effects of main factors and interaction.

| Treatment | TAA (mg g⁻¹) | EAA (mg g⁻¹) | NAA (mg g⁻¹) | EAA/TAA (%) |
|-----------|-------------|-------------|-------------|------------|
|           | 2012/2013   | 2013/2014   | 2014/2015   | 2012/2013  | 2013/2014  | 2014/2015  | 2012/2013  | 2013/2014  | 2014/2015  |
| I0        | 127.29      | 128.98      | 125.30      | 38.93      | 40.33      | 38.46      | 88.36      | 88.65      | 86.84      |
|           | 30.61      | 31.32      | 30.75      |
| I1        | 119.40      | 125.25      | 126.18      | 38.08      | 39.73      | 38.55      | 81.40      | 85.53      | 87.63      |
|           | 30.90      | 31.79      | 30.66      |
| I2        | 125.92      | 124.14      | 131.30      | 38.85      | 39.36      | 40.28      | 87.07      | 84.78      | 91.02      |
|           | 31.02      | 31.74      | 30.83      |

**F-test**

| I0×N0     | ns        | ns         | ns         | ns         | ns        | ns         | ns         | ns         | ns         |
| I0×N180   | **        | **         | **         | **         | **        | **         | **         | **         | **         |
| I0×N240   | **        | **         | **         | **         | **        | **         | **         | **         | **         |
| I0×N300   | **        | **         | **         | **         | **        | **         | **         | **         | **         |
| I1×N0     | 107.63b   | 136.60a    | 99.43b     | 33.46b     | 42.47a    | 31.33b     | 74.17b     | 94.13a     | 68.10b     |
| I1×N180   | 131.43a   | 104.27b    | 131.57a    | 40.10a     | 33.43b    | 40.13a     | 91.33a     | 70.83b     | 91.43a     |
| I1×N240   | 134.83a   | 137.20a    | 138.33a    | 40.90a     | 42.40a    | 40.57a     | 93.93a     | 94.80a     | 92.73a     |
| I1×N300   | 135.27a   | 137.87a    | 137.37a    | 41.27a     | 42.07a    | 42.62a     | 94.72a     | 91.74a     | 96.30a     |

**Grand mean**

| 124.23    | 126.13    | 127.60    | 38.62      | 39.81      | 39.10      | 85.61      | 86.32      | 88.50      |
| 30.84      | 31.62      | 30.75      |

**Note:** TAA: total amino acid; EAA: essential amino acid; NAA: non-essential amino acid. ns, not significant at P < 0.05; * Significant at P < 0.05; ** Significant at P < 0.01; Data in the same column with different letters indicate a significant difference at P < 0.05.

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affected EAAI in 2013/2014 and 2014/2015, due to the different effects of different nitrogen application treatments in each irrigation regime (Table 4). Irrigation and nitrogen application had a significant effect on PDCAAS, which increased with increasing irrigation, but decreased with increasing nitrogen application (Table 4). A two-way interaction between irrigation and nitrogen application was apparent for PDCAAS in 2013/2014 and 2014/2015, which ranged from 43.89 to 62.57% and 51.06 to 64.32%, respectively (Table 4).
Correlations among grain yield, protein content and amino acid composition

Significant correlations were observed among grain yield, protein content and amino acid composition (Table 5). Grain yield was positively correlated with protein, TAA and EAA content, but negatively with EAAI and PDCAAS (Pearson’s r = 0.511, 0.288, 0.296, -0.449 and -0.392, respectively). Close correlations were also found among protein content, TAA and EAA (0.805–0.991). Regression analysis further showed that the contents of EAA, NAA and TAA increased linearly with an increase in protein content (Fig 3). Similar slopes and correlation coefficients between grain protein and amino acid contents supported the view that higher EAA, NAA, and TAA amino acid contents are generally achieved under higher protein content. However, negative correlations were found between protein content and EAAI and PDCAAS (Pearson’s r = -0.362 and -0.579, respectively).

Table 5. Effects of irrigation and nitrogen application on EAAI and PDCAAS in wheat grain in 2012/2013, 2013/2014 and 2014/2015, and interactions between irrigation and nitrogen application; summary of F significance from analysis of variance of the effects of main factors and interactions.

| Treatment | EAAI (%) | PDCAAS (%) |
|-----------|----------|------------|
|           | 2012/2013| 2013/2014  | 2014/2015  | 2012/2013| 2013/2014| 2014/2015 |
| I0        | 58.70b   | 57.71      | 57.18b     | 51.99    | 53.00c   | 53.46b   |
| I1        | 61.81a   | 57.98      | 57.72b     | 54.47    | 56.55a   | 54.18b   |
| I2        | 60.21ab  | 57.12      | 61.58a     | 53.40    | 54.69b   | 56.54a   |
| F-test    | **       | ns         | **         | ns       | **       | **       |
| N0        | 61.01    | 64.29a     | 62.17a     | 55.74a   | 60.77a   | 61.15a   |
| N180      | 58.54    | 54.76b     | 57.31b     | 51.98b   | 51.97b   | 52.64b   |
| N240      | 61.34    | 56.18b     | 57.89b     | 53.51ab  | 53.24b   | 52.59b   |
| N300      | 60.07    | 55.20b     | 57.94b     | 51.92b   | 53.01b   | 52.53b   |
| F-test    | ns       | **         | **         | ns       | **       | **       |
| I0×N0     | 59.16    | 65.47a     | 58.47      | 53.30    | 62.57a   | 58.20a   |
| I0×N180   | 58.34    | 46.82c     | 56.33      | 51.57    | 43.89c   | 51.06b   |
| I0×N240   | 59.25    | 59.28b     | 57.01      | 51.78    | 51.13b   | 52.31b   |
| I0×N300   | 58.04    | 59.28b     | 56.92      | 51.32    | 54.43b   | 52.27b   |
| F-test    | ns       | **         | **         | ns       | **       | **       |
| I1×N0     | 61.60    | 65.81a     | 61.03a     | 54.76    | 61.75a   | 60.93a   |
| I1×N180   | 59.45    | 59.11b     | 56.64b     | 54.63    | 56.82b   | 53.37b   |
| I1×N240   | 63.65    | 59.11b     | 56.93b     | 55.20    | 59.54b   | 51.65bc  |
| I1×N300   | 62.53    | 47.89c     | 56.27b     | 53.28    | 48.08c   | 50.77c   |
| F-test    | ns       | **         | **         | ns       | **       | **       |
| I2×N0     | 62.28    | 61.58a     | 67.00a     | 59.16a   | 57.98a   | 64.32a   |
| I2×N180   | 57.81    | 58.33b     | 58.97b     | 49.74b   | 55.21a   | 53.48b   |
| I2×N240   | 61.11    | 50.14c     | 59.74b     | 53.56b   | 49.05b   | 53.80b   |
| I2×N300   | 59.63    | 58.43b     | 60.62b     | 51.15b   | 56.53a   | 54.54b   |
| F-test    | ns       | **         | **         | ns       | **       | **       |
| Grand mean| 60.24    | 57.60      | 58.83      | 53.29    | 54.75    | 54.73    |
| I×N (F-test) | ns     | **         | **         | ns       | **       | **       |
| CV (%)    | 7.78     | 5.279349   | 2.06       | 15.06    | 5.80612382 | 2.43    |

Note: EAAI: essential amino acid index; PDCAAS: protein digestibility-corrected amino acid score; ns, not significant at P < 0.05; * Significant at P < 0.05; ** Significant at P < 0.01; Data in the same column with different letters indicate a significant difference at P < 0.05.

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Discussion

Grain protein and amino acid composition

The grain protein content, comprised of gliadins and glutenins (storage proteins), as well as albumins and globulins (metabolic proteins), is an important determinant of grain quality in wheat. It is intensively affected by nitrogen application and irrigation. Coventry et al. [38] reported that the highest protein content in wheat grains was obtained with the lowest of four irrigation treatments. With increasing irrigation, the crude protein content of wheat grains was decreased from 14 to 9% in two growing seasons [39]. Excess watering reduced glutenins, high molecular weight (HMW) glutenins and the ratio of HMW to low molecular weight (LMW) glutenin subunits [40,41]. The lower protein content in wheat grains as a result of irrigation is caused by yield dilution effects on grain protein [42,43]. However, in the present study, protein content was not significantly different in wheat grains in the three irrigation treatments, although irrigation significantly improved grain yield and the ratio of EAA/TAA in 2013/2014 (Tables 2 and 3). This result indicated that there was no effect of yield dilution in our experiment. This is because all experiments were carried out in the Huanghuai area of North China, where wheat growth is usually stressed by water shortage, especially at the jointing and anthesis stages of development. Here, irrigation performed both once and twice promoted nutritional absorption from the soil, significantly increasing grain yield; however, there

Table 5. Correlations among grain yield, protein content, amino acid content, EAAI and PDCAAS in the three cropping seasons.

|            | Yield | Protein | TAA    | EAA    | EAAI   | PDCAAS |
|------------|-------|---------|--------|--------|--------|--------|
| Yield      | 1     |         |        |        |        |        |
| Protein    | 0.511*** | 1       |        |        |        |        |
| TAA        | 0.288** | 0.805*** | 1      |        |        |        |
| EAA        | 0.296** | 0.813*** | 0.991*** | 1     |        |        |
| EAAI       | -0.449*** | -0.362*** | 0.229*  | 0.230*  | 1     |
| PDCAAS     | -0.392*** | -0.579*** | 0.125  | -0.108 | 0.782*** | 1     |

Note:
* Significant at P < 0.05;
** Significant at P < 0.01;
*** Significant at P < 0.001

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Fig 3. Relationships between grain protein content and (a) essential amino acids (EAA), (b) non-essential amino acids (NAA) and (c) total amino acids (TAA) content.

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was little effect on protein content (Table 3). This finding further suggests that irrigation at jointing plus anthesis would be optimal in this region.

N application can have a significant effect on protein and amino acid composition in wheat grains. Increasing nitrogen application can increase protein content in wheat grains significantly [44,45], mainly by stimulating the accumulation of gliadins and glutenins [46,47]. Zhang et al. [48] reported that protein, Leu, Phy and TAA content in wheat grains were all significantly increased following nitrogen application at two sites. In the present study, the protein and amino acid content were improved by nitrogen application (Tables 2 and 3). Furthermore, a N rate of 240 kg N ha\(^{-1}\) significantly increased protein, TAA, EAA and NAA content in wheat grain compared with 180 kg N ha\(^{-1}\), but no further increases were found for these traits on treatment with 300 kg N ha\(^{-1}\), indicating that a N rate of 240 kg ha\(^{-1}\) was already sufficient to satisfy N uptake requirements from soil, and maintain protein accumulation in wheat grain in the experimental conditions. In fact, NAA accumulated to a greater extent in wheat grains than did EAA when nitrogen was increased, due to high levels of glutamate (Glu) and alanine (Ala) in NAA. Additionally, although no nitrogen treatment resulted in a higher proportion of EAA relative to TAA, this ratio was not significantly different following nitrogen treatments of 180, 240 and 300 kg N ha\(^{-1}\), suggesting EAA and TAA varying to a similar extent in response to N application (Table 3).

**Protein quality parameters**

The PDCAAS and CS of amino acids are used to evaluate the nutritive quality of protein. CS is affected by the wheat variety [13,49]; the CS of each EAA and the value of EAAI in wheat grains decrease significantly following insect infestation [50]. However, little is known about the effect of cultivation management on EAAI and PDCAAS under field conditions. In this study, EAAI and PDCAAS increased significantly with irrigation but decreased with N application (Fig 2). This suggests that irrigation improves the balance of amino acids, and therefore, the protein quality of wheat grains. Lys, the first limiting amino acid when calculating PDCAAS, significantly increased with increasing N application, consistent with the findings of Zhang et al. [48]. Michaelsen et al. [51] determined that the PDCAAS value of wheat for adults was 42–54%; however, in the current study, the range was 43.89–64.32%. This higher value was possibly caused by the fertile soil, suitable climate conditions or cultivar used.

**Correlation between yield and protein quality**

Determining the fine-scale relationship between wheat grain yield and the concomitant grain protein content would provide valuable information on how to optimize cultivation management. Gursoy et al. [52] showed a negative correlation between wheat grain yield and protein content \((r = -0.1177)\) under different tillage and residue management after cotton in three year field experiment. Furthermore, Li et al. [53] indicated that drought stress caused a reduction in yield but high grain protein content in 30 spring wheat varieties. However, in our study of different irrigation regimes and N application management, the correlation between yield and protein content was significantly positive (Table 5), mainly due to nitrogen management. Since nitrogen application is a crucial factor in wheat production, it results in a significant increase in yield and protein content compared to no N treatment [45,54], suggesting that both yield and protein content are increased to a comparable extent by nitrogen application. In addition, the nitrogen effect seems to be promoted by the irrigation regime [17,38], especially in semi-arid areas where the soil water is unable to meet the growth demands and irrigation therefore becomes important in terms of yield. This positive correlation was also observed by Nakano and Morita [55], who found that, compared to no N treatment, both yield and
grain protein content were higher under application of 4 and 2 g m$^{-2}$ of nitrogen at tillering and jointing. Furthermore, Tosti and Guiducci [56] also reported a positive effect on grain yield and protein after incorporation of faba bean into the soil (to improve N availability for the cereal component). Thus, to a certain extent, N application is an efficient way of increasing the protein content without causing yield reductions.

In the present study, although the EAAI and PDCAAS of the wheat grains were positively correlated ($r = 0.782$), they were negatively correlated with yield and grain protein content (Table 5). This suggests that with increasing grain yield and protein content, accumulation of EAA, especially Lys, is less than that of total protein, decreasing the balance of the amino acid composition and the overall utilization of wheat grain protein. That is, a contradiction exists between grain yield and protein quality when attempting to improve grain yield via irrigation or fertilizer management. An efficient approach such as breeding of high-Lys wheat cultivars or determining optimal cultivation management is therefore needed in order to improve these essential amino acids and the overall quality of wheat grains.

**Conclusions**

Both irrigation and nitrogen application significantly increased wheat grain yield. N application, but not irrigation, also had a significant and positive effect on grain protein content. The TAA, EAA and NAA content in grains also increased with increasing nitrogen, but no differences were observed under irrigation treatment in 2012/2013 and 2014/2015. In addition, EAAI and PDCAAS improved with irrigation but decreased with N application. Grain protein content was positively correlated with grain yield and contents of TAA and EAA, but negatively with EAAI and PDCAAS. Further analysis of the contradiction between yield and nutritional quality in wheat grains is now needed.

**Supporting information**

S1 File. Effects of irrigation and nitrogen application on 17 amino acids content in wheat grain in 2012/2013, 2013/2014 and 2014/2015, and interactions between irrigation and nitrogen application; summary of F significance from analysis of variance of the effects of main factors and interactions. Table A in S1 File: Asp, aspartic acid; Table B in S1 File: Thr, threonine; Table C in S1 File: Ser, serine; Table D in S1 File: Glu, glutamic acid; Table E in S1 File: Gly, glycine; Table F in S1 File: Ala, alanine; Table G in S1 File: Cys, cysteine; Table H in S1 File: Val, valine; Table I in S1 File: Met, methionine; Table J in S1 File: Ile, isoleucine; Table K in S1 File: Leu, leucine; Table L in S1 File: Tyr, tyrosine; Table M in S1 File: Phe, phenylalanine; Table N in S1 File: Lys, lysine; Table O in S1 File: His, histidine; Table P in S1 File: Arg, arginine; Table Q in S1 File: Pro, proline.

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References
1. Gao Y, Wu P, Zhao X, Wang Z (2014) Growth, yield, and nitrogen use in the wheat/maize intercropping system in an arid region of northwestern China. Field Crops Research 167: 19–30.
2. Shewry PR (2009) Wheat. Journal of Experimental Botany 60: 1537–1553. https://doi.org/10.1093/jxb/erp058 PMID: 19386614
3. Jalilian J, Modarres-Sanavy SAM, Saber Ali SF, Sadat-Asilian K (2012) Effects of the combination of beneficial microbes and nitrogen on sunflower seed yields and seed quality traits under different irrigation regimes. Field Crops Research 127: 26–34.
4. Shi R, Zhang Y, Chen X, Sun Q, Zhang F, Roemheld V, et al. (2010) Influence of long-term nitrogen fertilization on micronutrient density in grain of winter wheat (Triticum aestivum L.). Journal of Cereal Science 51: 165–170.
5. Anderson WK (2010) Closing the gap between actual and potential yield of rainfed wheat. The impacts of environment, management and cultivar. Field Crops Research 116: 14–22.
6. Makowski D, Nesme T, Papy F, Dore T (2014) Global agronomy, a new field of research. A review. Agronomy for Sustainable Development 34: 293–307.
7. Sissons M, Ovenden B, Adorada D, Milgate A (2014) Durum wheat quality in high-input irrigation systems in south-eastern Australia. Crop & Pasture Science 65: 411–422.
8. Martre P, Porter JR, Jamieson PD, Triboi E (2003) Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. Plant Physiology 133: 1959–1967. https://doi.org/10.1104/pp.103.030585 PMID: 14630962
9. Zhang M, Ma CY, Lv DW, Zhen SM, Li XH, Yan YM. (2014) Comparative Phosphoproteome Analysis of the Developing Grains in Bread Wheat (Triticum aestivum L.) under Well-Watered and Water-Deficit Conditions. Journal of Proteome Research 13: 4281–4297. https://doi.org/10.1021/pr500400t PMID: 25145454
10. Howarth JR, Parmar S, Jones J, Shepherd CE, Corol DI, Galster AM, et al. (2008) Co-ordinated expression of amino acid metabolism in response to N and S deficiency during wheat grain filling. Journal of Experimental Botany 59: 3675–3688. https://doi.org/10.1093/jxb/erm218 PMID: 18791197
11. Penas E, Martinez-Villaluenga C, Vidal-Casero C, Zielinski H, Frias J (2013) Protein Quality of Traditional Rye Breads and Ginger Cakes as Affected by the Incorporation of Flour with Different Extraction Rates. Polish Journal of Food and Nutrition Sciences 63: 5–10.
12. Millward DJ (2012) Amino acid scoring patterns for protein quality assessment. British Journal of Nutrition 108: S31–S43. https://doi.org/10.1017/S0007114512002462 PMID: 23107544
13. Konvalina P, Capouchova I, Stehno Z, Moudry J Jr., Moudry J (2011) Composition of essential amino acids in emmer wheat landraces and old and modern varieties of bread wheat. Journal of Food Agriculture & Environment 9: 193–197.
14. Wu XR, Chen ZH, Folk WR (2003) Enrichment of cereal protein lysine content by altered tRNA(lys) coding during protein synthesis. Plant biotechnology journal 1: 187–194. https://doi.org/10.1046/j.1467-7652.2003.00017.x PMID: 17156631
15. WHO/FAO/UNU Expert Consultation (2007) Protein and amino acid requirements in human nutrition.
WHO Technical reports Series, Geneva: World Health Organization 935.
16. Rajala A, Hakala K, Makela P, Muurinen S, Peltonen-Sainio P (2009) Spring wheat response to timing
of water deficit through sink and grain filling capacity. Field Crops Research 114: 263–271.
17. Ram H, Dadhwal V, Vashistt KK, Kaur H (2013) Grain yield and water use efficiency of wheat (Triticum
aestivum L.) in relation to irrigation levels and rice straw mulching in North West India. Agricultural
Water Management 128: 92–101.
18. Qiu GY, Wang L, He X, Zhang X, Chen S, Chen J, et al. (2008) Water use efficiency and evapotranspi-
ration of winter wheat and its response to irrigation regime in the north China plain. Agricultural and For-
est Meteorology 148: 1848–1859.
19. Zeleke KT, Nendel C (2016) Analysis of options for increasing wheat (Triticum aestivum L.) yield in
south-eastern Australia: The role of irrigation, cultivar choice and time of sowing. Agricultural Water
Management 166: 139–148.
20. Wang CY, Guo TC, Peng Y, Zhu YJ, Ma DY, Zhang CJ (2004) Effect of post-anthesis irrigation on grain
quality indices and yield in winter wheat (Triticum aestivum L.) (In chinese with English abstract). Acta
Agronomica Sinica 30: 1031–1035.
21. Rezaei M, Zehtab-Salmasi S, Najafi N, Ghassemi-Golezani K, Jalalikamal M (2010) Effects of water
deficit on nutrient content and grain protein of bread wheat genotypes. Journal of Food Agriculture &
Environment 8: 535–539.
22. Grahmann K, Verhulst N, Pena RJ, Buerkert A, Vargas-Rojas L, Govaerts B. (2014) Durum wheat (Triti-
cum durum L.) quality and yield as affected by tillage-straw management and nitrogen fertilization prac-
tice under furrow-irrigated conditions. Field Crops Research 164: 166–177.
23. Massoudifar O, Kodjouri FD, Mohammadi GN, Mirhadi MJ (2014) Effect of nitrogen fertilizer levels and
irrigation on quality characteristics in bread wheat (Triticum aestivum L.). Archives of Agronomy and
Soil Science 60: 925–934.
24. Chen C, Han G, He H, Westcott M (2011) Yield, Protein, and Remobilization of Water Soluble Carbohy-
drate and Nitrogen of Three Spring Wheat Cultivars as Influenced by Nitrogen Input. Agronomy Journal
103: 786–795.
25. Zhang LS, Zhang BJ, Wang PH, Zhao WM (2002) Effect of different contents of nitrogen application on
amino acids in process of wheat seed development. Acta Botanica Boreali-occidentalia Sinica 22: 646–
650.
26. Byers M, Bolton J (1979) Effects of nitrogen and sulphur fertilisers on the yield, N and S content, and
amino acid composition of the grain of spring wheat. Journal of the Science of Food and Agriculture 30:
251–263. PMID: 459435
27. Geesing D, Diacono M, Schmidhalter U (2014) Site-specific effects of variable water supply and nitro-
gen fertilisation on winter wheat. Journal of Plant Nutrition and Soil Science 177: 509–523.
28. Wang Y, Zhang X, Liu X, Zhang X, Shao L, Sun H, et al. (2013) The effects of nitrogen supply and water
regime on instantaneous WUE, time-integrated WUE and carbon isotope discrimination in winter
wheat. Field Crops Research 144: 236–244.
29. Bandyopadhyay KK, Misra AK, Ghosh PK, Hati KM, Mandal KG, Moahnty M. (2010) Effect of irrigation
and nitrogen application methods on input use efficiency of wheat under limited water supply in a Verti-
sol of Central India. Irrigation Science 28: 285–299.
30. Sepaskhah AR, Hosseini SN (2008) Effects of alternate furrow irrigation and nitrogen application rates
on yield and water- and nitrogen-use efficiency of winter wheat (Triticum aestivum L.). Plant Production
Science 11: 250–259.
31. Rodriguez-Felix F, Ramirez-Wong B, Isabel Torres-Chavez P, Alvarez-Aviles A, Moreno-Salazar S,
Eugenio Renteria-Martinez M, et al. (2014) Yellow berry, protein and agronomic characteristics in bread
wheat under different conditions of nitrogen and irrigation in Northeast Mexico. Pakistan Journal of Bot-
nany 46: 221–226.
32. Xu Z, Yu Z, Zhao J (2013) Theory and application for the promotion of wheat production in China: past,
present and future. Journal of the Science of Food and Agriculture 93: 2339–2350. https://doi.org/10.
1002/jsfa.6098 PMID: 23408419
33. Kooch Y, Samadzadeh B, Hosseini SM (2017) The effects of broad-leaved tree species on litter quality
and soil properties in a plain forest stand. Catena 150: 223–229.
34. Shao Y, Xie Y, Wang C, Yue J, Yao Y, Liu W, et al. (2016) Effects of different soil conservation tillage
approaches on soil nutrients, water use and wheat-maize yield in rainfed dry-land regions of North
China. European Journal of Agronomy 81: 37–45.
35. FAO/WHO Expert Consultation (1991) Food and Agricultural organization of the United Nations., FAO
Food and Nutrition Paper, Rome 5.
36. Schaafsma G (2012) Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. British Journal of Nutrition 108: S333–S336. https://doi.org/10.1017/S0007114512002541 PMID: 23107546

37. Tome D (2012) Criteria and markers for protein quality assessment—a review. British Journal of Nutrition 108: S222–S229. https://doi.org/10.1017/S0007114512002565 PMID: 23107532

38. Coventry DR, Yadav A, Poswal RS, Sharma RK, Gupta RK, Chhokar RS, et al. (2011) Irrigation and nitrogen scheduling as a requirement for optimising wheat yield and quality in Haryana, India. Field Crops Research 123: 80–88.

39. Waraich EA, Ahmad R, Saifullah, Ahmad S, Ahmad A (2010) Impact of water and nutrient management on the nutritional quality of wheat. Journal of Plant Nutrition 33: 640–653.

40. Jia D, Dai X, He M (2012) Polymerization of Glutenin during Grain Development and Quality Expression in Winter Wheat in Response to Irrigation Levels. Crop Science 52: 1816–1827.

41. Dai ZM, Xu TS, Li XG, Zhang H, Li Y, Zhang XL. (2016) Effect of different water supply on accumulation of high molecular weight glutenin subunits and glutenin macromolecules in near-isogenic wheat lines. Plant Soil and Environment 62: 53–59.

42. Pleijel H, Mortensen L, Fuhrer J, Ojanpäri K, Danielsson H (1999) Grain protein accumulation in relation to grain yield of spring wheat (Triticum aestivum L.) grown in open-top chambers with different concentrations of ozone, carbon dioxide and water availability. Agriculture Ecosystems & Environment 72: 265–270.

43. Taub DR, Miller B, Allen H (2008) Effects of elevated CO2 on the protein concentration of food crops: a meta-analysis. Global Change Biology 14: 565–575.

44. Marino S, Tognetti R, Alvino A (2011) Effects of varying nitrogen fertilization on crop yield and grain quality of emmer grown in a typical Mediterranean environment in central Italy. European Journal of Agronomy 34: 172–180.

45. Ercoli L, Masoni A, Pampagna S, Marietti M, Arduini I (2013) As durum wheat productivity is affected by nitrogen fertilisation management in Central Italy. European Journal of Agronomy 44: 38–45.

46. Wan Y, Gritsch CS, Hawkesford MJ, Shewry PR (2014) Effects of nitrogen nutrition on the synthesis and deposition of the omega-gliadins of wheat. Annals of Botany 113: 607–615. https://doi.org/10.1093/aob/mct291 PMID: 24344140

47. Fuertes-Mendizabal T, Gonzalez-Torralba J, Arregui LM, Gonzalez-Murua C, Begona Gonzalez-Moro M, Estavillo JM. (2013) Ammonium as sole N source improves grain quality in wheat. Journal of the Science of Food and Agriculture 93: 2162–2171. https://doi.org/10.1002/jsfa.6022 PMID: 23339023

48. Zhang M, Ma D, Wang C, Zhao H, Zhu Y, Guo T. (2016) Responses of amino acid composition to nitrogen application in high- and low-Protein wheat cultivars at two planting environments. Crop Science 56: 1277–1287.

49. Perez Conesa D, Ros Berruezo G, Periago Caston MJ (2002) Essential and non essential amino acid content of infant cereals in different stages of industrial processing and its relationship with chemical scores of protein quality. Archives latinoamericanos de nutricion 52: 193–202. PMID: 12184155

50. Jood S, Kapoor AC, Singh R (1995) Amino acid composition and chemical evaluation of protein quality of cereals as affected by insect infestation. Plant foods for human nutrition (Dordrecht, Netherlands) 48: 159–167.

51. Michaelsen KF, Hoppe C, Roos N, Kaestel P, Stoegaard M, Lauritzen L, et al. (2009) Choice of foods and ingredients for moderately malnourished children 6 months to 5 years of age. Food and Nutrition Bulletin 30: S343–S404. https://doi.org/10.1177/15648265090303S303 PMID: 19998864

52. Gursoy S, Sessiz A, Mahli SS (2010) Short-term effects of tillage and residue management following cotton on grain yield and quality of wheat. Field Crops Research 119: 260–268.

53. Li P, Chen J, Wu P (2011) Agronomic Characteristics and Grain Yield of 30 Spring Wheat Genotypes under Drought Stress and Nonstress Conditions. Agronomy Journal 103: 1619–1628.

54. Zhang Y, Dai X, Jia D, Li H, Wang Y, Li C, et al. (2016) Effects of plant density on grain yield, protein size distribution, and breadmaking quality of winter wheat grown under two nitrogen fertilisation rates. European Journal of Agronomy 73: 1–10.

55. Nakano H, Morita S (2009) Effects of Seeding Rate and Nitrogen Application Rate on Grain Yield and Protein Content of the Bread Wheat Cultivar ‘Minaminoa’ in Southwestern Japan. Plant Production Science 12: 109–115.

56. Tosti G, Guiducci M (2010) Durum wheat-faba bean temporary intercropping: Effects on nitrogen supply and wheat quality. European Journal of Agronomy 33: 157–165.