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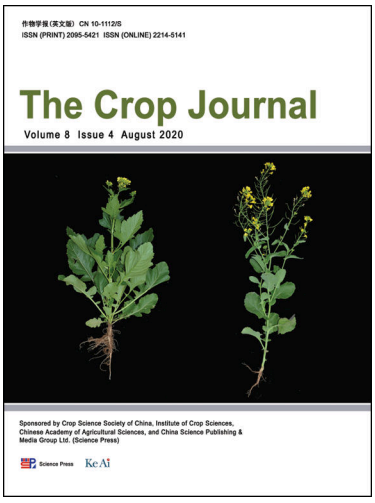

Effects of water deficit and high N fertilization on wheat storage protein synthesis, gluten secondary structure, and breadmaking quality

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PII: S2214-5141(21)00105-7
DOI: https://doi.org/10.1016/j.cj.2021.04.006
Reference: CJ 641

To appear in: *The Crop Journal*

Received Date: 4 January 2021
Revised Date: 11 March 2021
Accepted Date: 19 May 2021

Please cite this article as: J. Liu, J. Zhang, G. Zhu, D. Zhu, Y. Yan, Effects of water deficit and high N fertilization on wheat storage protein synthesis, gluten secondary structure, and breadmaking quality, *The Crop Journal* (2021), doi: https://doi.org/10.1016/j.cj.2021.04.006

Effects of water deficit and high N fertilization on wheat storage protein synthesis, gluten secondary structure, and breadmaking quality

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Abstract: The content and composition of wheat storage proteins are the major determinants of dough rheological properties and breadmaking quality and are influenced by cultivation conditions. This study aimed to investigate the effects of water deficit and high N-fertilizer application on wheat storage protein synthesis, gluten secondary structure, and breadmaking quality. Reverse-phase ultrahigh-performance liquid chromatography analysis showed that storage protein and gluten macropolymer accumulation was promoted under both independent applications and a combination of water-deficit and high N-fertilizer treatments. Fourier-transform infrared spectroscopy showed that water deficit and high N-fertilizer treatments generally improved protein secondary structure formation and lipid accumulation, and reduced flour moisture. In particular, high N-fertilizer application increased β-sheet content by 10.4% and the combination of water-deficit and high N-fertilizer treatments increased random coil content by 7.6%. These changes in gluten content and secondary structure led to improved dough rheological properties and breadmaking quality, including superior loaf internal structure, volume, and score. Our results demonstrate that moderately high N-fertilizer application under drought conditions can improve gluten accumulation, gluten secondary structure formation, and baking quality.

Keywords: Water deficit; High N-fertilizer; Storage proteins; Gluten structure; Breadmaking quality

1. Introduction

Wheat (*Triticum aestivum* L.) kernels contain 65%–75% starch and 12%–15% protein, which are synthesized and accumulated at about 10–30 days after flowering and strongly influence yield and quality formation [1,2]. Gliadins and glutenins are the major storage proteins deposited in wheat endosperm and confer respectively dough extensibility and elasticity [3]. Gliadins are monomeric and include α/β-, γ- and ω-gliadins, while glutenins are polymeric and consist of high- and low-molecular-weight glutenin subunits (HMW-GS and LMW-GS). Gliadins and glutenins can form gluten macropolymers (GMPs) linked by intra- and intermolecular disulfide bonds. These are among the largest protein molecules in nature [4]. Network formation by gluten polymerization contributes to the viscoelasticity of dough [5,6], permitting the processing of wheat flour into food products such as bread, noodles, and pasta [7,8].

During growth and development, wheat may encounter biotic and abiotic stresses that affect yield and quality formation, particularly at grain-filling stages [9]. As one of the most widespread abiotic stressors, drought severely affects wheat growth, grain development, and yield formation, especially at early grain-filling stages [10]. On the one hand, drought induces plant stomatal closure, inhibition of photosynthesis, and increased respiration [11] and results in accelerated grain filling and early maturing, reduced starch biosynthesis, and reduced grain weight and yield [12]. On the other hand, proteomic analysis has revealed [10,13,14] that water deficit induced the

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Received: 2021-01-04; Revised: 2021-03-11; Accepted: 2021-05-19.

accumulation of wheat storage proteins, improving breadmaking quality formation. Water deficit also led to changes of protein posttranslational modifications such as phosphorylation, involved in starch and protein synthesis and stress defense [15,16].

Nitrogen (N) is an essential element for plant growth and a component of organic compounds in plants. High-N fertilizer application generally improves grain metabolite, starch, and protein synthesis and breadmaking quality [17–19]. Wheat is generally cultivated in arid and semi-arid environments. The main Chinese wheat production area in northern China often suffers from severe drought owing to decreasing water supply and increasing drought frequency caused by climate change [20]. To alleviate losses in yields caused by drought conditions, moderately high-N fertilizer has generally been applied by local wheat farmers. A recent proteomic analysis [9] revealed that high-N fertilization under water-deficit conditions can alleviate wheat yield loss by increasing the accumulation of proteins involved in nitrogen and carbohydrate metabolism. However, there is still a lack of in-depth investigation of water-deficit and high-N fertilization effects, alone and in combination, on gluten protein accumulation, gluten secondary structure, and breadmaking quality.

The objective of the present study was to characterize the effects of independent and combined water deficit and high-N fertilizer on gluten protein accumulation, gluten secondary structure, dough rheological properties, and breadmaking quality in an elite Chinese bread wheat cultivar. The results were expected to establish the relationships between gluten accumulation and structure and function and shed light on the mechanisms of wheat gluten quality formation under various environmental conditions.

2. Materials and methods

2.1. Plant material and field treatments

The cultivar Jingdong 17 is widely cultivated in the northern winter wheat production area of China owing to its good adaptation and yield performance. A field experiment was performed at the experimental station of China Agricultural University, Wuqiao, Hebei province during the 2018 and 2019 wheat growing seasons. The mean rainfall of two consecutive wheat growth reasons at the experimental station was 125 mm. The 0–40 cm clay-loam soil contained 14.2 g kg^{-1} of organic matter, 1.02 g kg^{-1} of total nitrogen, 18.4 mg kg^{-1} of available phosphorus, and 102.8 mg kg^{-1} of available potassium.

In a previous study [21], the biomass of a wheat population and its spikelet number increased with N application amount in the range of 0–240 kg ha^{-1} of fertilizer urea $(NH_2)_2CO$. In this study, 240 and 120 kg ha^{-1} ureas were used as high-N and normal-N treatments, respectively. A randomized complete block design was used with four treatment groups: control group with well-watered irrigation and normal N (CK), water-deficit group without irrigation and with normal N (WD), high N with well-watered irrigation (HN) and combined water deficit and high N (WD+HN). Each group occupied 30 m^2 and three biological repeats, and an untreated zone 1 m in width between plots was maintained to minimize the effects of adjacent plots. Mature kernels were collected, dried, and stored at room temperature prior to use.

2.2. Basic quality parameter analysis

The basic quality parameters tested included protein content, gluten index, total gluten content, and GMP content. Seed protein content, gluten index, and total gluten content were determined, and size-exclusion high-performance liquid chromatography (SE-HPLC) was used to determine GMP content following Wu et al. [22].

2.3. RP-UPLC

Separation, characterization, and quantitation of seed gliadins and glutenins were performed using reverse-phase ultrahigh-performance liquid chromatography (RP-UPLC) on an Acquity UPLCTM instrument (Waters Corporation, Milford, Massachusetts, USA) with a Waters 300SB C18 column (1.7 μm). The extraction and RP-UPLC separation of gliadins followed Han et al. [23], while those of HMW-GS and LMW-GS followed Yan et al. [24] and Yu et al. [25], respectively.

2.4. ART-FTIR analysis of flour samples

An attenuated total reflectance (ATR)-Fourier-transform infrared spectroscopy (FTIR) spectrophotometer (VERTEX70_Bruker, Ettlingen, Germany) was used to determine differences in moisture, lipid, protein, and carbohydrate characteristics of wheat flour among treatments following Marti et al. [26]. The milled flour samples from four treatments were used for ART-FTIR. Background spectra (air) were collected prior to analysis of each sample. A wavelength range of 400–4000 cm^{-1} with an average of 32 scans at 4 cm^{-1} resolution was used, following Malalgoda et al. [27].

2.5. ART-FTIR analysis of dough samples

Dough samples were prepared in a Farinograph-AT (Brabender GmbH & Co. KG, Duisburg, Germany) equipped with a 50 g mixing bowl and MetaBridge software. All samples were prepared at optimal water absorption, and sufficient water was added to 50 g of flour to attain a consistency of 500 ± 20 Farinograph Units (FU). The temperature of the mixing bowl was kept at 30 °C using a temperature-controlled water bath and water added to the dough to reach the desired consistency was also kept at 30 °C. Dough samples were collected at dough development time, namely the time from the first addition of water to the point of maximum consistency range. Dough samples were collected with minimal additional physical manipulation, and fresh dough was used for FTIR analysis following Quayson et al. [28].

Dough samples were used to detect gluten secondary structures (the conformation of proteins in the dough samples) by ATR-FTIR. The FTIR spectrophotometer was equipped with a horizontal multi-reflectance diamond crystal accessory. The dough sample was firmly pressed against the crystal to remove air and to maximize contact. After the spectral test was completed, water vapor compensation and baseline correction were performed. The infrared spectra were analyzed using Omnic (version 9.0, Thermo Nicolet Inc, Waltham, MA, USA) and Peakfit (version 4.04, SPSS Inc., Chicago, IL, USA). During data analysis, reference H_2O–D_2O mixtures that matched the moisture content of the dough samples were obtained, vector-normalized, and used for the subtraction of water contribution in the amide I region at 1600–1700 cm^{-1} in the normalized spectra. To quantify the secondary structures of gluten protein in the amide I region, second-derivative spectra with a five-point Savitzky–Golay filter were used following Bock and Damodaran [29]. According to a recent study [27], the amide I band was assigned as follows: the spectral regions for β-sheets, random coils, α-helixes and β-turns were 1620–1644 cm^{-1}, 1644–1652 cm^{-1}, 1652–1660 cm^{-1} and 1660–1685 cm^{-1}, respectively. To determine the percentage of each secondary structure, the second-derivative area corresponding to each of the structures was calculated as a percentage of the total area of the amide I region.

2.6. Dough rheological properties and breadmaking quality analysis

Dough rheological properties were tested by Rapid Visco Analyzer (RVA) and Farinograph following Zhou et al. [30] and Zhen et al. [31]. RVA parameters included peak viscosity, final viscosity, breakdown, and setback using a RVA (Newport Scientific Series 3, Australia). Farinograph parameters detected with a 10 g Brabender Farinograph-E included water absorption, consistency, falling number, development time, stability time, and farinogram quality number (FQN). Breadmaking quality was tested following Sun et al. [32], including C-Cell parameters associated with bread inner structures using C-Cell image analysis software and equipment (Calibre Control International Ltd.; Warrington, UK), loaf volume, and appearance score.

2.7. Statistical analysis

Statistical analyses were performed with SPSS (version 19.0, SPSS Inc., Chicago, IL, USA). All data were subjected to one-way analysis of variance (ANOVA). Fisher's Least Significant Difference (LSD) test was used to determine significant differences at $P = 0.05$.

3. Results and discussion

3.1. Gluten protein accumulation changed under water deficit and high N-fertilizer

Protein content, total gluten content, gluten index, and GMP content were significantly increased under independent and combined water-deficit and high N-fertilizer treatments in comparison with the control group (Fig. 1A). Both total protein content and individual component accumulation showed distinct differences among treatments (Fig. 1B, C). The combined water-deficit and high N-fertilizer treatments significantly increased total gliadin content, but water-deficit treatment and high N-fertilizer treatment alone showed no clear effects. The accumulation of some gliadin components were significantly increased, including ω-gliadins under water deficit, α/β-gliadins under three treatments, and γ-gliadins under water deficit and combined treatments. However, γ-gliadins under water deficit were significantly decreased, and ω- and γ-gliadins under high N-fertilizer showed no significant change (Fig. 1B). In comparison with gliadins, glutenin accumulation was more strongly induced by independent and combined water-deficit and high N-fertilizer treatments (Fig. 1C). Both glutenin content and individual subunits, including total glutenin content, HMW-GS and LMW-GS content, and four individual HMW-GS (1Bx7+1By9 and 1Dx2+1Dy12), were significantly increased by three treatments.

Recent proteomic analyses [9,19] have shown that high N-fertilization application increases leaf photosynthesis and accumulation of proteins involved in nitrogen and carbohydrate metabolism, leading to increased starch and protein contents and grain yield. A previous study [33] showed that drought stress caused greater decline in the activities of key regulatory enzymes of wheat starch biosynthesis than in those of proteins, suggesting that carbohydrate metabolism is more sensitive to drought than nitrogen metabolism. We accordingly speculate that drought stress could result in a greater decrease in starch content and grain weight than in protein content, which could cause an increase in the relative content of grain protein (Fig. 1). Moderately high-N fertilization under water-deficit conditions not only alleviated losses in grain yield caused by water deficit, but increased gluten protein accumulation. Given that glutenin subunits are the major determinant of dough viscoelasticity, the increase of glutenins under independent and combined water-deficit and high N-fertilizer conditions could improve dough strength and baking quality.

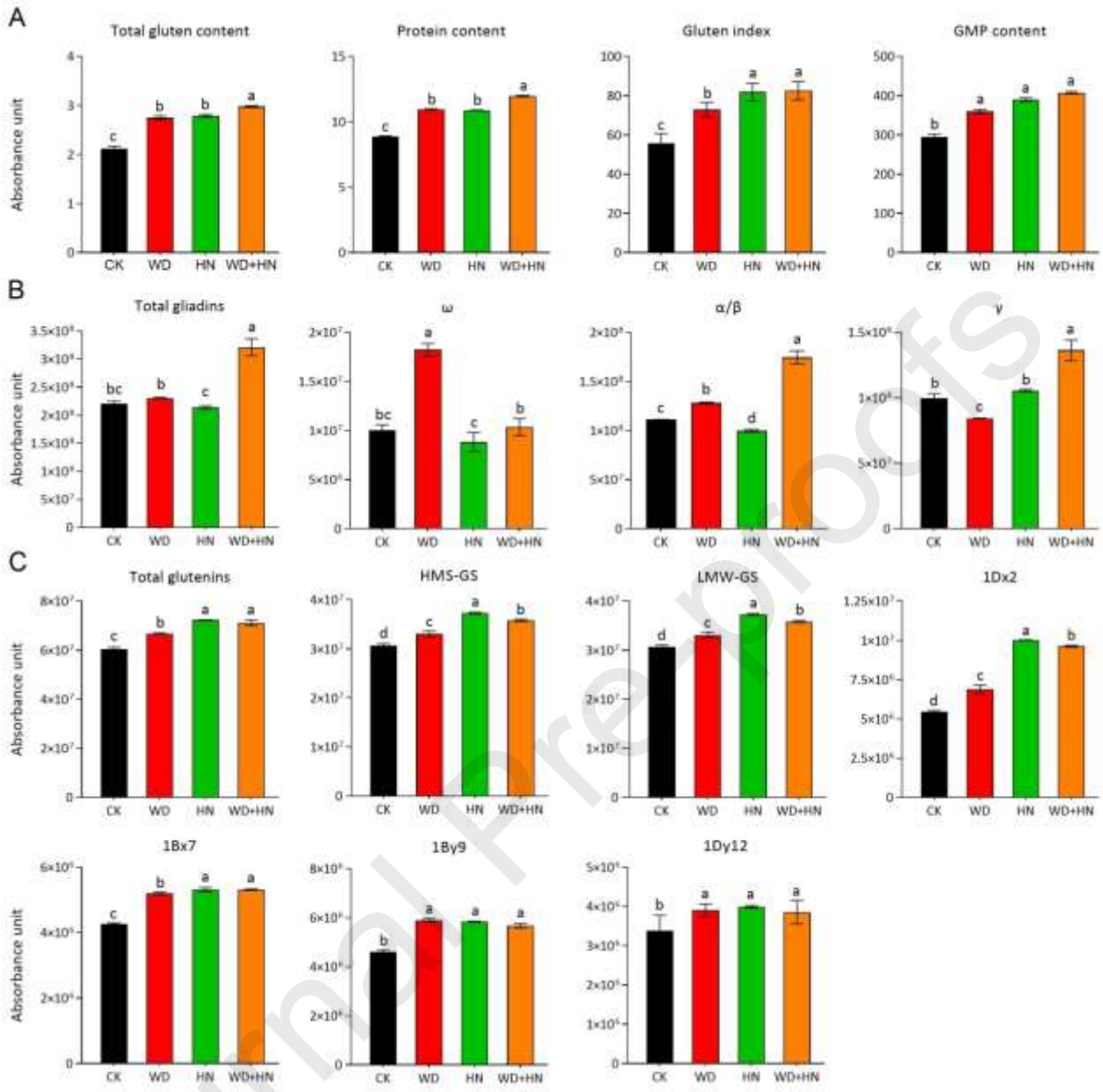

Fig. 1 – Changes in grain protein, gluten protein, and gluten macropolymer (GMP) content and storage protein accumulation in wheat cultivar Jingdong 17 under water-deficit (WD), high N-fertilizer (HN) and their combination (WD+HN).
(A) Protein content, total gluten content, gluten index, and GMP content. (B) Content of total gliadins, ω-gliadins, α/β-gliadins, and γ-gliadins. (C) Content of total glutenins, high-molecular-weight glutenin subunits (HMW-GS), low-molecular-weight glutenin subunits (LMW-GS), and individual HMW-GS (1Dx2, 1Bx7, 1By9, and 1Dy12).

3.2. Changes in wheat flour composition by ART-FTIR analysis

Flour composition changes under the experimental treatments were detected by ART-FTIR (Fig. 2A), including differences in carbohydrate, protein, lipid and moisture characteristics (Fig. 2B–E). In general, FTIR spectra of flour samples from different treatments showed similar patterns. However, slight differences in some regions representing flour-component characteristics were still present. Differences in peak intensity were observed in the 970–1170 cm^{-1} region associated with starch characteristics. The combined water deficit and high N-fertilizer treatment increased, and independent water-deficit and high N-fertilizer treatments reduced, the intensity of peaks

in this region (Fig. 2B). These changes may have resulted from differences in granule distribution of amylose and amylopectin characteristics that were affected by water-deficit and high N-fertilizer conditions [17]. In a previous study [34], FTIR spectra closely reflected starch granule distribution. It is also likely that starch damage during milling contributed to the observed differences as in the previous study [26].

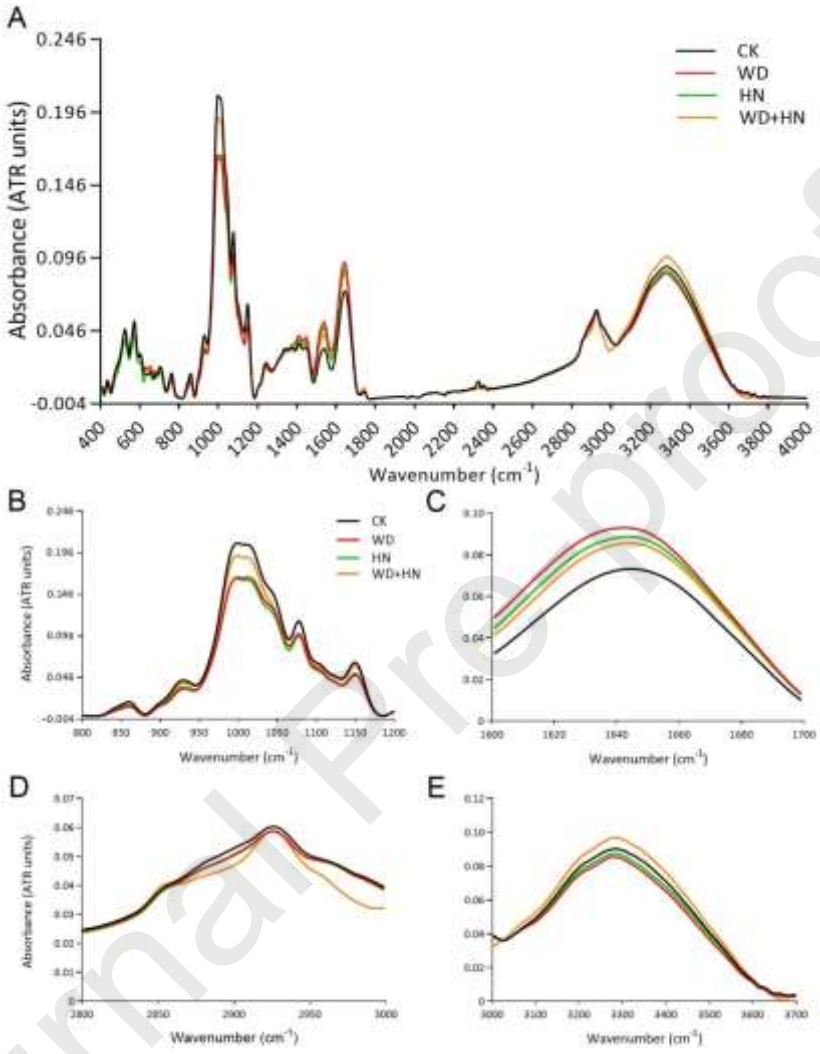

Fig. 2 – Changes of wheat flour compositions in wheat cultivar Jingdong 17 under water deficit (WD), high N fertilizer (HN), and their combination (WD+HN) by attenuated total-reflectance Fourier-transform infrared spectroscopy (ATR-FTIR).
(A) Comparison of ATR-FTIR spectra of wheat flour samples from different treatments. (B) Differences in carbohydrate (800–1200 cm^{-1}). (C) Differences in protein (1600–1700 cm^{-1}). (D) Differences in lipid (2800–3000 cm^{-1}). (E) Differences in moisture (3000–3700 cm^{-1}).

The peaks in the 1600–1700 cm^{-1} region are characteristic for protein secondary structural components, and the clear differences in peak intensity under different treatments were seen in the 1600–1660 cm^{-1} region (Fig. 2C). In comparison with the control group, both independent and combined water-deficit and high N-fertilizer treatments increased peak intensity in the 1600–1700 cm^{-1} region and increased protein secondary structure formation. These results are consistent with the increases in seed protein, gluten, GMP, and storage protein content under water-deficit and high N-fertilizer conditions (Fig. 1). The 2800–3000 cm^{-1} region corresponds to lipid component, and the differences in peak intensity were found in the 2850–2920 cm^{-1} and 2940–3000 cm^{-1} regions. Similar to protein secondary structure, both independent and combined water-deficit and high N-fertilizer treatments

increased flour lipid component synthesis (Fig. 2D). The peaks in the OH stretch region (3000–3700 cm^{-1}) are associated mostly with the moisture content of the samples. The flour samples from four treatments generally showed a similar FTIR spectrum with respect to both intensity and shape, but some differences in peak intensity in the 3011–3500 cm^{-1} region were still present. Water deficit and high N fertilizer independently, as well as their combination, led to a reduction in flour moisture content (Fig. 2E).

3.3. Gluten secondary structure changes by ART-FTIR

In this study, we used dough FTIR analysis to detect gluten secondary structure changes under several treatments (Fig. 3). In general, dough FTIR spectra from different treatments showed patterns similar in both peak shape and intensity (Fig. 3A). However, differences in peak intensity were observed in spectrum regions that represent particular secondary structure features (Fig. 3B). The peaks in the 1600–1700 cm^{-1} region represent a change in protein secondary structure characteristics. The spectral regions consist of 1620–1644 cm^{-1} for β-sheet, 1644–1652 cm^{-1} for random coil, 1652–1660 cm^{-1} for α-helix, and 1660–1685 cm^{-1} for β-turn structures [26,27]. High N-fertilizer application increased the absorbance intensity of four secondary structures in gluten protein. Water deficit slightly increased, and the combined treatments decreased, the absorbance intensity of gluten secondary structures. High N fertilizer alone and also the combined treatments increased β-sheets and random coils by 10.4% and 7.6%, respectively. The remaining treatments showed no significant effects on gluten secondary structure (Table 1).

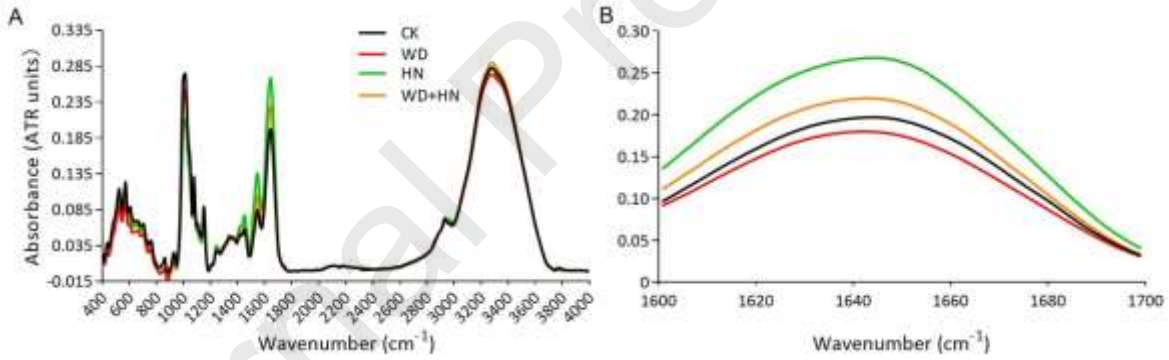

Fig. 3 – Gluten secondary structure changes in wheat cultivar Jingdong 17 under water deficit (WD), high N fertilizer (HN) ,and their combined treatment (WD+HN) by ART-FTIR.
(A) Comparison of ATR-FTIR spectra of wheat dough samples from different treatments. (B) Differences in gluten secondary structure (1600–1700 cm^{-1}) detected by ATR-FTIR.

Table 1 – Changes in gluten secondary structure under different treatments.

Treatment	β-sheet (%)	Random coil (%)	α-helix (%)	β-turn (%)
Control (CK)	39.52 ± 2.18 ^{b}	17.01 ± 1.03 ^{ab}	14.66 ± 0.96 ^{a}	28.81 ± 3.92 ^{a}
Water deficit (WD)	40.79 ± 1.42 ^{b}	16.83 ± 0.99 ^{ab}	14.75 ± 0.994 ^{a}	27.66 ± 0.84 ^{a}
High N fertilizer (HN)	43.62 ± 0.178 ^{a}	16.59 ± 0.11 ^{b}	13.88 ± 0.12 ^{a}	25.92 ± 0.21 ^{a}
WD+HN	39.95 ± 0.85 ^{b}	18.31 ± 0.56 ^{a}	15.28 ± 0.82 ^{a}	26.46 ± 0.53 ^{a}

Different letters in the same column indicate a significant difference at $P < 0.05$.

β-sheets are dominant in the secondary structures of wheat gluten proteins and are associated with a strong gluten network and the viscoelasticity of dough [35]. Glutenin subunits and gliadins can be combined in a certain ratio to form gluten. They provide a structural backbone to the gluten network in dough and endow the dough with

unique properties [36]. In particular, the central domain of HMW-GS consists of repetitive sequences rich in glutamine, proline, and glycine, which produce a series of overlapping reverse turns to form a β-turns and intermolecular β-sheets and confer gluten elasticity [37]. Because dough properties are closely associated with adequate formation of the gluten network [38], the increased β-sheets in the secondary structures of gluten protein under high N-fertilizer could improve the gluten network and increase dough viscoelasticity.

3.4. Changes in dough rheological properties

RVA and farinograph analyses were used to detect changes in dough rheological properties under several treatments (Table 1). Independent and combined water deficit and high N-fertilizer applications improved dough rheological properties, including increases in peak viscosity, final viscosity, setback, consistency, water absorption, development time, stability time, and FQN, and decreases in breakdown and softening degree. Falling number decreased and increased under water deficit and high N-fertilizer treatments, respectively, but no significant changes were found under the combined treatment.

Viscosity parameters are correlated with dough color, smoothness, firmness, processing quality, and score. Wheat with high peak viscosity shows better noodle quality, and the viscosity is positively correlated with the volume of steamed bread [39]. Dough with greater consistency generally has greater toughness, and water absorption is positively associated with dough elasticity. Both development time and stability time of dough are positively associated with dough strength. Stability time reflects the knead resistance of the dough, and these parameters were increased under different treatments, indicating that water-deficit and high N-fertilizer applications were beneficial for improving dough rheological properties.

FQN is positively correlated with the main indexes measured by mixograph and breadmaking quality [40]. Three treatments increased FQN by 8.70%–17.4% (Table 2). The increased FQN value was consistent with the improved dough rheological properties under both water-deficit and high nitrogen treatments. Thus, the FQN value could be used as an index to predict the quality of wheat flour and dough.

Table 2 – Comparative analysis of dough rheological properties under different treatments.

Rheological parameters	CK	Water deficit (WD)	High-N fertilizer (HN)	WD+HN
Peak viscosity	2718.23 ± 23.35 ^{d}	2856.12 ± 26.75 ^{b}	3894.00 ± 28.43 ^{a}	2825.62 ± 28.96 ^{c}
Final viscosity	3713.12 ± 31.92 ^{d}	3986.89 ± 35.32 ^{a}	3898.76 ± 33.78 ^{b}	3815.24 ± 36.75 ^{c}
Breakdown	623.78 ± 8.13 ^{a}	576.82 ± 10.42 ^{b}	524.73 ± 9.86 ^{c}	535.53 ± 9.51 ^{c}
Setback	1423.57 ± 25.74 ^{d}	1593.13 ± 2653 ^{b}	1512.74 ± 31.25 ^{c}	1612.61 ± 37.29 ^{a}
Consistency (FU)	491.12 ± 9.87 ^{b}	518.86 ± 8.79 ^{a}	523.47 ± 9.86 ^{a}	498.87 ± 8.97 ^{b}
Falling number	402.13 ± 4.78 ^{b}	378.65 ± 6.78 ^{c}	436.86 ± 7.74 ^{a}	412.23 ± 6.79 ^{b}
Softening degree (BU)	158.78 ± 8.56 ^{a}	132.67 ± 9.35 ^{b}	128.96 ± 8.63 ^{b}	135.23 ± 9.28 ^{b}
Water absorption (500 FU)	54.24 ± 0.37 ^{b}	57.67 ± 0.61 ^{a}	58.69 ± 0.78 ^{a}	57.89 ± 0.53 ^{a}
Development time (min)	3.21 ± 0.11 ^{c}	3.98 ± 0.13 ^{b}	3.76 ± 0.16 ^{b}	4.53 ± 0.16 ^{a}
Stability time (min)	3.52 ± 0.21 ^{c}	3.89 ± 0.25 ^{b}	3.84 ± 0.27 ^{b}	4.06 ± 0.13 ^{a}
Farinogram quality number (FQN)	46.00 ± 0.13 ^{c}	51.00 ± 1.12 ^{b}	50.00 ± 2.12 ^{b}	54.00 ± 0.45 ^{a}

Different letters in the same column indicate significant difference at P <0.05.

3.5. Breadmaking quality performance

Bread and steamed bread generally have a spongy internal texture structure that is considered a quality trait. In

addition to sensory evaluation, C-Cell image analysis technology can objectively and accurately evaluate the internal structure of the bread. We further measured breadmaking quality under different treatments, including the C-Cell parameters associated with bread internal structure and loaf parameters (Table 3; Fig. 4).

Table 3 – **Comparative analysis of C-Cell parameters and loaf parameters among treatments.**

Bread quality parameter	Control (CK)	Water deficit (WD)	High N-fertilizer (HN)	WD+HN
C-Cell parameters				
Slice area/px	251322.5 ± 679.58 ^{d}	263756.5 ± 558.76 ^{c}	287852.6 ± 849.62 ^{a}	278746.7 ± 578.63 ^{b}
Circumference/px	1821.42 ± 19.78 ^{c}	1996.54 ± 18.78 ^{b}	2085.52 ± 20.87 ^{a}	1996.79 ± 18.98 ^{b}
Slice brightness	125.87 ± 1.98 ^{c}	146.78 ± 1.86 ^{b}	158.97 ± 1.95 ^{a}	148.96 ± 1.73 ^{b}
Cell contrast	0.62 ± 0.01 ^{b}	0.78 ± 0.02 ^{a}	0.79 ± 0.02 ^{a}	0.76 ± 0.01 ^{a}
Cell quantity	2509.23 ± 22.86 ^{c}	2878.57 ± 25.45 ^{b}	3198.56 ± 38.71 ^{a}	2870.13 ± 25.44 ^{b}
Wall thickness/px	3.58 ± 0.05 ^{a}	3.21 ± 0.03 ^{b}	3.24 ± 0.02 ^{b}	3.22 ± 0.03 ^{b}
Cell diameter/px	18.98 ± 0.32 ^{a}	16.12 ± 0.38 ^{c}	17.13 ± 0.24 ^{b}	17.58 ± 0.25 ^{b}
Coarse cell volume	14.07 ± 0.35 ^{a}	11.24 ± 0.41 ^{b}	12.03 ± 0.29 ^{b}	12.08 ± 0.26 ^{b}
Average cell elongation	1.61 ± 0.02 ^{c}	1.79 ± 0.03 ^{a}	1.69 ± 0.01 ^{b}	1.71 ± 0.02 ^{b}
Attenuation ratio	54.12 ± 0.83 ^{c}	59.89 ± 0.74 ^{b}	63.78 ± 0.86 ^{a}	59.26 ± 0.82 ^{b}
Loaf parameters				
Loaf weight (g)	144.23 ± 1.76 ^{a}	145.78 ± 1.96 ^{a}	144.56 ± 1.68 ^{a}	144.87 ± 1.85 ^{a}
Loaf volume (mL)	526.78 ± 20.56 ^{c}	788.57 ± 17.5 6 ^{b}	868.75 ± 18.64 ^{a}	855.67 ± 15.76 ^{a}
Inner structure	20.00 ± 1.00 ^{c}	22.00 ± 1.00 ^{b}	26.00 ± 2.00 ^{a}	25.00 ± 1.00 ^{a}
Score (100)	51.00 ± 2.00 ^{d}	58.00 ± 1.00 ^{c}	65.00 ± 1.00 ^{a}	61.00 ± 2.00 ^{b}

Different letters in the same column indicate significant difference at $P < 0.05$.

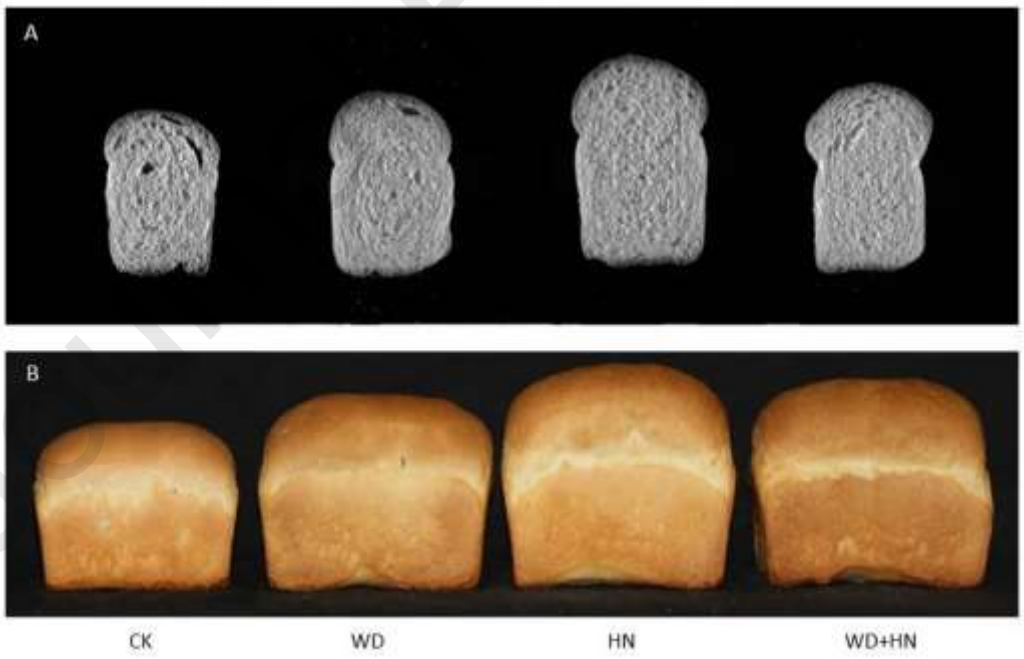

Fig. 4 – C-Cell image (A) and loaf appearance (B) of wheat cultivar Jingdong 17 under water-deficit (WD), high N-fertilizer (HN) and their combined treatment (WD+HN).

C-Cell parameters can be divided into two categories: slice information including slice area, circumference, sliced brightness, and cell contrast, and cell information including cell diameter, cell quantity, wall thickness,

coarse cell volume, average cell elongation, and attenuation ratio. According to the evaluation standard of baking quality of wheat flour bread in China (GB/T 16411–2008), the score of internal structure of bread accounted for 35% of the total score of bread. High-quality bread rolls are generally long in shape with fine and uniform cells and thin cell walls [41]. Independent and combined water-deficit and high N-fertilizer treatments increased slice area, circumference, sliced brightness, cell contrast, cell quantity, average cell elongation, and attenuation ratio, and reduced cell diameter, wall thickness, and coarse cell volume (Table 3). Thus, both water-deficit and high N-fertilizer treatments improved the internal structure of bread.

Loaf parameters, particularly loaf volume and score, are the most intuitive and important parameters for evaluating bread quality. Although loaf weight showed no significant change, loaf volume, inner structure and score increased under independent and combined water-deficit and high N-fertilizer conditions. In particular, high N-fertilizer and combined water-deficit and high N-fertilizer treatments increased loaf volume more than water deficit alone, by respectively 64.9% and 62.4% on average (Table 3). Thus, both water-deficit and high N-fertilizer treatments can improve the internal texture structure and appearance quality of wheat bread (Fig. 4).

4. Conclusions

Water-deficit and high N-fertilization treatments can either independently or synergistically promote storage protein and GMP accumulation. Water deficit and high N-fertilizer applications affected flour composition, generally improving protein secondary structure and lipid accumulation. In particular, high N fertilization under both water-deficit and irrigated conditions increased the content of β-sheets and random coils of gluten protein and improved gluten secondary structure. These changes in gluten content and secondary structures led to improved dough rheological properties and superior breadmaking quality.

Declaration of competing interest

Authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

Junxian Liu and Junwei Zhang performed all of the experiments, performed the data analysis, and wrote the paper. Genrui Zhu and Dong Zhu performed field experimental treatments and sample collection. Yueming Yan designed and supervised experiments and reviewed the paper. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

This research was financially supported by the National Natural Science Foundation of China (31771773 and 31971931) and the National Key Research and Development Program of China (2016YFD0100502).

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Declaration of Competing Interest

All the authors have no conflict of interest.