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Combined Effects of Starch Sucrose Content and Planting Density on Grain Protein Content of Winter Wheat (*Triticum aestivum* L.)

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Abstract Wheat (*Triticum aestivum* L.) is a staple crop worldwide, and yield improvement since the green revolution was attributed to chemical nitrogen (N) fertilizer application. We conducted field experiments in Yuncheng County, Shanxi Province, under planting density (2 million plants ha^{-1}, 2.25 million plants ha^{-1}, 2.5 million plants ha^{-1}) and topdressing nitrogen (12 kg ha^{-1}, 22 kg ha^{-1}, 32 kg ha^{-1}). The results shown that the starch content of grains with a density of 2.5 million plants ha^{-1} increased by 1.9%-7.5%, which improved the water absorption of flour and the processing quality of flour, but decreased the protein content, and gluten properties of grains. The nitrogen topdressing amount of 22 kg ha^{-1} wheat tiller increased by 6.3%-33.3%, and the dry matter accumulation increased by 1.0%-9.5%. The number of spikes increased by 3.7%-19.2%, and the yield increased by 3.7%-17.8% with the nitrogen topping amount of 22 kg ha^{-1} the protein content increased by 2.0%-4.2% with the nitrogen topping amount of 22 kg ha^{-1}, the starch content increased by 1.2%-7.2%, and the gluten index increased by 4.3%-21.5%.

Keywords: *albumin, protein content, planting density, soluble sugar, wheat*

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1. Introduction

Winter wheat (*Triticum aestivum* L.) farming accounts for one-third of the cultivated area in China. Approximately 40% of the cultivated land in the winter wheat, covering an area of 63 × 104 ha^{-1} [1]. Winter wheat production in this winter wheat is of importance in ensuring regional food security [2,3]. Planting density is an important factor affecting wheat population structure and yield formation. Appropriate seeding density can alleviate the competition between populations and individuals, contribute to the construction of a reasonable population structure, and lay a foundation for high yield and quality [4]. Found that appropriate seeding density can alleviate the competition between populations and individuals and contribute to the construction of a reasonable population structure. Studies have shown that planting density plays an important role in the regulation of the total stem number of wheat in the overwintering and greening-elongation stages, and the increase of planting density also leads to a gradual increase in the total stem number [5,6]. While the number of effective spike increases first and then decreases with the increase of planting density, while the percentage of tiller yield decreases. At a higher seeding amount, although the wheat population will reach a higher population number before jointing, the mortality rate and mortality rate will increase in the later stage, and this trend will become more significant with the increase of seeding amount, ultimately affecting the population quality [7,8]. Drought is the main limiting factor of winter wheat production in the winter wheat [9]. In different cultivation methods, the rate is an important factor of success, wheat production for seeding rate by changing the light between crop development process within the plant and plant, water and nutrient competition and greatly affect the use of environmental resources, lead to different population structure, ultimately affect grain yield, but also within the scope of control for farmers [10,11]. The imbalance of soil water and N supply is the main cause of low and unstable wheat yield in dry-land [12]. The application of N fertilizers can significantly increase grain yield and water use efficiency

(WUE) of winter wheat [13,14] showed that fertilizers can reduced the effect of soil moisture on productivity winter wheat soil while improving the wheat yield and WUE. The effects of water and N on crop yield are synergistic rather than individual. As the effects of water on yield and grain quality are influenced by N fertilizer, yield responses to N fertilizers vary with the annual precipitation level. A study on N application rate in the winter wheat for four consecutive years showed that when 180 kg N ha^{-1} was applied, wheat yield in the dry years increased by 14.0 % relative to no N application, whereas it increased by 32.8 % in the wet years [15].

Excessive N fertilization can have negative effects on crop yield and the environment [16]. Nowadays, scientists have realized that the factors that determine wheat quality are a complex concept, which are the result of the interaction of various factors inside the wheat grain, including the interaction between different types of proteins, between proteins and starch, and between proteins and lipids. Among them, the interaction between proteins is the most important. The research on the function of gluten protein mainly focuses on the following ideas: the effect of wheat high and low molecular weight glutenin subunits alone and mutually on wheat quality. Effects of low molecular weight glutenin and gliadin on wheat quality [17]. The excessive use of N fertilizers poses several negative effects on the environment [18]. A Study in the Loess Plateau has shown that with the use of controlled-release nitrogen fertilizers, the crop yield, NUE, and economic returns increased by 8.5%, 10.9%, and 11.3%, respectively [19]. Another study on N application rates in the Loess Plateau reported that the application of an appropriate amount of N fertilizer increased the content and composition of wheat proteins, leading to an improvement in baking quality of wheat flour [20]. With an increase in the N application rate, the investment on N fertilizer increases. N application should be determined based on the economic return [21]. Furthermore, a survey of farmers in dry farming areas in the Loess Plateau in 2011 showed that 42% of the farmers applied more than 200 kg ha^{-1} N, and achieved an average yield of 4500 kg ha^{-1} [22]. Apparently, the amount of N applied by farmers in the area exceeds the level of N required to achieve high yield. Considering the variety of agricultural practices, outlining an effective guidance on how to apply fertilizers according to varying annual precipitation for improved grain yield and quality as well as high water and fertilizer use efficiency in the winter wheat region of essential for the farmers and has been the focus of many researchers. Study on the relationship between gluten protein and wheat quality. The free amino acids transported by the vegetative organs gradually form the special protein components in wheat during the process of grain construction after wheat flowering. In this process, the changes of protein content and components have a great relationship with the grain quality of wheat. During wheat grain development, protein content showed a trend of "high-low-high". The low protein content in the middle of grain-filling is not only due to the fact that the synthesis rate of starch at this stage is faster than that of protein, but also related to the decrease of protein [23,24]. In this study the objects were as given; (i) To find the soluble sugar yield, (ii) The starch content of grains with a density of 2.5 million plants ha^{-1} increased by 1.9%-7.5%, which improved the water absorption of flour and the processing quality of flour, but decreased the protein content and gluten properties of grains. (iii) The density of 2.5 million plants ha^{-1} spikes increased by 14.1%-16.4%, and the yield increased by 11.6%-13.3%.

2. Materials and Methods

2.1. Description of Experimental Site

The experiment was carried out in the experimental site of Shanxi Agricultural University in Wenxi County, Shanxi Province (110°59′–111°37′E, 35°09′–35°34′N) in 2014–2017. Basic nutrients in the 0–20 cm layer.

2.1.1. Experimental Design and Field Management

A completely randomized design was used in the experiment with Basin, the planting density (2-million ha^{-1}, 2.25-million·ha^{-1}, 2.5-million ha^{-1}) and topdressing nitrogen (12 kg ha^{-1}, 22 kg ha^{-1}, 32 kg ha^{-1}) at the jointing stage replicates. The plot size was 4 m × 20 m. Before sowing, 150 kg P_2O_5 $ha^{-1}$and 150 kg K_2O ha^{-1}were evenly applied to the plots. Winter wheat seeds (Variety Yannong 1212) were sown with the N application rate of 97.5 kg ha^{-1} in early October each year. Sowing on October 17, 2020, using wide-space sowing (Select Xinbofeng rotary sowing machine, producing area Jishan County, Yuncheng County, Shanxi Province, complete rotary tillage, sowing, suppression and other operations at a time, straw stubble rotation tillage in 10-15 cm soil, forming a sowing depth of 3-5 cm, row spacing of 22-25 cm, width of 10-12 cm. Before sowing, the basal application of nitrogen, phosphorus and potassium was 150 kg ha^{-1}, P_2O_5 90 kg ha^{-1} and K_2O 36 kg ha^{-1}, respectively. Irrigation was 25 m^3/ mu at jointing stage, elongation and flowering stage, respectively. Water soluble nitrogen was 21 kg ha^{-1} at jointing stage. After flowering, potassium phosphate 1.5 kg ha^{-1} + Brassicin 10 ml ha^{-1} was sprayed on the leaves to prevent hot dry air and increase grain weight. Harvest on June 17, 2021.

2.1.2. Sucrose, Soluble Sugar and Starch

After flowering period listed growth consistent and the same day flowering of wheat spike and peeling grain were placed in the oven dried at 105°C for 20 mins and then at 80°C for 12 hours for dry weight. The quality and speed of weighing samples greatly affect the overall quality of the test. Then the grain was weighed and phenol method was used to determine the content of sucrose, and ketone color method was used to determine the total soluble total sugar content. H_2SO_4-H_2O_2-Phenol blue color method was used to determine the seed protein.

2.1.3. Grain to leaf area

Grain to leaf ratio was calculated according to [25], using Equations 1 and 2:

$$\text{Grain number to leaf area ratio} = \frac{\text{total number of grain par unit area}}{\text{total leaf are on the same plot at booting stage}}$$

$$\text{Grain weight to leaf ratio} = \frac{\text{grain weight (mg) par unit area}}{\text{total leaf are on the same plot at booting stage}}$$

2.1.4. Yield and Yield Components

At maturity, ten plants from each plot were randomly sampled from the inner rows for the determination of yield components such as ear number, seed number per ear and weight of thousand seed. Plot grain yield was determined by harvesting all plants in the area of 20 m^2, shelled using machine, and the grain was air-dried for the determination of grain yield.

2.1.5. Grain Protein and Its Components

The continuous extraction method was used to determine the protein components of grains, and the nitrogen content ×5.7 was the protein content. Repeat 3 times for each sample. The samples were dried at 105°C for 30 min and then weighed at 75°C. The seeds at maturity were naturally air-dried without drying. After grinding, nitrogen content of plant samples was determined by H_2SO_4-H_2O_2-indiophenol blue colorimetric method. [25].

Albumin: The 0.255g crushed sample was placed in a centrifuge tube, and added 2 mL water. After shaking, centrifugation was performed at 3000 r for 5 min. The supernatant was poured into the digestive tube, and repeated 3 times. Determination of nitrogen content by H_2SO_4-H_2O_2-indiophenol blue colorimetric method [25].

Globulin: Add 2 mL 10%NaCl to the residue in the centrifuge tube after extraction of aqueous solution, centrifuge at 3000 R for 5 min after shaking, and pour the supernatant into the digestive tube, repeat 3 times. Determination of nitrogen content by H_2SO_4-H_2O_2-indiophenol blue colorimetric method [25].

Gliadin: Add 2 mL 70% alcohol (calculated by weight ratio) to the residue after extraction of salt solution, then shake for 2 min, put the mixture in hot water at 80℃ for 30 min, take out the centrifuge tube, centrifuge at 3000 r after shaking for 5 min, Then, the supernatant was poured into the digestive tube, repeat 3 times. Determination of nitrogen content by H_2SO_4-H_2O_2-indiophenol blue colorimetric method [25].

Glutenin: Add 2 mL 0.2% NaOH was added to the residue after alcohol extraction, followed by centrifugation at 3000 R for 5 min after shaking, and the supernatant was poured into the digestive tube, repeated 3 times. Determination of nitrogen content by H_2SO_4-H_2O_2-indiophenol blue colorimetric method [25].

2.2. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using DPS and SAS 7.5 and the significant difference between treatment means were compared using least significant difference (LSD) test at P = 0.05. All graphs were drawn using Microsoft Excel 2010.

3. Results

3.1. Soluble Sugar, Sucrose and Starch Contents in Grains

Planting density and nitrogen topdressing at jointing stage had a significant effect on soluble sugar, sucrose and starch contents in wheat grains, but the interaction between density and nitrogen topdressing had no significant effect on soluble sugar, sucrose and starch contents in wheat grains (Table 1). The contents of soluble sugar, sucrose and starch in wheat with planting density of 2.5 million plants ha^{-1} were the highest, and the soluble sugar content increased by 4.5%-10.9%, sucrose content increased by 51.7%-21.9%, and starch content increased by 1.9%-7.4% compared to 2 million, and 2.25 million plants ha^{-1}. Compared to 12 and 32 kg ha^{-1}, the contents of soluble sugar, sucrose and starch in 22 kg ha^{-1} were the highest. The contents of soluble sugar, sucrose and starch increased by 2.8%-8.5%, 9.0%-22.5% and 1.2%-7.2% respectively. There was no significant difference in sucrose content between 2 million plants ha^{-1} and 22 and 32 kg ha^{-1} and no significant difference in soluble sugar and starch content between 2 million plants ha^{-1} and 32 kg ha^{-1}. There was no significant difference in starch content between 22 and 32 kg ha^{-1} at the planting density of 2.5 million plants. In conclusion, the planting density of 2.5 million plants ha^{-1} and the nitrogen rate of 22 kg ha^{-1} were beneficial to the accumulation of sugar and starch in wheat.

Table 1. Effects of planting density and nitrogen topdressing water and fertilizer on soluble sugar, sucrose and starch content of wheat

Planting density	NTJ (kg ha^{-1})	Soluble sugar content (mg g^{-1})	Sucrose content (mg g^{-1})	Starch content (%)
200	12	57.91 b	14.85 b	68.33 c
	22	62.52 a	18.96 a	73.64 a
	32	59.63 b	17.24 ab	71.54 b
	Mean	60.02 ± 0.34	17.07 ± 1.66	71.16 ± 0.47
225	12	61.82 b	18.35 c	73.86 b
	22	65.54 a	23.63 a	76.15 a
	32	63.73 ab	21.46 b	75.23 a
	Mean	63.73 ± 0.84	21.11 ± 0.26	75.03 ± 0.50
250	12	63.85 c	22.54 c	74.34 b
	22	69.73 a	28.82 a	78.55 a
	32	66.11 b	25.97 b	76.50 ab
	Mean	66.53 ± 0.44	25.73 ± 0.24	76.41 ± 0.50
F value				
Planting density (P)		85.64^{**}	237.95^{**}	75.17^{**}
NTJ (N)		45.16^{**}	85.26^{**}	38.86^{**}
P×N		0.94	1.34	2.04

NTJ Nitrogen topdressing at jointing stage. Values followed by the different small letters within a column are significant difference at the 0.05 level. * and ** denote a significant difference at 5% and 1%, respectively.

Table 2. Effects of planting density and nitrogen topdressing on nitrogen use efficiency of wheat with integrated water and fertilizer

Planting density	NTJ (kg ha^{-1})	Nitrogen uptake efficiency (kg kg^{-1})	Nitrogen use efficiency (kg kg^{-1})	Nitrogen use efficiency	Nitrogen use efficiency (kg kg^{-1})
200	12	1.20 b	48.28 c	0.63 b	57.92 b
	22	1.25 a	51.00 b	0.69 a	63.90 a
	32	1.13 c	51.96 a	0.69 a	58.51 b
	Mean	1.19 ± 0.01	50.42 ± 0.44	0.67 ± 0.01	60.11 ± 0.51
225	12	1.35 b	43.01 b	0.65 b	57.90 c
	22	1.39 a	47.89 a	0.66 a	66.79 a
	32	1.25 c	47.36 a	0.56 c	59.05 b
	Mean	1.33 ± 0.01	46.09 ± 0.46	0.62 ± 0.01	61.25 ± 0.61
250	12	1.50 b	47.11 a	0.64 b	70.66 b
	22	1.57 a	46.05 b	0.65 a	72.44 a
	32	1.40 c	46.37 b	0.64 b	65.11 c
	Mean	1.49 ± 0.01	46.51 ± 0.27	0.64 ± 0.01	69.40 ± 0.41
F value					
Planting density (P)		7501.55**	477.74**	699.39**	1265.89**
NTJ (N)		1827.41**	149.75**	506.89**	647.88**
P×N		16.97**	80.22**	700.27**	77.20**

NTJ: Nitrogen topdressing at jointing stage. Values followed by the different small letters within a column are significant difference at the 0.05 level. * and ** denote a significant difference at 5% and 1%, respectively.

3.2. Effects of Planting Density on Nitrogen Use Efficiency

The effects of planting density and nitrogen amount at jointing stage on nitrogen use efficiency of wheat were significant (Table 2). The N uptake and N partial productivity increased by 12.3%-25.1%, and N partial productivity increased by 13.3%-15.5%, respectively. The N use efficiency and N harvest index were the highest in wheat with planting density of 2.5 million plants ha^{-1} compared to that of 2 million and 2.25 million plants ha^{-1}. N use efficiency increased by 7.8%-8.6%, N harvest index increased by 3.6%-6.7%. Compared to 12 and 32 kg ha^{-1}, the N uptake, N harvest index and N partial productivity of 22 kg ha^{-1} were the highest. The N uptake and N harvest index increased by 3.5%-10.7% and 0.4%-15.1%, respectively. N use efficiency was the highest at 2 million plant density ha^{-1} and 22 kg ha^{-1}, but there was no significant difference between them and 32 kg ha^{-1}. N use efficiency was the highest when the planting density was 2.5 million plants ha^{-1} and the nitrogen application rate was 12 kg ha^{-1}. In conclusion, n use efficiency and N harvest index were the highest in 2 million plants ha^{-1}, N uptake and N partial productivity were the highest in 2.5 million plants ha^{-1}, and 22 kg ha^{-1} was beneficial to the improvement of N uptake, N harvest index and N partial productivity. However, the nitrogen use efficiency decreased with the increase of planting density.

3.3. Effects of planting Density on Grain Protein Content

The effects of planting density and nitrogen topdressing at jointing stage on the contents of protein and components except for the ratio of grain to alcohol were significant, but the interaction of density and nitrogen topdressing on the contents of protein and its components was not significant (Table 3). Compared to 225 and 2.5 million plants, 2 million plants ha^{-1} had the highest content of protein and its components, albumin content increased by 4.6%-8.1%, globulin content increased by 3.4%-12.9%, gliadin content increased by 2.1%-4.9%, and gluten content increased by 2.2%-4.2%. The protein content increased by 2.7%-6.2%, and the grain alcohol ratio was not significantly affected by planting density. Compared to 12 and 32 kg ha^{-1} the protein and component contents of 22 kg ha^{-1} were the highest. Albumin content increased by 2.6%-6.1%, globulin content increased by 3.9%-9.1%, and gliadin content increased by 1.3%-3.9%. The glutenin content increased by 0.9% to 2.8%, and the protein content increased by 2.0% to 4.2%. The albumin and gliadin contents of 2 million plants ha^{-1} showed no significant difference between nitrogen treatments of 22 and 32 kg ha^{-1}, and the glutenin contents of 2.25 million plants ha^{-1} shown no significant difference between nitrogen treatments of 22 and 32 kg ha^{-1}. There was no significant difference in globulin and glutenin contents between 22 and 32 kg ha^{-1} at 2.5 million plant densities. In conclusion, planting density of 2 million plants ha^{-1} and applying nitrogen rate of 22 kg ha^{-1} were beneficial to protein accumulation in grains.

3.4. Effects of Planting Density on Characteristics of Flour Gelatinization

The influence of planting density on the gelatinization characteristics of wheat flour reached a significant level, the influence of nitrogen topdressing amount on the water absorption rate, stability time and falling time of flour reached a significant level at jointing stage, and the influence of density and nitrogen topdressing interaction on formation time, stability time and falling value reached a significant level (Table 4). Compared to 2 million and 2.25 million plants ha^{-1}, the planting density of 2.5 million plants ha^{-1} had the highest water absorption rate and falling value, which increased by 0.4% to 1.7%, and the falling value increased by 4.4% to 4.8%. The planting density of 2 million plants ha^{-1} had the highest flour formation time and stable time, which increased by 5.3% to 7.4%. Stabilization time increased by 3.5-4.2%. Nitrogen topdressing had no significant effect on water absorption and formation time. Compared to 12 and 32 kg ha^{-1}, the stable time of 22 kg ha^{-1} was 0.6%-6.0%, and the landing value of 32 kg ha^{-1} was 11.2%-16.1%. There was no significant difference in water absorption and formation time between 12, 22 and 32 kg ha^{-1} at 2 million plant density, and no significant difference between 12 and 22 kg ha^{-1} at stable time. There was no significant difference between 22 and 32 kg ha^{-1} at 2.25 million plant density. There was no significant difference in water absorption between 22 and 32 kg ha^{-1} in the planting density of 2.5 million plants, and no significant difference in the formation time between 12 and 22 kg ha^{-1}. It can be seen that the a-amylase activity in flour was the highest when planting density was 2 million plants ha^{-1} and nitrogen fertilization rate was 32 kg ha^{-1}, water absorption rate was the highest when planting density was 2.5 million plants ha^{-1}, and stability time was the highest when nitrogen fertilization rate was 22 kg ha^{-1}.

Table 3. Effects of planting density and nitrogen topdressing on protein content of wheat with integrated water and fertilizer (%)

Planting density	NTJ (kg ha^{-1})	Albumin (%)	Globulin (%)	Gliadin (%)	Glutenin (%)	Glu/Gli	Total protein content
200	12	2.20 b	1.42 b	4.40 b	4.46 b	1.01 a	12.48 c
	22	2.32 a	1.53 a	4.58 a	4.59 a	1.00 a	13.02 a
	32	2.26 ab	1.47 b	4.52 a	4.51 b	1.00 a	12.76 b
	Mean	2.26 ± 0.03	1.47 ± 0.01	4.50 ± 0.01	4.52 ± 0.01	1.00 ± 0.01	12.75 ± 0.03
225	12	2.11 b	1.37 b	4.35 b	4.37 b	1.00 a	12.20 c
	22	2.22 a	1.48 a	4.48 a	4.48 a	1.00 a	12.66 a
	32	2.14 b	1.42 b	4.39 b	4.41 ab	1.00 a	12.36 b
	Mean	2.16 ± 0.01	1.42 ± 0.01	4.41 ± 0.03	4.42 ± 0.05	1.00 ± 0.01	12.41 ± 0.08
250	12	2.02 b	1.22 b	4.21 b	4.29 b	1.02 a	11.74 b
	22	2.15 a	1.35 a	4.36 a	4.37 a	1.00 b	12.23 a
	32	2.06 b	1.28 ab	4.26 b	4.33 ab	1.02 a	11.93 b
	Mean	2.08 ± 0.04	1.28 ± 0.01	4.28 ± 0.02	4.33 ± 0.04	1.01 ± 0.01	11.97 ± 0.10
F value							
Planting density (P)		51.84**	92.44**	87.10**	54.60**	3.11	197.96**
NTJ (N)		22.66**	32.51**	40.79**	17.40**	2.33	79.17**
P×N		0.19	0.11	1.14	0.37	0.94	0.43

NTJ: Nitrogen topdressing at jointing stage.Values followed by the different small letters within a column are significant difference at the 0.05 level. * and ** denote a significant difference at 5% and 1%, respectively.

Table 4. Effects of planting density and nitrogen topdressing on gelatinization characteristics of wheat flour with integrated water and fertilizer

Planting density	NTJ (kg ha^{-1})	Water absorption (%)	Development Time (min)	Stability (min)	falling value (s)
200	12	56.20 a	3.45 a	5.10 a	310.00 c
	22	56.60 a	3.35 a	5.10 a	329.00 b
	32	56.30 a	3.23 a	4.90 b	354.80 a
	Mean	56.40 ± 0.30	3.34 ± 0.12	5.00 ± 0.10	331.30 ± 7.10
225	12	56.80 b	2.91 b	4.70 c	317.00 b
	22	57.40 a	3.11 ab	5.00 a	323.00 b
	32	57.10 ab	3.22 a	4.80 b	357.00 a
	Mean	57.10 ± 0.20	3.01 ± 0.10	4.80 ± 0.10	332.30 ± 6.60
250	12	57.00 b	3.11 ab	4.80 b	317.00 c
	22	57.60 a	3.23 a	5.00 a	346.00 b
	32	57.40 a	3.02 b	4.80 b	378.00 a
	Mean	57.30 ± 0.20	3.12 ± 0.12	4.90 ± 0.10	347.00 ± 3.10
F value					
Planting density (P)		64.44**	15.33**	24.73**	29.13**
NTJ (N)		18.07**	1.54	24.71**	227.57**
P×N		0.61	6.45*	5.62**	5.73**

NTJ: Nitrogen topdressing at jointing stage.Values followed by the different small letters within a column are significant difference at the 0.05 level. * and ** denote a significant difference at 5% and 1%, respectively.

3.5. Effects of Planting Density on SPAD Value Analysis

The effects of planting density on the SPAD value of wheat before flowering were significant, the effects of nitrogen topdressing at jointing stage on the SPAD value of wheat at booting, flowering and 15 days after flowering were significant, and the interaction of density and nitrogen topdressing amount on the SPAD value of wheat at flowering and 15 days after flowering were significant (Table 5). The SPAD value at 15 days after booting, flowering and flowering was the highest at 2.5 million plants compared to 2 million and 2.25 million plants at 15 days after flowering, reaching 0.6%-1.3%. Compared to 12 and 32 kg ha^{-1}, the SPAD value at booting, flowering and 15 days after flowering was the highest at 22 kg ha^{-1}, reaching 0.2%-4.1%. There was no significant difference in SPAD value of 2 million plants at booting and flowering stage between 22 and 32 kg ha^{-1}. In conclusion, the SPAD value was the highest when the planting density was 2.5 million plants and the nitrogen rate was 22 kg ha^{-1}, which was beneficial to increase the photosynthetic capacity of wheat.

3.6. Effects of Planting Density on Wet Gluten Content and Gluten Index

The effects of planting density and nitrogen topdressing amount on dry gluten, wet gluten and gluten index were significant, and the interaction between density and nitrogen topdressing had significant effects on dry gluten and gluten index (Table 6). The planting density of 2 million plants ha^{-1} was higher than that of 225, and 2.5 million plants ha^{-1} in wet gluten content, dry gluten content and gluten index. The wet gluten content increased by 0.8% to 2.1%, dry gluten content increased by 4.1% to 6.6%, and gluten index increased by 1.7% to 6.2%. Compared to 12 and 32 kg ha^{-1}, the wet gluten content, dry gluten content and gluten index increased 3.9%-12.5%, 6.3%-18.2%, and 4.3%-21.5% respectively at 22 kg ha^{-1}. In conclusion, wheat can obtain better gluten characteristics when planting density was 2 million plants ha^{-1} and nitrogen rate is 22 kg ha^{-1}.

3.7. Effects of Planting Density on Flour Quality

The effects of planting density on wheat flour processing quality except final viscosity reached a significant level, the effects of nitrogen topdressing amount on maintaining viscosity, dilution value, final viscosity, peak time and gelatinization temperature reached a significant level at jointing stage, and the interaction between density and topdressing nitrogen had a significant level of influence on flour processing quality except peak time (Table 7). Compared to 2 million and 2.25 million plants ha^{-1} the planting density of 2.5 million plants ha^{-1} had the highest peak viscosity, retention viscosity, final viscosity, peak time and gelatinization temperature, with the peak viscosity increasing by 1.0% to 3.9%, retention viscosity increasing by 2.2% to 4.9%, and final viscosity increasing by 1.4% to 2.1%. Peak time increased by 1.1%-1.9%, gelatinization temperature increased by 0.1%-0.6%. There was no significant difference between the planting density of 2 million and 2.25 million plants ha^{-1}, the flour processing quality was the best, and the planting density of 2.5 million plants ha^{-1} with 22 kg ha^{-1} nitrogen fertilizer was the best. The interaction between planting density and nitrogen rate has a significant effect on flour processing quality. When planting density is 2.5 million plants ha^{-1} and nitrogen rate is 22 kg ha^{-1}, flour processing quality is better, when planting density is 2 and 2.25 million plants ha^{-1} and nitrogen rate is 12 and 32 kg ha^{-1}, flour processing quality is better.

Table 5. Effect of wheat seeding density and nitrogen topdressing amount on SPAD value with integrated water and fertilizer

Planting density	NTJ (kg ha^{-1})	JS	BS	AS	FS
200	12	56.60 a	54.81 b	57.61 b	59.20 b
	22	56.60 a	55.91 a	59.64 a	60.31 a
	32	56.60 a	55.82 a	58.82 a	59.40 b
	Mean	56.60 ± 0.40	55.51 ± 0.40	58.73 ± 0.72	59.67 ± 0.11
225	12	55.80 a	55.63 b	58.81 b	59.60 b
	22	55.80 a	56.32 a	60.42 a	60.92 a
	32	55.80 a	55.21 c	57.44 c	59.14 b
	Mean	55.80 ± 0.50	55.71 ± 0.20	58.92 ± 0.60	59.94 ± 0.25
250	12	56.20 a	55.84 b	59.24 b	59.02 b
	22	56.20 a	56.75 a	59.82 a	61.52 a
	32	56.20 a	55.70 b	59.37 b	59.41 b
	Mean	56.20 ± 0.60	56.11 ± 0.52	59.40 ± 0.22	60.02 ± 0.40
F value					
Planting density (P)		9.64**	8.85**	8.37**	1.75
NTJ (N)		——	24.16**	35.26**	51.26**
P×N		——	5.32	11.94**	3.94*

JS, BS, AS, and MS indicate jointing stage, booting stage, anthesis stage, and flowering stages NTJ: Nitrogen topdressing at jointing stage. Values followed by the different small letters within a column are significant difference at the 0.05 level. * and ** denote a significant difference at 5% and 1%, respectively.

Table 6. Effects of planting density and nitrogen topdressing on gluten characteristics of wheat with integrated water and fertilizer

Planting density	NTJ (kg ha^{-1})	Wet gluten content (%)	Dry gluten content (%)	Gluten index
200	12	29.46 c	10.62 b	54.92 c
	22	33.14 a	11.53 a	69.92 a
	32	31.15 b	10.84 b	59.53 b
	Mean	31.23 ± 0.61	11.03 ± 0.31	61.44 ± 1.24
225	12	28.82 c	10.33 b	54.99 c
	22	32.93 a	11.24 a	60.22 a
	32	31.13 b	10.16 b	57.65 b
	Mean	30.93 ± 0.84	10.55 ± 0.22	57.62 ± 1.23
250	12	29.12 c	9.31 c	55.67 c
	22	31.93 a	11.35 a	64.58 a
	32	30.64 b	10.22 b	61.00 b
	Mean	30.53 ± 0.64	10.33 ± 0.44	60.42 ± 1.00
F value				
Planting density (P)		3.73*	23.53**	175.97**
NTJ (N)		102.44**	82.47**	374.18**
P×N		1.56	8.05**	16.79**

NTJ Nitrogen topdressing at jointing stage. Values followed by the different small letters within a column are significant difference at the 0.05 level. * and ** denote a significant difference at 5% and 1%, respectively. NTJ Nitrogen topdressing at jointing stage.

Table 7. Effects of planting density and nitrogen topdressing on flour processing quality of wheat with integrated water and fertilizer

Planting density	NTJ (kg ha^{-1})	Peak viscosity	Trough viscosity	Breakdown	Setback	Final viscosity	Peak time	Pasting temper-nature
200	12	2506.00 a	1826.00 a	680.00 a	3304.00 b	1478.00 b	6.00 a	84.90 a
	22	2373.00 b	1727.00 b	646.00 b	3243.00 c	1516.00 ab	5.90 a	84.80 a
	32	2521.00 a	1832.00 a	689.00 a	3384.00 a	1552.00 a	5.90 a	84.80 a
	Mean	2466.71±46.72	1795.00±34.91	671.73±7.51	3310.34±18.43	1515.34±27.33	6.00±0.10	84.80±0.21
225	12	2583.00 a	1897.00 a	686.00 b	3383.00 a	1486.00 ab	6.10 a	85.50 a
	22	2582.00 b	1823.00 b	659.00 c	3494.00 b	1471.00 b	6.00 ab	85.60 a
	32	2546.00 a	1804.00 b	742.00 a	3322.00 b	1518.00 a	5.90 b	84.70 b
	Mean	2537.00±25.54	1841.30±43.41	695.74±6.63	3333.00±30.24	1491.74±24.53	6.00±0.10	85.30±0.12
250	12	2491.00 b	1848.00 c	643.00 c	3318.00 b	1470.00 b	6.10 a	86.40 a
	22	2657.00 a	1919.00 a	738.00 a	3471.00 a	1552.00 a	6.00 a	84.80 b
	32	2541.00 b	1879.00 b	662.00 b	3346.00 b	1467.00 b	6.10 a	84.80 b
	Mean	2563.00±52.82	1882.00±12.73	681.00±6.14	3378.34±20.76	1496.34±37.73	6.10±0.10	85.30±0.13
F value								
Planting density (P)		21.64**	28.36**	47.26**	26.55**	2.76	5.15*	46.56**
NTJ (N)		2.46	4.34*	64.06**	1.76	6.96**	4.26*	110.88**
P×N		22.75**	15.67**	228.04**	49.71**	8.84**	0.84	59.56**

NTJ Nitrogen topdressing at jointing stage. Values followed by the different small letters within a column are significant difference at the 0.05 level. * and ** denote a significant difference at 5% and 1%, respectively.

3.8. Effects of Planting Density on Yield and Components

The effects of planting density on spike number, grain number per spike and 1000-grain weight were significant, the effects of nitrogen fertilization on spike number and grain number per spike were significant, and the interaction between density and nitrogen fertilization on spike number and grain number per spike were significant (Table 8). Compared to 2 million and 2.25 million plants ha^{-1}, the number of spike and yield increased by 14.1%-16.4% and 11.6%, 13.3%, respectively. The 1000-grain weight decreased gradually with the increase of planting density. Compared to 12 and 32 kg ha^{-1}, 22 kg ha^{-1} had the highest spike number and yield, and spike number increased by 3.7-19.2% and yield increased by 3.7-17.8%. There was no significant difference in grain number per spike, and 1000-grain weight under different nitrogen treatments. In conclusion, spike number and yield were the highest when planting density was 2.5 million plants ha^{-1} and nitrogen rate was 22 kg ha^{-1}, and spike number was the main factor affecting wheat yield.

Table 8. Effects of planting density and nitrogen topdressing on yield and its components of wheat with integrated water and fertilizer

Planting density	NTJ ($kg\ ha^{-1}$)	Ear number ($10^4\ ha^{-1}$)	Grain number per spike	1000-grain Weight (g)	Yield ($kg\ ha^{-1}$)
200	12	471.04 c	44.94 a	60.75 a	10598.64 c
	22	583.05 a	47.35 a	60.16 a	12332.06 a
	32	548.07 b	43.76 a	58.97 a	11877.04 b
	Mean	534.06 ± 21.84	45.34 ± 3.05	59.95 ± 6.24	11602.61 ± 97.76
225	12	536.31 b	45.76 a	55.65 b	10595.64 c
	22	564.83 a	49.44 a	57.46 ab	12891.05 a
	32	545.34 b	46.96 a	59.71 a	11986.66 b
	Mean	548.83 ± 3.64	47.37 ± 6.14	57.64 ± 0.95	11824.47 ± 115.51
250	12	609.01 c	39.41 b	52.94 a	12931.44 c
	22	672.05 a	42.55 ab	56.10 a	13980.05 a
	32	636.06 b	41.36 ab	57.15 a	13217.05 b
	Mean	639.01 ± 14.50	41.16 ± 3.47	55.44 ± 3.55	13376.11 ± 77.95
F value					
Planting density (P)		1303.85**	174.37**	5.77*	3.04
NTJ (N)		1008.96**	61.15**	1.55	0.75
P×N		63.04**	8.76**	0.16	0.62

NTJ Nitrogen topdressing at jointing stage. Values followed by the different small letters within a column are significant difference at the 0.05 level. * and ** denote a significant difference at 5% and 1%, respectively.

Correlation analysis of plant water consumption and nitrogen accumulation with spike number, yield and components at maturity (Table 9). Under the sowing density of 2-2.5 million plants ha^{-1} and nitrogen rate of 12-32 kg ha^{-1}, the water consumption of wheat at each growth stage was significantly correlated with the number of wheat ears and yield, but negatively correlated with 1000-grain weight. There was a significant negative correlation between sowing, wintering and wintering jointing water consumption and grain number per panicle. Water consumption at each growth stage was negatively correlated with spikelet length and non-fertile spikelet, but positively correlated with fertile spike. The water consumption at each growth stage is closely related to spike number and yield, but not to spike traits at maturity stage.

Table 9. Relationship between water consumption at different growth stages and panicle characters, yield and components in wheat with the integration of water and fertilizer

Yield components	SS-WS	WS-JS	JS-AS	AS-MS
Ear number	0.87**	0.86**	0.91**	0.90**
Grain number per spike	-0.74*	-0.77**	-0.51	-0.43
1000-grain Weight	-0.66*	-0.67*	-0.64*	-0.68*
Yield ($kg\ ha^{-1}$)	0.79**	0.77**	0.88**	0.79**
Ear length	-0.55	-0.49	-0.38	-0.36
Fertile spikelet	0.23	0.37	0.48	0.32
Sterile spikelet	-0.18	-0.25	-0.36	-0.16

WS, JS, AS, and MS indicate sowing stage, wintering stage, jointing stage, anthesis stage, and maturity stages* and ** denote significant correlation at 5 % and 1 % probability levels, respectively. The same as below.

Under the sowing density of 2-2.5 million plants ha^{-1} and nitrogen rate of 12-32 kg ha^{-1}, the nitrogen accumulation of wheat at different growth stages was significantly correlated with the number of wheat ears and grain yield. There was a significant negative correlation between sowing wintering and wintering jointing nitrogen accumulation and 1000-grain weight. Nitrogen accumulation at each growth stage was negatively correlated with spikelet length and non-fertile spikelet, but positively correlated with fertile spikelet (Table 10). In conclusion, nitrogen accumulation at each growth stage was closely related to spike number and yield, but not to spike traits at maturity stage.

Table 10. Relationship between nitrogen accumulation and spike characters, yield and components in different growth stages of wheat with the integration of water and fertilizer.

Yield components	SS-WS	WS-JS	JS-AS	AS-MS
Ear number	0.91**	0.72*	0.93**	0.80**
Grain number per spike	-0.5	-0.25	-0.51	-0.18
1000-grain Weight	-0.72*	-0.66*	-0.59	-0.61
Yield ($kg\ ha^{-1}$)	0.83**	0.69*	0.90**	0.77**

WS, JS, AS, and MS indicate sowing stage, wintering stage, jointing stage, anthesis stage, and maturity stages * and ** denote significant correlation at 5 % and 1 % probability levels, respectively. The same as below.

3.9. Correlation Analysis of Plant Water Consumption and Nitrogen Accumulation with Grain Protein and Starch Contents and Gluten Index

When the sowing density of wheat was 2-2.5 million plants ha^{-1} and nitrogen rate was 12-32 kg ha^{-1}, the water consumption of sowing wintering and wintering was negatively correlated with the content of protein and its components in wheat grains. There was a significant positive correlation between starch content and water consumption of sowing wintering, joining-flowering, and blossoming-maturity plants (Table 11). There was a negative correlation between water consumption and protein content and a positive correlation between water consumption and starch content and gluten index. The with the increase of water consumption in each growth stage, the protein content of grain gradually decreased, but the starch content and gluten index gradually increased.

Table 11. Relationship between water consumption and protein, starch content and gluten index in different growth stages of wheat with the integration of water and fertilizer

Yield components	SS-WS	WS-JS	JS-AS	AS-MS
Albumin	-0.66*	-0.62	-0.52	-0.51
Globulin	-0.78**	-0.77**	-0.61	-0.57
Gliadin	-0.78**	-0.77**	-0.61	-0.57
Glutenin	-0.73*	-0.65*	-0.59	-0.58
Protein content	-0.73*	-0.68*	-0.57	-0.55
Starch content	0.68*	0.6	0.81**	0.86**
Gluten index	0.57	0.54	0.63	0.61

* and ** denote significant correlation at 5 % and 1 % probability levels, respectively. The same as below.

4. Discussion

4.1. Effects of Planting Density on Growth, Water and Nitrogen Uptake Utilization of Wheat

The number of spike increased by 14.1%-16.4%, and the yield increased by 11.6%-13.3%. The 1000-grain weight decreased gradually with the increase of planting density. Under the condition of high sowing amount, the population number increased, the light transmission was poor, and the water loss was less. However, due to the field shade, the population photosynthetic rate was low, which accelerated the leaf senescence, and the 1000-grain weight and grain number per spike were significantly decreased [26,27]. Not only single factors such as planting density and water and nitrogen application rate can affect crop yield and soil water and nitrogen, but also the interaction of water, nitrogen and density can affect water and nitrogen transport and distribution. It was found that "reducing nitrogen and increasing density" was a measure to improve nitrogen use efficiency and ensure crop yield. The planting density of wheat with water and fertilizer integration was 2-2.5 million plants kg ha^{-1}, and the plant water consumption, total water consumption, precipitation use efficiency, N use efficiency and water use efficiency were the highest at 22 kg ha^{-1}, and the plant water consumption increased by 2.1-9.2% compared to 12 and 32 kg ha^{-1} at 12 and 32 kg ha^{-1}. Increase total water consumption by 3.7-6.6%, precipitation use efficiency by 3.7-17.8%, N use efficiency by 3.7-17.8%, and water use efficiency by 0.9%-14.7%. [28] reported that the highest grain yield and higher economic re-turns and environmental benefits can be achieved at the optimal N application rate. such as red bean as the research object, set different planting density and fertilizer frequency, the effects of adzuki bean against each other both n uptake, the results showed that the amounts of accumulated nitrogen after phase density increases the flowers, the contribution rate of nitrogen assimilation and yield increase first and then, according to the frequency of reduced from 3 to 1 times, drum phase of the vegetative organs N have significantly improved. The contribution rate of translocation decreased significantly, and nitrogen accumulation increased by 4.3% to 24.5% during harvest.

4.2. Optimal Planting Density under Different Annual Improve Grain Yield Quality Wheat Production

Our results showed that increasing N up to the optimal N rate enhanced the total grain protein content and protein composition in years studied regardless of planting density. In the planting density of 225 ha^{-1} could increase the grain protein content by 2.5% relative to 200 ha^{-1}, and the application of 150 ha^{-1} in both normal and dry years could increase grain protein content by at least 3.8% relative to 200 ha^{-1} (Table 8). Similar to grain protein content, the starch content in grain was enhanced with increasing N up to the optimal rates in both wet and normal years. Optimal N application increased the water consumption of plants, resulting in increased grain protein and starch content. In the wet year, the higher starch content in grain was associated with the lower content of grain protein, whereas in the dry year, the lower starch content in the grain was associated with the higher content of grain protein (Table 1). This is a result of dilution effect as the grain protein content is relatively constant, an increase in grain starch content reduces the grain protein content [29]. In addition, the content of grain protein in planting density of 2-2.5 million plants ha^{-1} was higher than that in the (Table 7). A higher grain content results in not only a higher market price, leading to a higher economic return [30], but also a higher nutritional value for human consumption [31].

4.3. Effects of Planting Density and N Application Rates for Different Sustainable Wheat Production

The optimal N application rate in normal and dry years led to the highest WUE. However, in the wet year, the optimum N application rate resulted in the highest yield and water consumption during the growing period of crops, but the highest WUE was not achieved. The optimal N application rates under different precipitation conditions could also increase. By at least 18.0% relative to the lower N application rate. When the higher rate (210 kg N ha^{-1} in the wet year or 180 kg N ha^{-1} in the Nitrogen topdressing at jointing stage was reduced to a range from 12.9% to 36.8%. Excess N fertilization results in the accumulation of residual N in the soil [32,33]. The residual N could be lost due to runoff, erosion, and leaching, or through nitrification and volatilization [34,35]. The water consumption of wheat at each growth stage was closely related to dry matter accumulation and nitrogen accumulation at each growth stage. There was a significant correlation between plant water consumption and spike number and grain yield, and a significant negative correlation between plant water consumption and 1000-grain weight. There was a significant negative correlation between sowing to wintering and wintering to jointing water consumption and grain number per spike. Water consumption at each growth stage was negatively correlated with spike length and non-fertile spike, but positively correlated with fertile spikelet. With the increase of water consumption in each growth stage, the protein content of grains decreased gradually, but the starch content and gluten index increased gradually [36]. There was a significant correlation between plant nitrogen accumulation and dry matter accumulation at different growth stages. There was a significant correlation between plant nitrogen accumulation and wheat spike number and yield at each growth stage. There was a significant negative correlation between sowing to wintering and wintering to jointing nitrogen accumulation and 1000-grain weight. Nitrogen accumulation at each growth stage was negatively correlated with spikelet length and non-fertile spike, but positively correlated with fertile spikelet. The nitrogen accumulation during sowing and overwintering and wintering and jointing was closely related to grain protein content, and the protein content gradually decreased with the increase of nitrogen accumulation, while the nitrogen accumulation was closely related to starch content. Nitrogen accumulation and water absorption were highly coupled in wheat with water and fertilizer integration, resulting in a negative correlation between grain protein content and nitrogen accumulation [37]. In this study, it was found that the planting density of 2-2.5 million plants ha^{-1} of wheat with water and fertilizer was 2-2.5 million. Compared to 12 and 32 kg ha^{-1}, the planting density of 22 kg ha^{-1} had the highest spike length, the most fertile spikelet, the highest spike number and yield, and increased the spike length by 0.9% to 5.9%, and the fertile spikelet by 1.1% to 11.8%. Spike number 3.7%-19.2%, yield 3.7%-17.8%; Non-fertile spikelet was the highest at 32 kg ha^{-1}, reaching 4.0%-50.0%. There were no significant differences in panicle number and 1000-grain weight under different nitrogen supplemental levels. However, when the amount of chemical fertilizer exceeds a certain amount, crop yield will be reduced and the utilization rate of chemical fertilizer will be reduced.

5. Conclusions

The use of 2.5 million plant ha^{-1} seeding density and 22 kg ha^{-1} nitrogen topdressing for integrated water and fertilizer wheat is conducive to improving plant water and nitrogen use efficiency, improving yield and growth characteristics, and improving wheat quality. The high-yield and high-efficiency mechanism of water-fertilizer integrated wheat nitrogen accumulation, ear length and fertile spikelet, number of ears, grain numbers per spike, nitrogen, and grain protein, and its components in grains were soluble sugar, sucrose, starch, flour gelatinization characteristics and gluten characteristics, in addition to the final viscosity has significant effects of flour processing quality. The yield increased by 3.7%-17.8% with the nitrogen topping amount of 22 kg ha^{-1}, the protein content increased by 2.0%-4.2% with the nitrogen topping amount of 22 kg ha^{-1}, the starch content increased by 1.2%-7.2%, and the gluten index increased by 4.3% -21.5%.

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