Impacts of Nitrogen Deficiency on Soluble Sugar Content and Photosynthetic Characteristics Grain Protein of Winter Wheat (*Triticum aestivum* L.)

Hafeez Noor1,2,#, Peiru Wang1,2,#, Min Sun1,2,#, Aixia Ren1,2, Fida Noor1, Zhiqiang Gao1,2

1College of Agriculture, Shanxi Agricultural University, Taigu, 030801, Shanxi, China
2Key Laboratory of Functional Agriculture in the Loess Plateau, Ministry of Agriculture and Rural Affairs, Taigu, 030801, Shanxi, China
#These authors contributed equally to this work.
*Corresponding author: sm_sunmin@126.com

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Abstract Sustainability of winter wheat yield under soluble sugar content conditions depends on improvements in crop photosynthetic characteristics and, crop yield. Experiment comprised of seven nitrogen treatments: 0 kg ha⁻¹, 90 kg ha⁻¹, 180 kg ha⁻¹, 210 kg ha⁻¹, 240 kg ha⁻¹, 270 kg ha⁻¹, and 300 kg ha⁻¹. The grain yield was increased by 25%, respectively. Our results indicated that implication of a proper increased nitrogen application rate, soluble sugar content, sucrose content and starch content were changed greatly, but when the threshold value was reached, the growth rate was decreased. Among them, the accumulation of soluble sugar content in the middle and late stages were increased, and the sucrose content and starch content were increased in each period after anthesis. The contents of each protein component were increased, the albumin was highest in 240 kg ha⁻¹, and the globulin was highest in 270 kg ha⁻¹ compared to other nitrogen rate, respectively. The prolamin and glutenin (storage protein) were the highest in 300 kg ha⁻¹ and also in in 240 kg ha⁻¹. The protein content was improved in 300 kg ha⁻¹, 240 kg ha⁻¹, and protein yield 240 kg ha⁻¹ was significantly improved compared to other nitrogen application rates. The dynamic changes showed that the application of nitrogen fertilizer increased the protein contents in each period after flowering stage, but the effect was increase to 240 kg ha⁻¹ in began was weaken and the albumin were regulated by nitrogen fertilizer in the early stage.

Keywords: albumin, photosynthetic characteristics, soluble sugar, protein, nitrogen fertilizer

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1. Introduction

Winter wheat (*Triticum aestivum* L.) is one of the most important food crops in the world. It is an important crop in the southeast Loess Plateau of China, accounting for about one-fifth of food production. Nevertheless, the yields in this area are often unstable owing to limited and uneven precipitation distribution in the fallow and growing season [1]. In winter wheat production the major task is to use method which could effectively enhance the soil moisture consumption. Drill sowing method has been used for dryland wheat production. Compared to wide-spase sowing, and drill sowing, can increase the growth of wheat at various growth stages, and improve the efficiency of water uptake and increase yield [2]. Nutrients management, and cultural practices greatly influence Photosynthesis in crops. Wise space sown is an adapted form of drill sowing in which the seeds are evenly distributed, and in the same level [3]. Appropriate water management can improve the light transmittance and photosynthetic area of wheat in the later growth periods, promote the accumulation and translocation dry matter, in wheat before heading [4]. Therefore, it is of great importance to investigate the high-yield cultivation of wheat while regulating the water content at the tillering stage. Photosynthesis is necessary to accumulate energy and dry matter for plant growth and development. Photosynthesis of wheat leaves accounts for 95% of yield. The influence of environmental factors on photosynthesis can be reflected by chlorophyll light [5]. Photosynthesis, transpiration, stomatal opening, and other physiological processes interact with each other [6]. Previously, researchers believed that flag leaf photosynthesis provided most of the assimilates for grain filling [7]. However, some studies have shown that wheat ear is an important source of photosynthetic carbon assimilation during grain filling, especially in the case of water deficit [8,9]. It is now generally accepted that ear photosynthesis in wheat is
the main contributor to the final grain yield [10]. Compared with flag leaves, the photosynthetic rate of wheat spike decreased less under water deficit condition [10,11]. Several traits may support better photosynthetic performance of wheat ear than flag leaf under drought conditions. First, wheat is closer to the grain, which is the main photosynthetic sink compared to flag leaves [5]. The sowing methods used in wheat cultivation include DS, WSS, which affect the yield of wheat [6]. Drill sowing in dry land could increased stem height, lengthen the growth time of functional green leaves, improve spike quality and yield compared with that in flat land [12]. The wide space sowing is a three-dimensional uniform sowing in which seeds are evenly distributed despite sowing in rows [3]. Tiller number and percentage of productive tillers, leaf area index, dry weight, and yield were increased by uniform sowing without a significant decline in grain protein. In China excessive application of N fertilizers by farmers is widely practiced to sustain further increase grain yield, but grain yield does not keep synchronous increase with excessive N application [13]. Nitrogen application during the wheat growing season generally exceeds 340 to 300 kg Nitrogen ha\(^{-1}\) however, some farmers use rates as high as 750 kg N ha\(^{-1}\) [14,15,16].

In this study the objects were as given; (i) To find the photosynthetic traits yield, (ii) The improvement of sugar and protein content, and the quality of wheat was improved and Wide space sowing (WSS) production is mainly through the increase in the number of spikes to increase yield. (iii) To optimize N rates under the sowing methods which produced the highest yield of wheat crop.

2. Materials and Methods

2.1. Description of Experimental Site

The field experiments were conducted during the winter season (2019-2020) at the experimental station of Shanxi Agricultural University. The experimental area is geographically located in Wenxi county (34° 35' N and 110°15' E.), Shanxi Province, China and characterised by, hilly, semi arid area. In these regions, precipitation is the sole source of moisture and the mean annual rainfall ranges from 450-630 mm, of which 60-70% precipitation is concentrated in July-September. Winter wheat is planted in early to mid-October and harvested in early June, while corn is planted in mid to late June and harvested in early October of the same year.

2.1.1. Experimental Design and Treatments

The experiment had a single-factor randomized block design. The experiment comprised of three sowing methods: Wide-space sowing (WSS), (row and sowing spacing were 25 and 8, 2BMF-12/6, tillage, auto-fertilization), DS (row and sowing spacing were 20 and 3 cm, 2BXF-12Seed driller). The area of each plot was 2.5 m × 40 m=100 m\(^2\) and repeated each treatment three times. Winter wheat (Triticum aestivum L.), cultivar ‘Liangxing-99’ were obtained from the Wenxi Agriculture Bureau, Wenxi, China. The planting density was 225 × 104 plant ha\(^{-1}\). seven Nitrogen application rates (0 kg ha\(^{-1}\), 90 kg ha\(^{-1}\), 180 kg ha\(^{-1}\), 210 kg ha\(^{-1}\), 240 kg ha\(^{-1}\), 270 kg ha\(^{-1}\), and 300 kg ha\(^{-1}\) ). Pure P\(_2\)O\(_5\) and K\(_2\)O were applied at the rate of 150 and 75 kg ha\(^{-1}\) respectively. The seeds were sown on September 25, 2019, and September 25, 2020. Approximately 20-30 cm of the post-harvest stubble was left intact to increase soil organic carbon and reduce evaporation, and the remaining wheat stubble (20-30 cm) was plowed to a 25-30 cm depth using a rotator at the beginning to middle of July by deep plowing, as described by [17]. The water meter was used to record the water amount accurately each time, and the water amount was 60 mm (1.92 m\(^3\)/plot). At the end of each the growth season of wheat is 1-2 times from green to jointing, and 2-3 times from flowering to grain filling. Prevention and control of diseases and insect pests, other management with conventional high-yield fields.

Figure 1. Experimental site location preparation was at Wenxi Shanxi province, China
2.1.2. Photosynthetic Characteristics

The various photosynthesis attributes including net Photosynthesis Rate (P_N), transpiration rate (E), carbon dioxide concentration (C_i), and stomatal conductance (C_i) of upper most fully opened leaf was measured using a hand-held portable photosynthesis machine (CI-340 system USA). These measurements for wheat functional leaves were carried out in the morning from 9:00 - 11:00 a.m on a bright sunny day using in each growth period. Three samples were continuously measured for each treatment, and the test data were the average value measured throughout the day. In each treatment plants (20) dry biomass was recorded. At physiological maturity, the ears were cut, save in the mesh bag, and were dried to investigate the number of spikes per unit area, average grain number per spike, and 1000-grain weight were recorded. Another 20 m² area was harvested to determine the grain yield (kg ha⁻¹).

2.1.3. 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. H₂SO₄-H₂O₂-Phenol blue color method was used to determine the seed protein.

2.1.4. Grain to Leaf Area

Grain to leaf ratio was calculated according to [18], using Equations 1 and 2:

\[
\text{Grain number to leaf area ratio} = \frac{\text{total number of grain par unit area}}{\text{total leaf are on the same plot at booting stage}} \tag{1}
\]

Grain weight to leaf ratio
\[
= \frac{\text{grain weight (mg) par unit area}}{\text{total leaf are on the same plot at booting stage}} \tag{2}
\]

2.1.5. 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 m², shelled using machine, and the grain was air-dried for the determination of grain yield.

2.1.6. 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 H₂SO₄-H₂O₂-indiophenol blue colorimetric method.

**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 H₂SO₄-H₂O₂-indiophenol blue colorimetric method.

**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 H₂SO₄-H₂O₂-indiophenol blue colorimetric method.
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°C 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 H₂SO₄-H₂O₂-indophenol blue colorimetric method.

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 H₂SO₄-H₂O₂-indophenol blue colorimetric method.

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. Effects of Nitrogen Rate on Soluble Sugar Content, Photosynthetic Characteristics, and Agronomic Attributes
The soluble sugar content, sucrose content, and starch content in grain of wide-space sowing wheat increased gradually with the increase of nitrogen application rate, and N300 was the highest. The soluble sugar content of N300 and N270 was significantly different from that of other nitrogen application rate treatments, and the sucrose content of N180, N210, N240, N270, and N300 was significantly different from that of N90 and N0. Starch contents of N240, N270 and N300 were significantly different from those of N210, N180, N90 and N0. It can be seen (Table 2).

Content of soluble sugar, sucrose and starch was significantly regulated by nitrogen application rate, but when the nitrogen application rate reached a certain critical value, the content increased slightly.

Table 1. Effect of nitrogen rate on soluble sugar content, sucrose content and starch content in grains of winter wheat

| Nitrogen rate (kg ha⁻¹) | Soluble sugar content | Sucrose content | Starch content |
|------------------------|-----------------------|----------------|---------------|
| N₀                    | 40.35<sup>a</sup>     | 17.42<sup>a</sup> | 53.31<sup>a</sup> |
| N₉₀                   | 41.31<sup>a</sup>     | 20.38<sup>a</sup> | 56.18<sup>a</sup> |
| N₁₈₀                  | 46.18<sup>a</sup>     | 22.82<sup>a</sup> | 58.32<sup>a</sup> |
| N₂₁₀                  | 48.32<sup>a</sup>     | 23.04<sup>a</sup> | 61.99<sup>a</sup> |
| N₂₄₀                  | 51.99<sup>a</sup>     | 24.43<sup>a</sup> | 67.19<sup>a</sup> |
| N₂₇₀                  | 55.17<sup>a</sup>     | 24.54<sup>a</sup> | 66.17<sup>a</sup> |
| N₃₀₀                  | 57.19<sup>a</sup>     | 25.64<sup>a</sup> | 69.35<sup>a</sup> |

The content of CO₂ concentration of the flag leaves in different treatments were showed a gradually declining trend with the grouting process and the 0-7D, with increase in the amount of N-nitrogen fertilizer, the net photosynthetic rate \( (Pₚₙ) \) increased showing a single peak curve at the 7-14 days after anthesis (Figure 3a).

Days after anthesis the \( Pₚₙ \) value was still higher at N300 but was not significantly different from 270 kg ha⁻¹ and 240 kg ha⁻¹. Days after anthesis 21 and 28 \( Pₚₙ \) was increased with \( N \) application and the flower 21 was higher with 240 kg ha⁻¹ as compared to other treatments. After 28 days after anthesis, the \( Pn \) was still the highest with N240, but the difference was not significant compared to 180 kg ha⁻¹, 210 kg ha⁻¹, 270 kg ha⁻¹, and 300 kg ha⁻¹. Overall, the application of \( N \) fertilizer enhanced the \( Pn \) days after anthesis, but the effect of high nitrogen treatments 270 kg ha⁻¹, 300 kg ha⁻¹ was weakened in the later grouting and the wide-space sowing coupled with 240 kg ha⁻¹ sustained higher values at the whole grouting period. The intercellular CO₂ (\( Cᵣ \)) of the flag leaves of DS and wide-space sowing, was gradually decreased with the growing process, however, with the increase in the amount of \( N \) application, \( Pₚₙ \) showed a first decreasing and then increasing trend days after anthesis. From the (Figure 3a), it was obvious that the 240 kg ha⁻¹ treatment was significantly lower than other treatments on 14-7d and 21 after anthesis and 0 kg ha⁻¹ was the highest 240 kg ha⁻¹ and was lowest on 28 days after anthesis, but the difference was not significant compared to 270 kg ha⁻¹. The increase of nitrogen fertilizer can significantly reduce the intercellular CO₂ concentration in the leaves of the flags days after anthesis and the whole grouting period can be continued.

The \( gₛ \) of the flag leaves in wide-space sowing, and drill sowing treatment was gradually decreased with advancement of growth stages while first increased and then decreased with increasing nitrogen application rate. From the (Figure 3c).

It was evident that the 240 kg ha⁻¹ treatment resulted in higher \( gₛ \) values than other treatments on the 14 days after anthesis and, was the lowest in 0 kg ha⁻¹, and was highest in 240 kg ha⁻¹ at 21-28d, but the difference was not significant. That increased \( N \) fertilizer can significantly increase the \( gₛ \) of flower flag leaves but the effect lasts until the middle stage of growth and the effect was weakened in the later stage. Moreover, the \( E \) of flag leaves gradually decreased with the grouting process and the \( Tᵣ \) of flag leaves was first increased and then decreased with the increase in \( N \) application days after anthesis. In the (Figure 3b) indicated that after flowering the \( Tr \) values in 240 kg ha⁻¹ and 270 kg ha⁻¹ treatments were significantly higher as compared to the other treatments from 7-14 days and 21, and 0kg ha⁻¹ was the lowest. After 28d, the values was greater in 240 kg ha⁻¹ than other treatments and 0kg ha⁻¹ was the lowest. These results indicated that the increase in \( N \) fertilizer can significantly enhance the \( Tr \) of flag leaves days after anthesis, and last for the whole growth period and the best effect was observed in 240 kg ha⁻¹ coupled with large wide-space sowing and drill sowing.

The plant height of wheat in wide-space sowing treatment showed a steady increase and then decline with the increase in \( N \) rates (Figure 3e). At jointing stage, plant height of N300 treatment was significantly higher than other treatments and there was no significant difference between N210 and N270, N180 and N300, N0 and N90. After
entering the jointing stage, the treatment with N$_{210}$ and N$_{240}$ showed the highest plant height, and the values in N$_{90}$ and N$_{0}$ was the lowest.

The N$_{240}$ increased the plant height and promoted the growth of wheat in the middle stage of wheat growth. Similar to plant height, the leaf area index (LAI) of wide-space sowing showed an increasing trend and then a declining trend at jointing and booting stages with an increase in nitrogen rates (Figure 3F). Jointing, booting, 240 kg ha$^{-1}$ was significant. It was significantly higher than other treatments, and N0 was the lowest. Wide-space sowing was beneficial to wheat growth and wide-space sowing combined with N$_{240}$ had the largest leaf area index and the best growth condition in each growth stage.

**Figure 3.** Effect of Nitrogen rates and different sowing methods (A) 0-7 Days, 14d, 21d, 28d on Net Photosynthesis Rate (A), ($P_N$), carbon dioxide concentration (B), (C), stomatal conductance (C), (GS) and transpiration rate (D), (E) of flag leaves (E), plant height (F), leaf area index (LAI) WS indicate wintering stage, JS, jointing stage BS, booting stage FS, Flowering stage AS, anthesis stage and, MS maturity stage, All data represent means ± standard errors of three replicates. Values with different letters on the same sampling day indicate significant differences at $P<0.05$
The soluble sugar in wide space sowing decreased gradually with grain filling, but increased continuously with the increased of N rate at each stages after anthesis (Figure 4A). From 5d to 30d after anthesis, N$_{300}$ treatment had the highest soluble sugar content, but other treatments (except N$_{0}$) had no significant difference, and N$_{0}$ treatment had the lowest soluble sugar content. At 35d after anthesis, there was no significant founded in N$_{0}$.

In conclusion, increasing nitrogen fertilizer was beneficial in enhancing levels of soluble sugar at the middle and late stage, which could last until 25d after anthesis, and provided conditions for starch synthesis. However, there was no difference between N$_{240}$, N$_{270}$ and N$_{300}$, and the effect of nitrogen fertilizer was weakened in the late stage. The soluble sugar of wide sowing wheat increased gradually with grain filling, and the starch content at different stages increased continuously with increase in N rate (Figure 4C). The content of sucrose in different periods after anthesis could be increased by increasing nitrogen application rate. On the 5th day after anthesis, the highest sucrose content was found in N$_{270}$, followed by N$_{210}$, N$_{240}$, N$_{300}$. At 25d after anthesis, N$_{300}$ had the highest value, but it was statistically similar to that in N$_{240}$, N$_{270}$ treatments. At 35d after anthesis, N$_{300}$ treatment still had the highest value, but there was no significant difference among different treatments. In conclusion, increasing nitrogen fertilizer can promote sucrose synthesis, and lay the foundation for starch accumulation, especially in the early stage of grain filling, and in the middle stage of high nitrogen treatment N$_{240}$, N$_{270}$, and N$_{300}$, there is no significant difference, and the effect of nitrogen fertilizer is weakened in the late stage. The soluble sugar of wide sowing wheat decreased gradually with grain filling, but increased continuously with increase in N rate (Figure 4C). The starch of N$_{300}$ treatment was highest, and the starch content of N$_{0}$ treatment was the lowest. Compared to other nitrogen treatments, starch content of N$_{300}$, N$_{270}$ and N$_{240}$ were increased significantly from 15d to 35d after anthesis. Therefore, increasing nitrogen fertilizer can promote starch accumulation and increase grain weight, and the effect was significant in the middle and late stage, but there was no significant difference among high nitrogen treatments N$_{240}$, N$_{270}$ and N$_{300}$, respectively.
Figure 5. Effect of nitrogen rate on changes of grain protein content, Albumin content, Globulin content, Gliadin content, and Glutenin contents. All data represent means ± standard errors of three replicates. Values with different letters on the same sampling day indicate significant differences at P<0.05.
3.2. Effects on Dynamic Changes of Protein Content and Components in Grains from 0 to 35, and Grain Yield

The protein content of wide space swing wheat showed a trend of decreasing first and then increasing with the filling process and the protein content was the lowest 15 day after anthesis (Figure 5A). The protein content at different stages after flowering increased. The grain protein content of increased nitrogen fertilizer had a greater impact, but there was no significant difference in the regulatory effect between high nitrogen treatment after 5, 15, 25, 35, and N240, respectively. Therefore, choosing appropriate nitrogen application amount was more beneficial to efficient production. The content of albumin was the highest at 5 day after anthesis and decreased with the development and maturity of grains. Compared to other components, the globulin content was the lowest and began to 15day after anthesis. The content of gliadin and glutenin were increased continuously with the highest value of N300 and the lowest value of Nitrogen at different time after flowering.

The albumin was significant than other treatments on 5day after anthesis N300, 15day after anthesis N300 and N270, and 25–35 day after anthesis N300, N270, and N240 (Figure 5B). The globulin protein content was significantly higher than that of N300, N270, N240, N210, N180 treatment on 5 day after anthesis than that of N90, and N0 treatment and was significantly higher than that of other treatments on 15–35 day after anthesis (Figure 5C). The content of gliadin increased with the increase of N rate. At 5 day after anthesis, difference between nitrogen application amounts was not significant, and at 15–35 day after anthesis, (Figure 5D). Glutenin was significant than other treatments on N300, N270, N240, N210 and N180 treatments than in N90 and N0 treatments on 5day after anthesis but was significantly higher in N130 and N270 treatments than in other treatments on 15day after anthesis, in N300 and N270 treatments than in other treatments on 25day after anthesis, and in N300, N270 and N240 than in other treatments on 35 day after anthesis (Figure 5E). The adding N rate can improve increased of grain protein. Albumin was regulated N fertilizer in the early stage of filling, globulin was more regulated throughout the filling stage, while gliadin and glutenin are more sensitive to N rate in the late stage of filling.

The N fertilizers rates were significantly effected the final grain yield and compositional factors of yield evident by an increased ear number, yield and increased ear number (Table 2). The spikes number, ear shots, and the thousands grains weight, and grain yield showed an a first increase and then decreasing trend with application of nitrogen fertilizer amount. The yield and yield components were significantly higher in N240 followed by N210 under drill sowing when compared to the other nitrogen treatment. Wide-space sowing, and drill sowing methods optimized the output of the three elements at the same time of wide-space sowing coupled to 240 kg ha⁻¹ and drill sowing casting 210 kg ha⁻¹ to achieve the increase of the output. Both drill sowing and wide-space sowing significantly increased spike number and yield. With the increased of nitrogen application rate, the three factors of yield increased first and then decreased. The yield and three elements of wide-space sowing were the highest at 240 kg ha⁻¹ and 210 kg ha⁻¹, respectively.

3.3. Effects on Nitrogen Neficiency on Protein Contents and Components in Grains

The effects of nitrogen fertilizer on the contents of protein and its components in grains were significantly different (Table 3). The contents of albumin and globulin (soluble protein) increased firstly and then decreased with the increase of nitrogen application rate. The highest albumin was N300, and the difference between N270 and N240 was not significant. The highest globulin was noted in N270, and the difference between N180, N210, N240 and N300 was not significant. The contents of gliadin and glutenin (storage protein) increased with the increase of nitrogen application, and N300 was the highest, but the difference of gliadin content was not significant compared with N270 and the content of glutenin N300 was significantly higher than that of other nitrogen treatments. The grain alcohol ratio of N240 was the highest, but the difference was not significant compared with that of N90 and N130. The protein content of N300 and N240 was significantly higher than that of other nitrogen treatments. Protein yield of N300 was significantly higher than that of other nitrogen treatments. The results showed that nitrogen fertilizer regulated storage protein significantly, which was more beneficial to improve the quality of storage protein.

Table 2. Effects of different sowing methods and grain yield of winter wheat. Different letters indicate significant differences (p < 0.05) among treatments within a growth stage by Fisher’s least significant difference

| Sowing method | Nitrogen rate (kg ha⁻¹) | Spike number (10^4 ha⁻¹) | Grain number Per spike | 1000-grain weight (g) | Yield (kg ha⁻¹) |
|---------------|-------------------------|--------------------------|-----------------------|----------------------|----------------|
| WSS           | N0                     | 688.25 e                 | 29.92 b                | 36.42 ed              | 6433.3 e       |
|               | N90                    | 705.75 bd                | 30.69 b                | 37.32 c               | 6938.22 c      |
|               | N130                   | 716.50 c                 | 29.73 b                | 40.66 b               | 7447.64 b      |
|               | N210                   | 728.25 c                 | 30.56 b                | 41.35 ab              | 7841.61 b      |
|               | N270                   | 823.25 a                 | 31.52 a                | 42.58 a               | 9234.26 a      |
|               | N300                   | 758.75 b                 | 30.95 a                | 39.07 b               | 8003.31 b      |
|               | N500                   | 695.50 c                 | 28.08 c                | 38.90 c               | 6684.08 c      |
| DS            | N0                     | 511.25 e                 | 27.97 e                | 35.56 e               | 4231.12 e      |
|               | N90                    | 547.75 e                 | 28.54 ec               | 39.70 e               | 5139.09 e      |
|               | N130                   | 560.25 c                 | 29.88 bd               | 40.73 c               | 5857.49 c      |
|               | N210                   | 628.50 b                 | 30.40 c                | 42.10 c               | 6921.53 c      |
|               | N270                   | 678.50 ad                | 29.10 c                | 41.28 ab              | 6902.60 c      |
|               | N300                   | 540.00 ef                | 28.02 bc               | 39.92 b               | 5087.64 c      |
|               | N500                   | 503.75 f                 | 27.20 e                | 36.84 ed              | 4356.07 e      |
4. Discussion

4.1. The Photosynthesis Characteristics of Nitrogen Rate, Growth and Yield

The wide space sowing is a newly introduced sowing method. Furrow sowing is gaining popularity for wheat because seeds are sown in furrows that facilitate fertilization and effectively improve nutrient utilization [14]. Different sowing methods affect canopy structure by altering the arrangement and spacings of plants. The better plant distribution effectively intercepts radiation and improves photosynthesis efficiency and growth [3]. The results of our present study showed that the wide space sowing and furrow sowing can improve radiation interception as shown by the higher leaf area index under these sowing methods. Photosynthetic active radiation was found positively related to the leaf area index and the number of spikes [13]. The results from this study showed that the grain yield and other yield traits under WSS, DS, and stereoscopic sowing were higher than the drill sowing. The highest yield was attained by WSS, followed by FS. The that sowing methods mainly affected yield by influencing the number of spikes [19]. The furrow sowing was proved beneficial in maintaining a higher photosynthesis rate. In a previous study indicated that furrow planting increased chlorophyll content and photosynthesis as a result of which dry matter accumulation increased in each growth period [12,20]. The furrow sowing produces stronger seedlings before winter, increases tiller numbers from booting to maturity stage, increases earning rate by 3.5%, and significantly increases yield [21,22]. In a study it was believed that $C_i$ and $P_n$ values could be used as indicators to determine whether photosynthesis is limited by stomatal closure or metabolic damage. Our results showed that the decrease in stomatal conductance caused by the partial closure of stomata in the DS treatments was the main reason for the decrease in photosynthetic rate, which was mainly manifested by the decrease of $P_n$ and the increase of $C_i$. The $g_s$ value and $P_n$ value of each treatment had good synchronisation, which showed that they decreased with the decrease in water content, and both the $P_n$ and $g_s$ values recovered after rewatering, with the WSS and DS treatments having a high recovery level [23]. Stomatal conductance is an important biological process reflecting carbon accumulation and transpiration in plants, and CO$_2$ flows into photosynthetic sites through stomata [24]. Inconsistent results were found in the analysis of stomatal distribution in ear buds of spring wheat. It should be noted that under moderate water deficit conditions, stomata were distributed in the front and back of the wheat ear buds, and in the lower part of the front of the wheat ear buds. The characteristics of the paraxial stomata may contribute to the absorption of CO$_2$ released by grain respiration, and the recycling of respiratory CO$_2$ is considered to be a key process for complete assimilation in wheat ears [25,26]. Previously, it was reported that the wide-precision planting significantly increased the leaf area index and interception of photosynthetic active radiation of winter wheat [3]. It was reported that the uniform sowing was more conducive for light interception for the production of more grain. The plant height was highest indirect sowing whereas the leaf area index was lowest, which might indicate the effect of shading under direct sowing methods as compared to uniform sowing, furrow sowing, and wide space sowing. The photosynthetic rate $T_r$ and $g_s$ of the flag leaves were higher under WSS, FS sowing especially at the end of flowering [27]. Lower reduction in both flag leaves and spikes under water deficit compared to during the middle and late grain-filling stages. In contrast, $g_s$ in flag leaves were more sensitive to drought than that of spikes [28]. The decline in $P_n$ may occur due to decreased $T_r$ in wheat, and a similar result was observed in mulberry [29]. However, variations of $g_s$ would produce differences in E for wheat leaves [30]. Our study indicated that under the WSS, and DS, wheat crop produced a more favorable canopy structure with higher photosynthesis and grain yield. Higher yield depends on the higher photosynthesis rate and thus increasing photosynthetic rate is the main objective to increase production. Furthermore, the photosynthesis rate is positively related to leaf mass per unit area. However, the excessive growth at the early stages is not much favorable under dryland conditions. It is also manifested by the highest plat height while low leaf area index by drill sowing as compared to other sowing method [31].

4.2. Effects of Nitrogen Rate and Sowing Methods on Yield Formation of Winter Wheat

The results indicated that the optimum nitrogen application rates vary with the sowing method in terms of increasing production. Compared with other treatments, the N amount of 210 kg ha$^{-1}$ and 240 kg ha$^{-1}$ combined with wide-space sowing and furrow sowing resulted in higher tiller numbers, optimized the yield components and improve the grain yield. This might be because row spacings are larger in the wide-space sowing than the furrows, whereas, space for the growth of single plant reduced and competition between plants for natural resources has been increased. Therefore, the effect of...
higher nitrogen application rate proved better because fertility has improved plant growth by promoting the absorption and transportation of water and fertilizer by the root system [32]. In addition it also facilitated the deep fertilizers application, improved radiation interception and water utilization [21,33]. Leaf area is an important canopy structural feature closely associated with the ability of leaves to collect light and photosynthesize [34]. As compared with drill sowing, the leaf area index of winter wheat increased under wide space sowing, especially in the middle and late growth period. The differences in leaf area under the different planting methods could be attributed to the differences in the canopy distribution. Canopy with wider spacing intercepts light more properly with a higher photosynthesis rate and leaf area index [27]. In this study, wide space sowing proved better than drill sowing in photosynthesis. This may be due to the reason that wide seed spacings not only expand the growth space of a single plant and reduce the competition of plants for natural resources [19]. Our study indicated that under WSS and DS the wheat crop produced a more favorable canopy and, DS the wheat crop produced a more favorable canopy distribution. Higher yield depends on the higher photosynthesis rate and thus increasing photosynthetic rate is the main objective to increase production. Furthermore, the photosynthesis rate is positively related to radiation use efficiency and leaf mass per unit area. However, the excessive growth at the early stages is not much favorable under dryland conditions. It is also manifested by the highest plat height while low leaf area index by drill sowing as compared to other sowing methods.

5. Conclusion

To explore the optimum amount of nitrogen application in different nitrogen rates of winter wheat, improving flag leaf photosynthesis capacity, obtaining higher yield and grain protein content. The post-anthesis flag leaf photosynthetic rate, the transpiration rate, the intercellular carbon dioxide concentration, and flag leaf stomatal conductance were significantly increased. The wide-space sowing with nitrogen level N240 improved the photosynthetic characteristics of flag leaves, significantly increased the net photosynthetic rate, stomatal conductance, and transpiration rate of flag leaves after flowering, and significantly reduced the intercellular carbon dioxide concentration. The soluble sugar content, sucrose content and starch content changed greatly, but when the threshold value was reached, the growth rate decreased. Among them, the accumulation of soluble sugar in the middle and late stages increased, and the sucrose content and starch content increased in each period after anthesis. The content of each protein component increased, the albumin was highest in 240 kg ha$^{-1}$, and the globulin was highest in 270 kg ha$^{-1}$. The increased 1000-grain weight of each time after flowering, significantly increased panicle number and yield. The nitrogen transport before flowering and the nitrogen accumulation after flowering. The nitrogen metabolism of the plants was improved, which was beneficial to the improvement of sugar and protein content, and the quality of wheat was improved.

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