



Effects of sowing methods and nitrogen rates on photosynthetic characteristics, yield, and quality of winter wheat

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Abstract

Sustainability of winter wheat yield under dryland conditions depends on improvements in crop photosynthetic characteristics and crop yield. Therefore, studying the effects of different sowing methods and nitrogen rates on photosynthetic characteristics of flag leaves, nitrogen translocation, and yield could be an effective strategy to improve the crop yield. In this study, four nitrogen application concentrations and two sowing methods were used. The results showed that the photosynthetic rates were the highest at different stages of wide-space sowing (WSS) after flowering. Nitrogen concentration of 240 kg ha⁻¹ improved the photosynthetic characteristics and significantly increased the net photosynthesis, stomatal conductance, and transpiration rate after flowering and significantly reduced the intercellular CO₂ concentration compared to other nitrogen concentrations. Our overall findings suggested that WSS nitrogen treatment with 240 kg ha⁻¹ enhanced photosynthetic characteristics of flag leaves and nitrogen content of the plants leading to high yield under dryland conditions.

Keywords: CO₂; drill sowing; nitrogen fertilizer; photosynthetic characteristics; wide-space sowing.

Introduction

The yield of winter wheat (*Triticum aestivum* L.) in dryland areas is unstable and substantially lower than the average yield in other areas of China and other European countries. Stabilizing the yield of dryland wheat and improving the overall production and grain quality of dryland areas have always been the main task of research for cultivation work

in the arid regions of North China (Li *et al.* 2002, Ma *et al.* 2005). The Loess Plateau in China covers about 0.65 million km² area and has the population of 108 millions (Wang *et al.* 2016). The Loess Plateau has a semiarid climate with low and variable rainfall from 300–700 mm (Li *et al.* 2010). Nitrogen (N) deposition has dramatically altered terrestrial ecosystem properties and processes, such as plant nutrient cycling, photosynthetic carbon

Highlights

- Nitrogen fertilizer can significantly improve the net photosynthetic rate of winter wheat
- Different sowing methods can promote an increase of intercellular CO₂
- WSS with nitrogen concentration of 240 kg ha⁻¹ improves wheat yield in Loess Plateau area

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Abbreviations: AS – anthesis stages; C_i – substomatal CO₂ concentration; DS – drill sowing; E – transpiration rate; EFS – early grain-filling stage; g_s – stomatal conductance; LFS – late grain-filling stage; MFS – middle grain-filling stage, PA-M – precipitation anthesis to maturity; PF – precipitation fallow, PGT – precipitation growth total; PJ-A – precipitation jointing to anthesis; P_N – net photosynthetic rate; PS-J – precipitation sowing jointing; PT – precipitation total; WSS – wide-space sowing.

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assimilation, and species diversity (Liu *et al.* 2015, Mao *et al.* 2018). Under water stress, the grain-filling process is sustained mainly by photosynthesis in the upper parts of wheat plants, such as the flag leaves and wheat ears (Carr and Wardlaw 1965, Tambussi *et al.* 2005). Previously, researchers thought that photosynthesis of the flag leaves was supplying the majority of assimilates for grain filling (Evans *et al.* 1975). However, several studies showed that wheat ear is an important source of photosynthetic carbon assimilation during grain filling, especially if plants are under nitrogen fertilizer (Bort *et al.* 1994, Jia *et al.* 2015). Now it is accepted that nitrogen of ear photosynthesis is a major contributor to the final grain yield. It was proposed that high photosynthesis in wheat leaves is an important trait for nitrogen assimilation in wheat (Tambussi *et al.* 2007).

The relative stability of ear photosynthetic apparatus and relatively high photochemical efficiency might help in maintaining ear photosynthesis during the grain filling period in water-deficient plants (Martinez *et al.* 2003). There are several traits that likely support the superior photosynthetic performance of wheat ears compared to the flag leaves in wheat under drought conditions. The wheat ear is closer to the grain, which is the main photosynthetic sink (O'Brien *et al.* 1985, Tambussi *et al.* 2005). The stomata are micro valves which are responsible for gas exchange, specifically acquiring CO₂ from the air and transpiring moisture from a plant (Abbad *et al.* 2004). A xeromorphic anatomy has been detected in wheat ear glume increasing accumulation of CO₂ for photosynthesis (Teare *et al.* 1972). Photosynthesis in crops is changed by the addition of nitrogen and sowing is influenced, too. WSS is a modified form of drill sowing in which seeds are distributed evenly and in the same plane (Tao *et al.* 2018). In N-limited ecosystem, N deposition can satisfy plant N demand and stimulate photosynthetic capacity and plant growth (Peñuelas *et al.* 2013, Mao *et al.* 2018). The species-specific response to increased N deposition depends largely on their physiological adaptations in natural grassland community (Chen *et al.* 2005, Bubier *et al.* 2011). Different functional groups exhibit divergent photosynthetic capacity and nutrient cycling in response to altered N availability (Hikosaka 2004). The uniform sowing significantly increased the nitrogen rate and grain yield without a significant decline in grain protein (Bubier *et al.* 2011).

Fertilizers constitute an integral part of improved crop production technology. Nitrogen (N) is an essential mineral nutrient for plant growth, expands soil fertility and crop productivity (López-Bellido *et al.* 2012, Wang *et al.* 2012). A proper amount of N fertilizer application is considered a key for high crop production (Liang *et al.* 2019). An increase in grain yield is often associated with a low protein content (Triboi and Triboi-Blondel 2002). In China, farmers are excessively applying N fertilizers to sustain further increase grain yield, but grain yield does not keep a synchronous increase with the excessive N application (Meng *et al.* 2016). For dryland wheat production, the major task is to use a method which could effectively enhance the soil moisture consumption. Wide-

sowing method has been used for dryland wheat production and results have shown that compared to drill sowing, the WSS can increase the growth of wheat at various growth stages and improve the efficiency of water uptake and increase the yield (Sun *et al.* 2015).

The objective of this study was to find the best sowing method and optimize doses of nitrogen to increase the yield of winter wheat crop. Thus, we used two sowing methods, *i.e.*, wide-space sowing (WSS) and drill sowing (DS) and four nitrogen application concentrations set as 180, 210, 240, and 270 kg(N) ha⁻¹ (N180, N210, N240, N270, respectively) to analyze the photosynthetic characteristics and yield-related traits in winter wheat. Our findings showed that WSS with N240 enhances the photosynthetic characteristics of flag leaves and promotes high yield. Therefore, the WSS with N240 would be a valuable management practice to improve wheat yield in Loess Plateau area of China.

Materials and methods

Experimental site: The experiment was conducted outdoors at the Wenxi experimental site of Shanxi Agriculture University located in the southeastern part of the Loess Plateau, China (110.15°E and 34.35°N). It is a typical semiarid area with an altitude of 450–700 m. The average annual temperature is 11–13°C. The average annual rainfall is 450–630 mm, of which 60–70% precipitation is concentrated in July–September (Fig. 1). Winter wheat and maize are the main crops and they were harvested in early October of the same year.

Experimental design and treatments: The experiment, a typical winter wheat-summer fallow, started with the winter wheat, and ended when the winter wheat was harvested in June 2017, covering two successive wheat crops at the same experimental plot. The two factors split-plot design was adopted and two sowing methods were used, *i.e.*, wide-space sowing (WSS) and drill sowing (DS). An entire terraced field was divided into two parts. A different field

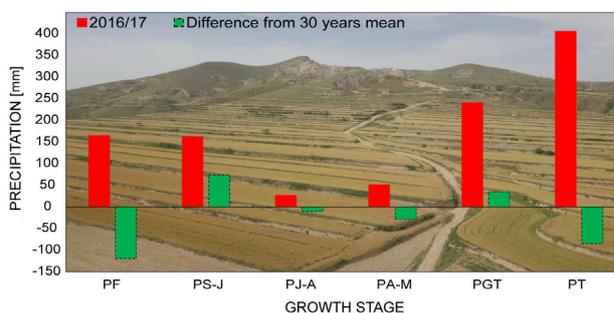


Fig. 1. Precipitation during study year (2016–2017) and difference in precipitation from average precipitation in last 36 years (1981–2017) in different growth stages of wheat at the experimental site in Wenxi, China. PF, PS-J, PJ-A, PA-M, PGT, and PT indicate the fallow precipitation and precipitation from sowing to jointing, jointing to anthesis, anthesis to maturity, precipitation growth total, and total precipitation, respectively.

was used each year. Seeds of the winter wheat (*Triticum aestivum* L.), cultivar 'Yunhan 20410', were obtained from the Shanxi Agriculture Bureau, Wenxi, China. Nitrogen was applied in four application concentrations set as 180, 210, 240, and 270 kg(N) ha⁻¹ (N180, N210, N240, N270, respectively). All treatments were replicated three times. The area of each plot was 30 m² (5 × 6 m). Winter wheat was sown in October in 2015 and 2017 and harvested in June of the following year. Stubble (about 25 cm high) was left in field after harvesting wheat. After the previous corn harvest, the straw was returned to the field. Before sowing, the basal application of phosphate fertilizer and potash fertilizer, 150 kg(P₂O₅) ha⁻¹ and 90 kg(K₂O) ha⁻¹, was applied to the soil. The nitrogen fertilizer was applied to the base ratio of 6:4, during the whole growing season, weed was well controlled by hand and no irrigation was applied in each experimental year. The planting density was 315 × 10⁴ plants ha⁻¹.

Photosynthetic characteristics: The net photosynthetic rate (P_N), transpiration rate (E), intercellular CO₂ concentration (C_i), and stomatal conductance (g_s) of the flag leaf were measured by LI-340 hand-held photosynthesis measurement system (LI-COR, Inc., Lincoln, Nebraska, USA) from 9:00 to 11:00 h. In order to compare photosynthetic characteristics of leaves with a similar developmental age, gas-exchange measurements of each cultivar were conducted immediately after the flag leaves fully expanded. The environmental conditions in the leaf chamber were tightly controlled by the gas-exchange instrument. Each measurement was repeated three times under the same conditions [irradiance of 200 μmol(photon) m⁻² s⁻¹; temperature of 26.7°C; relative humidity of 70.3%], which minimized the variations of gas-exchange parameters caused by different ambient environmental conditions. To minimize the diurnal variation of photosynthesis, the measurements were suspended when significant midday depression occurred.

For chlorophyll (Chl) extraction, ten leaf disks (0.7 cm in diameter) were obtained from the third upper leaves of the main stems of five plants from each plot at the pegging, pod-setting, pod-filling, and mature stages, respectively. Leaf disks were soaked in 15 ml of 95% ethanol for 48 h. The Chl contents were calculated as described by Li *et al.* (2000).

Determination of wet gluten content processing quality: The bromophenol blue water solution, isopropanol lactic acid mixture, and the settling values were determined by shock. The landing value was measured using the Landing Numerical Measurer (FN-IV). The *Micro Dough LAB*, a micro powder instrument was produced by a Botone (SCB) (Sweden) and it measured the fluidity of bread. The wet gluten content and gluten index were measured using the Gluten Index Meter (MJZ-II, China) quality analyzer. For quality analysis, dough mixed from 200 g of flour was divided into 0.25-g samples and the wet gluten content was calculated using the following formula (Noor *et al.* 2020a): wet gluten [%] = [(100% flour + 2 ml H₂O + 10% salt)/100] × 0.25 g flour.

The dry gluten was obtained by drying the wet gluten in an oven (TD5G, Hunan Xiang Li Scientific Instruments, Ltd., China) to constant mass at 100°C for 24 h using the air oven drying method. The dried gluten was left cool for 1 h before taking its mass as the dry gluten content. The percentage of dry gluten was calculated: dry gluten [%] = (mass of dry gluten [g]/0.25 g flour) × 100.

Determination of the spike number: Comparison of the spike of interest with the model spike occurs in an N-dimensional vector space, which dimensions are defined by the total spikelet number of the spike of interest. The geometrical difference in grain yield distributions along the spike (GYDAS) between the two spikes is based on the scalar product of these two vectors:

$$\cos a \left[\vec{a} \times \vec{b} \right] = \frac{\vec{a} \times \vec{b}}{|\vec{a}| |\vec{b}|}$$

Statistical analysis: The different data were subjected to analysis of variance (ANOVA) as split-plot design using DPS and SAS 9.0. Graphics were constructed using Microsoft Excel 2010. Mean values were calculated and significance of the difference between treatments was tested by LSD (least significant difference) method at the significance level of $P=0.05$.

Results

Photosynthetic characteristics: The concentration of CO₂ between the flag leaf cells after WSS and DS decreased gradually with the grouting process and from 7 to 28 d after anthesis, P_N increased with the amount of nitrogen applied showing a single peak increase trend. The P_N after 7–14 d of flowering was still the highest at N180 and not significantly different from N270 and N240. After flowering during 21–28 d, P_N increased with nitrogen application and after 21 d of flowering was higher at N240 with significant difference from other treatments (Fig. 2A). After flowering at 28 d, N240 was still the highest but the difference was not significant compared to N180, N210, and N270. The addition of nitrogen fertilizer can significantly improve the P_N of the flag leaves after flowering but the treatment effect of N270 in the later grouting was weaker and the WSS with N240 could sustain the whole grout period. C_i in the flag leaves decreased first and then increased with the increase of the nitrogen concentration at different stages after flowering. C_i under the N240 treatment was significantly lower than that of other treatments at 7–14 d and 21 d after flowering. C_i was the lowest in N240 treatment at 28 d after flowering, but the difference was not significant compared to N270 (Fig. 2B). Addition of nitrogen fertilizer after flowering could significantly reduce intercellular CO₂ concentration of the flag leaves and the whole grouting period of wheat could completely continue. The g_s of the flag leaves of wheat significantly decreased gradually with the process of growing in WSS and DS and the g_s of the flag leaves increased first and then decreased with an increase of nitrogen concentration at different stages after flowering. The g_s was significantly higher in

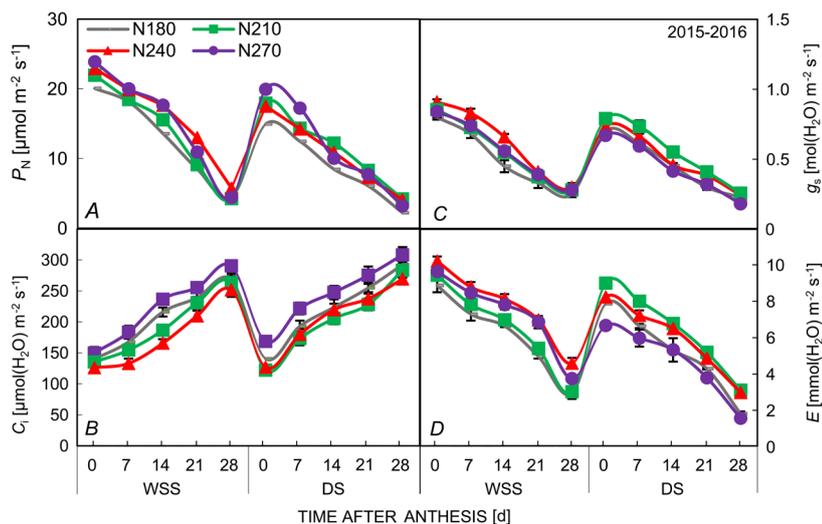


Fig. 2. Effect of sowing method on net photosynthesis (P_N) (A), substomatal CO_2 concentration (C_i) (B), stomatal conductance (g_s) (C), and transpiration rate (E) (D) in flag leaves of winter wheat after anthesis at different growth stages. WSS – wide-space sowing; DS – drill sowing.

N240 treatment compared to other treatments. The g_s was significantly the lowest in N240 at 14 d after flowering and the highest at 21–28 d in N240 but the difference there was not significant (Fig. 2C). The increased concentration of nitrogen fertilizer could significantly increase g_s of flower flag leaves, but the effect lasted until the middle stage of growth and then weakened in the later stage. The E of flag leaves decreased gradually with the grouting process and increased first and then decreased with the increase of nitrogen concentration at different stages after flowering. It can be seen from Fig. 2D that the E was significantly higher in N240 and N270 treatments than that of other treatments at 0–7, 14, and 21 d after flowering. After 28 d of flowering, E was significantly higher in N240 than that in other treatments. Increasing N fertilizer can significantly enhance E of flag leaves after flowering and the highest E was observed in N240 and WSS.

Correlation analysis: The correlation between photosynthetic characteristics of flag leaves and yield after anthesis was analyzed. Yield was positively correlated with the net photosynthetic rate of flag leaves at 21 and 28 d after anthesis. It was significantly positively correlated with the C_i of flag leaves 14 d after anthesis, and extremely significantly positively correlated with the C_i of flag leaves at 21 and 28 d after anthesis. The stomatal conductance of flag leaves was significantly positively correlated from 0 to 21 d after flowering. The transpiration rate of flag leaves in all post-anthesis periods was significantly positively correlated (Fig 3; Table 1S, *supplement*). It can be seen that WSS enhanced the net photosynthesis of flag leaves in the late stage of grain filling, reduced the intercellular carbon dioxide concentration of flag leaves in the late stage of grain filling, enhanced the stomatal conductance of flag leaves in the early stage of grain filling, and increased the transpiration rate of flag leaves in the early stage of grain filling, thus realizing the yield increase.

Effects of different sowing methods and N fertilizer rates on grain protein yield: The effect of nitrogen fertilizers on grain protein and components was significantly

different (Table 1). The contents of albumin and globulin (soluble protein) increased first and then decreased with the increase of nitrogen application. The highest value of albumin was in N240 and the difference between N240 and N270 was not significant. The contents of glutenin (storage protein) increased with the increase of nitrogen application and the highest content was observed in N240 and N270. The protein contents were significantly higher in N240 than that of other nitrogen applications treatments. Thus, it was obvious that N240 treatment regulated the storage protein and was more conducive to quality improvement.

Wheat yield and compositional factors were more common than regular strips with a significant increase in spike, yield, and an increased number of spikes (Table 2). The number of spikes, grain number per spike,

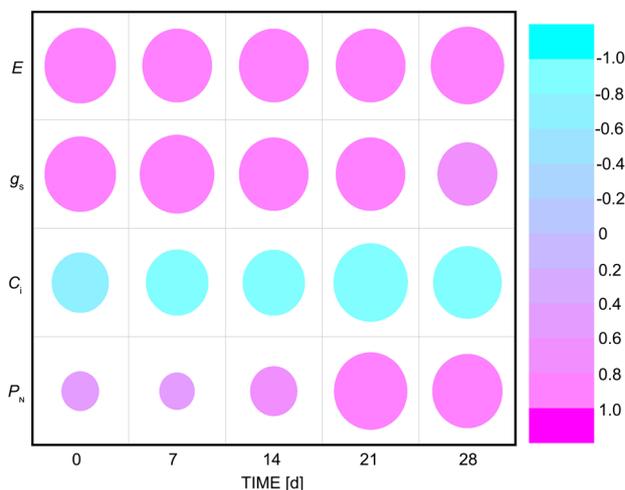


Fig. 3. Correlation coefficients between photosynthetic characteristics and yield in winter wheat. Net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), transpiration rate (E). Circle size and color bar indicate the correlation coefficients between photosynthetic characteristics and yield. Pink and blue color indicate the highest and lowest value of correlation coefficients, respectively.

the 1,000-grain mass, and yield showed the trend of first increasing and then decreasing with the increase of nitrogen fertilizers application. The yield and its three elements were the highest in N240 and spike number and yield were significantly different from other nitrogen treatments and under DS. The yield and its constituent elements were the highest in N210 and the spike and yield was significantly different from other nitrogen treatments. With the increased nitrogen rate, the three factors of yield increased first and then decreased. For WSS and DS, the yield and three elements were the highest at N240 and N210, respectively.

Effect of sowing method on the photosynthesis characteristics of the flag leaves:

Growth process of winter wheat after flowering on P_N of flag leaves showed a trend of a continuous decrease, with a large decrease in the early to middle grouting (Fig. 4A). The photosynthetic rates were the highest at different stages of WSS treatment. It can be seen that different sowing methods can promote the photosynthesis of flag leaves after flowering. Wide-space sowing especially promoted the early and middle stage of grouting. With the growth process of winter wheat after flowering, the E of flag leaves showed a trend of continuous decrease and the decrease was significant from the middle to the end of grouting (Fig. 4B). The E was the highest at different stages of WSS treatment. It can be seen that different sowing methods can promote transpiration of flag leaf after flowering, especially at the end of grouting. During the growth process of winter wheat after flowering, C_i showed a trend of a continuous increase, with a large increase in the early to middle

grouting (Fig. 4C). The intercellular CO_2 was the highest at different post-flowering stages of WSS treatment. It can be seen that different sowing methods can promote the increase of intercellular CO_2 in the middle and late stage of grout, among which the WSS can also promote the flowering stage and the early stage of grout. With the growth process of winter wheat after flowering, the g_s of flag leaves showed a trend of a continuous decrease, with a significant decrease from the initial stage to the middle stage of grouting (Fig. 4D). Compared to DS, the g_s in WSS at different stages after flowering increased by 40%. It can be seen that different sowing methods can improve the g_s of flag leaves after flowering, especially promote the late grouting. Among them, except for the early stage of grouting, the performance of WSS was the best.

Effects of sowing method on protein, protein components in grain and yield components at maturity stage:

The mature grain protein, albumin, gliadin, gluten, and protein yield were improved by WSS, as compared to DS (Table 3). The protein content and protein yield significantly increased by 13 and 42% under WSS as compared to DS, respectively. The grain protein, globulin, gliadin, and glutenin were the highest by DS method, while albumin and grain protein yield were the highest by WSS method.

The yield at WSS was higher than that of the DS (Table 4). The increase in number of spikes was 22% and the increase in grain yield was 25% in WSS as compared to DS. The difference between sowing methods was not significant on 1000-grain mass. The number of spikes and grain yield were the highest under WSS and the grain

Table 1. Effect of different nitrogen rate on grain protein and component contents of winter wheat at maturity stage. N180, N210, N240, and N270 denote nitrogen application concentrations of 180, 210, 240, and 270 kg(N) ha⁻¹, respectively. Values are means \pm SD ($n = 5$). Different letters denote significant differences at $p < 0.05$.

Nitrogen treatment	Albumin [%]	Globulin [%]	Gliadin [%]	Glutenin [%]	Glu/Gli	Protein [%]	Protein yield [kg ha ⁻¹]
N180	2.12 ^b	1.80 ^{Ab}	3.04 ^c	4.26 ^d	1.40 ^a	11.92 ^c	962.22 ^c
N210	2.20 ^b	1.80 ^{Ab}	3.94 ^b	5.24 ^c	1.33 ^b	13.40 ^b	1,050.77 ^b
N240	2.58 ^a	1.93 ^a	4.03 ^b	5.92 ^b	1.47 ^a	14.79 ^a	1,282.54 ^a
N270	2.46 ^a	1.95 ^a	4.30 ^a	5.81 ^b	1.35 ^b	14.03 ^b	1,087.29 ^b

Table 2. Effect of different sowing methods on yield component contents of winter wheat. DS – drill sowing; WSS – wide-space sowing. N180, N210, N240, and N270 denote nitrogen application concentrations of 180, 210, 240, and 270 kg(N) ha⁻¹, respectively. Values are means \pm SD ($n = 5$). Different letters denote significant differences at $p < 0.05$.

Sowing method	Nitrogen treatment	Spike number [$\times 10^4$ ha ⁻¹]	Grain number per spike	1000-grain mass [g]	Yield [kg ha ⁻¹]
WSS	N180	716.50 ^c	29.73 ^b	40.66 ^b	7,447.64 ^c
	N210	728.25 ^c	30.56 ^a	41.35 ^{Ab}	7,841.61 ^b
	N240	823.25 ^a	31.52 ^a	42.58 ^a	9,234.26 ^a
	N270	758.75 ^b	30.95 ^a	39.07 ^b	8,003.31 ^b
DS	N180	560.25 ^c	29.88 ^b	40.73 ^b	5,857.49 ^f
	N210	628.50 ^d	30.40 ^a	42.10 ^a	6,921.53 ^d
	N240	587.50 ^d	29.10 ^b	41.28 ^{ab}	6,092.60 ^f
	N270	540.00 ^c	28.02 ^{Bc}	39.92 ^b	5,087.64 ^g

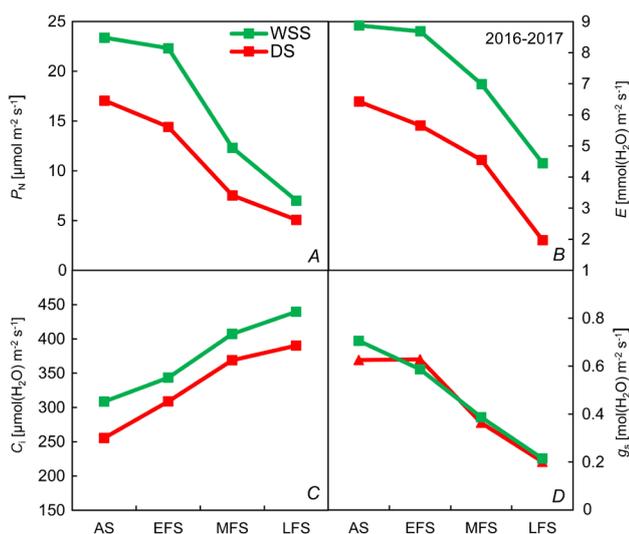


Fig. 4. Effects of sowing method on net photosynthesis (P_N) (A), transpiration rate (E) (B), substomatal CO_2 concentration (C_i) (C), and stomatal conductance (g_s) (D) in flag leaves of winter wheat after anthesis at different growth stages. AS, EFS, MFS, and LFS indicate anthesis stage, early grain-filling stage, middle grain-filling stage, and late grain-filling stage, respectively.

number per spike was significantly higher in WSS. It can be seen that different sowing methods mainly increase the yield by increasing the number of spikes.

Discussion

Effect of different nitrogen rate on sowing methods and photosynthesis characteristics of flag leaves after flowering: Increased photosynthesis improves the wheat yield (Parry *et al.* 2011), whereas N deficit can impair wheat development and yield-insufficient photosynthesis (Guerfel *et al.* 2009, Jia *et al.* 2015). In the present study, N-deficit conditions reduced the P_N of the flag leaves and spikes in winter wheat during the grain-filling period, and this effect was significant for the drought-sensitive sowing. Compared to the continuing decrease of P_N in the flag leaves, P_N in spikes changed steadily after anthesis under drought conditions. Tambussi *et al.* (2005) have shown that P_N of wheat spikes seems to be maintained under water stress compared to the flag leaves, which was consistent with our results. The reduction in the C_i , which we observed in the flag leaves, indicated earlier senescence during anthesis under N treatment, which may result from different sowing methods, but it can improve the P_N of flag leaves after flowering (Huseynova 2012). Conversely, the relative stability of nitrogen content in spike bracts might help sustain the duration of spike photosynthesis during the grain-filling period, particularly in drought-resistant sowing (Martinez *et al.* 2003). Stomatal conductance, an important biological process, reflects the carbon accumulation and transpiration in plants with CO_2 flowing into sites of photosynthesis by the stomata (Sikder *et al.* 2015). It is possible that g_s might play an important role

in the high P_N under both normal and nitrogen conditions (Farquhar and Sharkey 1982, Johnson *et al.* 1987). In the present study, g_s showed a lower reduction in both flag leaves and spikes under higher nitrogen compared to sowing during the middle and late grain-filling stages. In contrast, g_s in flag leaves was more sensitive to drought than that of spikes. Saecidi and Abdoli (2015) showed that the decline in P_N may occur due to decreased g_s in wheat, and a similar result was observed in mulberry (Lakshmi *et al.* 1996, Rodgers *et al.* 2012). In addition, variations in leaf E in wheat occurred due to differences in g_s (Morgan and LeCain 1991, Martin *et al.* 1994). In our study, sowing method reduced E of both flag leaves and spikes in winter wheat. There was a significant decline in E under drought conditions similarly as reported by van den Boogaard *et al.* (1997). It has been reported that the intercellular CO_2 concentration, transpiration rate, and net photosynthetic rate of wheat generally increased and became stable with the appropriate nitrogen application. Thus, nitrogen application with a proper dose could be beneficial for wheat during the grain-filling period. The results of this study showed that the photosynthetic indexes in the flag leaves of the broad drill with 240 kg(fertilizer) ha^{-1} all reached the optimal level which may be due to the appropriate nitrogen fertilizer amount promoting the synthesis of chlorophyll (Song *et al.* 2016). Photosynthetic rate, stomatal conductance, transpiration rate, and chlorophyll content are important components of photosynthetic characteristics of crops. Nitrogen is the main element of protein synthesis and important component of grain closely related to the life activities of crops and an indispensable nutrient-limiting factor in agricultural production. As a result, nitrogen has been applied extensively in agricultural production over the past few decades to increase wheat yields. However, some studies have shown that under the conditions of high nitrogen application, the photosynthetic rate of leaves decreased. Moreover, applying excessive amounts of nitrogen fertilizer in agriculture leads to the soil pollution, release of harmful gases into the atmosphere, and the eutrophication of waters (Wang and Li 2010, Noor *et al.* 2020b). Wide-space sowing (WSS) and nitrogen application N240 increased the g_s of flag leaves during the grain filling and significantly decreased it at post-anthesis. WSS with N240 was the best for wheat production and yield. In addition, the correlation between photosynthetic characteristics and yield of winter wheat after flowering showed that the WSS enhanced the flag leaf P_N in the late grain-filling stage and the flag leaf C_i in the early grain-filling stage, which increased the yield. The transpiration rate increased by 126%, the intercellular CO_2 concentration increased by 21%, and flag leaf stomatal conductance increased by 40% in WSS as compared to DS. The light transmittance of the large WSS and DS wheat population was significantly better than that of the traditional row spacing sowing wheat which could significantly adjust the net photosynthetic characteristics of the flower flag leaves. The drill sowing with 240 kg ha^{-1} improved the photosynthetic characteristics of flag leaves, significantly increased the net photosynthetic rate, stomatal conductance, and transpiration rate of flag leaves

Table 3. The protein components and protein yield of wheat under different sowing methods. Values are means \pm SD ($n = 5$). Different letters denote significant differences at $p < 0.05$.

	Wide-space sowing	Drill sowing
Albumin [%]	2.27 ^a	1.93 ^a
Globulin [%]	1.87 ^{Ab}	1.82 ^{Ab}
Gliadin [%]	4.18 ^a	3.52 ^b
Glutenin [%]	4.83 ^a	4.11 ^b
Protein content [%]	14.18 ^b	12.54 ^d
Protein yield [kg ha ⁻¹]	1,298.0 ^a	917.2 ^d

Table 4. Effects of different sowing methods on yield component contents of winter wheat. Values are means \pm SD ($n = 5$). Different letters denote significant differences at $p < 0.05$.

	Wide-space sowing	Drill sowing
Spike number [$\times 10^4$ ha ⁻¹]	850.9 ^a	695.0 ^d
Grain number per spike	31.26 ^a	31.01 ^{Ab}
1000-grain mass [g]	40.35 ^{Ab}	39.19 ^b
Yield [kg ha ⁻¹]	9,049.1 ^a	7,266.5 ^d

after flowering and significantly reduced the intercellular carbon dioxide concentration.

Effect of different sowing methods and nitrogen rate on nitrogen fertilizer: Increasing the amount of nitrogen fertilizer can increase the nitrogen content of wheat field tillage layer and promote nitrogen concentration in wheat plants. During maturity stage, the nitrogen in the range of 120 to 240 kg ha⁻¹ increased the accumulation of N in various organs but reduced the rate of nitrogen accumulation in wheat grain. According to Zhang *et al.* (2009), when nitrogen application increased before the operation, the accumulation of N in wheat organs at the maturity stage increased significantly but the nutrient in organs after flowering did not increase significantly. The results of this study also showed that the nitrogen accumulation before flowering increased and then decreased. The contribution rate of nitrogen to grain before flowering also increases and then decreases significantly with the increase of nitrogen accumulation. The reason for the analysis may be that water-soluble fertilizer needs to be increased synchronously with increasing the amount of nitrogen applied but the lack of water in wheat fields reduced the uptake of nitrogen fertilizer by the plant, which is not conducive to the accumulation of nitrogen in the plant (Chai and Xing 2010, Guo *et al.* 2018). At the same time, the response of wheat with a high gluten, medium gluten, and weak gluten content to N fertilizer was very different with increasing N application (Cao *et al.* 2005). To address this problem, many studies have focused on improving fertilization management by splitting up N applications, selecting a proper fertilization time, optimizing fertilizer application rate, and investigating new fertilizer types

that ensure an adequate amount of N available as required by the crop to maximize yields (Wang 1997, Yang *et al.* 2013). Better management practices and the appropriate use of N fertilizers are convenient and effective ways to meet crop N demands, as long as the timing and rate of applications meet the agronomic optimum that would ensure the desirable yield (Tilman *et al.* 2002, Stevens *et al.* 2005). At the same rate, splitting N applications and proper timing of N supply are critical for meeting plant needs and improving N uptake (Limon-Ortega *et al.* 2000). Previous studies found that the quantity of N180 derived from basal N was lower in grain than that in straw, whereas the N210 and N240 was higher in grain than that in straw (Yang *et al.* 2011).

The total protein content and different protein components of wheat grains increased significantly with the increase of nitrogen application. The analysis of the ratio of protein components to total protein in wheat seeds by nitrogen application in this experiment showed that the content of protein components increased, with the highest increase in albumin at 240 kg ha⁻¹, and the highest increase in globulin at 270 kg ha⁻¹ (Wang *et al.* 2015). The contents of gliadin and glutenin (storage protein) were the highest at N240. The protein content, and the protein yield were significantly higher at nitrogen concentration of 240 kg ha⁻¹. It was also found that albumin and globulin were more regulated by nitrogen fertilizer in the early stage of grouting, while gliadin and glutenin were more sensitive to nitrogen fertilizer in the late stage of grouting. However, it was also reported that grain globulin and gliadin increased with the increase of nitrogen application, while albumin and gluten content tended to decrease.

Effects of sowing methods on growth and yield characteristics of wheat: The wide-space sowing improved the light conditions for the population in the field, and at the same time, the suitable nitrogen application made the use of resources reasonable. The nutrient conditions were high in individual plants which developed larger green leaf area, and senescence of the whole wheat plant was delayed (Hu *et al.* 2016). Study showed that leaf photosynthesis was closely related to crop yield and leaf photosynthetic rate was an important reason for crop high yield while nitrogen fertilizer could enhance plants' ability to synthesize chlorophyll and was one of the most effective factors to regulate plant leaf photosynthetic capacity (Fuentes *et al.* 2003, Li *et al.* 2015). The relative water content of wheat at the grain-filling stage was in the range of 50–80%. With the increase of soil moisture, the protein content of grains showed a gradual decline (Zhang *et al.* 2006). We showed that the soil moisture content during the whole growth period was within the range of 50–80% of the maximum water-holding capacity in the field. As the soil moisture increased, the protein content of the grains gradually decreased. Under the conditions of this test, the water content in the growing period showed a significant positive correlation with the protein content of the grains. The wide-space sowing had a high water consumption in the growing period, high grain protein content, and high grain (Zhang *et al.* 2009). Research showed that

the use of wide-stubble sowing enabled robust growth of wheat individuals and significantly increased wheat grains, improved water absorption, wet gluten content, sedimentation value, and significantly increased wheat drought tolerance (Wang *et al.* 2016). The grain ratio is related to different growth stages, nitrogen accumulation prior to flowering, and wet gluten content. It can be seen that increasing the wide-space sowing is the most beneficial to improve the grain quality indicators, such as protein and component contents, grain number per spike, wet gluten content, while the nitrogen fertilizer was beneficial to improving the nutritional quality and processing quality of wheat. Increasing nitrogen fertilizer was beneficial to improving the content of protein.

Conclusions: Photosynthetic rate, stomatal conductance, and transpiration rate of flag leaves at different stages after flowering increased and the intercellular CO₂ concentration was reduced. Wide-space sowing with nitrogen of 240 kg ha⁻¹ improved photosynthetic characteristics of flag leaves and promoted the high yield. The photosynthetic rate was the highest in different stages of WSS after flowering; the number of spikes, 1,000-grain mass, and grain yield also increased in WSS as compared to DS. Therefore, wide-space sowing with nitrogen concentration of 240 kg ha⁻¹ could be an efficient strategy to improve wheat yield in Loess Plateau area.

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