Effects of Nitrogen Fertilizer Applications on Photosynthetic Production and Yield of *Japonica* Rice

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Abstract
Nitrogen management plays important roles in high-yielding rice production. In this two-year study, four rice cultivars were used to investigate the effects of the nitrogen application regime on yields, yield components, photosynthetic characteristics, and the dry matter accumulation and transformation of rice. Under a split-plot design, the main plots were assigned to five nitrogen application strategies, of which strategies 0 (N1), 165 (N2), 195 (N3), and 225 kg N ha^{-1} (N4) were applied as follows: 50% at basal, 35% at tillering and 15% at panicle initiation. In addition, strategy N5 was applied with 195 kg N ha^{-1} by 40% at basal, 15% at green-returning, 25% at the tillering stage, 15% at the panicle initiation stage, and 5% at the spikelet differentiation stage. Each main plot was sub-plotted by four experimental rice cultivars. Our results showed that all four cultivars obtained the highest yield with 195 kg N ha^{-1}application. The nitrogen treatments seemed to have a smaller effect on the number of spikelets per panicle and 1000-grains weight, but more influence on the number of panicles and seed-setting rate and eventually affected grain yield. Under the N5 treatment, high yield was obtained due to more effective panicles, more spikelets per panicle and a high seed-setting rate. Additionally, the N5 treatment caused a longer leaf stay-green duration and higher photosynthetic potential, and the leaf area decreased slower at the late stage. The N4 treatment increased the dry matter formation at the jointing stage and the N3 treatment accelerated the dry matter accumulation between the jointing and heading stages. The results suggested that under 195 kg N ha^{-1} nitrogen amount, a higher post-anthesis biomass could be gained, and the N5 treatment showed greater advantages from heading to maturity than the other treatments in the aspects of more dry matter accumulation at the late growth stage, a coordinated organ growth ratio, and a higher matter translocation rate from the stem and leaf to the panicle, thus benefiting yield formation.

Keywords Rice · Nitrogen application strategy · Yield · Photosynthetic production

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Introduction

Global agriculture faces the unprecedented challenge of feeding nine billion people by 2050 at low environmental costs (Chen et al., 2014; Godfray et al., 2010). Rice (*Oryza sativa* L.) is the foremost staple food in the world, contributing more than 21% to the calorific needs of the world population, and it plays a critical role in maintaining food security and human nutrition (Fitzgerald et al., 2009). According to the expected human population increase, China needs to produce approximately 20% more rice by 2030 to meet its domestic needs if rice consumption per capita is to be maintained at the current level (Peng et al., 2009). However, in the past ten years, rice yield stagnation has been observed, and much attention has been paid to some strategies to increase global crop production and two heated topics of these are expanding the cultivation area and increasing

the grain yield per unit area (Hou et al., 2019; Ray & Foley, 2013). However, the area of arable land for rice cultivation has decreased in recent years due to expanding urbanization and rapid industrialization (Cheng et al., 2007; Hou et al., 2019). Therefore, to ensure food security for the ever-increasing population, raising rice productivity per unit area remains a priority in China.

Nitrogen, an essential constituent of proteins, chlorophyll, and nucleic acids in plants, is an indispensable nutrient for rice growth that determines rice yield and greatly contributes to rice production (Liu et al., 2017; Zhou et al., 2019). In the last half century, rice grain yield has dramatically increased, mainly due to the increase in nutrient fertilizer input, especially N fertilizer (Peng et al., 2010; Zhang et al., 2019; Zhou et al., 2019). In China, farmers apply excessive amounts of N fertilizers due to their hope to sustain further grain yield increases (Yang et al., 2019). However, excessive N fertilizer application did not increase grain yield but did cause a series of environmental problems, including soil acidification, various air-pollution problems, increased greenhouse gas emissions, and eutrophication of surface water (Meng et al., 2016; Yi et al., 2020; Zhang et al., 2019). Therefore, exploring the optimization of N utilization management methods that can maintain and increase rice productivity and synergistically protect the environment is a major challenge. Recently, many optimized N fertilization management strategies have been developed to increase rice yield and the efficiency of N fertilizer application, such as site-specific N management (Peng et al., 2010), topdressing (Kamiji et al., 2011; Zhou et al., 2016), and integrated soil-crop system management (ISSM) (Fan et al., 2012). The core strategies for improving rice yield include increasing or reducing the amount or specifying the timing for N field application. For example, a nutrient management technology, Three-Control Technology (TCT) which was developed by the joint efforts of the Rice Research Institute of Guangdong Academy of Agricultural Science(GDRRI) (Guangdong, China) and the International Rice Research Institute (IRRI) (Manila, Philippines) combines both postponed fertilization times and reduced N input but achieves a more than 6% grain yield increase compared to the regular nitrogen fertilizer application (RFA) in field experiments. The TCT strategy adopts a "split" fertilization manner as in 40% for basal, 20% at mid-tillering, 30% at panicle-initiation, and 10% at heading (Wang et al., 2017). In this "split" manner, N fertilization is better controlled so that fertilizer usage can be lowered and the rice lodging-rate is reduced, which leads to benefits in both yields and environmental protection. Currently, this TCT fertilization strategy is widely adopted across Guangdong Province in China (Wang et al., 2017; Zhong et al., 2010).

Numerous previous studies involving split fertilization, such as topdressing in the late growth stage, and four or more doses of N fertilizer applied throughout the rice growing season, which improved crop yield and nitrogen utilization efficiency have been reported (Kamiji et al., 2011; Wang et al., 2017; Xu et al., 2015; Yi et al., 2020; Zhou et al., 2016). However, due to the complexity of the rice production environment and different topdressing times and frequencies, it is very difficult for farmers to estimate topdressing time and amount easily or conveniently; consistent results are difficult to obtain. Hence, under the current situation of rice production in China, and based on the combination of good cultivars and suitable management, it is imperative to simultaneously consider genotype and nitrogen fertilizer management practices to exploit the true yield potential of high-yielding rice cultivars and achieve a sustainable increase in rice productivity.

The grain yield of crops is based on dry matter accumulation by the photosynthesis of leaves post-anthesis and dry matter remobilization (Man et al., 2015), which is closely related to N fertilizer management methods. Increasing the dry matter accumulation and distributing dry matter into the grain as much as possible is the most effective way to increase crop yield (Xu et al., 2020). Leaf photosynthetic capacity is a key parameter determining crop yield. The amount of N application has a direct relation to photosynthesis, with higher nitrogen availability causing higher photosynthetic capacity and such a response to nitrogen translated in higher dry matter accumulation (Cechinand Valquilha 2019). Suitable N fertilizer management helps maintain the photosynthetic capacity of rice, which can promote dry matter accumulation in rice plants, thus increasing rice yield. Therefore, it is very significant to master N utilization management methods that affect photosynthetic production characteristics, and consequently, the grain yield. Large differences have been observed across rice species in response to nitrogen management strategies in terms of N utilization characteristics and metabolism and nutritional quality (Zhong et al., 2017; Zhou et al., 2018). Due to the lack of research regarding the physiological responses of rice to changes in N fertilization strategies, in this study, a two-year field experiment was conducted using four rice cultivars in Shenyang, Liaoning Province, China. The objective of this study was to investigate the effects of nitrogen fertilization management strategies on rice grain yield and photosynthetic production.

Materials and Methods

Site Description

The field experiment was carried out at the Teaching and Research Institute of Shenyang Agricultural University, Shenyang, Liaoning Province, China (41°48′ N, 123°24′

E) during the rice growing seasons of 2017 and 2018. Soil samples from the upper 20 cm layer were collected for soil analysis of properties before the tested fields were plowed in the first year; soil samples were air-dried, sieved, and analyzed in the laboratory using standard techniques. The soil had a brown loam texture with a pH of 6.8, organic matter of 30.6 g kg^{-1}, total N of 1.23 g kg^{-1}, available N of 78.6 mg kg^{-1}, Olsen P of 22.7 mg kg^{-1}, and available K of 118.6 mg kg^{-1}.

Tested Materials

The experimental materials used were four japonica rice cultivars Shendao47, Shendao6, Shendao11, and Shendao7. These four rice cultivars have been extensively used by local farmers for rice production. Rice seeds were pre-germinated each year, and then pre-germinated seeds were sown in a seedbed on April 19 in 2017 and April 21 in 2018. The seedlings with four leaves were manually transplanted into the paddy field on May 24 in 2017 and May 26 in 2018. Seedlings were transplanted at a density of 25 hills m^{-2} and performed at a spacing of 13.3 × 30.0 cm with three seedlings per hill.

Experimental Design and Crop Management

Experiments were arranged in a split-plot design with nitrogen treatments as the main plots and rice cultivars as the subplots with three replications. The plot size was 24 m^2 (5 m long, 4.8 m wide, and 16 rows at 30 cm row spacing). The treatments in each year were arranged in a split-plot design with three replications to give a total of 60 experimental units. Five N application treatments were used in this study, designated as follows: no N fertilizer application (N1), three conventional N fertilizer application rate treatments (N2, N3, and N4), and N5, which is commonly described as an "optimization" treatment. The total N amounts undre the three conventional N fertilizer application rates were 165 (N2), 195 (N3), and 225 kg ha^{-1} (N4), which were sequentially applied in the following manner: 50% as basal (1 day before transplanting), 35% at tillering (10 days after transplanting), and 15% at panicle initiation. The total N application amount

of N5 was 195 kg ha^{-1}, which was applied as follows: 40% as basal (1 day before transplanting), 15% at green turning (3 days after transplanting), 25% at tillering (10 days after transplanting), 15% at panicle initiation, and 5% at spikelet differentiation. The amount and date of N application for each treatment are listed in Table 1. All N fertilizers were applied in the form of urea. Phosphorus (75 kg P$_2$O$_5$ ha^{-1} as single superphosphate) and potassium (75 kg K$_2$O ha^{-1} as potassium sulfate) were applied and incorporated into all plots one day before transplanting as basal fertilizer. To prevent seepage between neighboring plots, the plots were separated by 50-cm-wide ridges with plastic film inserted into the soil at a depth of 20 cm. The field was flooded after transplanting, and a flood water depth of 3–5 cm was maintained until the tillering stage, and then the water was drained at the maximum tillering stage to reduce unproductive tillers. Rewatering occurred at the booting stage with a water layer of 3–5 cm until the heading stage; wetting–drying alternation irrigation was performed during grain filling duration; and the water was drained one week before maturity. Weeds, pest insects, and diseases were intensively controlled using chemicals to avoid biomass and yield losses (Zhou et al., 2019).

Measurement and Sampling

Chlorophyll Content (SPAD value)

At the jointing, heading, and filling stages, five plants in each plot were randomly chosen and the flag leaves or fully expanded leaves at the top plants were marked with a red spot to measure the chlorophyll content with a Minolta SPAD-502 chlorophyll meter. The upper, middle, and lower parts of each selected leaf were measured, and the average of the three readings was calculated as the chlorophyll content for the given leaf.

Photosynthetic Parameters and Leaf Area Index

At the heading, 20 days after heading, and maturity stages, five plants from five hills (the mean tiller numbers were used

Table 1 The timing and amount of N fertilizer application for different N treatments(kg ha^{-1})

Treatment	Total nitrogen	Basal fertilizer	Green turning fertilizer	Tillering fertilizer	Panicle fertilizer	Grain fertilizer
N1	0	0	0	0	0	0
N2	165	82.5	0	57.75	24.75	0
N3	195	97.5	0	68.25	29.25	0
N4	225	112.5	0	78.75	33.75	0
N5	195	78.0	29.25	48.75	29.25	9.75

for reference) in each plot were selected, and the flag leaf and the top second leaf were labeled with red thread to investigate photosynthesis and the leaf area index. Two labeled palnts were taken to measure photosynthesis. The photosynthesis was measured with a LI-6400 photosynthesis system (Li-Cor Inc., Lincoln, USA) during 9:00 a.m.-11:00 a.m. on a sunny and calm day. The parameters measured included net photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular CO_2 concentration (Ci) and were automatically recorded.

The above-mentioned five hills in each plot were taken to investigate leaf area. The total green leaf area of each labeled plant was measured by a leaf area meter (LI-300, Li-Cor Inc., Lincoln, NE, USA) to determine the leaf area index (LAI).

The photosynthetic potential ($\times 10^4$ m^2 d ha^{-1}) was calculated as follows:

Photosynthetic potential $= (L_1 + L_2)/2 \times (t_2 - t_1)$.

where L is leaf area and t is the measured time.

Dry Matter Accumulation

In each plot, five hill samples were taken to measure biomass at the jointing, heading, grain-filling, and maturity stages. Before sampling, the mean tiller numbers per hill of each plot were calculated. Then the mean tiller numbers were used for reference to choose the five representative hills from each plot. After recording the number of tillers, the plant samples were separated into leaves, stem-sheath, and panicles (when present). All samples were dried at 70 °C to a constant weight and weighed. The parameters related to dry matter accumulation and remobilization within the rice plants were calculated as follows:

Exportation percentage of the matter in the stem
− sheath (EPMSS, %)

$$
\begin{aligned}
= \ & (\text{dry matter of the stem} - \text{sheath at the heading stage} \\
& - \text{dry matter of the stem} - \text{sheath at maturity})\,/ \\
& \text{dry matter of the stem} \\
& - \text{sheath at the heading stage} \times 100
\end{aligned}
$$

Transformation percentage of the matter in the stem
− sheath (TPMSS, %)

$$
\begin{aligned}
= \ & (\text{dry matter of the stem} - \text{sheath at the heading stage} \\
& - \text{dry matter of the stem} - \text{sheath at maturity})\,/ \\
& \text{grain weight at maturity} \times 100
\end{aligned}
$$

Grain Yield and Yield Components

At maturity, six representative hills were sampled diagonally from the middle of each plot. The panicles were hand-threshed. Then grains were air-dried and then the filled spikelets were separated from unfilled spikelets by submerging them in 50% alcohol (Vwater:Valcohol/1:1). Three subsamples of 30 g filled grains were manually counted to determine the number of spikelets, 1000-grains weight (30 divided by the number of filled spikelets multiplied by 1000) was calculated. All unfilled spikelets were counted, and the total number of spikelets (filled and unfilled) was calculated (Zhou et al., 2019). Spikelets per panicle (total spikelets m^{-2}/panicles m^{-2}) and seed-setting rate ($100 \times$ filled spikelets m^{-2}/total spikelets m^{-2}) were calculated. The dry weights of leaves, stems, rachises, and filled and unfilled spikelets were measured after oven drying at 70 °C to a constant weight. Grain yield was determined from a 3.6 m^2 (the middle 4 rows by 3 m) area of each plot. The rice crops were harvested manually using

Table 2 Analysis of variance (ANOVA) for grain yield and yield related traits

Analysis of variance	F-value				
	Yield	Effective panicles m^{-2}	Spikelets per panicle	Seed-setting rate	1000-grains weight
Year(Y)	9.17[**]	0.59[ns]	8.19[**]	5.02[**]	3.89[*]
N application (N)	7.57[**]	9.11[**]	3.18[*]	6.23[**]	2.48[*]
Cultivar (C)	6.01[**]	4.30[*]	6.98[**]	4.58[**]	2.57[*]
Y×C	0.04[ns]	0.95[ns]	0.91[ns]	0.98[ns]	0.17[ns]
Y×N	0.61[ns]	1.16[ns]	1.04[ns]	1.01[ns]	1.34[ns]
C×N	3.48[*]	4.55[**]	0.21[ns]	4.15[**]	0.78[ns]
Y×C×N	0.87[ns]	1.24[ns]	0.41[ns]	0.32[ns]	1.20[ns]

*Significant at 0.05 level

**Significant at 0.01 level

ns, Not significant at 0.05 level

a sickle. Grain yields were adjusted to 14.5% moisture content.

Statistical Analysis

Analysis of variance (ANOVA) was performed using the GLM procedure in SAS (version 9.4, SAS Institute, Cary, NC, USA). Means of the treatments were compared using the least significant difference (LSD) test at the 0.05 probability level. ANOVA did not reveal any significant difference between years and nitrogen application, and between years and cultivar (Table 2). Similar results were observed under the same nitrogen application strategies and cultivars in each year; as a consequence, we reanalyzed the data with a reducing model by removing the insignificant factor, in this case, the Year factor. This article mainly analyzed data from 2017.

Results

Grain Yield and Yield Components

For the random factor, grain yield was significantly affected by the year (Table 2). For the fixed factors, the effect of cultivars on grain yield was significant. Different N application strategies significantly affected grain yield. Furthermore, the interactive effect between cultivar and N application strategy was significant on grain yield.

In terms of grain yield, different cultivars responded differently to nitrogen application treatments (Table 3). According to the average yield and its components under different nitrogen application treatments, the yield formation characteristics of the cultivars were different, as for Shendao47, Shendao6, Shendao11, and Shendao7, the number of effective panicles increased significantly, while the number of spikelets per panicle decreased, and the comprehensive yield was arranged from high to low. The results of variance analysis showed that there were significant differences in yield across all cultivars under different nitrogen application treatments, and the yield in 2018 was higher than that in 2017, but the trend for different treatments across the two years was similar. Cultivars significantly impacted grain yield, and the yields of erect panicle cultivars Shendao47 and Shedao6 were higher than those of curved panicle cultivars Shendao11 and Shendao7. Detailed analysis showed that under the same nitrogen application mode, the response of these four cultivars to the amount of nitrogen application was different, for Shendao47, grain yield significantly increased with the increase of nitrogen application amount,

and the average yield of N4 was 30.55% higher than that of N1 in both years; while Shendao6, Shendao11 and Shendao7 showed a trend of first increasing and then decreasing with the increase of nitrogen application amount. Through comparing the yield results of the N3 and N5 treatments under the same nitrogen application amount but different distribution proportions, the results showed that, the yields of Shendao47 and Shendao6 under the N5 treatment were significantly higher than that of N3, with average yield increases of 6.54% and 5.79%, respectively, in the two years. The yield of Shendao47 obtained the highest value at N3, and the yields of Shendao11 and Shendao7 were higher under the N5 treatment, while it was of nosignificant difference from that of N3. The N1 level consistently produced the lowest grain yields regardless of cultivar across both years.

The ANOVA for yield component traits including the number of effective panicles, number of spikelets per panicle, seed-setting rate, and 1000-grains weight is shown in Table 2. Generally, cultivar and N application treatment had significant effects on yield components. The interaction between cultivar and N application treatment had a significant effect on the number of effective panicles and seed-setting rate. In terms of grain yield components, the nitrogen application amount had a significant effect on the number of effective panicles per unit area, and there was a significant difference among treatments with different nitrogen application amounts. For all cultivars, the number of effective panicles per unit area increased significantly with the increase of N application amount. Compared with the N1 treatment without nitrogen application, the number of effective panicles in the N4 treatment with high nitrogen increased by approximately 60%. With the increase in the nitrogen application amount, the number of spikelets per panicle first increased and then decreased, while the seed-setting rate decreased significantly, and the seed-setting rate of Shendao47 and Shendao6 decreased more significantly under high amounts of nitrogen application. The 1000-grains weight of Shendao11 was less affected by the nitrogen application amount, while that of the other three cultivars decreased with the increase of nitrogen application amount, especially Shendao7. Grain yield components responded differently to the distribution proportion of nitrogen fertilizer. The number of effective panicles under the N5 treatment was slightly lower than that under the N3 treatment, but no significant difference was observed between the two treatments. The N5 treatment significantly increased the number of spikelets per panicle of Shendao47 and Shendao6, seed-setting rate and 1000-grains weight also increased to some extent, while this treatment had little effect on Shendao11 and Shendao7 (Table 3).

Table 3 Effect of different nitrogen treatments on grain yield and its components of rice

Year	Cultivar	Nitrogen treatment	Effective panicles m^{-2}	Spikelets per panicle	Seed setting rate (%)	1000-grains weight (g)	Grain yield (t ha^{-1})
2017	Shendao47	N1	261.7[d]	146.3[b]	88.6[a]	25.2[a]	8.13[d]
		N2	356.7[c]	149.2[ab]	81.1[b]	23.5[ab]	9.72[bc]
		N3	392.5[b]	152.6[ab]	77.2[bc]	22.8[b]	10.02[b]
		N4	425.0[a]	151.5[ab]	74.8[c]	22.5[b]	10.14[ab]
		N5	382.5[b]	158.3[a]	79.9[b]	23.5[ab]	10.71[a]
	Shendao6	N1	304.6[d]	107.5[c]	92.7[a]	25.9[a]	7.63[d]
		N2	369.9[c]	122.5[ab]	85.6[bc]	24.2[ab]	9.21[c]
		N3	436.4[b]	120.2[ab]	86.7[ab]	24.5[ab]	10.29[b]
		N4	484.3[a]	111.3[bc]	81.5[c]	23.8[b]	10.18[b]
		N5	423.9[b]	125.5[a]	87.1[ab]	24.6[ab]	10.87[a]
	Shendao11	N1	316.7[c]	96.6[a]	95.9[a]	26.2[a]	7.68[d]
		N2	428.7[b]	95.8[ab]	89.9[ab]	25.0[a]	8.86[c]
		N3	448.3[b]	98.3[a]	87.7[b]	25.2[a]	9.39[a]
		N4	496.0[a]	92.1[b]	84.6[b]	24.2[a]	9.17[b]
		N5	451.7[b]	97.1[a]	85.7[b]	25.7[a]	9.50[a]
	Shendao7	N1	395.8[d]	76.3[c]	92.7[a]	25.0[a]	6.81[c]
		N2	487.0[c]	80.6[ab]	88.2[ab]	23.4[a]	7.68[b]
		N3	565.8[b]	83.7[a]	88.4[ab]	22.2[b]	8.93[a]
		N4	594.8[a]	75.3[bc]	86.1[b]	22.0[b]	8.23[b]
		N5	552.8[bc]	82.7[ab]	90.1[ab]	22.7[ab]	8.52[ab]
2018	Shendao47	N1	263.2[d]	137.0[b]	93.8[a]	25.1[a]	8.22[d]
		N2	346.7[c]	151.8[a]	86.6[ab]	23.6[ab]	10.47[c]
		N3	366.6[b]	152.9[a]	85.7[ab]	23.9[ab]	11.13[b]
		N4	411.0[a]	151.7[a]	83.9[b]	23.1[b]	11.21[b]
		N5	357.0[b]	159.6[a]	88.5[ab]	24.2[ab]	11.82[a]
	Shendao6	N1	285.1[d]	115.2[b]	95.4[a]	25.7[a]	7.75[d]
		N2	382.2[c]	130.5[a]	92.4[a]	23.4[b]	10.26[c]
		N3	416.0[b]	126.7[a]	91.1[a]	23.9[ab]	10.92[b]
		N4	462.3[a]	119.5[b]	90.0[a]	23.2[b]	10.85[b]
		N5	420.5[b]	125.3[ab]	93.0[a]	24.4[ab]	11.57[a]
	Shendao11	N1	325.8[e]	93.2[ab]	96.9[a]	27.2[a]	7.95[c]
		N2	436.9[d]	92.4[ab]	93.9[a]	26.6[a]	9.65[b]
		N3	476.2[ab]	94.9[ab]	91.9[a]	25.7[a]	10.47[ab]
		N4	491.2[a]	90.2[b]	91.6[a]	25.1[a]	9.89[b]
		N5	459.5[bc]	97.8[a]	94.2[a]	25.5[a]	10.62[a]
	Shendao7	N1	382.5[d]	81.7[bc]	96.0[a]	24.7[a]	7.48[d]
		N2	533.1[c]	83.3[ab]	92.8[ab]	23.7[bc]	9.52[bc]
		N3	559.6[b]	85.9[a]	93.3[ab]	23.1[ab]	10.13[a]
		N4	621.6[a]	77.6[c]	87.4[b]	22.5[c]	9.12[c]
		N5	547.7[bc]	83.5[ab]	95.6[ab]	23.6[ab]	9.94[ab]

Values with a column followed by different letters are significantly different at p < 0.05; the same as below

Fig. 1 **SPAD** of rice under different nitrogen application treatments
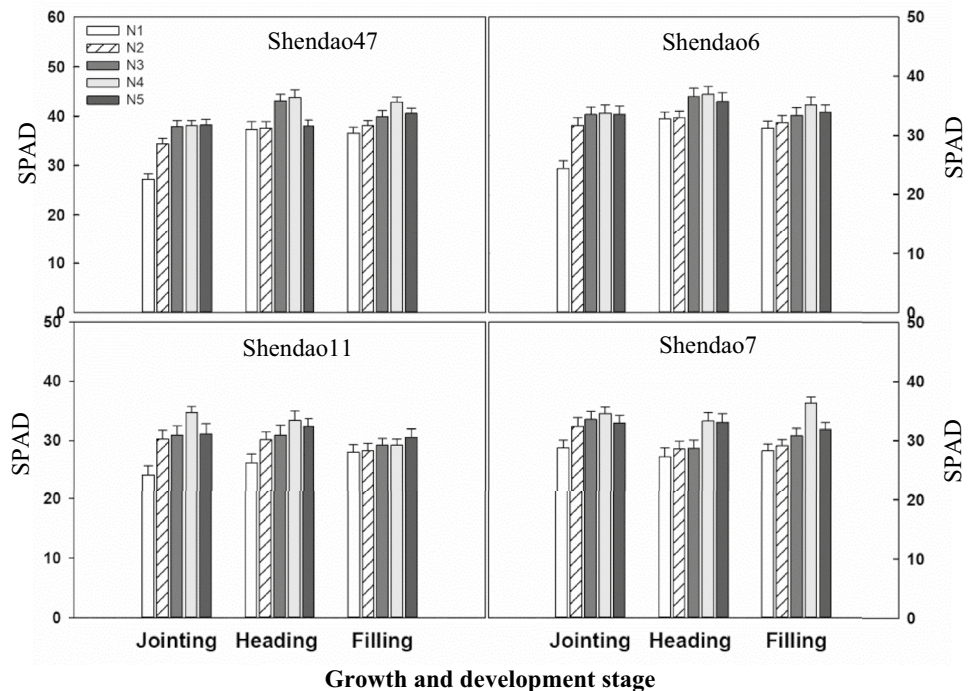
Photosynthetic Characteristics

Chlorophyll Content (SPAD value)

The chlorophyll content (SPAD value) of rice leaves increased with increasing of nitrogen application amount in the main stages of rice growth (Fig. 1). The difference in SPAD between the N3 and N5 treatments was small at the jointing stage. At the heading stage, the SPAD values of Shendao47 and Shendao6 for the N3 treatment were significantly higher than those for the N5 treatment; however for cultivars Shendao11 and Shendao7, the N3 SPAD values were lower than the N5 SPAD values. At the filling stage, the chlorophyll content of all four rice varieties under the N5 treatment was higher than that under the N3 treatment. Under the same nitrogen application amount, the N5 treatment showed a longer leaf stay-green duration than the N3 treatment.

Leaf area Index and Photosynthetic Potential

The increase in the nitrogen application amount promoted the increase in rice leaf area in the vegetative growth periods. At the jointing and heading stages, the leaf area index under the N4 treatment was the highest, followed by that under the N3 and N5 treatments. The leaf area index under the N2 and N1 treatments was relatively small. During the late growth stage, the leaf area index of each treatment began to decline, among which the leaf area of the N4 treatment

decreased rapidly, which was similar to that of N3 treatment 30 days after heading. The N5 treatment showed a certain leaf area duration ability, which was more obvious for Shendao47 and Shendao6; and was higher than that of the other treatments at 30 days after heading or maturity stage, while for Shendao11 and Shendao7, there was no significant difference among treatments N3, N4 and N5 (Table 4).

The photosynthetic potential of rice at different growth stages was significantly different under different nitrogen application treatments due to different leaf area indexes, growth periods and growth processes (Table 4). The photosynthetic potential from the jointing to heading stage and from heading to 30 days after heading was highest for the N4 treatment, followed by the N5 treatment, and the N1 treatment had the lowest photosynthetic potential. These differences were statistically significant. During the period from 30 days after heading to maturity, the photosynthetic potential of different cultivars of rice showed a similar trend as that of the leaf area index at the maturity stage.

Leaf Photosynthetic Parameters

The net photosynthetic rate (Pn) and other photosynthetic indexes of flag leaves and the second leaves from the top at the heading stage were significantly different under different nitrogen application treatments (Table 5). In terms of cultivars, the net photosynthetic rate, stomatal conductance and transpiration rate of Shendao47, Shendao6 and Shendao11 showed similar increasing patterns

Table 4 Leaf area index and photosynthetic potential of rice cultivars under different nitrogen treatments

Cultivar	Nitrogen treatment	Leaf area index				Photosynthetic potential		
		Jointing	Heading	30DAH	Maturity	Jointing-Heading	Heading-30DAH	30DAH-Maturity
Shendao 47	N1	2.05[c]	4.30[c]	1.74[d]	1.10[d]	79.4[c]	75.5[d]	35.5[d]
	N2	3.72[ab]	6.19[b]	2.94[c]	1.91[c]	123.8[b]	114.1[c]	60.6[c]
	N3	3.62[b]	6.58[a]	3.63[b]	2.71[ab]	127.5[ab]	127.6[b]	79.3[b]
	N4	3.86[a]	6.70[a]	3.76[b]	2.62[b]	132.1[a]	130.8[ab]	79.8[b]
	N5	3.78[ab]	6.56[a]	4.26[a]	2.93[a]	129.3[a]	135.3[a]	89.9[a]
Shendao 6	N1	2.13[d]	4.83[d]	2.03[d]	1.74[d]	87.0[d]	85.8[d]	47.1[d]
	N2	3.52[c]	6.04[c]	3.05[c]	2.11[c]	119.5[c]	113.6[c]	64.5[c]
	N3	3.71[bc]	6.57[ab]	3.56[b]	2.83[ab]	128.5[b]	126.6[b]	79.9[b]
	N4	4.03[a]	6.81[a]	4.20[a]	2.51[b]	135.4[a]	137.6[a]	83.9[ab]
	N5	3.86[ab]	6.45[b]	4.35[a]	2.95[a]	128.8[b]	135.0[a]	91.3[a]
Shendao 11	N1	2.16[c]	4.71[d]	2.43[b]	1.09[c]	85.8[d]	89.3[c]	44.0[c]
	N2	3.49[b]	5.58[c]	4.51[a]	1.32[b]	113.3[c]	126.1[b]	72.9[b]
	N3	3.65[ab]	6.17[b]	4.57[a]	2.71[a]	122.7[b]	134.3[ab]	91.0[a]
	N4	3.84[a]	6.61[a]	4.54[a]	2.90[a]	130.6[a]	139.4[a]	93.0[a]
	N5	3.75[a]	6.10[b]	4.41[a]	2.91[a]	123.2[b]	131.4[b]	91.5[a]
Shendao 7	N1	2.07[b]	5.09[c]	3.22[d]	2.03[b]	89.4[c]	103.9[c]	65.6[c]
	N2	3.72[a]	6.35[b]	5.04[c]	2.43[ab]	125.8[b]	142.4[b]	93.4[b]
	N3	3.65[a]	6.55[ab]	5.43[b]	2.61[ab]	127.6[ab]	149.8[ab]	100.5[a]
	N4	3.79[a]	6.78[a]	5.73[a]	2.54[ab]	132.2[a]	156.4[a]	103.4[a]
	N5	3.74[a]	6.53[ab]	5.49[ab]	2.74[a]	128.4[ab]	150.3[ab]	102.9[a]

30DAH refers to 30 days after heading

Table 5 Photosynthetic parameters of rice under different nitrogen treatments

Cultivar	Nitrogen treatment	Pn ($\mu mol\ m^{-2}\ s^{-1}$)		Cs ($mol\ m^{-2}\ s^{-1}$)		Ci ($\mu mol\ mol^{-1}$)		Tr ($mmol\ m^{-2}\ s^{-1}$)	
		FL	TSL	FL	TSL	FL	TSL	FL	TSL
Shendao 47	N1	16.37[d]	12.67[c]	0.604[a]	0.351[b]	284.5[a]	279.2[ab]	10.41[a]	8.17[b]
	N2	17.87[c]	12.76[c]	0.402[b]	0.377[b]	253.6[b]	284.9[a]	9.53[a]	8.47[b]
	N3	19.66[b]	14.97[bc]	0.489[ab]	0.388[b]	264.8[ab]	269.4[ab]	9.24[a]	8.63[b]
	N4	21.59[a]	19.25[a]	0.502[ab]	0.451[ab]	263.1[ab]	263.3[b]	10.42[a]	10.90[a]
	N5	20.80[ab]	17.52[ab]	0.441[ab]	0.588[a]	247.7[b]	283.0[ab]	10.61[a]	10.87[a]
Shendao 6	N1	18.22[c]	9.61[c]	0.529[a]	0.192[a]	277.1[a]	258.7[a]	9.64[a]	5.23[a]
	N2	19.14[bc]	11.20[bc]	0.393[c]	0.259[a]	254.7[b]	267.9[a]	8.65[c]	6.88[a]
	N3	20.01[ab]	12.11[ab]	0.468[b]	0.278[a]	260.5[b]	265.7[a]	9.03[bc]	7.20[a]
	N4	20.89[a]	13.11[a]	0.382[c]	0.283[a]	235.4[c]	258.1[a]	9.70[a]	7.67[a]
	N5	20.11[ab]	12.01[ab]	0.398[c]	0.306[a]	242.5[c]	274.5[a]	9.50[ab]	7.56[a]
Shendao 11	N1	13.89[c]	8.13[c]	0.274[c]	0.246[b]	247.1[a]	289.1[a]	6.04[c]	6.58[b]
	N2	18.53[b]	9.99[b]	0.372[b]	0.291[ab]	255.8[a]	286.7[a]	8.30[b]	7.15[ab]
	N3	19.00[b]	11.08[b]	0.305[bc]	0.227[b]	225.7[b]	261.0[b]	8.24[b]	6.33[b]
	N4	22.26[a]	15.76[a]	0.472[a]	0.330[a]	260.3[a]	259.2[b]	10.24[a]	8.82[a]
	N5	21.76[a]	14.40[a]	0.361[b]	0.358[a]	228.9[b]	269.8[b]	10.28[a]	8.87[a]
Shendao 7	N1	18.80[d]	10.05[c]	0.393[ab]	0.267[ab]	247.1[a]	280.2[a]	9.90[ab]	7.54[ab]
	N2	20.38[c]	10.44[c]	0.332[b]	0.290[ab]	224.1[b]	279.7[a]	8.70[b]	7.16[ab]
	N3	23.70[ab]	14.00[b]	0.510[a]	0.343[a]	259.1[a]	273.4[a]	11.01[a]	8.48[a]
	N4	24.88[a]	17.04[a]	0.441[ab]	0.333[a]	239.4[ab]	254.8[a]	10.98[a]	8.89[a]
	N5	22.77[b]	10.58[c]	0.515[a]	0.210[b]	259.2[a]	262.2[a]	10.39[a]	5.88[b]

Pn net photosynthetic rate, *Gs* stomatal conductance, *Ci* intercellular CO_2 concentration, *Tr* transpiration rate, *FL* flag leaf, *RSL* top second leaf

Table 6 Single stem and population dry matter weight at main stages of rice under different nitrogen treatments

Cultivar	Nitrogen treatment	Jointing		Heading		Filling		Maturity	
		DMWPS (g)	DMWP (t ha^{-1})	DMWPS (g)	DMWP (t ha^{-1})	DMWPS (g)	DMWP (t ha^{-1})	DMWPS (g)	DMWP (t ha^{-1})
Shendao 47	N1	1.49[a]	4.13[b]	3.67[a]	8.98[b]	5.39[a]	12.59[d]	6.48[a]	15.67[c]
	N2	1.53[a]	6.21[a]	3.05[b]	12.06[a]	4.66[ab]	16.87[b]	5.49[b]	22.13[b]
	N3	1.67[a]	6.27[a]	3.09[b]	12.52[a]	4.21[b]	18.50[a]	5.32[bc]	23.02[b]
	N4	1.50[a]	6.97[a]	3.03[b]	12.49[a]	4.10[b]	15.47[c]	5.10[c]	22.92[b]
	N5	1.49[a]	6.82[a]	2.92[b]	13.23[a]	4.64[ab]	18.49[a]	5.76[b]	24.98[a]
Shendao6	N1	1.32[a]	4.43[d]	2.78[a]	7.83[d]	4.27[a]	12.18[e]	5.53[a]	16.64[c]
	N2	1.63[a]	5.69[c]	2.63[a]	10.13[c]	4.09[ab]	13.83[d]	4.72[b]	17.62[c]
	N3	1.26[a]	5.98[bc]	2.59[a]	14.04[a]	3.61[c]	18.90[b]	4.33[b]	21.86[b]
	N4	1.22[a]	7.50[a]	2.47[a]	12.27[b]	3.42[c]	16.06[c]	4.30[b]	21.53[b]
	N5	1.30[a]	6.57[b]	2.69[a]	12.98[ab]	4.01[b]	20.19[a]	4.44[b]	23.92[a]
Shendao 11	N1	1.42[a]	4.72[b]	2.60[a]	8.14[b]	3.88[a]	11.90[d]	4.99[a]	17.39[c]
	N2	1.44[a]	7.01[a]	2.45[a]	11.88[a]	3.59[ab]	14.65[c]	4.58[ab]	21.58[b]
	N3	1.31[b]	7.26[a]	2.11[a]	12.28[a]	3.29[bc]	15.98[ab]	4.15[b]	22.12[ab]
	N4	1.41[ab]	7.37[a]	2.24[a]	11.16[a]	3.06[c]	15.67[b]	3.49[c]	22.05[ab]
	N5	1.39[ab]	6.63[a]	2.29[a]	10.93[a]	3.50[b]	16.79[a]	4.37[b]	22.58[a]
Shendao7	N1	1.01[a]	3.89[c]	2.03[a]	7.79[c]	2.96[a]	11.17[c]	3.61[a]	13.55[d]
	N2	1.08[a]	6.02[b]	1.87[ab]	8.81[bc]	2.33[c]	12.75[b]	3.27[ab]	17.67[c]
	N3	1.02[a]	6.32[b]	1.73[b]	12.37[a]	2.63[b]	18.81[a]	3.13[b]	24.15[a]
	N4	1.01[a]	7.59[a]	1.63[b]	9.63[b]	2.34[c]	17.91[a]	3.04[b]	20.90[b]
	N5	1.06[a]	6.14[b]	1.71[b]	9.78[b]	2.58[b]	13.28[b]	2.96[b]	20.41[b]

DMWPS dry matter weight of per stem, *DMWP* dry matter weight of population

increasing nitrogen application amounts, with a trend of N4 > N5 > N3 > N2 > N1. The differences between treatments were significant. However, the intercellular CO_2 concentration (Ci) showed the opposite trend. On the other hand, Shendao7 showed a slightly different pattern: the Pn of the N3 treatment was higher than that of the N5 treatment.

Dry Matter Accumulation and Translocation

Dry Matter Weight of Single Stem and Population at Main Growth Stages

There were significant differences in the dry matter weight per stem and population during the main growth stages under different nitrogen application treatments (Table 6). For the change in dry matter weight of a single stem, there was no significant difference between the different nitrogen application treatments at the jointing stage and heading stage. However, the dry matter weight per stem of rice at the filling stage and maturity stage decreased significantly with the increase of nitrogen application amount, the order of Shendao7 was N1 > N2 > N3 > N5 > N4, and that of Shendao47, Shendao6 and Shendao11 was N1 > N2 > N5 > N3 > N4. For the

change in the dry matter weight of the population, the dry matter accumulation of rice increased with the increase of nitrogen application rate at the jointing stage, and it was the highest under the N4 treatment. With continuous growth of the rice population, the dry matter accumulation rate of each nitrogen treatment changed, and it reached its highest value under the N3 treatment at the heading stage. There were differences in the dry matter weight of the population among different cultivars at the filling stage and mature stage. For Shendao7, this value was still the highest under the N3 treatment, while for Shendao47, Shendao6 and Shendao11, the highest values all appeared at the N5 treatment, which had significantly higher values than the other treatments. Overall, a nitrogen application amount of 195 kg ha^{-1} can result in higher biomass than other nitrogen application amounts.

Total Dry Matter in Main Growth Periods

The N4 treatment had the highest ratio of dry matter accumulation from the sowing to the jointing stage against the dry matter accumulation during the whole growth period in all rice cultivars (Table 7). From the jointing stage to the heading stage, there were significant differences in the dry matter accumulation of the population among the different

Table 7 Dry matter accumulation and its ratio to total dry matter in main growth periods of rice under different nitrogen treatments

Cultivar	Nitrogen treatment	Transplanting-Jointing		Jointing-Heading		Heading-Filling		Filling-Maturity	
		DMA (t ha^{-1})	RTDM (%)	DMA (t ha^{-1})	RTDM (%)	DMA (t ha^{-1})	RTDM (%)	DMA (t ha^{-1})	RTDM (%)
Shendao47	N1	4.13[b]	26.36	4.85[b]	30.95	3.61[c]	23.04	3.08[d]	19.66
	N2	6.21[a]	28.06	5.85[ab]	26.43	4.81[b]	21.74	5.26[b]	23.77
	N3	6.27[a]	27.24	6.25[a]	27.15	5.26[ab]	21.06	4.52[c]	19.64
	N4	6.97[a]	30.41	5.52[ab]	24.08	2.98[c]	13.00	7.45[a]	32.50
	N5	6.82[a]	27.30	6.41[a]	25.66	5.98[a]	25.98	6.49[ab]	25.98
Shendao6	N1	4.43[d]	26.62	3.40[c]	20.43	4.35[b]	26.14	4.46[ab]	26.80
	N2	5.69[c]	32.29	4.44[b]	25.20	3.70[b]	21.00	3.79[b]	21.51
	N3	5.98[bc]	27.36	8.06[a]	36.87	4.86[b]	22.23	2.96[c]	13.54
	N4	7.50[a]	34.84	4.77[b]	22.16	3.79[b]	17.60	5.47[a]	25.41
	N5	6.57[b]	27.47	6.41[ab]	26.80	7.21[a]	30.14	3.73[b]	15.59
Shendao11	N1	4.72[b]	27.14	3.42[b]	19.67	3.76[b]	21.62	5.49[b]	31.57
	N2	7.01[a]	32.48	4.87[ab]	22.57	2.77[c]	12.84	6.93[a]	32.11
	N3	7.26[a]	32.82	5.02[a]	22.69	3.70[b]	16.73	6.14[ab]	27.76
	N4	7.37[a]	33.42	3.79[b]	17.19	4.51[ab]	20.45	6.38[ab]	28.93
	N5	6.63[a]	29.36	4.30[ab]	19.04	5.86[a]	25.95	5.79[b]	25.64
Shendao7	N1	3.89[c]	28.71	3.90[b]	28.78	3.38[c]	24.94	2.38[c]	17.56
	N2	6.02[b]	34.07	2.79[cd]	15.79	3.94[c]	22.30	4.92[b]	27.84
	N3	6.32[b]	26.17	6.05[a]	25.05	6.44[b]	26.67	5.34[b]	22.11
	N4	7.59[a]	36.32	2.04[d]	9.76	8.28[a]	39.62	2.99[c]	14.31
	N5	6.14[b]	30.08	3.64[bc]	17.83	3.50[c]	17.15	7.13[a]	34.93

DMA dry matter accumulation, *RTDM* ratio to total dry matter

Table 8 Dry weight ratio of leaf, stem-sheath, panicle to total plant at middle and late stages of rice under different nitrogen treatments (%)

Cultivar	Nitrogen treatment	Ratio of leaf			Ratio of stem-sheath			Ratio of panicle		
		Heading	Filling	Maturity	Heading	Filling	Maturity	Heading	Filling	Maturity
Shendao47	N1	25.59[b]	14.90[b]	11.32[b]	62.09[a]	34.27[b]	31.84[a]	12.32[a]	50.83[a]	56.83[a]
	N2	31.45[a]	20.95[a]	14.82[a]	57.11[b]	40.37[a]	31.54[ab]	11.43[a]	38.68[b]	53.64[b]
	N3	32.22[a]	23.00[a]	14.92[a]	56.18[b]	39.80[a]	31.03[ab]	11.60[a]	37.19[b]	54.05[b]
	N4	31.79[a]	22.03[a]	14.91[a]	55.56[b]	37.59[a]	31.16[ab]	12.65[a]	40.37[b]	53.81[b]
	N5	33.00[a]	21.13[a]	15.02[a]	56.18[b]	40.16[a]	30.52[b]	10.82[a]	38.71[b]	54.56[b]
Shendao6	N1	24.41[c]	15.27[b]	11.45[c]	64.95[a]	42.02[a]	36.30[a]	10.64[a]	42.72[a]	52.26[a]
	N2	29.09[b]	19.39[ab]	13.77[b]	60.44[b]	41.09[a]	35.21[ab]	10.47[ab]	39.52[a]	51.02[ab]
	N3	33.61[a]	22.22[ab]	14.91[a]	57.38[c]	42.11[a]	33.31[b]	9.01[b]	35.67[a]	51.78[ab]
	N4	30.74[b]	19.32[ab]	14.33[ab]	59.30[b]	39.89[a]	35.84[ab]	9.96[ab]	40.78[a]	49.83[b]
	N5	30.52[b]	22.80[a]	13.54[b]	59.98[b]	38.79[a]	34.38[ab]	9.50[ab]	38.41[a]	52.09[ab]
Shendao11	N1	22.71[c]	12.72[b]	9.48[b]	63.77[a]	31.56[c]	31.61[a]	13.52[a]	55.71[a]	58.91[a]
	N2	27.80[b]	17.13[a]	11.36[ab]	58.83[bc]	34.43[b]	32.58[a]	13.36[a]	48.44[b]	56.05[b]
	N3	32.44[a]	18.00[a]	12.01[a]	55.26[c]	37.40[a]	32.44[a]	12.31[a]	44.60[c]	55.55[b]
	N4	25.72[bc]	16.25[a]	11.28[ab]	60.94[ab]	35.36[ab]	32.59[a]	13.34[a]	48.39[b]	56.13[b]
	N5	28.12[ab]	16.50[a]	11.14[ab]	58.79[bc]	35.31[ab]	32.81[a]	13.09[a]	48.19[b]	56.05[b]
Shendao7	N1	24.11[b]	14.60[b]	10.27[b]	63.08[a]	34.48[a]	35.37[ab]	12.81[a]	50.92[a]	54.37[a]
	N2	31.26[a]	19.01[a]	13.22[a]	57.09[b]	37.60[a]	33.96[b]	11.65[b]	43.39[b]	52.83[b]
	N3	30.48[a]	19.11[a]	12.02[a]	56.76[b]	38.52[a]	36.08[ab]	12.76[a]	42.37[b]	51.90[b]
	N4	31.12[a]	18.85[a]	13.00[a]	57.05[b]	37.48[a]	35.09[ab]	11.84[b]	43.67[b]	51.90[b]
	N5	31.59[a]	19.53[a]	12.12[a]	56.57[b]	38.39[a]	36.60[a]	11.84[b]	42.08[b]	51.28[b]

nitrogen treatments. When the nitrogen application amount was in the range of 0–195 kg ha^{-1}, the dry matter accumulation first increased and then decreased with an increasing nitrogen application amount. The ratio of dry matter accumulation during this period to that in the whole growth period was significantly different among the different nitrogen application treatments, and this difference was the most significant under the N3 treatment. From the heading stage to the filling stage, the dry matter accumulation is the same as that from the jointing stage to the heading stage. Shendao7 obtained the maximum value under the N4 treatment, and other cultivars achieved the highest value under the N5 treatment. The change trend of the dry matter accumulation ratio based on growth stage was similar to that of the total dry matter accumulation amount. From the filling stage to the maturity stage, the dry matter accumulation amounts of different cultivars were not consistent among the different nitrogen application treatments, and the ratios of dry matter accumulation to total dry matter accumulation in N1, N2, N3, N4, and N5 were 23.9%, 26.31%, 20.76%, 25.29% and 25.54%, respectively.

Dry Matter Distribution and Transformation

The dry matter distribution proportion in various organs at the middle and late stages of rice growth was influenced by nitrogen application treatment to different degrees (Table 8). The ratio for leaves was the largest at the heading stage; and gradually decreased with the growth process. The dry matter accumulation proportion of leaves under the high fertilizer treatment was higher at all stages. The ratio for leaves at the maturity stage of Shendao7 reached a maximum under the N2 treatment. Shendao47, Shendao6, and Shendao11 at the filling stage and mature stage reached their maximum values under the nitrogen application amount of 195 kg ha^{-1}. Compared with that for leaves, only the ratio of stem-sheaths in N1 the treatment without nitrogen application at the heading stage was significantly higher than those in the other nitrogen application treatments, and there was no significant difference among the different nitrogen application treatments at the other stages. For the dry matter ratio of rice panicles at different growth stages, different nitrogen application treatments had little effect on dry matter distribution proportion in rice panicles. The ratio of panicles in each stage achieved the highest value at the N1 treatment, and there was no significant difference among different nitrogen application treatments.

The exportation and transformation of photosynthetic dry matter stored in stems and sheaths before heading directly affect the formation of rice yield. There were significant differences in stem-sheath matter output and transformation among rice cultivars under different nitrogen application treatments (Fig. 2). For Shendao47, Shendao 6 and Shendao11, the maximum values of both the exportation percentage of the matter in stem-sheath (EPMSS) and the transformation percentage of the matter in stem-sheath (TPMSS) were appeared in the N5 treatment, while for Shendao7, the maximum values of EPMSS and TPMSS were appeared in the N3 treatment.
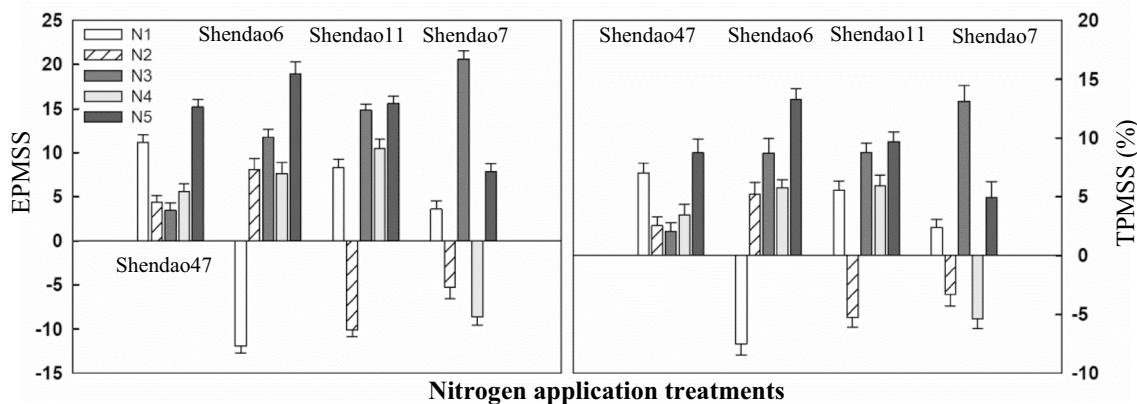

Fig. 2 Exportation percentage of the matter in stem-sheath (EPMSS) and transformation percentage of the matter in stem-sheath (TPMSS) under different nitrogen treatments. The negative values showed that only a small amount of material in the stem-sheath was transported to the panicle, and the distribution of dry matter in the mature stage was higher in the stem-sheath and lower in the panicle.It is because the supply capacity of source is greater than that of sink in the later stage (which may also be affected by the transport pathway of photosynthetic products from the leaf and stem-sheath to the grain), a part of photosynthetic products will accumulate in the stem-sheath The results showed that only a small amount of material in the leaf and stem-sheath was transported to the panicle, and the distribution of dry matter in the mature stage was higher in the leaf and stem-sheath and lower in the panicle, so the result was negative when the formula was used

Discussion

There have been a large number of studies carried out previously on the rice nitrogen-utilization characteristics and yield formation by manipulating the amount or the timing of the fertilization so to identify the optimal nitrogen application for yield improvement and pollution reduction. However, the results are inconsistent (Sun et al., 2012; Yi et al., 2020; Tan et al. 1981; Shi et al., 2008). There is a quadratic curve between rice yield and nitrogen application rate and high yields can be obtained at an optimal fertilization amount. Huang et al. (2006) used the nitrogen fertilizer test regression-equation and found that the appropriate nitrogen application rate for Liaojing 294 to reach a high yield was 190 kg ha^{-1}. In this study, grain yield increased with the increase in N fertilizer from 0 to 195 kg ha^{-1} for all the tested cultivars. However, the grain yields of Shendao6, Shendao11 and Shendao7 decreased with a further increase in N fertilizer from 195 to 225 kg ha^{-1}, while the Shendao47 yield continued to increase but at a decreasing increment for the given N level. These results suggest that the tolerance or response to excessive fertilizer of rice cultivars vary in terms yield. Sun et al. (2012) identified an optimum N application pattern that adopted stable early-stage application, an optimized middle-stage application and panicle-leaf topdressing. Additionally, Yi et al. (2020) suggested that delayed tillering fertilization from the 5.5-leaf stage to the 9-leaf stage could significantly increase rice yield. Furthermore, Shi et al. (2008) also found that the "split" of all-early-stage fertilization into early-and-later growth stages showed significantly improved performance with the same N application rate. In the present study, at the same nitrogen application level, the greatest grain yields of Shendao47, Shendao6, and Shendao11 were appeared with the N5 treatment, namely "N application at a later stage". Although the greatest yield of Shendao7 was observed with N5, the difference in grain yield between N3 and N5 was not significant. Since different rice cultivars, especially local rice cultivars, had different responses to fertilizer application management and ecological conditions, the nitrogen application strategies and fertilizer requirement characteristics of all cultivars should be further studied to guide rice production.

Rice grain yield was determined using effective panicles per unit area, spikelets per panicle, seed-setting rate, and grain weight. In rice production, nitrogen is applied to promote tillering and increase the amount of tillers at the early vegetative stages, and nitrogen topdressing is highly effective in maximizing spikelet production (Zhou et al., 2017). However, N application at an early stage significantly increased the number of rice tillers while the percentage of effective tillers decreased. Moreover, more effective tillers or a higher percentage of effective tillers was not always

obtained through greater nitrogen application (Deng et al., 2015). Previous studies have shown that large amounts of nitrogen application at early vegetative stages caused more ineffective tillers and decreased the panicle number and percentage of effective tillers of rice, even resulting in a serious nitrogen leaching risk (Deng et al., 2015; Peng et al., 2007; Qiao et al., 2013). Sun et al. (2012) reported that a reduction in nitrogen application at early stage and promote the proportion of panicle fertilizer could increased total number of spikelets, seed-setting rate and the percentage of effective tillers to some different extent and grain weight had little differences, thus enhancing grain yield. Shi et al. (2008) found that the N application pattern "Five-time application in three periods" significantly increased the number of panicles and filled grains per square meter. As a consequence, the total number of spikelets (sink size) increased, which was helpful to improve sink size and grain yield. The "Stable application at early stage large application at middle stage and satisfied application at late stage" pattern resulted in high yield by improving grain weight, seed-setting rate, and spikelets per panicle. Similarly, the split tillering fertilization (N5) in our study was also more conducive to regular tiller development and increased the number of effective panicles. Moreover, compared with the other treatments, the N5 treatment which reduced basal and tillering fertilizer and applied spikelet N fertilizer significantly increased seed-setting rate and grain weight and spikelets per panicle. Overall, the "split" N is applied to build an appropriate tiller population, improve the rice population quality, and then increase the effective panicles. Additionally, when applied to growing panicles, fertilizer is beneficial to promoting spikelet differentiation and inhibiting spikelet degeneration, consequently increasing filled grains per panicle (Deng et al., 2015; Kamiji et al., 2011).

Photosynthesis plays an important role in crop yield formation, providing nearly 70% of the material for crop yield (Manet al., 2015). The dry matter production process of crops comes from photosynthesis. It has been indicated that the improvement of the photosynthetic capacity of rice leaves is important for dry matter accumulation (Xu et al., 2020). An enhanced rice grain yield is consistently related to changes in photosynthetic characteristics. Photosynthetic capacity is significantly influenced by leaf senescence, which is a complex developmental process that involves chloroplast breakdown accompanied by chlorophyll degradation and the continuous loss of chloroplasts, causing leaf color changes from green to yellow (Zhou et al., 2017). Unfavorably delayed leaf senescence decreases the dry matter translocation rate from leaf sheaths to filling grains and results in an obvious decrease in grain yield. Moreover, maintaining a longer period of green leaf area, especially after anthesis when there is usually a rapid decrease in leaf area index, is another important way to improve crop photosynthesis and

hence biomass production (Richards, 2000). Previous studies have demonstrated that nitrogen nutrition management is of great significance to delay leaf senescence and prolong photosynthetic duration, which is associated with a longer grain filling duration and higher yields (Richards, 2000; Zakari et al., 2020). Yi et al. (2020) reported that delayed tillering fertilization increased the leaf area index after tillering and improved the canopy's photosynthetic capacity, thus promoting biomass production. Many studies have reported that N deficiency decreased the chlorophyll content and Pn rate in crop leaves, while sufficient N supply extended the longevity of functional leaves, hence the photosynthetic capacity and grain yield improved. Similarly, in this study, the chlorophyll content and Pn increased with the increase of N fertilizer application amount. While at the high N4 level, the leaf area decreased rapidly at late growth period. The duration of leaf photosynthetic activity shortened because of decreased light interception and the grain yield decreased. Furthermore, our results also showed that under the N5 treatment, the green leaf area maintained longer, the leaf area degradation after anthesis was slower, and the photosynthetic potential was higher, which were all beneficial to rice photosynthesis, thus increasing grain yield. These results suggested that it is vitally important that photosynthesis is enhanced during the grain filling stage aiming to increase grain yield by delaying leaf senescence and extending photosynthetic duration (Deng et al., 2015; Gaju et al., 2014).

The dry matter production of rice plants is a vitally important factor that determines the formation of rice grain yield (Ye et al., 2013). Dry matter production is the result of the accumulation and translocation of photosynthate in different plant organs, which is significantly affected by nitrogen management (Deng et al., 2015; Penget al., 2007; Qiao et al., 2013). N application could regulate stem-sheath quality to facilitate the transportation of photoassimilates to the grain sink, leading to an acceleration of the seed-setting rate and enhancement of grain weight (Yang et al., 2019). A large amount of N application at the tillering stage increases tillers but decreases the ear-bearing tiller rate. Excessive tillers in the early growth stage decrease the dry weight of a single stem. Furthermore, Peng et al. (2007) found that the ear-bearing tiller rate was significantly correlated with dry matter weight at early panicle initiation or its ratio to dry matter at the flowering stage. In the present study, there was no significant difference in the dry matter weight of a single stem among the different nitrogen application treatments at the jointing stage. The dry matter weight of a single stem of rice at the filling stage and maturity stage decreased significantly with increasing nitrogen application amount, mainly because the excessive tillers resulted in a excessively large population of rice and could not promote large panicles at booting.

Increasing the dry matter accumulation at maturity and improving the contribution of post-anthesis dry matter to grain can increase the grain yield (Man et al., 2015; Zhang et al., 2008). A higher photosynthesis rate post-anthesis, particularly at the middle and later grain filling stages promoted post-anthesis dry matter accumulation and grain filling, and hence increased the grain yield. In this study, Shendao47, Shendao6, and Shendao11 obtained highest grain yield under the N5 treatment and Shendao7 reached the highest grain yield under the N3 treatment, which was attributed to higher dry matter accumulation and post-anthesis dry matter accumulation. The results were in accordance with the findings of Deng et al. (2015), who reported that grain yield was significantly positively correlated with dry matter accumulation after the panicle initiation stage. Generally, high grain yield is based on abundant biomass yield, and grain yield is determined by the redistribution of dry matter accumulation in the vegetative organs pre-anthesis and the distribution of photosynthate post-anthesis. Optimal nitrogen application amounts and rational N application timing would be beneficial to improving rice population quality, and increasing dry matter accumulation. All the results suggest that split N fertilization could delay leaf senescence during the late stage (Gaju et al., 2014), improve rice population quality (Peng et al., 2007), and promote dry matter accumulation post-anthesis (Deng et al., 2015), which could increase the grain weight, filled grain percentage, and grain yield.

Conclusions

Optimal N fertilization management increased grain yield. The maximum yields of the four tested cultivars were appeared at the 195 kg ha^{-1} N application level, which can be used as a reference N application rate for high-yield rice production. The nitrogen treatments had a smaller effect on the number of spikelets per panicle; but more influence on the number of panicles and seed-setting rate and eventually grain yield. N fertilizer application increased the chlorophyll content and Pn. High yield was obtained due to more effective panicles, more spikelets per panicle and a high seed-setting rate at N5. Under the N5 treatment, the green leaf area maintained longer, the leaf area degradation after anthesis was slower, the photosynthetic potential was higher, and the dry matter accumulation post-anthesis was increased, which were all beneficial to rice photosynthesis, thus increasing grain yield. Our study identified a novel "split" and "balanced" nitrogen fertilization strategy that is advantageous to: yield formation by increasing the number of spikelets and 1000-grains weight; the photosynthetic potential by increasing the leaf area index and accumulation of photoassimilates, and the nitrogen cost by decreasing the number of ineffective tillers.

Declarations

Conflict of interest The authors declare no conflict of interest.

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