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RESEARCH

Effects of Dense Planting with Less Nitrogen Fertilization on Rice Yield and Nitrogen Use Efficiency in Northeast China

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Abstract

The mode of sparse planting with high N(nitrogen) application in Northeast China is not conducive to high and stable yields nor efficient use of resources. This study investigated if dense planting with less N fertilization in rice could improve nitrogen use efficiency (NUE) and increase yield in Northeast China. we conducted a field experiment using Jijing 88 and Tonghe 99 as planting material and setting three cropping modes of dense planting with less N (T1: 30 cm × 13.3 cm, 200 kg·ha^{-1}; T2: 25 cm × 13.3 cm, 175 kg·ha^{-1}; T3: 25 cm × 10 cm, 150 kg·ha^{-1}) and a local conventional cropping pattern (CK: 30 cm × 16.5 cm, 225 kg·ha^{-1}). The results showed that, when compared to CK, dense planting with less N application (T1, T2) increased the maximum tiller number, above-ground biomass and obtained higher panicle number per m^{-2} and grain filling rate. The maximum yields of Jijing 88 occurred with T1, which increased by 1.29% on average compared to CK; The maximum yields of Tonghe 99 occurred with T2, which increased by 3.50% on average compared to CK. The T1and T2 increased apparent recovery efficiency of nitrogen fertilizer (RE$_N$), agronomic nitrogen use efficiency (AE$_N$), internal nitrogen use efficiency (IE$_N$) compared to CK. Together, reducing N rate from 225 kg·ha^{-1} (CK) to 175 kg·ha^{-1} (T2) could obtained resulted generally equal or higher grain yield and higher NUE with increasing transplanting density from 30 cm × 16.5 cm (CK) to 25 cm × 13.3 cm (T2), and dense planting is a feasible strategy to reduce N input in Northeast China.

Keywords Rice · Dense planting · N application · Yield · NUE

Introduction

Rice is one of the important food crops in China, and increasing the yield has always been the main objective of rice breeding and cultivation. The yield of rice depends on the variety, environmental conditions, cultivation management, and the interactions between these three factors. It is particularly important to develop the yield potential of existing rice varieties through cultivation management measures when faced with the possibility of less available land and resource shortages due to a large population (Tilman et al., 2011). Northeast China is one of the most important Chinese rice cropping regions. Enhancing rice yield in Northeast China is the main strategy to increase the country's total rice yield and ensure its food security.

For rice production in Northeast China, considering the low temperature in the early growth stage, the cultivation model of sparse planting generally promotes an increase in late-growing tillers, delays the emergence time of population peak seedling number (Lin et al., 2011), and extends the tillering and growth periods, ultimately increases the

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probability of cold injury; on the other hand, the cultivation model of sparse planting and high nitrogen(N) may cause lodging and frequent occurrence of diseases and insect pests (Cu et al., 1996; Ye et al., 2012), adversely affect the improvement of rice yield. Sparse planting has become an important limiting factor in obtaining a stable and high yield of rice as well as nitrogen use efficiency (NUE) in Northeast China (Chen et al., 2014; Wu et al., 2013). Therefore, based on the existing mode of sparse planting, a moderate increase in transplanting density may be more conducive to achieve stable and high yields of rice in cold regions (Huang et al., 2013; Ma et al., 2013; Zhao et al., 2013a, 2013b).

The N is an important nutrient necessary for rice growth. Reasonable application of N fertilizer is an important cultivation measure to create high-yield rice populations and increase rice yield (Liu et al., 2016). In the past thirty years, N fertilizer has contributed significantly to the improvement of rice yield in China (Ju et al., 2015; Peng et al., 2010; Zhang et al., 2012a, 2012b). In Changchun, China, temperature rises slowly in spring, and the summer from July to August is the key period for rice growth, but weather is often rainy, with low temperature and light conditions, with the temperature dropping in early autumn (Liu et al., 2014). Such climatic conditions have a certain impact on the amount of N fertilizer operation in rice production. Therefore, improving the management of N fertilizer, using a relatively small amount of N to obtain a relatively high yield and achieving the coordination and unification of high yield and NUE, will be the fundamental in obtaining high-quality populations and improving NUE in rice field.

From the perspective of stable yield and improving NUE in rice field based on current sparse planting, dense planting with less N application may be beneficial in achieving high yields of rice and NUE in its cultivation in Northeast China. Strategies involving increased planting density and reducing the amount of N fertilizer to achieve high rice yields have attracted the attention of researchers (Chen et al., 2014; Mahamud et al., 2013; Zeng et al., 2012; Zhao et al., 2013a, 2013b). However, these studies generally integrate planting density or N reduction with other cultivation measures, but there are no systematic studies on the effect of dense planting with reduced basal N application on yield. Therefore, we conducted a field experiment using a local conventional cultivation mode (CK) and three treatments of dense planting with less N application in Changchun, Jilin province, China from 2017 to 2018. To provide a theoretical and practical basis for the establishment of high-yield and high-efficiency nutrient utilization cultivation systems, the growth and development characteristics, population quality, nutrient utilization efficiency were compared and analyzed.

Materials and Methods

Site Description

Field experiments were conducted from 2017 to 2019 in the rice experimental base. Soil characteristics for the 0–20 cm layer were 6.6 pH, 12.98 g·kg^{-1}soil organic matter, 0.38 g·kg^{-1} total N, 148.7 mg·kg^{-1} available N. The climate is a typical northern temperate continental monsoon climate with a mean annual temperature of about 5.27 °C and precipitation of 659.62 mm, and an average active accumulated temperature of 3289.1 °C and a frost-free period of 138 days from 2013 to 2017.

Experiment Design

The experiment had a completely randomized block design with three replications. Each plot was 24 m^2 in size. Two popular rice varieties with similar growth periods, Jijing 88 and Tonghe 99 were selected. Five cropping modes were involved in this experiment, including a local traditional cropping pattern (CK) and three cropping modes of dense planting with less N and non-N (N0) treatment (see Table 1). Dense planting was implied by increasing seedlings per hill, and a reduction in the total amount of N applied. The common planting density was 3 seedlings per hill. The application ratio of basal fertilizer/tiller fertilizer/ panicle fertilizer = 4:3:3. Base fertilizer and tiller fertilizer were applied 3 days before rice transplanting and on day 7 after rice transplanting, and panicle fertilizer was applied at panicle initiation. Phosphate fertilizer used was superphosphate, and the application rate was 50 kg P_2O_5 hm^{-2}; potassium fertilizer used was potassium chloride, the application rate was 75 kg K_2O hm^{-2}. Other management measures were carried out following local high-yield cultivation technology regulations. The detailed information about the planting density and N application was listed in Table 1.

Table 1 Planting density and N fertilization application amount and timing for each treatment

Treatment	N rate and timing (kg·ha^{-1})				Planting density
	Total	Basal	Tillering	Panicle initiation	
N0	0	0	0	0	30 cm × 16.5 cm
CK	225	90	67.5	67.5	30 cm × 16.5 cm
T1	200	80	60	60	30 cm × 13.3 cm
T2	175	70	52.5	52.5	25 cm × 13.3 cm
T3	150	60	45	45	25 cm × 10 cm

Sampling and Measurement

Determination of Maximum Tiller Number

After the rice was transplanted, two spots were designated for each plot, and 5 holes were marked in each spot to record the tiller dynamics. The investigation was stopped until the number of tillers decreased twice and the maximum number of tillers was recorded.

Determination of SPAD-Chlorophyll Values

For the determination of soil and plant analyzer development (SPAD) -chlorophyll value of the top leaf of rice, five rice plants were randomly selected from each plot at heading, filling and maturity stages. The leaf area of the five sampled plants was measured using a CCM-200 Plus Chlorophyll Content Meter.

Determination of Photosynthesis Rate

For measuring the net photosynthesis rate (Pn), five representative plants were selected from each treatment and the Pn values of top leaf was measured using a portable LI-6400 instrument (LI-COR Inc., Lincoln, NE, USA) at heading, filling and maturity stages. The measurements were carried out on a clear and sunny day from 9:00 to 11:00 a.m.

Dry Matter Weight and NUE

Five holes of representative plants were chosen in each plot at tillering, heading, and maturity stages according to the average number of stems and tillers in each plot, and fresh samples were oven-dried at 105 °C for 30 min and then at 80 °C to constant weight to determine the dry matter weight. Their total N content of the samples at maturity were determined by the Kjeldahl method using the Kjeldahl N determination apparatus (Kjel—tec8200, FOSS, Sweden).

Determination of Nitrate Reductase (NR) Activity and Glutamine Synthetase(GS) Activity

The top leaf of three plants were selected at at heading, filling and maturity stages. Some of leaves segments (middle position) were immediately frozen in liquid N_2 and then stored at – 80 °C for the determination of NR activity and GS activity. The NR activity was assayed using the standard protocol of Ali et al. (2007), The extraction for determination of GS enzymatic activity was assayed by the method of Lutt et al. (1999).

Yield and Yield Components

At harvest, 20 hills were sampled for each plot to determine the actual yield and yield components, which include the effective panicle number, spikelets per panicle, the number of real grains, seed-setting rate, and 1000- grain weight.

Calculations and Statistical Analysis

Apparent recovery efficiency of nitrogen fertilizer: (RE_N, %) = (the total N uptake in the N application area – the total N uptake in the area without N application)/the amount of N application × 100.

Internal nitrogen use efficiency: (IE_N, $kg{\cdot}kg^{-1}$) = the rice yield in the N application area /the total N uptake.

Agronomic nitrogen use efficiency: (AE_N, $kg{\cdot}kg^{-1}$) = (the rice yield in the N application area – the rice yield in the area without N application)/the amount of applied $N \times 100$.

The methods for calculating NUE were according to Ju et al. (2015).

All data were analyzed using Excel 2007 and SPSS version 16.0. There were three replicates in each experimental field. Differences between means were compared by analysis of variance (ANOVA). The means were tested with the least significance difference test at $P < 0.05$.

Results

Maximum Tiller Number(m^{-2}), Panicle Rate, and Above-Ground Biomass

Compared to CK, with increasing planting density and the reduction in N rate, the maximum tiller number of the rice population increased firstly and then decreased, and the panicle rate decreased (Table 2). Compared to CK, the T1 increased maximum tiller number by 14.0% (2017), 13.6% (2018) for Jijing 88 and 17.1% (2017), 18.8% (2018) for Tonghe 99; the T2 increased maximum tiller numbers of rice population by 15.8% (2017) and 14.9% (2018) for Jijing 88 and by 23.7% (2017) and 31.5% (2018) for Tonghe 99; the T3 decreased maximum tiller numbers for Jijing 88, but increased maximum tiller numbers by 21.8% (2017) and 29.0% (2018) for Tonghe 99. The maximum tiller number were found in T2 in both years, and no significant difference in maximum tiller number was found between T2 and T1. With increasing planting density and the reduction in N rate, the above-ground biomass of rice population increased firstly and then decreased compared to CK. The T1, and T2, the T3 significantly decreased above-ground biomass of rice

Table 2 Maximum tiller number, panicle rate, and above-ground biomass of each rice variety under different treatments

Year	Variety	Treatment	Maximum tiller number (m^{-2})	Panicle rate (%)	Above-ground biomass(kg·ha^{-1})		
					Tillering stage	Heading stage	Maturity stage
2017	Jijing 88	CK	570 b	77.4 a	173.7 a	833.9 a	1704.8 a
		T 1	650 a	76.6 a	180.8 a	847.8 a	1731.3 a
		T 2	660 a	67.9 a	183.5 a	837.2 a	1665.3 a
		T 3	560 b	65.4 a	157.2 b	788.2 a	1287.9 b
	Tonghe 99	CK	427 b	73.3 a	164.2 a	799.3 a	1649.8 a
		T 1	500 a	72.5 a	167.1 a	845.8 a	1706.5 a
		T 2	528 a	68.6 a	175.9 a	864.4 a	1743.6 a
		T 3	520 a	66.1 a	147.0 b	774.8 a	1371.5 b
2018	Jijing 88	CK	550 b	74.6 a	169.4 a	816.4 a	1605.1 a
		T 1	625 a	65.4 a	180.1 a	847.8 a	1729.3 a
		T 2	632 a	62.4 a	181.4 a	837.2 a	1665.4 a
		T 3	545 b	60.1 a	150.3 b	771.7 a	1210.1 b
	Tonghe 99	CK	400 b	72.0 a	160.4 a	753.6 a	1605.1 a
		T 1	475 a	69.7 a	160.8 a	830.3 a	1644.7 a
		T 2	526 a	61.9 a	172.8 a	843.8 a	1714.5 a
		T 3	516 a	58.3 a	138.3 b	728.0 a	1354.4 b

CK, 30 cm × 16.5 cm; 225 kg·ha^{-1}; T1, 30 cm × 13.3 cm; 200 kg·ha^{-1}; T2, 25 cm × 13.3 cm; 175 kg·ha^{-1}; T3, 25 cm × 10 cm; 150 kg·ha^{-1}.Values sharing same letters differ non-significantly ($P < 0.05$)

population at tillering and maturity stages, then, there were no significant different between T1, T2 and CK.

SPAD-Chlorophyll Values and Photosynthesis Rate

Compared to CK, with increasing planting density and the reduction in N rate, SPAD-chlorophyll values of the top leaf and photosynthesis rate decreased at heading, filling and maturity stages. Compared to CK, T1, and T2, the T3 significantly decreased SPAD-chlorophyll values of the top leaf for Jijing 88 and Tonghe 99 at heading, filling and maturity stages, however, there was no significant difference between T1, T2 and CK(Fig. 1). Compared to CK, T1, and T2, the T3 significantly decreased photosynthesis rate for Jijing 88 and Tonghe 99 at heading, filling and maturity stages, however, there was no significant difference between T1, T2 and CK (Fig. 2).

NR Activity and GS Activity

Compared to CK, with increasing planting density and the reduction in N rate, NR activity in the top leaf of Jijing 88 and Tonghe 99 decreased from heading to maturity stage, and compared to CK, T1, and T2, the T3 decreased NR activity in the top leaf for Jijing 88 and Tonghe 99 at heading, filling and maturity stages. Compared to CK, T1, and T2, the T3 significantly decreased GS activity in the top leaf for Jijing 88 and Tonghe 99 at heading, filling and maturity stages (Fig. 3).

Yields and Yield Components

Compared to the CK, with increasing planting density and reduction in basal N rate, the rice yield increased firstly and then decreased. Across three study years, the highest yield of Jijing 88 was found in T1, increased by 1.36%(2017),1.31% (2018) and 1.20% (2019), respectively, as compared to CK. the highest yield of Tonghe 99 was found in T2, increased by 3.68% (2017), 3.15% (2018) and 3.66% (2019), respectively, as compared to CK. However, no significant difference was found between T1, T2 and CK. The rice yield in T3 of two varieties were lower than for other treatment across three study years (Table 3).

Although the panicle number of rice population between the treatments varied with the tested varieties and years, a similar trend for panicle number per m^{-2} of dense planting with less N treatments was found (Table 3). Compared to CK, with increasing planting density and the reduction in N rate, panicle number per m^{-2} firstly increased and then decreased. Panicle number per m^{-2} of Jijing 88 was T1 > T2 > CK > T3, and the T1 increased by 2.80% on average compared to CK, but no significant difference was found between T1, T2 and CK. The T3 decreased panicle number per m^{-2} compared to T1, T2, and CK; the panicle number per m^{-2} of Tonghe 99 was T2 > T1 > T3 > CK, and the T2 increased by 18.36% on average compared to CK, but there was no significant difference between treatments.

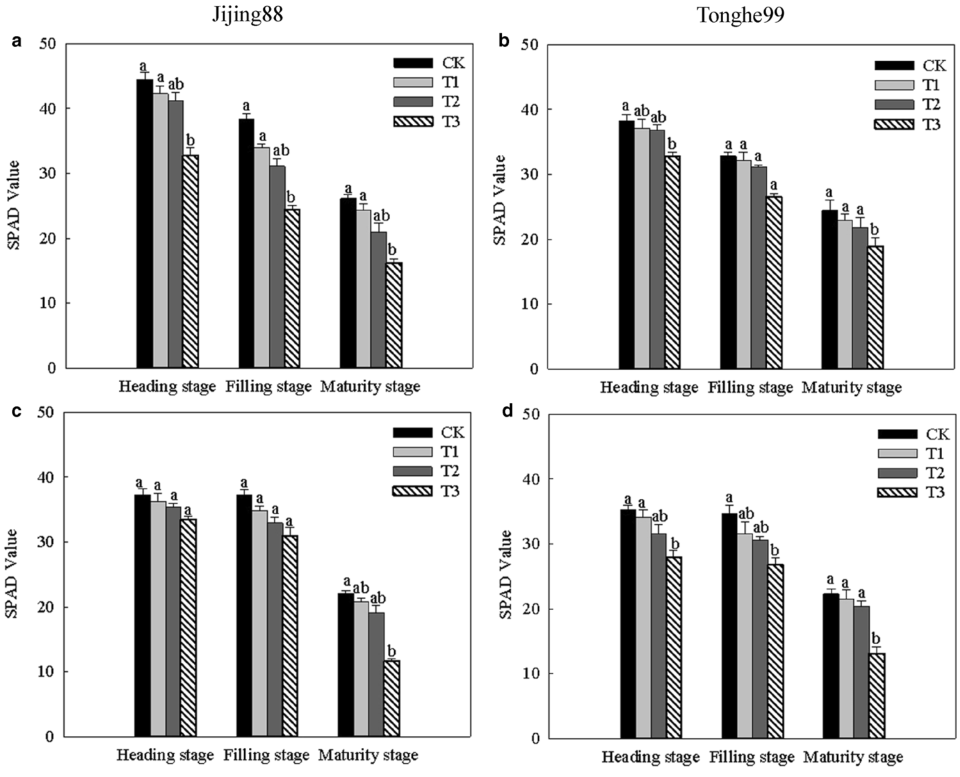

Fig. 1 effect of dense planting with less N application on top leaf of SPAD-chlorophyll values at different growth stages of Jijing88 and Tonghe99 in 2018 and 2019. **a**, **b**, 2017; **c**, **d**, 2018; CK, 30 cm × 16.5 cm; 225 kg·ha^{-1}; T1, 30 cm × 13.3 cm; 200 kg·ha^{-1}; T2, 25 cm × 13.3 cm; 175 kg·ha^{-1}; T3, 25 cm × 10 cm; 150 kg·ha^{-1}. Different letters within a growth stage indicate significant difference at $P < 0.05$

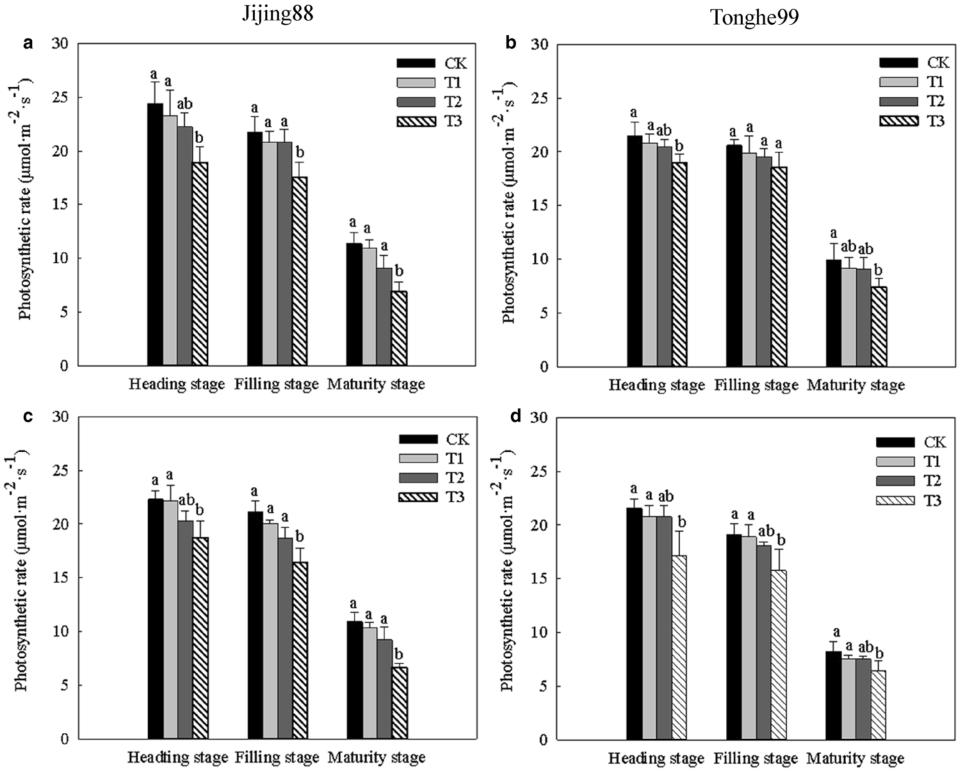

Fig. 2 effect of dense planting with less N application on top leaf of photosynthetic rate at different growth stages of Jijing88 and Tonghe99 in 2017 and 2018. **a**, **b**, 2017; **c**, **d**, 2018; CK, 30 cm × 16.5 cm; 225 kg·ha^{-1}; T1, 30 cm × 13.3 cm; 200 kg·ha^{-1}; T2, 25 cm × 13.3 cm; 175 kg·ha^{-1}; T3, 25 cm × 10 cm; 150 kg·ha^{-1}. Different letters within a growth stage indicate significant difference at $P < 0.05$

Compared to CK, with increasing planting density and the reduction in N rate, the number of spikelets per panicle decreased, the T1, T2, and T3 decreased spikelets per panicle by 5.40%, 17.65%, and 30.72% on average for Jijing 88, the T1, T2, and T3 decreased spikelets per panicle by 5.67%, 12.41%, and 31.65% on average for Tonghe 99. No

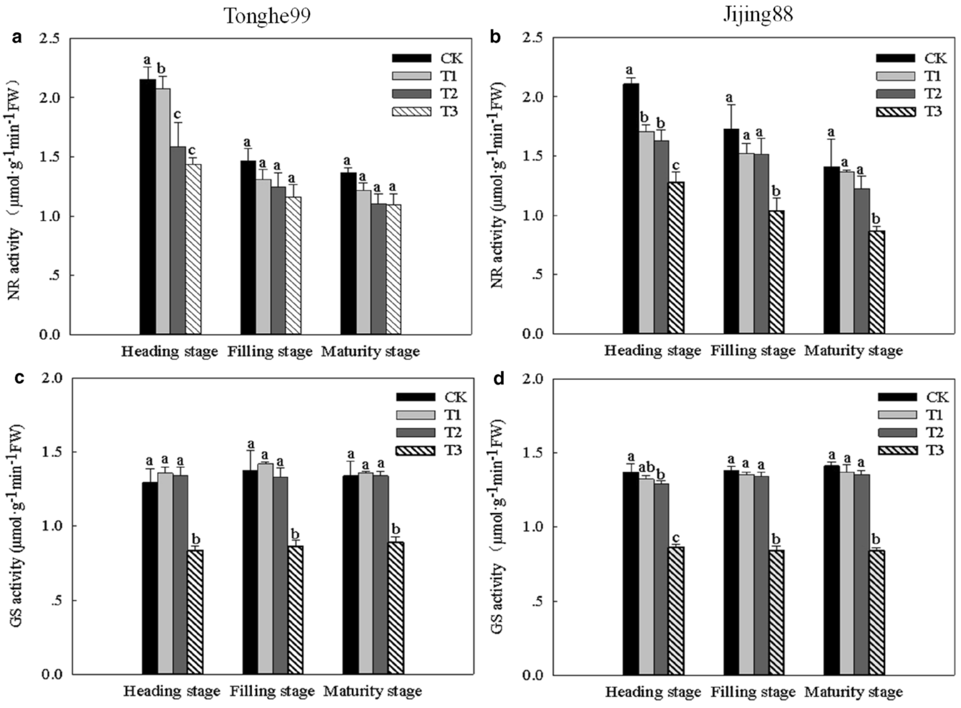

Fig. 3 effect of Dense planting with less N application on top leaf of NR activity and GS activity at different growth stages of Jijing88 and Tonghe99 in 2019. **a**, **b**, NR activity; **c**, **d**, GS activity; CK, 30 cm × 16.5 cm; 225 kg·ha^{-1}; T1, 30 cm × 13.3 cm; 200 kg·ha^{-1}; T2, 25 cm × 13.3 cm; 175 kg·ha^{-1}; T3, 25 cm × 10 cm; 150 kg·ha^{-1}. Different letters within a growth stage indicate significant difference at $P < 0.05$

Table 3 Differences in yields and yield components of rice under different treatments

Year	Variety	Treatment	Yield (kg·ha^{-1})	Panicles (/m^2)	Spikelets panicle^{-1}	Grain filling (%)	1000-grain-weight (g)
2017	Jijing 88	CK	9506.5 a	441.7 a	147.2 a	82.2 b	21.8 a
		T 1	9635.5 a	455.1 a	138.0 a	87.2 a	21.5 a
		T 2	9445.3 a	453.3 a	117.3 b	84.8 a	21.4 a
		T 3	8428.0 b	353.3 b	101.0 c	82.5 b	21.8 a
	Tonghe 99	CK	9394.2 a	321.1 a	182.0 a	82.7 b	23.0 a
		T 1	9553.2 a	362.5 a	170.3 a	84.0 ab	22.0 a
		T 2	9739.7 a	366.7 a	156.7 b	85.0 ab	23.4 a
		T 3	8349.1 b	345.2 a	121.0 c	85.6 a	23.3 a
2018	Jijing 88	CK	9451.5 a	430.3 a	142.4 a	82.4 b	21.7 a
		T 1	9574.9 a	440.1 a	133.7 a	87.1 a	21.8 a
		T 2	9363.1 a	437.1 a	116.5 b	84.5 a	21.5 a
		T 3	8243.7 b	352.5 b	100.2 c	82.6 b	21.9 a
	Tonghe 99	CK	9253.2 a	312.9 a	188.7 a	82.1 b	23.3 a
		T 1	9358.3 a	355.6 a	172.3 a	83.5 ab	23.2 a
		T 2	9544.4 a	357.5 a	165.3 b	84.7 ab	22.8 a
		T 3	8224.7 b	333.7 a	119.6 c	84.6 a	23.4 a
2019	Jijing 88	CK	8996.3 a	413.6 a	132.9 a	80.1 b	21.3 a
		T 1	9104.7a	426.4 a	127.8 a	86.2 a	21.6 a
		T 2	8907.2 a	423.3 a	113.7 b	85.2 a	21.4 a
		T 3	7891.0 b	311.1 b	91.5 c	81.0 b	21.6 a
	Tonghe 99	CK	8577.9 a	294.4 a	164.0 a	80.3 b	23.7 a
		T 1	8721.5 a	302.8 a	160.9 a	82.7 ab	23.3 a
		T 2	8891.6 a	321.7 a	146.1 b	85.4 ab	23.3 a
		T 3	7332.6 b	300.6 a	123.3 c	86.5 a	23.9 a

CK, 30 cm × 16.5 cm; 225 kg·ha^{-1}; T1, 30 cm × 13.3 cm; 200 kg·ha^{-1}; T2, 25 cm × 13.3 cm; 175 kg·ha^{-1}; T3, 25 cm × 10 cm; 150 kg·ha^{-1}.Values sharing same letters differ non-significantly ($P < 0.05$)

significant difference was found between T1 and CK, however, spikelets per panicle in T2 and T3 were significantly lower than CK.

Compared to CK, dense planting with less N application increased the grain filling, the T1, T2, and T3 increased grain filling by 6.47%, 4.02%, and 0.6% on average for Jijing 88, the T1, T2, and T3 decreased spikelets per panicle by 2.01%, 4.10%, and 4.76% on average for Tonghe 99. No significant difference was found between T1 and T2. Dense planting with less N application had no significant effect on the 1000-grain weight.

NUE

Dense planting with less N application application could increase NUE in rice field (Table 4). Compared to CK, the T1, T2 and T3 increased RE_N by 5.43%, 6.23%, 19.24% and 5.9%, 18.33%, 21.80% on average for Jijing 88 and Tonghe 99; significantly increased AE_N by 14.42%, 26.29%, 12.83% and 16.32%, 38.36%, 10.90% on average for Jijing 88 and Tonghe 99; significantly increased IE_N by 13.92%, 27.43%, 31.77% and 14.22%, 33.06%, 31.64% on average,for Jijing 88 and Tonghe 99, respectively.

Table 4 Apparent recovery efficiency of nitrogen fertilizer, agronomic nitrogen use efficiency and internal nitrogen use efficiency of each rice variety under different treatments

Year	Variety	Treatment	RE_N (%)	AE_N ($kg \cdot kg^{-1}$)	IE_N ($kg \cdot kg^{-1}$)
2017	Jijing 88	CK	35.2 c	21.1 c	42.3 d
		T1	41.5 bc	24.4 b	48.2 c
		T2	42.0 b	26.8 a	54.0 b
		T3	56.2 a	24.5 b	56.2 a
	Tonghe 99	CK	26.7 b	20.4 c	41.8 c
		T1	34.2 b	23.7 b	47.8 b
		T2	49.4 a	28.2 a	55.7 a
		T3	53.5 a	23.6 b	55.7 a
2018	Jijing 88	CK	34.5 c	21.2 c	42.0 d
		T1	40.7 bc	23.8 b	47.9 c
		T2	40.8 b	26.7 a	53.5 b
		T3	54.2 a	23.7 b	55.0 a
	Tonghe 99	CK	26.6 b	20.1 c	41.1 c
		T1	33.8 b	23.2 b	46.8 b
		T2	48.6 a	27.6 a	54.5 a
		T3	52.0 a	23.4 b	54.8 a
2019	Jijing 88	CK	31.8 c	18.9 c	40.0 d
		T1	35.6 bc	21.8 b	45.5 c
		T2	37.4 b	23.8 a	50.9 b
		T3	48.8 a	20.9 b	52.6 a
	Tonghe 99	CK	28.1 b	16.7 c	38.1 c
		T1	31.1 b	19.6 b	43.6 b
		T2	38.4 a	23.3 a	50.8 a
		T3	41.3 a	16.8 c	48.9 a

RE_N apparent recovery efficiency of nitrogen fertilizer, AE_N agronomic nitrogen use efficiency, IE_N internal nitrogen use efficiency, *CK* 30 cm × 16.5 cm; 225 $kg \cdot ha^{-1}$, *T1* 30 cm × 13.3 cm; 200 $kg \cdot ha^{-1}$, *T2* 25 cm × 13.3 cm, *175* $kg \cdot ha^{-1}$, *T3* 25 cm × 10 cm; 150 $kg \cdot ha^{-1}$

Values sharing same letters differ non-significantly ($P < 0.05$)

Discussion

Reducing external inputs of labor and N fertilizer, is a trend to rice production in China. Dense planting may be a recommended as a good cropping method to reduce N input in rice production, and little work has been reported to demonstrate its feasibility in rice. This study was conducted to determine the effects of dense planting with less N application on yield attributes and NUE. Across three study years, our results showed that reducing N rate from 225 to 150 $kg \cdot ha^{-1}$ resulted in significantly lower grain yield with a hill density of 25 cm × 10 cm (T3), however, rice yield were generally equal or higher under dense planting with less N application (T1, T2) than under CK (Table 3), indicates that moderately dense planting can reduce N input without sacrificing rice yield. This is consistent with that observed in rice (Zhu et al., 2016).

Significance for high yields is taking reasonable cultivation measures to improve dry matter accumulation and coordinate the relationship between individuals and populations in the middle and late growth stages (Fageria, 2007). Panicles number per unit area, spikelets per panicle, seed setting rate, and 1000-grain weight are directly related to rice yield (Sui et al., 2013; Zeng et al., 2012). Panicles number per unit area has mainly been determined by the maximum number of tillers before the jointing–booting stage and the nutritional level after the jointing–booting stage, and the grain filling formed after the jointing–booting stage. Density is the main factor for affecting the number of basic seedings and rice tillers, increasing the transplanting density might increase the number of stem tillers (Fan et al., 2012; Huang et al., 2018). Reducing the amount of N fertilizer might control the tiller number of the plant population (Yoshida., 1981; Zhong et al., 2003), and N reduction in the early stage could reduce ineffective tillers, which could reduce mutual shading among rice plants and is benefit to the biomass accumulation for grain filling(Zeng et al., 2012).In this study, the T1 and T2 significantly increased maximum tiller number per m^{-2} with unchanged panicle rate, and SPAD-chlorophyll values of the top leaf and photosynthesis rate at heading, filling and maturity stages had no significant difference over CK(Fig. 1).Thereby, equal or higher aboveground biomass, panicle number per m-2 and grain filling rate were found in T1 and T2 compared to the

CK, suggesting that the lower grain yield caused by reducing N rate was compensated for by increasing density.

At the same time, we also found that the response of the maximum tiller number of different varieties to the mode of dense planting with less N were different. Under the mode of dense planting with less N, the increase in the maximum tiller number for Jijing 88 was smaller than that of Tonghe 99 (Table 2). The tillering ability for Jijing 88 was stronger, and the effect of N application on tillering was strong (Dobermann, 2004), and the compensation effect of planting density on tiller number was also relatively small. However, Tonghe 99 belongs to the middle tillering ability variety, and the promotion effect of *N* application on its tillering was relatively small, so the compensation effect of planting density on the number of tillers was relatively enhanced, thereby, the maximum yields of Jijing 88 occurred with *T*, while the maximum yields of Tonghe 99 occurred with T2. Therefore, dense planting with less N application should be characterized in a quantified manner according to variety characteristics.

At present, the widely used sparse planting and high N cultivation mode had a high NUE in the early growth stage of rice (Huang et al., 2013). However, due to the low number of basic seedlings in the early stage and the weak N absorption capacity of rice plants in the early growth stage, a large amount of N was lost (Peng & Cassman, 1998; Peng et al., 2002). The activities of NR and GS are closely associated with plant N uptake (Li et al., 2018; Zhang et al., 2019). N application at panicle initiation is necessary to maintain NR and GS activities in the reproductive stage (Zhang, et al., 2013). In this study, there were no significant difference in NR activity and GS activity of the top leaf of rice at the filling stage and maturity stage between dense planting with less N application (T1 and T2) and CK. Moderate dense planting with less N application (T1 and T2) increased RE_N, AE_N, IE_N (Bhatia et al., 2012; Peng et al., 2010; Rehman et al., 2013; Sui et al., 2013; Pittelkow et al., 2013; Zhang et al., 2018). Even though RE_N, AE_N, IE_N in T3 was significantly higher than that of CK, excessive reduction in N application could cause decrease in rice yield.

Resent years, in rice production, the farmers basically follow the traditional management mode for field management (Huang & Zou, 2016). our results showed that dense planting is a recommended strategy to reduce N input (Huang et al., 2013), and the strategy may be more practical for machine-transplanted rice, because high planting density can be achieved easily with less labor by using machine transplanting. It can save economic costs, although the machine needs to be adjusted in the process of planting to save economic costs, so farmers will be willing to accept it.

Conclusions

In summary, compared with the conventional high-yield model, reducing N rate from 225 $kg{\cdot}ha^{-1}$ (CK) to 175 $kg{\cdot}ha^{-1}$(T2) could obtained resulted generally equal or higher grain yield and higher NUE with increasing transplanting density from 30 cm × 16.5 cm (CK) to 25 cm × 13.3 cm(T2). Dense planting with less N application is a comprehensive cultivation technology mode which should be combined and tested with a variety of characteristics, local climate conditions, and other factors. In production, we should also pay attention to preventing late lodging, premature aging caused by insufficient nutrients, and the occurrence of diseases and insect pests. Therefore, it is necessary to adjust measures to local conditions and varieties and combine with high-yield cultivation techniques and integrated pest control techniques to optimize field configuration and build a suitable population.

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Author Contributions XS and YG planned the experiments, SB and YL interpreted the results, QZ statistically analyzed the data and made illustrations, LG and WL made the write up. All authors discussed the results and commented on the manuscript. All authors have read and approved the final manuscript.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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