

SCIENTIFIC REPORTS



OPEN

Potential for high yield with increased seedling density and decreased N fertilizer application under seedling-throwing rice cultivation

Yang Liu^{1,2}, Chao Li⁴, Baohua Fang¹, Yong Fang⁵, Kailin Chen¹, Yuzhu Zhang³ & Haiqing Zhang²

Fertilizer application for rice production has increased significantly in southern China to raise yields, but has led to problems with lodging, quality decline and environmental pollution. Therefore, research on fertilizer-saving cultivation technologies for high-yielding rice is necessary. A two-factor experiment was conducted to evaluate the effects of seedling-addition treatment (SAT) and nitrogen-saving treatment (NST) on yield formation and nitrogen absorption of individual plants and plant groups under the seedling-throwing cultivation system. Numbers of spikelets per panicle and filled grains per panicle of individual plants declined under decreased nitrogen application, but was compensated by substantially increased effective panicles number and total number of glumous flowers under SAT. Under the optimal SAT–NST treatments of 18% less N fertilizer and 32% additional seedlings, yield increased 1.79% and 4.29% compared with that of conventional practice (CK) in 2015 and 2016, respectively. The mature-stage individual-plant biomass under SAT–NST treatments decreased by 27.46% and 20.49% compared with CK in 2015 and 2016, but plant-group biomass did not differ significantly (all $>16 \text{ t ha}^{-1}$). Under SAT–NST treatments, effective number of panicles was positively correlated with maximum number of seedlings ($r = 0.643$) and N absorption amount in the tillering stage ($r = 0.901$).

Rice (*Oryza sativa* L.) is one of the most important grain crops worldwide, especially in Asia¹. Over the next three decades, population growth and decreasing arable land area will pose a severe challenge to rice production in China, which is projected to use 68 million tons of fertilizer by 2030². To mitigate these problems, China's Ministry of Agriculture proposed guidelines to reduce the use of chemical fertilizers and pesticides to develop sustainable agricultural production. Nitrogen (N) is essential to achieve high rice yields. For example, Zhu *et al.*³ reported that the optimum quantity of N fertilizer to increase grain yield was 225–300 kg ha⁻¹. Fu *et al.*⁴ suggested that the N application rate for super rice should be increased by 12–14% to obtain high yield. These studies show that increases in rice yield rely heavily on excessive application of N fertilizer.

However, excessive N application significantly reduces the quality of rice, and leads to higher N loss and soil acidification^{5–7}. Plant density is one of the main factors that influence yield in rice, together with N availability^{8,9}. Zhu *et al.*¹⁰ observed that a moderate planting density combined with reduced basal application of N fertilizer can lead to rice crops with high yield and high N-use efficiency. Martinez *et al.*¹¹ observed that at high plant densities, phyllochrons were longer, panicle initiation occurred earlier, and the total number of leaves on the main stem decreased.

¹Hunan Rice Research Institute, Hunan Academy of Agricultural Sciences, Changsha, 410125, China. ²Southern Regional Collaborative Innovation Center for Grain and Oil Crops (CICGO), Hunan Agricultural University, Changsha, 410128, China. ³Hunan Hybrid Rice Research Center, Hunan Academy of Agricultural Sciences, Changsha, 410125, China. ⁴Hunan Soil and Fertilizer Research Institute, Hunan Academy of Agricultural Sciences, Changsha, 410125, China. ⁵Institute of Agricultural Biotechnology, Hunan Academy of Agricultural Sciences, Changsha, 410125, China. Yang Liu and Chao Li contributed equally. Correspondence and requests for materials should be addressed to Y.Z. (email: yuzhuzhang@hotmail.com) or H.Z. (email: hunanhongli@aliyun.com)

Year		2015			2016		
Treatment		Yield (t·ha ⁻¹)	Yield increased vs. CK (t·ha ⁻¹)	Increase ratio (%)	Yield (t·ha ⁻¹)	Yield increased vs. CK (t·ha ⁻¹)	Increase ratio (%)
SAT	T1	7.81bc	-0.56	-6.69	7.59b	-0.10	-1.30
	T2	7.85b	-0.52	-6.21	7.40b	-0.29	-3.77
NST	T3	7.84b	-0.53	-6.33	7.35b	-0.34	-4.42
	T4	7.30c	-1.07	-12.78	7.02c	-0.67	-8.71
SAT-NST	T5	8.22ab	-0.15	-1.79	7.59b	-0.10	-1.30
	T6	8.52a	0.15	1.79	8.02a	0.33	4.29
	T7	7.22c	-1.15	-13.74	7.16c	-0.53	-6.89
	T8	7.46c	-0.91	-10.87	7.21bc	-0.48	-6.24
CK		8.37a	—	—	7.69b	—	—

Table 1. Grain yield of early-season rice ‘Xiangwanxian 45’ under nitrogen fertilizer and plant density treatments in 2015 and 2016. Within the row for each location, means followed by the same letters are not significantly different according to LSD at $P = 0.05$.

Seedling-throwing is an alternative planting method for rice production popular in southern China because it may save on labor input and promote high yields. The seedling-throwing cultivation system also has potential benefits for reduction of fertilizer inputs. However, research into this cultivation practice is limited, especially with regard to its N-saving potential^{10,12}. The objectives of the present research were to develop high yield potential in the seedling-throwing rice cultivation system by means of an appropriate increase in seedling density and a concomitant decrease in N fertilizer input, and to explore the mechanisms for high yield and N conservation.

Results

Grain yield and yield components. Averaged across nine treatments, the grain yield were 7.84 t ha⁻¹ in 2015 and 7.45 t ha⁻¹ in 2016 (Table 1). The differences in grain yield among the treatments were significant, although the magnitude of the differences between 2015 and 2016 varied. The grain yield of the CK in 2015 and 2016 was 8.37 t ha⁻¹ and 7.69 t ha⁻¹, respectively. Under seedling-addition treatment (SAT) (T1 and T2) or nitrogen-saving treatment (NST) (T3 and T4) the grain yield was decreased compared with that of the CK: T1 and T2 yields decreased by 1.30–6.69%, and T3 and T4 yields decreased by 4.42–12.78%. The grain yield of combined SAT–NST treatments (T5, T7 and T8) decreased by 1.30–13.74%. But the grain yield of T6 increased by 1.79% and 4.29% compared with that of the CK in 2015 and 2016, respectively, but only the increase in 2016 was significant.

With regard to yield components for plant groups, the total number of spikelets and effective panicle number increased under SAT (T1 and T2) and decreased under NST (T3 and T4). Combined SAT–NST had a significant interaction effect on these two traits. The total number of spikelets in the T6 treatment was the highest among all treatments and exceeded that of the CK by 12.17% in 2015 and 5.82% in 2016. But in the T7 and T8 treatments declined significantly (Tables 2 and 3). The panicle number increased significantly under increased seedling density. Under combined SAT–NST, the panicle number in the T6 treatment was increased by 11.98%, 26.61%, 13.26% and 20.73% compared with that of T5, T7, T8 and CK in 2015 and by 8.30%, 18.22%, 12.91% and 14.76% in 2016, respectively.

With regard to yield components for individual plants, the number of spikelets per panicle and number of filled grains per panicle decreased significantly under SAT (T1 and T2) compared with those of the CK, but differences between NST (T3 and T4) and CK were less distinct. Combined SAT–NST had a significant interaction effect on these two yield components. Under SAT–NST, the number of spikelets per panicle and number of filled grains per panicle were lowest in the T6 treatment. The number of spikelets per panicle was 101.1 in 2015 and 105.4 in 2016, which was 7.00% and 7.78% less than that of the CK, respectively. The number of filled grains per panicle was 81.9 in 2015 and 88.7 in 2016, which was 11.84% and 8.18% less than that of the CK, respectively. SAT significantly affected the percentage of filled spikelets in 2015 and 2016, whereas NST significantly affected 1000-grain weight in 2015 and percentage of filled spikelets in 2016. However, no interaction effect on percentage of filled spikelets and 1000-grain weight under SAT–NST was observed.

Under combined SAT–NST treatment, the number of spikelets per panicle and number of filled grains per panicle decreased, but the total number of glumous flowers was increased mainly because the number of effective panicles greatly increased. The yield of early-season rice was positively correlated with the total number of glumous flowers ($r = 0.837$) and effective panicle number ($r = 0.724$) (Fig. 1A,B). On the other hand, the yield of early-season rice was negatively correlated with the number of spikelets per panicle, number of filled grains per panicle, percentage of filled spikelets and 1000-grain weight (Fig. 2A–D).

Individual and group biomass production. Significant differences in both individual biomass production (IBP) and group biomass production (GBP) under SAT and NST were observed. The interaction between SAT and NST on these traits was significant. The IBP declined under increased seedling density. Under conventional-practice N treatments, the IBP of T1 was higher 1.46 and 3.04 g plant⁻¹ than that of T2 in 2015 and 2016. Under 18% and 36% less N treatments, the IBP of T5 and T7 were higher than that of T6 and T8 respectively. The IBP of combined SAT–NST (T5, T6, T7 and T8) was significantly lower than that of the CK by 16.80–44.24% in 2015 and by 14.66–38.51% in 2016.

Treatment		Total Spikelets (10 ⁷ ·ha ⁻¹)	Panicle No. (10 ⁴ ·ha ⁻¹)	Spikelets per panicle	Filled grains per panicle	Spikelet filling percentage (%)	1000-grain weight (g)
SAT	T1	4.36ab	428.7b	101.6c	81.5d	80.2c	23.6ab
	T2	4.62a	460.6a	100.2c	74.4e	74.3d	23.5ab
NST	T3	4.08bc	374.0c	106.4ab	90.8ab	85.3ab	23.6ab
	T4	3.75c	345.1d	108.7a	94.6a	87.0a	23.2b
SAT-NST	T5	4.29ab	415.5bc	103.2bc	84.6c	82.0b	23.7ab
	T6	4.70a	465.3a	101.1c	81.9d	81.0b	23.2b
	T7	3.82c	367.5cd	103.9b	86.1bc	82.9b	23.6ab
	T8	4.17b	410.8b	101.4c	83.3cd	82.1b	23.4ab
CK		4.19b	385.4c	108.7a	92.9a	85.5ab	23.9a
Analysis of variance							
seedling (S)		*	**	*	**	*	ns
nitrogen (N)		*	*	ns	ns	ns	*
S × N		*	**	*	*	ns	ns

Table 2. Yield components of early-season rice ‘Xiangwanxian 45’ under nitrogen fertilizer and plant density treatments in 2015. Within the row for each location, means followed by the same letters are not significantly different according to LSD at $P = 0.05$. *Significant at the 0.05 level based on analysis of variance. **Significant at the 0.01 level based on analysis of variance. Ns denotes non-significance based on analysis of variance.

Treatment		Total Spikelets (10 ⁷ ·ha ⁻¹)	Panicle No. (10 ⁴ ·ha ⁻¹)	Spikelets per panicle	Filled grains per panicle	Spikelet filling percentage (%)	1000-grain weight (g)
SAT	T1	38.82b	358.8b	108.2b	89.7cd	82.9b	23.6ab
	T2	39.73ab	381.3a	104.2c	84.9e	81.5c	23.3b
NST	T3	36.36c	323.8d	112.3a	94.5b	84.1b	23.3b
	T4	35.69c	314.8e	113.4a	98.3a	86.7a	23.9a
SAT-NST	T5	38.58b	355.3b	108.6b	90.9c	83.6b	23.5ab
	T6	40.55a	384.8a	105.4c	88.7d	84.2b	23.1b
	T7	36.84c	325.5d	113.2a	93.6b	82.7b	23.5ab
	T8	36.39c	340.8bc	106.8bc	90.5c	84.7b	23.4b
CK		38.32b	335.3cd	114.3a	96.6ab	84.5b	23.5ab
Analysis of variance							
seedling (S)		ns	**	*	*	*	ns
nitrogen (N)		**	*	ns	ns	*	ns
S × N		*	**	*	*	ns	ns

Table 3. Yield components of early-season rice ‘Xiangwanxian 45’ under nitrogen fertilizer and plant density treatments in 2016. Within the row for each location, means followed by the same letters are not significantly different according to LSD at $P = 0.05$. *Significant at the 0.05 level based on analysis of variance. **Significant at the 0.01 level based on analysis of variance. Ns denotes non-significance based on analysis of variance.

The differences in GBP among all treatments differed from those observed for IBP. The GBP was elevated with increased seedling density (Table 4). Under conventional N treatments, the GBP of T2 was higher 1.38 and 0.87 t ha⁻¹ than that of T1 in 2015 and 2016. Under 18% and 36% less N treatments, the GBP of T6 and T8 were higher than T5 and T7 respectively. The difference in GBP between combined SAT–NST and the CK was more weakly significant than that observed for IBP, whereas the GBP of T5 and T6 were similar to or surpassed that of the CK.

Discussion

High-yield mechanism. As reported in previous studies^{13,14}, N fertilization and seedling density had no effect on aboveground biomass production. Other authors have concluded that N fertilization is needed to achieve maximum biomass production^{15–17}. In the present study, under the combined SAT–NST treatments the biomass of individual plants declined at the mature stage, but an increase in plant group biomass was achieved through an increase in seedling density, even though N consumption was reduced.

Suitable N application rates and plant spacing in rice fields may improve the growing environment of plants and produce higher grain yield⁹. Under N deficiency, yield components are reduced, especially panicle number per plant and per unit area¹⁸. By analyzing yield components we observed that reduction in the application quantity of N fertilizer changed yield components of individual plants (number of spikelets per panicle and number of filled grains per panicle) insignificantly, whereas a group index (panicle number) was reduced drastically. It is widely accepted that a high yield potential of rice requires a large number of effective panicles^{8,19}. However, the effective panicle number can be increased significantly by means of an appropriate increase in seedling density.

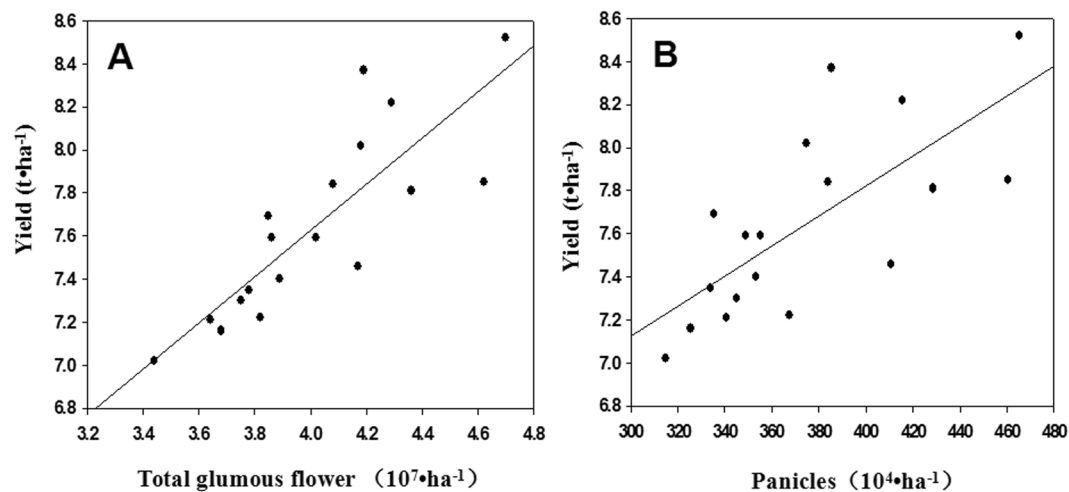


Figure 1. Correlations between total glumous flower, effective panicle number and grain yield. (A) Correlations between total glumous flower and grain yield. (B) Correlations between effective panicle number and grain yield.

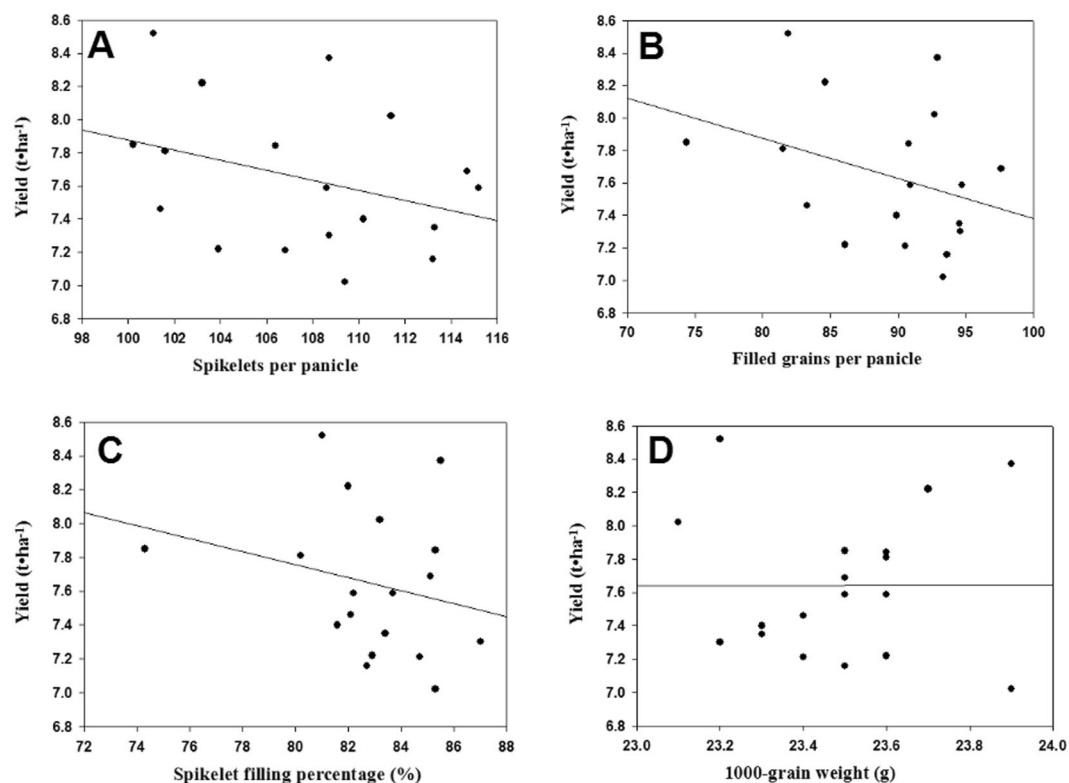


Figure 2. Correlations between spikelets per panicle, filled grains per panicle, spikelet filling percentage 1000-grain weight and grain yield. (A) Correlations between spikelets per panicle and grain yield. (B) Correlations between filled grains per panicle and grain yield. (C) Correlations between spikelet filling percentage and grain yield. (D) Correlations between 1000-grain weight and grain yield.

N-saving mechanism. Given that uniformity of N absorption at different development stages changes rapidly under different cultivation systems, biomass alone or tissue N concentration alone are not reliable indicators of plant N requirements²⁰ and may explain only slightly more than 60% of variability in total N accumulation in rice¹². In the present research, both biomass and tissue N concentration were measured to provide more reliable estimates of plant N absorption regularity under the seedling-throwing cultivation system. The N absorption amount (NAA) in the T1 to T8 treatments was lower than that of the CK by 6.7–34.8% over the entire growth period (Table 5). In addition, NAA was elevated with increase in seedling density and decrease in fertilizer N application rate. The highest N demand by rice plants is during the tillering stage, but a high application rate of N

Treatment		2015		2016	
		IBP (g/plant)	GBP (t·ha ⁻¹)	IBP (g/plant)	GBP (t·ha ⁻¹)
SAT	T1	42.31d	13.33b	49.77b	14.01c
	T2	40.85d	14.71b	46.73bc	14.88bc
NST	T3	57.11b	15.42ab	55.93a	15.10b
	T4	42.58d	12.50c	43.15c	12.65d
SAT-NST	T5	51.47c	16.05a	50.92b	16.04ab
	T6	44.87d	16.21a	47.44bc	17.08a
	T7	35.55e	11.97d	39.46d	12.43d
	T8	34.49e	12.41cd	36.69d	13.21cd
CK		61.86a	16.70a	59.67a	16.38a

Table 4. Dry matter weight of early-season rice ‘Xiangwanxian 45’ at mature stages under nitrogen fertilizer and plant density treatments in 2015 and 2016. Within the row for each location, means followed by the same letters are not significantly different according to LSD at $P = 0.05$.

Treatment and stage		NAA (kg·ha ⁻¹)				NAP (%)		
		SO-AT	AT-HD	HD-MA	Whole growth period	SO-AT	AT-HD	HD-MA
SAT	T1	41.3	65.4	40.8	147.5	28.00	44.34	27.66
	T2	47.0	69.7	41.4	158.1	29.73	44.09	26.19
NST	T3	31.6	51.3	47.1	130.0	24.31	39.46	36.23
	T4	26.3	41.7	42.0	110.0	23.91	37.91	38.18
SAT-NST	T5	38.0	55.6	62.2	155.8	24.39	35.69	39.92
	T6	51.7	43.4	64.0	159.1	32.50	27.28	40.23
	T7	48.7	21.8	40.0	110.5	44.07	19.73	36.20
	T8	49.8	19.6	41.6	111.0	44.86	17.66	37.48
CK		41.2	74.8	54.6	170.6	24.15	43.85	32.00

Table 5. Nitrogen absorption regularity of early-season rice ‘Xiangwanxian 45’ at different growth stages under nitrogen fertilizer and plant density treatments in 2016. SO-AT, sowing to active tillering; AT-HD, active tillering to full heading; HD-MA, full heading to maturity.

fertilizer as a basal dressing might cause considerable N loss because of the lower demand and uptake capacity of rice seedlings during early growth stages²¹. Under the optimal SAT-NST treatment (T6), 18% less N fertilizer was applied but a higher quantity of N was absorbed during sowing to tillering stage (Table 5) because of the increased seedling density, thus the treatment not only promoted seedling growth but also avoided excessive loss of fertilizer. Kamiji²² and Sui²³ suggested that topdressing with N fertilizer at the panicle initiation stage is an effective and necessary practice to enhance rice spikelet production to achieve high grain yield. Under the SAT-NST treatments, the highest NAA attained was 64.0 kg ha⁻¹ during the mature stage, which represents a N absorption proportion (NAP) of more than 40% in the entire growth period.

The seedling establishment phase is important for the productivity and profitability of rice²⁴. The development of a tiller primordium is influenced primarily by the amount of N supplied to the developing tiller. The total N content of the whole straw, leaves or stem may be a good indicator of the tillering activity of the plant because there are strong correlations between the N content of the leaves and that of the stem, and between the total- and soluble-N content in the culm²⁵. In the present study we observed a correlation between maximum number of seedlings and group NAA over the entire growth period under SAT-NST treatments (Table 6). The NAA in the periods from sowing to active tillering, and from active tillering to full heading was positively correlated with the number of effective panicles ($r = 0.643$) and the percentage of available tillers ($r = 0.619$), respectively. Furthermore, the maximum number of seedlings was positively correlated with effective number of panicles ($r = 0.901$) and percentage of available tillers ($r = -0.839$), which is consistent with previous research^{10,18}.

Conclusion

This study used the rice seedling-throwing cultivation system practiced in southern China to investigate the potential for attaining high yield under decreased N fertilizer input and increased seedling density with an early-season rice cultivar. By increasing the seedling density by 32% (to 36.0×10^4 seedlings ha⁻¹), N application can be reduced by 18% (to 135 kg N ha⁻¹) compared with the conventional seedling-throwing practice, but grain yield was not significantly affected. Under combined SAT-NST treatments, the biomass of an individual plant was less than that attained under the conventional practice, but the group biomass was more than 16.0 t ha⁻¹, which is identical to that achieved under the conventional cultivation system. The number of spikelets per panicle, number of filled grains per panicle and percentage of filled spikelets decreased when N fertilizer input was reduced, but the number of effective panicles and total number of glumous flowers increased considerably as a result of the

items	Absorptive amount of N			Maximum number of seedlings	Percentage of available tiller	Effective panicles
	SO-AT	AT-HD	HD-MA			
absorptive amount of N	SO-AT	—	<i>ns</i>	<i>ns</i>	<i>ns</i>	*
	AT-HD	$r = -0.307$	—	<i>ns</i>	*	<i>ns</i>
	HD-MA	$r = -0.144$	$r = 0.315$	—	<i>ns</i>	<i>ns</i>
the maximum number of seedlings	$r = 0.483$	$r = 0.349$	$r = 0.027$	—	**	**
percentage of available tiller	$r = -0.321$	$r = -0.618$	$r = 0.228$	$r = -0.839$	—	*
effective panicles	$r = 0.643$	$r = 0.229$	$r = 0.251$	$r = 0.901$	$r = -0.642$	—

Table 6. Pearson correlation coefficients between plant traits and nitrogen (N) absorption. SO-AT, from sowing to active tillering; AT-HD, from active tillering to full heading; HD-MA, from full heading to maturity. *Significant at the 0.05 level based on analysis of variance. **Significant at the 0.01 level based on analysis of variance. *ns* denotes non-significance based on analysis of variance

Treatment		N dosage (kg·ha ⁻¹)	Seedlings (10 ⁴ plant ha ⁻¹)	Compared with CK	
				Adding seedling	Saving N
seedling-addition treatment (SAT)	T1	165	31.3	16%	0%
	T2	165	35.6	32%	0%
nitrogen-saving treatment (NST)	T3	135	27.0	0%	18%
	T4	105	27.0	0%	36%
Both add seedling and save nitrogen (SAT-NST)	T5	135	31.3	16%	18%
	T6	135	35.6	32%	18%
	T7	105	31.3	16%	36%
	T8	105	35.6	32%	36%
Conventional cultivation	CK	165	27.0	—	—

Table 7. Details of the experimental design and treatments.

increased seedling density, which compensated for the decreased yield of individual plants. On the other hand, under SAT-NST treatments, an increased quantity of N is applied in the tillering and grain-filling stages, which favours a high yield by promoting tiller establishment and development into effective panicles. We conclude that, on the basis of the conventional seedling-throwing cultivation system, the N application rate can be decreased by about 18% to achieve high yield through an appropriate increase in seedling density. The present results provide a theoretical basis for seedling-throwing rice production and are applicable in southern China for the development of sustainable agriculture.

Materials and Methods

Field experiments. Field experiments were conducted in Yiyang County (24°03'52"N, 112°03'27"E, 70 m elevation), Hunan Province, China. 'Xiangwanxian 45', an inbred *indica* rice cultivar ('Zhouyou903' × 'Zhefu504'), was used as the experimental material. Treatments were arranged in a split-plot design with N treatments as the main plots and seedling density as subplots. The experiment was replicated three times, and the subplot size was 20 m². The three N fertilizer treatments were (i) conventional-practice N application (165 kg N ha⁻¹), (ii) 18% less N (135 kg N ha⁻¹) and (iii) 36% less N (105 kg N ha⁻¹). The three seedling density treatments were (i) conventional-practice density (27.0 × 10⁴ plants ha⁻¹), (ii) 16% additional seedlings (31.3 × 10⁴ plants ha⁻¹) and (iii) 32% additional seedlings (35.6 × 10⁴ plants ha⁻¹). Altogether, there were nine combinations of treatments, which were labeled T1 to T8 and CK (representing the conventional cultivation practice as the control); details of the treatments are listed in Table 7.

Pre-germinated seeds were planted in a seedling bowl-tray on 24 March 2015 and 28 March 2016. After leveling of the field, 5–6 seedlings per plant site were thrown into the puddle field on 20 April 2015 and 25 April 2016. When transplanting, the three seedling density treatments (27.0 × 10⁴, 31.3 × 10⁴ and 35.6 × 10⁴ plants ha⁻¹) were applied as densities of 15 × 24 cm, 16 × 20 cm and 16 × 20 cm, respectively. Approximately 60% of the N fertilizer was incorporated as basal fertilizer applied one day before seedling-throwing. The remaining N fertilizer was broadcast as urea at the tillering and booting stages, with 20% applied at each application. A total of 150 kg ha⁻¹ of potassium was applied to each plot in the form of potassium chloride, with 50% applied as a basal dressing and 50% as a topdressing at panicle initiation. A total of 75 kg ha⁻¹ of phosphorus was applied to each plot in the form of superphosphate, with 100% applied as a basal dressing. Except for mid-season drainage, the field was continuously flooded with 3–5 cm water depth until one week before the final harvest. Insects, weeds and diseases were controlled as required using standard practices to avoid yield loss.

Sampling and measurements. Five plants were sampled in each subplot at active tillering, booting (the fourth booting stage), full heading (when approximately 80% of the panicles had emerged from the flag leaf sheath) and maturity. Plants along the three border lines were excluded from sampling to avoid border effects. Plant samples were separated into stems, leaves and panicles. The dry weight of each organ was determined after oven-drying at 85 °C to a constant weight. Plants sampled at the maturity stage were hand-threshed after the panicles had been counted. We then measured individual indices: filled spikelets were separated from unfilled spikelets by submerging them in tap water; each subsample of 30 g filled grains and all unfilled spikelets were used to count the numbers of spikelets; the dry weight of straw (including the rachis) and numbers of filled and unfilled spikelets were determined after oven-drying at 70 °C to a constant weight; finally, the percentage of filled spikelets ($100 \times \text{filled spikelet number} / \text{total spikelet number}$) was calculated. The group indices of grain yield and effective panicle number were determined for the total area of each subplot and the yield adjusted to a moisture content of $0.14 \text{ g H}_2\text{O g}^{-1}$ fresh weight. The micro-Kjeldahl method was used to determine the N content of all plant samples.

Biomass production and N uptake were calculated using the following formulas.

“Individual” refers to one plant from among the 5–6 seedlings growing at one plant site. Individual biomass production (IBP) reflects the growth of individual plants and was calculated as:

$$\text{IBP} = \text{stem dry weight} + \text{leaf dry weight} + \text{panicle dry weight}$$

“Group” includes all plants growing within the same subplot. Group biomass production (GBP) reflects the yield potential within the plot and was calculated as:

$$\text{GBP} = \text{IBP} \times \text{plant number per hectare.}$$

Nitrogen uptake was calculated using the following formulas:

$$\text{N absorptive amount (NAA)} = \text{stem dry weight} \times \text{N content in stem} + \text{leaf dry weight} \times \text{N content in leaf} + \text{panicle dry weight} \times \text{N content in panicle}$$

$$\text{N absorption proportion (NAP)} = \text{N uptake in a certain period} / \text{N uptake in the entire growth period}$$

where a certain period includes three phases: SO–AT, sowing to active tillering; AT–HD, active tillering to full heading; and HD–MA, full heading to maturity.

Statistical analysis. Analysis of variance (ANOVA) was performed using Duncan’s multiple-range test to compare treatment means and significance was defined at $P < 0.05$. Pearson correlation coefficients were calculated using SPSS 10.0.

References

1. Areum, C., Ho, J. L., Bruce, R. H. & Srinivas, J. Effects of ripening temperature on starch structure and gelatinization, pasting, and cooking properties in rice (*Oryza sativa* L.). *Journal of Agricultural and Food Chemistry*. **63**(12), 3085–3093 (2015).
2. Yang, F. Present status of China’s fertilizer industry and analysis on market demand of fertilizer. *Phosphate & Compound Fertilizer*. **19**(2), 1–6 (2004).
3. Zhu, B. H. *et al.* Effects of nitrogen application rate on grain yield and quality of mechanical transplanting japonica rice Wuyunjing 27. *China Rice*. **21**, 63–66 (2015).
4. Fu, J. & Yang, J. C. Research advances in physiology of super rice under high-yielding cultivation. *Chinese Journal of Rice Science*. **25**, 343–348 (2011).
5. Zhu, D. W. *et al.* Effects of nitrogen level on yield and quality of japonica soft super rice. *Journal of Integrative Agriculture*. **16**(5), 1018–1027 (2017).
6. Carly, J. S., Nancy, B. & Dise, D. J. Gowing. Regional trends in soil acidification and exchangeable metal concentrations in relation to acid deposition rates. *Environmental Pollution* **157**(1), 313–319 (2009).
7. Zhang, D. *et al.* Effect of Post-Sowing Soil Compaction and Pre-Winter Irrigation on Dry Matter Translocation and Nitrogen Use Efficiency of Winter Wheat. *Journal of Triticeae Crops* (2017).
8. Huang, M. *et al.* Tillering responses of rice to plant density and nitrogen rate in a subtropical environment of southern China. *Field Crops Research* **149**(2), 187–192 (2013).
9. Clerget, B., Bueno, C., Domingo, A. J., Layaoen, H. L. & Vial, L. Leaf emergence, tillering, plant growth, and yield in response to plant density in a high-yielding aerobic rice crop. *Field Crops Research* **199**, 52–64 (2016).
10. Zhu, X. C. *et al.* Effects of dense planting with less basal N fertilization on rice yield, N use efficiency and greenhouse gas emissions. *International Journal of Agriculture & Biology*, **17**(6) (2015).
11. Martinez-Eixarch, M., Zhu, D. F., Catala-Fornier, M. D. M., Pla-Mayor, E. & Tomas-Navarro, N. Water, nitrogen and plant density affect the response of leaf appearance of direct seeded rice to thermal time. *Rice Science* **20**, 52–60 (2013).
12. Lin, X. Q. *et al.* Effect of plant density and nitrogen fertilizer rates on grain yield and nitrogen uptake of hybrid rice (*Oryza sativa* L.). *Cheminform* **29**(2), 6397–6400 (2009).
13. Christian, D. G., Riche, A. B. & Yates, N. E. Growth, yield and mineral content of *Miscanthus × giganteus* grown as a biofuel for 14 successive harvests. *Industrial Crops and Products*. **28**, 320–327 (2008).
14. Danalatos, N. G., Archontoulis, S. V. & Mitsios, I. Potential growth and biomass productivity of *Miscanthus × giganteus* as affected by plant density and N-fertilization in central Greece. *Biomass and Bioenergy*. **31**, 145–152 (2007).
15. Cosentino, S. L., Patane, C., Sanzone, E., Copani, V. & Foti, S. Effects of soil water content and nitrogen supply on the productivity of *Miscanthus × giganteus* Greef et Deu. In a Mediterranean environment. *Industrial Crops and Products* **25**, 75–88 (2007).
16. Atlin, G. N. *et al.* Developing rice cultivars for high-fertility upland systems in the Asian tropics. *Field Crops Research*. **97**, 43–52 (2006).
17. Lampayan, R. M. *et al.* Yield of aerobic rice in rainfed lowlands of the Philippines as affected by nitrogen management and row spacing. *Field Crops Research*. **116**(1), 165–174 (2010).

18. Huang, M., Zou, Y. B., Jiang, P., Xia, B. & Md Ibrahim, A. H. J. Relationship Between Grain Yield and Yield Components in Super Hybrid Rice. *Agricultural Sciences in China* **10**(10), 1537–1544 (2011).
19. Wang, F. & Peng, S. B. Yield potential and nitrogen use efficiency of China's super rice. *Journal of Integrative Agriculture*. **16**(5), 1000–1008 (2017).
20. Yuan, Z. *et al.* Indicators for diagnosing nitrogen status of rice based on chlorophyll meter readings. *Field Crops Research* **185**(185), 12–20 (2016).
21. Peng, S. B. *et al.* Improving Nitrogen Fertilization in Rice by Site-Specific N Management[M]//*Sustainable Agriculture Volume 2*. Springer Netherlands (2011).
22. Ding, C. *et al.* Nitrogen fertilizer increases spikelet number per panicle by enhancing cytokinin synthesis in rice. *Plant Cell Reports* **33**(2), 363–371 (2014).
23. Wu, Q. *et al.* Nitrogen use and rice yield formation response to zeolite and nitrogen coupling effects: Enhancement in nitrogen use efficiency. *Journal of Soil Science & Plant Nutrition* **16** (2016).
24. Chen, S. *et al.* Seasonal differences in the rice grain yield and nitrogen use efficiency response to seedling establishment methods in the Middle and Lower reaches of the Yangtze River in China. *Field Crops Research*. **205**, 157–169 (2017).
25. Tanaka, A. & Garcia, C. V. Studies on the relationship between tillering and nitrogen uptake of the rice plant. *Soil Science & Plant Nutrition*. **11**(1), 9–13 (1965).

Acknowledgements

This research was partly supported by grants from the National Science and Technology Plan of China (2013BAD07B11), and the National Key Research and Development Program of China (2016YFD0300507). We thank Robert McKenzie, PhD, from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

Author Contributions

Yuzhu Zhang and Haiqing Zhang designed the experiment. Yang Liu, Chao Li, Baohua Fang and Kailin Chen performed the experiment. Yang Liu and Yong Fang wrote the paper.

Additional Information

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2019