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

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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 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 2030⁵. 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.⁶ reported that the optimum quantity of N fertilizer to increase grain yield was 225–300 kg ha⁻¹. Fu et al.⁷ 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 acidification⁸–⁹. Plant density is one of the main factors that influence yield in rice, together with N availability⁸–⁹. 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.
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\(^\text{10,12}\). 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) declined by 1.30–3.74%. 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. The number of spikelets per panicle 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.

### 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 \(p = 0.05\).

| Year | 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       |                       |                                        |                  |                       |                                        |                  |
| 2015 | 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            |
| 2016 | T3        | 7.22c                 | −1.15                                  | −13.74           | 7.16c                 | −0.53                                  | −6.89            |
|      | T4        | 7.50c                 | −1.07                                  | −12.78           | 7.82c                 | −0.67                                  | −8.71            |
| 2015 | T5        | 8.22ab                | −0.15                                  | −1.79            | 7.59b                 | −0.10                                  | −1.30            |
| 2016 | T6        | 8.52a                 | 0.15                                   | 1.79             | 8.02a                 | 0.33                                   | 4.29             |
| 2015 | T7        | 8.66c                 | −0.91                                  | −10.87           | 7.21bc                | −0.48                                  | −6.24            |
| 2016 | T8        | 7.46c                 | —                                     | —                | 7.69b                 | —                                     | —                |
|      | SAT-NST   |                       |                                        |                  |                       |                                        |                  |
| 2015 | T5        | 8.37a                 | —                                     | —                | 7.69b                 | —                                     | —                |

**Notes:**

- **SAT:** seedling-addition treatment (T1 and T2).
- **NST:** nitrogen-saving treatment (T3 and T4).
- **SAT-NST:** combined SAT–NST treatments (T5, T7 and T8).
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 yield\(^9\). Under N deficiency, yield components are reduced, especially panicle number per plant and per unit area\(^8\). 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.

### 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 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 |
|            | 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 |
|            | 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     |

#### 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.

| 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 |
|            | 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 |
|            | 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 84.3b 23.5ab     |

Analysis of variance

- seedling (S) ns
- nitrogen (N) ** * * * ns
- S × N * ** * * ns

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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.
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 requirements and may explain only slightly more than 60% of variability in total N accumulation in rice. 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
fertilizer as a basal dressing might cause considerable N loss because of the lower demand and uptake capacity of rice seedlings during early growth stages\textsuperscript{21}. 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. Kamiji\textsuperscript{22} and Sui\textsuperscript{23} 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\textsuperscript{-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 rice\textsuperscript{24}. 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 culm\textsuperscript{25}. 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\textsuperscript{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 $\times 10^4$ seedlings ha\textsuperscript{-1}), N application can be reduced by 18% (to 135 kg N ha\textsuperscript{-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\textsuperscript{-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

### Table 4

| Treatment | IBP (g/plant) | GBP (t·ha\textsuperscript{-1}) |
|-----------|---------------|-------------------------------|
| SAT       |               |                               |
| T1        | 42.31d        | 13.33b                        |
| T2        | 40.85d        | 14.71b                        |
| NST       |               |                               |
| T3        | 57.11b        | 15.42ab                       |
| T4        | 42.58d        | 12.50c                        |
| SAT-NST   |               |                               |
| T5        | 51.47c        | 16.05a                        |
| T6        | 44.87d        | 16.21a                        |
| T7        | 35.55c        | 11.97d                        |
| T8        | 34.49e        | 12.41cd                       |
| CK        | 61.86a        | 16.70a                        |

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 $P = 0.05$.

### Table 5

| Treatment and stage | NAA (kg·ha\textsuperscript{-1}) | NAP (%) |
|---------------------|----------------------------------|---------|
| SAT                 | SO–AT 41.3 AT–HD 65.4 HD–MA 40.8 | 147.5   |
|                     | SAT-T1 65.4 AT–HD 40.8 HD–MA 41.4 | 158.1   |
|                     | SAT-NST T3 31.6 AT–HD 47.1 HD–MA 42.0 | 110.0   |
|                     | SAT-NST T5 26.3 AT–HD 41.7 HD–MA 42.0 | 147.5   |
|                     | SAT-T1 47.0 AT–HD 69.7 HD–MA 41.4 | 158.1   |
|                     | SAT-T2 51.6 AT–HD 47.1 HD–MA 42.0 | 110.0   |
|                     | SAT-T3 48.7 AT–HD 43.4 HD–MA 44.8 | 111.0   |
|                     | SAT-NST T5 41.2 AT–HD 74.8 HD–MA 44.6 | 170.6   |

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.
indica elevation), Hunan Province, China. ‘Xiangwanxian 45’, an inbred 32% additional seedlings (35.6
continuously flooded with 3–5 cm water depth until one week before the final harvest. Insects, weeds and diseases were
applied as densities of 15
were labeled T1 to T8 and CK (representing the conventional cultivation practice as the control); details of the
treatments are listed in Table 7.

When transplanting, the three seedling density treatments (27.0
of the field, 5–6 seedlings per plant site were thrown into the puddle field on 20 April 2015 and 25 April 2016.

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.

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°03’22”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 × filled spikelet number/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 g H2O 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:

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

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

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

Nitrogen uptake was calculated using the following formulas:

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

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

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 calcu-
lated using SPSS 10.0.

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**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.

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