Optimized nitrogen application increases rice yield by improving the quality of tillers

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ABSTRACT

The quantity and quality of tillers determine the yield of rice. In order to explore how optimized nitrogen fertilizer application (OFA) increases rice yield by affecting tiller growth, a pot experiment with three nitrogen treatments was performed on the basis of previous researches to investigate the growth and development of tillers. Results showed that under OFA, the emerging rate of secondary tillers and high leaf position tillers decreased, which increased with the number of primary tillers. The decrease of ineffective tillers increased the accumulation of biomass and nitrogen per tiller, which promoted the development of panicles. Compared with traditional nitrogen fertilizer application (TFA), the differentiated number of spikelets increased by 10.85%–21.70%, which led to the total number of filled spikelets increasing by 9.67%–18.95%, resulting in 9.6% increase in rice yield. Primary tillers, especially at the first, second, fifth, and sixth leaf positions, were the superior tillers in good quality, which made great contribution to rice yield and were significantly affected by nitrogen application. Making full use of the regulation effect of nitrogen on the quality of tillers will help to stabilize rice yield with less nitrogen input or increase rice yield without adding nitrogen input.
1. Introduction

Rice tillers are specialized branches bearing panicles and provide nutrients for panicle development (Hanada, 1993; Wang & Li, 2011). Therefore, the number of tillers and the quality of their growth directly determine the number and development of panicles, and ultimately affect the yield of rice, which is related to the world’s food security. The number of tillers is dynamic and adjustable and is easily affected by cultivation conditions, especially for plant nitrogen status (Zhong et al., 2002). Liu et al. (2011) discovered that nitrogen levels in the vegetative organs of 40 mg/L nitrogen-treated plants were significantly higher than those of the plants treated with 10 mg/L nitrogen, which promoted tiller bud germination. The content of plant hormones (e.g. auxin, cytokinin) and the gene expression levels (e.g. SP1 and SP3) are closely related to the emergence and growth of tillerings, all of which are affected by nitrogen level (Hu et al., 2020; Huang et al., 2019; Xu et al., 2015; Zha et al., 2019).

Researches have shown that tillers in different leaf positions made significant different contributions to rice yield (Karali et al., 2012; Lei et al., 2014; Li et al., 2011), indicating that the quality of tillers varies greatly among different leaf positions. Previous studies focused on the number of tillers, the development of spikelets, and their influencing factors, while few focused on the whole growth and development of tillers on their quality. Our preliminary experiment showed that optimized nitrogen fertilizer application (OFA) could significantly increase the ear-bearing percentage of tillers and grains per panicle (Zhou et al., 2016). We hypothesized that OFA increased rice yield by improving the quality of tillers. Therefore, in this study, the emergence and ear-bearing of different tillers, differentiation and degeneration of branches and spikelets, structure and seed setting characteristics of panicles on tillers of different grades and leaf position were investigated to (i) evaluate the disadvantages and advantage of different tillers and (ii) explore how OFA increases rice yield by affecting the quality of tillers.

2. Materials and methods

Experimental design

We performed a pot experiment laid out in a single-factor randomized block design. There were three nitrogen treatments: no nitrogen application (CK) as control, traditional nitrogen fertilizer application (TFA: base and tillering fertilizer proportions 70% and 30%, respectively), and OFA (35% applied at base, 15% at early tillering stage, 25% at jointing stage, and 25% at 15–20 days after jointing stage). The application time and proportion of nitrogen fertilizer in OFA were determined by 10 years of practical production experience, and two years of field experiments both on mechanical and manual transplanting hybrid indica rice, as reported by Zhou et al. (2016). The two nitrogen applications had the same total nitrogen amount of 1.2 g/pot. Phosphorus at 2.4 g P₂O₅ per pot was applied at base fertilizer, and potassium at 1.2 g K₂O per pot was applied at base fertilizer and jointing stage in all treatments.

Each pot was filled with 12.0 kg paddy topsoil that had been air dried and thoroughly mixed. The content of available nitrogen, phosphorus, potassium, and total nitrogen in the tested soil was 116.5, 13.02, 65.04, and 1580 mg/kg, respectively. Each treatment had 50 pots, with three replications. There were two rows in one replication, two pots were arranged side by side, and the interval between replications was 40 cm (Figure S1). To avoid the possibility of applied fertilizers being washed out by rain, pots were placed in a greenhouse. The meteorological conditions during the test are shown in Figure S2. The test plant was an indica hybrid rice cultivar, ‘Fyou498’ (‘F32A’ × ‘Shuhui498’), which has been planted in a large area of Sichuan Province. Seeds were sown on 18 April 2014; two seedlings were transplanted into each pot on 28 May, and harvested on 12 September of the same year. Pests were controlled according to standard recommendations, and other rice management practices were similar to those in the paddy field.

Indices and measurement methods

Tiller growth and decline

Leaf age was marked in the seedling bed. Twenty-two seedlings with similar leaf age and tillers were marked for each plot before transplanting into pots. A hang tag, marked with leaf order and level of tiller, was hung on each primary and secondary tiller when it came out. ‘P’ and ‘S’ represent primary and secondary tillers, respectively. ‘P-X’ and ‘S-X’ represent the primary and secondary tillers in the Xth leaf position of main stem, respectively. Tracking tiller growth and decay in real time and surveying the dynamics of stem growth and tillering were performed simultaneously. We calculated the following values:

Tiller emergence rate at each leaf position (%) = the number of tillers that actually emerged at this leaf position/the number of leaf positions investigated × 100

Percentage of ear-bearing tillers at each leaf position (%) = the number of ear-bearing tillers/the number of tillers actually emerged at this leaf position × 100.
Differentiation and degradation of spikelets and branches

At heading stage, panicles from six plants of each plot were separated into categories according to the marked hang tag to investigate the number of surviving, degenerated, and differentiated secondary branches and spikelets according to the trace method of Matsushima et al. (1978).

Dry matter and N accumulation

Plants from two representative pots of each plot were selected as samples at jointing stage (basal first internode elongation of 2 cm) and full heading stage (80% of the panicles were sprouting 1 cm). The samples were heated to 105°C for 60 min and then dried at 75°C to a constant weight to determine the dry matter content. The N content (%) was determined using the Kjeldahl method.

Determination of yield and its components

Rice was harvested at maturity and the yield of each plot was recorded. After measuring the moisture content and removing the impurities, the standard grain output of each pot was determined based on a 13.5% moisture content. Before harvesting, the number of effective panicles per plant had been determined using 22 pots of each plot, and tillers of 16 plants per plot were separated into categories according to the marked hang tag, and examined for panicle length, 1000-grain weight, seed setting percentage, and numbers of filled and unfilled grains per panicle separately.

Data analysis

The data were subjected to an analysis of variance, comparisons of treatments and varieties, and a mapping analysis. The bar diagrams were used to assess the differences and tendencies among the mean values of different attributes, and the comparisons were carried out using LSD’s multiple range tests at 0.05 levels.

3. Results

Tiller emergence and ear-bearing

Optimized nitrogen fertilizer application (OFA) can reduce the emergence of ineffective tillers and increase the ear-bearing tiller percentage (Figure 1 and Table S1). The total emergence number and rate of primary and secondary tillers of OFA were lower than those of TFA, especially for the secondary tillers in the first and third leaf positions, while the number and emergence rate of primary tillers at the fourth to seventh leaf positions increased, and the difference was significant at the seventh leaf position. The total number and rate of ear-bearing primary and secondary tillers of OFA were higher than those of TFA, especially for the primary tillers in the seventh, sixth, and second leaf positions. Compared with TFA, the leaf positions of the primary and secondary tiller emergence were all decreased by one under OFA condition (the tenth and eighth leaf position for primary and secondary tiller, respectively). On the whole, the effects of OFA on emerging and ear-bearing of secondary tillers were larger than those of primary tillers. Large amounts of base and tillering fertilizer under TFA promoted the growth of secondary tillers, but the percentage of barrier secondary tillers also increased.

Differentiation and development of spikelets

The number of filled grains was determined by the differentiation and development of spikelets, including degeneration, fertilization, and filling. The effects of nitrogen applications on the differentiation and development of spikelets in different leaf position panicles were not obvious (only had significant effect on the number of filled grains per panicle in a few leaf positions, as shown in Table S2), but that of panicles in different grades were significant difference (Figure 2). Results showed that 2.33% and 18.11% of the differentiated spikelets were degenerated and unfilled, respectively. Compared with TFA, the number of degenerated and unfilled spikelets of OFA was increased, but the differences were not significant, while the differentiated number of spikelets on panicles in the main stem, primary, and secondary tillers was significantly increased, which increased by 21.70%, 17.26%, and 10.85%, respectively. As a result, the number of filled spikelets increased by 9.67%–18.95%, which difference was significant in primary tillers. In summary, the increase in the number of differentiated spikelets with OFA treatment leads to an increase in the number of surviving spikelets, resulting in a significant increase in the number of filled spikelets.

Contribution of tillers to rice yield

The increase in the number of spikelets per panicle of OFA resulted in an increase in rice yield by 9.6% and 36.5%, compared with TFA and CK, respectively. The contribution of different tillers to the yield was significantly different. Tillers at different leaf positions contributed to rice yield in a bimodal curve pattern (Figure 3A). Tillers in the first, second, fifth, and sixth leaf positions made large contributions to the yield, each contributing more than 10% of the total. There was a low point at the third and fourth leaf positions, which may be due to
Figure 1. The emerging rate and ear-bearing percentage of primary and secondary tillers under different nitrogen treatments. The data is presented as a violin diagram. CK: no nitrogen application; TFA: traditional nitrogen fertilizer application; OFA: optimized nitrogen fertilizer application; P: primary tillers; S: secondary tillers.
transplantation injury. Overall, the number of emerging and ear-bearing tillers and the contribution to yield decreased with the leaf’s position from the base. Compared with TFA, the contribution rates of tillers in first, second, fifth, and sixth leaf positions of OFA increased, but those of others decreased. This indicates that OFA improved the contribution to the yield of superior leaf positions and played a role in making the strong stronger and the weak weaker. The contribution rate of tillers at different tiller categories also displayed the same function and advantage of OFA treatment (Figure 3B). In all treatments, the primary tillers had the highest contribution to yield at more than 60% (ranged from 63% to 69%), significantly higher than that of the

Figure 2. Differentiation and development of spikelets on tillers under different nitrogen treatments. AV: average value; CK: no nitrogen application; TFA: traditional nitrogen fertilizer application; OFA: optimized nitrogen fertilizer application.

Figure 3. Contribution of tillers to rice yield. CK: no nitrogen application; TFA: traditional nitrogen fertilizer application; OFA: optimized nitrogen fertilizer application; P: primary tillers; S: secondary tillers.
secondary tillers and the main stem. Even so, the contribution rate of primary tillers was still increased with OFA.

4. Discussion

The way for optimized nitrogen application increasing rice yield

Branching in rice, including tillers and panicle branches, is significantly affected by the application of nitrogen fertilizer. A significant positive correlation was found between the number of rice tillers and the concentration of NH$_4^+$ in the soil exists (Feng et al., 1993). Compared with OFA, the soil NH$_4^+$ concentration was maintained at a higher level with nitrogen fertilizers all applied as base and tillering fertilizer under TFA, which promoted the emergence of secondary tillers and high (tenth) leaf position tillers. However, these later-initiated tillers accumulated less biomass and ultimately failed to become effective tillers. Researchers have demonstrated that excessive tillering leads to high tiller abortion, small panicle size, and further reduced grain yield (Peng et al., 1994). Results of this study also showed that the ear-bearing tiller percentage of TFA, especially for the secondary tillers, decreased obviously, and the number of grains per panicle of TFA also significantly decreased, especially for panicles on primary tillers. Topdressing of nitrogen fertilizer to increase the nitrogen and biomass accumulation before heading could significantly increase the number of spikelets per panicle (Ding & Maruyama, 2004; Kamiji et al., 2011; Matsui & Kagata, 2002), while the constraint of resource or nutrient supply, especially for biomass and nitrogen, would inhibit rice spikelet differentiation and promote spikelet degeneration (Wang et al., 2012; Wang et al., 2018). Results of this study showed that the difference of dry matter and N accumulation per tiller and N content between TFA and OFA at the jointing stage was only 1.06%–4.04%, but that at heading stage was significant difference. Compared with TFA, dry matter and N accumulation per tiller and N content of OFA increased 8.0%, 31.53%, and 21.74%, respectively (Figure 4). It indicated that the accumulation of nitrogen in rice plants during panicle development stage was significantly increased by applying nitrogen fertilizer at jointing stage and 15–20 d after jointing stage, which promoted the production of dry matter, and ensured the nutrition needed for the development of young panicles, resulting in the increase in the number of differentiation of branches and spikelets. As discussed above, the way of OFA to increase rice yield can be summarized as follows (Figure 5): Emerging of secondary tillers and high leaf position tillers decreased, the ear-bearing percentage and the number of ear-bearing tillers (especially for the primary tillers at the second and fifth to seventh leaf positions) increased, which led to the increase in the dry matter and nitrogen accumulation for per tiller and promoted the better development of panicle (more surviving branches and spikelets), finally obtained higher yield.

Leaf position of superior-quality tillers and its utilization

Tillers are active and easy to control, and they are the basis for developing other components of the rice yield. Many studies have shown that tillers of
different grades and leaf positions have great differences in growth and their productivity, and there are obviously superior-quality tillers. For the manually transplanted rice in this study, primary tillers were the best: their contribution to yield was significantly higher than that of secondary tillers and main stems, a result that was similar to what has been reported by Li et al. (2011) and Lei et al. (2014). There are two common findings from studies of yield contribution of different tillers. First, the higher the leaf position of the tiller, the smaller the tiller’s contribution to yield. However, for manually and mechanically transplanted rice, the low-position tillers are easily affected by the seedling bed environment and transplantation injury, so the emergence rate and ear-bearing tiller percentage, or both, decreases, which reduces the superiority of the low-position tillers. For example, the first and second leaf positions of mechanically transplanted rice usually cannot grow tillers, and tillers before the fifth leaf position of manually transplanted rice always have a low peak. In this study, the third and fourth leaf positions were affected by transplantation injury – their contributions to yield decreased obviously, and the first, second, fifth, and sixth leaf positions were the superior tiller sites, as they all had high emergence rates, ear-bearing tiller percentages, and contribution rates. Second, there are 4–5 superior tiller positions, whatever the sowing or transplanting methods. Achieving high yields may involve changing the architecture of the plants, and an ideotype with only 3–4 tillers, each bearing

Figure 5. The way for optimized nitrogen application increasing rice yield.
a large number of spikelets in the panicles, has been introduced (Peng et al., 1999). Making full use of the superior leaf positions will help in breeding new plant types and make progress in rice production.

5. Conclusions

The quantity and quality of tillers determine the number and structure of panicles, which determine the yield of rice. Optimized nitrogen application improved the quality of tillers without decreasing the number of effective tillers. The improvement of tillering quality was shown as follows: the decrease of the emerging rate of secondary tillers and high leaf position tillers led to the increase of the ear-bearing percentage of tillers and the accumulation of dry matter and nitrogen per tillers, which promoted the development of panicles. The decrease of degenerated branches and the increase of differentiated secondary spikelets led to the increase in grains per panicle. Nitrogen application had the biggest influence on the growth and development of primary tillers, which made the greatest contribution to rice yield. Making full use of the regulation effect of nitrogen on the quality of tillers will help to stabilize rice yield with less nitrogen input or increase rice yield without adding nitrogen input.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by the cience and Technology Department of Sichuan Province [number 2021FYZ0005], and the National Natural Science Foundation of China (NSFC) [number U20A2022].

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