Heliyon 10 (2024) e34607

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Optimized nitrogen management improves grain yield of rice by regulating panicle architecture in South China

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ARTICLE INFO

Keywords:
Optimized nitrogen management
Panicle architecture
Rachis branch and floret
Differentiation
Degeneration

ABSTRACT

Optimized nitrogen (N) management (OPT), with reduced total N input and more N applied during panicle development, has been proved to increase grain yield of rice through panicle enlargement. However, the changes in panicle architecture and source of variation are not well understood. A hybrid rice variety named Tianyou 3618 was subjected to OPT and farmer's fertilizer practice (FFP) in early cropping seasons of 2016 and 2017. With 16.7 % less N input, OPT increased panicle size by 8.6 % and 27.4 %, and grain yield by 13.8 % and 12.3 % for 2016 and 2017, respectively. OPT had greater dry matter accumulation and N uptake from panicle initiation to heading, which bolstered panicle enlargement. The number of surviving florets per branch was quite constant under different N treatments for all primary, secondary, and tertiary branches, implying that panicle size was mainly determined by the number of branches rather than the number of florets per branch. Little change was observed between OPT and FFP in differentiation, degeneration and survival of primary branches and their florets. Surviving secondary and tertiary branches and their florets were significantly more under OPT than those under FFP. The increase in surviving secondary branches under OPT resulted from both enhanced differentiation and reduced degeneration. While the increase in surviving tertiary branches under OPT was merely from enhanced differentiation though their degeneration was also dramatically increased. Among the increased differentiated florets under OPT, 32.4%–36.3 % and 61.6%–67.7 % came from secondary and tertiary branches, respectively. Among the increased surviving florets under OPT, 62.2%–65.2 % and 32.5%–37.8 % came from secondary and tertiary branches, respectively. Both secondary branches and tertiary branches were principal contributors to the increase in panicle size of OPT. To our knowledge, this is the first report on the detailed changes in panicle architecture and their involvement in panicle enlargement and yield gain under OPT.

1. Introduction

Rice (*Oryza sativa* L.) is a major staple food crop and feeds more than half of the world's population [1]. As the largest rice producer,

Abbreviations: FFP, farmer's fertilizer practice; OPT, optimized N management; △DW, dry matter production from panicle initiation to heading; △TN, N uptake from panicle initiation to heading.

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https://doi.org/10.1016/j.heliyon.2024.e34607
Received 6 July 2023; Received in revised form 27 June 2024; Accepted 12 July 2024
Available online 18 July 2024

China contributes 27.9 % of the global rice production [2]. However, increasing global annual rice production by 32 % of current level would be sufficient to meet projected global rice demand by 2030 [3]. Rice yield is usually limited by the total number of spikelets per unit area (sink size) [4,5]. In addition to increasing the number of panicles, enlarging panicle size with more spikelets per panicle through breeding and crop management is an important approach to obtain sufficient sink size and thus increase the grain yield [6,7].

Nitrogen (N) fertilizer plays a key role in improvement of grain yield [8]. However, rice farmers in China generally apply most of N fertilizer as basal fertilizer or dressing during tillering stage, with little or no N fertilizer being applied during panicle development [9,10]. The overuse of N fertilizer and improper timing of application have resulted in low N use efficiency and serious environmental problems. Therefore, optimized N managements (OPT) were developed to achieve both higher yield and higher N use efficiency, such as site-specific N management [11], improved high-yielding cultivation [12], and "three controls" technology [13]. Among them, the "three controls" technology could effectively increase grain yield through increasing spikelets per panicle [14]. It has been officially recommended to rice farmers by the Ministry of Agriculture and Rural Affairs of China (2021–2023). Since 2008, more than 10 provinces (Guangdong, Guangxi, Jiangxi, Zhejiang, etc.) in south, southwest and Yangtze River Basin of China have been adopting the technology. The performance of OPT in increasing rice yield and income has been very consistent.

It has been well documented that the increase in spikelets per panicle (panicle size) through augmented N top-dressing during panicle formation contributed to the increased grain yield under OPT [15,16]. However, the mechanism underlying this process is still not well understood. Rice panicle consists of primary branches, secondary branches, and higher order branches [17]. In previous study, branching and florets observations focused on primary and secondary branches [18,19]. Very limited information about higher branches was reported. Rice panicle starts to develop after entering panicle initiation stage at around one month before anthesis. The primary, secondary, tertiary branches and their florets differentiate and grow sequentially. After pollen mother cell formation stage, degeneration of branches and florets occurs, which substantially reduces the number of spikelets on a panicle [20]. The number of spikelets observed at maturity is determined by the differentiation and degeneration of florets, relating to dry matter production and N accumulation during panicle formation. However, information on how to form a big panicle size under optimized N management is limited. Ding et al. [15] reported that application of N fertilizer near panicle initiation increased the number of spikelets per panicle because of more secondary branches and more florets on them. It is still unclear how the primary, secondary, or tertiary branches response to N management in their differentiation, degeneration, and survival. It's also unclear whether the enlarged panicle size comes from more differentiated florets or from less degenerated florets. Moreover, the contribution of primary, secondary, and higher order branches respectively to spikelets per panicle is not clarified yet. All these remain unclear by now. In addition, previous studies on panicle architecture mostly focused on primary and secondary branches [15,20,21]. No study on the role of tertiary branches in enlarging panicle size has been reported.

The objectives of the present study were (1) to investigate the effects of N management on the panicle architecture of rice, and (2) to evaluate the contribution of different grade of rachis branches and their florets in the large panicle formation under OPT and its carbohydrate and N supply basis. In this study, the government-recommended fertilizer management technology, namely "three controls" technology, was employed as OPT treatment, and the conventional farmer's fertilization practice (FFP) was used as control. Grain yield, yield components, dry matter production and N uptake were determined in this study. In particular, the panicle architecture was examined in detail, focusing on the differentiation, degeneration, and survival of primary, secondary, and tertiary branches, and florets on them.

2. Materials and method

2.1. Growth conditions and plant materials

Field experiments were conducted during early cropping seasons in 2016 and 2017 at Dafeng Experimental Station of Guangdong Academy of Agricultural Sciences, Guangzhou, Guangdong province, China (113°20′E, 23°08′N). The experimental site represents a typical double cropping rice area and is located at subtropical humid monsoon climate zone with an average frost-free period of 335–360 days. The early season is from April to July, and its daily mean temperature, relative humidity and precipitation are 26.7 °C, 83.5 % and 784.0 mm. The field soil developed from a lateritic red soil, which is extensively distributed in subtropical area of South China. The basic properties of A horizon soil (0–15 cm surface layer) are as follows: organic matter 41.34 g kg^{-1}, total N 1.62 g kg^{-1}, total P 1.06 g kg^{-1}, total K 15.99 g kg^{-1}, available N 82.56 mg kg^{-1}, available P 40.39 mg kg^{-1}, available K 58.69 mg kg^{-1} and pH 6.0.

The rice variety used in the experiments was Tianyou 3618, an indica hybrid combination with Tianfeng A as sterile line and Guanghui 3618 as restorer line. This variety was bred by Rice Research Institute of Guangdong Academy of Agricultural Sciences. The seeds used was manufactured by Guangdong Jindao Seed Industry Ltd.

Table 1
Timing and rate (kg N ha^{-1}) of N fertilizer application of two N treatments in the field experiments conducted in 2016 and 2017.

Treatment	Basal	Recovering	Early tillering	Mid-tillering	Late tillering	Primary branch differentiation	Total N rate
FFP	72	36	54	–	18	–	180
OPT	75	–	–	30	–	45	150

FFP, farmer's fertilizer practice; OPT, optimized N management. Recovering, early tillering, mid-tillering, and late tillering was 5, 10, 15, and 20 days after transplanting, respectively.

2.2. Experimental design and field management

The experiments were established using a randomized complete block design with two N treatments and three replications. The two N treatments were farmers' fertilizer practice (FFP) and optimized N management (OPT). Details of treatments are given in Table 1. In FFP, total fertilizer N input was 180 kg N ha^{-1} with 40 % as basal, 20 % at root recovering, 30 % at early tillering and 10 % at late tillering stage. In OPT, the "three controls" technology was employed, and its total fertilizer N input was 150 kg N ha^{-1} with 50 % as basal, 20 % at mid-tillering and 30 % at primary branch differentiation (PI). In comparison to FFP, OPT was 16.7 % lower in total N input. Inputs of phosphorus and potassium fertilizers were the same for both treatments. N fertilizer used in the study was urea (46 % N). All fertilizer P was applied as basal in the form of calcium superphosphate at 45 kg P_2O_5 ha^{-1}. Fertilizer K was applied in the form of potassium chloride at 135 kg K_2O ha^{-1}, with 50 % being applied as basal and 50 % at PI. The plot size of experiments was 32 m^2 (8 m × 4 m). All plots were separated by 0.2-m wide banks, which were covered with a double layered plastic film inserted into a 0.3-m depth in the soil to prevent fertilizer nutrients from penetrating into adjacent plots. Thirty-day-old seedlings were transplanted on 12 April at a hill spacing of 20 cm × 20 cm with two seedlings per hill. The irrigation and other field managements followed the local high-yielding practice. Weeds, insects, and diseases were controlled strictly to avoid yield losses.

2.3. Analysis of differentiation and degeneration of panicle branches and florets

Four hills of plant samples were randomly taken from each plot and the panicle architecture was determined at heading, when 50 % of the panicles fully emerged from the flag leaf sheath. For each panicle, the numbers of surviving and degenerated primary, secondary, and tertiary branches, and the surviving and degenerated florets on those branches were counted. The number of differentiated branches was the sum of the number of surviving and degenerated branches. Similarly, the number of differentiated florets was the sum of the number of surviving and degenerated florets. The pre-anthesis degeneration percentage of branch (%) was calculated as (number of degenerated branches/number of differentiated branches) × 100. The pre-anthesis degeneration percentage of floret (%) was calculated similarly. The degenerated branches and florets could be counted easily because their degenerated vestiges can be recognized obviously [17].

2.4. Dry matter accumulation, nitrogen uptake and grain yield measurement

At PI, heading and maturity, twelve hills of plants were randomly taken from each plot. The plants at PI and heading were separated into leaf blades and other parts including stems and leaf sheaths (and young panicles at heading). All samples were oven-dried at 105 °C for 30 min, then at 75 °C until a constant weight was reached. At maturity, the samples were separated into straw and panicles. Then the panicles were hand-threshed and separated into filled grain, unfilled grain, and rachis. The dry weight of these samples was determined after oven drying at 75 °C to a constant weight. Aboveground biomass was the sum of the dry weight of all these separated parts. N concentration of all plant parts at PI and heading was assayed using an Auto Analyzer 3 digital colorimeter (AA3, Bran + Luebbe, Germany). Aboveground N uptake was calculated by multiplying the dry weight by N concentration of each part and then summing them up.

At maturity, plants from a 5-m^2 area (125 hills) in each plot were harvested to determine grain yield, which was adjusted to 14 % moisture. Besides, 12-hill samples were taken diagonally from each plot to determine the yield components including the number of panicles per m^2, spikelets per panicle, filled grain rate and thousand grain weight.

2.5. Data analysis

Statistical analysis was performed using Statistix 8.0 (Analytical Software, Tallahassee, FL). In the analysis, treatment effects were separately assessed for each year. When ANOVA result showed a significant difference, multiple comparisons of means were performed using the Least Significant Difference method (LSD) with a 0.05 probability threshold.

Table 2
Grain yield and yield components of rice under two N treatments in the field experiments conducted in 2016 and 2017.

Treatment	Panicles per m^2	Spikelets per panicle	Filled grain rate (%)	Thousand grain weight (g)	Grain yield (t ha^{-1})	Total dry matter (t ha^{-1})
2016						
FFP	202.5 a	194.7 b	88.3 a	20.8 a	6.49 b	11.3 a
OPT	207.5 a	211.5 a	88.2 a	20.1 a	7.39 a	12.2 a
2017						
FFP	197.9 a	168.8 b	87.1 a	21.4 a	6.77 b	10.8 b
OPT	213.2 a	215.0 a	75.3 a	20.5 a	7.60 a	12.1 a

FFP, farmer's fertilizer practice; OPT, optimized N management.
Within a column for a given year, means followed by different letters are significantly different between FFP and OPT at the 0.05probability level according to *LSD*.

3. Results

3.1. Grain yield and yield components

Grain yield under OPT was significantly higher than that under FFP for both years (Table 2). The difference in grain yield between the two treatments was 13.8 % in 2016 and 12.3 % in 2017. Compared to that of FFP, the number of spikelets per panicle under OPT was significantly enhanced by 8.6 % in 2016 and 27.4 % in 2017. No significant differences between FFP and OPT were observed in the number of panicles per m^2, filled grain rate and thousand grain weight in both years. OPT had greatly more spikelets per m^2 than FFP (Fig. 1). Total aboveground dry matter at maturity was significantly greater under OPT in both years.

3.2. Characterization of panicle rachis branches

There was no significant difference between FFP and OPT in the number of primary branches in both years (Table 3). No degeneration of primary branches was observed in this study. Compared to that of FFP, the number of differentiated secondary branches under OPT was increased by 7.5 % and 7.1 % and the number of degenerated secondary branches was significantly reduced by 33.0 % and 41.7 % in 2016 and 2017, respectively. As a result, the number of surviving secondary branches under OPT was significantly greater than that under FFP, with the differences being 14.4 % and 22.9 % in 2016 and 2017, respectively. Degeneration percentage of secondary branches under OPT was significantly lower than that under FFP in both years.

The differences between FFP and OPT in tertiary branches were much greater than that in secondary branches (Table 3). All the differences between the two treatments in differentiated, degenerated, and surviving tertiary branches were significant. The number of differentiated tertiary branches under OPT was 5.0 and 6.6 times as that under FFP in 2016 and 2017, respectively. However, the number of degenerated tertiary branches under OPT was also more than five times that under FFP. The number of surviving tertiary branches under OPT was 4.9 and 7.4 times that under FFP in 2016 and 2017, respectively. The difference in degeneration percentage of tertiary branches was not significant between the two treatments in both years.

3.3. Characterization of panicle florets

There were no significant differences between FFP and OPT in the number of differentiated florets per panicle on both primary and secondary branches in both years (Table 4). However, the number of differentiated florets per panicle on tertiary branches under OPT was significantly higher than that under FFP. The total number of differentiated florets per panicle under OPT was 18.0 % and 20.5 % greater than that under FFP in 2016 and 2017, respectively. In the increment of total differentiated florets per panicle, 61.6%–67.7 % came from tertiary branches and 32.4%–36.3 % came from secondary branches.

As for the number of degenerated florets per panicle, no significant difference was observed on primary branches between FFP and OPT (Table 4). In both years, the number of degenerated florets under OPT was reduced on secondary branches but was increased on tertiary branches, indicating that OPT exerts an opposite impact on floret degeneration on secondary and tertiary branches. No significant difference existed in the number of total degenerated florets per panicle between the two treatments.

For the number of surviving florets per panicle, no significant difference was observed on primary branches between FFP and OPT (Table 4). Compared to that of FFP, the number of surviving florets on both secondary and tertiary branches was increased significantly under OPT. As a result, the total number of surviving florets on all branches under OPT was increased by 18.5 % and 27.6 % in 2016 and 2017, respectively. In the increased surviving florets per panicle under OPT, 62.2 %–65.2 % came from secondary branches, and 32.5%–37.8 % came from tertiary branches. There was no significant difference in the number of surviving florets per branch on either

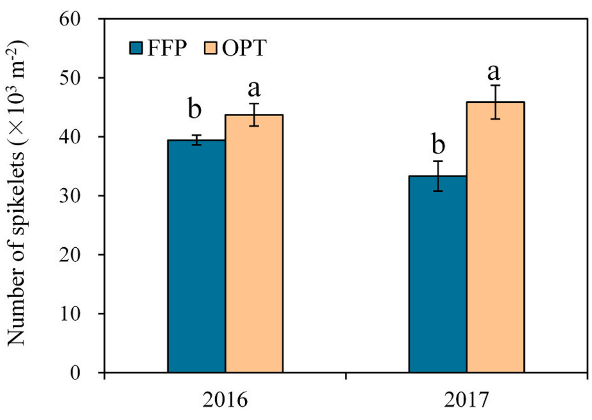

Fig. 1. Number of spikelets per m^2 under two N treatments in 2016 and 2017. FFP and OPT represent farmer's fertilizer practice and optimized N management, respectively. Vertical bars represent ±S.D. of the mean (n = 3). Different letters indicate significant difference between FFP and OPT at the 0.05 probability level within the same year.

Table 3
Number of differentiated, degenerated and surviving branches per panicle and degeneration percentage (DP) of branches under two N treatments in the field experiments conducted in 2016 and 2017.

Treatment	No. of primary branches	No. of secondary branches			DP of secondary branches (%)	No. of tertiary branches			DP of tertiary branches (%)
		Differentiated	Degenerated	Surviving		Differentiated	Degenerated	Surviving	
2016									
FFP	11.3 a	53.1 b	7.8 a	45.4 b	14.9 a	3.2 b	0.7 b	2.5 b	22.9 a
OPT	11.4 a	57.1 a	5.1 a	51.9 a	9.0 b	16.0 a	3.8 a	12.2 a	25.6 a
2017									
FFP	12.1 a	57.1 a	13.9 a	43.1 b	24.4 a	2.9 b	1.5 b	1.4 b	50.6 a
OPT	11.9 a	61.2 a	8.1 b	53.0 a	13.3 b	18.7 a	8.3 a	10.4 a	44.7 a

FFP, farmer's fertilizer practice; OPT, optimized N management.
Within a column for a given year, means followed by different letters are significantly different between FFP and OPT at the 0.05 probability level according to *LSD*.

Table 4
Number of differentiated, degenerated and surviving florets and degeneration percentage (DP) of florets on primary, secondary and tertiary branches and total florets per panicle of rice under two N treatments in the field experiments conducted in 2016 and 2017.

Floret position	Year	Treatment	No. of florets per panicle			DP (%)
			Differentiated	Degenerated	Surviving	
Primary branch	2016	FFP	62.3	0.73	61.6	1.18
		OPT	63.3	1.66	61.6	2.64
		Increment[b]	0.93ns	0.92ns	0.01ns	1.46ns
	2017	FFP	67.4	1.97	65.5	2.92
		OPT	67.3	0.83	66.5	1.23
		Increment	−0.03ns	−1.13ns	1.32ns	−1.69ns
Secondary branch	2016	FFP	182.8	28.68	154.1	15.54
		OPT	199.3	19.94	179.3	10.01
		Increment	16.51ns	−8.75ns	25.26[a]	−5.53ns
	2017	FFP	196.9	52.32	144.6	26.58
		OPT	214.8	32.17	182.6	15.09
		Increment	17.90ns	−20.15[a]	38.06[a]	−11.49ns
Tertiary branch	2016	FFP	7.34	3.14	4.20	41.3
		OPT	35.3	15.8	19.5	46.7
		Increment	28.00[a]	12.65[a]	15.34[a]	5.36ns
	2017	FFP	4.80	2.96	1.84	62.1
		OPT	42.2	21.4	20.8	50.8
		Increment	37.42[a]	18.43[a]	18.99[a]	−11.29[a]
All branches	2016	FFP	252.4	32.56	219.9	12.83
		OPT	297.9	37.39	260.5	12.54
		Increment	45.44[a]	4.83ns	40.61[a]	−0.29ns
	2017	FFP	269.1	57.24	211.9	21.28
		OPT	324.4	54.17	270.2	16.80
		Increment	55.29[a]	−3.07ns	58.37[a]	−4.48[a]

FFP, farmer's fertilizer practice; OPT, optimized N management.
Increment indicates the increased number of florets under OPT compared to that under FFP.
[a] Denotes significant difference between FFP and OPT at the 0.05 probability level within a row for a given year. ns stands for non-significant difference.

primary, secondary, or tertiary branches between FFP and OPT in both years (Fig. 2).

Distribution of differentiated, degenerated, and surviving florets on panicle branches was observed in both years (Fig. 3). More than 50 % of florets sat on secondary branches. The percentage of differentiated florets on primary and secondary branches was reduced significantly under OPT but was increased significantly on tertiary branches (Fig. 3A and B). The percentage of degenerated florets on primary branches was very low and its difference between the two treatments was not significant in both years (Fig. 3C and D). The percentage of degenerated florets on secondary branches was reduced significantly under OPT but was increased on tertiary branches in both years. There was no significant difference in percentage of surviving florets on secondary branches between FFP and OPT in both years (Fig. 3E and F). The percentage of surviving florets on primary branches was reduced significantly under OPT but was increased on tertiary branches in both years.

3.4. Dry matter accumulation and nitrogen uptake

As shown in Fig. 4, there was no significant difference between FFP and OPT in dry matter weight at PI for both years. The dry matter weight at heading under OPT was greater than that under FFP. Dry matter production from PI to heading (△DW) was 18.0 %

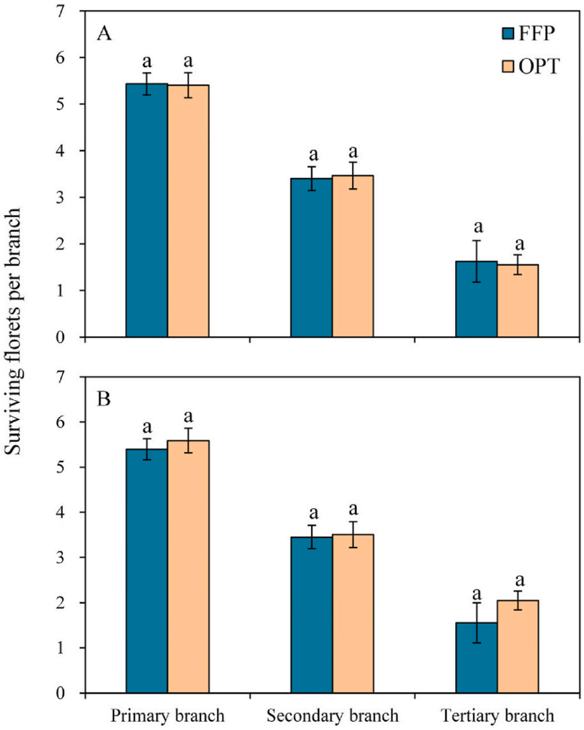

Fig. 2. Number of surviving florets per branch on primary, secondary, and tertiary rachis branches under two N treatments in 2016 (A) and 2017 (B). FFP and OPT represent farmer's fertilizer practice and optimized N management, respectively. Vertical bars represent ±S.D. of the mean (n = 3). Different letters indicate significant difference between FFP and OPT at the 0.05 probability level within the same year.

and 20.0 % greater under OPT in 2016 and 2017, respectively.

At PI, N uptake under OPT was significantly lower than that under FFP in both years. However, N uptake at heading was increased under OPT in both years. N uptake from PI to heading (△TN) was 156.7 % and 124.6 % higher under OPT in 2016 and 2017, respectively.

There was no significant difference in △DW per differentiated secondary floret between OPT and FFP (Table 5). OPT significantly increased △TN per differentiated secondary floret in both years. The △DW per differentiated tertiary floret and △TN per differentiated tertiary floret under OPT was 76.3%–88.9 % and 47.8%–78.9 % lower than that under FFP, respectively.

4. Discussion

In this study, grain yield was greatly increased under OPT in spite of a reduction in N input, which was consistent with the results from previous studies [13,14]. Although much less N was applied at early growth stage, the number of panicles per m^2 was not decreased because of higher percentage of productive tillers under OPT [13]. The panicle size was remarkably enlarged and filled grain rate was comparable under OPT with a considerable amount of N applied at panicle initiation stage (Table 2). Similar results have been reported previously [16,22–24]. Our results demonstrate again that optimized N management can enhance rice yield through enlarging panicle size. Although both rate and timing of N application were changed, timing is the main factor responsible to the large panicle size in OPT. N fertilization could increase the grain protein content and accumulation level of amyloplast molecular chaperones in rice grains [25,26]. So OPT with altered N top-dressing time enhanced the spikelet number for yield increments is likely related to the promoted total protein accumulation and starch synthesis.

In our study, the N treatment did not significantly affect the number of surviving florets per branch on either primary, secondary, or tertiary branches (Fig. 2), implying that this trait has a relatively high heritability and can hardly be affected by the environment and crop management practices [27]. Thus, it can be concluded that it is the number of surviving branches per panicle rather than the number of florets per branch that decides the final panicle size. It has been reported previously that varieties or mutants with larger panicles usually had more spikelets on secondary branches, while the number of their primary branches and florets on them were unchanged [20,21,28]. Panicle size was enlarged because of increased number of florets on secondary branches when N application was made just before panicle initiation [15]. In the present study, we found that the differentiation, degeneration and survival of primary branches and their florets were all not significantly altered by N treatment, while the number of surviving florets on secondary branches was increased under OPT (Tables 3 and 4). Moreover, we discovered that the number of differentiated and surviving florets on tertiary branches was substantially increased under OPT. About 2/3 of total additional differentiated florets and 1/3 of total additional surviving florets came from tertiary branches (Table 4). The percentage of surviving florets on tertiary branches was

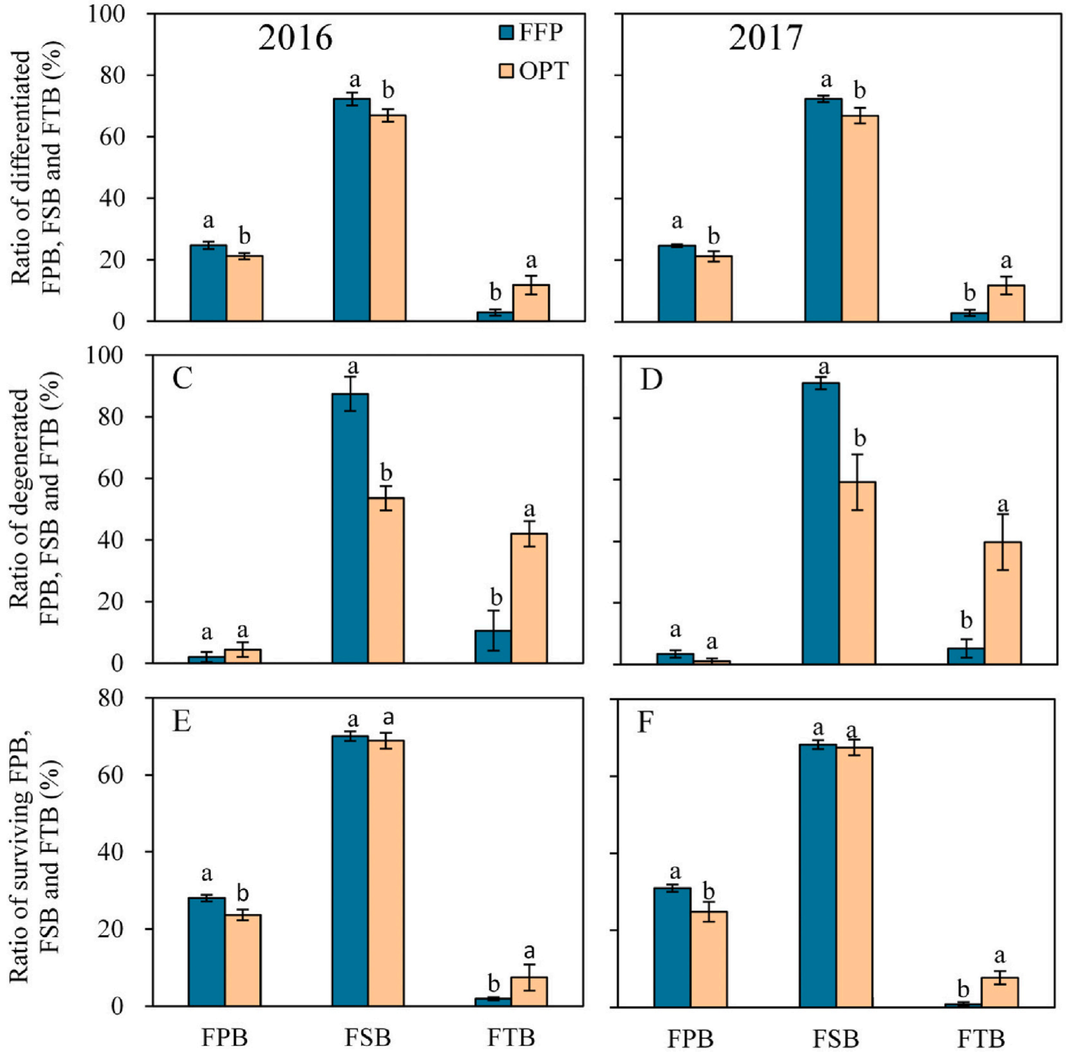

Fig. 3. Percentage of differentiated (A–B), degenerated (C–D), and surviving (E–F) florets on primary, secondary and tertiary branches (PB, SB, TB) under two N treatments in 2016 and 2017. FFP and OPT represent farmer's fertilizer practice and optimized N management, respectively. Vertical bars represent ±S.D. of the mean (n = 3). Different letters indicate significant difference between FFP and OPT at the 0.05 probability level within the same year.

remarkably higher under OPT (Fig. 3E and F). All these facts suggested that the tertiary branches and their florets contributed substantially to the enlarged panicle size under OPT. Although, only one hybrid rice variety was investigated in this study, we found similar results in another study using an inbred variety "Wushansimiao" [29]. Compared to FFP, OPT increased differentiated secondary and tertiary florets under OPT by 3.57 % and 121.1 %, respectively. The surviving secondary and tertiary florets under OPT were increased by 10.3 % and 307.1 %, respectively in compared with FFP. Consequently, panicle size of "Wushansimiao" was significantly enlarged and higher grain yield was obtained under OPT than FFP. Enhancing both differentiation and survival of tertiary branches is also essential for enlarging panicle size.

The surviving branches and their florets come from the difference between their differentiation and degeneration. Changes in either differentiation or degeneration can affect the final number of surviving branches and their florets. In this study, both surviving secondary and tertiary branches and their florets were enhanced under OPT. However, the source of variation was different for the secondary and tertiary branches. The increase in surviving secondary branches and their florets under OPT resulted from inhibition of degeneration, while the increase in surviving tertiary branches and their florets resulted from promotion of differentiation (Table 4). We found that the degeneration of tertiary branches and their florets was exacerbated under OPT (Tables 3 and 4). If the degeneration of tertiary branches and their florets could be reduced, then the sink size of rice could be further increased. Therefore, more studies should be focused on the degeneration of tertiary branches and their florets.

N is the most effective factor in spikelet differentiation and development. Larger panicle resulted by panicle N fertilizer can be explained by greater dry matter and N accumulation during panicle development [4,30]. Consistent with these researches, both △DW

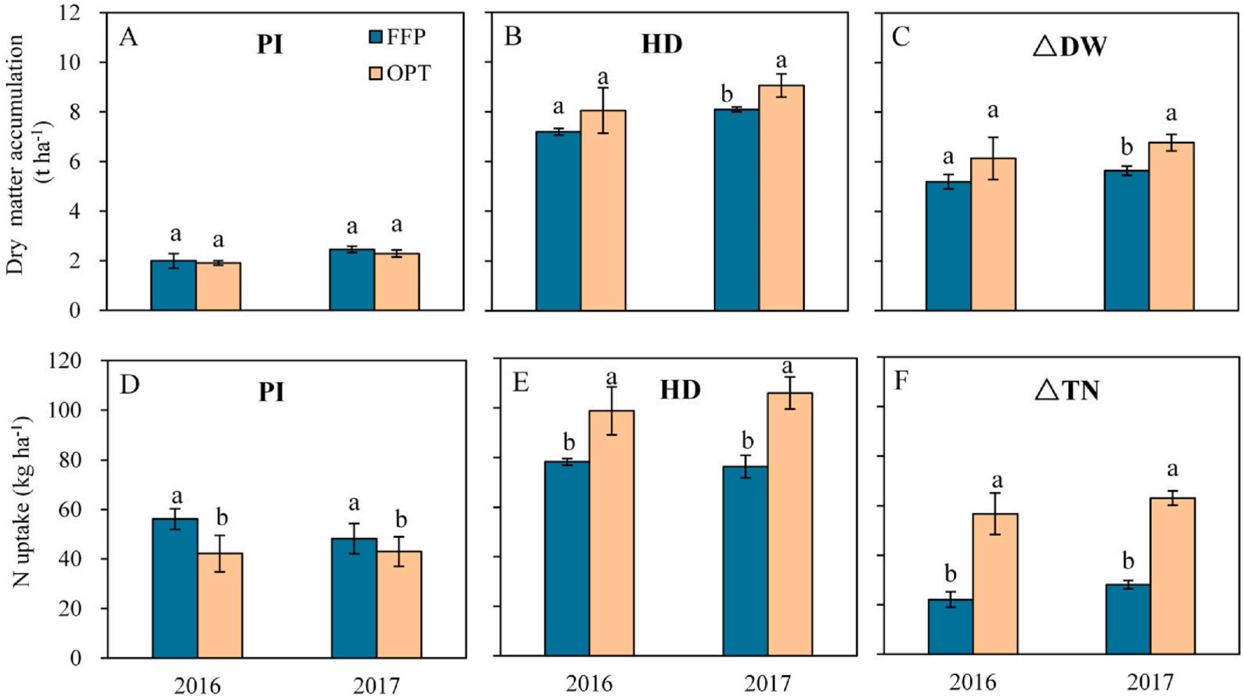

Fig. 4. Dry matter accumulation (A–C) and N uptake (D–F) at primary panicle branch differentiation (PI) and heading (HD), and from PI to HD (△DW, △TN) of rice under two N treatments in 2016 and 2017. FFP and OPT represent farmer's fertilizer practice and optimized N management, respectively. Vertical bars represent ±S.D. of the mean (n = 3). Different letters indicate significant difference between FFP and OPT at the 0.05 probability level within the same year.

Table 5
△DW per differentiated tertiary floret and △TN per differentiated tertiary floret of rice under FFP and OPT in the field experiments conducted in 2016 and 2017.

Year/ Treatment	△DW per differentiated secondary floret (mg floret^{-1})	△TN per differentiated secondary floret (mg floret^{-1})	△DW per differentiated tertiary floret (mg floret^{-1})	△TN per differentiated tertiary floret (mg floret^{-1})
2016				
FFP	14.11 a	0.06 b	372.18 a	1.55 a
OPT	14.81 a	0.14 a	88.36 b	0.81 b
2017				
FFP	14.51 a	0.07 b	705.89 a	3.46 a
OPT	14.84 a	0.14 a	78.39 b	0.73 b

FFP, farmer's fertilizer practice; OPT, optimized N management.
Within a column for a given year, means followed by different letters are significantly different between FFP and OPT at the 0.05 probability level according to *LSD*. △DW and △TN denote dry matter accumulation and N uptake from primary panicle branch differentiation to heading. △DW per differentiated floret = △DW/(number of differentiated florets per panicle × number of panicles per m^2), △TN per differentiated floret = △TN/(number of differentiated florets per panicle × number of panicles per m^2).

and △TN were greater under OPT (Fig. 4), which could bolster the formation of large panicles. In this study, the panicle fertilizer of N was top-dressed at the primary branch differentiation stage and began to be effective at the differentiation of secondary branches and their florets. Greater △DW and △TN under OPT not only inhibited the degeneration of secondary branches and their florets, but also promoted the differentiation of tertiary branches and their florets. However, the degeneration of tertiary branches and their florets were still much higher (Tables 3 and 4). OPT showed less △DW and △TN per differentiated tertiary floret compared to FFP (Table 5), suggesting that the increased dry matter and N nutrient was insufficient for survival of so many differentiated tertiary florets. In addition, sink efficiency may be an important reason for this problem. Spikelets locating on the basal part of panicle in rice have weaker physiological activity, which usually are poorly developed [31,32]. The tertiary branches/florets on the basal secondary branches should have weaker sink efficiency to compete nutrients.

N absorption was correlated with greater spikelet differentiation. However, the number of primary branches and their florets was not increased by the higher N content at panicle initiation under FFP. In other studies, panicle N fertilizer did have no significant effect on primary branches or their florets [15]. Increased N concentration in the stems did not promote spikelet differentiation [33]. Generally speaking, among the traits related to rice panicle, the heritability for primary branches and panicle length were very high

[34]. This explained why little change was observed between OPT and FFP in differentiation, degeneration and survival of primary branches and their florets.

It was reported that enlarged panicle size by N fertilizer was closely related to hormones which could stimulate the growth of rachis branches and florets. Ding et al. [15] found that N fertilizer increased number of flowers and branches by enhancing cytokinin synthesis in rice. In response to N treatment, enhanced brassinosteroids biosynthesis and signal transduction enhanced spikelet differentiation, reduced spikelet degeneration, and thus increased grain yield [35]. However, ethylene concentration in panicle was positively correlated to spikelet degeneration [36,37]. Accumulation of free-polyamines and ethylene in young panicles of rice was also related to the reduction in percentage of degenerated florets [38].

Panicle formation is controlled by specific genes related to branches and spikelets differentiation or degeneration [36,39–41]. Wu et al. [42] found that the novel N-responsive OsNLP3/4-OsRFL-OsMOC1 module that integrates N availability to regulate panicle architecture. Some functional genes of panicle development were differentially expressed in response to N fertilizer in rice, such as *OsCD1*, *OsCKX2/Gn1a* and *SP1*, maybe plays an important role in N fertilizer regulating spikelets per panicle in rice [43]. In another experiment, we found that *OsBAK1-6* encoding brassinosteroids signal pathway at the pollen mother cells meiosis stage was up-regulated by 5.1 folds under OPT (Fig. S1). However, almost all the existing studies focused on primary and secondary branches of the panicle. Very limited information is available in the literature regarding the differentiation, degeneration and survival of tertiary branches and their florets. Further studies are needed to understand the physiological and molecular mechanisms underlying the formation of enlarged panicle size under OPT, especially those related to tertiary branches.

To our knowledge, this is the first report on the detailed changes in panicle architecture and their involvement in panicle enlargement and yield gain under OPT, especially the contribution of tertiary branches to the enlarged panicle size. Nevertheless, limitations of the present study should be acknowledged. We generalized the conclusion using only one hybrid rice variety in this study. In addition, the mechanism underlying N fertilizer affecting different grade of rachis branches and their florets has not been clarified, although dry matter accumulation and N uptake partly explain the reasons. Further study needs to be conducted to elucidate the deeper mechanism at physiological, biochemical and molecular levels.

5. Conclusion

The grain yield was significantly increased through enlarged panicle size under OPT with 16.7 % less N input. Panicle size was mainly determined by the number of branches per panicle, rather than florets per branch. N management had little effect on the differentiation, degeneration and survival of primary branches and their florets. The difference in panicle size between OPT and FFP resulted mainly from increased surviving secondary and tertiary branches. Under OPT, the increase in surviving secondary branches number resulted from both enhanced differentiation and reduced degeneration, while the increase in tertiary branches number was merely from enhanced differentiation. Both secondary and tertiary branches were principal contributors to increase in panicle size. Greater dry matter production and N uptake from panicle initiation to heading was an important reason of increased branches and enlarged panicle size. To our knowledge, this is the first report on the detailed changes in panicle architecture and their involvement in panicle enlargement and yield gain under OPT.

Additional information

No additional information is available for this paper.

Funding

This study was supported by the Guangdong Modern Agricultural Industry Technology System for Rice (2023KJ105), the Guangdong Provincial Rural Revitalization Strategy Special Fund Seed Industry Revitalization Project (2022-NPY-00-005), the Team Construction Project of Agricultural Emerging Discipline of Guangdong Academy of Agricultural Sciences (202112TD), and the Special Cultivation Project for National Scientific and Technological Achievements from Guangdong Academy of Agricultural Sciences.

Data availability statement

Data included in article/supplementary material in the article.

CRediT authorship contribution statement

Xiangyu Hu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yanzhuo Liu:** Writing – review & editing, Writing – original draft. **Xuhua Zhong:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Rui Hu:** Methodology, Investigation. **Meijuan Li:** Writing – review & editing. **Bilin Peng:** Investigation. **Junfeng Pan:** Writing – original draft. **Kaiming Liang:** Writing – review & editing. **Youqiang Fu:** Writing – original draft. **Nongrong Huang:** Writing – original draft.

Declaration of competing interest

The author declared that there is no conflict of interest.

Acknowledgement

The authors would like to thank Mr. Guodong Zhang, Mr. Jinhua Liu, Ms. Fuxiu Zhang, Mr. Qingshui Liu and Mr. Jinxing Xu for their assistance in crop management and data collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34607.

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