Construction of Rice Under Dry Cultivation Dominant Population by Interaction of Seeding Rate and Nitrogen Rate

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Abstract

In order to construct the dominant population of rice under dry cultivation, the external characteristics of the population and the photosynthetic physiological characteristics of the upper three leaves were investigated. In this study, a double factor interaction method of seeding rate and nitrogen rate was used to construct the dominant population of rice under dry cultivation. We determined 195 $kg \cdot ha^{-1}$ seeding rate and 140 $kg \cdot ha^{-1}$ nitrogen rate as the appropriate configuration. To achieve high yield, high nitrogen utilization and moderate morphological characteristics by coordinating the comprehensive advantages of population spike number and spike grain number, and to increase the photosynthetic potential of the population by coordinating the reasonable distribution of light energy in the upper three leaves to construct the dominant population of rice under dry cultivation. Photosynthetic potential of 105.55$m^2 \cdot d/m^2$, light energy interception rate of 31.21% in the inverted second leaf and plant height of 101 cm at 10 days after flowering were important external characteristics of the dominant population of rice under dry cultivation. The ability of the inverted second leaf to intercept light energy is the basis for ensuring the photosynthesis of the population. The simultaneous coordination of photosynthetic enzyme activity, net photosynthetic rate and chlorophyll content in the inverted second leaf is an important physiological characteristic of the photosynthetically dominant group. The variation of photosynthetic physiological indexes in the inverted second leaf was characterized by ‘from genes to enzyme activity to net photosynthetic rate and chlorophyll, and the spatial variation was from the upper leaves to the lower leaves’. ‘ Protect the flag, promote the second, stabilize the third ’ (Maintaining photosynthetic capacity of the flag leaf , promoting photosynthetic enzyme activity in the inverted second leaf, and stabilizing photosynthetic gene expression in the inverted third leaf) is an important tool to construct the dominant population of rice under dry cultivation. These studies have significant implications for the future construction of dominant rice under dry cultivation in different regions and provide an important basis for the study of the regulatory mechanisms of photosynthetic pathways in different leaf positions.

1. Introduction

Rising temperatures and water scarcity due to climate change have become an urgent global challenge. Growing population, increasing agricultural water demand and decreasing available freshwater resources have exacerbated the impact of drought on agricultural production, and it is crucial to study sustainable agricultural production models. With the advancement of modern industry and agriculture, the concept of lighter production of crops with high yield, high efficiency, high quality, green, and adapted to mechanization and information technology is gaining popularity. Rice is at the core of food crops[1], and the exploration of lightly simplified cultivation technology for rice has received much attention from scholars at home and abroad[2–4]. Dry cultivation of rice is a kind of rice crop mode which is different from transplanted rice, water direct seeding, wet direct seeding and dry direct seeding water pipe. It is a rice crop mode that does not go through seedling and transplanting, and is directly sown under dry land preparation conditions, relying mainly on natural precipitation during the whole reproductive period, and only appropriately replenishing water during critical periods of water demand or in times of drought. Dry cultivation of rice has been rapidly developing in different rice regions at home and abroad by eliminating seedling breeding and transplanting, saving water resources, improving labor efficiency, optimizing land use efficiency, and adapting to mechanization. Seeding rate and nitrogen rate are important cultivation measures affecting rice yield formation and population quality composition[5,6]. Numerous previous studies have been done based on the effects of seeding and nitrogen variation on dryland crop populations such as cotton[7], oilseed rape[8], maize[9], and wheat[10]. In the light and simplified cultivation of rice mostly from the perspective of variety, sowing period, density, residues[11], fertilizers[12] and mechanized planting methods[13], it fully shows that the measures of each production link have a significant impact on the construction of dominant population of light simplified cultivation[14–16], and also reveals the importance of canopy photosynthetic capacity[17,18]. Previous studies mostly studied and optimized the characteristics of crop population from the perspective of single factor. At present, many scholars have constructed crop dominant population from the perspective of two factors and multi factor interaction[19,20]. The influence of photosynthetic coordination of different leaf positions on crop population has also attracted much attention. At present, there are few studies on the construction of rice under dry cultivation dominant population based on two factor interaction, and there are few reports on the photosynthetic characteristics of different leaf positions under the interaction of seeding rate and nitrogen rate in China. Therefore, in this study, Suijing 18 was used as the material to study the effects of different leaf position morphological characteristics, photosynthetic changes, yield and nitrogen absorption and utilization by means of the interaction between seeding rate and nitrogen rate, so as to clarify the suitable seeding rate and nitrogen rate allocation of the dominant population under dry cultivation, and to explore the photosynthetic physiological characteristics of upper three leaves in the dominant population under dry cultivation. The research results provide population optimization methods and breeding objectives for high-yielding and high-efficiency light-simplified cultivation patterns of rice under dry cultivation, and provide theoretical basis and technical support for the construction of high-light-efficient populations.

2. Results

Yield and yield components

The effects of seeding rate, nitrogen rate and the interaction effect of both on yield reached significant levels ($P = 0.001$;P0.01$P = 0.011$). The mean yield under each nitrogen rate was highest in the A4 population. The highest yielding combinations at each seeding rate were A1B5, A2B5, A3B4, A4B5, and A5B5 (Table 1), and the plants at A4 and A5 seeding rates were severely collapsed at the filling stage after nitrogen rate reached B4, with the A4B3 combination being highly yield and A2B5 > A3B4 > A4B3 > A1B5 > A5B3.

Table 1

Effects of interaction between seeding rate and nitrogen rate on yield and yield components of rice under dry cultivation condition. A: seeding rate, B: N rate. A1: 60kg·ha^{-1}, A2: 105kg·ha^{-1}, A3: 150kg·ha^{-1}, A4: 195kg·ha^{-1}, A5: 240kg·ha^{-1}; B1: 0kg·ha^{-1}, B2: 70kg·ha^{-1}, B3: 140kg·ha^{-1}, B4: 210kg·ha^{-1}, B5: 280kg·ha^{-1}. The value represents the mean ± SD of three replicates. Different letters with the same sowing amount indicate a significant difference at P < 0.05. * and ** indicate that the yield components are significantly influenced by the N rate, seeding rate and their interactions at 0.05 and 0.01 levels, and ns indicates "not significant". Same as below.

N rate (kg·ha^{-1})	Seeding rate(kg·ha^{-1})	Panicle number (×10^4 ha^{-1})	Grains per panicle	Filled grain rate(%)	1000-grain weight(g)	Grain yield (kg ha^{-1})
A1	B1	284.7 ± 10.1 a	69.5 ± 1.2 a	94.4 ± 1.1 a	25.2 ± 0.6 a	4700.8 ± 86.7 b
	B2	318.3 ± 17 a	83.1 ± 2.3 a	92.9 ± 1.7 a	26 ± 0.3 a	6395.7 ± 139 ab
	B3	370.3 ± 15.9 a	80.7 ± 1.2 a	93.1 ± 0.7 a	25 ± 0.5 a	6960.3 ± 174.2 ab
	B4	321.7 ± 5.6 a	76.5 ± 2.8 a	93.6 ± 0.5 a	26.2 ± 0.8 a	6049.9 ± 159.6 ab
	B5	390 ± 13.9 a	83.3 ± 1.2 a	95.4 ± 1.2 a	25.7 ± 1.8 a	7967.2 ± 231.7 a
	Mean	337.0	78.6	93.9	25.6	6414.8
A2	B1	221.7 ± 6.8 c	69 ± 2 a	91.6 ± 2 a	24.6 ± 0.6 a	3452.2 ± 159.2 c
	B2	417 ± 10.2 ab	77.1 ± 2.6 a	94.9 ± 1.1 a	24.9 ± 0.7 a	7587.7 ± 152.8 b
	B3	381.3 ± 18.9 b	80.9 ± 3.2 a	93.4 ± 1.7 a	26.1 ± 1.4 a	7512.3 ± 228.6 b
	B4	411.7 ± 17 ab	72.8 ± 1.9 a	95.8 ± 1 a	25.1 ± 2 a	7202.5 ± 85 b
	B5	511.7 ± 20.5 a	84.5 ± 1.1 a	93.5 ± 1.3 a	24.4 ± 1 a	9843.4 ± 202.9 a
	Mean	388.7	76.9	93.8	25.0	7119.6
A3	B1	267.3 ± 20.5 b	53.8 ± 3.1 c	94.9 ± 1.6 a	24.8 ± 0.6 a	3379.3 ± 167.1 c
	B2	285 ± 11.2 ab	68.7 ± 2.1 b	88.5 ± 1.5 a	26 ± 1.3 a	4497.5 ± 105 bc
	B3	407 ± 9.5 ab	75.5 ± 0.9 b	91.4 ± 0.2 a	24.4 ± 0.6 a	6846.6 ± 79.5 ab

N rate ($kg \cdot ha^{-1}$)	Seeding rate($kg \cdot ha^{-1}$)	Panicle number ($\times 10^4$ ha^{-1})	Grains per panicle	Filled grain rate(%)	1000-grain weight(g)	Grain yield ($kg\ ha^{-1}$)
	B4	447.7 ± 16.5 a	91.2 ± 3.8 a	90.4 ± 1.1 a	24 ± 0.6 a	8866.5 ± 181.2 a
	B5	417.3 ± 18.7 ab	79.5 ± 2.5 ab	93.4 ± 0.5 a	25.2 ± 1 a	7805.5 ± 208.4 a
	Mean	364.9	73.7	91.7	24.9	6279.1
A4	B1	340.7 ± 20.6 a	59.9 ± 3.4 d	90.6 ± 1.1 a	23.4 ± 0.7 b	4331.5 ± 189.4 c
	B2	504 ± 16.3 a	71.9 ± 2.4 bc	94 ± 2.4 a	25.2 ± 0.4 ab	8581.7 ± 157.3 ab
	B3	445.3 ± 21.8 a	81.6 ± 1.4 ab	93.2 ± 1.7 a	25.9 ± 1.1 a	8763.9 ± 207.3 ab
	B4	471.3 ± 24.5 a	67.9 ± 1.9 cd	93.5 ± 2.8 a	24.8 ± 1.6 ab	7426.4 ± 181 b
	B5	498 ± 11.5 a	87.3 ± 1.3 a	95.8 ± 1 a	25.2 ± 0.3 ab	10499.8 ± 230.4 a
	Mean	451.9	73.7	93.4	24.9	7920.7
A5	B1	497.7 ± 17.2 b	40 ± 1.4 b	91 ± 1.1 b	24.7 ± 1.6 a	4469.1 ± 216.8 a
	B2	421.7 ± 25.2 b	66.9 ± 3.3 a	94.6 ± 1.6 ab	25.2 ± 1.3 a	6710.7 ± 138 ab
	B3	512.3 ± 5.6 b	62.1 ± 2.5 a	95.9 ± 1.5 a	26 ± 0.2 a	7950.1 ± 221.2 a
	B4	491.7 ± 23.3 b	72.4 ± 3.3 a	91.5 ± 2.6 b	24.8 ± 1 a	8065 ± 207.4 a
	B5	619 ± 11.8 a	60.6 ± 2.1 a	91.5 ± 1.2 b	24.5 ± 1.3 a	8422.9 ± 129 a
	Mean	508.5	60.4	92.9	25.0	7123.6
Variance analysis	A	**	**	*	ns	**
	B	**	**	ns	ns	**
	A × B	*	**	**	ns	*

Increasing the seeding rate could increase the average level of population panicle number, while decreasing the average level of grains per panicle. The degree of response to nitrogen changes varied at different seeding rates, and increasing nitrogen rate at A1 and A2 seeding rates could increase panicle number and grains per panicle to obtain high yield. A3 seeding rate under B4 nitrogen rate to achieve the highest panicle number and grains per panicle to obtain high yield. The population grains per panicle was lower under A4 and A5 seeding rates, and the increase in nitrogen rate resulted in higher yields mainly by increasing the population panicle number.

External features

The mean levels of photosynthetic potential, plant height, and light energy interception were highest under the A4 population among the seeding rates. Photosynthetic potential increased with increasing nitrogen rate and was higher at B4 and B5 at all seeding rates, which was consistent with the pattern of yield trends.The average level of light energy interception rate of the population showed an inverted second leaf > flag leaf, with the highest values occurring in the range of B3-B5 at all seeding rates. Plant height showed an overall increasing trend with increasing nitrogen rate, compared to the increase at B1, which gradually increased with increasing seeding (Fig. 1)The coordination of photosynthetic potential, light energy interception capacity of different leaf positions and plant height of the A4B3 population under interaction conditions was reasonable. Photosynthetic potential of 105.55 $m^2 \cdot d/m^2$, 31.21% of light energy interception in inverted second leaf and plant height of 101 cm at 10 days after flowering were important external characteristic of the dominant population of rice under dry cultivation.

Net photosynthetic rate and chlorophyll content

With the increase of seeding rate, Pn and Chl in the upper three leaves showed a decreasing trend, and Pn and Chl in different leaf positions under each seeding rate were higher in the flag leaf and the inverted second leaf. The results showed that the Pn and Chl levels of population could be improved by increasing nitrogen rate, but the response degree of different leaf positions to nitrogen change was different under different seeding rates. When the seeding rate reached A4-A5, the continued increase in nitrogen rate after reaching B3 would cause the flag leaf Pn and Chl to show a decreasing or leveling off trend. The trend of Pn and Chl in the inverted second leaf and the inverted third leaf was the same. Under A3-A5 seeding rate, Pn reached a higher level at B4, and Chl was higher at B3-B4 level under A4-A5 seeding rate. With the continuous increase of nitrogen rate, Pn and Chl showed a stable or downward trend. In terms of the interaction between nitrogen rate and seeding rate, the highest values of Pn and Chl in three leaf positions were in the range of B3-B5. Under A4 seeding rate, the total Pn and Chl of A4B4 combination were the highest, and A4B3 could also reach a higher level (Fig. 2).

Rubisco and RCA activity

The average level of Rubisco activity was higher in the flag leaf and the inverted second leaf at all seeding rates. The RCA activity among leaf positions at A1-A4 seeding rate was higher in the inverted second leaf and the inverted third leaf and lower in the flag leaf. The activity of Rubisco and RCA was higher in the inverted second leaf at A4 seeding rate. Rubisco activity of the flag leaf under A1-A3 seeding rate was higher under B1 and B5 nitrogen rate. Under A4-A5 seeding rate, the Rubisco activity tended to decrease by continuing to increase the nitrogen rate after reaching B3-B4, and higher RCA activity could be achieved at the three-leaf position under B2 or B3 nitrogen rate. The overall Rubisco activity at the three-leaf position was higher in the A4B3 treatment with respect to the interaction between seeding rate and nitrogen rate. The overall RCA activity at the three-leaf position was higher in A4B2 and A4B3 treatments at A4 seeding rate (Fig. 3).

rbcL **and** rca **gene expression**

With increasing seeding, the total *rbcL* expression in the upper three leaves showed a decreasing and then increasing trend, and the *rca* expression showed a decreasing trend. The mean level of *rbcL* expression at each seeding rate was higher in the inverted third leaf and flag leaf. The mean level of *rca* expression under A1-A4 seeding rate showed higher levels in the inverted second leaf and the inverted third leaf and lower levels in the flag leaf among leaf positions. With regard to the interaction between seeding rate and nitrogen rate, the total *rbcL* expression at the three-leaf position of rice under dry cultivation was highest at B5 under A1 seeding rate and *rca* expression was highest at B4. The total expression of *rbcL* and *rca* at the three-leaf position was higher in B1 and B2 at A2, A3 and A5 seeding rates. The highest *rbcL* expression was reached at A4B3 treatment under A4 seeding rate (Fig. 4).

Nitrogen accumulation and utilization

The contradiction between high population nitrogen accumulation and low utilization can be reconciled by the interaction between seeding rate and nitrogen rate. The average levels of TNA, NRE and NAE in A1-A2 range showed an upward trend with the increase of seeding rate, while the average levels of A4-A5 showed a downward trend, which were higher at A2 and A4 and lower at A1, A3 and A5 under different seeding rates. There was no significant difference in TNA at B3 and B5 for A1 seeding rate, but the highest value of TNA was in B4-B5 under A2-A5 seeding rate. NRE was higher in the B2-B3 range under A1 and A2 seeding rates. NRE decreased with increasing nitrogen rate in the B4-B5 range at A3-A5 seeding rates. The NAE under A1 and A2 conditions was highest at B2 and lower at B4 and B5. NAE was lower under A4 and A5 seeding rates than under B4 and B5 conditions (Fig. 5).

3. Discussion

Panicle number, grains per panicle, filled grain rate and 1000-grain weight are the four components of yield. Increasing the number of spikes and grains on the basis of stable filled grain rate and 1000-grain weight was considered by previous authors as the key to increase rice yield[21–23]. Nitrogen and density control are the two most important crop management practices that significantly affect rice growth and yield formation[24–26]. In this study, effective panicle number and grains per panicle were positively correlated with yield (r = 0.750**; r = 0.632**), and the interaction of seeding rate and nitrogen rate had significant effect on panicle number and grains per panicle (P = 0.018; P < 0.01). The number of effective panicles could be increased by increasing nitrogen rate, and number of plants could be increased by increasing density.The increase of panicle number and grains per panicle restricted each other[27]. In this study, with the increase of seeding rate, the panicle number increased and grains per panicle decreased, and nitrogen rate applied to achieve the highest yield under each sowing rate was different, and the interaction between seeding rate and nitrogen rate can coordinate the comprehensive advantages of panicle number and grains per panicle, increase group storage capacity to achieve high yield.

In terms of the external characteristics of photosynthetic organs of dominant populations, studies have shown that reasonable sowing density can regulate the population structure of rice and alleviate the contradiction between individual development and population growth[28]. Increased nitrogen fertilization can increase the photosynthetic potential after tasseling[29]. In this study, the population photosynthetic potential was highly significantly and positively correlated with yield 10 days after flowering (r = 0.854**), and increasing the amount of seeding and nitrogen application could increase the population photosynthetic potential and enhance the population photosynthetic potential. Excessive N application can lead to excessive population density and plant collapse[30–32], and also reduces N fertilizer utilization, which is detrimental to ecological benefits[33–35]. In this study, severe collapse occurred in groups with excessive plant height at seeding rates of 195–240 kg·ha $^{-1}$ with nitrogen rate over 140 kg·ha $^{-1}$. The nitrogen agronomic utilization efficiency was the highest under the combination of seeding rate 195 kg·ha $^{-1}$ and nitrogen rate 140 kg·ha $^{-1}$. It has been shown that the plant shape of rice direct seeding at the flush stage is characterized by high leaf position, longer leaf length in the upper three leaves, smaller leaf-stalk angle, and external morphology exhibiting elongated leaf shape and compact plant shape[36]. Increasing N application and population density promotes plant growth and increases photosynthetic potential as well as interception of photosynthetically active radiation, which is an important prerequisite for a dominant population[37–39]. In this study, there was a two-way positive correlation between the photosynthetic potential of the population at 10 days after flowering, the light energy interception rate of the inverted second leaf and yield, indicating that the inverted second leaf played an important role in coordinating the light energy interception capacity of the population. It is unreasonable to excessively increase the seeding rate and nitrogen rate in conjunction with a comprehensive analysis of population photosynthetic organ morphology, plant height, collapse, and ecological benefits. The seeding rate of 195 kg·ha $^{-1}$ and the rate of 140 kg·ha $^{-1}$ of nitrogen can coordinate the interception of light energy by the inverted second leaf and thus improve the interception of light energy by the population, coordinate the photosynthetic potential of the population, and at the same time achieve the appropriate plant level to obtain a dominant population with no collapse, high yield and high ecological efficiency.

In terms of photosynthetic physiological characteristics of the dominant group, Pn level and Chl content are important indicators to evaluate the photosynthetic capacity of the crop[40,41]. In this experiment, Pn and Chl showed two highly significant positive correlations among leaf positions, with higher Pn and Chl in the flag leaf and the inverted second leaf. PN and Chl of the inverted second leaf had the highest correlation with yield (r = 0.488 * *; r = 0.679 * *). RCA activity can regulate Rubisco initial activity, Rubisco activity is highly positively correlated with Pn, and Rubisco activity decreases faster than photosynthesis and chlorophyll content during leaf senescence[42,43]. In this study, the inverted second leaf RCA enzyme activity was positively correlated with Rubisco enzyme activity to the highest degree, and the inverted second leaf Rubisco activity was positively correlated with Pn, and the flag leaf Pn and Chl were higher but not strongly synchronized with photosynthetic enzyme activity, indicating that the synergism between Pn and Rubisco is particularly important in photosynthetic physiological characteristics in addition to having higher Pn and Chl. As a key photosynthetic enzyme, Rubisco activity is affected by the external environment[44–45]. In this study, the activity of Rubisco was positively correlated with photosynthetic potential, the positive correlation between light energy interception rate and Rubisco enzyme activity was the highest in different leaf positions in the inverted second leaf. It indicates that the photosynthetic enzyme activity, net photosynthetic rate and chlorophyll content of the rice under dry cultivation the inverted second leaf 10 days after flowering were synchronized and coordinated, which in turn enhanced the yield potential of the population.

It has been found in photosynthesis-related genes that once plants are exposed to light, the transcript levels of *rbcL*, *rca* increase rapidly, and Rubisco activity increases similarly[46]. In this study, the changes in photosynthetic index values and the synergism among the indexes under dry cultivation in rice were related to leaf position, and the expression of *rbcL* gene in the inverted third leaf was positively correlated with Rubisco enzyme activity in the inverted second leaf. Positive correlation between *rca* gene expression and RCA activity in inverted second and inverted third leaf. The *rca* gene expression and RCA activity were lower and the synergism of gene, enzyme activity and net photosynthetic rate changes were lower in the flag leaf. Flag leaf of rice gradually senesce during tassel set, initially characterized by a decrease in photosynthetic rate and protein content, followed by a decrease in chlorophyll and RNA[47]. In this study, 10 days after flowering, the photosynthetic physiological characteristics of three leaves were high gene expression level in lower leaves, higher enzyme activity in middle leaves, and stable net photosynthetic rate and chlorophyll content in upper leaves. The change order of photosynthetic index was from gene to enzyme activity, then to net photosynthetic rate and chlorophyll, from the flag leaf to the inverted second leaf and then to the inverted third leaf.

Through the interaction of seeding rate and nitrogen rate, this study provided theoretical and practical basis for constructing the dominant population of rice under dry cultivation and formulating the breeding objectives and optimization system of rice under dry cultivation varieties, and provided a theoretical basis for coordinating the synchronization of genes, enzyme activities and photosynthetic indexes of the upper three leaves of rice under dry cultivation by means of genetic engineering and proteomics (Fig. 6).

4. Conclusions

There is a threshold value for photosynthetic potential and plant height 10 days after flowering in rice dry crop. Too large will lead to uncoordinated distribution of light energy in the upper three leaves of the population, large population competition, greed and late maturity leading to yield decline and collapse. the inverted second leaf are superior in light energy interception and have a greater impact on population photosynthetic coordination. Changes in photosynthetic physiological indicators were characterized as "from gene to enzyme activity to net photosynthetic rate and chlorophyll, and the spatial variation is from the upper leaf to the lower leaf. ‘ Protect the flag, promote the second, stabilize the third ’ (Maintaining photosynthetic capacity of the flag leaf, promoting photosynthetic enzyme activity in the inverted second leaf, and stabilizing photosynthetic gene expression in the inverted third leaf) is an important tool to construct the dominant population of rice under dry cultivation. This study showed that under the interaction of 195kg·ha $^{-1}$ seeding rate and 140·ha $^{-1}$ nitrogen rate could build a dominant population with coordinated light energy distribution in the upper three leaves, moderate plant size, large yield advantage and high nitrogen agronomic utilization under reciprocal crop, which can be used as a reference sowing rate and nitrogen application rate for rice under dry cultivation in central Jilin Province.

5. Materials And Methods

Test site and test materials

The trial was conducted in 2019 and 2020 at the National Crop Variety Validation Characterization Station on the campus of Jilin Agricultural University, Changchun, Jilin Province (125°39E, 44°46N), where the frost-free period was 135–140 days, The test material was Suijing 18.

Experimental design

The experiment used a two-factor split-zone design, with five levels of seed sowing at 60 kg·ha $^{-1}$ (A1), 105 kg·ha $^{-1}$ (A2), 150 kg·ha $^{-1}$ (A3), 195 kg·ha $^{-1}$ (A4) and 240 kg·ha $^{-1}$ (A5) in the main zone and five levels of nitrogen application at 0 kg·ha $^{-1}$ (B1), 70 kg·ha $^{-1}$ (B2), 140 kg·ha $^{-1}$ (B3), 210 kg·ha $^{-1}$ (B4) and 280 kg·ha $^{-1}$ (B5) in the secondary zone, respectively. The plot area was 20 m^2 with three field replications; the seeds were manually simulated mechanical strip sown. Seeded on May 6 both years, coated before sowing and dried in the shade to be non-sticky; the sowing row spacing was 25 cm in all treatments. 75 kg·ha $^{-1}$ each of phosphate (in P_2O_5) and potash (in K_2O) were used as basal fertilizer for each treatment and urea was used as basal fertilizer for nitrogen. Ridges were built around each treatment to prevent water and fertilizer loss. During the whole reproductive period, we mainly relied on natural rainfall, and only used sprinklers for uniform water replenishment during drought and critical water demand periods. Other field management measures were carried out according to the general high-yielding field model to ensure consistent management in all experimental plots.

Yield and yield components

Three rows of 4 m each were selected as survey points in each plot before harvest, and the average was used to calculate the effective number of spikes. Another 15 representative plants were taken for seed testing, and the number of spikes, fruit set rate and thousand grain weight were counted.

Net photosynthetic rate

Net photosynthetic rate (Pn) was measured 10 days after flowering on the main stem rapier leaves, the inverted second leaves, and the inverted third leaves using a Li−6400XT photosynthesizer with a built-in fixed light source and a light quantum density setting of 1200 μmol·m $^{-2}$·S $^{-1}$. The measurements were made between 9:00 and 11:30 a.m. on a clear, windless day, with three replicates of each treatment, and the mean values were calculated.

Chlorophyll

Three replicates of the fully expanded leaves of the main stem, the inverted second and third leaves were selected, and 0.1 g of fresh samples of cut and mixed leaves were extracted with 95% ethanol, and chlorophyll a and chlorophyll b contents (mg/g) were calculated after measuring the absorbance at 665 nm and 649 nm with a spectrophotometer.

Rubisco and RCA activity

The fresh leaves of upper three leaves on day 10 after flowering were used for the determination of Rubisco and RCA enzyme activities, and each treatment was replicated three times and entrusted to Qingdao Sci-Tech Quality Testing Co.

Rubisco enzyme activity assay: A solid phase antibody was made by coating a microtiter plate with purified plant Rubisco 1,5-diphosphate antibody. Plant Rubisco was added sequentially to the microtiter wells coated with the monoclonal antibody, and then combined with HRP-labeled Rubisco antibody to form an antibody-antigen-enzyme-labeled antibody complex, which was washed thoroughly and then colored with the substrate TMB. TMB is converted to blue by HRP enzyme and to the final yellow color by the action of acid. The shade of color was positively correlated with the plant Rubisco in the sample. The absorbance (OD) was measured at 450 nm using an enzyme standardizer, and the concentration of plant Rubisco activity in the samples was calculated by the standard curve.

RCA enzyme activity assay: Purified plant Rubisco activase (RCA) antibody was used to coat the microtiter plate to make a solid phase antibody. Plant RCA was added sequentially to the microtiter wells coated with the monoclonal antibody, and then combined with HRP-labeled RCA antibody to form an antibody-antigen-enzyme-labeled antibody complex, which was washed thoroughly and then colored with the substrate TMB. TMB is converted to blue by HRP enzyme catalysis and to a final yellow color by the action of acid. The shade of color was positively correlated with the plant RCA in the sample. The absorbance (OD value) was measured at 450 nm using an enzyme marker and the concentration of phyto-RCA activity in the samples was calculated from the standard curve.

rbcL **and** rca **gene expression**

Fresh leaves of upper three leaves from 10 days after flowering were taken for RNA extraction. A small amount of leaves were cut and ground in a pre-cooled mortar, and about 0.1 g of powder was weighed for RNA extraction. RNA extraction of the leaf material was performed using the Trizol method (TAKARA), followed by reverse transcription using a reverse transcription kit (TAKARA).The procedure was performed according to the instructions of TAKARA Trizol and PrimeScriptTMRT reagent Kit with gDNA Eraser. The cDNA was diluted 1:3 before being used for qPCR.

The primer sequences of the target genes rbcL, rca and the internal reference gene actin used in this study were obtained from the report of Hongling Tang[47], and the primer information is shown in (Table 2).

Table 2
Sequences of primers for Real-time quantitative PCR.

Gene name	Gene ID	Forward primer(5′-3′)	Reverse primer(5′-3′)
actin	X16280	GAGACCTTCAACACCCCTGCTA	ATCACCAGAGTCCAACACATTACCT
rbcL	D00207	CTTGAATGCGACTGCAGGTA	GAAGAAGTAGGCCGTTGTCG
rca	U74321	GACTGGTTCCTTCTACGGTTC	TGCTTGCTGTGCTCCTTG

The fluorescent quantitative PCR was measured using an ABI stepone plus real-time fluorescent quantitative PCR instrument with the TAKARA SYBRPremix Ex Taq II kit. The steps are as follows: All qPCR assays were performed with an ABI 7300 PCR system (Applied Biosystems) in a 20ul reaction volume, said reaction volume containing 1ul of template (10ng/lg DNA), SYBR Green PCR Mastermix and each primer. Apply the following hot procedures: A single DNA polymerase activation cycle at 95 °C–10min, followed by 40 amplification cycles at 95 °C–30s (denaturation step) and 60 °C–1min (annealing-extension step). The dissolution curve procedure was: 95 °C–15s, 60 °C–1min, 95 °C–15s. The technique was repeated three times. The standard curve was made by diluting the sample cDNA in a concentration gradient, and the reference gene was used as the standard for relative quantification by the $2^{-\triangle\triangle Ct}$ method when the amplification efficiency of the target gene of the reference gene was similar.

Nitrogen accumulation and nitrogen use efficiency

Samples were taken at full heading stage and mature stage. Three plants were selected from each plot after decomposition according to stems, leaves and panicles, and then were sterilized at 105 ℃ for 30 min, then dried to constant weight at 80 ℃. After grinding, the samples were digested with H_2SO_4-H_2O_2, and the nitrogen content of each organ sample was determined by Automatic Kjeldahl nitrogen analyzer.

Data calculation and statistical analysis

Since the data are basically the same for both years, the analysis is performed with 2020 data.

Nitrogen uptake utilization (%) = (nitrogen accumulation of plants in the nitrogen-applied zone - nitrogen accumulation of plants in the nitrogen-free zone)/nitrogen application × 100.

Nitrogen agronomic utilization rate (kg/kg) = (seed yield in nitrogen-applied area - seed yield in nitrogen-free area)/nitrogen application.

Photosynthetic potential ($m^2 \cdot d/m^2$)=$(L_1 + L_2)\times(t_2-t_1)/2$. where *L1* and *L2* are the leaf areas measured before and after, and *t1* and *t2* are the times of the two measurements before and after.

Light energy interception rate (%): light energy interception rate of sword leaves = (light intensity at sword leaves - light intensity at inverted second

leaves)/light intensity at sword leaves × 100, light energy interception rate of inverted second leaves = (light intensity at inverted second leaves - light intensity at inverted third leaves)/light intensity at inverted second leaves × 100.

Microsoft Excel 2017 was used for data entry and organization, SPSS 21.0 software was used for data analysis, and Origin was used for graphing.

Declarations

Acknowledgements

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Author contributions

Hao Jiang: Formal analysis, Writing—original draft preparation; Hao Jiang, Thobakgale Tebogo and Qingwang Su: Methodology; Zhihai Wu: Conceptualization; Yunzhe Li, Liwei Liu: Software; Baifeng Cang, Chenyang Bai, Jiayi Li, Dongchao Wang: Investigation; Ze Song, Meikang Wu: Data curation; Hao Jiang and Zhihai Wu: Writing—review and editing; Jingjing Cui and Xiaoshuang Wei: Supervision; Zhihai Wu: Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Competing Interests: Te authors declare no competing interests.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information file).

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Figures

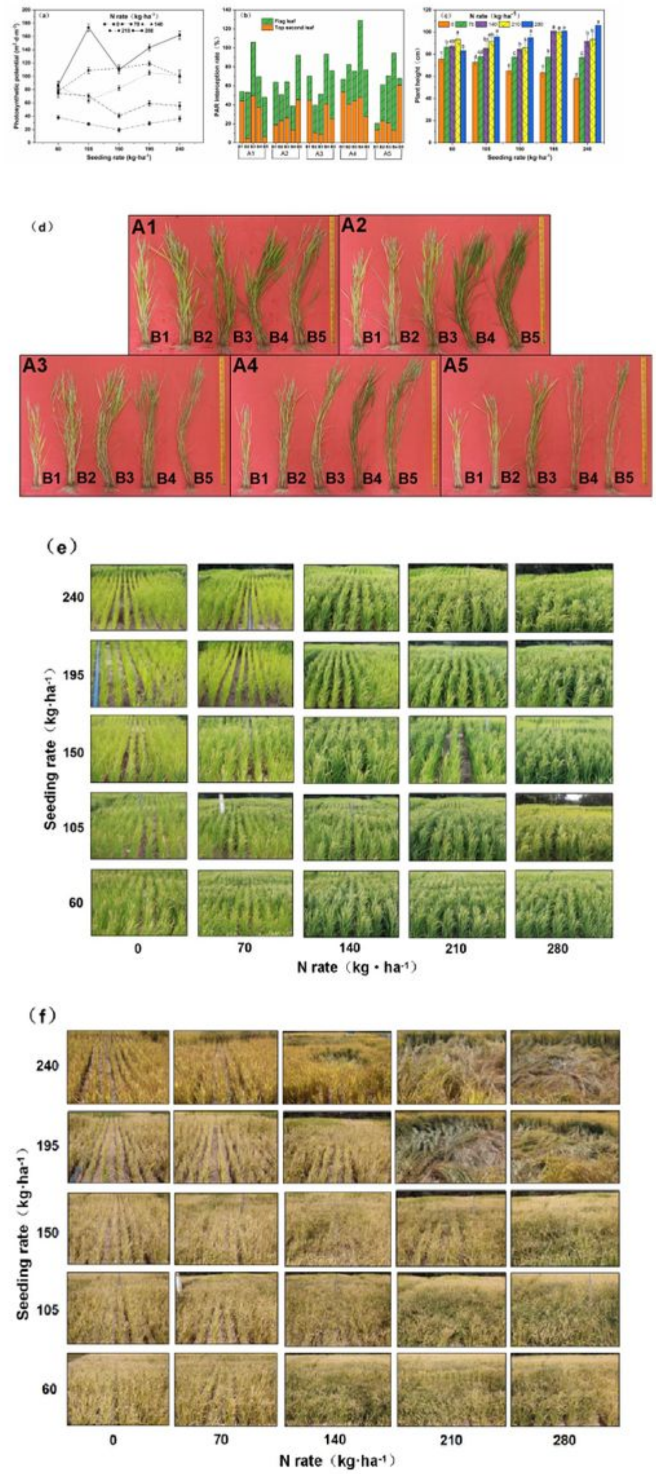

Figure 1

Effects of interaction between seeding rate and nitrogen rate on photosynthetic potential, PAR interception rate at different leaf positions and plant height at 10 days after flowering of rice under dry cultivation. (a) Photosynthetic potential; (b) Interception rate of light energy at different leaf positions; (c) Plant height; (d) Plant height pictures taken, use Origin software to process and merge the pictures. (e)

Rice growth after flowering under dry cultivation. (f) Growth of rice at mature stage under dry cultivation. Different letters with the same sowing amount indicate a significant difference at P<0.05.

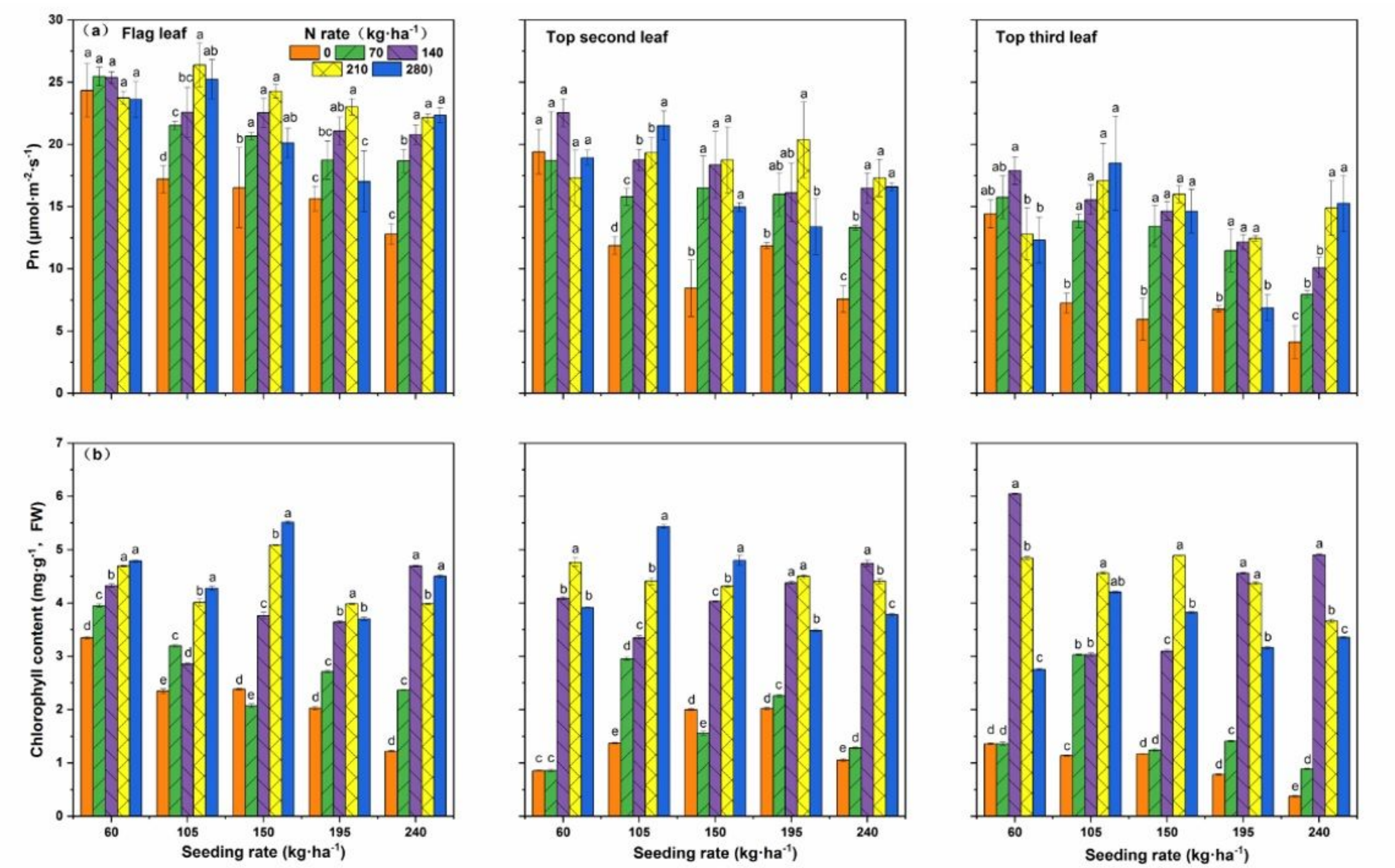

Figure 2

Effects of interaction between seeding rate and nitrogen rate on net photosynthetic rate and chlorophyll content of different leaf positions in rice population at 10 days after flowering under dry cultivation. (a) The net photosynthetic rate of the flag leaf; the first second leaf and the first third leaf; (b) the chlorophyll content of the flag leaf; the first second leaf and the first third leaf. Different letters with the same sowing amount indicate a significant difference at P<0.05.

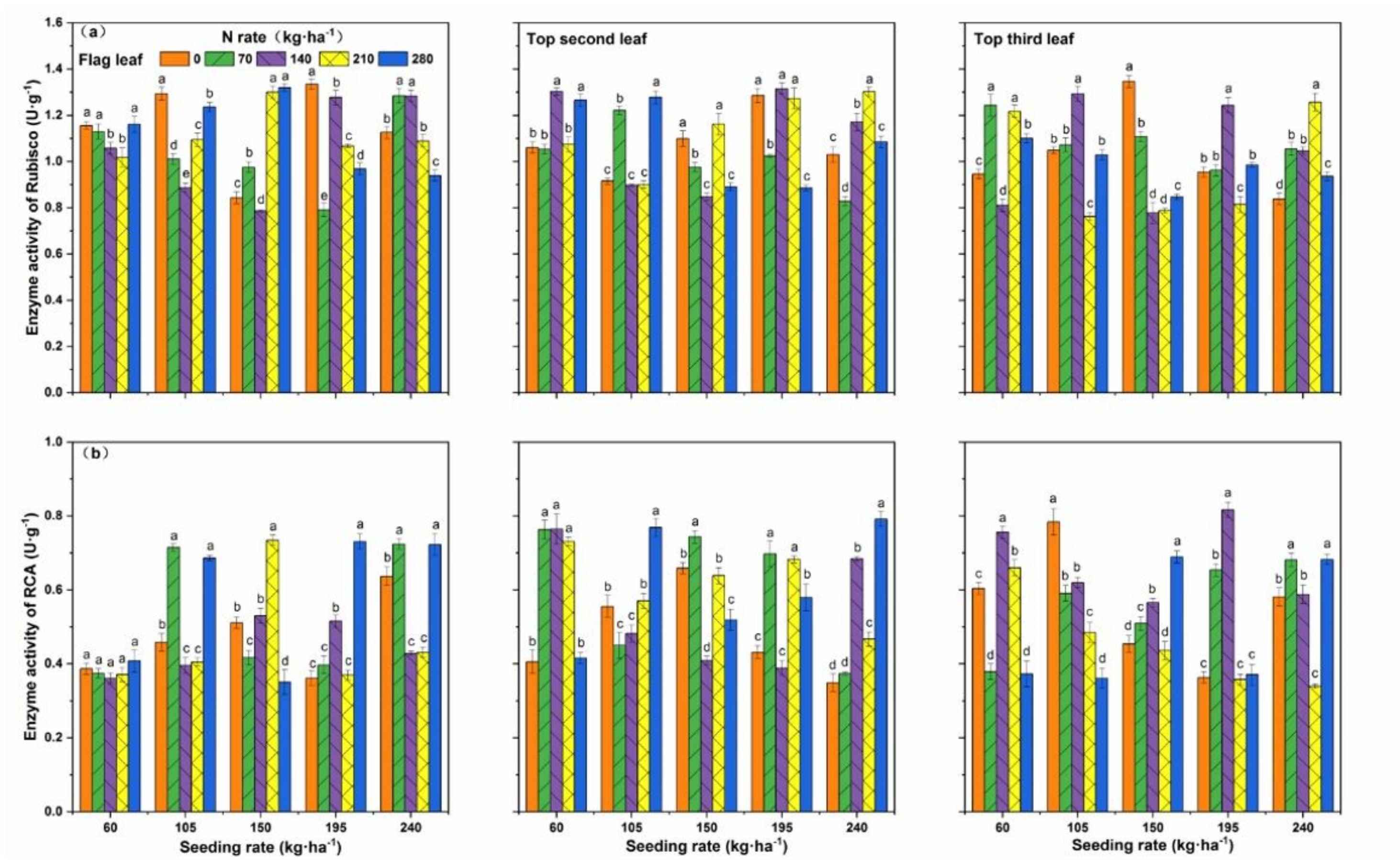

Figure 3

Effects of interaction between seeding rate and nitrogen rate on Rubisco and RCA activities in different leaf positions of rice population at 10 days after flowering under dry cultivation. (a) Flag leaf, first second leaf and first third leaf Rubisco activity; (b) Flag leaf, first second leaf and first third leaf RCA activity. Different letters with the same sowing amount indicate a significant difference at $P<0.05$.

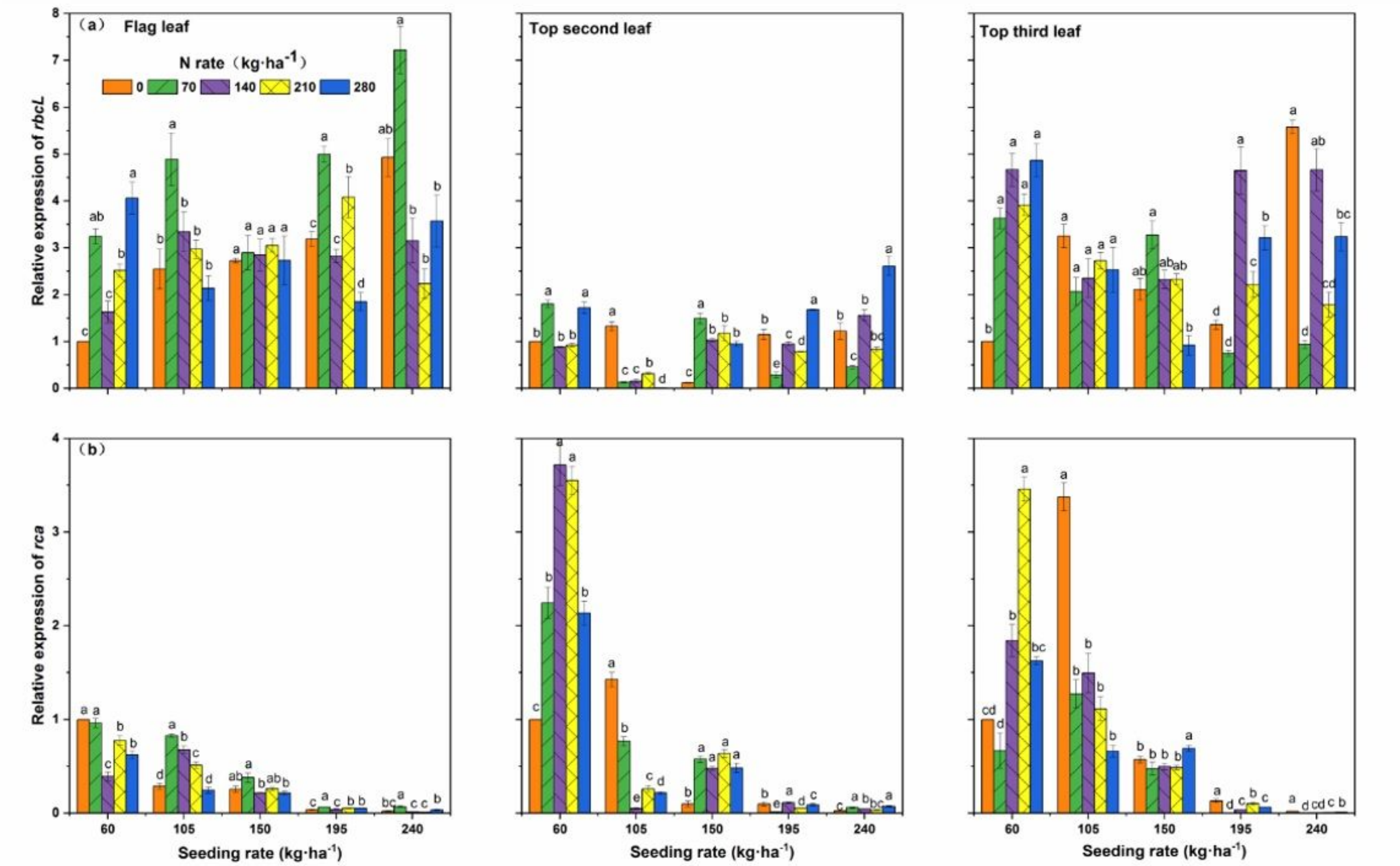

Figure 4

Effects of interaction between seeding rate and nitrogen rate on rbcL and rca gene expression in different leaf positions of rice population at 10 days after flowering under dry cultivation. (a) The expression level of rbcL gene in the flag leaf, the top two leaves and the top three leaves; (b) The expression level of the rca gene in the flag leaf; the top two leaves and the top three leaves. Different letters with the same sowing amount indicate a significant difference at P<0.05.

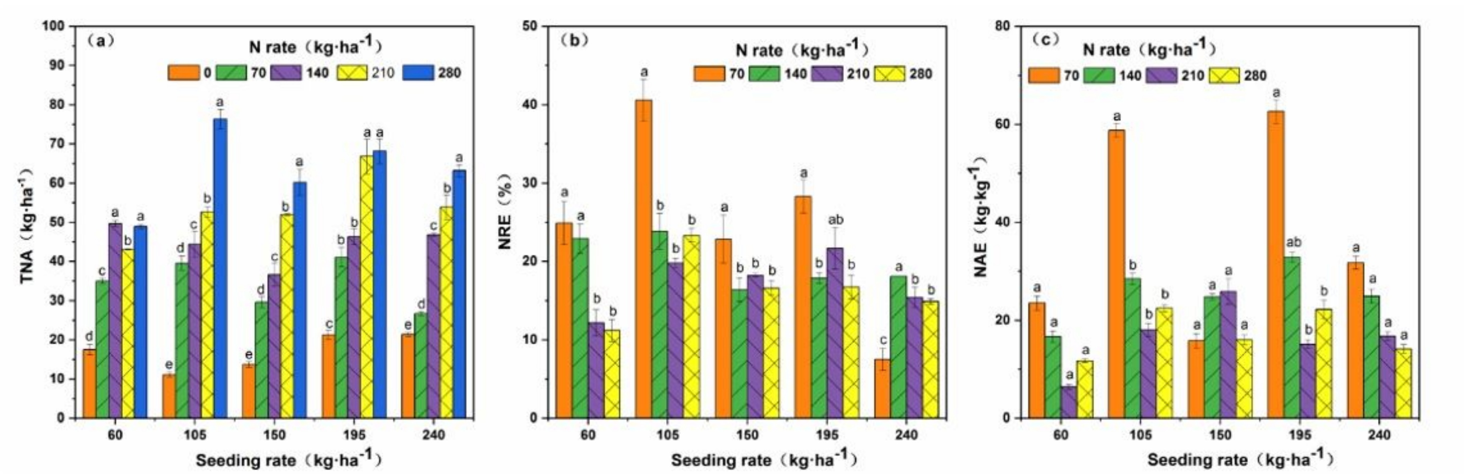

Figure 5

Effects of interaction between seeding rate and nitrogen rate on nitrogen accumulation and nitrogen use efficiency of rice under dry cultivation. TNA: total N accumulation; NRE: N recovery efficiency; NAE: N agronomic efficiency. Different letters with the same sowing amount indicate a significant difference at P<0.05.

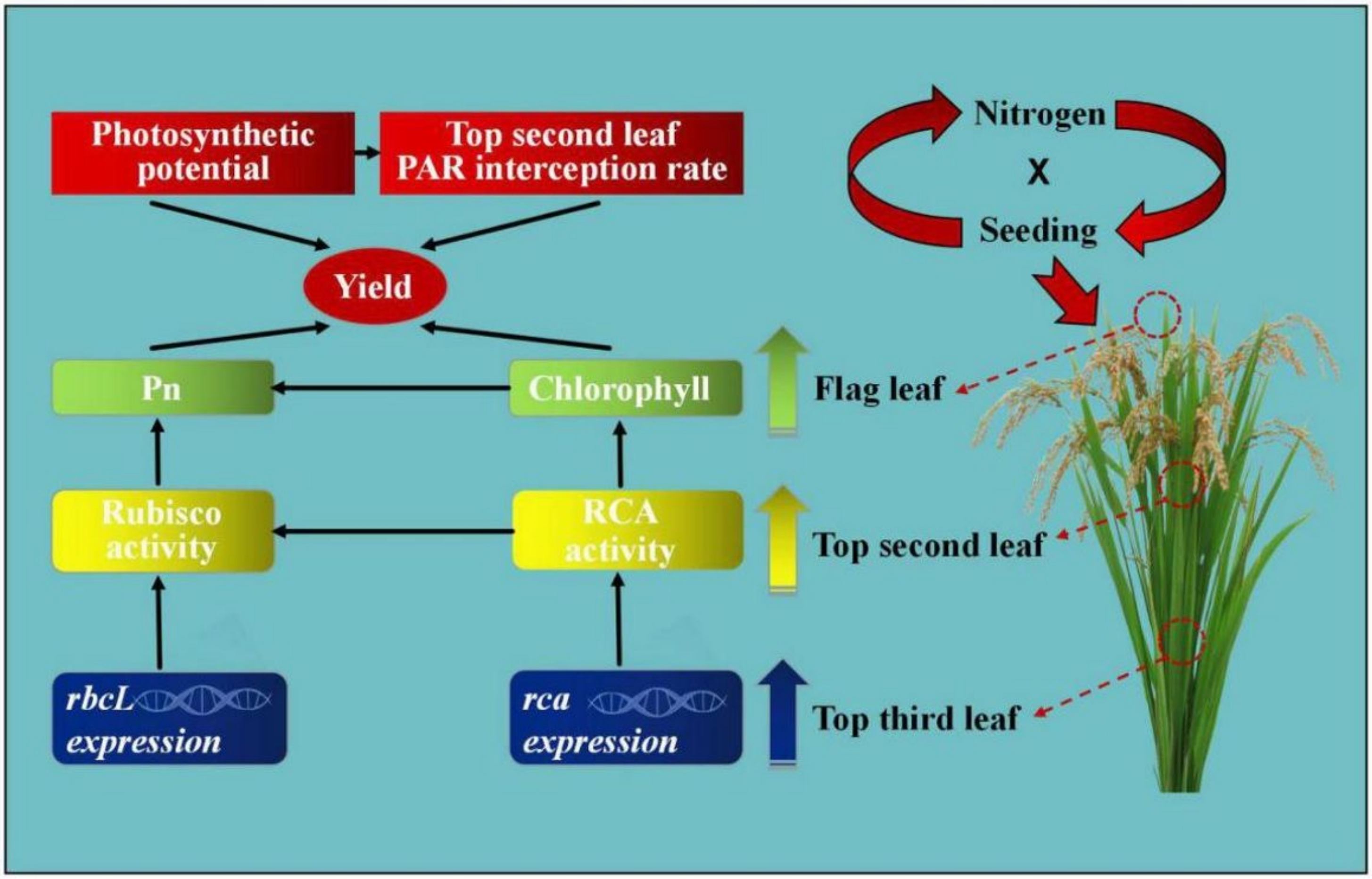

Figure 6

Mechanisms for building dominant populations of rice under dry cultivation.