

Physiological Basis of Photosynthetic Function and Senescence of Rice Leaves as Regulated by Controlled-Release Nitrogen Fertilizer

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Abstract: The physiological mechanism of photosynthetic function and senescence of rice leaves was studied by using early rice variety Baliangyou 100 and late rice variety Weiyou 46, treated with controlled-release nitrogen fertilizer (CRNF), urea and no nitrogen fertilizer. CRNF showed obvious effects on delaying the senescence and prolonging photosynthetic function duration of rice leaves. Compared with urea, CRNF could significantly increase the chlorophyll content of functional leaves in both early and late rice varieties, and this difference between the treatments became larger as rice growth progressed; CRNF increased the activities of active oxygen scavenging enzymes super oxide dismutase (SOD) and peroxidase (POD), and decreased the accumulation amount of malondialdehyde (MDA) in functional leaves during leaf aging; Photosynthetic rate of functional leaves in CRNF treatment was significantly higher than that in urea treatment. The result also indicated that CRNF could effectively regulate the contents of indole-3-acetic acid (IAA) and abscisic acid (ABA) in functional leaves; IAA content was higher and ABA content was lower in CRNF treatment than those in urea treatment. Therefore, application of CRNF could increase the rice yield significantly due to these physiological changes in the functional leaves.

Key words: controlled-release nitrogen fertilizer; rice; photosynthetic function; senescence; physiological mechanism

Premature senescence of rice leaves is one of the most important limiting factors for rice to get high yield, which is needed to be overcome urgently in rice production to prolong the photosynthetic function and delay leaf senescence. Some researchers considered that rice yield can increase by 2% in theory and by 1% in practice, if the life of rice functional leaves can be prolonged one day at maturity stage^[1,2]. Xu et al^[3] and Xiao et al^[4] demonstrated that increasing N nutrient applied could enhance the photosynthesis capacity, prolong rice leaf longevity and photosynthetic function duration. However, in farmers' practices, the recovery of nitrogen fertilizers applied was only 30–35%^[5], and most of nitrogen fertilizer applied were lost by leaching, erosion and runoff, or by gaseous emissions, which not only cause the waste of fertilizer nitrogen and energy resource, but also expose to a potential risk of ecological environments and food safety, such as greenhouse gas, floodwater, groundwater, soil and agricultural production contamination^[6].

Controlled-release nitrogen fertilizer is a kind of environment-friendly fertilizers coated with natural or

half-natural high molecular materials on the surface of general nitrogen fertilizer, from which release speed of nitrogen can be modulated and controlled. The studies in the recent years reported that controlled-release nitrogen fertilizer have many advantages compared with common nitrogen fertilizers. The fertilizer releases N in synchrony with plant requirement and can meet the N demands of rice through the whole growing season by a single application as basal fertilizer^[7]; Controlled-release fertilizer can not only increase rice yield and N recovery^[8-10], but also reduce the atmosphere and water contamination caused by excessive N through adjusting the N release models^[11, 12]. However, relatively few studies have been done on the effect of controlled-release nitrogen fertilizer on the senescence and photosynthetic function in rice leaves. The objectives of this study were to investigate the effect and its regulation mechanism from the point of photosynthetic property, the enzymatic activities of active oxygen scavenging systems and the endogenous hormones, and attempted to make it clear that the physiological mechanism of controlled-release fertilizer on increasing rice yield and N recovery.

MATERIALS AND METHODS

Experimental materials

The experiments were conducted at Gansha town, Changsha County, Hunan Province, China. Paddy soils were reddish yellow clayey loam soils derived from Quaternary red clay. Some chemical properties of the soils are listed in Table 1.

Controlled-release nitrogen fertilizers containing 42% N used in this experiment were prepared by the procedure described by the authors of this paper and produced by Hunan Xingxiang Science and Technology Development Co., LTD.

Rice varieties in the studies were early hybrid rice Baliangyou 100 and late hybrid rice Weiyou 45.

Experimental design

The experiments were conducted using late rice in 2002 and early rice in 2003 as materials. The treatments were: 1) control (no nitrogen applied, phosphate and potassium fertilizer applied, CK); 2) urea (nitrogen, phosphate and potassium fertilizer applied); 3) Controlled-release nitrogen fertilizer (nitrogen, phosphate and potassium fertilizer applied, CRNF). Nitrogen source used in urea treatment was urea and in CRNF treatment was controlled-release nitrogen fertilizer, respectively. $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and KCl were used as P and K sources, respectively. Fertilizers were applied at rates of N 90 kg/ha, P_2O_5 75 kg/ha, K_2O 118 kg/ha in early rice and N 105 kg/ha, P_2O_5 45 kg/ha, K_2O 126 kg/ha in late rice, respectively. All CRNF was applied in a lump as basal fertilizer one day before transplanting; Two times applications of urea were done (70% applied before transplanting, and 30% applied at 7–10 days after transplanting). All P_2O_5 and K_2O in the three treatments were applied as basal fertilizer before transplanting.

Urea (as basal fertilizers), CRNF, calcium super phosphate and KCl were incorporated to plots into 5 cm depth soil layer by an iron rake. Treatments were

replicated for three times and arranged in a randomized complete block design. Area of each plot was 20 m² in two years. Transplanting and harvesting for early rice were done on 15 April and 14 July, and for late rice were on 15 July and 25 October, respectively. Plant-row spacing was 20 cm × 20 cm. The managements of each treatment were totally same during the whole growth season of early and late rice.

Functional leaves for the first plant samples were collected in each replicated plot at booting stage, then following on the 8th, 16th, 24th and 32nd day after the first samples collected in early and late rice, respectively. Chlorophyll content (Chl), photosynthetic rate (P_n), malondialdehyde (MDA), super oxide dismutase (SOD), peroxidase (POD), IAA and ABA of each sample were assayed. Total N in plant and grain, total N, protein N, non-protein N and amino acid in brown rice at maturity stage were also analyzed. Rice straw and grain yields of each plot were measured at harvesting stage.

Plant analysis

Thirty rice plants were selected for chlorophyll content assay during the sampling time above-mentioned, and chlorophyll contents were measured directly using SPAD-502 (Japan). Ten rice plants were selected for P_n assay during the sampling stages above-mentioned, and P_n was measured *in vivo* using LI-6400 (made in USA) and expressed as CO_2 mg/(dm²·h). MDA was extracted with 5% (W/V) trichloroacetic acid and determined according to Lin et al.^[13]. For extraction of enzymes, leaf tissues were homogenized with 0.1 mol/L sodium phosphate buffer (pH 6.8) in a chilling pestle and mortar. The homogenate was centrifuged at 12 000 × g for 20 min and the supernatant was used for determination of enzyme activity. The whole extraction procedure was carried out at 4°C. SOD activity was assayed by NBT light reductive according to Wang et al.^[14] and expressed as U / mg protein. POD activity was determined by the methods according to Zhang^[15].

Table 1. Some chemical properties of soils in the experiment.

Rice type	pH	Organic matter (g/kg)	Total N (g/kg)	Alkali digested N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
Early rice	5.25	33.2	1.9	171.6	12.9	64.0
Late rice	6.30	29.9	1.8	155.9	18.0	60.0

IAA and ABA were measured by using LC-9A HPLC. The criterion reagents of IAA and ABA were bought from Fluka Company. Chemical properties of soils in the experiment, such as pH, organic matter, total N, total P, available N, available P and available K in soil and N contents in plants were analyzed by routine method^[16]. The measurements above mentioned were replicated for three times and gained the average. The data statistics was conducted by SPSS 10.

RESULTS

Effect of controlled-release nitrogen fertilizer on chlorophyll (Chl) contents and photosynthetic rate (P_n) of functional leaves

The chlorophyll contents in functional leaves can be treated as a credible index to measure photosynthetic function decline and senescence of rice. The Chl contents of functional leaves at different stages of early and late rice were shown in Table 2. In both early and late rice the Chl contents were presented as a declining tendency from booting stage to the 32nd day after booting stage. The results showed that the Chl contents in the treatments with N fertilizer application were higher than that in treatment with no N application. The Chl contents in urea and CRNF treatments of both early and late rice at every growth stages were considerably higher than that in CK. Comparison on Chl contents of functional leaves between urea and CRNF treatment, revealed that the Chl contents in CRNF treatment at the sampling stages were much higher than that in urea treatment at the corresponding stages, and the difference between

them became larger with rice development, and the similar tendencies were detected in both early and late rice. The Chl contents under CRNF treatment from the booting stage to the 32nd day after booting increased by 2.8%, 4.2%, 7.1%, 19.5%, and 29.4% in early rice, respectively, and those in late rice increased by 7.1%, 7.7%, 9.0%, 11.8% and 22.0%, respectively. The statistics analysis indicated the difference of Chl content between CRNF and urea treatment at every stages of early and late rice reached the significance level at $P < 0.05$ or $P < 0.01$, except for booting stage to the 8th day after booting in early rice. These results illuminated that, compared with urea application, the Chl contents can be significantly increased and photosynthesis function period can be prolonged by CRNF treatment, so CRNF is propitious to form and accumulate photosynthesis production, and this will help to increase rice yield. This was brought about by CRNF due to the synchronization between N release rates from controlled-release fertilizer and N uptake rates at every growth stage by rice. Previous research had made it out that the amount of N uptake by hybrid rice from booting stage to the 32nd day after booting is 31.7% and 31.6% of total N uptake at the early and late rice growth stage, respectively^[17]. While N amount released from CRNF during the corresponding period is 33.8% and 35.6% of total N applied, respectively^[18].

About 60–80% of the substances for rice grain filling come from photosynthesis after booting, and the intensity of photosynthesis of functional leaves during this period significantly affects 1000-grain weight and rice yield. The tendency of P_n of functional leaves in both early and late rice presented

Table 2. Effect of CRNF on Chl contents (SPAD value) in rice functional leaves.

Rice type	Treatment	Days after booting (d)				
		0	8	16	24	32
Early rice	CK	31.6 bB	30.9 bB	31.1 cB	28.7 bB	20.9 cB
	Urea	38.5 aA	35.7 aA	34.0 bAB	30.2 bB	23.5 bB
	CRNF	39.6 aA	37.2 aA	36.4 aA	36.1 aA	30.4 aA
Late rice	CK	35.1 bB	32.3 cB	30.4 cB	29.5 cB	20.7 bB
	Urea	36.7 bAB	35.2 bAB	34.3 bAB	32.1 bB	21.8 bB
	CRNF	39.3 aA	37.9 aA	37.4 aA	35.9 aA	26.6 aA

Data followed by different lowercase and uppercase letters indicate significance at 0.05 and 0.01 levels by Duncan's test (SSR), respectively.

slowly increase from booting stage to the 8th day after booting, and then began to decline (Fig. 1). N application significantly increased P_n of functional leaves compared with no N application. The effect of CRNF application on increasing P_n was much greater than that of urea application. From booting to the 32nd day after booting, the P_n in CRNF treatment increased by 21.6%, 15.5%, 11.5%, 16.2% and 16.1% in early rice, and by 9.5%, 16.4%, 14.5%, 23.1% and 8.6% in late rice over those in urea treatment, respectively. The results of statistic analysis suggested that the differences of P_n between urea and CRNF treatments at every growth stage all reached the significance at $P < 0.01$ in early rice and reached the significance $P < 0.05$ or $P < 0.01$ in late rice. The results demonstrated that CRNF application can improve the P_n of functional leaves after the booting stage, suggesting that CRNF application can enhance the photosynthesis intensity of functional leaves during this period, increase the photosynthesis production accumulation and enhance the grain filling and 1000-grain weight.

Effect of controlled-release nitrogen fertilizer on active oxygen scavenging systems of functional leaves

Malondialdehyde (MDA) content level is routinely used as an index of lipid peroxidation and its content can reflect lipid peroxidation level in plant cells and the cell damage extent. As shown in Fig. 2, MDA content increased with rice developmental progress. The noticeable effect of N fertilizer application on decreasing MDA content of functional leaves was evident at every stage of both early and late rice compared with no N fertilizer application.

The more marked effect on decreasing the MDA content was detected in CRNF treatment than that in urea treatment. For early rice, the difference between the two treatments at the 16th day and 24th day after booting reached the significance level at $P < 0.01$, respectively. At the stages, MDA content in CRNF treatment was higher by 26.4% and 20.0% than that in urea treatment. For late rice, the difference during the whole growth period except for the booting stage all reached the significance level at $P < 0.01$. This implied that controlled-release nitrogen fertilizer can reduce the accumulation of MDA content in functional leaves and alleviate the lipid peroxidation of functional leaves, which alleviated the hurt on bioplasm membrane system and maintained physiological function, thereby photosynthesis function duration of rice leaves was prolonged.

As shown in Fig. 2, SOD activity of function leaves in early and late rice changed with sampling time. The SOD activity increased from booting stage to the 8th day after booting and then began to decrease. SOD activity in urea treatment during the whole early rice growth stages was only a little higher than that in CK treatment and there were no obvious differences. While CRNF application significantly enhanced the SOD activity. The differences of SOD activities between urea and CRNF treatments at early rice growth stages except for booting stage were significant at $P < 0.05$ or $P < 0.01$ level, and those in CRNF treatment were higher by 19.0%, 14.0%, 35.8% and 25.6%, respectively. In late rice, SOD activities in the two N fertilizer treatments were both notably higher than those in CK treatment. From booting stage to the 16th day after booting, SOD activities increased

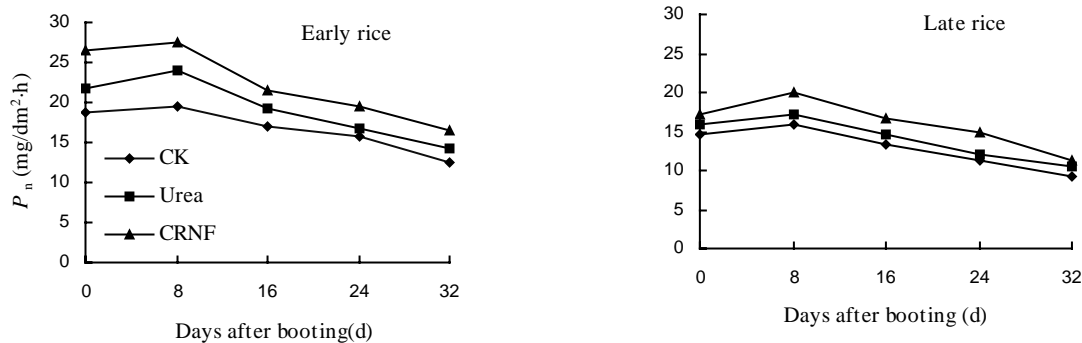


Fig. 1. Effect of CRNF on P_n in functional leaves of rice.

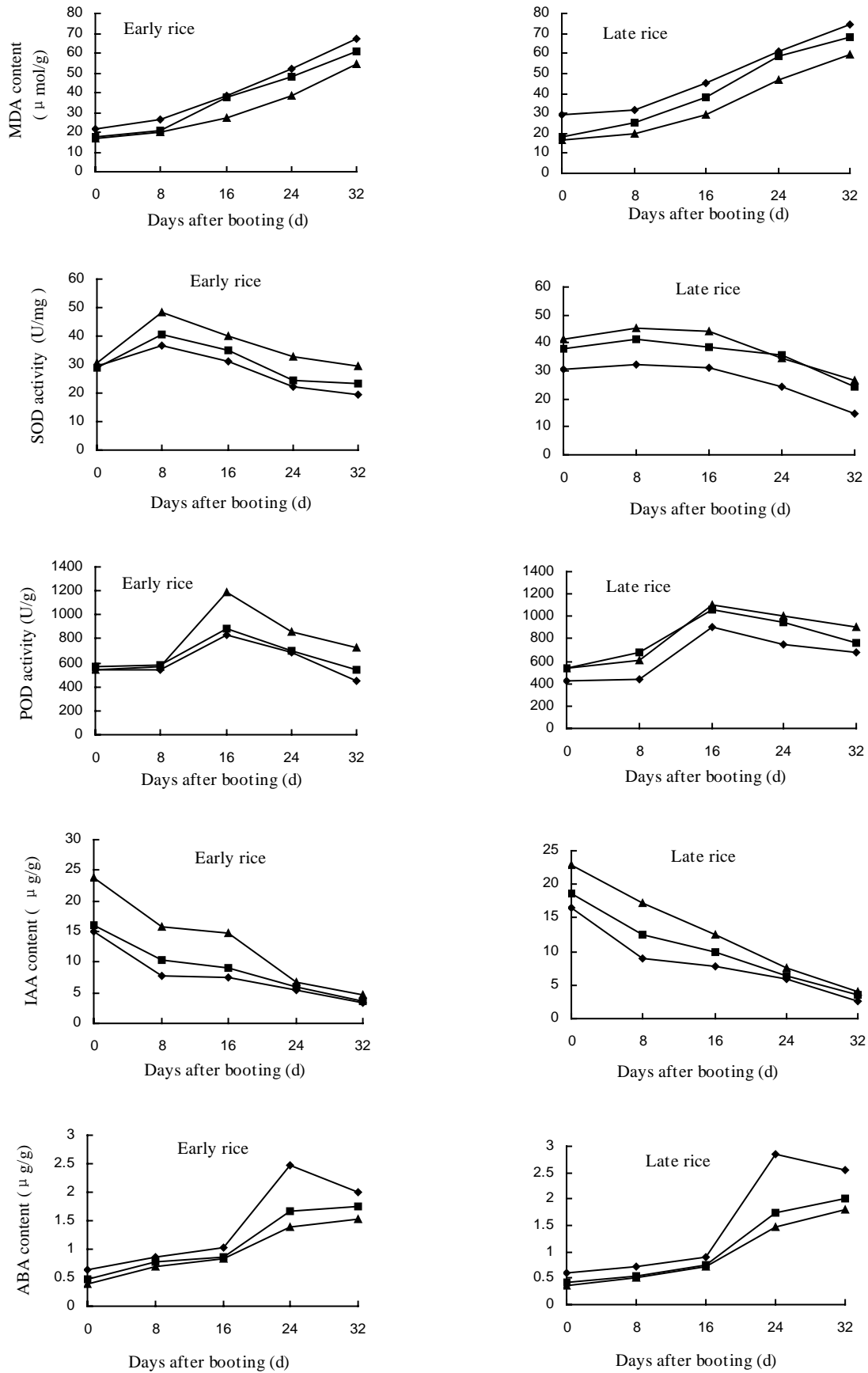


Fig. 2. Effect of CRNF on MDA, SOD, POD, IAA and ABA contents in rice functional leaves.

by 10.3%, 10.2% and 14.6% in CRNF treatment compared with those in urea treatment, respectively. There were no obvious differences between these two treatments during the 24th to 32nd day after booting. SOD is the first safeguard enzyme in active oxygen scavenging systems and its function is to scavenge the H_2O_2 in rice plant cells. This study illustrated that CRNF improved the SOD activity in rice functional leaves and enhanced its ability to scavenge H_2O_2 , and then reduced the peroxide accumulate in cells and hurt the bioplasm membranes.

POD is a kind of oxidation-reduction enzyme exists generally in plants with the function of catalyzing the H_2O_2 produced by SOD. This study demonstrated that there was no obvious effect on increasing POD activity after urea application in early rice, and the POD activity in urea treatment was only a little higher than those in CK treatment. The effect of CRNF on increasing POD activity was expressed at 16th day to 32nd day after booting, higher by 34.9%, 23.4% and 32.0% compared with urea treatment, respectively. The difference of POD activities in late rice between two N fertilizer application treatments and CK at sampling stages was significant at $P < 0.05$ or $P < 0.01$ level. There was no notable difference in POD activities between urea and CRNF treatments from the booting stage to the 16th day after booting, while enhanced by 17.0% and 19.0% in CRNF over urea and was significant at $P < 0.05$ during the 24th to 32nd day after booting.

Effect of controlled-release nitrogen fertilizer on IAA and ABA contents of functional leaves

Endogenous hormones in plants are one of the most important multitudinous factors affecting plant senescence. For instance, IAA is to delay the senescence and ABA is to accelerate the senescence. Fig. 2 showed that the change of IAA contents in functional leaves was presented to decline from the booting stage to the 32nd day after booting in both early and late rice. N fertilizer application improved IAA content at all rice growth stages, especially from the booting stage to the 16th day after booting. The effect of CRNF on improving IAA content was better than that of urea. Compared with urea treatment, IAA content in CRNF treatment increased by 28.4%,

38.0%, and 39.3% in early rice and 23.2%, 36.4% and 26.3% in late rice from the booting stage to the 16th day after booting, respectively. After then, IAA content decreased sharply, implying that the growth center had transferred to grains. Meanwhile high IAA content in functional leaves can help regulate photosynthesis, load, transportation and unloading the photosynthesis assimilation production. IAA content in CRNF treatment kept considerably higher than that in urea treatment and increased by 13.5% and 18.0% in early rice and 19.6% and 17.4% in late rice, respectively.

ABA content of functional leaves in early and late rice were shown in Fig. 2. ABA content in the CK treatment ascended from booting stage to the 24th day after booting and then began to decline. While the ABA contents in the two N fertilizer treatments kept rising from booting stage to the 32nd day after booting. ABA contents in CK treatment in early and late rice were all significantly higher than those in urea and CRNF, especially in the 24th to 32nd day after booting, increasing by 79.7% and 49.4% in early rice and 30.9% and 13.7% in late rice over CRNF and urea, respectively. There was no notably difference between urea and CRNF treatment from booting stage to 16th day after booting and the ABA content in urea treatment was only a little higher than that in CRNF. The larger effect of CRNF on decreasing ABA content were emerged on the 24th to 32nd day after booting and reduced by 16.9% and 13.1% in early rice and 16.0% and 10.4% in late rice compared with urea treatment, respectively. Higher ABA content in functional leaves at late rice growth stage will restrain the transport of photosynthesis production and accelerate the senescence of rice plant. This work illustrated that controlled-release nitrogen fertilizer can decrease ABA content in functional leaves at the late rice growth stage, so it is in favor for transferring photosynthesis production from rice leaves to grains and delaying leaf senescence.

Effect of controlled-release nitrogen fertilizer on rice yield and N recovery

Yields of early and late rice in CRNF treatment were gained 7482.5 kg/ha and 7700.0 kg/ha and increased by 9.5% and 6.9% over urea treatment,

Table 3. Comparison of yield, yield components and nitrogen recovery.

Rice type	Treatment	No. of panicles ($\times 10^4$ /ha)	No. of filled grains per panicle	1000-grain weight (g)	N recovery (%)	Yield (kg/ha)
Early rice	CK	114.00	56.2	25.35		5175.0
	Urea	165.00	66.6	23.57	30.3	6832.5
	CRNF	195.00	67.4	24.03	69.0	7482.5
Late rice	CK	165.00	131.2	23.89		5766.5
	Urea	225.00	141.8	24.64	41.2	7200.0
	CRNF	259.95	148.1	25.33	76.3	7700.0

respectively (Table 3). The yield differences reached significance at $P < 0.05$. Controlled-release nitrogen fertilizer increased rice yield were mainly due to that the number of panicles per hectare, the number of filled grains per panicle and 1000-grain weight in CRNF treatment were obviously higher than those in urea treatment. The N recoveries of CRNF application in early and late rice reached 69.0% and 76.3%, respectively, higher by 36.9 percent over urea application on average.

DISCUSSION

It is nitrogen fertilizer that is the largest nutrient applied in practice and N fertilizers usually used are urea and ammonium carbonate. Since urea and ammonium carbonate are easily soluble, N recovery of them for rice is low, with about 30–35% for urea and even lower for ammonium carbonate. The previous studies illustrated that controlled-release nitrogen fertilizer notably increased rice yield and N recovery, which was manufactured by coating natural and half natural materials, changing the diameter of film bore and adjusting the moisture characteristics of the alloy in tiny bore of coated film. In this study, the yield of early and late rice increased by 9.5% and 6.9%, respectively. N recoveries increased by 38.7 and 35.1 percent, respectively. The results are consistent with the previous research.

During the process of rice leaf senescence, Chl content and P_n declined incessantly, SOD and POD activities decreased and MDA content increased, therefore, large molecules such as protein, nucleic acid and lipid and so on were decomposed and bioplasm membranes were peroxidated. These

above-mentioned resulted in declined physiological functions^[3]. Some research demonstrated that more N fertilizer application may be benefit to delay leaf senescence and photosynthesis function declining, and can also improve the synthesis metabolism of active oxygen scavenging enzymes^[19]. The present research illuminated that, compared with urea, CRNF enhanced Chl contents and P_n in rice leaves at middle and late growth stages more effectively, and more photosynthesis productions were accumulated. Meanwhile high activity level of active oxygen scavenging enzymes such as SOD and POD were maintained and MDA accumulated in rice leaves was reduced, so the balance of active oxygen producing and scavenging was improved relatively. This perhaps was one of the main physiological reasons for CRNF to delay rice leaf senescence and photosynthesis function decline.

Endogenous hormones play an important role in rice senescence process, and the senescence is a result for the balance and harmony of many endogenous hormones. IAA and ABA are the main endogenous hormones affecting rice senescence. During middle and late growth phase, IAA content has a close relation with assimilation substance distribution and senescence delaying and it is a regulator for many factors restricting the reaction rate or quantity in assimilation, and it mainly embodied as follows: increasing sink activity and promoting leaves growth and so on. ABA can promote leaves senescence mainly due to it promotes protease and RNase activities and accelerates protein and nucleic acid decomposing. In this study, IAA contents in rice functional leaves increased significantly by CRNF applied, especially from booting stage to the 16th day

after booting, and increased by 28.4%, 38.0% and 39.3% in early rice and 23.2%, 36.4% and 26.3% in late rice, respectively. Meanwhile ABA contents in rice functional leaves decreased obviously, especially during the 24th to 32nd day, and decreased by 20.3% and 15.1% in early rice and 19.0% and 11.6% in late rice, respectively.

As described above, due to the synchronization between N release rates from controlled-release fertilizer and N uptake rates at different rice growth stages, controlled-release nitrogen fertilizer can modulate the photosynthesis characteristics, active oxygen scavenging system and endogenous hormones, and prolong functional period of rice leaves and increase rice yield.

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