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Effects of Nitrogen Fertilizer Treatments on Filling and Respiratory Rate of Caryopsis in Rice

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Abstract: An experiment was conducted to study the effects of nitrogen (N) rate and application time on grain filling and respiratory trait of caryopsis in two rice varieties, IR36 and Dali. The treatments were consisted of no N application topdressing at both tillering and booting stages (CK), 6 g/pot of N topdressing at the tillering stage and 2 g/pot of N topdressing at the booting stage, 2 g/pot of N topdressing at the tillering stage and 6 g/pot of N topdressing at the booting stage. The results showed that the proper utilization of N fertilizer can be helpful to maintain the higher water content, higher respiratory rate and higher dehydrogenase activity of rice caryopsis in late filling phase, and prolong the course for filling and maintaining higher respiratory rate and dehydrogenase activity of rice caryopsis. More N application at booting was more effective compared to more N application at tillering.
Key words: nitrogen fertilizer; water content; filling duration; respiratory rate; dehydrogenase activity; rice; caryopsis

The application of N fertilizer has been reported to have a great effect on filling of caryopsis. Many previous studies indicated that the dry matters of caryopsis were derived from photosynthates by tillers and leaves at two stages i.e. before and after panicle emergence [1-4]. Moreover, proper utilization of N fertilizer can improve N content and photosynthetic rate [5-7], delay leaf senescence [7-9], and increase the amount of dry matter for grain filling, thus improve the productivity of rice [5-10]. On the other hand, it has been reported that the amount and time of nitrogen application had tremendous effects on filling and dry matter accumulation in rice [11-19]. There has been a close relationship between grain filling and physiological activities of caryopsis at the filling stage [20-25]. Previously, it has been observed that the grain filling in rice ceased when the water content of caryopsis dropped to 20% [26-27] and respiratory rate to 0.05 μmol/ (mg • h) [28]. However, little work on the effects of N utilization on physiological activities of rice caryopsis at the filling stage has been done. Therefore, the present research is conducted to study the effect of N rate and application time on the physiological activities of caryopsis at the filling stage and to understand the mechanism involved in dry matter accumulation of rice under different N treatments.

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MATERIALS AND METHODS

Plant materials

The two rice varieties IR36 and Dali (*Oryza sativa* L.) were grown in pots on the experimental farm of Agricultural College of Yangzhou University during 2003 and 2004. The dimensions of the pots were 25 cm in diameter and 30 cm in height with the capacity of 15 kg of soil. The soil was sifted and mixed thoroughly before filled into pots. The chemical compositions of the soil were 32.5 g/kg organic matter, 83.8 mg/kg available N, 40.5 mg/kg available P, and 71.2 mg/kg available K. Urea was used as the source of N fertilizer. The experimental treatments were composed of: 1) CK, no N application at both tillering and booting stages; 2) HL, 6 g N per pot at the tillering stage and 2 g of N per pot at the booting stage; 3) LH, 2 g N per pot at the tillering stage and 6 g N at the booting stage. Three plants were grown in each pot, and each treatment had five replicates (Table 1).

Methods

Marking dates of caryopses development

At the flowering timing (about 11 a.m.), the

Table 1. Various nitrogen treatments to two rice varieties.

Variety	Treatment	N topdressing at the tillering stage (g/pot)	N topdressing at the booting stage (g/pot)
IR36	CK	0	0
	HL	6	2
	LH	2	6
Dali	CK	0	0
	HL	6	2
	LH	2	6

plants were tagged to record the flowering dates. The strong superior caryopses bloomed simultaneously were chosen for experimentation.

Measurement of fresh and dry weight and water content of caryopsis

After every three days, thirty superior grains were collected, the pericarps were stripped off to measure the fresh and dry weight after being dried at 90℃. Respiratory rate of the caryopsis was measured by the oxygen electrode method [29]. The ATP content of the caryopses was measured by bioluminescence [30], while the dehydrogenase activities were determined by tetrazolium (TTC) staining method [31].

RESULTS

Effects of various nitrogen treatments on caryopsis development and dynamic changes of water content in rice

During early stage of caryopsis development, in both varieties, the water content of caryopses was incredibly high with a rapid increase in fresh and dry weight, but later a gradual decrease has been noted in water content, and fresh and dry weight (Fig. 1).

The increase in the amount of N can improve the filling rate of caryopsis, while the increase in the diurnal fresh and dry weight can maintain higher water content of caryopses during the late filling stage, consequently prolonging the filling time and improving the weight of caryopses. It is clear from the Fig. 1, Fig. 2 that the LH treatment had higher effects than the HL treatment.

On fresh and dry weight of caryopsis

The application of N has shown a higher increase in fresh and dry weight of caryopses than the control treatment. Among both HL and LH treatments, the caryopsis weight of Dali was greater at the earlier stage under the treatment of HL, but lower at the later stage (*t*-test significant at 5%); The caryopsis weight

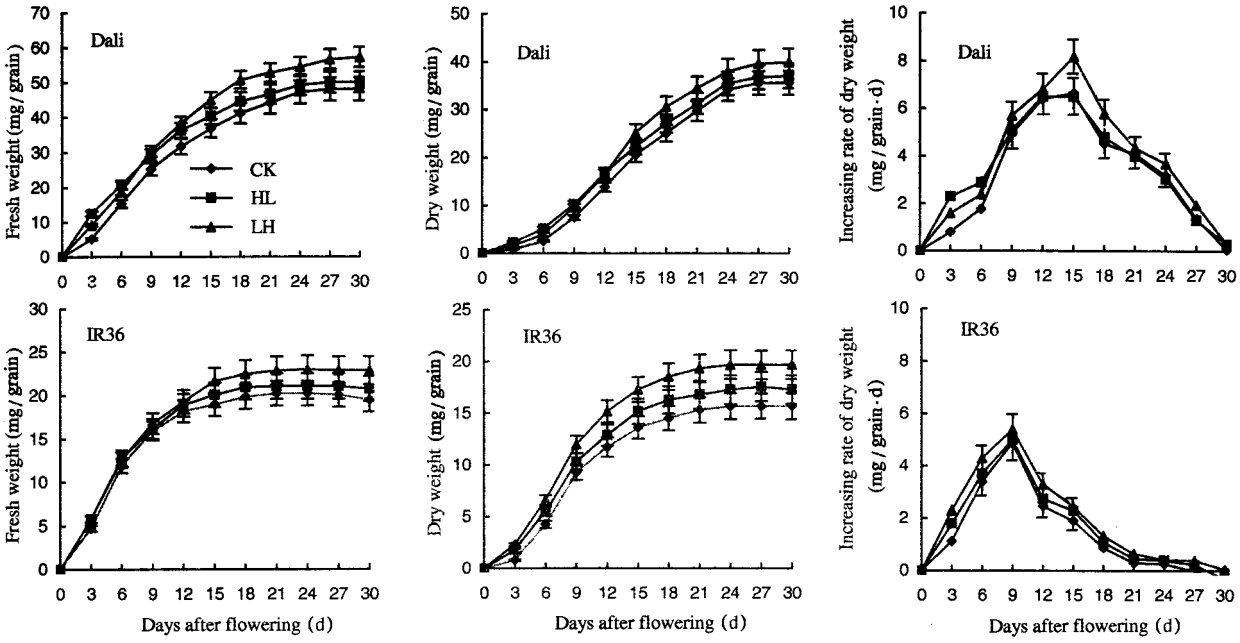

Fig. 1. Dynamic changes of fresh and dry weight and increasing rate of dry weight on rice caryopsis with various nitrogen treatments.

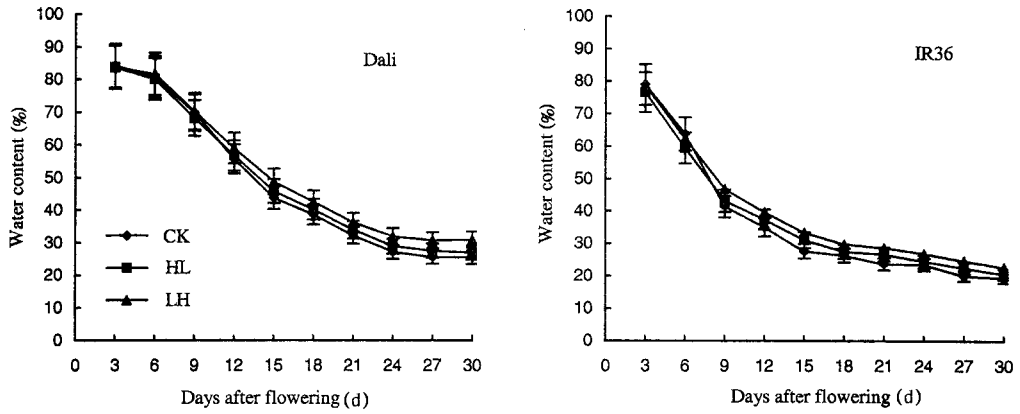

Fig. 2. Dynamic changes of water content in rice caryopsis with various nitrogen treatments.

of IR36 was not significantly affected at earlier, but the effect was significant at the later stage. Moreover the LH treatment had greater effect on caryopsis weight of IR36 than HL (*t*-test was significant); while the time at which the daily increasing rate in dry caryopsis weight reached maxium and the duration from filling to filling ceasing were shorter in IR36 than Dali, but on the whole the LH treatment had the highest rate (*t*-test significant at 5%).

On water content

Similar trend was found for the caryopsis water content of both varieties with the highest water content at the early filling stage and later a gradual decrease was noted. The filling rate had shown a close relationship with water content. It is obvious from comparing graphs in Fig. 2, that caryopses had a recorded highest filling rate (the deepest slope) when water content was 50–60%, afterwards, there was a gradual decrease in filling rate as the water content decreased, and finally the filling ceased when the water content reached 20%. This indicated that increasing the amount of N had no obvious effect on caryopsis water content at the earlier stage of caryopsis development, but at later stage the a considerable decrease has been observed in CK than in N application.

Effects of various nitrogen treatments on the respiratory rate and ATP content of caryopses

The Fig. 3 shows that at first the respiratory rates of caryopses increased and then decreased after attained the highest level. Similar trend was also found with filling rate. However, the N application has considerably increased the respiratory rate of the caryopses, especially when treated with LH (*t*-test significant at 5%). Among the N treatments the LH has shown the greatest effect on respiratory rate of caryopsis (Fig. 3), similar results were noted for respiratory rates based on caryopsis or fresh weight.

Synthesis of dry matter for grain filling is an energy consuming process, while respiration can release ATP, and supply adequate energy for synthesis of the dry matter. In previous study it has been reported that the ATP content had an important relationship with respiratory rate [31]. In our experiment we have noted that the ATP content of caryopses in N treated plants was higher than that in the control (CK). Similar trend was also noted for the respiratory rates, while LH showed the highest effects in both parameters (Fig. 3).

Effects of various nitrogen treatments on dehydrogenase activity of rice caryopsis

Dry matter of caryopsis is transferred via the vascular bundles in rachilla and accumulated in the dorsal part of the ovary before entering the aleuron and endosperm, and finally transformed into storage substance. The transport of organic matters and synthesis of storage substance are both energy dependent processes, which is transformed through respiration of various tissues in the caryopsis. The dehydrogenase activity of various tissues in caryopsis that indicates respiratory activities of various tissues in caryopsis could be determined by tetrazolium (TTC) staining method.

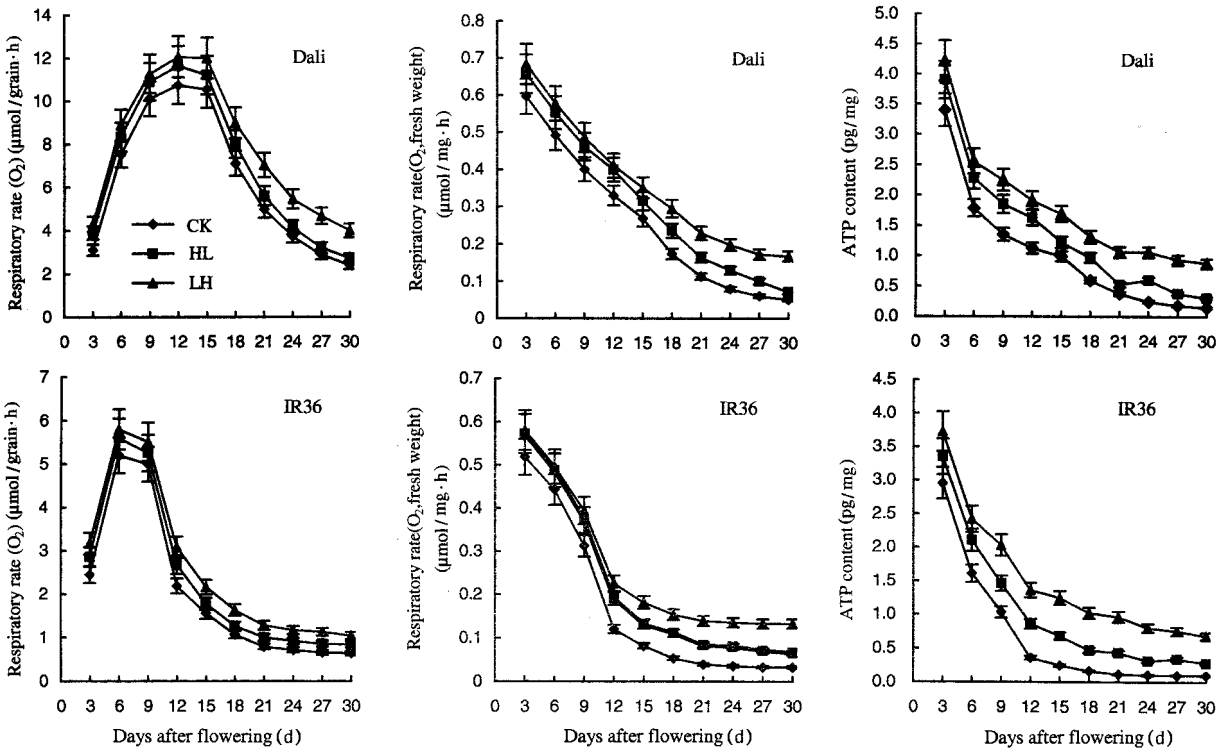

Fig. 3. Dynamic changes of respiratory rate and ATP content in rice caryopsis with various nitrogen treatments.

Table 2. The stain reaction with TTC in some tissues of rice caryopsis with various nitrogen treatments.

Tissue	Variety	Treatment	Days after flowering (d)										
			0	3	6	9	12	15	18	21	24	27	30
Endosperm	Dali	CK		+	++	++	++	++	+	+	-	-	-
		HL		+	++	++	++	++	++	+	-	-	-
		LH		+	++	++	++	++	++	+	+	-	-
	IR36	CK		+	++	+	+	-	-	-	-	-	-
		HL		+	++	++	+	-	-	-	-	-	-
		LH		+	++	++	+	+	-	-	-	-	-
Aleuron layer	Dali	CK		+	++	++	++	++	++	+	+	-	-
		HL		+	++	++	++	++	++	+	+	-	-
		LH		+	++	++	++	++	++	++	+	+	-
	IR36	CK		+	++	++	++	-	-	-	-	-	-
		HL		+	++	++	++	+	-	-	-	-	-
		LH		+	++	++	++	+	-	-	-	-	-
Vascular bundles in the dorsal part of ovary	Dali	CK	+	+	++	++	++	++	++	++	+	-	-
		HL	+	+	++	++	++	++	++	++	+	+	-
		LH	+	+	++	++	++	++	++	++	+	+	+
	IR36	CK	+	+	++	++	++	+	-	-	-	-	-
		HL	+	+	++	++	++	+	-	-	-	-	-
		LH	+	+	++	++	++	+	+	-	-	-	-
Vascular bundles in rachilla	Dali	CK	+	++	++	++	++	++	++	+	+	-	-
		HL	+	++	++	++	++	++	++	+	+	-	-
		LH	+	++	++	++	++	++	++	++	+	+	-
	IR36	CK	+	++	++	++	+	-	-	-	-	-	-
		HL	+	++	++	++	+	+	-	-	-	-	-
		LH	+	++	++	++	++	+	+	-	-	-	-

+, Lightly dyed; ++, Deeply dyed; -, Almost not dyed; Tissues which were deeply dyed have higher dehydrogenase activity.

As shown in Table 2, the current study showed that the vascular bundles in rachilla and in ovary wall had the dehydrogenase activity at the early flowering stage, however the activity decreased with the increase in starch accumulation and weight of caryopses, and disappeared after finished the filling of caryopses. Moreover, the dehydrogenase activities were also noted in the aleuron and endosperm when caryopsis was at the filling stage, but the activity was not detected after the filling of caryopses accomplished. Compared with the control treatment, in N treated plants the dehydrogenase activities in both tissues were improved by prolonging the activity duration. Table 2 indicates that the tissues under LH treatment had the greatest dehydrogenase activity in both varieties.

DISCUSSION

The proper utilization of N fertilizer could improve caryopsis weight of rice by improving the absorbing activity of the root in order to absorb more N from the soil. The increase in amount of N at the booting stage may provide sufficient N to rice plants during the late filling stage [33-34]. This could help lessen threats posed by catabolism of structural protein in stem and leaves [33], thereby extending the life-span of green leaves by maintenance of high photosynthetic rate [33].

In this study we have observed that the highest filling rate was recorded at 50–60% water content, which gradually decreased when the water content went below 50%, and ceased completely at 20% water content. The N application had a marked effect on water content of rice caryopsis during filling especially at the late filling stage. However, filling rate has a great relationship with water content [26], which has also been confirmed in this study (y=-0.056x^2+0.611x-11.008, R^2=0.7937). The HL treatment accelerated the filling of the caryopses and the decrease in water content, resulting in rapid maturity. While the LH treatment maintained higher respiratory rate and water content of caryopsis, which enabled the caryopsis cells to divide and enlarge continuously for acceptable of dry matter for grain filling, finally increasing the weight of caryopsis.

It can be recommended from the present findings that besides improving the leaf chlorophyll content for high photosynthetic function, N application can increase the caryopsis weight by keeping higher water content at the late caryopses development stage. Therefore, it can be concluded that the proper increase in N especially at the booting stage could be beneficial for rice productivity but care must be taken to avoid unfavorable-delayed senescence.

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