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Effects of Weak Light on Starch Accumulation and Starch Synthesis Enzyme Activities in Rice at the Grain Filling Stage

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Abstract: Dynamic changes of starch, amylose, sucrose contents and the activities of starch synthesis enzymes under shading treatments after flowering were studied using two rice varieties IR72 (indica) and Nipponbare (japonica) as materials. Under shading treatments, the starch, amylose and sucrose contents decreased, while ADP-glucose pyrophosphorylase (ADPGPPase) activity only changed a little, soluble starch synthase activity and granule bound starch synthase activity decreased, soluble starch branching enzyme (SSBE, Q-enzyme) activity and granule bound starch branching enzyme (GBSBE, Q-enzyme) activity increased, and starch debranching enzyme (DBE, R-enzyme) activity varied with varieties. Correlation analyses showed that the changes of starch content were positively and significantly correlated with the changes of sucrose content in the weak light. Both ADPGPPase activity and SSBE activity were positively and significantly correlated with starch accumulation rate. It was implied that the decline of starch synthase activities was related to the decrease of starch content and the increase of the activity of starch branching enzyme played an important role in the decrease of the ratio of amylose to the total starch under the weak light.

Key words: weak light; starch content; ADP-glucose pyrophosphorylase; starch synthase; starch branching enzyme; starch debranching enzyme; rice

Photosynthetic product in rice plant is transported to grains mainly in the form of sucrose. Sucrose in the grains eventually becomes starch through a series of enzymatic reactions [1-2]. The starch weight accounts for 90% of the brown rice [3-4], so the course of the rice grain filling is the biochemical process in which starch is mainly formed, and the factor of light is indispensable at the course. Much research has been conducted to study the influence of the light on the grain starch synthesis in rice, mostly on the starch accumulation [5-9], while less on the activities of starch synthesis enzymes. At present, there are so many reports on activities of starch synthesis enzymes [10-21], but fewer have been reported on the changes of activities of starch synthesis enzymes in rice under the weak light. Therefore, an experiment with shading treatment was conducted to reveal the influence of the weak light on the activities of starch synthesis enzymes in rice.

MATERIALS AND METHODS

Materials

Field experiments and indoor measurements were conducted at Graduate School of Agricultural and Life Sciences, the University of Tokyo in 2004. Two rice varieties IR72 and Nipponbare were used. The seeds were soaked and disinfected on the 8th May and sowed on the 10th May. Nipponbare bloomed on the 9th August and IR72 on the 17th August. One group was covered with shading nets after flowering, with a transmittance rate of 50%. The other group as control was not shaded.

Sampling method

The plants that grew uniformly were selected, the flowering spikelet at the bottom of the primary rachis branch that is on the top of panicle was marked with a black dot with a waterproof pen on the flowering day, and meanwhile, plastic cards were suspended on the panicle necks. Flowering dates were recorded on the cards and samples were taken once every 3 days. The samples were collected at 10:00-11:00 a.m. and immediately wrapped in aluminium foil and frozen in liquid nitrogen, then placed into a sealed plastic bag and stored at -80℃. The following determinations were made after taking all the samples with three replications.

Determination of carbohydrate

Three to eleven dehulled grains that had been

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marked were ground under 80% alcohol, followed by heating and centrifugation. The supernatant was transferred into a test tube with a screw lid. The sediment was added with 80% alcohol and centrifugated again, and the supernatant was transferred into the above test tube, and this course was repeated twice. For the supernatant solution, alcohol was removed with a centrifugation evaporator, then distilled water was added and the final supernatant after centrifugation was the sample for determination of sugar content. For the sediment, alcohol and water were removed with a centrifugation evaporator, followed by adding distilled water and incubating at 100℃, then the paste starch was transferred to a small plastic cup and the cup was added with acetate buffer (50 mmol/L, pH 4.6), Glucoamylase (84 U/mL buffer) and distilled water. The above paste starch was stored at 55℃ for 30 min after agitation, and then it was used as the sample for testing starch content after centrifugation.

Sucrose content

The sample (40 μL) was transferred into a cuvette, then S4 solution and distilled water were added. The S4 solution was consisted of 5 mg β-fructosidase and 1 mL acetate buffer (50 mmol/L, pH 4.6). After the sample being incubated at 30℃ for 1 h, sugar-testing solution was added and the initial value was measured under 340 nm using a spectrometer, followed by adding 1 μL HK/G6PDH and determining the absorbance of sucrose with three repeats.

Starch content

The sample (30 μL) and distilled water (total volume, 300 μL) were transferred into a cuvette, and then sugar-testing solution was added and its initial value was measured at 340 nm using a spectrometer. Finally 1 μL HK/G6PDH was added and the absorbance of starch were determined with three replications.

Amylose content

Iodine-based colorimetric method was adopted with three replications.

Assay of enzyme activities

Three marked grains were selected and dehulled, then GS-buffer, PVPP and 2-ME were added, followed by grinding in an ice bath. The supernatant solution was namely soluble enzyme solution after centrifugation for 10 min (2℃, 15 000 r/min). For the sediment, GS-buffer was added and supernatant was removed after centrifugation for 10 min, the operation was repeated again. The sediment left was granule-bound enzyme solution.

Activity of ADP-glucose pyrophosphorylase (*ADPGPPase*)

Reactive solution (pH 7.4, 100 mmol/L Hepes-NaOH, 3 mmol/L 3-PGA, 1.2 mmol/L ADPG, 3 mmol/L Na-PPi, 125 mmol/L $MgCl_2$, 200 mmol/L DTT) and enzyme solution were mixed in a test tube. The reaction lasted 20 min at 30℃ and ended in boiling water, followed by adding distilled water. The above solution was transferred into small test tubes, and then centrifuged (2℃, 15 000 r/min). The supernatant was transferred into cuvettes and then added with 15 μL 10 mg/mL NADP and measured at 340 nm using a spectrometer. Finally, 1 μL PGM and G6PDH were added and measurement was carried out.

Activity of soluble starch synthase (*SSS*)

Distilled water and enzyme solution were added to reaction solution (pH 7.4, 500 mmol/L Hepes-NaOH, 200 mmol/L DTT, 5 mmol/L ADPGlucose, 20 mg/mL Glycogen). The reaction lasted 40 min at 30℃ and ended in boiling water, followed by adding reactive solution (pH 7.4, 500 mmol/L Hepes-NaOH, 40 mmol/L PEP, 2 mol/L KCl, 200 mmol/L $MgCl_2$, Pyruvate kinase). The reaction lasted 30 min at 30℃ and ended in boiling water, followed by adding distilled water. The above reaction solution was transferred into small plastic test tubes and centrifugated at 2℃ and 15 000 r/min. The supernatant was transferred to cuvettes, followed by adding 500 mmol/L Hepes-NaOH (pH 7.4), 200 mmol/L $MgCl_2$, 100 mmol/L Glucose, 10 mg/mL NADP. The absorbance value was determined at 340 nm using a spectrometer. Finally 2 μL HK/G6PDH was added and absorbance value was measured.

Activity of granule bound starch synthase(*GBSS*)

The method was almost identical to that for assaying activity of SSS.

Activity of soluble starch branching enzyme (*SSBE*)

Hepes-NaOH (pH 7.4), AMP, G1P, phosphorylase,

distilled water and enzyme solution were added into test tubes. The reaction lasted 30 min at 30℃ with vibration; HCl was added to end the reaction, and then DMSO, 0.1% iodine and 1% potassium iodide were added. The above reactive solution was transferred into a cuvette and the absorbance was read at 540 nm.

Activity of granule bound starch branching enzyme (*GBSBE*)

The method was similar with that described in the assay of the activity of SSBE.

Activity of starch debranching enzyme (*DBE*)

Distilled water and enzyme solution were added into reaction solution involving 500 mmol/L Mes-NaOH (pH 6.2) and 40 mg/mL Pulluan. After reaction being lasted 20 min at 30℃ and ended in boiling water, Somogyi-Nelson D solution was added and reaction lasted 20 min in boiling water, followed by adding Somogyi-Nelson C solution. The supernatant was taken and diluted after centrifugation and absorbance was read at 520 nm using a spectrometer.

Assays of the enzymes activities above were repeated 3 times and the spectrometer used was Beckman DU800. The software, Excel 2000, was adopted to analyze data and make graphs. Correlation analyses were made by SPSS 11.0.

RESULTS

Dynamic changes of starch content in rice grains at the grain filling stage

The dynamic changes of the starch content in the two varieties at grain filling stage were shown in Fig. 1.

The starch content of IR72 and Nipponbare declined under the weak light. It was averagely decreased by 0.637 mg/grain in IR72 and by 0.812 mg/grain in Nipponbare. However, the two varieties had different decline trends. The starch content of IR72 under the shaded treatment was even lower than the control at the early stage while the difference between the shading and the control was diminished at the late stage. As for the starch content of Nipponbare, the difference between the shading and the control was small at the early stage and the content under the shaded treatment was much lower at the middle and late stages.

Dynamic changes of amylose content

The dynamic changes of the amylose content of the two varieties were shown in Fig. 1. The amylose contents of IR72 and Nipponbare were decreased under the weak light, with average decrease by 1.554 and 1.432 percent point in IR72 and Nipponbare, respectively.

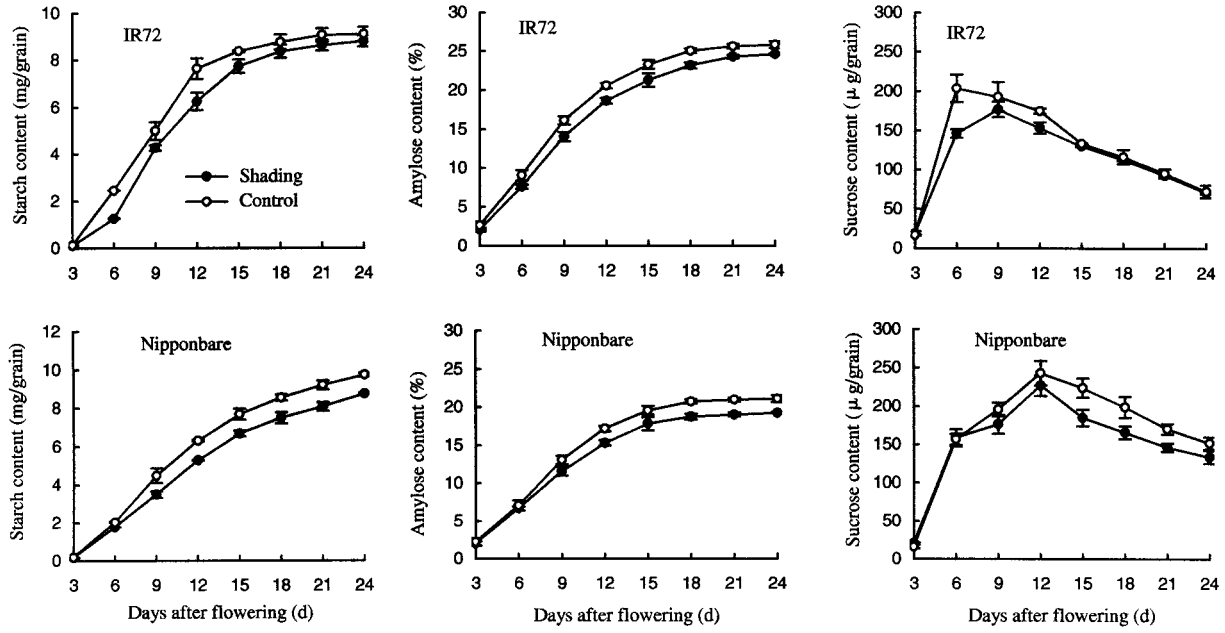

Fig. 1. Starch, amylose, and sucrose contents in rice grain after flowering.

Dynamic changes of sucrose content

The dynamic changes of the sucrose content of the two varieties at the grain filling stage were shown in Fig. 1. The sucrose content of IR72 and Nipponbare fell under the weak light. IR72 was averagely decreased by 12.969 μg/grain and Nipponbare by 17.783 μg/grain. But the two varieties had different modes. The sucrose content of IR72 under shaded was even lower than that under the control at the early stage while the content under shading treatment was fundamentally identical with that under the control at the late stage. The sucrose content of Nipponbare under shaded was similar with that under the control at the early stage while the content under shading treatment was even lower at the middle and late stages. The sucrose in grains is mainly imported from 'source' organs, so the main reason for the decrease of the sucrose content was due to the abilities of the sucrose synthesis of the 'source' organs and the sucrose transference were influenced under the weak light.

Changes of the activity of ADPG

The dynamic changes of the activity of ADPG at the grain filling stage were shown in Fig. 2. Compared with the control, the activity of ADPG in IR72 did not have obvious difference under the weak light, and the activity of ADPG in Nipponbare changed a little and its maximum value and the value at the late stage were a bit higher under the weak light than the control.

Changes of the activity of SSS

As shown in Fig. 2, the activity of SSS in IR72 and Nipponbare were much lower under the weak light than the control. With ADPG or UDPG as the glucose donor, starch synthase catalyzes glucose of ADPG to be transferred to α-1, 4 connected glucose chain, and then the chain is made to add a glucose unit to synthesize amylose. Therefore weak light was not favorable to the amylose synthesis.

Changes of the activity of GBSS

For the activity of GBSS, IR72 and Nipponbare showed lower GBSS activity under the weak light than the control (Fig. 2), suggesting that weak light was not helpful for the amylose synthesis.

Changes of the activity of SSBE

Compared with the control, the activity of ADPG in the two varieties was higher under the weak light (Fig. 3), indicating weak light was favorable to the amylopectin synthesis.

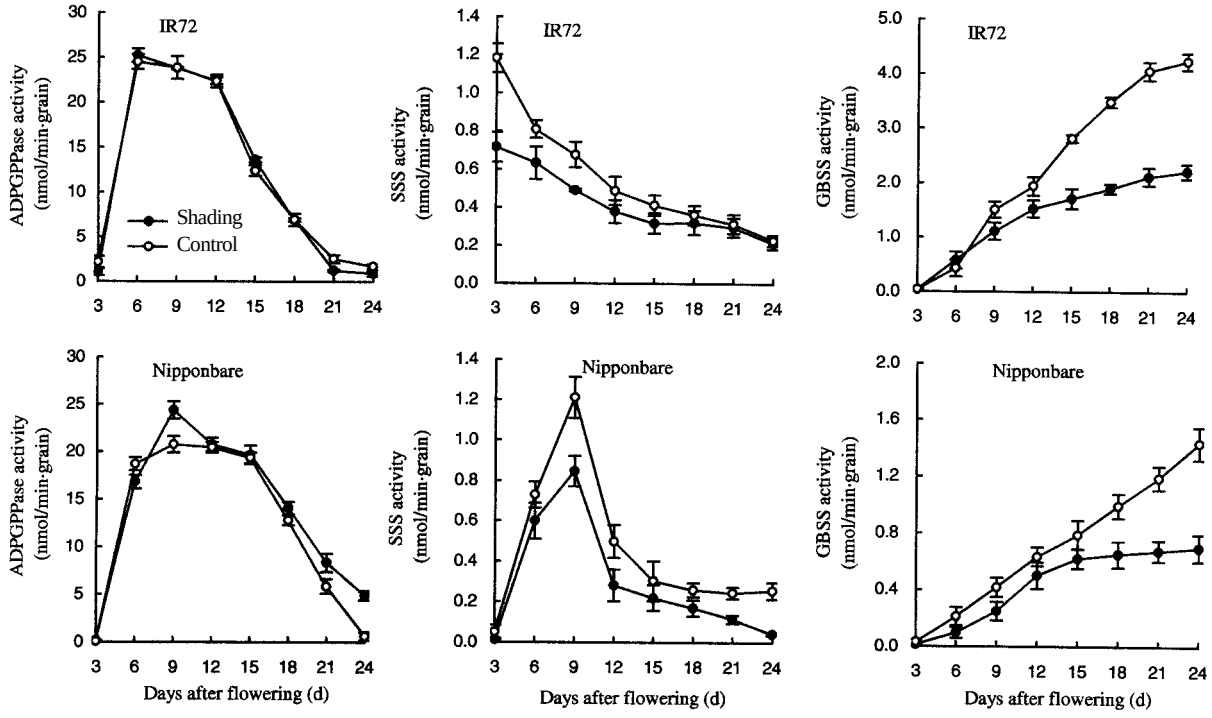

Fig. 2. Changes of ADPGPPase, soluble starch synthase (SSS) and granule-bound starch synthase (GBSS) activities in rice grain after flowering.

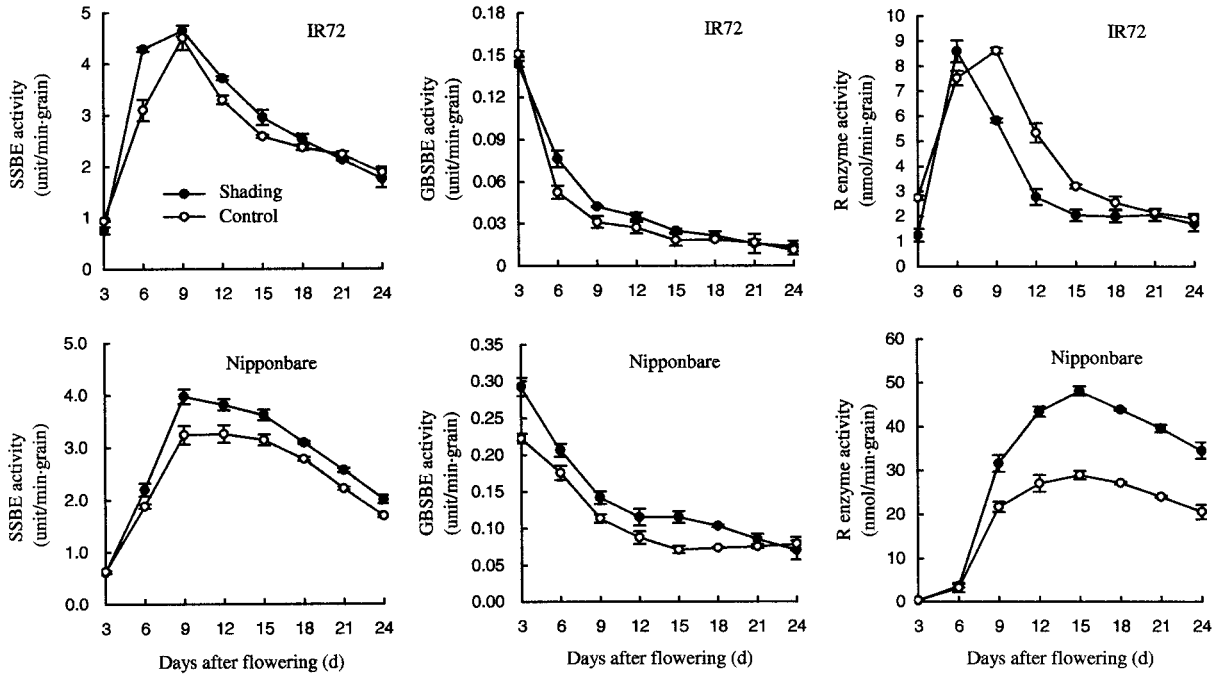

Fig. 3. Changes of soluble starch branching enzyme (SSBE), granule bound starch branching enzyme (GBSBE) and starch debranching enzyme (R enzyme) activities in rice grain after flowering.

Changes of the activity of GBSBE

The dynamic changes of the activity of GBSBE in the two varieties were shown in Fig. 3. The activity of GBSBE was higher under the weak light than the control, implying that the weak light was favorable to amylopectin synthesis.

Changes of the activity of DBE (R-enzyme)

As shown in Fig. 3, the two varieties displayed differently. Under the weak light, the activity of DBE in IR72 was much lower than that under the control while that in Nipponbare was much higher at the late stage. This suggested that weak light was not favorable to the degradation of the amylopectin in IR72 but was advantageous for the degradation of the amylopectin in Nipponbare.

Correlation of the activities of starch synthesis enzymes and starch accumulation rate

The correlations between ADPGPPase, SSS, GBSS, SSBE, GBSBE, DBE and grain starch accumulation rate were analyzed and the results were listed in Table 1.

As shown in Table 1, the activities of ADPGPPase and SSBE in the two varieties were positively and significantly correlated with starch accumulation rate. The activities of GBSBE and GBSS were not significantly associated with starch accumulation rate. The correlation between activity of SSS and starch accumulation rate varied with different varieties (Nipponbare showed significantly positive correlation while IR72 did not). The correlation between DBE and starch accumulation rate indicated that Nipponbare did not have significant correlation under the weak light or normal light; IR72 did not have significant correlation under the weak light and showed significantly positive correlation under the normal light.

DISCUSSION

In this study, we found a significant correlation between the activities of ADPGPPase, starch branching enzyme and starch accumulation rate, which was in agreement with the results obtained from different crops. For example, Kumar and Simgh [22] reported that there was significant correlation between the activity of ADPGPPase and starch accumulation rate in wheat. Doehlert et al [23-25] also thought so through the research on corn. Zhao et al [26] considered that the activities of ADPGPPase, starch synthase and starch branching

Table 1. Correlation coefficients of ADPGPPase, SSS, GBSS, SSBE, GBSBE, DBE activities with starch accumulation rate.

Varieties	Enzymes involving in starch synthesis	Treatment	Coefficient
IR72	ADPGPPase	Shading	0.8513**
		Control	0.9750**
	Soluble starch synthase (SSS)	Shading	0.0960
		Control	0.1949
	Granule bound starch synthase (GBSS)	Shading	-0.1093
		Control	-0.4867
	Soluble starch branching enzyme (SSBE)	Shading	0.8605**
		Control	0.8532**
	Granule bound starch branching enzyme (GBSBE)	Shading	-0.1700
		Control	-0.1447
	Starch debranching enzyme (DBE)	Shading	0.5089
		Control	0.9178**
Nipponbare	ADPGPPase	Shading	0.9386**
		Control	0.9132**
	Soluble starch synthase (SSS)	Shading	0.7763*
		Control	0.9121**
	Granule bound starch synthase (GBSS)	Shading	-0.0753
		Control	-0.3292
	Soluble starch branching enzyme (SSBE)	Shading	0.7796*
		Control	0.7126*
	Granule bound starch branching enzyme (GBSBE)	Shading	-0.2033
		Control	-0.1173
	Starch debranching enzyme (DBE)	Shading	0.2400
		Control	0.2026

*,** Significant at the 0.05 and 0.01 levels, respectively.

enzyme were significantly correlated with starch accumulation rate in rice. Nakamura and Yuki [27], Umemoto [28] found the activities of ADPGPPase and starch branching enzyme were significantly correlated with starch accumulation rate in rice. So we might draw a conclusion that the activities of ADPGPPase and starch branching enzyme were directly related with starch accumulation rate and starch synthesis and they were key enzymes during starch synthesis course in rice grain.

The sucrose content in grains decreased and starch synthesis amount declined under the conditions of shading. The differences in sucrose content and starch synthesis amount under the two treatments were significant and their correlation coefficients were 0.7762* for IR72 and 0.8613** for Nipponbare. This showed the decline of the sucrose content was one of the important reasons for the decrease of starch synthesis. Meanwhile we knew that a series of changes happened to starch synthesis enzymes under the shaded treatments, indicating that light intensity had obvious influence on the activities of starch synthesis enzymes, but it had various effects on different kinds of the starch synthesis enzymes. For example, the activity of ADPGPPase changed only a little, the activity of starch synthase displayed the tendency of decrease, the activity of starch branching enzyme showed the trend of increase and the activity of starch debranching enzyme varied with different varieties. Judging from the functions of starch synthase during starch synthesis process, the decrease of the activities of starch synthase was relevant to the decline of the amount of starch synthesis under the shading. According to starch synthase, the activity of SSS was weaker than that of GBSS. Therefore, we deduced that GBSS had more influence on starch synthesis compared to SSS.

The ratio of amylose content to the total starch content displayed the trend of decrease under the conditions of shading. Obviously, it was related to the changes of the activity of starch branching enzyme. The increase of the activity of starch branching enzyme promoted amylose to change into amylopectin, which caused the decrease of amylose content. Namely the increase of the activity of starch branching enzyme was

the main reason for the decline of the ratio of amylose content to total starch amount. According to the activity of starch branching enzyme, the activity of SSBE was high while the activity of GBSBE was low and their activities difference reached 20-30 times. Therefore, we inferred that the decrease of amylose or the synthesis of amylopectin mainly depend on SSBE. R-enzyme is a debranching enzyme, it mainly hydrolyze branch of ultimate dextrin or α-1, 6-glucosidic bond of periphery branch of amylopectin molecule and played an important role in the degradation of amylopectin. But the functions and effects of R-enzyme were quite finite during grain filling process and amylopectin forming course in grains. We could see from the research that R-enzyme in IR72 decreased while R-enzyme in Nipponbare increased. Although R-enzyme in Nipponbare increased, amylose content still decreased. This showed that the function of the increase in the R-enzyme activity could not compensate the influence of the increase in starch branching enzyme activity on amylose. In other words, starch branching enzyme had much more influence on amylose than R-enzyme. Having used rice mutant to conduct research, Nakamura et al [29-30] reached a conclusion that R-enzyme was relevant to the sophisticated structure of amylopectin in starch.

As for starch synthase, this study showed different varieties had different correlations between starch synthase and starch accumulation rate. The important role of starch synthase in starch synthesis course needs further exploration.

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