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Effect of High Temperature on Sucrose Content and Sucrose Cleaving Enzyme Activity in Rice Grain During the Filling Stage

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Abstract: Dynamic changes of sucrose, fructose, glucose contents and differences in activities of sucrose synthase, vacuolar invertase, and cell wall bound invertase in rice grain after flowering stage were studied under natural and high temperatures by using two japonica rice varieties Koshihikari and Sasanishiki. In rice grains, the sucrose synthase activity was higher than that of invertase, which was significantly correlated with starch accumulation rate, indicating that the sucrose synthase played an important role in sucrose degradation and starch synthesis. Under high temperature, the significant increase in grain sucrose content without any increase in fructose and glucose contents, suggested that the high temperature treatment enhanced sucrose accumulation, while diminished sucrose degradation in rice grains. Compared with the control plants, the decrease in activities of sucrose synthase, vacuolar invertase, and cell wall bound invertase with high temperature treated plants indicated that the deceleration of sucrose-degradation was related to the decrease in activities of sucrose synthase and invertase.

Key words: high temperature; sucrose content; sucrose synthase; vacuolar invertase; cell wall bound invertase; rice grain

Global warming is due to continuous increase in greenhouse effect caused by human productive activities. Much attention has been paid to the influence brought by the increase of temperature on crop growth. Many researches in China and other countries are investigating the rise of high temperature on rice in recent 10 years. These researches are mainly focused on the effect of temperature on rice quality and physiological status, endosperm shape, and granulose structures etc [1-7]. However, few works have been done on starch content and starch synthesis enzyme activities under different temperatures [8-12]. The mechanism in changes of sucrose content and sucrose cleaving enzyme activities have not been very clear up to date.

The degradation of sucrose in grains is the first step in the process of starch synthesis. Sucrose came from the origin organs in grains produces glucose, fructose and UDPG through enzymolysis, and it eventually becomes starch by a series of enzymatic reactions [13-14]. The starch in grains approximately accounts for 90% of the total brown rice weight [15-16], thus the rice grain-filling process is a biochemical process, where sucrose decomposition and starch synthesis usually take place. Ascertaining the effects of temperatures on sucrose degradation and sucrose cleaving enzyme activities will plays an important part to fully understand the mechanism of grain starch synthesis. Therefore, in current study the role of high temperature on sucrose quantity and sucrose cleaving enzyme activity was investigated.

MATERIALS AND METHODS

During the experiment two rice varieties Koshihikari and Sasanishiki were used. Koshihikari and Sasanishiki, which were bred in 1956 and 1963 respectively, have good eating quality and were grown as main local varieties in Japan for a long time. In 2002, the cultivated areas of the two varieties took the first and the tenth place respectively in Japan. The field experiments were conducted on the research farm in the University of Tokyo, and measurements were carried out at the Crop Science Laboratory of the University of Tokyo in 2004. The seeds were sowed

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on the 15 April and the seedling were transplanted on 11 May. The flowering day of Koshihikari and Sasanishiki were 24 July and 21 July, respectively. A 2.5-meter-high semi-enclosed greenhouse (the upper part was covered with transparent film, and 0.8 meter to the bottom was not closed), was built on the farm on the flowering day for high temperature treatment. The outside temperature treatment was regarded as control. Automatic temperature testing instruments were set in the treatment inside and outside greenhouse. The temperatures were recorded every 30 min. The day (from 7:00 a.m. to 7:00 p.m.) and night (from 7:00 p.m. to 7:00 a.m. next day) temperatures of the greenhouse were 2℃ and 0.3℃ higher than those outside greenhouse.

Sampling method

The uniformly grown plants were selected and the flowering spikelets at the bottom of the primary rachis branches on the top of panicles was marked with a waterproof pen during the flowering stage. Meanwhile, each panicle was covered by a plastic label. The flowering dates were recorded on the labels and panicles were sampled once every six days (for Koshihikari, the sampling interval between the first and the second time was 3 days). The samples were collected at 10:00-11:00 a.m. and then immediately wrapped in aluminium foil and frozen in liquid nitrogen, then placed into a sealed plastic bag and stored at -80℃. The following measurements were conducted after sampling with three replications.

Determination of carbohydrate

Three to eleven marked dehulled grains 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 supernatant was transferred into the test tube, and this course was repeated twice. For the supernatant, 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 were 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, which was used as the sample for testing of starch content after centrifugation.

Test of glucose and fructose contents

The sugar test solution was added to sample that was diluted by 10 times. The components of sugar test solution were 150 mmol/L HEPES (pH 7.4), 10 mmol/L $MgSO_4$, 3 mmol/L NADP, 10 mmol/L ATP-Na. The initial value was tested by a spectrometer at 340 nm, followed by addition of 1 μL HK/G6PDH and the absorbance value of the glucose was determined, then 1 μL phospho-glu-isomelase was added and the absorbance value of the fructose was measured after agitation.

Test of sucrose content

The sample (40 μL) was transferred into a cuvette, then S4 solution and distilled water were added. The S4 solution consisted of 5 mg β-fructosidase and 1 mL acetate buffer (50 mmol/L, pH 4.6). After the sample was 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 addition of 1 μL HK/G6PDH for determining of the absorbance value of sucrose with three replications.

Analysis of starch

The samples (30 μL) and distilled water (270 μL) were transferred into a cuvette and sugar test solution (200 μL) was added and the initial value was measured at 340 nm by a spectrometer. Finally, 1 μL HK/G6PDH was added and the absorbance value of starch were recorded with three replications.

Assay of enzyme activities

Three marked grains were selected and dehulled. Then added GS-buffer, PVPP and 2-ME, followed by ground in an ice bath. Finally centrifugated for 10 min (2℃, 15000 r/min) to get the supernatant solution (i. e. vacuolar enzyme solution). For the sediment,

GS-buffer was added and the supernatant was removed after centrifugation for 10 min, the operation was repeated again. The sediment left was cell wall granule-bound enzyme solution.

Sucrose synthase (SS) activity

Reaction solution (pH 7.4, 500 mmol/L Hepes-NaOH, 200 mmol/L $MgCl_2$, 100 mmol/L fructose, 50 mmol/L UDPG) and enzyme solution were added to the test tubes and the tubes were placed into waterbath at 0℃. The reaction was made at 30℃ for 30 min and ended after adding 70 μL of 1 mol/L NaOH. Then the test tubes were kept in a boiling water bath for 10 min and kept in a water bath at 80℃ for 8 min after adding 0.1%(*w/v*) resorcinol and 30% HCl. Finally, the solution was transferred into a cuvette and the absorbance values were determined at 520 nm using a spectrometer.

Vacuolar invertase (VCI) activity

Reaction solution (pH 4.6, 40 μL 1 mol/L acetate buffer, 10 μL 1 mol/L sucrose, 120 μL distilled water and 30 μL enzyme solution) was transferred into test tubes and the tubes were placed onto ice waterbath. The reaction was made at 30℃ for 30 min and ended at the boiling waterbath. Distilled water was added to the above solution and centrifuged. Then the supernatant and sugar test solution (pH 7.4, HEPES 150 mmol/L, $MgSO_4$ 10 mmol/L, NADP 3 mmol/L, 10 mmol/L ATP-Na) were added to the cuvette and the measurements were carried out at 340 nm using a spectrometer, followed by another measurements after adding 2 μL HK/G6PDH.

Cell wall bound invertase (CWI) activity

The method was almost identical to that for assaying activity of VCI with three replications and the spectrometer Beckman DU800 was used.

The Excel 2000 software was used to analyze data and make graphs. Correlation analyses were made by SPSS 11.0.

RESULTS

Dynamic changes of sucrose content and its cleaving products in rice grains

Dynamic changes of sucrose content

The dynamic changes in sucrose content of rice grains during the filling stage were shown in Fig. 1. An increasing trend has been shown in sucrose content with an average increase of 51.027 μg/grain and 20.806 μg/grain in Koshihikari and Sasanishiki, respectively, under the high temperature. The increase of sucrose content in grains indicated the enhancement of sucrose synthesis in origin organs or the deceleration of sucrose cleaving in grains.

Dynamic changes of fructose content

Under the high temperature, the fructose content of Koshihikari approached the control at the early stage, while it was lower at middle stage and higher at later stage compared with the control. The average value was decreased by 0.590 μg/grain compared with the control (Fig. 2). It has been noted that the fructose content in Sasanishiki was higher than that in the control and increased with an average of 2.130 μg/grain during the whole filling period under the high temperature (Fig. 2).

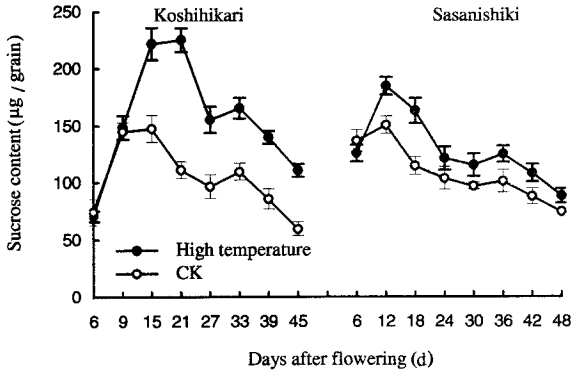


Fig. 1. Changes of sucrose content in rice grains.

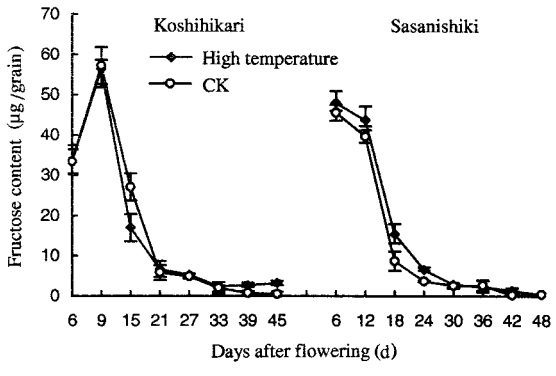


Fig. 2. Changes of fructose content in rice grains.

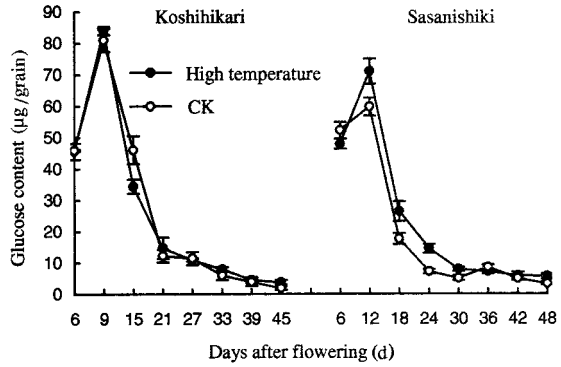


Fig. 3. Changes of glucose content in rice grains.

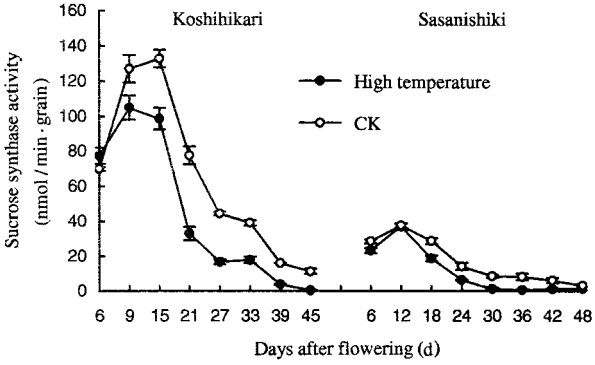
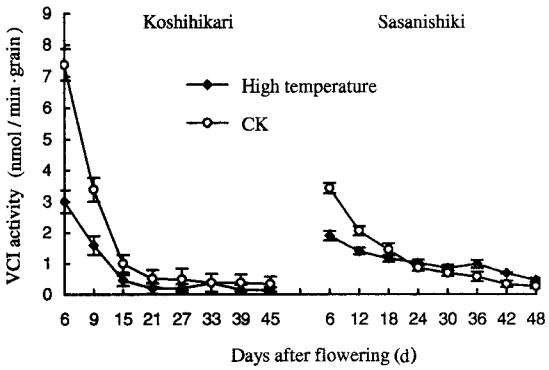


Fig. 4. Changes of sucrose synthase activity in rice grains.



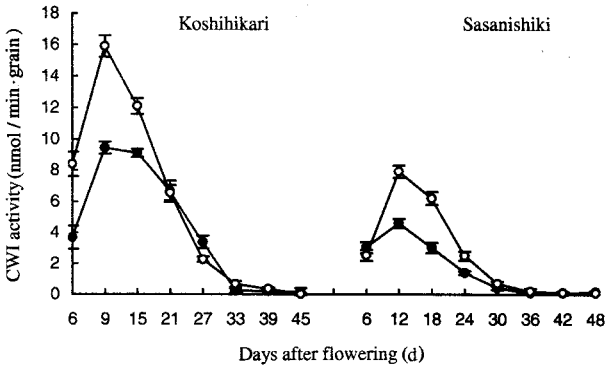


Fig. 5. Changes of vacuolar invertase(VCI) and cell wall bound invertase (CWI) activities in rice grains.

Dynamic changes of glucose content

It could be seen from Fig. 3 that, the glucose content of Koshihikari was declined under the high temperature with an average of 0.305 μg/grain, but the glucose content in Sasanishiki increased with an average of 3.479 μg/grain, compared with the control plants.

Changes of sucrose cleaving enzyme activities in rice grains

Sucrose is degraded mainly by two kinds of enzymes, namely sucrose synthase and invertase. The sucrose synthase reactions are reversible, while invertase reactions are irreversible.

Changes of sucrose synthase activity

The main function of sucrose synthase is to catalyze the sucrose cleaving to produce UDPG and fructose and provide glycosyl for starch synthesis rather than sucrose synthesis during grain filling course of rice [17-18]. The changes of sucrose synthase activity during the grain filling period have been shown in Fig. 4. It showed that the sucrose synthase activity in grains of the two varieties was even lower under the high temperature than that of the control, suggesting that the ability of decomposing sucrose through sucrose synthase pathway had the tendency of decline on the whole under the high temperature. According to Fig. 4, there was evident difference between values of sucrose synthase activity in the two varieties and SS values of Koshihikari were higher than those of Sasanishiki.

Changes of invertase activity

Invertase is also named sucrose enzyme and its function is to produce glucose and fructose by hydrolyzing sucrose. The changes of VCI and CWI activities in the two varieties were shown in Fig. 5. It is clear from the figures that the VCI activity of Koshihikari dropped, compared to the control. The VCI activity of Sasanishiki was lower at the early

Table 1. Coefficients of correlation between sucrose synthase, invertase activities with starch accumulation rate.

Variety	Sucrose cleaving enzyme	Treatment	Correlation coefficients with starch filling rate
Koshihikari	Sucrose synthase	High temperature	0.8407**
		Control	0.8522**
	Vacuolar invertase	High temperature	0.1632
		Control	0.0756
	Cell wall bound invertase	High temperature	0.8887**
		Control	0.7430*
Sasanishiki	Sucrose synthase	High temperature	0.9218**
		Control	0.8774**
	Vacuolar invertase	High temperature	0.5373
		Control	0.6899
	Cell wall bound invertase	High temperature	0.9411**
		Control	0.8335*

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

phase of active grain filling and higher at the late stage under the high temperature than the control. However, high temperature decreased CWI activity in rice grains compared with the control, indicating that the high temperature is unfavorable for the degradation of sucrose.

Correlation analysis between sucrose synthase, invertase activities and starch accumulation rate

The correlations between sucrose synthase, invertase activities and starch accumulation rate were analyzed and the results were listed in Table 1.

The data for sucrose synthase activity showed a positive and highly significant correlation with starch accumulation rate (Table 1). Meanwhile the CWI activity was positively and significantly related to starch accumulation rate, but VCI activity was not significantly correlated with starch accumulation rate under the high or normal temperature. The above results indicated that the sucrose synthase and CWI had important influences on sucrose cleaving and starch synthesis.

DISCUSSION

Sucrose synthase catalyzes the cleavage of sucrose to UDPG and fructose, and the invertase to glucose and fructose in the process of sucrose degradation. These two kinds of enzymes are all involved in sucrose degradation. Shannon et al [19] and Sung et al [20] reported that the invertase was significantly correlated with the starch accumulation rate in corn grains, and potato tubers, respectively. In this study, the sucrose synthase activity in rice grain was even higher than invertase activity, and significantly related to the starch accumulation rate, indicating that the sucrose synthase was the main pathway for sucrose degradation. The above finding was consistent with the results of Wang et al [21].

Under the high temperature, both the sucrose synthase and invertase activities declined, suggesting that the two pathways of sucrose cleaving were affected disadvantageously. Based on the invertase activity, the CWI activity was significantly high and correlated with the starch accumulation rate, while VCI activity was low and not significantly relevant to the starch accumulation rate. Therefore, it can be concluded that CWI performed larger functions than VCI in the course of sucrose cleaving and starch accumulation.

The current investigation showed that high temperature treatment obviously enhanced the sucrose content in rice grains without increase in sucrose cleaving products, i.e. glucose and fructose contents. However, the sucrose content in Koshihikari was augmented by an average of 51.027 μg/grain, glucose and fructose contents totally decreased by an average of 0.895 μg/grain; On the other hand, for Sasanishiki, the sucrose content increased by an average of 20.806 μg/grain, while the total contents of glucose and fructose were only enhanced by an average of 5.609 μg/grain. It indicated that the sucrose degradation was restrained and the cleaving rate was slowed down in high temperature. The reduction of the sucrose synthase and invertase activities suggested that the declines in sucrose synthase and invertase activities

are the main reason for the fall of sucrose cleaving rate under high temperature.

It's interesting that glucose content was higher than fructose content in rice grains under high or natural temperature. Generally, equal amount of glucose and fructose is produced when sucrose is hydrolyzed by invertase, and fructose can also be produced as sucrose is hydrolyzed by sucrose synthase, therefore the fructose content should not be lower than glucose content. Based on the phenomenon that glucose content was higher than fructose content, it can be suggested that enzymatic reactions are more favorable for the conversion of fructose in grains, making it possible that fructokinase plays more active role than hexokinase, therefore, the transformation rate of fructose was higher than that of glucose. It also support the view that sucrose synthase played an important role in sucrose degradation.

It can be concluded from the current finding that the sucrose synthase played a quite important role in the process of sucrose cleaving in rice grain. Moreover, the sucrose content increased distinctly while glucose and fructose contents were not augmented correspondingly under the high temperature, suggesting that the sucrose cleaving was repressed and the sucrose-cleaving rate was decelerated. Thus it can be assumed that there must be come linkage between the deceleration of sucrose cleaving rate and the decline of sucrose synthase, invertase activities.

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