Rice Science, 2006, 13(1): 43－50
http://www.ricescience.org

Relationship Between Variation in Activities of Key Enzymes Related to Starch Synthesis During Grain Filling Period and Quality of Eating and Cooking in Rice

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Abstract: Four japonica rice varieties with significant differences in quality of eating and cooking were used in the experiment. The varieties showed differences in amylose and amylopectin contents at different grain filling stages, which were attributed to the accumulative speed of starch at different grain filling stages. During grain filling period, the varieties had no difference in the time when the activities of ADPglocose pyrophosphorylase (AGPP) and soluble starch synthesis (SSS) reached a maximum, but had difference in the time when the activity of starch branching enzyme (SBE) reached a maximum, in which the inferior quality varieties were earlier than the high quality ones, and high quality varieties still kept high enzyme activities at the late stage of grain filling. The correlation and correlative degree between AGPP, SSS, SBE and amylose content, amylopectin content, taste meter value, and RVA properties varied with the different stages of grain filling. The correlation between SSS activity and taste meter value was not significant during the whole period of grain filling, but the activities of AGPP and SBE had significant or highly significant correlation with taste meter value. It was helpful for improving quality of eating and cooking of japonica rice to use the materials with low enzyme activity at the early stage of grain filling or high enzyme activity at the late stage as parents.
Key words: japonica rice; grain filling; starch synthesis enzymes; cooking quality; eating quality

Eating or cooking quality is one of the important quality of rice. Amylose content, RVA properties and taste meter value are three important indexes for evaluating cooking and eating quality of rice, which are determined by the chemical and physical compositions of starch [1-5]. The ratio of starch content to the weight of brown rice is higher than 90%. So the process of rice grain filling is described as the process of the starch synthesis and accumulation. The present studies showed that three enzymes including ADP glucose pyrophosphorylase (EC 2.7.7.21, AGPP), soluble starch synthase (EC 2.4.1.21, SSS), starch branching enzyme (EC 2.4.1.18, SBE) are considered as key enzymes involved in grain starch synthesis and play an important regulation role in the starch synthesis and accumulation. There are many research reports at home and abroad about variation in activities of the key enzymes related to starch synthesis during grain filling in different rice cultivars, on their relationships with starch accumulation and grain filling, and with amylose content, gel consistent and alkali value. However, little has been reported about the relationship between enzymatic activities and cooking quality including taste meter value, RVA properties, etc. In this study, four japonica varieties with different cooking and eating quality were used to investigate the relationship between cooking and eating quality and the activities of key enzymes involved in starch synthesis. This study aimed to reveal the biochemical mechanism of good quality formation in rice and provide the theories for rice cultivation and breeding.

Received: 12 October 2005; **Accepted:** 20 December 2005
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MATERIALS AND METHODS

Materials and treatments

The field experiment was conducted at Xiangfang Farm of Northeast Agricultural University in 2002. Four japonica cultivars with different eating and cooking quality were used, including Shuiludao 1, Dongnong 416, Fuzihikari, Toukei 180. The experiment was laid out in a split plot design with three replications, with nitrogen fertilizer levels in the main plot and varieties in the sub-plot. According to the amount of nitrogen fertilizer applied in the local

area (90-100 kg/ha), two nitrogen fertilizer treatments were adopted, which were 135 kg and 45 kg N per hectare respectively. Half of the total nitrogen fertilizer was applied as basal dose, and the remaining was applied in two equal splits (at the initial tillering stage and before the panicle initiation stage, respectively). All treatments were fertilized with 45 kg P_2O_5 per ha, as basal dose, and 45 kg K_2O per hectare, half as basal dose and the remaining was applied at the maximum tillering stage.

The experimental materials were sown on 6 April and transplanted on 22 May. There were four 5 m-length rows for each experiment. Seedlings were transplanted at a spacing of 10 cm × 30 cm, and with 2 seedlings per hill. Manage the experimental field as usual.

The panicles that grew unanimously and headed on the same day were marked with labels in each treatment at the heading stage. Panicle samples were collected since the 12th day after heading and at 6-day interval. And at each sampling time, the panicles that had been marked were collected at 9:00-9:30 a.m. and immediately frozen in liquid nitrogen, and then kept in a refrigerator (-20℃). Five grains in the middle of panicles were adopted for determination of the enzyme activities. The rest grains in the panicles were dried for starch determination. When harvested, the experimental materials in the same plot were collected together, and air-dried for three months. The brown rice was filtrated with a sieve of 1.9 mm, and processed into milled rice with an automatic rice polisher in the rate of milled rice output 90%. And then milled rice was ground to flour through a sieve for quality analysis.

Methods

Enzymes extraction

Thirty dehulled rice grains (embryo removed) were weighted for enzyme extraction. The grains were ground with a freezed homogenizer in the buffer containing Hepes–NaOH (pH 7.6), 5 mmol/L $MgCl_2$, 5 mmol/L DTT, 2% (*W*/*V*) PVP. The homogenate was centrifuged at 10 000 × *g* for 10 min, and the supernatants were used for the enzyme assay.

Determination of AGPP activity

Enzyme preparation (100 μL) was added into 550 μL reaction solution containing 100 mmol/L Hepes-NaOH (pH 7.4), 1.2 mmol/L ADPG, 3 mmol/L PPi, 5 mmol /L $MgCl_2$ and 4 mmol/L DTT. The reaction lasted for 20 min and was stopped by placing the reaction system into a boiling-water bath for 30 s. The suspension was centrifuged at 10 000 × *g* for 10 min. The supernatant solution (500 μL) was mixed with 100 μL reaction solution containing 6 mmol/L NADP, 1.5 IU glucophosphomutase, 1.5 IU glucose 6-phosphate dehydrogenase. The reaction was conducted at 30℃ for 10 min, and then 2 mL buffer was added into the mixture. The enzymatic activity was measured as absorbance of 340 nm. One unit of enzymatic activity was defined as causing 0.01 OD increase in 1 min.

Determination of SSS activity

Crude enzyme preparation (100 μL) was added into 180 μL mixture solution containing 50 mmol/L Hepes-NaOH (pH 7.4), 1.6 mmol/L ADPG, 0.7 mg amylopectin, 15 mmol/L DTT. The reaction was conducted at 30℃ for 10 min, then stopped by placing the reaction system into a boiling-water bath. After cooled in iced-water bath, the suspension was appended with 100 μL reaction solution containing 50 mmol/L Hepes-NaOH (pH 7.4), 4 mmol/L PEP, 200 mmol/L KCl, 10 mmol/L $MgCl_2$, and 1.2 U pyruvate kinase, and placed into a boiling-water bath for 30 s to ensure inactivation of the enzyme, and centrifuged at 10 000 × *g* for 5 min.

The supernatant (350 μL) was mixed with 300 μL reaction solution containing 50 mmol/L Hepes-NaOH (pH 7.4), 10 mmol/L glucose, 20 mmol/L $MgCl_2$, 2 mmol/L NADP, 1.4 U hexokinase and 0.35 U glucose 6-phosphate dehydrogenase. The reaction was conducted at 30℃ for 10 min, and then appended with 2 mL buffer solution. The enzyme activity was measured by its absorbance at 340 nm. One unit of enzyme activity was defined as causing 0.01 OD increase in 1 min.

Determination of SBE activity

Fifteen dehulled grains (embryo removed) were weighed for enzyme extraction. The grains were ground with a freezed homogenizer in 6 mL 0.05

mol/L citric acid buffer (pH 7.0), the homogenate was centrifuged at 10 000 × *g* for 10 min, and the supernatants were used for the assay of enzymes. Supernatant (1 mL) was mixed with 1 mL 0.2 mol/L citric acid buffer (pH 7.0) and placed into a water bath (37℃) for 40 min. The reaction was stopped by addition of 1 mL 1 mol/L CCl_3COOH. The reaction system was appended with 1 mL iodine solution and calibrated to 50 mL. The enzymatic activity was determined at 660 nm and contrasted with zero. Enzymatic activity was described by OD_{660} decrease percentage.

Determination of starch content and protein content in rice grain

Amylose and starch content were measured by the method of He Zhao-fan (1981), and amylopectin content was calculated by subtracting amylose from starch content. Protein content was determined by semi-micro Kieldahl method and the conversion coefficient is 5.95.

Determination of taste meter value

Taste meter value was measured by a Rice Taste Degree Meter (TOYOMATA-90B). Thirty-three gram milled rice was boiling for 10 min in a container, then cooled for 1 min. The rice was measured in an electromagnetic wave instrument. The data were used to evaluate the eating quality of rice. Relative taste meter value of rice was evaluated contrasting with the full marks 100.

Analysis of RVA properties

RVA properties were measured with a Rapid Visco Analyzer developed by Newport Scientific Corporation. Three gram milled rice flour of each sample was weighed into the aluminum canister, to which 25 mL of distilled water was added. The idle temperature was set to 50℃, and the following 13 min test profiles were run: 50℃ held for 1.0 min, ascended to 95℃ in 3.9 min, held at 95℃ for 2.4 min, descended to 50℃ in 3.9 min, and held at 50℃ for 1.8 min.

RVA profile was described by six parameters, i.e. peak viscosity (PKV), hot paste viscosity (HPV) or trough viscosity, cool paste viscosity (CPV) or final viscosity, breakdown (defined as subtracting the HPV from PKV), setback (defined as subtracting PKV from CPV) and consistence (defined as subtracting HPV from CPV). All the viscosity parameters were expressed in Rapid Visco Units (RVU).

Above determination of enzymes activities and rice quality were conducted with two replications, and the means of replication were used for statistics. The analysis was carried out according to the data from high nitrogen fertilizer treatment except the relevant analysis between the three enzymes activities and cooking and eating quality, which were according to the data from high and low nitrogen treatments.

RESULTS

Comparison of eating and cooking quality among rice cultivars with different quality

Both protein and amylose contents are important factors affecting the eating and cooking quality of rice. Taste meter value serves as an important and direct index in estimating the eating quality of rice instead of sensory test. It has been considered that the cultivars with high taste meter value have better eating quality than those with low taste meter value.

As showed in Table 1, protein contents of Shuiludao 1 and Dongnong 416 were significantly higher than those of Fuzihikari and Toukei 180. There was significant difference in amylose content among tested cultivars. Dongnong 416 had the highest amylose content and Toukei 180 had the lowest, and there was difference of 4.3 percent point between them. The taste meter values of Toukei 180 and Fuzihikari were significant higher than those of Shuiludao 1 and Dongnong 416. The results showed that the tested cultivars had significant or very

Table 1. Protein content, amylose content and taste meter value of rice in different cultivars.

Cultivar	Protein content (%)	Amylose content (%)	Taste meter value
Shuiludao 1	8.75 aA	15.83 cB	50.6 bB
Dongnong 416	7.87 bB	17.79 aA	53.8 bB
Fuzhihikari	6.89 cC	17.09 bA	69.4 aA
Toukei 180	6.73 cC	13.49 dC	64.1 aA

Within a column, values followed by uppercase and lowercase letters mean significant difference at 1% and 5% levels, respectively.

significant difference in amylose and protein contents and taste meter value. According to the taste meter values, the tested cultivars could be divided into two groups: good quality cultivars, Fuzihikari and Toukei 180 and inferior ones, Shuiludao 1 and Dongnong 416.

Comparison of amylose and amylopectin accumulation in grain filling

The dynamic of amylose and amylopectin accumulation in grain filling was showed in Fig. 1. The accumulation rate of amylose and amylopectin appeared fast from initial heading to 24 days after heading, and then became slow. The accumulation content of amylose and amylopectin in this period was 76.99-91.31% and 85.41-91.22% of final amount of rice grain, respectively. The results showed that the period from initial heading to 24 days after heading was the main period for accumulating amylose and amylopectin.

The accumulation amounts and rates of amylose and amylopectin were different at different grain filling stages among cultivars with various rice quality. During the period from initial heading to 20 days after heading, amylose contents and accumulation rates of Shuiludao 1 and Dongnong 416 were higher than those of Fuzihikari and Toukei 180. However, on 20 days after heading the accumulation amounts and rates of amylose and amypection of Fuzihikari was higher than those of Shuiludao 1. Toukei 180 with low amylose content and high quality has lower accumulation rate and content than the other cultivars during grain filling period. Amylopectin accumulation amount of Shuiludao 1 and Dongnong 416 were higher than those of Fuzihikari and Toukei 180 from initial heading to 12 days after heading, but it was reversed after 15 or 20 days of heading. The result showed that the difference of grain amylose and amylopectin contents occurred in different periods of grain filling and was represented as the starch accumulation rate in different periods. Lower quality cultivars had higher amylose content and accumulate rate at the early filling stage, and it was reversed at the late filling stage. Therefore, high amylose accumulation rate at the early stage of grain filling should be a disadvantage for the formation of good rice quality.

Comparison of activities of key enzymes involved in starch synthesis in different quality cultivars

For the four tested cultivars, the activities of AGPP, SSS, and SBE trended to increase gradually to a peak value, thereafter descended during grain filling stage (Table 2). Different cultivars had different enzymes activities at the same filling stage and difference in the time when enzyme activities reached a maximum.

For Shuiludao 1, the maximum of AGPP activity displayed on 12 days after heading, which was earlier than that for the other three cultivars (on 18 days after heading). The peak value of SSS in these four tested cultivars showed a similar tendency. The time when SBE reached a maximum varied with different cultivars. Shuiludao 1 and Dongnong 416 reached the peak on 18 days after heading; however, Fuzihikari and Toukei 180 reached the peak on 24 days after heading.

The ANOVA analysis showed that *F* values of the

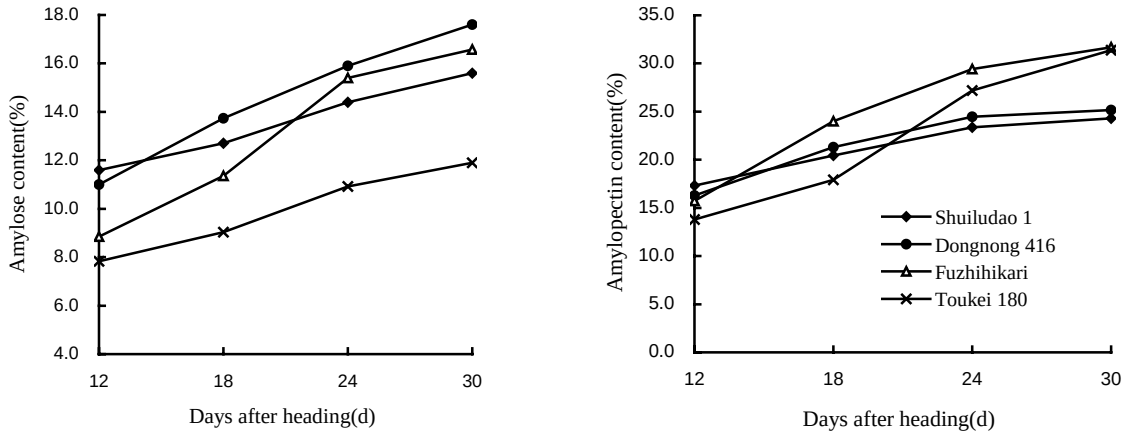

Fig. 1. Changes of amylose and amylopectin contents in rice grain at the filling stage.

Table 2. Changes of AGPP, SSS and SBE activity during grain filling period. U/grain·min

Cultivar	AGPP				SSS				SBE			
	12 d	18 d	24 d	30 d	12 d	18 d	24 d	30 d	12 d	18 d	24 d	30 d
Shuiludao 1	4.90 aA	4.38 dC	3.53 dC	2.56 bB	3.08 aA	3.97 aA	3.28 aA	2.26 bB	23.8 aA	33.6 bB	21.8 dD	8.6 cC
Dongnong 416	4.51 bA	5.56 cB	4.20 cB	2.48 bB	2.68 bB	3.95 aA	3.15 aA	2.36 bB	21.0 bA	37.3 aA	25.9 cC	12.8 bB
Fuzihikari	3.38 cB	5.94 bA	5.03 bA	3.57 aA	2.85 bB	4.29 aA	3.74 aA	3.01 aA	20.1 cB	27.2 cC	32.9 bB	22.1 aA
Toukei 180	3.06 dC	6.67 aA	5.16 aA	3.59 aA	2.26 cC	4.49 aA	3.64 aA	2.88 aA	17.1 dC	24.2 dD	40.6 aA	22.7 aA

Uppercase and lowercase letters indicate significant difference at 1% and 5% levels, respectively.

Table 3. Coefficients of correlation between activities of enzymes and starch content at different grain filling stages.

Type of starch	Type of enzyme	Days after heading			
		12 d	18 d	24 d	30 d
Amylose content	AGPP	0.447	0.222	-0.194	-0.597
	SSS	0.328	-0.910**	-0.212	-0.169
	SBE	0.572	-0.582	-0.500	-0.754*
Amylopectin content	AGPP	-0.786*	0.688	0.599	0.805**
	SSS	-0.577	0.135	-0.167	0.743*
	SBE	-0.672	-0.845**	0.669	0.694

* and ** indicate significant difference at 0.05 and 0.01 levels, respectively.

three enzyme activities in different quality cultivars were significant at 0.05 or 0.01 levels except the *F* value of SSS on 18 and 24 days after heading. The range of *F* values was from 10.02 to 41.13.

Multiple comparisons of enzymatic activities between cultivars with different rice quality were carried out by SSR method and the results were listed in Table 2. On 12 days after heading the activities of AGPP and SBE in order were Shuiludao 1> Dongnong 416 > Fuzihikari > Toukei 180 and the difference among them was significant, and activity of SSS showed that Shuiludao 1 > Fuzihikari > Dongnong 416 > Toukei 180; the difference between Fuzihikari and Dongnong 416 was not significant. On 18 days after heading, activity of AGPP was Toukei 180 > Fuzihikari> Dongnong 416 > Shuiludao 1, and as to SBE the result was Dongnong 416 > Shuiludao 1 > Fuzihikari > Toukei 180, and the difference among them was significant. On 24 days after heading, AGPP and SBE displayed as follows: Toukei 180 > Fuzihikari > Dongnong 416 > Shuiludao 1, and the difference among them was significant. On 30 days after heading, the activities of these three key enzymes in Toukei 180 and Fuzihikari were remarkably higher than those of Dongnong 416 and Shuiludao 1. The results showed that high enzyme activity was an important physiological characteristic for cultivars with good quality.

Relationship between enzyme activities and eating and cooking quality in rice

Simple correlation coefficients between the three key enzymes activities and starch content, taste value and RVA profile were calculated according to the data from high and low nitrogen treatments and showed in Tables 3, 4, 5.

As shown in Table 3, the activities of SSS on 18 days after heading and SBE on 30 days after heading were very significantly and negatively correlated with amylose content, respectively. The activities of AGPP on 12 days after heading and SBE on 18 days after heading were significantly and negatively correlated with amylopectin content. The activities of AGPP and SSS on 30 days after heading were significantly and positively correlated with amylopectin content. The correlation between the three key enzyme activities and amylose content, amylopectin content didn't reach a significant level in other periods of grain filling.

On 12 days after heading, the activities of AGPP and SBE were correlated negatively with taste meter value (Table 4), while positively correlated on 24 d and 30 days after heading. The three key enzymes had no significant correlation with taste meter value on 18 days after heading. SSS activity had no correlation with taste meter value during the whole filling period. The results showed that high activity of AGPP and SBE at early filling stage was disadvantaged to the formation of good quality of rice. On the contrary, high enzyme activities in the middle and late filling period were helpful for the formation of good quality rice.

Table 4. Coefficients of correlation between activities of enzymes and taste meter value at different grain filling stages.

Enzyme	Days after heading			
	12 d	18 d	24 d	30 d
AGPP	-0.801*	0.141	0.887**	0.898**
SSS	-0.605	-0.349	0.558	0.695
SBE	-0.832**	-0.172	0.951**	0.797*

* and ** indicate significant difference at 0.05 and 0.01 levels, respectively.

As listed in Table 5, on 12 days after heading, the activities of the three key enzymes were significantly and positively correlated with final viscosity, setback, and consistency and the activities of AGPP and SBE were negatively correlated with breakdown at 1% and 5% level, respectively. On 18 days after heading the activity of AGPP was very significantly and positively correlated with peak viscosity and setback, and negatively with trough viscosity and final viscosity; SSS activity was negatively correlated with peak viscosity and setback, positively with final viscosity; SBE activity was significantly and negatively correlated with peak viscosity, and significantly and positively with trough viscosity and final viscosity. On 24 days after heading, the activity of SBE was positively correlated with peak viscosity and breakdown, and negatively correlated with consistency and setback. On 30 days after heading, the activity of AGPP was positively correlated with breakdown, and negatively correlated with consistency; the activity of SBE was positively correlated with breakdown. The correlation between the three key enzymes and RVA profile in other periods didn't reach a significant level. It was suggested that high activities of AGPP and SBE at the early filling stage was disadvantaged to the formation of good viscosity characters. However, high enzyme activities in the middle and late grain filling period were helpful for the formation of good viscosity characters in rice.

From Table 5, we also knew that the middle period of grain filling (18 days after heading) was a critical period, in which the correlation between enzyme activities and eating and cooking quality characters changed not only in degree but also in direction of the correlation. For example, during 12-18

Table 5. Coefficients of correlation between enzymatic activities and RVA properties at different grain filling stages of rice.

Days after heading	Enzyme	Peak viscosity	Trough viscosity	Final viscosity	Breakdown	Setback	Consistency
12 d	AGPP	-0.473	0.470	0.709*	-0.715*	0.727*	0.761*
	SSS	-0.315	0.684	0.908**	-0.644	0.883**	0.792*
	SBE	-0.623	0.452	0.783*	-0.867**	0.840**	0.903**
18 d	AGPP	0.834**	-0.947**	-0.895**	-0.248	0.809**	0.330
	SSS	-0.772*	0.554	0.784*	-0.431	-0.806*	-0.700
	SBE	-0.871**	0.727*	0.751*	0.051	-0.630	-0.267
24 d	AGPP	0.322	-0.327	-0.499	0.490	-0.513	-0.528
	SSS	0.176	-0.222	-0.302	0.287	-0.297	-0.308
	SBE	0.810**	-0.203	-0.674	0.954**	-0.830**	-0.949**
30 d	AGPP	0.692	-0.097	-0.421	0.780*	-0.535	-0.709*
	SSS	0.385	-0.226	-0.310	0.511	-0.306	-0.444
	SBE	0.610	-0.190	-0.465	0.735*	-0.543	-0.687

* and ** indicate significant difference at 0.05 and 0.01 levels, respectively.

days after heading, SBE activity was negatively correlated with taste meter value, but the result was reversed during 24-30 days. SBE was negatively correlated with breakdown on 12 days after heading, but during 18-30 days the correlation between them changed from not significantly and positively to very significantly and positively correlated.

DISCUSSION

Many studies have been conducted on the relationship between key enzyme activities and starch content and structure [15-19]. However, the knowledge to the function of the three key enzymes on starch synthesis was different. Zhao et al [15] reported that at the early filling stage the activity of starch synthase was negatively correlated with gel consistency and alkali value and positively correlated with amylose content; at the middle and late stage of grain filling, the activity of SBE was significantly and positively correlated with gel consistency and alkali value and negatively correlated with amylose content. Our experiment results illustrated that the relationships between enzymatic activities and starch content, taste value and RVA properties were different with types and expression time of enzymes during grain filling. At the early, middle and late stages of grain filling, the activities of AGPP and SBE were significantly correlated with taste meter value. But the activity of SSS had no significant correlation with taste value during grain filling period. During grain filling period the activities of AGPP and SBE have more significant correlation with RVA profiles than that of SSS. Therefore, AGPP and SBE played more important roles in the formation of rice eating quality. The enzyme activities were regulated by hormone content and the balance between different hormones and at different filling stage the three key enzymes had different effects on rice quality. So we could regulate key enzyme activities involved in the starch synthesis through the methods of cultivation and chemical controls and enhance the eating and cooking quality of rice. For example, improving the activities of AGPP and SBE at the middle and late grain filling stages could enhance the cooking and eating quality of rice.

In addition, the results of our experiment showed that the accumulation of amylose ended at the late grain filling stage, but the activities of AGPP and SBE were significantly and positively correlated with taste meter value in this period. The results elucidated that AGPP and SBE could regulate the eating and cooking quality through controlling the fine structure of starch instead of amylose content.

The grain weight is one of the factors determining grain yield. Yang et al [14] pointed out that, in the period of 3-12 days after flowering, the activities of AGPP, SSS and SBE were positively correlated with grain weight and grain filling rate; at the middle and late stages of grain filling (13-39 days after flowering) were negatively correlated with grain filling rate and grain weight at different significance levels. In this essay, at the early grain filling stage (12 days after heading) high activities of AGPP and SBE would be disadvantaged to the formation of the good eating and cooking quality in rice, and at the middle and late filling stages they were helpful to form good eating and cooking quality. So we might draw a conclusion that high activities of AGPP and SBE at the early grain filling stage will enhance rice yield through increasing grain weight but degrade the eating and cooking quality of rice. The result might to a certain extent explain why the cultivars with high quality have low yield and in the contrary the cultivars with high yield will have poor quality. Therefore in rice breeding, we should distinct the choice of high yield cultivars with high quality ones in order to meet the needs of domestic and international markets.

ACKNOWLEDGEMENTS

This research was supported by the Key Research Project of the Department of Education of Heilongjiang Province, China (10S11Z002), the Natural Science Foundation of Heilongjiang Province, China (C01-10) and the Rice Science Foundation Project of China (0003219).

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