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Effect of Temperature at Grain Filling Stage on Activities of Key Enzymes Related to Starch Synthesis and Grain Quality of Rice

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Abstract: Three japonica rice varieties with different cooking and eating quality were grown at high temperature in the greenhouse and natural field. Effects of temperature at the grain filling stage on these varieties were investigated in terms of the activities of key enzymes related to starch synthesis and cooking and eating quality of rice grain. The high temperature at the grain filling stage increased protein content, and decreased amylose content and taste meter value of rice; inferior grain quality varieties showed a greater magnitude of the increase or decrease than the superior ones. Reaction of rapid visco analyser profiles to the temperature varied with rice varieties. The activities of adenosine diphosphoglucose pyrophosphorylase (AGPP), soluble starch synthase (SSS) and starch branching enzyme (SBE) gradually increased to a peak value, and thereafter declined as grain filling progressed. Enzyme activities in different varieties differed in a same filling stage, and also in the time when the enzyme activity reached a maximum. AGPP and SSS were insensitive to the environmental temperature, but SBE was comparatively sensitive to the temperature, and its activity declined when temperature was too high or too low.
Key words: japonica rice; grain filling stage; temperature; starch synthesis enzymes; cooking quality; eating quality

Rice grain is consumed mostly as cooked rice, and thus cooking and eating properties of rice grain are important quality traits. Amylose content (AC), starch rapid visco analyser (RVA) properties and taste meter value, which are of much importance in physical-chemical properties when cooking and eating quality is evaluated, are affected by chemical components and physical properties of endosperm in grain, and therefore they have close relationship with cooking and eating quality [1-3]. Since starch occupies 90% or more of brown rice weight, the grain filling is characterized by synthesis and accumulation of starch, which is catalyzed by enzymes. The photosynthetic products synthesized in source organs are transported to grain in the form of sucrose, and then catalyzed by enzymes to synthesize starch. Therefore, the activities of the enzymes related to starch synthesis are closely associated with amylose content, starch RVA properties, taste meter value and other cooking and eating properties. It has been shown that in the photosynthetic or non-photosynthetic organs of higher plants, adenosine diphosphoglucose pyrophosphorylase (EC 2.7.7.21, AGPP), soluble starch synthase (EC 2.4.1.21, SSS) and starch branching enzymes (EC 2.4.1.18, SBE) are key enzymes for starch synthesis metabolism, regulating starch synthesis and accumulation in rice grain [4-8]. There have been many research reports available for changes in activities of the key enzymes related to starch synthesis during grain filling in different rice varieties, relationships between the activity with starch accumulation and grain filling [4-12], effects of the temperature at the filling stage on rice cooking and eating quality, and on the activities of the key enzymes related to starch synthesis metabolism and starch RVA properties [13-15], and so on. Since the temperature plays a very crucial role in rice yield and quality, it is of great significance to characterize the effects of temperature on activities of the key enzymes related to starch synthesis and rice grain cooking and eating quality so as to fully understand how temperature affects the cooking and eating quality. In this report we describe the effects of the temperature at the grain filling stage on activities of the key enzymes related to starch synthesis and the quality of cooking and eating in the three japonica rice with different cooking and eating quality. The objective of this study is to determine the relationships between temperature with

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superior grain quality and the grain biochemical characteristics so as to provide theoretical basis for breeding and cultivating high quality rice.

MATERIALS AND METHODS

Materials and temperature treatments

The experiment materials were three japonica rice varieties, Fukei 180, Fujihikari, and Dongnong 415, which were different in the quality of cooking and eating. The pot experiment was conducted in 2003 in Agricultural College, Northeast Agricultural University, Harbin, China. In this experiment, there were two temperature treatments, one was high temperature in greenhouse (average 24.5℃, the highest 30.1℃/the lowest 18.9℃) and the other was natural temperature outdoor (average 21.3℃, the highest 25.7℃/the lowest 16.9℃). There were eight pots (25 cm×25 cm) for each variety. The rice was sown on April 6 and transplanted on May 25. Upon seedling recovery from transplanting, seedlings were thinned to four (of uniform growth) in each pot where $NH_4H_2PO_4$ and urea of 1 g were applied respectively and the same amount fertilizers were used at the maximum tillering stage. At the full heading of each cultivar, the four pots were laid in outdoor and the other four in the greenhouse for 30 d and then were moved to outdoor waiting for harvest.

Samples and harvest

At the heading stage, for each treatment the panicles heading on the same day were tagged with labels. The panicles were sampled from the 10th day after heading and once every 5 d at 9:00-9:30 a.m. and immediately frozen in liquid nitrogen, and then kept in a refrigerator (-20℃) until used. The grains in the middle of the panicle, which milked unanimously by and large, were used to determine the enzyme activities. When harvested, the grains of each variety in the same treatment were collected, and air-dried for three months. The brown rice was sieved with 1.9-mm sieve, and processed into milled rice with an automatic rice miller at the milled rice rate 90%. And then the milled rice was ground to flour through a sieve for quality trait analysis.

Determination methods

The extractions of AGPP and SSS were referred to the method of Liang et al [6], and enzyme reaction were undertaken according to the method of Nakamura [16]. The extraction and activity assay of SBE were according to the method of Li [17]. Amylose content was measured according to the standard methods promulgated by Ministry of Agriculture of the People’s Republic of China. Protein content in rice grain was determined with Kjeldahl method and converting coefficient is 5.95. Taste meter value was measured by Test Rice Taste Degree Meter (TOYOMATA-90B, Japan). The RVA paste viscosity was determined by a Rapid Visco Analyser (RVA, model 4D, Newport Scientific, Japan).

Above determination of enzyme activities and rice quality were conducted with two replications, and the means of replications were used for statistics.

RESULTS

Effects of temperature during grain filling period on cooking and eating quality of rice grain

Protein and amylose content are main chemical components that affect the cooking and eating quality of rice grain, and if their contents are too high, the cooking and eating quality of rice will be poor. 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 varieties with high taste meter value have better eating quality than those with low taste meter value. RVA properties indicate reaction of rice flour or starch to heat, which is an important index in evaluating rice starch pasting viscosity. Effects of the temperature on protein and amylose content, taste meter value and RVA profiles were presented in Table 1.

As shown in Table 1, under natural temperature, protein content in Dongnong 415 increased by 1.06 and 1.11 percentage points as compared with that of Fukei 180 and Fujihikari, respectively. Amylose content in Fujihikari increased by 4.21 percentage points as compared with that of Fukei 180. Taste meter value of Fukei 180 and Fujihikari increased by 12 and 13, respectively, as compared with that of Dongnong 415. From these data, we concluded that there were difference in amylose and protein contents and taste meter values among the tested varieties. According to the taste meter value, the varieties could be classified into two groups, i.e. high quality varieties, Fukei 180 and Fujihikari, and low quality Dongnong 415, which was consistent with sensory test result.

Table 1 also showed that high temperature during grain filling made protein content increase and amylose content and taste meter value decrease. But the extent of increase or decrease varied with the varieties. The range of variation of Dongnong 415 was wider than that of Fukei 180 and Fujihikari. All these indicated that the temperature influenced the low quality varieties greater on grain protein and amylose content and taste meter value than the high quality ones.

Table 2 showed that under natural temperature conditions, peak viscosity and breakdown of Fukei 180 and Fujihikari were higher than those of Dongnong 415, and setback was obviously lower than that of Dongnong 415. Compared with normal temperature, final viscosity and breakdown of all the three varieties declined and peak viscosity of Fukei 180 and Fujihikari descended but that of Dongnong 415 increased, and setback of Fukei 180 increased and that of Fujihikari and Dongnong 415 decreased at the high temperature. These illustrated that effects of the temperature during grain filling and ripening period on RVA profiles varied with varieties and RVA parameters. Therefore, the temperature during grain filling at which the best starch viscosity profiles formed varied with varieties.

Variation in activities of key enzymes related to starch synthesis during grain filling and ripening period

For these three varieties, activities of AGPP, SSS and SBE during grain filling stage trended to increase gradually to a peak value, thereafter declined (Fig.1). Different varieties had different enzyme activities at the same filling stage and differed in the time when enzymes activities reached a maximum.

For Fukei 180, the maximum of AGPP activity appeared at 20 d after heading, which was later than the other two varieties. For the peak value of SSS activity, Fujihikari appeared at 15 d after heading, and earlier than the other two varieties. The peak of SBE activity occurred at 20 d after heading for these three varieties.

Comparing the activities of these three key enzymes in the tested varieties, we found that AGPP and SBE in Fukei 180 showed higher activities than the other two varieties at whole filling stage, and SSS in Dongnong 415 performed strongest except that its maximum was lower than that in Fukei 180. In addition, the activity of SBE in Fujihikari was stronger than that in Dongnong 415 from 25 days later

Table 1. Effects of the temperature at the filling stage on the protein content, amylose content and taste meter value in rice grain.

Variety	Protein content (%)			Amylose content (%)			Taste meter value		
	H	L	H－L	H	L	H－L	H	L	H－L
Fukei 180	7.66	6.80	+0.86	9.20	9.84	-0.64	73.0	74.3	-1.3
Fujihikari	7.95	6.75	+1.20	13.20	14.05	-0.85	70.0	73.3	-3.3
Dongnong 415	9.01	7.86	+1.15	11.67	12.68	-1.01	51.0	61.3	-10.3

L and H indicate natural temperature and high temperature, respectively, which is the same as in Table 2.

Table 2. Effects of the temperature at the filling stage on rice RVA properties.

Variety	Peak viscosity			Final viscosity			Breakdown			Setback		
	H	L	H－L	H	L	H－L	H	L	H－L	H	L	H－L
Fukei 180	235.8	382.2	-146.4	130.4	249.0	-118.9	149.5	215.2	-65.7	-105.5	-133.2	27.7
Fujihikari	293.6	325.9	-32.3	270.5	320.2	-49.7	115.7	133.5	-17.8	-23.2	-5.7	-17.5
Dongnong 415	299.6	288.4	11.2	306.8	312.8	-6.0	90.6	102.4	-11.8	7.2	24.3	-17.1

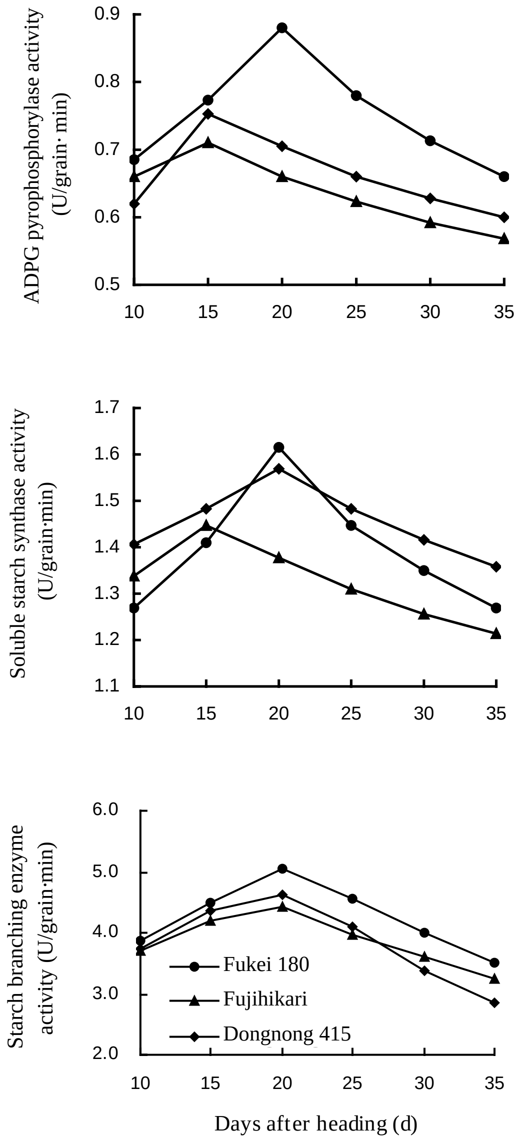

Fig. 1. Variations of enzyme activity of AGPP, SSS and SBE during grain filling period.

after heading.

Reaction of activities of key enzymes related to grain starch synthesis to the temperatures at the filling stage

In order to understand how temperature influences the biochemical characteristics of cooking and eating quality of rice grain, variation in activities of AGPP, SSS, and SBE in Dongnong 415 at the 20 d after heading were determined under the different reaction temperatures of 20℃, 25℃, 30℃, 35℃, and 40℃. Fig. 2 showed that the activity of SSS increased with the reaction temperature rising, and reached a peak at 35℃ after which the activity descended

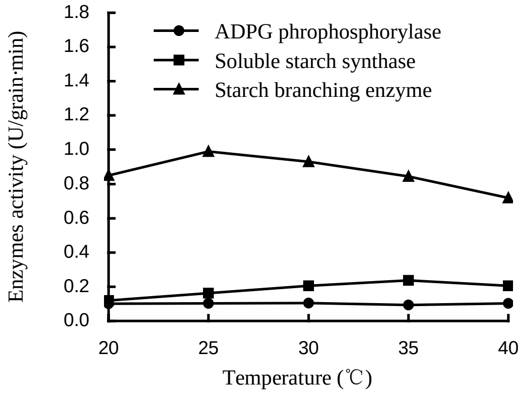

Fig. 2. Relationship between temperature and activity changes of AGPP, SSS and SBE in grains of Dongnong 415.

gradually, but its range of variation was narrow. The activity of AGPP almost didn't change under different reaction temperatures. The activity of SBE also increased with the reaction temperature increasing, and get to a maximum at 25℃, after which the activity fell with temperature rising, and its range of variation was wide. So, we can conclude that AGPP and SSS were insensitive to temperature, but SBE was sensitive to temperature, its activity was affected by both low and high temperatures.

Effects of the temperature on activities of key enzymes related to starch synthesis during the whole grain filling period

It was shown in Table 3 that during the whole grain filling for high or low quality varieties, the activities of AGPP and SSS were higher at the high temperature than that at natural temperature. SBE activity showed difference in different stages of grain filling, and at the early stage, for Fukei 180 from initial heading to 20 d after heading, and for Fujihikari and Dongnong 415 from initial heading to 15 d after heading, SBE activity at natural temperature was weaker than that at high temperature. Henceforth for these three varieties, SBE activity at natural temperature was stronger than that at high temperature. Analysis above made it clear that high temperature at the filling stage enhanced the activities of AGPP and SSS.

Table 3. Comparison on activities of AGPP , SSS and SBE at different temperatures. U/grain·min

Type of enzyme	Variety	Days after heading					
		10 d	15 d	20 d	25 d	30 d	35 d
ADPG pyrophosphorylase (AGPP)	Fukei 180	0.020	0.065	0.065	0.065	0.043	0.052
	Fujihikari	0.060	0.030	0.020	0.042	0.042	0.043
	Dongnong 415	0.002	0.025	0.046	0.056	0.049	0.053
Soluble starch synthase (SSS)	Fukei 180	0.100	0.090	0.050	0.050	0.020	0.041
	Fujihikari	0.003	0.010	0.013	0.040	0.016	0.052
	Dongnong 415	0.020	0.040	0.030	0.128	0.172	0.256
Starch branching enzyme (SBE)	Fukei 180	0.069	0.185	0.277	−0.380	−0.270	−0.273
	Fujihikari	0.448	0.220	−0.108	−0.360	−0.220	−0.170
	Dongnong 415	0.130	0.228	−0.312	−1.135	−1.095	−0.580

Data in the table are the difference values of the enzyme activity between high temperature and natural temperature.

DISCUSSION

In our experiment, effects of temperature at the grain filling stage on activities of key enzymes related to rice grain starch synthesis were studied under the conditions of high temperature in glass greenhouse and natural temperature outdoor. Because glass can absorb some ultraviolet ray, there was difference in the quantity of ultraviolet ray between inside and outside glasshouse, which may influence the experiment results. Some previous studies [18] have shown that ultraviolet ray mainly affects plant morphological formation or causes plants to die, and had no more influence on plant growth in natural conditions. Ultraviolet ray that get to the ground is no longer the limiting factor for rice development and growth, and ultraviolet ray has less effect on rice photosynthesis than temperature. So, in present trial the effects of temperature were the chief factor. Therefore the results of our experiment were enough to explain the relationships between the temperature at the grain filling stage and activities of key enzymes related to rice grain starch synthesis and cooking and eating quality.

Our results indicated that the activity of AGPP was steady under the condition of different reaction temperatures, and the activity of SSS got to a peak at about 35℃ and had little change at different temperatures, but the activity of SBE varied considerably while reaching its maximum at about 25℃, which was in agreement with the study of Cheng et al [15]. Furthermore, during the whole grain filling period the activities of AGPP and SSS were always higher at high temperature than those at natural temperature, and SBE varied with the changes of filling period and temperature. We can find that the most favorable temperature to produce high quality rice is at about 25℃ for high quality japonica rice, the same as the temperature at which SBE keeps the highest activity. It is reasonable to conclude that SBE maybe is the regulation focus by which the temperature at the grain filling stage exerts influence on rice cooking and eating quality.

Being mainly governed by genetic factor, rice cooking and eating quality is also regulated by climate factors, and temperature at the grain filling stage is of the main one [11-14]. It was obvious from our results that temperature at the grain filling stage had remarkable effects on rice amylose and protein contents and taste meter value, as well as breakdown. With the temperature increasing, protein content increased and taste meter value and breakdown descended at the same time while amylose content decreased, which made rice cooking and eating quality be inferior. High temperature promotes amino acids to move from culms and leaves to grain and grain protein synthesis, resulting in an increase in grain protein content [19]. Therefore, high temperature exerts adverse influence on cooking and eating properties of rice grain via enhancing protein content not by way of the variation in amylose content.

In addition, the results of our experiment demonstrated that cooking and eating properties of the tested variety responded differently to the temperature at the grain filling stage, so different varieties need temperatures at the grain filling stage to form superior cooking and eating properties. When cultivating superior quality rice, we should arrange varieties reasonably: take measures suited to local conditions according to the temperature requirement of different varieties and climatic character, and select suitable seeding and transplanting date so as to make superior varieties be in the optimum temperature conditions when grain is filling. Furthermore, when breeding superior quality varieties we should choose suitable environments according to breeding objective so that hybrid progeny will be selected efficiently with optimum selection results.

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