

Changes in Activities of Glutamine Synthetase during Grain Filling and Their Relation to Rice Quality

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Abstract: Four japonica rice varieties differed in cooking and eating qualities were used in a pot experiment to study the relationship between the activities of glutamine synthetase during grain filling and rice quality. The activities of glutamine synthetase gradually increased and then declined as a single peak curve in the course of grain filling. The 15th day after heading was a turning point, before which the enzymatic activities in the inferior rice varieties with high protein content were higher than those in the superior rice variety with low protein content, and after which it was converse. The activity of glutamine synthetase in grain was correlated with the taste meter value, peak viscosity and breakdown negatively at the early stage of grain filling whereas positively at the middle and late stages. Moreover, it was correlated with the protein content of rice grain and setback positively at the early stage and negatively at the middle and late stages. The correlation degree varied with the course of grain filling. From 15 days to 20 days after heading was a critical stage, in which the direction of correlation between the activity of glutamine synthetase and taste meter value and RVA properties of rice changed.

Key words: japonica rice; grain filling; glutamine synthetase; enzyme activity; nitrogen content; protein content; cooking and eating quality

Nitrogen assimilation is an important physiological process for plant growth and development. Inorganic nitrogen could be assimilated by plants into the forms of glutamine and glutamic acid. Glutamine synthetase is a key enzyme for nitrogen assimilation^[1], which regulates nitrogen metabolism. Synthesis and transformation of amino acid could be promoted by improving the activity of glutamine synthetase, thus enhancing nitrogen movement in the metabolism. The activity of glutamine synthetase is positively correlated with the amount of protein nitrogen in single rice grain at significant levels^[2]. In previous studies, some researchers had studied the effect of chemicals on the activity of glutamine synthetase. For example, carboxymethyl chitosan could enhance the activities of glutamine synthetase in rice leaves at the heading stage so as to increase the amounts of total nitrogen and protein nitrogen in rice grain^[3]. Uniconazole could

increase the glutamine synthetase activity and protein content in rice grain at the grain filling stage^[2]. Applying more phosphate or potash fertilizer could improve the activities of glutamine synthetase in leaves and grains, thereby enhanced protein synthesis and metabolism in rice leaves^[4]. More nitrogen application in the whole growth period or at the booting stage could increase the amounts of total nitrogen, protein nitrogen, non-protein nitrogen and the activities of glutamine synthetase in leaves and grains of rice^[5]. Moreover, increasing nitrogen application could also improve the activity of glutamine synthetase in flag leaf of wheat^[6].

It is generally accepted that nitrogenous compounds are carried from vegetative organs to grains in the form of amino acid or acid amide and synthesized into protein in grains. There exists a close relation between rice protein synthesis and nitrogen metabolism, and protein is one of the important factors affecting the cooking and eating properties of rice^[7-9]. Therefore, the variation of glutamine synthetase activity should be connected with cooking and eating qualities of rice.

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However, few reports have been found on the relationship between variation of glutamine synthetase activity in grain and cooking and eating qualities of rice. This study tried to make clear the variation of glutamine synthetase activity in rice grain at the grain filling stage and its relation to cooking and eating qualities of rice to provide the theoretical basis for breeding and cultivating rice varieties with good rice quality and high yield.

MATERIALS AND METHODS

Plant materials and experimental design

A pot experiment was conducted in 2004 at the Agricultural College, Northeast Agricultural University, Harbin, China. Four japonica rice varieties with different cooking and eating qualities, including Shuiludao 1, Toukei 180, Fuzihikari and Dongnong 415 were used. The materials were sown on 6 April and transplanted on 25 May, with five pots (20 cm in diameter and 20 cm in height) for each variety (as a group or replication) and three replications. At the seedlings recovery from transplanting, seedlings were thinned to four plants (with uniform growth) in each pot. For a pot, 1 g $\text{NH}_4\text{H}_2\text{PO}_4$ and 1 g urea were applied before transplanting and 1 g urea was topdressed at the maximum tillering stage.

Twenty panicles that grew synchronously and headed on the same day were labeled in each replication of each variety at the heading stage. Panicle samples were collected since the 10th day after heading at a 5-day interval. At each sampling time, the marked panicles were collected at 9:00-9:30 a.m. and immediately frozen in liquid nitrogen, and then kept in a refrigerator (-20°C). The grains in the middle of panicles were adopted for determination of glutamine synthetase activity. The remaining grains in the panicles were used for determining total nitrogen content.

At harvesting, the grains of each variety in the same replication were collected together, and air-dried for three months. The brown rice was sieved with a sieve of 1.9 mm, and processed into milled rice with an automatic rice polisher at the rate of 90% of milled rice output. The milled rice was ground to flour for quality analysis.

Determination method of nitrogen content, protein content, and rice quality

Total nitrogen and protein contents in rice grains were determined by the semi-micro Kjeldahl method and the conversion coefficient was 5.95. Amylose content was measured according to the standard method promulgated by the Ministry of Agriculture of the People's Republic of China. Taste meter value and RVA properties were measured according to the method described by Shen et al^[10].

Assay of glutamine synthetase activity

The glutamine synthetase activity was determined according to *Experimental Instruction in Plant Physiology* edited by the Academy of Crop Science of Korea Rural Development Administration with some modifications.

Enzyme extraction

Fifteen dehulled rice grain (embryo removed) were weighed for enzyme extraction. The grains were ground with a frozen homogenizer in 5 mL buffer solution pre-cooled in ice (containing 0.05 mol/L HEPES, 0.01 mol/L cysteine, 5% polyvinylpyrrolidone, pH was adjusted to 7.0 with NaOH) and the homogenate was poured into a centrifugal tube. Later, the homogenizer was rinsed with the buffer solution (5 mL) and then poured into the centrifugal tube. The centrifugal tube with all the homogenate was centrifuged at $12\,000 \times g$ for 10 min (4°C). The supernatant was used for the enzyme assay.

Determination of glutamine synthetase activity

Crude enzyme preparation (0.8 mL) was added into 2.2 mL reaction solution containing 0.6 mL buffer, 0.6 mol/L MgSO_4 (0.2 mL), 1.2 mol/L *L*-monosodium glutamate (0.8 mL, pH 7.0), 0.06 mol/L ATP (0.4 mL), 1:1 mixture solution (0.2 mL) of hydroxylamine (0.7 mol/L) and NaOH (1.0 mol/L). The reaction was conducted at 40°C for 30 min, then stopped by adding stop solution (0.8 mL) with a 1:1:1 proportion of 10% FeCl_3 , 24% CCl_3COOH (dissolved in 0.2 mol/L HCl solution), and 50% HCl. Ten minutes later, the OD value was read at 540 nm.

RESULTS

Comparisons of eating and cooking qualities among rice varieties

Protein content is an important factor affecting the eating and cooking qualities of rice, and taste meter value serves as an important and direct index in estimating the eating quality of rice instead of sensory test. Varieties with high taste meter values are commonly considered to have better eating quality than those with low taste meter values. Moreover, RVA property is an important physical index reflecting viscosity properties of rice. As showed in Table 1, protein content and setback of Shuiludao 1 and Dongnong 415 were significantly higher than those of Fujihikari and Toukei 180, and taste meter values, peak viscosities, breakdowns of Shuiludao 1 and Dongnong 415 were significantly lower than those of Fujihikari and Toukei 180.

According to the quality properties above mentioned, the tested varieties could be divided into two groups, i.e. superior quality varieties, Fujihikari and Toukei 180 and inferior ones, Shuiludao 1 and Dongnong 415, which coincided with sensory test results.

Variations of glutamine synthetase activity and total nitrogen content in rice grain

For the four tested varieties, glutamine synthetase activities tended to increase gradually to peak values, thereafter descended (Table 2). Different varieties had various enzyme activities at the same time and peak time when the enzyme activity reached maximum value. For Shuiludao 1 and Dongnong 415, the activity of glutamine synthetase peaked at 15 d after heading (DAH), which was earlier than that of Toukei 180 and Fujihikari (at 20 DAH). In term of enzyme activity, glutamine synthetase activities in Shuiludao 1 and Dongnong 415 were higher than those in Fujihikari and Toukei 180 before the 15th day after heading and glutamine synthetase activity in Shuiludao 1 was remarkably higher than that in the other three varieties. However, the glutamine synthetase activity in Toukei 180 was obviously higher than that in the other three varieties after 15 DAH. This illustrated that the varieties with different rice qualities had differences in the time and degree of nitrogen metabolism.

As shown in Table 3, the variations of total nitrogen contents in grains of the four tested varieties showed a similar tendency at the filling stage, i.e. total nitrogen content increased rapidly at the early filling stage and then did slightly after 25 DAH. During the

Table 1. Protein contents, amylose contents, taste meter values and RVA properties of different varieties.

Variety	Protein content (%)	Taste meter value	Peak viscosity (RVU)	Breakdown (RVU)	Setback (RVU)
Shuiludao 1	7.26 bAB	55.0 cC	309.1 cC	103.9 cC	32.6 aA
Toukei 180	6.80 cCB	74.3 aA	382.2 aA	215.2 aA	-133.2 dD
Fujihikari	6.75 cC	73.3 aA	325.9 bB	133.5 bB	-5.7 cC
Dongnong 415	7.86 aA	61.3 bB	288.5 dD	102.7 cC	24.3 bA

Within a column, values followed by the common uppercase and lowercase letters mean no significant difference at 1% and 5% levels, respectively.

Table 2. Multiple comparison of glutamine synthetase activities in rice grains at the filling stage.

Variety	Glutamine synthetase activity (OD/grain · h)					
	10 DAH	15 DAH	20 DAH	25 DAH	30 DAH	35 DAH
Shuiludao 1	0.0271 aA	0.0301 aA	0.0243 aA	0.0184 bBC	0.0143 bcBCD	0.0115 bcB
Toukei 180	0.0156 cC	0.0211 cCD	0.0255 aA	0.0225 aA	0.0187 aA	0.0151 aA
Fujihikari	0.0184 bBC	0.0224 bB	0.0252 aA	0.0202 bAB	0.0160 bAB	0.0126 bAB
Dongnong 415	0.0202 bB	0.0241 bB	0.0199 bB	0.0160 cCD	0.0128 cD	0.0102 cB

Within a column, values followed by the common uppercase and lowercase letters mean no significant difference at 1% and 5% levels, respectively.

DAH, Days after heading.

Table 3. Multiple comparison of nitrogen contents in rice grains at the filling stage.

Variety	Nitrogen content in rice grain (mg/grain)					
	10 DAH	15 DAH	20 DAH	25 DAH	30 DAH	35 DAH
Shuiludao 1	24.87 aA	33.69 aA	37.67 aA	41.10 aA	42.36 aA	42.22 aA
Toukei 180	17.03 cC	25.90 cC	33.45 bBC	36.49 bB	37.03 cB	36.49 cB
Fujihikari	20.57 bB	29.16 bBC	35.06 bAC	37.57 bB	38.28 bcB	38.10 bcB
Dongnong 415	22.19 bAB	32.20 aAB	35.86 abAC	38.42 bAB	39.71 bAB	39.71 bAB

Within a column, values followed by the common uppercase and lowercase letters mean no significant difference at 1% and 5% levels, respectively.

DAH, Days after heading.

whole filling period, total nitrogen contents in Shuiludao 1 and Dongnong 415 were higher than those in Toukei 180 and Fujihikari. The results indicated that the rate and amount of total nitrogen accumulation in the varieties with high protein content were always higher than those in varieties with low protein content.

Relationship between glutamine synthetase activity and cooking and eating qualities in rice

The simple correlation coefficients between glutamine synthetase activity and cooking and eating properties were calculated according to the data from the four tested varieties. As shown in Table 4, glutamine synthetase activity in rice grain was negatively correlated with taste meter value, peak viscosity, breakdown, and positively correlated with protein content and setback at the early filling stage (before 15 DAH), but the results were reversed at the middle and late filling stages. The correlation between the enzyme activity and cooking and eating properties changed with the course of grain filling. For example, the correlation of the enzyme activity with peak viscosity varied from not significant to significant at

5% or 1% levels. This suggested that glutamine synthetase activity had a certain relation with cooking and eating qualities, and high activity of glutamine synthetase at the early filling stage was disadvantageous to improvement for cooking and eating qualities of rice. The 15-20 days after heading were a critical period, in which the correlations between glutamine synthetase activity and taste meter value or RVA profile characteristics changed in the direction of the correlation.

DISCUSSION

Rice quality is closely related to carbon, nitrogen and fat metabolisms of the plants, which are affected by the synthesis and transportation of photosynthetic products, variations of grain filling and key enzyme activity^[11]. Glutamine synthetase is a multi-functional enzyme in the center of nitrogen metabolism, and rice typically displays the preference to ammonium. These suggest glutamine synthetase should play a key role in rice nitrogen nutrition and rice grain yield. Sun et al^[12] reported that glutamine synthetase activity in the function leaves of rice at the maximum tillering stage

Table 4. Coefficients of correlation between glutamine synthetase activity and protein content, taste meter value, RVA properties at different grain filling stages.

Days after heading	Protein content	Taste meter value	Peak viscosity	Breakdown	Setback
10	0.4061	-0.9201	-0.6365	-0.7511	0.7614
15	0.3885	-0.9219	-0.5656	-0.6817	0.6894
20	-0.9610*	0.5047	0.7375	0.5899	-0.5237
25	-0.9204	0.7536	0.9598*	0.8943	-0.8546
30	-0.8616	0.7864	0.9870*	0.9493*	-0.9201
35	-0.8316	0.7512	0.9954**	0.9600*	-0.9347

*, **Significant at 5% and 1% levels, respectively.

was significantly and negatively correlated with total grain number and filled grain number and weight per panicle. At the booting stage, it was significantly and negatively correlated with 1000-grain weight, and significantly and positively correlated with the number and weight of filled grains per panicle. At the heading stage, the activity was significantly and positively correlated with total grain number per panicle. Zhu et al.^[13] noted that glutamine synthetase activities in function leaves of rice varieties with high protein content were higher than those of the varieties with low protein content. Tang et al.^[14] found that a rice variety Chaofengzao 1 with high yield and protein content showed high contents of total nitrogen, protein nitrogen and non-protein nitrogen and activity of glutamine synthetase in leaves and grains at the late ripening stage, resulting in improvement in protein content and grain yield. Our results showed that the variation of glutamine synthetase activity in rice grain during grain filling was a single-peak curve, that is, glutamine synthetase activity gradually increased to a peak then gradually descended. This was consistent with the result of Yang et al.^[2]. Variations of glutamine synthetase activity and total nitrogen content in grains were out of phase during grain filling. At the early filling stage glutamine synthetase activities in the rice varieties with high protein content were higher than those in the varieties with low protein content, and it was reverse entirely at the middle and late filling stages. Total nitrogen contents in the rice grains of the varieties with high protein content were higher than those of the varieties with low protein content in the whole grain filling period. This suggested that rice varieties with different protein contents might differ in nitrogen metabolism in grains at the grain filling stage, which need to be further studied.

In our study, the simple correlation analysis showed that at the early filling stage glutamine synthetase activity in grain was negatively correlated with taste meter value, peak viscosity and breakdown, and at the middle and late filling stages they were positively correlated. Moreover, the glutamine synthetase activity at the early filling stage was positively correlated with protein content and setback, whereas at the middle and late filling stages they were negatively correlated. Though there were no more

tested varieties in this study and some correlations did not reach the significant level, correlation coefficients were high and some coefficients of correlation reached the significant level. Tang et al.^[5, 15] also reported that more nitrogen fertilizer and low plant density could increase glutamine synthetase activity in leaves and grains, and grain protein content. These results suggested that glutamine synthetase activity had a certain relation to cooking and eating qualities of rice. Since protein content has a close relation to rice cooking and eating qualities, so glutamine synthetase might affect cooking and eating qualities by regulating protein synthesis metabolism. In view of an important regulatory role of glutamine synthetase on rice yield and quality, to study the regulation mechanisms is very significant theoretically and practically for rice cultivation and breeding.

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