Activities of Enzymes for Sucrose-Starch Conversion in Developing Endosperm of Rice and Their Association with Grain Filling in Extra-Heavy Panicle Types

Tsuneo Kato¹, Dai Shinmura² and Ayano Taniguchi¹

¹School of Biology-Oriented Science and Technology, Kinki University, Kinokawa, Wakayama 649-6493, Japan; ²School of Bioresources, Hiroshima Prefectural University, Shobara, Hiroshima 727-0023, Japan

Abstract: Rice cultivars with numerous spikelets per panicle (extra-heavy panicle types) frequently fail to exhibit their high yield potential due to low grain filling. Existing genetic variation in grain filling, however, opens possibilities for genetic improvement for this trait. We studied the correlation between grain filling and the activities of enzymes for sucrose-starch conversion in developing endosperm. The activity of sucrose synthase (EC 2.4.1.13, SuSy) and ADP-glucose pyrophosphorylase (EC 2.7.7.27, AGPase), were measured in three extra-heavy panicle types and a standard cultivar grown at two locations under different environmental conditions. The proportions of grains with definite specific gravities and the rate of grain filling were adopted as the parameters related to grain filling. AGPase activity, but not that of SuSy, was consistently correlated to high proportions of high-density grains (specific gravity > 1.20) and high rates of grain growth in spikelets, particularly in those on secondary branches in which low grain filling is the rule. Such correlation was also detected in spikelets on primary branches which generally show better grain filling, but only early stages. Therefore, a high activity of AGPase might contribute to the reduction of the sucrose concentration by accelerating sucrose metabolism at the developing seed, a sink terminus of the phloem. Thus the sink-directed phloem transport of sucrose would be promoted, resulting in improved grain filling of extra-heavy panicle types. SuSy would play some roles in such a cultivar difference in grain filling, but depending on environments.

Key words: ADP-glucose pyrophosphorylase, Developing endosperm, Extra-heavy panicle type, Grain filling, Inferior spikelet, Rice, Sink activity, Sucrose synthase.

In rice and other cereal crops, grain yield can be defined as the product of yield sink capacity and filling efficiency (Kato and Takeda, 1996). Breeding efforts have expanded yield sink capacity, the maximum size of sink organs to be harvested, mainly by increasing the number of spikelets per panicle. As a result, cultivars with numerous spikelets in each panicle and extra-heavy panicle types, such as hybrid rice and the New Plant Type of the International Rice Research Institute (Khush, 1996; Peng et al., 1999), have become available. The extra-heavy panicle types, however, do not always exhibit their high yield potential, since their degree of grain filling generally is low (Peng et al., 1999; Liang et al., 2001; Yang et al., 2002). The genetic improvement of grain filling, therefore, should be a key target to achieve consistently high yield in extra-heavy panicle types (Peng et al., 1999). Significant genetic variation in the degree of grain filling, on the other hand, exists among extra-heavy panicle types of rice (Yamamoto et al., 1990; Kato and Tamaki, 1999; Peng et al., 1999), which is an indispensable resource for the genetic improvement of grain filling. The basis of the genetic variation in grain filling, however, remained unclear.

Grain filling depends on sucrose transport from source leaves to the endosperm. Sustained sucrose transport requires a steep gradient of turgor between sources and sinks which ultimately is maintained by differential sucrose concentrations between sources and sinks. Generally, sieve tube contents will move towards the sinks which reduce turgor most efficiently through sucrose unloading (Sturm and Tang, 1999; Patrick and Offer, 2001; Benacch and Soltani, 2002). Therefore, variability in the capacity to reduce the sucrose concentration in sieve tubes close to developing endosperm appears a plausible cause for the variation in the rate of grain-filling process, and consequently in the degree of grain filling observed in extra-heavy panicle types of rice.

Several types of sucrose transporters in cell membranes of phloem cells are responsible for unloading sucrose into the apoplast of developing
rice endosperm (Matsukura et al., 2000; Furbank et al., 2001). The sucrose unloaded is removed through sucrose-starch conversion, maintaining the sucrose concentration low (Sturm and Tang, 1999). Sucrose is converted successively to UDPglucose, glucose-1-phosphate, ADPglucose, and finally to starch, by sucrose synthase (EC 2.4.1.13, SuSy), UDPglucose pyrophosphorylase (EC 2.7.7.9), ADPglucose pyrophosphorylase (EC 2.7.7.27, AGPase), and several enzymes related to starch synthesis, respectively (Perez et al., 1975; Nakamura et al., 1989; Emes et al., 2003).

The objective of this study was to elucidate the relation between these enzymes and the efficiency of grain filling, particularly in extra-heavy panicle types showing different degrees of grain filling. Two enzyme activities for sucrose-starch conversion in developing rice endosperm, SuSy and AGPase, were examined. As a final result of grain filling, the proportions of grains with a specific gravity higher than 1.06 (filled grains) was measured as a general parameter of the final result of grain filling. The proportion of grains with a specific gravity higher than 1.20 (high-density grains) was also measured, since this parameter would indicate critically a high efficiency of grain filling (Venkateswarlu et al., 1988). In addition, the rate of the grain-filling process after anthesis was evaluated as another parameter of the efficiency of grain filling, because it should be related directly to the transport of photosynthates. The position of the spikelets examined in a panicle was also taken into account, as the efficiency of grain filling is strongly dependent on the spikelet position in a panicle (Mohapatra et al., 1993; Ishimaru et al., 2003).

Materials and Methods

1. Plant materials and cultivation

Plants were grown on paddy fields at the School of Bioresources, Hiroshima Prefectural University, Shobara, Hiroshima, Japan (34°40’N, 132°58’E, 295 m above sea level) in 2001, and at the School of Biology-Oriented Science and Technology, Kinku University, Kinokawa, Wakayama, Japan (34°17’N, 135°20’E, 97 m above sea level) in 2003. Table 1 shows the average daily temperature and monthly sunshine hours during June, July, August and September, the growing period after transplanting. These meteorological data were cited from the web site of the Japan Meteorological Agency (http://www.data.jma.go.jp/obd/stats/etrn/index.php) accessed date, 30 April 2006, verified date, 18 March 2007.

At both locations, three rice (Oryza sativa L.) cultivars of the extra-heavy panicle type (Akenohoshi, Takanari and Nanjing 11) and one standard cultivar (Koshihikari) were grown. Akenohoshi is a japonica-type cultivar developed from an indica-japonica cross in Japan in 1985, and tends to show low grain filling (Kato, 2004). Nanjing 11 is an indica cultivar developed in China in 1967, with relatively high grain filling. Takanari is an indica-type cultivar developed by crossing Miyang 42 and Miyang 25 in Japan in 1990; it shows a level of grain filling intermediate between Akenohoshi and Nanjing 11. Koshihikari is a typical japonica cultivar and one of the most widely cultivated in Japan.

Seeds of the four cultivars were sown in nursery boxes on 4 May and 9 May in 2001 and 2003, respectively. Juvenile plants were transplanted into paddy fields on 7 June and 10 June in 2001 and 2003, respectively. Cultivars were arranged according to a completely randomized design with two replications. Each plot per cultivar/replication consisted of five rows and 40 hills (2001) or 25 hills (2003) per row; there were three plants per hill. Distances between rows and hills were 30 cm and 15 cm, respectively. At the time of transplanting, nitrogen, phosphorous and potassium were applied at 5 : 8 : 8 g m\(^{-2}\) and 6 : 6 : 6 g m\(^{-2}\) in 2001 and 2003, respectively. The same elements were applied again at 2 : 0 : 0 g m\(^{-2}\), 3 : 2 : 2 g m\(^{-2}\) and 2 : 0 : 0 g m\(^{-2}\) on 27 June, 16 July and 25 July, respectively, in 2001, and at the rates of 3 : 0 : 0 g m\(^{-2}\) and 3 : 4 : 4 g m\(^{-2}\) on 27 June and 22 July, respectively, in 2003. Standard cultivation practices were applied for irrigation, pest control, etc.

2. Grain-filling process

Heading dates of tagged individual panicles at the second and fourth rows of each plot were recorded. Five panicles were collected from each plot every fifth day starting five days after heading (DAH) until 50 DAH, and were dried at 80°C overnight. Spikelets were sorted into two groups according to position on a panicle, namely spikelets on primary branches (PB spikelets) and those on secondary branches (SB spikelets). After another drying period of two days at 70°C, fertilized unhulled grains were weighed. Rates of grain filling were estimated from these data using a two-line regression model (Kato, 1989).

3. Enzyme extraction and assay

In 2001 and 2003, several panicles were collected from one replication of each cultivar at 10, 15 and 20 DAH. Panicles were kept on ice and were immediately transferred into the laboratory, where spikelets were classified into those on PB and SB, frozen in liquid nitrogen and stored at −80°C until enzyme extraction. About 0.2 g endosperm with pericarp were isolated from frozen spikelets, divided equally into four 1.5 mL tubes, and ground thoroughly with 0.8 mL extraction buffer each (50 mM HEPES/NaOH pH 7.5, 10 mM MgCl\(_2\), 2 mM EDTA, 50 mM 2-mercaptoethanol, 12.5% glycerol, and 5% PVP-40). After centrifugation at 18,800×g for 20 min, the supernatant was dialyzed against 1mM HEPES/NaOH (pH 7.5) overnight using a dialyzing cellulose tube (Cellu-Sep T2, MWCO...
Dialyzed samples were stored at −80°C until activity assay. Most steps were performed at 4°C or on ice. The extraction was repeated three times for each cultivar and sampling time.

**SuSy activity** was measured by assessing sucrose cleavage using the methods of Sung et al. (1989) and Kato (1995) with slight modifications: 0.8 U of UDPglucose pyrophosphorylase (U8501, Sigma Chemical Co., MO, USA) was included in the enzyme reaction mixture as an additional coupling enzyme. A final concentration of 125 mM sucrose was added as a substrate. AGPase-mediated synthesis of glucose-1-phosphate from ADPglucose was measured using the method of Nakamura and Yuki (1992). ADPglucose was added at 1.25 mM final concentration. In both the SuSy and AGPase assays, the glucose-1-phosphate produced was converted to 6-phosphogluconolactone via glucose-6-phosphate by phosphoglucomutase (P3397, Sigma Chemical Co., MO, USA) and glucose-6-phosphate dehydrogenase (G5885, Sigma Chemical Co., MO, USA). All enzymatic reactions were performed in a cuvette at 30°C. NADPH production in the last step was monitored as absorbance at 340 nm with a spectrophotometer (UV-1700, Shimazu Co., Kyoto, Japan). Protein concentration in each enzyme sample was determined using the Protein Assay Kit (Bio-Rad Laboratories, Inc., CA, USA) with BSA as a standard. Enzyme activity was measured once for every sample and expressed as mU (mg protein)^{-1}. One enzyme unit (U) is defined as the change of substrate quantity per min (μmol min^{-1}) in the enzymatic reaction at 30°C.

**4. Grain filling and other agronomic characters**

Heading dates were noted in all plots for the plants in the third row which had not been sampled for monitoring the grain-filling process, and the length of the longest culm, length of the panicle on the longest culm, and the number of panicles per hill were determined for each hill at maturity. Six panicles were sampled from each hill and dried at room temperature. Five of the six panicles were threshed by hand, and spikelets were classified into PB spikelets and SB spikelets. The proportions of spikelets showing specific gravities of >1.06 and >1.20 were determined using NaCl solutions of these specific gravities to obtain the proportions of filled grain and high-density grain, respectively. Finally, the number of spikelets was counted in the sixth panicle.

**Results**

**1. Meteorological conditions and agronomic characters**

Table 1 shows that the plants cultivated in 6000-8000, Membrane Filtration Products, Inc., TX, USA). Dialyzed samples were stored at −80°C until activity assay. Most steps were performed at 4°C or on ice. The extraction was repeated three times for each cultivar and sampling time.

**SuSy activity** was measured by assessing sucrose cleavage using the methods of Sung et al. (1989) and Kato (1995) with slight modifications: 0.8 U of UDPglucose pyrophosphorylase (U8501, Sigma Chemical Co., MO, USA) was included in the enzyme reaction mixture as an additional coupling enzyme. A final concentration of 125 mM sucrose was added as a substrate. AGPase-mediated synthesis of glucose-1-phosphate from ADPglucose was measured using the method of Nakamura and Yuki (1992). ADPglucose was added at 1.25 mM final concentration. In both the SuSy and AGPase assays, the glucose-1-phosphate produced was converted to 6-phosphogluconolactone via glucose-6-phosphate by phosphoglucomutase (P3397, Sigma Chemical Co., MO, USA) and glucose-6-phosphate dehydrogenase (G5885, Sigma Chemical Co., MO, USA). All enzymatic reactions were performed in a cuvette at 30°C. NADPH production in the last step was monitored as absorbance at 340 nm with a spectrophotometer (UV-1700, Shimazu Co., Kyoto, Japan). Protein concentration in each enzyme sample was determined using the Protein Assay Kit (Bio-Rad Laboratories, Inc., CA, USA) with BSA as a standard. Enzyme activity was measured once for every sample and expressed as mU (mg protein)^{-1}. One enzyme unit (U) is defined as the change of substrate quantity per min (μmol min^{-1}) in the enzymatic reaction at 30°C.

| Meteorological element | Month | Hiroshima 2001 | Wakayama 2003 |
|------------------------|-------|----------------|---------------|
| Average daily temperature (°C) | June | 20.5 | 23.2 |
|                         | July  | 24.7 | 25.4 |
|                         | August | 24.4 | 27.9 |
|                         | September | 19.6 | 25.5 |
| Monthly sunshine hours | June  | 91.0 | 123.3 |
|                         | July  | 153.7 | 144.9 |
|                         | August | 178.0 | 215.7 |
|                         | September | 140.6 | 221.5 |

Table 2. Agronomic characters in the four rice cultivars at the two locations.

| Cultivar | Days to heading | Panicles per hill | Culm length (cm) | Panicle length (cm) | Spikelets per panicle |
|----------|-----------------|-------------------|------------------|---------------------|-----------------------|
|          | Hiroshima 2001 |                   |                  |                     |                       |
| Koshihikari | 97.0^d       | 21.5^d         | 93.5^d          | 20.5^d              | 127.9^d              |
| Akenohoshi  | 109.0^a      | 15.5^a         | 75.7^b         | 19.6^b              | 167.4^d              |
| Takanari   | 106.5^b      | 15.0^b         | 64.6^c         | 23.2^b              | 192.3^c              |
| Nanjing 11 | 99.0^d       | 14.1^b         | 72.6^b         | 27.0^b              | 212.3^c              |
|          | Wakayama 2003 |                   |                  |                     |                       |
| Koshihikari | 93.0^a       | 14.6^b         | 80.4^a         | 20.3^b              | 105.9^b              |
| Akenohoshi  | 105.5^c      | 10.7^b         | 71.4^b         | 22.4^b              | 219.3^c              |
| Takanari   | 104.5^b      | 13.7^a         | 66.9^b         | 21.4^a              | 179.9^c              |
| Nanjing 11 | 94.5^b       | 12.8^a         | 60.7^b         | 26.4^a              | 181.1^c              |

Means with identical superscript letter within a column in the same location are not significantly different (df=4, P<0.05) according to F-protected Fisher’s LSD. Absence of superscript letters indicates that no significant differences were detected among cultivars in the initial ANOVA.
Wakayama, 2003, were exposed to higher temperature and longer periods of sunshine than those in Hiroshima, 2001, during their growth period after transplanting, particularly in September. These differences between the two locations might cause the difference in the expression of some agronomic traits (Table 2). The patterns of cultivar-specific agronomic traits, on the other hand, were mostly similar at both locations. Though Akenohoshi showed slightly fewer spikelets per panicle in Hiroshima, 2001, the three extra-heavy panicle types all developed significantly more spikelets per panicle than Koshihikari at both locations. Only Koshihikari in Hiroshima, 2001 showed a slight lodging (data not shown). The number of days to heading varied by 12 between the cultivars at a given location (Table 2). The cumulative temperatures from heading were higher in the early heading two cultivars, Koshihikari and Nanjing 11 in Hiroshima, 2001 (Fig. 1). However, the reverse results for the cultivar difference were obtained in Wakayama, 2003.

Table 3. Proportions of filled grain and high-density grain, and the rate of grain filling of the four rice cultivars at the two locations.

| Cultivar   | Proportion of filled grain (%) | Proportion of high-density grain (%) | Rate of grain filling (mg day\(^{-1}\) grain\(^{-1}\)) |
|------------|-------------------------------|-------------------------------------|-----------------------------------------------|
|            | PB\(^1\) spikelet | SB\(^1\) spikelet | PB spikelet | SB spikelet | PB spikelet | SB spikelet |
|------------|-----------------|-----------------|------------|-------------|------------|-------------|
| Hiroshima 2001 |                 |                 |            |              |            |              |
| Koshihikari | 57.4\(^b\)     | 36.7\(^c\)     | 0.0\(^b\)  | 0.0\(^c\)   | 0.90\(^c\) | 0.68\(^b\)   |
| Akenohoshi | 82.4\(^a\)     | 59.4\(^b\)     | 0.0\(^c\)  | 0.0\(^b\)   | 0.70\(^b\) | 0.55\(^a\)   |
| Takanari   | 66.8\(^ab\)    | 48.3\(^b\)     | 2.3\(^b\)  | 1.2\(^b\)   | 0.67\(^b\) | 0.49\(^b\)   |
| Nanjing 11 | 81.4\(^a\)     | 60.5\(^a\)     | 17.7\(^a\) | 9.3\(^a\)   | 1.03\(^a\) | 0.72\(^b\)   |
| Wakayama 2003 |                 |                 |            |              |            |              |
| Koshihikari | 64.4            | 42.2            | 0.0\(^b\)  | 0.0\(^b\)   | 1.11\(^c\) | 0.86\(^b\)   |
| Akenohoshi | 73.8            | 57.3            | 0.0\(^b\)  | 0.0\(^b\)   | 1.33\(^b\) | 0.89\(^a\)   |
| Takanari   | 78.1            | 52.4            | 18.0\(^b\) | 8.8\(^b\)   | 1.22\(^b\) | 1.05\(^a\)   |
| Nanjing 11 | 81.5            | 54.0            | 15.8\(^b\) | 6.9\(^b\)   | 1.60\(^c\) | 1.41\(^a\)   |

Means with identical superscript letter within a column in the same location are not significantly different (df=4, P<0.05) according to F-protected Fisher’s LSD. Absence of superscript letters indicates that no significant differences were detected among cultivars in the initial ANOVA.

\(^1\) PB, primary branch; SB, secondary branch.
2. Grain filling and grain-filling process

In Hiroshima, 2001, significant differences between cultivars in the proportion of filled grain were detected in both groups (PB and SB) of spikelets, but not in Wakayama, 2003. The lower proportion in Koshihikari might partly be due to its slight lodging as mentioned above. In contrast, the proportions of high-density grain and the rates of grain filling consistently showed significant differences between cultivars for both spikelet groups at both Hiroshima and Wakayama. Nanjing 11 and Takanari produced many high-density grains at both spikelet positions, but Akenohoshi failed to do so (Table 3). Generally, Nanjing 11 showed higher rates of grain filling than the other cultivars. Particularly in the experiments of 2003, these differences tended to be significant (Table 3).

3. Enzyme activity

The activity of SuSy showed significant differences among cultivars at most stages in Hiroshima, 2001 (Fig. 2A and B). In particular, Nanjing 11 and Takanari showed significantly higher SuSy activity in both spikelet positions at 10 DAH than Akenohoshi. In Wakayama, 2003, a few significant differences were detected between the four cultivars, but the differences in enzyme activity could hardly be associated with the differences in grain filling (Fig. 2C and D). The low SuSy activities in SB spikelets of Akenohoshi and Koshihikari observed at 10 DAH in Hiroshima, 2001, were not supported by the data of the experiment performed in Wakayama, 2003. The activity of SuSy gradually declined with the progress of grain filling in most of the cultivars in Wakayama, 2003, while no such a common trend became evident in Hiroshima, 2001.

In contrast, the activity of AGPase followed basically similar patterns at both locations and in all four cultivars: it gradually declined after heading in PB spikelets, but showed a peak at 15 DAH in SB spikelets (Fig. 3). Notably, SB spikelets of Nanjing 11 showed the highest activity of all cultivars at most stages in both experiments (Fig. 3B and D), and these differences were significant at 15 and 20 DAH in Hiroshima, 2001.
Differences also were significant between Nanjing 11 and Akenohoshi at 10 and 15 DAH in Wakayama, 2003, but not between Nanjing 11 and Takanari (Fig. 3D). In PB spikelets, Nanjing 11 also showed significantly higher AGPase activity than the other cultivars at 10 DAH in Hiroshima, 2001 (Fig. 3A), but not in Wakayama, 2003 (Fig. 3C).

To demonstrate clearly the associations between those enzyme activities and the parameters of grain filling, correlation coefficients were calculated in each spikelet position, each sampling time and each location (Table 4). Though most of the coefficients were not significantly different from zero due to the low degrees of freedom, AGPase activity was closely and positively correlated with the proportion of high-density grain and the rate of grain filling for SB spikelets in all sampling times and at both locations. The AGPase activity for PB spikelets also showed high and positive correlations with the above two parameters of grain filling at both locations, but only at early sampling stages. In contrast to the case of AGPase, SuSy activity showed high correlations in several cases at only one of the two locations (Table 4). Several high correlation coefficients for SuSy activity were negative.

**Discussion**

In experiments at both locations, AGPase activity in inferior spikelets (SB spikelets) tended to be higher in Nanjing 11 than in other cultivars at most developmental stages of endosperm (Fig. 3). This cultivar also produced in most cases higher AGPase activity than the other cultivars at 10 DAH in Hiroshima, 2001 (Fig. 3A), but not in Wakayama, 2003 (Fig. 3C).
in early developmental stages (Table 4), although no significant difference among cultivars for AGPase activity was detected in Wakayama, 2003 (Fig. 3C). On the contrary, SuSy activity was correlated in a few cases to the parameters of grain filling, whereas not consistently between the two locations tested (Fig. 2 and Table 4).

Nanjing 11 headed earlier to heading than Akenohoshi at both locations (Table 2). In Hiroshima, 2001, Nanjing 11 was grown certainly under higher temperature conditions during grain filling period than Akenohoshi, whereas in Wakayama, 2003, the former was exposed lower temperature during the same period than the latter (Fig. 1). This result suggests that the difference in temperature conditions would not completely explain the differences in AGPase activity and other grain filling parameters between Nanjing 11 and Akenohoshi. Cheng et al. (2005) examined the activities of several enzymes in developing rice endosperm of two cultivars with different amylose contents under different temperature conditions. They revealed in both cultivars that under a high temperature SuSy activity was higher in early developmental stages, whereas the activity became lower in middle to later stages. AGPase activity showed similar responses to temperature, but not prominent compared with SuSy activity. The variation in temperature condition might partly cause the inconsistency of the cultivar difference in SuSy activity (Table 4). However, AGPase obviously showed the consistent results in spite of the temperature variation. These results suggest that variation in grain filling between the three extra-heavy panicle cultivars was attributable to varying levels of the activity of AGPase in inferior spikelets more consistently than SuSy.

Several studies have been made on the association between enzyme activities in sink organs and yield components. Liang et al. (2001) examined several factors including enzyme activities to explain why grain filling rates are lowered in SB spikelets of indica-japonica F1 hybrids, as compared with ordinary japonica cultivars. They concluded that the activities of both AGPase and SuSy, and also the number of endosperm cells, determined the lower sink strength of one indica-japonica F1 hybrid cultivar. Couce and Gravois (2006) measured the activity of SuSy in developing endosperm of several rice cultivars and breeding lines. Though they did not examine AGPase activity, they demonstrated that the activity of SuSy in developing endosperm might be a useful indicator for the selection for higher grain yield. Ishimaru

### Table 4. Correlation coefficients between sucrose synthase (SuSy) or ADPglucose pyrophosphorylase (AGPase) activity and the parameters of grain filling for the four cultivars at the two locations.

| Enzyme | Spikelet | DAH | Hiroshima 2001 | Wakayama 2003 | Hiroshima 2001 | Wakayama 2003 | Hiroshima 2001 | Wakayama 2003 |
|--------|----------|-----|----------------|--------------|----------------|--------------|----------------|--------------|
|        |          |     | Proportion of filled grain | Proportion of high-density grain | Rate of grain filling | Proportion of filled grain | Proportion of high-density grain | Rate of grain filling |
|        |          |     | 10  | 15  | 20  | 10  | 15  | 20  | 10  | 15  | 20  |
| SuSy   | PB       |     | 0.521 | 0.156 | 0.325 | 0.122 | 0.320 | 0.065 | 0.333 | 0.105 | 0.395 | 0.290 | 0.142 | 0.452 | 0.217 |
|        | SB       |     | 0.909 | 0.147 | 0.664 | 0.113 | 0.864 | 0.679 | 0.265 | 0.125 | 0.247 | 0.590 | 0.655 | 0.351 | 0.449 |
| AGPase | PB       |     | 0.362 | 0.498 | 0.613 | 0.115 | 0.879 | 0.486 | 0.669 | 0.412 | 0.417 | 0.781 | 0.821 | 0.886 | 0.714** |
|        | SB       |     | 0.626 | 0.505 | 0.717 | 0.015 | 0.704 | 0.850 | 0.638 | 0.031 | 0.098 | 0.840 | 0.841 | 0.994* | 0.633* |

*, ** Significant correlation coefficients at the 0.05 and 0.01 probability levels, respectively (df=2 for 10, 15 and 20 DAH, and df=10 for all sampling times).

1PB, primary branch; SB, secondary branch.

2“all” means all sampling times examined.
et al. (2005) compared the expression of genes encoding carbohydrate-metabolizing enzymes between developing superior and inferior spikelets of rice. They demonstrated that the inferior spikelets showed weak and/or delayed expression of the genes for enzymes including AGPase and SuSy. In developing pods of mung bean (Vigna radiata L.), Chopra et al. (2005) demonstrated that SuSy, AGPase, and also UDPglucose pyrophosphorylase appeared to control the conversion of sucrose to ADPglucose and to regulate the sink strength. In several extra-heavy panicle types of rice, the present study revealed that AGPase, and partly SuSy depending on environment, could play an enhancing role with respect to grain filling.

AGPase is considered to catalyze a rate-limiting step in starch synthesis in several crops, and is an allosteric enzyme; its activity is increased by 3-phoshoglyceral acid and inhibited by inorganic phosphate. Smidansky et al. (2003) described a transgenic rice line in which seed yield and biomass were increased through transformation with a gene encoding a modified maize AGPase that was insensitive to inorganic phosphate. Similar results were obtained from analogous experiments in potato (Sweetlove et al., 1996), wheat (Smidansky et al., 2002; Meyer et al., 2004, 2007), rice (Sakulsingharoj et al., 2004) and maize (Wang et al., 2007). Sweetlove et al. (1996), on the other hand, reported that the transformed modified AGPase gene did not affect the starch content of tubers in spite of the increase in AGPase activity. The present study emphasized that natural genetic variation, not a genetically modified form, in AGPase activity might contribute to the improvement of grain filling in extra-heavy panicle rice cultivars. More detailed studies using genetically segregating populations are needed to clarify the nature of the correlation of AGPase activity, and also of SuSy activity, with grain filling in rice extra-heavy panicle types, as well as the relationships between sink and source strengths.

Acknowledgements

We sincerely thank A. Horibata and Y. Ohnishi, School of Biology-Oriented Science and Technology, Kinki University, and Dr. K. Irifune, School of Bioresources, Hiroshima Prefectural University, for their valuable help.

References

Bencall, P. and Soltani, F. 2002. Source-sink partitioning. Do we need Münch? J. Exp. Bot. 53 : 1919-1928.
Cheng, F., Zhong, L., Zhao, N., Liu, Y. and Zhang, G. 2005. Temperature induced changes in the starch components and biosynthetic enzymes of two rice varieties. Plant Growth Regul. 46 : 87-95.
Chopra, J., Kaur, N. and Gupta, A.K. 2005. Role of enzymes of sucrose-starch conversion in seed sink strength in mung bean. Biol. Plant. 49 : 561-565.
Counce, P.A. and Gravois, K.A. 2006. Sucrose synthase activity as a potential indicator of high rice grain yield. Crop Sci. 46 : 1501-1507.
Emes, M.J., Bowsher, C.G., Hedley, C., Burrell, M.M., Scrase-Field, E.S.F. and Tetlow, I.J. 2003. Starch synthesis and carbon partitioning in developing endosperm. J. Exp. Bot. 54 : 59-575.
Furbank, R.T., Scofield, G.N., Hirose, T., Wang, X.D., Patrick, J.W. and Offler, C.E. 2001. Cellular localization and function of a sucrose transporter OsSUT1 in developing rice grains. Aust. J. Plant Physiol. 28 : 1187-1196.
Ishimaru, T., Matsuda, T., Ohsugi, R. and Yamagishi, T. 2003. Morphological development of rice caryopses located at the different positions in a panicle from early to middle stage of grain filling. Funct. Plant Biol. 30 : 1139-1149.
Ishimaru, T., Hirose, T., Matsuda, T., Goto, A., Takahashi, K., Sasaki, H., Terao, T., Ishii, R., Ohsugi, R. and Yamagishi, T. 2005. Expression patterns of genes encoding carbohydrate-metabolizing enzymes and their relationship to grain filling in rice (Oryza sativa L.) : Comparison of caryopses located at different positions in a panicle. Plant Cell Physiol. 46 : 620-628.
Kato, T. 1989. Relationship between grain-filling process and sink capacity in rice (Oryza sativa L.). Jpn. J. Breed. 39 : 431-438.
Kato, T. 1995. Change of sucrose synthase activity in developing endosperm of rice cultivars. Crop Sci. 35 : 827-831.
Kato, T. and Takeda, K. 1996. Associations among characters related to yield sink capacity in space-planted rice. Crop Sci. 36 : 1135-1139.
Kato, T. and Tamaki, M. 1999. Grain filling characteristics of rice cultivars with numerous spikelets in a panicle. In T. Horie et al. eds., Proc. Int. Symp. “World Food Security and Crop Production Technologies for Tomorrow”. Graduate School of Agriculture, Kyoto University, Kyoto. 356-357.
Kato, T. 2004. Effect of spikelet removal on the grain filling of Akenohoshi, a rice cultivar with numerous spikelets in a panicle. J. Agric. Sci. 142 : 177-181.
Khush, G.S. 1996. Prospects and approaches to increasing the genetic yield potential of rice. In R.E. Everson et al. eds., “Rice Research in Asia. Progress and Properties”. CAB International, Wallingford. 59-71.
Liang, J., Zhang, J. and Cao, X. 2001. Grain sink strength may be related to the poor grain filling of indica-japonica rice (Oryza sativa L.) hybrids. Physiol. Plant. 112 : 470-477.
Matsukura, C., Saitoh, T., Hirose, T., Ohsugi, R., Perata, P. and Yamaguchi, J. 2000. Sugar uptake and transport in rice embry. Expression of companion cell-specific sucrose transporter (OsSUT1) induced by sugar and light. Plant Physiol. 124 : 85-93.
Meyer, F.D., Smidansky, E.D., Beecher, B., Greene, T.W. and Giroux, M.J. 2004. The maize Sh2r6hs ADP-glucose pyrophosphorylase (AGP) large subunit confers enhanced AGP properties in transgenic wheat (Triticum aestivum). Plant Sci. 167 : 899-911.
Meyer, F.D., Talbert, L.E., Martin, J.M., Lanning, S.P., Greene, T.W. and Giroux, M.J. 2007. Field evaluation of transgenic wheat expressing a modified ADP-glucose pyrophosphorylase large subunit. Crop Sci. 47 : 336-342.
Mohapatra, P.K., Patel, R. and Sahu, S.K. 1993. Time of
flowering affects grain quality and spikelet partitioning within the rice panicle. Aust. J. Plant Physiol. 20 : 231-241.
Nakamura, Y., Yuki, K., Park, S.Y. and Ohta, T. 1989. Carbohydrate metabolism in the developing endosperm of rice grains. Plant Cell Physiol. 30 : 833-839.
Nakamura, Y. and Yuki, K. 1992. Changes in enzyme activities associated with carbohydrate metabolism during the development of rice endosperm. Plant Sci. 82 : 15-20.
Patrick, J.W. and Offler, C.E. 2001. Compartmentation of transport and transfer events in developing seeds. J. Exp. Bot. 52 : 551-564.
Peng, S., Cassman, K.G., Virmani, S.S., Sheehy, J. and Khush, G.S. 1999. Yield potential trends of tropical since the release of IR8 and the challenge of increasing rice yield potential. Crop Sci. 39 : 1552-1559.
Perez, C.M., Perdon, A.A., Resurreccion, A.P., Villareal, R.M. and Juliano, B.O. 1975. Enzymes of carbohydrate metabolism in the developing rice grain. Plant Physiol. 56 : 579-583.
Sakulsingharoj, C., Choi, S.B., Hwang, S.K., Edwards, G.E., Bork, J., Meyer, C.R., Preiss, J. and Okita, T.W. 2004. Engineering starch biosynthesis for increasing rice seed weight : the role of the cytoplasmic ADP-glucose pyrophosphorylase. Plant Sci. 167 : 1323-1333.
Smidansky, E.D., Clancy, M., Meyer, F.D., Lanning, S.P., Blake, N.K. and Talbert, L.E. 2002. Enhanced ADP-glucose pyrophosphorylase activity in wheat endosperm increases seed yield. Proc. Natl. Acad. Sci. U.S.A. 99 : 1724-1729.
Smidansky, E.D., Martin, J.M., Hannah, L.C., Fischer, A.M. and Giroux, M.J. 2003. Seed yield and plant biomass increases in rice are conferred by deregulation of endosperm ADP-glucose pyrophosphorylase. Planta 216 : 656-664.
Sturm, A. and Tang, G.Q. 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. Trends in Plant Sci. 4 : 401-407.
Sung, S.J.S., Xu, D.P. and Black, C.C. 1989. Identification of actively filling sucrose sinks. Plant Physiol. 89 : 1117-1121.
Sweetlove, L.J., Burrell, M.M. and ap Rees, T. 1996. Starch metabolism in tubers of transgenic potato (Solanum tuberosum) with increased ADPglucose pyrophosphorylase. Biochem. J. 320 : 493-498.
Venkateswarlu, B., Vegara, B.S., Parao, F.T. and Visperas, R.M. 1988. Enhancing grain yield potentials in rice by increasing the number of high density grains. Philipp. J. Crop Sci. 11 : 145-152.
Wang, Z., Chen, X., Wang, J., Liu, T., Liu, Y., Zhao, L. and Wang, G. 2007. Increasing maize seed weight by enhancing the cytoplasmic ADP-glucose pyrophosphorylase activity in transgenic maize plants. Plant Cell Tissue Organ Cult. 88 : 83-92.
Yamamoto, Y., Yoshida, T., Enomoto, T. and Yoshikawa, G. 1990. Characteristics for the efficiency of spikelet production and the ripening in high-yielding japonica-indica hybrid and semidwarf indica rice varieties. Jpn. J. Crop Sci. 60 : 365-372*.
Yang, J., Peng, S., Zhang, Z., Wang, Z., Visperas, R.M. and Zhu, Q. 2002. Grain and dry matter yields and partitioning of assimilates in japonica/indica hybrid rice. Crop Sci. 42 : 766-772.

* in Japanese with English summary.