Sucrose-metabolism enzymes in developing rice endosperm: Their relations to grain filling of rice cultivars with extra-heavy panicles

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Abstract

Rice cultivars with numerous spikelets in a panicle (extra-heavy panicles) have been developed aiming at higher yield derived from higher capacity of photoassimilates storage. However, these cultivars did not realized the original object in many cases due to the increase in their number of spikelets on secondary branches (SB), which showed relatively poor grain filling. On the other hand, some cultivars showed some better grain filling even in the spikelet on SB. This study examined the variation in the activities of sucrose synthase and ADPglucose pyrophosphorylase (AGPase) both of which are important for the sucrose-to-starch conversion in developing endosperm and may consequently concern the variation in grain filling, among rice cultivars with extra-heavy panicles. The results indicated lower activities of both enzymes in then poor grain filling cultivar Akenohoshi, particularly in the spikelet on SB in the early stage of development. In contrast, the enzyme activities were higher in the better grain filling cultivar, Nanjing 11. Nucleotide sequence polymorphisms were also searched for 5' regulatory region of AGPase large subunit gene among the same cultivars. There were several nucleotide differences among cultivars. These results suggest that the structural differences in those genes encoding sucrose-metabolism enzymes should cause the change of gene expression and enzyme activity. These changes would, at least partly, be relevant to the variation in grain sink strength, and consequently in grain filling among rice cultivars with extra-heavy panicles.

Media summary

Good grain filling of a rice cultivar with extra-heavy panicles was associated with its higher activities of sucrose-metabolism enzymes in developing endosperm.

Key Words

Filled grain percentage, high-density grain percentage, rate of grain filling, ADPglucose pyrophosphorylase, sucrose synthase, nucleotide sequence polymorphism.

Introduction

Various types of rice (*Oryza sativa* L.) cultivars with numerous spikelets in a panicle (extra-heavy panicles) have been developed aiming at higher grain yield (Peng et al. 1999). In some cases, however, these cultivars did not attain an expected high yield due to the increase in the spikelet on secondary branches (SB) which showed poorer grain filling than the spikelet on primary branches (PB) (Peng et al. 1999). On the other hand, better grain filling was also found even in the "inferior" spikelet on SB depending on cultivars with extra-heavy panicles (Yamamoto et al. 1991). This indicates that the poor grain filling found in the cultivars with extra-heavy panicles could be genetically improved through utilizing available genetic resources. The improvement of grain filling in these cultivars should, thus, be an important issue to realize their huge yield potential.

One plausible cause for the poor grain filling is the lower ability to attract photoassimilates from source organs after anthesis (Yang et al. 2002). Sucrose, which is a translocated form of photoassimilates in cereals, is cleaved into fructose and UDPglucose after the unloading from phloem terminus to sink organs. Then, the latter product is converted to glucose-1-phosphate, ADPglucose, and finally to starch in endosperm cells by several enzymes (Perez et al. 1975; Nakamura et al. 1989). The lower ability for the attraction of photoassimilates would be partly attributable to the lower activity of some of those sucrose-

metabolism enzymes in developing endosperm (Liang et al. 2001). This study, therefore, determined the activities of two major enzymes, sucrose synthase (EC2.4.1.13, SuSase), which catalyses the sucrose cleavage, and ADPglucose pyrophosphorylase (EC2.7.7.27, AGPase), which appears to be a key enzyme for starch synthesis (Smidansky et al. 2002), in developing endosperm at different growth stages. Different positions of spikelets, spikelets on PB and on SB, were also taken into account in this enzyme assay, due to their different degrees of grain filling. Several rice cultivars with extra-heavy panicles showing different degrees of grain filling were used as materials. Moreover, polymorphisms in DNA sequence were exploited for the regulatory region of the gene encoding AGPase among examined cultivars, in order to explain the cultivar difference in the activity of AGPase.

Methods

Plant materials

Rice cultivars, Nanjing 11, Takanari, Akenohoshi, and Koshihikari, were used. The former three have extra-heavy panicles and the last has a panicle of ordinary size in Japanese cultivars. Generally, the degrees of grain filling were descendent in the order of Nanjing 11 (good grain filling), Takanari, and Akenohoshi (poor grain filling), among the cultivars with extra-heavy panicles. Koshihikari was used as a standard cultivar. The days to sowing to heading were similar among the four cultivars, ranging from 97 to 109 days.

They were sown in nursery boxes at 4 May 2001, and transplanted into a paddy field of Hiroshima Prefectural University at 7 June, according to a randomised block design with two replications. A plot consisted of five rows, with 40 hills per row and three plants per hill. The distance between hills was 15cm and between rows was 30cm apart. Total amounts of nitrogen, phosphorous, and potassium in the fertilizer applied in the paddy field were 12, 10, and 10 g/m^2, respectively. After maturity, several agronomic traits were measured.

Grain filling process and degree of grain filling

After recording heading date of each panicle, five panicles were sampled and dried at every five days from five days after heading (DAH) through 50 DAH. The fertilized spikelets on those five panicles were collected separately depending on their positions in a panicle: spikelets on PB and on SB. Average weight of single spikelet of each cultivar, replication, and spikelet position was obtained. From these data, grain-filling rate and grain-filling duration were estimated from the method of two-line regression (Kato 1989).

After maturity, about 100 panicles were harvested from the centre of each plot. All spikelets on those panicles were collected depending on their positions as above: spikelets on PB and on SB. They were measured for their filled grain percentage (percentage of spikelets with specific gravities more than 1.06) and high-density grain percentage (percentage of spikelets with specific gravities more than 1.20, Venkateswarlu et al. 1986), by NaCl solutions with corresponding specific gravities.

Enzyme extraction and assay

At 10, 15, and 20 DAH, which roughly corresponded to the early, middle, and late stage of grain growth at spikelets on PB, respectively, several panicles were sampled from the same plots as above. Immediately after sampling, spikelets on PB and on SB were frozen separately with liquid nitrogen and stored in -80℃ until used. After removing hulls and embryos, endosperms were homogenized in an extraction buffer with a mortar and pestle. Crude extract of enzymes was obtained under about 4℃, according to a modified method of Sung et al. (1989). The activities of SuSase and AGPase were assayed according to the procedures of Sung et al. (1989) and Nakamura et al. (1989), respectively. Protein contents were determined by the dye-binding method with bovine serum albumin as a standard.

Determination of DNA sequence

To obtain the DNA sequence for the regulatory region of the gene encoding the large subunit of AGPase, some databases were searched with BLAST based on a known sequence for the coding region of this gene (Accession no. U66041). The sequence of a rice genomic DNA PAC clone in chromosome 8 (Accession no. AP004459) was found out as an available object which showed complete homology and involved a long upstream region of the AGPase gene. Based on this sequence, two kinds of 19- or 20-mer primer pairs were designed. They can amplify two fragments of about 1kbp through PCR, which partly overlap to each other. DNA for a template was extracted from etiolated seedling leaves of the four cultivars each with DNeasy Plant Mini Kit (QIAGEN Inc., USA). PCR was conducted using KOD-Puls DNA polymerase (TOYOBO, Japan). The amplified DNA fragments were separated by electrophoresis using 2.0 % agarose gel, extracted with QIAquik Gel Extraction Kit (QIAGEN Inc., USA), and purified by ethanol precipitation. The obtained DNA was sequenced using the same primers as above with a DNA sequencer (CEQ 2000, BECKMAN COULTER Inc., USA). All procedures used for DNA extraction, amplification, collection from gel, and sequencing were conducted according to the respective manufacture's instructions.

Results

In both spikelet positions, three cultivars with extra-heavy panicles had significantly higher numbers of spikelets than a cultivar with ordinary panicles, Koshihikari (Table 1). Also, there were significant differences among those cultivars with extra-heavy panicles, particularly for the spikelet on SB. One of the cultivars with extra-heavy panicles, Nanjing 11, showed a significantly higher high-density grain percentage, a higher rate of grain filling, and a shorter duration of grain filling, than Akenohoshi in most cases (Table 1). Of the two spikelet positions, the spikelet of PB showed higher filled grain and high-density grain percentages, higher rates of grain filling, and shorter durations of grain filling than the spikelet on SB in most cultivars.

The activities of SuSase were not significantly different among three cultivars with extra-heavy panicles at the spikelet on PB (Fig. 1A). On the other hand at the spikelets on SB, Nanjing 11 showing a higher degree of grain filling, and also Takanari, exhibited significantly higher SuSase activities at 10 and 15 DAH compared with Akenohoshi, which showed a lower degree of grain filling (Fig. 1B). These significantly higher enzyme activities of Nanjing 11 compared with Akenohoshi were also detected in AGPase of the spikelet on PB at 10 DAH, and of the spikelet on SB at all stages examined (Fig. 1C, 1D).

In Nanjing 11, and partly in Takanari, there were no apparent differences in both enzyme activities between spikelets on PB and on SB (Fig. 1). On the other hand, Akenohoshi, and also Koshihikari, showed obviously lower activities of SuSase and AGPase at the spikelet on SB compared with PB, particularly at an initial stage of grain growth.

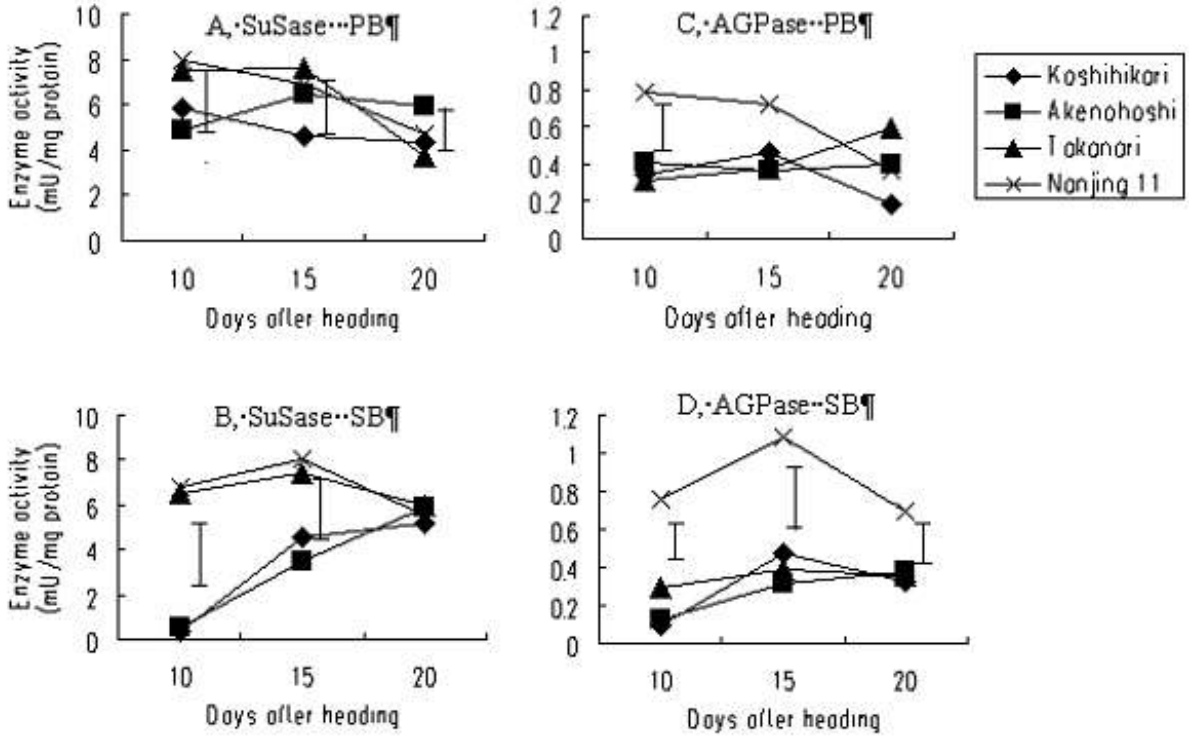

Figure 1. Changes of the two sucrose-metabolism enzymes (SuSase and AGPase) in developing endosperms on different spikelet positions (PB and SB) of four rice cultivars.
Vertical bars in the figures are LSD (*P*<0.05) at the respective periods.

For about 800 nucleotides (nt) upstream from the transcription start point (tsp) of the AGPase large-subunit gene (Anderson et al. 1991), Nanjing 11 showed three regions which differed to those of the other three cultivars examined: deletion of CC at 114th nt from tsp, deletion of one T in a ninefold T at 61st nt, and substitution of GC for TG at -261st nt. No polymorphisms were observed among the other three cultivars (Fig. 2).

Table 1. Grain filling characteristics of the four rice cultivars used.

Spikelet position	Cultivar	Filled grain %	High-density grain%	Grain-filling rate (mg/day/spikelet)	Grain-filling duration(days)	Spikelets/ panicle
Primary branch	Koshihikari	57.4	0.0	0.90	23.8	62.6
	Akenohoshi	82.4	0.0	0.70	31.2	72.0
	Takanari	66.8	2.3	0.67	28.3	83.3
	Nanjing 11	81.7	17.7	1.03	23.5	85.1
Secondary branch	Koshihikari	36.7	0.0	0.68	26.9	65.3
	Akenohoshi	59.4	0.0	0.55	32.3	95.4
	Takanari	48.3	1.2	0.49	35.4	109.2
	Nanjing 11	60.5	9.3	0.72	29.3	127.4

Values with a common letter were not significantly different to each other in the same spikelet position (*P*<0.05).

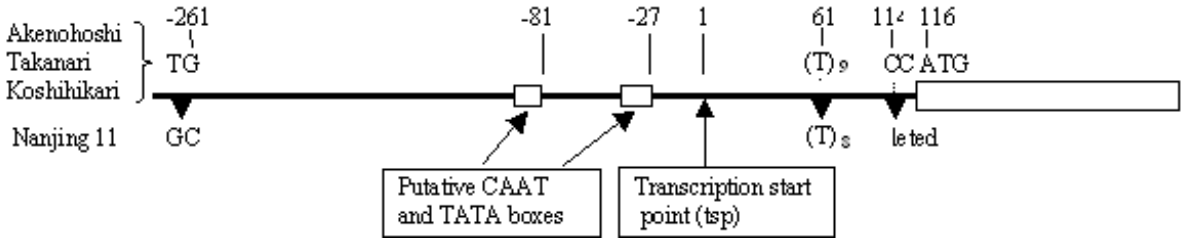

Figure 2. Nucleotide polymorphisms among four rice cultivars in the 5' non-coding region of the AGPase large-subunit gene.
Numbers on the upper line indicate the nucleotide orders from tsp (=1). The tsp and putative CAAT and TATA boxes were quoted from Anderson et al. (1991).

Conclusion

The present results clearly indicated that Akenohoshi, which had spikelets with lower specific gravities, and showed lower rates of grain filling at both spikelet positions, also showed lower activities of two sucrose-metabolism enzymes in developing endosperm. On the contrary, Nanjing 11 had spikelets with higher specific gravities, and showed higher rates of grain filling and higher activities of the above enzymes. The enzymatic characteristics of Nanjing 11 might be attributed to the structural differences in the regulatory region of AGPase gene. The lower grain sink strength of cultivars with extra-heavy panicles, together with other internal factors, might cause their lower grain filling and should be one of the targets in the genetic improvement of grain filling, and finally of grain yield for those cultivars.

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