Chinese Agron. J. 2: 183-192 (1992)

Carbohydrate metabolism enzymes in developing rice grains differing in grain-filling characteristics

Meng Lei Wei and Jih Min Sung

Department of Agronomy, National Chung Hsing University, Taichung, Taiwan, Republic of China

Abstract. Large-grain rice cultivar (1000-grain weight > 40 g) is known to accumulate less starch per unit grain dry weight. The reason for this phenomenon is still not known. This study is aimed at evaluating the changes in the activity of grain enzymes involved in sucrose to starch conversion in two rice cultivars differing in grain-filling characteristics. The results indicated that significant differences existed between cultivars in the accumulation of grain carbohydrates. Cultivar differences also existed for the activity of sucrose to starch conversion enzymes. The lower starch accumulation rate per unit grain dry weight of large-grain cultivar appears to be related to the lower activity of sucrose to starch conversion enzymes, expressed on grain dry weight basis, during the rapid phase of starch accumulation.

Key words: Carbohydrate metabolism, Rice grain, Sucrose, Starch synthesis enzyme.

不同穀粒充實特性水稻品種之發育中穀粒碳水化合物代謝酵素

魏夢麗　宋濟民

中興大學農藝系

摘要：大粒種水稻（千粒重＞40克）已知在每單位穀粒乾重所累積之澱粉較少，造成這種現象之原因目前仍不十分清楚。本研究主要在調查二種穀粒充實特性相異之水稻品種，其穀粒內負責蔗糖與澱粉轉化之各種酵素活性變化。試驗結果發現品種間就穀粒所累積之碳水化合物以及負責蔗糖與澱粉轉化之各種酵素活性而言，確實有顯着之差異。大粒型品種較低之澱粉累積速率似乎與其在澱粉快速累積時期所測得之低蔗糖與澱粉轉化酵素活性有關。

關鍵語：碳水化合物代謝、水稻穀粒、蔗糖、澱粉合成酵素。

INTRODUCTION

Large-grain rice cultivars (1,000-grain weight > 40 g) have been introduced into Taiwan because of their high yield potential (Wei et al. 1984). Despite their greater grain volume, those large-grain cultivars tend to have considerable opacity in the endosperm, caused by the loose packing of starch and protein particles, resulting in a lower market value. This undesirable grain characteristic could be attributed to either the low starch synthesis ability of the grain, or to the decreased sucrose supply to the grain, or both. Wei et al. (1988) reported that the cessation of dry weight accumulation for a Large-grain cultivar occurred despite ample sucrose supply to the grain. Similar responses were also attainable for CO_2-enriched large-grain rice cultivar (Chen, unpublished data). Thus, the undesirable grain-filling characteristic of the large-grain cultivar is most likely caused by the low starch biosynthesis of the grain during grain development. The lower starch accumulation per unit grain dry weight for this type of cultivar (Wei et al. 1984) seems to favor this notion.

Changes in the activity of starch synthesis enzymes have been analyzed during grain development in cereal crops (Doehlert and Lambert 1991, Doehlert et al. 1988, Kumar and Singh 1980, Nakamura et al. 1989, Ou-Lee and Setter 1985). It is generally accepted that the activity of these enzymes could control grain growth, provided that the supply of sucrose is not limiting (Dale and Housley 1986, Doehlert and Lambert 1991, Doehlert et al. 1988, Singh and Asthir 1988, Singh and Juliano 1977). The basic objective of this study is to investigate the carbohydrate metabolism in the grain portion of two solution-cultured rice cultivars differing in grain-filling characteristics, with particular emphasis on the characterization of changes in the activity of sucrose to starch conversion enzymes.

MATERIALS AND METHODS

Grains of rice (*Oryza sativa* L.) cultivar Pegonil (1,000-grain weight is 42 g) and Tainung 67 (1,000-grain weight is 25 g) were soaked in deionized H_2O at 25°C in darkness. After 24 h the grains were rinsed with deionized H_2O, and germinated in a plastic box (60×30×3 cm) filled with soil. The 3-leaf stage seedlings were transferred to plastic pots (40×60×30 cm) containing an ammonium based nutrient solution (contained 40 mg $N \cdot L^{-1}$, 10 mg $P \cdot L^{-1}$, 40 mg $K \cdot L^{-1}$, 40 mg $Ca \cdot L^{-1}$, 40 mg $Mg \cdot L^{-1}$, 0.5 mg $Mn \cdot L^{-1}$, 0.05 mg $Mo \cdot L^{-1}$, 0.2 mg $B \cdot L^{-1}$, 0.01 mg $Zn \cdot L^{-1}$, 0.01 mg $Cu \cdot L^{-1}$, 2 mg $Fe \cdot L^{-1}$, and 50 mg $Si \cdot L^{-1}$) which was changed weekly (Sung and Lo 1990). Solutions was continuously aerated to maintain sufficient oxygen and to provide solution mixing. Developing grains were tagged at anthesis and sampled periodically from 7 days after anthesis to physiological maturity (30 days after anthesis).

Ten panicles harvested randomly from each replication were brought to the laboratory. One hundred grains removed randomly from upper 1/3 portion of the panicle were immediately dehulled and kept at −70°C. Subsamples for enzyme assay (30 grains) were hand-homogenized at 4°C in a mortar and pestle with 50 mM hydroxyethyl piperazinyl ethanesulfonic acid (HEPES)-NaOH buffer (pH 7.5) containing 5 mM $MgCl_2$ and 1 mM dithiothreitol (DTT). The homogenate was certrifuged at 3,000 × g for 20 min at 4°C and the supernatant served as the enzyme preparation for uridine diphosphate-glucose pyrophosphorylase (UDPG-PPase) and adenosine diphosphate-glucose pyrophosphorylase (ADPG-PPase) assays. Part of the supernatant was dialyzed at 4°C for 24 h. The dialyzed solution

was used as the sourc eof sucrose synthase, soluble invertase and PPi-phosphofructokinase (PPi-PFK). The residue was washed 3 times with extracting buffer and centrifuged as before, and the supernatant was decanted. Five mL of extraction buffer (pH 7.5), containing 50 mM HEPES-NaOH, 5 mM $MgCl_2$, 1 mM DTT and 1 M NaCl, was added to the residue and centrifuged at 3,000 × g for 20 min at 4°C. The supernatant was dialyzed for 24 h and then used as the enzyme preparation for insoluble invertase. Soluble protein of enzyme preparations was determined using Bio-Rad protein reagent (Bio-Rad Laboratories 1977).

Sucrose synthase and PPi-PFK were assayed using the procedures detailed by Doehlert et al. (1988). Soluble and insoluble invertase were assayed according to Doehlert and Felker (1987). ADPG- and UDPG-PPase were assayed spectrophotometrically as described by Rocher et al. (1989). One unit of activity was defined as the activity necessary to produce 1 μmol of product in 1 min at 25°C.

Subsamples of dehulled grains were oven-dried at 70°C for 48 h, weighed and ground to a homogeneous flour. The flour (0.2 g) was placed in a test tube and to which 1 mL of water was added, and the mixture was shaken at 25°C for 4 h. After centrifugation (550 x g, 15 min), the supernatant was used for glucose determination (Trinder 1969). Sucrose was determined by the amount of glucose released following hydrolysis with 20 IU of invertase (Sigma Chemical, St. Louis, MO) at 30°C for 30 min. Starch was extracted from the residue with 9.2 N $HClO_4$ and analyzed by the anthrone method (Morris 1948).

The experimental design was a randomized complete block design with four replicates. All data were subjected to an analysis of variance and when a significant ($p<0.05$) F ratio occurred for treatment effect, a least significant difference (LSD) was calculated.

RESULTS AND DISCUSSION

Grain dry weight appeared to increase in a curvilinear manner for these two cultivars between 7 and 30 days after anthesis (DAA), although the number of sampling of data was not sufficient to characterize their growth curves (Fig. 1A). As was expected, Pegonil appeared to have a greater rate of dry weight accumulation than did Tainung 67 ($p<0.05$). This is consistent with earlier reports (Wei et al. 1984, Wei et al. 1988). Glucose, sucrose and starch content on a grain basis had distinct developmental patterns. In both cultivars, glucose had its highest content at 14 DAA, and declined thereafter (Fig. 1B). Sucrose content for Pegonil also peaked at 14 DAA, which was 7 days later than Tainung 67 (Fig. 1C). Only little starch was present in 7-day-old rice grains. Subsequently it increased from 4 to 5 mg per grain at 7 DAA to 14 to 24 mg per grain at 30 DAA (Fig. 1D). The parallel response in starch content and grain dry weight indicates these two physiological traits are closely coupled (Singh and Juliano 1977).

Significant differences ($p<0.05$) existed between cultivars in carbohydrate content (Fig. 1B, C and D). Cultivar Pegonil tended to accumulate more carbohydrates than Tainung 67, possibly due to its larger grain volume in comparison with Tainung 67 (Wei et al. 1984, Wei et al. 1988).

Carbohydrate content of the grains, expressed on a dry weight basis, was affected by sampling stages and cultivars (Fig. 2). Both cultivars showed that glucose and sucrose were present in relatively higher concentration at earlier stages of grain development and decreased subsequently throughout the sampling period (Fig. 2A). The concentration of glucose remained lower than sucrose during grain aging (Fig. 2B). This is in agreement

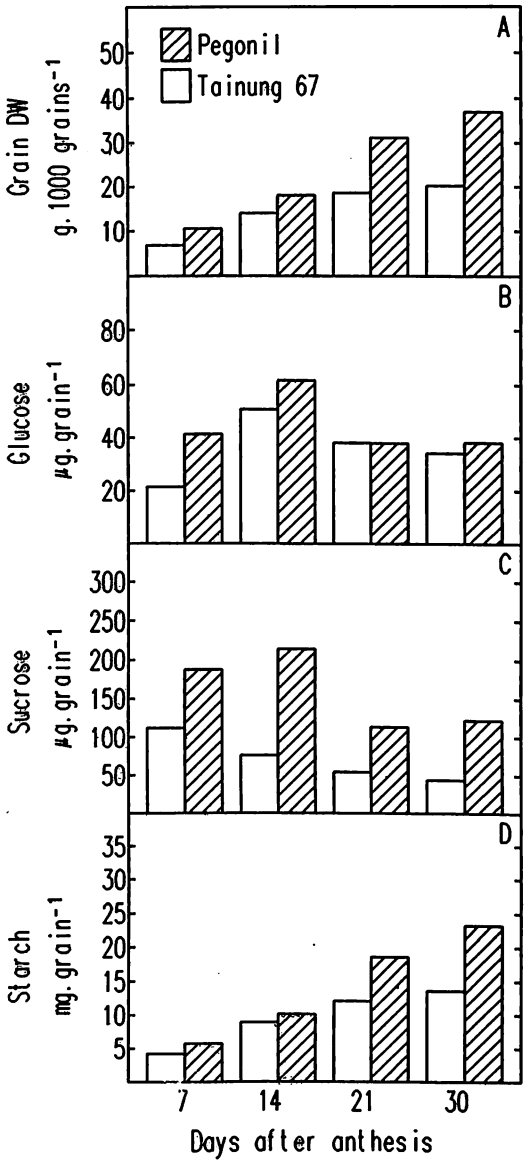

Fig. 1. Changes of grain dry weight, glucose, sucrose, and starch contents, expressed on grain basis, for two rice cultivars differing in grain-filling characteristics. Vertical bar above histograms was $LSD_{0.05}$

with another report (Smyth et al. 1986). With the decreases in sugar concentration, there was a concomitant increase in starch concentration in the grains (Fig. 2C). Cultivar Pegonil tended to have lower starch concentration during the early period of grain development ($P<0.05$), even though it had higher starch content per grain in comparison with Tainung 67 (Fig. 1D).

Fig. 3A shows sucrose synthase activity on grain basis at different sampling stages. Activity was detected in the first sampling (7 DAA), with subsequent increase until 14 DAA, followed by a decline till grain maturity (30 DAA). Invertase activity followed the pattern of

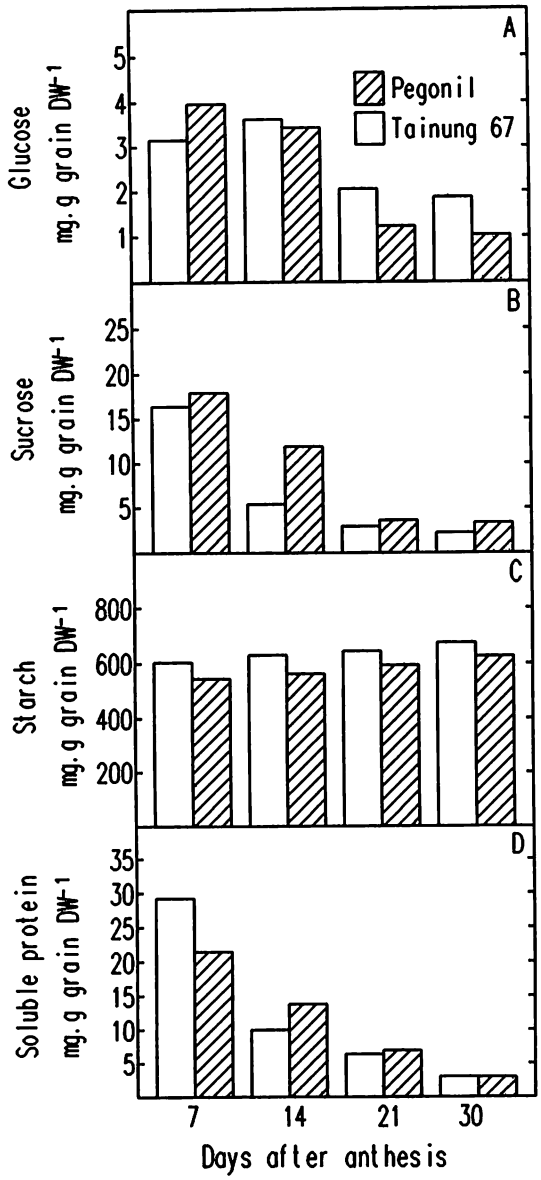

Fig. 2. Changes of glucose, sucrose and starch contents, expressed on grain dry weight basis, for two rice cultivars differing in grain-filling characteristics. Vertical bar above histograms was $LSD_{0.05}$.

sucrose synthase. Both soluble (Fig. 3B) and insoluble invertases (Fig. 3C) were higher in the grain activity at early sampling stages (7 and 14 DAA), but dropped abruptly thereafter. Nevertheless, activity was still detectable at grain maturity. This result differs from the invertase pattern for wheat grains (Kumar and Singh 1980), in which the activity disappeared during late stages of grain filling. Activity of sucrose synthase when compared to invertase was high ($p<0.05$), indicating this enzyme plays a significant role in sucrose to starch conversion, as suggested by other studies (Chan et al. 1990, Smyth and Prescott 1989). Similar conclusions were also drawn in other cereal crops (Dale and Housley 1986, Keeling et al. 1988). However, invertase's role in the initial stages of grain growth should not be over-

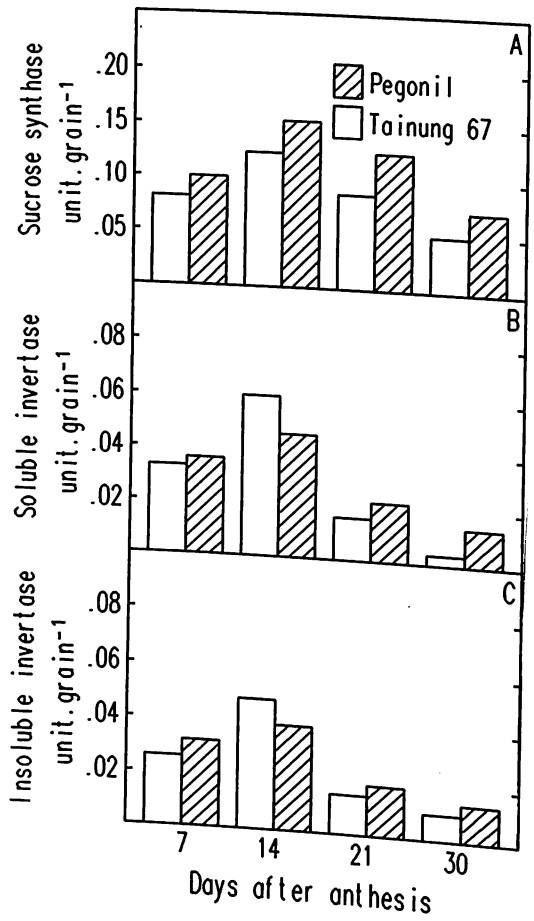

Fig. 3. Changes of sucrose synthase, soluble invertase, and insoluble invertase activities, expressed on grain basis, for two rice cultivars differing in grain-filling characteristics. Vertical bar above histograms was $LSD_{0.05}$.

looked, as it might be coordinate with sucrose synthase, during the early period of grain development, to support starch biosynthesis. Such a mechanism has been reported in maize and wheat grains (Dale and Housley 1986, Doehlert and Felker 1987). Alternatively, they might provide the substrates for respiration to support the grain growth. Significant differences existed between cultivars in sucrose synthase and invertase activities expressed on grain basis (Fig. 3), with Pegonil grains hydrolyzing more sucrose than Tainung 67 (except for the invertase activity sampled at 14 DAA).

PPi-PFK catalyzes the conversion of Fructose-6-phosphate to Fructose-1,6-bis phosphate, which is then further cleaved to triose phosphate before entering amyloplasts for starch synthesis (Doehlert et al. 1988, Keeling et al. 1988, Nakamura et al. 1989). In this study, PPi-PFK grain activity peaked at 7 DAA, and then declined thereafter (Fig. 4A). Cultivar Pegonil had the advantage over Tainung 67 ($P<0.05$) in PPi-PFK activity per grain.

UDPG-PPase grain activities measured at earlier stages of grain growth (Fig. 4B) were relatively low in comparison with other cereals (Doehlert and Lambert 1991, Kumar and Singh 1980, Ou-Lee and Setter 1985). Activity peaked at 14 DAA, which coincided with sucrose synthase activity, then declined. ADPG-PPase followed the pattern of UDPG-PPase (Fig. 4C), but the activity was less than UDPG-PPase. Other research studies (Nakamura

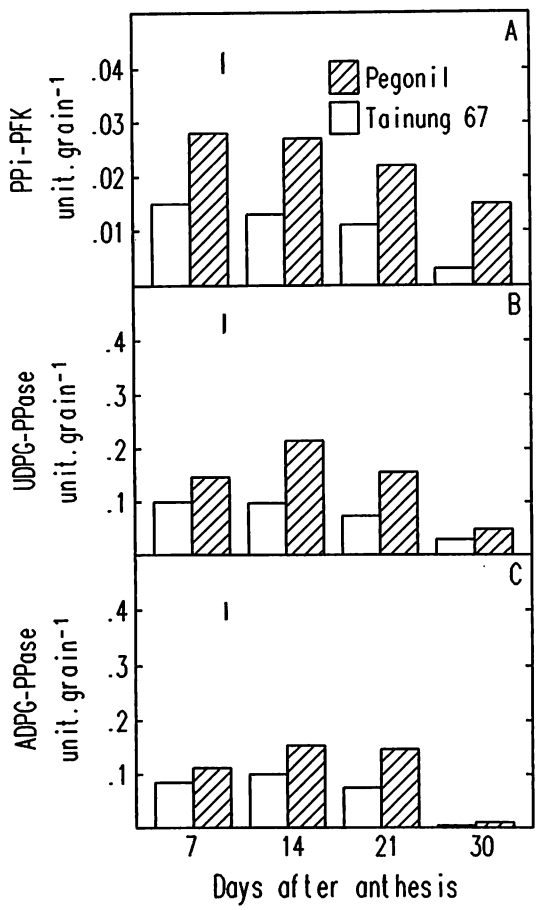

Fig. 4. Changes of PPi-PFK, UDPG-PPase, and ADPG-PPase activities, expressed on grain basis, for two rice cultivars differing in grain-filling characteristics. Vertical bar above histograms was $LSD_{0.05}$.

et al. 1989, Singh and Juliano 1977) also came to the same conclusion. Changes in UDPG-PPase and ADPG-PPase activities between cultivars paralleled grain size, with larger grains having higher activity (Fig. 4B and C).

In this study, enzyme activities were also examined on a grain dry weight basis to identify developmental patterns which might escape detection on a grain basis. As shown in Fig. 5 and Fig. 6, all the sucrose to starch conversion enzymes showed the highest activity during initial sampling, when starch was accumulating at the highest rate (starch accumulation rates were calculated for each sampling interval from the starch content data) (Fig. 2D). With the decline in starch accumulation rate following grain aging, there was a concomitant decrease in the activities of these enzymes. The data in Fig. 5 and Fig. 6 further revealed that the changes in enzymes activity (Fig. 5 and Fig. 6) paralleled soluble protein content on a dry weight basis (Fig. 5D). Thus, the differential response of enzyme activity (on a dry weight basis) appears to be associated with the quantitative changes in enzymes per se. Nevertheless, the reduced sucrose synthase activity in rice grains was probably not attributable to enzyme deterioration, because the synthesis of sucrose synthase protein has been shown to proceed till grain maturity (Chan et al. 1990). Reduced sucrose synthase activity is presumably due to decreased sucrose availability (Fig. 2B), or to the loss of

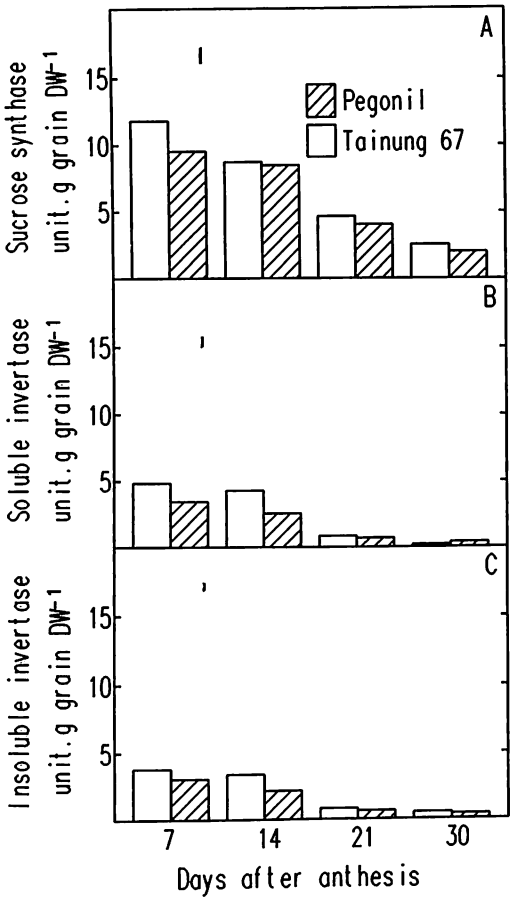

Fig. 5. Changes of sucrose synthase, soluble invertase, and insoluble invertase activities, and soluble protein, expressed on grain dry weight basis, for two rice cultivars differing in grain-filling characteristics. Vertical bar above histograms was $LSD_{0.05}$.

enzyme activation.

ADPG-PPase is a key regulatory enzyme for starch synthesis (Doehlert and Lambert 1991, Nakamura et al. 1989, Ou-Lee and Setter 1985, Singh and Juliano 1977). In this study, correlations were found between ADPG-PPase and sucrose synthase (r values were and 0.980** and 0.982** for Pegonil and Tainung 67, respectively), suggesting that the enzymes are coordinately expressed in rice grain. As shown in Fig. 5A, the activities of sucrose synthase, expressed on a grain dry weight basis, for Pegonil was lower than that for Tainung 67 ($p<0.05$). Similar cultivar response was also observed on ADPG-PPase for 7-day-old grain (Fig. 6C), when starch was accumulated at the highest rate (Fig. 2D). These results seem to indicate that, on a grain dry weight basis, Pegonil is less efficient at metabolizing sucrose, on per unit grain dry weight basis, to support starch synthesis in comparison with Tainung 67. With reduced sucrose synthase (Fig. 5A) and ADPG-PPase (Fig. 6C) activities, a reduction in starch accumulation per unit grain dry weight for Pegonil (Fig. 2D) would be anticipated.

In conclusion, the present data reveal that the large-grain cultivar Pegonil is capable of accumulating more carbohydrate in the developing grain, due to its greater grain volume. But it accumulated less starch per unit grain dry weight. The undesirable grain-fill in the

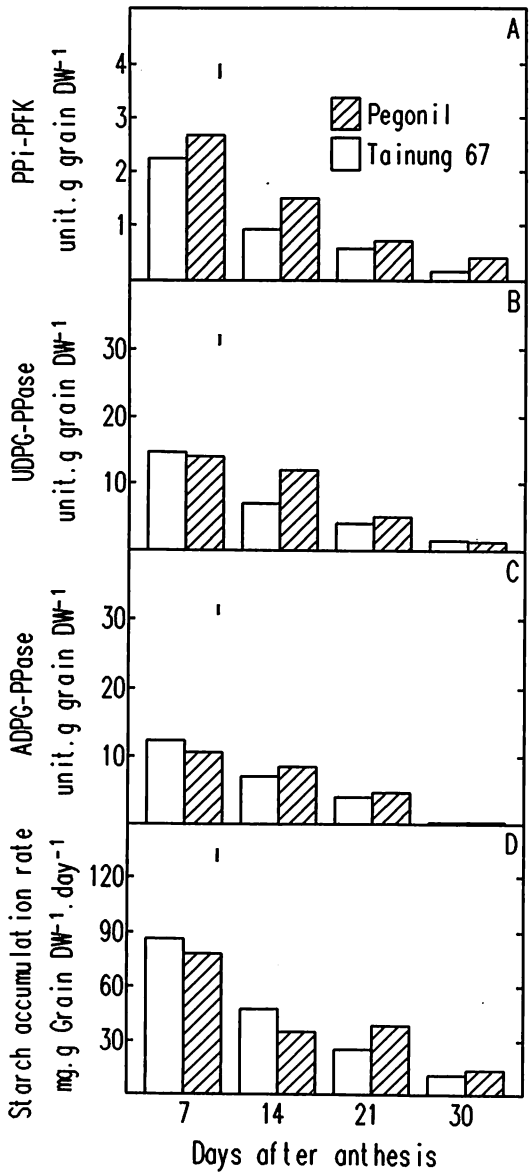

Fig. 6. Changes of PP-PFK, UDPG-PPase, and ADPG-PPase, and starch accumulation rate, expressed on grain dry weight basis, for two rice cultivars differing in grain-filling characteristics. Vertical bar above histograms was $LSD_{0.05}$.

large-grain cultivar Pegonil is mainly associated with the lower sucrose synthase and ADPG-PPase activities on grain dry weight basis, resulting in a lower starch accumulation per unit grain dry weight.

Acknowledgements. The authors thank Council of Agriculture for financial supports.

REFERENCES

Bio-Rad Laboratories. (1977) Bio-Rad protein assay. Tech. Bull. 1051. Bio-Rad Lab, Richmond, CA.

Chan, H. Y., Ling, T. Y., Juang, R. H., Ting, I. N., Sung, H. Y. and Su, J. C. (1990) Sucrose synthase in rice plants. Growth-associated changes in tissue specific distributions. *Plant Physiol.* 94:1456-1461.

Dale, E. M. and Housley, T. L. (1986) Sucrose synthase activity in developing wheat endosperms differing in maximum weight. *Plant Physiol.* 82:7-10.

Doehlert, D. C. and Felker, F. C. (1987) Characterization and distribution of invertase activity in developing maize (*Zea mays*) kernels. *Physiol. Plant.* 70:51-57.

Doehlert, D. C. and Lambert, R. J. (1991) Metabolic characteristics associated with starch, protein, and oil deposition in developing maize kernels. *Crop Sci.* 31:151-157.

Doehlert, D. C., Kuo, T. M. and Felker, F. C. Felker. (1988) Enzymes of sucrose and hexose metabolism in developing kernels of two inbreds of maize. *Plant Physiol.* 86:1013-1019.

Keeling, P. L., Wood, J. R., Tyson, R. H. and Bridges, I. G. (1988) Starch biosynthesis in developing wheat grain. Evidence against the direct involvement of triose phosphates in the metabolic pathway. *Plant Physiol.* 87:311-319.

Kumar, R. and Singh, R. (1980) The relationship of starch metabolism to grain size in wheat. *Phytochemistry* 19:2299-2303.

Morris, D. L. (1948) Quantitative determination of carbohydrates with dry-wood's anthrone reagent. *Science* 107:254-255.

Nakamura, Y., Yuki, K., Park, S. Y. and Ohya T. (1989) Carbohydrate metabolism in the developing endosperm of rice grains. *Plant Cell Physiol.* 30:833-839.

Ou-Lee, T. M. and Setter, T. L. (1985) Enzyme activities of starch and sucrose pathways and growth of apical and basal maize kernels. *Plant Physiol.* 79:848-851.

Rocher, J. P., Prioul, J. L., Lecharny, A., Reyee, A. and Joussaume, M. (1989) Genetic variability in carbon fixation, sucrose-P-synthase and ADP glucose pyrophosphorylase in maize plants of differing growth rate. *Plant Physiol.* 89:416-420.

Singh, R. and Asthir, B. (1988) Import of sucrose and its transformation to starch in the developing sorghum caryopsis. *Physiol. Plant.* 74:58-65.

Singh, R. and Juliano, B.O. (1977) Free sugars in relation to starch accumulation in developing rice grain. *Plant Physiol.* 59:417-421.

Smyth, D. A. and Prescott, H. E., Jr. (1989) Sugar content and activity of sucrose metabolism enzymes in milled rice grain. *Plant Physiol.* 89:893-896.

Smyth, D. A., Repetto, B. M. and Seidel, N. E. (1986) Cultivar differences in soluble sugar content of mature rice grain. *Physiol. Plant.* 68:367-374.

Sung, F. J. M. and Lo, W. S. (1990) Growth responses of rice in ammonium-based nutrient solution with variable calcium supply. *Plant Soil* 125:239-244.

Trinder, P. (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.* 6:24.

Wei, M. L., Kuo, Y. C. and Liu, D. J. (1984) Physiological studies of rice tiller. II. Productivity of varieties differing in grain volume. *Agric. Res. China* 33:12-23.

Wei, M. L., Kuo, Y. C. and Liu, D. J. (1988) Relationship of grain weight variation to grain yield and the related physiological characteristics in rice. *J. Agric. Assoc. China* 142:26-41.

(Received May 22, 1992 and accepted May 29, 1992)