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ORIGINAL PAPER

Comparison of starch accumulation and enzyme activity in grains of wheat cultivars differing in kernel type

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Abstract It is generally accepted that sucrose synthase (SuSy), ADP-glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS), granule-bound starch synthases (GBSS) and starch branching enzyme (SBE) play a key role in starch synthesis in wheat grains. Starch synthesis in wheat grains is influenced by genotype and environment. However, what is not known is the degree of variation in enzyme activity during starch accumulation of wheat cultivars differing in kernel types. The present study was carried out to characterize the changing activities of key enzymes during grain filling in two kernel type winter wheat cultivars. Results showed that starch accumulation rate (SAR) and activities of SuSy, AGPase, SSS, GBSS and SBE in large kernel types were significantly higher than those in small kernel types. The soil water deficit experienced during the course of the experiment led to an increase at early grain-filling period and decrease during late grain-filling, respectively, in SAR and activities of key enzymes involved in starch synthesis, especially SuSy, AGPase, SSS, and SBE. Water deficit enhanced grain starch accumulation in small kernel types. It suggests that rainfed treatment increase physiological activities during early grain-filling and promote starch accumulation in small kernel types. The simulation with Richards' equation showed that it was accumulation duration and SAR that determined the starch accumulation in large kernel types. Compared with small kernel types, plants of large kernel types maintained longer filling duration, higher SAR and greater activities of related enzymes during mid and late grain-filling. These observations suggest stronger sink activities in large kernel types at a later stage of development. Consequently, large kernel types have advantages over the small kernel types in terms of the amount of starch accumulation at mid and late stage, but are sensitive to water deficit.

Keywords Wheat (*Triticum aestivum* L.) · Kernel type · Irrigation · Sucrose synthase · ADP-glucose pyrophosphorylase · Starch synthase · Starch branching enzyme

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Introduction

In wheat (*Triticum aestivum* L.), starch is a major component of grain yield, accounting for 65% to 75% of the final dry weight of the grain (Dale and Housley 1986; Hurkman et al. 2003; Stark et al. 1992), and an important factor affecting the processing quality of wheat. Starch synthesis in wheat grains has been studied extensively. It has been reported that the enzymes involved in starch synthesis and starch accumulation, including sucrose synthase (SuSy; EC 2.4.1.13), ADP-glucose pyrophosphorylase (AGPase; EC 2.7.7.27), starch synthase (SSS and GBSS; EC 2.4.1.21), and starch branching enzyme (SBE; EC 2.4.1.18), play key roles in starch synthesis and accumulation in wheat grain (Yang et al. 2004; Edurne et al. 2003; Emes et al. 2003; Okita 1992). It is evident that cultivars of different kernel-type vary in grain development. Large kernel types generally have more endospermic cells and longer filling duration than small kernel types. Zhao et al. (2003) reported that increased photosynthate production in large kernel type cultivars during mid-late grain filling period could provide sufficient substrate for grain development. In addition, improved conversion and utilisation of assimilates in sink tissues resulted in higher starch accumulation and filling rates in grains at mid-late stage. The peaks of starch accumulation rate (SAR), photosynthetic rate, sucrose content and activities of related enzymes in large kernel types occur at mid-late filling stage and last longer (Gao et al. 2003; Wang 2004). Studies have revealed that activities of enzymes involved in starch synthesis in wheat are both developmentally and environmentally determined (Jenner et al. 1991; Hawker and Jenner 1993; Jenner 1994). Ercoli et al. (2008) showed that grain yield, dry matter, N accumulation and remobilization were all negatively affected by water stress during grain filling. Maximum activities of SSS, GBSS and SBE in winter wheat grains are reached at mid grain-filling stage and remained high under optimum water status but decreased significantly under severe or moderate drought stress (Xu et al. 2003). The starch from wheat grown under water stress conditions show lower amylose content, lipid content and pasting temperature, and higher peak viscosity, final viscosity and setback at 15 DPA (Singh et al. 2008). Ahmadi and Baker (2001 reported that reduction in grain growth rate of water-stressed wheat plants resulted from decreased SSS activity, whereas growth cessation is due mainly to the inactivation of AGPase. However, a better understanding of the mechanism for starch accumulation and its component variation would be helpful both to improve processing quality and to promote grain yield in wheat. So far there is limited information available on the comparison of starch accumulation and accompanying enzyme activities in grains between irrigated and rainfed wheat plants with varying kernel types.

The objective of this study was to investigate any differences that may be found in starch accumulation of wheat cultivars differing in kernel types and field-grown in irrigated and rainfed conditions. The activities of SuSy, AGPase, SSS, GBSS and SBE in relation to starch accumulation in two contrasting wheat cultivars, in terms of kernel type, were therefore investigated.

Materials and methods

Plant materials

Two kernel type winter wheat (*Triticum aestivum* L.) cultivars, large kernel type, SN710331 and WM8, and small kernel type, LM21 and JN17, were used (Table 1).

Experiment description

The experiment was carried out on the experimental farms of Shandong Agricultural University (36°N, 117°E), Tai'an, and the Research Institute of Agricultural Science, (37°N, 116°E), Dezhou, PR China, respectively, in the growing season of 2004–2005. In Tai'an, the 0–20 cm soil layer contained 71.5 mg kg^{-1} available nitrogen, 12.5 mg kg^{-1} available phosphate and 80.5 mg kg^{-1} available potassium. In Dezhou, the soil contained available nitrogen-phosphate- potassium at 62, 8.5 and 122.5 mg kg^{-1}, respectively. Before planting, fertilizer was applied at 6 kg m^{-2} farmyard manure, 11.2 g m^{-2} N, 2.7 g m^{-2} P and 7.9 g m^{-2} K. 11.2 g m^{-2} N was also used as a top dressing at the stem elongation stage of wheat in two experimental locations. Two contrasting water regimes (irrigated and rainfed) were used at both experimental sites. The experiment was a 4 × 2 (four cultivars and two water regimes) factorial design with eight treatments. Each of the treatments had three plots as repetitions in a complete randomized block design. Plot dimension was 3 m × 3 m, and plots were separated by a ridge wrapped with plastic film. Under irrigated conditions, the crop was flood-irrigated twice

Table 1 Grain yield components and starch accumulation for the tested wheat cultivars field-grown in two water regimes in two experimental sites

Site	Treatment	Cultivars	Spike number (spike m^{-2})	Grain number (grain $spike^{-1}$)	Kernel weight (mg $grain^{-1}$)	Starch accumulation (mg $grain^{-1}$)
Tai'an	Irrigated condition	SN710331	447 d[a]	36.2 cd	65.8 a	44.9 a
		WM8	455 d	44.4 a	51.9 b	35.1 c
		LM21	636 bc	43.7 a	36.9 d	26.0 de
		JN17	801 a	34.6 de	36.8 d	25.3 e
	Rainfed condition	SN710331	392 d	35.6 d	64.8 a	41.7 b
		WM8	424 d	39.1 bc	53.4 b	34.2 c
		LM21	576 c	42.1 ab	39.5 cd	26.9 de
		JN17	696 b	32.0 e	41.1 c	27.6 d
Dezhou	Irrigated condition	SN710331	416 c	37.9 ab	58.7 b	39.0 a
		WM8	384 c	42.9 a	51.8 c	34.0 b
		LM21	586 ab	37.6 ab	41.9 e	28.9 c
		JN17	613 a	39.7 a	37.8 f	25.4 e
	Rainfed condition	SN710331	336 d	33.0 b	60.9 a	38.5 a
		WM8	318 d	41.4 a	52.7 c	33.9 b
		LM21	553 b	37.4 ab	43.4 d	29.2 c
		JN17	593 a	37.9 ab	40.4 e	26.6 d

[a] Different letters indicate statistical significance at the $P = 0.05$ level of probability

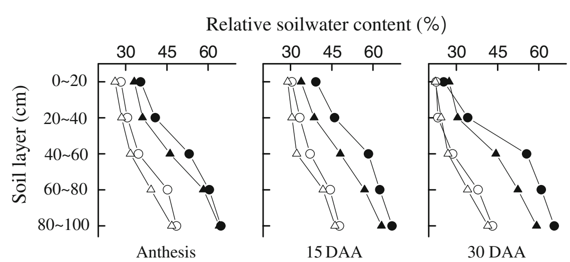

Fig. 1 The relative soil water content (%) after anthesis in Tai'an (●, Irrigated; ○, Rainfed) and Dezhou (▲, Irrigated; △, Rainfed) experimental sites

after sowing (17 December and 8 April) with 750 m^3 hm^{-2} amount of applied water at each date. The total rainfall during the wheat growing period was 201 mm and 92.2 mm in Tai'an and Dezhou, respectively. The moisture content in the soil is shown in Fig. 1. Plants were sown on 6 and 15 October in Tai'an and Dezhou, respectively, at a density of 180 seedling m^{-2}. Normal crop farming practices were implemented to minimize pest, disease and weed incidence.

Plant sampling

Spikes that flowered on the same day were labeled with thread. From 7 days after anthesis (DAA), 30 of the labeled spikes were sampled at 7-day intervals until maturity. These spikes were inactivated at 105°C for 10 min and then dried at 80°C for measurement of grain weight and starch content. On the same sampling date, 10 of the labeled spikes were frozen in liquid nitrogen for 2 min and then stored at −40°C until assayed for enzyme activities. From each plot all plants (except from the guard area surrounding the plot) from a 4 m^2 site were harvested at maturity for the determination of grain yield. Yield components, i.e. the spikes per square meter, grain number per spike, kernel weight and starch accumulation were determined from plants harvested.

Determination of amylose and amylopectin contents

Amylose and amylopectin contents in wheat grains were determined with a coupled spectrophotometer assay (Jiang et al. 2003). For the determination, 100 mg of milled grains were stirred with 10 ml of 0.5 M KOH for 30 min at 90°C and then diluted to a volume of 50 ml with distilled water. From this, 2.5 ml was removed to a fresh tube containing 20 ml distilled water. This solution was adjusted to pH 3.5 with 0.1 M HCl, and 500 μl of I_2-KI reagent added.

Finally, this solution was diluted to a final volume of 50 ml with distilled water. After standing for 20 min, the mixture's absorbance was measured with a TU-1901 spectrophotometer at 473, 554, 633 and 741 nm.

The processes of starch accumulation in the grain were fitted by Richards' (1959) growth equation as described by Yang et al. (2004):

$$W = A/\left(1 + Be^{-kt}\right)^{1/N} \tag{1}$$

The SAR was calculated as the derivative of Eq. 1:

$$G = AKBe^{-k}/\left(1 + Be^{-kt}\right)^{(N+1)/N} \tag{2}$$

where W is the starch weight (mg), A is the final starch weight (mg), t is the time after anthesis (d), and B, K and N are coefficients determined by regression. The active starch accumulation duration was defined as the days when W was from 5% (t_1) to 95% (t_2) of A. An average SAR during this period was therefore calculated from t_1 to t_2.

Enzyme assay

Preparation of enzyme extracts

The preparation procedure was similar to the method of Nakamura et al. (1989). For the assays of enzymes, 5–10 frozen grains were weighed and homogenized with a pestle in a pre-cooled mortar containing 10 ml ice-cooled extraction buffer (50 mM Hepes-NaOH [pH 7.5], 2 mM KCl, 5 mM EDTA, 1 mM DTT [Dithiothreitol], 1% (w/v) PVP [polyvinylpyrrolidone-30]). An aliquot of the homogenate (30 µl) was mixed with 1.8 ml extraction buffer and then centrifuged at 2000*g* at 0–4°C. The resulting pellet was suspended in 2 ml of the extraction buffer and this used for the GBSS activity assay. The remainder of the homogenate was centrifuged at 10,000*g* at 0–4°C for 10 min, and the resulting supernatant was used as the preparation of SuSy, AGPase, SSS, and SBE.

All enzyme assays were optimized for pH and substrate concentration and were shown to be within the linear phase with respect to incubation time and protein concentration. Protein content was determined according to Zhang and Qu (2003), using bovine serum albumin as standard. During assay, the background values were routinely taken as the activities detected with a reaction time of zero (the enzymes were denatured immediately after their addition to the reaction mixtures).

SuSy (EC 2.4.1.13)

The assay was carried out according to the method of Wardlaw and Willenbrink (1994). The reaction mixture (110 µl) contained 50 mM Hepes-NaOH (pH 7.5), 100 mM fructose, 100 mM UDPglucose, and 50 mM $MgCl_2$. The reaction was started by adding 50 µl of crude enzyme extract. After incubating at 30°C for 30 min, the reaction was stopped by boiling for 1 min. After adding 200 µl of 2 M NaOH, the solution was incubated for 10 min at 100°C. After cooling, 2 ml of 30% (v/v) HCl and 1 ml of 0.1% (w/v) resorcin were added and the solution incubated for 10 min at 80°C. Finally, the formation of UDPglucose-dependent sucrose catalysed by SuSy was determined by measuring the absorbance at 480 nm with a TU-1901 spectrophotometer.

AGPase (EC 2.7.7.27)

The assay protocol was carried out according to the method of Nakamura et al. (1989). The reaction mixture (400 µl) contained 50 mM Hepes-NaOH (pH 7.5), 1.2 mM ADPG, 5 mM PPi, 6 mM $MgCl_2$, and 3 mM DTT. The reaction was started by adding 50 µl of crude enzyme extract. After incubating at 30°C for 20 min, the reaction was stopped by boiling for 1 min. After cooling, 100 µl of 6 mM $NADP^+$, 0.08 IU phosphoglucomutase, 0.07 IU glucose-6-phosphate dehydrogenase, and 300 µl of 50 mM Hepes-NaOH (pH 7.5) were added and the solution incubated for 10 min at 30°C. The activity was determined by measuring the increase in absorbance at 340 nm.

SSS and GBSS (EC 2.4.1.21)

The assay was similar to the procedure of Nakamura et al. (1989). The reaction mixture (final volume 400 µl) comprised 1.6 mM ADPG, 0.7 mg amylopectin, and 15 mM DTT all in 50 mM Hepes-NaOH (pH 7.5) with 50 µl of crude enzyme used to start the reaction. After 20 min, the reaction was stopped by boiling for 1 min. To the mixture was added 200 µl of a solution comprising 50 mM Hepes-NaOH (pH 7.5), 4 mM PEP (phosphoenolpyruvate), 200 mM KCl, 10 mM $MgCl_2$, 1.2 IU pyruvate kinase, and further incubated for 20 min at 30°C. The resulting solution was heated in a boiling-water bath for 1 min and mixed with 400 µl of a solution of 50 mM Hepes-NaOH (pH 7.5), 10 mM Glucose, 20 mM $MgCl_2$, 2 mM $NADP^+$. The enzymic activity was measured as the increase in absorbance at 340 nm after the addition of 1.4 IU hexokinase and 0.35 IU G6P dehydrogenase.

SBE (EC 2.4.1.18)

The assay was carried out according to the method of Li et al. (1997). The reaction mixture contained 50 mM Hepes-NaOH (pH 7.5) and 7.5 g l^{-1} soluble starch (final volume 1.45 ml). The reaction was started by adding 50 µl of crude enzyme extract. After incubating at 37°C for 40 min, the reaction was stopped by boiling for 1 min. The activity was assayed spectrophotometrically at 660 nm after the addition of 150 µl of 0.2% I_2-KI.

Statistical analysis

All measurements were replicated, and data were analyzed for variance using SAS statistical analysis package (version 8.0; SAS Institute, Cary, NC). Means and standard deviations were calculated for individual measurements on each sampling date. Differences among means were tested using least significant difference ($P < 0.05$). The enzymic activities in wheat grains from both experimental sites were measured, and results were very similar. Only the Tai'an experimental site was reported in this paper because of limited space.

Results

Accumulation of amylose and amylopectin in single grain

The accumulation of both amylose and amylopectin in large kernel types, SN710331 and WM8, were much higher than in small kernel types ($P = 0.01$), LM21 and JN17, in both irrigated and rainfed conditions at two experimental sites (Figs. 2, 3). The average amylose weight per kernel in the large kernel types of both treatments was 24.9% higher than that in the small kernel types, while that of amylopectin was 33.1% higher. The simulation with Richards' equation demonstrated evidently higher SAR and longer active duration in the large kernel types than in the small kernel types (Table 2). The results indicate that starch accumulation duration and SAR are predominant factors responsible for final starch accumulation in the large kernel types.

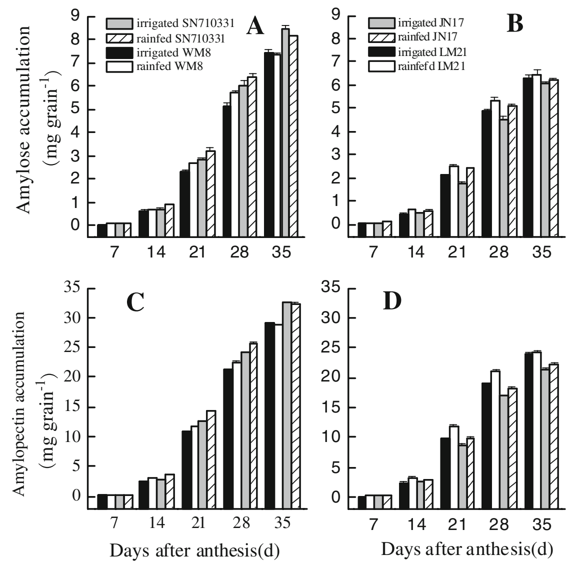

Fig. 2 Amylose and amylopectin accumulation in grains of large kernel types (**a**, **c**) and small kernel types wheat (**b**, **d**) grown in Tai'an experimental site

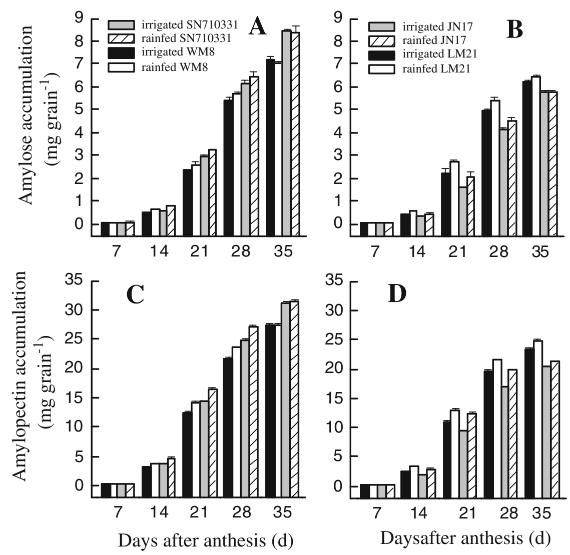

Fig. 3 Amylose and amylopectin accumulation in grains of large kernel types (**a**, **c**) and small kernel types wheat (**b**, **d**) grown in Dezhou experimental site

Table 2 Starch accumulation parameters in grains of wheat field-grown in two water regimes in two sites

Site	Treatment	Cultivars	Time of max. SAR (days)	Active duration (days)	SAR (mg grain^{-1} days^{-1})
Tai'an	Irrigated condition	SN710331	22.91	35.93	1.48
		WM8	23.60	35.80	1.32
		LM21	22.70	26.84	1.25
		JN17	23.32	27.56	1.10
	Rainfed condition	SN710331	21.48	30.28	1.54
		WM8	22.52	28.99	1.41
		LM21	21.68	23.41	1.36
		JN17	22.15	26.04	1.17
Dezhou	Irrigated condition	SN710331	21.12	33.81	1.42
		WM8	21.50	32.07	1.29
		LM21	21.66	24.74	1.28
		JN17	21.65	26.48	1.09
	Rainfed condition	SN710331	20.32	26.80	1.60
		WM8	20.58	25.75	1.43
		LM21	20.52	23.77	1.37
		JN17	19.97	22.27	1.28

The active duration and SAR exhibited variable responses with soil moisture. They were enhanced by a water deficit, suggesting that drought conditions were responsible for higher grain-filling rates and shorter grain-filling duration. The responses to water deficit differed greatly between the two kernel type cultivars. Under rainfed conditions, the small kernel types showed significantly higher accumulation of both amylose and amylopectin throughout the grain-filling period compared with those under irrigated conditions (Figs. 2b, d and 3b, d). A similar pattern was observed in the large kernel types at 7–28 DAA, but the final accumulation of amylose and amylopectin was lower than those under irrigated conditions (Figs. 2a, c and 3a, c). Along the lines of the characterization of starch accumulation at the mature stage (Table 1), the results of starch accumulation at the mature stage show clearly that irrigation was more beneficial to the accumulation of amylose and amylopectin in large kernel types.

Accumulation rate of starch

The SAR on both kernel types followed a similar pattern under both irrigated and rainfed conditions at both Tai'an and Dezhou (Figs. 4, 5, respectively). It rose gradually and then declined after reaching a maximum. From 25 DAA, the SAR in the large kernel types was markedly higher than those in the small kernel types, averaging 30.2% higher, which indicated that the higher starch accumulation in the large kernel types was mainly due to the higher SAR during mid-late grain-filling period.

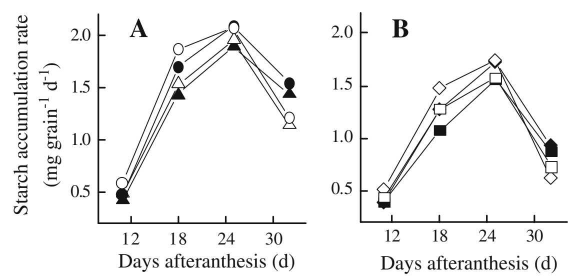

Fig. 4 SAR in grains of large kernel types (△, WM8; ○, SN710331 in **a**) and small kernel types wheat (◇, LM21; □, JN17 in **b**) grown under irrigated (black symbols) and rainfed (white symbols) conditions in Tai'an experimental site

Compared with irrigated plants, enhanced and reduced SARs were observed in grains of plants subjected to soil water deficit in both kernel types at early and mid-late filling stage, respectively; however, the magnitude of increase at the early stage differed in the two kernel types. The increased SAR in the small kernel types at 18 DAA (17.0%) was markedly higher than that in the large kernel types

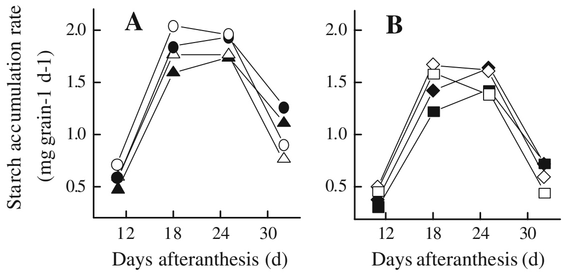

Fig. 5 SAR in grains of large kernel types (△, WM8; ○, SN710331 in **a**) and small kernel types wheat (◇, LM21; □, JN17 in **b**) grown under irrigated (black symbols) and rainfed (white symbols) conditions in Dezhou experimental site

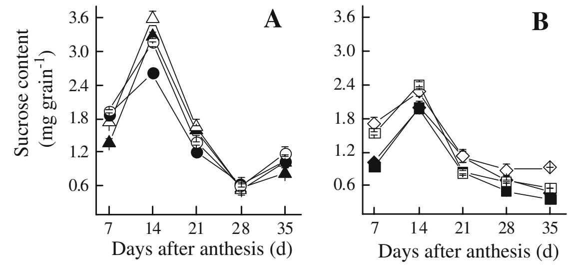

Fig. 7 Sucrose content in grains of large kernel types (△, WM8; ○, SN710331 in **a**) and small kernel types wheat (◇, LM21; □, JN17 in **b**) grown under irrigated (black symbols) and rainfed (white symbols) conditions in Tai'an experimental site

(8.6%). The results suggest that the rainfed conditions during the experimental period at both sites were more beneficial for starch accumulation in the small kernel types at the early filling stage.

SuSy activity

The SuSy activities in grains of two kernel types exhibited single-peak curves during grain filling, yet the peak value and the peak-occurring time were significantly different (Fig. 6). Compared with the small kernel types, the peaks of the large kernel types increased by 57.4% and 37.7%, respectively, under irrigated and rainfed conditions.

With the rainfed treatment, SuSy activity in the small kernels was higher during the early filling period, and then declined gradually to values lower than those in irrigated treatment after 21 DAA, whereas the SuSy activity in the large kernels was significantly lower than those in irrigated treatment from 14 DAA. These results suggest that water deficit enhanced the capacity of plants to break down and take up of sucrose in the small kernel types during early grain filling. However, the irrigated treatment markedly improved the ability during mid and late grain filling, especially in the large kernel types.

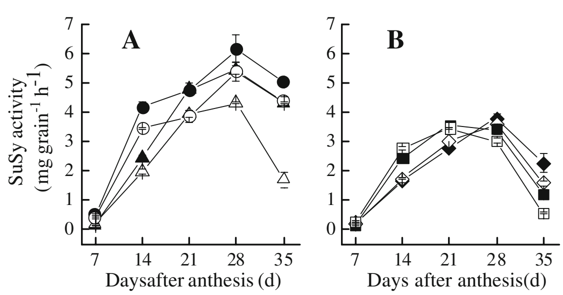

Fig. 6 SuSy activity in grains of large kernel types (△, WM8; ○, SN710331 in **a**) and small kernel types wheat (◇, LM21; □, JN17 in **b**) grown under irrigated (black symbols) and rainfed (white symbols) conditions in Tai'an experimental site

Sucrose content

The average sucrose content during grain filling was 39.1% higher in grains of the large kernel types than in the small kernel types under irrigated and rainfed conditions (Fig. 7). Water deficit led to enhanced sucrose content in grains of both small kernel and large kernel types. Hence, it could be concluded that large kernel types had stronger capacity of supplying and utilizing assimilate than small kernel types along the lines of the sucrose content and SuSy activities.

AGPase activity

The AGPase activities in grains of both kernel types showed single peak curves with peaks at 28 DAA under irrigated and rainfed conditions; however, there existed an obvious difference in peaks between the two kernel types (Fig. 8a, b). During 7–14 DAA, the AGPase activities increased by 16.8% in the large kernels than those in the small kernels, yet the former were 55.0% higher than the latter during 21–35 DAA, indicating that large kernel types had the better capacity to supply ADPG than small kernel types during mid-late grain filling.

In comparison with irrigated conditions, AGPase activities in small kernels under rainfed conditions were higher before 21 DAA and decreased rapidly after 28 DAA, and were significantly lower in large kernels from 21 DAA. The results indicate that water deficit contributed to the enhancement of AGPase

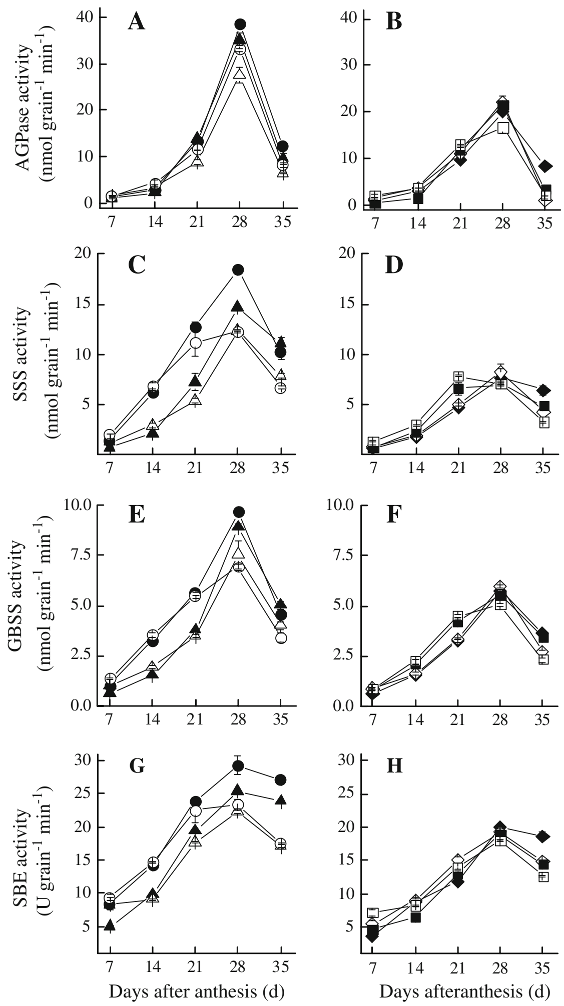

Fig. 8 AGPase, SSS, GBSS and SBE activities in grains of large kernel types (△, WM8; ○, SN710331 in **a**, **c**, **e**, **g**) and small kernel types wheat (◇, LM21; □, JN17 in **b**, **d**, **f**, **h**) grown under irrigated (black symbols) and rainfed (white symbols) conditions in Tai'an experimental site

activity during early-mid grain filling, especially for the small kernel types; whereas water supply increased ADPG significantly during mid-late grain filling, particularly for the large kernel types.

SSS and GBSS activity

During grain filling, both SSS and GBSS activities examined in two kernel types presented single peak curves, while their maximum peak value and time at which it was reached were different between the four cultivars (Fig. 8c–f). The mean SSS activities of the large kernel types were 79.7% higher than those of the small kernel types. The GBSS activity in grains of the two kernel types showed a similar pattern throughout the filling period, i.e. reached the highest peak at 28 DAA and then decreased rapidly (Fig. 8e, f). In comparison with small kernel types, large kernel types possessed higher GBSS activity peak value and maintained higher GBSS activity during late grain-filling period.

Compared with the irrigated treatment, the SSS and GBSS activities reduced by 19.0% and 12.3% in the large kernels, whereas increased by 0.4% and reduced by 4.2% in the small kernels, respectively, under rainfed treatment.

The above results indicate that large kernel types had advantages over the small kernel types in terms of the starch accumulation, but tended to be more responsive to water supply, leading to a significant decrease in activities of enzymes involved in starch synthesis under rainfed conditions. In contrast to large kernel types, the response to water deficit in small kernel types was not as sensitive.

SBE activity

Under both irrigated and rainfed conditions, the SBE activity in the grains of two kernel types exhibited single peak curves with peaks at 28 DAA, which differed markedly between the two kernel types cultivars. The average SBE activities of the large kernel types were 44.5% higher than that of the small kernel types (Fig. 8g, h). The two kernels types responded differently to water supply. The mean SBE activities in the large kernels under rainfed conditions were 13.1% lower than that under irrigated conditions. The SBE activities in the small kernels increased and decreased during early-mid (before 21 DAA) and late (after 28 DAA) grain-filling stage, respectively, under water deficit conditions (Fig. 8h); however the magnitudes of the decrease were lower than those in large kernel types. This infer that irrigation may benefit the SBE activities in large kernel types during mid-late filling period, yet small kernel types were more suitable for rainfed conditions.

Discussion and conclusion

It is generally accepted that grain-filling rates in cereals is mainly determined by sink strength. The sink strength can be described as the product of sink size and sink activity. Sink size is a physical restraint that includes cell number and cell size. Sink activity is a physiological restraint that includes multiple factors and key enzymes involved in carbohydrate utilization and storage (Liang et al. 2001; Wang et al. 2003). The present results suggest that (1) activities of SuSy, AGPase, SSS, GBSS and SBE in large kernel types were slightly and significantly higher than those in small kernel types during early and mid-late filling period, respectively, which was in accordance with previous reports (Zhao et al. 2003; Gao et al. 2003), (2) the simulation with Richards' equation showed that it was accumulation duration and SAR rather than the initiation time of starch accumulation that determined the starch accumulation in large kernel types. Along the lines of the characterization of enzyme activities, it is believed that the physiological mechanism for higher starch accumulation in large kernel types was the more vigorous sink activities during mid-late period.

As shown in Fig. 8, the activities of enzymes in large kernel types were higher than those in small kernel types during mid-late grain-filling period (21–35 DAA). On the other hand, the SAR and accumulation of amylose and amylopectin in the large kernel types were higher than those in the small kernel types as early as initial filling stage, indicating that more endosperm cells (sink capacity) might have played a major role at early grain-filling stage in grains of large kernel types, whereas the higher starch accumulation resulted mainly from higher enzymes activities at mid-late grain-filling stage. However, the extent to which the amount of endosperm cells contributed to the grain growth requires further investigations in wheat cultivars differing in kernel types.

The present results reveal that the activities of enzymes involved in starch synthesis were enhanced by water deficit under rainfed conditions, which conform to previous reports. Mild drought were found to shorten grain duration, increase grain-filling rates and accelerate remobilization of non-structural reserve carbohydrates to grains, but severe drought significantly reduced the enzymes' activities (Xu et al. 2003; Yang et al. 2000, 2001). Furthermore, the present study implies that the effect of soil water status on the activities of enzymes varied with grain-filling stage. The rainfed conditions led to increase and decrease during early and late grain-filling stage in activities of SuSy, AGPase, SSS and SBE, respectively, which in good agreement with the SAR in grains. It can be concluded that the reduced activities of enzymes involved in starch synthesis, other than the shortage of sucrose was the overwhelming factor to limit the starch accumulation under rainfed conditions during late filling period.

Planting conditions were found to have an effect on the two kernels types assessed. Irrigated and rainfed conditions led to a significant increase and decrease, respectively, in activities of key enzymes in relation to starch accumulation in large kernel types during mid-late grain filling, indicating that large kernel types were sensitive to water supply. On the other hand, the activities of key enzymes in small kernel types also decreased under water deficit conditions during mid-late period; however the magnitudes of the decrease were lower than those in large kernel types. This infers that small kernel types were insensitive to water deficit. Therefore, it can be conclude that irrigation is beneficial for the production potential in large kernel types, whereas small kernel types were the better choice for rainfed cultivation.

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