

ACTIVITIES OF KEY ENZYMES INVOLVED IN STARCH ACCUMULATION IN SORGHUM (*SORGHUM BICOLOR* L. MOENCH) GRAINS WITH DIFFERENT STARCH CONTENTS

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Abstract

Starch accumulation and the seven metabolic enzyme activities in sorghum grains with different starch contents were investigated under field conditions in Shenyang Agricultural University. The results indicated that the starch accumulation in Tieza17 (high starch content variety) and Liaoza11 (low starch content variety) grains could be fitted with logistic equation. The activities of sucrose synthase (SS), soluble starch synthase (SSS) and uridine diphosphate glucose pyrophosphorylase (UGPase) in Tieza17 were similar to those in Liaoza11. Adenosine diphosphate glucose pyrophosphorylase (AGPase) and granule-bound starch synthase (GBSS) activities in Tieza17 grains were consistently higher than those in Liaoza11 grains. Starch branching enzyme (SBE) and starch debranching enzyme (DBE) activities increased or decreased significantly between the two varieties at different filling stages. It is suggested that the differences in the activities of AGPase, GBSS, SBE and DBE and their tradeoff played an important role in regulating starch accumulation in sorghum grains.

Introduction

Sorghum is considered as a multipurpose crop that provides food, feed, and raw materials for wine, fuel and industrial starch (Wang *et al.* 2015a). Starch is the main component of sorghum grains, which determines the quality of sorghum grains and has an important influence on grain yield, palatability and grain brewing (Xiao *et al.* 2019).

The raw material for starch synthesis originates with the synthesis of sucrose in the leaves (Chen *et al.* 2019). Sucrose is transported to storage organs (such as grains) via the phloem, and starch is then synthesized by a series of enzymatic reactions. Seven enzymes *viz.* sucrose synthase (SS), UDP-glucose pyrophosphorylase (UGPase), ADP-glucose pyrophosphorylase (AGPase), soluble starch synthetase (SSS), granule-bound starch synthase (GBSS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) are known to play critical role in starch synthesis. Fettke and Steup (2011) have shown that ADPG is a direct precursor to starch synthesis. A starch synthase catalyzes the reaction of ADPG with a starch primer and transfers a glucose molecule onto a starch primer to extend the starch chain. Starch synthases include SSS and GBSS. SBE has dual functions (Han *et al.* 2019). On the one hand, it can cleave the glucan linked by alpha-1,4 glycosides, including amylose and amylopectin straight chains. On the other hand, SBE can connect the cleaved short chains through the alpha-1,6 glycosides to the receptor chain, thus

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forming a branched sugar chain (Zeeman *et al.* 2010). SBE is the key enzyme affecting starch composition and structure in grains. DBE, which cleaves the alpha-1,6 glycosidic bond of amylopectin, can modify the structure of starch (Park *et al.* 2014). DBE mainly hydrolyzes the branch point of the limiting dextrin or the alpha-1,6 glycosides of the branching point of amylopectin and plays an important role in the decomposition of amylopectin. Although the effects of the environment on the activity of starch synthetases have been documented (Yi *et al.* 2014), but the information on role of these key enzymes in the starch synthesis of sorghum especially with different starch contents in field condition is not available.

Thus the present study was aimed to investigate the activities of the key enzymes involved in starch biosynthesis in sorghum and determine whether and how the changes in these enzyme activities were related to starch accumulation in two sorghum varieties differing in grain starch contents. The results may provide insights into the mechanisms by which starch accumulation is regulated in sorghum.

Materials and Methods

Field experiments were conducted at the experimental base of Shenyang Agricultural University in 2012 and 2013, respectively, in a completely randomized block design with three replicates per treatment. Two sorghum varieties, Liaozal1, with a low starch content, and Tieza17, with a high starch content, were used as test materials. The plot area was 18 m², the row spacing was 60 cm, the plant spacing was 18.5 cm, and the planting density was 90000 plants/ha. Diammonium phosphate (225 kg/ha) was used as the base fertilizer during sowing, and urea (225 kg/ha) was applied as the top dressing at the jointing stage. The sowing date was 6 May, and the harvest date was 25 September in 2012 and 2013.

Flowering plants of uniform size and age were selected and labeled. Three panicles were sampled every 7 days after flowering, and 15 grains from the upper and middle panicles were selected and weighed. The grains were frozen with liquid nitrogen and stored in a freezer at -70°C for enzyme activity determination. The rest of the grains were kept in kraft paper bags. The grains were killed at 105°C for 1 to 2 hrs and then dried to constant weight at 80°C for the determination of starch accumulation.

The amylose content and amylopectin content in the sorghum grains were spectrophotometrically determined by the double-wavelength method (He 1985).

Total starch content (%) = amylose content (%) + amylopectin content (%)

Starch accumulation (mg/seed) = starch content (%) × grain weight (mg/seed)

Taking the days after flowering (t) as the independent variable and starch accumulation (W) as the dependent variable, the process of starch accumulation in grains was simulated by the logistic equation: $W=K/(1+ae^{-bt})$, where K is the maximum value of accumulation, and a and b are parameters. Starch accumulation characteristic parameters were calculated according to the method of Yi *et al.* (2014).

The sucrose content was determined by the resorcinol method (Zhang *et al.* 2003). The preparation procedure was described by Cheng *et al.* (2003). A frozen sample of 15 grains was homogenized with a pestle in a precooled mortar containing 5 ml of 50 mmol/l Hepes-NaOH (pH 7.5), 28 mmol/l MgCl₂, 2 mmol/l EDTA, 10 g/l PVP-30, and 1 mmol/l DTT and was centrifuged at 2000 g at 4°C. The supernatant was then collected for the determination of AGPase, UGPase, SSS, SBE, DBE, and SS activities. The precipitate was suspended in 5 ml buffer solution and then used for the determination of GBSS activity. The method for determining activities of these enzymes was followed by Cheng *et al.* (2003).

Data were statistically analyzed with SPSS 19.0. As the data from both years showed the same tendencies, they were averaged. Means were compared by Duncan's new multiple range test at the significance level of 0.05 (Yi *et al.* 2014). The logistic curve equation of starch accumulation (W) with days after flowering (t) was fitted with CurveExpert software V1.3.

Results and Discussion

The accumulation of starch in the sorghum grains varied in an "S" curve with days after flowering (Fig. 1); therefore, the process was fitted by a logistic equation (Table 1). During the grain-filling stage, the accumulation of total starch, amylose and amylopectin in Tieza17 was consistently higher than that in Liaoza11. The maximum starch accumulation of total starch, amylose and amylopectin in Tieza17 and Liaoza11 was 29.60, 4.86 and 25.50 mg/seed, and 16.91, 2.66 and 14.39 mg/seed, respectively. The C_0 , V_{mean} and V_{max} of Tieza17 were higher than those of Liaoza11 (Table 2).

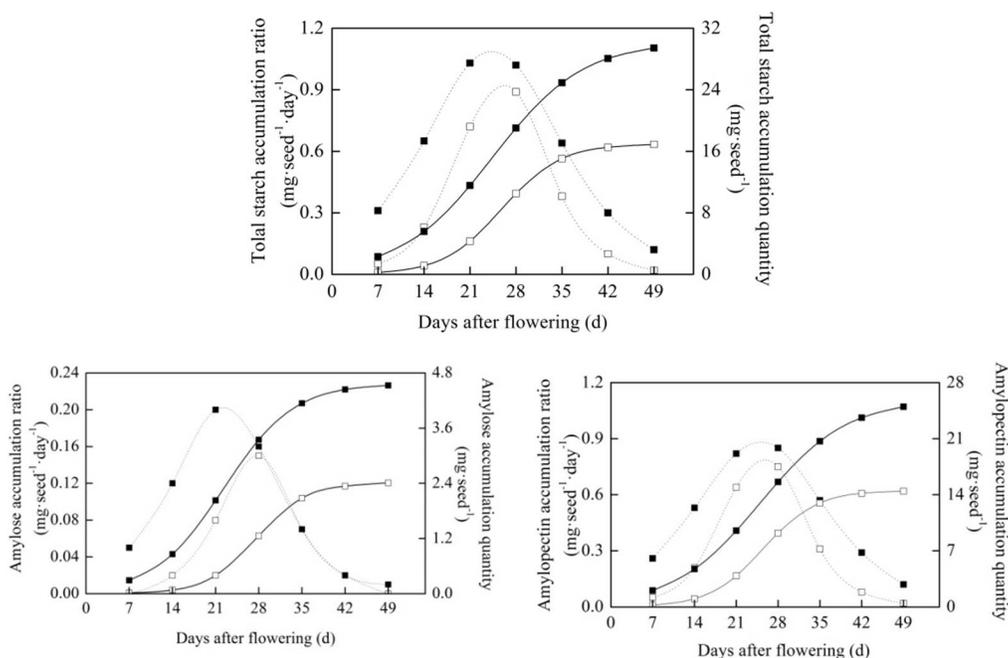


Fig. 1. Accumulation quantity and ratio of total starch, amylose and amylopectin in grains of two sorghum cultivars. Solid frames represent Tieza17, hollow frames represent Liaoza11. Dotted lines represent total starch accumulation ratio, solid lines represent total starch accumulation quantity.

The sucrose content in the two sorghum varieties decreased (Fig. 2). From the 21st day after flowering, the sucrose content in Liaoza11 was significantly higher than that in Tieza17, indicating that the metabolism of sucrose degradation in the high-starch sorghum grains was robust, which provided a good foundation for the accumulation of starch in grains.

Activities of SS and UGPase in Liaoza11 and Tieza17 were both reached a peak 21 days after flowering (Fig. 3). The change in SS and UGPase in the two sorghum varieties was consistent and basically synchronized with the starch accumulation rate, indicating that SS and UGPase were closely related to starch accumulation, but the differences in the activities of the two enzymes

were not significant between the two varieties, suggesting that they were not the reasons for the differences in starch accumulation in the two varieties, which was partly consistent with the findings of Ke *et al.* (2020) who found the UGPase activity was not different between sorghum varieties especially during early filling stage.

Table 1. Logistic equation of total starch accumulation, amylose accumulation and amylopectin accumulation quantity in sorghum grains.

Starch accumulation	Varieties	Logistic equation	Standard error	Correlated coefficient
Total starch accumulation	Tieza17	$W=29.60/(1+33.32e^{-0.145t})$	1.266	0.996**
	Liaozal1	$W=16.91/(1+343.2e^{-0.222t})$	1.082	0.992**
Amylose accumulation	Tieza17	$W=4.86/(1+38.09e^{-0.155t})$	0.380	0.987**
	Liaozal1	$W=2.66/(1+870.9e^{-0.240t})$	0.191	0.991**
Amylopectin accumulation	Tieza17	$W=25.50/(1+24.63e^{-0.123t})$	1.795	0.985**
	Liaozal1	$W=14.39/(1+297.5e^{-0.219t})$	0.835	0.993**

W, accumulation quantity; t, days after flowering; **, significant at 0.01 level.

Table 2. Starch accumulation characteristic parameters in sorghum grains.

Starch accumulation characteristic parameters	Total starch accumulation quantity		Amylose accumulation quantity		Amylopectin accumulation quantity	
	Liaozal1	Tieza17	Liaozal1	Tieza17	Liaozal1	Tieza17
C_0	0.049	0.863	0.003	0.142	0.048	1.043
T (d)	47.00	55.89	47.47	53.41	47.05	56.04
V_{mean} (mg/seed/d)	0.360	0.530	0.056	0.091	0.306	0.456
T_{Vmax} (d)	26.30	24.19	28.28	23.22	26.04	25.85
V_{max} (mg/seed/d)	0.939	1.073	0.159	0.187	0.787	0.788

C_0 : initial potential, T: accumulation duration, V_{mean} : mean accumulation rate, T_{Vmax} : time reaching the maximum accumulation rate, V_{max} : maximum accumulation rate.

AGPase is generally considered to be a key enzyme in the biosynthesis of starch and a rate-limiting enzyme in starch synthesis (Li *et al.* 2011, Tuncel and Okita 2013). Stark *et al.* (1992) reported that the AGPase gene was transformed into potato, and the starch content of transgenic potato increased by 30%. In the present experiment, the dynamic change in AGPase activity was consistent with the change in the starch accumulation rate and grain-filling rate. In addition, the difference in AGPase activities in the two varieties was significant during the whole filling stage, indicating that AGPase is the key enzyme that induced differences in the starch between the two sorghum varieties.

There was no significant difference in SSS activity between the two varieties. The SBE activity in Liaozal1 was higher than that in Tieza17 before 21 days after flowering, while the SBE activity in Tieza17 was significantly higher than that in Liaozal1 after 28 days after flowering (Fig. 3). The DBE activity in Liaozal1 was significantly higher than that in Tieza17 21 days after flowering. The main function of GBSS is to catalyze the synthesis of amylose (Zeeman *et al.* 2010). In this study, the activity of GBSS in Tieza17 was significantly higher than that in Liaozal1

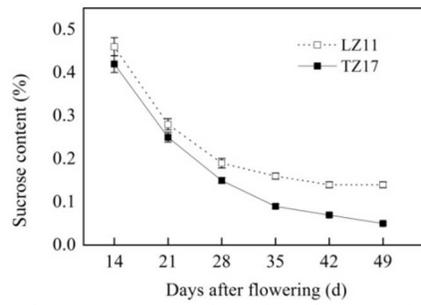


Fig. 2. Changes in sucrose content in grains of two sorghum cultivars. LZ11, Liaoza11; TZ17, Tieza17.

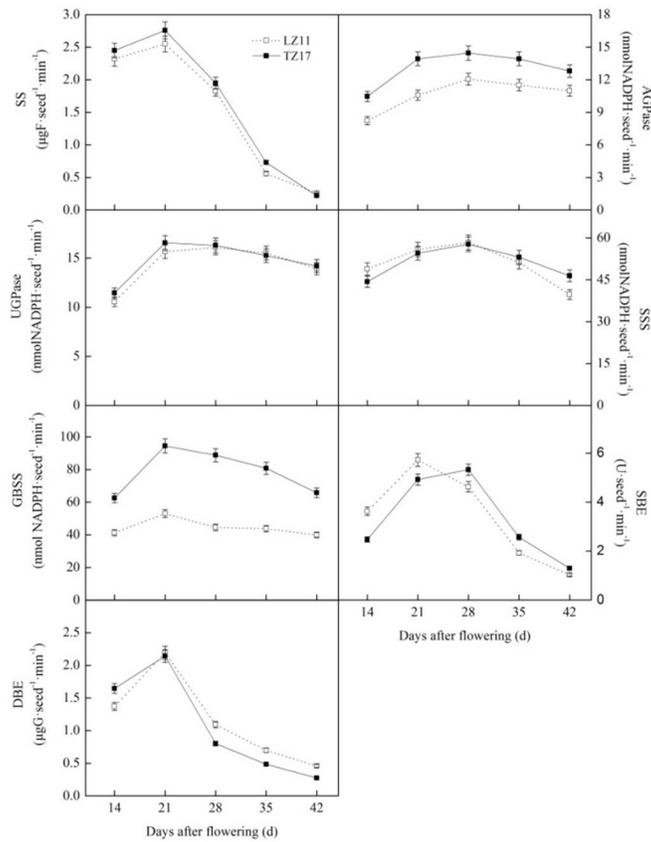


Fig. 3. Changes in activities of the enzymes involved in starch synthesis in grains of two sorghum cultivars. LZ11, Liaoza11; TZ17, Tieza17.

throughout the whole filling stage, but the difference in the amylose content in the two varieties was mainly observed at the early stage of grain filling, *i.e.*, during the first 28 days after flowering. This difference was mainly due to the relatively high activity of SBE in the Tieza17 grains at the late filling stage and the relatively low activity of DBE, which resulted in the difference in amylopectin accumulation and the amylopectin accumulation rate in both varieties. Li *et al.* (2005)

reported that the increase in DBE activity could not compensate for the effect of SBE activity on amylose, *i.e.*, the effect of SBE on the amylose content during grain formation was much higher than that of DBE on the amylose content. At the same time, it was also shown that although the difference in GBSS between the varieties was marked, the role of GBSS in starch synthesis at the late stage of filling was not as obvious as that of other enzymes due to the relatively low amylose content in the grains. It is suggested that the starch accumulation rate in grains and the accumulation of its components were not necessarily related to the activity of GBSS, SBE or DBE but were closely related to the tradeoff between the activities of these enzymes.

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