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RESERVE CARBOHYDRATES FROM KERNELS OF SUGARY AND SUGARY ENHANCER MAIZE

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Key Word Index—*Zea mays*; Gramineae; seed; starch; phytoglycogen; carbohydrate; sorbitol.

Abstract—Mature seeds of 'sugary' (*su*) and 'sugary–sugary enhancer' (*su–se*) maize inbreds were compared with respect to amount and properties of starch and glycogen. Sugars and sorbitol were also determined. Sucrose and maltose were elevated in the *su–se* seeds, starch was reduced and phytoglycogen was within the range expected for an *su* line. The *se* trait did not result in altered structure of the starch or phytoglycogen. Hence, it is unlikely that the observed increase in maltose resulted from premature action of amylases on starch and phytoglycogen during seed maturation.

INTRODUCTION

Earlier reports in this series were concerned with characterizing the 'sugary enhancer' (*se*) trait found in the 'sugary' (*su*) maize inbred IL677a. Comparisons of immature kernels revealed that IL677a possessed a higher sucrose level than other *su* lines [1–3]. IL677a was unique in that maltose appeared during seed maturation and increased until it comprised several percent of the weight of mature dry seeds. The other *su* lines tested contained only a trace of maltose, and the maltose level tended to drop as the seeds matured. After a genetic study indicated that *se* was a single gene recessive modifier of *su* [2], *se* was transferred successfully into a series of *su* inbreds [4]. Immature seeds of IL677a had the level of water-soluble polysaccharide expected of an *su* line, but starch was reduced [1]. Hence, the *se* trait resembles other high sugar mutants, such as *sh2* and *bt2*, in that sucrose is elevated and starch is reduced. However, *se* differs markedly from shrunken and brittle mutants, which accumulate neither phytoglycogen [5, 6] nor maltose [2].

The metabolic basis for *se* gene action has not been established. If a blockage in starch synthesis is involved, the cause is not reduced activity of ADP-glucose pyrophosphorylase [1], in contrast to shrunken and brittle [7, 8]. Premature action of α- and β-amylase activities in maturing *se* seeds could reduce the starch content and explain the appearance of maltose. In that case the starch remaining in mature seeds should show evidence of degradation, and water-soluble polysaccharides might consist of starch fragments rather than phytoglycogen. Phytoglycogen is a metabolic end-product since its structure precludes conversion to amylopectin [9]. Hence, premature action of β-amylase during maturation should shorten the outer chains of any phytoglycogen found in mature IL677a seeds. The present work was conducted to test these ideas.

RESULTS AND DISCUSSION

This study revealed that mature seeds of IL677a do not have reduced phytoglycogen, but there is less starch and higher total sugars compared to a typical *su* inbred (IL451b) when values are expressed as a percentage of dry wt (Fig. 1, bottom). These results confirm and extend data from earlier work [1] which utilized immature seeds. The carbohydrate differences between the two inbreds are seen

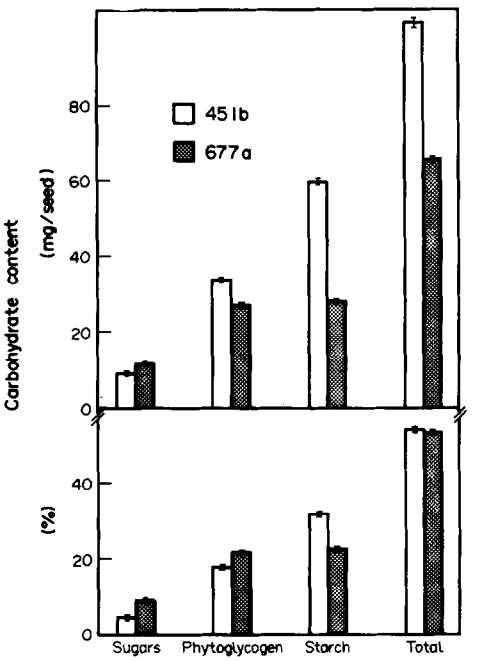

Fig. 1. Content of total sugars, phytoglycogen and starch in mature dry seeds of the *su* inbred lines IL451b and IL677a. Average seed weight was 187 mg/seed for IL451b and 123 mg/seed for IL677a. Sequential extraction with 80% ethanol, 10% ethanol, and 90% DMSO provided the fractions designated sugars, phytoglycogen and starch. The carbohydrate content of each fraction was determined and the values expressed as mg carbohydrate per seed (top) or percentage of seed dry wt (bottom).

more clearly when results are expressed as mg/seed (Fig. 1, top). The IL677a seeds were 34% lighter than IL451b. *Ca* 50% of this reduction was due to reduced starch, with relatively little effect on phytoglycogen content per seed. The analysis of individual sugars (Fig. 2) confirmed that this sample of IL677a seed resembled the mature seed lots, analysed earlier [2, 3], in having high maltose and sucrose compared to other *su* inbreds. Sorbitol was present in both maize lines, in agreement with a recent report concerned with this hexitol [10].

Similar profiles were obtained when the water-soluble polysaccharides from the two *su* inbreds were compared using gel filtration (Fig. 3). Two distinct size classes of phytoglycogen were observed, with the first eluting in fractions 10–13 and the second in fractions 14–30. The water-soluble polysaccharide of an *su* conversion of IA453 was earlier reported to contain the same two classes of phytoglycogen, designated I and II in order of elution [11]. Water-soluble polysaccharide from the two inbreds was further characterized with respect to average chain length, percentage of conversion to maltose and iodine spectrum (Table 1). The iodine spectra were identical, and maximum absorption occurred at the wavelength expected for phytoglycogen [11, 12]. Close agreement to expected values was also obtained for the two inbreds regarding average chain length [11, 13, 14], and size of the β-amylase limit dextrin was not reduced in IL677a.

Starch samples from the two inbreds were similar, and each contained two size classes of molecules that were separable by gel filtration (Fig. 4). The broad smaller class (fractions 20–40) corresponds to amylose, and the sharply defined peak eluting between fractions 10 and 15 corresponds to amylopectin. A portion of the class I phytoglycogen is sedimented by high speed centrifugation [11, 15], so the high MW fractions of Fig. 4 are expected to contain some phytoglycogen. IL451b has an increased proportion of this material compared to IL677a as well as a lower proportion of class I phytoglycogen in the water-soluble fraction (Fig. 3). This difference between inbreds probably is caused by a slight difference in solubility of the phytoglycogen. Differences of similar magnitude were also observed among phytoglycogens of various *su* lines [11].

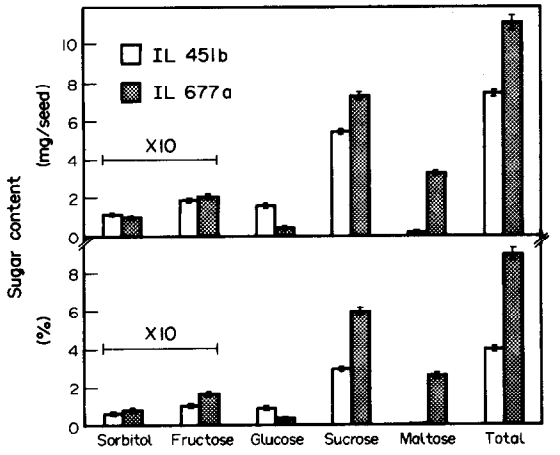

Fig. 2. Sugar and sorbitol content of mature dry seeds of IL451b and IL677a. The 80% ethanol fraction from Fig. 1 was analysed by GC, and details are given in the text.

Table 1. Characterization of water-soluble polysaccharide extracted from seed of IL451b and IL677a maize

Line	Average chain length	Conversion to maltose by β-amylase	Iodine spectra A_{max} (nm)	
			$-CaCl_2^*$	$+CaCl_2^*$
IL451b	14.0	48%	460	450
IL677a	13.6	45%	460	450

*Iodine reagent made in double distilled water ($-CaCl_2$) or saturated calcium chloride ($+CaCl_2$).

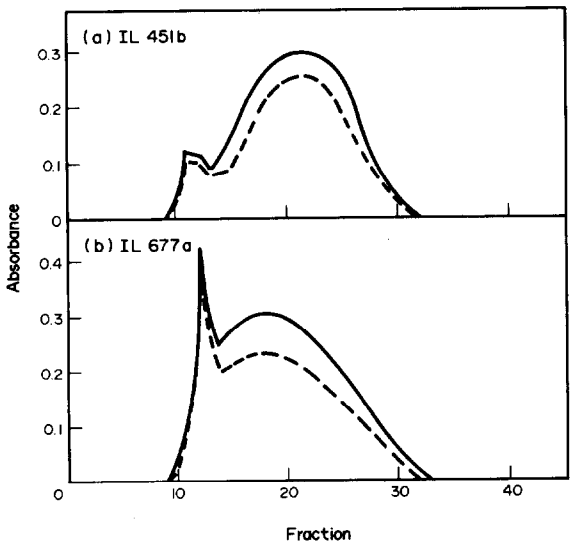

Fig. 3. Gel filtration of phytoglycogen from seeds of IL451b and IL677a. The 10% ethanol fractions from Fig. 1 were used. Samples were prepared and fractionated on Biogel $A_{50\,m}$ columns. *A* values are given for 480 (——) and 540 nm (– – –).

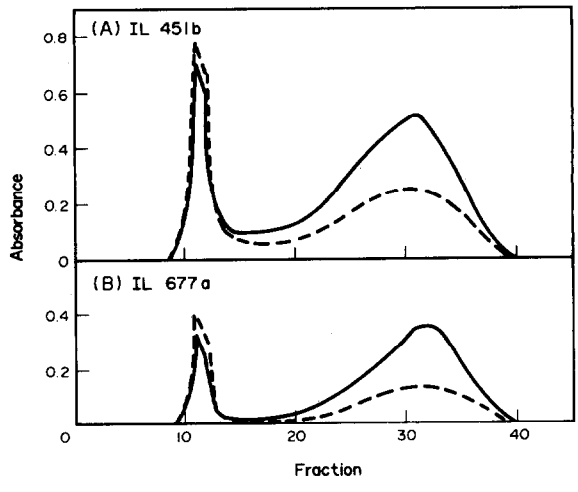

Fig. 4. Gel filtration of starch from seeds of IL451b and IL677a. The 90% DMSO fractions from Fig. 1 were chromatographed on Biogel $A_{50\,m}$ columns. *A* values are given for 540 (– – –) and 660 (——) nm.

The results presented above show clearly that seeds of IL677a possess starch and phytoglycogen with properties typical of the *su* genotype. Premature action of α-amylase is an unlikely cause of reduced starch in IL677a seeds, since partially degraded starch was not found in the dimethyl sulfoxide extract, and none was detectable in the water-soluble polysaccharide. Since the outer chains of IL677a phytoglycogen were not shortened, it is also unlikely that premature action of β-amylase is responsible for the increase in maltose that occurs during maturation. Hence, it is likely that maltose is an early product of the hexoses moving into IL677a endosperm, but this idea remains to be confirmed experimentally. It will also be important to establish whether sucrose and maltose increase together due to diversion of hexose from starch synthesis or whether increased synthesis and turnover of one disaccharide leads to accumulation of the other. A satisfactory explanation of why these disaccharides are elevated must take into account the observation that accumulation of only one of the two reserve polysaccharides is adversely affected.

EXPERIMENTAL

Mature dry seeds of the inbreds IL677a and IL451b, produced in 1979 at Urbana, Illinois, were obtained from Professor A. M. Rhodes. Sugars, water-soluble polysaccharides and starch were obtained by sequential extraction with 80% (v/v) EtOH, 10% (v/v) EtOH and 90% (v/v) dimethyl sulfoxide (DMSO) [1, 3, 16]. The seeds were ground in a Wiley mill equipped with a 20-mesh screen, and duplicate 1.5 g samples of each variety were blended for 2 min at top speed in a Sorvall Omnimixer with 25 ml hot 80% EtOH. Homogenates were centrifuged (13 000 *g*, 10 min, 7°), supernatant fluids were decanted and pellets were rinsed with three additional portions of hot 80% EtOH. The supernatant fluids from each sample were combined and made to a final vol. of 100 ml. Each pellet was suspended in 25 ml 10% EtOH and stored overnight at 3° to allow water-soluble materials to dissolve. The suspensions were clarified by centrifugation, the pellets immediately rinsed with three additional portions of 10% EtOH, and the combined supernatants made to 100 ml. The remaining pellets were suspended in 20 ml 90% DMSO and incubated for 2 hr at 70° with constant stirring. Insoluble materials were removed by centrifugation (13 000 *g*, 10 min, 20°), the 70° incubation was repeated twice with 15 ml portions of 90% DMSO, and the combined supernatants were made to a final vol. of 50 ml. Total carbohydrate content of the various extracts was determined by the PhOH–H_2SO_4 procedure [17] with D-glucose as the standard. Individual sugars and sorbitol in the 80% EtOH extracts were determined by GC of their TMS derivatives [3, 10]. Polysaccharides in the 10% EtOH extracts and 90% DMSO extracts were fractionated by gel filtration on 1.5 × 30 cm columns containing Biogel $A_{50\,m}$ [11]. The columns were equilibrated before use with 0.01 M NaOH that contained 0.02% sodium azide, and the same soln was used to elute polysaccharides. Starch and phytoglycogen samples were ppted with EtOH and dissolved in 0.01 M NaOH in preparation for CC. Polysaccharide samples (5 mg in 0.5 or 1.0 ml of soln) were loaded onto the columns, and 1.0-ml fractions were collected. Polysaccharides were located in the fractions using iodine reagent prepared with double distilled H_2O or satd $CaCl_2$ [12].

Polysaccharides (5–10 mg/ml) were treated with β-amylase (Calbiochem, La Jolla, CA) or *Pseudomonas* isoamylase (Hayabashi Biochemical Labs., Okayama, Japan), using standard exptal conditions [18, 19], and the reactions were stopped by boiling for 10 min. Total carbohydrate was determined by the anthrone procedure [20] and reducing sugars by Nelson's procedure [21]. Percentage of conversion to maltose was calculated from results of β-amylase digestions, and average chain lengths were calculated from results of isoamylase digestions.

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