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Analysis of Endosperm Sugars in a Sweet Corn Inbred (Illinois 677a) Which Contains the Sugary Enhancer (*se*) Gene and Comparison of *se* with Other Corn Genotypes[1]

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ABSTRACT

The endosperm sugars of a new corn (*Zea mays* L.) mutant, sugary enhancer (*se*), were analyzed by gas-liquid chromatography and were compared with sugars of other genotypes. Illinois 677a, a sugary (*su*) inbred containing the *se* gene, was high in sucrose and was distinguished from all of the other genotypes by its high maltose content. During kernel development, the maltose content of IL677a increased to 3.28% dry weight at 40 days postpollination and remained high at the dry mature stage, whereas 'Silver Queen,' a high quality sugary (*su*) hybrid not possessing the *se* gene, showed no such trend in maltose accumulation. Sucrose, fructose, and glucose decreased during kernel development in 'Silver Queen' and IL677a from 19 days postpollination until the dry mature stage. The slow drying characteristic and the reduced starch content previously reported for maturing seeds of IL677a may be related to the maltose accumulation reported here.

Sweetness and good texture are two important characteristics of a high quality vegetable corn. The two most frequently used commercial mutants are *su* and *sh2*.[2] The *su* genotype accumulates large amounts of phytoglycogen (13), a factor in the texture of the edible product, but produces a lower level of endosperm sugars than the *sh2* mutant which is low in phytoglycogen (4, 11).

A new mutant, *se*, was discovered to have characteristics that distinguish it from previously known maize mutants (7, 8). The *se* gene is a recessive modifier of the *su* gene (1, 7). Illinois 677a, an *su se* inbred, has a sugar content comparable to that of *sh2*, yet its phytoglycogen content equals that of *su Se* genotypes (8). The kernels of IL677a were also lighter in color and slower to dry than normal sugary kernels (6, 8).

The present study was initiated to characterize further the changes in sugars of IL677a during kernel development and the manner in which *se* gene is expressed. GLC was used as a rapid and quantitative technique for the analysis of maize sugars.

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[2] Abbreviations: *su:* sugary; *sh2:* shrunken-2; *se:* sugary enhancer; *ae:* amylose extender; *du:* dull; *h:* horny; *wx:* waxy; *wx*^{a}*:* waxy-a; *Su:* starchy; *o2:* opaque-2; *fl:* floury; *fl2:* floury-2; bt2: brittle-2; DAP: days after pollination; TMS: trimethylsilyl; TSIM: trimethylsilylimidazole; RT: retention time.

MATERIALS AND METHODS

Reagents. Column-packing materials, sugar standards, and sugar derivatives were from Pierce Biochemical Co., Rockford, Ill. The reagents were Stox-Oxime Internal Standard Reagent (25 mg hydroxylamine hydrochloride/ml pyridine and 6 mg phenyl-β-D-glucopyranoside/ml as internal standard), *N*-trimethylsilylimidazole, and Sugar Standard Calibration Mixture-Group I (10 mg each of D-fructose, D-glucose, and sucrose/ml pyridine). The stock concentration of all other sugars for gas chromatography was 1 mg/ml in water, the standards for paper chromatography were 10 mg/ml, and all were stored at −18 C. Columns were packed with 3% OV-17 (80/100 mesh; Chromosorb W [HP]).

Plant Material. Various maize (*Zea mays* L.) lines were grown as the seed sources of endosperm materials. The source of *su se* was IL677a, a selection from a three-way cross of (Bolivia 1035 × IL44b) × IL442a. The sources of *su Se* were 'Silver Queen,' a high quality hybrid and the inbred IL451b. Other endosperm mutants were backcross recoveries of IL451b.

Hand-pollinated ears were picked, husked, and promptly frozen in liquid N_2 in the field. Ears were then placed on dry ice and transported to cold storage at −18 C. For the weekly developmental series, all ears of 'Silver Queen' and IL677a were pollinated on July 31, 1976, and two ears/genotype were harvested at weekly intervals beginning at 19 DAP. All other ears were harvested at predetermined stages of development and frozen for later analysis. In all crosses the ear parent is designated first.

Extraction and Analysis of Corn Sugars and Sugar Standards. Approximately 5 g of frozen kernels or 3 g of dry kernels from the middle of the ear were weighed and ground in a Sorvall Omni-Mixer at maximum speed with 25 ml of boiling 80% ethanol (8). Corn harvested sooner than 30 DAP was ground 2 min and corn harvested past 30 DAP was ground 5 min. Dry kernels were softened for 5 min in boiling 80% ethanol prior to grinding. The extract was centrifuged at 17,000*g* for 6 min at 20 C and the supernatant fluid poured off and saved. The pellet was then resuspended with 25 ml of boiling 80% ethanol and the procedure repeated three times. Four extractions were sufficient for complete recovery of the sugars at all stages of development (6). The four supernatant fluids were combined and diluted to 100 ml. A portion of that extract (0.1 ml) was evaporated overnight in a 0.3-ml vial at 70 C. Upon removal from the oven, the vials were immediately capped with a septum, cooled, and stored at room temperature until derivatization. Two extractions were made per ear for the developmental series of IL677a and 'Silver Queen,' with one extraction made per ear otherwise.

Oximes of the reducing sugars were prepared by addition of 0.1 ml of the Pierce Stox-Oxime Reagent to the dry samples in the vials (12). The samples were then heated for 15 min at 70 C and cooled to room temperature. TMS-sugars were formed by the

addition of 0.1 ml TSIM, shaken vigorously for 30 s, and left to incubate for 15 min at room temperature (2, 15). Two derivatizations were made per extraction. After vigorous mixing, 1 μl of the derivatized sugars was injected into a Hewlett Packard model 5830 gas chromatograph. Due to the instability of the derivatives, all chromatograms were done within 3 h of completion of silylation. For the developmental sequence, two injections were made per derivatization. Otherwise, only one injection was made per derivatization.

Figure 1 shows the standard program used for all analyses reported in this paper. This temperature program separated all of the corn sugars in the shortest possible time yet maintained adequate resolution of the sugar peaks. Standard curves were prepared for the sugars actually found in corn extracts, and the detector response was determined for each sugar relative to the internal standard. The gas chromatograph was programed with the detector response, expected retention time of each sugar, and the dry weight of each corn sample. At the end of the run, the instrument computed and automatically printed all of the numerical values given in the inset of Figure 1.

The observed retention times (min) of the TMS-sugar-oximes on 3% OV-17 were: D-fructose, 3.00; L-sorbose, 3.11; D-glucose, 3.64; D-galactose, 3.99 and 4.21; D-mannose, 3.94 and 4.26; phenyl-β-D-glucopyranoside (internal standard), 7.87; sucrose, 9.54; trehalose, 10.32; lactose, 10.44; maltose, 10.85; turanose, 10.85; gentiobiose, 11.54 and 11.69; isomaltose, 11.65, 12.11, and 12.31; melibiose, 12.53 and 12.83; and raffinose, 16.31. The detector response (area units) for the sugars found in the corn extracts relative to the internal standard (μg/μl) was: D-fructose, 1.54; D-glucose, 1.65; sucrose, 1.28; and maltose, 1.13. The standard curves of glucose, fructose, sucrose, and maltose were linear from 0.05 to 10.00 μg/μl.

Paper Chromatography. Descending paper chromatography was run on Whatman No. 1 paper in a solvent system of 1-butanol-ethanol-water (40:11:10, v/v) (9). One μl of each of the sugar standards and the 10-fold concentrated corn extracts was applied at the origin. After development (50 h) and drying, the sugar spots were visualized by the silver nitrate method (14). Distances (cm) of the various sugar standards from the origin were: maltose, 4.8; sucrose, 7.5; turanose, 7.6; glucose, 13.4; and fructose, 15.8.

RESULTS AND DISCUSSION

Illinois 677a and 'Silver Queen' were compared for changes in sugar content, beginning at 19 DAP and continuing to the dry mature stage (Fig. 2). Glucose, sucrose, and fructose continually decreased in both from 19 DAP to the dry mature stage. The glucose level of 'Silver Queen' was initially 3.5 to 4 times higher than that of IL677a, but decreased more rapidly until at the dry mature kernel stage it was essentially the same in both—less than 1% dry weight. The initial fructose content of 'Silver Queen' was 2.5 times higher than that of IL677a but both decreased to less than 0.5% dry weight at the dry mature stage. Fructose was higher than glucose in IL677a until 54 DAP and in 'Silver Queen' until 41 DAP.

Sucrose, unlike the hexoses, was nearly five times higher in IL677a than in 'Silver Queen' at 19 DAP and remained higher throughout the maturation process. Even when the kernels were dry, the sucrose content of IL677a was 7.28% dry weight, nearly 2.5 times that of 'Silver Queen.'

The large peak with a retention time of 10.85 min (Fig. 1) was first observed in extracts of IL677a during this experiment. Of the many hexoses and disaccharides for which retention times were determined, only maltose and turanose corresponded to the unknown peak. None of the other sugars tested were found in any of the corn extracts. Further experiments proved that the unknown peak in IL677a was maltose and not turanose. Extracts from dry mature kernels of IL677a, 451b *su* and 451b *sh2* were chromatographed on paper with 1-butanol-ethanol-H_2O. Only IL677a produced a sugar spot with the same mobility as the maltose standard. The suspected maltose spot in IL677a was eluted from the paper and studied further. When part of the eluate was derivatized, only the peak at 10.85 min was seen on the gas chromatogram. Treating

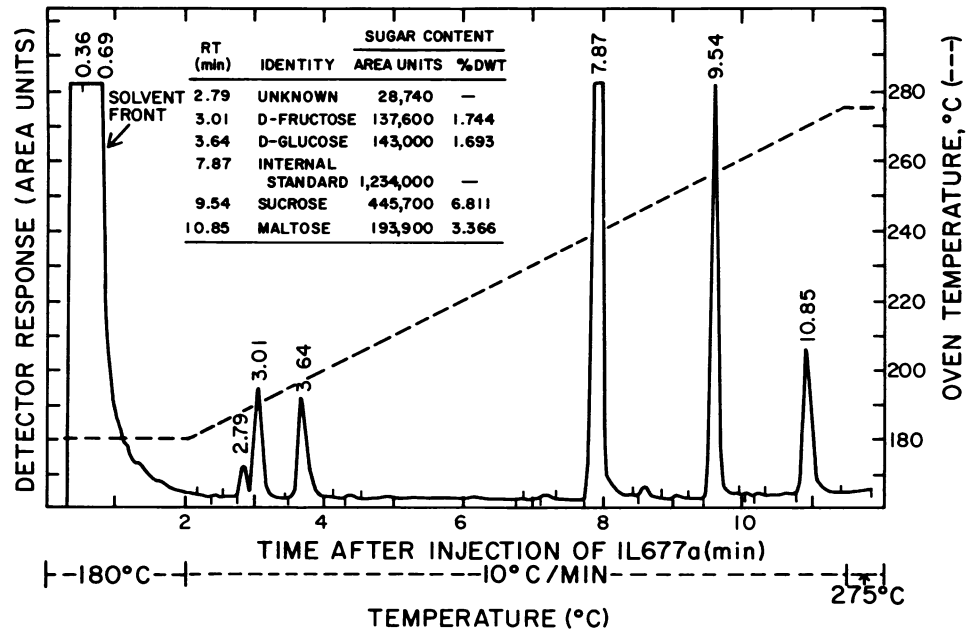

FIG. 1. Gas chromatogram of TMS-sugar derivatives of seeds from IL677a at 41 days postpollination. The small vertical lines on either side of each sugar peak indicate the duration of integration. Column: 183 cm × 3.2 mm i.d., glass, packed with 3% OV-17 on (80/100 mesh Chromosorb W [HP]). Oven temperature program: isothermal at 180 C for 2 min; programed at 10 C/min to 275 C; isothermal for 0.4 min. Flame ionization detector temperature: 300 C. Injection port temperature: 285 C. Carrier gas: helium (24.4 g/cm² pressure) at 20 cc/min. FID gases: hydrogen, 7.3 g/cm² pressure; air, 11.7 g/cm² pressure.

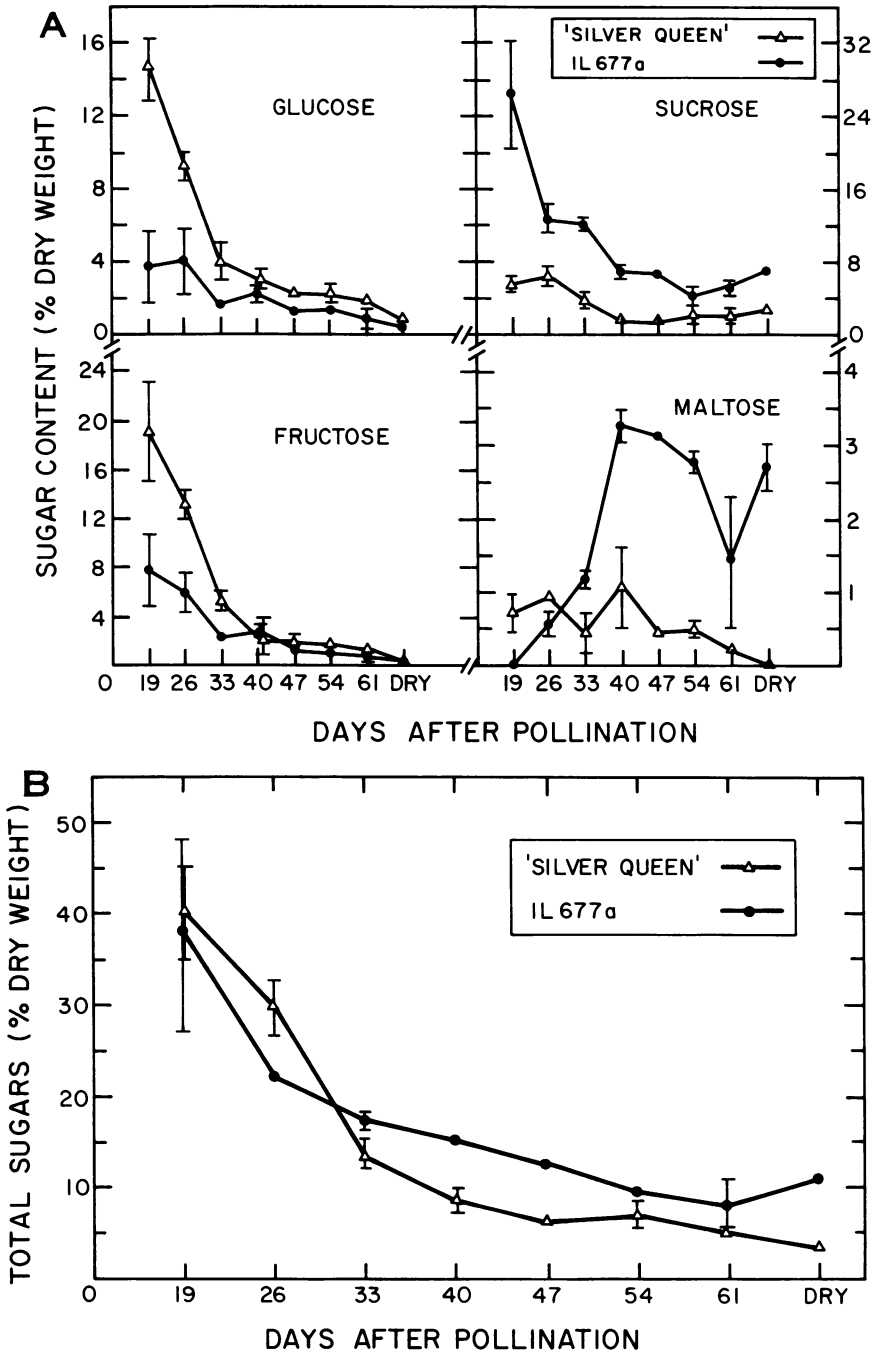

FIG. 2. Sugar content of maturing seeds of IL677a and 'Silver Queen.' Vertical bars indicate the difference between duplicate ears. A: individual sugars; B: total sugars.

the eluate with α-glucosidase caused the expected amount of glucose to appear, and no other new compound was seen on the gas chromatogram. When oxime formation was omitted from the derivatization procedure, the retention times and area ratios of the two anomeric peaks were essentially identical for the TMS-maltose standard (10.14 and 10.40 min and a ratio of 0.93) and the suspected TMS-maltose of IL677a (10.12 and 10.38 min and a ratio of 0.97).

A sharp contrast was found in the developmental patterns of maltose content in IL677a and 'Silver Queen.' No maltose was detected in IL677a at 19 DAP, but the level increased rapidly to 3.28% dry weight at 40 DAP (the stage just prior to shriveling of kernels in the field) and remained high thereafter. 'Silver Queen' contained some maltose at the earliest stage measured, but the maltose level decreased over time until none was detected in the dry seed. Analysis of fresh and frozen ears from the 1977 plantings confirmed the unusual trend in maltose content seen in IL677a. Liquid endosperm removed from kernels still on the IL677a plant also contained maltose. These additional experiments indicated that maltose was not a by-product of faulty extraction procedures or improper freezing technique.

The discovery of large quantities of maltose in IL677a was unexpected. Maltose has twice been reported to be present in the developing corn endosperm but only in minor quantities (4, 13) compared to the values reported here for IL677a. Specific mutants and mutant combinations varied in their maltose content, *su* having no maltose (4). Maltose is, in fact, rarely found as a major sugar constituent in any fruits or vegetables (10).

The large accumulation of maltose in IL677a could be due to β-amylase action on starch in the endosperm of the developing kernel. No evidence in this study indicated the presence of maltotriose or higher maltodextrins, which are characteristic end products of α-amylase. β-Amylase has been detected in the endosperm of developing and mature maize kernels, although no data on changes during maturation have been reported (3, 5, 16). Further investigation may reveal that the reduced starch content caused by the *se* gene is due to enhanced starch degradation rather than a reduction in rate of biosynthesis.

The total sugar content of both 'Silver Queen' and IL677a decreased from 19 DAP until the dry mature stage (Fig. 2B). The total sugar content of 'Silver Queen' was higher than that of IL677a prior to 33 DAP when maltose began dramatically increasing.

In general, the changes in individual sugars (glucose, fructose, and sucrose) and the total sugars reported here are consistent with published data (4, 8, 11). The gas chromatography data of this study confirm the colorimetric work on IL677a by Gonzales *et al.* (8). The colorimetric analysis did not reveal maltose.

The slow drying nature of IL677a and its extended period of edibility could result from an osmotic retention of moisture caused by the genotype's unusually high level of soluble carbohydrates (sugars and phytoglycogen). Maltose accumulation is greatest just prior to the time of kernel shriveling, and this accumulation may be a major factor in causing IL677a to dry more slowly than other genotypes.

The effect of the *se* gene on sugars of developing maize kernels was studied with F1 ears of IL677a crosses harvested at 24 DAP. All genotypes were in an *su* background, and the endosperm dosage of *se* varied from 0 to 3. The results are summarized in Table I. IL677a possessed a higher level of sucrose and maltose than all of the other genotypes. The *se se se* endosperm (IL677a) was higher for all sugars than any of the *Se Se Se* endosperms. When comparisons are made between reciprocal crosses of the same parentage, all *Se se se* endosperms were higher in sucrose, maltose, and total sugars than *Se Se se* endosperms. In the *Se se se* endosperm, the presence of one dose of *o2* or *f12* also enhanced sucrose, maltose, and total sugar accumulation more than in any of the other crosses. Principal component analysis which summarizes trends in similarity among variables and individuals was performed on this data. The results showed clearly that *se se se*

Table I. Sugars of IL677a, IL451b, IL451b f1, IL451b f12, IL451b o2 and reciprocal crosses at 24 days post-pollination.

Genotype[a]	Ear	se[b]	Percent dry weight				
			Frc	Glc	Suc	Malt	Total Sugars
677a	1	3	2.05	1.27	14.94	0.63	18.89
	2	3	3.10	1.61	18.01	0.70	23.42
	3	3	1.99	0.96	17.08	0.70	20.73
451b	1	0	1.03	0.44	5.99	0.05	7.51
	2	0	0.79	0.33	6.20	0.02	7.34
	3	0	1.02	0.54	5.88	0.02	7.46
	4	0	1.03	0.61	6.15	0.09	7.88
451b f1	1	0	1.11	0.40	7.27	0.02	8.80
	2	0	0.81	0.21	6.82	0.05	7.89
	3	0	0.87	0.29	8.54	0.02	9.72
451b f12	1	0	0.90	0.29	8.24	0.10	9.53
	2	0	0.95	0.21	10.54	0.20	11.90
	3	0	0.58	0.22	6.24	0.13	7.17
	4	0	0.81	0.21	10.34	0.18	11.54
451b o2	1	0	0.94	0.32	9.06	0.04	10.36
	2	0	1.03	0.19	8.27	0.02	9.51
451b X 677a	1	1	1.01	0.60	6.30	0.04	7.95
	2	1	1.19	0.69	5.56	0.03	7.47
677a X 451b	1	2	1.34	1.03	8.64	0.23	11.24
	2	2	1.37	0.83	9.35	0.21	11.76
451b f1 X 677a	1	1	0.91	0.46	6.60	0.01	7.98
	2	1	2.73	1.30	8.21	0.01	12.25
677a X 451b f1	1	2	1.31	0.85	9.17	0.06	11.39
451b f12 X 677a	1	1	1.08	0.84	4.78	0.06	6.76
	2	1	1.10	0.72	5.79	0.12	7.73
677a X 451b f12	1	2	1.45	1.20	11.02	0.31	13.98
	2	2	1.88	1.48	14.44	0.30	18.10
451b o2 X 677a	1	1	1.40	1.37	4.87	0.00	7.64
	2	1	1.82	1.24	6.12	0.03	9.21
677a X 451b o2	1	2	2.24	1.59	12.06	0.29	16.18
	2	2	2.72	2.20	12.56	0.52	18.00

[a] All genotypes are in an su background; female parent designated first.

[b] Refers to the number of doses of se in the triploid endosperm.

endosperms were distinct from all other endosperm populations (6). In addition, *Se se se* endosperms were distinctly different from both homozygous endosperms. So although *se* is recessive to *Se*, the dominance of the latter is not complete.

The *se se* genotype is distinguished from other common mutant genotypes at the edible stage due to its accumulation of sugars, particularly maltose. The *se* gene may, with further study, aid in elucidating the pathways of starch and phytoglycogen metabolism in maize.

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