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Fluorometric Sucrose Evaluation for Sugar Beet

DANIELE TREBBI[†] AND J. MITCHELL MCGRATH*,[§]

Department of Crop and Soil Sciences, Plant Breeding and Genetics Graduate Program, Michigan State University, East Lansing, Michigan 48824-1325, and Sugarbeet and Bean Research Unit, USDA-ARS, 494 PSSB, Michigan State University, East Lansing, Michigan 48824-1325

Sucrose is the economic product from sugar beet. Disease resistance is often available in low-sucrose genotypes and, prior to the deployment of such novel genes as available into the cultivated spectrum, selection for increased sucrose content is required during introgression. The objective of this work was to evaluate a relatively rapid and inexpensive enzymatic−fluorometric microtiter plate assay for sucrose quantification in sugar beet root dry matter, both for progeny testing in the greenhouse and for evaluation of field-grown mother roots. As determined using HPLC, sucrose content in diverse populations of sugar and table beet assayed over various developmental stages ranged from 0.213 to 2.416 mmol g^{-1} of dry matter, and these values were used as references for both refractometry and enzymatic−fluorometric assay. As expected, refractometric analysis generally overestimated sucrose content. Enzymatic−fluorometric analyses were reasonably well correlated with HPLC results for young greenhouse-grown root tissues ($R^2 = 0.976$), and less so with older field-grown roots ($R^2 = 0.605$), for unknown reasons. Enzymatic−fluorometric assays may be best deployed for progeny testing of young seedlings.

KEYWORDS: HPLC; fluorometry; enzymatic assay; glucose; sucrose; fructose; raffinose; sugar analysis; Amplex Red; resorufin

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) has been selected for high sucrose content as a source of sweetener in human diets for the past two centuries; sucrose in fresh beets increased from ∼6% [fresh weight (FW)] in early selections to >18% (FW) in many modern hybrids (*1*). Public sugar beet breeding programs generally focus on improving germplasm with resistance to biotic and abiotic stress. Introgression of favorable alleles and novel genes from lower sucrose content wild genotypes, usually accomplished through backcross breeding, invariably reduces sucrose content in elite germplasm. Reselection for sucrose content, while maintaining characters of interest during back-crossing, is required in the return to agronomic and economic performance, and here efficiencies in the selection process are needed.

The generally accepted method for industrial sugar quantification has been polarimetry (*2*, *3*). Polarimetry can be cumbersome, especially during selection in early generations when seed quantity and quality may be limited, because a relatively large number of beets (∼10−15) are needed for adequate juice sample volumes, and measurements can be skewed by optical activity of other sugars, particularly glucose and raffinose (*4*). Refractometry is frequently used to estimate soluble solid content in crude extracts (*5*), but the lack of sucrose specificity and additional interfering compounds (*6*) make refractometry less suitable than polarimetry for determining the sucrose content in sugar beets.

Both polarimetry and refractometry are available for analyses of unprocessed beets; however, water content in fresh sugar beet roots (75−80%) has a major influence on sucrose content as well as root yield. Sucrose content in fresh roots has been consistently (negatively) correlated with root yield across elite breeding lines and over multiple environments (*7*−*9*); however, this relationship has been suggested to be a pseudocorrelation (*10*). If so, breeding and selection methods geared toward analyses of sucrose and non-sucrose dry matter may be expected to reveal additional opportunities for genetic gains, as well as perhaps assist in the return to elite breeding line status from crosses with wild and unadapted germplasm.

Methods to examine sucrose as a proportion or as a total yield of dry matter (DM) in sugar beet roots have received some attention (*11*, *12*), but it is not routinely practiced because an extra processing step is required (i.e., drying the tissues) and sugar factories operate exclusively on fresh postharvest materials. Among analytical methods to view sucrose as a proportion of DM, highest sensitivity and specificity are achieved by chromatographic analyses [e.g., high-performance liquid chromatography (HPLC)] (*13*). Time and labor costs for sample preparation and sequential analyses (∼12 min per sample) limit the use of HPLC to analyses of relatively small populations (*14*). Enzymatic assays, based on phosphorylation or hydrolysis

* Corresponding author [telephone (517) 353-9262; fax (517) 337-6782; e-mail mitchmcg@msu.edu].
[†] Michigan State University.
[§] U.S. Department of Agriculture.

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Table 1. Mean Dry Matter Sucrose Contents Measured with HPLC and EFA in Five Germplasm Lines at Seven Developmental Points

entry	WAE[a]	no. of samples	no. of roots/sample[b]	HPLC %	SD[c]	HPLC mmol g^{-1}	SD	EFA[d] %	SD	EFA mmol g^{-1}	SD
SR96	3	2	58 + 54	10.75	1.19	0.31	0.03	10.30	0.78	0.30	0.02
	4	2	27 + 25	27.10	0.29	0.79	0.01	26.00	0.34	0.76	0.01
	5	2	6 + 5	38.48	0.53	1.12	0.02	37.25	0.92	1.09	0.03
	6	2	3	48.07	2.53	1.40	0.07	48.39	0.91	1.41	0.03
	7	2	3	57.70	0.95	1.69	0.03	55.86	1.20	1.63	0.04
	9	2	3	58.19	2.13	1.70	0.06	58.95	0.74	1.72	0.02
	18	10	0.5[e]	68.08	4.50	1.99	0.13	66.42	8.38	1.94	0.24
USH20	3	2	47 + 45	10.64	2.11	0.31	0.06	10.05	1.06	0.29	0.03
	4	2	24 + 22	23.84	2.42	0.70	0.07	24.39	0.29	0.71	0.01
	5	2	10 + 7	48.36	16.32	1.41	0.48	47.94	16.64	1.40	0.49
	6	2	3	55.75	3.70	1.63	0.11	54.57	2.09	1.59	0.06
	7	2	3	53.55	1.07	1.56	0.03	58.05	3.04	1.70	0.09
	9	2	3	58.07	4.10	1.70	0.12	59.08	6.75	1.73	0.20
	18	10	0.5[e]	69.12	3.56	2.02	0.10	69.17	3.13	2.02	0.09
C869	3	2	45 + 42	10.13	0.01	0.30	0.00	8.99	0.48	0.26	0.01
	4	2	18 + 16	30.56	0.70	0.89	0.02	29.74	1.34	0.87	0.04
	5	2	6 + 5	38.18	4.93	1.12	0.14	38.22	3.50	1.12	0.10
	6	2	3	49.10	2.11	1.43	0.06	48.42	0.53	1.41	0.02
	7	2	3	58.23	0.89	1.70	0.03	64.35	7.12	1.88	0.21
	9	2	3	55.32	2.69	1.62	0.08	62.96	6.17	1.84	0.18
	18	10	0.5[e]	65.10	3.19	1.90	0.09	67.88	6.76	1.98	0.20
W357B	3	2	52 + 47	8.04	0.92	0.23	0.03	6.50	1.04	0.19	0.03
	4	2	27 + 24	14.83	0.23	0.43	0.01	12.62	1.05	0.37	0.03
	5	2	7	35.30	1.50	1.03	0.04	34.60	2.31	1.01	0.07
	6	2	5	42.62	2.11	1.25	0.06	40.34	1.06	1.18	0.03
	7	2	3	48.44	4.59	1.42	0.13	46.41	5.89	1.36	0.17
	9	2	3	62.92	22.18	1.84	0.65	62.95	20.08	1.84	0.59
	18	10	0.5[e]	60.92	1.71	1.78	0.05	57.64	4.39	1.68	0.13
F3 population	19	54	6	61.62	7.18	1.80	0.21	58.40	6.84	1.71	0.20

[a] Weeks after emergence. [b] Different numbers of roots were used in each sample. [c] Standard deviation [d] Enzymatic fluorescence assay. [e] Two samples were taken per root.

of sucrose, have been used to determine DM sucrose content in plant tissues (*15*, *16*). Their application, particularly in microtiter plate formats (*17*−*19*), allows an ability to enhance breeding efficiency both by increasing the number of samples examined, but perhaps more importantly, by routinely assessing DM sucrose content in sugar beet individuals and populations. Here we report the development, evaluation, and potential limitations of a fluorogenic, multiplate-format enzymatic assay for DM sucrose quantification of sugar beet taproots. The enzymatic−fluorogenic assay (EFA) may be useful when high sensitivity is needed or when processing time needs to be minimized, but may be subject to as yet undetermined artifacts when mature, field-grown, roots are sampled.

The purpose of this work was to (i) ascertain the sensitivity of EFA for sucrose content determination in immature beet roots, which could aid in the characterization of the sucrose accumulation process during plant development and perhaps allow earlier selection for acceptable mature root sucrose content; (ii) assess the feasibility and utility of EFA for determining sucrose content in a wide range of germplasm typically encountered in a beet breeding program; and (iii) assess the practicality of EFA for rapid DM sucrose determination. Ultimately, the later aim will need to address a separate problem area, that of rapidly sampling and dehydrating many individual beet roots, to measure and select for sucrose content as a proportion of the total dry matter of the root.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Sugar beets C869, USH20, and SR96 and table (red) beet W357B (*20*−*23*) were greenhouse grown in 0.25 m^2 wooden boxes with 15 cm soil depth, 8−16 dark−light cycle, at 15−20 °C, and irrigated daily with fertilization twice a month, for 9 weeks after emergence (WAE). These lines and F_3 plants derived from the cross C869 × W357B were also grown at Michigan State University Agronomy Farm, East Lansing, MI, during 2002 and 2003 using standard agronomic practices. Samples were obtained from roots collected weekly from the 3rd through the 9th WAE from greenhouse-grown plants (48 samples) and from roots harvested at 18 (2003) or 19 (2002) WAE from field-grown plants (94 samples).

Sugar Extraction. Plants were harvested, leaves removed, roots washed, and weighed, and 10 g of root tissue was frozen in liquid nitrogen and stored at −80 °C. For younger roots, progressively fewer roots were needed at weekly intervals, ranging from 58 roots per accession at 3 weeks to 3 roots at 9 weeks of age (**Table 1**). Samples of older roots (18 and 19 WAE) were obtained from a 2 cm thick transverse section at the widest part of the root. For greenhouse-grown samples, two replications were done of the complete experiment (e.g., biological replication). For field-grown samples, one section from each root was taken, and two samples from this root were analyzed independently (e.g., technical replication). Following the method of Spackman and Cobb (*19*), samples were lyophilized to dryness (held at <1 mTorr for at least 3 h) and reweighed to determine water content and then ground to a fine powder with a mortar and pestle. Pulverized dried tissue (100 mg) was resuspended in 4 mL of 80% ethanol in a 5 mL fluted-cap tube (USA Scientifics, Inc.), placed horizontally on an orbital shaker (50 rpm) at 40 °C for 16 h, and the suspension was centrifuged at 3000*g* for 10 min to obtain the clarified ethanol sugar extraction solution. The clarified ethanol sugar extract was used directly for EFA, and the same solution was dried and resuspended for HPLC and refractometer analyses.

Chromatography and Refractometry. An aliquot (1.5 mL) of the clarified ethanol sugar extract was vacuum dried, the pellet was resuspended in 1.5 mL of high-resistivity water (18 MΩ cm^{-1}), and the solution was passed through a 0.2 μm filter (Puradisc 25 TF, Whatman). An aliquot of the water-resuspended sugar extract (0.3 mL) was analyzed with a Rudolph J157 automatic refractometer (Rudolph Research Analytical), read at 589.3 nm (20 °C). The remaining aliquot of water-resuspended sugar extract (1.2 mL) was used for HPLC analyses with a 6.5 mm × 300 mm steel cartridge Waters Sugar-PakI

carbohydrate column (WAT085188, Waters Co., Milford, MA). The mobile phase was 134 μM Na_2Ca EDTA set at a constant flow of 0.5 mL min^{-1}, 90 °C, 12 min run time, and quantified with a Waters 410 differential refractometer held at 35 °C, as per the manufacturer's literature (*24*). Concentration standards for sucrose (2.92−46.74 mM), glucose and fructose (0.22−4.44 mM), raffinose (0.13−1.34 mM), and stachyose (0.12−1.20 mM) were used to generate standard curves. System control and data management were accomplished using Empower Chromatography Manager software (Waters Co.).

Enzymatic−Fluorometric Assay. In summary, sucrose was hydrolyzed via invertase, followed by conversion of D-glucose to D-gluconolactone by the action of glucose oxidase, with concomitant evolution of H_2O_2. Hydrogen peroxide, in the presence of horseradish peroxidase, reacted with Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine, Molecular Probes, Eugene, OR) to generate resorufin (*25*), a red-fluorescent oxidation product with absorption and emission maxima of 563 and 587 nm, respectively (*26*). These reagents were included in the Amplex Red glucose/glucose oxidase assay kit (A-22189, Molecular Probes) and were deployed according to the manufacturer's instructions. The reaction stoichiometry of sucrose-derived glucose and resorufin is 1:1; thus, quantification of resorufin is directly proportional to glucose in the sample.

Specifically, in deep-well microtiter plates with three replicates for each sample, 100 μL of the clarified ethanol sugar extract was added to 1.9 mL of reaction buffer (50 mM NaH_2PO_4, pH 7.5) for native glucose determination. From this, 100 μL was transferred to a second well for sucrose determination, to which was added 100 μL of freshly prepared 10 mg mL^{-1} invertase (I-4504, Sigma) in 100 mM sodium acetate, pH 4.5 (*27*). Plates were sealed with aluminum foil (Microseal "F" Foil, MJ Research, Waltham, MA) and incubated at 55 °C for 90 min, and 1 mL of reaction buffer was added to neutralize the pH. Native glucose and sucrose-derived glucose measures were quantified in 96-well-format plates (Fluorotrac600, black, 96-well flat-bottom plate, Greiner Labortechnik) by adding 4 μL of sample to 46 μL of reaction buffer. Also included in each plate were glucose standards (1, 2, 4, 8, and 16 μM in reaction buffer; 50 μL) to generate calibration curves. Fifty microliters of oxidant solution was then added (prepared per the manufacturer's directions, containing 100 μM Amplex Red, 0.2 unit mL^{-1} horseradish peroxidase, and 2 units mL^{-1} glucose oxidase, Molecular Probes), and plates were incubated at room temperature protected from light for 30 min. A Wallace VICTOR^2 V plate reader (Perkin-Elmer, Inc., Wellesley, MA) was used in the stabilized energy mode with D531 excitation/D572 emission filters to measure fluorescence. Native sucrose content was initially calculated by subtracting native glucose readings from sucrose-derived glucose amounts estimated from hydrolyzed sucrose but later abandoned because of low native glucose concentrations in all tested samples relative to sucrose content (see Results) and to minimize the expense of running duplicate Amplex Red reactions; all results reported here were considered to be sucrose-derived glucose.

Analyses. All statistical treatments were performed using JMP version 5.0.1 (SAS Institute). The goal of these comparisons was to compare refractometric and EFA results for their ability to predict HPLC-determined sucrose contents; thus, HPLC results were used as the baseline for comparison.

RESULTS

HPLC analysis of 142 samples derived from 1046 roots taken from 3 to 19 weeks of age showed a wide range of sucrose contents, from 0.23 to 2.02 mmol g^{-1} of DM (**Figure 1**; **Table 1**). During development, sucrose content was least at the earliest stages of growth, with an average of 0.29 mmol g^{-1} of DM at 3 weeks, increasing with age to an average of 1.89 mmol g^{-1} of DM at 18−19 weeks. Sucrose content was least in table beet W357B and greatest in sugar beet, as expected, although variation among sugar beets was not as high as expected on the basis of their fresh weight sucrose values (ca. 15% for USH20 and C869 versus 18% for SR96; data not shown). F_3 plants derived from sugar beet × table beet cross, sampled only

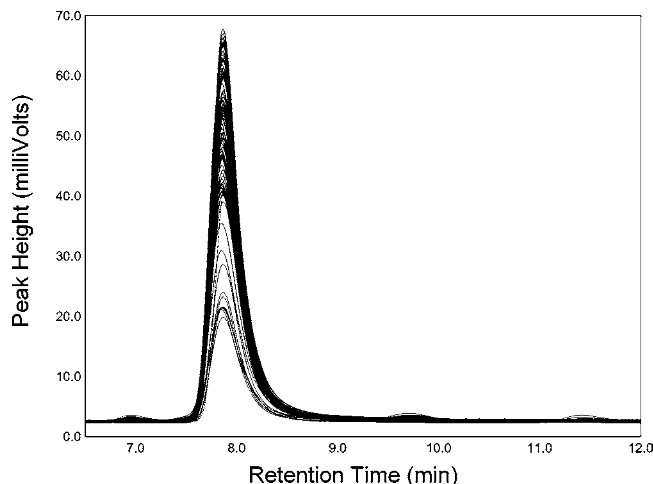

Figure 1. Range of 142 overlaid HPLC chromatograms analyzed for carbohydrate composition. Peaks at 6.94 (±0.02), 7.86 (±0.01), 9.72 (±0.02), and 11.43 (±0.07) min represent raffinose, sucrose, glucose, and fructose, respectively. Sugar concentrations were determined from peak areas.

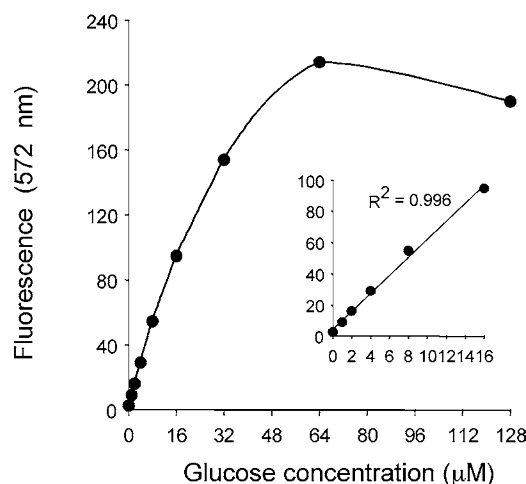

Figure 2. Determination of optimal range of glucose concentrations for a standard curve. (Inset) Glucose concentration range (0–16 mM) selected for the standard curve.

from field-grown individuals at 19 weeks of age, had intermediate sucrose content values, as expected. In all germplasm considered, low amounts of glucose and fructose were consistently detected by HPLC (from 5.5 to 44.4 μmol g^{-1} of DM) at the earliest stage of development and in only 15% of the samples at root maturity (5.5−83.3 μmol g^{-1} of DM). Raffinose (3.9−13.9 μmol g^{-1} of DM) was present only at root maturity and was observed in all lines. Stachyose was not detected.

Enzymatic−Fluorescence Assay. Samples for which the carbohydrate content was determined via HPLC were retested using an enzymatic-based assay with fluorometric detection of glucose. Briefly, sucrose was hydrolyzed to glucose and fructose using invertase, and then total glucose content was assayed using a resorufin-based detection assay. In a standard 2-fold dilution series of glucose (0−128 μM), fluorometric signal strength increased over the interval of glucose concentrations from 0 to 64 μM, but decreased at 128 μM (**Figure 2**). Near linearity was seen in the 0−16 μM glucose range ($R^2 = 0.996$), and this was chosen as the target interval for the standard curve for sucrose EFA of sugar beet.

Sucrose content of sugar beet exceeded 2.4 mmol g^{-1} of DM in some HPLC-analyzed samples from individual roots, and here

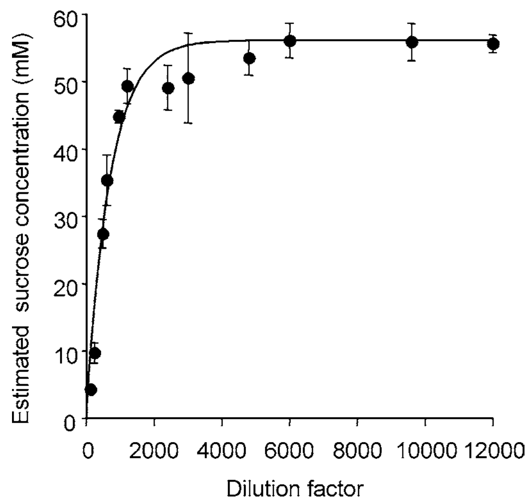

Figure 3. Determination of sugar extract dilution optimum from a standard 60 mM sucrose solution. Bars represent ± SD of four replications.

sucrose ethanol extract concentrations were calculated to be as high as 60 mM. Dilution of the extracts was thus necessary, and a dilution series (240, 480, 600, 960, 1200, 2400, 3000, 4800, 6000, 9600, and 12000-fold) of a standard 60 mM sucrose solution, replicated four times, was used to identify the more appropriate dilution ranges required to maintain assay linearity (**Figure 3**). Dilutions lower than 3000-fold underestimated sucrose concentration, presumably through saturation of the assay's enzyme kinetics. Dilution factors of 6000 and above gave equivalent estimates, and 6000-fold dilution of sample extracts was chosen for sugar beet sucrose EFA.

Invertase-hydrolyzed sucrose and glucose standard solutions, each ranging from 1 to 16 μM, were tested individually. Both sucrose and glucose showed a high linear correlation ($R^2 = 0.985$; $p < 0.0001$) between estimated and expected results, with their slopes and intercepts not significantly different from 1 and 0, respectively. Fructose and raffinose standard solutions, each ranging from 1 to 16 μM, were tested individually after invertase treatment to test their native reactivity in the assay. Fructose and raffinose were not detected across the range of concentrations tested.

Comparison of Analytical Methods. Overall, bivariate analyses comparing matched pairs of individual readings (by method) showed that sucrose content determined by HPLC was correlated with both refractometric ($R^2 = 0.950$; $p < 0.0001$) and EFA ($R^2 = 0.920$; $p < 0.0001$). Refractometry was relatively imprecise and overestimated HPLC-determined sucrose content by ~0.2 mmol g^{-1} (as dry weight), with both slope and intercept of its regression line ($y = 0.880x + 0.451$) being significantly different from 1 and 0, respectively (**Figure 4A**). EFA and HPLC sugar determinations showed comparable estimates of sucrose content, and the slope and intercept of its linear regression ($y = 0.977x - 0.003$) did not significantly differ from 1 and 0, respectively (**Figure 4B**). Correlations of mean sucrose values (based on variety and WAE, **Figure 5**) compared between methods were slightly higher than individual matched-pairs ($R^2 = 0.985$; $p < 0.0001$ for EFA).

Differences in the magnitude of variation observed with EFA between younger, lower sucrose, greenhouse-grown roots and mature, higher sucrose, field-grown roots were observed. The source of this uncertainty (e.g., as related to either age, sucrose content, or location) could not be estimated from these data. Bivariate statistics for younger, greenhouse-grown, roots indicated a high correlation between EFA and HPLC results ($R^2 = 0.976$; $p < 0.0001$; $y = 1.049x - 0.053$), but this correlation

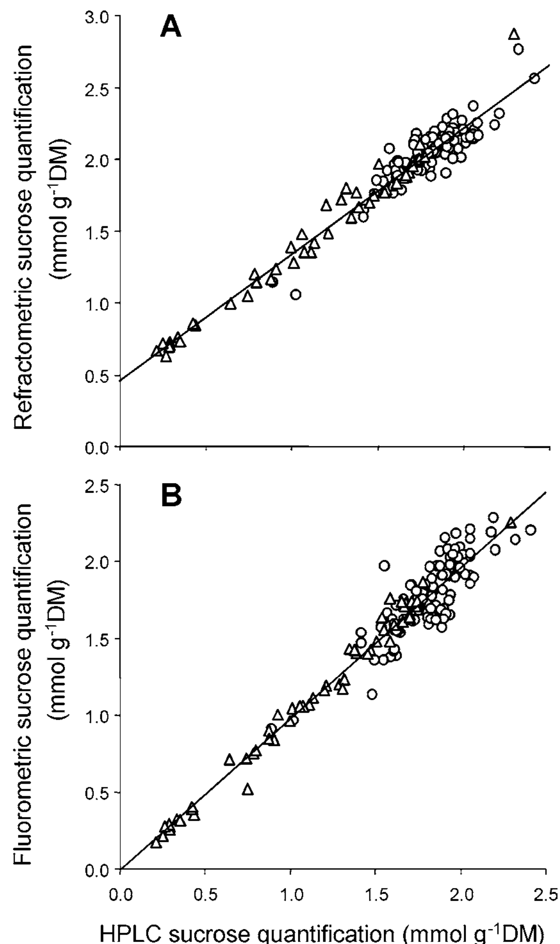

Figure 4. Comparison of sucrose quantification methods: (**A**) correlation between sucrose contents on dry matter (DM) basis estimated with chromatographic (HPLC) and refractometric analyses ($R^2 = 0.950$; $y = 0.880x + 0.451$); (**B**) correlation between sucrose contents on DM basis estimated with HPLC and fluorometric analyses ($R^2 = 0.920$; $y = 0.977x - 0.003$). Triangles and circles represent young, greenhouse-grown, and mature, field-grown, samples, respectively.

degenerated for older, field-grown, roots ($R^2 = 0.605$; $p < 0.0001$; $y = 1.016x - 0.092$) when these groups were considered separately. For field-grown roots, mean DM sucrose determined with HPLC was 1.84 mmol g^{-1} [standard deviation (SD) = 0.181; confidence interval $(CI)_{0.95} = 1.80-1.87$], whereas from the EFA average of three replications the mean DM sucrose was 1.77 mmol g^{-1} (SD = 0.236; $CI_{0.95} = 1.73-1.82$). For field-grown roots, EFA underestimated HPLC sucrose values by 0.06 mmol g^{-1} (as dry weight) (SD = 0.149; $CI_{0.95} = 0.03-0.09$).

All field-grown genotypes demonstrated a level of uncertainty with EFA; thus, there was no obvious varietal component, although correlations (all with $p < 0.0001$) between HPLC and EFA were higher with USH20 and W357B ($R^2 > 0.97$), lower with SR96 ($R^2 > 0.95$), and low or very low with C869 and the F3 population ($R^2 = 0.938$ and 0.500, respectively). With the exception of USH20, the only commercial hybrid in this dataset, this progression follows an increasing level of genetic diversity (e.g., increased heterozygosity) within the populations. Root color did not appear to influence correlations, as white roots showed as much variability as red roots ($n = 83$, $R^2 = 0.928$, versus $n = 62$, $R^2 = 0.910$, respectively).

In general, three replications of each EFA were performed, and variability among replicates was present. Bivariate analyses

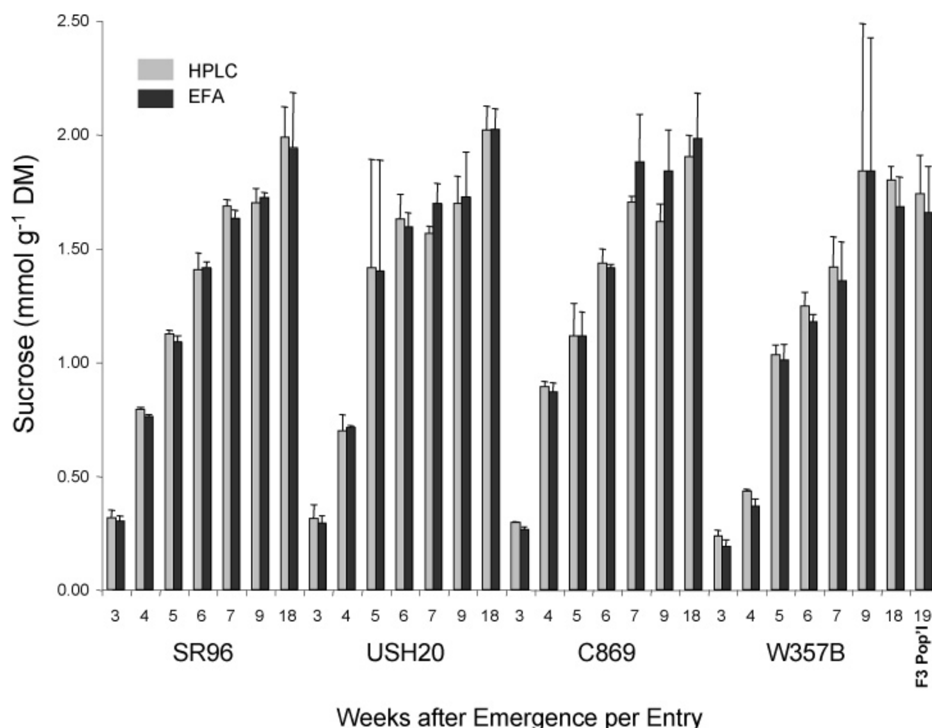

Figure 5. Mean sucrose content comparison between methods (HPLC and EFA) for each line at each sampled time point (data from **Table 1**). Error bars are SD.

between replicates of greenhouse-grown roots indicated less variation (Rep1 versus Rep2, $R^2 = 0.994$; Rep 2 versus Rep3, $R^2 = 0.997$; Rep1 versus Rep3, $R^2 = 0.995$; all with $p < 0.0001$). In contrast, variability in results from replicated samples of field-grown roots was higher (Rep1 versus Rep2, $R^2 = 0.792$; Rep 2 versus Rep3, $R^2 = 0.881$; Rep1 versus Rep3, $R^2 = 0.895$; all with $p < 0.0001$). Increased variability in estimated sucrose content using EFA observed in mature, field-grown, roots relative to young, greenhouse-grown, roots was not expected, and from measurements to date, no clear trends to explain this difference were evident.

DISCUSSION

Three methods to determine sucrose content in dry sugar beet root tissues (refractometry, chromatography, and EFA) were compared, specifically to validate the EFA reported here for a microtiter plate format. Time and labor savings of such an assay could be substantial in a breeding program examining thousands of individuals, if validated, and concomitantly allow an estimation of sucrose content expressed as a proportion of total DM content. Progeny tests for sucrose content in young plants could assist breeders by obviating field screening in early-generation breeding materials, because sucrose accumulation appears to be initiated early in growth and varietal sucrose content may be approximated by as few as 10 weeks after emergence (*28*, *29*). The relative straightforward nature of the EFA assay suggests semiautomation could increase sample throughput.

Reasonable sucrose content estimates were obtained using the EFA within a wide range of sample dilutions (4800−12000-fold), indicating high sensitivity. Enzymatic digestions were considered a potential source of error. For glucose oxidase, results obtained with different incubation times were uniform within the range of 30−80 min (data not shown). For invertase, initial times and temperatures of incubation were chosen to allow the reaction to proceed to near completion, and results suggested this was the case. An additional concern was the low pH optima for invertase activity, which is usually different from that needed during detection of liberated glucose and requires an extra step for pH adjustment (*30*). In the assay here, the pH was raised from 4.5 to neutrality during the required dilution of the extract, saving one processing step.

As expected, refractometry was not suitable for precise sucrose determination. The correlation was reasonable for a rapid estimation, but it overestimated HPLC-determined sucrose content. EFA and HPLC results were well correlated in the early development of the sugar beet root, which might be important in progeny testing of young plants when sufficient tissue mass has not yet accumulated for reliable polarimetric analyses or when optically active compounds such as free glucose and raffinose could interfere with polarimetric determinations (*13*). In older field-grown roots with higher sucrose content, the correlation between EFA and HPLC results was less convincing.

It is unclear exactly the source of higher variability between field-grown EFA and HPLC results. Field-grown samples consisted of single roots, whereas greenhouse-grown roots were pooled samples, and genetic heterogeneity is prevalent in beet germplasm (*31*). However, this cannot explain variation within samples extracted from the same source. Variability was relatively high among replicated readings of the same field-grown sample but did not account for all of the variability observed among field-grown roots, suggesting some inter-root or interpopulation influence on the overall variation; however, no statistically informative relationships were uncovered. Variability among field-grown roots for the presence or absence of enzyme inhibitors of invertase, glucose oxidase, and horseradish peroxidase might explain these results, as could variability in roots for inhibitors of fluorescence per se (e.g., quenching). The detection of raffinose in only field-grown roots suggests the possibility that unique biochemicals are produced in these conditions, although raffinose appeared to have little or no effect itself on the detection procedures. Variability introduced by

dilution or other technical errors seems to be unlikely because these operations were the same as used with greenhouse-grown roots.

In summary, caution should be applied in using EFA for sucrose determination of field-grown beet roots. Enzymatic−colorimetric assays (*15*−*19*) may be less sensitive to the postulated interference seen using EFA but were not performed here because sensitivity to predict sucrose in young roots as a potential criterion for early selection was a higher priority. Greenhouse-grown beets, particularly young beets (<10 weeks old), appeared to be less sensitive to unknown influences contributing to variability introduced in the assay. The level of precision afforded by the enzymatic−fluorometric methods was not ideal, but the magnitude of the differences (estimated as 1% sucrose FW) was not so large as to render the method useless for breeding purposes, especially during backcross breeding of unadapted materials with elite breeding lines. The sources of variability among field-grown roots are puzzling, and their experimental determination may reveal growth-environment properties not previously appreciated. Conversely, increased dilution of sample extracts may be one trivial approach to minimize observed uncertainties.

ABBREVIATIONS USED

DM, dry matter; FW, fresh weight; SD, standard deviation; EFA, enzymatic−fluorometric assay; HPLC, high-performance liquid chromatography.

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