Sucrose Concentration and Taproot-Leaf Weight Ratio Relationships Among Seedlings of a Few Sugarbeet (*Beta vulgaris* L.) Breeding Lines

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INTRODUCTION

Sucrose concentration and size of taproot contribute importantly to the economic yield of sugarbeet. Thus, individual plants identified as having genetically controlled higher sucrose concentration and larger taproots, which are used as breeding stock, should improve economic yield. In the past, however, most of the improvements in yield were based on mean performance of breeding lines, when crossed and progeny tested for a full growing-season in the field. Although identifying genetically superior individual plants of a breeding line was difficult and often confounded by the marked environmental variability in field tests, breeders isolated breeding lines with higher sucrose concentration and demonstrated that sucrose concentration was genetically controlled. More recently, sucrose concentration has been reported to be inversely related to mean cell size in the tap root and to taproot weight of sugarbeet breeding lines and cultivars (3, 7).

Selection of sugarbeet seedlings for extremes in taproot-leaf weight ratio (TLWR) demonstrated that individual seedlings of a breeding line may have considerable variability of TLWR which is under genetic control and it can significantly affect both taproot size and root yield per hectare (9, 10). Sugarbeet populations selected for a high TLWR may have significantly higher sucrose concentration than low-TLWR or unselected populations when grown in

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field tests (6, 10; Theurer and Doney, unpublished). Up to 2.5-fold variation in sucrose concentration in taproots within a single breeding line was observed in an experiment involving 15 seedlings per line and three breeding lines grown in a single controlled environment chamber[1].

Objectives of this study were to 1) confirm the large range in sucrose concentration in seedling taproots of a breeding line grown in a well controlled environment; 2) determine relationships between sucrose concentration, osmotic concentration, and other juice constituents; 3) determine seedling sucrose concentrations of the female parents and their progenies; 4) stimulate further research to determine the physiological basis for the marked difference in taproot sucrose concentration among individual seedlings; and 5) determine the relationship between sucrose concentration and TLWR in breeding lines.

MATERIALS AND METHODS

The experiments were conducted in Controlled Environment Chambers, M-2 model, built in Chagrin Falls, Ohio[2]. The environment was 14 h of incandescent and fluorescent light, photosynthetic photon flux density about 500 $\mu mol \cdot s^{-1} \cdot m^{-2}$; relative humidity 65 $\pm$ 2%; and ambient CO_2 concentration. Temperature in Experiment 1 and 2 was 27°C day and 16° night. In Experiment 3, temperature was a constant 23°C for the first 16 days and then 25° day and 20° night for the remaining 25 days. In Experiment 4, temperature was a constant 22°C.

Breeding lines in Table 1 prefixed by "L" were developed by the U.S. Department of Agriculture at the Logan, Utah station for the western United States and those with "EL" were developed at the East Lansing, Michigan station for use in the Great Lakes area, thus they are of

[1] Doney, D. L., R. A. Wyse, F. W. Snyder and G. E. Carlson, unpublished data.

[2] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Table 1. Brief description of sugarbeet populations used in the controlled environment experiments.

Experiment	Population designation and characteristics
1 L53 x L19	Bulked seed of breeding line having high taproot sucrose concentration.
1 EL40-203	Seed from a single female having low TLWR, polycrossed by low TLWR plants.
2 EL40-104	Seed from a single female having high TLWR, polycrossed by high TLWR plants.
3 EL40-104	Same as in Experiment 2.
3 L19	Bulked seed of an inbreed breeding line having high taproot sucrose concentration.
4 EL40-104	Four seedlings selected for high sucrose concentration, mean = 114.9 ± 3.6 mg sucrose per g root, grown and polycrossed in isolation. Progeny of each female evaluated for sucrose concentration. See Table 3.
4 EL40-104	Three seedlings selected for low sucrose concentration, mean = 89.2 ± 2.5 mg sucrose per g root, grown and polycrossed in isolation. Progeny of each female evaluated for sucrose concentration. See Table 3.

rather diverse genetic background.

The seedlings, one per 20 cm diameter plastic pot, were grown in vermiculite. They received a complete, full strength mineral nutrient solution (9) to cause flushing once daily as small seedlings and twice daily as larger seedlings. Age (days post-emergence) of the plants at harvest is given in Tables 2 and 3. Plants were harvested rapidly after removal from the growth chamber. Fresh weights of leaf laminae and taproot plus hypocotyl (fibrous roots carefully removed) were obtained. The hypocotyl or crown was removed from the taproot. The true taproot was weighed and immediately frozen in liquid nitrogen and stored in a freezer.

In preparation for sucrose analysis, the frozen taproot was fractured into pieces and homogenized with 2 ml of solution (12.5 g of $Al_2(SO4)_3 \cdot 18\ H_2O$ per liter) per gram of tissue in a Kimematica Polytron homogenizer[2] at half speed for 30 seconds. After centrifugation, the liquid was decanted into a vial and refrozen until analyzed. Sucrose was analyzed by the van Handel method (5). The

osmotic concentration of the juice, corrected for the aluminum sulfate and dilution, was determined by Peltier thermocouple psychrometry (2). The juice of Experiment 1 was analyzed for nitrogen and phosphorus using a Technicon autoanalyzer (1) and for potassium and sodium by atomic absorption.

The values for TLWR were obtained from the taproot plus hypocotyl fresh weight divided by the leaf blade fresh weight. Correlations of sucrose concentration in the seedling taproot versus the TLWR of each seedling were determined for two Logan breeding lines and four populations of TLWR selections out of East Lansing breeding line EL40.

RESULTS

Sucrose Concentration

The marked variation in taproot sucrose concentration among seedlings within populations and breeding lines (L53 x L19, L19) grown in a controlled environment has been confirmed (Table 2). With the exception of parent 7 in Table 3, progenies from a single female plant have a coefficient of variation about half the size of that of progenies grown from bulked seed of a number of females, e.g., L53 x L19 and L19. Progeny of EL40-203 had a large coefficient of variation, in spite of being derived from a single female plant (Table 2).

The mean osmotic concentrations of L53 x L19 and EL40-203 did not differ, yet mean sucrose concentrations and mean nitrogen content of the juices differed by more than two standard deviations (Table 4). The sum of the molar equivalents of the identified constituents of the juice of each breeding line also differed considerably, 0.368 for L53 x L19, 0.275 for EL40-203 (Table 4). Among the six taproot constituents examined in Table 4, only sucrose concentration for breeding line EL40-203 correlated significantly with potassium, $r = 0.529^{**}$. The sum of the five analyzed solutes failed to correlate significantly with osmotic concentration; 0.000 NS for L53 x L19, and 0.420 NS, (0.423 for 5%), for EL40-203. Progenies of

Table 2. Variation in taproot sucrose concentration among seedlings within each sugarbeet population.

Expt. no.	Population	Age at harvest[#]	No. of replic.	Sucrose conc. mg/ root Mean	SD	Coefficient variation as %	No. of fold range in Sucrose conc.	Sucrose/ Seedling taproot
1	L53 x L19	28	23	48.1 ±	9.3	19	2.07	5.24
1	EL40-203	28	23	30.2 ±	8.0	26	2.73	5.41
2	EL40-104	47	25	100.0 ±	8.6	9	1.39	-
3	L19	37	23	52.5 ±	14.2	27	3.00	-
3	EL40-104	35	5	53.2 ±	2.0	4	1.11	-

[#] Days post-emergence.

Table 3. Relation of taproot sucrose concentration in the seedling female parent, EL40-104, to that of the seedling progeny. Experiment 4.

Parent no.		Sucrose concentration		Coefficient of variation as %
		Parent[#]	Progeny[##]	
		mg sucrose/g root		
High group				
22		120.0	63.0 ± 5.9	9
7		114.0	64.9 ± 11.5	18
20		114.0	61.4 ± 5.7	9
27		111.6	59.1 ± 5.0	8
	Mean	114.9 ± 3.6	62.1 ± 2.5	
Low group				
7		91.2	64.6 ± 7.0	11
26		90.0	54.8 ± 5.5	10
16		86.4	58.6 ± 6.3	11
	Mean	89.2 ± 2.5	59.3 ± 4.9	

[#] Age 47 days post-emergence.
[##] Mean for 7 seedlings; age 27 days post-emergence.

Table 4. Molar equivalents of osmotic concentration and certain constituents in taproots of seedling sugarbeets grown for 28 days post-emergence in a controlled environment. Experiment 1.

Population	Osomtic conc.	Sucrose	Nitrogen	Potassium	Phosphorus	Sodium
L53 x L19	0.567 ±0.173[#]	0.141 ±0.028	0.150 ±0.018	0.056 ±0.012	0.019 ±0.002	0.002 -
EL40-203	0.564 ±0.255[#]	0.088 ±0.024	0.103 ±0.012	0.057 ±0.010	0.025 ±0.004	0.003 -

[#] Standard deviation.

EL40-104 with the highest sucrose concentrations were compared with those having the lowest sucrose concentrations (Table 5). Those with the highest sucrose had lower osmotic concentrations than those with the lowest sucrose (Table 5). Grouping the individuals according to the highest or the lowest sucrose concentration did not produce a significant correlation between sucrose concentration and osmotic concentration (Table 5).

Plants in Experiment 1, harvested at 28 days post-emergence, were of the following sizes: population L53 x L19; leaves (>1 cm in length) per plant, 17 to 22; mean leaf blade fresh weight in grams, 69.8 ± 13.3; mean taproot plus hypocotyl fresh weight 9.8 ± 2.7; taproot fresh weight ranged from 3.8 to 14.8; population EL40-203;

Table 5. Comparison of sucrose and osmotic concentrations in taproots of seedling sugarbeet progenies of high-TLWR plant EL40-104 at 27 days post-emergence in a controlled environment. Experiment 4.

Sucrose concentration classification	Molar equivalents of#		Correlation coefficient
	Sucrose	Osmotic conc.	
Highest	0.214 ± 0.015	0.495 ± 0.067	-0.017 NS
Lowest	0.150 ± 0.005	0.620 ± 0.112	-0.513 NS

Mean for seven seedlings.

leaves per plant, 13 to 20; mean leaf blade fresh weight, 51.2 ± 8.4; mean taproot plus hypocotyl fresh weight, 3.8 ± 1.2; taproot fresh weight ranged from 2.0 to 6.1 grams. Leaf blade weight correlated significantly with taproot weight, r = 0.639** for L53 x L19 and r = 0.696** for E140-203. In contrast, at this stage of development, sucrose concentration failed to correlate significantly with either taproot weight or leaf blade weight in both populations.

Relation Between Sucrose Concentration and TLWR

The coefficients of variation (CV) for sucrose concentration and TLWR in Table 6 indicate rather large variation in each trait among seedlings of most of these populations. Sucrose concentration correlated significantly with TLWR in three of the six populations examined (Table 6). Mean sucrose concentration in the six populations correlated significantly with mean TLWR, r = 0.947** (Table 6).

DISCUSSION

Sucrose concentration is primarily an additive genetically controlled trait in sugarbeet. A carefully controlled environment is required to evaluate the genetically controlled variation among individual plants. Because of the relatively uniform light and good environmental control in these experiments, we believe that the marked variation in sucrose concentration among the seedlings of a breeding line indicates a sizable genetic component which is contributing to the variation. The 25 plants of line L19 grown in Experiment 3 provide evidence for this assertion. Two sets of seedlings in adjacent pots had

Table 6. Mean sucrose concentration and mean TLWR for six sugarbeet populations and their correlation coefficents.

Expt. no.	Population	Sucrose concentration Molar equivalents	CV	TLWR Mean	CV	df	Correlation coefficient
			%		%		
1	EL40-203	0.088 ± 0.023	26	0.075 ± 0.016	21	21	0.484@
1	L53 x L19	0.141 ± 0.027	19	0.142 ± 0.033	23	21	0.364 NS
2	EL40-104	0.291 ± 0.025	9	0.547 ± 0.112	20	23	0.542**
3	L19	0.153 ± 0.041	27	0.211 ± 0.054	26	21	0.693**
4	Progenies of high sucrose parents#	0.181 ± 0.022	12	0.237 ± 0.024	10	25	0.237 NS
4	Progenies of low sucrose parents#	0.173 ± 0.021	12	0.203 ± 0.036	18	19	0.013 NS

Significant: @ 2% level, ** 1% level.

Selected out of female parent EL40-104.

33.6 versus 84.0 and 28.0 versus 72.7 mg sucrose per gram fresh weight of taproot, which is a 2.5- and a 2.6-fold difference in sucrose concentration of adjacent plants. At harvest (37 days post-emergence), mean leaf blade weight of these plants was 69.6 ± 10.9 g and mean taproot plus hypocotyl fresh weight was 14.8 ± 4.5. Line L19 has had some inbreeding, thus, breeders consider it to be inbreed. Our data, however, suggest that the genetically controlled sucrose concentration of L19 at this stage of development may be more variable than previously assumed.

Wyse[3] has observed that sucrose accumulation may be minimal in very young seedling taproots and that the time when sucrose accumulation is initiated may vary somewhat. Unfortunately, these early experiments did not have a precise index, other than age, to compare the stages of plant development. Since plant size varies according to the environment provided and taproot size also varies among and within breeding lines and cultivars, it is essential to use a more precise index for stage of development in future studies. The best index of development would be the plastochron index (4). A less satisfactory, but probably adequate, indicator of plant development would be to record the total number of leaves (larger than 1 cm) developed during the period of growth until the plant is harvested. If axillary leaves develop, they should not be included in the count of leaves to indicate stage of development.

Theurer[4] has followed taproot sucrose accumulation in sugarbeet breeding lines throughout the growing season. He observed that young seedlings of the high sucrose line, L19, had a lower sucrose concentration than seedlings of some other breeding lines, but later in the season, the L19 plants accumulated sucrose more rapidly and had the highest sucrose concentration by the end of the growing season. Based on Theurer's observation, seedlings of different breeding lines cannot be assumed to maintain their

[3]Personal communication from R. A. Wyse.

[4]Personal communication from J. C. Theurer.

rank order for sucrose concentration over the growing season. At present, however, evidence is lacking as to whether individual seedlings of breeding line L19 with higher sucrose concentration will maintain their rank order throughout the growing season. Vegetative propagules or ramets of individual plants of L19 and a few other breeding lines could provide numbers of plants of a given genotype (8), which could be harvested sequentially over the growing season to determine more precisely the patterns of genetic control of sucrose accumulation.

The marked variations in sucrose concentration and the failure of osmotic concentration to correlate either with sucrose or the sum of the analyzed constituents suggests a possible divergence in time of initiation and/or in rate of sucrose accumulation or even a diversity of the physiological mechanism(s) of accumulation operating in the various seedlings. The sum of the solutes which were analyzed constituted 65 percent of the osmotic concentration of breeding line L53 x L19, but only 49 percent of line EL40-203, suggests a possible physiological difference between the two breeding lines. An understanding of the genetically controlled physiological basis of these differences may provide the sugarbeet breeder with a powerful tool to breed sugarbeets with greater recoverable sucrose.

Relation Between Sucrose Concentration and TLWR

The large coefficients of variation for sucrose concentration and TLWR and the divergent correlations between sucrose concentration and TLWR suggest that each sugarbeet plant used in the breeding program must be selected for high sucrose content and for high TLWR to maximize economic yield.

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