Selection for Potential Sucrose Concentration in 7-Day-Old Sugarbeet Seedlings

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INTRODUCTION

Sugarbeet (*Beta vulgaris* L.) breeding practices have generally attempted to increase root yields while maintaining or increasing sucrose concentration. Unfortunately, a negative correlation exists between root yield and sucrose concentration. Selection for high root yield has generally led to a reduction in sucrose concentration and/or selection for high sucrose has generally resulted in reduced root yields (Doney, 1979; Doney et al. 1981; Hecker, 1967; Oldemeyer, 1975; Powers, 1957; Tasuda and Hosokawa, 1969). We found this negative association to be largely due to the opposite affect of cell size on root yield and sucrose concentration; i.e., large celled genotypes produce large roots (high root yields) and are low in sucrose concentration, whereas small-celled genotypes produce small roots (low root yields) and are high in sucrose percentage (Doney et al., 1981; Doney and Theurer, 1983). Ideally, genotypes with rapid cell division rate and small cells (i.e. many small cells) would give high root yields and high sucrose concentrations.

A seedling selection criterion (Doney and Theurer, 1976) developed at this laboratory has been effective in increasing root yield; however, we found that the large selections (higher root yield) were larger because they were producing both more and larger cells (Doney et al.,

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1981). The new selections were higher in root yield but lower in sucrose percentage. This hypocotyl diameter selection criterion, although effective, confounded the two cellular parameters, cell size and cell number. We theorized that if we could determine a given genotypes's relative cell size in very young plants, then the cellular parameters (size or number) responsible for large hypocotyl diameter at 21 days of age could be ascertained. This study was undertaken to develop a method to easily and rapidly identify genetic differences in cell size of very young plants. The study included the response of environmental and seed quality effects on selection and the evaluation of potential selection progress in heterozygous populations.

MATERIALS AND METHODS

Cortex cell size and number were determined from cross sections of roots of plants seven days post-planting. Only those plants that had emerged by six days post-planting were sampled. Root tissue cross sections were taken from approximately 30 plants of each genotype, fixed in formalin-alcohol-acetic acid (FAA), and dehydrated in a graded ethanol series. The sections were embedded and stained with safranin and fast green by standard techniques. Mean cell diameter was determined by counting the number of cortex cells intersecting a line drawn across the root cross section and dividing that number into the length of the line. Longitudinal root sections showed the cortex cells to be spherical in shape; therfore, cell size was calculated as:

$$V = 4/3\ \pi r^3$$

where V = cell volume
r = mean cortex cell radius

Seed for hypocotyl diameter (HD) measurements was planted 1.9 cm deep in vermiculite and watered with either tap water or nutrient solution daily. Hypocotyl diameter measurements were made on seedling 6, 7, 9, 11, 14 and 28 days after planting. Plants emerging after day six post-planting were discarded and not used for subsequent measurements.

Genetic materials used to evaluate the relationship between potential sucrose concentration and hypocotyl diameter of seven-day-old plants were commercial and experimental hybrids, and inbreds; all of which had been previously grown and evaluated for sucrose percentage in replicated field trials.

Seed quality tests were conducted on two hybrids GW45 and GW115, obtained from The Great Western Sugar Co., and two hybrids, AH10 and AH12, obtained from The Amalgamated Sugar Co. Seed sizes tested were 7-8/64, 8-9/64, and 9-10/64 inches for the AH10 and AH12 hybrids, and 6-7/64, 7-8/64, 8-9/64, and 9-10/64 inches for the GW45 and GW115 hybrids.

Four highly heterogenous open-pollinated populations (g237, h537, f1 and f354), were used for individual plant selection. Individual plants (7 days post-planting) having hypocotyl diameters greater than one standard deviation above and one standard deviation below the population mean were selected from each population.

All plants selected for each of the large and small hypocotyl diameter populations were interpollinated in separate isolation chambers for seed increase. The resulting populations were tested for sucrose percentage in replicated field trials.

Individual plant selections were also made in populations f354 and h537 for large and small hypocotyl diameter at 7 and 28 days post-planting. In these selection studies, one seed was planted in each of 500 vermiculite-filled clear, plastic pots (4.5 cm by 10.5 cm) for each population. Seven days after planting, each plant hypocotyl was measured and recorded without removing the plant from the pot. At 28 days post-planting, all plants were removed from their respective pots, their hypocotyl diameters remeasured, and plants for the four combinations of large and small hypocotyl diameters at 7 and 28 days were saved for seed production. All plants of each selection parameter or combination of parameters designated at 7 and 28 days were grown in separate isolation chambers for seed

production and field testing.

Field plots consisted of two rows, 56 cm apart and 12 m in length. All field trials were replicated six times. At harvest, all beets from each plot were machine harvested after the removal of the top and crown below the lower leaf scar. They were than weighed for root yield. Two random 10-beet samples were selected from each plot for determination of sucrose percentage.

RESULTS AND DISCUSSION

Cortex Cells and True Root Cells

Under our controlled greenhouse conditions, seedlings begin emerging at about 5 days post-planting. The root-hypocotyl tissue of emerging plants is largely cortex cells with a very small core of meristamatic tissue. This center core of meristamatic tissue differentiates into the primary and secondary cambial rings. At 14 to 16 days post-planting, the primary cambial ring, from which true root cells begin forming, is visible. At about 35 days of age, all the cambial rings are formed and functioning. During this phase of root expansion the cortex cells expand but do not divide. At about 35 days post-planting, the cortex tissue splits from the true root and is sloughed off. All genotypes lay down about the same number of cortex cells at germination (numerous unpublished observations and calculations by the author). The cortex cells protect the meristamatic tissue until differentiation is complete. Even though cortex cells are not permanent cells, the differences in cell size due to the genotype of the developing embryo are already manifest in the cortex cells. The large hypocotyl diameter (large-HD) selections from our earlier hypocotyl diameter selection studies (Doney and Theurer, 1976) were larger at 28 days post-planting than the small hypocotyl diameter (small-HD) selections, primarily due to larger cells (Table 1). The cortex cells of the large-HD selections at 7 days post-planting were also larger than the cortex cells of the small-HD selections. The large-HD selections with large cortex and true root cells were lower in sucrose percen-

Table 1. Cortex and true root cell size (volume) at 7 and 28 days post-planting, respectively, and harvest sucrose percentage for large and small sugarbeet hypocotyl diameter selections.

Hypocotyl diameter selection†	Cell size (volume) Cortex cells (7 days)	True root cells (28 days)	Harvest Sucrose
	cm^3 X 10-8	cm^3 X 10-8	%
Large	9.4	8.1	14.5
Small	6.2	6.1	15.4
LSD 0.05	0.5	0.4	0.6

†Selection based on hypocotyl diameter of 28-day-old plants.

tage than the small-HD selections (Table 1).

Two genotypes with extreme differences in sucrose percentage potentials showed highly significant differences in cortex cell size (Table 2). The low sucrose genotype, Blanca, had cortex cells three-fold larger than the high sucrose genotype, L19.

Table 2. Cortex cell size at 6 days post-planting and harvest sucrose percentage of two sugarbeet cultivars with wide differences in sucrose percentage potential.

Genotype	Cortex cell size	Harvest sucrose
	cm X 10^{-8}	%
Blanca	3.10	9.0
L19	1.03	18.0
LSD 0.05	0.49	0.7

The resultant conclusions were: 1) the hypocotyl-root tissue of emerging sugarbeet seedlings is largely cortex cells, 2) all genotypes lay down about the same number of cortex cells at germination, 3) cortex cells do not divide, and 4) cortex cell size of emerging sugarbeet plants is determined by the genotype of the developing embryo. We theorized, therefore, that a simple measure of the diameter of the hypocotyl of very young plants should render a relative measure of genetic differences in true root cell size and an indirect measure of sucrose concentration potential.

Correlation of Sucrose Percentage and Hypocotyl Diameter

of Young Plants

To evaluate whether the HD of very young plants is a relative measure of sucrose concentration, we measured the HD of plants 7 days post-planting in three sets of genetic material that had been previously evaluated for sucrose concentration in replicated field trials. Six inbred lines ranging in harvest sucrose percentages from 15.2 to 18.4 gave a significant negative correlation of -0.68 between sucrose percentages and HD values of 7-day-old plants (Table 3). Another test of 14 hybrids with sucrose

Table 3. Hypocotyl diameters of sugarbeet plants 7 days post planting, and harvest sucrose percentages for six inbreds.

Inbred	Hypocotyl diameter	Harvest sucrose
	mm	%
L19	0.706	18.4
L53	0.775	18.0
L37	0.696	17.7
L10	0.796	17.7
L29	0.795	17.1
L17	0.820	15.2
LSD 0.05	0.025	0.6

percentages ranging from 14.9 to 18.1 showed a similar relationship (r = -0.77) (Table 4). An additional set of 25 genotypes of commercial and experimental hybrids and inbreds was tested. A significant negative correlation of -0.78 was obtained between the HD values of 7-day-old plants and harvest sucrose percentage in this test. These data substantiated the premise that a simple measurement of the HD of newly emerged seedlings gives a measure of relative cell sizes and, indirectly, of sucrose percentages.

A major concern was the effect of environmental factors such as seed quality, nutrition, photosynthesis, etc., on the HD of these very young plants. Several tests were conducted to evaluate these possible influences as well as to develop more precision in identifying genetic differences. The effect of plant age on HD was studied in nine genotypes from widely different genetic backgrounds

Table 4. Hypocotyl diameter of sugarbeet plants 7 days post planting, and harvest sucrose percentages for 14 genotypes

Hybrid	Hypocotyl diameter	Harvest sucrose
	mm	%
46F3	0.772	18.1
ACH14	0.774	17.4
6F4	0.737	16.9
GWD2	0.805	16.9
Beta 1345	0.818	16.8
d148	0.823	16.5
c27	0.787	16.4
6F5	0.787	16.3
UI#8	0.831	16.1
UI#51	0.843	15.7
USH20	0.836	15.6
UI#50	0.879	15.5
HH22	0.836	15.3
gl	0.889	14.9
LSD 0.05	0.028	0.6

Table 5. Hypocotyl diameters of nine sugarbeet genotypes from 5 to 16 days post planting.

	Hypocotyl diameter				
	Days post planting				
Genotype	5	6	7	8	16
	mm				
gl	-	0.742	0.739	0.752	0.803
HH22	0.729	-	0.762	0.762	0.767
UI8	0.673	0.701	0.770	0.732	0.749
C27	0.655	0.635	0.696	0.643	0.683
GWD2	0.681	0.726	0.711	0.693	0.721
Beta 1345	0.693	0.685	0.701	0.711	0.757
ACH14	0.678	0.681	0.676	0.676	0.678
46F3	0.665	0.660	0.681	0.665	0.688
L19	0.594	0.620	0.632	0.630	0.655
LSD 0.05	0.015	0.015	0.020	0.023	0.023
Mean	0.671	0.681	0.709	0.696	0.721
Correlation with harvest sucrose %	-0.74	-0.86	-0.87	-0.70	-0.86

(Table 5). The ranking of the nine genotypes in HD did not change significantly from 5 to 16 days post-planting (Table 5). The hypocotyl diameter growth was very slow during this period of time; however after day 16 (data not shown), the expansion accelerated. This corresponded with

primary cambial ring development and the onset of true root cell formation. Significant negative correlations between HD values and harvest sucrose percentages were obtained at each measurement.

Seed quality was evaluated by testing different seed sizes from the same seedlot of four different cultivars. Plants grown from each seed size of hybrids GW45 and GW115 were measured for HD at 7, 9, 11, 14, and 28 days and hybrids AH10 and AH12 were measured at 9, 11, 14, and 28 days post-planting. The large-seed sizes, 8-9/64 and 9-10/64 inches, gave significantly larger HDs than the small-seed sizes, 6-7/64 and 7-8/64 inches (Table 6). The

Table 6. Hypocotyl diameter of different seed sizes of four different sugarbeet hybrids from 7 to 28 days post-planting.

Seed size	Days post-planting				
	7	9	11	14	28
inches			mm		
			GW45		
6-7/64	0.683 b	0.709 c	0.746 b	0.805 b	3.302 a
7-8/64	0.731 b	0.759 b	0.757 ab	0.843 a	3.340 a
8-9/64	0.777 a	0.739 b	0.777 a	0.869 a	3.228 a
9-10/64	0.765 a	0.782 a	0.772 ab	0.866 a	3.302 a
Mean	0.739	0.747	0.762	0.841	3.292
			AH10		
7-8/64		0.709 b	0.731 b	0.820 b	3.632 a
8-9/64		0.719 b	0.815 a	0.858 a	3.556 a
9-10/64		0.747 a	0.808 a	0.826 b	3.657 a
Mean		0.724	0.785	0.833	3.622
			GW115		
6-7/64	0.754 b	0.704 b	0.762 a	0.808 b	3.546 a
7-8/64	0.772 a	0.744 a	0.767 a	0.813 b	3.358 b
8-9/64	0.785 a	0.764 a	0.772 a	0.864 a	3.345 b
9-10/64		0.767 a	0.800 a	0.836 b	3.485 a
Mean		0.744	0.780	0.828	3.434
			AH12		
7-8/64		0.711 c	0.759 b	0.787 b	3.104 a
8-9/64		0.803 a	0.858 a	0.848 a	3.106 a
9-10/64		0.749 b	0.843 a	0.803 b	3.152 a
Mean		0.754	0.820	0.813	3.122

Values followed by the same letter are not significantly diferent at p = 0.05.

differences in HD values between the large- and small-seed sizes was greatest in the youngest plants, (7 and 9 days) and diminished as the plants grew. By the time the plants were 28 days old, the differences in HD values between seed sizes had disappeared. There were, however, significant differences between hybrids at 28 days post planting (Table 6). The initial differences were probably due to differences in available nourishment for germination and early growth in the different seed sizes. As the plants grew and began manufacturing photosynthate from their true leaves, genetic differences became the predominating factor and overcame differences due to seed size.

The above studies were conducted in vermiculite and watered with tap water. Since the nutrition available to the seed appears to have a significant effect on the HD, we anticipated that if ample nutrients were available at the onset of germination, the difference in seed size might be diminished. We tested a number of genotypes for this nutrition effect by comparing tap water vs. nutrient solution added at the time of planting. The HD values of plants receiving nutrient solution were significantly larger at 6 days than those of plants watered with tap water (Table 7). The ranking of the genotypes, however,

Table 7. Hypocotyl diameters at 6 days post-planting of nine sugarbeet genotypes watered daily with nutrient and tap water.

Genotype	Hypocotyl diameter of plants 6-days post-planting	
	Nutrient	tap water
	mm	mm
g1	0.874	0.742
HH22	0.950	0.762
UI#8	0.856	0.701
C27	0.775	0.635
GWD2	0.836	0.726
Beta 1345	0.790	0.686
ACH14	0.775	0.681
46F3	0.770	0.660
L19	0.721	0.620
Mean	0.815	0.691
LSD 0.05	0.020	0.018

r (nutrient vs tap water) = 0.93

did not change (r = 0.93). As the plants grew, differences become greater (data not shown).

As soon as the plants emerge, (4 to 5 days) the cotyledons begin photosynthesizing. Photosynthesis is accelerated as the true leaves appear at about 14 days. This early photosynthesis in the cotyledons could affect HD values, especially, as the plants mature (as shown above). This influence was evaluated by covering half of the pots of 21 genotypes with black plastic. Seven days after planting, the plants covered with black plastic had essentially the same HD's as the uncovered plants (Table 8). As the plants grew, this difference became greater (data not shown); however, the ranking of the genotypes did not change, r = 0.92 (Table 8).

Table 8. Hypocotyl diameters at 7 days post-planting of 21 sugarbeet genotypes grown under dark and light conditions.

Genotype	Hypocotyl diameter of plants 7 days post-planting	
	Dark	Light
	mm	mm
gl	0.904	0.889
UI#50	0.881	0.879
USH20	0.866	0.836
d148	0.846	0.823
GWD2	0.838	0.805
HH22	0.838	0.820
C17	0.823	0.813
UI#8	0.820	0.830
Beta 1345	0.818	0.820
US#51	0.805	0.843
C27	0.790	0.762
6F5	0.787	0.805
28F1	0.782	0.775
L10	0.780	0.795
L53	0.775	0.785
L29	0.767	0.795
46F3	0.762	0.772
6F4	0.757	0.737
ACH14	0.744	0.756
L19	0.706	0.739
L37	0.693	0.696
Mean	0.800	0.797
LSD 0.05	0.018	0.018

r (Dark vs Light) = 0.92

Selection Studies

Selection experiments were conducted on plants 7 days post-planting that had been grown in vermiculite watered with nutrient solution. The results reported herein are one cycle of mass selection in four different highly heterozygous open-pollinated populations. In every population, the small-HD selection gave a higher sucrose percentage than the parent population; however, none was significantly higher (Table 9). The large-HD selections generally had a sucrose percentage below their respective parents. Two large-HD (L91 and the large-HD composite of h537) had a significantly lower sucrose percentage than their parents. Selections L91 and i63 were selected in different tests but tested in the same replicated field trial and may account for the difference in sucrose percentage (Table 9). Parent population f354 and its set of selections were tested in 1981 and 1982 (Table 10). Both years, the small-HD selection was higher in sucrose percentage than the parent, with the increase being significant in 1982. The sucrose percentage of the large-HD selection was lower in 1981 and slightly higher in 1982 than the parent (Table 10).

Selection progress was in the expected direction in each of the four populations; however, few selections differed significantly from the parent. Continued cycles of selection and a more conscientious effort to control seed quality might significantly increase sucrose concentration.

In populations f354 and h357, efforts were made to combine the HD at 7 days and the HD at 28 days technique. Ideally, those plants with small HD values at 7 days and large HD values at 28 days should be producing many small cells. These genotypes should, therefore, be high in both sucrose percentage and root yield. It was difficult to find plants with those ideal parameters (small HD at 7 days and large HD at 28 days); however, sufficient plants were identified with these ideal characteristics to produce a new selection in each population. These ideal

Table 9. Harvest sucrose percentages for large and small hypocotyl diameter 1st cycle mass selection populations from three sugarbeet heterozygous populations.

Genotype	Hypocotyl diameter selection†	Harvest sucrose
		%
L63	Small selection	15.9
g237	Parent	15.2
L33	Large selection	15.3
LSD 0.05		1.1
ml66	Small selection	13.0
h537	Parent	12.9
ml39	Large selection	12.9
LSD 0.05		0.5
Composit of 3 selections*	Small selection	14.9
h537	Parent	14.6
Composit of 3 selections*	Large Selection	14.2
LSD 0.05		0.4
L89	Small selection	16.3
f1	Parent	16.0
i63	Large selection	15.5
L91	Large selection	13.5
LSD 0.05		0.6

†Determined on plants seven days post planting.
*Different than the ml66 and ml39 selections

Table 10. Sugarbeet harvest sucrose percentages determined from 1981 and 1982 field tests for small and large-hypocotyl diameter first cycle mass selections and the parent population f354.

Genotype	Hypocotyl diameter† selection	Harvest sucrose 1981	1982
		%	%
L240	Small selection	15.6	15.1
f354	Parent	15.5	14.6
L241	Large selection	15.0	14.7
LSD 0.05		0.9	0.5

†Determined on plants seven days post-planting

first cycle mass selections, along with other combinations of these two parameters, were evaluated in replicated field trials (Table 11). The new ideal selections were

Table 11. Sugarbeet harvest root yields and sucrose percentages of selections made for large- and small-hypocotyl diameter at 7 and 28 post-planting and the parent populations.

Genotype	Hypocotyl diameter selection 7 days	28 days	Sucrose	Root yield
			%	t/ha
L92	Small	Small	16.	50.9
L64	Small	Large	15.7	50.2
f354	Parent	Parent	16.1	53.3
LSD 0.05			0.6	6.0
h357-2	Small	Small	15.1	31.0
h537-6	Small	Large	14.6	29.8
h537	Parent	Parent	14.6	32.8
h537-1	Large	Large	14.8	32.1
h537-57	Large	Small	14.0	28.7
LSD 0.05			0.4	2.5

not superior to the parent populations. There was a trend for the small-HD at 7 days to have higher sugar percentages than the large-HD at 7 days. The only selection that responded significantly was the least ideal selection (h537-57). This selection had a large HD at 7 days and a small HD at 28 days and was significantly lower in sucrose percentage and root yield than the parent (Table 11).

The results reported in this paper and in earlier research (Doney and Theurer, 1976) suggest that independently selecting for HD at 7 days will increase sucrose percentages or at 28 days will effectively increase root yield. However, combining the two appears to be ineffective. Low heritability for each selection criterion would render the heritability of the combined criteria too low to identify superior genetic deviates. Selection pressure for one criterion would nullify or mask the ability to exert significant selection pressure on the other criterion.

SUMMARY

Cell size has been shown to be inversely related to sucrose concentration; i.e., genotypes with large root cells are low in sucrose concentration. Potential sucrose concentration could be determined in young plants if the relative root cell size could be quickly and easily determined. This research was undertaken to develop a quick and easy method to determine relative root cell size. It was found that all genotypes lay down the same number of cortex cells at germination and that the genetic potential for cell size is already apparent in the cortex cells. A simple measurement of the hypocotyl diameter (HD) of newly germinated seedlings should, therefore, give a relative measurement of the genetic cell size and potential sucrose concentration. Three sets of genetically different material gave correlations between harvest sucrose concentrations and HD values of 7-day-old plants of from -0.68 to -0.78. Nutrition and light did not affect the ranking of genotypes; however, seed size did influence the HD and tended to confound the correlations. Selections were made for large and small HD values of 7-day-old plants in four highly heterozygous populations. In all four populations, the small HD selections gave higher sucrose concentrations (significantly higher in two populations) than did the large HD selections. All small HD selections had higher (but not significant) sucrose concentrations than their respective parent populations. Combining the HD values of the 7-day-old selection technique (for high sucrose concentration) with the HD values of the 28-day-old selection technique (for high root yield) was not effective. Low heritability for each selection criterion rendered the combined criteria too low to identify superior genetic deviates. Selection pressure for one criterion nullified or masked the ability to exert significant selection pressure on the other criterion.

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