Seedling Physiology and Sugarbeet Yield*

D. L. DONEY**

Received for publication April 23, 1979

In sugarbeet, we have a most unique crop plant to study because of the growth phase and plant part that is of economic importance.

The physiologist likes to divide yield into biological yield (BY) and economic yield (EY). BY is total dry matter produced in the growing season, whereas EY is the total dry matter of economic importance. In many crop plants, the EY involves the reproductive growth phase and is somewhat unrelated to the BY; however, in sugarbeet the EY involves the vegetative growth phase and is very closely related to the BY. This makes the investigation of the BY somewhat easier.

Very little differentiation takes place during the vegetative growth phase. The major differentiation between the time of germination and harvest takes place in the first few weeks of growth. Therefore, our studies of sugarbeet yield can be focused on growth and the growth processes.

Most differentiation takes place in the first 30 days of growth. Germination takes place between 3 and 5 days after planting, depending on temperature. At about 3 days the germinating seed sends out a radicle, and by 5 days the cotyledons emerge. Growth is very slow for the next 5 to 7 days until true leaves are formed. The first true leaves begin emerging at about 10 to 12 days after planting and emerge at the rate of about 2 to 4 per week for the rest of the growing season. By the time the plants are 30 days old, they have 6 to 10 true leaves.

---

*Cooperative investigations of Agricultural Research, Science and Education Admin., U.S. Department of Agriculture; the Beet Sugar Development Foundation; and the Utah Agricultural Experiment Station. Approved as Journal Paper No. 2329, Utah State Agricultural Exp. Sta., Logan, UT.

**Research Geneticist, USDA, Science and Education Administration, Agricultural Research, Crops Research Laboratory, UMC 63, Utah State University, Logan, UT 84322

The root doesn't begin to thicken until the first true leaves are formed. When the radical first emerges from the germinating seedling, it is composed of mostly cortex material with a center core of undifferentiated meristamatic tissue. The number of cortex cells does not increase with expansion of the root, but the cells grow in size and eventually break and are sluffed off as the true root grows.

Differentiation begins immediately in the core, although it seems rather slow at first (Figure 1). In about 10 to 12 days when the first true leaves are forming, vascular material (Figure 2) can be seen in the core as well as the beginning of the primary cambial layer. This gives rise to the secondary cambial layer by about 18 days (Figure 3).

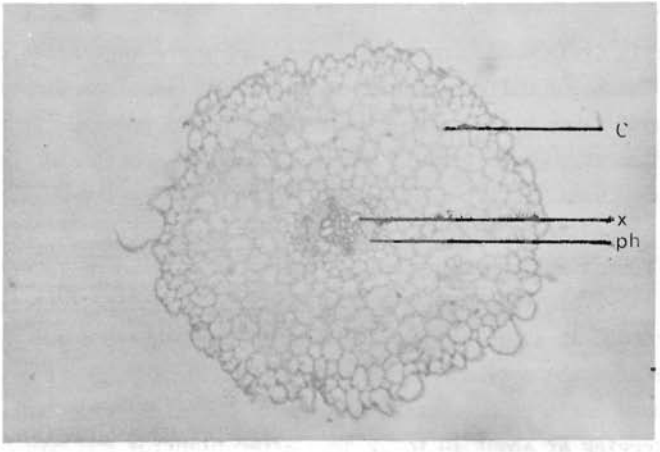

Figure 1. Cross section of 9-day old sugarbeet root. C = cortex; ph = phloem; x = xylem; x 150.

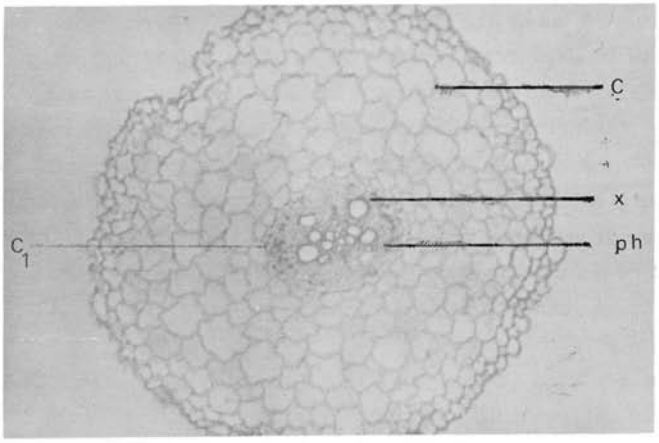

Figure 2. Cross section of 13-day-old sugarbeet root. C = cortex; ph = phloem; x = xylem; C_1 = primary cambium; x 143.

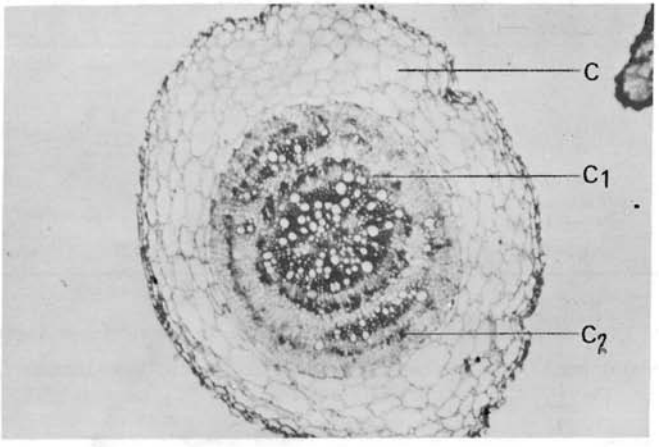

Figure 3. Cross section of 20-day-old sugarbeet root. C = cortex; C_1 = primary cambium; C_2 = secondary cambium; x 83.

All cell division takes place at the cambial layers from which the new cells differentiate into xylem, phloem, and storage parenchyma cells. The secondary cambial layer gives rise to the third cambial layer and so on until all the rings are formed, which occurs at about 30 to 40 days or when the root is about 1.0 to 1.5 cm in diameter (1). From then on growth is cell division and cell expansion, taking place simultaneously in all rings. The genetic identity of a sugarbeet plant has been attained by this time. Its ring number, cell size, photosynthate partitioning, and vigor in relation to other genotypes have already been determined. This means we should be able to measure important growth parameters in the seedling stage rather than waiting until harvest time.

Dr. Snyder reported (this issue) that he was able to select plants genetically different in their partitioning of photosynthate at a rather young age. Once the genetic relationship for partitioning of photosynthate occurs, it changes very little throughout the remainder of the growing season. For example, two inbreds (L19 and L10) differ in their partitioning, as indicated by their root/shoot ratio (Table 1).

Table 1. Root/shoot ratio of inbreds L19 and L10 from July 1 to September 8.

	Root/Shoot Ratio		
	L19	L10	L19 as % of L10
July 1	0.158	0.241	66
July 28	0.419	0.661	64
August 18	0.692	1.125	62
September 8	0.890	1.364	65

From July 1 to September 8, the relationship between these inbreds in root/shoot remained constant although the ratio was increasing for both.

The difference in root/shoot ratio between genotypes GWD2 and L19 is small; yet, this genetic difference can be detected in plants 10 days after planting (Figure 4). The relationship between these genotypes remains constant although the ratio changes with time. It decreases for the first 15 days, levels off between 20 and 30 days, then begins increasing and continues to increase throughout the remainder of the growing season. The leaves grow more rapidly at first until the root is about 1 cm in diameter, which is about the time all the rings are formed. Then growth of the root increases. As more meristematic tissue is formed in the root, more photosynthate is demanded for cell division and growth. However, relative genetic partitioning is determined as soon as the first true leaves begin manufacturing food.

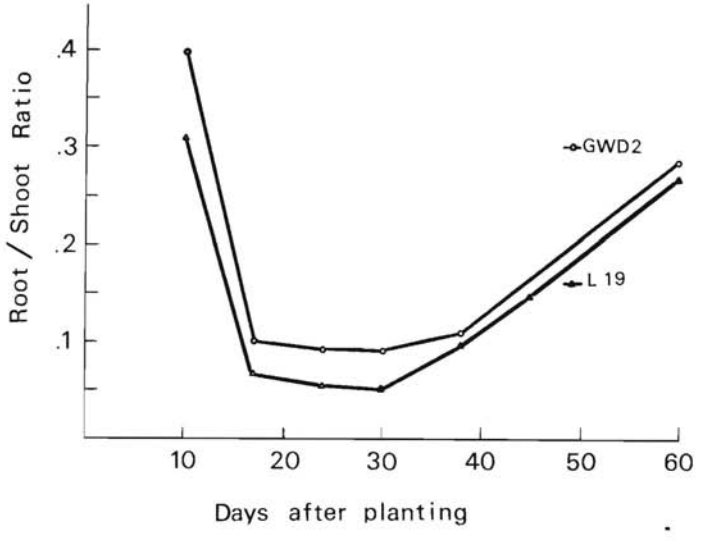

Figure 4. Root/shoot ratio of genotypes GWD2 and L19 from 10 to 60 days after planting.

The relative percent dry matter of leaves is also determined very early (Figure 5). At 10 days, genetic differences among genotypes L19, GWD2, and Blanca in percent dry matter of the leaves were already evident. These differences remained throughout the growing

season. The relative percent dry matter of the roots followed a similar pattern (Figure 6); however, genetic differences were not evident until about 15 days. The percent dry matter in the root increased more rapidly than in the leaves.

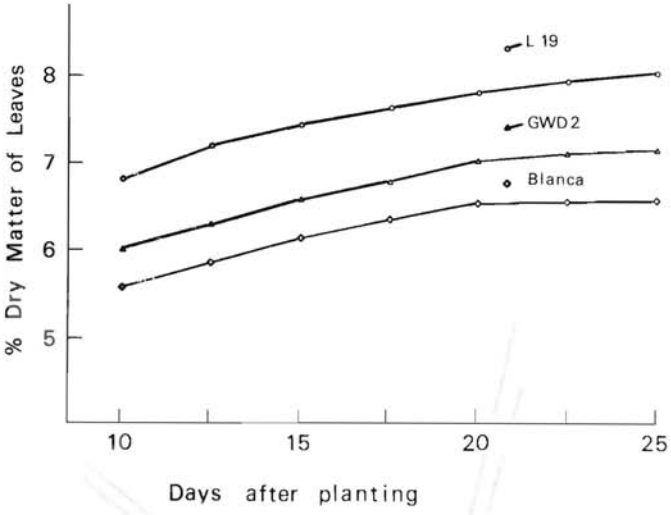

Figure 5. Percent dry matter of leaves of genotypes L19, GWD2, and Blanca from 10 to 25 days after planting.

Genetic differences in root diameter are also established very early. Two genotypes, Blanca and L19, gave significant differences as early as 5 days (Figure 7).

These results lead me to believe that we can determine the potential of a given genotype in vigor, growth, and sugar production at a very young age. The keys are: 1) control of the environmental variation, and 2) knowledge of the parameters to measure.

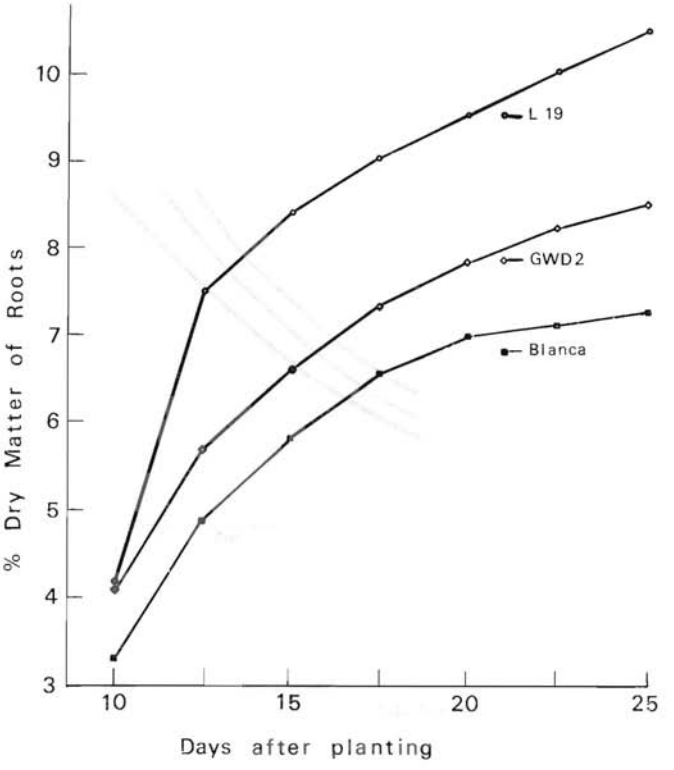

Figure 6. Percent dry matter of roots of genotypes L19, GWD2, and Blanca from 10 to 25 days after planting.

We have found that the environmental variation for root weight is generally greater in the seedling stage than in mature plants (Table 2). The coefficient of variation of a uniform hybrid was about 10 percent greater for seedling root yield than for root yield of mature plants.

Many workers have recognized the desirability and potential of measurement of seedling parameters. A very brief summary of some of the attempts to correlate seedling characters with yield and sugar production is given in Table 3.

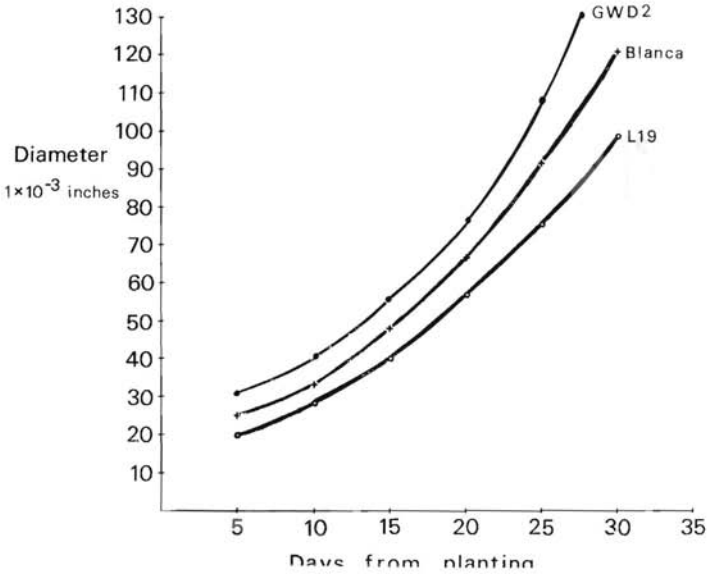

Figure 7. Root diameter of genotypes Blanca and L19 from 5 to 35 days after planting.

Pannonhalmi (14) in Hungary studied the effect of irradiation of the seed and reported a positive effect on yield. The effect of seed size has been reported to influence yield by three workers: two from USSR (8, 13) and one from Ireland (10). The effect of germination on yield has generally given negative results (3, 6, 8, 15); only one worker (8) has reported a positive effect. All workers (5, 6, 9, 15, 18) who have studied effects of seedling root weight on yield report a positive effect on root yield. Root diameter has been shown to be highly correlated with root yield by Shimamoto of Japan (16, 17) and myself (6). One worker in Belgium (7) reported a correlation of peroxidase activity in seedlings with percent sugar, and finally a Russian (4) has reported that seed treated with ultrasonic sound germinated sooner, and the seedlings grew more rapidly than untreated seed.

Table 2. Coefficient of variation of a uniform hybrid for root weight of mature roots and 3-week-old seedlings.

Age	Measurement	CV
5 months	Root weight	21.5%
3 weeks	Root weight	31.0%
3 weeks	Hypocotyl diameter	9.5%

Table 3. Seedling parameters and their influence on growth and yield.

Seedling Parameter	Researcher	Country	Influence	
			Positive	Negative
X-Irradiation on growth	Pannonhalmi (14)	Hungary	x	
Seed size on yield	Efremov (8)	USSR	x	
" " " "	MacLachlan (10)	Ireland	x	
" " " "	Muratov (13)	USSR	x	
Seed germ. on yield	Rostel (15)	E. Germany		x
" " " "	Battle (3)	England		x
" " " "	Efremov (8)	USSR	x	
" " " "	Doney (6)	USA		x
Seedling root wt. on yield	Kulenev (9)	Bulgaria	x	
D i t t o	Rostel (15)	E. Germany	x	
D i t t o	Buzanov (5)	USSR	x	
D i t t o	Doney (6)	USA	x	
D i t t o	Snyder (18)	USA	x	
Root diam. on yield	Shimamoto (16,17)	Japan	x	
" " " "	Doney (6)	USA	x	
Peroxidase on % sugar	Dubucq (7)	Belgium	x	
Ultrasonic sound on growth	Bulavin (4)	USSR	x	

We have studied a number of seedling characteristics in our lab. Several years ago we found that root diameter gave us a better correlation with harvest yield than the other morphological factors studied. A Japanese worker, Shimamoto (16, 17), had earlier reported that in young plants, root diameter gave a better correlation with harvest yield than root length. One reason for this better relationship with yield is the cone shape of the sugarbeet. An increase in the diameter of a cone has a greater influence on the total volume of a cone than a similar increase in the length. We were able to show that this relationship was true in plants as young as 3 weeks old (6). We originally measured the hypocotyl because we were saving the plants, but we have since found that better measurements can be made by pulling the plant and measuring the area of greatest expansion. A detailed description of our technique is given in Appendix I.

Over the past few years, we have conducted numerous tests to compare our hypocotyl diameter rankings with the ranked yields in replicated field trials (Table 4). These comparisons gave correlations from -0.70 to 0.91; however, most ranged from 0.60 to 0.90. Poor correlations generally resulted from poor field trials (Tests 7, 8, and 12). In Test 3, lines were not significantly different for hypocotyl diameter or harvest root yield; therefore, the correlation for Test 3 has little meaning. Entries in test 8 and 9 were identical except they were grown at different locations. Unknown residual fertilizer effects were observed in Test 8. This resulted in a very high coefficient of variation and a non-significant correlation (0.34) for root yield between these two field trials. The poor correlation for Test 15 is difficult to explain. The field trial had excellent precision. The greenhouse trials were conducted to verify the hypocotyl diameter rankings and they were identical.

In general, however, relative root yield can be predicted by measuring the hypocotyl diameter of 3-week-old seedlings. Our correlations are as good or better than variety trial correlations for root yield between locations.

Table 4. Correlations of hypocotyl diameter with harvest root yield obtained in replicated field trials.

Year	Test	(r)	No. of Entries	Description
1973	1	0.60	18	Diallel (no inbreds)
"	2	0.70	18	Diallel
1974	3	0.10	12	O.P. lines (no difference between lines)
"	4	0.73	15	O.P. lines
"	5	0.76	12	Hybrids
1975	6	0.90	6	O.P. lines
1976	7	0.27	10	Sugar Sel. (3 reps.)
"	8	0.16	20	Hybrids
"	9	0.60	20	Hybrids (same hybrids as Test 8)
"	10	0.88	26	Diallel (inbreds included)
"	11	0.78	7	Commercial Hybrids (Am. Crystal)
"	12	-0.70	7	Sugar Sel. (Single-row plots)
1977	13	0.60	25	Hybrids (TASCO)
"	14	0.74	9	Sugar Sel.
"	15	0.04	7	Hybrids (Great Western)
"	16	0.91	11	Sugar Sel.

The hypocotyl diameter selection technique was further evaluated as a selection tool in two separate experiments involving open-pollinated lines and hybrids. In the first experiment a series of lines from an open-pollinated population was measured for seedling hypocotyl diameter. Seed from those plants with hypocotyls of large diameters were pooled into Population 1006, and seed from those plants with hypocotyls of small diameters were pooled into Population 1005. These two resultant populations were tested in a replicated field trial and results are given in Figure 8. There was a 20 percent difference between the two populations in hypocotyl diameter. The large hypocotyl diameter population (1006) yielded 30 percent greater than the small hypocotyl diameter population (1005) (Figure 8). A significant decrease in sucrose percentage was observed in the large hypocotyl diameter population; however, it still produced significantly more total sucrose. The second experiment was from a group of hybrids (having a common female parent)

selected for large and small hypocotyl diameter. The large hypocotyl diameter hybrids significantly outyielded the small hypocotyl diameter hybrids for both root weight and gross sucrose (Table 5). The sucrose percentage was not affected by the selection procedure.

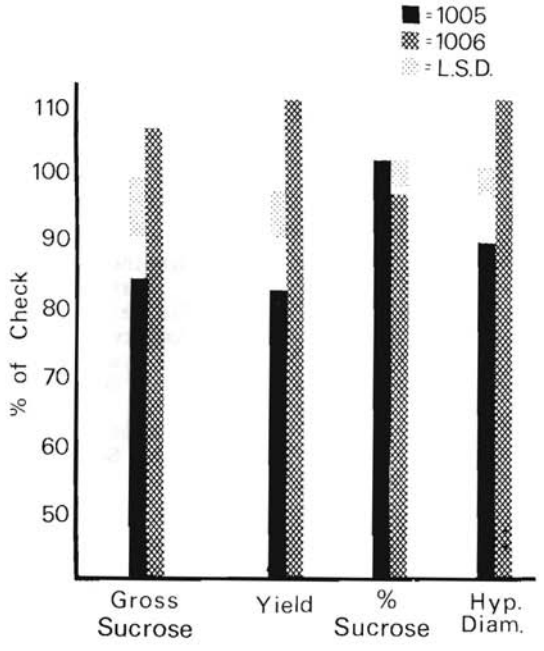

Figure 8. Gross sucrose, root yield, percent sucrose, and hypocotyl diameter of a large hypocotyl diameter population (1006) and a small hypocotyl diameter population (1005). Data are presented as a percent of a check variety.

Table 5. Gross sucrose, root yield, and percent sucrose for hybrids selected for large and small hypocotyl diameter.

Hybrids	Gross Sucrose	Tons/ Acre	Percent Sucrose
Large hypocotyl diameter	5839	21.1	13.8
Small hypocotyl diameter	4910	17.9	13.7
LSD at 0.05	870	2.6	0.7

If this technique is to be of value, it must be useful in a breeding program. We have, therefore, adapted it into a recurrent selection breeding program (Figure 9). This program takes only 1 year per cycle, while the conventional recurrent selection breeding method takes 3 to 4 years. Seed is space-planted in the field in July. At harvest time, about September 15, a selection is made for sucrose percentage. Selected beets are cut in half and one half placed in the coldroom for thermal induction. At the same time, stecklings of a CMS tester are placed in the coldroom for induction. Around December 15 these half-beets and the CMS tester plants are brought from the coldroom and individually crossed. The other half-root is then thermally induced. The testcross progeny harvested from the CMS tester is then tested for hypocotyl diameter. The parents (other half) of the best progenies (largest hypocotyl diameter) are intercrossed to produce the selection population.

In order to determine the achieved progress in one cycle of selection (1 year), we crossed the new selection population and the parent population to the CMS tester (L53 CMS). This resulted in four test populations (Table 6). A comparison between the parent testcross and the new population testcross indicates the effect on combining ability. Progress, per se, is indicated in the comparison between the parent and the new population.

From about 200 beets, 17 were selected whose progenies averaged 7 percent better than the parent progeny mean. The achieved progress depends on the heritability and correlation with root yield. A heritability of 1.00 and a correlation of 1.00 would result in an increase of 7 percent in root yield (Table 6 - Predicted Yield). Based on earlier estimates (6), we would expect a 3 to 4 percent increase in root yield.

These four populations were tested in the greenhouse for hypocotyl diameter and also in replicated field trials. The new population testcross gave a 5 percent increase in hypocotyl diameter and a 2 percent increase in root yield over the parent population testcross (Table 6). The combining ability effect was about what was expected for hypocotyl diameter but a little lower than expected for root yield.

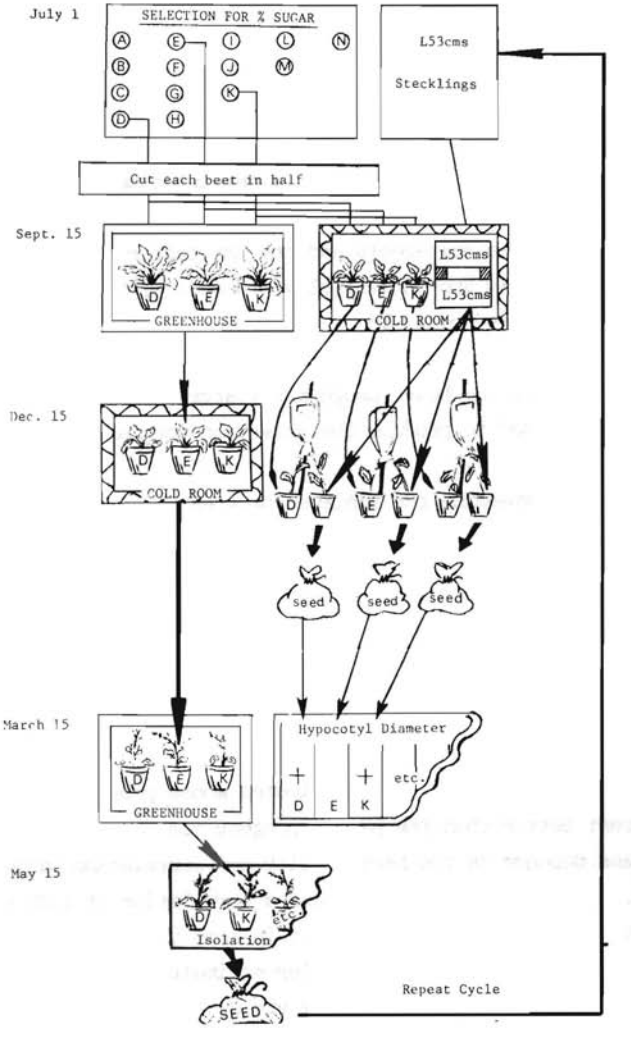

Figure 9. Flow diagram of a recurrent selection breeding method for sugarbeet using the hypocotyl diameter technique as a selection criterion for yield combining ability.

Table 6. Hypocotyl diameter and field data for parent population testcross, new selection population testcross, parent population and new selection population. Data are in percent of parent.

Population	Hypocotyl Diameter		Field Data		
	*Predicted Yield	Greenhouse Test	Root Yield	% Sugar	Gross Sugar
L53CMS x Parent Pop.	100	100	100	100	100
L53CMS x New Sel. Pop.	107	105	102	99	101
Parent Population		100	100	100	100
New Selection Pop.		111	110	95	104
LSD 0.05	5	4	6	3	7

*Mean hypocotyl diameter of the selected plants over the parent population mean based on hypocotyl diameter progeny tests.

The new population exceeded the parent population by 11 percent for hypocotyl diameter and 10 percent for root yield. This increase was accompanied by a significant decrease in sugar percentage. This points out the need to consider sugar concentration in any breeding program. These selections were based only on hypocotyl diameter without regard to sugar percentage. For this reason we have incorporated the sugar selection step in the recurrent selection method mentioned earlier (Figure 9). This step was added after the first cycle of selection and, at present, we haven't determined its effectiveness.

There ought to be other ways of determining sugar potential in the seedling stage. Some of the methods might be osmotic pressure, cell size, ring number, or ring width. The osmotic pressure is easily measured in the seedling stage, as is ring number and ring width. However, in a breeding program where it is necessary to evaluate a large number of plants, the feasibility of these methods is questionable.

Several workers have reported a good correlation between cell size and percent sugar (2, 12, 11); however, measurement of cell size poses a difficult problem. Counting cells in a grid or across a plane of a cross section is very tedious and very difficult,

considering the many sizes and shapes one observes in a cross section. Cell size can also be determined by separating the cells with the use of macerating enzymes and counting repeated samples of cells. This method is rather sophisticated and time consuming. It would not be practical in a breeding program. Another suggestion would be to scan for cell wall material either in a densitometer or IR analyzer from thin cross sections. We are not sure how effective or practical these methods would be.

In summary, many of the genetic differences in the growth processes are established in very young beets. Therefore, we ought to be able to improve sugar production by selecting for some of the important growth and sugar parameters in the seedling or young-plant stage. The key is to be able to control the environmental variation and to know what parameters to select.

In our greenhouse technique, we have been able to control much of the environmental variation. We have also shown that selection by use of the hypocotyl diameter of seedlings is effective in improving root yield. Some other important parameters for measurement might be photosynthate partitioning, root diameter, osmotic pressure, and cell size. There also might be other more important parameters of which we are currently unaware. At present, research in this area shows promise.

LITERATURE CITED

(1) Artschwager, Ernst. 1926. Anatomy of the vegetative organs of the sugar beet. J. Agr. Res. 33:143-176.

(2) Artschwager, Ernst. 1930. A study of the structure of sugar beets in relation to sugar content and type. J. Agr. Res. 40:867-915.

(3) Battle, J. P. and W. J. Whittington. 1971. Genetic variability in time to germination of sugar-beet clusters. J. Agric. Sci. Cam. 76:27-32.

(4) Bulavin, A. I., L. N. Sitenko. 1974. The reaction of polyhybrid and parental forms of sugar beet plants to ultrasonic treatment of the seed. Vestn. Khar'kov. un'ta, biologiya. 105:76-78. From Ref. Zhurnal, Abst. 7.55.260.

(5) Buzanov, I. F., K. A. Makovetskii, N. G. Budkovskaya, L. S. Pilipchenko, N. I. Semenenko. 1972. Effect of single selection of sugarbeet for developmental vigor of the root system and tops on yield and quality of seed. In Osnovn. Vyvody Nauch-issled. rabot VNII sakhar. svekly po sokhar. Suckle za 1968. g. 62-65. From Ref. Zhurnal Abst. 10.55.565.

(6) Doney, D. L. and J. C. Theurer. 1976. Hypocotyl diameter as a predictive selection criterion in sugarbeet. Crop Sci. 16:513-515.

(7) Dubucq, M., M. Bouchet, and T. Gaspar. 1973. Peroxidase activity and isoperoxidases in sugarbeet. J. International Inst. for Sugar Res. 6:109-116.

(8) Efremov, A. E. 1968. The importance of fruit size and seed size in breeding monogerm sugarbeet. Sci. Trans. Breed. Agrotech. Mech. Cult. Sugar Beet, Grain, Pulse Crops. L'gov. Exp-breed. Sta. 2:29-34. From Ref. Zhurnal; Abst. 1.55.174.

(9) Kulenev, D. 1967. Studies of the efficacy of selection in summer crops of high-sugar varieties of sugarbeet by their initial growth rate. News Sci-res. Ivanov Inst. sug-beet Sumen:41-48. From Bulg. Sci. Lit. Abst. 1114.

(10) MacLachlan, J. B. 1972. Effect of seed size on yield of monogerm sugar beet (Beta vulgaris). Irish J. of Agric. Res. 11:233-236.

(11) Milford, G. F. J. 1973. The growth and development of the storage root of sugar beet. Ann. Appl. Biol. 75:427-438.

(12) Milford, G. F. J. 1976. Sugar concentration in sugarbeet; Varietal differences and the effects of soil type and planting density on the size of the root cells. Ann. Appl. Biol. 83:251-257.

(13) Muratov, M. KH., I. A. Grankina. 1974. The productivity of different seed-ball fractions of intervarietal sugarbeet hybrids. Kazakstan anyl sǎruasylyk gylymynyn habarsysy. 2:32-35. From Ref. Zhurnal; Abst. 7.55.794.

(14) Pannonhalmi, J. 1968. Stimulation of emergence in sugar beets differing in degree of ploidy by means of x irradiation. Mezogazd. Novenyterm. Kutatoint. Kozl. 4:145-160.

(15) Rostel, H. J. 1971. Possibilities of early diagnosis in sugarbeet breeding. Arch. Zuchtungsforschung 1 (1). From Landwirtschaftliches Zentralblatt. Abst. 1966.

(16) Shimamoto, Y. and S. Hosokawa. 1969. Genetic variability of certain characters at various growth stages in sugar beet. Jap. J. Breeding 19:100-105.

(17) Shimamoto, Y. and S. Hosokawa. 1967. Statistical genetical analysis of root growth in sugarbeet. I. Changes in genetical parameters with growth. Bull. Sugar Beet Res. Suppl. No. 9:118-122.

(18) Snyder, F. W. 1975. Leaf and root accretion by sugarbeet seedlings in relation to yield. J. Amer. Soc. Sugar Beet Technol. 18:204-213.

APPENDIX I

HYPOCOTYL DIAMETER TECHNIQUE FOR PREDICTING ROOT YIELD

The key to prediction of root yield from seedling hypocotyl diameter is control of environmental variation. The more vigorous genotypes will expand in root diameter more rapidly than the less vigorous genotypes. Control of environmental variation will determine how well we can detect true genetic differences. This requires extreme care since root weight measurements of seedlings usually have a larger environmental error than those of mature plants. In our experiments, we have been able to exert excellent control for much of the environmental variation and, thus, predict the harvest root yield fairly well by the following techniques:

1. Type of Container Used. Clear plastic 185 ml vials, 45 mm diameter by 105 mm deep. These can be obtained for about 8¢ each. A hole is drilled into the bottom for drainage.
2. Planting. The vials are filled with vermiculite and compressed to 1 inch (25.4 mm) from the top. Two seeds are placed in the center and covered with 1 inch (25.4 mm) of vermiculite. The vermiculite is wet down very carefully, making sure to wet completely but not to overflowing.
3. Bedding. Planting takes place on Thursday. The plants begin emerging on Tuesday, and all plants that have emerged by Wednesday are saved. The remainder are discarded. We start with 36 pots per line and end up with about 30 plants per line. Because the number is not the same for all lines, we use a completely randomized design (CRD). On Wednesday, all the saved plants are placed in a moist sand bed in a CRD. The pots are spaced on 3-inch (7.62 CM) centers. A 3' x 29' (1 m x 6 m) bed will hold about 880 pots. Pots are thinned to one plant per pot.

   Holes for the pots are made by inverting a plastic vial, pressing it into the sand and withdrawing the sand. With moist mortar sand, this can be done rather easily and quickly. The sand is kept moist by watering two to three times a week. This maintains the root zone temperature at 20 C $\pm$ 1.

4. Nutrients. Each plant receives 10 ml of nutrient solution daily (except on weekends). A diluter-dispenser, adjusted to deliver 2-10 ml aliquats at each pump, is used to apply the nutrient solution. This allows two plants to be watered at a time. Using this method, 1500-1800 plants can be watered per hour.
5. Rotation. There is still about a 15-20 percent gradient in light intensity over the bed. To compensate for this variation in light, the plants are rotated twice a week from front to rear and left to right.
6. Temperature. Root zone temperature is 20 C $\pm$ 1 and air temperature is 24 C $\pm$ 6. There are greater fluctuations in air temperature in the greehouse during the summer than in the winter; therefore, our results are best in the winter months.
7. Measurements. Plants are measured 19 days after emergence. The best time to measure is when the hypocotyl diameter is about 0.1 inch. As the plant gets larger, the cortex of the hypocotyl splits and is unsymetrical. Measurement is made by a spring-loaded microcaliper calibrated in 1/1000 of an inch. The plants are pulled and the largest part of the hypocotyl-taproot tissue measured (excluding the crown). Some hypocotyls are not round; therefore, all plants are measured in two directions (180°) and the average recorded.
8. Preserving Plants. If it is desirable to save individual plants, the leaves are trimmed back and the plant repotted. Survival rate at this stage of growth is about 80 to 90 percent. The survival rate of smaller plants is much less.
9. Precision. A uniform hybrid is included in every test as a measure of the environmental variation and as a standard. The coefficient of variation of this standard runs between 7 and 9 percent. Significant differences are between 4 and 5 thousandths of an inch. Each test consists of 25 lines and two checks as standards.

The more vigorous genotypes at the seedling stage are generally more vigorous throughout the growing season and are the highest yielding.