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Anatomical characteristics of hypocotyl of sugar beets different in sugar content

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Abstract. Six seedling hypocotyl anatomical characters of sugar beet diploid lines and triploid hybrids were measured. Root yield and sugar content of these lines and hybrids were evaluated in replicated field trials. Some of the studied hypocotyl characters: the diameter of the central core, the diameter of parenchymatic cells outside the central core and the width of xylem, correlated negatively with sugar content and positively with root yield. This suggests that these parameters can be used in preliminary selection of sugar-beet breeding material. Introducing such criteria into the breeding process could speed up the selection and reduce the number of expensive field trials.

Key words: *Beta vulgaris* L., cell size, central core, root yield, sucrose concentration, vascular tissue.

An increase of sugar yield, which depends on root yield and sucrose concentration, is the most important goal in sugar-beet breeding. However, there is a negative correlation between these two characters. The increase of root yield usually results in reduction of sucrose concentration and vice versa (HECKER 1967, DONEY 1979, 1984b, WISE 1979, DONEY et al. 1981).

Evaluation of new breeding materials involves usually replicated field trials which are expensive and time-consuming. Identification of genotypes superior for potential total sugar yield already in the seedling stage could significantly accelerate the selection and variety improvement.

A number of sugar-beet seedling characters such as respiration rate, root length, root weight and dry matter, stomata number on the first true leaf,

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osmotic pressure potential, ring number and density, diameters of taproot and hypocotyl were investigated (DONEY 1979, 1984a, b, DONEY, THEURER 1976, 1990, IWATA, TSUDA 1986). Most of them, however, showed no relationship with field root yield and/or sugar content. DONEY and THEURER (1976) suggested that hypocotyl diameter of 3-week-old seedling is the most efficient feature, and the increased root yield can be achieved by selecting seedlings with a large hypocotyl diameter.

DONEY (1984a) assumed that the cellular parameters (size and number) are responsible for a large hypocotyl. The inverse relationship was found between the root parenchymatic cell size in very young plants and sucrose concentration in mature root (DONEY et al. 1981, DONEY 1983, 1984a, b, DONEY, THEURER 1983, ŚLIWIŃSKA 1991) i. e. large-celled genotypes produce large roots and have a low sucrose concentration, whereas small-celled genotypes produce small roots and have a high sucrose concentration.

The anatomy of vegetative organs of sugar beet was studied by ARTSCHWAGER (1926). ARTSCHWAGER (1930) and MILFORD (1973) investigated the relation between the structure of the mature sugar-beet root and sugar content. There is, however, very little information in the literature about the relation between seedling anatomy and yield parameters.

This paper presents results of the study on some anatomical characters of hypocotyl, and their relation to root yield and sugar content. The usefulness of these parameters in preliminary selection of sugar beets has alsobeen tested.

Material and methods

Nine diploid lines and nine triploid hybrids from MARIBO Seed (Denmark) were investigated. Diploid lines 1, 2 and 3 were Z-types with a high sugar content and low root yield. Line 4, 5, 6, 7 and 8 were N-types with a medium sugar content and root yield, whereas line 9 had a very high root yield (EE-type). Triploid hybrids 1, 2 and 3 represented the Z-type; 4, 5 and 6 the N-type, and 7, 8 and 9 – the EE-type. Diploid lines 1 to 6 were multigerm and other entries were monogerm.

Seeds were sown in 0.5 l plastic pots and grown under the controlled conditions in a growth chamber (16h light, 20°C / 8h dark, 18°C). At growth stages I, II and III with 4-6, 8-10 and 12-14 leaves, respectively, 30 seedlings (about 3, 5 and 7 week-old, respectively) were taken each time from every line and hybrid. Seedlings with detached leaves were fixed for 24h in 20% ethanol, then for 24h in 40% ethanol and finally stored in 60% ethanol.

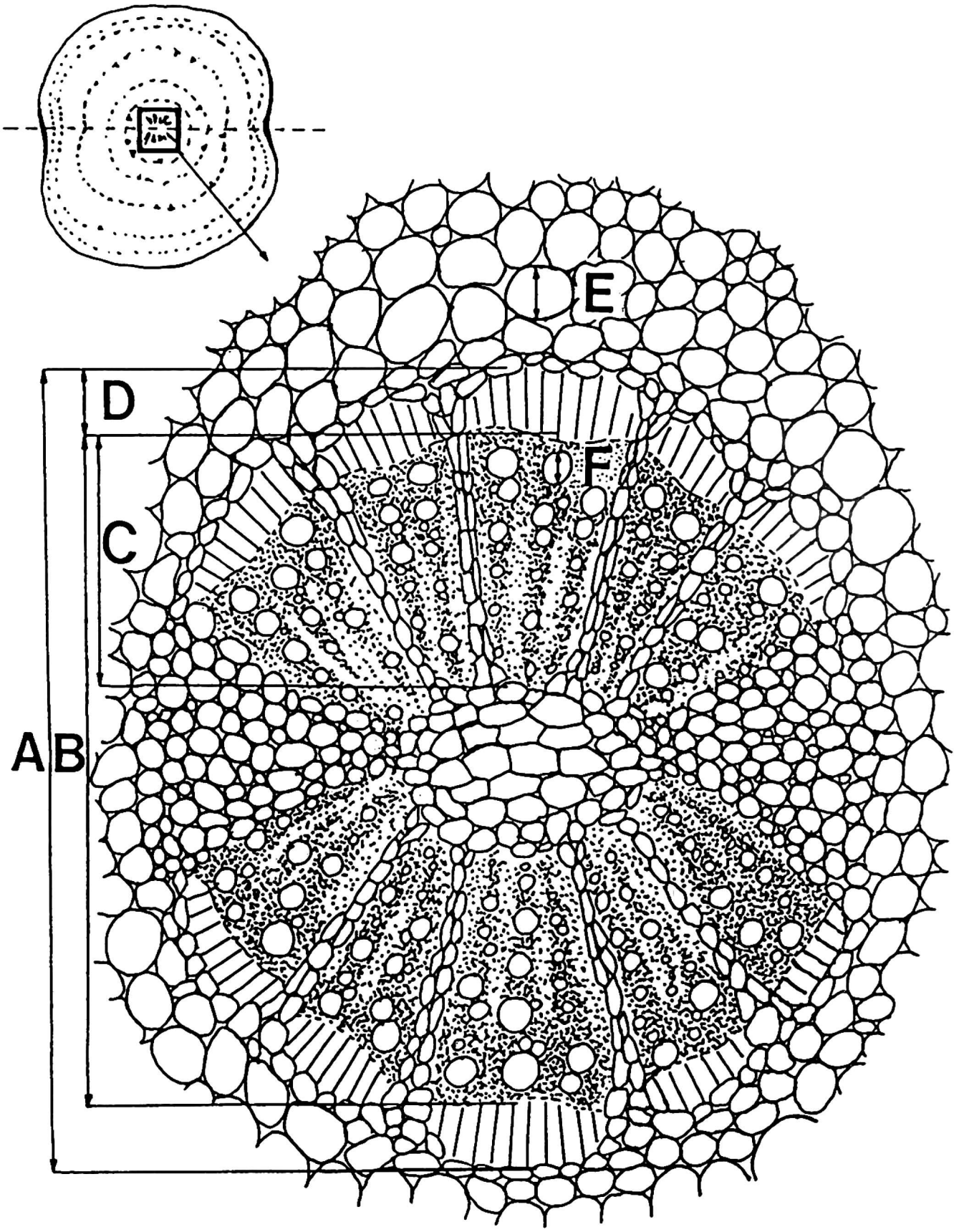

Fig. 1. A schema of cross section through the sugar-beet seedling hypocotyl
A – diameter of the central core, B – diameter of the central core to the cambium, C – width of xylem in central core, D – width of phloem in central core, E – diameter of parenchymatic cells outside the central core, F – diameter of tracheids of the central core xylem

Microtome cross sections were made from the hypocotyl and stained with safranin. Measurements made by a Biolar microscope with a micrometric scale covered following six characters (Fig. 1): A – diameter of the central core, B – diameter of the central core to the cambium (without phloem), C – width of xylem in central core, D – width of phloem in central core, E – diameter of parenchymatic cells outside the central core (five cells along the hypocotyl radius between the central core and the first ring were selected for each plant), F – diameter of tracheids of the central core xylem (five tracheids along the xylem were selected for each plant).

All the measurements were made perpendicularly the axis joining the bundles. Our preliminary investigation showed that owing to such a way of measurement the characters were more stable, more suitable for exact measuring, and correlated better with root yield and sugar content than characters measured along the axis.

Root yield and sugar percentage were determined in replicated field trials performed at experimental fields of MARIBO Seed in Denmark in 1992. For each character a one-factor analysis of variance was made. The analyses of correlation and regression for obtained results were performed.

Results

Significant differences between the tested diploid lines or triploid hybrids were observed in most of the examined characters (Tables 1 and 2; in all tables lines and hybrids were numbered according to decreasing sugar content). The tendency of an enlarged size of tissues and cells with a decreasing sugar content was evident. These enlargements especially concerned the diameters of the central core (A and B) and the diameter of parenchymatic cells (E), in particular at the second growth stage.

The root yield and sucrose content of the tested genotypes are given in Table 3. Monogerm lines 7 and 8 yielded relatively less, as compared to multigerm lines. Triploid hybrids proved to be more variable in sugar content than diploid lines. This explains a higher variation within triploid seedling characteristics, as compared to the characteristics of diploid seedlings.

Relevant coefficients were calculated to check correlation between the characters of seedlings and mature roots (Table 4). For triploid hybrids, the 4-6 leaf stage appeared to be too early to find significant correlations between the examined traits. For diploid lines, the diameter of the central core (A and B) and the diameter of parenchymatic cells outside the central core (E) were

Table 1. Hypocotyl characters (denotations: see Fig.1) of nine diploid sugar-beet lines at three growth stages (Duncan's test)

Line No.	Hypocotyl characters (μm) A	B	C	D	E	F
			Growth stage I (4-6 leaves)			
1	1076 d[1)]	845 d	275 c	116 e	29.37 d	24.18 bc
2	1235 c	934 cd	285 c	140 bcd	36.70 bc	23.52 bcd
3	1374 abc	1060 b	303 bc	147 abcd	39.08 ab	21.58 d
4	1440 ab	1051 bc	344 a	164 a	36.60 bc	25.38 ab
5	1319 bc	1070 ab	301 bc	132 de	33.03 cd	26.76 a
6	1406 ab	1090 ab	332 ab	152 abc	34.82 bc	25.36 ab
7	1322 bc	1035 bc	305 bc	138 cd	33.45 cd	23.72 bcd
8	1504 a	1170 a	329 ab	159 ab	37.50 abc	23.39 bcd
9	1466 ab	1175 a	305 bc	140 bcd	41.34 a	22.93 cd
			Growth stage II (8-10 leaves)			
1	1543 c	1234 c	414 b	139 b	38.98 c	27.85 abc
2	1569 bc	1264 bc	427 b	138 b	40.94 c	26.02 bc
3	1616 bc	1301 bc	450 ab	146 b	42.38 bc	27.61 abc
4	1624 bc	1241 c	461 ab	177 a	45.54 bc	27.53 abc
5	1599 bc	1304 bc	409 b	130 b	41.25 c	30.25 a
6	1786 ab	1446 ab	464 ab	141 b	42.18 bc	26.84 bc
7	1739 abc	1447 ab	447 ab	134 b	43.60 bc	25.35 c
8	1745 abc	1408 abc	458 ab	150 b	48.63 ab	28.67 ab
9	1917 a	1589 a	504 a	133 b	53.31 a	25.83 bc
			Growth stage III (12-14 leaves)			
1	2240 bc	1766 b	650 bc	230 ab	65.70 ab	26.66 ns[2)]
2	2342 b	1800 b	684 b	260 a	63.62 ab	30.52
3	2141 bc	1723 b	598 bc	197 abc	57.70 bc	27.40
4	2466 ab	1900 b	697 b	253 a	66.38 ab	28.36
5	2295 bc	1860 b	600 bc	163 c	41.63 cd	26.70
6	2160 bc	1827 b	600 bc	162 c	47.86 bcd	26.60
7	1839 c	1585 b	531 c	142 c	37.52 d	25.62
8	2299 bc	1969 b	699 b	174 bc	49.87 bcd	26.54
9	2837 a	2391 a	851 a	205 abc	80.46 a	27.64

[1)] values in a column for a particular growth stage followed by the same letters are not significantlly different at p=0.05

[2)] non significant differentiation

correlated with sugar content at that early development stage. At the second and third growth stages a significant negative correlation between sugar content in mature root and hypocotyl characters such as diameter of the central core (A and B), width of xylem (C) and diameter of parenchymatic cells (E) was found for both ploidy levels. At the same time these characters were positively correlated with root yield.

Table 2. Hypocotyl characters (denotations: see Fig. 1) of nine triploid sugar-beet hybrids at three growth stages (Duncan's test)

Hybrid No.	Hypocotyl characters (μm) A	B	C	D	E	F
			Growth stage I (4-6 leaves)			
1	1452 e[1)]	1028 d	414 d	203 a	42.02 f	31.15 ab
2	1667 cd	1255 bc	426 d	170 cde	56.95 a	33.10 a
3	1609 d	1208 c	441 bcd	169 de	46.90 e	31.17 ab
4	1671 cd	1265 bc	430 cd	188 abcd	51.13 cd	27.83 c
5	1721 bcd	1281 bc	468 abc	192 ab	53.91 abc	32.46 ab
6	1947 a	1440 a	481 a	203 a	53.02 bc	28.22 c
7	1812 b	1428 a	438 cd	158 e	56.05 ab	26.82 c
8	1785 bc	1354 ab	470 ab	189 abc	57.28 a	30.99 b
9	1671 cd	1243 c	461 abcd	186 bcd	48.10 de	32.54 a
			Growth stage II (8-10 leaves)			
1	2129 c	1562 d	612 de	254 abc	50.60 d	27.16 de
2	2309 bc	1801 bcd	654 cd	233 c	54.02 cd	28.21 cde
3	2136 c	1601 d	624 de	272 a	57.41 c	28.86 bc
4	2278 bc	1706 cd	576 e	252 abc	57.70 c	26.80 e
5	2464 b	1848 bc	706 bc	266 ab	66.04 b	33.21 a
6	2470 b	1985 b	708 bc	250 abc	69.25 b	28.51 bcd
7	3207 a	2657 a	777 a	237 bc	80.45 a	29.43 bc
8	3257 a	2630 a	763 ab	275 a	77.81 a	29.59 bc
9	3175 a	2515 a	732 ab	240 bc	80.70 a	29.76 b
			Growth stage III (12-14 leaves)			
1	2404 cd	1714 c	712 de	303 ns[2)]	78.19 bc	32.91 a
2	2723 bc	2088 b	675 e	264	69.75 d	29.95 c
3	2303 d	1728 c	693 e	286	65.22 d	32.40 a
4	2645 cd	1892 bc	725 de	277	74.85 c	30.18 bc
5	2766 bc	2063 b	833 bc	296	74.96 c	31.73 abc
6	2825 b	2118 b	796 cd	302	75.28 c	31.80 ab
7	3220 a	2594 a	892 b	261	93.91 a	32.30 a
8	3196 a	2647 a	938 a	305	77.77 bc	31.64 abc
9	3248 a	2595 a	1008 a	281	81.23 b	30.40 bc

[1)] values in a column for a particular growth stage followed by the same letters are not significantly different at p=0.05

[2)] non significant differentiation

Additional calculation was made to prove the usefulness of the anatomical hypocotyl character measurements in practical breeding, when differences between the tested lines or hybrids are rather small. Correlations between the chosen hypocotyl characters and sugar content were estimated after elimination of the line 9 and the hybrids 7, 8 and 9 with extremely low sugar content and high root yield (Table 6).

Table 3. Root yield and sugar content of nine diploid lines and nine triploid hybrids of sugar beet – data from replicated field trials

Diploid lines			Triploid hybrids		
No.	Sugar content %	Root yield t/ha	No.	Sugar content %	Root yield t/ha
1	17.82	53.63	1	17.38	41.62
2	17.79	53.93	2	17.13	43.00
3	17.66	50.20	3	17.08	44.39
4	17.29	55.30	4	16.81	42.95
5	17.16	53.10	5	16.72	40.92
6	17.08	49.37	6	16.69	40.93
7	16.98	49.38	7	11.60	65.65
8	16.01	53.50	8	11.49	68.44
9	10.40	79.20	9	11.35	69.14

Table 4. Coefficients of correlation between hypocotyl characters (denotations: see Fig. 1) and yield parameters for nine diploid lines and nine triploid hybrids of sugar beet at three growth stages (I – 4-6 leaves, II – 8-10 leaves, III – 12-14 leaves)

Hypocotyl character	Growth stage	Root yield		Sugar content	
		Diploid lines	Triploid hybrids	Diploid lines	Triploid hybrids
A	I	0.297	0.235	–0.485*	–0.336
	II	0.603**	0.947**	–0.821**	–0.980**
	III	0.871**	0.847**	–0.728**	–0.902**
B	I	0.392	0.339	–0.612**	–0.432
	II	0.578*	0.931**	–0.808**	–0.968**
	III	0.922**	0.898**	–0.897**	–0.936**
C	I	–0.064	0.264	–0.082	–0.345
	II	0.648**	0.739**	–0.775**	–0.804**
	III	0.892**	0.858**	–0.782**	–0.903**
D	I	–0.066	–0.332	–0.045	0.294
	II	–0.152	–0.099	0.234	0.117
	III	0.225	–0.175	0.087	0.158
E	I	0.564*	0.274	–0.616**	–0.334
	II	0.779**	0.846**	–0.906**	–0.906**
	III	0.740**	0.634**	–0.530*	–0.699**
F	I	–0.213	–0.064	0.251	0.130
	II	–0.270	0.184	0.302	–0.250
	III	0.200	–0.068	0.036	0.054

* significant at p=0.05

** significant at p=0.01 (Student's t test)

Table 5. Equations of linear regression for yield parameters and hypocotyl characters (denotations: see Fig. 1) for nine diploid lines and nine triploid hybrids of sugar beet at two growth stages (II – 8-10 leaves, III – 12-14 leaves)

Hypocotyl characters (x)	Growth stage	Root yield (y)	r^2 (%)	Sugar content (y)	r^2 (%)
		Diploid lines			
A	II	–	76	y = 42.9101–0.0157x	67
	III	y = –13.1400+0.0299x		y = 30.9812–0.0063x	53
B	II	–	85	y = 37.8543–0.0157x	65
	III	y = –15.5469+0.0379x		y = 33.9642–0.0094x	81
C	II	–	79	y = 44.3988–0.0623x	60
	III	y = –3.4254+0.0894x		y = 29.5392–0.0200x	61
E	II	y = –16.0185+1.6255x	61	y = 37.4975–0.4794x	82
	III	y = 27.0677+0.4973x	55	–	
		Triploid hybrids			
A	II	y = –15.8635+0.0256x	90	y = 29.9785–0.0057x	96
	III	y = –36.9695+0.0312x	72	y = 35.2328–0.0071x	81
B	II	y = –3.6781+0.0268x	87	y = 27.3156–0.0060x	94
	III	y = –16.5326+0.0312x	81	y = 30.2412–0.0070x	88
C	II	y = –41.1831+0.1345x	55	y = 36.6341–0.0314x	65
	III	y = –24.3292+0.0930x	74	y = 32.1340–0.0210x	82
E	II	y = –10.2138+0.9242x	72	y = 29.1843–0.2128x	82
	III	–		y = 33.6829–0.2415x	49

Table 6. Coefficients of correlation between sugar content and hypocotyl characters: diameter of central core to the cambium, width of xylem and diameter of parenchymatic cells for eight diploid lines (1-8) and six triploid hybrids (1-6) at two growth stages

Hypocotyl characters	Growth stage	Sugar content	
		Diploid lines	Triploid hybrids
Diameter of central core to the cambium	4-6 leaves	–0.809**	–0.873**
	8-10 leaves	–0.680**	–0.776**
Width of xylem	4-6 leaves	–0.611**	–0.837**
	8-10 leaves	–0.472	–0.501
Diameter of parenchymatic cells	4-6 leaves	–0.237	–0.613*
	8-10 leaves	–0.904**	–0.908**

* significant at p=0.05

** significant at p=0.01 (Student's t test)

Although some of the estimated values became lower, it was found that in the case of less differentiated material the correlation coefficients for the diameter of central core (B) and for width of xylem (C) were still significant even at the earliest growth stage tested. Previous calculation (Table 4) not always proved such correlation probably because the initial growth rate of high root yield type is different from that of the N- and Z-types.

An analysis of regression indicated linear relationship between yield parameters and some hypocotyl characters (Table 5).

Discussion

The studies on the growth of beet root as a sugar accumulating organ showed that sugar concentration was closely related to anatomical and developmental characteristics (MILFORD 1973). The sugar-beet root is differentiated into a complete set of cambial rings in the first 30-35 days of growth. All the rings are formed by the time when the root is 1-1.5 cm in diameter (ARTSCHWAGER 1926).

Our investigation proved that genetic differences are determined when the plant is very young. For economic and practical reasons possibly the earliest growth stage is desirable for testing, i.e. the 4-6 leaf stage and/or 8-10 leaf stage should be recommended. Characters of older seedlings show significant correlation with yield parameters as well. However, in plants growing in a growth chamber for over five weeks the development of new leaves was inhibited, and increment of root diameters was unequal.

The diameter of central core, width of xylem and diameter of parenchymatic cells appeared to be the most efficient criteria. The reason why the size of xylem seems to be a better criterion than the size of phloem is probably the constitution of these tissues. Xylem is composed of three dead constituents, namely tracheids, vessels and xylem plates, and of one alive constituent, i. e. xylem parenchyma. In phloem three constituents are alive – sieve tubes, companion cells and phloem parenchyma – and only sieve plates are dead. Dimensions of alive constituents of bundles are modified by squeezing during plant growth, which explains a higher variation in the size of phloem, as compared to xylem. For the same reason the estimation of the diameter of central core without phloem (B) is recommended rather than that with phloem (A). The size of tracheids, however, correlated neither with root yield nor with sugar content. There was a great variation in the diameter of tracheids along

the xylem on the same cross section which was caused by the bundle growth (different age of cells). At the same time the mean values of this character were very similar in different genotypes.

The regression analysis confirmed the results of DONEY (1983) and DONEY and THEURER (1983), showing that a relationship between seedling cell size and mature root yield characters is not always linear (Table 5). So, it is not advisable to rely only on one character.

The results obtained in this work suggest the usefulness of preliminary selection based on simultaneous measurements of the diameter of the central core and the width of xylem in the hypocotyl of seedlings at the 4-6 leaf stage or on those of the diameter of central core and parenchymatic cells in the hypocotyl of seedlings at the 8-10 leaf stage. It will reduce the cost of sugar-beet breeding process due to an early elimination of undesirable materials low in sugar content and due to a decrease of the number of lines and hybrids tested in the field trials.

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