A STUDY OF THE SOLIDS, SUGAR AND SWEETNESS CONTENT OF SELECTED INBRED CARROT LINES AND THEIR HYBRIDS

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ABSTRACT

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By

Susan Ann Engstrom Bittenbender

Inbred carrot lines and their single cross hybrids developed at Michigan State University were examined for differences in solids, sugar and sweetness content. Gas-liquid chromatography was used to quantify the sucrose, alpha-glucose, beta-glucose and fructose found to occur in the carrot roots. A taste panel was used to evaluate relative sweetness. Total and soluble solids content was also determined.

Among the inbred and hybrid lines, total and soluble solids and total sugars were mutually and positively correlated. Among the inbreds total sugar varied directly with each individual sugar. Among the hybrids total sugar was related only to sucrose. Correlations involving sweetness were evident among the hybrids.

The inbred MSU 6000 contained more solids and sugars than the other inbreds. Hybrid solids and sugars content suggested heritability of these traits. Other parent lines also appeared to influence the sugar and solids content.

The data suggested that both nuclear and cytoplasmic control and interactions of these controls may be responsible for sugar content in the hybrids.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
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to my husband

Skip

He has provided me with steadfast support as

a fellow horticulturalist and as a friend.

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INTRODUCTION

Michigan ranks third in crop value among the top eighteen carrot producing states (Whitaker et al. 1970). This is an improvement over the situation that existed about twenty-five years ago - at which time Michigan had relatively few acres in carrot production and was not producing a high quality root (Carlton 1958). Recent improvements can be attributed in part to successful trials and releases of lines and hybrids developed at Michigan State University.

Cultivar improvement has been characterized by uniform shape, size and interior and exterior root color. In cross-section the improved carrot root exhibits higher phloem to xylem area ratios, uniform orange color and the absence of yellow or watery cores. A sweeter taste has also been associated with the newer genotypes, particularly among the hybrids.

Compared with other vegetables, the carrot is high in food production efficiency (MacGillivray et al. 1942). Standard cultivars of carrots in 100 gram portions supply 200% of the adult recommended daily allowance (RDA) of provitamin A and percentages of other nutrient allowances slightly below or comparable to many common vegetables (Watt and Merril 1963). A recent study on advanced breeding lines showed that 256 to 330% of the adult RDA of provitamin A may be obtained from a 55 gram fresh portion (Leveille _et al_. 1974). Thus it is reasonable to attempt to widen the acceptance of this vegetable in the human diet.

Carrots now rank ninth in annual commercial production value on a nation-wide basis (Whitaker _et al_. 1970). This may be partially explained by consumer preference, although it also reflects the relative low cost of carrots to the consumer.

Improvement in carrot taste properties is one method of enhancing the acceptability of carrots. Carrot taste is characterized by bitter, oily and sweet components in a relatively bland flavor background. Some of the compounds responsible for these properties have been identified and assayed (Sondheimer 1957, Otsuka and Take 1969, Heatherbell _et al_. 1971 and Alabran and Mabrouk 1973). Sweetness is particularly important to cultivar improvement, as demonstrated by the fact that small, sweet carrots command a higher price on the market.

Accordingly this study was undertaken to determine differences in total and/or individual sugars between inbred carrot lines and among selected F_1 hybrids of these lines developed at Michigan State University. Differences in sugar content could be used as a criterion of further selection for sweet tasting carrots.

LITERATURE REVIEW

The sweeter naturally occurring sugars are fructose, sucrose and glucose. When sucrose is given a sweetening power value of 100, fructose and glucose have the relative values 173 and 74, respectively (Guthrie 1971). The sweetness of fructose, however, will vary depending on the presence of tautomeric isomers (Shallenberger 1971). Maltose has a sweetening value of 33 when sucrose is set at 100 (Green 1971).

Carrots contain both reducing and nonreducing sugars. The reducing sugars range from 0.1 to 5% and the nonreducing sugars range from 2.5 to 8.5% on a fresh weight basis (Hasselbring 1927, Werner 1941, Carlton and Peterson 1963, Sistrunk _et al_. 1967, Otsuka and Take 1969, Ēngel' _et al_. 1970, Alabran and Mabrouk 1973 and Phan and Hsu 1973). Therefore total sugars vary from 2.6 to 13.5%.

The ratio of nonreducing sugars to reducing sugars has been reported as 2:1 for both xylem and phloem of harvested roots of 'Imperator 11' (Phan and Hsu 1973). Earlier work, however, showed a higher ratio of 4:1 in the phloem and approximately 2:1 in the xylem of mature 'Nantes' and 'Red-Cored Chantenay' roots (Werner 1941).

The sugar content of carrot roots has been shown to depend upon the stage of root maturity (Yamaguchi et al. 1952 and Phan and Hsu 1973), morphological source of the tissue (Werner 1941 and Phan and Hsu 1973), cultivar (Werner 1941, Carlton and Peterson 1963, Sistrunk et al. 1967 and Kraut 1974) and environmental location (Leveille et al. 1974).

Individual mono- and disaccharides which comprise the reducing and nonreducing sugars have been reported. Alabran and Mabrouk (1973) determined by gas-liquid chromatography that an 'Imperator' strain of carrots contained 3.4% sucrose, 1.5% alpha-glucose, 1.9% beta-glucose, 1.1% fructose and 0.4% of one unknown sugar. Otsuka and Take (1969) found that carrots of an unnamed cultivar contained 2.4% sucrose, 2.1% maltose and 0.4% glucose. Moderate amounts of arabinose have been reported in addition to sucrose, fructose and glucose in Egyptian-grown 'Chantenay' carrots (Wali and Hassan 1966).

A postharvest decline in total sugar content was reported for 'Imperator 11' (Phan et al. 1973) and for 'Nantes' and 'Chantenay' carrots (Werner 1941). Phan et al. (1973) did note a rise in total sugars at the sixteenth week due to the appearance of raffinose. The ratio of nonreducing to reducing sugars has also been shown to decrease following harvest and during storage (Platenius 1932, Werner, 1941, and Phan et al. 1973).

It has been suggested that invertase activity is partly responsible for the postharvest changes in sugar content (Platenius 1932 and Phan et al. 1973). Increased activity of the tricarboxylic acid cycle has also been indicated (Phan et al. 1973). This suggests metabolic conversion of the sugar carbon skeleton. Inversion of sucrose alone may not impair and may even improve sweet taste properties during storage since the resulting monosaccharides contribute to sweetness. TCA activity, however, could remove a percentage of the sugars altogether. Deterioration or preservation of sweetness would depend on the relative magnitudes of these two activities.

It is suggested that sweet taste properties may be best preserved in roots initially containing relatively high levels of sucrose and/or fructose. Such roots would be more salable immediately after harvest and following periods of cold storage.

Hasselbring (1927) found no cultivar of carrot to predominate in sugar content. Carlton (1958) reported greater variability within than among three standard cultivars; 'Nantes', 'Imperator' (two seed lots) and 'Gold Pak'. The cultivars did differ, however, in reducing sugar content. In an inbreeding experiment Carlton and Peterson (1963) were successful in reducing within cultivar variation. They reported significant differences in sugar content among the S_2 progeny of a 'Nantes' and of three 'Long Chantenay' seed lots. The roots were chosen individually according to their differing total sugar content for the S_1 generation and according to percent soluble solids for the S_2 generation. More recently Kraut (1974) determined significant differences among advanced carrot breeding lines.

Carlton and Peterson (1963) showed a high positive correlation between soluble solids and sugars on an individual root basis and proposed that refractometer readings could be used to screen for high sugar roots. This technique would require destruction of part of the root to be used for seed. Since stecklings are commonly stored intact and are 'cut' for selection in the spring or after vernalization is satisfied, such treatment could cause a rise in the susceptibility to fungal and bacterial attacks.

Another screening technique has been demonstrated by Bassett (1974). Brine solutions were used to determine the relative specific gravities of individual carrot roots. Specific gravity has been shown to correlate significantly with sugar content (Bassett 1973). This approach does not damage the root and may prove valuable for screening material on an individual root basis or by pedigree.

The present study did not deal with these screening methods but was designed to assess the status of some currently available advanced carrot lines in terms of their sugar content *per se*. The sugar assay gives direct information about sugar content on a mean and single root basis. This may be important since the correlative methods, although proven quite reliable, have r values varying between 0.75 and

0.96 (Carlton and Peterson 1963 and Bassett 1973). Small differences in breeding material may not be detected easily by these methods. Caution in analysis of and selection between genotypes, such as in a diallel study, would be required since it has been established that the regression relationship of the above-mentioned traits varied with genotype (Carlton and Peterson 1963).

Analysis using gas chromatography is particularly useful since the reducing sugar fructose can be quantified directly. High fructose concentrations are desirable from a sweetness standpoint.

Previous to the early 1960's standard methods of carbohydrate analysis consisted of titrimetric procedures, paper and thin layer chromatography and polarimetric determinations. The formation of volatile trimethylsilyl sugar derivatives (Sweeley *et al*. 1963) has permitted a direct and more accurate look at the individual sugars in tissue systems via a single analysis. Comprehensive reviews and reports are available on the gas-liquid chromatography of carbohydrates (Sweeley *et al*. 1966, Holligan and Drew 1970, Holligan 1970, Birch 1973 and Dutton 1973).

MATERIALS AND METHODS

The plant material consisted of six inbred lines and twenty-five hybrids developed at Michigan State University. The carrots were harvested at Grant, Michigan on 16 October, 1974. Six randomly selected, representative roots from each line were stored at 4°C for three weeks before being removed to 1°C lockers. Over a six day period they were removed from storage for sample preparation. Roots were sliced cross-sectionally into three equal lengths. The top was returned to storage for subsequent seed production, the center was reserved for subsequent sugar and solids analysis and the tip portion was tested organoleptically by a six member taste panel.

The center portion was sliced into two millimeter discs. Thirty grams of discs selected randomly from each portion were weighed and frozen at -10°C. The samples were lyophilized in an automatic Virtis unit at a platen temperature of 60°C, a condensor temperature of -60°C and a vacuum of less than 5.0 μm. The freeze-dried samples were weighed, ground through a #40 mesh screen in a Wiley Mill and collected in four ounce jars. These jars were kept under desiccation at -10°C. Refreezing was chosen since preliminary observations with test material indicated that room temperature storage resulted in loss of sugars.

Total solids were determined by taking the weight of lyophilized discs as a percentage of the recorded fresh weight. Percent soluble solids were determined from a reconstituted carrot slurry of 6.67% dry matter. One gram of dry carrot powder was allowed to stand in 14 milliliters of water for five minutes with periodic agitation to insure complete hydration. The slurry was filtered through 15 centimeter Whatman #5 filter paper. Three milliliters of the filtrate were collected and capped in one dram screw cap vials. Readings were taken from a Valentine Precision Model 350A refractometer. The following formula was used to calculate theoretical soluble solids values for fresh weight of each root:

(percent total solids) x [(measured soluble solids)/(6.67)] = soluble solids of fresh tissue

Preliminary experiments on carbohydrate content of the dried, ground tissue indicated that 98°C distilled water (Carlton 1958), 98°C 80% ethanol (AOAC 1975) and room-temperature distilled water (Waldron _et al_. 1948) can be used interchangably as solvents. A five minute magnetic stirring extraction in room-temperature water proved as effective as the longer official procedures for extracting carbohydrates. The stirring procedure was chosen because of its convenience and speed.

Preliminary use of the gas-liquid chromatograph and the mass spectrometer indicated that no contaminating or cochromatographing materials were present in the unpurified carrot filtrate. The sample preparation procedure used follows:

One gram of carrot powder was weighed into a 150 milliliter beaker. One-half gram of $CaCO_3$ and 100 milliliters of distilled water were added. The solution was placed over a magnetic stirrer for five minutes and filtered through a 15 centimeter Whatman #5 filter paper. One milliliter of the filtrate was pipetted into a one-half dram vial. One milliliter of a known concentration of internal standard was added and the vial was placed under 0.07 atmospheres at 50°C for 20 hours. The dried samples were stored in glass desiccators.

Trimethylsilyl derivatives were prepared using Tri-sil-'Z' (Pierce Chemical Co.) and allowed to stand for eight hours prior to injection into the chromatograph. A Beckman GC 4 chromatograph equipped with a flame ionization detector was used to quantify the sugars. A 1.83 meter glass column with a 6.25 millimeter outer diameter and a 2.00 millimeter inner diameter was packed with 3% SE-30 on Chromosorb WHP 80/100 mesh (Supelco, Inc.). This gave satisfactory resolution among sugar and internal standard (hexa + (0 - TMS) - sorbitol) peaks. A temperature program permitted elution of the monosaccharide and sorbitol derivatives within ten minutes at 185°C and elution of the sucrose derivative at 255°C after five minutes.

RESULTS AND DISCUSSION

Preliminary analysis of the dried carrot tissue identified the sugars fructose, alpha-glucose, beta-glucose and sucrose. Their respective retention times were 5.0 minutes, 6.5 minutes, 9.7 minutes and 20.2 minutes. The inbred lines contained on the average 1.20% fructose, 0.40% alpha-glucose, 0.68% beta-glucose and 4.54% sucrose. The hybrids contained 0.91% fructose, 0.32% alpha-glucose, 0.54% beta-glucose and 5.38% sucrose. The alpha and beta anomers of glucose were combined for the subsequent statistical analyses since chemical equilibrium during the extraction and trimethylsilylation invariably occurred (Sweeley *et al*. 1966).

Previously unidentified peaks eluted intermittently during the course of analysis. The first occurred on the trailing solvent peak at 2.0 minutes. It has been tentatively identified as arabinose.

The second peak appeared as an ascending shoulder on the fructose peak. Mass spectrometry analysis revealed that only the TMS-derivative or isomeric forms of this derivative were present. Two very small peaks followed the main fructose peak in original and sample fructose preparations. Ellis (1969) reported that this fructose derivative when dissolved in hexamethyldisiloxane (HMDSO) will deliver four peaks due to anomeric and ring isomer formations. The relative retention times reported for these peaks corresponded well with those observed in this study.

HMDSO is a reaction by-product of sugar derivatization in the presence of water. It is likely that HMDSO was formed in the carrot sample sirups. It's probable formation is offered as an explanation for these peaks. Individually these peaks interfered negligibly or not at all with sugar quantification.

Code numbers used for inbred and hybrid lines in subsequent tables are presented in Table 1.

Table 2 contains means of the sugar and solids measurements made upon the six inbred lines. The inbreds differed significantly in total solids, soluble solids, total sugar, fructose and glucose content. These differences were due to the line MSU 6000 which contained the largest amounts of these components. The other five inbreds did not differ significantly from each other.

Similar results were reported by Kraut (1974) where MSU 6000 contained higher total sugar than did MSU 5986 in a 1972 Texas planting and more than MSU 5931 in a 1973 Texas planting. Kraut (1974) also showed that MSU 6000 was higher in soluble solids content than MSU 872 in a 1973 Idaho planting.

In Table 2 the order of magnitude of means prevails rather consistently for all measurements except sucrose and sweetness. Correlation data among these measurements are

Table 1. Breeding Material Codes.

Code	Identification
Parents	
6000	MSU 6000
5986	MSU 5986
872	MSU 872
1302	MSU 1302
9541	MSU 9541
5931	MSU 5931
Hybrids[z]	
6000 x 872	MSU 6000 pollinated by MSU 872

[z]Only one code example is given for the hybrids. All other hybrid codes follow this pattern.

Table 2. Mean Sugar and Solids Content and Sweetness Values Associated with Roots of Six Inbred Carrot Lines.

Parent Pedigree	Percent Total Solids	Percent Soluble Solids	Total Sugar[x]	Sucrose	Fructose	Glucose	Sweetness[y]
6000	15.09 a[z]	12.47 a	87.26 a	46.21	22.18 a	18.87 a	3.00
5986	12.52 b	9.61 ab	67.58 b	45.87	11.01 b	10.71 b	2.67
872	12.48 b	10.18 ab	65.56 b	43.53	11.62 ab	10.42 b	3.33
1302	12.09 b	8.33 b	63.30 b	42.67	10.63 b	10.00 b	3.50
9541	11.74 b	9.43 ab	63.59 b	44.90	9.71 b	8.99 b	2.83
5931	11.34 b	8.52 b	62.95 b	49.35	7.38 b	6.23 b	2.33
Grand Mean	12.54	9.76	68.37	45.42	12.09	10.86	2.94
Coefficients of Variation	8.12%	21.08%	13.39%	18.00%	32.97%	30.98%	52.52%

[x] All sugar values are presented as mg/g fresh tissue.

[y] Sweetness values were determined on a 0 to 10 scale.

[z] Tukey's HSD test was used to determine significant differences among means at the 0.05 level.

presented in Table 3. Total solids, soluble solids and total sugars all showed significant positive correlations with each other. This stands in agreement with previous reports on carrot tissue (Werner 1941, Carlton and Peterson 1963, Bassett 1973 and Bassett 1974). Of the individual sugars, fructose and glucose correlated more closely than sucrose with the total and soluble solids. This pattern of correlation is also evident when the individual sugars are compared with total sugar. This is of importance since total and soluble solids and/or total sugars may be used as a basis for selection without the expense of any one of the sugars involved. This finding is in contrast to that reported by Carlton and Peterson (1963) who showed that total sugar, as well as total and soluble solids and sucrose, levels were inversely correlated with reducing sugar content in 'Long Chantenay' carrots.

Among the individual sugars reported here the relationships are apparently random with the exception of fructose and glucose. These two sugars show a strong positive correlation of 0.97 (Table 3).

None of the measurements correlated with the panel sweetness ratings at the 95% confidence level. However, total sugars correlated with sweetness at the 90% confidence level. Multiple regression analysis indicated that fructose and sucrose expressed as percentages of the total solids were related to sweetness. The R value was 0.32 and the regression was significant only at the 84% confidence level.

Table 3. Simple Correlations Among Measurements Made upon Roots of Six Inbred Carrot Lines and Twenty-five F_1 Hybrid Combinations.

	Inbreds						
Hybrids	Total Solids	Soluble Solids	Total Sugar	Sucrose	Fructose	Glucose	Sweetness
Total Solids		0.84**	0.77**	0.45**	0.65**	0.66**	0.18
Soluble Solids	0.79**		0.82**	0.49**	0.55**	0.56**	0.14
Total Sugar	0.87**	0.76**		0.47**	0.77**	0.76**	0.29
Sucrose	0.81**	0.71**	0.85**		-0.20	-0.20	0.20
Fructose	-0.20**	-0.20**	-0.10	-0.60**		0.97**	0.19
Glucose	-0.25**	-0.22**	-0.12	-0.62**	0.95**		0.15
Sweetness	0.14	0.21**	0.23**	0.08	0.18**	0.20**	

**r values so noted are significantly different from zero at the 0.05 confidence level.

The predictive equation follows:

$$\text{sweetness} = -4.67 + 0.02\ (\%\ \text{fructose}) = 0.01\ (\%\ \text{sucrose})$$

The lack of large and significant correlations was unexpected as was the absence of significant differences in sweetness content (Table 2). This was due in part to palate fatigue suffered due to the many samples per panelist.

Means of the sugar and solids measurements taken on the twenty-five hybrid combinations appear in Table 4. The hybrids contained slightly more total sugar and more percent sugar due to sucrose than the inbred lines. The hybrids differed significantly for all traits except sweetness. Some of these differences were found in total and soluble solids, total sugars and sucrose content. They were due in general to the high and low hybrid combinations MSU 6000 x MSU 872, MSU 5986 x MSU 872, MSU 1302 x MSU 6000, MSU 5931 x MSU 6000, MSU 6000 x MSU 1302, MSU 6000 x MSU 9541, MSU 1302 x MSU 5931, MSU 5986 x MSU 9541 and MSU 5931 x MSU 5986. Some of the hybrid combinations responsible for significant differences in fructose and glucose content (Table 4) are not among those listed above. This indicates that the reducing sugars do not vary directly with the solids, total sugar and sucrose content.

The differences among the hybrid combinations remain rather parallel for total and soluble solids, total sugars and sucrose. Fructose and glucose levels also varied together. Correlation coefficients for these relationships are shown in Table 3. Among the hybrids total and soluble

Table 4. Mean Sugar and Solids Content and Sweetness Values Associated with Roots of Twenty-five Carrot F_1 Hybrids.

Hybrid Pedigree	Percent Total Solids	Percent Soluble Solids	Total[x] Sugar	Sucrose	Fructose	Glucose	Sweetness[y]
6000 x 872	15.19a[z]	11.54a	83.38a	70.37a	6.62 bc	6.39 b	4.67
5986 x 872	14.41ab	11.01ab	83.22a	64.09ab	9.84abc	9.29ab	3.17
1302 x 6000	13.91abc	11.39ab	82.79ab	63.18ab	10.11abc	9.45ab	2.83
9541 x 5986	13.60abcd	10.19ab	77.95abc	64.90a	7.00abc	6.05 b	4.17
5931 x 6000	13.26abcde	10.56ab	77.60abc	56.15abcd	11.48abc	9.98ab	5.67
6000 x 1302	13.25abcde	10.79ab	74.35abc	61.78abc	6.47 c	5.60 b	3.33
6000 x 9541	13.18abcde	9.92ab	74.16abc	53.80abcd	10.42abc	9.94ab	4.17
5986 x 5931	13.13abcde	9.58ab	73.22abcd	56.73abcd	8.66abc	7.83ab	3.33
872 x 5931	12.90abcde	10.13ab	75.73abc	62.77ab	6.64 bc	6.32 b	3.00
1302 x 872	12.90abcde	9.82ab	72.46abcd	56.74abcd	7.90abc	7.83ab	3.50
6000 x 5986	12.80 bcde	10.08ab	68.32abcd	54.39abcd	7.37abc	6.57 b	2.17
5931 x 872	12.61 bcde	9.21ab	70.27abcd	44.98 bcd	12.22ab	13.06a	4.67
872 x 1302	12.53 bcde	9.05ab	74.07abc	55.35abcd	9.30abc	9.42ab	4.67
9541 x 1302	12.49 bcde	10.01ab	72.80abcd	52.53abcd	10.11abc	10.16ab	3.00
1302 x 9541	12.29 bcde	9.92ab	68.20abcd	49.40 bcd	9.65abc	9.15ab	3.83

Table 4 (cont'd.).

1302 x 5986	12.25 bcde	9.16ab	65.52 bcd	50.57abcd	8.05abc	6.90ab	3.67
5931 x 9541	12.17 bcde	9.78ab	68.94abcd	54.35abcd	7.64abc	6.96ab	4.17
872 x 5986	12.12 bcde	9.25ab	67.10 bcd	49.08 bcd	9.27abc	8.75ab	4.17
872 x 9541	12.05 cde	8.83ab	64.57 cd	40.89 d	12.45a	11.23ab	4.00
5986 x 6000	12.02 cde	9.17ab	69.35abcd	47.49 bcd	11.25abc	10.62ab	4.17
5931 x 1302	11.89 cde	7.83 b	65.91 bcd	49.22 bcd	8.49abc	8.20ab	3.83
5986 x 1302	11.78 cde	7.82 b	63.09 cd	41.03 cd	11.28abc	10.78ab	3.00
1302 x 5931	11.75 cde	8.57ab	71.97abcd	56.01abcd	8.07abc	7.90ab	4.17
5986 x 9541	11.55 de	8.54ab	62.57 cd	43.57 bcd	9.99abc	9.07ab	3.67
5931 x 5986	11.07 e	8.04ab	58.21 d	44.96 bcd	6.90abc	6.37 b	3.33
Grand Means	12.68	9.61	71.43	53.77	9.11	8.55	3.77
Coefficients of Variation	8.60%	17.03%	10.22%	17.85%	29.17%	33.65%	53.68%

[x] All sugar values are expressed as mg/g fresh tissue.

[y] Sweetness values were determined on a 0 to 10 scale.

[z] Tukey's HSD test was used to determine significant differences among means at the 0.05 level.

solids and total sugars were mutually and positively correlated. Of the individual sugars sucrose was positively correlated with these traits and was responsible for the agreement between the solids data and total sugar. Data in Table 3 also indicated that as total sugars increase in the hybrids the percentage due to sucrose also increased.

Although the reducing sugars were again positively correlated, negative correlations existed between the reducing sugars and sucrose. This suggests antagonism between their respective formations. The reducing sugars are also inversely correlated with total solids, and soluble solids, while a random relationship exists between the reducing and total sugars. The pattern of inverse correlations among these hybrids is similar to that reported by Carlton and Peterson (1963) for inbred carrots. These researchers discussed the association of high reducing sugars with low total solids and fiber content. Such an association brings both sweet and tender components together which is valuable in the fresh market situation. Hybridization techniques considering this association may provide an efficient means to create new cultivars with these traits.

Among the hybrids sweetness appeared to be related to soluble solids, total sugars, fructose and glucose. Fructose was the sweetest sugar present in the carrots and glucose, although about one-third as sweet, was directly related to fructose concentration. Since sucrose was inversely correlated with the reducing sugars, its contribution to sweetness was probably reflected in the total sugar correlation value. Multiple regression analysis failed to explain sweet taste on the basis of all four of these traits. However sucrose and alpha-glucose expressed on a fresh weight basis may be used to predict sweetness in the following equation:

$$\text{sweetness} = -1.98 + 0.06\ (\text{sucrose}) + 0.76\ (\text{alpha-glucose})$$

The R value for this equation was 0.33 and the regression was significant at the 95% confidence level.

Difficulty was encountered in looking for parental influence upon hybrid traits partly because the relationships among solids and sugars differed in the inbred and hybrid groups. Another difficulty arose due to lack of sufficient hybrid combinations to complete a 6 x 6 diallel study. Seed was unavailable for five combinations, three of which, unfortunately, involved MSU 6000. However, as shown in Table 5, the general combining ability total and reducing sugar means did not change appreciably between the complete 4 x 4 diallel and the larger incomplete diallel. It was evident that all but one of the seven progeny of MSU 6000 fell above the average for total sugars and those traits positively correlated with total sugars (Table 4). For purposes of discussion, total sugars shall be considered representative of these traits.

As shown in Table 5, MSU 6000 behaved equally well for total sugars whether it was used as a pollinator or as a seed parent. In both capacities MSU 6000 ranked high among

Table 5. General Combining Ability Means of Six Parent Carrot Lines Based on Total and Reducing Sugar Content.

Parent Line	4 x 4 Complete Diallel		6 x 6 Incomplete Diallel	
	Total Sugar[z]	Reducing Sugar	Total Sugar	Reducing Sugar
6000	--	--	75.10	14.97
	--	--	76.60	20.96
5986	73.20	19.23	70.30	19.71
	63.60	15.41	67.40	14.64
872	72.30	16.57	70.40	18.35
	75.30	20.05	77.40	18.29
1302	70.00	15.55	72.20	16.81
	67.70	19.16	70.10	18.06
9541	--	--	75.40	16.66
	--	--	67.70	19.13
5931	64.80	18.41	68.20	18.25
	73.60[y]	15.14[x]	73.60[y]	15.14[x]

[x,y]Means followed by the same letter are based on the same values.

[z]All sugar means are presented as mg/g fresh tissue.

the general combining ability means for total sugars. MSU 6000 as a pollinator was characterized by high reducing sugar content as well. This is of importance because reducing sugars were not related to the total sugars and will therefore not necessarily appear in higher amounts as total sugars increased in the hybrid combinations. MSU 6000 as a female resulted in very low reducing sugar content.

Combinations involving MSU 1302 also showed little differences in total or reducing sugar when this parent was used as a female or as a male. In both cases the sugar contents were average.

The largest difference in total sugars as influenced by male and female parentage were exhibited by lines MSU 872 and MSU 9541. MSU 872 had a strong positive influence upon total sugar when used as a pollinator. This may be of greater general importance than the influence of MSU 6000 as a pollinator since the former was based upon four combinations whereas the latter involved only three. The strength of MSU 872 as a female parent, also based on four combinations, was slightly below average for total sugars and about average for reducing sugars.

MSU 9541 occurred as a pollinator in five combinations and resulted in below average total sugar content. These five combinations were quite variable as shown in Table 4. However, among the female general combining ability means, MSU 9541 ranked highest in total sugars. Here it occurred in only two combinations both of which were above the grand mean. Reducing sugar content for this line as a seed parent and as a pollinator was near the average.

Five combinations involving MSU 5986 as a pollinator showed that it was consistently weak in total sugars in both the complete and incomplete diallel situations. In only one of the five combinations did the total sugar content fall above the grand mean. The influence of MSU 5986 as a female on the total sugar content was average. MSU 5986 was also responsible for a large sex-related difference in reducing sugar content. The female influence was positive whereas the male influence was negative.

MSU 5931 had the poorest female influence on total sugar content. Its performance as a pollinator was slightly above average, although the reducing sugar content was lower than average.

Reciprocal differences among the hybrids are evident in Table 4. These may be used as specific examples to clarify what has been stated previously about parental influences. MSU 5986 x MSU 872 was significantly higher in total sugar content than MSU 872 x MSU 5986. Since both of these parent lines served as average females it is suggested that this reciprocal difference was due to the exchange of pollinators.

A difference between MSU 9541 x MSU 5986 and the reciprocal cross falls slightly short of significance at the 95% confidence level. This difference was, however, significant at the 90% confidence level. This exchange of weaker pollinators suggests that MSU 9541 was a strong female contributor to total sugar content.

The total sugar content of MSU 5986 x MSU 5931 was significantly different from that of its reciprocal at the 90% level. The former combination involved two average parents but the latter involved both a poorer pollinator (MSU 5986) and a poorer female (MSU 5931). Accordingly the latter combination had the lowest total sugar value among the hybrid group.

SUMMARY AND CONCLUSIONS

In conclusion the following phenomena are evident from this parent-hybrid study on the solids, sugar and sweetness content in carrots.

Differences in total and soluble solids and total and reducing sugars existed. MSU 6000 was significantly higher in quantities of solids and total and reducing sugars than were the other lines. The reducing sugars were not correlated with sucrose content, but all other solids and sugar measurements were mutually and positively correlated.

Significant differences in solids and sugar content were apparent among the hybrids. Some of these differences were due to superior quality of the combinations in which MSU 6000 was one of the parents. MSU 6000 was the only parent line that served as a positive influence as both a male and female parent. This behavior suggests that these outstanding qualities were transmitted genetically and that the MSU 6000 cytoplasmic factors were not strongly influential.

Although the other inbreds did not show significant differences in the inbred analysis, their general combining ability means did suggest genetic influence upon the hybrids. Another strong pollinating line, MSU 872, and a strong female parent, MSU 9541, were found. However, in these cases the positive influence was associated with the sex of the parent. This suggests that more than one mechanism of inheritance most likely involves cytoplasmic influences. Interactions between cytoplasm and the genome are also likely since significant reciprocal differences occurred among the hybrids.

Positive correlations existed among the traits measured in the inbred lines, but negative correlations were found in the hybrids. Although no explanation is readily available for the inverse relationship between the reducing sugars and the solids and sucrose contents in the hybrids it may be of use in the breeding of sweet and tender fresh market carrots.

The sweetness values were generated in hopes of relating them to specific sugars or combinations of sugars or synergisms among sugars in the fresh carrot. Such relationships would provide useful breeding bases. However, the sweetness data could not be readily interpreted for the inbred group. Simple and multiple regressions showed significance among the hybrids where soluble solids and total and reducing sugars and sucrose were related to sweetness. The correlation coefficients were quite small, however.

This parent-hybrid study has served as an extension of the nutrition analyses of advanced carrot breeding material recently begun at Michigan State University. This study has also provided a basis for the comparison of several more years work on this subject. Repetition over location will also provide information on the type of variation involved in sugar make-up of this breeding material.

Thorough assessment of the combining ability of the inbred lines rests upon completion of the full diallel design. In this manner statistical significance may be attached to the general combining ability information. The five combinations required for this completion are now available.

Recommendations

Recommendations are offered in order that future experiments involving this plant material may be more successful and meaningful.

The results of this study are not as tenable as they might be were one to complete the 6 x 6 diallel. Five missing combinations made analysis of heritability by standard methods impossible. Statistical significance could not be attached to the combining ability means.

In preparation for analysis the center third of each root was used. Riddle and MacGillivray (1966) noted that total solids decreased from the top to the bottom third of the carrot root. It has already been documented that carrot sugar content is positively correlated with total solids. Since all carrot roots were sampled in the same manner, the differences observed should not have been affected by the total solids phenomena. However, future workers may wish to consider alternative sampling procedures.

The statistical analyses reported here were characterized by relatively high coefficients of variation. Preliminary analyses of sugar content showed a 1.0 to 2.0% error between injections into the gas chromatograph with the use of an internal standard. This reflects the within-machine variation.

Error between subsamples of the same material rose to 4% indicating that improvement of technique is in order. The employment of an automatic pipette is strongly suggested for the placement of sample and standard volumes in the vials.

The statistical design itself contributed most greatly to random error. Blocks, though employed during analysis, were absent in the field. Relationships of sweetness values with the sugar analyses will be more realistic if block effects can be removed during simple and multiple regression. It is likely that multiple range tests will also show a greater number of differences among means. One may also wish to consider increasing the number of replicates (blocks) from six to a larger number if labor is available.

Optimum conditions during the taste panel analysis were not met. Two violations are of concern - the volume of samples per panelist and the absence of a reference carrot. Spencer (1971) offered a discussion of taste panels and sweetness measurements. Since future workers will presumably plant a complete diallel, this will increase, not decrease the number of samples per panelist (block). The use of incomplete block designs would solve this problem.

Analysis of dried carrot product is preferable to fresh because of the storage and handling advantages. Sugars may be selectively removed by respiration during hot air drying (Smith 1969). It has been reported that sugar losses may also occur in such tissues due to non-enzymatic browning (McWeeny 1973). Substantial sugar losses seen in hot air-dried preliminary sample tissue that was stored at room temperature are presumable due to one or both of these activities. Sugar loss was greatly circumvented by the use of the freeze dryer, but enzymes which hydrolyze sucrose are not inactivated during this process (Smith 1969). Preliminary experiments indicated that enzyme activity was not apparent during the extraction of sugars, but this does not preclude the generation of invert sugar during other handling of the lyophilized sample. The freeze drying itself employed a commercial procedure of heating at 60°C at the end of the operation. Enzyme activity here is not impossible.

The appearance of invert sugar may be of special interest since fructose has been entirely absent in some reports on the sugar content on freshly analyzed carrot roots (Platenius 1934 and Otsuka and Take 1969). Although fructose-lacking freeze-dried carrots have been recently analyzed by gas chromatography (Bittenbender 1975), future workers may wish to investigate the possibility of artificially induced fructose content in this type of sample.

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