Breeding of Sugar Beets With Reference to Sodium, Sucrose, and Raffinose Content

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In recent years, new techniques and laboratory equipment suitable for mass operation in sugar beet chemical analysis have been developed. For example: flame spectrophotometry for the determination of various cations and the paper chromatographic technique for the determination of raffinose and other melassigenic substances.

The literature before 1930 contains numerous references to non-sugars but very little data applied directly to a breeding program. Such references were briefly reviewed by Doxtator and Calton (2). Later references using chemical analysis other than sucrose in the breeding phases of the research work have been summarized by Wood (5).

Many investigators (1, 2, 3, 4, 5) have applied selection pressure for low sodium content of individual roots. All have shown that the sodium content was significantly correlated with sucrose but in a negative relationship. All have shown by progeny tests that significant reductions in sodium could be accomplished by mass selection. In the majority of such tests, significantly higher sucrose percentages have been experienced. However, in very few investigations has a low sodium selection produced progenies which significantly out yielded their original parents in pounds sucrose per acre.

Wood (5) reported on progeny tests of roots selected for high and low raffinose content. He concluded that the raffinose content of beets could be significantly reduced by mass selection and that this character may be rather simply inherited.

The objectives of the present investigations were to separate the original population into groups with distinctly different quantities of tested chemical constituents and to determine the effectiveness of using low sodium as a criterion in selecting for higher sucrose content.

Methods and Materials

Approximately 1,200 roots of an elite stock of American No. 4 were selected during the fall of 1953. These roots were selected phenotypically for size, shape, good crowns, etc. Each root was weighed, sampled, and analyzed for sucrose, raffinose, sodium, galactinol, and inositol.

Table 1 shows the correlation coefficients of the various characteristics studied in the original selected population. Sucrose and sodium were reported as percent of beet, while raffinose, galactinol, and inositol were percent on dry substance; the weight was reported in pounds.

These roots were separated into three main groups of approximately 400 roots each. The three groups represented a random mixture of the original variety and did not differ greatly from each other as every beet had an equal chance of being selected for any one of the three groups.

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[2] Numbers in parentheses refer to literature cited.

Table 1.—Correlation Coefficients of 1046 Roots Selected from an Elite Stock of American No. 1.

	Raffinose	Sodium	Galactinol	Inositol	Weight
Sucrose	—.1412	—.7115	—.3483	—.1612	—.2322
Raffinose	....	.0964	.0663	.1412	—.0096
Sodium	....	....	.2473	.1122	.1909
Galactinol	....	....	....	—.0021	.0068
Inositol	....	....	....	....	.0845

r = .062 for significance at the 5 percent level.
r = .081 for significance at the 1 percent level.

Each of these main groups were again divided into two smaller subgroups, altogether, making a total of six small groups. A seventh group was added by selecting approximately every 35th beet regardless of its physical and/or chemical characteristics as a check selection with which the other groups could be compared. This group was labeled 54-407.

The first group was subdivided by selecting paired roots with the same sucrose content, but one (54-408) having a high sodium content and the other (54-409) having a low sodium content. The second group was subdivided by selecting for high and low raffinose content. These were given pedigree numbers of 54-410 and 54-411 respectively.

In the last group the beets were divided into two groups, odd and even numbers. Thirty-one roots were selected within the even numbers on a combined basis of both sucrose and sodium percent. The coefficient used for this selection was sucrose percentage times (one minus sodium content). Within the odd numbers, thirty beets were selected on the basis of high sugar only. These were given the numbers of 54-412 and 54-413 respectively.

All other characteristics were recorded but only the ones mentioned above were used in differentiating this population into the various subgroups.

The number of roots selected for each group, the general means, and standard errors of means of each character studied for each group, and for the entire population, are shown in Table 2.

Table 2.—Means, Standard Errors, and Number of Roots of the Entire Population, and of the Seven Selected Groups for Various Chemical Characteristics.

Pedigree Number	Criteria for Selection	No. Roots Selected	Weight in Pounds	Percent Sucrose	Percent Sodium	Percent Raffinose
54-407	Check	32	2.63±.15	13.18±.28	.1129±.0095	.434±.0249
54-408	High Sucrose—High Na.	32	2.28±.12	15.68±.11	.0916±.0055	.426±.0332
54-409	High Sucrose—Low Na.	32	2.09±.10	15.67±.11	.0341±.0017	.458±.0390
54-410	High Raffinose	38	2.58±.18	12.74±.40	.1043±.0087	.841±.0211
54-411	Low Raffinose	39	2.53±.15	13.71±.26	.0898±.0074	.158±.0062
54-412	A Coef. of Na. and Sucrose	31	2.07±.09	15.82±.12	.0495±.0035	.387±.0272
54-413	High Sucrose	30	2.24±.12	15.92±.13	.0606±.0048	.478±.0414
Entire Population		1046	2.53±.03	13.69±.08	.0972±.0015	.457±.0061

Roots of each of the above seven groups were space isolated in the spring of 1954 and produced seed that fall. Seed from these seven groups plus seed of the original parental stock were planted in an 8x8 Latin Square test at Rocky Ford, Colorado, on April 25, 1955. Plots were 4-rows wide and 35-feet long. The two center rows were harvested and three 10-beet samples were taken at random from the harvested roots.

Three weeks later border rows of five of the selections were harvested and two 10-beet samples were taken for chemical analysis. The objective of the second harvest was to determine whether or not the raffinose content increased while the beets were still growing in the late fall.

Results and Discussion

The first harvest results of the 1955 replicated progeny test of the various chemical selected strains are shown in Table 3, along with the original parent. All seven of the chemical selections originated from the same parental variety and only one mass selection was made to differentiate these strains into their respective sub-populations.

Highly significant differences were obtained among the various groups for every attribute listed in Table 3. This was as expected as selection pressures were applied in opposite directions for some of the chemical characteristics such as raffinose.

Table 3.—Stand, Yield, and Chemical Results of the Parent and Seven Selected Chemical Strains.

Pedigree Number	Criteria for Selection	Pounds Sucrose Per Acre	Tons Per Acre	Percent Sucrose	Percent Na.	Percent Raffinose	Number of Beets Per 100 Feet
54-407	Check	8251	26.93	15.32	.0572	.214	84.1
54-408	High Sucrose- High Na.	7868	24.71	16.01	.0554	.248	101.2
54-409	High Sucrose- Low Na.	8137	25.32	16.05	.0314	.273	96.1
54-410	High Raffinose	6838	22.06	15.48	.0468	.336	93.7
54-411	Low Raffinose	7714	24.64	15.66	.0421	.164	94.5
54-412	A Coef. of Na. and Sucrose	8151	27.15	15.64	.0401	.217	87.8
54-413	High Sucrose	7951	24.66	16.05	.0405	.237	94.1
Parent	American No. 1	7225	23.62	15.24	.0534	.250	101.0
General Mean		7804	24.89	15.68	.0459	.242	94.1
LSD 5% Pt.		638	2.07	.46	.0084	.048	7.8
LSD 1% Pt.		853	2.77	.62	.0113	.064	10.4
C. V.		2.87%	2.92%	1.03%	6.52%	6.90%	2.90%

Certain paired comparisons of the various strains should be considered because most of the groups were selected with this in mind. The comparison of the parental variety with the check (54-407) shows a highly significant increase for tons and pounds sucrose per acre in favor of 54-407. Strain 54-407 represents a random mixture of the selected roots from the parental variety. If fiducial limits were calculated for the means in Table 2 for selection 54-407 and the entire population, they would overlap. This indicates that selection 54-407 was a satisfactory sample of the entire population of 1046 root selected from the parental American No. 1 variety.

The results in Table 3 show that the progeny (54-407), with one phenotypic selection for large well-shaped roots, bred true and the root yield was significantly increased over the parental American No. 1 variety. There were no significant differences between these strains for any of the chemical characters studied. Although significant differences were detected in stand, it did not seem to affect either the yield of tons and/or sucrose percentage. Evidently the plants were sufficiently spaced to have adequate competition.

Because selection 54-407 was part of the same population of selected beets which made up the other strains in this test and because it was significantly higher than the parental strain for tons, and pounds sucrose per acre, it should be used as the check in the various comparisons to be discussed.

One of the objectives of the experiment was to select for high and low raffinose content and then test the progenies to see if they bred true. In Table 2, the means for raffinose content for selected groups 54-410 and 54-411 were significantly different from each other. If fiducial limits were calculated at the one percent level, the raffinose means for these two groups would not overlap. The selected roots of 54-410 also contained highly significantly more raffinose than the check 54-407, while the roots of 54-411 were significantly below the check.

The progeny results in Table 3 show these two selections differ significantly at the one percent level for percent raffinose. The check selection with a mean of .214 was significantly higher at the five percent level than 54-411 with a mean of .164. However, the check was significantly lower at the one percent level than the high raffinose selection 54-412. The first cycle of selection indicated that these chemicals were hereditable and greater advances could be made in breeding for a higher raffinose variety than for a lower raffinose strain.

The correlation coefficients in Table 1 show a negative relation for raffinose with sucrose and weight, however, the latter was not significant and the value was so low that weight and raffinose could be considered independent of each other. Sodium had a low positive correlation which was expected because of its negative relationship with sucrose. The means of the selected roots in Table 2 verified these correlations as the low raffinose selection (54-411) was lower in sodium and higher in sucrose percentage than 54-410; weight was approximately the same. However, none of these means, including the check, were significantly different at the five percent point.

The progeny test of Table 3 also confirms the above correlation with the high raffinose selection, 54-410 being slightly higher in sodium and lower in sucrose and weight than the low raffinose selection. The progeny of 54-411 produced significantly more tons and pounds sucrose per acre than 54-410. This might indicate that the negative correlation between raffinose and weight was stronger than the value obtained in Table 2. It also may have been due to environmental conditions. Another possibility was that either extremely high or low raffinose content was detrimental, as the check yielded significantly higher tons than these extreme raffinose selections. The check was significantly higher than the low raffinose selection at the five percent level and the high raffinose selection at the one percent level. It also was significantly higher in sodium percentage and slightly lower in sucrose.

The border rows of five of the selections were harvested three weeks later and two 10-beet samples were taken for chemical analysis. These are shown in Table 4.

Table 4.—Chemical Results of the Late Harvest of the Five Strains.[1]

Pedigree Number	Criteria for Selection	Percent Suc.	Percent Na.	Percent Raff.	Increase of Raffinose in 3 Weeks
54-408	High Sucrose—High Na.	17.08	.0455	.300	21.0%
54-409	High Sucrose—Low Na.	17.06	.0296	.353	29.3%
54-412	A Coef. of Na. and Sucrose	16.20	.0419	.300	21.0%
54-413	High Sucrose	17.16	.0372	.314	32.5%
Parent	American No. 1	16.29	.0510	.318	27.2%
General Mean		16.76	.0411	.317	29.6%
LSD 5% Pt.		NS	.0139	.029	....
LSD 1% Pt.		NS	.0188	.039	....
C. V.		2.01%	11.71%	3.12%	

[1] Analyzed as a randomized block design with eight replications and five varieties.

The raffinose data was analyzed by assuming a split plot design with dates as the sub-plots and varieties as the main plots. Highly significant differences were found among the varieties and the F value for dates was 76.4. In order to be significant at the one percent level the F value only needed to be 7.4. The date X variety interaction was very small and non-significant.

What caused this increase in raffinose we do not know, but we assume that the colder weather had some effect. The mean minimum temperature for two weeks before the first harvest beginning October 1 was 40.9 degrees, while the minimum mean temperature between the two harvests was 30.3 degrees Fahrenheit. There were only three days which were 32 degrees or below before the first harvest, and eleven days which were 32 degrees or below between the two harvests. The dates of the two harvests were October 14 and November 4.

Additional experiments will be necessary to determine the effect of environment on the accumulation of raffinose.

The remainder of the test was designed to determine the effectiveness of using sodium in the breeding program as a criterion in selecting for higher sucrose content.

The fact that sodium and sucrose were negatively correlated to a very high degree, as shown in Table 1, suggests the possibility of using both of these characteristics to select for high sucrose content. The hypothesis is, that the mechanism of the beet was such that low sodium, per se, directly or indirectly, tended to bring about a higher concentration of sucrose or visa versa.

The comparison of strains 54-408 and 54-409 were selected with the same sucrose content but with a high and low sodium content. The means in Table 2 indicate the groups were selected in that manner as the sodium content of these groups was significantly different, but all other characteristics were the same.

The results of the progeny test are given in Table 3. Two main points should be considered; first, the sucrose content of these two groups were within .04 percent of each other in Table 3, which was the average of 24 samples, and with .02 percent of each other in Table 4, which was the mean of 16 samples. The objective of holding sucrose constant during the selecting of mother roots was fully realized in the progeny test. The second point to consider was the sodium content. The results of the progeny test in Table 3 show the mean sodium percentage for 54-408 as .0554 and for 54-409 as .0314. These means were significantly different at the one percent level. Similar results are shown in Table 4. No other significant differences were detected for these two groups. In comparing these two selections (54-408 and 54-409) with the check we find differences among the following characteristics: (A) The sucrose content of both selections was significantly higher than the check at the one percent level, (B) selection 54-408 was significantly lower than the check in tons per acre, (C) selection 54-409 was highly significantly lower in sodium percent than either the check or selection 54-408, ad (D) selection 54-409 was significantly higher in raffinose than the check.

The fact that the low sodium selection did not increase the sucrose content indicates that these two chemical mechanisms were not as directly related as first thought, and that sugar beet varieties could be bred with a high sodium and a high sucrose content. However, a strict interpretation of the sucrose-sodium correlations in Table 1 shows only 50 percent of the factors which affect low sodium have an effect on high sucrose. Perhaps the environmental factors have great effect on these two characters. Again additional investigations are needed to study the inheritance of sucrose and sodium. Such experiments also should be designed to study the environmental effect and the genetic-environmental interactions.

The last comparison was designed to test two methods of selection for high sucrose content. Group 54-412 was selected on the basis of a sodium-sucrose coefficient while group 54-413 was selected only on the basis of high sucrose. If low sodium was a factor in selecting for high sucrose, then the progeny of group 54-412 should contain a higher percent of sucrose than group 54-413.

Again if fiducial limits at the five percent point were calculated for the means of groups 54-412 and 54-413 in Table 2, all would overlap. This would indicate that the selected roots of these two groups were part of the same population for all characteristics shown in Table 2.

The results of the progenies from these two groups are shown in Table 3. The sodium content of these two groups were approximately equal. Therefore, selection for high sucrose was just as effective for lowering the sodium content as selection on a sucrose-sodium coefficient. The sucrose means were 15.64 for group 54-412 and 16.05 for group 54-413. The difference between these means was .41 while .46 was needed for significance. Although we can not say that these populations are different at the five percent point, it is certainly questionable, whether they are estimates of the same population. The results shown in Table 4 also favor selection 54-413 over 54-412 for higher sucrose content, but again it was not statistically significant. However, over 40 samples were taken from these strains and the trend of 54-413 was slightly higher in sucrose. A comparison of the check 54-407 with these two selections shows 54-413 to be significantly higher in sucrose content than the check, while selection 54-412 was not statistically different from the check. If this comparison were used, it would indicate that selecting for high sucrose content was more effective than using a sodium-sucrose coefficient in obtaining progenies with a higher sucrose content.

The weight factor between these groups was in favor of group 54-412. The mean ton yield of group 54-412 was 27.15 while group 54-413 yielded only 24.66. This difference was significant at the five percent level. Very few investigators in the past have reported an increase in weight in low sodium selections, and the correlations in Table 1 show that such a result is unlikely. However, the data in this test in Table 3 show higher yield in tons for both of the low sodium selections (54-409 and 54-412) when compared to their respective counterpart (54-408 and 54-409). When groups 54-412 and 54-413 were compared for gross sucrose per acre, no significant differences could be detected. A "t" test was applied to find the level of significance for these two groups in pounds sucrose per acre. A "t" value of .756 with 14 degrees of freedom was calculated. The probability that these means were different was less than 40 percent.

Three points should be considered from the results of the sodium test. First, the sodium content was heritable and a low sodium variety of sugar beet can be bred if such was desirable from the processing view point. Second, it was possible to breed for various levels of sodium without effecting the sucrose content. Third, the selection based on sucrose only produced progenies which were slightly higher, although not significantly, in sucrose than the progenies of a sucrose-sodium selection.

In view of the above facts it seems at the present time that sodium was actually of little value in the breeding program and that a high sucrose selection could be accomplished without selecting for low sodium. Also, the fact that sucrose percentage was measured with more precision than sodium, as shown by the C. V. values, emphasized the need of sucrose reading without combining it with a sodium reading which has a large variance.

Summary

Approximately 1,200 roots of an elite stock of American No. 1 were selected in the field in 1953. These roots were subdivided into seven groups. Six of the groups were selected for distinctly different chemical contents and the seventh group represented a random mixture of the 1,200 selected roots.

From the data submitted in this report the following conclusions were drawn.

1. It was possible to select roots with high and low raffinose content and these bred true in progeny tests.

2. The raffinose content of selections in the field was found to have increased approximately 30 percent in three weeks. Lower minimum mean temperatures were suggested as one possibility for causing this increase.

3. Paired roots were selected with the same sucrose content but with one having a high sodium content and the other having a low sodium content. Progenies from these selections bed true, i.e., both had equal sucrose content but significantly different sodium percentages. This indicated that sodium, per se, had little effect on the sucrose content and that the negative correlation of sucrose and sodium could be easily broken.

4. Progenies selected only on the basis of their sucrose content had a higher sucrose content than progenies selected on the basis of a sucrose-sodium coefficient.

5. In view of the data at the present time it seems that sodium actually is of little value in the breeding program, unless a low sodium variety is extremely important from the processing viewpoint.

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