Relationship Between Root Weight, Glutamic Acid and Sucrose Content of Inbred Lines of Sugar Beets[1]

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Studies on the glutamic acid content of commercial varieties of sugar beets under diverse environmental conditions have indicated the value of this measurement in distinguishing the physiological processes involved in the growth and storage mechanism of the beet (1, 2, 3) .[3] The enormous variations found in glutamic acid content from beet to beet (4) suggested that genetics might have an important bearing on this characteristic. If this could be established and a series of inbred lines investigated, correlations might be revealed between sucrose, glutamic acid and weight which were not apparent in the more or less heterogeneous material from commercial varieties. Better knowledge of these physiological relationships should lead to a more productive breeding program.

In 1947, eight beets each from eight inbred lines, self-pollinated for several generations, were sampled by removing a wedge-shaped sample of pulp from each beet. In order to obtain an adequate sample for analysis, the beets selected were above average size for their line, but they were all sufficiently homozygous for leaf, petiole and root characteristics to be representative of the line.

The heritability of glutamic acid was clearly evident even in this small series. There were highly significant differences between lines in glutamic acid as well as sucrose (Table 1) . The tremendous variation previously noted in the glutamic acid content of individual beets from commercial lines was reduced in some of these strains by more than 50 percent. There was a similarity in glutamic acid content of closely related lines 300, 301 and 304, and in lines 324 and 327. The relatively high glutamic acid of the latter lines and the low content of 322 was confirmed in a subsequent planting in 1949 of seed from one selfed plant out of each of these lines (5) . Although there was some tendency for lines high in sucrose to have high glutamic acid and small weight, there was no correlation between glutamic acid and weight.

In 1950, a much larger series of inbred lines was selected. Ten beets from each of 43 lines, self-pollinated from four to 10 generations, were stored in the root cellar at Fort Collins until February when they were packed in wet sphagnum moss and shipped to Woodland. The beets were in excellent condition on arrival. They were weighed and sucrose and glutamic acid determinations (4) made on isopropanol extracts of the pulp obtained from a diagonal coring through each beet.

The beets ranged in size from 0.46 to 2.71 pounds; in sucrose percentage from 11.3 to 18.9, and in glutamic acid from 0.03 to 1.05 percent. The range of individual glutamic acid values within a line was extremely nar-

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[3] Numbers in parentheses refer to literature cited.

row in some lines and enormously great in others. This variation did not seem to be influenced by the degree of inbreeding, since lines self-pollinated eight to 10 generations did not generally show less variation than those selfed four or five generations. Part of the variation could probably be due to the pronounced effect of soil nitrogen on glutamic acid and to a lesser extent on sucrose and weight, but this effect cannot be measured in an unreplicated experiment.

In spite of these large individual variations, wide differences between lines were evident in glutamic acid as well as sucrose and weight. Statistical calculations of significant differences between lines require data which follow a normal frequency distribution and have a variance independent of the mean. This was the case for sucrose, and Figure 1 indicates a nearly

Table 1.—Comparison of Inbred Lines Grown in Colorado in 1947.

Line	Wt. in Lbs.	% Sucrose	% GA
296	1.81	13.7	.18
300	1.01	13.7	.09
301	1.08	16.0	.22
304	1.14	16.0	.12
308	1.36	11.3	.06
322	1.62	10.1	.09
324	1.23	15.0	.32
327	1.26	14.3	.51
Sig. Dif. 5% Point	19%	1.2	66%

homogeneous variance. As previously reported (4), and as is clearly illustrated in Figure 3, the range of variation in glutamic acid within lines increases as the average glutamic acid content of the line increases, up to approximately 0.3 percent. Beyond this value it approaches a normal distribution. The logarithmic transformation of the data previously recommended is somewhat insufficient in this experiment below 0.3 percent and excessive above that level.

A transformation yielding a variance unaffected by the mean was accomplished by a procedure similar to that described by Bartlett (5). The arithmetic mean glutamic acid values for each line were plotted against the reciprocal of the standard deviation and a smooth curve drawn, extrapolating this to the error of the assay at 0 percent glutamic acid. This curve was integrated numerically to give $f(G) = \int_0^G (1/\sigma G)\, dG$ l e 2). This transformation yielded a variance independent of the mean and reduced heterogeneity greatly, although wide discrepancies from a homogeneous variation remained (Figure 4). This relationship should prove useful for trans-

Xable 2.—Transformation Function for Glutamic Acid.

$$f(G) = \int_0^G \frac{dg}{\sigma_G}$$

% GA	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
.00	0	2.09	3.39	3.93	4.14	4.32	4.48	4.65	4.75	4.86
.10	4.95	5.03	5.10	5.17	5.23	5.28	5.32	5.36	5.40	5.44
.20	5.47	5.50	5.53	5.55	5.58	5.60	5.62	5.64	5.66	5.68
.30	5.70	5.71	5.75	5.74	5.76	5.77	5.78	5.79	5.80	5.82
.40	5.83	5.84	5.85	5.86	5.87	5.88	5.89	5.90	5.91	5.92

When % GA > 0.425, $f(G) = 5.427 + \%\ GA$

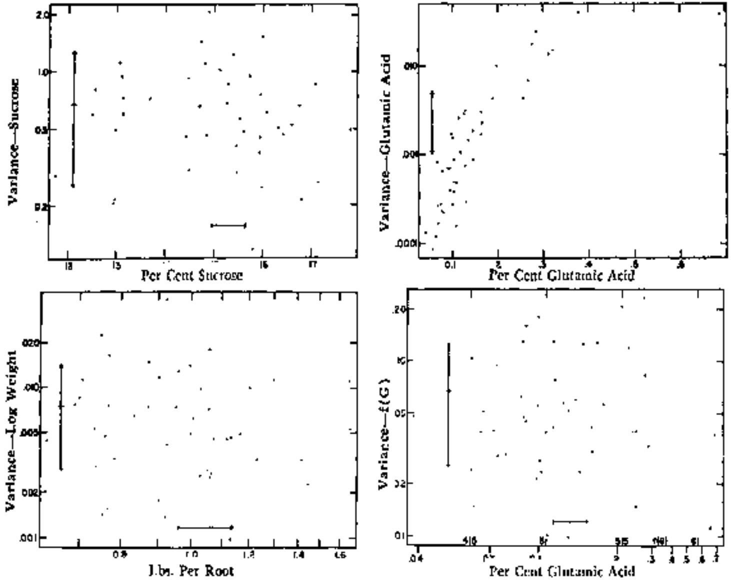
Figures 14. Relationship between mean and variance of sucrose, glutamic acid, and weight values for 10 beets from each of 43 inbred lines of beets.

Figure 1.

Figure 3.

Figure 2.

Figure 4.



Each point represents the variation of the individual values around the mean of their respective inbred line. The vertical arrows indicate the range of variance which would be expected at the 95 percent interval for a ten-beet sample from a normal population; cross bar indicates the average variance obtained for the 41 lines. Horizontal arrows show the size of the least significant difference (5 percent level) for comparison of two means; cross bar denotes general average of all lines. Variance for sucrose is nearly independent of mean (Figure 1, upper left). Increasing variance of glutamic acid with increasing mean glutamic acid (Figure 3, upper right) indicates the necessity for transformation of scale of this measurement (Figure 4, lower right"). **Tigure 2, lower left, indicates that log transformation is adequate for weight.**

forming experimental data on glutamic acid whenever there is a wide range of values. It has the disadvantage of requiring that the data be expressed in terms of the transformation function of glutamic acid [f (G)] rather than in percent units for the application of confidence limits.

Data on root weight also required transformation—but use of the logarithm was sufficient (Figure 2) to eliminate any relation between variability and average size. Variability was again much larger than expected from the normal curve of error.

With glutamic acid and weight measurements transformed to give as homogeneous error variation as feasible, it is possible to evaluate differences between the various lines. In Table 3, the lines have been divided into 10 groups based on a 1 to 10 rating from high to low sucrose, glutamic acid and weight.[4] Within each sucrose rank, the lines have been evaluated from the standpoint of all three measurements. The L. S. D. for each measurement is indicated and in general there is a significant difference only between alternate rankings. More than normal variations within any one line have been indicated also. These do not seem to be correlated with the number of generations the line was self-pollinated, which is shown in the last column of the table. Lines high in glutamic acid appear to have more variation in sucrose than do low glutamic acid lines (Figure 5) . This correlation helps to explain the slightly high or low variability of a few strains, but the implications of such a correlation are not yet clear.

Only three lines, 997, 694 and 981, were above average in all three measurements. All of the beets in 997 had outstandingly high glutamic acid, averaging 0.69 percent, which is more than double that of the next highest line, and 18 times that of the lowest GA line.

There were highly significant differences between lines in sucrose, glutamic acid and weight (F values: Sucrose 31.39, glutamic acid 24.72, weight 13.3; D. F. 42/387) . The range of glutamic acid values in the lines within one rank of sucrose was very large. This indicated absence of correlation should permit the selection of high sugar lines of either high or low glutamic acid.

The general pattern of correlation between the measured variables is as follows:

	DF	^{r}SG	^{r}SW	^{r}GW
Between lines	41	—.23 n.s.	—.34[1]	+.23 n.s.***
Within lines	386	—.25[2]	—.12[1]	+.21[2]

[1] Significant difference at the *5%* level.
[2] At the 0.1 percent level.

The small, significant, negative correlation of sucrose—weight and probably the method of selection used in the breeding of these strains prevents extremely high sugar—high weight and low sugar—low weight combinations. These excepted, the low level and non-significance of the correlations of GA—sucrose and GA—weight between lines indicates that it should be possible to obtain almost any type of combination of sucrose—GA—weight desired.

* In terms of standard deviation the range of glutamic acid was found to be about equal to that of sucrose but that of weight was smaller. Accordingly, in order to make significant differences in rank equal in all three measurements, weight is rated 2 to 8 instead of 1 to 10.

Within lines, glutamic acid was correlated negatively with sucrose and positively with weight. Both of these are highly significant, but they are also very small and do not invalidate the aforesaid conclusions. On the other hand, this does not infer that control of the three measurements is by any means independent. Nine of the lines showed three types of deviation from the average pattern of correlation. Extreme examples of these three types are as follows:

Strain	DF	r_{SG}	r_{SW}	r_{GW}
731	8	-.90***	—.85**	+.94**
721	8	—.51 n.s.	+.81**	—.72*
975	8	+.83**	+.37 n.s.	+.28 n.s.

The pattern of correlation shown by 731 also appeared in lines 695, 706, and 730 and is an extreme case of the usual pattern. It is readily identifiable as caused by variations in nitrogen fertility and is the major component of the variation between beets within a line. Possibly, a few of the beets in these lines were quite nitrogen-deficient and the average sugar reported is too high and glutamic acid low.

Weight may be positively correlated with sucrose as in 721 when it is negatively correlated with glutamic acid and is identifiable as caused by changes in growth potential (2) at low nitrogen status such as might be caused by differences in competition.

Glutamic acid may be positively correlated with sucrose as in line 975 if there is no correlation with weight; otherwise, sucrose and glutamic acid are negatively correlated (Figure 6). The pattern shown by 975 also appeared in 727, 733 and 905 and its cause is not apparent. It is similar to effects observed in commercial beets resulting from changes in storage potential or in growth potential at high nitrogen status (2, 3).

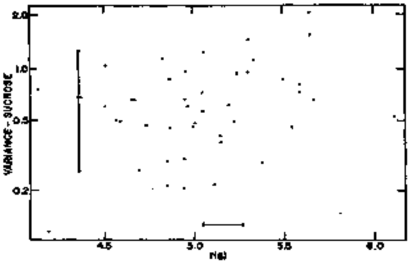


Figure 5. Sucrose variance increases with increasing mean glutamic acid of inbred line. Each point represents the variation of the individual values around the mean of their respective inbred line. The vertical arrows indicate the range of variance which would be expected at the 95 percent interval for a ten-beet sample from a normal population; cross bar indicates the average variance obtained for the 43 lines. Horizontal arrows show the size of the least significant difference (5 percent level) for comparison of two means; cross bar denotes general average of all lines.

Table 3.—Comparison of Lines Ranked According to Sucrose, **Glutamic Acid and Weight.**

1950 No.	Rating SGW[1]	Percent Sucrose	Percent Glutamic Acid	Weight	Total Selfings
955	1-8-7	17.8	.07	0.77	S4
706	2-4-7	17.1	.21[3]	0.76	S4[5]
975	2-8-7	17.1	.08	.74	S9
837	2-7-5	16.8	.09[3]	1.03	S8
997	3-1-5	16.6	.69	1.06	S4
793	3-7-4	16.4	.08	1.15	S6
730	3-8-7	16.7	.07	.71	S5
786	3-8-7	16.3	.07	.77[3]	S7
694	4-3-5	16.0[3]	.28	1.01	S4
905	4-5-6	16.1	.13	.92[4]	S8
981	4-5-5	15.9	.13	1.00	S9
701	4-7-6	16.0	.09[3]	.90	S7
727	4-10-5	16.0	.04	1.06	S8
673	4-6-2	15.6	.11	1.65	S7[5]
978	4-6-8	15.7	.10	.63	S9
832	4-6-4	15.5	.11[3]	1.12	S8
715	4-10-7	15.8	.04	.69	S9
779	4-5-7	15.5	.13	.70	S8
849	4-3-6	15.4	.11	1.13	S6
712	4-7-6	15.5	.09	.92	S8
695	5-3-6	14.9[3]	.27[4]	.92	S4
950	5-5-5	14.8	.16[3]	1.03	S4[5]
692	5-8-5	15.2	.07	1.42	XS4
876	5-7-7	15.3	.09	.79	XS5
691	5-6-7	14.9	.11	.74	XS4
716	5-8-7	15.1	.06	.76	S8
810	6-3-5	14.7	.29	1.05	S8
807	6-4-6	14.4	.23	.90	S8
683	6-5-2	14.5	.14	1.65	XS4
784	6-5-5	14.7[3]	.16	1.00	S8[5]
689	6-6-6	14.7	.10[3]	.87[3]	S4
838[2]	6-6-7	14.5	.10	.77	XS5
731	7-6-5	13.7	.11	.96	S9
831[2]	8-3-6	13.1	.25	.87	XS5
677	8-5-4	13.1	.16	1.17	S4[5]
770	8-5-3	13.0	.14	1.45	S10
733	8-6-5	13.0	.12	1.05	S9
756	8-6-4	13.0	.10	1.20	S10
757	8-6-5	13.1	.10	1.07	S10
752	8-7-7	13.1	.09	.75	S10
846[2]	9-3-5	12.6	.25	.95	XS5
721	9-8-5	12.5	.06	1.06[3]	S8
891[2]	10-4-4	11.7	.17	1.23	XS5
LSD 5% Point		0.7	36%	19%	

[1] Rating SGW: Rating Sucrose, Glutamic Acid, Weight.
[2] Red beets, or originated from a cross with a red garden beet.
[3] Greater than average variation within strains.
[4] Extremely large variation.
[5] Obvious outpollenation found or suspected in one or more root generations of **the line.**

It seems likely that within lines the true picture of the correlations between sucrose, glutamic acid and weight is generally obscured by at least three important environmental factors operating simultaneously, any one of

TABLE 4. - COMPARISON QF RELATED LINES

LINE	ORIGIN	ROOT GENERATION SEPARATED	TOTAL SELFINGS	STRAIN NO.	GA %	SUCROSE %	WT. (LB)
		1947 STRAINS					
A.	FLAT FOLIAGE	S3—R3	S5	324	.31	15.0	1.25
		S3—R3	S5	327	.50	14.3	1.26
B.	FLAT FOLIAGE	S5—R5	S8	300	.09	13.7[1]	1.01
		S5—R5	S8	304	.12	16.0	1.14
		S5—R5	S8	301	.22	16.0	1.08
		1950 STRAINS					
C.	CESENA	S1—R1	S4	689	.10[1]	14.7	.87
		S1—R1—S3—R3	S4	695	.27	14.9	.92
		S1—R1—S3—R3	S4	694	.28	16.0[1]	1.01
D.	HILLESHOG	?S2—R2	S4	997	.69[1]	16.6	1.06[1]
		?S2—R2	S4	706	.21	17.1	.76
E.	UNKNOWN	S3—R3	S9	715	.04	15.8	.69
		S3—R3—S7—R7	S9	731	.11	13.7	.96
		S3—R3—S7—R7	S9	733	12	13.0	1.05
F.	PIONEER S2 F4(S3) UNKNOWN RED	XS1—S4—R4	S5	831	.25	13.1	.87[1]
		XS1—S4—R4	S5	846	.25	12.6	.95
		XS1—S4—R4	S5	891	.17[1]	11.7[1]	1.23[1]
G.	FLAT FOLIAGE COMMERCIAL	S5—R5—S7—R7	S8	807	.23	14.4	.90
		S5—R5—S7—R7	S8	810	.29	14.7	1.05
		S5—R5—S7—R7	S8	832	.11	15.5	1.12
		S5—R5—S7—R7	S8	849	.11	15.4	1.13
		S5—R5—S7—R7	S8	837	.09	16.8[1]	1.03
H.	FREDRIKSEN FF	S6—R6	S10	770	.14[1]	13.0	1.45[1]
		S6—R6	S10	752	.09	13.1	.75
		S6—R6—S9—R9	S10	756	.10	13.0	1.20
		S6—R6—S9—R9	S10	757	.10	13.1	1.07
J.	FLAT FOLIAGE COMMERCIAL	S7—R7	S9	975	.08	17.1[1]	.74
		S7—R7—S8—R8	S9	978	.10	16.7	.63
		S7—R7—S8—R8	S9	981	.13	16.9	1.00[1]

[1] SIGNIFICANT DIFFERENCE 5 % POINT
S-SEED GENERATION
R-ROOT GENERATION

which would produce large measurable correlations if the other two remained. constant.

Several of the 43 lines in the 1950 series were more or less closely related. Twenty-three diverse lines established from a common ancestor of succeeding generations were produced from seven of the original lines. The pronounced likeness between closely related lines in glutamic acid as well as sucrose and weight is evident in Table 4. The lines have been listed in order from SI to S7, depending upon the first generation at which separation of lines took place. The mark, [1], denotes significant differences between lines in any one characteristic. There were significant differences between lines in E, F and G (as indicated by differences in all three characteristics) where separation took place at the S_3, S_4 or S_5 root generation, respectively. Subsequent separation at the S7 root generation in G or in J did not result in significantly different lines. The close relationships of the lines high in glutamic acid (over 0.2 percent) is particularly interesting. The two high lines in 1947 were from a common origin; the eight lines in 1950 were derived from four original lines, two of which also had relatively high sucrose. This general close agreement between related lines in glutamic acid as well as sucrose constitutes good evidence for the heritability of this characteristic.

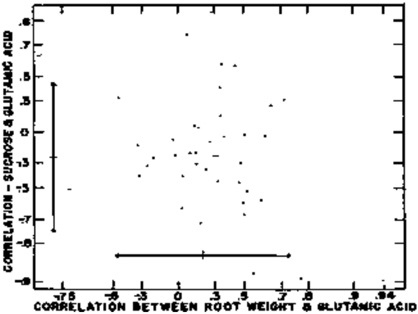


Figure 6. Points represent correlations between root weight and glutamic acid and between sucrose and glutamic acid within each of 43 inbred lines of sugar beets. Each arrow indicates the 95 percent interval of expected correlations if 10 beet samples were taken from a normal population with a correlation equal to the average correlation (marked with cross bar) found within lines measured .

Summary

Ten beets each from 43 inbred sugar beet lines grown in Colorado in 1950 were weighed and analyzed individually for glutamic acid and sucrose. The glutamic acid values, which varied from 0.03 to 1.05 percent, showed a remarkable relationship between mean and variance. A table of values of a transformation function is presented which practically eliminates this difficulty and facilitates the analysis of variance of glutamic acid data.

The inbred lines have been classified on the basis of a 1 to 10 rating from high to low sucrose, glutamic acid and weight. There were significant differences between the lines in glutamic acid as well as sucrose and weight. There was no correlation between lines in glutamic acid and sucrose, or

glutamic acid and weight which should make possible the selection of high sucrose lines of either high or low glutamic acid.

Within lines, there were small but highly significant correlations; negative, for glutamic acid and sucrose; and positive, for glutamic acid and weight. Factors, probably environmental, appear to be operating to balance these correlations and prevent, at the present time, a true measure of existing relationships.

The likeness between closely related lines is very striking and the heritable character of glutamic acid is strongly indicated.

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