Some Chemical-Genetic Studies Pertaining to Quality in Sugar Beets (Beta vulgaris L.) [1, 4]

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Quality of the sugar beets delivered to the factories for processing has become of considerable concern in many sugar-beet producing areas of the United States. In some areas the percentage sucrose is so low that it is questionable whether factories can continue to operate at a profit. It is thought that the application of increased amounts of nitrogenous fertilizers may be responsible at least in part for the decrease noted in percentage sucrose. The purpose of this article is to report the results from some chemical-genetic studies pertaining to quality in sugar beets (*Beta vulgaris* L.). Particular emphasis will be placed on the interrelations of percentage sucrose, total nitrogen, betaine and glutamic acid.

Experimental Considerations

The design of the experiment is depicted by the tabulations listed in Table 1. There are two treatments, fertilized and non-fertilized. The fertilized plots received a surface application of 100 pounds of available nitrogen (N) and 250 pounds of available phosphorus (P_2O_5) per acre on April 4, 1956. The fertilizer was cultivated under with a rototiller. The experiment was planted on April 10 and 11. On June 26, another 100 pounds of N per acre were drilled in the center of each space between rows of the fertilized plots. The fertilizer treatments are split blocks of replications and are randomized within each replication. The six populations are randomized within each treatment. The locations refer to the position of the eight plants harvested from each plot. For more details about the experimental design see (7) [5].

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[5] Numbers in parentheses refer to literature cited.

Table 1.—Sources of variation designated as main effects, and number of each.

Main effects	Number
Replications	40
Populations	6
Treatments	2
Locations	8

The materials used in the study are populations and fertilizer treatments. The populations are A54-1, A54-1BB, 50-406BB, 50-406, F_1 hybrid, and 52-307. A54-1 is a commercial variety and A54-1BB resulted from seed harvested from 25 mother beets of A54-1. The 25 mother beets giving rise to population A54-1BB were grown in an isolated seed plot along with 25 mother beets from each of 22 other populations. This isolation plot was composed of 25 rows in each of which one mother beet from each population occurred at random, making a total of 23 mother beets per row. Seed was harvested from all mother beets of A54-1 on an individual plant basis and then was bulked to provide population A54-1BB. Seed saved from mother beets of 50-406 and handled in a similar manner produced the population designated as 50-406BB. Hence, since 50-406 is an inbred, 50-406BB is a topcross. Populations 50-406 and 52-307 are inbreds and the F_1 hybrid population resulted from crossing them.

The methods used in analyzing the data have been reported in previous articles (4, 5, 6, 7). All the analyses for total nitrogen, betaine and glutamic acid are from thin juice samples. These three characters are reported as milligrams per 100 milliliters of thin juice equated to a refractometer reading of 10. The method of determining percentage of sucrose is standard with the sugar companies and is well known. The data for glutamic acid were transformed to logarithms for calculating variances, covariances, regressions and correlation coefficients. However, the means for glutamic acid as presented in the tables are calculated from the data on the arithmetic scale.

The statistical constants employed in studying the data are means, variances, covariances, regressions and correlation coefficients.

Both the environmental and genetic variabilities are subject to a certain amount of control. The environmental variability may be controlled to some extent by cultural and fertilizer practices and the genetic variability by breeding varieties and hybrids giving the desired response to these cultural and fertilizer practices. The purpose of this article is to report the results from

studies of the environmental and genetic variances in an attempt to learn to what extent the environment and genotype can be molded to suit the requirements of the beet sugar industry and to learn what procedures to follow to accomplish the desired results.

Method of Chemical Analysis

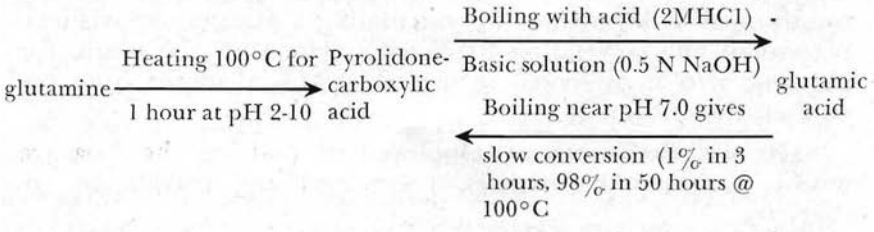
The method of determining total nitrogen was reported by Payne et al., see (4).

Betaine

Quantitative determinations of betaine (trimethylglycine) in thin beet juice samples were made by a procedure described by Focht, Schmidt and Dowling (1). Aliquots of 5 ml of thin beet juice samples were transferred at room temperature in 30 ml beakers. The beakers were placed in the refrigerator at 3°C for about 30 minutes. By experimentation, it was found that adjustment of the sample to a pH 1.0 was not necessary, and since the thin beet juice samples were not discolored, they were not filtered through carbon. The samples were carried through the procedure outlined by Focht et al., with the above changes. The milligrams of betaine per ml of sample were determined by comparing the readings obtained on the Beckman spectrophotometer to the readings on a standard curve.

Glutamic Acid

The determination of glutamic acid content of thin juice was indirectly the determination of glutamine in the original sugar beet. During the oxalation process of preparing the thin juice, some of the glutamine is converted to pyrolidonecarboxylic acid (PCA) and some to glutamic acid, Wilson and Cannan (13).



Since it is more difficult to determine total glutamine and PCA content than glutamic acid, we chose to hydrolyze the existing glutamine and PCA to glutamic acid and determine the total glutamic acid.

Several methods of determining glutamic acid (2, 3, 9, 10) in thin juice samples were investigated. A chromatographic method

was chosen. Several different hydrolytic procedures were tried on the thin juice samples (8, 12, 13). In each procedure, the hydrolyzing mixture was heated in a water bath for different time intervals at boiling temperature (94°C).

Hydrolysis with 2-4 N HCl caused the formation of a brown precipitate and a brown-colored solution which interfered with spotting for chomatographic study.

Hydrolysis in a very basic solution showed good conversion to glutamic acid, but the high alkaline salt content caused tailing and irregularly shaped spots on the chromatogram.

Alkaline hydrolysis in 0.5 N NaOH heated for 6 hours in the water bath showed complete conversion to glutamic acid on all samples except those with very high glutamine. The PCA conversion was about 98% complete at this time. The hydrolysate was satisfactory for chromatographic purposes.

Method

One ml of the thin juice sample was transferred into a graduated tube (15 ml centrifuge tube), and 0.1 ml of 5 N NaOH was added and the mixture was heated in a water bath at boiling temperature (94°C) for 6 hours. The water level of the bath was maintained so that the digestion mixture in the tube was always completely immersed. Also during the digestion a few drops of distilled water were added at intervals to maintain a volume of approximately 1.0 ml. After 6 hours, the samples were removed, cooled to room temperature, and made up to a volume of 2.0 ml with distilled water for spotting.

For one dimensional chromatograms, 5 microliters of the diluted hydrolyzed sample were placed on Whatman No. 1 filter paper (20 cm x 20 cm). The solvent used was 80 percent phenol (Merck-reagent grade). The chromatographing was continued until the solvent nearly reached the top of the paper (about 6 hours). The papers were then removed, hung in a hood[6] to dry over night at room temperature, then dipped in 0.3 percent ninhydrin in 95 percent ethyl alcohol to reveal the spots, and dried again, away from sunlight.

After 18 hours, the dried, stained papers were cut and passed through the Spinco Analytrol which automatically measures the density of the spot and records the measurement in square centimeters. The concentration of glutamic acid in mg per ml was determined by comparison of the sample readings to a standard curve made from samples of known concentration.

[6] Contamination by some fumes may cause discoloration to the stained papers later, if care is not taken while chromatographs dry.

Comparison of Warburg and Chromatographic Methods for Total Glutamic Acid Determination

To check the accuracy of the chromatographic method for determination of glutamic acid quantitatively, eight thin juice samples, two known samples of glutamine and glutamic acid, and two thin juice samples plus known amounts of glutamine and glutamic acid were hydrolyzed and analyzed by the Warburg method (11) and by the chromatographic method. The comparative results are shown below:

Sample no.	Content	I Warburg (mg per ml)	II Chromatograph (mg per ml)	Deviation of method II from method I in (mg per ml)	Percent deviation
1	Glutamine 1 mg/ml	0.965 mg/ml	0.98 mg/ml	+0.015	1.6
2	Glutamic Acid 1 mg/ml	0.965	0.99	+0.025	2.6
3	Thin Juice	0.43	0.48	+0.05	11.6
4	Thin Juice	0.885	0.96	+0.075	8.5
5	Thin Juice	0.685	0.76	+0.075	11.0
6	Thin Juice	0.815	0.83	+0.015	1.8
7	Thin Juice	0.49	0.52	+0.03	6.1
8	Thin Juice	0.825	0.82	—0.005	0.6
9	Thin Juice	0.715	0.72	+0.005	0.7
10	Thin Juice	0.72	0.70	—0.02	2.8
11	Sample 3+ 0.4 mg/ml Glutamine	0.78	0.81	+0.03	3.8
12	Sample 3 + 0.4 mg/ml Glutamic Acid	0.83	0.88	0.05	6.0
			Average % difference		4.75%

Since the laboratory facilities could not use the Warburg method on 4300 samples, the chromatographic method was used.

Results

An examination of Table 2 reveals that there are differences between populations and fertilizer treatments for percentage sucrose, total nitrogen, betaine and glutamic acid. Also from other data there were found to be marked differences between replications for all of these characters. For example, replication group 33 to 40 was found to be low in percentage sucrose and high in total nitrogen, betaine and glutamic acid. The differences between fertilizer treatments and replications are attributable to environment. The differences between populations contribute primarily to genetic variability. However, there is a negligible amount in this experiment of environmental variability included with the genetic variability due to differences between population.

Each population for each fertilizer treatment is composed of 320 plants. It is apparent that the differences between plants comprise the total variability for populations within fertilizer treatments. In turn this total variability of a given population

Table 2.—The means and their standard errors for percentage sucrose, nitrogen, betaine and glutamic acid, population genetic studies 1956, fertilized and non fertilized.

Populaton and treatment	Sucrose	Nitrogen[1]	Betaine[1]	Glutamic acid[1]
A54-1	%	mg	mg	mg
Fertilized	16.8±0.10	46.8±1.44	116.6±1.53	80.1±3.98
Non-fertilized	17.9±0.10	18.8±0.91	89.8±1.48	12.8±1.87
A54-1BB				
Fertilized	16.7±0.12	44.8±1.42	115.1±1.49	41.4±2.79
Non-fertilized	17.8±0.11	16.9±0.73	85.5±1.62	7.6±0.88
50-406BB				
Fertilized	17.3±0.10	33.6±1.32	106.1±1.33	45.8±3.50
Non-fertilized	17.6±0.09	12.6±0.62	69.9±1.40	6.4±0.93
50-406				
Fertilized	16.1±0.09	31.2±0.82	125.3±1.97	17.3±1.35
Non-fertilized	17.4±0.08	14.6±0.59	73.4±1.55	8.0±0.92
F_1				
Fertilized	17.6±0.09	21.3±0.58	101.3±1.20	18.0±1.18
Non-fertilized	17.6±0.07	9.8±0.31	51.3±1.14	3.5±0.28
52-307				
Fertilized	16.6±0.09	18.6±0.46	108.7±1.22	10.0±0.50
Non-fertilized	16.5±0.10	11.1±0.28	79.8±1.38	3.0±0.14

[1] Milligrams per 100 milliliters of thin juice equated to a refractometer reading of 10.

is composed of two parts, namely, that due to environmental variability within the experimental area and that due to heritable differences between plants. The latter is termed genetic variability and arises from the fact that some plants have different genotypes. The results from the studies within populations and fertilizer treatments will be considered first.

Environmental Variability

The correlation coefficients and the percentages of the variances accounted for by regression are given in Table 3. They are averages derived from the data for the inbreds 50-406, 52-307, and their F_1 hybrid. Hence the correlation coefficients and regressions measure the relations between characters attributable to environmental variability. The data show the relations between percentage sucrose and total nitrogen, betaine and glutamic acid

Table 3.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, average of 50-406, F_1, and 52-307, environmental.[1].

Characters correlated	Correlation coefficient r	% accounted for by regression
Fertilized		
Sucrose vs. nitrogen	—0.48	23.0
Sucrose vs. betaine	—0.09	0.8
Sucrose vs. glutamic acid	—0.28	7.8
Non-fertilized		
Sucrose vs. nitrogen	—0.30	9.0
Sucrose vs. betaine	0.03	0.1
Sucrose vs. glutamic acid	—0.25	6.2

[1] At the 0.05 level r = 0.06 and at the 0.01 level r = 0.08. These are approximations.

on the fertilized and non-fertilized plots. The relations between sucrose and nitrogen, and sucrose and glutamic acid are negative on both the fertilized and non-fertilized plots. There seems to be very little relation, if any, between sucrose and betaine. The percentages of the variances accounted for by regression are not great, the largest amount being 23.0 percent for sucrose and nitrogen.

The corresponding relations for nitrogen, betaine and glutamic acid are given in Table 4. The relations are all positive and the correlation coefficients are considerably larger than those involving sucrose. The highest correlation coefficients are for glutamic acid and nitrogen and the lowest are for betaine and glutamic acid. The percentages of the variances accounted for by regression range from 16 to 46.2.

Table 4.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, average of 50-406, F_1, and 52-307, environmental.[1]

Characters correlated	Correlation coefficient r	% accounted for by regression
Fertilized		
Betaine vs. nitrogen	0.47	22.1
Glutamic acid vs. nitrogen	0.68	46.2
Betaine vs. Glutamic acid	0.40	16.0
Non-fertilized		
Betaine vs. nitrogen	0.65	42.2
Glutamic acid vs. nitrogen	0.66	43.6
Betaine vs. glutamic acid	0.49	24.0

[1] At the 0.05 level r = 0.06 and at the 0.01 level r = 0.08. These are approximations.

The conclusions that can be drawn from Tables 3 and 4 are that, as regards individual plants, percentage sucrose is largely independent of total nitrogen, betaine and glutamic acid. This would indicate that there are environmental conditions which allow individual beets to have fair amounts of nitrogen, betaine and glutamic acid and still be fairly high in percentage sucrose. If the agronomist can learn what these environmental conditions are, it may be possible to follow cultural and fertilizer practices that would result in higher percentage sucrose in beets delivered to the factory.

The correlation coefficients for glutamic acid and nitrogen are positive and fairly high. It seems that more difficulty will be involved in controlling the environment so that the commercial crop harvested is low in one of these two characters and high in the other. Manipulation of the environment may lead to low betaine and high glutamic acid or vice versa as only 16.0 percent of the total variance of one on the other is accounted for by regression on the fertilized plots, and only 24.0 percent on the non-fertilized plots.

The correlation coefficients and percentages of the variances accounted for by regression for the two inbreds and the F_1 are given in Table 5. The data are for the fertilized and non-fertilized plots.

For the fertilized plots and for 50-406, sucrose is most closely associated with nitrogen, 32.5 percent of the variance being accounted for by regression. The only statistically significant correlation involving sucrose and betaine is for inbred 50-406. Again the relation is negative. However, only 6.2 percent of the variance is accounted for by regression.

Table 5.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, environmental.[1]

Population and characters correlated	Fertilized		Non-fertilized	
	Correlation coefficient r	% accounted for by regression	Correlation coefficient r	% accounted for by regression
50-406				
Sucrose vs. nitrogen	—0.57	32.5	—0.53	28.1
Sucrose vs. betaine	—0.25	6.2	—0.30	9.0
Sucrose vs. glutamic acid	—0.39	15.2	—0.37	13.7
F_1				
Sucrose vs. nitrogen	—0.54	29.2	—0.36	13.0
Sucrose vs. betaine	—0.01	0.0	—0.04	0.2
Sucrose vs. glutamic acid	—0.31	9.6	—0.37	13.7
52-307				
Sucrose vs. nitrogen	—0.31	9.6	0.03	0.1
Sucrose vs. betaine	0.09	0.8	0.38	14.4
Sucrose vs. glutamic acid	—0.11	1.2	—0.06	0.4

[1] At the 0.05 level $r = 0.11$ and at the 0.01 level $r = 0.15$.

The corresponding data for the non-fertilized plots are given in Table 5 also. For 50-406, sucrose is negatively correlated with nitrogen, betaine and glutamic acid and all the correlation coefficients are significantly different from zero. This is quite a contrast with the correlation coefficients for 52-307. For 52-307 the only statistically significant correlation coefficient is between sucrose and betain and it is positive. Hence all populations do not show the same relations between sucrose and nitrogen, betaine and glutamic acid. Stated genetically there are interactions between genotypes and the environments. However, in no case is more than 28.1 percent of the variance of sucrose accounted for by regression.

The corresponding correlation coefficients showing the closeness of the environmental relations for nitrogen, betaine and glutamic acid on the fertilized plots are given in Table 6. The correlation coefficients are all positive and significantly higher for 52-307 than for 50-406 and the F_1 hybrid. However, that for glutamic acid and nitrogen is not significantly so at the 0.05 level.

Again there are interactions between populations (genotypes) and the environment. For betaine and nitrogen, the percentage of the variance accounted for by regression is more than twice as great for 52-307 than it is for the other two populations. For betaine and glutamic acid, regression accounts for approximately four times as much of the variance in 52-307 as it does in the other two populations.

Table 6.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, environmental.[1]

Population and characters correlated	Fertilized		Non-fertilized	
	Correlation coefficient r	% accounted for by regression	Correlation coefficient r	% accounted for by regression
50-406				
Betaine vs. nitrogen	0.44	19.4	0.73	53.3
Glutamic acid vs. nitrogen	0.65	42.2	0.79	62.4
Betaine vs. glutamic acid	0.32	10.2	0.68	46.2
F_1				
Betaine vs. nitrogen	0.40	16.0	0.58	33.6
Glutamic acid vs. nitrogen	0.71	50.4	0.62	38.4
Betaine vs. glutamic acid	0.34	11.6	0.37	13.7
52-307				
Betaine vs. nitrogen	0.65	42.2	0.59	34.8
Glutamic acid vs. nitrogen	0.74	54.8	0.47	22.1
Betaine vs. glutamic acid	0.65	42.2	0.32	10.2

[1] At the 0.05 level $r = 0.11$ and at the 0.01 level $r = 0.15$.

The corresponding data for the non-fertilized plots are given in Table 6 also. Here the relations are reversed as the higher correlation coefficients are found within population 50-406 as compared with 52-307. The percentage of the variance accounted for by regression is considerably higher for 50-406. Comparing the data for the two fertilizer treatments it can be seen that there is a distinct genotype-environment interaction.

In addition to the data for individual plants within the inbreds and F_1 hybrid, the differences between fertilizer treatments and the differences between replications provide a means of studying the environmental inter-relations between sucrose and nitrogen, betaine and glutamic acid, and between nitrogen, betaine and glutamic acid. The combined data for the fertilized and non-fertilized plots are given in Table 7. The correlation coefficients for sucrose and nitrogen, and sucrose and glutamic acid are extremely high, a little over 80 percent of the environmental variance being accounted for by regression. The relation is negative. Also the relation between glutamic acid and nitrogen is extremely high and positive, 91.5 percent of the environmental variability being accounted for by regression. This means that those cultural and fertilizer practices which tended to increase nitrogen and the

nitrogenous compounds on the average correspondingly decreased sucrose. On the other hand, cultural and fertilizer practices which tended to increase total nitrogen also tended to increase correspondingly betaine and glutamic acid. These data definitely point out the advantages of using sound fertilizer and cultural practices. Much can be accomplished toward increasing the percentage of sucrose in the sugar beets delivered to the factory by so doing.

Table 7.—Correlation coefficients and percentages of the variances accounted for by regression, between replications, environmental.[1]

Characters correlated	Correlation coefficient r	% accounted for by regression
Sucrose vs. nitrogen	—0.91	83.2
Sucrose vs. betaine	—0.70	48.7
Sucrose vs. glutamic acid	—0.90	81.4
Betaine vs. nitrogen	0.88	76.7
Glutamic acid vs. nitrogen	0.96	91.5
Betaine vs. glutamic acid	0.84	70.4

[1] At the 0.05 level r = 0.30 and at the 0.01 level r = 0.39.

Genetic Variability

It has been seen that much can be accomplished by controlling the environment. Next, the bearing the data have on the interrelations between percentage sucrose, total nitrogen, betaine and glutamic acid and between total nitrogen, betaine and glutamic acid will be considered. Again the correlation coefficients and regressions will be studied in connection with the genetic variances to determine these relations.

The data for the individual plants are given in Table 8. These are averages over the three segregating populations. Percentage sucrose is negatively associated with total nitrogen, betaine and glutamic acid on the fertilized plots and with nitrogen on the non-fertilized plots. The associations between sucrose and nitrogen and between sucrose and betaine on the fertilized plots do not differ materially.

Table 8.—Correlation coefficients and percentage of the variances accounted for by regression, between plants average of A54-1, A54-1BB and 50-406BB, genetic.[1]

Characters correlated	Correlation coefficient r	% accounted for by regression
Fertilized		
Sucrose vs. nitrogen	0.30	9.0
Sucrose vs. betaine	—0.34	11.6
Sucrose vs. glutamic acid	—0.20	4.0
Non-fertilized		
Sucrose vs. nitrogen	0.32	10.2
Sucrose vs. betaine	0.06	0.4
Sucrose vs. glutamic acid	0.05	0.2

[1] At the 0.05 level r = 0.06 and at the 0.01 level r = 0.08. These are approximations.

Corresponding data for total nitrogen, betaine and glutamic acid are listed in Table 9. Again these values are over-all averages for the segregating populations. On the fertilized plots the correlation coefficients are positive and highest for betaine and total nitrogen, next highest for glutamic acid and nitrogen and lowest for betaine and glutamic acid. On the non-fertilized plots the correlation coefficients are highest for glutamic acid and nitrogen and lowest for betaine and glutamic acid.

Table 9.—Correlation coefficients and percentage of the variances accounted for by regression, between plants, average of A54-1, A54-1BB and 50-406BB, genetic.[1]

Characters correlated	Correlation coefficient r	% accounted for by regression
Fertilized		
Betaine vs. nitrogen	0.66	43.6
Glutamic acid vs. nitrogen	0.45	20.2
Betaine vs. glutamic acid	0.04	0.2
Non-fertilized		
Betaine vs. nitrogen	0.39	15.2
Glutamic acid vs. nitrogen	0.73	53.3
Betaine vs. glutamic acid	0.30	9.0

[1] At the 0.05 level r = 0.06 and at the 0.01 level r = 0.08. These are approximations.

The data for each of the three segregating populations are listed in Table 10. On the fertilized plots the highest correlation coefficients involving the genetic variances and covariances are for population 50-406BB. They are all negative. The same is true of population A54-1BB, except that the correlation coefficients are not nearly so high. In population A54-1BB the greatest amount of the variance accounted for by regression is 9.0 percent and it is for sucrose and nitrogen. The only significant correlation coefficient for A54-1 is between sucrose and betaine. Clearly, populations differ in the closeness of the relation between sucrose and total nitrogen and between sucrose and the nitrogenous-compounds as regards the genetic variability on the fertilized plots. It should be much easier to breed high sucrose at the higher fertility level working with A54-1 than to do so by working with 50-406BB.

The data in Table 10 show that on the non-fertilized plots the relation between sucrose and total nitrogen is negative for all three segregating populations. This is also true of sucrose and betaine and sucrose and glutamic acid for A54-1. The relations between sucrose and these two nitrogenous compounds are positive and significant for A54-1BB. For population 50-406BB the correlation coefficients are not significantly different from zero. Comparing the correlation coefficients for populations and treatments reveals that again there is a decided genotype-environment

Table 10.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, genetic.[1]

Population and characters correlated	Fertilized		Non-fertilized	
	Correlation coefficient r	% accounted for by regression	Correlation coefficient r	% accounted for by regression
A54-1				
Sucrose vs. nitrogen	0.04	0.2	—0.52	27.0
Sucrose vs. betaine	—0.42	17.6	—0.14	2.0
Sucrose vs. glutamic acid	—0.04	0.2	—0.27	7.3
A54-1BB				
Sucrose vs. nitrogen	—0.30	9.0	—0.12	1.4
Sucrose vs. betaine	—0.13	1.7	0.20	4.0
Sucrose vs. glutamic acid	—0.27	7.3	0.30	9.0
50-406BB				
Sucrose vs. nitrogen	—0.63	40.0	—0.32	10.2
Sucrose vs. betaine	—0.81	65.6	0.03	0.1
Sucrose vs. glutamic acid	—0.83	68.9	0.04	0.2

[1] At the 0.05 level r = 0.11 and at the 0.01 level r = 0.15. These are approximations.

interaction. In no case are the percentages accounted for by regression large.

The data showing the genetic relations between total nitrogen, betaine and glutamic acid for the fertilized and non-fertilized plots are listed in Table 11. On the fertilized plots correlation coefficients are positive and highest for 50-406BB. The association is particularly high between total nitrogen and glutamic acid for populations A54-1BB and 50-406BB. In all three segregating populations the correlation coefficients are positive and rather high for total nitrogen and betaine. For populations A54-1 and A54-1BB the correlation coefficients for betaine and glutamic acid are not significantly different from zero.

Table 11.—Correlation coefficients and percentages of the variances accounted for by regression, between plants, genetic.[1]

Population and character correlated	Fertilized		Non-fertilized	
	Correlation coefficient r	% accounted for by regression	Correlation coefficient r	% accounted for by regression
A54-1				
Betaine vs. nitrogen	0.64	41.0	0.69	47.6
Glutamic acid vs. nitrogen	0.23	5.3	0.80	64.0
Betaine vs. glutamic acid	—0.02	0.0	0.63	39.7
A54-1BB				
Betaine vs. nitrogen	0.61	37.2	0.16	2.6
Glutamic acid vs. nitrogen	0.90	81.0	0.64	41.0
Betaine vs. glutamic acid	—0.03	0.1	—0.35	12.2
50-406BB				
Betaine vs. nitrogen	0.86	74.0	0.28	7.8
Glutamic acid vs. nitrogen	1.00	100.0	0.69	47.6
Betaine vs. glutamic acid	0.42	17.6	0.54	29.2

[1] At the 0.05 level r = 0.11 and at the 0.01 level r = 0.15. These are approximations.

The data showing the genetic relations between total nitrogen, betaine and glutamic acid for the non-fertilized plots are given in Table 11 also. The correlation coefficients are higher for A54-1 than they are for 50-406BB. This is the reverse of what was true on the fertilized plots. The values are lowest for A54-1BB, being negative for betaine and glutamic acid. Again there are decided genotype-environment interactions as evidenced by comparing the percentages of the variances accounted for by regression for populations and fertilizer treatments.

Comparisons between the environmental and genetic correlation coefficients and percentages of the variances accounted for by regression between replications and between populations are given in Table 12. The relations between percentage sucrose and total nitrogen, and percentage sucrose and glutamic acid are extremely high for environmental. In both cases better than 80 percent of the environmental variability of percentage sucrose is accounted for by regression. The relation between sucrose and betaine is also negative and fairly close. This means that cultural and fertilizer practices have to be such as not to result in excess amounts of nitrogen and nitrogenous compounds if beets high in percentage sucrose are to be produced. These are environmental influences and are subject to considerable immediate control.

Table 12.—Correlation coefficients and percentages of the variances accounted for by regression between replications and between populations, environmental and genetic.

Character and variability	Correlation coefficient r	% accounted for by regression
Sucrose vs. nitrogen		
Environmental[1]	—0.91	83.2
Genetic[2]	0.28	7.9
Sucrose vs. betaine		
Environmental[1]	0.70	48.7
Genetic[2]	0.46	21.3
Sucrose vs. glutamic acid		
Environmental[1]	0.90	81.4
Genetic[2]	0.50	25.0

[1] At the 0.05 level r = 0.30 and at the 0.01 level r = 0.39.
[2] At the 0.05 level r = 0.11 and at the 0.01 level r = 0.15. These are approximations.

The data for the variability due to genetic causes present a different picture. The correlation coefficients are fluctuating about zero showing that probably very little of the genetic variances are accounted for by regression. In the studies of the genetic variances and covariances within and between segregating populations the degrees of freedom are sufficient to give a high degree of reliability. It will be remembered that for these data in populations A54-1 and A54-1BB in no case was more than 27 percent of

the genetic variability accounted for by regression (see Table 10). This was true for both the fertilized and non-fertilized plots. However for the segregating population 50-406BB on the fertilized plots the correlation coefficients were negative and as much as 69 percent of the variance of sucrose was accounted for by regression.

The corresponding environmental and genetic data involving the correlation coefficients and the percentage of the variances accounted for by regression are given in Table 13 for total nitrogen, betaine and glutamic acid. The associations are close and positive as regards both the environmental and the genetic components of variability. The exception is the genetic variability involving betaine and glutamic acid. Here only 27 percent of the genetic variance is accounted for by regression.

Table 13.—Correlation coefficients and percentages of the variances accounted for by regression, environmental and genetic.

Characters and variability	Correlation coefficient r	% accounted for by regression
Betaine vs. nitrogen		
Environmental[1]	0.88	76.7
Genetic[2]	0.72	51.9
Glutamic acid vs. nitrogen		
Environmental[1]	0.96	91.5
Genetic[2]	0.95	90.4
Betaine vs. glutamic acid		
Environmental[1]	0.84	70.4
Genetic[2]	0.52	27.2

[1] At the 0.05 level r = 0.30 and at the 0.01 level r = 0.39.
[2] At the 0.05 level r = 0.11 and at the 0.01 level r = 0.15. These are approximations.

Discussion

These researches show that much can be accomplished to improve the quality of sugar beets by cultural and fertilizer practices. Also they show that much can be accomplished by the plant breeder. However, to capitalize on the maximum potentials of the cultural and fertilizer practices and on the improved hybrids or strains produced by the plant breeder, the two must be combined into one effort. That is, proper cultural and fertilizer practices must be accompanied by the use of hybrids or varieties bred to give the desired performance under the cultural and fertilizer practices found to give the best results. To appreciate fully this statement let us again consider the data listed in Table 2.

From a comparison of the fertilized and non-fertilized plots of Table 2, it is clear that without exception the fertilizer used has increased the total nitrogen, betaine and glutamic acid in all six populations. These increases have been accompanied by significant decreases in percentage sucrose in three of the six popula-

tions but such is not true for the other three. Two of the three populations showing no significant decrease in percentage sucrose are hybrids and the other is inbred 52-307. The two hybrids have high percentage sucrose on both the fertilized and the non-fertilized plots, whereas, the inbred, comparatively speaking, has low percentage sucrose on both the fertilized and non-fertilized plots. It is clear that the two hybrids are capable of producing high sucrose at the higher fertility level.

Of considerable importance is the reason why the two hybrids are capable of producing high sucrose at the high fertility level, whereas, the commercial variety A54-1 and the broad genetic base A54-1BB derived from it are not capable of so doing. Consider further the data for A54-1 and the two hybrid populations 50-406BB and the F_1 (50-406 x 52-307) grown on the fertilized plots. The F_1 is lower in concentrations of total nitrogen, betaine and glutamic acid. These data indicate that the concentrations of nitrogen and the two nitrogenous compounds are rather closely associated with percentages of sucrose and hence probably of considerable importance.

This raises the question as to whether all genotypes react the same. The data are presented in Table 14. On the fertilized plots the data for A54-1 are 46.8, 116.6 and 80.1 and the percentage sucrose is 16.8, the data for 50-406 are 31.2, 125.3 and 17.3 and the percentage sucrose is 16.1, and finally the data for the topcross (50-406BB) involving this inbred are 33.6, 106.1 and 45.8 and the percentage sucrose is 17.3. These comparisons show that some genotypes have lower concentrations of betaine in the thin juice than others but do not differ greatly in the concentration of total nitrogen. Since 50-406 is high in betaine and 50-406BB is not, this might indicate betaine is more closely associated with low sucrose. However, this is not substantiated by the study of the environmental variances and covariances, as less of the environmental variance of percentage sucrose is accounted for by regression of sucrose on betaine than by sucrose on total nitrogen and sucrose on glutamic acid. The data as a whole seem to indicate that the two hybrids capable of producing high sucrose at the higher fertility level do so because the absorption, translocation and metabolic processes are such as to result in different concentrations of total nitrogen and total nitrogenous compounds in the thin juice as compared with the commercial variety and the inbred.

The data in Table 15 provide some information as to why the F_1 hybrid is capable of producing high percentage sucrose at the higher fertility level. Consider first percentage sucrose. The

Table 14.—Means and their standard errors for percentage sucrose, nitrogen, betaine and glutamic acid for A54-1, 50-406BB and 50-406.

Population and treatment	Sucrose	Nitrogen	Betaine	Glutamic acid
	%	mg	mg	mg
A54-1				
Fertilized	16.8±0.10	46.8±1.44	116.6±1.53	80.1±3.98
Non-fertilized	17.9±0.10	18.8±0.91	89.8±1.48	12.8±1.87
50-406BB				
Fertilized	17.3±0.10	33.6±1.32	106.1±1.33	45.8±3.50
Non-fertilized	17.6±0.09	12.6±0.62	69.9±1.40	6.4±0.93
50-406				
Fertilized	16.1±0.09	31.2±0.82	125.3±1.97	17.3±1.35
Non-fertilized	17.4±0.08	14.6±0.59	73.4±1.55	8.0±0.92

fact that 50-406 produced 17.4 percent sucrose on the non-fertilized plots shows that it has the potential for high sucrose production. That is, it has genes capable of conditioning high sucrose under the proper environment. On the other hand 52-307 does not have the genes for conditioning high sucrose but does have the genes that keep total nitrogen, betaine and glutamic acid low in the thin juice at time of harvest. The F_1 hybrid between 50-406 and 52-307 inherits genes for high sucrose production from 50-406 that are at least phenotypically dominant and genes from 52-307 that are phenotypically dominant for low total nitrogen and low betaine in the thin juice. Hence, the absorption, translocation and metabolic processes of the F_1 hybrid result in high percentage of sucrose at both fertility levels.

Table 15.—Means and their standard errors for percentage sucrose, nitrogen, betaine and glutamic acid for the two inbreds and their F_1 hybrid.

Population and treatment	Sucrose	Nitrogen	Betaine	Glutamic acid
	%	mg	mg	mg
50-406				
Fertilized	16.1±0.09	31.2±0.82	125.3±1.97	17.3±1.35
Non-fertilized	17.4±0.08	14.6±0.59	73.4±1.55	8.0±0.92
F_1				
Fertilized	17.6±0.09	21.3±0.58	101.3±1.20	18.0±1.18
Non-fertilized	17.6±0.07	9.8±0.31	51.3±1.14	3.5±0.28
52-307				
Fertilized	16.6±0.09	18.6±0.46	108.7±1.22	10.0±0.50
Non-fertilized	16.5±0.10	11.1±0.28	79.8±1.38	3.0±0.14

The data on the correlation coefficients and the percentage of the variances accounted for by regression show that there are interactions between genotypes and the environments. Further, the studies show that these interactions are such that it should be possible to make decided progress in improving the quality of the beets delivered to the factory through the joint efforts of chemists, soil scientists and plant breeders. Simply stated, sugar

beets can be bred which, if grown under specified and attainable cultural and fertilizer practices, will have the desired chemical constituents to meet the requirements for high processing quality.

Summary and Conclusions

(1) In this study there were three segregating and three non-segregating populations. They were grown at two soil fertility levels and there were 40 replications. The populations were randomized within treatments and replications.

(2) This design of the experiment provided a measure of environmental and genetic variability. There were three measures of the environmental variability: that due to differences between replications; that due to differences between plants in the non-segregating populations; and that due to differences between fertilizer treatments. There are two measures of the genetic variability: that due to differences between plants having different genotypes in the segregating populations; and that due to differences between means of the populations. The variances and covariances of the non-segregating populations furnish an estimate of the environmental variances and covariances of the segregating generations and hence provide a means of estimating the genetic variances and genetic covariances for these generations.

(3) In this experiment the differences between populations, soil fertility levels and replications for the characters percentage sucrose, total nitrogen, betaine and glutamic acid are statistically significant. This can be shown by tests (see Table 2). Such being the case these data should provide information on the interrelation of these characters. Variances, covariances, correlation coefficients and regression are calculated and used to study the interrelations of the characters.

(4) A high proportion of the environmental variance of percentage of sucrose was found to be negatively associated with total nitrogen, betaine, and glutamic acid. This showed that an increase in the applications of nitrogen-containing fertilizers resulted in an increase of total nitrogen, betaine and glutamic acid in the thin juice. Certain concentrations of these nitrogen constituents were accompanied by a decrease in percentage sucrose. The concentrations at which this decrease in percentage sucrose occurred were found to differ with populations. That is, there is a genotype environment interaction.

(5) The study of the genetic variability showed that the relations between percentage sucrose and total nitrogen, betaine and glutamic acid are much less marked. The genotype environment interaction was substantiated. This showed that genotypes differ in the amount of nitrogen constituents in the thin juice

and that this difference between genotypes is not necessarily the same for the two fertility levels.

(6) A study of the environmental variability for total nitrogen, betaine and glutamic acid revealed that all are positively associated and that the relation was closest for total nitrogen and glutamic acid. Glutamic acid and betaine were not nearly so closely associated. It seems that it would not be possible by altering cultural and fertilizer practices to vary total nitrogen much without affecting glutamic acid to some extent and in the same direction.

(7) A study of the genetic variability for the nitrogen constituents showed that total nitrogen, betaine and glutamic acid are positively associated and to nearly the same extent as found for the environmental variability. Again, betaine and glutamic acid are the least closely associated. Hence breeding programs designed to do so should be able to recombine these latter two nitrogenous compounds in different amounts. This finding may have considerable practical application to the breeding of populations of sugar beets for the production of both sugar and monosodium glutamate.

(8) The data indicate that decided increases in percentage sucrose can be obtained on high fertility soils by breeding populations of sugar beets adapted to growing under these conditions. In this study on an average such populations had lower concentrations of nitrogenous constituents in the thin juice. A study of the means showed that the two hybrids were such populations and were capable of producing high percentage sucrose at higher fertility levels.

Literature Cited

(1) FOCHT, ROBERT L., SCHMIDT, FRED H., and DOWLING, BASIL B. 1956. Colorimetric determination of betaine in glutamate process end liquor. J. Agr. and Food Chem. 4: 546-548.

(2) MARIANI, E. 1951. Paper partition chromatographic analysis and its application in sugar manufacture. Industria Saccarifera Italiana 44: 234-244.

(3) MARIANI, E. and TORRACA, G. 1951. Chromatographic investigation of amino acids in the by-products of sugar manufacture. Industria Saccarifera Italiana 44: 245-250.

(4) PAYNE, MERLE G., POWERS, LEROY, and MAAG, GRACE W. 1959. Population genetic studies on the total nitrogen in sugar beets (*Beta vulgaris* L.). J. Am. Soc. Sugar Beet Technol. X (7): 631-646.

(5) POWERS, LEROY, ROBERTSON, D. W., and CLARK, A. G. 1958. Estimation by the partitioning method of numbers and proportions of genetic deviates in certain classes of frequency distributions. J. Am. Soc. Sugar Beet Technol. IX (8): 677-696.

(6) POWERS, LEROY, ROBERTSON, D. W., and REMMENGA, E. E. 1958. Estimation of the environmental variances and testing reliability of residual variances for weight per root in sugar beets. J. Am. Soc. Sugar Beet Technol. IX (8) : 697-708.

(7) POWERS, LEROY, ROBERTSON, D. W., WHITNEY, R. S., and SCHMEHL, W. R. 1958. Population genetic studies with sugar beets (*Beta vulgaris* L.) at different levels of soil fertility. J. Am. Soc. Sugar Beet Tech. IX (8) : 637-676.

(8) PUCHER, G. W. and VICKERY, H. B. 1940. A method for determining glutamine in plant tissues. Ind. Eng. Chem. Anal. Ed. 12: 27-29.

(9) SEIDMAN, M. and BLISH, M. J. 1957. Quantitative determination of L-glutamic acid by L-glutamic acid decarboxylase (from E. *Coli*.). J. Ag. and Food Chem. 5: 448-452.

(10) STARK, J. B., GOODBAN, A. E., and OWENS, H. S. 1951. Paper chromatography of organic acids. Anal. Chem. 23: 413-415.

(11) UMBREIT, W. W., BURRIS, R. H. and STAUFFER, J. F. 1957. Manometric Techniques. Burgess Publishing Company, Minneapolis, Minnesota. Ed. 3, rev. 339pp.

(12) VICKERY, H. B., PUCHER, G. W., CLARK, H. E., CHIBNALL, A. C. and WESTALL, R. G. 1935. The determination of glutamine in the presence of asparagine. Biochem. J. 29: 2710-2720.

(13) WILSON, HILDEGARDE and CANNAN, R. KEITH. 1937. The glutamic acid-pyrolidonecarboxylic acid system. J. Biol. Chem. 119: 309-331.