Selection for Low and High Aspartic Acid and Glutamine in Sugar Beets

R. E. FINKNER, C. W. DOXTATOR, P. C. HANZAS AND R. H. HELMERICK[1]

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In recent years there has been an increasing widespread and copious use of nitrogen fertilizer in sugar beet production. In most cases this has resulted in an increase in beet yield along with low sucrose content. Impurities "or nonsugars" have increased, causing a low extraction of sugar per ton of beets and an increased production of molasses. The increases of amino acids and of other nitrogenous compounds in the beet are the major factors contributing to the reduction of the quality of beet juice for sugar extraction.

The objectives of the present investigations were: To determine if certain amino acids could be increased or decreased by ordinary mass selection; to ascertain if these selections reacted the same under different nitrogen levels of fertility; and to determine how these selections affected other chemical compounds in the beets.

The classical protein and oil selection experiments on corn conducted at Illinois have demonstrated that chemical composition of plants was in part under genetic control (10)[2]. Selections in sugar beets for high and low quantities of chemicals such as sucrose, sodium, potassium, galactinol, raffinose, and purity have been successful as determined by progeny tests. Many investigators (1, 2, 3, 4, 17, 19) 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 or increases in sodium content could be accomplished by mass selection. Wood (17) 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. Later Wood et al. (18) studied the inheritance of raffinose production in sugar beets and reported that the number of effective factor pairs involved in the production of raffinose between the two parents used was about five; at least one was isodirectional and all were equal in magnitude. In the crosses studied neither dominance, nor heterosis, nor linkage appeared to be involved. Quantitatively, the factors for raffinose production in the two parents followed an arithmetic

[1] Manager Research Station, Plant Breeder, Research Chemist and Plant Breeder, respectively, American Crystal Sugar Company, Rocky Ford, Colorado.

[2] Numbers in parentheses refer to literature cited.

scale and consequently were additive. Finkner et al. (4, 6) also studied beets selected for high and low raffinose content. They found through progeny tests that these selections bred true for high or low raffinose content. They also studied these selections under different harvest dates and storage conditions. Again it was found that the high selections remained high and the low selections stayed low.

Powers et al. (14) summarized much of the recent data concerning selection for high and low sodium and raffinose contents. They also reported on selections for thin juice purity and sucrose content. The data for thin juice purity showed that selection for greater purity has resulted in an increase in this character.

Agricultural scientists are aware that the soil has long been recognized as the basis of agricultural production. In the elucidation of the chemodynamics of soil plant complex it has been shown by several investigators on several crops that the addition of fertilizer to the soil can change the chemical composition of plants. Hac et al. (7) and Walker et al. (15) studied the effect of nitrogen fertilizer on the glutamic acid content in sugar beets. They found that the application of nitrogen fertilizer caused an increase in glutamic acid of beets. Walker and Hac (16) observed that as the soil moisture increased under both furrow and sprinkler irrigation, nitrogen fertilizer increasingly stimulated yield and glutamic acid content of beets. Haddock et al. (8) showed that several nitrogen constituents, especially glutamine, increased with nitrogen fertilizer applications. Finkner et al. (5) presented data using three different levels of nitrogen applications and found that the amino acid content of the beets increased as the rates of nitrogen increased. In a study of nine different amino acids and the total amino acid content Finkner et al. (5) showed a significant linear increase response to nitrogen application.

Payne et al. (11, 12) made population genetic studies pertaining to nitrogen compounds in sugar beets and concluded that varieties of beets could be bred which would contain lower amounts of nitrogen constituents even when grown on high fertility soils.

Materials and Methods

The variety used in this experiment was SLC 24, a self-sterile monogerm. A total of 2,272 beets was selected in 1958 by the unit block method similar to that developed by Powers (13). The selection unit block was 35 feet long and 11 feet wide. It differed from Powers' method in that no inbreds or F_1 hybrids were planted to measure the environmental variation.

Each individual beet was weighed, sampled and analyzed for sucrose, aspartic acid and glutamine. The number of roots selected from each unit was recorded and the range and mean calculated. The standard deviation of each unit block was estimated by utilizing the formula, Range/s = mean ratio, a short-cut method described in Snedecor's "Statistical Methods", 5th Edition, Table 2.2.2, page 38.

Based on these estimated standard deviations, selections were made within the unit block for beets which were higher or lower than the block mean for aspartic acid and glutamine. Beets selected for high aspartic acid were at least twice the standard deviation higher than the block mean and the beets selected for low aspartic acid were at least 1.3 times the standard deviation below the block mean. The selection deviation values used for glutamine were 2.3 times the standard deviation for the high selection and 1.1 times the standard deviation below the block means for the low selection. A random selection of approximately every 25th root from the total population was saved and considered the check.

Although sugar and weight were recorded for the individual roots, selections were based on the amount of the two amino acids. The amino acids were determined by a paper chromatographic procedure reported by Hanzas (9). All paper chromatographic determinations are reported as percent on dry substance.

The number of roots selected for each group, the pedigree numbers, the general means for each character studied for each group, and for the entire population, are shown in Table 1.

Table 1.—Means of weight, chemical characteristics and number of roots for five amino acid selections.

Pedigree number	Character	No. beets selected	Individual root data			
			Wt. Lbs.	% Suc.	% Asp. A.*	% Gluta.
59-407	Low Aspartic Acid	59	2.7	11.0	.10	.14
59-408	High Aspartic Acid	81	2.7	11.0	.44	.87
59-409	Low Glutamine	68	2.7	11.4	.15	.14
59-410	High Glutamine	81	2.6	11.3	.39	1.54
59-411	Random Selection	84	2.4	11.1	.22	.43
Entire Population (2,272 Beets)			2.5	10.8	.22	.44

From the above table it will be seen that the high and low selections for aspartic acid and glutamine were greatly different, but in weight and sucrose percents they were similar.

Roots of each of the five groups were space isolated in the spring of 1959 and produced seed that fall. In 1960 the five seed lots were planted in a split plot replicated test at Rocky Ford, Colorado, and at East Grand Forks, Minnesota. In these tests the three main plots were the levels of nitrogen, and the selections were the subplots. The plots were single rows with a commercial variety planted on each side to give uniform competition for each selection. The selections were replicated six times in each test.

Plots were harvested for weight, sucrose and other juice quality characters. Paper chromatography was used to determine amino acids, total amino acid, galactinol[3], and raffinose. Total nitrogen was determined by a modified micro-Kjeldahl nesslerization (11). Sodium and potassium were determined by the flame spectrophotometer. Sugar and purity were analyzed by standard sugar analysis procedures.

Experimental Results

Remarkable differences were obtained in the progeny tests of the five amino acid selections. Very reliable differences between the high and low amino acid selections were obtained for all characters tested at one or the other, or both locations, except for sucrose percent. The results of these progeny tests under different nitrogen levels are shown in Table 2 for the Rocky Ford test and Table 3 for the East Grand Forks test. It should be noted that the levels of nitrogen fertilizer used were different for each test as the soils at East Grand Forks contained much more organic matter than the Rocky Ford soils.

There was a total of four significant variety $\times$ nitrogen interactions in both tests. Only one of these was highly significant; the others were possibly chance deviations. The results indicate that the varieties, in general, reacted similarly in the three different soil nitrogen environments. In the Rocky Ford test the addition of nitrogen had only minor effects on the characters studied, as significant differences between rates were detected for only seven characters from a total of twenty. These differences also were significant only at the five percent level. In the East Grand Forks test the addition of nitrogen had a greater effect on these characters than the test at Rocky Ford as ten significant differences were detected and many of these were highly significant. The addition of nitrogen significantly decreased the percent sucrose and increased the total amino acid content in each test.

Selections for high and low aspartic acid and glutamine contents significantly separated the original populations into distinct groups. In sugar per acre, tonnage, sucrose and purity, the selections gave varying results. The low aspartic acid selection (59-407) increased root yield in both tests, but the stands also

[3] Recent improvements in technique indicate that the galactinol values may be too high.

Table 2.—Stand, yield and chemical results of five amino acid selections planted at Rocky Ford, Colorado, at three different nitrogen fertility levels.

Selection No.	Character	No. Roots per 35'	Lbs. Sugar per acre	Tons Beets per acre	Percent on beet: sucrose	Percent on beet: % K	Percent on beet: % Na.	Pct. purity	Percent on dry substance: Raffinose	Galactinol	Asp. A.[1]	Glut. A.	Aspara	Gluta.	Glycine	G.A.B.A.	Alanine	Valine	Leucines	Total amino acids	Total Nitrogen
59-407	Low asp. A.	54.9	4030	17.02	11.80	.212	.243	82.6	.466	.443	.130	.067	.097	.545	.077	.200	.050	.058	.066	1.29	1.01
59-408	Hi Asp. A.	46.1	3583	14.40	12.53	.206	.190	83.9	.488	.388	.153	.065	.139	.733	.099	.220	.074	.062	.075	1.64	1.06
59-409	Low gluta.	52.0	3814	15.11	12.60	.191	.203	85.7	.422	.395	.115	.055	.073	.488	.072	.198	.047	.049	.055	1.16	0.95
59-410	Hi gluta.	45.2	3811	15.16	12.56	.220	.196	83.6	.488	.410	.165	.063	.152	.838	.114	.243	.082	.076	.097	1.84	1.11
59-411	Random sel.	46.7	3468	14.72	11.83	.229	.223	82.5	.453	.429	.143	.070	.117	.647	.084	.221	.063	.063	.074	1.49	1.03
Sign. diff. (19:1)		5.7	378	1.38	NS	.014	.018	1.5	NS	.039	.016	NS	.017	.095	.013	.023	.011	.009	.009	.14	.06
Sign. diff. (99:1)		7.5	NS	1.84	NS	.018	.024	2.0	NS	NS	.022	NS	.023	.113	.017	.031	.015	.013	.012	.18	.08
Nitrogen Rates																					
0		49.8	3851	15.26	12.64	.199	.196	84.20	.425	.379	.132	.058	.106	.588	.079	.210	.055	.056	.066	1.36	1.00
75		48.6	3797	15.38	12.37	.212	.206	84.28	.474	.424	.137	.060	.113	.643	.093	.215	.066	.060	.070	1.47	1.00
150		48.6	3575	15.20	11.78	.223	.232	82.44	.444	.436	.154	.074	.127	.719	.095	.225	.069	.069	.084	1.62	1.09
Sign. diff. (19:1)		NS	NS	NS	0.50	NS	.019	NS	NS	.042	NS	.011	NS	.097	NS	NS	NS	NS	.012	.17	NS
Sign. diff (99:1)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Variety x nitrogen interaction		NS	NS	NS	NS	NS	*	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Amino acids listed in order:
Aspartic acid
Glutamic acid
Asparagine
Glutamine
Glycine
Gamma amino butyric acid
Alanine
Valine
Leucines

Table 3.—Stand, yield and chemical results of five amino acid selections planted at East Grand Forks, Minnesota, at three different nitrogen fertility levels.

Selection No.	Character	No. Roots per 35'	Lbs. Sugar per acre	Tons per acre	Percent on beet: sucrose	Percent on beet: % K	Percent on beet: % Na.	Pct. purity	Percent on dry substance: Raffinose	Galactinol	Asp. A.[1]	Glut. A.	Aspara.	Gluta.	Glycine	G.A.B.A.	Alanine	Valine	Leucine:	Total amino acids	Total Nitrogen
59-407	Low asp. A.	29.4	5428	17.29	15.86	.134	.073	87.4	.234	.241	.162	.041	.137	0.72	.094	.168	.044	.073	.115	1.54	1.34
59-408	Hi asp. A.	26.4	4620	14.45	15.98	.137	.057	86.9	.203	.198	.187	.042	.200	1.00	.151	.183	.066	.097	.151	2.06	1.51
59-409	Low gluta.	28.4	5118	15.88	16.18	.128	.064	87.2	.220	.223	.174	.037	.132	0.65	.110	.171	.048	.073	.112	1.48	1.33
59-410	Hi gluta.	28.2	4805	15.09	15.90	.139	.063	85.5	.193	.209	.189	.042	.212	1.04	.189	.197	.086	.122	.168	2.22	1.72
59-411	Random sel.	26.6	4647	14.66	15.82	.144	.066	85.8	.230	.207	.161	.053	.188	0.94	.138	.182	.059	.091	.142	1.93	1.49
Sign. diff. (19:1)		NS	467	1.48	NS	.007	.007	1.3	.024	.026	NS	.010	.027	.16	.021	.015	.009	.014	.017	.21	.12
Sign. diff. (99:1)		NS	621	1.98	NS	.009	.009	NS	.032	NS	NS	NS	.036	.22	.028	.020	.012	.019	.022	.28	.16
Nitrogen rates																					
0		27.0	4825	14.86	16.20	.130	.060	87.3	.211	.222	.158	.035	.149	0.87	.115	.176	.054	.077	.126	1.74	1.37
50		28.7	4990	15.51	16.11	.134	.066	86.8	.208	.217	.169	.041	.169	0.80	.131	.177	.056	.088	.132	1.75	1.46
100		27.7	4955	15.96	15.54	.145	.068	85.1	.230	.208	.197	.052	.202	0.94	.163	.188	.071	.108	.154	2.06	1.61
Sign. diff. (19:1)		NS	NS	NS	.54	NS	NS	1.1	NS	NS	.016	.011	.021	NS	.022	NS	.006	.013	.014	.21	NS
Sign. diff. (99:1)		NS	NS	NS	NS	NS	NS	1.5	NS	NS	.022	NS	.030	NS	.031	NS	.009	.019	.019	NS	NS
Variety x nitrogen interaction		NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS

[1] Amino acids listed in order:
Aspartic acid
Glutamic acid
Asparagine
Glutamine
Glycine
Gamma amino butyric acid
Alanine
Valine
Leucines

were at least ten percent higher than the check (59-411) in both tests. This increase in stand may have helped to increase the yield. The low glutamine selection (59-409) with a slight increase in stand, showed only slight increases in yield and sugar per acre, and was not significantly above the check for any of these characters. The low selections were higher in all of these desirable characters than the high amino acid selections. Selecting for high or low aspartic acid or glutamine did not affect the percent sucrose. Purity of juice was significantly improved by low glutamine selection in both tests, and to a lesser degree by the aspartic acid selection.

The raffinose and galactinol contents were changed by the selection for low and high amino acids. In the East Grand Forks test, the high selection of both amino acids significantly decreased the raffinose content. In the Rocky Ford test the selections were not significantly different for raffinose content. The galactinol content was significantly increased in the East Grand Forks test by selecting for low aspartic acid, however in the Rocky Ford test the high selection significantly lowered the galactinol amount. Therefore it appears that selections for high and low aspartic acid decreased and increased the galactinol content, respectively, in these varieties. The glutamine selections were not significantly different from each other for galactinol content but the results for the East Grand Forks test were similar to the trend mentioned above for the aspartic acid selections. In the Rocky Ford test the glutamine selections were not significantly different for galactinol content but the general trend was reversed.

The sodium and potassium results were different for the two amino acid selections. The low glutamine selection reduced the potassium content significantly in both tests, but did not affect the sodium content. The low aspartic acid selection increased the sodium content significantly while the high aspartic acid decreased the sodium content significantly, but did not affect potassium. These results were obtained in both tests.

There were nine amino acids evaluated plus total amino acids and total nitrogen in the progeny tests. The selections for low and high aspactic acid contents shifted all nine amino acids in their respective directions of selection, i.e., all amino acids had a tendency to increase in the high selection and to decrease in the low selection. The same was true for the glutamine selection except that the separations were greater in magnitude. There were slight variations from these results but none reached the significant level. It can be concluded therefore, that selection for a reduction or an increase of these two amino acids, reduced or increased all amino acids. The glutamine selections were more effective than the aspartic acid selections in shifting the populations. Furthermore, total nitrogen in the beet juices of these low selections was significantly lower than the check, while the total nitrogen in the high glutamine selection was significantly higher than the check.

Discussion of Results

It is evident from the data that the aspartic acid and/or glutamine content can be increased or decreased in the root by selection pressure. Such pressure affected the nitrogen metabolism of the plants as there was a general increase or decrease of the total amino acid content and the total nitrogen content, depending on the direction of the selection pressure.

The effects of the selection pressure applied in these tests were not completely confined to the nitrogen-containing compounds. Selection of aspartic acid significantly affected the sodium content while the selection applied to glutamine significantly changed the potassium content. These were the only two mineral elements studied. Shifts in amounts of other elements also probably occurred.

Changes were noted in the carbohydrates studied. The high amino acid selections significantly decreased the raffinose content and a similar trend was apparent for galactinol. Sucrose content remained unchanged.

The general trend was for purities to increase with the lowering of amino acid content. This was to be expected, as a large part of the nonsugars are nitrogenous compounds. Considering all the characters studied in these tests, the selection for low amino acid content was beneficial by increasing sugar yield and juice quality, but not beneficial by causing an increase in raffinose, galactinol and sodium. These three latter constituents have a tendency to lower beet juice quality. This was overbalanced however, by the beneficial effects of the lower amino acid content as reflected in the purity data.

Considering the aspartic acid selections and the glutamine selections, it appears that either one could be used satisfactorily to improve beet varieties, although the glutamine selection was slightly more effective in spreading the populations into separate groups. In a breeding program the selection pressure applied against glutamine would be just as effective as selecting against both aspartic acid and glutamine at the same time. The low glutamine selection also had lesser detrimental effects as it did not significantly change the sodium, raffinose or galactinol content of the beets. On the other hand increases in tonnage and sugar per acre were definitely associated with low aspartic acid selections.

It had been postulated that a decrease in one amino acid might cause an increase in some of the other amino acids of the beet. In these tests, there was no striking evidence of this occurring. If one amino acid was reduced or increased by selection, then all amino acids had a tendency to be reduced or increased.

It would be interesting to know more about the physiology of the sugar beet plant, and the chemical pathways of the many metabolic systems. In this investigation, amino acid selections caused changes in the concentration of some carbohydrates and also of the mineral elements studied. Why and how, in the various metabolic systems, does selection for high or low amino acids affect the carbohydrate physiology or the utilization of minerals? The data presented show that it does happen but additional basic physiological studies are needed to elucidate these interlocking metabolic systems.

The varieties responded to the fertilizer treatments as was expected, i.e., when more nitrogen was applied the increase in amino acids and nitrogenous compounds was greater. If the plant breeder develops varieties which are low in amino acids under moderate levels of nitrogen, these plant breeding advances can be offset by applying excessive amounts of nitrogen. Therefore good agricultural practices must be adhered to for improved varieties to work most efficiently.

Summary

(1) Selection for high and low aspartic acid and glutamine contents of sugar beets caused an increase or a decrease respectively in all nine amino acids, as well as total amino acid content and total nitrogen content. Purity of juice was significantly improved by the low glutamine selection in both tests, and to a lesser degree by the aspartic acid selection.

(2) Low glutamine selection reduced the potassium content significantly in both tests but did not greatly affect the sodium content. The low aspartic acid selection increased the sodium content significantly while the high aspartic acid selection decreased the sodium content, but the different aspartic acid selections had little effect upon potassium. Low aspartic acid selections also increased yield but percent sugar was not significantly affected by an amino acid selection.

(3) Differences were obtained between different nitrogen applications but selection $\times$ nitrogen interactions were not important.

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