Relation of Certain Amino Acids to Other Impurity and Quality Characteristics of Sugarbeet[1]

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In 1966 and 1967 we conducted studies to determine the levels of certain amino acids of sugarbeet with different genetic backgrounds that were exposed to different nitrogen fertility levels. We studied the relationships of these amino acids to several impurity compounds and correlated them with each other and with some yield factors to find information as to where efforts for quality improvement might be most effective.

Sugarbeet quality is a general term intended to describe the relative processing characteristics of beets or the ease and completeness of sucrose recovery from the raw product. Anything that interferes with recovery of white sugar is considered undesirable. The increased use of nitrogen fertilizers in the production of sugarbeet emphasizes the importance of well coordinated chemical-genetic and soils studies as they pertain to processing quality.

Recent emphasis in studying quality has been on thin juice purity and individual impurity components. Currently, thin juice purity in experimental materials is determined on laboratory thin juice by the method developed by Brown and Serro (1)[4] and modified by Carruthers and Oldfield (2). This phosphated thin juice does not differ from factory second carbonation juice with respect to major impurity components. Carruthers and Oldfield (2) report that 70% of thin juice nonsugars are potassium and sodium salts, amino acids and betaine, and that they are not present in equal quantities.

The free amino acids remaining in the thin juice are an important source of nonsugars. Carruthers and Oldfield (2) state that one-half of the nitrogen in purified juice originates as amino

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[4]Numbers in parentheses refer to literature cited.

acids, with 50 to 80% of this from glutamine which has both an amide and amino nitrogen group. Rorabaugh and Norman (11) rank the amino acids second to chloride and carbonate salts in deleterious effect on sucrose crystallization rate. The amino acids in peptides or proteins are almost entirely removed in the juice purification leaving the free amino acids which carry through to the thin juice. These free amino acids are estimated by Silin (12) to be about 0.2% of the total beet (fresh weight basis).

Amino acids have been measured in total and also individually (12) in various beet juices, and 14 have been reported to be in sugarbeet. These individual quantitative determinations have been made by reflectance density of amino acid spots on ninhydrin developed paper chromatograms. A quantitative study of the amino acids in sugarbeet has not previously been made on an amino acid analyzer. One of the purposes of the research leading to this work has been to analyze quantitatively for individual amino acids by using an amino acid analyzer, and to determine the effect of genotype and nitrogen fertilization on the quantity and proportion of the amino acids.

Glutamine accounts for 50 to 80% of all nitrogen originating from amino acids (2). Glutamine is readily changed to pyrrolidone carboxylic acid (PCA) and glutamic acid (to a lesser extent) during juice purification and sucrose crystallization, so that it represents 10 to 17% of nonsugars in the molasses. PCA is particularly important in the later stages of the factory process (2). Since the amino acids are such an important impurity component, it is necessary that their intra-and inter-relationships with other impurity components be established along with the effect of genotype and nitrogen fertility.

The case against glutamine, PCA, and glutamic acid has two facets, since they are not only major contributors to sugar loss into the molasses, but also contribute to processing difficulties caused by deamidation of glutamine. This lowers the buffering capacity of juice and its alkalinity. This is true for anything that increases the ratio of certain nitrogenous compounds to inorganic cations; because with such a ratio, the available alkalinity in the clarified juice is inadequate for achievement of low lime salts, and maintenance of pH in the sugar end unless large amounts of soda ash are added. The addition of soda ash increases molasses.

Plants are able to synthesize 18 amino acids and two amides and to form the constituents common to most proteins. About one hundred other amino and imino acids have limited distributions in high plants, but for the most part are not incorporated

into proteins. Undoubtedly, ammonia forms the major, and possibly the only, inorganic nitrogen compound utilized directly for amino acid biosynthesis. Recent studies, using N^{15} —labeled forms of ammonia, nitrate and elementary nitrogen, have confirmed that nitrogen rapidly enters glutamic acid and glutamine molecules. Aspartic acid, alanine, arginine and other amino acids are more slowly labeled irrespective of the type of inorganic nitrogen nutrient supplied. A kinetic treatment of N^{15}-ammonia incorporation into α-amino groups of free and protein-bound amino acids has established that glutamic acid, and apparently glutamine, form the only primary products of assimilation. Alanine and aspartic acid have been shown to be secondary products of nitrogen incorporation.

Materials and Methods

Our study consisted of laboratory and statistical analyses of 36 yield, quality, and leaf component characters from 12 genetic populations at three nitrogen fertility levels over a two-year period. The experiments were grown under irrigation at the Colorado State University Agronomy Research Center in 1966 and 1967. In both years planting was done April 10-15, and harvesting on October 10-15.

In 1966, three populations were grown in a split-plot design with 10 replications. Thin juice amino acid determinations were made only on the first five replications. In 1967, 10 replications of 11 different genetic populations were grown, two of which were the same ones grown in 1966. In 1967, we determined eight characters on all 11 populations and 28 characters on three populations (Table 1). In both years nitrogen fertility treatments were main plots and populations were subplots.

The experimental areas each year had a uniform application of 20 pounds of actual nitrogen and 100 pounds of phosphorus pentoxide (P_2O_5) prior to fall plowing barley stubble. Nitrogen as ammonium nitrate (NH_4NO_3) was applied the following spring and harrowed into the treatment plots. Nitrogen treatments in 1966 were 0 pounds, 100 pounds (preplant); and 250 pounds (100 pounds, preplant, and 150 pounds, side dressed, after thinning but prior to July 14). The only difference in 1967 was that the middle treament was 125 instead of 100 pounds. The treatment without nitrogen was clearly nitrogen deficient all season; the 100 and 125 pound application of nitrogen in both years resulted in deficiency symptoms just prior to harvest; the 250 pound application showed no deficiency symptoms.

Three populations were grown in 1966, two F_1 hybrids and GW 359-52R (an open pollinated multigerm former commercial variety). One of the F_1's and GW 359-52R were included in the

1967 experiment along with nine other F_1, inbred and special populations.

In both 1966 and 1967, we determined root yield and sucrose content on the roots from which phosphated thin juice was later recovered (at time of purity determination). This thin juice, according to Carruthers and Oldfield (2), is equivalent to the factory second carbonation juice; it receives no further purification and the processor must contend with all remaining juice impurities in the extraction and refining process. We determined impurity components on this thin juice and did a complete analysis

Table 1.—Characters determined and their units.

1966		1967	
Roots		Roots	
Plot weight	kgs/size plot	Plot weight	kgs/size plot
Sucrose	%	Sucrose	%
Recoverable sucrose	kgs	Recoverable sucrose	kgs/size plot
Pressed Juice		Thin Juice	
Conductivity	millimhos/cm[2]	Purity	%
		Potassium	mg/100ml
Thin Juice		Sodium	mg/100ml
Purity	%	Total nitrogen	mg/100ml
Potassium	mg/100ml	Betaine[2]	mg/100ml
Sodium	mg/100ml	NO_3 nitrogen	mg/100ml
Total nitrogen	mg/100ml	Amino nitrogen[2]	mg/100ml
8 amino acids[1] (asp, glu, gly, ala, val, ileu, leu, lys).	μ moles/ml	9 amino acids[2] (asp, glu, gly, ala, val, ileu, leu, tyr, lys).	μ moles/ml
		Leaves (Dried)[2]	
		Copper	mg/100g
		Cobalt	mg/100g
		Calcium	mg/100g
		Magnesium	mg/100g
		Iron	mg/100g
		Nickel	mg/100g
		Leaves (Fresh)[2]	
		11 amino acids (asp, ser, glu, pro, ala, val, ileu, leu, tyr, phe, lys)	μ moles/g
		Fresh root (Pulp)[3]	μ moles/g
		12 amino acids (asp, glu, gly, ala, val, ileu, leu, tyr, phe, lys, his, arg)	

[1] Determinations made on reps 1 through 5.
[2] Determinations made only on populations 1, 2 and 6.
[3] Partial analysis made on 1 to 4 reps of populations 1, 2 and 6.

for the free amino acids using the Technicon Amino Acid Analyzer[5]. In 1967, we prepared samples for amino acid analyses from fresh leaves and roots as soon after collection as possible. We used acid digested dried leaf samples for the determination of several metallic ions. We collected leaf samples just prior to root harvest. We selected the first mature, fully expanded leaves, when going from the inner part of the crown to the outer part. There are usually several leaves of this type on each plant.

In each plot we sampled and pooled one leaf from each of 20 plants which helped compensate for sampling errors which were inherent in the technique. The leaves were carefully stacked, tip to tip and petiole to petiole, and placed in a plastic bag with an identification tag. A transverse center section was removed from each sample for the amino acid analysis. The remaining portions of each sample were dried for metal analyses. A complete list of the determinations made on the different tissues is outlined in Table 1. Table 2 lists the populations used in 1966 and 1967, along with some of the characteristics of the populations.

Table 2.—Populations used in 1966 and 1967 and some of their characteristics.

Year and population		Characteristics
1966		
1.	GW 359-52R	Open pollinated commercial multigerm variety adapted in the Colorado plains; high yield, relatively high sucrose and thin juice purity.
2.	52-305CMS × 54-458,F1	Single cross hybrid; relatively low sucrose and thin juice purity; high thin juice nitrogen.
3.	52-430 × 52-307,F1	Single cross hybrid; relatively low thin juice nitrogen.
1967[1]		
1.	52-430 × 52-307,F1	Same as population 3 in 1966.
2.	52-305CMS × 52-407,F1	Single cross hybrid; low sucrose and purity; high thin juice nitrogen.
3.	52-430 × 54-346,F1	Single cross hybrid; high sucrose and purity.
5.	A59-2	High glutamic acid selection from American Crystal Sugar Co.
6.	GW 359-52R	Same as population 1 in 1966.
7.	52-305CMS	Inbred used in F1's.
8.	52-430	Inbred used in F1's.
9.	52-407	Inbred used in F1's.
10.	54-346	Inbred used in F1's.
11.	34	Inbred; curly top resistant, bolting resistant.
12.	Ovana	Open pollinated white fodder beet.

[1] Population 4 in original planting plan was deleted from study because of poor emergence and stand.

[5]Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable.

The sucrose content was determined using the standard Sachs Le Docte cold digestion method. Recoverable sucrose was calculated using an equation developed by the Great Western Sugar Company (7). Plot weight was determined in 1967 on a single row 19-foot plot bordered on each side by a uniform competitor, in 1966 on second row of 19-foot four row plots. Conductivity was determined on pressed juice by using a solubridge apparatus that measured conductance in millimhos/cm. Purity was determined on phosphated thin juice (2). The total nitrogen determination was based on the method reported by Payne *et al.* (8). Quantitative determinations of betaine were made by a procedure described by Focht *et al.* (3), modified by Payne *et al.* (9).

We determined sodium and potassium on a Baird-Atomic flame photometer. An atomic absorption spectrophotometer (Perkin-Elmer Model 290) was used to determine the other metallic ions in the samples. These metal analyses, with the exception of calcium and magnesium, were carried out according to the procedures outlined in Perkin-Elmer's Analytical Methods for Atomic Absorption Spectrophotometry (1966), as modified by Harrison and Andre (6).

Leaves for amino acid analyses were harvested on a plot basis and the samples were prepared immediately after harvest. A 25 g transverse section cut from the center of the stacked leaves was placed in 100 ml of 10% sulfosalicylic acid solution (w/v) and ground for 5 minutes in a Waring blender. The sulfosalicyclic acid denatured the proteins present in the sample. After grinding, the samples were allowed to stand until the liquid layer separated from the foam.

A 10 ml sample was withdrawn from the liquid layer with a pipette and centrifuged for 10 minutes. This sample was adjusted to a pH of 2.0 with 40% NaOH. The sample could have been analyzed at once or frozen (at -20°C) for later analysis. Frozen samples were warmed to room temperature before being placed on the amino acid analyzer. Any sediment in the sample was removed by centrifugation before being placed on the columns. Depending on the sample, 0.5 to 1.5 ml was used for the analysis. Norleucine was originally used as the internal standard for each chromatogram but our samples contained dihydroxyphenyl alanine (DOPA) which affected the area of the norleucine peak. For an internal standard we used L-α-amino-β-quanidinopropionic acid. This internal standard was made by taking a 2.5 micromole/ml solution of L-α-amino-β-quanidinopropionic acid in 0.01N HCl and adjusting the pH to 2.0. Ten ml of this solution plus 5 ml of 62.5% sucrose were placed in a 25 ml volumetric flask and diluted to volume with 0.01N HCl.

The samples were chromatographed on a two-column Technicon Amino Acid Autoanalyzer. The 140 cm. × 6 mm. columns were filled with Technicon's type B, 8% cross-linked sulfonated resin beads. The column temperature was maintained at 56.6° C. Gradient buffers, ranging in pH from 2.875 to 5.00, were pumped through the columns, along with the sample, under a pressure of 250-300 psi for 21 hours. Before analysis of a sample, three or four duplicate runs were made using 0.2 ml of Technicon's 18 amino acid standard solution plus 0.2 ml of the internal standard on each column. The 18 amino acid standard was diluted from 2.5 micromoles per ml to 1.0 micromole per ml by using 2.0 ml of the standard, 2.0 ml of 0.01N HCl and 1.0 ml of 62.5% sucrose solution. The areas were calculated on the standard curves, and the average area for each amino acid was used to compute the amount of the individual amino acids found in the samples by comparison to the standard areas (13). The internal standard (0.2 ml) was also used on each column with each sample analyzed, so that adjustments could be made for any variation between analyses due to possible changes in the sensitivity of the color producing reagents. Some changes (4, 10) were made in the procedure along with the arranging of the modules so that two 21-hour analysis could be run simultaneously (5).

One of our objectives was to study the individual amino acids in beets of different genetic backgrounds while exposed to different fertility levels. In order to get a measurable amount of some of the other amino acids and keep the glutamic acid curve on the paper, the analyzer worked best under the conditions of concentration and pH described above for a single run. By this procedure we were able to study a primary amino acid product and some secondary amino acid products of nitrogen assimilation.

The autoanalyzer system was modified so that the ninhydrin and hydrazine sulfate were kept separate until they had passed through the proportioning pump. They were completely dissolved in each other in the first mixing coil in discrete units separated by nitrogen. In like manner, the sample was mixed in this solution in a second mixing coil before going through the reaction bath where it was heated to 90°C for approximately 24 minutes for color reaction to take place.

This procedure determined the following amino acids in micromoles per gram of fresh leaf: aspartic acid (asp), threonine (thr), serine (ser), glutamic acid (glu), proline (pro), glycine (gly), alanine (ala), valine (val), isovaline (ival), cystine (cys-S-S-cys), methionine (met), isoleucine (ileu), leucine (leu), tyrosine

(tyr), phenylalanine (phe), ornithine (orn), lysine (lys), histidine (his), tryptophan (try) and arginine (arg). Glutamine and asparagine were also present but not measurable. Unknown amino acids with peaks appearing between alanine and glycine and between ornithine and lysine are being studied. Ammonia was not measured.

A composite root pulp sample was prepared on a plot basis from the individual beets with a rasp. A sample was taken from the crown to the tail of each individual root in the plot, and the composite sample was mixed before taking out a 25 g sample of the pulp which was immediately placed in 100 ml of 10% sulfosalicylic acid solution and ground for 5 minutes. The ground root sample was then treated in the same way as the leaf samples. The stored samples were brought to room temperature and centrifuged before a 1 ml sample was withdrawn and analyzed on the Technicon amino acid analyzer. Micromoles of amino acids per gram of root sample were calculated against standards as before.

The thin juice was prepared according to Carruthers and Oldfield (2). The thin juice was adjusted to a pH of 2 and the samples were frozen until they were put on the amino acid analyzer. A thin juice sample of 0.5 ml was chromatographed along with the internal standard. The results were compared to amino acid standards and reported as micromoles per milliliter.

Results

The experimental results will be presented for each year, and relationships between years will be injected wherever pertinent. Also, discussion necessary to maintenance of continuity will be included with the results.

Year 1966

Means for plot characters and thin juice characters in 1966 along with multiple range tests for differences among all means are shown in Table 3. The hybrid 52-430 × 52-307, F_1 (pop. 3) had relatively low root yield and was apparently unable to benefit from the additional nitrogen in the 250 pound per acre treatment. The high rate was about 150 pounds in excess of the commercially recommended rate. This same hybrid declined in sucrose with increasing nitrogen but attained its highest purity at the 100 pound nitrogen rate. Hence, this genotype had a considerably different nitrogen response from the commercial variety (pop. 1) and the other hybrid (pop. 2). Populations 1 and 2 were rather typical in their nitrogen response. Recoverable sucrose was maximum at 100 pounds of nitrogen. In populations 1 and 2 the additional root yield at 250 pounds of nitrogen was more than offset by lower sucrose and purity.

Among the thin juice impurity components in hybrid population 3, we found that potassium and sodium were not much different from populations 1 and 2. However, the total thin juice nitrogen at 250 pounds of nitrogen per acre was lower than in populations 1 and 2. In fact, total nitrogen did not increase with the additional 150 pounds of nitrogen. This F_1 genotype was not capable of utilizing the additional nitrogen which functioned more as a repressor or inhibitor than growth stimulator. This peculiarity in total thin juice nitrogen of population 3 was partly a reflection of the amino acids in the thin juice. In the case of population 3 there was no significant change in any of the amino acids in going from 100 to 250 pounds of nitrogen. The absence of change may mean that the nitrogen incorporation into the secondary products was deterred or that the process was slowed down. The amount of glutamic acid reported in thin juice is not the amount of glutamic acid in the root, because at the pH of the sample, and with time, some glutamine changes to pyrolidone carboxylic acid (PCA) and, to a lesser extent, to glutamic acid. Also, the change of asparagine to aspartic acid goes on under these conditions to a minor extent. In populations 1 and 2 there was a marked increase in all eight amino acids from both nitrogen increases. This in turn was reflected in the population means where population 3 never had a higher content of any amino acid than the other two populations. Amino acid quantities increased significantly with the increase in nitrogen fertilization. Thus, the individual amino acids left in the thin juice increased with an additional supply of nitrogen but maximized under abundant nitrogen at some point that was genotype dependent.

The eight amino acids listed in Table 3 were those present in the thin juice in measurable quantity and were not occluded on the chromatogram.

In all samples we had difficulty separating the amino acid peaks in the threonine-serine area of the chromatograms when we used the buffer system and temperature necessary to obtain separation of the other amino acids in a single run. The medium sized threonine peak was usually occluded to the larger peak in the serine position. Therefore we could not accurately calculate the amount of threonine present in the sample. The large peak in the serine position often showed two, sometimes three, closely occluded peaks of different sizes. We attempted to identify these occluded peaks and their respective positions by use of known amino acids. As a result we believe them to be serine, asparagine,

Table 3.—Means and tests of differences for plot and thin juice characteristics in 1966

Population &/or nitrogen treatment	Root weight (kg/plot)	Sucrose %	Conduct. (millimhos)	Thin juice purity%	Recov. suc. (kg/plot)	Thin juice K	Thin juice Na (mg/100ml)	Thin juice N
1. CW 359-52R								
0 lbs N	7.070a[1]	18.16a	0.88b	96.880a	1.1925a	64.854b	11.745b	26.709c
100 lbs N	7.735a	17.31b	0.96b	96.080a	1.2257ab	63.706b	14.848b	33.733b
250 lbs N	8.005a	15.56c	1.42a	92.616b	1.0530b	86.895a	32.192a	54.562a
2. 52-305CMS × 54-458,F1								
0 lbs N	6.585b	17.40a	0.75c	96.755a	1.0679a	63.277b	8.841b	25.017c
100 lbs N	7.240ab	16.75a	0.97b	95.551a	1.1015ab	65.531b	13.150b	35.123b
250 lbs N	7.575a	14.96b	1.50a	91.579b	0.9423b	95.818a	27.342a	62.952a
3. 52-480 × 52-307,F1								
0 lbs N	5.135a	17.11a	0.73b	94.429a	0.7767a	56.011b	11.307b	24.259b
100 lbs N	5.615a	16.62a	0.83b	95.522a	0.8502a	61.079b	15.612b	36.064a
250 lbs N	4.770a	14.11b	1.32a	94.084a	0.5961b	73.129a	41.157a	41.792a
Population means								
1	7.603a	17.01a	1.09a	95.194a	1.1587a	71.812a	19.595b	38.335a
2	7.133a	16.37b	1.07a	94.628a	1.0382b	74.042a	16.611c	41.031a
3	5.183b	15.07c	0.95b	94.678a	0.7411c	65.406b	22.695a	34.032b
N Treatment means								
0 lbs	6.263b	17.57a	0.79c	96.021a	1.0121a	61.374b	10.631c	25.322c
100 lbs	6.873a	16.89b	0.92b	95.720a	1.0661a	62.772b	14.547b	34.972b
250 lbs	6.785a[2]	14.89c	1.41a	92.760b	0.8656b	85.114a	33.724a	53.102a

[1] Means followed by the same letter do not differ significantly ($P<0.05$)

Table 3—(Continued)

Population &/or nitrogen treatment	Amino acids in thin juice (μ moles/ml)							
	asp	glu	gly	ala	val	ileu	leu	lys
1. GW 359-52R								
0 lbs N	0.342c	0.760c	0.034c	0.136b	0.078c	0.122b	0.116b	0.022b
100 lbs N	0.512b	1.074b	0.062b	0.246b	0.130b	0.198b	0.204b	0.032b
250 lbs N	0.866a	1.608a	0.112a	0.662a	0.290a	0.430a	0.508a	0.064a
2. 52-305CMS × 54-458,F1								
0 lbs N	0.422b	0.954b	0.048b	0.154a	0.086b	0.136b	0.144b	0.030b
100 lbs N	0.460b	1.088b	0.048b	0.168a	0.114b	0.206b	0.208b	0.034b
250 lbs N	1.082a	2.066a	0.090a	0.280a	0.198a	0.390a	0.538a	0.070a
3. 52-430 × 52-307,F1								
0 lbs N	0.268b	0.894b	0.034a	0.088a	0.056a	0.058b	0.070a	0.022a
100 lbs N	0.468a	1.292a	0.044a	0.154a	0.094a	0.148ab	0.156a	0.032a
250 lbs N	0.554a	1.374a	0.038a	0.150a	0.084a	0.126a	0.142a	0.030a
Population								
1	0.573a	1.147b	0.069a	0.348a	0.166a	0.250a	0.276a	0.039a
2	0.655a	1.369a	0.062a	0.201b	0.133b	0.244a	0.297a	0.045a
3	0.430b	1.187b	0.039b	0.131b	0.078c	0.111b	0.123b	0.028b
N Treatment								
0 lbs	0.344c	0.869c	0.039c	0.126b	0.073c	0.105c	0.110c	0.025b
100 lbs	0.480b	1.151b	0.051b	0.189b	0.113b	0.184b	0.189b	0.033b
250 lbs	0.834a	1.683a	0.080a	0.364a	0.191a	0.315a	0.396a	0.055a

and glutamine. In thin juice and root samples the largest occluded peak seems to be glutamine. We hope with further work on this problem that we can find a method to separate these peaks so that each can be measured accurately. Other amino acids detected in trace quantities were proline, cystine, methionine, tyrosine, phenylalanine, arginine, histidine and tryptophan. Any quantity with an optical density reading on the chromatogram charts of less than 0.020 was considered to be a trace.

Among the eight prevalent measurable amino acids, aspartic and glutamic acids were most abundant and there was roughly twice as much glutamic as aspartic acid. This was as expected because glutamic acid and glutamine are primary products of nitrogen assimilation and aspartic acid and alanine are secondary products of nitrogen assimilation. In populations 1 and 2 at 250 pounds of nitrogen, alanine, valine, isoleucine and leucine were present in quantities which we consider significant in relation to quality; however, this was not true in population 3. In population 3 only glutamic and aspartic acids were present in significant quantities.

It should be remembered that these determinations were made on thin juice which had been purified and the amino acids present would have been primarily free of soluble amino acids. The amino acid content of thin juice is not necessarily a reflection of amino acids in the raw juice or root. Also, free amino acids in an organism tend to be very labile and represent a metabolic pool which may vary considerably, being a function of both environment and genotype. The amino acids in thin juice would be expected to be less labile than in leaf tissue.

Thin juice samples came from roots harvested at different hours of the day, since it usually takes several hours to harvest an experiment. They were sampled at slightly different lengths of time after harvest, since sampling in an experiment also takes several hours. However, the relationships among amino acids and with other characters, as shown by correlations, were quite constant and did not show a lot of random or unaccountable variation. This constancy was also reflected in the analyses of variance by the total absence of population × nitrogen × replication interaction for any of the amino acids.

The difference in amino acids was generally greater due to nitrogen treatment than to population effect, at least for combinations selected. There were increasing amounts of amino acids with increasing nitrogen except in population 3 as pointed out

previously. This increase appeared to be generally nonlinear, depending on both the particular amino acid and the population. There was a significant population × nitrogen interaction for all 8 amino acids, usually due to population 3 at 250 pounds of nitrogen. This represents a differential genetic response to increased nitrogen. The direction of the response in population 3 was desirable, but this appeared to have resulted from the inability of this genotype to utilize higher nitrogen rather than utilizing it in a more efficient and desirable manner.

Simple correlations among individual thin juice amino acids in 1966 were high and significant within populations (52 of 54 r values were significant, the nonsignificant r's were all in pop. 3) particularly within populations 1 and 2. This was probably due to the rather direct response of all amino acids to nitrogen fertilization. The same type correlations within nitrogen treatments were not as high, particularly with glutamic acid. This latter fact was interesting since glutamic acid was the most abundant. This general reduction in correlation was a reflection of the secondary importance of the genotype compared to nitrogen fertility level in influencing the quantity of amino acids.

The simple correlations for all characters within populations and within nitrogen treatments for 1966 were not tabulated, therefore only the information derived from these correlations will be discussed. Correlations within nitrogen treatments within populations were generally not significant, due to only three degrees of freedom for testing r. An examination of relations was made on the 1967 amino acid data where complete data were available on 10 replications instead of only five.

Year 1967

Data from the 1967 experiment were more extensive than those in 1966. Eleven populations were included and eight characters (noted in Table 1) determined on each. On populations 1, 2 and 6 a total of 36 determinations were made, all on 10 replications within the three nitrogen fertility levels. Means for the eight characters are shown in Table 4. Population 1 was the same F_1 hybrid that appeared as population 3 in 1966 and population 6 (GW 359-52R) was the same as population 1 in 1966. Populations 7 through 11 were inbred lines most of which were parents of the F_1 populations 1, 2 and 3. Population 5 (A59-2) was an increase of selections for high glutamic acid from *Beta vulgaris* × *Beta macrocarpa*, F_4. Population 12 (Ovana was a fodder beet developed in Europe from a sugarbeet × fodder beet cross. It would be expected to be of poor quality by sugarbeet standards.

Table 4.—Means and tests of differences for plot and thin juice characters on all 11 populations in 1967.

Pop. &/or N treatment		Suc.	Root weight	Pur.	Rec. suc.	Thin juice			
						K	Na	N	NO_3-N
1.	0# N	15.66a[1]	8.05a	92.48a	1.059a	94.5b	31.2a	49.4b	7.22b
	125# N	15.47a	8.48a	92.28a	1.109a	97.8b	33.8a	60.5b	13.01ab
	250# N	14.24b	8.74a	90.27a	0.998a	126.7a	43.0a	74.4a	17.79a
2.	0# N	15.74a	12.55b	92.92a	1.681ab	121.2b	30.5b	52.3c	12.91b
	125# N	14.96a	15.11a	90.10b	1.795a	133.7b	42.8a	69.6b	17.21ab
	250# N	13.64b	14.68a	89.56b	1.576b	155.4a	48.8a	83.4a	24.47a
3.	0# N	15.78a	10.79ab	93.30a	1.446a	77.3b	28.0b	29.7b	6.46b
	125# N	15.21a	10.00b	93.29a	1.299a	89.7ab	39.3ab	44.6a	14.36a
	250# N	14.16b	12.07a	92.01a	1.434a	102.0a	43.7a	52.2a	16.44a
5.	0# N	14.66a	12.54b	91.66a	1.529a	98.9b	41.2b	54.1c	12.69b
	125# N	14.20a	14.36a	90.20ab	1.615a	111.4b	44.3ab	70.5b	15.57b
	250# N	12.96b	15.66a	88.32b	1.538a	134.8a	55.0a	89.2a	27.12a
6.	0# N	15.45a	13.42a	93.15a	1.782a	97.4c	32.3b	43.5c	9.76c
	125# N	14.33b	14.50a	90.22b	1.666ab	114.1b	52.9a	67.7b	17.19b
	250# N	13.50c	14.24a	89.05b	1.488b	135.5a	61.2a	83.8a	27.60a
7.	0# N	16.35a	4.93a	93.36a	0.690a	98.1c	14.7a	50.5c	5.09b
	125# N	15.62a	5.69a	91.15a	0.740a	117.3b	21.4a	66.3b	11.17ab
	250# N	14.22b	5.56a	88.67b	0.611a	144.7a	19.9a	90.5a	15.65a
8.	0# N	14.89a	6.19a	92.17a	0.758a	98.9c	34.0b	45.8b	7.13b
	125# N	13.54b	5.24a	89.35b	0.559ab	118.3b	47.6a	55.3b	11.84b
	250# N	13.12b	4.64a	87.81b	0.461b	146.3a	45.1ab	71.6a	19.40a
9.	0# N	14.77a	5.81a	92.51a	0.721a	101.4b	30.8b	35.6b	7.52c
	125# N	13.50b	6.86a	89.61b	0.727a	115.0b	56.2a	60.2a	19.91b
	250# N	12.67c	6.10a	88.08b	0.587a	135.6a	57.1a	67.8a	27.89a

[1] Means followed by the same letter, within populations, do not differ significantly ($P<0.05$).

Table 4—(Continued)

Pop. &/or N treatment		Suc.	Root weight	Pur.	Rec. suc.	Thin juice K	Thin juice Na	Thin juice N	Thin juice NO_3-N
10.	0# N	15.67a	4.35a	95.74a	0.609a	73.3b	20.4b	30.1b	3.81b
	125# N	14.92a	4.55a	91.87b	0.564a	80.6ab	32.2ab	36.6ab	6.92ab
	250# N	14.03b	5.04a	93.11b	0.607a	91.5a	43.5a	48.2a	13.75a
11.	0# N	15.88a	7.40b	92.90a	0.984a	85.6b	24.6a	40.7c	5.47b
	125# N	14.89b	9.66a	91.18ab	1.186a	97.5b	33.0a	66.4b	8.95ab
	250# N	14.19b	10.08a	89.36b	1.122a	126.8a	30.4a	86.3a	13.60a
12.	0# N	8.86a	18.66b	83.80a	1.045a	156.9c	84.4b	76.7b	35.83c
	125# N	8.25a	21.60a	81.00b	1.038a	217.6b	115.3a	106.5a	59.18b
	250# N	7.31b	21.84a	77.59c	0.803b	249.8a	121.2a	105.1a	76.26a
Population	1	15.12ab	8.42d	91.68bc	1.055cd	106.3dc	36.0cd	61.4cde	12.68c
	2	14.78bc	14.11b	90.86cd	1.684a	136.8b	40.7bc	68.4bc	18.20b
	3	15.05ab	10.95c	92.87ab	1.393b	89.7f	37.0cd	42.2f	12.42c
	5	13.94dc	14.19b	90.06d	1.561a	115.1cd	46.9b	71.2b	18.46b
	6	14.43cd	14.05b	90.81cd	1.645a	115.7cd	24.4e	65.0bcd	18.19b
	7	15.40a	5.59ef	91.06cd	0.681e	120.0c	9.3f	69.1bc	10.64c
	8	13.85e	5.36ef	89.77d	0.593e	121.2c	42.2bc	57.6de	12.79c
	9	13.65e	6.26c	90.07d	0.679e	117.3c	48.0b	54.6e	18.44b
	10	14.87bc	4.64f	93.58a	0.593e	81.8f	32.0d	38.3f	8.16c
	11	14.99ab	9.04d	91.14cd	1.097c	103.3e	29.3de	64.5bcd	9.34c
	12	8.14f	20.70a	80.80e	0.962d	208.1a	107.0a	96.1a	57.09a
N Trmt.	0# N	14.88a	9.52b	92.18a	1.119a	100.3c	33.8c	46.2c	10.36c
	125# N	14.08b	10.55a	90.02b	1.118a	117.5b	47.2b	64.0b	17.76b
	250# N	13.09c	10.78a	88.53c	1.020b	140.8a	51.7a	77.5a	25.45a

There were significant differences in sucrose content among populations, nitrogen treatments and nitrogen treatments within every population. Hence, without exception sucrose declined with increased nitrogen fertilization. There was no population by nitrogen treatment interaction; sucrose declined at the same rate in all populations.

In the case of root weight there were considerable differences among populations; the fodder beet had high yield while the inbreds had typically low yield. The mean for all populations within the 125 and 250 pound nitrogen treatment was not different, but both were greater than the 0 treatment. So on the average there was little or no yield response beyond 125 pounds of nitrogen. There was a significant population × treatment interaction; not all populations responded in the same manner to increasing nitrogen. This was particularly true of populations 2, 6, 7 and 8. This interaction was of interest because it indicated that there were genotypes which were more capable of utilizing the available nitrogen to increase root yield.

Thin juice apparent purity was significantly different from one population to another; this was of most interest in the hybrid and commercial populations. With increasing nitrogen, purity declined, in the same nonlinear fashion as sucrose content. There was no interaction of population × treatment indicating the dominating effect of nitrogen environment over genotype.

Recoverable sucrose was a function of weight, sucrose and purity, but weight was the dominant factor. Recoverable sucrose is more useful than gross sucrose since extractable sucrose is the economically valuable product. There were significant differences among populations with 2 and 6 producing the highest quantity. Because of the rapid decline in sucrose and purity and less rapid increase in root weight, recoverable sucrose was not different at 0 and 125 pounds of nitrogen, and both were higher than at 250 pounds. Within populations recoverable sucrose at 250 pounds of nitrogen was either the same or less than at 125 pounds. Therefore, 250 pounds of N would represent an economic loss at the levels of available N in the check treatment for this study. The amount of available N initially in the soil is an important factor to consider in determining the rate of fertilizer N to recommend in order to prevent a negative effect on recoverable sucrose. Among the 11 populations there was no interaction of populations and N treatments for recoverable sucrose.

The thin juice is probably the most important plant material for analysis of nonsugars, since this is considered to be the equivalent of second carbonation juice in the factory (2). All nonsu-

crose compounds in this juice persist through the sucrose extraction process and terminate in the molasses. Seven characters, in addition to nine amino acids, were determined on thin juice in 1967. Purity of this juice was mentioned above. Purity is an index or ratio indicating the portion of total soluble solids in the juice which is sucrose.

Potassium is a rather important cation in the thin juice. With respect to potassium content, there were significant differences among nitrogen treatments and among populations. There was also a significant population by nitrogen treatment interaction. Although potassium increased in every population with increased nitrogen fertilization, it did not increase at the same rate. Sodium in the thin juice behaved in the same manner as potassium.

Total nitrogen in the thin juice was significantly different for nitrogen treatments and for populations but their interaction was not significant. Hence, total nitrogen increased the same in all populations with increasing nitrogen fertilization. However, there was considerable difference among populations which represents the potential for developing populations with low total nitrogen along with other desirable characters.

Nitrate (NO_3) nitrogen in the thin juice was the only other character measured on all 11 populations. Nitrate (NO_3) nitrogen represents a form in which nitrogen is commonly translocated. There were significant differences in NO_3 among populations, among nitrogen treatments and among nitrogen treatments within populations (interaction). Averaged across the entire experiment about 6% of the total juice nitrogen was contained in NO_3. This compares with about 4% of the total thin juice nitrogen contained in glutamic acid. The total nitrogen contribution of a compound is not a good measure of its melassigenic power. Rather the effect of the compound on the sucrose crystallization rate and the effect on the pH or buffering capacity of the juice are the factors that determine how an impurity affects the recovery of sugar. It is generally considered that the nitrite-nitrate-nitrogen content of various factory juices changes little and goes through the factory unchanged with only slight elimination.

Means of 28 characters measured on three populations are listed in Table 5. The thin juice and leaf characters were determined on all 10 replications, but the root amino acids were measured on 1 to 4 replications and should be considered less reliable. Multiple range tests have been calculated across N treatments, across populations and across N treatments within populations. Within any group means followed by the same letter do not differ significantly.

Table 5.—Means and tests of differences for thin juice, leaf, and root characters measured on 3 populations in 1967.

Pop. &/or N treatment		Amino N	beta-ine	Thin juice								
				asp	glu	gly	ala	val	ileu	leu	tyr	lys
1.	0# N	14.84b[1]	169.3b	0.995a	1.886b	0.084a	0.175a	0.077a	0.147a	0.163a	0.056a	0.032a
	125# N	18.74ab	180.9b	1.147a	2.159b	0.086a	0.167a	0.089a	0.0202a	0.206a	0.110a	0.026a
	250# N	22.97a	205.2a	1.226a	2.484a	0.085a	0.229a	0.091a	0.202a	0.223a	0.154a	0.031a
2.	0# N	13.08c	168.1b	1.018b	1.551b	0.063b	0.112b	0.059b	0.143b	0.118b	0.086b	0.022b
	125# N	19.45b	180.4ab	1.305ab	1.911a	0.085ab	0.175ab	0.119a	0.216a	0.280a	0.164b	0.039a
	250# N	24.56a	190.4a	1.367a	2.208a	0.106a	0.216a	0.125a	0.301a	0.337a	0.277a	0.041a
6.	0# N	14.52c	122.8b	0.777b	1.312b	0.086b	0.213b	0.121b	0.200c	0.217c	0.115c	0.042b
	125# N	23.72b	133.7ab	1.146a	1.906a	0.115a	0.487a	0.182a	0.315b	0.346b	0.333b	0.052ab
	250# N	29.75a	142.6a	1.423a	2.056a	0.166a	0.572a	0.216a	0.401a	0.450a	0.467a	0.057a
Population												
	1	18.85b	185.1a	1.110a	2.176a	0.085b	0.190b	0.086b	0.184c	0.197b	0.107c	0.030b
	2	19.03b	179.6a	1.230a	1.890b	0.085b	0.168b	0.101b	0.230b	0.255b	0.176b	0.034b
	6	22.66a	133.0b	1.115a	1.758b	0.132a	0.434a	0.173a	0.306a	0.338a	0.305a	0.050a
N Treatment												
	0# N	14.14c	153.4c	0.917c	1.583c	0.078c	0.177c	0.086c	0.163c	0.176c	0.086c	0.032b
	125# N	20.64b	165.0b	1.199b	1.992b	0.105b	0.276b	0.130b	0.254b	0.277b	0.202b	0.039a
	250# N	25.76a	179.4a	1.339a	2.249a	0.119a	0.339a	0.144a	0.302a	0.337a	0.299a	0.043a

[1] Means followed by the same letter do not differ significantly ($P<0.05$).

Table 5—(Continued)

Pop. &/or N treatment		Dried leaf					
		Cu	Co	Ca	Mg	Fe	Ni
1.	0# N	1.897a	0.732a	383.95a	347.08a	23.559a	1.275ab
	125# N	2.150a	0.708a	415.57a	436.12a	26.811a	1.155b
	250# N	2.212a	0.756a	490.29a	498.97a	23.571a	1.305a
2.	0# N	4.260a	0.804a	759.78a	584.59a	25.684a	1.230a
	125# N	1.811b	0.720a	757.46a	688.02a	24.710a	1.245a
	250# N	1.756b	0.768a	710.72a	733.73a	20.921a	1.200a
6.	0# N	2.033a	0.804a	600.63a	464.70a	25.233a	1.200a
	125# N	1.896a	0.732a	527.96a	450.61a	25.013a	1.290a
	250# N	1.711a	0.708a	565.43a	554.59a	29.448a	1.215a
Population							
	1	2.086a	0.732a	429.94c	427.39b	24.647a	1.245a
	2	2.609a	0.764a	742.65a	668.78a	23.772a	1.225a
	6	1.880a	0.748a	564.67b	489.97b	26.565a	1.235a
N Treatment							
	0# N	2.730a	0.780a	581.45a	465.46c	24.825a	1.235a
	125# N	1.952b	0.720b	566.99a	524.92b	25.511a	1.230a
	250# N	1.893b	0.744b	588.81a	595.76a	24.647a	1.240a

Table 5—(Continued)

Pop. &/or N treatment		Fresh leaf										
		asp	ser*	glu	pro	ala	val	ileu	leu	tyr	phe	lys
1.	0# N	0.344c	0.569b	0.328c	0.208c	0.792b	0.129b	0.072c	0.076b	0.072c	0.071b	0.040b
	125# N	0.428b	0.560b	0.376b	0.276b	0.784b	0.144a	0.084b	0.088a	0.080b	0.084a	0.044a
	250# N	0.532a	0.740a	0.412a	0.352a	0.872a	0.140a	0.132a	0.088a	0.108a	0.084a	0.040b
2.	0# N	0.496c	0.468c	0.320b	0.300c	0.852b	0.120c	0.048c	0.072b	0.056b	0.044b	0.044c
	125# N	0.560b	0.548b	0.352a	0.396b	0.872b	0.144b	0.084a	0.088a	0.068a	0.048a	0.058a
	250# N	0.628a	0.600a	0.344a	0.668a	0.932a	0.156a	0.068b	0.088a	0.064a	0.052a	0.048b
6.	0# N	0.356c	0.538c	0.340c	0.164c	0.804c	0.128c	0.064c	0.084b	0.064b	0.051c	0.040b
	125# N	0.476b	0.580b	0.380b	0.236b	0.852b	0.140b	0.072b	0.088a	0.068b	0.062a	0.044a
	250# N	0.612a	0.712a	0.436a	0.296a	0.904a	0.148a	0.112a	0.088a	0.100a	0.056b	0.044a
Population												
	1	0.435c	0.625a	0.372b	0.279b	0.816c	0.138a	0.096a	0.084b	0.087a	0.080a	0.041c
	2	0.561a	0.539b	0.339c	0.455a	0.885a	0.141a	0.067c	0.083b	0.063c	0.048c	0.048a
	6	0.481b	0.612a	0.385a	0.232c	0.853b	0.139a	0.083b	0.087a	0.077b	0.057b	0.043b
N Treatment												
	0# N	0.399c	0.523c	0.329c	0.224c	0.816c	0.125c	0.061c	0.077b	0.064c	0.056b	0.041c
	125# N	0.488b	0.563b	0.369b	0.303b	0.836b	0.143b	0.080b	0.088a	0.072b	0.065a	0.047a
	250# N	0.591a	0.684a	0.397a	0.439a	0.903a	0.148a	0.104a	0.088a	0.091a	0.063a	0.044b

* Probably includes asparagine and glutamine.

Table 5—(Continued)

Pop. &/or N treatment	Fresh Root											
	asp	glu	gly	ala	val	ileu	leu	tyr	phe	lys	his	arg
1. 0# N	1.760	2.480	.040	.280	.160	.240	.280	.040	.040	.120	.080	.160
125# N	1.440	2.120	.040	.200	.160	.200	.240	.040	.040	.080	.040	.120
250# N	2.560	3.520	.080	.360	.200	.320	.404	.240	.080	.160	.080	.320
2. 0# N												
125# N	2.480	2.680	.040	.240	.200	.400	.520	.320		.120	.040	.080
250# N	2.640	3.240	.080	.360	.240	.480	.560	.480	.080	.160	.080	.160
6. 0# N	1.900	2.232	.080	.480	.260	.480	.460	.288	.068	.112	.060	.100
125# N	1.760	2.028	.100	.592	.248	.392	.440	.340	.060	.120	.040	.100
250# N	2.272	2.848	.140	.860	.352	.620	.688	.780	.080	.152	.080	.160
Population												
1	1.920	2.708	.052	.280	.172	.252	.308	.108	.052	.120	.068	.200
2	2.560	2.960	.060	.300	.220	.440	.540	.400	.080	.140	.060	.120
6	1.976	2.368	.108	.644	.288	.472	.528	.468	.068	.128	.060	.120
N Treatment												
0# N	1.872	2.280	.072	.440	.240	.376	.424	.240	.060	.112	.064	.112
125# N	1.828	2.152	.080	.468	.228	.360	.420	.288	.056	.112	.040	.100
250# N	2.380	3.028	.120	.692	.308	.548	.620	.640	.080	.152	.080	.188

Looking at thin juice amino N, there were significant differences due to N treatments both within and across populations and also differences due to population. Differences due to N treatments were greater than those due to populations. The proportion of amino N in the total N went up slightly from 0 to 250 pounds of N, 30.6 to 33.2%. This proportion varies more for populations, 27.8% for population 2 to 34.9% for population 6. Since amino N is one of the most deleterious type of nitrogenous compounds, the proportion of amino N in the total N could be an important consideration. Population 2 at 27.8% would be a more desirable genetic population than 6 at 34.9%.

Betaine was present in quite large quantity in the thin juice. It has low melassigenic power but was present in such large quantity that it became important. There were significant differences in betaine quantity among populations, N treatments and N treatments within populations. Betaine always increased with increasing nitrogen, but the differences due to genotype (populations) were greater than that induced by N treatment. The nitrogen in betaine in this experiment constituted about 39% of the total N at 0 pounds nitrogen down to 30% and 27% for 125 and 250 pounds, respectively. Hence, as available nitrogen increased, proportionately less was found in the form of betaine. This was unfortunate since the other more melassigenic compounds increased proportionately. The proportion that betaine N was of total N was 35%, 31% and 24% for populations 1, 2 and 6, respectively. The proportions were not greatly different from N treatments so one can say that genotype and nitrogen environment had about equal effect on the proportion of nitrogen tied up in betaine. It may be fortunate that such a large part of the thin juice nitrogen was present in betaine since betaine is one of the least noxious, with respect to sucrose recovery, of the nitrogen containing compounds, but unfortunate that the proportion of betaine declined with increased nitrogen fertilization.

The free amino acids present in measurable quantity in the thin juice are listed in Table 5 Glutamic and aspartic acids were the quantitatively important ones. Serine, threonine, asparagine and glutamine (not measurable because of occlusion) were less than glutamic but probably higher than aspartic acid. The other seven amino acids appeared in small quantity but jointly they became important. Seven other amino acids (methionine, phenylalanine, histidine, tryptophan, arginine, proline, ornithine) were present in trace quantities as well as three unknowns, one of which appeared in appreciable quantity.

Almost without exception the amino acids increased quantitatively with increased nitrogen fertilization. The amount of increase was dependent on genotype which led to the significant population by N treatment interaction in six of the nine measured amino acids. Nitrogen treatments caused a significant change in every case while aspartic acid was the only case in which populations showed no significant difference. Hence, the free amino acid content of thin juice was a function of available nitrogen while the rate of change was a function of genotype.

For example, aspartic acid increased by 83% in population 6 in going from 0 to 250 pounds N while in population 1 and 2 it increased only 28% and 34% respectively. Tyrosine was most responsive to increased N; it increased 175%, 222% and 306% in going from 0 to 250 pounds N in populations 1, 2 and 6 respectively. Tyrosine was rather minor in quantity, however. In general, populations 2 and 6 increased more rapidly in amino acids than did population 1, but the latter population was one which responded least to increased N in 1967 and hardly at all in 1966. It was a genotype which lacked the capacity to utilize nitrogen.

Even though one expects the quantity of free amino acids in a plant to be quite labile, those in the thin juice were very consistent and reflected quite distinctly the N treatment and population effect. Apparently the free amino acid content in the root at harvest time was quite stable.

Only limited data are reported for amino acids in fresh root. These amino acid analyses were made from samples of pulp or brei. Unfortunately, very few replications were sampled so that there were no errors or tests of differences for these means in Table 5. Twelve amino acids were present in measurable quantity including phenylalanine, histidine and arginine which were not present in thin juice. Those nine amino acids present in both thin juice and root rank quantitatively in exactly the same order in both sample sources, the only difference being that the amino acids in the root were only 30% to 40% as much per gram of pulp as per milliliter of thin juice. This could be expected since all proteins and insoluble solids that were present in the pulp were removed in the thin juice purification process; the thin juice can be considered more concentrated with respect to free amino acids.

The quantities of the three additional amino acids (phenylalanine, histidine and arginine) were insignificant. All the amino acids in the root responded to N treatment and population in virtually the same way as in thin juice. Again, tyrosine showed

the greatest response to nitrogen. It appeared that either root tissue or thin juice could be used equally well to determine the relative quantity of free amino acids in beet roots.

Leaves were also harvested just prior to root harvest. Samples for amino acid analyses were prepared from fresh leaves immediately after harvest. Dried leaf samples were used for analyses for metallic ions (or metals).

There were 11 free amino acids present in measurable quantity in fresh leaves. In comparison with thin juice, glycine was not present in measurable quantity, but serine, proline and phenylalanine were present and measurable. The quantitative rank of amino acids in fresh leaves was quite different from thin juice or root. Alanine was most abundant followed by serine (possibly with asparagine and glutamine), aspartic acid, glutamic acid, proline and valine in that order. With minor exceptions, the quantity of leaf amino acids increased with increased nitrogen fertilizer. The response was, on the average, slightly less than in thin juice.

There appeared to be little relationship between population rank for leaf and thin juice amino acids. It appeared that the relative quantities of leaf amino acids would not serve well as indicators of root or thin juice amino acids. One would expect free amino acids in the leaf to be a rather dynamic metabolic pool, changing over the season, from light to darkness, and even from hour to hour. No direct measure of this quality was made; but relatively large error mean squares for all the leaf amino acids indicated that there was more plot to plot variability than for thin juice amino acids.

The dried portions of the leaf samples were analyzed for copper, cobalt, calcium, iron, nickel and magnesium. There were no meaningful or consistent differences in quantity of these elements due either to N treatments or populations. These elements in the leaves did not appear to provide any useful information relative to quality and genotype performance.

In order to establish the relationships of 36 characters for which an orthogonal set was available, simple correlations of all 36 were calculated. Also, correlations of the 8 characters measured on 11 populations were calculated but not tabulated. Among the 8 characters across all 11 populations it was interesting to note that nitrate-nitrogen (NO_3-N) in the thin juice was more highly correlated with sucrose, root weight, purity, potassium and sodium than was total nitrogen in thin juice. These same correlations within populations across N treatments were about the same for NO_3-N and total N, except for population 12 which was the fodder beet. In this population NO_3-N was much more

closely related to the other six characters than was total N. This was particularly true for sucrose, root yield, purity and recoverable sugar. This must indicate that many times some of the nitrogen included in total N must be in a form which is not so detrimental to sucrose accumulation and purity.

Simple correlation coefficients gave some idea of the effect of nitrogen fertilizer on relationships of various characters across a fairly large sample of genotypes. Root weight was negatively correlated with sucrose to about the same degree in all N treatments (-0.61**, -0.56**, and -0.60**). The same was true for sucrose with purity (0.76**, 0.82**, and 0.83**) and weight with purity (-0.58**, -0.54**, and -0.50**). Recoverable sucrose was related to weight at 0, 125, and 250 pounds N (0.69**, 0.66**, and 0.62**). It was related to purity only at 250 pounds N (0.24*) and was related to sucrose only at 125 and 250 pounds of N (0.20* and 0.22*).

Thin juice K maintains the same correlations with other characters across all N treatments. It was not correlated with recoverable sucrose, nor were sodium, total N and NO_3-N correlated with recoverable sucrose at any of the N levels. Correlations of Na with the other characters were not greatly changed by N treatment. Correlations of total thin juice nitrogen with weight, sucrose, and K were highest at 125 pounds N, but correlations with purity and Na declined with increased nitrogen fertilizer. Nitrate-nitrogen in the thin juice was, with minor exception, most highly correlated with all other characters at all N treatments.

The correlations of NO_3-N with the other seven characters did not change with change in N treatment, except NO_3-N with total N where the correlation was greatly reduced at 250 pounds of N, from 0.82** and 0.83** at 0 and 125 pounds of N to 0.44** at 250 pounds. At this level of excess available nitrogen, NO_3-N was no longer a strong indicator of the nitrogen balance and relationship of nitrogenous compounds in the plant. It was of some concern that recoverable sugar was so poorly correlated with thin juice potassium, sodium, total N and NO_3-N, since net sugar was the character of ultimate commercial interest.

A few of these correlations were significant within populations, particularly populations 6 and 12 (data not shown). Correlations within N treatments within populations were calculated but had only 8 degrees of freedom for testing. Within the populations of greatest interest there was little difference in correlations from within population and within N treatment. Hence, the correlations were little affected by population × N treatment interaction.

Correlations of all 36 characters which were determined only on populations 1, 2 and 6 were calculated, but not tabulated within the entire experiment, within populations within N treatments, and within N treatments within populations.

Since correlations among the first eight characters already have been discussed over all 11 populations, only their relationship with the 28 additional characters will now be discussed. Sucrose was negatively correlated with all amino acids in both the thin juice and fresh leaves Among these correlations, those with thin juice amino acids were generally higher than those with leaf amino acids, except for aspartic and glutamic acids. This was of interest since aspartic and glutamic were the most abundant amino acids in the thin juice. Sucrose content was not significantly correlated with betaine, leaf metals and leaf phenylalanine and lysine.

Root yield correlations across all three populations and the three N treatments were positive and significant with all thin juice impurities except betaine, glutamic acid and lysine. Root yield correlations with leaf metals did not appear to be important; with leaf amino acids they were generally weaker than with thin juice amino acids. Correlations of thin juice purity with thin juice amino acids, other nonsugars and leaf amino acids were consistently significant and negative except for leaf valine, phenylalanine and lysine. Only magnesium among the leaf metals was correlated with purity (-.30**).

Recoverable sucrose was noticeable by its lack of correlation with most of the major juice impurities as well as sucrose and purity. Recoverable sucrose was largely determined by root yield, hence, tends to be related to other characters much like root yield was, only weaker. Thin juice potassium was positively correlated with most characters except the leaf metals, sucrose, and purity. Sodium followed the pattern of potassium quite closely even though it was correlated only 0.53**. Amino N in the thin juice was most highly correlated with thin juice nitrogen, sodium, isoleucine, leucine and NO_3-N in that order. It was correlated with all leaf amino acids except lysine, but not as highly as with the thin juice amino acids. Nitrate-nitrogen was more highly related with weight, as well as thin juice potassium, sodium, alanine, valine, tyrosine and lysine than was total N, althought it was less highly correlated than amino N in most cases.

It appeared that thin juice amino N was generally a better determinant of root yield, sucrose, purity and recoverable sucrose than was thin juice total N and NO_3-N. However, it was still only weakly related to recoverable sucrose. Betaine, although generally present in large quantity in thin juice, was not very

highly related to any of the characters in the study. The most important measurable thin juice amino acids quantitatively were aspartic and glutamic acids. Those of minor importance were alanine, isoleucine, leucine and tyrosine, while glycine, valine and lysine were present in very small quantity, even with 250 pounds of N. Glutamine, serine and threonine are likely important, but were not measurable under conditions of this experiment.

Comparing aspartic and glutamic acids, glutamic acid had the highest correlation with recoverable sucrose and betaine while aspartic acid was most correlated with root weight, purity, potassium, sodium, total N, glycine, alanine, valine, isoleucine, leucine, tyrosine and lysine. Thin juice aspartic and glutamic acids were not generally as highly correlated with the leaf amino acids; of the two, glutamic generally had the highest correlation.

When these same correlations were calculated within populations some differences were noted. Namely, the correlations of aspartic and glutamic acids were generally weakest for population 1 and strongest for 6. Also, glutamic acid had in general higher correlations with all variables up through 18 than did aspartic acid. Hence, use of one amino acid as an indicator for other characters may be applicable only within specific populations. These same type correlations within N treatments showed that aspartic and glutamic acids were equally related with weight, sucrose, purity, recoverable sucrose, potassium, sodium, total N, amino N and betaine, but the relationships became weaker with increasing nitrogen. With the other thin juice amino acids, aspartic was more highly correlated than glutamic. These correlations were also reduced with increasing nitrogen.

In dried leaf samples the metals copper, cobalt, calcium, magnesium, iron and nickel were present in greatly different quantities. Calcium and magnesium were abundant relative to copper, cobalt, iron and nickel. There were very few significant correlations of these metals with any of the other characters.

The amino acids in fresh leaves at harvest had quite frequent correlations with other characters. The two most conspicuous observations about their correlations were that the frequency of significant correlations was sharply reduced with increasing N fertilization, and the quantity of a particular amino acid in the leaf was rarely correlated with the quantity of the same amino acid in the thin juice.

Discussion

The response of population 3 (an F_1 hybrid) in 1966 to increasing nitrogen fertilizer was somewhat unusual. It represent-

ed a marked genotype difference from populations 1 and 2 which seemed to respond rather typically. With increasing fertilizer N from adequate (100 lbs) to excess (250 lbs) the purified thin juice extracted from the roots of population 3 did not increase significantly in total N or any of the amino acids. This was a desirable genotype response with respect to quality, but it was accompanied by an unacceptably low root yield. Apparently, the individual amino acids in the thin juice quantitatively maximize at some point with increasing fertilizer nitrogen. If a high performing genotype had a low maximizing point this would be a very desirable characteristic. However, it would seem physiologically unlikely that this maximization point would be sufficiently low in high yielding genotypes. There may also be a year or location interaction with genotype in this case. An interaction with years was indicated in 1967 when this same F_1 hybrid did not maximize as soon or as decisively with increasing N fertilizer as in 1966.

From the sample of four genotypes analyzed for thin juice amino acids (1966 and 1967) it would appear that all amino acids present in measurable quantity increased with increasing available soil nitrogen to some maximum or plateau which was genotype dependent. The three populations with acceptable yield characteristics did not reach this point even with 250 pounds of fertilizer N. The increase in quantities of amino acids was not linear, however. They appeared to increase more between 0 and 100 or 125 pounds than between 100 or 125 and 250 pounds of nitrogen.

The rate of increase was also different for the different amino acids. For instance, in population 6, 1967, aspartic acid increased proportionately more than glutamic acid but the opposite was true for population 1. For population 2 the increase rates of aspartic and glutamic acids were about the same.

There was considerable difference in thin juice amino acids due to year. Part of this difference might have been due to differences in residual nitrogen in the fields in which the 1966 and 1967 experiments were grown, but it was very doubtful that most of the year difference can be attributed to this source.

The most abundant measurable amino acid in the thin juice was glutamic acid followed by aspartic. Threonine, serine, and probably asparagine and glutamine were present, but were occluded, and therefore not measurable under conditions of this experiment. All of the others were present in minor quantity, but glutamic acid, alanine, valine, isoleucine, leucine, tyrosine and lysine did contribute 2.83% of the total thin juice N in 1967 compared to 2.58% for aspartic acid and 4.34% for glutamic

acid; that was the average for all three populations over all N treatments.

Although the study was not designed to quantitatively analyze the thin juice for all nitrogenous compounds, some comparisons can be made. As fertilizer N was increased, the proportion of total thin juice N (accounted for by the amino acids, amino N and betaine) declined, while that of NO_3-N increased. From 65 to 71% of total N was accounted for within populations and 68 to 75% within N treatments. There remained unmeasured specific nitrogenous compounds which accounted for 25% or more of the total N. This seems quite high unless there was considerable unmeasured glutamine, asparagine, threonine, serine, pyrolidine carboxylic acid (PCA), and ammonia in the thin juice. Purines, pyrimidines and nucleosides probably make minor contributions to total N. No complete nitrogen analysis of this type laboratory thin juice has ever been published. Probably no analysis has ever been undertaken; but one should be, since it appears that this phosphated thin juice is being used increasingly in beet quality evaluation.

The differences in thin juice amino acid content due to N treatment were generally greater than that due to genotype. From the limited sample of genotypes one would conclude that amino acid content can be shifted and regulated much more by nitrogen culture than by genotype. The dominance of nitrogen environment over genotype was also evident in the sucrose content and thin juice purity in 1966 and 1967. In this study it appeared that genotype could do relatively little to overcome the effects of nitrogen on sucrose content and the various nitrogenous impurities. However, in previous work, two hybrids were found capable of producing high sucrose with low concentrations of total nitrogen in the thin juice the low total nitrogen appears not to be caused by amino acids.

There were no populations among the four analyzed for amino acids which demonstrated the possibility of finding or developing a high performing genotype with low thin juice amino acids. A poor performing genotype (population 3 in 1966 and 1 in 1967) did have lower thin juice amino acids and small response to increasing N fertilizer, but this was probably part of the reason for its poor performance.

Other thin juice impurities were quite deleterious but none so much as amino nitrogen compounds. Carruthers and Oldfield (2) rate potassium as 0.25 as deleterious. Potassium increased with increased nitrogen fertilization but at different rates in difference populations. Also, the increase of potassium was not linear in relation to N fertilizer

Sodium, which is considered by Carruthers and Oldfield (2) to be more detrimental than potassium, was rated by them as 0.35 as melassigenic as amino N. The anions with which potassium and sodium are associated are perhaps quite important. The chloride determinations in this study have not been analyzed at this time. Carbonates were very labile and difficult to measure quantitatively. It would seem that these anions plus the sulfates may all be quite melassigenic and warrant further study. On the other hand they may necessarily be associated with potassium and sodium, so that a measure of potassium and sodium would provide as much quality information as a measure of both cations and anions. Potassium and sodium are easy to measure quantitatively relative to chlorides and particularly carbonates. The individual cation and anion relationships need to be studied before the anion content can be ignored as it has been in the past.

Betaine was the one nitrogenous compound measured whose quantity was affected more by genotype than by N fertilization. This was encouraging from the standpoint of breeding, since relatively low betaine genotypes in all nitrogen environments should be synthesizable. It was not apparent from this study that a betaine reduction was compensated for by any other melassigenic compound. It is a trimethyl product of glycine. It would be a valuable by-product of beet sugar refining if a commercial use for it were found.

Comparisons of free amino acids in the root tissue and thin juice indicated that either all free amino acids were retained in the thin juice process or the individual amino acids were eliminated in equal proportions. The amino acids ranked the same quantitatively in root and thin juice. This was not true of free amino acids in the leaves. Alanine and serine were most abundant in fresh leaf but were minor in both the root and thin juice. From this study it appeared that relative quantities of leaf amino acids would be of little value as indicators of root and thin juice amino acids, root quality or sucrose yield.

Since several of the metals measured in the leaves are present in some of the essential enzymes, we hoped that they might be related in some way to quality or yield characters. However, no useful relationships were found. As a matter of fact, very few significant correlations of any type were found in relation to these metals. From this study it appeared that quantities of copper, cobalt, calcium, magnesium, iron and nickel in the leaves at harvest were of little value in determining beet quality or sucrose yield.

Nitrate-nitrogen in the thin juice seemed to be of more importance than has been reported previously. Nitrate-nitrogen

was most highly correlated with the yield characters, potassium and sodium. This occurred in spite of the fact that it accounts for only 3.5 to 7.6% of total N in sugarbeet. This compared with much higher values for amino N and betaine. In the fodder beet NO_3-N accounted for more than twice as much of the total N, 13.4.%. Without exception the proportion of total N contributed by NO_3-N increased with increased N fertilization. This was the opposite of betaine and most of the amino acids.

We saw that the nitrogenous compounds make varying contributions to total N in the thin juice dependent on the nitrogen status of the soil and the genotype. Averaged over the three populations studied, amino N and NO_3-N increased in proportion to the total thin juice N while betaine, aspartic and glutamic acids decreased. A sizable portion of the total N and amino N was unaccounted for by the nitrogen-containing compounds analyzed.

In the thin juice it appeared that aspartic and glutamic are the only measurable amino acids worthy of much consideration although glutamine, asparagine, serine and threonine should also be measured. The other seven present in the thin juice would be important if considered as a group, but they were affected somewhat differently by genotype and N fertilizer. Hence, they did not respond as a group and would have to be treated individually with respect to fertilization and selection.

Summary

The quantity of some individual amino acids was determined in sugarbeet of different genetic backgrounds and at different nitrogen fertility levels. The relationships of impurity components with each other and with yield factors was determined to obtain new information about where efforts for quality improvement might be most effective.

The study consisted of laboratory and statistical analyses of 36 yield, quality, and leaf component characters from 12 genetic populations, at three nitrogen fertility levels, over 2 years. Only two populations were common to both years, and not all populations were analyzed for all characters.

The data of both years and of four genotypes showed that those measurable amino acids in the thin juice (equivalent to factory second carbonation juice) increased with increasing available soil nitrogen. There was an indication that they may reach some maximum or plateau which was genotype dependent. Every amino acid was present in larger quantity in 1967, which might be explained by a year interaction or by residual soil nitrogen. The most abundant amino acids in thin juice were glutamic acid and then aspartic acid, although asparagine, glutamine, PCA, serine and threonine may have been abundant but were not

measurable under conditions of this experiment. Glutamic and aspartic acids were the primary products of nitrogen assimilation.

The study was not designed to analyze the thin juice quantitatively for all nitrogenous compounds. Some comparisons can, however, be made:

1. The differences in thin juice amino acid content due to nitrogen treatment were greater than that due to genotype, indicating that amino acid content can be shifted more by nitrogen culture than by genotypes.
2. It appeared from limited evidence that genotype could do little to overcome the detrimental effects of excess nitrogen fertilization.
3. As nitrogen treatment was increased, the proportion of total thin juice nitrogen accounted for by most of the amino acids, amino nitrogen and betaine declined while that of nitrate-nitrogen increased.
4. From 65 to 71% of nitrogen was accounted for within populations and 68 to 75% within nitrogen treatments
5. Unmeasured specific nitrogenous compounds accounted for 25% or more of the total nitrogen. This seems high unless there was decomposition of glutamine and asparagine.

There was no high performing genotype (high weight sucrose) with low thin juice amino acids. In previous work we found two genotypes that had low total nitrogen in the thin juice at harvest. This low total nitrogen appeared not to be caused by amino acids.

The amino acids ranked the same quantitatively in roots and thin juice. This was not true of amino acids in the leaves. There appeared to be little useful relationship of leaf amino acids and quality or yield characters.

Nitrate-nitrogen in thin juice seemed to be of more importance than has been reported previously. It was highly correlated with yield characters, K and Na. It was apparent that the nitrogenous compounds measured make varying contributions to total nitrogen in the thin juice, dependent on the nitrogen status of the soil and genotype. There was a portion of the total nitrogen and amino nitrogen which was unaccounted for.

Sodium and potassium increased in every population with increased nitrogen fertilization, but at different rates in different populations. Betaine was the one nitrogenous compound whose quantity was affected more by genotype than by nitrogen fertilization. Genotypes with low betaine should be synthesizable. Quantitites of copper, cobalt, calcium, magnesium, iron and nickel in the leaves at harvest were of little value in determining beet quality or sucrose yield.

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