Sand Culture Experiments With Sugar Beets

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Glutamine is the probable precursor of glutamic acid in sugar beets, and the amount present in the beet is dependent largely upon the available soil nitrogen. It has been possible to measure the effects of various amounts of nitrogen fertilizer upon the glutamic acid content of beets grown in the field and to obtain a fairly accurate evaluation of this response (1, 2).[2] However, due to the extreme variability found in the glutamic acid of individual beets grown in the same field, prohibitive numbers of beets are required for exact quantitative measurements. A substantial reduction in this variation was sought in order that reasonably sized experiments could be undertaken to study factors altering the response to fertilizers.

Beets grown under controlled nutrient conditions seemed the best approach to this problem. Preliminary experiments with growth in pots of hand mixed soil with limiting supplies of nutrient or growth in solution culture held little promise. Growth in sand with complete chemical nutrient solutions have been highly satisfactory, however, and the results of a two-year study with this type of culture will be reported here. In these studies, the nutrient environment was maintained as constant as possible by frequent applications of freshly prepared solutions used *in* sufficient amounts at each application to flush out any old nutrient present. This procedure should also limit any effect of nitrifying bacteria on the nutrient.

The purpose of the first year's study, which was carried out with commercial seed, variety U. S. No. 33, was to determine the effect of a constant nutritional environment upon sugar beets grown in pots of sand outdoors in full sunlight during the regular beet growing season in California. The same nutrient solutions were used during the entire growth period. The effects of varying amounts and concentrations of nitrogen upon weight, sugar and glutamic acid were determined.

The second year, seed from two strongly contrasting inbred lines of beets was used instead of a commercial variety. The experiment was set up in a factorial design to study the effects of: 1, two levels of nitrogen; 2, ammonia vs. nitrate; and 3, high sodium vs. high potash treatments. At harvest, individual root and top weights were obtained and analyses were carried out for sugar, glutamic acid (1), Na, K (3), Ca (4), Mg (5), Mn (6), and soluble inorganic salts.

Procedure

The beets were grown in 12-inch unglazed flower pots containing 34 pounds of 20-mesh sand. The sand used retained about 2,000 ml. of nutrient and 1,900 cc. of free air space after 20 minutes drainage. Regular applications of 4.5 liters of freshly prepared nutrient solution were carried out during the entire growth period by flooding .the sand surface. Each of the

[1] Research Division International Minerals & Chemical Corporation, Woodland, California.
[2] Numbers in parentheses refer to literature cited.

various nutrients used was prepared by mixing one volume of each of three stock solutions with 1,200 volumes of well water. The required amounts of each stock solution were measured out accurately well in advance of actual use.

The stock solutions were prepared to contain the desired amount of nutrient in a minimum volume of stable solution. Their compositions are shown in Tables 1 and 2.

***Solution** 1* contains sufficient sulfuric acid to produce a pH of 6.1 in the final dilute nutrient, all of the sodium over 5.2 mM in the final nutrient, and the trace heavy metals and magnesium. The well water used in these experiments provided sufficient magnesium.

Solution 2 provides a large excess of calcium and chloride so that alterations may be made in ammonium and nitrate without great effect on the former nutrients.

***Solution** 3* provides high levels of phosphate, potassium or sodium, and the trace anions. The final nutrients are saturated with iron phosphate and supersaturated with dicalcium phosphate. Their composition in regard to individual nutrients is shown in Table 2, together with the known constituents of the well water used in these experiments.

The seeds were planted 6 to 8 per pot about May first and thinned gradually to one beet per pot about 7 weeks after planting. Germination was excellent, but some trouble was encountered due to seedling disease and to burning of the young leaves at the sand surface during the hot weather. Harvests were made the middle of November.

1948 Experiment

This introductory experiment, to study sand culture techniques and to determine the effects of both amount of nitrogen in the nutrient solutions and rate of nutrient application, tested the following four treaments:

Treatment	Nutrient	Applications per day	Conc, of N mM/L	Amt. of N Est. mM/day	Number of Beets Treated
1	I	2	1	4	7
2	II	2	2	8	7
3	II	1	2	4	6
4	III	1	4	8	6

At the alternate times when treatments 3 and 4 were not applied, sufficient well water adjusted to pH 6.1 with sulfuric acid was used to barely saturate the sand. Watering or nutrient application at least twice daily was found to be necessary to prevent wilting.

*Effects of **Amount** of **Nitrogen:*** At both levels of nitrogen used, the young beets (6 weeks after planting) showed symptoms of nitrogen deficiency. They contained only about 0.1% glutamic acid, the leaves were yellowish, and significant differences in leaf length were evident between the beets grown at the two amounts of nitrogen furnished. The difference disappeared during the subsequent rapid growth period, and the leaves slowly became dark green and remained green.

At harvest, corings of each beet were analyzed for sucrose and glutamic acid (1). The only significant results obtained were those assignable to

Table 1.—Stock Solutions[1]

Nutrient	1948 1	1948 2	1948 3	1948 X	1949 A	1949 B	1949 C	1949 D	1949 E	1949 F	1949 G	1949 H
Solution 1												
H_2SO_4	191.5	191.5	191.5	191.5	191.5	191.5	131.0	131.0	131.0	131.0	191.5	191.5
Metals[2]	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
$Na_2SO_4.10H_2O$	0	0	0	0	0	0	348	348	348	348	0	0
Solution 2												
$CaCl_2.2H_2O$	529	529	529[4]	529	529	529	529	529	529	529	529[4]	529
NH_4Cl	0	0	0	0	0	48.2	48.2	0	0	96.3	96.3	0
$Ca(NO_3)_2.4H_2O$	0	0	0	850	70.8	0	0	70.8	141.7	0	0	141.7
NH_4NO_3	50.1	100.3	200.6	200.6	0	0	0	0	0	0	0	0
Solution 3												
$K_2HPO_4.3H_2O$	822	822	822	822	822	822	0	0	0	0	822	822
KH_2PO_4	163	163	163	163	163	163	0	0	0	0	163	163
$NaH_2PO_4.H_2O$	0	0	0	0	0	0	464	464	464	464	0	0
$Na_2HPO_4.12H_2O$	0	0	0	0	0	0	516	516	516	516	0	0
H_3BO_3	2.22	2.22	2.22	2.22	2.22	2.22	2.22	2.22	2.22	2.22	2.22	2.22
$(NH_4)_6Mo_7O_{24}.4H_2O$	0	0	0	.022	.022	.022	.022	.022	.022	.022	.022	.022

[1] H_2SO_4 and Metals in ml/L, all others in gm/L

[2] Metals: $FeSO_4$ 54.8, $AlCl_3.6H_2O$ 32.2, ZnO .448, $CuCl_2.2H_2O$ 1.94, $MnSO_4.2H_2O$ 27.6 gm/L and H_2SO_4 10 ml/L

[3] This Solution saturated at 15°C

[4] This Solution saturated at 45°C

amount of nitrogen furnished as shown as shown in the following table:

Amount of N mM per day	Root Wt. Lbs.	Top Wt. Lbs.	Sucrose Percent	Glutamic Acid Percent
4	4.09	1.20	15.4	0.50
8	5.15	1.52	13.5	0.72
L.S.D. 5%	**1.02**	(0.44)	1.2	**0.15**

Effect** of **Rate of Application** of **N: Rate of nutrient application had no appreciable effect *in* itself or in altering the response to amount of nitrogen available. Twice daily application appeared to be preferable; the results were more uniform, and the time required for application of nutrient was actually less than that necessary for careful watering. The quality of the beets obtained indicates that a substantial fraction of all nitrogen available was absorbed by the beets.

Table 2.—Concentration of Nutrients

Millimols per Liter of Final Solutions

Nutrient	1	2	3	X	A	B	C	D	E	F	G	H	Well water
Ca	3	3	3	3	3.25	3	3	3.25	3.5	3	3	3.5	1.5
Cl	6	6	6	0	6	7	7	6	6	8	8	6	2.6
NH_4	0.5	1	2	2	0	.75	.75	0	0	1.5	1.5	0	
NO_3	0.5	1	2	8	.5	0	0	.5	1	0	0	1	
K	7	7	7	7	7	7	0	0	0	0	7	7	
Na	0	0	0	0	0	0	7	7	7	7	0	0	
SO_4	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	0.6
PO_4	4	4	4	4	4	4	4	4	4	4	4	4	
Mg	0	0	0	0	0	0	0	0	0	0	0	0	1.3

Parts per million Final Solution

Fe	Al	Mn	Cu	Zn	B	Mo
.5	**.1**	.25	.02	.01	**.3**	.01

Comparison with Field Grown Beets: The large root weight and extraordinary glutamic acid content of these beets grown in sand culture indicates that except during very early growth even the lower amount of nitrogen furnished was much greater than that which would produce the average type of sugar beet in the field. On the other hand, sucrose was very high for such high nitrogen beets, indicating that the primary difference from field-grown beets was in improvement of the storage potential (2) rather than in the amount of available nitrogen.

In further contrast to field-grown beets, those beets growing at a given and constant level of nitrogen showed a considerable negative correlation (r = —0.474) between glutamic acid and root weight, which provides strong direct evidence that glutamine acts as storage nitrogen. The magnitude of the response is such that a 10% increase in size under constant nitrogen nutrient results in a *decrease* of 0.032% glutamic acid, or 5% of the average glutamic acid. This variation in size reflects a faster growth of the beets chiefly attributable to variations in vigor. Such growth involves protein formation at the expense of the stored glutamine.

On the other hand, a 10% increase in size resulting from an increased nitrogen supply is accompanied by an *increase* of 0.103% glutamic acid, or 17% of the average glutamic acid. This increase in glutamic acid must represent a net change after using some of the absorbed nitrogen for growth.

At a given nitrogen level, there was no correlation between sucrose and either weight or glutamic acid.

The highly unusual nature of these beets makes it impossible to draw definite conclusions as to reduction of variability resulting from the experimental environment. Glutamic acid shows a coefficient of variation of 30%, which is reduced to 26% when the relationship to beet weight is taken into account. This is an enormous improvement in reproducibility over the usual value of 100% for field beets. However, a considerable part of the decrease in variability could be caused by the high N level rather than by constancy of environment.

Table 3.—Comparison of Leaf and Storage Type Beets Grown in Sand Culture 1949.

Variety	Leaf Type	Storage Type	L.S.D.
Root Wt. in Pounds	0.68	**1.18**	0.20
Top Wt. in Pounds	**.66**	**.16**	**.15**
Percent Sucrose	**11.6**	15.6	1.2
Percent Glutamic Acid	0.22	0.88	0.07
Percent Soluble Inorganic Salts	**1.26**	**.59**	.11
Percent Sodium	.102	.024	.031
Percent Potassium	**.40**	**.18**	**.05**
Percent Magnesium	.059	.050	.010
Percent Calcium	.048	.036	.012
ppm. Manganese	**31**	20	**6**

Root weights were obviously much more constant than for field beets, especially the lack of very large and small beets. The weights had a coefficient of variation of 31%, in contrast to field beets with 50% or more. Much of this improvement could be due also to high nitrogen level, or to lack of competition. Sucrose (coefficient of variation of 10%) showed no improvement over field beets.

1949 Experiment

This experiment was again concerned with the effects of amount of available nitrogen. In addition, differences in response to type of nitrogen (ammonium salts vs. nitrate) , the effects of high potash in contrast to high sodium nutrient, and the differences between two inbred strains of beets were investigated.

Both varieties of beets used were in the seventh generation of inbreeding, chiefly by selling[3]. The parent generations of these beets had shown characteristically different top growth and root shape, and both varieties appeared to be nearly homozygous in these conformations. Analyses had indicated highly significant differences between the varieties in sucrose and glutamic acid content. For purposes oE identification here, the two varieties have been designated as "leaf type" and "storage type." The leaf type variety was characterized by heavy, upright leaf growth. The leaves were

[3] Seed was obtained through the kindness of Mr. G. W. Deming, Agronomist, U.S.D.A., at Fort Collins, Colorado.

relatively large, long, noncrinkled, and shiny bright green *in* color. The roots were tapering, low in sucrose and glutamic acid content. The storage type variety had less heavy, more open, low, flat top growth. The petioles were short and thin; the leaves only slightly longer than wide, slightly crinkled and medium dark, dull green in color. The roots were rounded in shape, high in sucrose and glutamic acid content.

Sixteen pots of each variety (planted alternately in a single row) were grown 9 weeks on high nitrogen nutrient, solution X. At the final thinning, the beets appeared to have an excellent nitrogen status. The nitrogen in the nutrient was reduced at this time, and four pots of each variety received solutions A, B, G, and H. At 11 weeks of age, sodium was substituted for potash in half of the beets at each nitrogen level, making eight nutrient treatments A to H (see Table 1) , which were used throughout the remainder of the experiment.

Table 4.—Comparison of High Potash vs. High Sodium Treatments.

Treatment	Potash	Sodium	L.S.D.
Percent Sodium	0.017	0.109	0.031
Percent Potassium	.37	.85	.11
Percent Magnesium	.064	.045	.011
Persent Soluble Inorganic Salts	**1.01**	.85	.11

Growth of many of the leaf type beets was impaired seriously by attacks of the sugar beet crown-borer *(Hulstia undulatella,* Clemens) . The crown-borer was finally controlled by an occasional application of 0.3 gm. of 50% DDT suspended *in* the nutrient solution. The leaf type variety was also selectively but not seriously attacked by aphids, diabroticas, and caterpillars.

Effects of ***Variety:*** Definite differences between the leaf and storage type varieties are apparent from the average results at harvest given in Table 3. The lower root weight and higher Mg, Ca, and Mn contents (which are closely correlated with weight in the leaf type beets) were evidently caused by the selective insect damage. The larger top weight and potassium content of the leaf type variety were reached in spite of the damage, which tended to reduce them. Insect damage could have been responsible only in small part of the much higher sodium and .inorganic salt and lower sucrose and glutamic acid content of these beets.

Effects of Sodium vs. Potash Treatments: Substitution of sodium for potash in the nutrient 11 weeks after planting produced the significant changes in Na, K, Mg, and soluble inorganic salts shown in Table 4. It also produced certain minor alterations in the larger nitrogen and ammonia effects on sucrose and glutamic acid. The decreases in Mg. and soluble inorganic salts noted were caused by the low potash supply—both measurements were in general closely correlated to the potassium content.

Variety of beet had considerable effect upon the changes produced by sodium nutrient. Inorganic composition was influenced by both nutrient and genetics. Potassium and soluble inorganic salts show this effect relatively simply:

Variety Treatment	Leaf Type Potash	Leaf Type Sodium	Storage Type Potash	Storage Type Sodium	L.S.D.
Percent K	0.55	.25	.20	.17	.06
Percent Soluble Inorg. Salts	1.41	1.12	.60	.58	.15

Thus the effects of genetics and nutrient upon percent K are much more than additive; one must be adequate for demonstration of the other. Variations in inorganic salt content follow variations in K content except in the sodium-treated leaf type beets. Effects on % Mg and % Ca somewhat similar to those on percent K are discussed with the ammonia effects.

Percent Na is higher in the leaf type beets and is increased by sodium in the nutrient as is percent K by potash in the nutrient, but the situation

Table 5.—Effect of Type and Level of Nitrogen.

	Treatment	Millimols Nitrogen per Day 2N[1]	3A	4N	6A	L.S.D.
Percent Sodium—Leaf Type	K	0.024	026	.011	.020	.121
	Na	.057	192	.190	.294	
Percent Sodium—Storage Type	K	.019	014	.007	.012	.012
	Na	.028	036	.044	.036	
Percent Glutamic Acid	K	.43	46	.54	.72	.12
	Na	.42	66	.47	.68	
Root Wt. in Pounds		.96	84	.79	1.13	28

[1] Nitrogen supplied as nitrate (N); as ammonium salts (A)

is further complicated by a strong stimulation by nitrogen level for sodium absorption in the presence of sufficient sodium in the nutrient (Table 5) . This stimulation is nearly proportional to the nitrogen content independent of the form of nitrogen, and is much stronger in the leaf type beets.

In the absence of sufficient sodium in the nutrient, percent Na is affected quite differently. In this case, the higher Na of the leaf type beets is explainable by insect damage (the larger, less damaged beets had the same Na content as the storage type beets) . Increase in nitrate lowers instead of raising the percent Na, while ammonia nitrogen has little effect. The leaf type beets appeared to have a much more extensive system of root hairs than the storage type beets. This may be the major explanation of the generally higher mineral uptake of this strain.

Effects of Nitrogen Level: Increasing the level of ammonia resulted in the expected rise in root weight, while increasing nitrate did not (Table 5) . This is the only significant effect on root weight noted aside from that of variety or insect damage. An increase in weight with increasing nitrogen level could be expected in the leaf type beets with their low glutamic acid content, but the variations in weight (coef. var. 43%) caused by the insect damage may have masked the effect. In the storage type beets, the glutamic acid was so high even at the low level of nitrogen that no significant increase in weight could be expected, although the coefficient of variation in this variety was reduced to 19%. This reduction in variation over the commercial variety (31%) of the previous year is probably due to both homozygosity and the higher glutamic acid content.

Sucrose and top weight showed no consistent response to nutrient or significant differences in nutrient effects between the two varieties. Sucrose values in the leaf type beets were too low to give much of a nitrogen response; the coefficient of variation was 24%. In the storage type beets, the coefficient of variation was only 3% and the quite significant results are discussed later.

Glutamic acid was strongly increased by nitrogen level (Table 5). There was some tendency for this response to be greater for the leaf type beets, which had lower glutamic acid content.

Effects of Nitrates vs. Ammonium Salts: Beets grown with ammonia as the source of nitrogen in the presence of high sodium showed a considerably greater increase in glutamic acid than those grown at an equal nitrogen level on nitrate. In the potash-treated beets, the advantage of

Table 6.—Effect of Variety and Type and Level of Nitrogen

Variety Treatment		Leaf Type		Storage Type		L.S.D.
			N	A	N	
Percent Magnesium	K Treatment	0.072	**.072**	.070	.042	.022
	Na Treatment	.068	**.031**	.050	.033	
Percent Calcium		.059	**.037**	.035	.036	**.015**

[1] Nitrogen supplied as ammonium salts (A); as nitrate (N)

ammonia disappeared, the glutamic acid values being directly proportional to the nitrogen level. The absorption of ammonia appears to be repressed by high potash.

Ammonia also produced effects on Mg, Ca, and Mn content which appear to be independent of the ammonia concentration (Table 6). Ammonia is as effective as potash in raising magnesium content. Either would produce the maximum magnesium in the leaf type beets, but both are required to produce the maximum *in* the storage type beets. Beets receiving ammonia averaged 31 ppm Mn, those receiving nitrate 20 ppm; L.S.D. 6 ppm. Ammonia increased the calcium content only in the leaf type beets (Table 6).

Storage Type Beets: The generally greater accuracy obtained in the insect-resistant storage type beets elicited further information of considerable interest, although it may be applicable only to this extreme type of beet.

Potassium and root weight were highly correlated (r= +0.83). When the effects due to variations in root weight were eliminated by equalizing the root weights, the following significant effects of potash became evident: as in the leaf type beets, a definite increase *in* potassium content occurred on high potash nutrient which had been concealed by variations in root weights in the storage type beets. Moreover, there was considerably greater absorption of potassium in the beets receiving nitrate than in those receiving ammonia, and greater absorption at the higher nitrate level than at the low level. Increasing the ammonia, on the other hand, decreased the potassium content of the beets:

Millimols Nitrogen Per Day		Nitrate 2	4	Ammonia 3	6	L.S.D.
% Potassium (1-lb. roots)	K Treatment	0.20	.28	.18	.14	0.08
	Na Treatment	.07	.16	.13	.08	

Glutamic acid, after adjustment to equal root weights, gave the following comparable results:

Millimols Nitrogen Per Day		Nitrate 2	4	Ammonia 3	6	L.S.D.
% Glutamic Acid (1-lb. Roots)	K Treatment	0.76	.93	.83	.86	0.20
	Na Treatment	.68	.81	.97	.90	

The adjustment to equal weight removes most of the effect of increasing-nitrogen level on glutamic acid, but brings out more strongly the tendency for a high potash level to stimulate nitrate absorption and to inhibit ammonia absorption as measured by the resulting glutamic acid values.

Sucrose in the storage type beets with their extraordinarily small tops decreased with increasing size of the root and increased with the increasing size of top (R = 0.80) . Equalization of both root and top weights made even more significant the increase in sugar in the beets on the high K nutrient, and the rise in this increase with nitrogen level.

Millimols Nitrogen per Day		Nitrate 2	4	Ammonia 3	6	L.S.D.
Percent Sucrose (1 lb. root, 0.15 lb. top)	K Treatment	15.6	15.6	16.7	16.9	0.8
	Na Treatment	16.7	14.9	15.5	15.0	

Positive and nearly significant partial correlations of sucrose with both sodium (0.54) and soluble inorganic salts (0.69) at equal root and top weights indicate that the effect of potash is attributable to its greater power in raising the total salt content.

The effect of potash *in* raising the sugar content indicates the possibility of an economic optimum in salt content similar to that of nitrogen level. The extremely low inorganic salts in this line of inbreds probably contributed to the detection of this effect.

Smaller but also significant is the advantage in percent sucrose of ammonia over nitrate at equivalent nitrogen levels. This is particularly true for the high potash nutrient beets and may be attributed to the slowing of ammonia absorption by potash.

These beets also showed a high correlation (—0.76) between sucrose and glutamic acid. At equal levels of glutamic acid both ammonia, as opposed to nitrate, and potash, as opposed to sodium, raised the sucrose content quite significantly. This effect of ammonia in raising the storage potential (2) of beets may be explained plausibly on the basis of diversion of photosynthetic energy from nitrate reduction to sucrose formation.

The fraction of soil nitrogen actually available in the form of ammonia may thus be of considerable importance in estimating the proper nitrogen usage for a particular field.

Summary

Both commercial and inbred varieties of sugar beets have been grown very successfully *in* pots of sand with complete chemical nutrient solutions. The nutrient supply was maintained at the desired levels by frequent renewal with freshly prepared solutions. Amount, type, and rate of application of nitrogen, and high sodium vs. high potash nutrients, were studied. Their effects upon variety, root weight, sucrose, glutamic acid, Na, K, Mg, Ca, and Mn are discussed.

For commercial varieties 2 millimols of nitrate distributed in two applications per day is recommended after growth is well established. Younger beets require more nitrogen. The ammonium form of nitrogen seems to produce superior beets to nitrate, but for comparison with field beets, and possibly for more uniform results, nitrate is recommended.

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