Changes in the Concentration of Amino Acids in Sugar Beet Plants Induced by Virus Yellows

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Introduction

Investigations have shown that changes in the concentration of certain amino acids take place in the leaves of susceptible sugar beet plants infected with a virulent strain of the curly top virus (2)[2]. The studies reported here were concerned with the changes in the concentration of certain amino acids in the leaves of sugar beet plants infected with a virulent strain of beet yellows virus.

Methods

In the first test, 20 plants of sugar beet variety US 75, growing in 6-inch pots in the greenhouse, were selected for uniformity. Ten plants were inoculated in the 6 to 8-leaf stage with a severe strain of the yellows virus. The remaining plants served as controls. The plants were fertilized bi-weekly with Hoagland's solution containing 210 p.p.m. of nitrogen until they reached the 12 to 14-leaf stage, at which time leaf samples were taken.

The plants were watered late in the afternoon to make certain they were not under stress when leaf samples were taken the following morning. Leaf samples, representing three ages of leaves, were taken from each plant as follows: (a) The two youngest mature leaves, showing the typical chronic symptoms of thickening and yellowing, were taken from each of the diseased plants for individual plant analysis. Leaves of the same age were taken from the healthy plants for the controls. These leaves were not senescent but were designated as "old leaves" for contrast. (b) Two leaves which had just reached full size were taken from each of the diseased and healthy plants and designated as "mature leaves." (c) Two leaves which were approximately one-half fully expanded were also taken from the diseased and healthy plants and designated as "young leaves." The midribs were removed and the juice expressed from the quick-frozen blades at a pressure of 5000 pounds. The juice was preserved by the addition of a thymol crystal and a small amount of phenyl mercuric nitrate and stored at 10° F. The samples were thawed, shaken thoroughly, and centrifuged before aliquots were removed for analysis.

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[2] Numbers in parentheses refer to literature cited.

A second test was conducted to determine whether similar changes in the concentration of the same amino acids occur in yellows-affected leaves of sugar beets grown in the field. In this test, the amino acids were determined in only the mature, healthy and diseased, leaves taken from 123 breeders selections showing a range of reaction to yellows. One ¾-inch disk was removed from each of 10 mature leaves taken from different healthy plants of the same breeders selection and composited. Leaves of comparable age, showing the chronic symptoms of virus yellows, were sampled in a similar manner. The inoculated plants were growing in a row adjacent to the healthy plants.

The amino acids were identified by two-dimensional paper chromatography. The technique was a modification of the methods given by Block, Le Strange, and Zweig (1) and by Goodban, Stark, and Owens (3). For quantitative work one-dimensional papergrams were run by the ascending method using Whatman No. 3MM paper. The solvents were normal butanol-acetic acid-water (4:1:5), phenol saturated with water, and 2, 6-lutidine-ethanol-water (2:2:1) to which was added 12.5 ml. of diethylamine per liter of solvent. The concentration of cystine, alanine, tyrosine, r-aminobutyric acid, phenylalinine, and "the leucines" was determined using the butanol solvent, and valine and methionine were determined using the lutidine solvent. The concentration of the remaining amino acids was determined using water-saturated phenol.

Each leaf sample was spotted in triplicate with two healthy and 2 corresponding diseased samples on each sheet of paper. Two-microliter spots were used for the amino acids determined in phenol as the solvent, and 3 and 5 microliter spots were used for the amino acids run in the butanol and lutidine solvents, respectively. Five and 10 microliter spots were required to identify certain of the amino acids on 2-dimensional papergrams. The solvents were allowed to ascend the paper 18 to 19 cm. which required approximately 7½ hours at 25° C.

A standard 200 mg. % solution was prepared for each amino acid and dilutions made to give a series of concentrations of 100, 50, 25, 12.5, and 6.25 mg. %. Four 2-microliter spots of each dilution were placed on papers and run concomitantly in the same cabinet with the leaf samples.

The papers were dried overnight in a 10 cu. ft. oven holding a tray of water and maintained at 45° C. The papers were dipped in 0.3% ninhydrin in redistilled ethanol. The color was allowed to develop for a period of 4½ hours in an incubator maintained at 45° C. The relative humidity was maintained at approximately

50 percent with a moisture tray and a stream of preconditioned air passing through the incubator at a rate sufficient to replace the air three times during the color-developing period. The background color of the papergrams was very light and uniform, the transmission density being only slightly greater than that of the untreated paper.

The relative density of the spots of the standards was determined by a photovolt transmission densitometer unit using an aperture 4 mm. in diameter. The mean transmission density of the 4 replicated spots, for each of the concentrations, was plotted against the log of the concentration of the amino acid and a standard curve drawn. A densitometer reading, of the area having the maximum density, of each leaf-sample spot was made and the concentration of the amino acid taken from its standard curve.

Table 1.—The Concentration of Certain Amino Acids in the Expressed Juice of Leaves of Three Ages of Individual Healthy and Yellows-Affected Sugar Beet Plants Grown in the Greenhouse.[1]

	Concentration of Amino Acids (Milligram Percent)					
	Old Leaves		Mature Leaves		Young Leaves	
Amino Acid	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Aspartic Acid	28.9	16.4	32.8	27.6	27.4	18.5
Glutamic Acid	46.5	32.9	48.5	32.2	36.2	27.9
Citrulline	25.7	83.8	27.0	50.0	31.3	32.6
"Leucines"	9.7	25.7	4.7	11.2	9.5	12.3
Threonine	4.7	21.1	3.6	6.6	5.8	5.2
p-Alanine	19.7	30.0	12.4	17.0	22.3	21.0
r-aminobutyric	11.8	15.2	12.4	19.0	17.5	18.3
Valine	12.1	21.7	10.0	15.0	8.1	9.8
Methionine	13.4	18.3	9.0	10.5	7.7	8.0
Tyrosine	16.1	17.0	14.0	13.1	36.2	37.9
Alanine	6.1	6.0	6.0	7.1	10.1	10.2
Cystine	6.3	4.4	7.1	7.2	10.3	9.3
Serine	2.7	4.1	4.4	5.4	6.6	6.4
Glycine	2.3	2.2	4.1	7.6	—	—

[1] Mean values for 10 plants.

Results

Table 1 shows the mean concentration of the amino acids determined in three ages of leaves from 10 healthy and 10 diseased plants grown in the greenhouse. Aspartic acid and glutamic acid were the only amino acids determined which showed

a decrease in concentration in the diseased leaves. The decrease occurred in all ages of leaves but not to the same extent. In the old and mature leaves, citrulline, the "leucines," threonine, p-alanine, r-aminobutyric, alanine, valine, and methionine increased in concentration, whereas the concentration of serine, tyrosine, cystine, and glycine remained unchanged. The "leucines" were the only amino acids to show an increase in concentration in all ages of leaves. The young leaves appeared to be affected least by the disease. Except for the decrease in the concentration of aspartic and glutamic acids and the increase in the "leucines," the concentrations of the remaining amino acids were unchanged. Arginine, histidine, and lysine were also found to be present, the highest concentration being found in the old leaves. Of the three, arginine appeared to be present in highest concentration. In some samples the concentration reached 3 mg. percent.

Figure 1.—Papergram, developed in water-saturated phenol, of juice expressed from mature leaves of 3 healthy plants and juice from leaves of comparable age from 3 plants having yellows showing. Aspartic acid (asp.), glutamic acid (glut), serine (ser), citrulline (cit), and -aminobutyric acid (gaba). Other amino acids are present with r-aminobutyric acid. H-Healthy leaf, D-Diseased leaf.

Figure 1 is a typical papergram, developed in water-saturated phenol, showing spots of certain amino acids in the juice expressed from healthy mature beet leaves and from juice expressed from leaves of similar age showing the chronic symptoms of yellows.

A relationship which may be useful in diagnosing the disease in individual plants is a ratio of the amino acids which decrease to those that increase in the leaves as a result of the disease. For example, if the ratio $\frac{\text{aspartic acid plus glutamic acid}}{\text{citrulline plus alanine}}$ (alinine calculated as citrulline) is calculated for the leaves of healthy and diseased plants, values are obtained which are different. The amino acid ratios are shown in Table 2 for three ages of leaves from 10 healthy and 10 diseased plants grown in the greenhouse. The greatest difference in the amino acid ratio between the healthy and diseased plants was obtained with the leaves old enough to show chronic symptoms of the disease.

Table 2.—The Amino Acid Ratio[1] in the Expressed Juice from Leaves of Three Ages from Healthy and Yellows-Affected Sugar Beet Plants Grown in the Greenhouse.

Plant	Old Leaves		Mature Leaves		Young Leaves	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio
1	3.03	0.67	2.63	2.66	2.00	1.12
2	2.00	0.34	2.59	0.94	1.12	0.75
3	1.58	0.76	2.14	0.56	0.96	0.62
4	2.52	0.35	3.70	0.88	1.02	0.87
5	1.75	0.36	2.42	0.67	1.67	1.29
6	2.42	0.44	2.86	1.06	1.94	1.29
7	1.89	0.59	1.65	1.10	1.56	0.79
8	1.90	0.42	1.14	0.63	0.88	0.95
9	2.34	0.37	1.80	1.23	1.63	1.09
10	1.17	0.35	1.19	0.79	1.31	1.06
Mean	2.06	0.46	2.21	1.05	1.41	0.98

[1] $\frac{\text{Aspartic Acid + Glutamic Acid}}{\text{Citrulline + Alanine}}$

In this test, the mean ratio for the old leaves of the healthy plants was approximately 4.5 times greater than the ratio for the old leaves of diseased plants. The ratios did not overlap.

The wide variation in the amino acid ratio of the leaves showing the chronic symptoms indicated that all plants were not affected by the disease to the same extent. In view of this vari-

ation in the amino acid ratio among individual plants, it appears that it may be possible to select plants by an amino acid ratio that may have a higher degree of resistance to virus yellows.

In the second test, which was conducted on field-grown plants, a highly significant reduction in the concentration of aspartic acid and glutamic acid also occurred in the leaves of the diseased plants. The mean reduction in concentration for these amino acids was 47 and 40 percent, respectively. The concentration of citrulline and alanine increased 37 percent in the same diseased plants over that of the healthy plants.

The amino acid ratio separated the healthy and diseased plants into two groups. The mean amino acid ratio for the healthy plants was 1.41 as compared with 0.62 for the diseased plants. Based on analysis of leaves of healthy plants, only 9 of the 123 selections had ratios which would classify them as diseased plants. Conversely, in an analysis of leaves of diseased plants, only 8 of the 123 selections had ratios which fell in the range covered by the healthy plants.

Of the 123 breeders selections, four were of the type which remain relatively green even though the plants are stunted by the disease. These selections are designated by some breeders as "green selections." The mean amino acid ratio for these "green selections" was 1.60 and 0.57 for the healthy and inoculated plants respectively. It appears, therefore, that the changes in the concentration of the amino acids, as a result of the disease, may not be directly correlated with the yellowing of the infected plants.

Summary

The concentration of certain amino acids was determined, by paper chromatography, in the juice expressed from three different ages of leaves taken from healthy and from yellows-infected beet plants grown in the greenhouse and from mature leaves taken from sugar beet selections grown in the field. The concentration of aspartic acid and glutamic acid decreased in all ages of leaves, whereas all the other amino acids determined either increased or remained unchanged. The greatest changes in the concentration of the amino acids occurred in the old leaves that showed the typical chronic symptoms of the disease. In the young leaves, aside from a reduction in the concentration of aspartic and glutamic acids, only the "leucines" increased. The concentrations of the remaining amino acids were unchanged. Differences in the concentration of certain amino acids in the leaves of healthy and yellow-affected sugar beet plants were found

to be sufficiently high and constant to suggest that a ratio of the amino acids involved may be useful as an aid in diagnosis.

A considerable variation in the concentration of certain amino acids occurred among sugar beet plants affected with yellows. This indicates that it may be possible to select individual plants which may have resistance to sugar beet yellows on the basis of the changes in the concentration of certain amino acids in the mature leaves showing the chronic symptoms of the disease.

Literature Cited

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