Changes in the Concentration of Amino Acids in the Leaves of Sugar Beet Plants Affected with Curly Top

J. M. FIFE[1]

Introduction

Previous investigations (1)[2] have shown that striking differences occur in the relative concentration of certain amino acids in the juice expressed from the leaves of healthy and curly-top-diseased sugar beets. The work reported here summarizes some of the findings obtained at this laboratory on the amino acids found (and the relative amounts of certain ones) in the leaves of healthy, susceptible sugar beets. These are compared to the findings in comparable leaves of plants affected with a severe strain of the curly-top virus.

Preparation of Samples

A variety of sugar beets, susceptible to curly top, was grown in the greenhouse in six-inch pots in highly fertile soil. The plants, four per pot, were inoculated with a virulent strain of curly-top virus as early as the two-leaf and as late as the six-leaf stage, depending upon the experiment, by means of viruliferous beet leafhoppers. The uninoculated plants, thinned to two per pot, were cared for in the same manner and made up the controls. The plants were fertilized immediately after inoculation and at frequent intervals until the leaf samples were taken.

The samples were taken after the disease had reached a maximum degree of severity. This was usually four to five weeks after inoculation, depending upon the age of the plants at the time of inoculation. The oldest leaf showing severe symptoms of curly top, together with all of the younger leaves on the plant, was taken for a sample. Leaves of the same age range were taken from the healthy control plants at the same time. Leaves were taken from a minimum of 20 to as many as 100 diseased plants for each sample of diseased leaves and from smaller numbers of healthy plants for the control sample.

The petioles were removed at the base of the leaf and the juice expressed from the washed, quick-frozen blades to 6000 p.s.i. The juice was filtered through a mat of celite and preserved with thymol and phenyl mercuric nitrate and stored at 10° F. The samples were filtered again immediately before using. Eleven such pairs of samples were prepared over a period of two years.

Analysis by Paper Chromatography

The number of amino acids and their relative amounts were determined by 2-dimensional paper chromatography by the ascending method. Normal butanol-acetic acid-water (4:1:5), phenol-water (4:1) and 2,6-lutidine (200

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[1] Chemist, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Salinas, California.

[2] Numbers in parentheses refer to literature cited.

ml.), 95 percent ethanol (200 ml.), water (100 ml.) and diethyl amine (6.25 ml.) were the solvents used. As much as 10 /μl of the cleared juice was spotted on 11 x 11 Whatman Nos. 1, 3, and 52 papers for identification purposes.

For quantitative studies, 1-dimensional papergrams were used for all amino acids except histidine, lysine, γ-aminobutyric and glycine. These amino acids, except the last, were determined by 2-directional papergrams using phenol followed by the lutidine solvent. Gamma-aminobutyric acid was separated by dusting the line of ascent of the acids in phenol with basic copper carbonate followed by the lutidine solvent which did not contain the diethyl amine. Glycine was determined using the butanol solvent followed by lutidine. The butanol solvent alone was used to determine valine, the "leucines" and cystine while the lutidine solvent was used to determine threonine and as a check upon the "leucines" and valine. The phenol solvent was used to determine aspartic acid, glutamic acid, alanine, and argenine. The concentration of argenine was so high, relative to that of histidine and lysine, that only 1 /μl of juice was sufficient for a determination of the former and without interference from the other two acids.

Duplicate spots of 1 to 3 /μl of the juice, depending upon the amino acid being determined, of each pair of samples of healthy and diseased leaves were placed side by side 2 cm. apart on all three papers.

The same volume of the standards, shown in Table 1, was replicated four times with each concentration on separate sheets of each paper and run concomitantly in the same cabinet with each set of unidirectional papers.

The papers were developed at a temperature of 23° C., allowing the solvents to ascend, in most cases, 18 to 20 cm. above the origin. The developed papergrams were dried overnight in a circulating oven at 40° C., then dipped into a 0.3-percent solution of ninhydrin in 95 percent ethanol. Color development took place during a six-hour period in an incubator maintained at 40° C., and a relative humidity of 45 percent by passing preconditioned air through the incubator at a rate sufficient to replace the air at least once every two hours.

Table 1.—Composition of Amino Acid Solutions Used as Standards.

Amino Acid	1	2	3	4
			Mg. %	
Aspartic	140	70	35	17.5
Glutamic	140	70	35	17.5
Argenine	140	70	35	17.5
Histidine	200	100	50	25
Lysine	200	100	50	25
Valine	200	100	50	25
Leucine	200	100	50	25
Serine	200	100	50	25
Threonine	200	100	50	25
Gamma-Aminobutyric	200	100	50	25
Glycine	100	50	25	12.5
Cystine	200	100	50	25

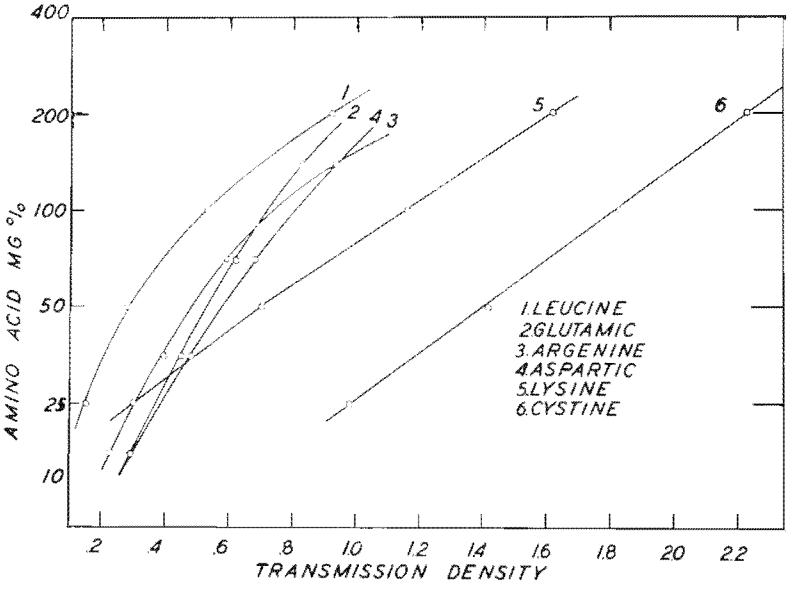

Figure 1.—Typical standard curves obtained by plotting log of amino acid concentration against transmission density.

The relative density of the spots was determined the following morning by a photovolt transmission densitometer unit using a 4-mm. aperture. The transmission density values were corrected for the background value of the paper. The log concentration of the standard amino acids was plotted against the mean "transmission density" of the four spots. Typical curves are shown in Figure 1. The curves were usually straight lines for the three lowest concentrations, but the curves of some of the amino acids tended to flatten somewhat above the 100 mg. percent concentration. The concentration of each amino acid was determined on all three papers (Whatman Nos. 1, 3, and 52) and the papergrams compared as to quality for quantitative purposes. The paper which gave the best separation and sharpest spots was selected for each amino acid for the final determinations. Duplicate spots of each sample on the same paper and duplicate papers were run for the data shown in Table 2.

Table 2 shows the mean concentration of the principal amino acids in the juice expressed from healthy and curly-top-diseased sugar beet leaves in 11 separate experiments, together with the mean ratio of diseased to healthy for each amino acid. The concentration of each amino acid varied somewhat in the 11 samples of healthy juice. This variation could be due to one or more of several factors, such as the difference in the fertility level maintained in the different sets of plants, the age of the plants at the time of inoculation, the variation in the time interval between inoculation, and the taking of the samples, or the time of the year the plants were grown.

Table 2.—Concentration of Certain Amino Acids in the Expressed Juice of Healthy and Diseased Sugar Beet Leaves.

Amino acid	Leaf Juice		
	Healthy Mg. %	Diseased Mg. %	Ratio D/H
Aspartic	35[1]	54[1]	1.55
	41[2]	28[2]	0.67
Glutamic	61[1]	93[1]	1.53
	168[2]	53[2]	0.49
Argenine	50	165	3.3
Lysine	8	26	3.0
Histidine	9	14	1.6
"Leucines"	18	30	1.6
Valine	35	72	2.0
Serine	44	70	1.6
Gamma-Aminobutyric	18	29	1.5
Threonine	23	34	1.4
Glycine	12	44	3.6
Alanine	37	70	1.9
Cystine	10	10	1.0

[1] Mean value of samples taken at 10:00 a.m.

[2] Mean value of samples taken at 4:00 p.m.

With the exception of cystine, all the amino acids shown in the table were present in greater concentration in the diseased than in the healthy sample, in 10 of the 11 pairs of samples analyzed. Why the one pair of samples did not follow this same pattern in all respects is not known.

The concentration of aspartic and glutamic acids was found to be influenced by the time of day the samples were taken. These amino acids were higher in the diseased leaves than in the healthy leaves, only in the six samples taken in the morning. The mean ratio of diseased to healthy was 1.55 and 1.53 for aspartic and glutamic acids, respectively. The five samples taken at 4:00 p.m. show that, during the day, aspartic and glutamic acids decreased sharply in diseased leaves while at the same time they increased in the healthy leaves to a degree which completely reversed the ratios found in the samples taken in the morning. The ratio of diseased to healthy for the samples taken at 4:00 p.m. was 0.67 and 0.49 for aspartic and glutamic acids, respectively. From this, it appears that the ability of the beet leaf to utilize these acids during the day is not greatly affected by the disease.

The most striking difference observed is the accumulation of the basic amino acids, argenine, histidine, and lysine in the leaves of the diseased plants. Argenine makes up by far the major portion of this group. The lowest ratio of argenine found in the diseased leaves to that in the healthy was 1.6, while ratios of 4.0, 5.9, 8.1, and in one case, 13.6 were found. In the last instance, however, the argenine in the healthy juice was only 10 mg. percent, which was the lowest value obtained. Glycine accumulated in diseased leaves until the concentration was 3.6 times greater than that found in the healthy leaves. The concentration of glycine, however, was relatively low in the healthy leaves in comparison with that of argenine.

In addition to the amino acids listed in the table, tyrosine, phenylalanine, and methionine have been found in the juice expressed from healthy and diseased sugar beet leaves. At least three other amino acids were present in small amounts. These have not been identified. At least one of the unidentified acids is either a beta or a gamma-amino acid.

Summary

Certain amino acids tend to accumulate in the leaves of susceptible sugar beet plants affected with a severe strain of the curly-top virus. Arginine appears to accumulate to the greatest extent in the diseased leaves relative to that in comparable healthy leaves. A mean of all the determinations showed that the concentration of arginine was approximately three-fold greater in the leaves of diseased beet plants than in healthy leaves. Aspartic and glutamic acids were present in greater concentration in diseased leaves in the samples taken in the morning, whereas the reverse was true for the samples taken late in the afternoon. The average ratio for all the amino acids in the diseased leaves to that in the healthy leaves was greater than 2.0. This indicates that, in general, the amino acids as a whole, tend to accumulate in the leaves of susceptible sugar beets when inoculated with a severe strain of the curly-top virus, to about double that found in comparable healthy leaves. At least three unidentified amino acids are present in the leaves of healthy and diseased beet leaves, one of which appears to be a beta or a gamma-amino acid.

Reference

(1) FIFE, J. M. 1954. Chromatography as a method of attack on the problem of the chemical nature of resistance of sugar beets to curly top. Proc. Amer. Soc. Sugar Beet Tech. 8 (1) :207-211.