Changes in Concentration of Three Amino Acids, in Mature Leaves of Sugar Beet Plants, Produced by Mass Selection from a Population Infected With Beet Yellows

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The concentration of certain free amino acids is greatly altered in the mature leaves of sugar beet plants with chronic symptoms of beet yellows (3)[2] and of beet western yellows (1). In this study, only the leaves that had just reached full maturity, either healthy or beet yellows-inoculated, were analyzed. In the leaves of some plants infected with beet yellows, the concentration of aspartic acid and of glutamic acid decrease as much as 70% as compared to that in healthy control leaves. In the same leaves, the concentration of glutamine sometimes was more than double that found in the healthy controls. As a result of these concentration changes, the amino acid ratios (concentration: $\frac{\text{aspartic acid} + \text{glutamic acid})}{\text{glutamine}}$ were quite different. In one test, conducted in the greenhouse under controlled nutritional conditions, the amino acid ratio varied from 1.00 to 3.00 in control leaves taken from different plants. In the beet yellows-infected leaves, the ratio varied from 0.34 to 0.67. Extensive tests, involving 121 breeder's selections, were made on leaves of plants growing in the field in 1957. The mean amino acid ratios were 1.41 and 0.59 for the uninoculated and inoculated plants, respectively.

Plants from 28 breeder's selections were tested the following year. The mean ratios were 1.26 and 0.38 for the uninoculated and inoculated plants, respectively.

The amino acid ratio was determined in leaves of individual plants of a large population inoculated with beet yellows virus. These plants were grown under controlled nutritional conditions in the greenhouse. Leaves of a very low percentage of the plants with chronic symptoms of the disease had concentrations of the three amino acids, more nearly like those in leaves of healthy plants even though the leaves tested were showing

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[2] Numbers in parentheses refer to literature cited.

the chronic symptoms of the disease. The calculated amino acid ratios were, therefore, equal to that of some healthy plants. The data suggested that the degree to which the concentrations of these amino acids are changed (as indicated by the amino acid ratio) in the leaves of infected plants may be used as an aid in identifying those plants which have resistance to beet yellows and possibly to beet western yellows as well.

There is ample evidence that the concentration of certain chemical constituents in the sugar beet root are, to a certain degree, under genetic control. Mass selections in sugar beets for high and for low concentrations of chemicals such as, galactinol, sucrose, raffinose, potassium and sodium have been successful as shown by progeny tests.

Finkner et al. (4) have made a recent comprehensive review of the literature pertaining to changes, in the chemical composition of the sugar beet root, resulting from selection pressure for certain of the chemical compounds. These investigators also made selections for high and low aspartic acid and for glutamine content of sugar beet roots grown in the field. The selections resulted in roots of the progeny having a higher or lower content of these two amino acids, respectively. The total amino acid content and total nitrogen were affected similarly. These investigations also showed that selection for low glutamine resulted in a significant reduction in the potassium content of the roots. Selection for a low aspartic acid content resulted in an increase in the yield of the roots but had no significant effect on the percent sucrose in the roots.

Changes in the concentrations of the amino acids in the leaves of healthy and of beet yellows-infected plants, resulting from selection pressure, have not been reported. The first objective of this investigation was to determine whether the concentrations of these three amino acids could be shifted, in the leaves of both healthy and beet yellows-inoculated plants, by ordinary mass selection from a population of sugar beet plants infected with beet yellows. The second was to determine whether the changes in the concentrations of the amino acids that occurred, caused a shift in the amino acid ratio.

Methods

The first selections were made from a population of approximately 1,000 plants. They were grown in five large boxes in the greenhouse. The boxes were lined with polyethylene sheeting and filled with sterilized sand. Seed of variety US 75 were planted on 6-inch centers and thinned to one plant. They were

watered with Hoagland's solution at half-strength. The plants were inoculated in the early 4-leaf stage with a virulent strain (Strain 5) of the beet yellows virus.

Leaf samples were taken from each plant 42 days after inoculation. The sample consisted of the two leaves which had just reached full maturity and which showed the chronic symptoms of the disease. Every other plant was removed 57 days after inoculation and its root weight recorded. The remainder of the plants were harvested 85 days after inoculation.

After the midribs had been removed, the leaf samples were quick-frozen. The juice was expressed from the thawed tissue by applying a total pressure of 5,000 pounds. The concentration of aspartic acid, glutamic acid and glutamine[3] was determined by paper chromatography using the ascending method with water-saturated phenol as the solvent. The concentrations of the ninhydrin-stained amino acids were determined by measuring the density of the spots with a Photovolt Densitometer (2).

Plants were selected from the population and placed in either one of two groups, depending upon their root weight and their amino acid ratio. Group 1 included those plants having a root weight greater than the mean of the population from which they were selected by at least twice the standard deviation and an amino acid ratio equal to or greater than the mean of the population. Only 28 plants fulfilled both of these requirements. These plants were given 120 days of photo-thermal induction and then placed in isolation for an open-pollinated seed increase. The seed from each individual plant was harvested separately and considered a selection, or a line.

Group 2 included only 10 plants. Each plant had a root weight greater than the mean of the population from which they were taken. These plants had an amino acid ratio greater than the mean of the total population by at least twice the standard deviation. Note that the selection pressure for the amino acid ratio was much greater for the plants of Group 2 than for plants of Group 1. The plants of Group 2 were given photo-thermal induction and placed in isolation for an open-pollinated seed increase. This seed also was harvested on an individual plant basis and each one considered as a selection.

The second successive selections were made from a population of approximately 1,000 plants. Methods used in growing the plants were essentially the same as described for making the first selections, except that the plants were grown in pots.

[3] The amino acid labeled citrulline (Figure 1) of an earlier report has been shown, by further tests, to be mainly that of glutamine. Although both citrulline and alanine have been shown to be present in the extracts tested, the concentrations of these two amino acids are insignificant compared to that of glutamine.

Twenty-four plants were grown from seed of each of the 28 selections from Group 1 and of the 10 selections from Group 2. Plants of the parent variety (US 75) and a breeder's selection (No. 663) were included for test purposes. The plants were inoculated with the same virulent strain of the beet yellows virus used to inoculate the plant population from which the first selection was made. Leaf samples were taken and root weights recorded. The selection pressure, applied for both the amino acid ratio and for the root weight, was arbitrarily set at two standard deviations greater than the mean of the population. Only 10 plants fulfilled both these requirements. They were given photo-thermal induction, then placed in isolation for an open-pollinated seed increase. The seed from each plant was harvested separately. Each seed lot was considered a selection, as before.

A greenhouse test was then conducted to determine if the selection pressure, applied for a high amino acid ratio, changed the concentrations of the amino acids in leaves of plants of the progeny. Seven first selections, four of the second successive selections, and the parent (US 75) were tested as follows:

Twenty-five plants of each of the selections were grown in a sand-sponge-rock mixture, with one plant per 1-gallon can, and watered with Hoagland's solution at half-strength. The plants were inoculated, in the early 4-leaf stage, with the same virulent strain of the virus. The same number of uninoculated plants of each of the selections were grown for controls. Leaf samples were taken from all plants when the inoculated plants reached the chronic stage of the disease. The concentrations of the three amino acids involved were determined and the amino acid ratios calculated. The data were analyzed by analysis of variance.

Results and Discussion

Selection pressure, applied for a high amino acid ratio, caused highly significant changes in the concentration of the amino acids in leaves of plants of the progeny, provided the plants were infected with beet yellows (Table 1). In every case where the difference between the selection and the parent was significant, the concentrations of both aspartic acid and glutamic acid were higher in the leaves of the selections than in the leaves of the parent. The concentration of glutamine was lower in leaves of the selections than in leaves of the parent in every case. These concentration changes were toward that in the leaves of healthy plants. In every case where the amino acid ratio was significantly different from that of the parent, it was higher in

Table 1.—Progeny test of plants selected for a high amino acid ratio in mature leaves of beet yellows-infected plants. The concentration of three free amino acids in leaves of beet yellows-infected plants of the parent and of plants of first and second successive selections.

Sibs of 1st & 2nd suc. selections	Selection pressure applied for amino acid ratio	Root wt.	Amino acid conc. Asp.	Glut.	Glut NH_2	Asp. + Glut. / Glut NH_2
			Mg. %	Mg. %	Mg. %	Ratio
US 75	Parent		3.9	8.6	49.2	0.26
DS3 1st	$> \bar{X}$	$> \bar{X} + 2s$	8.7**	7.3	37.5	0.43*
DS22 1st			4.9	7.0	51	0.23
DS23 1st			5.0	9.3	58	0.25
DS24 1st			19.3**	16.8**	56	0.48**
DS9 1st			8.3**	7.9	37 *	0.44*
DS7 1st			7.6*	7.9	40	0.37
DR6 1st	$> \bar{X} + 2s$	$> \bar{X}$	11.8**	9.7	23.6**	0.91**
RS9 2nd Suc.	$> \bar{X} + 2s$	$> \bar{X} + 2s$	16.8**	12.1**	36 *	0.66**
RS3 2nd Suc.			8.2**	10.7	24.8**	0.77**
RS2 2nd Suc.			12.5**	14.9**	33.8*	0.81**
RS5 2nd Suc.			12.0**	14.2**	29.8**	0.88**
Significant difference		(99:1)	3.2	3.4	16.1	0.22
		(19:1)	2.5	2.6	12.2	0.17

** Significant at the 1% level.
* Significant at the 5% level.

the infected leaves of plants of the progeny than in infected leaves of the parent. The shift in the amino acid ratio as a result of selection pressure was, therefore, toward that in leaves of the healthy plants.

The mean amino acid ratio (0.78), for the 4 second successive selections, was 3-fold greater than that of the parent (0.26).

The performance of one selection (DR-6) indicates that the same differences in the concentration of the amino acids, and the resulting amino acid ratio, may be obtained in a first selection provided the selection pressure is great enough.

Selection pressure, for a high amino acid ratio, did not result in consistent changes in the concentrations of the amino acids in leaves of healthy plants of the progeny (Table 2). The shift in the amino acid ratios followed no consistent pattern relative to that of the parent.

The defensive mechanism established in plants of the progeny against changes in the concentrations of these amino acids, in leaves of plants under the stress of beet yellows, may be correlated with resistance to the disease. Promising first and second successive selections are being field tested for resistance to beet yellows. The results will be reported later.

Table 2.—Progeny test of plants selected for a high amino acid ratio in mature leaves of plants infected with beet yellows. The concentration of three free amino acids in leaves of healthy plants of the parent and of first and second successive selections.

Sibs of 1st & 2nd suc. selections	Selection pressure applied for amino acid ratio	Root wt.	Amino acid conc. Asp.	Glut.	Glut NH_2	Asp. ÷ Glut. Glut NH_2
			Mg. %	Mg. %	Mg. %	Ratio
US 75	Parent		18.7	33.9	38.6	1.36
DS3 1st	$> \bar{X}$	$> \bar{X} + 2s$	21.3	45.5**	21.0**	3.19**
DS22 1st			16.2	30.0	18.5**	2.50**
DS23 1st			18.4	28.1	33.4	1.40
DS24 1st			20.0	29.6	51 *	0.98**
DS9 1st			17.3	30.5	17.5**	2.74**
DS7 1st			18.8	31.6	38.7	1.30
DR6 1st	$> \bar{X} + 2s$	$> \bar{X}$	21.0	30.3	50 *	1.02*
RS9 2nd Suc.	$> \bar{X} + 2s$	$> \bar{X} + 2s$	18.5	30.4	36.6	1.34
RS3 2nd Suc.			15.7*	38.4	68 **	0.78**
RS2 2nd Suc.			16.9	48.2**	47.0	1.38
RS5 2nd Suc.			42.1**	52 **	55 **	1.69*
Significant difference		(99:1)	3.7	9.5	14.4	0.37
		(19:1)	2.8	7.2	10.9	0.28

** Significant at the 1% level.
* Significant at the 5% level.

Summary

Progeny tests have shown that the concentrations of aspartic acid, glutamic acid and glutamine are changed in the leaves of sugar beet plants by selection pressure for a high amino acid ratio $\frac{(\text{aspartic} + \text{glutamic})}{\text{glutamine}}$ from a population of plants infected with beet yellows. The evidence is clearly shown by analysis of newly-matured leaves of plants of the progeny, as compared to leaves of the parent, provided the plants are infected with beet yellows. The concentration of aspartic acid and of glutamic acid increased while that of glutamine decreased in the infected leaves of plants of the progeny relative to the concentrations of these amino acids in leaves of infected plants of the parent. These changes in concentration resulted in a higher amino acid ratio. In beet yellows-infected plants, the shift in the concentration of these amino acids, in leaves of plants of the progeny, was toward that found in healthy control plants of the parent.

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