Title
Quantitative Determination of the Free Amino Acids and Amides in Roots and Leaves of Healthy and Exocortis-Infected Citrus sinensis Osbeck on Poncirus trifoliata Raf.

Journal
International Organization of Citrus Virologists Conference Proceedings (1957-2010), 3(3)

ISSN
2313-5123

Authors
Feldman, A. W.
Hanks, R. W.

Publication Date
1965

DOI
10.5070/C50kh3s6hf

Peer reviewed

A. W. FELDMAN and R. W. HANKS

Quantitative Determination of the Free Amino Acids and Amides in Roots and Leaves of Healthy and Exocortis-Infected Citrus Sinensis Osbeck on Poncirus Trifoliata Raf.

During the past ten years, there has been increasing emphasis on the use of chromatography as a tool for studying host-pathogen responses. Many of these investigations have dealt with the amino acid and amide composition of herbaceous plants and have, in general, indicated that certain amino acids are increased following pathogenesis (1-3, 7-11). With certain diseases, particularly strap-leaf of Chrysanthemum (14), Western X Disease of peaches (5, 6), and frenching of tobacco (12), specific amino acids have been implicated as being directly responsible for the symptoms produced. There have been few studies on the amino acid and amide composition of either roots or leaves of virus-infected fruit trees (5, 6) and, to date, no work of this nature has been reported on citrus. Our understanding of the nature of the biochemical response of citrus to virus disease is quite limited, and it is believed that more knowledge on the biochemical patterns could be valuable in interpreting the significances of the metabolic aberrations and their effect on host response.

The purpose of this investigation was to compare the quantitative levels of the free amino acids and amides in both the roots and leaves of healthy and exocortis-infected *Citrus sinensis* Osbeck 'Hamlin' on *Poncirus trifoliata* Raf. Data are also presented on the percentage of each constituent in the composition of the amino acid pool as a consideration in interpreting the extent of any amino acid imbalance on the physiological syndrome.

Materials and Methods

The plants used in these investigations were kindly supplied by Mr. G. G. Norman, Citrus Budwood Registration Office, Florida Department of Agriculture. Spring flush leaves (recently matured) and feeder root samples were collected in late March, 1963, from each of three field-grown healthy (designated as H-1, H-2, and H-3) and three exocortis-infected (designated as E-1, E-2, and E-3) trees of *C. sinensis* on *P. trifoliata*. The budwood for these trees was obtained from six different sources. All had been indexed and found to be free of tristeza, xyloporosis, and psorosis viruses. Specific information about tree condition, size, year budded, exocortis symptoms, and color test data for each of these source trees is listed in Table 1.

TABLE 1. General characteristics of the source trees used for amino acid analyses

Tree No.	Year Budded	First Exocortis Symptom	Color Test[e]	Condition and Size of Tree at Sampling			
				Vigor	DAB[a]	DBB[b]	Ratio[c]
E-1	4/55[d]	9/61	+	Fair	60	63	1-1
E-2	4/55[d]	9/61	+	Fair	30	57	1-2
E-3	4/55[d]	9/61	+	Fair	45	81	1-2
H-1	4/55	....	—	Good	72	110	3-4
H-2	4/55	....	—	Good	76	104	3-4
H-3	4/55	....	—	Fair	60	71	1 1

[a]Diameter of stem above bud in mm. [b]Diameter of root below bud in mm.
[c]Ratio DAB/DBB. [d]Rebudded 6/56.
[e]Phloroglucinol—HC1 color test for presence (+) or absence (—) of exocortis (March, 1963).

Twenty-five grams fresh weight each of leaves and feeder roots were collected from each tree. The amino acids were extracted with 200 ml of 80 per cent ethanol for three minutes in a Waring Blendor and for four hours on a shaking machine, after which the brei was filtered through sintered glass. Extracts were stored at 4°C. until processed for chromatography (8). Details on procedures for desalting, two-dimensional paper chromatography, solvent systems, and for quantitative analyses of each amino acid and amide have been published elsewhere (8). The reported data are based on at least four chromatograms on each leaf and root sample.

Results

Leaves.—Twenty ninhydrin-positive materials were found, of which

18 were identified (Table 2). Pipecolic acid and unknowns A and B were three materials found in the leaves, but not in the roots. Aspartic acid, glutamic acid, serine, glycine, threonine, glutamine, alanine, γ-aminobutyric acid, leucines, pipecolic acid, unknowns A and B, ethanolamine, and proline were the most prevalent in both plant groups. Proline accounted for more than 60 per cent of the total free amino acids and amides. Asparagine, tyrosine, valine-methionine, methionine sulfoxide, arginine, and lysine were found in small amounts.

The total free amino acid content of the healthy leaves averaged 2597 μg/g whereas leaves from exocortis-infected plants averaged 1774 μg/g. Leaves from plants H-1 and H-2 averaged approximately 74 per cent more free amino acids and amides than those obtained from leaves of the three exocortis-infected plants (E-1, E-2, E-3). One healthy plant (H-3) was found to have a total free amino acid and amide content similar to that of the exocortis-infected plants. The diameter of the rootstock and trunk and the vigor of tree H-3 were also comparable to the exocortis group of plants (Table 1). This healthy plant did not have exocortis symptoms and had been determined to be exocortis-free on the basis of the phloroglucinol color test.

Despite the higher amounts of the total free amino acids and amides in the healthy leaves, the percentage of these amino acids and amides in the pool was remarkably similar to the percentage composition of the corresponding amino acid and amide pool in the leaves of exocortis-infected plants (Table 2).

Roots.—Seventeen ninhydrin-positive materials were found in the citrus roots (Table 2). Aspartic acid, glutamic acid, serine, glycine, glutamine, alanine, γ-aminobutyric acid, leucines, ethanolamine, asparagine, and proline were the most prevalent in both healthy and diseased plant groups. Proline was the most abundant and accounted for approximately 40 per cent of the total free amino acids and amides. In contrast to the leaves, which had less than 1 per cent asparagine, the roots had approximately 20 per cent, so that asparagine and proline combined accounted for more than 60 per cent of the total free amino acids and amides in the roots. Threonine, tyrosine, valine-methionine, methionine sulfoxide, arginine, and lysine were also found in small amounts.

The total amount of free amino acids and amides was essentially the same (average 865 μg/g) in the roots of the healthy trees as in the exocortis-infected trees. The percentage of each of the amino acids and amides, with the exception of asparagine, which was approximately 6 per cent higher in the healthy plants, was also virtually the same for both healthy and diseased plant groups (Table 2)

Discussion

The two to eight years that are required for symptoms of exocortis virus to develop on the trifoliate rootstock suggest a gradual biochemical change in the host even though ray cells of the rootstock may begin to show color production with phloroglucinol-HC1 approximately two years

TABLE 2. FREE AMINO ACIDS AND AMIDES IN THE LEAVES AND ROOTS OF HEALTHY AND EXOCORTIS-INFECTED C. SINENSIS (VAR. HAMLIN) ON P. TRIFOLIATA ROOTSTOCK. (μG/G FRESH WEIGHT OF TISSUES)

Amino Acid	Leaves							
	Healthy				Exocortis-Infected			
	Tree No.			Avg.	Tree No.			Avg.
	H-1	H-2	H-3	%	E-1	E-2	E-3	%
Aspartic Acid	50	40	20	1.4	8	5	5	0.3
Glutamic Acid	65	60	35	2.0	15	25	33	1.4
Serine	140	100	50	3.7	38	58	68	3.1
Asparagine	40	20	10	0.9	10	20	20	0.9
Glycine	50	30	20	1.3	5	20	50	1.4
Threonine	30	35	20	1.0	15	25	28	1.3
Glutamine	160	120	60	4.4	43	85	73	3.8
Alanine	155	140	100	5.0	70	100	100	5.1
Υ-aminobutyric Acid	200	185	120	6.5	120	160	180	8.6
Proline	2200	1760	1080	64.7	630	1300	1320	61.2
Tyrosine	30	20	10	0.8	10	10	10	0.6
Valine-Methionine	20	20	20	0.8	15	23	23	1.1
Leucines	30	35	30	1.2	33	48	45	2.4
Pipecolic Acid	30	20	30	1.0	28	40	20	1.6
Methionine Sulfoxide	6	6	6	0.2	3	5	3	0.2
Arginine	10	10	6	0.3	5	5	10	0.4
Lysine	10	6	6	0.3	5	5	10	0.4
Ethanolamine	40	45	45	1.7	40	48	35	2.3
Unknown A[a]	40	30	30	1.3	35	45	25	2.0
Unknown B[b]	30	40	35	1.3	45	35	25	2.0
Totals	3336	2722	1733	99.8	1173	2062	2083	100.1

Amino Acid	Roots							
	Healthy				Exocortis-Infected			
	Tree No.			Avg.	Tree No.			Avg.
	H-1	H-2	H-3	%	E-1	E-2	E-3	%
Aspartic Acid	28	18	18	2.3	23	18	20	2.5
Glutamic Acid	70	45	55	6.1	80	40	45	6.8
Serine	20	10	10	1.4	18	10	10	1.5
Asparagine	310	130	280	26.0	230	105	145	19.8
Glycine	20	10	5	1.3	5	5	5	0.6
Threonine	10	5	5	0.7	10	5	5	0.8
Glutamine	20	20	23	2.3	23	20	15	2.4
Alanine	48	40	50	5.0	50	40	40	5.4
Υ-aminobutyric Acid	93	85	65	8.7	80	70	70	9.1
Proline	320	200	545	38.4	450	280	280	41.6
Tyrosine	15	5	5	0.9	10	5	10	1.0
Valine-Methionine	8	8	8	0.8	28	15	15	2.4
Leucines	23	18	20	2.2	20	10	13	1.7
Methionine Sulfoxide	3	3	—	0.3	3	2	—	0.3
Arginine	15	5	5	0.9	10	10	10	1.2
Lysine	10	5	5	0.7	10	5	10	1.0
Ethanolamine	20	25	18	2.3	15	15	15	1.9
Totals	1033	632	1117	100.3	1065	655	708	100.5

[a]Rf Butanol-acetic acid-water 0.54 and phenol-ammonia-water 0.75.
[b]Rf Butanol-acetic acid-water 0.68 and phenol-ammonia-water 0.91.

after infection (4). These changes in the root apparently do not alter the amino acid metabolism in the stock since neither the total free amino acids and amides nor the percentage composition of these constituents is changed. The effect on the free amino acids in exocortis-infected plants appears to be confined to the scion in which only the absolute amounts of the free amino acids and amides were reduced, in this case, by approximately 50 per cent. Since the percentage of these constituents is essentially the same for the healthy and virus-infected plants, we believe that there is actually no imbalance in the composition of the amino acid pool of the exocortis-infected plants.

Since the percentage of the free amino acids and amides noted in the healthy and exocortis-infected trees is within the range observed for other citrus species (8, 13), the present data would tend to support the proposal that there may be little or no girdling in the exocortis-infected trees. Girdled citrus trees tend to accumulate proline and arginine in the leaves with a concomitant decrease in proline in the roots (13).

It is evident from these investigations that additional studies on other biochemical aspects such as polyphenols, protein amino acids, and organic acids using various stock-scion combinations should be considered for interpreting the biochemical response of citrus to virus diseases.

Literature Cited

1. ALLISON, R. M. 1953. Effect of leaf roll virus infection on the soluble nitrogen composition of potato tubers. Nature 171: 573.
2. BENEDICT, W. G., and HILDEBRAND, A. A. 1958. The application of chromatographic methods to a study of the susceptibility of soybean to stem canker. Can. J. Plant Sci. 38: 155-163.
3. BURTON, C. L., and DEZEEUW, D. J. 1961. Free amino acid constitutions of healthy and scab-infected cucumber foliage. Phytopathology 51: 776-777.
4. CHILDS, J. F. L., NORMAN, G. G., and EICHHORN, J. L. 1958. A color test for exocortis infection in Poncirus trifoliata. Phytopathology 48: 426-432.
5. DIENER, T. O., and WEAVER, M. L. 1957. On the significances of proline and pipecolic acid accumulation in Western X diseased peach leaves. Phytopathology 47: 8.
6. DIENER, T. O. 1960. Free amino acids and amides in healthy and virus infected cherry and peach leaves. Phytopathology 50: 141-145.
7. FIFE, J. M. 1956. Changes in the concentration of amino acids in the leaves of sugar beet plants affected with curlytop. J. Am. Soc. Sugar Beet Technologists 9: 207-211.
8. HANKS, R. W., and FELDMAN, A. W. 1963. Comparison of free amino acids and amides in roots of healthy and Radopholus similis-infected grapefruit seedlings. Phytopathology 53: 419-422.
9. KRUSBERG, L. R. 1961. Studies on the culturing and parasitism of plant-parasitic nematodes, in particular Ditylenchus dipsaci and Aphelenchoides ritzambosi on alfalfa tissue. Nematologica 6: 181-200.
10. SELMAN, I. W., BRIERLEY, M. R., PEGG, G. F., and HILL, T. A. 1961. Changes in the free amino acids and amides in tomato plants inoculated with tomato spotted wilt virus. Ann. Appl. Biol. 49: 601-615.
11. SHAW, M., and COLOTELLO, N. 1961. The physiology of host-parasite relations. VII. The effect of stem rust on the nitrogen and amino acids in wheat leaves. Can. J. Botany 39: 1351-1372.
12. STEINBERG, R. A. 1952. Frenching symptoms produced in Nicotiana tabacum and N. rustica with optical isomers of isoleucine, leucine, and Bacillus cereus toxin. Plant Phys. 27: 302-308.
13. STEWART, I. 1961. Nitrogen transformations in citrus trees. Proc. Soil Crop Sci. Soc. Florida 21: 272-282.
14. WOLTZ, S. S. 1963. Growth-modifying and antimetabolite effects of amino acids on chrysanthemum. Plant Phys. 38: 93-99.

This paper is the Florida Agricultural Experiment Stations' Journal Series No. 1701.