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Effect of Root Aeration on Amino Acid Levels in Cotton Plants[1]

Gene Guinn and L. A. Brinkerhoff[2]

ABSTRACT

Oxygen deficiency in the rooting medium altered amino acid levels in roots, xylem sap, and leaves of cotton plants (*Gossypium hirsutum* L.). In general, poor root aeration increased amino acid levels in roots and sap, but decreased amino acid levels in leaves. Poor root aeration greatly increased alanine and also increased serine, gamma-aminobutyric acid, glutamic acid, and glutamine contents of roots and xylem sap. On the other hand, poor root aeration had almost no effect on asparagine content of roots and decreased asparagine contents of xylem sap and leaves.

Aditional index words: **Alanine, Gamma-aminobutyric acid, Asparagine, Aspartic acid, Glutamic acid, Glutamine, Oxygen deficiency, Serine, Verticillium wilt, Xylem sap.**

SEVERAL workers have reported changes in amino acid composition of plant tissues in response to oxygen deficiency. Near anaerobic conditions greatly increased the level of gamma-aminobutyric acid (GABA) in leaves (8, 10, 12, 14) and tissue cultures (15). Alanine also increased in radish and turnip leaves deprived of oxygen, but not as much as GABA (12, 14). Less attention has been given to effects of oxygen deficiency in roots even though roots are more frequently subjected to oxygen deficiency under field conditions than are leaves. Dubinina (4) reported that poor root aeration caused accumulation of several organic and amino acids in roots of pumpkin, tomato, and willow. Grineva (6) found that oxygen deficiency not only caused amino acids to accumulate but also caused roots to excrete sugars, amino acids, and organic acids.

In this paper we report the effects of poor root aeration on the levels of certain amino acids and amides in roots, xylem sap, and leaves of cotton plants.

MATERIALS AND METHODS

Plant Culture

Cotton seeds (*Gossypium hirsutum* L. 'Westburn') were germinated in vermiculite, selected for uniformity, and transferred to a complete nutrient solution modified from that of Arnon and Hoagland (1). Iron was supplied as ferric EDTA. Continu-

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[2] Plant Physiologist and Plant Pathologist, Crops Research Division, and Department of Botany and Plant Pathology, Oklahoma State University, Stillwater, Okla. 74074.

ous forced aeration was provided to roots of all plants until time for treatment. Three groups of plants were cultured in a fiberglass greenhouse to provide (a) roots and leaves for amino acid analyses, (b) xylem sap samples, and (c) roots for NAD and NADH assays. Four plants were grown in each 12-liter polyethylene container and treatments were replicated at least four times, except that xylem sap samples were pooled from 32 plants in each treatment.

The first group of plants was cultured during September. Nutrient solutions were renewed after 20 days and aeration in half the containers was discontinued the next day. Plants were harvested 1, 4, and 7 days after aeration was discontinued. A Yellow Springs Instrument Co.[3] oxygen monitor was used to estimate the relative concentrations of oxygen in the aerated and non-aerated nutrient solutions just prior to each harvest. Roots and leaves were rinsed successively in tap and distilled water, removed from the plants, and lyophilized. The dried tissues were stored over $CaCl_2$ at —26 C and ground to pass a 60-mesh screen before analysis.

Plants from which xylem sap was collected were grown during May and June. Nutrient solutions were renewed 21 and 31 days after transfer of seedlings. Aeration was discontinued to half the plants at the time of the second renewal and xylem sap was collected 4 days later. Plants were decapitated just below the cotyledonary node, the bark peeled back, the stumps rinsed with deionized water, and short pieces of latex tubing fitted over the stumps to serve as reservoirs. Sap was removed from these reservoirs every 30 min and transferred to a freezer where it was stored until amino acids could be chromatographed.

Plants for NAD and NADH assay were cultured during late October, November, and early December. Roots were harvested 4 days after aeration was discontinued to half the plants. In order to minimize oxidation of NADH, roots were drained briefly, rinsed successively in ice-cold tap and distilled water, placed in paper sacks, immersed in liquid nitrogen, and lyophilized.

Amino Acids: Extraction, Purification, Chromatography

Amino acids were removed from 500-mg portions of dry tissue by three successive 15-min extractions with 15-ml portions of 70% ethanol at 80 C. Ethanol was removed *in vacuo* and the aqueous residue made to 20 ml with water and shaken with 5 ml of chloroform to remove residual lipids and protein (11). After centrifugation the upper phase was purified on a column of Dowex 50W[3] by a modification of the method of Thompson et al. (13). We used an 0.8- by 15-cm column of 50- to 100-mesh resin in the H^+ form and eluted amino acids from this with 75 ml of 2*N* NH_4OH. The NH_3 was removed *in vacuo* and the residue dissolved in 2 ml of water and shaken with 1 ml of chloroform.

Ten-ml portions of xylem sap were purified on Dowex 50W as outlined above for extracts from roots and leaves.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

Amino acids were separated by two-dimensional descending paper chromatography. A mixture of 1-butanol, acetic acid, and water (60:15:25, v:v:v) was used in the first dimension and an 80% aqueous solution of fused phenol was used in the second dimension. The papers were dried thoroughly then dipped through a 0.5% (w:v) solution of ninhydrin in acetone and placed in a moist oven for 15 min at 90 C. Eight amino acids were identified by co-chromatography with standards. (Glycine is not listed in the tables because of the small amounts found.) Although other spots were present they either could not be identified or appeared too faint to quantitate. Color was eluted from identified spots with 50% ethanol and absorbance determined at approximately 560 nm in a colorimeter.

NAD and NADH

Attempts were made to measure nicotinamide adenine dinucleotide in the oxidized (NAD) and reduced (NADH) forms. Dry root samples were extracted with 0.1 *N* HCl or 0.1 *N* NaOH, the extracts neutralized, and the rate of reduction of dichlorophenolindophenol measured in the presence of NAD diaphorase, ethanol, and yeast alcohol dehydrogenase (16).

RESULTS AND DISCUSSION

Oxygen level in the non-aerated nutrient solutions dropped to about 15% of the level in aerated solutions within 24 hours and remained at this level during the remainder of the 7-day experimental period.

Roots and xylem sap showed striking changes in amino acid composition in response to oxygen deficiency in the nutrient solution (Tables 1 and 2). A number of factors may have been responsible for these changes, including a decreased rate of protein synthesis, a decreased flow of xylem sap, and an accumulation of precursor organic acids.

It seems likely that ATP content would decrease in oxygen-deficient roots because of a decrease in rate of oxidative phosphorylation. Since RNA and protein synthesis require nucleotide triphosphates (7), a decrease in rate of oxidative phosphorylation should slow protein synthesis and permit free amino acids to accumulate.

Xylem sap exuded much less rapidly from non-aerated than from aerated roots. Only 39 ml of sap were collected from non-aerated plants compared with 103 ml from the aerated plants. Therefore, the slower flow rate could partially account for the higher concentration of amino acids in xylem sap of non-aerated plants and could have contributed to accumulation in roots because of less rapid removal. (However, flow rate caused by root pressure may be unrelated to flow rate caused by transpiration in intact plants.)

Table 1. Amino acids in roots as influenced by two levels of root aeration.*

Root treatment	Asparagine	Glutamine	Aspartate	Glutamate	Serine	Alanine	GABA
			After 1 day				
Aerated	26 ± 0.26	0.7 ± .03	10.6 ± .05	8.5 ± .18	4.4 ± .08	2.0 ± 0.11	0.8 ± .07
Not aerated	36 ± 0.90	1.0 ± .08	11.4 ± .54	15.2 ± .32	9.8 ± .39	16.8 ± 2.36	5.8 ± .13
			After 4 days				
Aerated	33 ± 0.78	0.6 ± .02	15.3 ± .86	11.4 ± .35	4.2 ± .04	2.3 ± 0.08	1.1 ± .12
Not aerated	34 ± 1.30	1.9 ± .11	14.6 ± .14	17.0 ± .32	14.0 ± .49	33.6 ± 2.19	5.8 ± .22
			After 7 days				
Aerated	29 ± 1.36	1.3 ± .07	12.5 ± .24	9.7 ± .34	5.4 ± .17	4.0 ± 0.38	1.2 ± .08
Not aerated	31 ± 1.00	5.4 ± .56	11.4 ± .60	16.2 ± .21	21.8 ± .73	77.0 ± 0.70	5.6 ± .33

* Amino acid concentrations are expressed as micromoles per g dry wt of root tissue and are averages of four replications. Standard errors are shown.

Table 2. Amino acids in xylem sap as influenced by two levels of root aeration.*

Root treatment	Asparagine	Glutamine	Aspartate	Glutamate	Serine	Alanine	GABA
Aerated	2,770 ± 339	84 ± 6.4	302 ± 20	46 ± 4.4	79 ± 4.8	96 ± 61	24 ± 1.4
Not aerated	2,213 ± 144	530 ± 48.0	924 ± 78	148 ± 2.8	1,350 ± 60.0	4,724 ± 166	778 ± 74.0

* Sap was pooled from 32 plants in each aeration treatment and was collected four days after aeration was discontinued in the not aerated treatment. Amino acid concentrations are expressed as micromoles per liter of exuded sap and are averages of four determinations. Standard deviations are shown.

Table 3. Amino acids in leaves as influenced by two levels of root aeration.*

Root treatment	Asparagine	Glutamine	Aspartate	Glutamate	Serine	Alanine	GABA
				After 1 day			
Aerated	12.2 ± .99	4.0 ± .38	10.0 ± .30	14.8 ± .46	4.4 ± .19	6.8 ± .42	1.2 ± .08
Not aerated	8.0 ± .55	1.4 ± .10	8.0 ± .28	12.8 ± .29	3.0 ± .07	4.8 ± .14	0.7 ± .04
				After 4 days			
Aerated	21.2 ± .75	4.2 ± .40	14.8 ± .26	19.4 ± .38	6.8 ± .12	5.6 ± .19	2.4 ± .26
Not aerated	8.2 ± .08	0.4 ± .03	8.6 ± .42	12.2 ± .49	3.2 ± .11	2.6 ± .19	0.7 ± .08
				After 7 days			
Aerated	5.7 ± .83	0.8 ± .11	5.2 ± .21	5.0 ± .08	1.7 ± .08	2.2 ± .08	0.6 ± .04
Not aerated	2.0 ± .17	0.6 ± .05	5.3 ± .25	4.8 ± .25	1.8 ± .06	2.2 ± .10	0.6 ± .02

* Amino acid concentrations are expressed as micromoles per g dry wt of leaf tissue and are averages of four replications. Standard errors are shown.

In general, there appeared to be a relationship between accumulation of amino acids and the number of oxidative steps required for production of their precursor organic acids. Alanine accumulated more than other amino acids. Pyruvic acid, a precursor of alanine, is less oxidized than precursors of other amino acids. Serine, which can be derived from hydroxypyruvic acid (5), also accumulated in roots and xylem sap. Glutamic acid, gamma-aminobutyric acid (GABA), and glutamine can all be derived from alpha-ketoglutaric acid (5, 10, 12), which is two oxidative steps removed from pyruvic acid. These amino acids did accumulate, but not nearly as much as did alanine. Aspartic acid did not accumulate in roots. Oxaloacetic acid is a precursor of aspartic acid and, if formed by normal operation of the citric acid cycle, requires more oxidative steps for formation than any of the other alpha-keto acids which serve as precursors for amino acid synthesis. Asparagine did not accumulate appreciably in non-aerated roots and was the only amino acid which decreased in xylem sap from non-aerated roots. Since the pathway for asparagine biosynthesis is still in doubt, a logical explanation cannot be given for asparagine decreases, although a decreased level of ATP may have been a factor. Asparagine was the major form of organic nitrogen in xylem sap of aerated plants but was exceeded by alanine when roots were subjected to oxygen deficiency (Table 2).

In contrast to the effects of poor root aeration on amino acid composition of roots and xylem sap, poor root aeration tended to cause a decrease in amino acid content of leaves (Table 3). Asparagine content of leaves was markedly decreased by depriving roots of oxygen, possibly because of a decreased rate of transport from roots to leaves.

Because of the apparent relationship between oxidation level of precursor organic acids and accumulation of various amino acids in oxygen-deficient roots, we suspected that the ratio of NAD:NADH shifted toward the reduced form in response to oxygen deficiency. An accumulation of NADH should promote nitrate reduction and a decrease in NAD should cause pyruvic and alpha-ketoglutaric acids to accumulate. Dubinina (4) found that an oxygen deficiency caused pyruvic, malic, and succinic acids to accumulate in roots. However, we were not able to detect NADH in either aerated or non-aerated cotton roots. Results obtained with internal standards indicated that NaOH and KOH extracted enzyme inhibitors from cotton roots which strongly interfered with the assay for NADH. It is also possible that NADH was oxidized to NAD during harvest even though precautions were taken to prevent such oxidation. Therefore, the apparent abscence of NADH in roots may not have been true for oxygen-deficient roots prior to harvest.

The striking increases in alanine content of roots and xylem sap and substantial increases in other amino acids may affect development of Verticillium wilt, and possibly other diseases, when cotton roots are subjected to oxygen deficiency. We found that both xylem sap and root homogenate from oxygen-deficient roots supported better growth of *Verticillium albo-atrum* Reinke and Berth than similar solutions from well-aerated roots. Malca et al. (9) reported that a number of amino acids, including GABA and alanine, gave better growth of *V. albo-atrum* than inorganic nitrogen. Christie (3) found that three isolates of Verticillium grew better with aspartic acid, glutamic acid, or alanine than with other amino acids as sources of nitrogen. Booth (2) recently reported that a susceptible line of cotton exuded more alanine from roots than did a Verticillium-tolerant line.

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