Plant Physiol. (1986) 82, 724–728
0032-0889/86/82/0724/05/$01.00/0

Effects of Water Stress on the Organic Acid and Carbohydrate Compositions of Cotton Plants

Received for publication October 25, 1985 and in revised form July 9, 1986

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ABSTRACT

Two photoperiodic cotton (*Gossypium hirsutum* L.) strains (T185 and T466) which had been empirically selected because of poor performance and two strains (T25 and T256) selected because of enhanced performance under field water stress were evaluated for stress-induced changes in their organic acids and carbohydrates. Profiles and quantitation of organic acids and carbohydrates from aqueous extractions of cotton leaf tissue were determined by high performance liquid chromatography. In all cases, the water-stressed plants showed two to five times greater amounts of organic acids and carbohydrates over the values determined for the irrigated samples. Under stress, sucrose accumulation was observed in wilting strains (poor performers) probably related to rate of translocation out of the leaf. The most dramatic response to water stress was the accumulation of citric acid in strains T25 and T256 as compared to T185 and T466. Citric/malic acid ratios for both the irrigated and water-stressed samples of T25 and T256 were twice those of T185 and T466.

Organic acids and carbohydrates have been implicated in various roles in the metabolic and physiological responses of plants to water stress. Early papers by Eaton and Ergle (7–9) reported that, at low water levels, carbohydrates accumulated in cotton plants which had complete fruit loads. In cotton leaves, drought caused large reductions in starch concentrations, variable effects on sucrose, and an increase in hexose sugar. The gains in sugars were substantial on a relative basis but were minor in actual amounts. They concluded that drought appears to depress carbohydrate utilization by the cotton plant to a greater extent than it does photosynthesis (7).

Malic, citric, and oxalic acids are frequently found in large amounts (several percent of the dry weight of the tissue) in mature tissues or organs of plants (4). For cotton, higher concentrations of organic acids are found in the leaves than in other vegetative parts, frequently at 10% of the basis of dry weight (8). The major acids accounting for a total content of approximately 10% of the dry weight of cotton leaves were identified as citric, malic, and oxalic, with malic usually present in the greatest amount. It was reported that drought caused large reductions in concentrations of citric acid with gains in malic acid but with overall maintenance of the level of total organic acids (8). Despite the assumption that carbohydrates are precursors of the organic acids, Ergle's group failed to find consistent correlations between the carbohydrate and organic acid data.

Tissue analysis of several osmotically active solutes in cotton by Cutler and Rains (6) indicated that soluble sugars and malate accumulate to about the same levels (on dry weight basis) in both stress-conditioned and unconditioned plant exposed to stress. Citric was the only other acid present in cotton leaves in measurable quantities. Accumulation could not account for the turgor change. Radin *et al.* (13–15) studied the water relations of cotton plants under nitrogen deficiency. Osmotic potentials were slightly lower in nitrogen-deficient leaves. The difference in solute concentrations was not from organic acids which were almost unchanged. Sugar concentrations could account for only about 25% of the difference. Only malic and citric acids were present in more than trace quantities.

Ackerson (1–3) studied osmoregulation in response to water stress with emphasis on the role of specific photosynthetically derived solutes and their relation to osmotic adjustment for water stress-acclimated and nonacclimated cotton plants. Acclimated plants exhibited lower rates of photosynthesis than did nonacclimated plants when leaves were fully turgid. Acclimated plants had more glucose than nonacclimated plants with the pattern of accumulation dependent upon leaf age. Sucrose accumulation in response to decreasing water potential also depended on leaf age. Acclimated young leaves exported sucrose, whereas nonacclimated leaves of the same age accumulated sucrose at the same leaf water potential. Older leaves of both acclimated and nonacclimated plants accumulated sucrose as plants became stressed during the day (2).

This study investigates the effect of water stress upon the organic acid and carbohydrate compositions of leaf samples from four strains of field-grown cotton to determine which metabolic components, if any, are correlated with enhanced performance under water stress.

MATERIALS AND METHODS

Four strains of photoperiodic cottons (*Gossypium hirsutum* L.) were selected for study under drought conditions because of their range of responses to water stress, their morphological character, and their diverse root systems observed during several years of testing. Nonflowering stocks were used to reduce the variability in growth associated with changing sink-source relationships with boll development (10, 11). The nonflowering strains were chosen from the world collection of approximately 1400 strains of *G. hirsutum* L. maintained by the USDA-ARS cotton program at College Station, TX. The Texas designations of the strains analyzed in this study are T25, T185, T256, and T466. The strain T25, which performs well under dryland conditions, has a fibrous root system with a short tap root and

numerous lateral branches, has small leaves, and tends to retain leaf turgor (10, 12). The strain T256, which grows taller than T25, has a long tap root with numerous laterals, has medium leaves, and is also nonwilting. In contrast, T185 has an intermediate root system (both tap and laterals), has large leaves, and wilts in response to water stress. T466 also has a poor root system, has small leaves, and wilts during periods of water stress.

The four nonflowering (photoperiodic) cotton strains were planted in randomized complete block designs in Lubbock, TX. The soil type was an Acuff fine sandy loam soil (fine-loamy, mixed, thermic, Aridic Paleustoll). Three replications of each strain were planted on May 17, 1983. The four strains were planted in five 3 m rows with 1 m between rows. Plants were thinned after emergence to 11 plants/m. The two soil-water regimes consisted of fully irrigated and dryland tests located in the same field about 30 m apart. The strains in the dryland treatment were planted in a rainout shelter to prevent the addition of supplemental water by rainfall. Following a preplant irrigation there was no further irrigation of the dryland plot during the study. The irrigated plants, which were located in plots outside the confines of the rainout shelter, were watered weekly to field capacity with a drip irrigation system. The three center rows were used for analysis.

Leaf samples from both irrigated and dryland plots were harvested at 108 DAP[1] for analysis of organic acid and carbohydrate compositions. The fully expanded leaves were randomly selected from each of the four strains and stored at −80°C until sampling and extraction.

Samples were taken randomly from the leaves with a cork borer for consistent leaf area. A sample of 12 leaf discs was used for each extraction with two replicate extractions for each strain. The leaf discs were pulverized in liquid N_2 in the presence of PVPP (Sigma)[2] for removal of phenolics using an amount approximately equivalent to the fresh weight. Following addition of 3 ml of deionized H_2O, the samples were homogenized. Subsequent filtration into sample vials completed preparation for HPLC analysis.

Comparisons of HPLC profiles of organic acids and carbohydrates were used to assess the effectiveness of extraction procedures. Fresh leaf tissue was extracted by: (a) ambient deionized H_2O followed by homogenization, (b) 80°C water followed by homogenization, then maintenance at 80°C for 10 min. Dried leaf tissue was treated as in (a). Sucrose levels were not significantly different in any of these cases. The ambient water extraction gave the most complete extraction of all components as well as being the simplest in operation. Throughout sampling, preparation, and storage, samples were maintained at −80°C as much as possible to minimize sucrose inversion.

A Beckman microprocessor controlled HPLC system was used with operation conditions of column temperature at 40°C, isocratic mode with dilute H_2SO_4 (pH = 2.1), and a BioRad Organic Acid Column HPX-87H. The detectors employed were RI (Beckman model 156) and a variable wavelength UV-visible with rapid scanning (Beckman model 165). The UV channels were set at 210 and 254 nm with spectral scan (190–700 nm) of peaks detected on the 210 nm channel. Comparison of spectral scans was used for identification. Data analysis was by computer employing Laboratory Automation System (Hewlett Packard model 3357). Two replicate runs were made by HPLC for each extraction. Quantitation of components was based upon peak area calculations related to standard curves determined from at least three concentrations of standards.

A fresh weight was measured for each individual sample of leaf discs. The percentage dry weights of the samples were determined from duplicate but separate samples. Samples were weighed to obtain the fresh weight, then microwaved to dryness, stored in a desiccator, and then weighed.

Statistical analysis was carried using the SAS by analysis of variance. Duncan's multiple range test was also run where appropriate.

RESULTS AND DISCUSSION

The effects of water deficits on the water status and development of the photoperiodic cotton strains were monitored throughout the growing season by measurement of: (a) changes in leaf water status, and (b) reduction in plant growth. In conjunction with these analyses, measurements of organic acid and carbohydrate levels in the leaves of the four strains were determined.

Changes in Leaf Water Status. The midday leaf water status of the photoperiodic cotton strains was monitored at 50 and 106 DAP. The water status data for two of the strains are presented in Table I. Approximately a 1.5-fold difference in leaf water potential occurred between the irrigated and dryland treatment at 106 DAP. Throughout the course of this experiment, the effects of soil water deficits on leaf water status were enhanced in the T185 material compared with T25. The T25 turgor potentials for both the irrigated and dryland treatments remained relatively constant between treatments, with a reduction in the turgor potential from 0.82 to 0.45 MPa from 50 to 106 DAP (Table I). The T185 potentials exhibited a greater decline between 50 and 106 DAP with an average decrease from 0.75 to 0.12 MPa. The leaf water status data show the development of water stress on the two photoperiodic cotton strains. T256 water status closely resembled that of T25; and T466 water status followed that of T185 (data not shown).

Stress-Induced Changes in Plant Morphology. Water stress effects were observed for all four strains in morphological responses measured in the field. Overall, major reductions were noted with water stress for height (65–80%), leaf number (60–80%), leaf area index (75–85%), fresh weight (75–80%), and dry weight (70–80%). The range of response generally varied according to strain. Interestingly enough, comparisons of strains at a given age under dryland conditions indicate that all four have approximately the same total leaf area despite the range of height and leaf size distributions because those strains with small leaves have greater numbers of leaves than do the larger leafed strains. Notwithstanding the equivalent total leaf areas, biomass production reflected differences in acclimation. The strains T25 and T256 had shoot dry weights of 1830 and 1815 kg/ha for dryland conditions, while the strains T185 and T466 had shoot dry weights of 1290 and 1520 kg/ha. T25 and T256 also appear to perform better under stress than do T185 and T466 by maintaining turgor of leaves (not wilting).

Comparisons of the fresh weights and percentage dry weights were made for the strains under both irrigated (control) and dryland (stressed) conditions in Table II. The dryland samples had greater fresh weights than the irrigated samples and corresponding larger percentage dry matter accumulation. These results appear to contrast Ackerson's data (2) where the ratios of dry weight/fresh weight (or % dry weight) were smaller for acclimated plants than for control (nonacclimated) plants. However, it should be pointed out that the acclimated plants in Ackerson's study had been subjected to a series of water deficits, each followed by irrigation, rather than subjected to continuous, increasing water-deficit conditions of this study (5). Quisenberry

[1] Abbreviations: DAP, days after planting; PVPP, polyvinylpolypyrrolidone; SAS, Statistical Analysis System; LAI, leaf area index; RI, refractive index.

[2] Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the United States Department of Agriculture over others not mentioned.

Table I. *Midday Water Status of Two Photoperiodic Cotton Strains under Irrigated and Dryland Conditions*
Standard deviations (±) are indicated

Treatment	DAP					
	50			106		
	Water potential	Osmotic potential	Turgor potential	Water potential	Osmotic potential	Turgor potential
	MPa					
Irrigated						
T25	−0.45 ± 0.03	−1.29 ± 0.06	0.84 ± 0.05	−1.34 ± 0.12	−1.73 ± 0.07	0.39 ± 0.07
T185	−0.67 ± 0.07	−1.51 ± 0.06	0.84 ± 0.06	−1.60 ± 0.12	−1.72 ± 0.04	0.12 ± 0.10
Dryland						
T25	−0.83 ± 0.03	−1.63 ± 0.07	0.81 ± 0.05	−1.89 ± 0.08	−2.38 ± 0.11	0.49 ± 0.08
T185	−0.94 ± 0.05	−1.64 ± 0.09	0.65 ± 0.04	−2.38 ± 0.17	−2.51 ± 0.19	0.12 ± 0.08

Table II. *Fresh and Dry Weights of Samples*
Means of duplicate samples. Standard deviations (±) are indicated.

Strain	Condition	Fresh Wt[a]	Dry Wt
		g	%
T25	Irrigated	0.0959 ± 0.0069	23.7 ± 0.8
T25	Dryland	0.1841 ± 0.0040	25.5 ± 1.3
T185	Irrigated	0.1114 ± 0.0013	22.2 ± 0.9
T185	Dryland	0.1670 ± 0.0017	32.5 ± 0.4
T256	Irrigated	0.1411 ± 0.0017	25.8 ± 1.6
T256	Dryland	0.1671 ± 0.0037	33.5 ± 1.8
T466	Irrigated	0.1339 ± 0.0071	23.9 ± 1.1
T466	Dryland	0.1961 ± 0.0015	29.2 ± 0.8

[a] 12 leaf discs; total leaf surface area = 6.04 cm^2.

(11) observed that some cotton strains did produce proportionally higher dry weights under stress in a study of exotic strains similar to those reported here. In this study, measurement of percent dry matter of the whole plant (above ground) gave values which parallel those for the percent dry weight of the leaf discs.

Identification and quantitation of the five major components are given based upon the dry weight of sample (Table III). The water-stressed plants showed two to five times greater total amounts of organic acids and carbohydrates (malic, citric, and oxalic acids, glucose, and sucrose) over the values determined for the irrigated samples based upon leaf dry weight (Table III), area and fresh weight (data not shown). The levels of glucose and malate are in the same range with stress conditions as suggested by Cutler's observations (6).

The most striking result is the increase in material in response to water stress. The greatest increases are in the citric and 'oxalic' acid peaks (Table III). Oxalic acid is only a portion of the peak located at the retention time of 7 min. This peak can include any material that is excluded from the column and may be higher mol wt materials and/or oppositely charged materials. Approximation of the amount of material present has been made by quantitation based on oxalic acid. The phenolics have been removed in the cleanup procedures. Subsequent study employing octadecyl solid phase extraction columns has indicated the presence of nonpolar structures eluting under the excluded peaks which may be lipid components. Attempts to differentiate and further identify these materials are currently underway. A strong UV absorption associated with this peak tends to eliminate simple oligosaccharides which do not possess chromophoric groups. The amount of oxalic acid and accompanying materials increased with water stress.

The major objective of this study was to determine if chemical compositional parameters could be correlated with field performance. Statistical analysis of the data using Duncan's procedure is given in Table IV. It can be seen that the values for sucrose, citric acid, malic acid, total carbohydrates, and total organic acids are not significantly different for T25 and T256 but are significantly different from T185 and T466, thus falling into two groups. The level of oxalic acid is the only clear exception to this observation. Based upon the previously mentioned problems in the oxalic acid determination, the validity of this discrepancy must be questioned. These results can be used to classify the strains into groups that correspond with groupings observed in the field with respect to 'enhanced' (T25 and T256) *versus* 'poor' (T185 and T466) drought performance in maintaining leaf turgidity (Table I). The most complete group correspondence was for the dry weight basis: the organic acid components and sucrose, as well as the total amounts of carbohydrates and organic acids, grouped the strains as indicated for the drought stressed samples. The differences in the strain comparisons for citric acid at each condition should be noted; none of the strains are

Table III. *Quantitation on Dry Weight Basis*
Means of replicate runs of duplicate extractions.

	T25		T185		T256		T466	
	Irrigated	Dry	Irrigated	Dry	Irrigated	Dry	Irrigated	Dry
	mg/g dry wt							
Sucrose	6.2	6.4	8.4	28.1	4.7	5.4	16.7	29.2
Glucose	16.8	22.7	17.0	22.4	14.7	24.8	13.2	18.6
Citric acid	33.3	74.4	31.8	45.7	29.0	87.9	20.7	34.9
Malic acid	18.4	14.8	46.7	27.7	11.3	20.6	37.4	31.1
Oxalic Acid + excluded material	7.2	84.9	6.1	62.9	20.4	55.7	5.7	56.2
Total carbohydrates	23.0	29.1	25.3	50.5	19.3	30.2	29.9	47.8
Total organic acids	58.9	174.1	84.5	136.3	60.7	164.2	63.7	122.1
Total	81.8	203.2	109.8	186.7	80.0	194.3	93.6	169.8

Table IV. *Statistical Analysis of Organic Acid and Carbohydrate Levels by Duncan's Procedure*

Means not significantly different are connected by a straight line (α = 0.05). Designation by strain which are ranked by level from high to low (left to right).

	Dry Weight Basis			
	High			Low
Irrigated				
Sucrose	T466	T185	T25	T256
Glucose	T185	T25	T256	T466
Citric Acid	T25	T185	T256	T466
Malic Acid	T185	T466	T25	T256
Total Carbohydrates	T466	T185	T25	T256
Total Organic Acids	T185	T466	T256	T25
Total	T185	T466	T25	T256
Dryland				
Sucrose	T466	T185	T25	T256
Glucose	T256	T25	T185	T466
Citric Acid	T256	T25	T185	T466
Malic Acid	T466	T185	T256	T25
"Oxalic" Acid	T25	T185	T256	T466
Total Carbohydrates	T185	T466	T256	T25
Total Organic Acids	T25	T256	T185	T466
Total	T25	T256	T185	T466

Table V. *Characterization of Varieties by Organic Acid Composition: Ratio of Citric to Malic Acids*

Strain	Condition	Citric:Malic	Citric:Malic
		mg/g dry wt basis	*μmole/g dry wt basis*
T25	Irrigated	1.82a[a]	1.26
T25	Dryland	5.10c	3.51
T185	Irrigated	0.67b	0.48
T185	Dryland	1.65b	1.15
T256	Irrigated	2.57a	1.79
T256	Dryland	4.28c	2.98
T466	Irrigated	0.56b	0.39
T466	Dryland	1.12b	0.78

[a] Means followed by different letters are statistically different at α = 0.05 by the Duncan's multiple range test.

significantly different for the irrigated conditions (for any calculation basis), while they are all significantly different or fall into the group classification for the dry condition. Malic acid levels for the controls for leaf area and fresh weight group according to performance but not for the stressed. The malic acid levels for the controls on a dry weight basis are statistically different for each strain but fall into the above-cited groups for the stressed samples. It cannot be discounted that solute differences among strains could be due to different levels of stress because, as noted, water potentials differ among strains.

Varying effects have been discussed with respect to the citric and malic acid contents so that an effort was made to further characterize the strains for any possible systematic compositional changes. The ratios of citric to malic acids were calculated and are listed in Table V for each strain under both conditions. Since the ratios are essentially the same for fresh weight, dry weight, and leaf area measurements, only one set of values is given. Statistical pairwise comparisons (Duncan's Multiple Range Test) indicate significant difference between the ratios for the irrigated and the dryland samples for T25 and T256 but no difference for T185 and T466. Additionally, at each condition (both irrigated and dryland), the strains were grouped into the same sets as for enhanced and poor drought performance.

How the enhanced drought performers are acclimating to the water stress can be summarized in the following way: Sucrose levels are the same for the irrigated and stressed. This may indicate that transport is continuing. The age of the plants (108 DAP) would seemingly preclude this possibility, that it must be remembered that these are nonflowering strains so that the source-sink relationships, *i.e.* mature developed bolls normally seen at this age, are not occurring. There are increases (30–60% of the control) in the glucose levels, possibly indicating solute accumulation. The effects in the glucose and sucrose composition are summed in the total carbohydrate composition which showed the increases mentioned with stress. It is difficult to assess the importance of these factors because, as Eaton and Ergle (7) have pointed out, the relative increases are significant, but the actual amounts are minor at approximately 2% of dry weight for irrigated rising to approximately 3% for stressed. It must be remembered that no account has been taken for compartmentalization or location of these components within the leaf tissue since these extractions have been made from ground-up tissue. Presumably, the changes in concentration, if occurring in a very small unit, could have significant impact.

Organic acid compositions for those strains that are enhanced performers under water-deficit conditions show more dramatic influences of stress. There are variable effects in malic acid levels but large increase (2–3×) in citric acid with stress. This change could represent osmoregulation. It should be noted that for T25 and T256 there are differences in individual responses, but the ratio of citric to malic was not significantly different for the two strains compared for either condition. The magnitudes of the values have increased with stress and are, therefore, different. The means of the ratios of citric to malic for irrigated and dryland conditions for T25 and T256 are almost identical (3.46–3.43). Under stress the sum of citric and malic acids increases 1.7 to 2.7 times. The oxalic acid component has many unknown factors included and, not surprisingly, does not correlate, but the total organic acid level including these components reflects 6% level for irrigated increasing by 10 to 11% to 16.4 to 17.4% of dry weight with stress. To reiterate, the overall total increase with stress is due to the organic acid pool size rather than the small increase from the glucose accumulation.

In contrast, the strains which are poor performers have accumulated sucrose compared to the irrigated controls (and the enhanced performers), possibly indicating changes in transport functioning in the manner of Ackerson's observations (2). Glucose levels have slightly increased just as with the enhanced performers making for total carbohydrate increases of approximately 2%. The major difference is in the organic acid makeup where citric has increased (although smaller than for the enhanced performers) and the malic acid decreased. Ratios of citric/malic acids are less than unity for irrigated and greater than unity for stressed, but are not significantly different for the two strains or for irrigated and stressed. The sum of malic and citric with stress response corresponds more closely with Eaton and Ergle's (8) observation of no change with stress. Thus, the overall change in organic acid for the poor drought performers is a 5.1 to 5.8% increase, totalling only 7.6% change.

The measurement of levels of chemical components within the leaf reflect the performance of the whole plant under water stress for different strains of cotton in the field. Thus, the validity of this approach to study the effects of stress-induced modifications in chemical compositions of crop plants to gain understand-

ing of mechanisms of acclimation behavior is confirmed by this preliminary study. These observations require further exploration to develop fully the potential implications of this system.

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