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Changes in Free and Conjugated Indole 3-Acetic Acid and Abscisic Acid in Young Cotton Fruits and Their Abscission Zones in Relation to Fruit Retention during and after Moisture Stress

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ABSTRACT

Experiments were conducted with field-grown cotton (*Gossypium hirsutum* L.) in 1985 and 1986 to determine effects of water deficit on levels of conjugated indole 3-acetic acid (IAA) and abscisic acid (ABA) in young fruits (bolls) and their abscission zones in relation to boll retention. Tissues were harvested three times during an irrigation cycle in 1985. They were harvested twice during an irrigation cycle and once after irrigation in 1986 to determine extent of recoveries of measured parameters. As reported earlier, the free IAA content of abscission zones decreased with moisture stress. Irrigation caused a partial recovery in free IAA content of abscission zones and caused a partial recovery in rate of boll retention. In contrast to free IAA, conjugated IAA increased with water deficit, both in 3-day-old bolls and in their abscission zones. Bolls contained much more ester IAA than their abscission zones. Some, but not all, of the increase in ester IAA in bolls during moisture stress could have come from a conversion of amide-linked IAA. Amide IAA decreased slightly during stress and increased after irrigation, but the concentration was low relative to ester IAA. Free and conjugated ABA both increased during stress and decreased after irrigation. However, the concentration of conjugated ABA remained relatively high in abscission zones. Ester IAA, being more resistant than free IAA to enzymic destruction during stress, may hasten recovery of fruit retention after relief of stress by providing a source of free IAA in abscission zones to inhibit continued abscission.

Water deficit decreases cotton fruit (boll) retention (12–15, 20), probably because it increases ethylene production (11), increases the ABA content of bolls and their abscission zones (12, 14), and decreases the IAA content of boll abscission zones (14). IAA delays or prevents abscission (2), in part because it prevents the synthesis of the specific cellulase in the abscission zone that can cause abscission (1, 19, 21).

The drought-induced decrease in the IAA content of abscission zones could result from decreased synthesis, decreased transport to the abscission zones, increased rate of destruction, or an increased rate of conversion of IAA to other forms such as esters and amides. Plant water deficits reduced the basipetal transport of IAA in cotyledonary and leaf petiole sections taken from cotton seedlings (9, 10), but no such measurements have been made in fruit peduncles. Evidence was also reported that water deficit may increase the rate of destruction of IAA by increasing the activity of IAA oxidase (8). Beyer and Morgan (4) reported that ethylene decreased transport of IAA, increased the rate of decarboxylation, and caused a significant increase in IAA metabolites. These effects could indirectly result from a water deficit because water deficit increases ethylene production in young cotton bolls (11).

A relatively small percentage of all forms of IAA occurs as free IAA in most plants; most of the IAA is conjugated with sugars and amino acids (7). Cohen and Bandurski (7) suggested four roles for IAA conjugates: (a) transport of IAA, (b) storage and subsequent reuse of IAA, (c) protection of IAA from enzymic destruction, and (d) the homeostatic control of the concentration of IAA in the plant. These postulated roles indicate that IAA conjugates may be important, especially in hastening the recovery of plants from stresses such as a temporary water deficit.

In contrast to IAA conjugates, ABA conjugates are thought to be stable end products (5) that are sequestered in the vacuoles (6). Other workers, however, suggested that conjugated ABA may serve as a reservoir for the release of free ABA (17, 18).

The purpose of the investigations reported here was to determine the influence of water deficit on the concentrations of the conjugated forms of IAA and ABA in young cotton bolls and their abscission zones. The free and conjugated forms of IAA and ABA were also measured after relief of stress to determine the extent to which they returned to original levels after irrigation.

MATERIALS AND METHODS

Plant Culture. Cotton (*Gossypium hirsutum* L. cv Deltapine 61)[1] seeds were planted on April 8, 1985 and on April 1, 1986 in a field at the Western Cotton Research Laboratory in Phoenix. After germination, seedlings were thinned to about 99,000 plants per ha. Some of the results from the 1985 experiment were reported earlier (14). The 1985 experiment was conducted to compare thinned and defruited plants with control plants through an irrigation cycle. Additional results from the control plots will be reported here. The 1986 experiment was part of a test that included nitrogen as a variable. Urea was applied to the control plots on May 28 to give about 168 Kg of N ha^{-1}. No fertilizer was applied to the low-N plots. Each plot was four rows (4 m) wide by 29 m long. Plots were separated by berms to prevent irrigation water and N from moving from one plot to another. Treatments were replicated four times in a randomized

[1] Names of products are included for the benefit of the reader and do not imply endorsement or preferential treatment by the United States Department of Agriculture.

complete block design.

Flowers were tagged on the day of anthesis with dated tags in both center rows of each plot. Bolls and their abscission zones were harvested 3 d later from one of the center rows in 1985, and from one center row and part of the other in 1986. An 8-m segment of one of the center rows was used for determination of boll retention. Three harvests were made during the July 3 to July 18 irrigation cycle in 1985. Flowers were tagged on July 5, 9, and 12; 3-d-old bolls were harvested on July 8, 12, and 15. Two harvests were made during the June 13 to June 27 irrigation cycle in 1986. Flowers were tagged on June 13 and June 23 and 3-d-old bolls were harvested on June 16 and June 26. The plots were irrigated on June 27 and flowers were tagged that day to determine changes in fruit retention and ABA and IAA concentrations after relief of stress. Bolls and their abscission zones were harvested June 30, 3 d after irrigation. Tagged bolls in the 8-m segments used for determination of boll retention were not disturbed until October. Tags on retained bolls were then collected and used to calculate boll retention rates for the different flowering dates.

To estimate midday leaf water potentials, two leaves were harvested from each plot between 1200 and 1400 h, placed in plastic bags in an ice chest, and taken to a nearby laboratory where xylem water potentials were estimated with a pressure chamber.

Plant Material. Bolls and their abscission zones were harvested between 0800 and 0900 h as reported earlier. In 1985, peduncles and fruiting branches were cut within about 2 mm of the separation layer of abscission zones, but in 1986 they were cut about 1 mm from the separation layer. Bolls and abscission zones were quickly frozen in aluminum pans at −80°C, lyophilized, weighed, ground to pass a 40-mesh screen, and stored in sealed vials at −80°C. As an additional precaution in 1986, vials with plant material were flushed with N_2 (injected through rubber septa) before they were sealed. This procedure was repeated each time the vials were opened to remove plant samples. The vials were kept out of the freezer only long enough to warm above the dew point before samples were removed for analyses and the vials were flushed with N_2 and returned to the freezer.

ABA and IAA Analyses. ABA and IAA were extracted, purified, and estimated by HPLC as described earlier (14, 16). Briefly, the method was as follows; Dry 200-mg samples were extracted with 80% methanol that contained antioxidants and internal standards of ^{14}C-ABA and ^{14}C-IAA. Methanol was removed with a rotary evaporator at about 35°C. Hexane was used to extract Chl and lipids before the pH was adjusted to 8 with K_2HPO_4 or higher with KOH and NaOH for alkaline hydrolysis of conjugated IAA and ABA. To determine conjugated ABA and ester IAA, samples were hydrolyzed in 1 N KOH at 25°C, 60 min (3). These conditions were just as effective as pH 11 for 1 h at 60°C for hydrolysis of conjugated ABA. Some impurities were extracted with ethyl acetate and the pH then adjusted to 2.8 with H_3PO_4. In 1985, the acidic solution was passed through a C18 cartridge to remove ABA and IAA from the aqueous solution. The cartridge was rinsed with 10 ml of 1 mM HCl, ABA and IAA were eluted with 15 ml of 0.02 N NH_4OH, and the pH of the effluent was quickly adjusted to 2.8 with H_3PO_4. ABA and IAA were then extracted into ether. Because recoveries were sometimes less than 50% with that procedure, ABA and IAA were partitioned into ether before they were passed through the C18 cartridge in 1986. In that variation, ether was removed *in vacuo*, the residue was dissolved in 0.5 ml of methanol, 5 ml of 1 mM HCl were added, and the mixture was loaded onto a C18 cartridge. The cartridge was rinsed with 10 ml of water before ABA and IAA were eluted with 5 ml of pure methanol, which was a more effective eluent than 0.02 N NH_4OH. The methanol was removed *in vacuo* at 35°C. The residue was dissolved in acetonitrile and filtered through a 0.2 μm nylon filter with centrifugation into a 1.5-ml conical tube. Acetonitrile was evaporated with a stream of N_2.

Boll samples harvested in 1986 were also hydrolyzed in 7 N NaOH under N_2 for 3 h at 100°C to determine amide IAA (3). After hydrolysis, the pH was adjusted to about 8.8 with 6 N H_3PO_4 while cooling in water. Some of the impurities were extracted with two 10-ml portions of ethyl acetate and one 10-ml portion of hexane before the pH was adjusted to 2.8. IAA was then extracted into ether and further purified on a C18 cartridge as outlined above for the 1986 procedure.

The samples were then purified by HPLC on 250 × 4.6-mm columns packed with 5-μm spherical particles. The ABA and IAA fractions were collected separately as they eluted from the column. The samples were first purified on an old strong anion exchange (SAX) column developed with 80% methanol-0.02 N acetic acid. In 1986, the IAA fraction was further purified on a second, newer, SAX column developed with 80% methanol-0.1 N acetic acid. The ABA and IAA fractions were concentrated to the aqueous phase with a stream of N_2 and separately loaded onto a C18 column developed with 50% methanol-0.02 N acetic acid. ABA was detected by absorbance at 254 nm and IAA was detected by natural fluorescence at 254 nm excitation and 360 nm emission. IAA eluted from the C18 column as a single fluorescent peak after purification on two SAX columns. Internal standards of ^{14}C-ABA and ^{14}C-IAA, added at the start of extraction, were used to estimate recoveries. All values were corrected for losses and for contributions by internal standards.

Free ABA and free IAA were determined first. Free plus conjugated ABA and free plus ester IAA were then determined by subjecting another set of samples to mild alkaline hydrolysis (1 N KOH for 1 h at 25°C). Total IAA (free plus ester plus amide) was measured in another set of samples that were hydrolyzed in 7 N NaOH 3 h at about 99°C. Ester IAA and conjugated ABA were estimated by subtracting the values of free IAA and ABA from those obtained by mild alkaline hydrolysis. Amide IAA was calculated as the difference between results obtained by mild and strong alkaline hydrolysis.

RESULTS AND DISCUSSION

Water deficit did not affect ester IAA the same way it affected free IAA. Whereas the concentration of free IAA in 3-d-old bolls and their abscission zones decreased with water deficit, the concentration of ester IAA increased (Tables I and II). Furthermore, the increases in ester IAA were greater than the decreases in free IAA. The ester content of bolls was much higher than that of abscission zones, as might be expected if IAA synthesized in developing ovules is the major source of IAA in fruit abscission zones. Furthermore, the concentrations of ester IAA were much greater than those of free IAA, especially in the bolls. The concentration of free IAA in abscission zones increased after stress was relieved by irrigation on June 27 (Table II, the June 30 column) concomitant with increases in boll retention. To our surprise, however, the free IAA content of bolls did not increase after irrigation but, instead, continued to decrease. The ester IAA content of bolls also decreased after irrigation. The ester IAA content of bolls may decrease during the season; it was lower in July of 1985 (Table I) than in June of 1986 (Tables II) and was lower on June 30 than on June 16. Results in the low-N plots (not shown) were similar to those in the controls (Table II) in 1986.

The mechanism by which ester IAA increased as plants became stressed for moisture is not known. We measured amide IAA in bolls to see if it might be the source. Although the concentration of amide IAA in bolls may have decreased with moisture stress, the decrease was not enough to account for the increase in ester IAA (Table II). In contrast to ester IAA, amide IAA increased

Table I. *Changes in Water Potential, Boll Retention, and Free and Conjugated IAA and ABA in 3-d-old Bolls and Their Abscission Zones Through an Irrigation Cycle in 1985*

Plots were irrigated July 3 and 18. See text for details. Data are averages of four replications ± SE.

Measurements	Harvest Date		
	July 8	July 12	July 15
Xylem water potential, MPa	−1.69 ± 0.11		−2.90 ± 0.09
Boll retention, %	74 ± 4	51 ± 2	3 ± 1
IAA in bolls		*ng g^{-1} dry wt*	
Free	105 ± 6	114 ± 2	61 ± 4
Ester	493 ± 41	808 ± 45	868 ± 123
Sum	598 ± 43	922 ± 43	930 ± 127
IAA in abscission zones		*ng g^{-1} dry wt*	
Free	91 ± 2	64 ± 1	36 ± 4
Ester	17 ± 8	97 ± 7	241 ± 12
Sum	108 ± 8	162 ± 7	276 ± 9
ABA in bolls		*μg g^{-1} dry wt*	
Free	2.63 ± 0.27	3.42 ± 0.15	6.40 ± 0.04
Conjugated	0.92 ± 0.33	0.93 ± 0.21	2.85 ± 0.44
Sum	3.55 ± 0.36	4.33 ± 0.18	9.25 ± 0.43
ABA in abscission zones		*μg g^{-1} dry wt*	
Free	0.51 ± 0.10	1.38 ± 0.06	1.81 ± 0.03
Conjugated	1.79 ± 0.07	2.17 ± 0.21	2.15 ± 0.14
Sum	2.30 ± 0.08	3.55 ± 0.21	3.96 ± 0.12

Table II. *Changes in Water Potential, Boll Retention, and Free and Conjugated IAA and ABA in 3-d-old Bolls and Their Abscission Zones Through an Irrigation Cycle in High-N Plots in 1986*

Plots were irrigated July 13 and 27. Data are averages of four replications ± SE.

Measurements	Harvest Date		
	June 16	June 26	June 30
Xylem water potential, MPa	−1.80 ± 0.08	−2.81 ± 0.09	−1.82 ± 0.07
Boll retention, %	96 ± 2	27 ± 9	78 ± 6
IAA in bolls		*ng g^{-1} dry wt*	
Free	120 ± 9	93 ± 11	57 ± 5
Ester	1422 ± 63	1625 ± 83	471 ± 41
Amide	234 ± 47	168 ± 75	360 ± 12
Total	1776 ± 91	1876 ± 135	888 ± 41
IAA in abscission zones		*ng g^{-1} dry wt*	
Free	72 ± 2	43 ± 3	61 ± 4
Ester	57 ± 4	221 ± 17	185 ± 12
Sum	129 ± 1	264 ± 18	246 ± 11
ABA in bolls		*μg g^{-1} dry wt*	
Free	1.88 ± 0.07	6.62 ± 0.44	2.61 ± 0.13
Conjugated	1.69 ± 0.08	2.36 ± 0.29	1.54 ± 0.06
Total	3.57 ± 0.12	8.98 ± 0.15	4.15 ± 0.08
ABA in abscission zones		*μg g^{-1} dry wt*	
Free	0.45 ± 0.05	1.71 ± 0.06	0.51 ± 0.03
Conjugated	0.99 ± 0.04	2.70 ± 0.17	2.37 ± 0.04
Total	1.44 ± 0.09	4.42 ± 0.11	2.88 ± 0.03

after stress was relieved by irrigation. But, the total IAA content of bolls was lower after irrigation than before (Table II).

Although abscission zones contained much less ester IAA than bolls, moisture stress caused a larger (percentage) increase in ester IAA in abscission zones than it did in bolls (Tables I and II). If ester IAA moves from bolls into their abscission zones, these results indicate that moisture stress may stimulate, rather than inhibit, such movement. This is just the opposite of what has been shown for transport of free IAA in petioles (9, 10). If ester IAA is more resistant to enzymic destruction than free IAA (7), and is readily converted to free IAA (7), the increase in ester IAA content of abscission zones may facilitate a rapid recovery of fruit retention after relief of stress. Free IAA content of abscission zones and boll retention both increased after irrigation (Table II). The correlation coefficient of boll retention percentage *versus* free IAA content of abscission zones was 0.83 in 1985 and 0.68 in 1986.

In contrast to the results obtained for IAA conjugates, water deficit had the expected effects on free and conjugated ABA. The concentration of conjugated ABA in bolls was lower than that of free ABA (Tables I and II) and did not increase as quickly during an irrigation cycle as did free ABA (Table I). Both, however, increased in bolls and their abscission zones with water deficit. Conjugated ABA in bolls decreased after irrigation, but the conjugated ABA content of abscission zones remained high (Table II).

As in 1985 (14), there appeared to be a negative relationship between free ABA and boll retention in 1986 (Table II). Correlation coefficients (r values) were −0.83 and −0.85 for free ABA content of bolls and abscission zones, respectively, *versus* boll

retention percentage. Ethylene production in young bolls also increases with water deficit (11), possibly as a direct response to the stress or as an indirect response through a stimulation of ethylene production by ABA. In either case, increased ethylene production should decrease boll retention.

The roles suggested by Cohen and Bandurski (7) for IAA conjugates (transport, storage and subsequent reuse, protection from enzymic destruction, and the homeostatic control of IAA); the high concentrations of ester IAA found in fruits and their abscission zones; and the fact that the concentration of ester IAA increased with moisture stress, all point to an important role of ester IAA during and after periods of water deficit. The tendency to produce and transport esterified IAA to abscission zones may hasten the rate of recovery of fruit retention after relief of moisture stress by providing a readily available source of free IAA to inhibit continued abscission. Boll retention increases immediately after irrigation early in the season (15).

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