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Influence of Water Deficits on the Abscisic Acid and Indole-3-Acetic Acid Contents of Cotton Flower Buds and Flowers

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ABSTRACT

A field experiment was conducted during the summer of 1988 to test the hypothesis that water deficit affects the abscisic acid (ABA) and indole acetic acid (IAA) concentrations in cotton (*Gossypium hirsutum* L.) flower buds in ways that predispose young fruits (bolls) that subsequently develop from them to increased abscission rates. Water deficit had little effect on the ABA content of flower buds but increased the ABA content of flowers as much as 66%. Water deficit decreased the concentrations of free and conjugated IAA in flower buds during the first irrigation cycle but increased them during the second cycle. Flowers contained much less IAA than buds. Water deficit slightly increased the conjugated IAA content of flowers but had no effect on the concentration of free IAA in flowers. Because water deficit slightly increased the ABA content but did not decrease the IAA content of flowers, any carry-over effect of water deficit on young boll shedding might have been caused by changes in ABA but not from changes in IAA.

It has been known for many years that water deficit increases the shedding rate of young cotton (*Gossypium hirsutum* L.) bolls (20). Our earlier research showed that moisture stress increased the rate of ethylene evolution from young bolls (11), increased their ABA content (12–14), and decreased their free IAA content (13, 14). These changes in hormonal concentrations were in directions that should increase abscission. Large flower buds, however, contain high concentrations of free and amide-linked IAA (15, 16) and are resistant to abscission just before they reach anthesis (7, 9, 21, 23). The high concentrations of IAA in large flower buds decreased rapidly to low levels at anthesis and remained low in young bolls for at least 4 d after anthesis (15, 16). Low levels of IAA in young bolls are probably a major factor in the high probability of shedding during the young-boll stage of development.

Even though large flower buds contain such high concentrations of IAA that they are unlikely to abscise, we postulated that water deficit might decrease their IAA content and that the subsequent sharp decline in IAA, as they reach anthesis, might lead to critically low concentrations in flowers and young bolls. Likewise, water deficit might cause ABA to accumulate in buds and flowers. If such changes occur in response to water deficit stress, they could have a carry-over effect and increase the probability of shedding of young bolls that develop from them even after irrigation. To test this hypothesis, we measured the ABA and IAA contents of flower buds (about 3 d preanthesis) and flowers through two irrigation cycles.

MATERIALS AND METHODS

Plant Material

Cotton (*Gossypium hirsutum* L.) 'Deltapine 77' seeds were planted in a field at the Western Cotton Research Laboratory in Phoenix April 28 in rows spaced 1 m apart, and the soil was irrigated the next day. On May 16, the seedlings were thinned to about 10 per m of row to give a population of about 100,000 plants per ha. About 125 mm of water was applied on May 17. Berms were constructed to divide the area into eight plots, each of which was four rows wide by 30 m long. Two nitrogen levels were replicated four times in a randomized complete block. All plots were irrigated with about 125 mm of water each date on June 2, 25, July 13, and July 28. Rain occurred on July 29 and August 1 (8.6 and 5.1 mm, respectively).

Ten flower buds and 10 white flowers (at anthesis) were harvested in each plot from the first node of fruiting branches on June 29, July 7, 12, 18, and 27, and on August 3. Flower buds were harvested from fruiting branches that were one mainstem node above the fruiting branch with a white flower, and were assumed to be 3 d preanthesis (because the interval between anthesis of flowers on successive fruiting branches is about 3 d). Flower buds and flowers were rinsed in cold deionized water, placed in labeled paper sacks, quickly frozen in aluminum pans at −80°C, freeze-dried, and stored at −80°C under nitrogen until they could be analyzed.

Water potentials of the uppermost fully expanded mainstem leaf of four plants per plot were measured on the day of each harvest with a pressure chamber (25). The leaves were harvested between 12:30 and 2 PM, placed in plastic bags which were then placed in a moist cooler, and transported to a nearby laboratory for water potential measurements.

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Analysis of ABA and IAA

Free ABA and free and total IAA were analyzed by HPLC by a modification of procedures reported earlier (15–17). Fifty to 200-mg samples of lyophilized tissue were extracted overnight in 80% methanol (free ABA and free IAA) or in 70% acetone (for total IAA) that contained 100 mg Na ascorbate and 200 mg butylated hydroxytoluene per L as antioxidants. Samples were reduced to the aqueous phase by rotary flash evaporation and lipids were removed with three 10-mL portions of hexane. The pH of samples used for estimation of free ABA and IAA was never alkaline but was adjusted to 2.8 with 0.5 M H_3PO_4 after lipids were extracted with hexane.

Conjugated (amide-linked plus ester) forms of IAA were hydrolyzed (1) by adding 3 g of NaOH pellets to 10 mL of aqueous extract in a 50-mL polypropylene centrifuge tube. The tube was immediately capped loosely with a cover (DuPont Sorvall[2] 03268) fitted with a rubber septum (inserted in a hole drilled in the cover). A small-diameter stainless-steel syringe needle was inserted through the septum and used to flush the tube with N_2. After air was displaced by the N_2 (1–2 min), the cover was pushed tightly into the tube while N_2 was still flowing, the needle was withdrawn, and the NaOH pellets were dissolved by vortexing. Each container was flushed with N_2 and sealed before the contents were mixed, and before NaOH was added to the next sample. A 1-mL plastic syringe was attached to each tube by inserting its needle through the septum. The syringe contained a small steel ball (an air rifle BB or a stainless-steel ball available from Small Parts, Inc., 6891 N.E. Third Ave., P. O. Box 381736, Miami, FL 33238) that served as a check valve to release pressure during incubation without admitting air. The samples were then placed in a covered boiling water bath for 3 h. After incubation the tubes were cooled in water before they were opened. The pH of the strong alkaline digest was adjusted to 2.8 with 5 M H_3PO_4 while cooling in water.

Hydrolyzed and free IAA and ABA were extracted from the acidified aqueous phase with CH_2Cl_2, concentrated to an aqueous phase, and partially purified with nylon filters and C18 cartridges as reported earlier (6, 16). Further purification and quantification were achieved by sequential HPLC on strong anion exchange and C18 columns (17).

GC-SIM-MS

For confirmation of identity and amount of ABA and IAA, some samples were shipped to Weslaco, TX, analyzed by gas chromatography-selected ion monitoring-mass spectrometry (GC-SIM-MS), and the results compared with those obtained by HPLC. Ring-labeled [$^{13}C_6$]IAA (5) and [$^2H_6$]ABA (24) were added as internal standards at the start of extraction and the samples then subjected to the same extraction and bulk purification as outlined above. After elution of ABA and IAA from the C18 cartridge with methanol, they were methylated with diazomethane (4) and analyzed with a Hewlett-Packard 5970 GC-MS as described earlier (6).

[2] The use of trade names is for identification only and does not imply endorsement or preferential treatment over other brands that may also be suitable.

Table I. *Leaf Water Potentials and ABA Concentrations in Flower Buds and Flowers as Influenced by Irrigation*

Flowers (at anthesis) and flower buds (about 3 d preanthesis) were harvested from the first nodes of successive fruiting branches. Data are averages of four replications ± SE.

Date	Leaf Water Potential	Flower Buds	Flowers
	−MPa	*μg ABA g^{-1} dry wt*	
June 25 (irrigated)			
June 29	1.44 ± 0.02	1.10 ± 0.03	0.90 ± 0.03
July 7	1.60 ± 0.03	1.34 ± 0.03	1.26 ± 0.04
July 12	2.74 ± 0.06	1.29 ± 0.03	1.36 ± 0.15
July 13 (irrigated)			
July 18	1.48 ± 0.06	1.11 ± 0.10	0.85 ± 0.04
July 27	2.42 ± 0.07	1.35 ± 0.04	1.41 ± 0.11
July 28 (irrigated)			
August 3	1.49 ± 0.06	1.03 ± 0.04	0.84 ± 0.01

RESULTS

Nitrogen had relatively little effect on ABA and IAA contents of flower buds and flowers; results were similar in the high-N and low-N plots. Therefore, only results from the high-N plots will be reported here.

The free ABA contents of flower buds and flowers changed some during irrigation cycles: as expected ABA increased with water deficit stress and decreased after irrigation (Table I). The concentration of ABA changed more in flowers than it did in buds, but the changes were not great even in flowers. The maximum increase was 66% in flowers between July 18 and 27.

Flower buds sampled about 3 d preanthesis contained very high concentrations of IAA (Table II). However, response to stress differed from the first to the second irrigation cycle. Conjugated IAA in flower buds decreased with stress before the July 13 irrigation, but increased with stress to a very high level before the July 28 irrigation (Table II). The IAA content of flower buds apparently increased with water-deficit stress late in the fruiting cycle as the plants entered cutout (a hiatus in growth and flowering). In preliminary observations in 1987, we found even higher concentrations of IAA in flower buds at the second node of fruiting branches or stressed 'DPL 61' plants that had almost stopped flowering. Total IAA in those flower buds increased from 12 μg/g dry weight on July 9 to 80 μg/g on July 16 as the leaf water potential decreased from −1.88 to −2.71 MPa in 1987. We did not, however, measure changes during an earlier irrigation cycle in 1987. The high concentrations of IAA indicated by HPLC were confirmed in representative samples both years by GC-SIM-MS.

Water deficits may cause an increase in the concentration of amide-linked IAA because of changes in other hormones. Chang and Jacobs (3) reported that pretreatment of *Coleus* petioles with either ABA or an endogenous 'senescence factor' (possibly 1-aminocyclopropane-1-carboxylic acid) decreased free IAA and increased IAA-aspartate. Beyer and Morgan (2) found that cotton stem sections rapidly converted ^{14}C-IAA to ^{14}C-IAA-aspartate and other metabolites. Pretreatment with ethylene apparently increased the amount of conversion. Epstein (8) reported that ethephon increased the concentration

Table II. *Concentrations of Free and Conjugated IAA in Flower Buds and Flowers as Influenced by Irrigation*

Flowers (at anthesis) and flower buds (3 d preanthesis) were harvested from the first nodes of successive fruiting branches. Plants were stressed by July 12 and 27 (see Table I for leaf water potentials). Data are averages of four replications ± SE.

Date	Flower Buds	Flowers	Flower Buds	Flowers
	μg conjugated IAA g^{-1} dry wt		μg free IAA g^{-1} dry wt	
June 25 (irrigated)				
June 29	31.83 ± 4.42	5.78 ± 0.70	2.57 ± 0.41	0.10 ± 0.01
July 7	40.19 ± 6.26	6.59 ± 0.46	2.38 ± 0.13	0.12 ± 0.01
July 12	15.92 ± 2.79	8.20 ± 0.92	0.57 ± 0.02	0.12 ± 0.00
July 13 (irrigated)				
July 18	24.28 ± 3.75	3.14 ± 0.70	1.37 ± 0.16	0.13 ± 0.01
July 27	63.09 ± 3.73	5.06 ± 0.69	2.11 ± 0.31	0.13 ± 0.01
July 28 (irrigated)				
August 3	20.30 ± 2.48	2.66 ± 0.61	0.74 ± 0.09	0.12 ± 0.01

of amide-linked IAA in olive leaves without, however, affecting the concentration of free IAA. Since water deficit increases ethylene production in petioles (22) and young bolls (11) it might also increase ethylene production in flower buds and indirectly affect the formation of IAA-aspartate through its effect on ethylene (or ABA). Ethylene production also increased in young bolls as cotton plants entered cutout (10). Cytokinins, on the other hand, may inhibit the conjugation of IAA. Lau and Yang (19) reported that kinetin greatly suppressed the conjugation of IAA in mung bean seedlings. Water deficit decreases cytokinin activity in shoots (18) and, thus, might permit increased conjugation of IAA if it decreases cytokinin activity in flower buds. As reported by Epstein (8) for olive leaves, we found that increased conjugation of IAA in flower buds did not occur at the expense of free IAA because both increased from July 12 to 27 (Table II).

Stress had relatively little effect on the IAA content of flowers (Table II). The trend, however, was for conjugated IAA to increase with stress during both irrigation cycles. It increased from 5.78 to 8.20 μg/g during the first irrigation cycle, and from 3.14 to 5.06 μg/g during the second. This is somewhat similar to results we obtained earlier with bolls in which ester IAA increased with stress even though free IAA decreased (14).

Free IAA in flower buds responded to stress in much the same way as total IAA; it decreased with stress during the first irrigation cycle, but increased with stress during the second (Table II). These changes, however, probably do not carry over to young bolls because the free IAA content of flowers showed no response to stress and was independent of the wide fluctuations in the free IAA content of flower buds (Table II). The results indicate that remarkable decreases in both free and conjugated IAA occurred during the 3 d before anthesis. Flowers contained much less IAA than 3-d preanthesis buds, but plant water status had no apparent effect on the consistently low level of free IAA in flowers (Table II, last column).

Although water deficit slightly increased the ABA content (Table I), it had no effect on the free IAA content of flowers (Table II). Therefore, we conclude that the pronounced changes in the concentrations of free and conjugated IAA In flower buds had no carry-over effect on the free IAA content of flowers (and young bolls since the fruiting form must pass through the flowering stage to become a boll) and that there is probably little effect of moisture stress during the late flower-bud stage on shedding of young bolls that subsequently develop from those buds after irrigation. If there is any effect, it might be due, at least in part, to the small increases in the ABA content of flowers. But, because water deficit did not decrease the IAA content of flowers, any carry-over effect on young boll abscission is not likely due to preanthesis changes in IAA.

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