Plant Physiol. (1988) 87, 629–631
0032-0889/88/87/0629/03/$01.00/0

Changes in Abscisic Acid and Indoleacetic Acid before and after Anthesis Relative to Changes in Abscission Rates of Cotton Fruiting Forms

Received for publication December 7, 1987 and in revised form March 9, 1988

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) fruiting forms exhibit pronounced changes, with age, in their probability of abscission. Large floral buds rarely abscise, but after anthesis the young fruits (bolls) have a high probability of abscising. Abscission rate reaches a peak about 5 to 6 days after anthesis and then gradually decreases. An experiment was conducted to try to determine the reason for the rapid and pronounced increase in probability of abscission just after anthesis. Cotton was grown in the field and fruiting forms of various ages from 9 days before to 9 days after anthesis were all harvested the same day and subsequently analyzed for ABA and IAA. The concentration of ABA decreased slightly at anthesis and increased gradually thereafter. In contrast, the concentration of IAA was high before anthesis and then decreased at anthesis to about one-fifth the previous concentration. IAA remained low for at least 4 days after anthesis and then increased rapidly between 7 and 9 days after anthesis. The high concentration of IAA in floral buds before anthesis is probably a major factor in their resistance to abscission. Likewise, the low concentration of IAA at anthesis and for about 4 days thereafter may promote fruit abscission during the young boll stage.

Young cotton (*Gossypium hirsutum* L.) fruiting forms exhibit remarkable changes in susceptibility to abscission during their development. Probability of abscission is very low just before anthesis and high for a few days after anthesis. Ewing (6), referring to floral buds as squares and fruit as bolls, noted that, "apart from insect damage, shedding of squares is rare and shedding of open flowers is extremely rare. It is in the young boll stage that our shedding principally occurs." Likewise, McNamara *et al.* (14) reported that water deficit caused young bolls to shed and that there was a strong tendency for the flower buds to develop into flowers and then shed as young bolls rather than for the large flower buds to shed. Eaton and Ergle (5) stated that it is extremely rare to find large floral buds shedding prior to anthesis. Probability of abscission increases shortly after anthesis. Maximum rates of abscission occur between 5 and 6 d after anthesis (8, 14) and gradually decline thereafter to near zero at 20 d (7, 8).

No hypotheses have been proposed to explain the low abscission rate of large flower buds, but hormonal balance is a likely factor. Chatterjee (3) reported that levels of IAA-like compounds were highest in the epicalyx of flower buds and lowest in the epicalyx of flowers. In contrast, Luckwill (13) stated, "in those plants which have a normal sequence of fruit development, the ovary, before fertilization, contains either very small amounts of free auxin or none at all; but after gametic union (fertilization), relatively large amounts of free auxin may be extracted." Hänisch ten Cate *et al.* (10) reported 3- to 4-fold increases in IAA content of *Begonia* flowers during their opening. Koning (11) reported a 10-fold increase in the level of free auxin in *Gaillardia* disk flowers just before anthesis and filament elongation. He reported finding the very high concentration of 574 μg of IAA per g fresh weight of flowers when no pollinators were active. He cited several studies which indicated that the auxin level increases just before flower opening and anthesis and then drops to a very low level. Rodgers (15) estimated the concentration of IAA-like substances in cotton fruits of various ages, but he did not examine floral buds.

Davis and Addicott (4) reported increasing amounts of ABA per fruit during the first 10 d after anthesis and suggested that the changing hormonal balance was a cause of fruit abscission, but they did not measure the ABA content of flower buds nor the IAA content of flower buds or fruits.

Because IAA has long been recognized as an inhibitor of abscission (1, 2) and because ABA has been reported to be a promoter of abscission (1), we conducted an experiment to determine concentrations of ABA and IAA in flower buds, flowers, and young fruits during stages of development from 9 d before to 9 d after anthesis.

MATERIALS AND METHODS

Plant Material. Cotton (*Gossypium hirsutum* L. 'DPL 61') was planted in a field at the Western Cotton Research Laboratory in Phoenix on April 8, 1987. Plants were irrigated on April 15, May 6, June 5, and about every 2 weeks thereafter. Seedlings were thinned to about 99,000 ha^{-1} in rows that were 1 m apart. Urea was applied on June 4 to give 168 kg of N ha^{-1}. White flowers (at anthesis) were tagged on June 17, 19, 22, and 24 for subsequent harvest on June 26 to give bolls that were 9, 7, 4, and 2 d old, respectively. Flower buds and white flowers were also harvested on June 26. Age of flower buds was estimated by their location. Because the time interval between successive fruiting branches is approximately 3 d, flower buds harvested at the first node of fruiting branches that were one, two, or three mainstem nodes above a fruiting branch with a white flower at the first node were considered to be 3, 6, or 9 d preanthesis, respectively. Twenty flower buds or flowers were harvested in each age group in each of four replications. Twelve to 15 fruits were harvested in each age group. The fruiting forms were immediately rinsed in cold deionized water and stored in a cold room until harvesting was completed at about 10 AM. Fruits were cut open to facilitate drying, and all fruiting forms were quickly frozen in aluminum pans at −80°C and then lyophilized. The

Table I. *ABA and IAA Contents of Cotton Flower Buds, Flowers, and Young Fruits from 9 Days before Anthesis Until 9 Days after Anthesis*

Fruiting forms were all harvested the same day and were harvested only from the first node of fruiting branches. Days before anthesis were estimated by position of fruiting branches on the main stem relative to the fruiting branch with an open white flower. Data are averages of four replications ± SE.

Days before or after Anthesis	Concentration		Ratio ABA/IAA	Amount per Fruit	
	ABA	IAA		ABA	IAA
d	*μg/g*	*ng/g*	*mol/mol*	*ng/fruit*	*ng/fruit*
−9	1.40 ± 0.06	386 ± 20	2.42 ± 0.09	178 ± 6	49 ± 3
−6	1.25 ± 0.07	617 ± 52	1.36 ± 0.04	227 ± 10	112 ± 7
−3	1.24 ± 0.03	516 ± 57	1.67 ± 0.22	279 ± 6	116 ± 13
0	0.89 ± 0.02	116 ± 6	5.16 ± 0.29	314 ± 4	41 ± 3
2	1.16 ± 0.04	120 ± 3	6.48 ± 0.41	263 ± 8	27 ± 1
4	1.97 ± 0.02	112 ± 3	11.74 ± 0.26	603 ± 10	34 ± 1
7	2.38 ± 0.12	152 ± 2	10.39 ± 0.62	1468 ± 88	94 ± 4
9	2.41 ± 0.13	240 ± 5	6.71 ± 0.38	2297 ± 93	229 ± 8

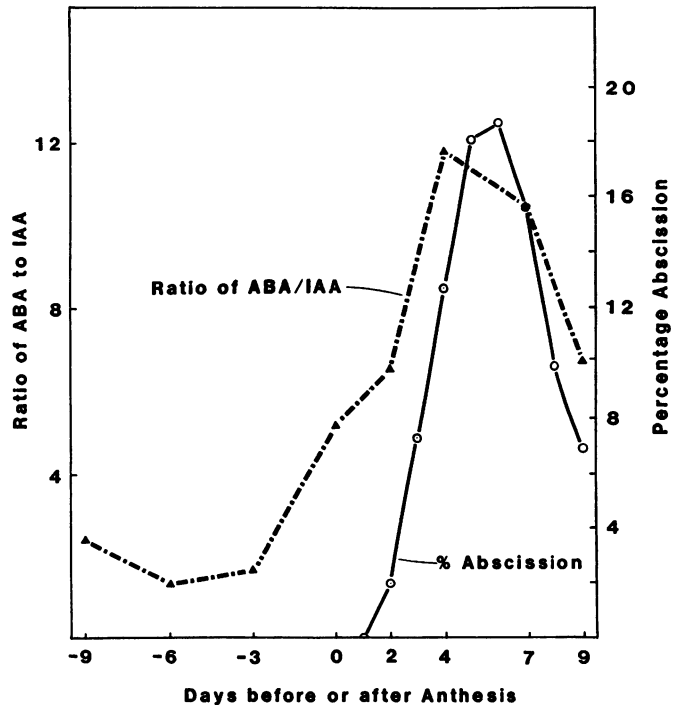

FIG. 1. Ratio (mol/mol) of ABA to IAA in fruiting forms before and after anthesis compared with abscission. The abscission rate data were redrawn from Guinn (8) and were obtained with the same cultivar under field conditions at the same location.

dry forms were weighed, ground to pass a 40-mesh screen, and stored in vials (flushed with N_2) at −80°C.

ABA and IAA Analyses. ABA and IAA were purified and measured by a modification of a procedure reported earlier (9). Samples of 500 mg each were extracted overnight at 4°C with gentle stirring in 30 ml of 80% methanol that contained antioxidants and radioactive internal standards (about 5,000 dpm each of ^{14}C-ABA and ^{14}C-IAA). The samples and four 10-ml rinses (with 80% methanol containing antioxidants) were filtered through Whatman No. 1[1] paper with suction. Methanol was then removed by rotary flash evaporation at 40°C. Lipids were removed from the aqueous residue by extracting twice with 10-ml portions of hexane. The pH was adjusted to 2.8 with dilute H_3PO_4. ABA and IAA were extracted from the acidified aqueous samples with three 10-ml portions of dichloromethane. The dichloromethane was removed by rotary flash evaporation, and the residue was dissolved in 5 ml of 1 mM HCl. Each sample was loaded onto a C_{18} cartridge (Waters Sep-Pak) which was then rinsed with 5 ml of water. ABA and IAA were eluted with 5 ml of methanol. Methanol was removed by rotary flash evaporation and the aqueous residue was extracted with two 5-ml portions of dichloromethane that were then loaded, in sequence, onto a silica cartridge. ABA and IAA were eluted from the silica cartridge with 5 ml of methanol. The methanol was removed by rotary flash evaporation, and the samples were dissolved in 1 ml of acetonitrile (to prevent possible methylation during storage), filtered with centrifugation through 0.2 μm nylon filters, and stored overnight (or longer when necessary) in a freezer.

The samples were concentrated to about 50 μl under a stream of N_2 and further purified by HPLC, first on a strong anion exchange (SAX) column nd then on a C_{18} column. The strong anion exchange column was developed with 80% methanol-0.05 N acetic acid at 1.5 ml min^{-1}. The ABA and IAA fractions were collected separately, evaporated to the aqueous phase under a stream of N_2, and loaded onto a 25-cm C_{18} column (Adsorbosphere C_{18} HS, 5 μm, Alltech Associates). The samples were eluted isocratically with 60% methanol-0.02 N acetic acid at 0.7 ml min^{-1}. ABA was detected by absorbance at 254 nm, and IAA was detected by native fluorescence at 254 nm excitation and 360 nm emission. ABA and IAA were the major UV-absorbing and fluorescent peaks, respectively, and they were well separated from other, smaller peaks. The fractions containing ABA and IAA were collected separately in scintillation vials and counted in a liquid scintillation spectrometer to determine recoveries of internal standards. Peak heights were corrected for losses and for contributions by the internal standards. Recoveries averaged about 60% for IAA and about 80% for ABA.

RESULTS AND DISCUSSION

Flower buds contained a high concentration of IAA, but IAA decreased to a minimum at anthesis (or 2 d after anthesis when expressed as amount per fruit). The concentration of IAA remained low until 4 d postanthesis and then increased (Table I). Because IAA inhibits abscission (1, 2), the high concentration of IAA in flower buds could be an important factor in their resistance to abscission, although not necessarily the only factor. An increase in ethylene production at anthesis (12) is another likely cause of an increase in abscission after anthesis. Although Lipe and Morgan (12) showed only one data point before anthesis for

[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.

each of two fruiting forms, ethylene production was much less before than at anthesis. Therefore, the ethylene stimulus for abscission increased at anthesis. ABA, on the other hand, decreased slightly at anthesis and then increased gradually thereafter (Table I).

Davis and Addicott (4) reported increasing amounts of ABA per fruit to a maximum at 10 d after anthesis and correlated the increase in ABA with increased abscission. Our values for amount of ABA per fruit (Table I) agree closely with theirs even though we used a different cultivar. But, they showed fruit abscission as cumulative values rather than daily values. Therefore, maximum amount of ABA per fruit occurred after the maximum rate of fruit abscission. In fact, it occurred at a time when the proportion of fruits that were abscising was decreasing rapidly. Because of the time required for synthesis, secretion, and action of hydrolytic enzymes (cellulase and pectinase) in the abscission zone, a change in hormonal balance must precede a change in abscission rate if it is to be regarded as the cause of that change. Fruit abscission rate usually reaches a maximum 5 to 6 d after anthesis (8, 14), well before the maximum amount of ABA per fruit (4) and also before the maximum concentration of ABA (Table I).

Although Davis and Addicott (4) indicated that ABA is a factor in fruit abscission, they pointed out that the balance between hormones may be more important than the concentration of any one hormone. We calculated the ratio of ABA to IAA in fruits of different ages and plotted the results in Figure 1. Earlier data for abscission rates for fruits of various ages (Fig. 6 of Ref. 8) were also plotted in Figure 1. Although daily values for the ABA to IAA ratio were not obtained, it is apparent that an increase in the ABA to IAA ratio preceded an increase in abscission rate with increasing boll age. A decline in the ABA to IAA ratio, due to a more rapid increase in IAA than ABA (Table I), coincided with a decline in fruit abscission rate.

A rapid decrease, with age, in the capacity for ethylene production is another likely cause for the decreasing rate of fruit abscission with increasing fruit age (7). Guinn (7) reported that older fruits are not able to produce as much ethylene as young fruits and that ethylene evolution decreased with increasing fruit age beyond 6 d postanthesis when cotton plants were stressed by placing them in dim light. He suggested that the decreasing capacity of older fruits to produce ethylene is one cause of the decline in abscission rates as cotton fruits become older. Although the ratio of ABA to IAA may be important, it appears that an increase in IAA content (Table I) and a decrease in ethylene production (7, 12) are more likely causes of the decrease in fruit abscission than changes in ABA because ABA was increasing (Ref. 4; Table I) at an age when fruit abscission rate was already decreasing (Fig. 1).

The high concentration of IAA in flower buds before anthesis is probably a major factor in their resistance to abscission. Likewise, the low concentration of IAA at anthesis and for about 4 d thereafter is probably a major factor in the high probability of fruit abscission at the young boll stage. Loss of the corolla was not the cause of the decrease in concentration of IAA because the IAA content was already low on the day of anthesis (Table I, day 0), approximately 2 d before the corolla abscised. The reason for the rapid decline in IAA content at anthesis is not known and is to be the subject of future investigations.

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