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Fluctuation in Levels of Endogenous Plant Hormones in Ovules of Normal and Mutant Cotton during Flowering and Their Relation to Fiber Development

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Abstract. The contents of indole-3-acetic acid (IAA), gibberellins (GAs), abscisic acid (ABA), and cytokinins were determined in ovules of normal cotton (Tm-1) and a kind of fiber differentiation mutant (Xin) before and after flowering by enzyme-linked immunosorbent assays. It was found that 24 h before flowering, a peak of IAA content was observed in ovules of Tm-1, whereas in ovules of Xin, a low level of IAA was determined. From −1 day (1 day before flowering) to +3 days (3 days after flowering), GA_{1+3} levels in ovules of Xin were 40–70% lower than those of Tm-1; GA_{4+7} levels were very low, and there was no visible difference in GA_{4+7} content between normal and mutant cotton. The ABA content in ovule of Tm-1 decreased by 70% 3 days after flowering, whereas that of Xin only decreased by 20%. The levels of cytokinins in ovules of Tm-1 decreased after flowering, and those of Xin kept up a steady increase.

Key Words. Plant hormones—Cotton ovule—Mutant—Fiber development

The cotton fiber is a single epidermal cell that begins to elongate from the ovule surface at anthesis (Beasley 1973). A number of research studies have focused on the effects of exogenous plant hormones on fiber development, since both fertilized and unfertilized cotton ovules could be successfully cultured in vitro (Beasley 1971, 1973, Beasley and Ting 1973, 1974). It has been found that exogenously applied indole-3-acetic acid (IAA) and gibberellic (GA_3) could enhance the differentiation of fiber and promote its elongation, whereas abscisic acid (ABA) and cytokinins inhibited the fiber growth (Beasley and Ting 1973, 1974; Chen et al. 1988; Shen et al. 1978; Wang et al. 1985; Zhang 1982; Zheng and Xu 1982). By comparison, less is known about the endogenous levels of plant hormones in ovules during fiber development. The fiber differentiation mutant, without any lints and fuzz, may provide an approach for elucidating the role of plant hormones in fiber growth and development. Thus, the contents of endogenous hormones in ovules of normal and mutant cotton were determined and compared, and their relation to fiber development is discussed.

Materials and Methods

Plants

Cotton (*Gossypium hirsutum* L.) ovules in normal (Tm-1) and mutant (Xin) plants were collected 3 days and 1 day before flowering, and 0, 1, 3, 5, and 8 days after flowering. Fresh samples (200–1,000 mg) were weighed and then placed into liquid nitrogen and kept at −20°C until extraction for hormone analysis.

Extraction and Measurement of Plant Hormones by ELISAs

Extraction and purification of plant hormones prior to immunoassay have been described previously (Weiler 1986; Zhang et al. 1995; Zheng and Zhou 1995). The main steps are: extraction of homogenized samples in cold 80% (v/v) aqueous methanol at a rate of 5 ml/g FW overnight at 4°C with butylated hydroxytoluene (10 mg/liter) to prevent oxidation. The supernatant was collected after centrifugation at 10,000 × g (4°C) for 20 min. Then the crude extract was passed through a C_{18} Sep-Pak cartridge (Waters, Milford, MA). The efflux was collected,

Abbrevations: IAA, indole-3-acetic acid; GA, gibberellin; ABA, abscisic acid; ELISA, enzyme-linked immunosorbent assay; FW, fresh weight; PBS, phosphate-buffered saline; iPA, isopentenyladenosine; ZR, zeatin riboside; DHZR, dihydrozeatin riboside; CTK, cytokinin.

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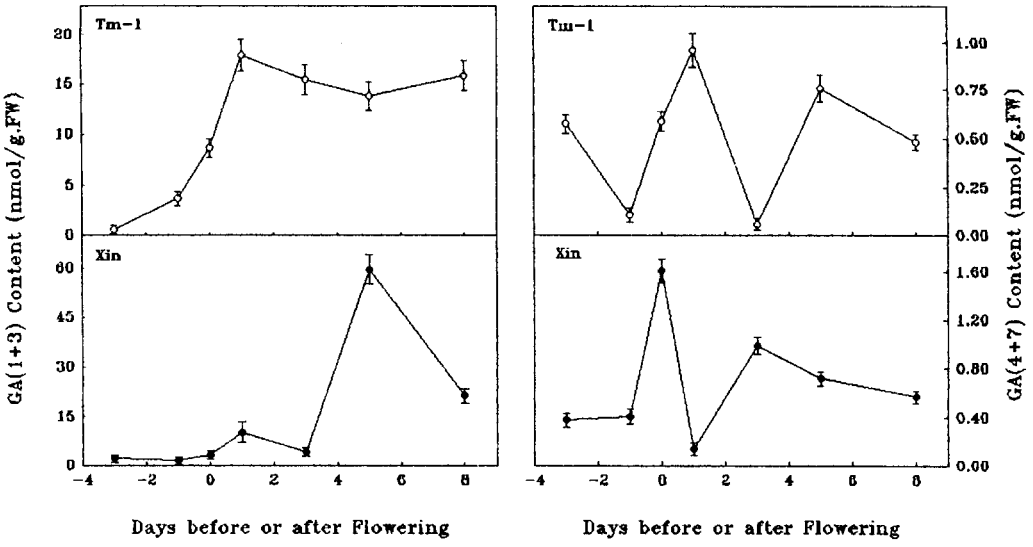

Fig. 1. Changes in endogenous GA levels in the ovules of normal (Tm-1) and mutant cotton (Xin) during flowering. Shown are the averages ± S.E. of three replicates. Days before and after flowering are shown as negative and positive numbers, respectively.

and 400 μL of it was taken out and dried with a stream of N_2; the residue was dissolved in 400 μL of PBS (0.01 M, pH 7.4) to subject to iPA, ZR, and DHZR ELISAs, respectively. Another 600 μL aliquot of the filtrate was taken and dried with a steam of N_2, and the residue was dissolved in 200 μL of PBS (0.01 M, pH 9.2), adjusted to pH 8.5, then partitioned three times with an equal volume of ethyl acetate. The remaining water phase was adjusted to pH 2.5 and extracted three times with an equal volume of ethyl acetate again. The extracts (ethyl acetate phase) were pooled and dried with a gentle stream of N_2; the residue was either redissolved in 200 μL of PBS (0.01 M, pH 7.4) to subject to GA_{1+3} ELISA or redissolved in 200 μL of 100% methanol for methylation with ethereal diazomethane and taken up with 400 μL of PBS for GA_{4+7}, IAA, and ABA ELISAs, respectively.

The procedures of direct ELISA measurement based on monoclonal antibodies that showed highly specific immunoreactivity with GA_{1+3}, GA_{4+7}, and ABA, respectively, have been described by Zheng and Zhou (1995), Zheng et al. (1995), and Zhou et al. (1996).

Indirect ELISA measurements using polyclonal antibodies against IAA, iPAs, ZRs, and DHZRs, respectively, have been described by Zhang et al. (1990), Chen et al. (1992), Chen and Zhou (1996), and Wang et al. (1994).

Both direct and indirect ELISAs were performed with microtitration plates (Nunc). Each hormone was determined three times on the same extract, and all samples were assayed in triplicate. The S.E. was calculated.

Results

Changes of Endogenous GA Levels in Cotton Ovules During Flowering

Fluctuation patterns of GA_{1+3} levels in ovules of normal and mutant cotton are shown in Fig. 1. The content of GA_{1+3} in the ovules of normal cotton was about 0.6–18.0 nmol/g FW, and the highest content of GA_{1+3} was observed 1 day after flowering (+1 day). However, the content of GA_{1+3} in the mutant (Xin) was 40–70% lower than that in normal (Tm-1) from −1 day (1 day before flowering) to +3 days (3 days after flowering), and the peak of GA_{1+3} content was observed at +5 days, which was 60 nmol/g FW. On the other hand, when flowering, the GA_{1+3} content in ovules increased by 135% in Tm-1 and 120% in Xin within 24 h. After flowering, the GA_{1+3} content increased by 107% within 24 h and kept up a high level in Tm-1; in ovules of Xin, an obvious decrease in GA_{1+3} content was observed at +8 days.

The contents of GA_{4+7} in ovules of normal and mutant cotton were determined at a similar level, and they were relatively low compared with that of GA_{1+3}, with the highest being about 1.0 nmol/g FW in Tm-1 and 1.6 nmol/g FW in Xin (Fig. 1). The peaks of GA_{4+7} levels in ovules of Tm-1 were observed at +1 and 5 days, whereas those of Xin were observed at 0 and +3 days, respectively.

Changes of Endogenous IAA Level in Cotton Ovules during Flowering

Twenty-four h before flowering, the content of IAA in the ovules of normal cotton was about eightfold higher than that at 0 days, whereas IAA in mutant cotton was determined to be at a low level (Fig. 2). Then, the IAA content decreased gradually until +5 days in Tm-1. However, from −3 to +3 days, the fluctuation pattern of the IAA level in the ovules of Xin was just opposite that of Tm-1.

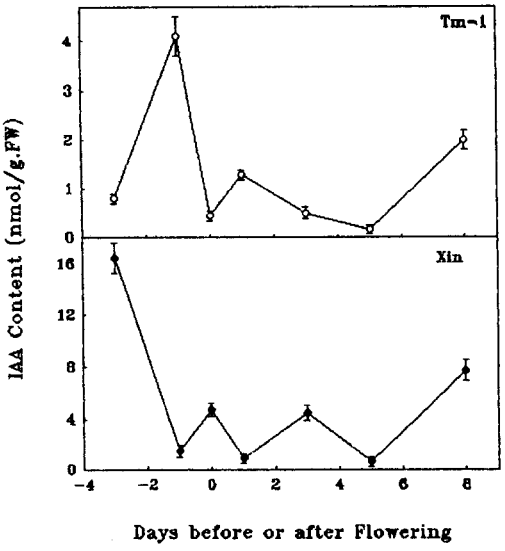

Fig. 2. Changes in endogenous IAA levels in the ovules of normal (Tm-1) and mutant cotton (Xin) during flowering. Shown are the averages ± S.E. of three replicates. Days before and after flowering are shown as negative and positive numbers, respectively.

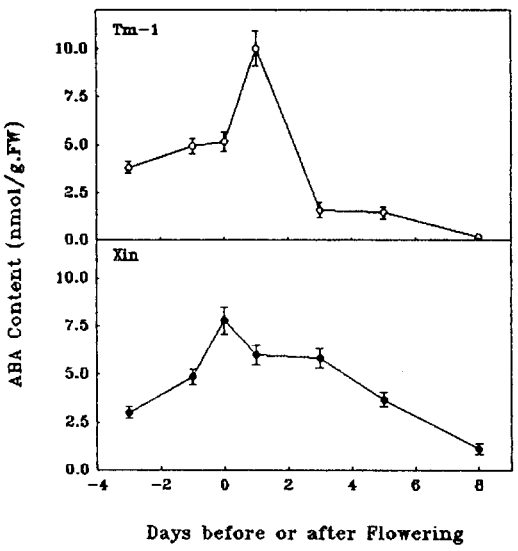

Fig. 3. Changes in endogenous ABA levels in the ovules of normal (Tm-1) and mutant cotton (Xin) during flowering. Shown are the averages ± S.E. of three replicates. Days before and after flowering are shown as negative and positive numbers, respectively.

Changes of Endogenous ABA Level in Cotton Ovules during Flowering

The content of ABA in the ovules of normal and mutant cotton was determined at a similar level with the highest being 10 and 7.8 nmol/g FW, respectively (Fig. 3). The fluctuation patterns of ABA levels in the ovules of normal and mutant cotton were almost similar except that the peak of ABA content was observed at +1 day in Tm-1, whereas that of Xin was observed at 0 days. However, the ABA content in the ovule of Tm-1 decreased by more than 70% 3 days after flowering, whereas that of Xin only decreased by 20%.

Changes of Endogenous CTK Levels in Cotton Ovules during Flowering

CTKs were the most abundant among the measured hormones, especially in the ovules of mutant cotton (Fig. 4), and the ZR levels were relatively low compared with those of iPA and DHZR, both in normal and in mutant cotton. The fluctuation patterns of iPA levels in the ovules of normal and mutant cotton were very different. The peak of iPA content was observed at −1 day in Tm-1, then it decreased by almost 95% when flowering and decreased by 90% 24 h after flowering. Eight days after flowering it decreased to 0.2 nmol/g FW. However, in the mutant the iPA content increased from −3 to +8 days. It increased almost 10- and 15-fold within 48 h at +3 and +5 days, respectively.

The change of ZR levels in ovules of mutant cotton was similar to that of iPA, whereas in Tm-1 no obvious change was observed, with the range of ZR content being 1.8 to 3.7 nmol/g FW before +8 days.

The change of DHZR levels in the ovules of mutant cotton was also similar to that of iPA except that a significant increase was observed at +1 day, when it increased more than 12-fold within 24 h. In Tm-1 the DHZR content increased 6.5-fold within 24 h before flowering, and the peak that was observed 1 day after flowering was 33 nmol/g FW. Then, it decreased by 85% within 48 h, and it was only 1.0 nmol/g FW at +8 days.

Discussion

It is known that the initiation of fiber starts before flowering and fertilization and that fibers elongate during anthesis (Beasley 1973; Stewart 1975). Therefore, the measurement of endogenous levels of hormones before fertilization was considered important for probing the process of fiber initiation. We do not know yet exactly which regulators control fiber initiation. In vitro, IAA could promote fiber production compared with GA in unfertilized ovules, whereas with fertilized ovules, exogenously applied IAA only showed a weak effect (Beasley and Ting 1973, 1974; Chen et al. 1988; Shen et al. 1978; Zhang 1982), indicating that a high IAA level before fertilization is more crucial. In this study, 24 h before flowering a peak of IAA content in the ovules of Tm-1 is observed, whereas IAA is determined to be at a low level in the ovules of Xin, suggesting that a high level of IAA

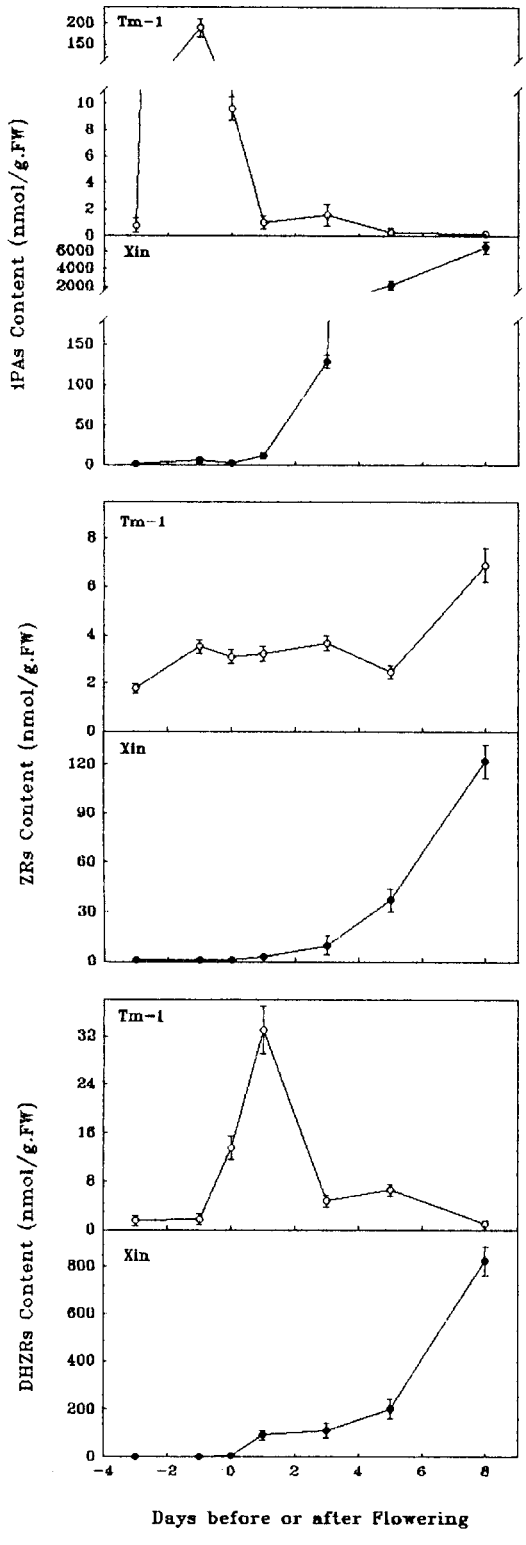

Fig. 4. Changes in endogenous CTK levels in the ovules of normal (Tm-1) and mutant cotton (Xin) during flowering. Shown are the averages ± S.E. of three replicates. Days before and after flowering are shown as negative and positive numbers, respectively.

in ovules just before flowering may function as a kind of stimulus for the initiation of fiber.

Since the content of GA_{4+7} is very low compared with that of GA_{1+3}, and there is no visible difference in the GA_{4+7} content between mutant and normal cotton, 13-nonhydroxy GAs are not the main GAs involved in fiber elongation. Because GA_{1+3} maintains a high level in Tm-1 after flowering while the GA_{1+3} content decreases obviously from +5 days in Xin, it may provide for the following fiber elongation. IAA may associate with GAs to stimulate the growth of the fiber cell, just like its effect on elongation of other kinds of cells (Bergfeld et al. 1988; Sakoda et al. 1992; Shibaoka 1991, 1994; Zandomeni and Schopter 1993), since at the elongation stage (5 days after flowering), a trend of increase in its content is found. In vitro we found that IAA could induce a marked stimulation of fiber production from cotton ovules; and when IAA and GA_3 were applied simultaneously, the total fiber units recorded showed the effects to be additive (data not shown).

Interestingly, DHZRs (DHZ and DHZR) in ovules are determined at a higher level than ZRs, which is just opposite the result in other organs or tissues of other species (Zhang et al. 1994; Chen et al. 1996; Wang et al. 1994). On the other hand, the difference of iPA and DHZR content in the ovules of normal and mutant cotton is somewhat similar, and extremely high levels in the ovules of mutant cotton have been determined. These results indicate that the high content of CTKs may be the main factor that inhibits fiber elongation in the mutant, since the high concentration of CTKs could inhibit fiber production (Beasley 1973; Beasley and Ting 1973, 1974; Chen et al. 1988; Zhang 1982) in both fertilized and unfertilized ovules in vitro.

In vitro, ABA reduced the capacity of the ovule to produce fibers in the presence of IAA or GA_3 (Beasley and Ting 1974). A low level of endogenous ABA seems to be essential for fiber growth. It is found that at the fiber elongation stage, the ABA level decreases gradually in normal cotton ovules. The decrease in the ABA content in the ovules of mutant cotton is also observed, but such a decrease is slow.

Based on above results of endogenous hormone and the previous exogenous hormones we discussed, we suggest that a high level of IAA before flowering may initiate the fiber production, and 13-hydroxy-GAs may provide for the fiber elongation, whereas high contents of ABA and cytokinins inhibit its growth.

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