J Plant Growth Regul (1998) 17:1–5

Journal of
Plant
Growth
Regulation

Short Communication

Potential Role of Abscisic Acid in Cotton Fiber and Ovule Development

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Received June 25, 1997; accepted October 15, 1997

Abstract. Fibers and ovules of a cotton cultivar (*Gossypium hirsutum* L. Trambak-108) were analyzed for growth and free abscisic acid (ABA) content by indirect enzyme immunoassay. An inverse correlation between fiber elongation and ABA content was observed. In the seed, accumulation of ABA was observed during secondary thickening and the maturation phase. The potential role of ABA in fiber and seed development is discussed.

Key Words. Abscisic acid—Cotton (*Gossypium hirsutum*) fiber—Cotton seed—Indirect enzyme immunoassay

Cotton is grown around the world in tropical and subtropical regions. Each year about 16 million tons of cotton fiber and about 33 million tons of cotton seeds are produced worldwide (Firoozabady 1989). New varieties of cotton must be developed to meet the needs of textile industries and the demand of cotton producers. Yield improvement, fiber quality, uniform maturity, short stature, and earliness are the traits upon which cotton breeders have placed much emphasis. The objective of genetic improvement in cotton is to develop cultivars with high yield potential, improved fiber and seed quality, and other agronomic characteristics in addition to an increased level of resistance to pests.

Cotton fiber length and its dry weight per seed are two parameters of commercial importance. Fiber length is the best criterion for spinning value and determines the yarn strength and the finest yarn size into which the cotton can be spun (Rusca 1970). Fiber dry weight per seed, on the other hand, is an important determinant of fiber yield, and any advancement in this component is likely to improve productivity (Bharadwaj 1988, Butany et al. 1968).

In recent years, cotton seeds have yielded high grade oil and protein concentrates for human consumption, and this has highlighted the importance of research on the improvement of cotton seeds. Sucrose translocated from the leaves accumulates as cellulose in mature fibers, whereas the seed accumulates high concentrations of protein and lipid.

Considerable evidence indicates that phytohormones play a decisive role in fiber development (Beasley and Ting 1973, 1974, Naithani et al. 1982). In in vitro culture of ovules it was observed that indoleacetic acid (IAA) plays an important role in fiber development, whereas abscisic acid (ABA) inhibits it (Beasley and Ting 1973). Addicott (1982) observed that the ABA content was higher in mature cotton fruits than in young healthy fruits. However, no detailed studies have been made on the role of ABA in fiber and ovule development.

In the present study, the ABA content in fiber and ovules was analyzed during the entire period of growth and development. The aim of our research was to define parameters regulating cotton fiber and ovule development. In the future this may help to identify gene(s) of interest for selection criteria of better yielding varieties or for genetic manipulation.

Because phytohormones like ABA are synthesized in small quantities, a sensitive and accurate immunoassay was developed previously for ABA analysis in plant tissue (Merterns et al. 1983, Weiler 1980). In those studies enzyme immunoassay (EIA) determination of ABA was not significantly different from that determined by high performance liquid chromatography (HPLC) or gas chro-

Abbreviations: IAA, indoleacetic acid; ABA, abscisic acid; EIA, Enzyme immunoassay; HPLC, high performance liquid chromatography; GC, gas chromatography; ELISA, enzyme-linked immunosorbent assay; DPA, days postanthesis.

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matography (GC) analysis. The advantage of EIA for hormone analysis is the ability to use crude plant extracts without sacrificing sensitivity and selectivity. To amplify the reaction, an indirect enzyme-linked immunosorbent assay (ELISA) technique was used to quantify endogenous levels of ABA in developing cotton fibers and ovules.

Materials and Methods

Seeds of cotton cultivar (*Gossypium hirsutum* L. cv. Trambak-108) were grown in the field. The cultural practices, including irrigation and application of fertilizers and insecticides, were conducted to maximize the lint yield. On the day of anthesis, each individual flower was tagged, and healthy bolls were harvested for the analysis of fiber and seed growth in terms of fiber length, fresh weight, dry weight, and estimation of ABA in both fiber and seed. To minimize the effect of environmental variations, data were collected from flowers that had bloomed during as narrow a period as possible.

Fiber Length Measurements

Fiber length was determined by the method of Gipson and Ray (1969). A locule from a boll was placed in boiling water to allow the seeds to separate from each other, and each seed was placed on the convex surface of a watch glass. The fibers were streamed out with a jet of water. The length of the fiber was measured to the nearest mm, from the rounded side of the seed, adjacent to the chalazal end.

Fresh Weight and Dry Weight Measurements

Fibers were removed manually from the seed with a scalpel without removing the seed coat. Seeds from four locules of four bolls were used for fresh and dry weight measurements. Freshly separated fibers and seeds were weighed before and after oven drying to a constant weight at 70°C to obtain data on fresh and dry weights. The water content was determined by the difference in the fresh and dry weight at a given time. The mean dry weight and water content per seed ± S.D. was calculated.

Freshly harvested bolls were opened with a scalpel, and fibers were separated from the ovule and stored in a freezer before use. During the early stages it was difficult to separate the fibers from the ovules, so the intact ovules were taken for analysis; at day 9 after anthesis and subsequently the separated fibers were used. The frozen material was crushed in the dark in 80% chilled methanol (Tahara et al. 1991). The supernatant was collected after centrifugation at 10,000 × *g* for 10 min and concentrated to 0.5 mL in dark.

Statistical Analysis

The sets of data on dry weight and water content of fiber and seed were fitted to an appropriate polynomial curve using the equation of the form:

$$y\ (\text{mg}) = a + bx + cx^2 + dx^3 \ldots xgx^6$$

where x is day after anthesis; y is mg fresh weight, dry weight, or water content; and $a, b, c, \ldots g$ are constants.

The rate curves were obtained by differentiating the best-fit equation.

Preparation of Polyclonal Antibodies against ABA

Polyclonal antibodies were developed using a modified method described earlier (Weiler 1980). ± *cis-trans*-ABA (Sigma) was conjugated to a carrier protein bovine serum albumin before immunization. Three rabbits (nearly 14 weeks old) were immunized by intramuscular injection. Booster injections were given monthly to raise the antibody titer. The presence of specific IgGs against ABA was tested by an indirect ELISA technique, using an internal standards and known concentrations of ABA.

Procedure for ELISA

To avoid cross-reactivity with the conjugated protein, ABA-ovalbumin conjugate was used to immobilize antigen on a polystyrene microtiter tray. Various concentrations of conjugate (ranging from 50 to 500 ng) were passively adsorbed to the wells. The conjugate was diluted with coating buffer (10 mm carbonate buffer, pH 9.7). The plate was incubated at 37°C for 3 h and then washed several times with washing buffer (phosphate-buffered saline containing 0.05% Tween 20 and 0.5% ovalbumin, pH 7.2).

Various dilutions of antiserum were added to the wells and incubated for 1 h at 37°C. Unbound rabbit serum was removed by repeated washing with the washing buffer.

Second antibody, antirabbit goat IgG conjugated to peroxidase (1: 1,500 in phosphate-buffered saline, 0.03 M, pH 6.4) was added to each well and incubated for 1 h at 37°C. All wells were washed again, and substrate solution containing 20 mM guaiacol, H_2O_2 (3%), and 0.03 mM phosphate buffer, pH 6.4 (2:1:3) was added. The resulting color was read at 492 nm with an ELISA plate reader. For estimation of ABA in unknown samples, different concentrations of samples were mixed with the antibody (with or without internal standards) before addition into the wells. Similarly, known concentrations of ABA were used for standard curve preparation. All estimations were done in triplicate and reported as mean values ± S.D.

Results and Discussion

Data of fiber length and fiber dry weight were fitted to polynomial equations of different degrees, and the best-fit equation was determined statistically by performing a *t*-test for different r^2 values. The data on fiber length and dry weight were explained adequately by a fourth degree polynomial equation (Fig. 1).

Fiber initials, which started elongation soon after anthesis, entered the linear phase of elongation after 9 days postanthesis (DPA) and continued to elongate up to 30 DPA when maximum length was attained. In subsequent periods there was no significant change in fiber length (Fig. 1*a*). The rate of fiber elongation per day reached a maximum on day 15, followed by a gradual decrease, and the minimum rate of elongation was recorded on day 36 (Fig. 1*a*).

Fiber dry weight showed a sigmodial pattern with a definite lag phase during the early period of fiber growth. Fiber initials entered the linear phase of dry matter ac-

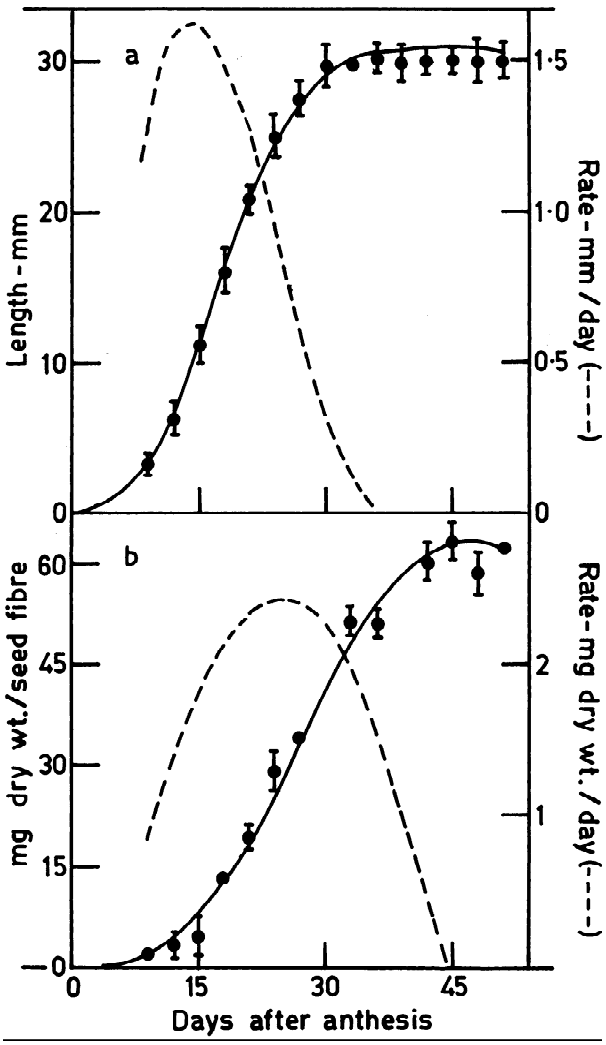

Fig. 1. Fiber growth curve derived from polynomial equations. *Panel a,* changes in length (*continuous line*) and a rate of fiber elongation (*broken line*). *Panel b,* fiber dry weight (*continuous line*) and rate of dry matter accumulation (*broken line*). *Vertical bars* represent ± S.D.

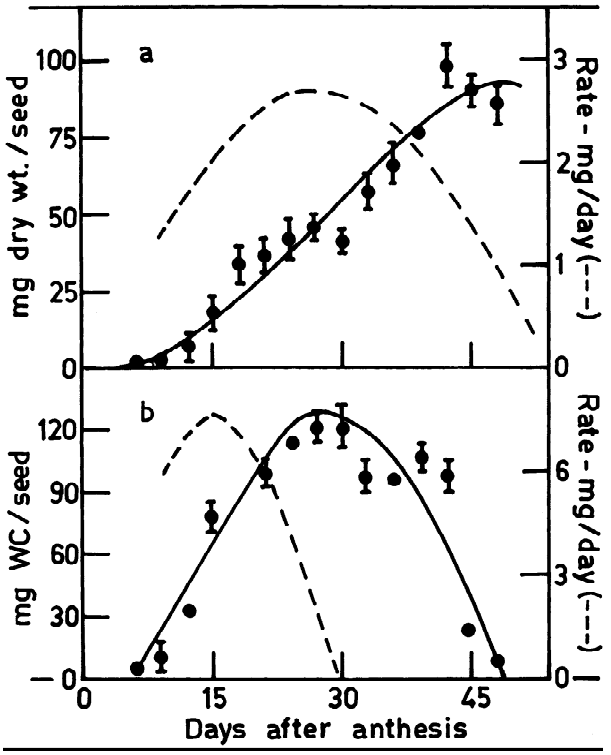

Fig. 2. Seed growth curve derived from polynomial equations. *Panel a,* change in seed dry weight (*continuous line*) and rate of dry matter accumulation (*broken line*). *Panel b,* water content (*continuous line*) and rate of water uptake (*broken line*) against boll age. *Vertical bars* represent ± S.D.

cumulation around 9 DPA. The fiber attained maximum dry weight at 48 DPA, when the boll opened. The rate curve of dry matter accumulation per day recorded a maximum rate during 27–33 DPA (Fig. 1*b*). Growth analysis of cotton fiber, with respect to its length and dry weight, has been reported earlier from this laboratory (Jasdanwala et al. 1977, Naithani et al. 1982, Thaker et al. 1986, 1989, 1996). In all of these studies it was shown that the development of cotton fiber can be divided into four distinct phases: initiation, elongation, secondary thickening, and maturation, and that there was a clear-cut overlap between various phases.

Data on seed dry weight and water content were also fitted to polynomial equations (Fig. 2). The trend in dry matter accumulation showed an initial lag up to 12 DPA after which it entered a linear phase of dry matter accumulation, the maximum dry weight being recorded on day 48. Subsequently, a slight decrease in weight was recorded (Fig. 2*a*). The rate of dry matter accumulation was highest during 24–30 DPA. Water content was low up to 3–5 DPA, increasing until 33 DPA, and declining thereafter (Fig. 2*b*). The change of water content was maximum on day 15 and decreased gradually. The minimum rate of water content was recorded on day 30.

Changes in free ABA levels in fiber are presented in Fig. 3. It was observed that the ABA content increased up to 24 days, then decreased up to 30 days, after which they increased gradually with a peak at 39 days. In subsequent periods, the ABA content decreased slightly. Thus ABA showed an inverse correlation with the rate of fiber elongation. Additionally, when the fiber achieved maximum rate of dry matter accumulation (on day 30), the free ABA content was relatively low and increased with the decrease in the rate of dry matter accumulation. An inverse relationship between internal ABA levels and cell elongation in roots and coleoptiles of rice has been reported (Lee et al. 1994). A negative correlation between ABA levels in the elongation zone of maize root

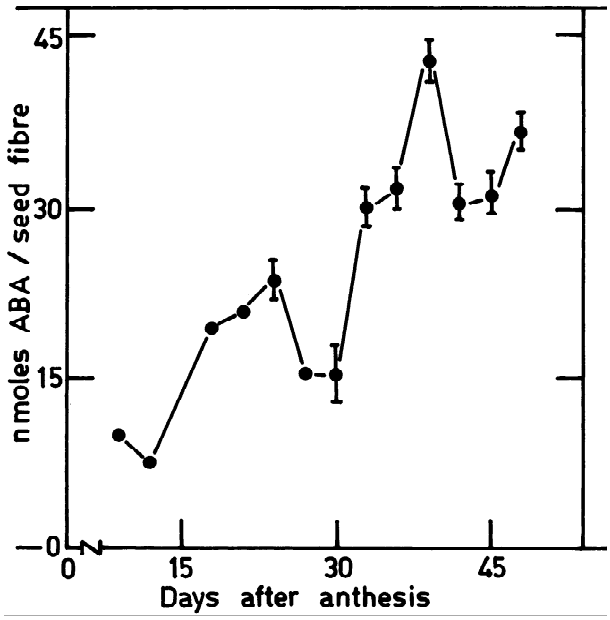

Fig. 3. Changes in ABA content in fibers against boll age. Each data point is the mean of three replicates estimated by an indirect ELISA. *Vertical bars* represent ± S.D.

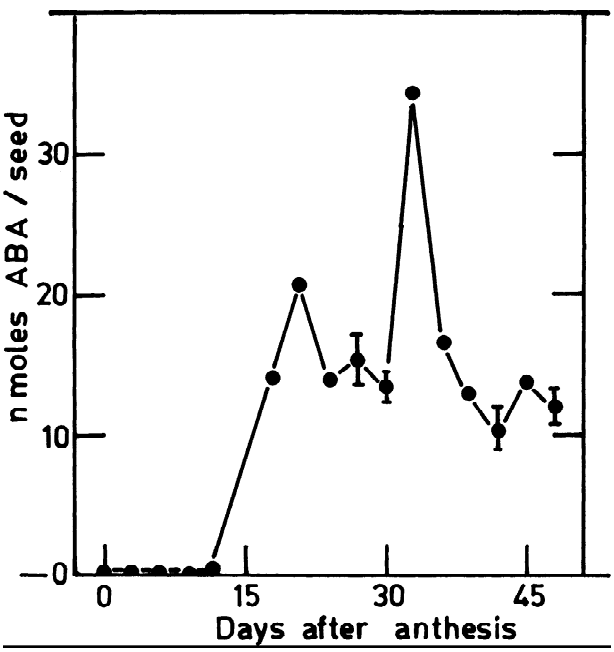

Fig. 4. Changes in ABA content in ovules against boll age. Each data point is the mean of three replicates estimated by an indirect ELISA. *Vertical bars* represent ± S.D.

and the growth rate of the root has also been reported (Pilet and Saugy 1987). Similarly, the role of ABA in inhibition of shoot elongation of *Scirpus mucronatus* has also been reported (Lee et al. 1996). The inverse relation with fiber growth observed in the present study also supports the view that ABA may be one of the factors that influences fiber growth and development.

In developing cotton seed, ABA content was negligible during the early phases of seed development but began to increase from day 15 (Fig. 4). The peak was observed on day 33 and decreased gradually until boll opening. Except for the peak at day 33, the ABA content remained almost unchanged during the seed development phase. Therefore, the ABA content may not have any specific regulatory role in dry matter accumulation, but increased concentrations at the later part of seed development may participate in maturation of the seed. Embryo development ceases during the maturation phase of the seeds of most angiosperms. During this phase seeds become desiccated and enter a period of arrested development which serves to prevent them from germinating in unfavorable conditions. Endogenous ABA levels are thought to participate in this regulation (Moore 1989).

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