HORMONAL REGULATION OF FIBER ELONGATION, AND THE ENZYMATIC HYDROLYSIS OF ABSCISIC ACID CONJUGATE IN DEVELOPING COTTON (*Gossypium arboreum* L.) FIBRES

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Abstract

Changes in endogenous levels of IAA, GA and ABA during *in vivo* fiber growth of *Gossypium arboreum* L. (cv. LD 327) showed an over-riding influence of ABA in limiting the rate of fibre elongation. The fibre growth of unfertilized cotton ovules cultured *in vitro* was elevated by the application of fluridone, which markedly decreased the ABA level concomitant with an increase in the levels of growth promoters (IAA, GA_3) in cultured ovules. The enzymatic hydrolysis of β-glucopyranosyl ester of ABA by β-glucosidase and esterase were studied in fibres at 15 and 35 days after anthesis. Since the activity levels of two hydrolases did not correlate with the free ABA contents at two stages of fibre growth, it is suggested that the high content of free ABA at 15 days after anthesis (DAA) was not a consequence of the increased activity of the ABA-Glc splitting enzymes. Our data suggests that ABA conjugate is the final product of the ABA metabolism under different stages of fibre growth.

Introduction

The available evidence indicates that hormones play a decisive role in fibre development in cotton. It has been shown that IAA is essential for the elongation of fibre initials, whereas gibberellic acid (GA) was involved in the expansion of the ovule (Dhindsa, 1978; Nayyar et al., 1989). Levels of IAA or the rate of IAA degradation may regulate the termination of fibre elongation and the beginning of secondary wall synthesis (Basra and Malik, 1984). The level of ABA in cotton seeds is extremely high during the period of rapid fibre elongation and it continues to be high even after fibre elongation has terminated (Nayyar et al., 1989). In another study, seeds treated with fluridone, which inhibited ABA production, stimulated fibre length (Nayyar et al., 1989) in *Gossypium arboreum.*

The β-D-glucopyranosyl ester of abscisic acid (ABA-Glc) is the chief conjugate of the plant hormone assayed in many plants as an endogenous substance or as a metabolite of exogenously applied ABA. Even though the physiological function of the conjugate is not yet known, it appears to be much more a final product of ABA metabolism than a storage or transport form (Zeevaart, 1983), being restricted to plant cell vacuoles (Lehmann and Glund, 1986). Recently, Lehmann and Vlasov (1988) have investigated the enzymic hydrolysis of abscisic acid conjugate. The intent of the present investigation was to secure information on fiber elongation, endogenous levels of hormones, the behaviour of conjugated ABA during fibre elongation and secondary wall thickening phase. The latter was done by investigating hydrolysing enzymes in soluble proteins from fibre and characterization of the nature of the concerned enzymes.

Materials and Methods

Plants of *Gossypium arboreum* L. (cv. LD 327) were raised in the field according to the recommended practices for fertilizer application, plant protection, weed control and irrigation schedules to optimize seed cotton yield under field conditions. On the day of anthesis, the flowers were tagged in the morning and bolls were sampled at different days after anthesis (DAA).

Fibre length was evaluated as fibre length determination from seeds taken from bolls of appropriate ages. Bolls were harvested at 5 day intervals between 5 and 40 DAA and length of fibres was measured according to Gipson and Ray (1969). Individual fibres were separated from the seeds at 10, 15, 20, 25, 30 and 35 DAA, and used for the extraction, purification, derivatisation and determination of endogenous levels of IAA, GA, and ABA by GLC method (see Nayyar et al., 1989).

For *in vitro* studies, unfertilized ovules excised from ovaries were collected within a few hours after anthesis. A set of 10-15 ovules scooped from the same ovary were cultured in Beasley and Ting (1974) medium with the following modifications: 120 mM glucose, alpha-naphthalene acetic acid (5 microM) and gibberellic acid (0.5 microM). An appropriate concentration of fluridone was added to the medium.

For the study of beta-glucosidase and esterase, seed with adhering fibres at 15 and 35 DAA were taken incubated in Hepes buffer (20 mM, pH 7.5) for 3 h at 28 degrees C with continuous aeration. Various chemicals e.g. ABA (10 microM) and fluridone (5 ppm) were incorporated into the medium. After 3 h incubation, the fibres were separated from the seeds and extracted for the preparation of soluble activities of beta-glucosidase and esterase. Beta-glucosidase activity was determined as given in Sharma et al. (1981) using p-nitrophenyl-beta-D-glucopyranoside as substrate, the activity of esterase was assayed by a modification of the method described by Amador and Wacker (1965) using alpha-naphthyl acetate as a substrate.

Results and Discussion

The development of cotton fibres is divisible into four phases: (i) initiation, (ii) elongation, (iii) secondary wall

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deposition and (iv) maturation. The fibre initiation begins a day or two after anthesis, and fibre initials enter into elongation phase immediately (Nayyar et al, 1989). In the present investigation, the fibres of *Gossypium arboreum* L. (cv. LD-327) elongated rapidly during the period of 5 to 15 DAA and thereafter their growth rate declined (Fig. 1). By the 15 DAA nearly 85 to 90% of the final fibre length was attained, however, between 20 and 25 DAA, fibre elongation ceased.

Changes in endogenous levels of IAA, GA and ABA in fibres at various days after anthesis are shown in Fig. 2. The level of IAA at 10 and 15 DAA were comparatively lower than at 20 through 30 DAA. The level of IAA did not correlate with the rate of fibre elongation. Maximum levels of GA were estimated at 16 DAA amongst the stages studied and did not show a precise relationship with the rate of fibre elongation. The ABA level was several fold higher than IAA and GA and was highest at this stage. Apparently, high ABA acts as a signal for the decline of the elongation phase and initiation of the secondary thickening phase.

Soluble protein fractions were investigated with regard to their hydrolysing activities. The enzymes studied were beta-glucosidase (substrate: p-nitrophenyl-beta-glucoside), esterase (substrate: alpha-naphthyl acetate). The data plotted in Figs. 3 and 4 showed the activities of hydrolysing enzymes at 15 and 35 DAA. The activities of esterase and beta-glucosidase were highest at 35 DAA. Due to the lack of any definitive correlation between the activity level of two hydrolysing enzymes, and ABA content at two stages of fibre growth, it is reasonable to assume that the high content of ABA at 15 DAA was not a consequence of the enhancing acticity of ABA-Glc splitting enzymes at that stage. Addition of 10 microM ABA in the medium decreased the activities of the ABA-Glc hydrolysing enzymes in the soluble protein fraction. Possibly, the added ABA was not metabolized to either acidic compounds or to conjugated ABA, hence, indicating it specific influence on the activity of the two hydrolyzing enzymes.

We also attempted to investigate the kind of enzymes having ABA-Glc hydrolysing activities. Consequently, the activities for splitting of ABA-Glc, PNP-beta-GLc (glucosidase) and alpha-naphthyl acetate (esterase) were assayed. Comparing Figs. 3 and 4, where ABA (10 microM) and fluridone (5 ppm) were added, the activity pattern of two enzymes made interesting revelations. From these results, we infer that ABA conjugate was hydrolyzed by beta-glucosidase as well as esterase. We also investigated the influence of fluridone, a reported inhibitor of ABA biosynthesis (see Nayyar et al, 1989), on the level of hormones in ovules 20 days after culture. The data given below shows a marked decrease over the controls.

Table 1. Effects of fluridone (5 ppm) on growth hormones content (micrograms per gram of tissue fresh weight) 20 days after culturing.

Treatment	IAA	GA_3	ABA
Control	0.50	1.20	0.90
Fluridone	1.92	1.46	0.40

From our studies it is apparent that at 35 DAA, the content of ABA decreased sharply. In cultured ovules addition of fluridone markedly decreased the ABA content. If the decrease of free ABA was a consequence of ABA-Glc conjugation and under the assumption that the enzyme activities in soluble protein fraction indicated that the *in vivo* status of the fibres, we would expect alterations in the activity levels of the involved enzymes. Interestingly, we observed such changes in the controls at 35 DAA, and also in the cultures with added fluridone (Figs. 3, 4). It is thus reasonable to assume that the decrease of free ABA observed at 35 DAA was a consequence of the enhancing activity of ABA-Glc splitting enzymes in the analyzed proteins. Contrarily, the addition of ABA markedly decreased the activities of the two enzymes toward PNP-beta-GLc and alpha-naphthyl acetate in the soluble protein fraction of fibres.

Present studies are the first efforts to investigate the possible function of ABA-Glc in cotton fibres at enzymic levels and how they relate to fibre growth. Our data suggest that ABA conjugate is the final product of ABA metabolism during fibre development.

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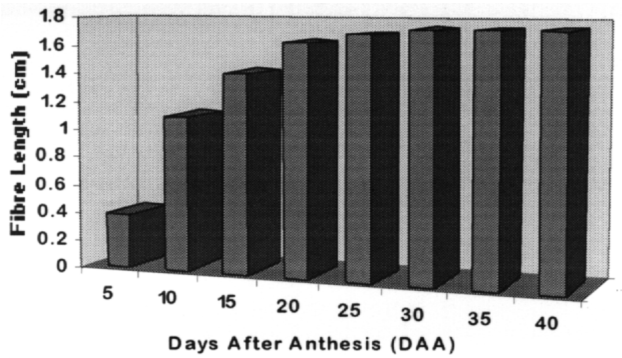

Figure 1. Fibre Length During Cotton Fibre Development

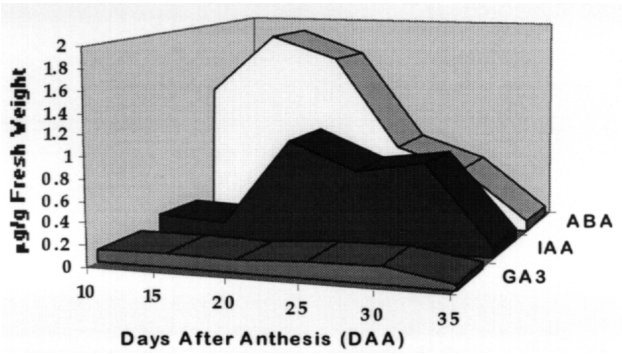

Figure 2. Hormonal Level During Cotton Fibre Development

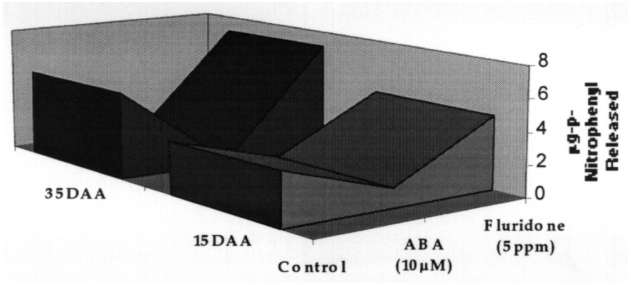

Figure 3. Influence of ABA and Fluridone on β-Glucosidase Activity (µg-p-Nitrophenyl Release/h/mg protein)

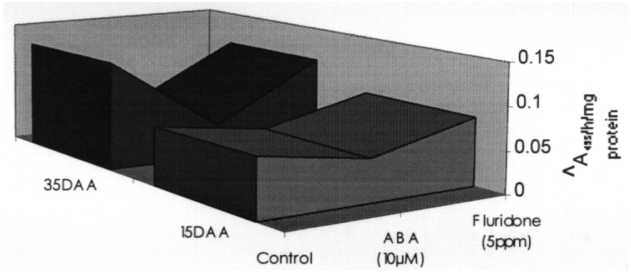

Figure 4. Influence of ABA and Fluridone on Esterase Activity (^A496/h/mg protein)