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HORMONAL REGULATION OF FIBRE ELONGATION AND THE ENZYMIC HYDROLYSIS OF ABSCISIC ACID CONJUGATE IN DEVELOPING COTTON (*GOSSYPIUM ARBOREUM* L.) FIBRES

PUSHP SHARMA* AND C.P. MALIK

Department of Botany, Punjab Agricultural University, Ludhiana-141 004, Punjab

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SUMMARY

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

Key words: **Abscisic acid conjugate, fibre elongation, *Gossypium*, hormones, ovule culture**

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_3) was involved in the expansion of the ovule (Singh *et al.* 1995, Gokani and Thaker 2002a, Malik 2004). 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). Changes in both IAA and ABA levels seemed not to be closely related with the rate of cellulose accumulation, but there was still a relationship between the ratio of ABA and IAA and secondary wall thickening (You-Ming *et al.* 2001). 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 had terminated (Nayyar *et al.* 1989). In another study, seed treated with fluridone, which inhibited ABA production, stimulated fibre length (Nayyar *et al.* 1989, Sharma and Malik 2005) in *G. arboreum*.

The B-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 precise physiological function of the conjugate is not known yet it appears to be much more a final product of ABA metabolism than a storage or transport form (Zeevaart 1983, Gokani and Thaker 2002) being restricted to plant cell vacuoles (Lehmann and Glund 1986). Lehmann and Vlasov (1988) have investigated the enzymic hydrolysis of abscisic acid conjugate.

*Corresponding author, E-mail: pushp20@yahoo.com, Present address: Department of Plant Breeding and Genetics, Punjab Agricultural Unversity, Ludhiana-141 004, Punjab.

The intent of present investigation was to secure information on fibre elongation, endogenous levels of hormones, the behaviour of conjugated ABA during fibre elongation and secondary wall thickening phase. The latter was done by investigating hydrolyzing enzymes in soluble proteins from fibres 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 elongation 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 days after anthesis (DAA) and length of fibre was measured according to Gipson and Ray (1969). Individual fibres were separated from the seeds at 10, 15, 20, 25, 30, 35 DAA and used for the extraction, purification, derivatization and determination of endogenous levels of IAA. GA_3 and ABA by GLC method (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 was cultured in the Beasley and Ting (1974) medium with the following modifications: 120 mM glucose, α-naphthalene acetic acid (5μM) and gibberellic acid (0.5 μM). An appropriate concentration of fluridone (1-methyl-3-phenyl-5 (3-trifuromethyl-phenyl)-4 (1H)-pyridinone) was 5 mg l^{-1}.

For the study of β-glucosidase and esterase, seed with adhering fibres at 15 and 35 DAA were taken and incubated in Hepes buffer (20 mM, pH 7.5) for 3 h at 28°C with continous aeration. Various chemicals, e.g. ABA (10 μM) and fluridone (5 ppm) were incorporated in the medium. After 3h incubation, the fibres were separated from the seeds and extracted for the preparation of soluble activities of β-glucosidase and esterase. β-glucosidase activity was determined as described by Sharma *et al* (1981) using p-nitrophenyl-β D-glucopyronoside as substrate. The activity of esterase was assayed by a modification of the method described by Amador and Wacker (1965) using α-napthyl acetate as a substrate. Proteins were estimated by the method of Lowry *et al.* (1951). The results represent averages of three experiments. The standard error was within the range of ±6%.

RESULTS AND DISCUSSION

The development of cotton fibre is divisible into four phase : (i) initiation, (ii) elongation, (iii) secondary wall thickening and (iv) maturation. The fibre initiation begins a day or two after anthesis and the fibre initials enter into elongation phase immediately (Nayyar *et al.* 1989, Ruan *et al.* 2001). In the present investigation, the fibres of *Gossypium arboreum* L. cv. LD 327 elongated rapidly during 5-15 DAA and thereafter their growth rate declined (Table 1). By day 15, nearly 85% to 90% of the final fibre length was attained and between 20-25 DAA, fibre elongation ceased.

Table 1. Fibre length (cm) at various days after anthesis (DAA)

Days after anthesis (DAA)	Fibre length (cm)
5	0.38 ± 0.02
10	1.1 ± 0.06
15	1.42 ± 0.04
20	1.64 ± 0.08
25	1.70 ± 0.04
30	1.74 ± 0.02
35	1.74 ± 0.03
40	1.74 ± 0.06

Changes in endogenous levels of IAA, GA_3 and ABA in fibres at various days after anthesis are depicted in Table 2. The level of IAA at 10 and 15 DAA were comparatively lower than at 20, 25 and 30 DAA. Maximum level of GA_3 was estimated at 30 DAA amongst the stages studied. The levels of both IAA and GA_3 did not show a precise relationship with the rate of fibre elongation. The ABA level was several fold higher than IAA and GA_3 upto 15 DAA and was highest at

this stage. Apparently, high ABA acts as a signal for the decline of elongation phase and initiation of secondary thickening phase.

Table 2. Endogenous levels of hormones in fibres at various days after anthesis

Days after anthesis (DAA)	Hormones contents (µg g^{-1} fw)		
	IAA	GA_3	ABA
10	0.20	0.12	1.30
15	0.16	0.13	1.90
20	1.00	0.11	0.67
25	0.75	0.15	0.80
30	0.85	0.16	0.60
35	0.10	0.05	0.10

Soluble protein fractions were investigated with regard to their hydrolyzing activities. The enzymes studied were β-glucosidase (substrate : PNP-B-Glc), esterase (substrate : -α naphthyl acetate). The data set in Table 3 shows the soluble fraction of proteins from fibres at two stages of development, i.e. 15 and 35 DAA. The activities of esterase and β-glucosidase were highest during 35 DAA. Due to the lack of any definite correlation between the activity level of two hydrolyzing enzymes, and ABA contents at two stages of fibre growth, it is reasonable to assume that the high content of free ABA at 15 DAA was not a consequence of the enhancing activity of the ABA-Glc splitting enzymes at that stage.

Table 3. Activity of hydrolyzing enzymes in the fractions of soluble protein of cotton fibres at two developmental stages

Treatments	Soluble protein			
	Esterase (A 495 h^{-1} mg $protein^{-1}$)		β-glucosidase (µg-p-nitrophenyl released h^{-1} mg $protein^{-1}$)	
	DAA		DAA	
	15	35	15	35
Control	0.07	0.13	3.26	5.48
ABA @ 10µM	0.05	0.04	1.26	0.22
Fluridone @ 5 ppm	0.08	0.11	4.65	7.01

Addition of 10 µM ABA in the medium decreased the activity of the ABA-Glc hydrolyzing enzymes in the soluble protein fraction. Possibly the added ABA was not metabolized to either acidic compounds or to conjugated ABA, hence indicating its specific influence on the activity of the two hydrolyzing enzymes. We also attempted to investigate the kind of enzymes having ABA-Glc hydrolyzing activities. Consequently the activities for splitting of ABA-Glc, PNP-B-Glc (glucosidase) and -α naphthyl acetate (esterase) were assayed. Comparison of data in Table 3, where ABA (10 µM) and fluridone (5 ppm) were added, the activity pattern of two enzymes made interesting revealation. From these results, we infer that ABA conjugate was hydrolyzed by β-glucosidase as well as esterase. We also investigated the influence of fluridone, a reported inhibitor of ABA biosynthesis (Nayyar *et al.* 1998, Sharma and Malik 2005) on the level of hormones in ovules 20 days after culture. The data showed a marked decreased in ABA while IAA and GA_3 content increased over the control (Table 4).

Table 4. Influence of fluridone on the level of hormones in ovules 20 days after culture

	Hormone (µg g^{-1} fw)		
	IAA	GA_3	ABA
Control	0.50	1.20	0.9
Fluridone (Flu) treatment	1.92	1.46	0.4

From our studies, it is apparent that at 35 DAA, the content of ABA decreased sharply. In tissue culture, with added Flu, ABA decreased markedly. If the decrease of free ABA was a consequence of ABA-Glc conjugation and under the assumption that the activity of the enzymes in the protein of soluble fraction indicated the *in vivo* status of the fibres, we would accept alterations in the activity level of the enzyme involved. Interestingly, we observed such changes in the control at 35 DAA and also in cultures with added Flu (Table 3). It is, thus, reasonable to assume that the decrease

of the free ABA observed during 35 DAA was a consequence of the enhancing activity of the ABA-Glc-splitting enzymes in the proteins analysed. Contrarily, the addition of ABA markedly decreased the activities of the two enzymes towards PNP-B-Glc and α-naphtyl acetate in the soluble protein fraction of fibres.

Present studies showed the possible function of ABA-Glc in cotton fibres at enzymic level pertinent to fibre growth. Our data suggest that ABA conjugate is final product of the ABA metabolism under the different stages of fibre growth. A positive correlation between final fibre length, free and conjugated IAA and phenyl acetic acid (PAA) content was observed by Gokani and Thaker (2002b).

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