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Regulation of Differential Fibre Development in Cotton by Endogenous Plant Growth Regulators

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The endogenous levels of IAA, GA_3 and ABA were determined at initiation, elongation, secondary thickening and maturation phases of fibre development in two *Gossypium* cultivars varying in fibre length. The short staple cultiver *(G. arboreum)* as compared with long staple cultivar *(G. hirsutum)* possessed high IAA and GA_3 levels before fertilization and just after fertilization, while converse was true during active phase of fibre elongation. The amount of ABA was considerably higher at all phases of fibre development in short staple cultivar than long staple cultivar. Analysis of correlation coefficients and regression values indicated the ABA involvement in regulation of extent of fibre elongation in genetically different cotton cultivars. The observations are discussed in the light of possible functions of phytohormones during fibre development.

Key Words: Cotton, *Gossypium arboreum, G. hirsutum,* Fibre Development, Phytohormones

Introduction

On the basis of growth analysis, cotton fibre development is divisible into four phases: (i) initiation, (ii) elongation, (iii) secondary thickening, and (iv) maturation (Naithani et al. 1982). The fibre initiation starts a day before up to a day or two after anthesis and the initials enter into elongation phase immediately. The final length of a cotton fibre is the product of the rate of elongation per day and the total period of elongation. Variability in the rate and period of elongation and secondary wall deposition exists among different cotton varieties. The mechanisms regulating the termination of elongation and initiation of secondary wall thickening are largely unknown. Previous studies from our laboratory (c.f. Basra & Malik 1984) have suggested number of probable mechanisms which considerably affect various phases of fibre development.

Considerable evidence indicates that phytohormones play a decisive role in cotton fibre development. Growth substances implicated in cell elongation have been detected in cotton fibres and seem to play a significant role in regulating their development (Bhardwaj & Lad 1977, Naithani et al. 1982, Bhardwaj & Verma 1985). On the basis of *in vitro* ovule culture studies, Beasley and Ting (1973) concluded that the fertilized isolated cotton ovules appear to be (i) deficient in their capacity to synthesize optimal levels of gibberellins, (ii) sufficient in their production of cytokinins, (iii) optimal or near optimal in the production of auxin (IAA), and (iv) ABA was not essential for fibre elongation. Hence a diminution of its effective concentration concomitant with and perhaps dependent upon an increase in IAA and GA_3 following fertilization would permit ovular and fibre growth.

Though mean fibre length varies in genotypes of cultivated species of *Gossypium* yet physiological and biochemical changes underlying differential fibre growth are not well understood. Since involvement of phytohormones in cotton fibre development has been repeatedly suggested (Beasley & Ting 1973, Bhardwaj & Lad 1977), it is highly probable that these have a decisive function in regulating differential fibre growth. A perusal of available literature indicates that very little information exists on this aspect. Therefore, the present study was initiated to investigate variations in endogenous phytohormones during the four phases of fibre development (as mentioned above) in two cottons.

Materials and Methods

The field trial of the cotton crop (*Gossypium hirsutum* L. cv. F 414 (a long staple type) and *Gossypium arboreum* L. cv. LD 230 (a short staple type) was laid according to the recommended practices for fertilizer application, plant protection, weed control and irrigations to optimize lint yield under field conditions. During the peak season about 200 flowers were tagged in each variety on the day of anthesis (designated as day 0) in the morning and bolls were collected at various pre- (2 days) and post-anthesis stages (2-35 days).

The fibres were difficult to separate from ovules up to 5 days after anthesis and hence the young ovules/seeds were used as such for analysis. At day 10 and subsequent periods, fibres removed from seeds were used. The endogenous concentrations of phytohormones were extracted, purified and quantified using GLC, as detailed by Sethi and Singh (1983).

Fibre length was determined with callipers using the method of Gipson and Ray (1969) measuring from ovule epidermis to the tips of fibre produced on the chalazal end of ten randomly selected seeds per boll from 5 different bolls. For dry matter analysis, fibres were carefully excised from seeds and were washed several times with 80% (v/v) ethanol and dried in oven for 2 days at 80°C before weighing.

Correlation coefficients, regression analysis and standard error were worked out using the methods suggested by Panse and Sukhatme (1978).

Results and Discussion

The endogenous level of IAA in the ovules before fertilization (2 days before anthesis) remained low and then it increased sharply after anthesis in the long staple (LS) cultivar (figure 1). The rise was almost continuous till 3 DAA, except a slight decline at one day after anthesis (DAA). A steep decrease in IAA level was noticeable in the following stages. A similar pattern was observed in short staple (SS) cultivar, where the IAA level increased till 2 DAA and declined sharply at the succeeding stages. According to Beasley (1973), auxin(s) is the major hormone produced after fertilization. Studies of Naithani et al. (1982) based on bioassay techniques are confirmed by our observations. During initial stages of fibre growth compared with LS-cultivar, the SS-cultivar possessed higher levels of IAA whereafter a reverse pattern was discerned (figure 1). It seems that early decline of IAA levels in SS-cultivar may be considerably affecting the subsequent fibre elongation. Contrastly, the LS-cultivar maintained relatively higher IAA levels which might be responsible for stimulation of rate of fibre extension. Figure 1 shows a different pattern of gibberellins in both the cultivars. The amount of GA_3 was lowest compared with the other phytohormones examined. In general, both the cultivars had low amounts of GA_3 immediately after anthesis. In the SS-cultivar, GA_3 concentration declined till 1 DAA, whereas in LS-cultivar the decrease was noticeable till 2 DAA. These findings are supported by the observations of Beasley and Ting (1973) who suggested that fertilized cotton ovules were deficient in their capacity to synthesize optimal level of gibberellins. In LS-cultivar, the GA_3 level increased after 2 days, whereas in SS-cultivar a reverse trend was evident. GA_3 has been implicated in ovule growth (Dhindsa 1978) and increase in GA_3 contents in the LS-cultivar at later stages of fibre growth might be helping in sustaining rapid seed growth to keep pace with increased fibre growth during the same period. However, in SS-cultivar, low levels of GA_3 and IAA indicate relatively slower development of ovules and fibres. Earlier, Beasley (1977) suggested that relatively distinct differences in the length of two fibre types could be due to sequential 'perception' and relative amounts of effective endogenous auxins and gibberellins. In our studies also the relative concentrations of IAA and GA_3 in ovules at different periods possibly regulate the rate of fibre elongation in the two cotton cultivars.

The ABA level, compared with those of IAA and GA_3 was maximum during the entire period of ovule and

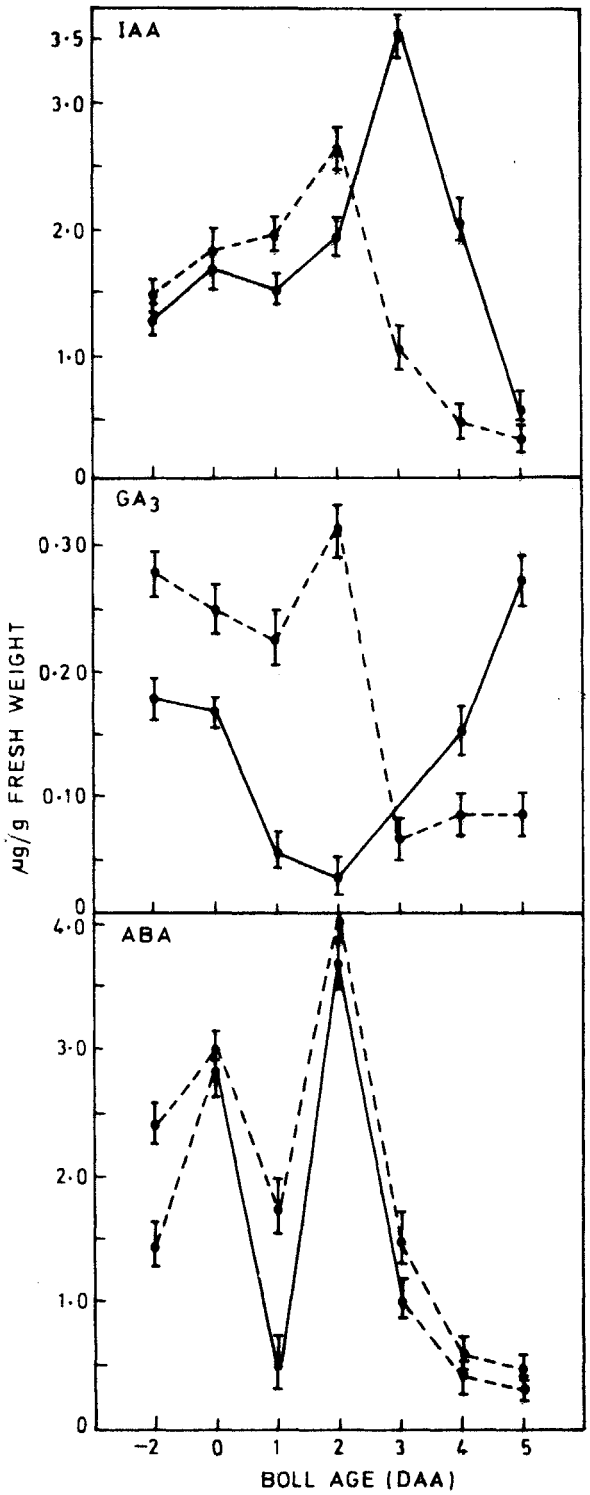

Figure 1 Endogenous levels of phytohormones in ovules and seeds in long staple (•—•) and short staple (•···•) cotton versus bollage. Vertical bars represent standard error

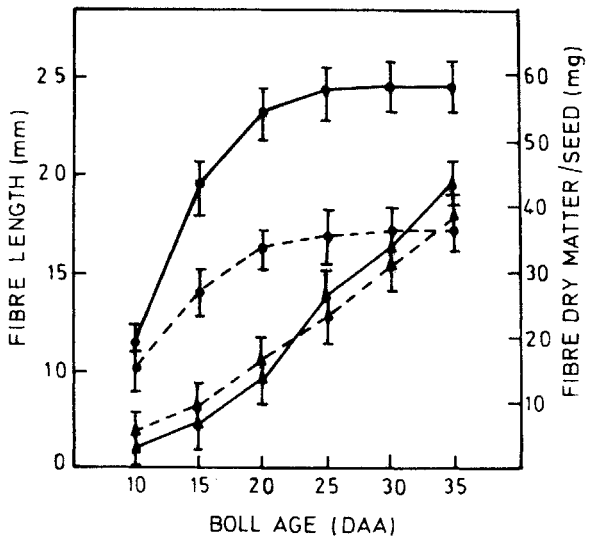

Figure 2 Fibre length and fibre dry matter per seed in long staple (•—•) and short staple (•···•) cotton versus bollage. Vertical bars represent standard error

fibre growth (figure 1). In both the cultivars, ABA content increased from 2 days before anthesis till the day of anthesis. A sharp decrease in its level was observed following anthesis succeeded by a second peak at 2 DAA in the two cultivars. At the following stages, the ABA level declined and was minimum at 5 DAA in both the cultivars. In the SS-cultivar compared with its counterpart ABA level was higher at all stages of ovule and fibre growth (figure 1). The present data reveal that the ovule and fibre growth is initiated as a result of decline in ABA concentration following fertilization. A second rise in ABA level at 2 DAA in both the cultivars probably provides threshold level of this phytohormone before seeds and their associated fibres achieve active growth. Another possibility appears to be requirement of specific ratio of ABA with IAA and GA_3, during that period. Beasley and Ting (1973) suggested that active ovular and fibre growth is dependent upon decrease in ABA concentration with concomitant increase in IAA and GA_3. From our studies it seems that an adequate ratio between IAA, ABA and GA_3 needs to be maintained for their suitable interaction.

Analysis of fibre length from 10 DAA onwards indicated active fibre elongation between 10 to 20 DAA,

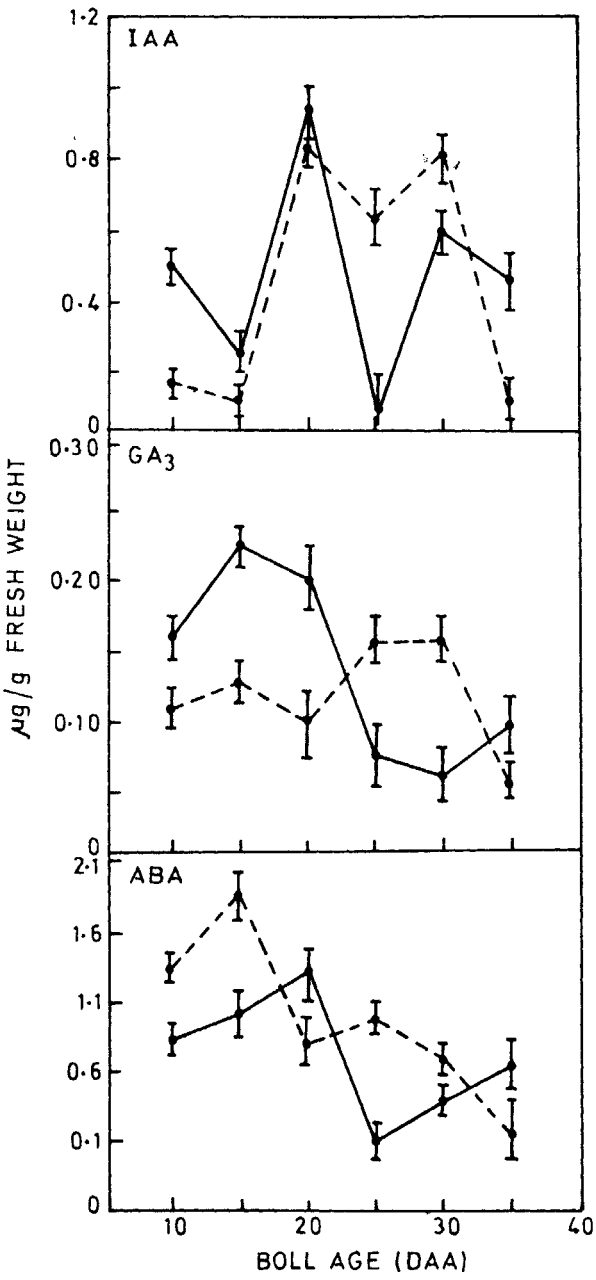

Figure 3 Endogenous levels of phytohormones in isolated fibres in long staple (●—●) and short staple (●···●) cotton versus bollage. Vertical bars represent standard error.

whereafter it slowed down in both the cultivars (figure 2). The dry matter of fibres exhibited a sigmoid pattern (figure 2). The SS-cultivar accumulated more of dry matter than LS-cultivar during the period of rapid fibre growth, whereafter the trend was conversed. The fibres almost ceased to elongate at 25 DAA, however, the dry matter accumulation continued indicating the initiation of secondary thickening phase.

The endogenous levels of IAA and GA_3 in the isolated fibres did not show any relationship with the rate of fibre elongation (figures 2,3). Both the phytohormones were at low level at 10 and 15 DAA compared with the later stages. Nevertheless, the IAA and GA_3 concentrations during active fibre growth were relatively more in LS-cultivar than SS-cultivar. Naithani et al. (1982) also failed to observe any relationship between IAA and rate of fibre elongation. The ABA level was several fold higher than IAA and GA_3 during the phase of fibre development (figure 3). The SS-cultivar compared with LS-cultivar possessed high ABA during rapid fibre growth (figure 3). The amount of ABA was highest at 15 and 20 DAA in SS- and LS-cultivar, respectively. Thus the results of the present study clearly suggest that fibre elongation in both the cultivars was retarded by high ABA concentration at the above described stages of fibre growth. Thus, high ABA apparently acts as a signal for the decline of elongation phase and initiation of secondary thickening phase. In SS-cultivar, this signal seemingly emanates earlier (at 15 DAA or early) and resulted in reduced final fibre length than LS-cultivar where 20 DAA stage appeared to be more appropriate for retarding fibre elongation. Thus, differences in endogenous levels of ABA possibly account for the variation in fibre length of two cottons.

The influence of ABA on fibre growth was also corroborated by correlation and regression analysis of these phytohormones with mean length values of isolated fibres. The fibre length in the two cotton cultivars was positively and non-significantly correlated with IAA and GA_3 (table 1). The correlation coefficient between fibre length and ABA was negative in both the cultivars but highly significant in SS-cultivar. The R^2 values for this correlation also varied greatly between the two cultivars. To further investigate the involvement of ABA in fibre growth, isolated fertilized ovules of SS-cultivar were cultured *in vitro* in Beasley medium sup-

plemented with either ABA or fluridone (inhibitor of ABA biosynthesis) (Nayyar et al. 1989). Addition of fluridone significantly stimulated fibre growth, whereas ABA retarded it LS-cultivar.

Table 1 *Correlation coefficients and regression values in long staple (LS) and short staple (SS) cotton*

Correlation between	*LS*	*SS*
Fibre length x IAA	0.4254	0.1007
	(10.7)	(10.7)
Fibre length x ABA	—0.0556	—0.9567*
	(1.2)	(79.4)
Fibre length x GA_3	—0.1150	0.3273
	(10.3)	(14.4)

*Significant at 1% level

Figures in parenthesis indicate R^2 (%) values for the respective correlations.

Correlative associations with cotton (varying in fibre length).

The present studies clearly demonstrate the role of ABA in the regulation of differential fibre length in the two cotton cultivars. Our observations are in sharp contrast to the other workers (Bhardwaj & Lad 1977, Naithani et al. 1982, Bhardwaj & Verma 1985) where emphasis was laid on the regulatory role of auxins and gibberellins in the determination of fibre length of various *Gossypium* spp. Our studies also indicate that the four phases of fibre development are regulated by interaction of ABA with IAA and GA_3. It is suggested that proper manipulation of these phytohormones, either physiologically or genetically, could alter the various phases of fibre development leading to desired fibre length.

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