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# Accumulation of abscisic acid in cotton fibre and seed of normal and abnormal bolls

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## SUMMARY

Cotton (*Gossypium hirsutum* L.) yield and quality is affected by altered fruiting patterns with progress in season. The present study was conducted to analyse normal and altered (abnormal) boll (fruit) development at maturation phase. Both normal and abnormal bolls of the same age groups were analysed for growth in terms of dry weight, water content and endogenous abscisic acid (ABA) content of fibre and seed. Endogenous level of ABA was estimated by using antibodies raised against ABA–protein conjugate. To amplify the reaction, indirect ELISA (Enzyme Linked Immuno Sorbent Assay) was performed. A marked decrease in dry matter accumulation (DMA) of seed and fibre was observed in abnormal bolls as the season progressed. Fibre from the abnormal bolls showed marked variation in endogenous ABA content, however, in abnormal seeds water content and endogenous abscisic acid content showed significant variation compared to that of normal bolls. From the results, it is concluded that a marked decrease in seed dry weight may be because of a decrease in water content and accumulation of higher endogenous abscisic acid content, whereas, the major reason for reduced fibre weight may be due to accumulation of endogenous abscisic acid.

## INTRODUCTION

Cotton is grown around the world in tropical and subtropical regions. About 16 million tons of cotton fibre and 33 million tons of cottonseeds are produced every year worldwide (Firoozabady 1989). Cotton is an indeterminate crop in which vegetative and reproductive structures compete for available photosynthates throughout the growing season (Mauney 1986).

In general, the cotton plant produces a great many more potential fruiting sites than it matures as fruits. Cotton plants shed up to 70% of all the fruiting structures initiated from sympodial branches, during various periods in the reproductive stages of development (Peoples & Matthews 1981). Growth and flowering slow or stop and fruit retention decreases as fruit load increases. Several environmental and biological events have a direct influence on boll retention (Guinn 1982*b*). The abscission of young bolls could result from competition for nutrients, a change in hormonal status or both (Guinn 1982*b*). It is also reported that boll abscission is stimulated by water deficit (Guinn 1985). ABA has multiple physiological effects on growth and development of plants. It is an effective growth inhibitor in various plant species. It reduces growth by inhibiting cell division (Albanell *et al*. 1985) and cell elongation (Doss *et al*. 1983). Circumstantial evidence also suggests a casual role of ABA in boll abscission. Water deficit increases the ABA content of cotton leaves (Radin & Hendrix 1988), boll (Guinn 1982*a*) and boll retention (Davis & Addicott 1972).

In seeds of most angiosperms, embryo development ceases during the maturation phase, during which seeds become desiccated and enter a period of arrested development preventing them from germinating during unfavourable conditions. Endogenous ABA levels are thought to participate in this regulation (Moore 1989). ABA is known to create a protective mechanism towards water stress and other stresses (Tanino *et al*. 1990; Ingram & Bartels 1996).

From earlier studies on cotton boll development, it was observed that endogenous ABA content correlates inversely with elongation growth of fibre and dry matter accumulation phase of developing cottonseed (Gokani *et al*. 1998). In the present investigation, cotton cultivar *Gossypium hirsutum* (cv. RCH-2) was studied for abnormal boll development during the years 1998 and 1999. Abnormal growth of bolls was considered as reduced growth (size of the boll) and aborted ovules (or locules as a whole) in the bolls.

Because phytohormones like ABA are synthesized

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in small quantities, a sensitive and accurate immunoassay was developed earlier for ABA analysis in plant tissue (Weiler 1980; Mertens *et al.* 1983). In those studies EIA (Enzyme Immuno Assay) results for the determination of ABA were not significantly different from that of HPLC or GC analysis. The advantage of EIA for hormone analysis is the ability to use crude plant extract without sacrificing sensitivity and selectivity. To amplify the reaction, an indirect ELISA was used to quantify endogenous ABA (Gokani *et al.* 1998) from fibres and seeds of the normal and abnormal bolls.

## MATERIALS AND METHODS

Seeds of cotton cultivar RCH-2 were grown in the field. Cultural practices including irrigation, application of fertilizers and insecticides were conducted to optimize the lint yield. On the day of anthesis, each individual flower was tagged and grouped as healthy bolls and the bolls showing abnormal features like reduced growth, late maturation, presence of aborted locule and abnormal shape. Both types of bolls, of the same age, having normal and altered growth, were harvested for estimation of endogenous abscisic acid content, and growth in terms of dry weight of fibres and seeds, their water content and fibre length. To minimize the effects of environmental variations, data were collected from flowers that bloomed during as narrow a period as possible.

### *Fibre length measurement*

Fibre length was determined by the method of Gipson & 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 a convex surface of a watch glass. Fibres were streamed out with a jet of water. Length of the fibre was measured to the nearest mm, from the rounded side of the seed, adjacent to the chalazal end. The final values were taken as the mean of 20–25 replicates.

### *Fresh and dry weights measurement*

Fibres were removed manually from the seed without removing the seed coat. Fibres from the different locules from three bolls were used for dry weight measurement. Freshly separated fibres were weighed before and after oven drying to a constant weight at 70 °C to obtain data on fresh and dry weights. Water content for each age group was calculated as the difference between fresh and dry weight. Mean values of three replicates with $\pm$SD were plotted.

### *Sample preparation for abscisic acid estimation*

Frozen fibre samples (300–500 mg fresh weight) were powdered in a mortar pre-chilled with liquid nitrogen and mixed with 5 ml of 80% methanol (v/v) containing 100 mg/l ascorbic acid as an antioxidant. The mixture was stirred properly and incubated for 48 h at 4 °C. The mixture was centrifuged at 10000 ***g*** for 10 min at 0 °C and supernatant collected. Pellets were washed twice and the pooled supernatant, concentrated by vacuum drying. These extracts were used as sample source for estimation of abscisic acid.

### *Raising of antibodies against ABA*

#### *Preparation of ABA–BSA and ABA–casein conjugate*

Abscisic acid (132 mg) was dissolved in 3 ml of DMF (N-N,dimethyl formamide):distilled water (2:1) and added drop wise in 250 mg BSA dissolved in distilled water and adjusted to the pH 8·5. After addition of ABA, the pH was readjusted to 8·0 with 1 N NaOH. N-ethyl-n-(3-dimethylaminopropyl)-carbodiimide hydrochloride (210 mg) was added to the ABA–BSA mixture in 4 portions within 90 min. The complete preparation was then stirred constantly for 19 h in dark at 4 °C. It was then dialysed against tap water for 4 days and stored at 0 °C.

#### *Immunization and separation of IgG*

The ABA–BSA conjugate was mixed with an equal amount of Freund's complete adjuvant and injected to rabbits, in triplicate, by intramuscular route. Booster doses were given periodically to raise the titer. Animals were bled and the serum separated. IgG was collected by passing the serum through DEAE-cellulose column pre-equilibrated with 0·01 M phosphate buffer (pH 8). The purified IgG was concentrated to the original volume of serum, and used for ELISA test after appropriate dilution. The purified IgG against ABA–BSA conjugate was tested for cross-reactivity with other plant growth regulators. The reaction of IgG with injected conjugate was considered as maximum reaction. However, no significant reaction of separated IgG with other PGRs were observed (data not presented).

### *Estimation of abscisic acid content*

Abscisic acid contents were estimated by indirect ELISA using anti rabbit-goat IgG, tagged with peroxidase (Gokani *et al.* 1998). In brief, to avoid reaction with tagged protein, ABA–casein conjugate was coated on the ELISA plate followed by the coating with antibodies against ABA–BSA conjugate, previously mixed with different samples. The mixture of samples (with or without internal standards) and IgG (antibody) were incubated at 37 °C for 1 h and at 4 °C for 3 h prior to the coating on ELISA plate to facilitate maximum binding reaction of antigen (ABA) present in the sample with antibody. The plate was

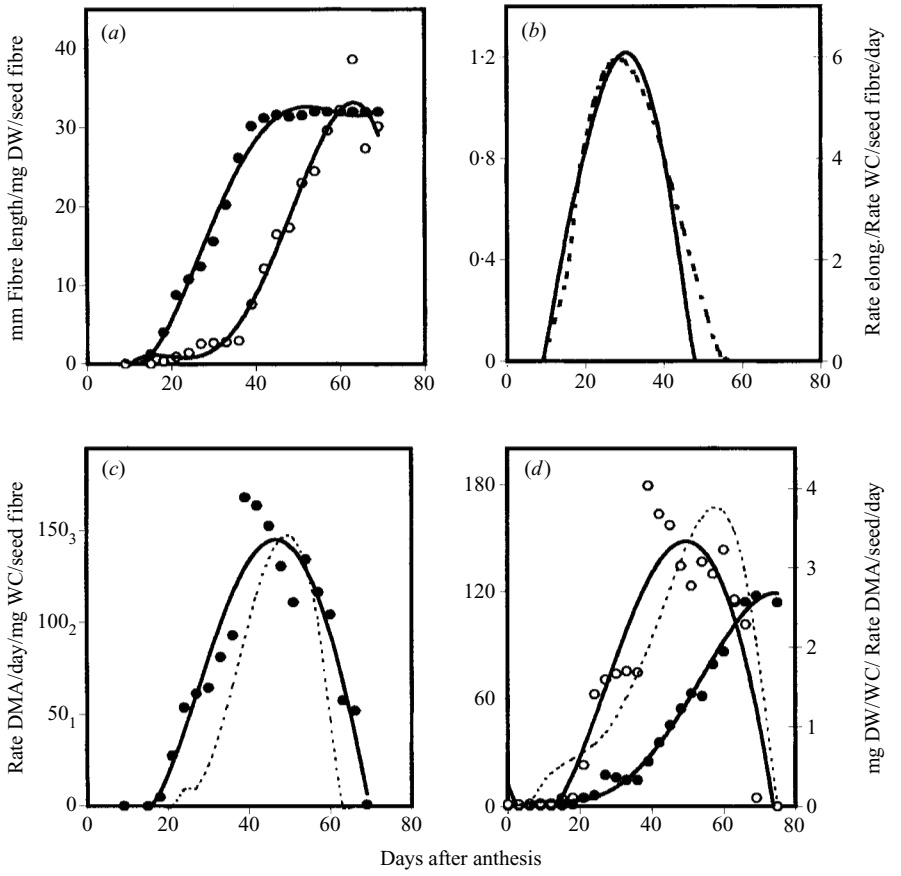


Fig. 1. Changes in different growth parameters of fibre and seed from normal bolls of cotton cultivar RCH-2 against boll age. (*a*) fibre length (●) and fibre dry weight (○); (*b*) rate of fibre elongation (– – –) and rate of fibre water content (——); (*c*) rate of fibre dry matter accumulation (DMA, – – –) and fibre water content (WC, ●); and (*d*) seed dry weight (DW, ●), seed water content (WC, ○) and rate of seed dry matter accumulation (DMA, – – –).

then coated with anti rabbit IgG tagged with peroxidase. After each coating ELISA plate was washed thoroughly with phosphate buffer saline containing 0·05% Tween-20. Colour was developed using O-phenylene diamine and reaction was terminated by addition of 6 N sulphuric acid.

Assay values were obtained as the mean of nine replicates, three replicates from three different extracts of the same age group. Relative binding values were calculated as B/Bo, where B and Bo are the values of absorbance in the presence and absence of internal standard or sample, respectively. To test sensitivity of the assay, each sample was mixed with a known concentration of abscisic acid (50–150 ng) as an internal standard before reacting with the antibodies. The values that fell on a standard curve, prepared for each particular plate, were taken.

The experiment was repeated during 1998 and 1999. Mean values are taken and *t*-test was performed for each data set of normal and abnormal bolls.

## RESULTS AND DISCUSSION

### *Growth analysis*

#### *Normal boll*

Data of dry matter accumulation and water content of fibre and seed and fibre length were fitted to polynomial equations. The bestfit equation was determined statistically by performing a *t*-test for different $R^2$ values.

Fibre length showed a typical sigmoidal pattern (Fig. 1*a*). After a lag for about 6–9 days fibre elongation entered into a linear phase and continued up to 48 days (32 mm, Fig. 1*a*), in the subsequent period fibre length stabilized. The maximum rate of fibre elongation was observed on day 27 (1·18 mm/day, Fig. 1*b*).

Data on dry matter accumulation showed that dry matter accumulation started after the fibre had attained maximum rate of elongation (27 days) and

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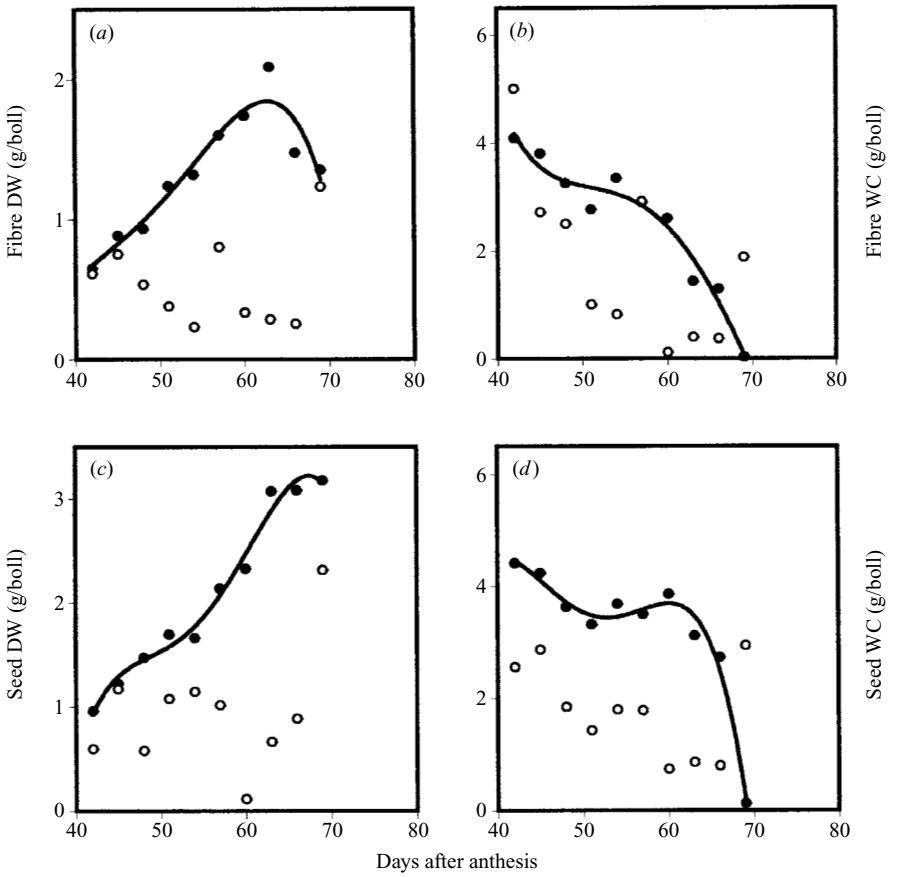


Fig. 2. Changes in dry weight (DW, *a*, *c*) and water content (WC, *b*, *d*) of fibre (*a*, *b*) and seed (*c*, *d*) in normal (●) and abnormal (○) bolls of cotton cultivar RCH-2 against boll age.

continued to accumulate till day 63 (77·31 mg/seedfibre, Fig. 1*a*). Maximum rate of dry matter accumulation was observed on 48 DPA (2·92 mg/seedfibre/day, Fig. 1*c*).

Water content of the fibres increased rapidly during elongation growth of the fibres, and attained a peak (179·3 mg/seedfibre) when fibres reached their maximum rate of dry matter accumulation (Fig. 1*c*). Maximum rate of water content was recorded around 27–30 DPA (6·1 mg/seedfibre/day) when fibres achieved their maximum rate of elongation.

From the above results cotton fibre development can be divided into four distinct phases i.e. (i) initiation (0–9 days), (ii) elongation (9–36 DPA), (iii) secondary thickening (27–48 days), and (iv) maturation (42 days onwards). Data of water content showed close correlation ($r = 0{\cdot}86$) with rate of dry matter accumulation while rate of water uptake showed a close correlation with rate of fibre elongation ($r = 0{\cdot}97$, Fig. 1*b*).

From seed growth analysis it was observed that after a lag of 18 days seed continued to accumulate dry matter and maximum dry weight (117·64 mg/seed) was observed on day 69. The maximum rate of dry matter accumulation (3·75 mg/seed/day) was observed on 57 DPA and declined thereafter.

From this growth analysis seed development is divided into (i) cell division phase (0–21 days), (ii) rapid dry matter accumulation phase (21–45 days), and (iii) maturation phase (45 days onwards).

Major components of cotton yield are (i) number of bolls per plant, (ii) number of seeds per boll and (iii) fibres per seed. Any abnormalities in these parameters may decrease the yield significantly. In this experiment, we have observed that nearly 20–30% of the bolls showed abnormal characters near the time of boll opening. As compared to the normal bolls, abnormal bolls of the same age groups showed a remarkable decrease in the number of fully developed seeds ($P < 0{\cdot}01$).

*Abnormal growth*

Abnormality in the genotype was quite frequent in the later stages of development (42 DPA onwards).

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Table 1. *Comparison of normal and abnormal bolls represented with* ± SE (*14* DF)

| Variable | Mean | |
|---|---|---|
| | Normal | Abnormal |
| Number of seeds/boll | 27·0 ± 1·13 | 13·2 ± 5·12 |
| Fibre dry weight (g/boll) | 1·37 ± 0·198 | 0·57 ± 0·235 |
| Fibre water content (g/boll) | 2·42 ± 0·417 | 2·10 ± 0·486 |
| Seed dry weight (g/boll) | 2·19 ± 0·293 | 1·08 ± 0·482 |
| Seed water content (g/boll) | 3·24 ± 0·279 | 2·02 ± 0·182 |
| Fibre ABA content (μg/seed) | 0·83 ± 0·025 | 1·85 ± 0·105 |
| Seed ABA content (μg/seed) | 1·80 ± 0·113 | 2·47 ± 0·154 |

Therefore, in Fig. 2, only data of the later stages are presented where nearly 20–25% of the bolls showed abnormal growth. Maximum fibre dry weight of normal bolls was recorded at 63 DPA (2·1 g/boll), while at the same time the dry weight of fibres from abnormal bolls was only 0·42 g (Fig. 2*a*, $P < 0{\cdot}01$%). Similarly, maximum seed dry weight from normal bolls was recorded on 69 DPA (3·2 g/boll), whereas at the same time bolls having abnormal characteristics had 2·3 g/boll dry weight (Fig. 2*c*, $P < 0{\cdot}01$%). In general, significant decrease in dry weight of seed and fibre was observed in abnormal bolls as compared to normal bolls (Table 1). Similarly, amount of water content was also low in seeds and fibres from abnormal bolls (Fig. 2*b*, *d*). Final fibre length of normal and abnormal bolls showed no significant difference.

Dry matter accumulation in seeds and fibres of normal and abnormal bolls showed significant variation (Fig. 1*a*, *b*, Table 1). A clear reduction, in dry matter accumulation of seeds (51%) and fibres (59%) as compared to normal bolls, was observed. The total production of bolls has been reported to be the most important contributor to the yield in both modern and absolute cultivars (Wells & Meredith 1984*b*). Continued growth of plant parts after boll initiation, a decrease in the number of bolls (Guinn 1982; Wells & Meredith 1984*a*) and a reduction in boll weight in those maturing near the end of the season (Meredith & Bridge 1973) have been reported. In the present study also, reduction in growth in terms of decrease in seed and fibre dry weight of the bolls was observed with progress in season. This may be because of various plant parts acting as sinks that compete with each other for available assimilates (Guinn 1982*c*).

Data of water content of seed and fibre of the abnormal and normal bolls showed marked variation in seed (*c.* 48%, $P < 0{\cdot}01$) as compared to the fibres (*c.* 14%, Table 1). In three cotton genotypes, variation in final seed weight and fibre cell elongation showed close parallels with water content (Rabadia *et al.* 1999). In the present study, fibres attained maximum

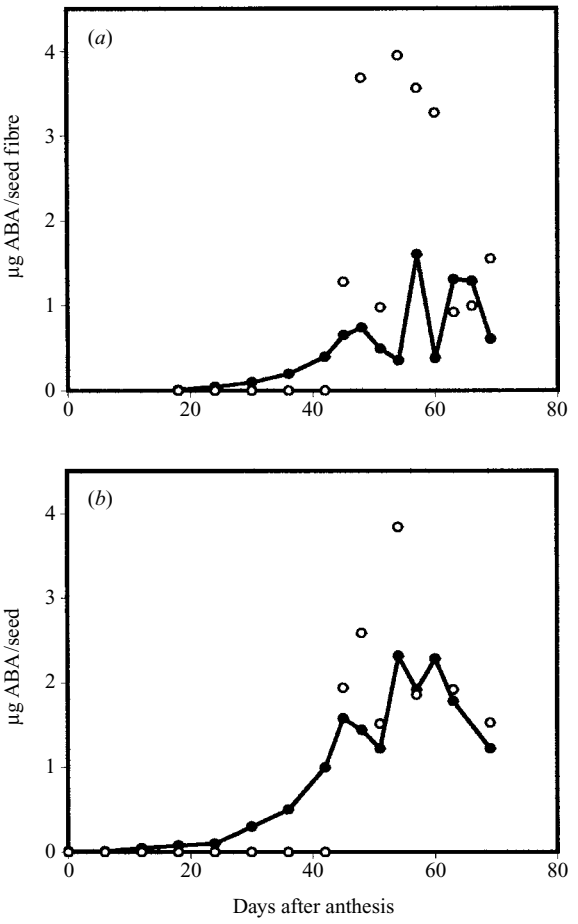


Fig. 3. Changes in endogenous abscisic acid (ABA) content of fibre (*a*) and seed (*b*) in normal (●—●) and abnormal (○) bolls of cotton cultivar RCH-2 against boll age.

length during early period of development and no significant change in fibre length was observed between normal and abnormal bolls. Similarly, fibre water content of normal and abnormal bolls does not differ remarkably as it does in seeds of normal and abnormal bolls. Further, in abnormal seeds dry matter accumulation showed remarkable decrease with decrease in water content suggesting thereby that water content strongly correlates with dry matter accumulation. Water status has multifunctional regulation in seed development. For example, it regulates (i) metabolic rates of the cell (Bewley & Black 1993), (ii) osmotic potential of developing seed (Saab & Obendrof 1989) and (iii) cell size of the sink tissues (Rabadia *et al.* 1999; Thaker 1999). A decrease in any one of the above-stated functions during desiccation could lead to cessation of dry matter accumulation (Westage 1994).

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*Abscisic acid*

Abscisic acid content of seed and fibre from abnormal bolls was remarkably higher than in the normal bolls of the same age (Fig. 3, Table 1). In the dry matter accumulation phase of seeds and fibre ABA content was more than double in most of the abnormal bolls than the normal boll. Maximum ABA content of normal fibres/seed was 1·6 μg at 57 DPA, whereas at the same time, in abnormal fibres ABA content was 3·7 μg (Fig. 3*a*, $P < 0{\cdot}01$). Similarly, maximum ABA content in the normal seed was at 54 DPA (2·3 μg/seed) as compared to 3·8 μg/seed of abnormal boll (Fig. 3*b*, $P < 0{\cdot}01$).

The abnormal fibres and seeds accumulated more ABA than normal bolls (Fig. 3). Considerable evidence has accumulated over the past several years that ABA levels rise sharply during maturation of cotton and other seeds (Rock & Quatrano 1995; Gokani *et al.* 1998). In normal bolls ABA content increased considerably during the later developmental period of seed and fibre (Fig. 3). In fibre, endogenous ABA levels increased after the fibre had attained its maximum length.

Higher levels of ABA in seeds and fibres of abnormal bolls in the present study suggest that ABA might have a role in reduced growth. ABA functions are antagonistic to the effect of growth promoters (Pilet 1975). For instance, enriching winter wheat plants with ABA greatly reduced grain dry weight (Borkovec & Prochazaka 1990). Ober *et al.* (1991) clearly showed that ABA reduces the resource mobilizing ability of the sink in the lupin and maize, respectively. It was also reported that ABA application reduces the GA-induced sucrose uptake in excised veins of *Pisum sativum* (Estruch *et al.* 1989). Additionally, it has been also reported that number of cells in maize decreased with application of ABA (Myers *et al.* 1990). Remarkable accumulation of ABA in abnormal bolls suggests its inhibitory role in boll development.

Although fibre cell elongation and secondary thickening are two independent processes, elongation growth determines the final length of the fibre while secondary thickening takes place on the elongated fibre. Both these parameters are useful for the textile industries (Rusca 1970). Mature cotton fibre is the purest form of cellulose (*c.* 99%) deposited during secondary thickening phase, which determines the strength of the fibre (Meinert & Delamer 1977; Ryser 1985). In normal and abnormal fibres, rapid ABA accumulation was observed after the fibres had completed their elongation growth. Therefore, in abnormal bolls although ABA content was higher as compared to normal bolls, final length of the fibre was not affected. However, excessive accumulation of ABA in fibres of abnormal bolls during the secondary thickening phase may reduce the cellulose deposition on the fibre and hence fibre strength (Timpa *et al.* 1991). Further, excessive accumulation of endogenous ABA level and reduction of water content of seeds have reduced dry matter accumulation of seeds remarkably, suggesting thereby the inhibitory role of ABA in cotton fibre and seed development.

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