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PHYSIOLOGY

Increasing Reversal Frequency in *Gossypium hirsutum* L. 'MD51' through Exogenous Application of Plant Hormones

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INTERPRETIVE SUMMARY

Cotton fiber strength is a textile trait directly affected by the organization of the secondary cell wall. The dead, dried secondary cell walls of fibers are processed into textiles. Within the wall, cellulose microfibrils are helically oriented around the fiber wall. In the secondary walls of cotton fibers, microfibrils routinely reverse their gyre. This is a unique occurrence found only in cotton fibers and has been referred to as a *reversal point*. Reversal points are thought to create weak areas in the secondary wall where fibers will probably break. As the secondary wall develops, the number of reversal points increases and microfibril orientation appears to change. The reversal appearance changes with the deposition of subsequent layers of secondary wall. Early in secondary wall development, cellulose microfibrils are oriented in a shallow-pitch helix and reversal points exhibit an abrupt change in the orientation of microfibrils (*Z reversals*). Later in development, microfibrils exhibit a steep-pitch helical orientation and reversals exhibit a more gradual change in microfibril orientation (*S reversals*). As the secondary wall develops, there are significant increases in total reversal frequency and in the frequency of S reversals. The frequency of Z reversals decreases with subsequent development of the secondary wall.

As fibers develop, the total number of reversals increases. Exogenous application of the hormone gibberellic acid increases the total reversal frequency and significantly affects reversal type. Application of indole-3-acetic acid has no effect on reversal frequency over untreated fibers but significantly affects reversal type. At 50 d post-anthesis, almost all reversals observed are S reversals, and both hormone-treated fibers have significantly fewer Z reversals than untreated fibers.

This study demonstrates that topical application of plant hormones to developing bolls alters reversal appearance and frequency. Changes in reversal patterns may lead to improvements in fiber textile traits.

ABSTRACT

Reversal points in cotton fibers may compromise fiber strength by generating regions of wall weakness. When viewed with polarized light, two types of reversals are observed: S and Z. This study investigated the effects of exogenously applied plant hormones on reversal frequency and type during secondary wall development in *Gossypium hirsutum* L. 'MD51'. The data indicate that reversal frequency increases and microfibril orientation changes as the secondary wall is deposited and walls thicken. Application of gibberellic acid ($C_{19}H_{22}O_6$) (1.0 mg L^{-1}) significantly increased total reversal frequency, whereas indole-3-acetic acid ($C_{10}H_9NO_2$) (0.1 mg L^{-1}) had no effect. Fibers treated with both gibberellic acid and indole-3-acetic acid exhibited significantly fewer Z reversals than untreated fibers at 50 d post-anthesis. During secondary wall synthesis, a relationship may exist between fiber elongation, reversal frequency, and reversal type. Our observations are consistent with previous suggestions that reversal points may be localized areas of cell expansion, arising from growth that occurs after the secondary wall is deposited. New insight into the factors that affect reversal frequency may be used to make a positive impact on textile properties.

Fiber strength is measured as either the ability of a single fiber, or bundles of fibers, to resist breakage under stress (Wakeham and Spicer, 1951; Betrabet et al., 1963; Raes et al., 1968; Egle and Grant, 1970). Fiber strength is affected directly by the organization of the cell wall. Reversal points may decrease single-fiber strength by generating weakness within the wall (Wakeham and Spicer,

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1951; Betrabet et al., 1963; Raes et al., 1968; Hearle and Sparrow, 1971). Alternatively, reversal points may induce changes in the convolution of mature dried fibers, thereby enhancing interactions among fibers within a bundle, which increases fiber bundle strength (Hsieh, 1999).

Reversals are produced when helically oriented wall microfibrils reverse their gyre. Two types of reversals have been identified (Wakeham and Spicer, 1951; Raes et al., 1968; Waterkeyn, et al., 1975; Seagull, 1986, 1993). *Z reversals* (Fig. 1) contain microfibrils whose orientation appears to change abruptly, thus giving the appearance in polarized light of an abrupt change in direction of the microfibril pattern. Z reversals are most evident early in the development of the secondary cell wall (Seagull, 1986). *S reversals* (Fig. 2) are found later in secondary wall development and appear to exhibit microfibril patterns with a more gradual change in orientation (Seagull, 1986). Reversal frequency increases during fiber development (Hebert and Boylston, 1984), suggesting that the development of reversals may be physiologically controlled.

Plant hormones control growth and development by effecting change in division, expansion, and differentiation of cells (Fosket, 1994). In cotton fiber, gibberellic acid (Sigma Chemical, St. Louis, MO) and indole-3-acetic acid (Sigma Chemical) are specifically required for elongation and secondary wall development (Delanghe, 1986). The exogenous application of indole-3-acetic acid to cotton ovules is necessary for ovule growth and for increases in fiber length and weight *in vitro* (Beasley and Ting, 1973; Dhindsa, 1978). Also, gibberellic acid controls fiber elongation (Jasdanwala et al., 1977). Exogenous application of gibberellic acid induces significant increases in fiber length during secondary wall synthesis (Seagull et al., 2000a). A review by Wilkins and Jernstedt (1999) reported that the ratio of indole-3-acetic acid to abscisic acid changes in the transition from primary to secondary wall synthesis; however, this finding may be coincidental and not causally related to secondary wall development. Specific effects of hormones on wall synthesis and/or organization have yet to be characterized fully.

Wall synthesis and fiber elongation are directly related processes in which cell size and shape are determined by the deposition and organization of

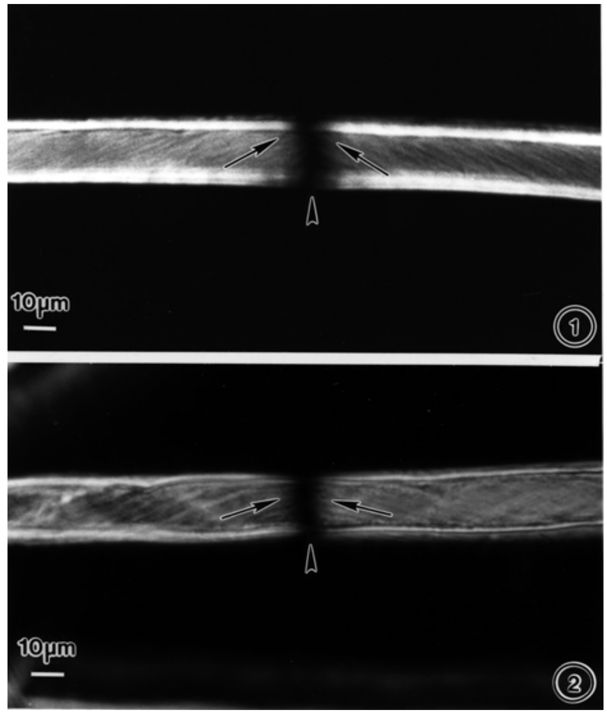

Fig. 1 and 2. Polarized light micrographs of (1) Z reversal from a 30-d post-anthesis, untreated cotton fiber with a microfibril pattern that exhibits an abrupt change in orientation, and (2) S reversal from an indole-3-acetic acid-treated, 50-d post-anthesis fiber with microfibrils that exhibit a more gradual change in orientation (arrows indicate the orientation of microfibrils; arrowheads indicate the reversal point).

the cell wall. Elongation is the result of turgor pressure-driven cell expansion causing the wall to stretch. A major unresolved question regarding fiber growth relates to the observation of simultaneous fiber elongation and secondary wall synthesis (Meinert and Delmer, 1977). Initial explanations relied on the perception that cotton fibers elongated via tip synthesis (reviewed by Seagull, 1990). Recent data on fiber ultrastructure show that organelles such as Golgi and secretory vesicles are disbursed throughout the fiber, an observation that is more consistent with a diffuse growth mechanism (Seagull, 1995; Tiwari and Wilkins, 1995). The difference between these two mechanisms is merely the location where additional wall and plasmalemma are incorporated in the fiber as elongation occurs during secondary wall synthesis.

Raes et al. (1968) suggested that fiber elongation during secondary wall synthesis was localized to reversal points. If true, then alterations in fiber elongation should be accompanied by changes in reversal frequency.

This study investigated whether reversal frequency and type are altered by exogenous application of gibberellic acid or indole-3-acetic acid to fibers grown *in plantae*. Given the relationship between reversals and fiber strength, the ability to alter reversal frequency could be used to improve the textile properties of cotton.

MATERIALS AND METHODS

Plant Growth

Gossypium hirsutum L. MD51 cotton plants were grown in a 2- by 3.1-m growth chamber, illuminated with a 1000-W sodium vapor lamp (Venture Lighting International, Solon, OH) on a 16/8-h, light/dark cycle at a temperature of 30/25ºC. Plants were grown in 20-L pots of BMX soilless potting mixture (Premier Horticulture, Rivière-du-Loup, Quebec). Plants were watered as needed and fertilized once a month with Miracle-Gro (The Scotts Co., Marysville, OH) general-purpose fertilizer.

Hormone Treatment

Stock solutions of gibberellic acid, 1.0 mg mL^{-1}, in 0.2% ethanol (C_2H_5OH) (Sigma Chemical, St. Louis, MO) and indole-3-acetic acid, 1.0 mg mL^{-1} in 0.2 *N* potassium hydroxide (KOH) (Sigma Chemical), were prepared and diluted with tap water to final concentrations of 1.0 mg L^{-1} (0.3 μM) gibberellic acid and 0.1 mg L^{-1} (0.57 μM) indole-3-acetic acid. Three drops of one treatment type were placed directly onto each boll with a Pasteur pipette, beginning on the day of anthesis and continuing daily until 5 d post-anthesis. Untreated fibers had no treatment. Tap water was not used because a previous study indicated no significant difference between water application on bolls and no treatment (Seagull et al., 2000b). Flowers were tagged on the day of anthesis and bolls were harvested at 30, 40, or 50 d post-anthesis. Bolls were quickly opened and then seeds and fibers placed in a fixative solution of 25% acetic acid (CH_3COOH) and 75% methanol (CH_3OH).

Reversal Analysis

Five ovules, randomly chosen, from four different bolls on four different plants were collected at 30, 40, and 50 d post-anthesis for each treatment type (untreated, indole-3-acetic acid-treated, or gibberellic acid-treated). Samples of whole fibers, removed from the boll, were teased apart on a microscope slide containing a mix of 50:50 glycerol [$C_3H_5(OH)_3$] and water. Reversal points were examined with a polarizing light microscope with a 6.3× polarizing objective lens (Aus Jena, Germany) and a calibrated ocular micrometer (VWR Scientific, South Plainfield, NJ). The number of reversals along three consecutive lengths of the ocular micrometer (4.695 mm) in the middle region of the fiber was counted and converted to reversals per centimeter of fiber length. A 50× polarizing objective lens was used to determine the S or Z reversal type. Approximately 3000 reversal points from 50 fibers for each age, randomly selected from different bolls, ovules, and plants, were counted. Repeated measures ANOVA were employed to determine the statistical significance within treatment groups over time, followed by separate one-way ANOVA with Post Hoc Tukey Tests within and between groups ($p <$ 0.05 was deemed significant).

RESULTS AND DISCUSSION

This study examined whether exogenous application of indole-3-acetic acid and gibberellic acid can alter reversal formation in plant grown fibers of *Gossypium hirsutum* L. MD51. Fiber strength is affected by variations of cellulose deposition. Reversal points are one such variation thought to contribute areas of weakness within the cotton fiber wall (Wakeham and Spicer, 1951; Betrabet et al., 1963; Raes et al., 1968; Egle and Grant, 1970).

Two types of reversal points, S and Z, occur during secondary wall development (Wakeham and Spicer, 1951; Raes et al., 1968; Waterkeyn et al., 1975; Seagull, 1986, 1993). During normal development, the total reversal frequency (per centimeter) increases (Fig. 3), the frequency of S reversals increases (Fig. 4), and the frequency of Z reversals remains the same (Fig. 5).

Indole-3-acetic acid and untreated fibers, within their respective groups, exhibit a significant increase in total reversals only at 40 to 50 d post-anthesis (Table 1). Indole-3-acetic acid has no effect on total reversal frequency (Table 1) and S reversal frequency (Table 2) relative to control fibers. The

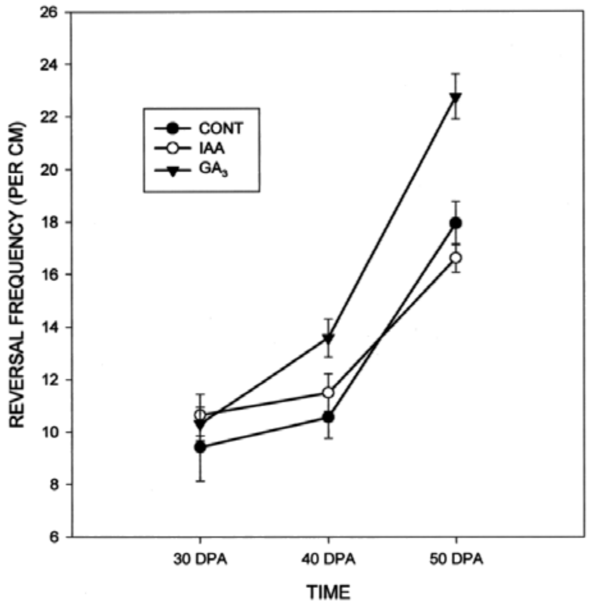

Fig. 3. The effects of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) on total reversal frequency. Gibberellic-treated fibers exhibit significantly more reversals per centimeter than indole-3-acetic-treated and untreated fibers. DPA = d post-anthesis; CONT = control.

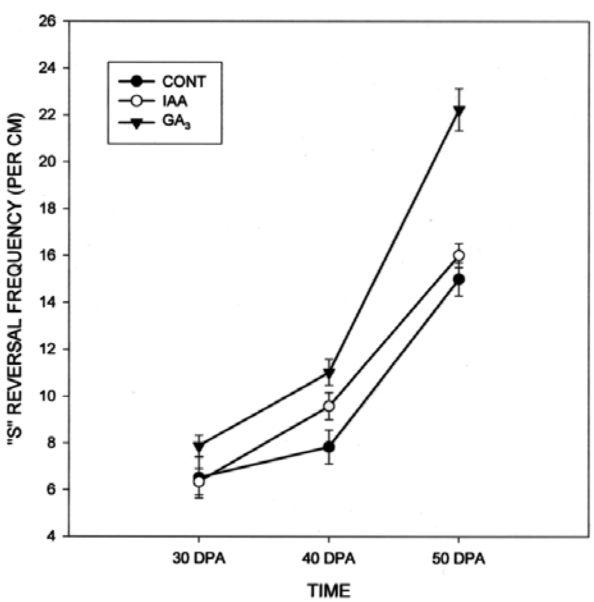

Fig. 4. The effects of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) on the development of S reversals. The S reversal frequency in both treated and untreated fibers increases over time. DPA = d post-anthesis; CONT = control.

total frequency of Z reversals decreases, although this decrease is not consistently significant with increasing age (Table 3).

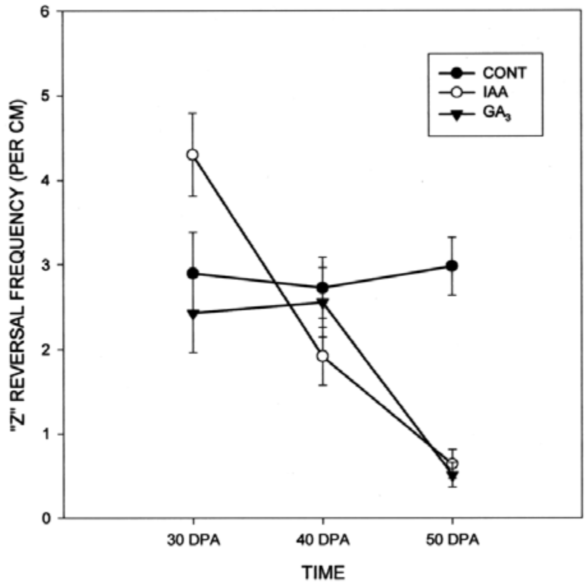

Fig. 5. The effects of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) on the development of Z reversals. A decrease is observed in the frequency of Z reversals over time in both hormone treatments. Untreated fibers appear not to exhibit a decrease in Z reversal frequency over time. DPA = d post-anthesis; CONT = control.

Table 1. Statistical analysis (ANOVA) of total reversal frequency among three groups of cotton fibers: control (CONT), or treated with either indole-3-acetic acid (IAA) or gibberellic acid (GA_3). GA_3-treated fibers had significantly more reversals than IAA-treated and untreated fibers. IAA-treated fibers trended toward more reversals but the increase was not significant.

DPA†	CONT‡,§	±SE	IAA‡,§	±SE	GA_3‡,§	±SE
30	9.415aA	1.280	10.650aA	0.786	10.309aA	0.642
40	10.565aA	0.810	11.502aA	0.720	13.589bB	0.720
50	17.938bA	0.828	16.614bA	0.548	22.748cB	0.862

$P < 0.05$ = statistically significant.
† DPA = d post-anthesis.
‡ Lowercase letters are comparisons within columns (age effects).
§ Uppercase letters are comparisons of within rows (treatment effects).

With the application of gibberellic acid, the total number of reversals significantly increases at 30 to 40 d post-anthesis and at 40 to 50 d post-anthesis (Table 1). Throughout development, the frequency of S reversal points increases (Fig. 4) and Z reversal points decreases (Fig. 5), although the changes in frequency of Z reversals is significant

Table 2. Statistical analysis (ANOVA) of the increase in S reversal frequency among three groups of cotton fibers: control (CONT), or treated with either indole-3-acetic acid (IAA) or gibberellic acid (GA_3). The frequency of S reversals occurred earlier with hormone treatment. Treated fibers had significant increases in S reversals at 30 to 50 d post-anthesis (DPA), whereas untreated fibers exhibited a significant increase at 40 to 50 DPA. GA_3 application had significant increases over untreated and IAA-treated fibers at 30 to 50 DPA.

DPA	CONT†,‡	±SE	IAA†,‡	±SE	GA_3†,‡	±SE
30	6.518aA	0.879	6.347aA	0.563	7.881aB	0.448
40	7.838aA	0.724	9.585bA	0.585	11.033bB§	0.557
50	14.995ba	0.712	16.018cA	0.506	22.237cB	0.903

***P* < 0.05 = statistically significant.**

† Lowercase letters are comparisons within columns (age effects).

‡ Uppercase letters are comparisons within rows (treatment effects).

§ Difference between IAA and GA_3 not significant.

Table 3. Statistical analysis (ANOVA) of the decrease in Z reversal frequency among three groups of cotton fibers: control (CONT), or treated with either indole-3-acetic acid (IAA) or gibberellic acid (GA_3). The trend of Z reversals is to decrease over time. IAA-treated fibers exhibit a significant decrease at 30 to 50 d post-anthesis (DPA). GA_3 treatment results in a significant decrease at 40 to 50 DPA. The decrease is not consistently significant between groups.

DPA	CONT†,‡	±SE	IAA†,‡	±SE	GA_3†,‡	±SE
30	2.897aA	0.486	4.303aB	0.489	2.428aA	0.464
40	2.726aA	0.360	1.917bA	0.340	2.556aA	0.413
50	2.982aA	0.344	0.639cB	0.175	0.511bB	0.143

***P* < 0.05 = statistically significant.**

† Lowercase letters are comparisons within columns (age effects).

‡ Uppercase letters are comparisons within rows (treatment effects).

only between 40 and 50 d post-anthesis and not between 30 and 40 d post-anthesis (Table 3). The observed decrease in the frequency of Z reversals indicates that Z reversals disappear or are converted into S reversals. The observed increase in total reversal frequency (Fig. 3) indicates the addition of reversals. Z reversals in the wall might develop into S reversals with subsequent wall deposition. Newly formed reversals might be deposited into the wall either in the S or Z type because Z reversal points are evident at all ages of fiber development.

Gibberellic acid-treated fibers exhibit a significantly greater increase in reversal frequency over indole-3-acetic acid-treated or untreated fibers at 40 and 50 d post-anthesis (Table 1) and a significantly greater increase in S reversals throughout the study period (Table 2). These data indicate that in addition to total reversal frequency, reversal type is influenced by the application of gibberellic acid.

Hebert and Boylston (1984) proposed the existence of only one type of reversal, with its appearance changing during subsequent wall deposition. Data presented here are not in agreement with this interpretation. If we assume that wall deposition occurs evenly over the length of the fiber, then the orientation of wall microfibrils should be uniform along the length of the fiber. This would not produce the observed S and Z reversals on the same fiber (Fig. 6). Alternatively, we believe that reversal type is related to wall microfibril orientation in the wall, that is, Z reversals are made when microfibrils are deposited in a shallow-pitch helical pattern, and S reversals occur when microfibrils are deposited in a more steeply pitched helical pattern. Thus the observed simultaneous presence of Z and S reversal points indicates that wall microfibrils are simultaneously deposited with different orientations in different regions of the fiber. If a reversal started as Z when microfibrils were oriented in a shallow pitch, subsequent wall deposition or perhaps elongation of the wall would orient them to a steep pitch and cause reversals to appear as S.

Reversals are generated by changes in helical gyre of cellulose microfibrils in the secondary wall, and microfibril orientations are controlled by changes in microtubule patterns (Yatsu and Jacks, 1981; Seagull, 1986, 1992). In the present study, reversal frequency increased significantly with the exogenous application of the hormone gibberellic acid (Fig. 3). Therefore, the current data suggest that

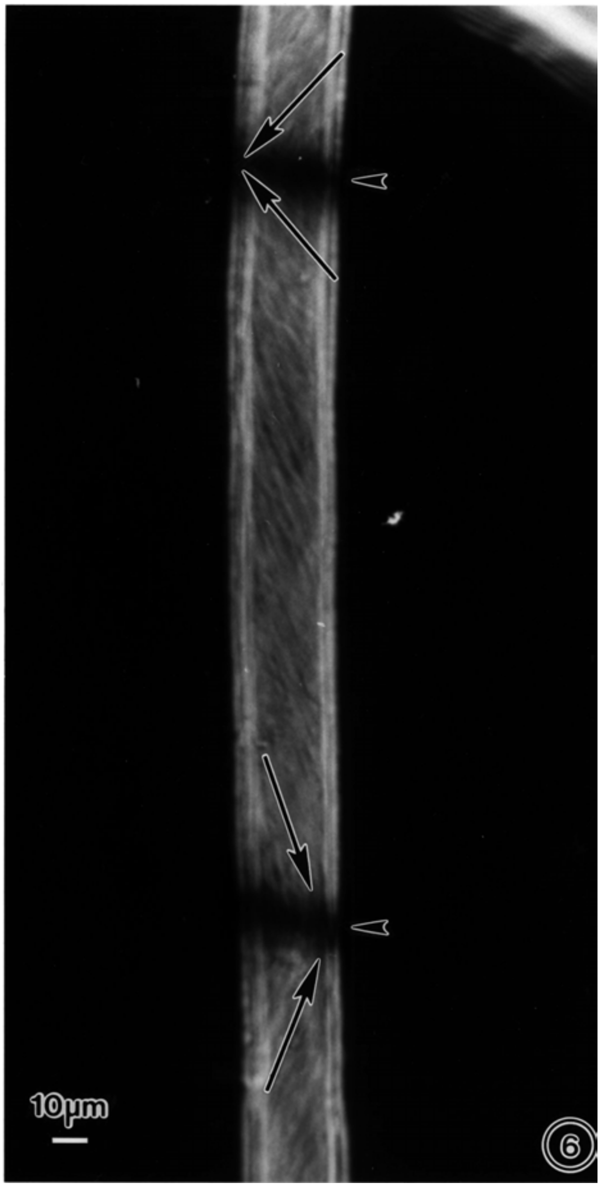

Fig. 6. An indole-3-acetic acid-treated fiber at 50 d post-anthesis exhibits both a Z reversal (top) and an S reversal (bottom). Arrows indicate microfibril patterns and arrowheads indicate the reversal point.

gibberellic acid may alter microtubule arrays in cotton fibers. This is consistent with other literature that indicates that alteration of microtubules may be one mechanism by which hormones alter plant cells (Seagull, 1992).

Hebert and Boylston (1984) reported that reversal frequency increases as fibers mature. The findings of our study confirm those observations. Also, Hebert and Boylston suggested that the frequency of reversals is regulated by genetic composition. Data from our study indicate that reversal frequency can also be manipulated by the addition of hormones to the developing ovaries.

Previously, Raes et al. (1968) suggested that reversal points might be areas of fiber elongation. Reversal points may represent localized regions of cell expansion arising from growth that occurred after the deposition of the secondary wall. Although elongation occurs mainly during primary wall synthesis, there is an overlap between secondary wall synthesis and fiber elongation (Meinert and Delmer, 1977; Delange, 1986; Seagull et al., 2000a,b). If elongation during secondary wall synthesis occurs at reversal points, then one would hypothesize that increases in elongation during the secondary wall synthesis phase should be accompanied by increases in reversal frequency.

The mechanisms by which gibberellic acid and indole-3-acetic acid mediate their effects are unknown at this time. Both indole-3-acetic acid and gibberellic acid induce many types of plant cells to expand (Fosket, 1994). In cotton fiber, increased indole-3-acetic acid levels were found to coincide with secondary wall synthesis (Jasdanwala et al., 1977). Perhaps the combined effects of increased wall extensibility, along with increasing osmotic potential, results in localized regions of growth after secondary wall synthesis begins.
Gibberellic acid is essential for fiber elongation on fertilized ovules *in vitro*, whereas indole-3-acetic acid has little effect (Beasley and Ting, 1973; reviewed by Basra and Saha, 1999). *In vivo*, gibberellic acid produced significant increases in fiber length (Oliveri and Seagull, 2000; Seagull et al., 2000a). In the current study, gibberellic acid treatment resulted in an increase in reversal frequency. Concurrent increases in both fiber length (Oliveri and Seagull, 2000; Seagull et al., 2000a) and reversal frequency (this report) support the hypothesis of Raes et al. (1968) that fiber elongation during secondary wall synthesis and the formation of reversals may be correlated. The precise mechanisms by which reversal points occur are poorly understood at this time. It is hoped that continued research might yield a better understanding of the biological and physical processes involved.

CONCLUSIONS

Throughout secondary wall development, two types of reversals were observed: S and Z. As the secondary wall develops and reversal frequency increases, a greater percentage of reversals are S. The increase in S reversals is consonant with the transition in microfibril pattern from shallow- to steep-pitch helical.

During secondary wall deposition, fiber elongation continues. The reversal point may be a localized area of cell expansion that arises from growth that occurs after the secondary wall is deposited.

Hormone-induced increases in reversal frequency (Fig. 3) and fiber length (Oliveri and Seagull, 2000; Seagull et al., 2000a) support the hypothesis of Raes et al. (1968) that reversal points may represent localized regions of fiber elongation during secondary wall synthesis. Generally in plant cells, secondary wall synthesis occurs after cell expansion has ended. Cotton fibers appear to be an exception, with cell elongation continuing during secondary wall synthesis. If reversal points represent localized regions of cell elongation, then one would predict our observation of concomitant increases in fiber length and reversal frequency.

Many questions remain unanswered concerning the formation of reversals and their possible involvement in cell elongation. The more we understand about the structure and physical properties of the secondary wall, the better our opportunity to manipulate change for improving cotton quality.

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