The *Journal of Cotton Science 8:105–111 (2004)*
http://journal.cotton.org, © The Cotton Foundation 2004

MOLECULAR BIOLOGY AND PHYSIOLOGY

Pre- and Post-Anthesis Application of Exogenous Hormones Alters Fiber Production in *Gossypium hirsutum* L. Cultivar Maxxa GTO

Robert W. Seagull* and Sofia Giavalis

ABSTRACT

In recent years, crop yield in cotton has remained constant, suggesting that some type of yield plateau has been reached within the existing cotton germplasm. This study examines the effect of exogenous application of indole-3-acetic acid (IAA) or gibberellic acid-3 (GA_3) on the numbers of fibers produced per ovule. IAA or GA_3 was applied exogenously to either pre- or post-anthesis squares and flowers. Fiber number per ovule was determined over the first 5 d of fiber development. Both pre- and post-anthesis treatment with IAA resulted in significant increases in the number of fibers per ovule, with post-anthesis treatments resulting in the largest increase (58%) in fiber number. Pre-anthesis treatment with GA_3 resulted in an increase in fiber number, whereas post-anthesis treatment with GA_3 resulted in a significant decrease in fiber production. Hormone treatment did not affect ovule length or width, and thus increases in fiber number most likely are due to increases in the proportion of epidermal cells that develop into fibers.

The economic importance of cotton has resulted in extensive studies of fiber growth and development. Improvements in fiber yield remain a major goal in the efforts to improve economic competitiveness of the U.S. cotton industry. Increases in crop yield resulting from cotton breeding programs have leveled-off in recent years (Calhoun and Bowman, 1999) leading to the suggestion that a yield plateau may have been reached with the current cotton germplasm (Meredith, 2000). Within a specific genotype, considerable variation in yield can be observed in plants grown under different physiological conditions (Lewis, 2000). Through the manipulation of physiological processes in cotton, it may be possible to increase yield in the existing germplasm. The five approaches to increasing cotton crop yield outlined by Lewis (1992) remain valid today. Crop yield can be improved by increasing the number of plants per acre, the number of bolls per plant, the number of seeds per boll, the number of fibers per seed, or the weight of individual fibers. This manuscript focuses on improving crop yield by increasing the number of fibers produced per ovule (seed).

Cotton fibers are the result of a terminal differentiation sequence that begins with ovule epidermal cells. Fibers exhibit three overlapping stages of development: initiation, elongation and secondary wall thickening, which is followed by a maturation phase that ends with fiber death (Jasdanwala et al., 1977; Wilkins and Jernstedt, 1999). Fiber initiation occurs in two morphological phases, a spherical expansion above the epidermis (ballooning), followed by elongation in which the rounded cells develop a tapered end (Stewart, 1975). Not all cells of the epidermis develop into fibers. Between 10% (Ryser, 1999) and 25 % (Beasley, 1975; Stewart, 1975) of epidermal cells become fibers. The factors that regulate the number of fibers produced per ovule remain unclear.

Morphological fiber initiation coincides with flower anthesis. Data on the timing of fiber initiation varies, but most reports indicate initiation begins several hours to several days before anthesis (Berlin, 1986; Graves and Stewart, 1988; Joshi et al., 1967; Ramsey and Berlin, 1976; Stewart, 1975). It appears that developmental cues for fiber production are present over a long period of time, so there may be a very long "window of opportunity" over which to manipulate development to increase fiber production. Genetic techniques have expanded our understanding of the events leading to fiber development and have identified a pre-anthesis stage where epidermal cells undergo changes in gene and protein expression in preparation for fiber expansion (Graves and Stewart, 1988; Wilkins and Jernstedt, 1999). Epidermal cells on pre-anthesis ovules are "primed" for fiber devel-

R. W. Seagull and S. Giavalis, Department of Biology, Hofstra University, Hempstead, NY, 11549
*Corresponding author: Robert.W.Seagull@Hofstra.edu

opment, and await hormone signals associated with anthesis (Graves and Stewart, 1988).

Several studies indicate that fiber initiation is influenced by physiological changes in the plant. Fiber number per ovule is affected by agricultural practices, such as changes in planting date (Bowman et al., 2000; Lewis, 2000), and environmental factors, such as changes in mean minimum daily temperature (Lewis, 2000). Numerous studies of the possible involvement of plant hormones in fiber development have been conducted (Basra and Saha, 1999; Gialvalis and Seagull, 2001; Momtaz, 1998), which have been aided by the development of a cotton ovule culture technique by Beasley (Beasley, 1971; Beasley and Ting, 1973; 1974). Analyses of fiber development *in vitro* indicate that unfertilized ovules require the addition of indole-3-acetic acid (IAA) and gibberellic acid 3 (GA_3) for ovule growth and fiber development. If ovules are fertilized before culturing, then exogenous IAA has a minimal affect on fiber development, but exogenous GA_3 is required for ovule growth. Other plant hormones, such as cytokinins and abscisic acid have an inhibitory affect on fiber development (Beasley and Ting, 1973; 1974). Studies using fibers grown *in plantae* usually examine the elongation phase of fiber development (Basra and Saha, 1999). Analysis of hormone content in young ovules and fibers grown *in plantae* indicates that auxin levels are high initially (0 d post-anthesis) and then drop dramatically through 8 d post-anthesis (Chen et al., 1996; John, 1994; 1999; Nayyar et al., 1989). This research supports previous observations of Jasdanwala et al. (1980) indicating an increase in IAA levels is important for epidermal cells to differentiate into fibers.

Although it is clear that ovules grown *in vitro* produce fibers, differences in wall chemistry and protein profiles between fibers grown *in vitro* and *in vivo* have been reported (Davidonis, 1999; Meinert and Delmer, 1977; Turley, 1998). Other differences between fibers grown *in vitro* and *in vivo* include differences in fiber length, secondary wall synthesis, and the presence of cell division (Kim and Triplett, 2001). To date, the effects of the culturing techniques on the number of fibers produced per ovule remains undescribed.

Previous publications, examining the effects of hormones on fiber development, often used a staining/de-staining technique for quantifying fiber production (Beasley et al., 1974). This approach examines fibers after 2 wk growth, thus changes in fiber production are a combination of changes in fiber number and individual fiber growth. To specifically examine changes in fiber number per ovule, a quantitative technique was modified from Van't Hof (1998). This technique provides accurate data on changes in fiber number per ovule (Gilavalis and Seagull, 2001; Seagull, 1999) The current study extends our previously published observations on the effects of hormones on fiber initiation from ovules grown *in vitro* (Gialvalis and Seagull, 2001). To obtain a better understanding of the role of hormones in fiber production *in plantae*, exogenous hormones were applied to developing flowers and bolls of a commercial cultivar of cotton (*G. hirsutum* L.). The objective was to determine if exogenous application of plant hormones before and after anthesis could alter the number of fibers produced per seed.

MATERIALS AND METHODS

Plant growth. *Gossypium hirsutum* cv. Maxxa GTO was grown in 20-L pots of Pro Mix potting mixture (Premier Horticulture, Riviere du Loup, Quebec). Plants were grown in a greenhouse with a day–night temperature cycle of 32°C/26°C. Plants were watered daily and fertilized monthly with Miracle Gro general-purpose fertilizer (Scotts Co., Marysville, OH), per manufacturer's instructions.

Hormone application. Stock solutions of gibberellic acid 3 (GA_3), and indole-3-acetic acid (IAA) (Sigma-Aldrich Chemical; St Louis, MO) were prepared by dissolving the appropriate amount of chemical in ethanol for GA_3 or KOH for IAA to yield a final concentration of 1.0 mg/mL. Treatment solutions were prepared by diluting hormone stock solutions with water to provide working concentrations of 1.0 mg/L (2.9×10^{-6} M) GA_3; 0.1 mg/L (5.7×10^{-6} M) IAA. Water was used as the control. Because gibberellic acid stock solutions contained ethanol and indole-3-acetic acid stocks contained KOH, all treatment solutions (including the water control) were adjusted with appropriate amounts of ethanol and KOH to yield a final concentration of 0.02% ethanol and 5×10^{-5}M KOH. Ten drops of hormone solution or water were applied using a Pasteur pipette.

Pre-anthesis treatment started as soon as the squares were detected. Developing squares were treated every other day with the appropriate solution until the day of anthesis. Flowers were harvested from the plant on a specific day post-anthesis, start-

ing from 0 d post-anthesis through 5 d post-anthesis. During treatment, the flower bracts were gently pulled back to facilitate the application of the solution directly to the developing flower.

In post-anthesis treatment, flowers were treated with the appropriate solution starting at 0 d post-anthesis. Treatments were applied everyday until the day of harvest. Flowers were harvested from 0 d post-anthesis through 5 d post-anthesis.

Fiber counts. Ovules from at least three flowers were collected for each post-anthesis sample and fixed in Farmer's fixative [methanol: acetic acid (3:1)] for at least 1 h. To facilitate fiber counting, ovules were macerated using the techniques of Seagull (1998). Briefly, the ovules were rinsed with water and placed in 5N HCl for 45-60 minutes, rinsed with water, and soaked in Schiff's reagent for 1 h. After another rinse in water, the ovules were stored in 45% acetic acid.

To separate the cells of the ovule, seeds were ground with a Pellet Pestle tissue grinder (VWR Scientific; Bridgeport, NJ) in Eppendorf tubes. Fibers were counted using a Fuchs-Rosenthal counting chamber (VWR Scientific; Bridgeport, NJ). Twelve random samples from six ovules were counted for each treatment.

Ovule measurements. The length and width of fixed ovules (as above) was measured using an Olympus XZS (Olympus Optical; Tokyo, Japan) dissecting microscope equipped with a drawing tube. Back-lighting the ovules provided sufficient contrast to easily distinguish the boundary of ovules covered with cotton fibers. Twenty ovules per sample were measured.

Statistical analysis. A two-way ANOVA was performed using SYSTAT 8.0 (Systat Software, Inc.; Point Richmond, CA). As a result of a non-normal distribution of the data, Bonferroni adjustments were applied. Compared data points at $P \leq 0.10$ were considered significantly different.

RESULTS AND DISCUSSION

The effects of exogenous hormone application on two developmental points of time, pre-anthesis (from square initiation to flower opening) and post-anthesis (first 5 d after flower opening) were studied. Fiber development was assessed by examining the first stage of fiber growth, initiation. Initial experiments were done with *G. hirsutum* cultivar Maxxa GTO to determine if control treatments (0.02% ethanol and 5 X 10^{-5}M KOH) altered fiber initiation. Neither pre- nor post-anthesis treatment with the control solution (0.02% ethanol in 5 X 10^{-5}M KOH) had an effect (between-treatment comparisons at all ages were not significantly different, $P > 0.5$) on the number of fibers produced per ovule (Figure 1). In untreated ovules, fiber numbers increased each day between 0 and 5 d post-anthesis, reaching a maximum of approximately 16,000 fibers per ovule. (Figure 1). On 0 d post-anthesis, untreated ovules contain 1813 ± 218 fiber initials per ovule, ovules treated at pre-anthesis contain 1850 ± 200 fibers, and ovules treated at post-anthesis contain 1760 ± 130 fibers. Ovules treated at both pre- and post-anthesis exhibited significant increases in fiber numbers from 0 through 5 d post-anthesis (Figure 1, Table 1).

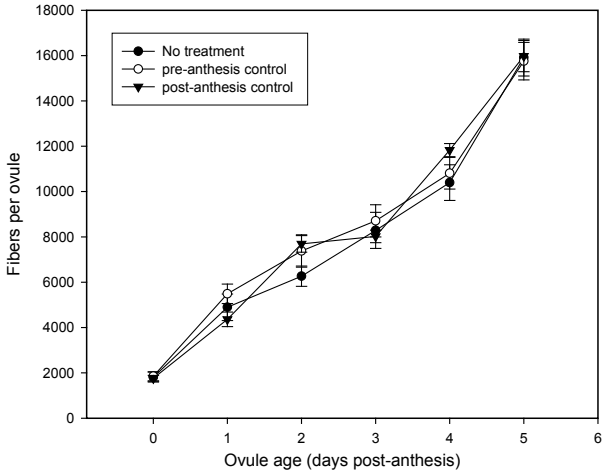

Figure 1. Effects of control treatments on fiber initiation in *G. hirsutum* cv. Maxxa.

Table 1. Statistical analysis (2-way ANOVA) of fiber number per ovule among six different hormone treatments through 6 days of development

Days post-anthesis	Fibers per ovule[z]					
	Pre-anthesis treatment			Post-anthesis treatment		
	Control	IAA	GA_3	Control	IAA	GA_3
0	a, A	a, B	a, C	a, A	a, A	a, A
1	b, A	b, B	b, C	b, A	b, D	b, A
2	c, A	c, B	c, A	c, A	c, B	c, A
3	d, A	d, B	d, A	d, C	d, B	d, C
4	e, A	e, B	e, C	e, A	e, D	e, A
5	f, A	f, B	f, C	f, A	f, D	f, E

[z] **Lower case letters within columns represent comparisons of age effects. Upper case letters between columns represent comparisons of treatment effects. Same letter indicates no significant difference at $P \leq 0.10$.**

Fiber initiation begins on or before the day of anthesis, as indicated by the number of fibers evident at 0 d post-anthesis (Figure 1). These data agree with previous reports of fiber production starting before the day of anthesis (Berlin, 1986; Graves and Stewart, 1988; Joshi et al, 1967, Ramsey and Berlin, 1976; Stewart, 1975). Fibers observed at this time ranged from epidermal cells that exhibit ballooning to short fibers with tapered tips (unpublished data).

For ovules receiving pre-anthesis treatment, at 0 d post-anthesis the GA_3–treated ovules contained significantly fewer fibers than either the control or IAA-treated ovules. At 0 d post-anthesis, IAA-treated ovules contained significantly more fibers than GA_3-treated ovules but significantly fewer fibers than control ovules. (Figure 2, Table 1). By 5 d post-anthesis, both IAA- or GA_3-ovules had significantly greater numbers of fibers than control-treated ovules (Figure 2; Table 1). Pre-anthesis treatment with IAA resulted in the greatest number of fibers.

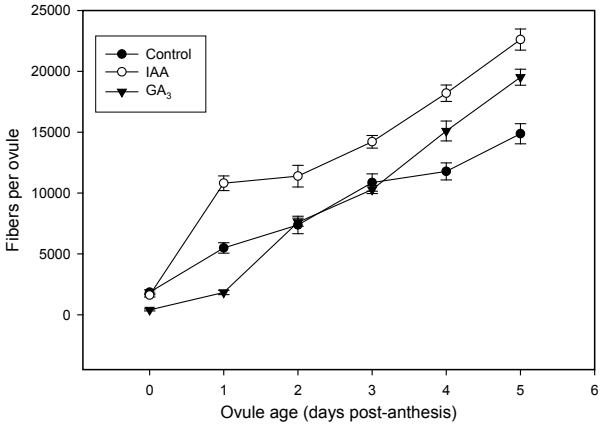

Figure 2. Effects of pre-anthesis treatments with IAA and GA_3 on fiber initiation in *G. hirsutum* cv. Maxxa.

In ovules treated post-anthesis, 5 d exposure to IAA resulted in a significant increase in fiber number compared with control-treated ovules (Figure 3; Table 1). Post-anthesis treatment with GA_3 resulted in a significant decrease in fiber production by 5 d post-anthesis compared with the control, but there were no significant differences between control and GA_3-treated ovules at earlier stages (i.e. 0 to 4 d post-anthesis) (Figure 3; Table 1).

A comparison of pre- and post-anthesis treatments indicates that GA_3 was most effective when applied pre-anthesis, with pre-anthesis ovules exhibiting an approximately 40% increase in fiber number at 5d post-anthesis relative to ovules treated post-anthesis. Treatment with IAA was most effective in increasing fiber number when applied post-anthesis (approximately 12% increase relative to pre-anthesis treatment). Overall, the most effective treatment for increasing fiber number was the post-anthesis treatment with IAA, which produced an approximate 12% increase over the next highest value, pre-anthesis treatment with GA_3, and an approximate 59% increase above the comparable (post-anthesis treatment) control ovules (Table 1).

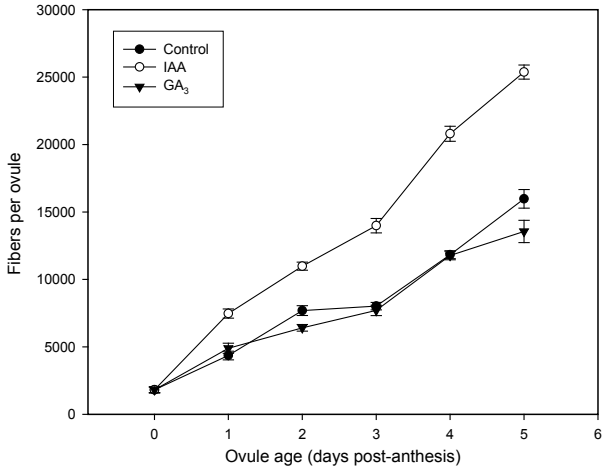

Figure 3. Effects of post-anthesis treatments with IAA and GA_3 on fiber initiation in *G. hirsutum* cv. Maxxa.

Previous literature on endogenous levels of GA_3 is somewhat contradictory. Comparing wild-type and mutant lines of *G. hirsutum*, Chen et al. (1996) reported very low endogenous levels of GA_1 and GA_3 in wild-type cotton immediately before anthesis (2 and 3 d before flowering) and then dramatically rise, reaching a somewhat steady level by 1 d post-anthesis. In the same study, a fiberless mutant exhibited low GA_1 and GA_3 levels until 5 d post-anthesis. These data indicate that increasing levels of GA at anthesis may be important for fiber initiation. In a study by Nayyar et al. (1989), GA_3 levels were high at pre-anthesis (2 d before flowering) and then decreased through 2 d post-anthesis, followed by an increase through 5 d post-anthesis. Data from their study indicate that low levels of GA_3 in early post-anthesis ovules coincide with fiber initiation. In this study, increased levels of GA_3 during pre-anthesis resulted in an increased fiber production, whereas increased levels of GA_3 during post-anthesis resulted in decreased fiber production. These observations are more consistent with the data of Nayyar et al. (1989), indicating that high levels of GA_3 during pre-anthesis may induce increased fiber production. Maintaining that high level with post-anthesis exogenous applica-

tion of GA_3 becomes inhibitory, as indicated by the decreased fiber number observed at 5 d post-anthesis in the ovules treated post-anthesis (Figure 3).

Previously published literature on endogenous IAA levels in cotton ovules during the fiber initiation stage is also contradictory. In a comparison of a wild-type cotton and a fiberless mutant, the wild-type exhibited low IAA levels during pre-anthesis (4 d before flowering) with a peak IAA level at -2 d post-anthesis, followed by a dramatic return to low levels by 0 d post-anthesis (Chen et al., 1996). Fiberless mutants exhibit high pre-anthesis (4 d before flowering) levels of IAA followed by a dramatic drop at -1 d post-anthesis to a low level that remained constant 8 d post-anthesis. Similar work by Nayyar et al. (1989) indicated that IAA levels were low during pre- and early post-anthesis (2 d before flowering through 2 d post-anthesis), reaching a maximum at 3 d post-anthesis and then rapidly declining through 5 d post-anthesis. An examination of free IAA content in several commercial cultivars of cotton indicates that IAA levels are high at anthesis (3 d before flowering) and then decrease (John, 1994). All these studies demonstrate a peak in IAA levels near the day of anthesis, followed by a rapid decline. The observed increase in fiber production with exogenous IAA application in our study may be the result of maintaining high IAA levels after anthesis.

Increases in fiber number per ovule could result from increases in the proportion of epidermal cells that become fibers, from increases in the total number of epidermal cells available to become fibers, or from some combination of the two. Hormone treatments have no significant affect on fiber diameter over the experimental period (unpublished data), so if the observed increase in fiber number is the result of an increase in the total number of epidermal cells, then increases in fiber number should correspond with an increase in the size of the ovules. Neither hormone treatment had an affect on ovule length or width (Figure 4). Statistical comparisons indicate no significant differences (data not shown). Since ovule length and width were unaffected by hormone treatment, we assume that these ovules have approximately the same number of epidermal cells. Combining these observations with the observed increase in fiber number per ovule indicates that the increase in fiber production observed with hormone treatment is the result in an increase in the proportion of epidermal cells that become fibers.

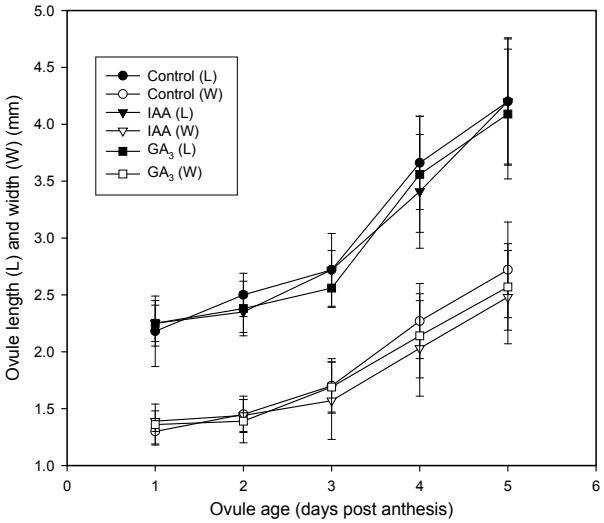

Figure 4. Effects of post-anthesis treatments with IAA and GA_3 on length (L) and width (W) of ovules of *G. hirsutum* cv. Maxxa.

In this study, exogenous hormone application clearly has an impact on *in vivo* fiber production. Pre-anthesis treatment with hormones alters post-anthesis fiber production (Figure 2), which supports the notion of pre-anthesis events having an impact on later fiber production (Wilkins and Jernstedt, 1999). Increasing the levels of IAA early in fiber development (0-5 d post-anthesis) resulted in the largest increase in fiber numbers, suggesting the morphogenic pathways for epidermal cells remain flexible at this stage. These data are consistent with the possibility of increasing cotton fiber yield through minor changes in regulatory patterns. Changes that result in increases in endogenous IAA levels during the first 5 d post-anthesis could result in significant increases in fiber production.

ACKNOWLEDGEMENTS

Supported by a Core Development Grant from Cotton Incorporated. We thank Mr. Dennis Burke, Biology Laboratory Director at Hofstra University, without whose assistance this work could not have been completed.

REFERENCES

Basra, A.S. and S. Saha. 1999. Growth regulation of cotton fibers. p. 47-63. *In* A.S. Basra (ed.) Cotton Fibers: Developmental Biology, Quality Improvement and Textile Processing. Food Products Press, an imprinting of Haworth Press, Inc., New York.

Beasley, C.A. 1971. In vitro culture of fertilized cotton ovules. BioScience 21:906-907.

Beasley, C.A. 1975. Developmental morphology of cotton flowers and seed as seen with the scanning electron microscope. Am. J. Bot. 62:584-592.

Beasley, C.A. and I.P. Ting. 1973. The effects of plant growth substances on the *in vitro* fiber development from fertilized cotton ovules. Am. J. Bot. 60:130-139.

Beasley, C.A. and I.P. Ting. 1974. Effects of plant growth substances on the *in vitro* fiber development from unfertilized cotton ovules. Am. J. Bot. 61:188-194.

Beasley, C.A., E.H. Birnbaum, W.M. Dugger, and I.P. Ting. 1974. A quantitative procedure for estimating cotton fiber growth. Stain Technol. 49:85-92.

Berlin, J.D. 1986. The outer epidermis of the cotton seed. p. 375-414. *In* J.R. Mauney, and J.McD. Stuart, (eds.) Cotton Physiology. Cotton Foundation, Memphis TN.

Bowman, D.T., G.A. Van Esbroeck, J. Van't Hof, and J.M. Jividen. 2000. Fiber cell initials in upland cotton. p. 46-62. *In* Proc. Genetic Control of Cotton Fiber and Seed Quality Workshop, San Antonio, TX. 5-6 Dec. 2000. Cotton Incorporated, Cary, NC.

Calhoun, D.S. and D.T. Bowman. 1999. Techniques for development of new cultivars. p. 361 – 414. *In* C.W. Smith and J.T. Cothren (eds.) Cotton Origin, History, Technology and Production. John Wiley and Sons, Inc. New York.

Chen, J.G., X.M. Du, and X. Zhou. 1996. Fluctuation in levels of endogenous plant hormones in ovules of normal and mutant cotton during flowering and their relation to fiber development. J. Plant Growth Regul. 15:173-177.

Davidonis, G.H. 1999. Cotton fibers in vitro. p. 65 – 84. *In* A.S. Basra (ed) Cotton Fibers: Developmental Biology, Quality Improvement and Textile Processing. Food Products Press, an imprinting of Haworth Press, Inc., New York.

Gialvalis, S., and R.W. Seagull. 2001. Plant hormones alter fiber initiation in unfertilized, cultured ovules of *Gossypium hirsutum*. J. Cotton Sci. 5:252-258 [Online]. Available at http://cotton.journal.org/2001/issue4/pdf/07.pdf

Graves, D.A., and J. McD. Stewart. 1988. Chronology of the differentiation of cotton (*Gossypim hirsutum* L.) fiber cells. Planta 175: 254-258.

Jasdanwala. R.T., Y.D. Singh, and J.J. Chinoy. 1977. Auxin metabolism in developing cotton hairs. J. Exp. Bot. 28:1111-1116.

Jasdanwala. R.T., Y.D. Singh, and J.J. Chinoy. 1980. Changes in components related to auxin turnover during cotton fiber development. Biol. Pflanzen 55:23-36.

John, M.E. 1994. Progress in genetic engineering of cotton for fiber modifications. p. 14 -17. *In* G.A. Constable, and N.W. Forrester (eds.) Proc. World Cotton Research Confer. 1: Challenging the future. Brisbane, Australia, 13-17 Feb. 1994. Int. Cotton Advisory Committee, Washington, DC.

John, M.E. 1999. Genetic Engineering strategies for cotton fiber modification. p. 271-292. *In* A.S. Basra (ed.) Cotton Fibers: Developmental Biology, Quality Improvement and Textile Processing. Food Products Press, an imprinting of Haworth Press, Inc., New York.

Joshi, P.C., A.M. Wadhwani, and B.M. Johri. 1967. Morphological and embryological studies of *Gossypium* L. Proc. Natl. Inst. Sci. India 33:37-93.

Kim, H.J., and B.A. Triplett. 2001. Cotton fiber growth *in planta* and *in vitro*. Models for plant cell elongation and cell wall biogenesis. Plant Physiol. 127:1361-1366.

Lewis, H. 1992. Future horizons in cotton research. p. 4-18. *In* The Proceedings of the Cotton Fiber Cellulose: Structure, Function, and Utilization Conference, Savannah, GA. 28-31 Oct. 1992. Cotton Inc., Raleigh, NC.

Lewis, H. 2000. Environmental regulation of yield and quality components in upland cotton. p. 8-36. *In* Proceedings Genetic Control Cotton Fiber and Seed Quality Workshop, San Antonio, TX. 5-6 Dec. 2000. Cotton Inc., Cary, NC.

Meinert, M.C., and D.P. Delmer. 1977. Changes in the biochemical composition of the cell wall of the cotton fiber during development. Plant Physiol. 59:1088-1097.

Meredith, W.R., Jr. 2000. Cotton yield progress-Why has it reached a plateau? Better Crops 84:6-9 [Online]. Available at http://www.back-to-basics.net/fertilityfacts/pdf_files/cotton_yield_progress.pdf (verified 3 May 2004).

Momtaz, O.A. 1998. Effect of plant growth regulators on in vitro fiber development from unfertilized and fertilized Egyptian cotton ovules. Plant Growth Regul. 25:159-164.

Nayyar, H., K. Kaur, C.P. Malik, and A.S. Basra. 1989. Regulation of differential fiber development in cotton by endogenous plant growth regulators. Proc. Indian Acad. Sci. 55:463-468.

Ramsey, J.C., and J. D. Berlin. 1976. Ultrastructure of early stages of cotton fiber differentiation. Bot. Gaz. 137:11-19.

Ryser, U. 1999. Cotton fiber initiation and histodifferentiation. p. 1-45. *In* A.S. Basra A.S. (ed.) Cotton Fibers: Developmental Biology, Quality Improvement and Textile Processing. Food Products Press, an imprinting of Haworth Press, Inc., New York.

Seagull, R.W. 1998. Cytoskeletal stability affects cotton fiber initiation. Int. J. Plant Sci. 159:590-595.

Seagull, R.W. 1999. Fiber development. p. 32 – 55. *In* R. Seagull and P. Alspaugh (eds.) Cotton Fiber Development and Processing: An Illustrated Overview. Int. Textile Center, Lubbock TX.

Stewart, J. McD. 1975. Fiber initiation on the cotton ovule (*Gossypium hirsutum*). Am. J. Bot. 62:723-730.

Turley, R.B. 1998. Cotton fiber cell development: Comparison of protein profiles from field and culture grown ovules. p. 1462. *In* Proc. Beltwide Cotton Conf., San Diego, CA. 5-9 Jan. 1998. Natl. Cotton Counc. Am, Memphis, TN.

Van't Hof, J. 1998. Production of micronucleoli and onset of cotton fiber growth. Planta 205:561-566.

Wilkins, T.A. and Jernstedt, J.A. 1999. Molecular genetics of developing cotton fibers. p. 231-269. *In* A.S. Basra (ed) Cotton Fibers: Developmental Biology, Quality Improvement and Textile Processing. Food Products Press, an imprinting of Haworth Press, Inc., New York.