The *Journal of Cotton Science 22:12–23 (2018)*
http://journal.cotton.org, © The Cotton Foundation 2018

BREEDING AND GENETICS

History of Cotton Fiber Bioscience Research at USDA-ARS Southern Regional Research Center

David D. Fang* and Hee Jin Kim

ABSTRACT

Improving fiber quality has been an important breeding goal for cotton breeders. Better understanding of fiber development helps cotton scientists to devise a strategy for crop improvement either through marker-assisted selection or via manipulation of fiber genes. USDA-ARS Southern Regional Research Center (SRRC) in New Orleans, LA has a long history of studying postharvest fiber chemistry and physics, but research on cotton fiber development in planta began only in 1985. During the period of 1985 to 2008, cotton fiber bioscience research at SRRC was led by Barbara Triplett whose research focused on dissecting cotton fiber development at the level of gene processes and testing hypotheses about the functional roles of specific genes or cohorts of coordinately regulated genes in important fiber traits. Following stakeholders' recommendations, the cotton fiber bioscience research unit (CFBRU) was established in 2007 at SRRC. Currently, research projects at the CFBRU are focusing on 1) in-depth research to understand the basic biology of cotton fiber development, 2) genetically mapping fiber quality and yield quantitative trait loci, and 3) using the obtained information in breeding to improve cotton fiber quality. Major recent accomplishments include, but are not limited to, identification of causative genes for Ligon-lintless 1 and immature fiber mutations and identification of stable large-effect fiber quantitative trait loci and their application in practical breeding.

The United States Department of Agriculture (USDA) Agricultural Research Service (ARS) Southern Regional Research Center (SRRC) in New Orleans, LA is one of four regional agricultural research centers established by the Agricultural Adjustment Act (Farm Bill) of 1938. It is located immediately adjacent to the City Park. Since its inception, cotton research has been a strong focus at SRRC. In the 1950s, Benerito and her team developed cross-linking technologies that led to the invention of wrinkle-free and fire retardant cotton fabrics that greatly benefitted the cotton industry (Benerito et al., 1966). By the early 1980s, more than 80% of the research projects at SRRC focused on cotton, ranging from chemical modification and physical characterization of cotton fiber to measurement of fiber quality attributes. However, until 1985, no research had been conducted to understand the basic biology of cotton fiber development in planta, that is, how cotton fiber develops from an ovule epidermal cell to a 30- to 35-mm long hollow tube of cellulose within a 60-day period.

COTTON FIBER BIOSCIENCE RESEARCH GROUP ACTIVITIES AND ACCOMPLISHMENTS BETWEEN 1985 AND 2008

With the advent of plant molecular biology techniques along with the tissue culture method development for regenerating transgenic cotton from a Coker cultivar (Davidonis and Hamilton, 1983), the cotton fiber bioscience research group (CFBRG) was founded in 1985 at USDA-ARS-SRRC with a mission to focus on a molecular genetics approach for fiber quality improvement (Triplett and Kim, 2006). This research group first belonged to the Fiber Science and Technology Research Unit, and was reorganized a few times during the first decade. Within the research group, the laboratory of Barbara Triplett was called Fiber Bioscience and in the 1990s was renamed Cotton Fiber Bioscience. There were several other scientists in the research group, but the authors are not fully aware of their detailed research activities in the field of cotton fiber bioscience. Thus, in this paper, the authors describe only the research activities, progress, and perspectives of the labora-

D.D. Fang* and H.J. Kim, Cotton Fiber Bioscience Research Unit, USDA-ARS-SRRC, 1100 Robert E. Lee Blvd, New Orleans, LA 70124
*Corresponding author: david.fang@ars.usda.gov

tory led by Triplett, who played a pivotal role in the establishment of the Cotton Fiber Bioscience Research Unit.

Triplett (Fig. 1) joined USDA-ARS-SRRC in 1983 as a Research Plant Physiologist with expertise on molecular biology and plant developmental biology. During her tenure at the SRRC her main objectives were: 1) understanding cotton fiber development at the level of gene processes, 2) testing hypotheses about the functional roles of specific genes or cohorts of coordinately regulated genes in important fiber traits, and 3) transfer of genetic and molecular strategies for fiber improvement to cooperating cotton geneticists for implementation and testing. To achieve these objectives, she established extensive cooperative research networks with ARS and non-ARS cotton scientists.

Figure 1. Research Plant Physiologist Barbara Triplett served at Southern Regional Research Center of the Agricultural Research Service of the United States Department of Agriculture for 25 years (1983-2008). Left: The photo in which Triplett examined a cotton hairy root culture appeared in *AgResearch Magazine* (Peabody, 2005). Right: A front image of USDA-ARS-SRRC located in New Orleans, LA.

Early Cotton Fiber Development Research. In the late 1980s and early 1990s, Triplett investigated relationships of fiber structure, morphology, and development of Upland cotton with fiber physical and mechanical properties. She compared cotton fiber development between wild type and fiber mutants grown in planta and in vitro (Triplett et al., 1989). She characterized cotton fiber mutant lines including Ligon-lintless 1 (Li_1), naked seed (N_1), pilose (H_2), and immature fiber (*BTL*) that were identified previously by other ARS scientists in Texas (Kohel et al., 1974). She further determined genetic effects of the mutants on yarn properties (Triplett, 1990). She, with other cell biologists and chemists, also studied how development of the primary cell wall (PCW) and secondary cell wall (SCW) impacted fiber elongation and cellulose biosynthesis (Fig. 2A-2D) (Goynes et al., 1995; Pesacreta et al., 1997; Wakelyn et al., 2007). She determined the molecular weight distributions of developing cotton fibers at various stages by gel-permeation chromatography, and showed that the PCW of the elongating fibers had lower molecular weights than the SCW of the thickening fibers (Timpa and Triplett, 1993). She was recognized as an expert on cotton research as evidenced by an invitation to write a cotton fiber development review in *Plant Physiology* (Kim and Triplett, 2001), which has become a highly cited cotton article.

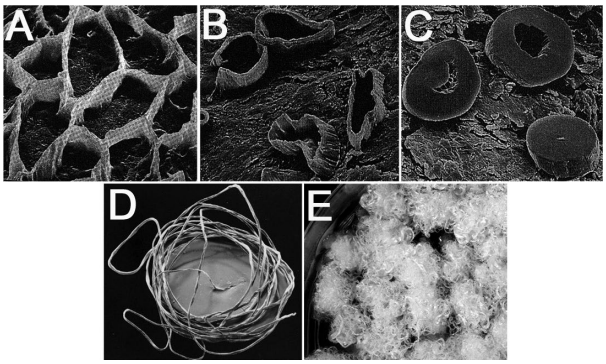

Figure 2. Cotton development in planta and in vitro. A-C. Cotton fiber development in planta. Secondary cell wall development was monitored from cross-sectioned fibers (*G. hirsutum* TM-1) at 15 days post anthesis (DPA) (A), 20 DPA (B), and 40 DPA (C) by scanning election microscope. D. A single and whole fiber was coiled and photographed. E. Cotton ovule culture at 21 DPA.

Triplett further optimized conditions for the cotton ovule culture system (Fig. 2E) that was developed originally by Beasley and Ting (1973) and utilized it as a useful tool for characterizing fiber development (Kim and Triplett, 2001; Triplett, 2000; Triplett et al., 1989), identifying extra cellular peroxidase activities (Mellon and Triplett, 1989; Triplett and Mellon, 1992), characterizing cell wall polymers (Triplett and Timpa, 1995), and testing phytohormonal effects (Triplett et al., 2008a).

In the early1990s, Triplett recruited a visiting scientist, Robert Seagull and post-doctoral associates, David Dixon and John Andersland, to research characterization of fiber cytoskeletal changes that influence fiber elongation and strength (Andersland and Triplett, 2000; Andersland et al., 1998; Dixon et al., 1994, 2000). Her research team showed a relationship between the cotton fiber cytoskeleton and cell wall organization by microscopic evaluation of developing fibers (Fig. 3A and 3B) and identified tubulin proteins from developing cotton fibers.

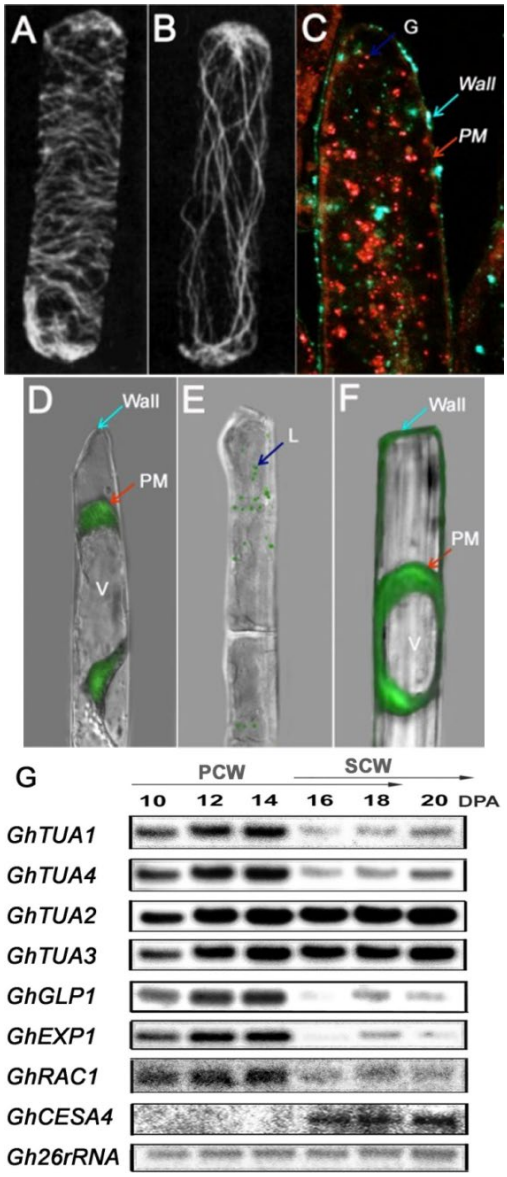

Figure 3. Molecular analyses of cotton fiber genes. A. Immunofluorescent staining of fiber microtubules that were perpendicular to the long axis of the fiber cell. B. Immunofluorescent staining of fiber microtubules that were parallel to the long axis of the fiber cell. C. Simultaneous immune-localization of *Gh*GLP1 (green spots and lines) and Golgi bodies (red spots) in cotton fibers. D-F. Subcellular localization of cytosolic *Gh*CSD1 (D), plastic *Gh*CSD2 (E), and extracellular *Gh*CSD3 (F). The CSDs were fused with green fluorescent protein (GFP), and expressed in the plasmolyzed *Arabidopsis* roots. G. Northern blot analyses of cotton fiber genes. Transcripts encoding *α- tubulin 1, 2, 3,* and *4*; *germin-like protein GLP1, expansin EXP1, Rac/Rop GTPase RAC1, cellulose synthase catalytic subunit CESA4,* and 26 ribosomal RNA (a loading control) were developmentally regulated. PCW, primary cell wall stage; SCW, secondary cell wall stage.

Molecular Analyses of Cotton Fiber Development. Triplett recruited David Whittaker and Hee Jin Kim as post-doctoral molecular biologists to the group in 1997 to initiate cotton molecular biology research. Whittaker determined α-tubulin genes (*GhTUA1, 2, 3* and *4* in Fig. 3G) that were regulated during cotton fiber development (Whittaker and Triplett, 1999). Kim used a differential display technique to identify cotton fiber genes that were developmentally or genetically regulated. As a result, the team characterized small GTPases (Charalambopoulos et al., 1999; Kim and Triplett, 2004a; Kim et al., 2000), GhGLP1 germin-like protein (Kim et al., 2004; Kim and Triplett, 2004b), Mn superoxide dismutase (Kim and Triplett, 1998), and three different types of Cu/Zn superoxide dismutases (GhCSDs) regulating reactive oxygen species (ROS) and mediating cell wall growth at various subcellular locations (Kim and Triplett, 2008; Kim et al., 2008) (Fig. 3C-3G).

In addition, cellulose synthase catalytic subunit (CESA) genes specifically associated with SCW thickening processes from Upland cotton were identified and characterized (Fig. 3G). *GhCesA4* (Kim et al., 2002) and two *GhCesA2* genes (Kim et al., 2012; Lee et al., 2013) located on two different subgenomes were identified from allotetraploid cotton. Their temporal and spatial expressions were determined in transgenic *Arabidopsis* and cotton tissues (Kim et al., 2011) **(**Fig. 4).

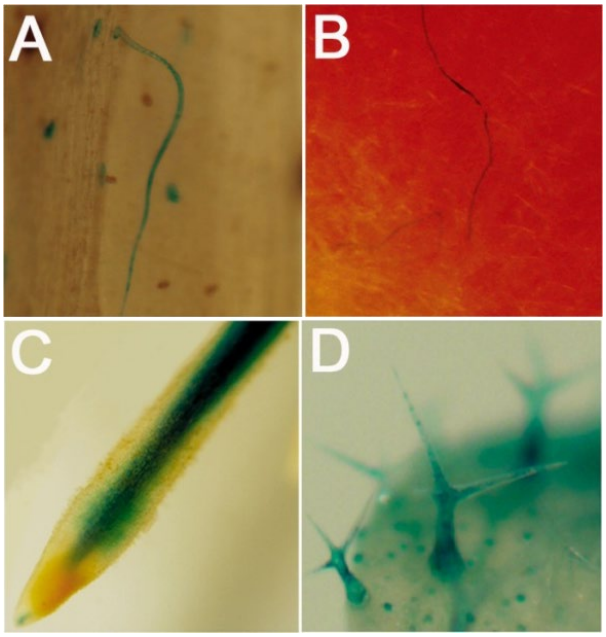

Figure 4. Functional analyses of Upland cotton *cellulose synthase catalytic subunit 4* (*GhCESS4*). A GUS reporter regulating *GhCESA4* promoter generated blue color in cotton stem trichome (A), developing fiber in cotton ovule culture (B), cotton hairy root culture (C), and *Arabidopsis* leaf trichome (D).

Growth with ARS and Extramural Funding. During the 2000s, the CFBRG grew as Triplett received multiple grants awarded by National Aeronautics and Space Administration (NASA), National Science Foundation (NSF), Cotton Incorporated, Louisiana Governor's Biotechnology Initiative, U.S.-Egypt Science & Technology Program, and USDA-Former Soviet Union Scientific Cooperation Program in addition to the ARS research project. With extramural research funds, Triplett recruited Duane Smith and Osama Hassan as visiting professors, and Sunran Kim, Fredrick James, and Doug Hinchliffe as new postdoctoral cellular and molecular biologists along with multiple graduate students and undergraduate students from University of New Orleans and Xavier University. To determine the effect of gravity on cotton fiber development for the NASA fundamental space biology, the conditions for cotton ovule culture system were further optimized and characterized (Triplett, 2000; Triplett and Johnson, 1999). The culture condition was later applied to dissecting fiber initiation process for the NSF research project (Kim et al., 2015; Pang et al., 2009; Yang et al., 2006). The cotton oligonucleotide microarray chips developed with the NSF project were used for identifying cotton fiber genes responsible for superior fiber strength of a cotton line MD52ne with ARS cotton breeder, William R. (Bill) Meredith at Stoneville, MS (Hinchliffe et al., 2010; Meredith, 2005a). Triplett and her graduate student, Stephanie Moss, supported by Louisiana Governor's Biotechnology Initiative introduced *Agrobacterium rhizogenes*-induced cotton hairy root system into the cotton research community. The cotton hairy roots were used for producing gossypol (Frankfater et al., 2009; Peabody, 2005; Triplett et al., 2008b), studying nematode resistance (Wubben et al., 2009), and localizing the cellulose synthase activities (Kim et al., 2011). Triplett was also involved in sequencing the first diploid cotton (*Gossypium raimondii* Ulbrich) genome that became the reference sequence for Upland cotton species (Chen et al., 2007; Paterson et al., 2012).

In the midst of the group's growth, in August 2005 the SRRC suffered grave damage as a result of Hurricane Katrina (Peabody and Durham, 2007). Due to extensive flooding and mold growth by a subsequent levee failure, all biological specimens were lost, and the CFBRG team relocated to the Department of Soil and Crop Science at Texas A&M University (TAMU) with help from David Stelly and Wayne Smith. During the recovery period from the Hurricane (Sept. 2005-July 2006), the team recollected cotton samples from an ARS cotton field of Russell Kohel in Texas, and re-initiated genomic research with collaborations of Stelly, Hongbin Zhang, and Hisashi Koiwa from TAMU and Z. Jeffrey Chen from the University of Texas in Austin (Kang et al., 2008; Kim et al., 2011, 2012, 2015; Lee et al., 2013).

CREATION OF THE COTTON FIBER BIOSCIENCE RESEARCH UNIT

In the late 1990s and early 2000s, the U.S. cotton industry entered into a period with both challenge and opportunity. The introduction of transgenic herbicide tolerance and bud worm/boll worm resistant cotton cultivars and their quick adoption by cotton growers revolutionized the U.S. cotton industry. However, fiber quality of U.S. cotton was not improved, and actually declined in certain popular cultivars such as DP555BR. During the same time, the U.S. cotton industry changed dramatically, transitioning from primarily domestic consumption of the raw product to predominantly production for export. Offshore cotton textile mills often have more stringent requirements in fiber quality due to spinning methodologies used. To remain competitive, the U.S. must produce high quality cotton that meets the demands of the global textile industry. In lieu of this demand, the ARS National Program Leader, John Radin and SRRC director, Patrick Jordan, organized a stakeholders' meeting at SRRC in 2004. The attendees included representatives from the National Cotton Council of America, Cotton Incorporated, cotton seed companies such as Bayer CropScience, textile mills, cotton exporters, apparel companies, and consumer products companies such as Procter & Gamble. The stakeholders recommended that ARS strengthen research to better understand cotton fiber development, and in turn to use the gained information to improve fiber quality through breeding. In October 2006, the second stakeholders' meeting was held at SRRC. This time, the stakeholders recommended that ARS create a new research unit. On 23 April 2007, ARS management officially approved the creation of the Cotton Fiber Bioscience Research Unit (CFBRU) at SRRC in New Orleans, LA. Jordan merged the laboratories of two cotton physiologists (Triplett and Gayle Davidonis) to start the CFBRU. The unit mission was to address fiber quality at the molecular level and modify and improve cotton fiber using molecular, genomic, and biotechnological approaches. Specific research areas include biology of fiber development, gene expression in the fiber, genetic and genomic knowledge of value-added traits, breeding and selection tools,

and innovative methods to enable value-capture from improved cotton fiber traits or products. Following the retirements of Davidonis and Triplett, the newly established CFBRU had a period of a few months without a scientist until David Fang joined the unit as the Research Leader in June, 2009.

CFBRU ACTIVITIES AND ACCOMPLISHMENTS SINCE 2009

After Fang became the research leader of the CFBRU, he first focused on hiring scientists to establish a research team. He hired Hee Jin Kim as a permanent scientist in April, 2010. In July, 2010, Marina Naoumkina was hired as the third scientist. Prior to joining CFBRU, Naoumkina was a staff research scientist at the Noble Foundation working on *Medicago truncatula* Gaertner. A postdoctoral research associate, Doug Hinchliffe, also remained a member of the research team for a year. On 14 May, 2018, Gregory Thyssen was hired as a Research Computational Biologist. Thyssen has been with CFBRU and Cotton Chemistry and Utilization Research Unit at SRRC since 20, May, 2012.

Fang's vision for the CFBRU includes: 1) conducting more in-depth research to understand the basic biology of cotton fiber development, 2) genetically-mapping fiber quality and yield quantitative trait loci (QTL), and 3) using the obtained information in breeding to improve cotton fiber quality. During the past eight years, most of our research activities were centered on these three areas. Following are description of our research projects and accomplishments.

Use of *Li*$_1$ and *Li*$_2$ Mutants to Study Cotton Fiber Elongation. Triplett and her team had been studying cotton fiber growth using fiber mutants in vitro and in planta (Kim and Triplett, 2001). We continued to use fiber mutants to investigate fiber elongation at the molecular level. There are numerous fiber mutants available (Percy et al., 2015). These naturally occurring mutants and their wild-type near isogenic lines (NILs) provide a unique and powerful model system to study cotton fibers at various stages of development including initiation, elongation, and secondary cell wall biosynthesis. Of these fiber mutants, two of them, Ligon-lintless 1 (*Li*$_1$) and Ligon-lintless 2 (*Li*$_2$) (Fig. 5) cause extremely short fiber (< 6 mm), and both are controlled by one single dominant gene designated as *Li*$_1$ and *Li*$_2$, respectively. Unlike the *Li*$_1$ mutant, which exhibits pleiotropy in the form of severely stunted and deformed plants in both the homozygous dominant and heterozygous states (Kohel, 1972), the *Li*$_2$ mutant plants appear healthy and morphologically identical to the homozygous recessive wild-type plants with the exception of shorter seed fibers (Narbuth and Kohel, 1990). In cooperation with Rickie Turley at USDA-ARS in Stoneville, MS, we used the *Li*$_2$ mutant to study fiber elongation. Using a combined functional and structural genomics approach, we mapped the *Li*$_2$ gene within an interval of 1.39 cM (Hinchliffe et al., 2011b). To further narrow down the *Li*$_2$ locus region, we used multiple high-throughput sequencing approaches to identify the relevant region of reference sequence and identify single nucleotide polymorphisms (SNPs) near the *Li*$_2$ locus. We performed RNAseq on 8 DPA fiber cells from the *Li*$_2$ mutant and its wild-type NIL DP5690. We identified SNPs that would result in non-synonymous substitutions to amino acid sequences of annotated genes. Next, we sequenced total DNA from pools of F_2 plants, using a super-bulked segregant analysis sequencing (sBSAseq) approach. The sBSAseq predicted 82 non-synonymous SNPs in a 3-Mb region that included the region identified by RNAseq. We designed subgenome-specific SNP markers and tested them in an F_2 population of 1733 individuals to construct a genetic map. Our resulting genetic interval contains only one gene, an aquaporin, which is highly expressed in wild-type fibers and is significantly under-expressed in elongating *Li*$_2$ fiber cells (Thyssen et al., 2014b). We transformed the aquaporin gene into Coker 312 to confirm its functionality. Although cotton fiber is shorter in the transgenic cotton than the wild type, it is not as short as the *Li*$_2$ mutant. This leads us to suspect that the aquaporin might not be the causative gene of *Li*$_2$ mutation. Efforts to determine the identity of *Li*$_2$ gene is in progress in the CFB research unit.

Figure 5. Phenotypes of cotton line DP5690 (WT) and short fiber mutant lines (*Li*$_1$, *Li*$_2$ and *liy*), and immature mutant (*im*).

In parallel with mapping efforts, CFBRU scientists also analyzed the metabolomics profiles between *Li*$_2$ mutant and its wild-type NIL (Naoumkina et al., 2013). A significant reduction of detected free sugars, sugar alcohols, sugar acids, and sugar phosphates were observed in mutant fibers. Biological processes associated with carbohydrate biosynthesis, cell wall loosening, and cytoskeleton were down-regulated in *Li*$_2$ fibers.

In 2011, the *Li*$_1$ mutant was re-introduced into our research program. Although the *Li*$_1$ mutation was discovered in 1929 (Kohel et al., 1974), the causative gene was never identified. We used an F_2 population of 2553 progeny to map the *Li*$_1$ locus within an interval of 10.4 cM on chromosome 22 (Gilbert et al., 2013). To narrow down the interval, we sequenced the whole genomes of two F_2 DNA bulks (homozygous wild type and homozygous mutant type), each bulk consisting of 100 F_2 plants. We mapped the *Li*$_1$ locus in a region of 255 kb (Thyssen et al., 2015). Recently, we identified the causative mutation of the dominant dwarf *Li*$_1$ short-fiber mutant as a single Gly65Val amino acid substitution in a polymerization domain of an actin gene, GhACT_LI1 (Gh_D04G0865) (Thyssen et al., 2017). Virus-induced gene silencing experiments confirmed its functionality.

Use of Immature Mutant to Study Cotton Fiber Fineness and Maturity. Cotton fiber fineness and maturity measured indirectly as micronaire (MIC) are important properties of fiber quality. Yet they have been difficult subjects for investigation. We used the immature mutant (*im*) to study this fiber quality (Fig. 5 and 6). We showed that the lower MIC of fibers in the *im* mutant was due to the lower degree of fiber cell wall thickening as compared to the TM-1 fibers. We mapped the *im* locus at an interval of 0.45 cM on chromosome 3 (Kim et al., 2013a). Later, we further increased our population size to 2837 F_2 progeny, and identified a 22-bp deletion in a pentatricopeptide repeat (PPR) gene as the cause of immature fiber mutation (Thyssen et al., 2016). This frame-shift mutation results in a transcript with two long open reading frames: one containing the N-terminal transit peptide that targets mitochondria, the other containing only the RNA-binding PPR domains, suggesting that a functional PPR protein cannot be targeted to mitochondria in the immature mutant. In separate research, we (Kim et al., 2013b) showed that energy deprivation symptoms such as the reduced levels of cell growth process (cell wall biosynthesis, protein synthesis, and sucrose metabolism) and the elevated levels of the recycling process (starch degradation, transports, and metal ion binding) occurred in the immature mutant as compared to its wild type. This further indicates the role of mitochondria in affecting fiber cell wall thickness.

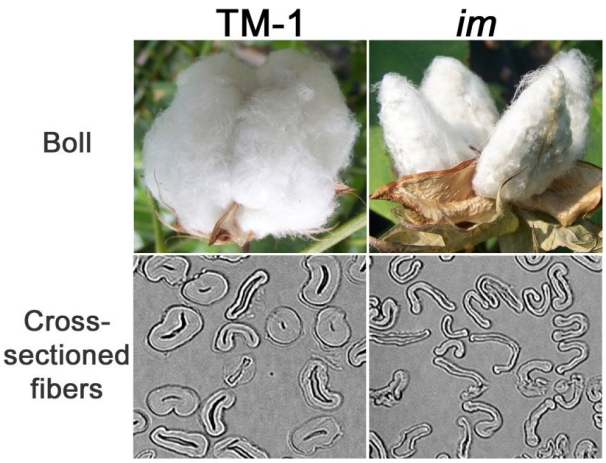

Figure 6. Comparisons of cotton bolls and cross-sectioned images between TM-1 and its near isogenic immature fiber (*im*) mutant line.

DNA Marker Development and High-Density Genetic Map Construction. DNA markers and high-density genetic maps are essential tools for trait QTL identification and molecular breeding. Before joining CFBRU, Fang developed several thousand microsatellite markers with prefixes DPL, DC, COT, C2, or SHIN (Xiao et al., 2009). After joining CFBRU, he continued efforts to develop DNA markers. Using genotyping-by-sequencing method, the CFBRU developed approximately 6000 intraspecific polymorphic SNP markers (Islam et al., 2015). In cooperation with Stelly and other scientists in the cotton community, approximately 2300 of our SNP markers were included in the 63K SNP array (Hulse-Kemp et al., 2015).

We assisted John Yu at USDA-ARS, College Station, TX to construct a TM-1×3-79 high-density genetic map (Yu et al., 2012b) and later augmented this map (Fang and Yu, 2012). In cooperation with Anna Blenda at Clemson University, SC and Jean-Marc Lacape at CIRAD, France, we constructed the first cotton high-density consensus genetic map that contains 8254 loci (Blenda et al., 2012).

Identification of Fiber QTL. We are using three approaches to identify fiber, yield, and other agronomic trait QTL, that is, a bi-parental mapping population, a multi-parent advanced generation inter-cross (MAGIC) population, and genome-wide association study (GWAS) using historical national cotton variety test data.

Fiber QTL identification using MD52ne × MD90ne population. MD52ne is a high strength cotton line developed by Meredith. It has approximately 10 to 25% higher fiber bundle strength than its recurrent parent MD90ne (Meredith, 2005b). Previously, Triplett and her team used these two lines to identify genes regulating this superior trait (Hinchliffe et al., 2010). We also suggested that the increased fiber bundle strength in MD52ne could be due to its earlier entrance from fiber elongation into the second cell wall thickening (Hinchliffe et al., 2011a). Using an F_2 population of MD52ne × MD90ne, we identified one stable strength QTL on chromosome 3, and length and short fiber content QTL on chromosome 14 and 24 (Islam et al., 2014). We further confirmed these QTLs using an F_3 population, and developed closer SNP markers flanking these QTLs (Islam et al., 2016c). Although we do not know the genes contributing to the longer and stronger fiber in MD52ne, we suggested that ethylene and its phytohormonal network might promote the fiber elongation, whereas receptor-like kinases might regulate fiber cell wall assembly and strength (Islam et al., 2016a).

In cooperation with Michael Gore at USDA-ARS, Maricopa, AZ, we identified yield component and fiber QTL using TM-1 × NM24016 population (Gore et al., 2014).

Fiber and agronomic QTL identification using a MAGIC population. The MAGIC population was developed by Johnie Jenkins and Jack McCarty at USDA-ARS in Starkville, MS, via a half-diallel crossing scheme between 11 parents (Jenkins et al., 2008). The 550 RILs we are using are C_5S_6. The entire MAGIC population and their 11 parents were planted in replicate in 2009 to 2011 in Starkville, MS and in 2014 to 2016 in Starkville and Stoneville, MS and Florence, SC to obtain fiber quality measurements. Yield data were also obtained from 2014 to 2016 plantings. We screened the 11 parental varieties with 15538 microsatellite markers, and identified 2132 polymorphic ones. Using the fiber data obtained from 2009 to 2011 in Starkville, MS, we identified 131 fiber QTLs and 37 QTL clusters. Two major QTL clusters were observed on chromosomes 7 and 16 (Fang et al., 2014). We further mapped fiber QTL with additional 6071 SNP markers. We identified and validated one QTL cluster associated with four fiber quality traits on chromosome 7. Gene expression and amino acid substitution analysis suggested that a regeneration of bulb biogenesis 1 (*GhRBB1_A07*) gene is a candidate for superior fiber quality in Upland cotton. (Islam et al., 2016b). Recently, we deep sequenced the 11 parents at 20× coverage and skim sequenced the 550 RILs at 2.5× coverage. We identified 1,520,684 SNPs and 327,509 indels among the 11 parents. Of them, 849,070 SNPs and 83,810 indels segregated in the MAGIC RIL population. We are using these markers to identify yield and other trait QTL (data not published).

Among the 11 parental varieties, Paymaster HS26 was found susceptible to the herbicide Envoke®, whereas the other 10 are tolerant. After spraying this herbicide, McCarty observed that 20 RILs were damaged by this herbicide. He developed an F_2 population of STV474 × HS26. We used this F_2 population to map the susceptible gene on chromosome 20 (Thyssen et al., 2014a).

GWAS using historical data from National Cotton Variety Tests (NCVT). Under the leadership of Jenkins, we are cooperating with three Chinese scientists to re-sequence the whole genomes of 362 cotton varieties that were included in the NCVT at different times. Our strategy is to conduct GWAS for fiber quality, yield, and seed compositions using the historical NCVT data. We expect to identify trait QTL via this approach.

Characterization of Cotton Germplasm Using Molecular Markers. To better utilize germplasm for breeding, it is important to characterize the germplasm first. We genotyped 193 Upland cotton cultivars collected from 26 countries using 448 microsatellite markers. Analysis of unique marker allele numbers indicated that modern U.S. Upland cotton has been losing its genetic diversity during the past century (Fang et al., 2013). Later we participated in a larger project to characterize a quarter (approximately 2500 accessions) of the entire U.S. cotton germplasm collection in College Station, TX. This cooperative effort resulted in several publications (Hinze et al., 2015, 2016, 2017; Yu et al., 2012a).

Use of DNA Markers in Breeding. As mentioned above, a strong positive association between the *GhRBB1* gene on chromosome 7 and fiber quality (strength, length, uniformity, and short fiber content) was observed in the MAGIC population. We developed a DNA marker to detect the *GhRBB1* gene. By selecting the favorable allele of this gene, multiple fiber quality traits can be significantly improved. Our preliminary results showed that this association remains in Acala-type varieties. We are using this marker to make a selection by cooperating with Jenkins. We are also cooperating with Linghe Zeng in Stoneville,

MS to validate the fiber strength QTL from cotton line MD52ne in different genetic backgrounds, and use the markers in breeding once confirmed.

FUTURE PERSPECTIVES

As the CFBRU enters its 10th anniversary in 2017, we are facing both challenges and opportunities. In one aspect, the federal budget has been flat since sequestration began in 2013.We are in a state of financial constraint that impedes us from pursuing other projects such as exploiting superior fiber traits from related *Gossypium* species. In another aspect, improving fiber quality through molecular and genomic approaches has gained great attention from both private and public sectors since the 1990s. Breeders are more receptive to use DNA markers to assist selection as long as the marker-trait association is stable and the effect is relatively significant. During the past decade, many fiber QTLs were identified by scientists around the world. However, few of them are actually being implemented in practical breeding. To fully and successfully implement molecular and genomic technologies in practical breeding, the future of our research will focus on the following: 1) validate the already-identified fiber QTLs in more genetic backgrounds, and identify QTLs that have moderate to high effects. These QTLs in turn will gain priority to be used in practical breeding; 2) identify stable yield and yield component QTLs; 3) confirm the functionality of fiber genes, and exploit their feasibility to improve fiber quality via biotechnology; 4) investigate genome-selection and their applicability in cotton breeding; and 5) develop a SNP chip that contains informative SNP markers associated with traits.

ACKNOWLEDGMENTS

This project was supported by USDA-ARS CRIS project #6054-21000-017-00D. Authors acknowledge Barbara Triplett, Devron Thibodeaux, Alfred French, Michael Dowd, and Thomas Ed Cleveland who shared their memories of the early CFBRG history. We greatly appreciate the contributions of current and former CFBRU employees: Marina Naoumkina, Gregory Thyssen, Doug Hinchliffe, Md Sarifu Islam, Hong Moon, Matthew Gilbert, Xianliang Song, Ping Li; Chris Florane, and Tracy Condon. Our thanks also go to many cooperators in the cotton community and program sponsor Cotton Incorporated. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture that is an equal opportunity provider and employer.

REFERENCES

Andersland, J.M., D.C. Dixon, R.W. Seagull, and B.A.Triplett. 1998. Isolation and characterization of cytoskeletons from cotton fiber cytoplasts. In Vitro Cell. Devel. Biol.-Plant 34:173–180.

Andersland, J.M., and B.A. Triplett. 2000. Selective extraction of cotton fiber cytoplasts to identify cytoskeletal-associated proteins. Plant Physiol. Biochem. 38:193–199.

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

Benerito, R.R., J.B. McKelvey, and R.J. Berni. 1966. A new theory of resiliency in cotton. Textile Res. J. 36:251–264.

Blenda, A., D.D. Fang, J.F. Rami, O. Garsmeur, F. Luo, and J.M. Lacape. 2012. A high density consensus genetic map of tetraploid cotton that integrates multiple component maps through molecular marker redundancy check. PLoS ONE 7:e45739.

Charalambopoulos, J., H. Kim, and B.Triplett. 1999. Characterization of two cDNs encoding Rab small GTP-binding proteins from cotton (*Gossypium hirsutum* L.), PGR99-149. Plant Physiol. 121:685.

Chen, Z.J., B.E.Scheffler, E.Dennis, B.A.Triplett, T. Zhang, W. Guo, X. Chen, D.M. Stelly, P.D. Rabinowicz, and C.D.Town. 2007. Toward sequencing cotton (*Gossypium*) genomes. Plant Physiol. 145:1303–1310.

Davidonis, G.H., and R.H. Hamilton. 1983. Plant regeneration from callus tissue of *Gossypium hirsutum* L.. Plant Sci. Letters 32:89–93.

Dixon, D.C., W.R. Meredith Jr., and B.A.Triplett. 2000. An Assessment of α-tubulin isotype modification in developing cotton fiber. Intern. J. Plant Sci. 161:63–67.

Dixon, D.C., R.W. Seagull, and B.A.Triplett. 1994. Changes in the accumulation of α-and β-tubulin isotypes during cotton fiber development. Plant Physiol. 105:1347–1353.

Fang, D.D., L.L. Hinze, R.G. Percy, P. Li, D. Deng, and G.Thyssen. 2013. A microsatellite-based genome-wide analysis of genetic diversity and linkage disequilibrium in Upland cotton (*Gossypium hirsutum* L.) cultivars from major cotton-growing countries. Euphytica 191:391–401.

Fang, D.D., J.N. Jenkins, D.D. Deng, J.C. McCarty, P. Li, and J. Wu. 2014. Quantitative trait loci analysis of fiber quality traits using a random-mated recombinant inbred population in Upland cotton (*Gossypium hirsutum* L.). BMC Genomics 15:397.

Fang, D.D., and J.Z. Yu. 2012. Addition of 455 microsatellite marker loci to the high-density *Gossypium hirsutum* TM-1 x *G. barbadense* 3-79 genetic map. J. Cotton Sci. 16:229–248.

Frankfater, C.R., M.K. Dowd, and B.A.Triplett. 2009. Effect of elicitors on the production of gossypol and methylated gossypol in cotton hairy roots. Plant Cell, Tissue Organ Culture 98:341–349.

Gilbert, M.K., R.B. Turley, H.J. Kim, P. Li, G. Thyssen, Y. Tang, C.D. Delhom, M. Naoumkina, and D.D.Fang. 2013. Transcript profiling by microarray and marker analysis of the short cotton (*Gossypium hirsutum* L.) fiber mutant Ligon lintless-1 (Li1). BMC Genomics 14:403.

Gore, M.A., D.D. Fang, J.A. Poland, J. Zhang, R.G. Percy, R.G. Cantrell, G. Thyssen, and A.E.Lipka. 2014. Linkage map construction and quantitative trait locus analysis of agronomic and fiber quality traits in cotton. Plant Genome 7:1–8.

Goynes, W., Ingber B., and B. Triplett. 1995. Cotton fiber secondary wall development—time versus thickness. Textile Res. J. 65:400–408.

Hinchliffe, D.J., W.R. Meredith, C.D. Delhom, D.P. Thibodeaux, and D.D. Fang. 2011a. Elevated growing degree days influence transition stage timing during cotton (*Gossypium hirsutum* L.) fiber development and result in increased fiber strength. Crop Sci. 51:1683–1692.

Hinchliffe, D.J., W.R. Meredith, K.M. Yeater, H.J. Kim, A.W. Woodward, Z.J. Chen, and B.A. Triplett. 2010. Near-isogenic cotton germplasm lines that differ in fiber-bundle strength have temporal differences in fiber gene expression patterns as revealed by comparative high-throughput profiling. Theor. Appl. Genetics 120:1347–1366.

Hinchliffe, D.J., R.B. Turley, M. Naoumkina, H.J. Kim, Y. Tang, K.M. Yeater, P. Li, and D.D. Fang. 2011b. A combined functional and structural genomics approach identified an EST-SSR marker with complete linkage to the Ligon lintless-2 genetic locus in cotton (*Gossypium hirsutum* L.). BMC Genomics 12:445.

Hinze, L.L., D.D. Fang, M.A. Gore, B.E. Scheffler, J.Z. Yu, J. Frelichowski, and R.G. Percy. 2015. Molecular characterization of the Gossypium Diversity Reference Set of the US National Cotton Germplasm Collection. Theor. Appl. Genetics 128:313–327.

Hinze, L.L., E. Gazave, M.A. Gore, D.D. Fang, B.E. Scheffler, J.Z. Yu, D.C. Jones, J. Frelichowski, and R.G. Percy. 2016. Genetic diversity of the two commercial tetraploid cotton species in the Gossypium diversity reference set. J. Heredity 107:274–286.

Hinze, L.L., A.M. Hulse-Kemp, I.W. Wilson, Q.H. Zhu, D.J. Llewellyn, J.M. Taylor, A. Spriggs, D.D. Fang, M. Ulloa, J.J. Burke, M. Giband, J.M. Lacape, A. Van Deynze, J.A. Udall, J.A. Scheffler, S. Hague, J.F. Wendel, A.E. Pepper, J. Frelichowski, C.T. Lawley, D.C. Jones, R.G. Percy, and D.M. Stelly. 2017. Diversity analysis of cotton (*Gossypium hirsutum* L.) germplasm using the Cotton-SNP63K Array. BMC Plant Biol. 17:37.

Hulse-Kemp, A.M., J. Lemm, J. Plieske, H. Ashrafi, R. Buyyarapu, D.D. Fang, J. Frelichowski, M. Giband, S. Hague, L.L. Hinze, K.J. Kochan, P.K. Riggs, J.A. Scheffler, J.A. Udall, M. Ulloa, S.S. Wang, Q.H. Zhu, S.K. Bag, A. Bhardwaj, J.J. Burke, R.L. Byers, M. Claverie, M.A. Gore, D.B. Harker, M.S. Islam, J.N. Jenkins, D.C. Jones, J.M. Lacape, D.J. Llewellyn, R.G. Percy, A.E. Pepper, J.A. Poland, K. Mohan Rai, S.V. Sawant, S.K. Singh, A. Spriggs, J.M. Taylor, F. Wang, S.M. Yourstone, X. Zheng, C.T. Lawley, M.W. Ganal, A. Van Deynze, I.W. Wilson, and D.M. Stelly. 2015. Development of a 63K SNP array for cotton and high-density mapping of intraspecific and interspecific populations of Gossypium spp. Gene|Genome|Genetics 5:1187–209.

Islam, M.S., D.D. Fang, G.N. Thyssen, C.D. Delhom, Y. Liu, and H.J. Kim. 2016a. Comparative fiber property and transcriptome analyses reveal key genes potentially related to high fiber strength in cotton (*Gossypium hirsutum* L.) line MD52ne. BMC Plant Biol. 16:36.

Islam, M.S., G.N. Thyssen, J.N. Jenkins, and D.D. Fang. 2015. Detection, validation, and application of genotyping-by-sequencing based single nucleotide polymorphisms in Upland cotton. Plant Genome 8:1–10.

Islam, M.S., G.N. Thyssen, J.N. Jenkins, L. Zeng, C.D. Delhom, J.C. McCarty, D.D. Deng, D.J. Hinchliffe, D.C. Jones, and D.D. Fang. 2016b. A MAGIC population-based genome-wide association study reveals functional association of GhRBB1_A07 gene with superior fiber quality in cotton. BMC Genomics 17:903.

Islam, M.S., L. Zeng, C.D. Delhom, X. Song, H. Kim, P. Li, and D.D. Fang. 2014. Identification of cotton fiber quality quantitative trait loci using intraspecific crosses derived from two near-isogenic lines differing in fiber bundle strength. Molec. Breeding 34:373–384.

Islam, M.S., L. Zeng, G.N. Thyssen, C.D. Delhom, H.J. Kim, P. Li, and D.D. Fang. 2016c. Mapping by sequencing in cotton (*Gossypium hirsutum*) line MD52ne identified candidate genes for fiber strength and its related quality attributes. Theor. Appl. Genetics 129:1071–86.

Jenkins, J.N., J.C. McCarty, O.A. Gutierrez, R.W. Hayes, D.T. Bowman, C.E. Watson, D.C. Jones. 2008. Registration of RMUP-C5, a random mated population of upland cotton germplasm. J. Plant Registr. 2:239–242.

Kang, J.S., J. Frank, C.H. Kang, H. Kajiura, M. Vikram, A. Ueda, S. Kim, J.D. Bahk, B.A. Triplett, K. Fujiyama, S.Y. Lee, and H. Koiwa. 2008. Salt tolerance of *Arabidopsis thaliana* requires maturation of N-glycosylated proteins in the Golgi apparatus. Proc. Nat. Acad. Sci. 105:5933–5938.

Kim, H.J., J. Charalambopoulos, and B. Triplett. 2000. Characterization of a cDNA encoding a Rac-like GTP-binding protein from cotton (*Gossypium hirsutum* L.) Plant Gene Register. Plant Physiol. 122:291.

Kim, H.J., D. Hinchliffe, B. Triplett, K. Yeater, H. Moon, M. Gilbert, G. Thyssen, R. Turley, D. Fang. 2015. Phytohormonal networks promote differentiation of fiber initials on pre-anthesis cotton ovules grown i*n vitro* and *in planta*. PLoS ONE 10(4):e0125046.

Kim, H.J., N. Kato, S. Kim, B. Triplett. 2008. Cu/Zn superoxide dismutases in developing cotton fibers: evidence for an extracellular form. Planta 228:281–292.

Kim, H.J., H.S. Moon, C.D. Delhom, L. Zeng, and D.D. Fang. 2013a. Molecular markers associated with the immature fiber (im) gene affecting the degree of fiber cell wall thickening in cotton (*Gossypium hirsutum* L.). Theor. Appl. Genetics 126:23–31.

Kim, H.J., N. Murai, D.D. Fang, B.A. Triplett. 2011. Functional analysis of *Gossypium hirsutum* cellulose synthase catalytic subunit 4 promoter in transgenic Arabidopsis and cotton tissues. Plant Sci. 180:323–332.

Kim, H.J., T.C. Pesacreta, and B.A. Triplett. 2004. Cotton-fiber germin-like protein. II: Immunolocalization, purification, and functional analysis. Planta 218:525–535.

Kim, H.J., Y. Tang, H.S. Moon, C.D. Delhom, and D.D. Fang. 2013b. Functional analyses of cotton (*Gossypium hirsutum* L.) immature fiber (*im*) mutant infer that fiber cell wall development is associated with stress responses. BMC Genomics 14:889.

Kim, H.J., and B.A. Triplett. 2008. Involvement of extracellular Cu/Zn superoxide dismutase in cotton fiber primary and secondary cell wall biosynthesis. Plant Signaling Behav. 3:1119–1121.

Kim, H.J., and B.A. Triplett. 1998. Characterization of a manganese superoxide dismutase from cotton fiber. PGR98-171. Plant Physiol. 118:712.

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.

Kim, H.J., and B.A. Triplett. 2004a. Characterization of GhRac1 GTPase expressed in developing cotton (Gossypium hirsutum L.) fibers. Biochimica et Biophysica Acta (BBA) - Gene Struct. Expression 1679:214–221.

Kim, H.J., and B.A. Triplett. 2004b. Cotton fiber germin-like protein. I. Molecular cloning and gene expression. Planta 218:516–524.

Kim, H.J., B.A. Triplett, H.-B. Zhang, M.-K. Lee, D.J. Hinchliffe, P. Li, and D.D. Fang. 2012. Cloning and characterization of homeologous cellulose synthase catalytic subunit 2 genes from allotetraploid cotton (*Gossypium hirsutum* L.). Gene 494:181–189.

Kim, H.J., M.Y. Williams, and B.A.Triplett. 2002. A novel expression assay system for fiber-specific promoters in developing cotton fibers. Plant Molec. Biol. Reporter 20:7–18.

Kohel, R.J. 1972. Linkage tests in Upland cotton, *Gossypium hirsutum* L. II. Crop Sci. 12:66–69.

Kohel, R.J., J.E. Quisenberry, and C.R. Benedict. 1974. Fiber elongation and dry weight changes in mutant lines of cotton. Crop Sci. 14:471–474.

Lee, M.-K., Y. Zhang, M. Zhang, M. Goebel, H.J. Kim, B.A. Triplett, D.M. Stelly, and H.-B. Zhang. 2013. Construction of a plant-transformation-competent BIBAC library and genome sequence analysis of polyploid Upland cotton (*Gossypium hirsutum* L.). BMC Genomics 14:208.

Mellon, J.E., and B.A. Triplett. 1989. De novo synthesis of peroxidase in cotton ovule culture medium. Physiol. Plantarum 77:302–307.

Meredith, W.R. 2005a. Registration of MD 52ne high fiber quality cotton germplasm and recurrent parent MD 90ne. Crop Sci. 45:807–808.

Meredith, W.R. 2005b. Minimum number of genes controlling cotton fiber strength in a backcross population. Crop Sci. 45:1114–1119.

Naoumkina, M., D.J. Hinchliffe, R.B. Turley, J.M. Bland, and D.D. Fang. 2013. Integrated metabolomics and genomics analysis provides new insights into the fiber elongation process in Ligon lintless-2 mutant cotton (*Gossypium hirsutum* L.). BMC Genomics 14:155.

Narbuth, E.V., and R.J. Kohel. 1990. Inheritance and linkage analysis of a new fiber mutant in cotton. J. Heredity 81:131–133.

Pang, M., A.W. Woodward, V. Agarwal, X. Guan, M. Ha, V. Ramachandran, X. Chen, B.A. Triplett, D.M. Stelly, and Z.J. Chen. 2009. Genome-wide analysis reveals rapid and dynamic changes in miRNA and siRNA sequence and expression during ovule and fiber development in allotetraploid cotton (*Gossypium hirsutum* L.). Genome Biol. 10:R122.

Paterson, A.H., J.F. Wendel, H. Gundlach, H. Guo, J. Jenkins, D. Jin, D. Llewellyn, K.C. Showmaker, S. Shu, J. Udall, M.-J. Yoo, R. Byers, W. Chen, A. Doron-Faigenboim, M.V. Duke, L. Gong, J. Grimwood, C. Grover, K. Grupp, G. Hu, T.-h. Lee, J. Li, L. Lin, T. Liu, B.S. Marier, J.T. Page, A.W. Roberts, E. Romanel, W.S. Sanders, E. Szadkowski, X. Tan, H. Tang, C. Xu, J. Wang, Z. Wang, D. Zhang, L. Zhang, H. Ashrafi, F. Bedon, J.E. Bowers, C.L. Brubaker, P.W. Chee, S. Das, A.R. Gingle, C.H. Haigler, D. Harker, L.V. Hoffmann, R. Hovav, D.C. Jones, C. Lemke, S. Mansoor, M.U. Rahman, L.N. Rainville, A. Rambani, U.K. Reddy, J.-K. Rong, Y. Saranga, B.E. Scheffler, J.A. Scheffler, D.M. Stelly, B.A. Triplett, A. Van Deynze, M.F.S. Vaslin, V.N. Waghmare, S.A. Walford, R.J.Wright, E.A. Zaki, T. Zhang, E.S. Dennis, K.F.X. Mayer, D.G. Peterson, D.S. Rokhsar, and X. Wang. 2012. Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. Nature 492:423–427.

Peabody, E. 2005. Rooting for gossypol. Agric. Res. 53:12–14.

Peabody, E., and S. Durham. 2007. USDA facility celebrates full recovery from Katrina, Research News, Agricultural Research Service, Beltsville, Maryland.

Percy, R., B. Hendon, E. Bechere, and D. Auld. 2015. Qualitative genetics and utilization of mutants. p. 155–186 *In* D. D. Fang and R. Percy (Eds.), Cotton (2nd Ed.), ASA-CSSA-SSSA, Madison, WI.

Pesacreta, T.C., L.C. Carlson, and B.A. Triplett. 1997. Atomic force microscopy of cotton fiber cell wall surfaces in air and water: quantitative and qualitative aspects. Planta 202:435–442.

Thyssen, G., J.C. McCarty, P. Li, J.N. Jenkins, and D.D. Fang. 2014a. Genetic mapping of non-target-site resistance to a sulfonylurea herbicide (Envoke) in Upland cotton (*Gossypium hirsutum* L.). Molec. Breeding 33:341–348.

Thyssen, G.N., D.D. Fang, R.B. Turley, C. Florane, P. Li, and M. Naoumkina. 2014b. Next generation genetic mapping of the Ligon-lintless-2 (Li2) locus in upland cotton (*Gossypium hirsutum* L.). Theor. Appl. Genetics 127:2183–2192.

Thyssen, G.N., D.D. Fang, R.B. Turley, C. Florane, P. Li, and M. Naoumkina. 2015. Mapping-by-sequencing of Ligon-lintless-1 (Li1) reveals a cluster of neighboring genes with correlated expression in developing fibers of Upland cotton (*Gossypium hirsutum* L.). Theor. Appl. Genetics 128:1703–1712.

Thyssen, G.N., D.D. Fang, R.B. Turley, C.B. Florane, P. Li, C.P. Mattison, and M. Naoumkina. 2017. A Gly65Val substitution in an actin, GhACT_LI1, disrupts cell polarity and F-actin organization resulting in dwarf, lintless cotton plants. Plant J. 90:111–121.

Thyssen, G.N., D.D. Fang, L. Zeng, X. Song, C.D. Delhom, T.L. Condon, P. Li, and H.J. Kim. 2016. The immature fiber mutant phenotype of cotton (*Gossypium hirsutum*) Is linked to a 22-bp frame-shift deletion in a mitochondria targeted pentatricopeptide repeat gene. Gene|Genome|Genetics 6:1627–1633.

Timpa, J.D., and B.A. Triplett. 1993. Analysis of cell-wall polymers during cotton fiber development. Planta 189:101–108.

Triplett, B.A., and H.J. Kim. 2006. Using cotton fiber development to discover how plant cells grow. p. 153–163 *In* T. Hayashi (Ed.), The Science and Lore of the Plant Cell Wall. Universal Publishers, Boca Raton, FL.

Triplett, B.A. 1990. Evaluation of fiber and yam from three cotton fiber mutant lines. Textile Res. J. 60:143–148.

Triplett, B.A. 2000. Cotton ovule culture: a tool for basic biology, biotechnology and cotton improvement. In Vitro Cell. Develop. Biol.-Plant 36:93–101.

Triplett, B.A., W.H. Busch, and W.R. Goynes. 1989. Ovule and suspension culture of a cotton fiber development mutant. In Vitro Cell. Develop. Biol.-Plant 25:197–200.

Triplett, B.A., and D.S. Johnson. 1999. Adding gelling agents to cotton ovule culture media leads to subtle changes in fiber development. In Vitro Cell. Develop. Biol.-Plant 35:265–270.

Triplett, B.A., H.J. Kim, D. Hinchliffe, S.-H. Sze, P. Thaxton, D. Stelly, and Z.J. Chen. 2008a. Phytohormone regulation of cotton fiber development in vitro. p. 153–156 *In* Z. Xu, et al. (Eds.), Biotechnology and Sustainable Agriculture 2006 and Beyond, Springer Verlag, NewYork, NY.

Triplett, B.A., and J.E. Mellon. 1992. Purification and characterization of anionic peroxidases from cotton (*Gossypium hirsutum* L.). Plant Sci. 81:147–154.

Triplett, B.A., S.C. Moss, J.M. Bland, M.K. Dowd, and G.C. Phillips. 2008b. Induction of hairy root cultures from *Gossypium hirsutum* and *Gossypium barbadense* to produce gossypol and related compounds. In Vitro Cell. Develop. Biol.-Plant 44:508–517.

Triplett, B.A., and J.D.Timpa. 1995. Characterization of cell-wall polymers from cotton ovule culture fiber cells by gel permeation chromatography. In Vitro Cell. Develop. Biol.-Plant 31:171–175.

Wakelyn, P.J., N.R. Bertoniere, A.D. French, D.P. Thibodeaux, B.A. Triplett, M.-A. Rousselle, W.R. Goynes Jr, J.V. Edwards, L. Hunter, D.D. McAlister, and G.R.Gamble. 2007. Cotton Fiber Chemistry and Technology. CRC Press, New York, NY.

Whittaker, D.J., and B.A.Triplett. 1999. Gene-specific changes in α-tubulin transcript accumulation in developing cotton fibers. Plant Physiol. 121:181–188.

Wubben, M.J., F.E. Callahan, B.A. Triplett, and J.N.Jenkins. 2009. Phenotypic and molecular evaluation of cotton hairy roots as a model system for studying nematode resistance. Plant Cell Rep. 28:1399–1409.

Xiao, J., K. Wu, D.D. Fang, D.M. Stelly, J. Yu, and R.G.Cantrell. 2009. New SSR markers for use in cotton (*Gossypium* spp.) improvement. J. Cotton Sci. 13:75–157.

Yang, S.S., F. Cheung, J.J. Lee, M. Ha, N.E. Wei, S.H. Sze, D.M. Stelly, P. Thaxton, B.A. Triplett, and C.D.Town. 2006. Accumulation of genome-specific transcripts, transcription factors and phytohormonal regulators during early stages of fiber cell development in allotetraploid cotton. Plant J. 47:761–775.

Yu, J.Z., D.D. Fang, R.J. Kohel, M. Ulloa, L.L. Hinze, R.G. Percy, J. Zhang, P. Chee, B.E. Scheffler, and D.C. Jones. 2012a. Development of a core set of SSR markers for the characterization of *Gossypium* germplasm. Euphytica 187:203–213.

Yu, J.Z., R.J. Kohel, D.D. Fang, J. Cho, A. Van Deynze, M. Ulloa, S.M. Hoffman, A.E. Pepper, D.M. Stelly, J.N. Jenkins, S. Saha, S.P. Kumpatla, M.R. Shah, W.V. Hugie, and R.G.Percy. 2012b. A high-density simple sequence repeat and single nucleotide polymorphism genetic map of the tetraploid cotton genome. Gene|Genome|Genetics 2:43–58.