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Review

Genetic and Molecular Regulation of Cotton Fiber Initiation and Elongation

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Abstract: Cotton fiber, a crucial and sustainable resource for global textile production, undergoes a complex five-stage developmental process, encompassing initiation, elongation, transition, secondary cell wall biosynthesis, and maturation. These elongated single-cell fibers originate from the outer ovule epidermis. The development of cotton fibers involves intricate changes in gene expression and physiological processes, resulting in a nearly pure cellulose product that is vital for the global cotton industry. Decoding the genes associated with fiber development enhances our understanding of cotton fiber mechanisms and facilitates the cultivation of varieties with enhanced quality. In recent decades, advanced omics approaches, including genomics, transcriptomics, and proteomics, have played a pivotal role in identifying the genes and gene products linked to cotton fiber development, including the MYB transcription factor family, which coordinates cotton fiber development. Molecular studies have revealed the transcription factors, like MYB, WRKY, Homeodomain Leucine Zipper (HD-ZIP), and basic helix–loop–helix (bHLH), influencing fiber initiation and elongation. The intricate interplay of phytohormones, like auxin, gibberellic acid (GA), brassinosteroids (BRs), jasmonic acid (JA), ethylene, abscisic acid (ABA), and cytokinin, is explored, providing a comprehensive perspective on the shaping of cotton fibers. Numerous candidate genes and cellular processes affecting various aspects of fiber development hold promise for genetic engineering or marker-assisted breeding to improve fiber quality. This review presents a comprehensive overview of key achievements in cotton molecular biology, with a specific emphasis on recent advancements in understanding the transcription factors and phytohormones involved in cotton fiber initiation and elongation.

Keywords: cotton fiber; initiation; elongation; transcription factors; phytohormones

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1. Introduction

Cotton (*Gossypium* spp.) is a globally cultivated and economically significant fiber crop, playing a pivotal role in the textile industry and making substantial contributions to the global economy. The quality of cotton fibers directly influences the excellence of cotton-based textiles. Derived from single-celled epidermal seed trichomes produced by four domesticated species within the *Gossypium* genus, cotton stands as the most widely utilized plant textile globally. The genus, encompassing over 50 species spread across the tropics and subtropics, exhibits rich species diversity and morphological variation [1]. This diversity, especially in the context of the domestication experiment involving four species and two ploidy states within *Gossypium*, serves as a natural and powerful system for studying genetic, genomic, and genotype-to-phenotype transitions. Notably, *Gossypium hirsutum* cotton exhibits exceptional adaptability to various environmental conditions. It is renowned for its capacity to yield substantial quantities of high-quality fiber, constituting approximately 95% of all planted cotton [2]. At the same time, *Gossypium barbadense* produces luxury textiles with fine, lengthy, and robust fibers [3]. Additionally, *Gossypium hirsutum* requires fewer resources and displays enhanced resistance to pests and diseases compared

with *Gossypium barbadense.* Despite domesticated diploids producing inferior fibers, they persist in local cultivation due to their adaptation to specific regional conditions [3].

Each cotton fiber is a single, elongated cotton seed coat epidermal cell. The differentiation and development of cotton fiber cells are highly complex and are divided into five overlapping stages: fiber initiation [days post-anthesis (DPA), −1~3 DPA], fiber cell elongation (1~16 DPA), transition (16~20 DPA), secondary cell wall (SCW) synthesis (20~40 DPA), and fiber cell maturation (40~50 DAP) [4–6] (Figure 1). In *Gossypium hirsutum*, long lint fibers primarily initiate before or on the day of anthesis, while ovule epidermal cells starting at or after 3 DPA produce shorter fibers known as linters or fuzz (Figure 1B). Fiber mutants with inhibited fiber initial growth have demonstrated that the first detectable sign of fiber initiation occurs on the day of anthesis (0 DPA), with approximately 25% of epidermal cells contributing to fiber initials [7–9]. Transition is a distinct developmental stage that occurs between primary and secondary wall synthesis. Over several days, a stage-specific transcriptome underpins the distinctive cellular and biochemical status of the fiber cell. The galacturonosyltransferases (GAUTs) gene family, which is a critical participant in the pectin synthesis pathway, plays an important role in elongation, transition, and cell wall synthesis [10]. The often mentioned 'maturation' phase of fiber development is not well characterized. After SCW cellulose synthesis stops, the fiber continues to dehydrate within the closed boll, and the lateral packing of cellulose increases (Figure 1A). Morphological and embryological studies, coupled with advanced microscopy techniques, have significantly contributed to our understanding of cotton fiber development. Vibrational sum frequency generation, attenuated total refection infrared (ATR-IR), Fourier transform Raman (FT-Raman) spectroscopy, and X-ray diffraction (XRD) were all used to study the cellulose component in mature and air-dried fibers from two species, *Gossypium hirsutum* and *Gossypium barbadense* [11].

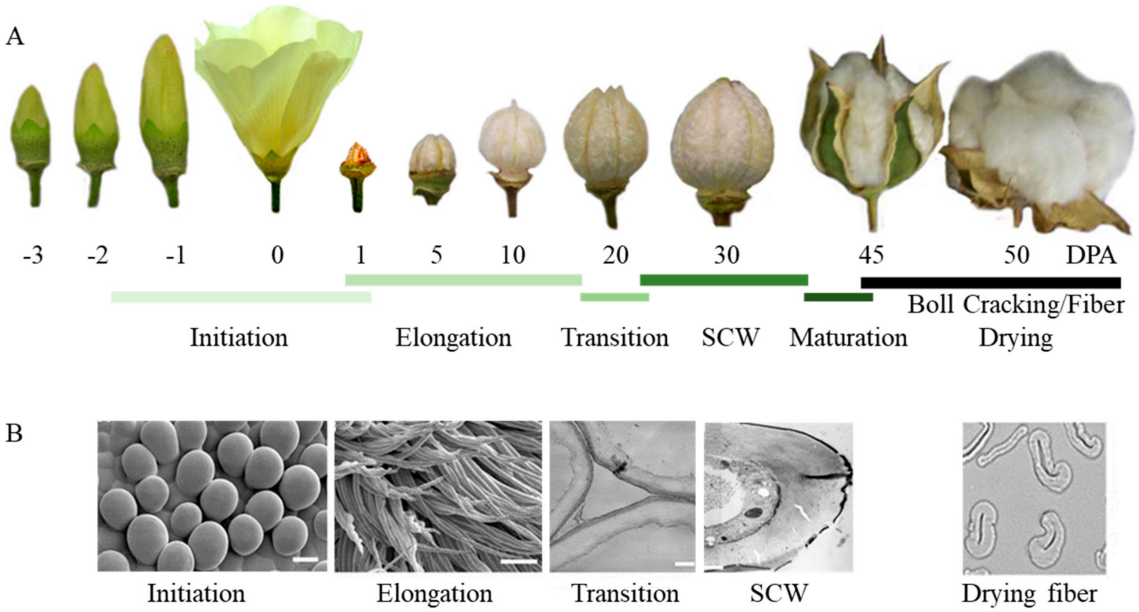

Figure 1. Cotton fiber development. (**A**) Progression of cotton flower and boll development. Fiber developments are classified into five stages, including initiation, elongation, transition, secondary cell wall (SCW) biosynthesis, and maturation. DPA, days post-anthesis. Adapted with permission from Fang et al. [12]. (**B**) Images of fibers at progressive developmental stages: SEM of fiber initials (bar = 10 μm) and fibers during early elongation (bar = 100 μm); TEM of the first stage of fiber thickening during the transition stage (three adjacent fibers are shown; bar = 300 nm) and near the end of secondary cell wall synthesis (bar = 1 μm); and light micrograph of cross-sectioned mature, dried, fibers. Images excerpted with permission from Figure 1 in Stiff and Haigler [6].

Understanding the intricate processes of initiation and elongation in cotton fibers involves the coordination of multiple genes and pathways. The integration of next-generation sequencing technology, including mRNA sequencing and genome sequencing with innovative phenotype evaluation methods, has emerged as a transformative tool in comprehending the biological mechanisms governing cotton fiber development. For example, the 102 *TBL* (*Trichome Birefringence Like*) gene family members were evaluated for their involvement in fiber development from *Gossypium hirsutum*, with two *GhTBL* genes (*GhTBL7* and *GhTBL58*) showing differential expression in the fiber at 10 DPA. Silencing these genes resulted in a significant reduction in the fiber length at 10 DPA, suggesting a potential role for these genes in fiber elongation [13]. Recently, a total of 143 *GhHMAs* (heavy metal-binding domain) were detected by genome-wide identification in *Gossypium hirsutum*. A gene expression profile provided essential clues about the function of *GhHMA* genes in cotton fiber development and the response to various abiotic stresses. *GhHMA26* was predominantly expressed in 10 DPA fiber cells, and the relative expression was higher than any other *GhHMAs*, which indicates that *GhHMA26* may positively regulate fiber elongation [14]. Genome-wide analysis of the serine carboxypeptidase-like protein family reveals that *Ga09G1039* is involved in fiber elongation in cotton with the overexpression of *Ga09G1039* significantly increasing the length of stem trichomes [15]. RNA sequencing (RNA-seq) data and N6-methyladenosine sequencing (m6A-seq) data showed that methylation of m6A affected the mRNA stability of these fiber elongation-related genes, including the transcription factor *GhMYB44*. The overexpression of GhMYB44 reduced fiber elongation, whereas the silencing of *GhMYB44* produced longer fibers [16]. Comparative phosphor-proteomic analysis between two cotton varieties, *J7-1* and *J14-1*, revealed that phosphorylation of sucrose synthase *GhSUS2* by Ca^{2+}-dependent protein kinases *GhCPK84/93* affects cotton fiber development. Moreover, ABA could promote the transcription and translation of *GhCPK84* and *GhCPK93*, thereby enhancing the phosphorylation of GhSUS2 to impede fiber elongation [17]. A genome-wide association study (GWAS) identified a ~6.2 kb insertion, larINDELFZ, positioned at the end of chromosome 8 in fuzzless *Gossypium arboreum*. Comprising a ~5.0 kb repetitive sequence and a ~1.2 kb fragment translocated from chromosome 12, this remote insertion was predicted to function as an enhancer located ~18 kb upstream of the dominant repressor GaFZ (Ga08G0121). This finding unveiled a novel regulator of fiber/trichome development, shedding light on the significance of noncoding sequences in cotton. The large-fragment insertion activates the GaFZ gene and is associated with fuzz and trichome reduction in *Gossypium arboretum* [18]. Genome-wide exploration identified a total of 125, 73, and 71 full-length *Catharanthus roseus receptor-like kinase 1-like* (*CrRLK1L*) family genes in *Gossypium hirsutum*, *Gossypium arboreum*, and *Gossypium raimondii*, respectively, with some of the *GhCrRLK1Ls* preferentially expressed in fibers at the different stages. *GhCrRLK1L104* was highly expressed in fibers at 30 DPA, and the overexpression of *GhCrRLK1L104* in *Arabidopsis* increased the trichome length, which indicates its function in cell elongation [19].

Recently, fiber osmoregulation has emerged as a crucial player in the regulation of cotton fiber initiation and elongation. A two-year field experiment was conducted to determine whether potassium ameliorates *Gossypium hirsutum* fiber length by regulating osmotic and K^+/Na^+ homeostasis under salt stress [20]. Another study [21] showed that low soil available phosphorus (AP) contents (P_0: 3 ± 0.5; P_1: 6 ± 0.5 mg kg^{-1}) inhibited the fiber cell elongation, leading to a reduction in the maximum velocity of fiber elongation (V_{Lmax}) and fiber length. This was mainly due to lower malate content and V-H^+-PPase activities [21]. A cell wall–localized β-1,3-glucanase, GhGLU18, was found to promote fiber elongation and cell wall thickening by degrading callose and enhancing polysaccharide metabolism [22]. Calcium is also involved in fiber development. An in vitro ovule culture demonstrated that Ca^{2+} rescued the shorter-fiber phenotype of *GhIQD10* overexpression lines. *GhIQD10* was expressed mainly in the transition period of cotton fiber development. *GhIQD10* interacted with *GhCaM7* and the interaction was inhibited by Ca^{2+} [23].

Phytohormones, including gibberellin acid (GA), auxin, cytokinin, brassinosteroids (BRs), abscisic acid (ABA), ethylene, jasmonic acid (JA), cytokinin, salicylic acid, and strigolactone (SL), are small endogenous signaling molecules in plants [24]. Many of these hormones directly participate in fiber initiation and elongation. For example, strigolactones are a class of carotenoid-derived plant hormones that modulate cotton fiber elongation and secondary cell wall thickening. The endogenous SLs were significantly higher in fibers at 20 DPA. Exogenous SLs significantly increased fiber length and cell wall thickness [25]. The fiber-specific expression of *GhOR1Del*, a positive regulator of carotenoid accumulation, was found to upregulate the carotenoid level in cotton fiber and simultaneously increase the contents of carotenoids, ABA, and ethylene in elongating fibers [26].

This manuscript provides a comprehensive review of the essential roles played by various transcription factors, such as MYB, WRKY, HD-ZIP, and bHLH transcription factors, and phytohormones, including auxin, GA, BR, JA, ethylene, ABA, and cytokinin, during fiber initiation and elongation. The review offers an in-depth analysis of their contributions to cotton fiber development, emphasizing their interplay and regulatory mechanisms. Understanding these factors holds immense potential for the advancement of our knowledge and the optimization of cotton cultivation for improved fiber quality and yield.

2. Fiberless Mutants Identification

Cotton fibers originate from single cells within embryo epidermal cells, with only 25~30% of them ultimately developing into fibers [5]. The initiation and final development of fibers play a crucial role in determining cotton yield. Stewart observed a fiber density of approximately 3300 fibers per mm^2, with the ratio of fiber initials to total epidermal cells being 1:3.7 at anthesis [27]. Two types of fibers exist: lint fibers and fuzz fibers, with lint fibers possessing higher economic value. Interestingly, there is no observable phenotype difference when both types of fibers initiate on the epidermal surface [28]. Lint fibers initiate on or a day before the day of anthesis (−1~0 DPA) and elongate to 2~3.5 cm, while fuzz fibers initiate at 3~5 DPA and only reach around 5~10 mm in length [27].

Several fiberless or fuzzless mutants, including four dominant (*Li1*, *Li2*, *N1*, and *Fbl*) and three recessive (*n2*, *sma-4* (*ha*), and *sma-4* (*fz*)) mutants, have been studied to identify genes and understand their interactions in the molecular mechanism of fiber initiation and elongation [29]. By crossing fuzzless and/or lintless mutants, the *N1*, *n2*, *Li3*, and *Li4* loci were identified to control the presence or absence of lint or fuzz. For instance, *N1N1* confers the presence of fuzz, *n2n2* inhibits fuzz initiation and development, and the duplicate gene pairs *Li3Li3* and *Li4Li4* determine the presence of lint. Homozygosity for *li3li3* and *li4li4* may also inhibit fuzz development [30]. Comparative scanning electron microscopy studies of fiber development in a normal TM-1 genotype and the near-isogenic *Li1* mutant at 1 DAP and 3 DAP revealed minimal differences during the early stages, suggesting that *Li1* gene expression occurs later, probably during the elongation phase [31]. Cross-pollination of *N1*, *n2*, and *n3* in upland cotton lines produced fiberless seeds, such as *MD17* and *SL1-7-1* [32]. The mutant exhibited lower short fiber content and better yarn quality than the wild-type cultivar [33]. The *Li2* short fiber mutation is located within a terminal deletion of chromosome 18 in cotton [34]. siRNA-induced silencing of a family of *RanBP1*s inhibits the elongation of cotton fiber cells in the *Li2* mutant [35].

XZ142FLM, a natural fiberless mutant with well-studied genes that are responsible for the fiberless phenotype, stands in contrast to *GhVIN1-RNAi* (*GhVIN1i*), one of the few fiberless cotton lines associated with sugar metabolism and signaling generated through reverse genetics. Comparative transcriptome analysis between the natural fiberless mutant *XZ142FLM* and the transgenic fiberless line *GhVIN1i*, obtained by RNAi silencing of *GhVIN1*, identified common differentially expressed genes (DEGs) in ovules during fiber initiation. The respective DEGs were enriched in several identical pathways related to fiber initiation, revealing shared molecular regulatory networks controlling fiber initiation [36]. Several genes related to fiber initiation or elongation, such as *GhMML3*, *GhVIN1*, *GhMYB25*,

GhHD-1, and *GhHOX3*, exhibited similar expression patterns in mutant *XZ142FLM* and the RNAi mutant *GhVIN1i* during fiber early development. This suggests the operation of similar mechanisms for fiber initiation in these two fiberless lines. The study sheds light on the regulatory networks mediated by *GhMML3* and *GhVIN1* in controlling fiber initiation in cotton [36].

In a study investigating natural antisense transcripts and siRNA control over fiber development, the researchers discovered that small RNA derived from the *GhMML3_A12* locus can induce self-cleavage of *GhMML3_A12* mRNA, leading to the production of naked seeds and the subsequent inhibition of lint fiber in *N1* plants [37]. Employing a map-based cloning strategy for the first time in tetraploid cotton, they successfully cloned the naked seed mutant gene (*N1*) encoding a *MYBMIXTA-like transcription factor 3* (*MML3*)/*GhMYB25-like* on chromosome A12, known as *GhMML3_A12*, associated with fuzz fiber development [37]. Phenotypic and genotypic analysis of MYB25-like alleles in cottons exhibiting various fiber phenotypes and their crossed progeny revealed that both *MYB25-like_At* and *MYB25-like_Dt* are linked to lint development. Fuzz development, on the other hand, is primarily determined by the expression level of *MYB25-like_Dt* at approximately 3 DPA, making *MYB25-like_Dt* a strong candidate for *N2*. Recently, cotton microtubule-associated protein *GhMAP20L5* was reported to mediate fiber elongation through the interaction with the tubulin *GhTUB13*. In the RNA-silencing plants, *GhMAP20L5* expression was repressed by at least 28.1% in comparison with its null plants at different development stages (0 DPA, 6 DPA, 12 DPA, and 18 DPA), which resulted in a reduction in the fiber elongation rate, fiber length, and lint percentage [37].

3. Transcriptional Regulation

3.1. MYB Transcription Factors

MYB family genes play pivotal roles across diverse biological processes, including cell cycle control, hormone signaling, secondary metabolism, meristem formation, cellular morphogenesis, and responses to abiotic stress, among others. In the context of cotton, MYB has been established as a key regulator of fiber development. A study by Suo et al. [38] identified 55 MYBs in developing cotton ovules both before and after fiber initiation (−3 DPA to 3 DPA). *GhMYB25*, identified through transcriptome comparisons between wild-type and fiberless cotton mutants, is implicated in this developmental pathway [28,39]. *GhMYB25* is expressed in fiber initials during the initiation and elongation stages. Both *GhMML3* (*GhMYB25-like*) and *GhMYB25* were downregulated in the outer integument of 0 DPA ovules in fuzzless or fiberless mutants, indicating their close relation to fiber initiation [40] (Figure 2). Mutants with RNAi-induced silencing of *GhMYB25* exhibited shifts in the timing of rapid fiber elongation, resulting in the formation of shorter fibers. Additionally, noticeable reductions in trichomes on various plant parts and decreased seed production were observed. *GhMYB25* was also identified in the diploid species *Gossypium arboreum* (A2 genome), *Gossypium raimondii* (D5 genome), and the allotetraploid *Gossypium hirsutum cv. Coker 315* (AD genome). Its suppression resulted in fewer fiber initials with delayed expansion, while overexpression led to more fiber initials without altering the final fiber length. In the regulatory cascade controlling fiber initiation, *GhMYB25-like* acts upstream of *GhMYB25* [41]. *GhMYB25-like* expression was reduced in *fl* mutant ovules, resulting in fiberless seeds, but overexpression did not increase fiber initials. RNAi of *GhMYB25-like* resulted in fiberless cotton seeds without affecting trichome development elsewhere [41].

The R2R3 MYB family transcription factor, *GhMYB109*, shares structural similarities with *Arabidopsis AtGL1*, a known regulator of leaf trichome development [42]. Further analysis of *GhMYB109* expression in cotton fiber initial cells and elongating fibers suggested its direct involvement in fiber initiation and elongation [38] (Figure 2). Transgenic reporter gene analysis confirmed the fiber-specific expression of a 2 kb *GhMYB109* promoter, while antisense-mediated suppression of *GhMYB109* led to a substantial reduction in fiber length [42]. *GhMYB109* operates downstream of initiation-related *GhMYB25-like* and

GhMYB25 [41]. Antisense suppression of *GhMYB109* leads to a reduction in fiber length. Furthermore, the diminished expression of *GhMYB109* is associated with the suppression of genes involved in fiber elongation, such as *GhACO1* and *GhACO2*, which contribute to ethylene (ET) biosynthesis, and *GhTUB1* and *GhACT1*, encoding cytoskeletal proteins. Consequently, it is likely that *GhMYB109* acts upstream of phytohormonal and cytoskeletal changes during fiber elongation [40]. An R3-MYB gene, *GhCPC*, was identified through cDNA microarray analysis. The overexpression of *GhCPC* not only delayed fiber initiation but also resulted in a significant reduction in fiber length. Experiments suggest that *GhCPC* may negatively regulate cotton fiber initiation and early elongation through a potential CPC-MYC1-TTG1/4 complex [43].

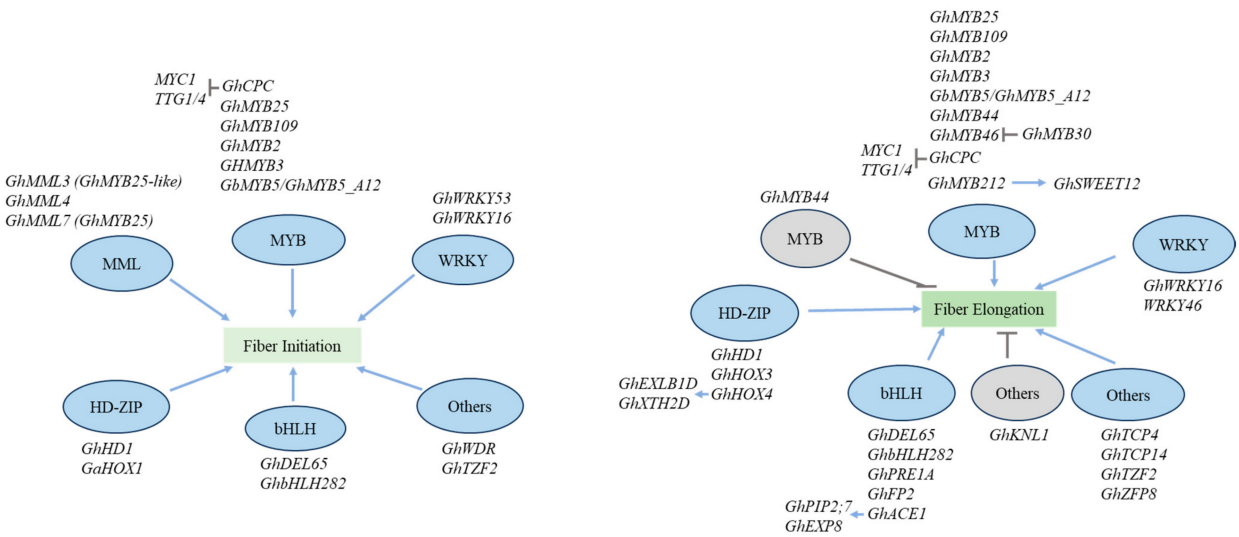

Figure 2. Key transcription factors that regulate fiber initiation and elongation. The light blue color and arrows indicate the positive regulation, and the light grey color and bars represent the negative regulation.

Guan et al. [44] proposed a specific pairing of a transcription factor and its downstream target based on the co-expression of *GhMYB2* and *GhRDL1* in *Arabidopsis*. This co-expression induced ectopic seed and silique trichomes. *GhMYB2* functioned similarly to *Arabidopsis GL1*, which targets the *GhRDL1* promoter and positively regulates fiber development (Figure 2). A variant within the *MYB5_A12* homolog has been classified within the *GL1* functional group. During the fiber initiation stage (−3 to 0 DPA) and the early elongation stage (5 to 10 DPA) in fibers, the transcript levels of *MYB5_A12* were higher in *G. barbadense* compared to *Gossypium hirsutum*. Both *GhMYB5_A12* and *GbMYB5_A12* were identified as contributors to the fiber development network, interacting with *EGL3* and *HOX3* [45]. *GhMYB30* has been established as a key regulator in fiber development through multiple pathways. It was identified as a crucial regulator of *GhMUR3*, responsible for encoding a xyloglucan galactosyltransferase essential for xyloglucan synthesis during fiber elongation; *GhMYB30* was also found to directly bind to the *GhMUR3* promoter, activating GhMUR3 expression (Figure 2). Additionally, *GhMYB46* was confirmed as a target gene of *GhMYB30* through EMSA, with *GhMYB46* exhibiting a significant increase in *GhMYB30*-silenced lines, indicating the inhibitory role of *GhMYB30* on *GhMYB46* expression during fiber development [46] (Figure 2).

Sucrose serves as a direct carbon source during fiber elongation. In a study investigating the role of *GhMYB212* in regulating sucrose transportation into expanding fibers, *GhMYB212* RNAi plants (*GhMYB212i*) exhibited reduced accumulation of sucrose and glucose in developing fibers, resulting in shorter fibers and a lower lint index. RNA-seq and protein–DNA binding assays indicated that *GhMYB212* was closely linked to sucrose and starch transportation and metabolism pathways, directly controlling the expression of

the sucrose transporter gene *GhSWEET12* [47]. Interestingly, exogenous sucrose supplementation in ovule cultures did not rescue the shorter-fiber phenotype of *GhMYB212i*. Current investigations support the notion that *GhMYB212* functions as the primary regulator of fiber elongation by controlling the expression of *GhSWEET12* [48] (Figure 2). m6A, the most abundant methylation modification in eukaryotic mRNA, showed elevated expression levels in the short fiber mutants *Li2*. The methylation of m6A affected the mRNA stability of numerous genes related to fiber elongation, including the transcription factor *GhMYB44*. Overexpression of *GhMYB44* led to reduced fiber elongation, while the silencing of *GhMYB44* resulted in the production of longer fibers [16] (Figure 2).

3.2. MYBMIXTA-like (MML) Transcription Factors

Transcription factors, particularly *MYBMIXTA-like* (*MML*), *GhMML3*, *GhMML4*, and *GhMML7*, play central roles in regulating fiber initiation [49] (Figure 2). Evidence suggests that MIXTA-like MYB transcription factors, such as *GhMML4* and *GhMML7*, act as master regulators of cotton fiber initiation.

GhMML3, identified as a crucial regulator of fiber initiation, was found to be downregulated in the fiberless mutant *XZ142FLM*, which specifically governs fiber initiation [41] (Figure 2). Positioned in tandem with *GhMML3* is *GhMML4_D12*, another MIXTA gene responsible for fuzz fiber development. These closely related MIXTA genes play a pivotal role in directing the production of fiber initiation in two distinct cell forms: lint and fuzz fibers. It is suggested that they might influence the same metabolic pathway in different cell types. Recent map-based cloning analyses have confirmed the significant role of *GhMML3* in fiber initiation in various fiber mutants, including *N1NSM*, *n2NSM* (fuzzless but linted), and fiberless *XZ142FLM*. Specifically, Wan et al. [37] localized the mutated dominant *N1* gene of the fuzzless mutant *N1NSM* to chromosome A12, the same locus as *GhMML3_At*. Virus-induced gene silencing of *GhMML3_At* resulted in little to no fuzz fibers in cotton, indicating that *GhMML3_At* primarily regulates fuzz fiber initiation [37].

Through map-based cloning, the fuzzless mutant *N1* was mapped to the locus linked to the *MYBMIXTA-like TF 3* (*MML3*)/*GhMYB25-like* gene (located on chromosome A12) with fuzz development [37]. Further studies revealed that this locus contains two MML genes, *MML3* and *MML4*, arranged tandemly to control fuzz initiation [37] (Figure 2). The lint fiber development gene (*Li3*) encodes *GhMML4_D12* on chromosome D12. Decreasing the expression of *GhMML4_D12* in *n2NSM* plants resulted in a significant reduction in epidermal cell prominence and lint fiber production [50]. Zhu et al. (2018), analyzing interspecific populations, suggested that *GhMML3_Dt* is the best candidate allele for the mutated recessive *n2* gene and mainly regulates fuzz fiber development, while both *GhMML3_At* and *GhMML3_Dt* are associated with lint fiber development [51].

3.3. WRKY Transcription Factors

As one of the most extensive transcription factor families in plants, WRKY transcription factors play essential roles in cotton fiber development by regulating genes associated with fiber growth and quality. Studies have underscored the significance of WRKY genes across the initiation, elongation, and maturation phases, showcasing their regulatory functions. In an extensive comparative study conducted by Ding et al. [52], WRKY transcription factor genes linked to fiber development were identified in the diploid cotton species *Gossypium raimondii* and *Gossypium arboreum*. A total of 112 WRKY genes were pinpointed in *Gossypium raimondii*, while 109 WRKY genes were identified in *Gossypium arboreum*. Transcriptome analysis revealed the involvement of many WRKY genes in specific fiber development processes and displayed distinct expression patterns between the two species [52]. One such gene, *GhWRKY53*, was identified as being fiber initiation-related (Figure 2). The heterologous expression of *GhWRKY53* in *Arabidopsis* significantly increased trichome density. A yeast two-hybrid experiment identified twelve proteins interacting with *GhWRKY53* from the cotton fiber cDNA library. These findings establish a foundation for further exploration of *GhWRKY53*'s role in cotton fiber development and offer new

targets for studying putative group III WRKY genes in *Gossypium hirsutum* [53]. In another study by Wang et al. [54], the investigation focused on *GhWRKY16*, a WRKY transcription factor in cotton. Experiments demonstrated that *GhWRKY16* positively regulated both fiber initiation and elongation (Figure 2). Silencing *GhWRKY16* in transgenic cotton lines resulted in a significant reduction in fiber protrusions on the ovule and shorter fibers compared to the wild type. During the early stages of fiber development, *GhWRKY16* was found to directly bind to the promoters of genes such as *GhHOX3*, *GhMYB109*, *GhCesA6D-D11*, and *GhMYB25*. The phosphorylation of *GhWRKY16* by *GhMPK3-1* was identified as essential for the transcriptional activation of downstream genes during the cotton fiber development process [54].

3.4. HD-ZIP Transcription Factors

HD-Zip proteins exhibit diverse and sometimes overlapping functions, spanning stress responses to morphogenesis and development. A decline in *GhHD1* transcripts was associated with delayed fiber initiation and impaired trichome formation, while its overexpression led to enhanced fiber initiation with no discernible effect on leaf trichomes [41]. Through a genome-wide analysis, 13 HD-ZIP IV genes were identified in *Gossypium arboreum* and 26 in *Gossypium hirsutum* [55]. Among these, three genes encoding transcription factors, *GhHOX1*, *GhHOX2*, and *GhHOX3*, were isolated from both cotton species [56]. *GaHOX1* serves as a homolog of *Arabidopsis GLABRA2* (*GL2*), a gene essential for cell expansion, branching, and cell wall maturation in leaf trichomes [54]. As a member of the class IV homeodomain-leucine zipper (HD-ZIP) family of transcription factors, *GaHOX1* exhibits robust expression in various *Gossypium arboreum* tissues, with peak expression in 0 DPA ovule epidermal cells and 1 DPA fibers (Figure 2). In *Gossypium hirsutum*, its highest expression occurs during early fiber elongation. *GaHOX1* demonstrated a potential role in fiber initiation by complementing the trichome-less phenotype of the *Arabidopsis gl2-2* mutant [56].

Experimental findings from transgenic cotton plants revealed that *GhHOX3* may serve as a central regulator of fiber elongation. Silencing this gene resulted in a reduction in fiber length of up to 80%, yielding fuzz-like short fibers on the seeds. Conversely, overexpression of *GhHOX3* led to an extension of fiber length [57]. *GhHOX3* controls cotton fiber elongation in a hormone GA-dependent manner (Figure 2). In the presence of GA, the HOX3 protein interacts with the HD-ZIP protein (GhHD1) and the GA repressor DELLA protein (GhSLR1). The GhHOX3-GhHD1 complex exhibits higher transcriptional activity, while *GhSLR1* competitively binds to *GhHOX3*, impeding the transcriptional activation necessary for transmitting the GA signal for fiber cell elongation [55,58] (Figure 2).

Additionally, *GhHOX4* has been identified as playing a crucial role in fiber elongation. Overexpressing *GhHOX4* in cotton results in longer fibers, whereas transgenic cotton with silenced *GhHOX4* displays a "shorter fiber" phenotype compared to the wild type. *GhHOX4* directly activates two target genes, *GhEXLB1D* and *GhXTH2D*, promoting fiber elongation. Conversely, phosphatidic acid (PA), associated with cell signaling and metabolism, interacts with *GhHOX4*, hindering fiber elongation (Figure 2). These findings suggest that *GhHOX4* positively regulates fiber elongation, while PA may play a role in the transition from fiber elongation to secondary cell wall formation by negatively modulating *GhHOX4* in cotton [59].

3.5. bHLH Transcription Factors

Basic helix–loop–helix (bHLH) transcription factors play pivotal roles in regulating plant cell cycle and elongation processes. GhDEL65, a bHLH protein derived from *Gossypium hirsutum*, is a functional homolog of *Arabidopsis GLABRA3* (*GL3*) and an enhancer of *GLABRA3* (*EGL3*), contributing to the regulation of fiber development. *GhDEL65* transcripts were detected in 0~1 DPA ovules, with notable abundance in 3 DPA fibers, indicating a potential role in early fiber development [60] (Figure 2). Ectopic expression of *GhDEL65* in the *Arabidopsis gl3egl3* double mutant partially rescued trichome development, confirming

GhDEL65's involvement in fiber development. Interaction studies revealed *GhDEL65*'s association with cotton R2R3 MYB transcription factors *GhMYB2* and *GhMYB3*, as well as the WD40 protein *GhTTG3*, suggesting the existence of a MYB-bHLH-WD40 protein complex in cotton fiber cells [60] (Figure 2). Both allotetraploid cottons, *Gossypium hirsutum* and *Gossypium barbadense*, contain two subgenomes, At and Dt. In tetraploid cotton, the bHLH transcription factor *GhPRE1A*, expressed specifically in fiber cells from its At subgenome, is presumed to play a role in regulating fiber elongation. Simultaneously, its Dt subgenome homolog remains inactive due to a TATA-box fragment deletion in its promoter region [61]. A recently identified gene, *GhbHLH282*, not only plays a role in regulating fiber development but is also involved in brassinosteroid signaling [62].

Two additional bHLH transcription factors, *fiber-related protein 2* (*GhFP2*) and *ACTIVATOR FOR CELL ELONGATION 1* (*GhACE1*), have been reported to interact with each other, positively influencing fiber elongation [63] (Figure 2). The overexpression of *GhFP2* in cotton hindered fiber elongation, resulting in shortened fiber length. Conversely, the suppression of *GhFP2* expression in cotton promoted fiber development, leading to longer fibers compared to the wild type. *GhACE1* promotes fiber elongation by activating the expression of *GhPIP2;7* and *GhEXP8*, but its transcriptional activation on downstream genes may be impeded by *GhFP2* [63].

3.6. Other Transcription Factors

Additionally, several other transcription factor genes, such as *TCP*, *WD40*, *KNOX*, *CCCH Zinc Finger*, and C_2H_2 genes, play crucial roles in controlling the fiber development in cotton. In a recent discovery, miR319-regulated CIN-type TCP genes, such as *GhTCP4*, were identified for their role in modulating the elongation of cotton fiber cells [64] (Figure 2). *GhTCP4* interacts antagonistically with *GhHOX3*, influencing the growth of cotton fiber elongation (Figure 2). During the initial stages of cotton fiber development, miR319 is abundant, maintaining its target TCPs at low levels, while *GhHOX3* actively promotes the elongation of fiber cells. The overexpression of a miR319-resistant form results in reduced fiber cell elongation and shorter fibers [64]. A MYB–basic helix–loop–helix (bHLH)–WD40 gene, *GhWDR*, can rescue *Arabidopsis* trichome mutant *ttg1* and interact with *GhMML4* (*GhMML4_D12*) to regulate spinnable lint production [65]. The cotton class II KNOX, *GhKNL1*, acts as a transcriptional repressor in fiber development. Silencing *GhKNL1* in transgenic cotton led to longer fibers with thicker secondary cell walls, whereas dominant repression transgenic lines of *GhKNL1* exhibited the opposite fiber phenotype compared to controls (Figure 2). Moreover, *GhKNL1* was found to suppress the expression of *GhEXPA2D/4A-1/4D-1/13A* by binding to their promoters, thereby regulating cotton fiber elongation [66] (Figure 2). In another notable finding, a novel tandem CCCH Zinc Finger (TZF), *GhTZF2*, was discovered to regulate cotton fiber development. *GhTZF2* exhibited high expression in ovule cells during the very early stages of fiber development, and knockout lines of *GhTZF2* produced significantly shorter fibers with thinner cell walls, underscoring its crucial role in the modulation of cotton fiber development [36]. Recently, Liu et al. [67] found that Zinc Finger Protein8 (GhZFP8) encoding a C2H2 transcription factor were abundant at 3, 6, and 30 DAP during the fiber elongation. The interference of GhZFP8 inhibited the boll expansion and fibers elongation [67] (Figure 2).

3.7. Omic Tools for Studying the Fiber Initiation and Elongation

Through two-dimensional gel electrophoresis, a total of 235 proteins displayed varying abundance between 5 and 25 DPA, forming 4 distinct abundance patterns throughout fiber development [68]. Contributing to the regulation of the dynamic fiber proteome, particularly in protein degradation, a cotton RING-type ubiquitin ligase (E3), GhRING1, was identified. The transcriptional activity of GhRING1 increased from 5 to 15 DPA, followed by a decrease from 15 to 23 DPA. Additionally, recombinant GhRING1 exhibited ubiquitin ligase activity in vitro [5,6,69]. To investigate the molecular mechanisms underlying cotton fiber elongation, a combination of microarray technology and quantitative real-time PCR

(qRT-PCR) was employed to analyze the DEGs in the *Li1* mutant compared to the wild type. A total of 1915 DEGs were identified, with 984 upregulated genes and 931 downregulated genes, reaching their peak expression at 5 DPA. Among these, numerous transcription factors, including zinc finger, MYB, and basic-leucine zipper, as well as hormones (ACO and ABP) and other proteins, were identified. This comprehensive analysis contributes to a better understanding of the molecular basis of early fiber elongation [70].

By a genome-wide comparative transcriptome analysis using the Affymetrix cotton GeneChip in isogenic *fuzzy-lintless* (*Fl*) and normal *fuzzy linted* (*FL*) lines of *Gossypium arboreum* at 0 and 10 DPA, a multitude of transcription factors involved in fiber initiation and elongation were identified. Specifically, at 0 dpa, transcription factors such as *AP2-EREBP*, *C2H2*, *C3H*, *HB*, and *WRKY* were found to be upregulated. Conversely, at 10 dpa in the mutant *Fl* line, the transcription factors, including *AP2-EREBP*, *AUX/IAA*, *bHLH*, *C2H2*, *C3H*, *HB*, *MYB*, *NAC*, *Orphans*, *PLATZ*, and *WRKY*, were downregulated [71]. Through the re-mapping of over 380 cotton RNAseq datasets using consistent mapping strategies that encompass approximately 400-fold coverage of the genome, a total of 47 transcription factor binding sites were identified, with Dof, GATA, and ZF-HD exhibiting prominent binding frequencies across all developmental stages. Notably, the binding frequency of Homeodomain, MYB/Myb related, NAC, and WRKY TFs was relatively higher during the initiation and development phases of fiber growth [72].

Employing single-cell RNA sequencing (scRNA-seq), a single-cell assay for transposase-accessible chromatin with high-throughput sequencing (scATAC-seq), and laser capture microdissection (LCM) coupled with RNA-seq (LCM-seq), Wang et al. [73] effectively characterized the transcriptome of fiber cells and established the developmental trajectory of the fiber cell lineage in cotton ovules during the primary developmental stage. Notably, the findings indicate that the small peptide GhRALF1 and the transcription factor *GhTCP14* rhythmically control fiber cell growth, potentially through the modulation of hormone signaling, extracellular pH, and the metabolism of mitochondria and protein translation [73].

By utilizing DNase I hypersensitive sites sequencing (DNase-Seq) and RNA-seq data, a high-confidence regulatory network was constructed for cotton ovules at 0 and 3 DPA, as well as fibers at 8, 12, 15, and 18 DPA. Distinct chromatin accessibilities were observed in the ovules (0 and 3 DPA) compared to the fiber elongation stages (8, 12, 15, and 18 DPA). The researchers constructed a robust regulatory network of transcription factors associated with ovule and fiber development, leveraging chromatin accessibility and gene co-expression networks. Within this network, the identification of a novel transcription factor, *WRKY46*, emerged, suggesting its potential role in shaping fiber development by regulating lignin content [74] (Figure 2). The analysis of cis-regulatory modules also revealed the impact of hormones on fiber development, emphasizing the regulatory divergence of transcription factor motifs. This study unveiled dynamic chromatin accessibility during ovule and fiber developmental stages, highlighting the involvement of numerous transcription factors in fiber development [74].

4. Phytohormonal Regulation

Phytohormones play pivotal roles as small endogenous signaling molecules in plants, and their influence on cotton fiber development has been a subject of considerable research. Among these hormones, auxin, gibberellin (GA), jasmonic acid (JA), ethylene, cytokinin, abscisic acid (ABA), and brassinosteroids (BRs) have been identified as key regulators in various stages of fiber development. Auxin, ethylene, GA, JA, and BR have been identified as positive regulators, playing crucial roles in fiber initiation and elongation processes. Conversely, CK and ABA exert a negative influence on fiber growth. In in vitro culturing of cotton ovules, the addition of exogenous auxin and GA induces fiber initiation, emphasizing its positive regulatory role. Conversely, abscisic acid inhibits fiber growth in this system. Notably, both auxin and GA have the capacity to overcome the inhibitory effects on total fiber production induced by ABA [75]. This intricate interplay between

various phytohormones highlights the complexity of the regulatory networks governing cotton fiber development. Understanding these hormone-mediated pathways is crucial for devising strategies to optimize fiber yield and quality in cotton cultivation.

4.1. Auxin

During the development of cotton ovules, the concentration of indole-3-acetic acid (IAA) exhibits distinct temporal dynamics. Prior to anthesis, there is a notable surge in IAA levels, peaking at 2 DPA, followed by a gradual decrease to approximately one-fifth of the initial concentration. Subsequently, a rapid increase is observed between 7~9 DPA [76]. Maintaining a consistent supply of IAA within this critical time frame has been shown to yield significant effects on fiber development. Chen and Guan [77] reported that such supplementation resulted in an increase in the number of fiber cell initials by 14–19% while concurrently reducing the number of fuzz fibers. To investigate the potential causal link between auxin accumulation around anthesis and lint fiber development, Zhang et al. [78] employed the FBP7 promoter, an epidermal-specific promoter active in the cotton ovule epidermis from −2 to 10 DPA. The transgenic plants carrying *FBP7::iaaL* demonstrated a significant reduction in the number of fiber initials in 0 DPA ovules, providing experimental evidence that verifies the crucial role of IAA in initiating fiber cells. The utilization of the synthetic response element DR5, consisting of a tandem direct repeat of 11 bp containing the auxin-responsive element TGTCTC, has provided insights into the dynamics of IAA accumulation in cotton ovules during fiber cell differentiation and initiation (from −2 to 2 DPA). The expression of DR5::GUS indicates that IAA accumulation in fiber initials commences before flower opening [79]. These findings underscore the intricate regulatory network involving the Aux/IAA gene family, auxin response factors (ARFs), and IAA accumulation in cotton fiber development. In a study by Gokani and Thaker [77], an analysis of fibers was conducted on three cotton cultivars: *Gossypium hirsutum* hybrid-4 (H-4), hybrid-8 (H-8), and *Gossypium arboreum* G.Cot-15. This analysis revealed the involvement of auxin in fiber elongation, both in vitro and in vivo [80].

ARFs represent essential components in the auxin signaling pathway, orchestrating the expression of early auxin-responsive genes through binding to auxin response elements (AuxRE). In *Gossypium hirsutum*, the expression pattern of ARFs showed significant upregulation in the *GhARF2* and *GhARF18* subfamilies during the fiber initiation stage (Figure 3). Subsequent yeast one-hybrid studies confirmed the involvement of six downstream transcription factors in this regulatory network [81]. *GhARF2b* exhibits specific expression in developing ovules and fibers. When *GhARF2b* is overexpressed using a fiber-specific promoter, it hinders fiber cell elongation while simultaneously promoting initiation. Conversely, the downregulation of *GhARF2b* through RNAi leads to a reduction in the number of fibers but an increase in their length [82].

The Aux/IAA gene family plays a crucial role in interacting with auxin response factors to coordinate the regulation of auxin response genes throughout plant development. Han et al. [83] conducted a study identifying nine *GhAux* genes, ranging from *GhAux1* to *GhAux9*. Notably, *GhAux4*, *GhAux5*, *GhAux6*, and *GhAux7* exhibit preferential expression in ovules on the day of anthesis, while *GhAux1*, *GhAux2*, and *GhAux3* are abundantly expressed in vegetative organs. Additionally, *GhAux8* and *GhIAA16* display preferential expression during the fiber developmental stages, with *GhAux8* prominent in the early fiber elongation stages and *GhIAA16* during the fiber initiation and secondary cell wall thickening stages [83] (Figure 3). Expression profiling further strengthens the pivotal role of auxin in the elongation of fiber cells. Specifically, the expression of the auxin binding protein *GhABP* significantly increases by approximately 59-fold from 0 to 10 DPA. Additional analyses reveal that *GhABP* expression is exclusive to elongated fibroblasts, with no presence in undifferentiated epidermal cells [84] (Figure 3).

Auxin is known to activate Rac-like GTPases and TCP transcription factors, which, in turn, trigger the expression of auxin-responsive genes in plants [85,86]. Specifically, in the context of cotton fiber development, the Rac genes *GhRacA* and *GhRacB* exhibit elevated

accumulation during the initiation and elongation stages of seed fibers (Figures 2 and 3). This observation suggests a significant involvement of *GhRacA* and *GhRacB* in the early phases of fiber development, potentially acting as key mediators in the auxin-stimulated processes [85,87]. The introduction of exogenous indole-3-acetic acid (IAA) specifically triggers increased expression of *GhTCP14*, which is prominently present during the fiber cell initiation and elongation stages [86] (Figures 2 and 3). *GhTCP14*, in turn, directly binds to the promoters of genes encoding crucial components, such as the auxin uptake carrier (AUX1), the auxin response protein (IAA3), and the auxin efflux carrier (PIN2). This direct interaction suggests that *GhTCP14* likely plays a role in the IAA-mediated differentiation and elongation of cotton fiber cells, providing a key connection between TCP transcription factors and the intricate processes involved in cotton fiber development [86].

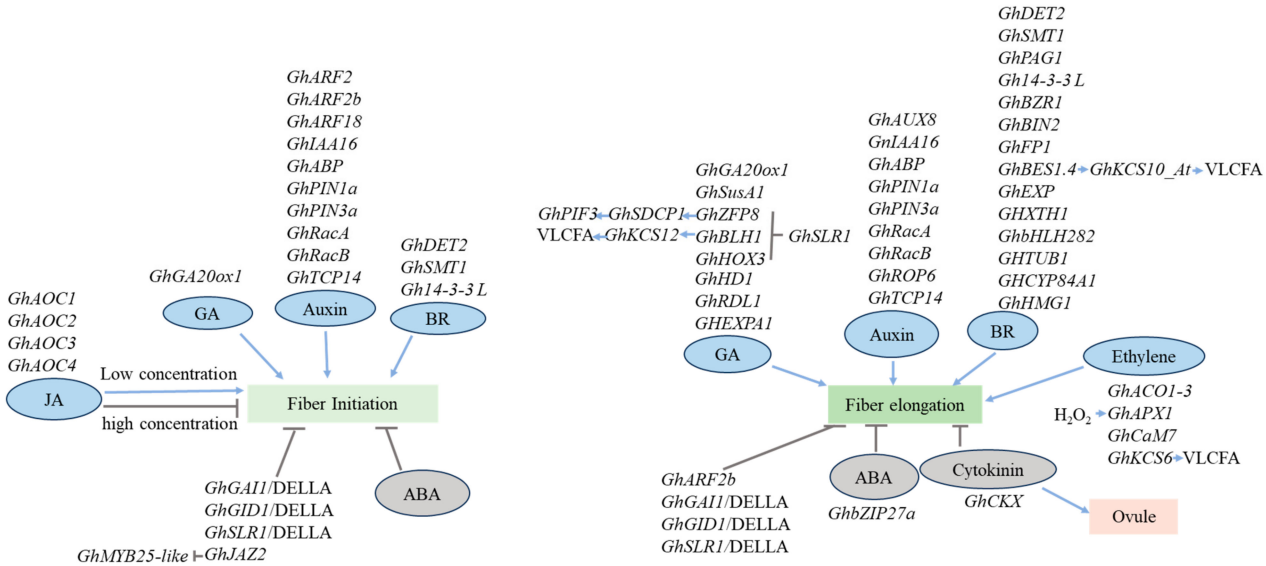

Figure 3. Phytohormone regulation of fiber initiation and elongation. The light blue color and arrows indicate the positive regulation, and the light grey color and bars represent the negative regulation. GA, gibberellic acid; BRs, brassinosteroids, JA, jasmonic acid; ABA, abscisic acid. ABA and cytokinin both have negative regulation effect on fiber initiation and elongation. Cytokinin promotes the growth of ovule.

Polar auxin transport, primarily facilitated by PIN-FORMED (PIN) proteins, plays a crucial role in auxin-triggered organogenesis across various plant species. In cotton, the mRNA of *GhPIN3a* was identified in the outer integument of ovules, encompassing fiber cells. Suppression of *GhPIN1a* was found to hinder both fiber initiation and elongation, emphasizing the significance of GhPIN-mediated auxin transport in the accumulation of fiber-specific auxin in cotton [88] (Figure 3). Recent investigations have highlighted the cell-specific degradation of *GhPIN3a*, a process guiding the establishment of the auxin gradient in cotton ovule epidermal cells. This degradation is linked to the preferential expression of *GhROP6* GTPase in fiber cells. *GhROP6*, in turn, reduces the abundance of *GhPIN3a* at the plasma membrane and facilitates intracellular proteolysis of *GhPIN3a*. Overexpression and activation of *GhROP6* lead to enhanced cell elongation, resulting in a significant improvement in cotton fiber length [89] (Figure 3).

4.2. Gibberellic Acid (GA)

GA serves as a pivotal plant hormone regulating diverse developmental processes, including root and stem elongation, seed germination, trichome development, flowering, and fruit ripening. Like auxin, GA exhibits a positive influence on cotton fiber initiation and elongation. The application of exogenous GAs has been shown to promote both fiber initiation and elongation in cultured ovules. In contrast, the introduction of the GA

biosynthesis inhibitor paclobutrazol leads to a reduction in the number and length of fibers compared to controls without hormone treatment [90–93]. The endogenous level of GA3, a bioactive gibberellic acid form, is notably higher in long-staple cotton varieties compared to medium- and short-fiber varieties [94].

Transcriptomic analyses have unveiled the upregulation of genes involved in GA metabolism and signaling, such as those encoding *GA 20-oxidase* (*GA20ox*) during fiber initiation and elongation (Figure 3). The overexpression of *GhGA20ox1* in cotton significantly increases the endogenous GA content in cotton fiber at 0 DPA and 10 DPA. This enhancement correlates with the increasing of fiber numbers during the initiation and early elongation between 0 DPA and 3 DPA [95]. The sucrose synthase gene *GhSusA1* exhibits significantly higher transcript levels and sucrose synthase activity when *GA 20-oxidase* is overexpressed in cotton fibers, along with the increased expression of *GhSusA1* in sucrose synthase genes, thereby promoting cotton fiber cell development [95].

DELLA proteins interact with key regulatory proteins and transcription factors, functioning as negative regulators in the GA signaling pathway. These interactions serve to dampen their transcriptional activation or prevent them from binding the target genes [96]. In cotton, the DELLA protein GhGAI1 is transcribed at higher levels in the fuzzless–lintless mutant *XZ142FLM* ovules between −1 DPA and 3 DPA than in its wild-type siblings, indicating a negative regulatory role during the early stages of cotton fiber development [97]. DELLA proteins degrade upon binding to *GID1* in the presence of GA, releasing essential transcription factors and promoting the expression of GA-responsive genes [96,98]. GhSLR1 and GhGID1, two DELLA proteins, show specific expressions in cotton fibers, and their interaction is activated in response to GA. Ectopic expression of *GhSLR1* in *Arabidopsis* enhances the expression of GA-responsive genes and results in a dwarf phenotype [94,99]. In conditions of low GA levels, *GhSLR1* interacts specifically with the transcription factor *GhHOX3*, a pivotal player in the GA signaling pathway, suppressing the regulation of target genes [57]. Conversely, during high GA levels, the degradation of *GhSLR1* protein liberates *GhHOX3*, facilitating its interaction with *GhHD1*. This interaction triggers the expression of *GhRDL1* and *GhEXPA1*, thereby contributing to cotton fiber elongation [57]. Recently, it was discovered that the degradation of *GhSLR1* leads to the activation of two transcription factors, *GhBLH1* and *GhZFP8*, which then promote fiber elongation [100]. *GhBLH1* binds to *GhKCS12* promoter and activates its expression to enhance VLCFA biosynthesis [100]. *GhZFP8* activates the *GhSDCP1*, which will upregulate the expression of the *GhPIF3* gene associated with fiber elongation [100].

4.3. Brassinosteroids (BRs)

BRs, categorized as steroid hormones in plants, assume crucial roles in cotton fiber development. The application of a low concentration of brassinolide, a bioactive BR derived from plants, notably enhances fiber cell elongation in cotton. Conversely, the use of brassinazole (BRZ), a BR biosynthesis inhibitor, significantly impedes fiber cell development in vitro. In this context, brassinolide (BL) has been observed to stimulate the expression of genes associated with elongation, such as *EXP*, *XTH*, *AGP*, and *GhTUB1*, while brassinazole (BRZ) has the opposite effect, inhibiting their expression [101] (Figure 3). Furthermore, the application of finasteride, a steroid 5α-reductase inhibitor, significantly hinders fiber elongation. Notably, this inhibitory effect can be reversed by the application of BRs, highlighting the regulatory role of BRs in cotton fiber development [102]. Applying brassinazole externally to cotton floral buds results in significant abnormalities in fiber cell differentiation. Steroid reduction, a crucial step in brassinosteroid (BR) biosynthesis catalyzed by steroid 5α-reductase (DET), is recognized as a major rate-limiting process. The expression levels of *GhSMT1* and *GhDET2* mRNA in cotton ovules increase from 0 DPA to 10 DPA and subsequently decrease at 20 DPA ovules of the fiberless mutant compared to the wild type [103]. *GhDET2*, a cotton steroid 5α-reductase, exhibits heightened expression levels during both fiber cell initiation and elongation phases (Figure 3). The silencing of cotton *GhDET2* inhibits fiber initiation, while the upregulation of *GhDET2* expression

driven by the seed coat-specific promoter pFBP7 enhances fiber numbers [102]. Additional genes related to BR biosynthesis, such as GhPAG1, play a role in cotton fiber development by modulating the concentration of endogenous BRs [104]. The *pag1* mutant, characterized by dwarfism and diminished fiber length, attributed to a pronounced inhibition of cell elongation and expansion. The application of BL effectively restored its growth and promoted fiber elongation [104].

The overexpression of *Gh14-3-3 L* in cotton promotes fiber elongation, resulting in enhanced mature fiber length, while silencing *Gh14-3-3 L* significantly hampers the initiation and elongation of fiber cells [105] (Figure 3). This reduction in fiber length can be partially restored by the exogenous application of brassinosteroids (BRs). Subsequent investigations revealed that external BR application induces the expression of *brassinazole-resistant 1* (GhBZR1), and the phosphorylation of BZR1 by GhBIN2 kinase facilitates its binding to an acidic regulatory protein Gh14-3-3. Moreover, *Gh14-3-3 L* was found to interact with *GhBZR1*, and the *14-3-3*-regulated *GhBZR1* directly binds to the promoters of *GhXTH1* and *GhEXP*, thereby regulating gene expression during the fiber cell elongation stage [105] (Figure 3).

Brassinosteroids (BRs) also play a role in regulating fiber growth through the involvement of the transcription factors. The cotton bHLH protein *GhFP1* has been identified as a positive regulator of fiber elongation (Figure 3). Transgenic cotton overexpressing *GhFP1* displayed significantly longer fiber length compared to the wild type, while the suppression of *GhFP1* expression hindered fiber elongation. Notably, the expression of brassinosteroid (BR)-related genes was markedly upregulated in fibers of GhFP1-overexpressing cotton but downregulated in *GhFP1*-silenced fibers. The BR content in the transgenic fibers exhibited significant alterations compared to that in the wild type [106]. Brassinosteroids (BRs) play a crucial role in plant growth and development through the action of *BRI1-EMS-SUPPRESSOR1* (*BES1*)/*BRASSINAZOLE-RESISTANT1* (*BZR1*) transcription factors (Figure 3). In-depth insights into the regulation of cotton fiber development by *GhBES1.4* were obtained through combined analysis of DAP-seq and RNA-seq data from *GhBES1.4* overexpression and RNAi lines. *GhBES1.4* overexpression positively influenced fiber elongation, while *GhBES1.4* silencing resulted in reduced fiber length. The integrated approach of GWAS, RNA-seq, and DAP-seq identified seven genes directly regulated by *GhBES1.4*, including *Cytochrome P450 84A1* (*GhCYP84A1*) and *3-hydroxy-3-methylglutaryl-coenzyme A reductase 1* (*GhHMG1*), both promoting cotton fiber elongation [107]. Furthermore, BR controls cotton fiber elongation by modulating very-long-chain fatty acid (VLCFA) biosynthesis. *GhBES1.4* regulates endogenous VLCFA contents and directly binds to *BR RESPONSE ELEMENTS* (*BRREs*) in the *GhKCS10_At* promoter region (Figure 3). This interaction regulates *GhKCS10_At* expression, leading to increased endogenous VLCFA contents. GhKCS10_At overexpression promotes cotton fiber elongation, while GhKCS10_At silencing inhibits fiber growth, emphasizing GhKCS10_At's positive regulatory role in fiber elongation [108].

4.4. Jasmonic Acid (JA)

The influence of JA on fiber initiation follows a dose-dependent pattern. Cotton ovules cultivated in a medium containing 0.001 μM JA exhibited increased fiber cell initiation, while a higher concentration of 2.5 μM inhibited fiber initiation. This suggests that maintaining an optimal concentration of JA is crucial for effective fiber initiation [109] (Figure 3).

In exploring the molecular aspects, four members of the allene-oxide cyclase (AOC) family, integral to JA biosynthesis, were concurrently upregulated during fiber initiation, particularly at −1 DAP in linted–fuzzed TM-1 compared to other tissues and organs (Figure 3). Real-time quantitative PCR analysis of different fiber mutants indicated that the expression levels of four JA biosynthesis enzymes, the AOC genes (*GhAOC1~GhAOC4*), were higher in −1 DPA ovules of fiberless mutants compared to linted–fuzzless and linted–fuzzed lines. These genes exhibited increased expression under JA treatment, with predominant expression in −3~1 DPA ovules, particularly at −1 DPA, signifying their

crucial regulatory role during fiber initiation [110]. This aligns with the outcomes observed in in vitro ovule cultures with different concentrations of JA.

The JASMONATE ZIM-DOMAIN (JAZ) protein stands out as a pivotal inhibitory factor in the jasmonic acid (JA) signaling pathway. Specifically, *GhJAZ2* exhibits heightened expression during the fiber initiation stage, and the upregulation of GhJAZ2 expression in cotton has been found to impede fiber initials [111] (Figure 3). This suppression is achieved through the interaction of *GhJAZ2* with *GhMYB25-like*, leading to the inhibition of *GhMYB25-like* activity. Furthermore, GhJAZ2 interacts with various proteins, including *GhGL1*, *GhMYC2*, and *GhWD40* (Figure 3), which constitute the core components of the WD-repeat/bHLH/MYB transcriptional complex, known to be integral to fiber development. This interaction adds a layer of complexity to the understanding of the regulatory mechanisms underlying cotton fiber development [111].

4.5. Ethylene

Ethylene biosynthesis emerges as a pivotal pathway upregulated during cotton fiber cell elongation, as revealed by physiological and gene expression analyses [103]. Gene expression profiling experiments highlighted the heightened expression of *1-Aminocyclopropane*-1-Carboxylic Acid Oxidase (*ACO1-ACO3*) genes, which are crucial for ethylene production, particularly during the 10–15 DPA phase of fiber elongation. Experiments involving the exogenous application of ethylene reported increased fiber cell expansion, while the ethylene inhibitor 2-aminoethoxyvinyl glycine (AVG) hindered fiber growth [103] (Figure 3). These findings underscore the significant role of ethylene in supporting cotton fiber growth and elongation. Moreover, ethylene is proposed to enhance cell elongation by upregulating the expression of tubulin, sucrose synthase, and expansin genes [103].

Ethylene appears to play a dual role in fostering fiber elongation by facilitating the generation of hydrogen peroxide (H_2O_2), identified as a reactive oxygen species (ROS) with a substantial impact on in vitro fiber cell elongation [112] (Figure 3). Ascorbate peroxidase (APX), an enzyme responsible for scavenging ROS, participates in regulating intracellular ROS levels [113]. In the context of wild-type cotton, *GhAPX1* exhibits heightened expression in 5 DPA fiber cells compared to ovules in the *fl* mutant [114]. The application of exogenous H_2O_2 significantly triggers the transcription of GhAPX1 and augments APX activity [112]. Furthermore, ethylene, when externally applied, stimulates the production of H_2O_2 during fiber elongation, suggesting a downstream connection between H_2O_2-induced cotton fiber elongation and the ethylene signaling pathway. It becomes apparent that ROS regulate the accumulation of Ca^{2+}, thereby fostering fiber elongation, primarily by promoting ethylene production [115]. The overexpression of calmodulin *GhCaM7* has been identified as a promoter of fiber elongation, whereas GhCaM7 RNAi plants exhibit delayed fiber initiation and inhibited fiber elongation [116] (Figure 3). These findings collectively underscore the intricate interplay of ethylene, ROS, and calmodulin in regulating essential processes during cotton fiber development.

Saturated very-long-chain fatty acids (VLCFAs) play an important role in orchestrating the regulation of cotton fiber growth by promoting ethylene production [117]. Ethylene can counteract the inhibition of fiber cell elongation induced by 2-chloro-N-[ethoxymethyl]-N-[2-ethyl-6-methyl-phenyl]-acetamide (ACE), an inhibitor of VLCFA biosynthesis. Remarkably, VLCFAs are unable to overcome the inhibition caused by AVG. In vitro application of C24:0 fatty acids has been shown to induce a substantial increase in ACO transcripts, resulting in a significant surge in ethylene production. The overexpression of *KCS6*, a pivotal gene in VLCFA biosynthesis within Upland cotton, yields a remarkable increase in the final length of the fiber (approximately 6.0–12%), suggesting that VLCFAs may operate upstream in the ethylene pathway [115]. These findings highlight the intricate interconnection between VLCFAs, ethylene production, and the regulation of the essential processes governing cotton fiber development.

4.6. Abscisic Acid (ABA)

Past investigations have demonstrated that the application of ABA in vitro not only hinders the initiation of cotton fiber cells but also impedes the elongation of cotton fibers [118] (Figure 3). The application of exogenous abscisic acid (ABA) in vitro was found to inhibit cotton fiber development, while treatment with an ABA inhibitor (ABAI) promoted fiber development [119]. In a recent investigation, the physiological changes and proteomic profiles of *Gossypium hirsutum* ovules were examined after 20 days of ABA or ABAI treatment. The results revealed significant alterations compared to the control, with the fiber length notably reduced under ABA treatment and increased under ABAI treatment [119]. This inhibitory effect is linked to an elevation in ABA levels. Analyzing the endogenous ABA content in different fiber cells revealed a gradual increase during the initiation and elongation stages of fiber cells (0–10 DPA), followed by a decline in the period of rapid elongation (10–20 DPA). Subsequently, ABA levels returned to their original low state during the maturation stage (30–50 DPA) [120]. Research findings indicate an elevated level of ABA in mature cotton fruits compared to their younger, healthy counterparts [121]. In line with these discoveries, the concentration of ABA in fruits exhibits a low point at the time of anthesis, decreasing over the subsequent two days. It then undergoes a substantial increase, reaching up to 15-fold between 2 DPA and 10 DPA, before decreasing again and becoming undetectable up to 30 DPA. From 30 to 50 DPA, there is a renewed surge in ABA levels [5]. These findings collectively underscore the intricate regulatory role of ABA at various stages of cotton fiber development. Throughout development, the ABA content in short-staple fibers consistently surpasses that in long-staple fibers [122]. Furthermore, the levels of endogenous ABA in cotton ovules exhibit a positive correlation with short fiber production [123]. The short fiber cotton mutant *Li1* exhibits a significantly higher accumulation of ABA in 0 DPA ovules compared to the wild type [124]. At the early fiber initiation stage in the *Xu142 fl* mutant, a notable increase in ABA levels was observed [125]. This aligns with the previously mentioned correlation between endogenous ABA levels in cotton ovules and short fiber production. Additionally, the short fiber mutant *Li2* exhibited a significantly higher deposition of ABA in 0 DPA ovules compared to the wild type [124]. These findings collectively lead to the conclusion that there exists an inverse relationship between ABA content and fiber length. Dasani and Thaker conducted a comprehensive analysis of fibers from three cotton cultivars, *Gossypium hirsutum hybrid-4* (*H-4*), *hybrid-8* (*H8*), and *Gossypium arboreum G.Cot-15*, to elucidate the role of ABA in fiber elongation under both in vitro and in vivo conditions [126].

Recently, within transgenic cotton featuring the fiber-specific promoter proSCFP driving the expression of *GhOR1Del*, a positive regulator of carotenoid accumulation, notable alterations in hormonal content were observed during fiber elongation. Specifically, there was an increase in both abscisic acid (ABA) and ethylene levels in the elongating fibers. The downstream regulator of ABA, *GhbZIP27a*, demonstrated a capacity to stimulate the expression of the ethylene synthase gene *GhACO3* by binding to its promoter. This interaction strongly suggests that ABA plays a role in promoting fiber elongation by augmenting the production of ethylene [26].

A comprehensive proteomic analysis identified a total of 7321 proteins, including 365 and 69 differentially abundant proteins in the ABA versus control and ABAI versus control comparisons, respectively. Notably, proteins associated with phenylpropanoid biosynthesis were upregulated after ABA treatment, suggesting a crucial role of this pathway in the response to ABA. Additionally, three auxin-related proteins were upregulated, indicating the potential involvement of auxin in the regulation of fiber development under ABAI treatment. Furthermore, the enrichment of indole alkaloid biosynthesis in the ABAI group hinted at a possible promotion of fiber elongation [119]. These findings highlight the highly interconnected network of ABA, its inhibitor, and the diverse molecular pathways influencing cotton fiber development at both the physiological and the proteomic levels.

4.7. Cytokinin

Cytokinin plays a pivotal role in regulating various aspects of plant development, including cell division, tissue and organ senescence, and apical dominance. While cytokinin is initially present at a relatively low concentration in unfertilized ovules, its levels progressively rise after anthesis. Notably, the introduction of exogenous cytokinin into ovule culture medium has been found to significantly enhance ovule growth, but concurrently, it inhibits the elongation of fiber cells [118] (Figure 3). Interestingly, the accumulation of endogenous cytokinin is predominantly observed in wild-type ovules, with limited presence in fiber cells [127]. To modulate cytokinin levels, one approach involved the overexpression of isopentenyl transferase (IPT), a rate-limiting enzyme crucial in cytokinin biosynthesis [128]. This experiment provided insights into the intricate balance required for cytokinin regulation and its differential impact on ovule and fiber cell development in plants. Cytokinin oxidase/dehydrogenase (CKX), a pivotal enzyme responsible for the cleavage of the unsaturated side chain of cytokinin N6, is a significant negative regulator in cytokinin metabolism. Suppressing *CKX* expression was shown to elevate endogenous cytokinin levels in plants [129]. Utilizing RNAi technology to silence *GhCKX* transcripts in transgenic cotton plants resulted in a notable increase in seed number and a slight enhancement in fiber yield [130]. The enhanced expression of the *GhIPT* gene in cotton, whether controlled by the CaMV35S promoter or a seed-specific promoter, leads to a substantial increase in cytokinin accumulation. Surprisingly, this heightened cytokinin level does not exhibit any discernible impact on fiber yield or quality [49,51]. In summary, cytokinin exhibits a crucial role in ovule development; yet, paradoxically, it appears to play a negative role in the growth of fiber cells in cotton. These findings highlight the intricate and context-dependent influence of cytokinin in multiple aspects of cotton plant physiology.

5. Conclusions

Gaining insight into the underlying genetic and molecular mechanisms of fiber initiation and elongation in cotton is a crucial step to further success in breeding higher quality cotton fiber and elevating the overall fiber yield. An in-depth exploration of the intricate mechanisms dictating fiber development enables researchers and breeders to develop improved cultivars with traits modified at the genomic level. The significance of fiber length and strength cannot be overstated, as they serve as the paramount parameters shaping the quality of cotton and thereby exerting a direct effect on the performance and quality of textiles derived from these fibers. The onset of fiber initiation is intricately linked to lint formation, directly impacting the overall cotton yield. Among the various molecular mechanisms governing fiber development, transcriptional regulation and phytohormonal regulation emerge as the two key players guiding this intricate process. *Gossypium barbadense* is known for producing higher quality fibers but typically has lower yields compared to *Gossypium hirsutum*. A critical research direction involves enhancing fiber quality through a combination of traditional and molecular breeding techniques, with a focus on developing the longest, finest, and strongest fibers.

Regarding the early stages of cotton fiber development (initiation and elongation), an important question arises: Can the primary formation and initial development of trichomes be effectively analyzed using molecular and cellular biological methods to observe both horizontal and vertical division patterns of wool fibers and villous fibers? Additionally, what is the impact of the balance between the auxin inflow vector *AUX1* and the efflux vectors (*PIN1*, *PIN2*, and *PIN3*) on the initiation and elongation processes of cotton fibers?

Considering climate change, it is essential to explore the effects of abiotic and biotic stress on the fiber initiation process and the genes controlled under stress conditions. Future research should address the following: (1) How can cotton breeders control these genes to improve fiber quality or produce high-quality fibers under stress conditions? (2) How can breeders use this information to enhance their cotton varieties? (3) How can breeders control the initiation of short fuzz lint to transform it into long lint, thereby increasing fiber quality? (4) What is the impact of these genes on other economic traits such as yield and

maturity? (5) How does the genotype × environment interaction significantly affect cotton fiber development?

It is imperative to delve into the molecular networks orchestrating fiber initiation, elongation, and the subsequent maturation of secondary walls in cotton fibers. Transcriptional regulation to control gene expression and phytohormonal regulation, of plant hormones, constitute the backbone of these interrelated processes. Unraveling the genetic factors and signaling pathways associated with these mechanisms is indispensable for deciphering the genetic architecture that underlies optimal fiber length and strength. By understanding the interplay between these molecular elements, researchers can develop more targeted breeding strategies, leveraging this knowledge to produce cotton cultivars with superior fiber attributes. The identification of key genes and pathways through advanced molecular techniques facilitates the selection of cotton varieties that exhibit the desired fiber characteristics.

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