ARTICLE

COMPARATIVE STUDIES ON TWO DIPLOID COTTON GENOMES REVEALS FUNCTIONAL DIFFERENCES OF BASIC HELIX-LOOP-HELIX PROTEINS IN ARABIDOPSIS TRICHOME INITIATION

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ABSTRACT

Background: *The cultivated tetraploid cotton species (AD genomes) was originated from two ancestral diploid species (A- and D-genomes). While the ancestral A-genome species produce spinnable fibers, the D- genome species do not. Cotton fibers are unicellular trichomes originating from seed coat epidermal cells, and currently there is an immense interest in understanding the process of fiber initiation and development. Current knowledge demonstrates that there is a great of deal of resemblance in initiation mechanism between by Arabidopsis trichome and cotton fiber.* ***Aims****: In this study, we performed comparative functional studies between A-genome and D-genome species in cotton by using Arabidopsis trichome initiation as a model.* ***Methodology****: Four cotton genes TTG3, MYB2, DEL61 and DEL65 were amplified from A-genome and D-genome species, and transformed into their homolog trichomeless mutants Arabidopsis ttg1, gl1, and gl3egl3, respectively.* ***Results****: Our data indicated that the transgenic plants expressing TTG3 and MYB2 genes from A-genome and D-genome species complement the ttg1 and gl1 mutants, respectively. We also discovered complete absences of two functional basic helix loop helix (bHLH) proteins (DEL65/DEL61) in D- diploid species and one (DEL65) that is functional in A-genome species, but not from D-genome species. This observation is consistent with the natural phenomenon of spinnable fiber production in A- genome species and absence in D-genome species.* ***Conclusions****: These results suggested that MYB2, TTG3 and DEL65, when expressed in Arabidopsis, regulated the regulatory network genes during the trichome initiation process.*

KEY WORDS
Arabidopsis trichome, MYB2, TTG3, DEL65, DEL61

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INTRODUCTION

In position-dependent cell fate determination and pattern formation in *Arabidopsis* trichomes have been well-studied. Trichomes are unicellular single-celled structures emerging and differentiating in the leaf epidermal cells. After cell divisions, these structures subsequently become independent of each other [1]. The mature Arabidopsis leaf trichome consists of a stalk and three to four branches, and its function can vary from trapping herbivorous insects, dispersing seeds, reducing transpiration, to protecting the plants from ultraviolet radiation. The morphogenesis of a trichome is characterized by a series of six phases [2], starting with the introduction of trichome initial followed by subsequent radial expansion and completing with the promotion of completely developing trichome. Other cellular activities connected with the trichome maturation from phase one to phase six include endo-reduplication of the nuclear DNA to an average of 32–64C (the ploidy level of original un-replicated cells is 2C), vacuolization during the transition, and the development of surface papillae during phase one through four, phase four to five, phase five and six, respectively [1].

The initiation of *Arabidopsis* single-celled trichome from leaf epidermal cells presents a useful tool to study the genetic pathways and regulatory signals in cell fate regulation [3, 4]. Genetic and molecular research have elucidated trichome development by a transcriptional and regulatory network controlled by trichome activating and suppressing genes. Over thirty genes have been isolated accounting for diverse aspects in trichome formation including trichome initiation, spacing, size, and morphology. Three groups of proteins have been showed to participate in a trimeric complex to promote trichome initiation, including the WD40 protein TRANSPARENT TESTA GLABRA1 (*TTG1*) [4-8], the R2R3 MYB-related transcription factor GLABRA1 (*GL1*) [9], and the basic helix-loop-helix (bHLH)-like transcription factors GL3 and their functionally redundant ENHANCER OF GL3 (*EGL3*) [10, 11]. Upon the assembly of these proteins, the trichome trimeric complex activates transcription of its direct downstream gene glabrous2 (*GL2*) encoding a homeodomain-leucine zipper protein. *GL2* is documented as the primary target gene of the trichome patterning machinery and is accountable for regulating trichome initiation on *Arabidopsis* leaves [1, 3, 12]. Together with *GL2*, *WRKY* transcription factor *TTG2* and cell cycle gene SIAMESE (*SIM*) are also upregulated. *TTG2* is strongly expressed during trichome patterning and differentiation, while SIM controls endo-replication, a process essential for trichome development.

The GL1-GL3/EGL3-TTG1 complex also upregulates a number of homologous R3 single repeat MYB genes that partially redundantly function as trichome initiation suppressors. These include TRIPTYCHON (*TRY*) [13], CAPRICE (*CPC*), ENHANCER OF TRY AND CPC1 (*ETC1*), *ETC2*, *ETC3* [14], TRICHOMELESS1 (*TCL1*), and *TCL2* [15]. It has been proposed that these repressors render the trimeric complex inactive by the competition with GL1 for binding site with GL3, forming the inert complex R3 MYB INHIBITOR-GL3/EGL3-TTG1. The binding and competition with GL1 differ substantially among these repressors. Binding assays suggest that *TRY* shows the strongest binding affinity, while CPC is the most dominant competitor for binding of GL1 to GL3 [16, 17].

Cotton (*Gossypium* spp.) is regarded as one of the most influential crop plants widely cultivated for textile production. Of fifty-two members in *Gossypium* genus, there are 46 diploids (2n = 2X = 26), five well-

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established tetraploids, and one purported tetraploid species (2n = 4X = 52). It has been proposed that diploid cotton species may have originated from a common ancestor that subsequently evolved and diversified into eight monophyletic groups denoted as A–G, and K [18-20]. Approximately 1–2 million years ago, there was a spontaneous and interspecific hybridization event between A and D diploids and subsequent polyploidization that introduced a new allotetraploid (AD) lineage. Two diploid *G. herbaceum* (A1), *G. arboretum* (A2) and two allotetraploid Upland cotton species, *Gossypium hirsutum* L (AD1), and Sealand cotton, *G. barbadense* (AD2), are dominating in more than 70 countries and have had significant influence on global economic development [21, 22] . Interestingly, *G. raimondii* (D5) contributes the D-genome of the allotetraploid cottons, yet it does not confer spinnable fibers production as the A-genome donors do (*G. arboreum, G. herbaceum*) [19].

Cotton fibers are seed trichomes. Since both cotton fiber and *Arabidopsis* trichome are single-celled structures differentiated from the ovule and leaf epidermal cells, respectively, it is suggested that these two species could share analogous mechanisms for mediating cell fate determination in trichomes. Compared with the *Arabidopsis* trichome, the underlying mechanism of cotton fiber initiation formation remains elusive. Most of the recent research on cotton fiber development focus on genomic and transcriptomic profiles during the cell elongation stage and secondary wall biosynthesis stage [23-27]. However, the mechanism controlling these pathways still needs to be elucidated.

Previous reports also characterized the importance of transcription factors in cotton fiber developmental pathways. So far, dozens of cotton genes encoding numerous classes of transcription factors have been characterized and found to be upregulated in developing fiber cells. Additionally, many of these cotton genes exhibited high protein sequence similarities to *Arabidopsis* trichome regulators [4, 28]. Ectopic expression of *GaMYB2* from *G. arboreum*, which is homologous to *AtGL1*, rescues the trichomeless phenotype of the *Arabidopsis* gl1 T-DNA mutant and induces a single trichome from the epidermis of *Arabidopsis* seeds, suggesting that *GaMYB2* is a functional homolog of *GL1* [29-31]. Additionally, homologs of *Arabidopsis GL3*, *TTG1, CPC, TRY* and *GL2* (*GaDEL65*, *GaTTG1*, *GaCPC*, *GaTRY*, and *GaHOX1*, respectively) were also isolated from *G. arboreum* and functionally characterized using the *Arabidopsis* trichome model system [31-33]. The four WD-repeat AtTTG1-like genes *GhTTG1–GhTTG4* from the Dt subgenome of the upland cotton *G. hirsutum* have been identified to be constantly expressed in some tissues, such as ovules and fibers [34].

In this paper, we tested if the cotton genes activate the *Arabidopsis* trimeric complex similarly to the *Arabidopsis* genes in initiating the trichome. Our results indicated that transgenic lines with *MYB2*, *TTG3* and *DEL65* from diploid genomes A and D complemented the trichomeless phenotype of gl1-1, ttg1-1 and gl3-1 egl3-77439, respectively. We also analyzed the gene expression of the downstream targets of the trichome initiation complex in three different trichomeless mutants, ttg1-1, gl1-1 and gl3-1 egl3-77439 and complemented lines with their cotton homologs *TTG3*, *MYB2* and *DEL65*, respectively. Our quantitative PCR showed that in transgenic lines with *MYB2*, *TTG3* and *DEL65*, trichome-positive regulators *GL2*, *TTG2*, *SIM*, and *HDG11* were up-regulated while the regulation of trichome suppressors *TRY*, *TCL1*, *ETC1*, and *CPC* were downregulated with the over-production of trichomes on leaves. These results represented a similar regulatory network in trichome formation in *Arabidopsis* transgenic lines complemented with homologous cotton genes.

MATERIALS AND METHODS

Plant materials and growth conditions

The trichomeles *Arabidopsis* gl3-1 egl3-77439 (Kanamycin resistant) double mutant (CS6516), and two single EMS mutants ttg1-1 (CS89) and gl1-1 (CS1644) were previously described by Esch et al. (2003), Humphries et al. (2005), and Guan et al. (2014), respectively [34-36]. All the seeds were obtained from *Arabidopsis* Biological Resource Center (Ohio State University, Columbus, OH). *Arabidopsis* seeds were surface- sterilized by the vapor-phase sterilization method described by Clough and Bent (1998) [37]. Seeds were transferred into 1.5µl tubes, which were subsequently placed in a desiccator jar. Prior to sealing the desiccator, a beaker containing 250ml bleach and 5ml HCl was positioned in the desiccator. Sterilization was carried out 12 hours in the fume hood. Once seeds were collected, they were plated on Murashige and Skoog (MS) medium containing 0.8% phytogel. After vernalization for 2 days by placing in the dark at 4 °C, seeds were finally transferred to a growth chamber with the following environmental conditions: 22 °C, light intensity of 130-150 Em^{-2s-1}, 16:8h, light: dark photoperiod and relative humidity of 80% as described previously [38]. Seven days after germination, seedlings were transplanted to soil and grown until maturity in the same temperature and light conditions. Antibiotic selections were performed by supplementing the MS medium with Kanamycin (50mg.ml^{-1}) or Hygromycin (50mg.ml^{-1}) or Basta (50mg.ml^{-1}).

Cloning of *DEL65*, *TTG3* and *MYB2*

To prepare the 35S::*DEL65*, 35S::*DEL61* and 35S::*TTG3* genomic constructs from cotton genomes A (A1, A2) and D (D1, D2, D9), the entire genomic DNA regions of *DEL65*, *DEL61* and *TTG3* were amplified by PCR and then cloned in pMDC32 vectors. For cloning *DEL65* and *DEL61*, the forward and reverse primers were engineered with AscI and PacI restriction sites with the following sequences [Table A1]: GaDEL65-F/ GaDEL65-R; GaDEL61-F/ GaDEL61-R. For cloning *TTG3*, the forward and reverse primers were engineered with XhoI and NotI restriction sites with the following sequences: GaTTG3-F/ GaTTG3-R: The PCR products were subsequently inserted into CaMV 35S expression cassette of pMDC32 vectors. The *MYB2* sequence from

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A and D cotton diploid species was isolated from cotton genomic DNA by the primer pair of XhoI-GhMYB2-F/ NotI-GhMYB2-R. The amplified products were subsequently ligated into the pBARN vector and sequenced.

Genetic complementation

The pMDC32 and pBARN vectors harboring *DEL61*, *DEL65*, *TTG3* and *MYB2* genes were electroporated into *Agrobacterium tumefaciens* GV3101, respectively. Prior to plant transformation, these constructs were verified by sequencing. *A. tumefaciens*-mediated transformation of *Arabidopsis* plants homozygous for double mutant for gl3-1 egl3-77439, homozygous single mutant for gl1-1 and homozygous single mutant for ttg1-1 were performed by the floral dip method with constructs 35S::*DEL65* or 35S::*DEL61*, 35S:: *MYB2*, and 35S:: *TTG3*, respectively [37]. The transgenic seeds were screened on plates containing both Hygromycin and Cefotaxime for selection. For 35S::*MYB2* construct transformation, the transgenic seeds were screened on plates containing Basta for selection. The resistant seedlings were transplanted to soil and phenotypically analyzed.

Phenotypic analyses and microscopy

Arabidopsis wild type and transgenic leaves were collected at the 15-day rosette stage and examined under an Olympus SZ61 industrial microscope. Images were taken by 5-megapixel digital color camera Olympus UC50 (Japan).

RNA extraction, cDNA synthesis and quantitative PCR

Transgenic plants with trichome recovery phenotype were subjected to RNA extraction. Total RNA from *Arabidopsis* wild type, mutants and transgenic plants were extracted from 100 mg three-week-old leaf tissues using SpectrumTM Plant Total RNA Kit (Sigma-USA) in accordance with the manufacturer's instructions. For synthesis of the first strand cDNA, RNA was treated with RNase-free DNase I (Sigma, USA) to eliminate genomic DNA, and two µg of total DNA-free RNA were used to synthesize first strand cDNA with iScriptTM Reverse Transcription Supermix RT-qPCR (Bio-Rad, USA) in accordance with the manufacturer's instructions.

For Real time quantitative PCR, double-strand cDNA samples were diluted with water to 0.025 to 0.005 times depending on the concentration of the first-strand cDNA samples. Eight downstream target genes- *GL2* (Q186_GL2_F/Q186_GL2_R), *HDG11* (Q204_HDG11_F/Q205_HDG11_R), *SIM* (Q202_SIM_F/Q203_SIM_R), *TTG2* (Q188_TTG2_F/Q189_TTG2_R), *CPC* (Q180_CPC_F/Q181_CPC_R), *ETC1* (Q184_ETC1_F/Q185_ETC1_R), *TCL1* (Q200_TCL1_F/Q201_TCL1_R), and *TRY* (Q182_TCL1_F/Q201_TCL1_R) were amplified. Primers of target gene and control gene ACTIN (Q9At-Actin-F/Q10At-Actin-R) were listed [Table A1]. Quantitative PCR was conducted with FastStart DNA Green Master (Roche-USA) in accordance with the manufacturer's instructions, and a Roche Real-time detection system Light Cycler 96 was used to detect and determine the differential expression of the studied genes. Quantitative PCR data were analyzed by using ΔΔCt method [39].

Table 1: Primer sequences

Primer	Sequence
GaDEL65-F	GCTTGGCGCGCCATGTCTACTGGAGTTCAACATCAAG
GaDEL65-R	GGCCTTAATTAATCAACACTTGCCAGCAATTCTTTGC
GaDEL61-F	ATCGCTCGAGATGGCTACTACCGGGGTTCAAAATCAAG
GaDEL61-R	ATCGGCGGCCGCCTAAAAAGATTGTTTTACCCTTGATTTTATAGTCACAG
GaTTG3-F	GCCGCTCGAGATGGAGAATTCAACTCAAGAATCCCACCTG
GaTTG3-R	ATCGGCGGCCGCTCAAACTTTGAGAAGCTGCAATTTGTTGG
*Xho*I-*GhMYB2*-F	ACTGGCGGCCGCATGGCTCCAAAGAAGGATGGAGT
*Not*I-*GhMYB2*-R	ACTGCTCGAGTTATACCATTGCTAATGGATCC
Q9*At-Actin*-F	GCACCCTGTTCTTCTTACCG
Q10*At-Actin*-R	AACCCTCGTAGATTGGCACA
Q180_*CPC*_F	CAAGGCTTCTTGTTCCGAAG
Q181_*CPC*_R	GCCGTGTTTCATAAGCCAAT
Q182_*TRY*_F	TGTCGGTGATAGGTGGGATT
Q183_*TRY*_R	GACGGTGAGGCTTGGTATGT
Q184_*ETC1*_F	CCAACCATTGTTGCCTCTTC
Q185_*ETC1*_R	TCATCACCCAAAACCTCTCA

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Q186_*GL2*_F	CCCCTCTGGATTCTCAATCA
Q187_*GL2*_R	GACGAGGTTTGTCACGGATT
Q188_*TTG2*_F	GAAGCAGGAGTATCGCAAGG
Q189_*TTG2*_R	GATCATCACTCGCTCGTTCA
Q200_*TCL1*_F	AAGAAGAGTGGTGGGACGTG
Q201_*TCL1*_R	TGATGAGGAGAACCCCACTC
Q202_*SIM*_F	CTTTACACGTCGACCCACTC
Q203_*SIM*_R	CATACTTGTGCATGTGCCTCT
Q204_*HDG11*_F	ATATGGAGTCGGTGGAAACG
Q205_*HDG11*_R	GCATTGAAGGCAAAAGAAGG

RESULTS

Complementation studies

Transgenic plants expressing *TTG3* and *MYB2* genes from A and D diploids complemented the ttg1-1 and gl1-1 mutants, respectively. However, ectopic expression of *DEL65* from the A diploid rescued the gl3-1 egl3-77439 mutant, while over expression of *DEL65* from the D- diploid did not recover the double mutant phenotype [Fig. 1]. This observation was consistent with the natural phenomenon of spinnable fiber production in the A- genome species and absence in the D- genome species. Since these results were from one each of these species, we have characterized the *DEL65* from other available A and D diploid species. Genomic DNA of *DEL65* was cloned from A1 (*G. herbaceum*), A2 (*G. arboreum*), D1 (*G. thurberi*), D2 (*G. armourianum*), and D9 (*G. laxum*) species and transformed into the gl3-1 egl3-77439 double mutant. Conclusively, *TTG3* and *MYB2* from both A and D genomes species, and only *DEL65* from A diploid species complemented the trichomeless phenotype.

Fig. 1: From top to bottom: Complementing assays on trichome phenotype were performed on *ttg1-1* single mutant, *gl1-1* single mutant, and *gl3-1 egl3-77439* double with *TTG3*, *MYB2*, and *DEL61*, *DEL65*, respectively. Arrows indicate trichome initiation on first true leaves of transgenic plants.

Since *Arabidopsis* has functionally redundant bHLH proteins (GL3 and EGL3) involved in trichome initiation, the cotton diploids might contain functionally redundant proteins contributing to fiber formation. Genome wide analysis for the presence of DEL65 homolog in A and D diploid was performed and we found the presence of a

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closely-related protein, a single copy *DEL61* in both genomes (A-genome: accession number GCA_00642285.2 from position 95853928 bp to position 95856825 bp and D-genome: accession number PRJNA82769 from position 1682788 bp to position 1685967 bp). The *DEL61* was amplified from A and D diploid species independently, and subsequently transformed into gl3-1 egl3-77439 double mutants. Interestingly, the DEL61 from both diploids did not complement the trichomeless phenotype of the double mutant [Fig. 1]. Taken together, our complementing assays illustrated that the lack of spinnable fiber production in the D- diploids could be attributed to the complete absence of functional *DEL65* and *DEL61*. The non-functionality of these two bHLH proteins may not be the only reason to explain why D- genome does not confer spinnable fiber production, but it could be one of the key factors missing in D diploid species.

Conclusively, based on the *Arabidopsis* trichome model system, it demonstrated the functional differences in DEL65 between A and D diploid species. Sequence comparison demonstrated that there is 96.7% homology in *DEL65*-A and *DEL65*-D at DNA level and yet *DEL65*-D is not functional, therefore, it is highly fascinating to investigate the molecular basis for functional differences of DEL65 in future studies.

Gene expression analysis

Trichomes are well patterned on *Arabidopsis* leaves due to the lateral inhibition mechanism [13] while there is no apparent pattern in fiber formation on cotton seed. Our complementing assays illustrated that transgenic lines with *MYB2*, *TTG3* from genome- A and -D, *DEL65* from genome- A rescued the trichomeless phenotype of gl1-1, ttg1-1, gl3-1 egl3-77439, respectively. However, trichome initiation in transgenic lines with one cotton gene in the *Arabidopsis* trimeric complex still reflected defined pattern on *Arabidopsis* leaves [Fig. 1].

In order to answer the question of pattern difference, we conducted quantitative PCR to observe if there is any difference in gene expression of downstream target genes responsible for trichome promoting regulated by trimeric complex in wild type, trichomeless mutants, and transgenic lines from cotton genomes A and D. Eight candidate target genes were chosen from published data [40].

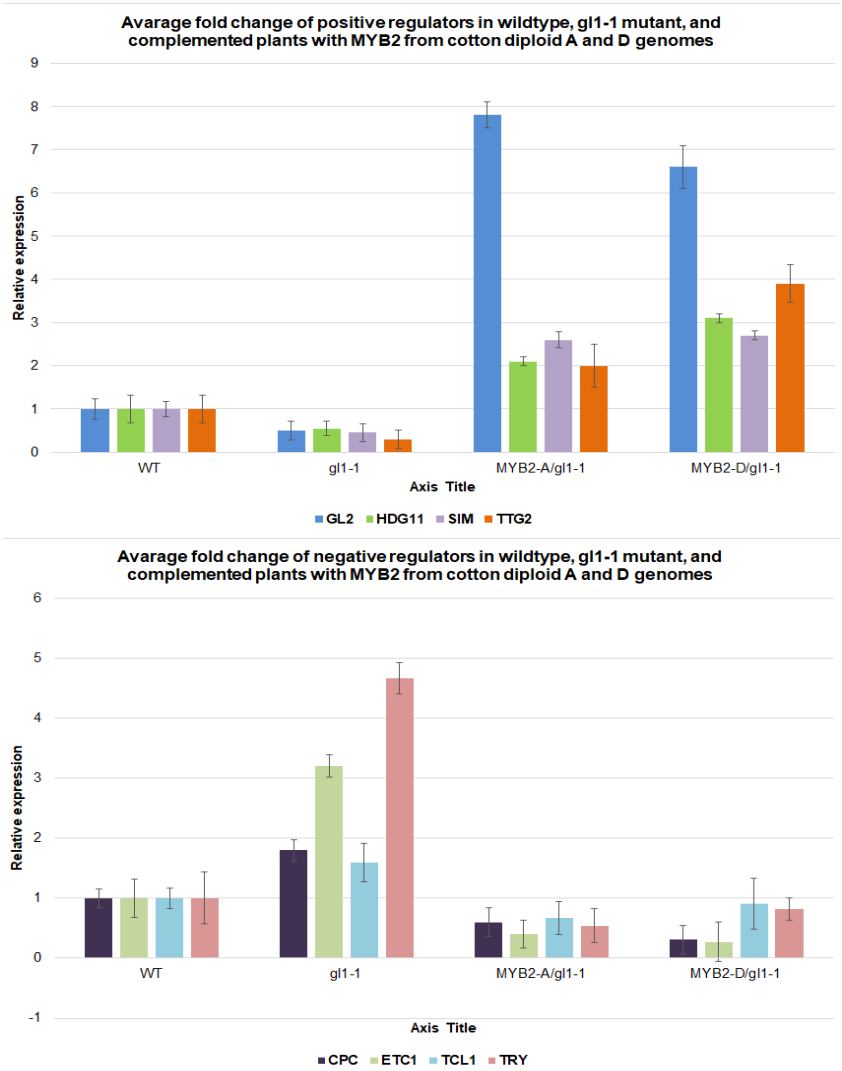

Fig. 2: Transcript levels for individual gene copies of the four trichome negative genes and four positive regulatory genes in Arabidopsis wild type, gl1-1, 35S::*MYB2*-A/ gl1-1 and 35S::*MYB2*-A/ gl1-1

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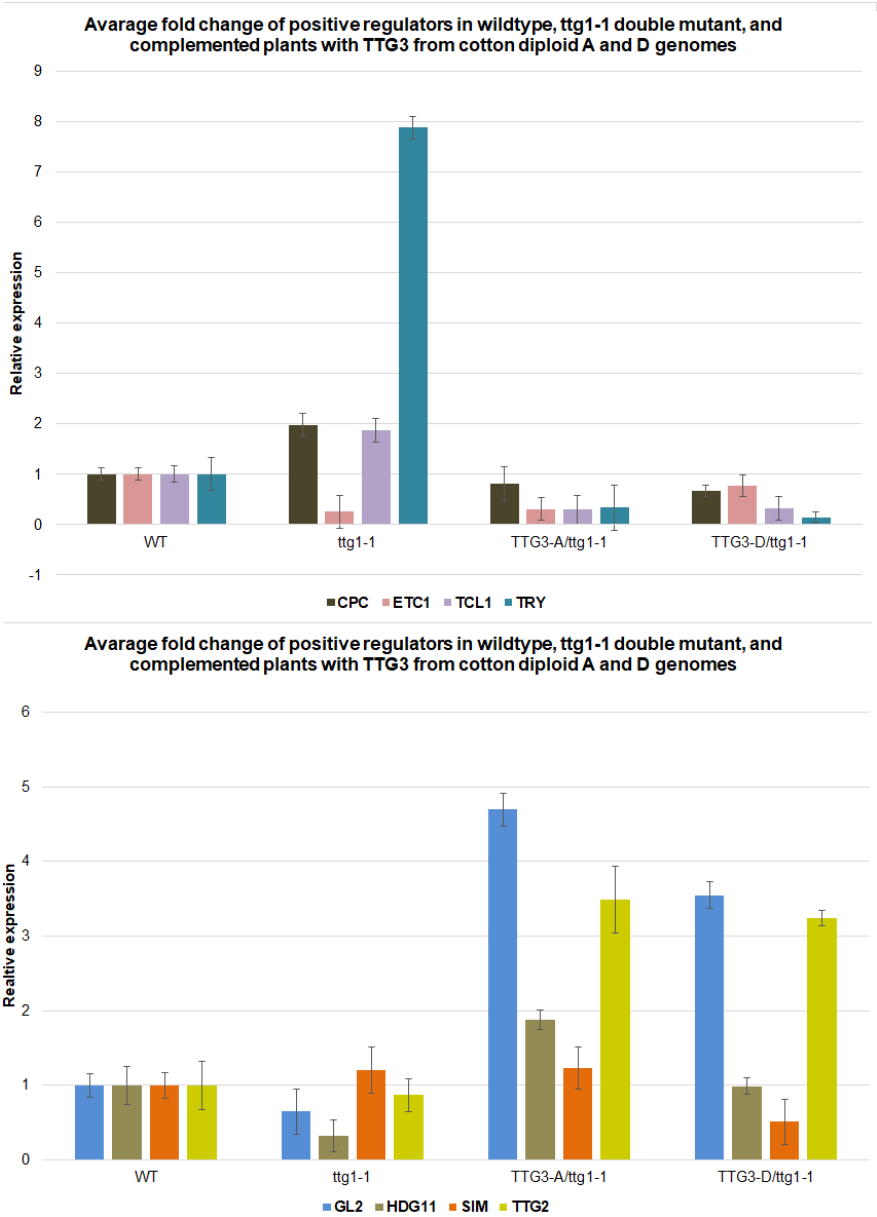

Fig. 3: Transcript levels for individual gene copies of the four trichome negative regulatory genes and four positive regulatory genes in *Arabidopsis* wild type, double mutant ttg1-1, 35S::*TTG3*-A/ ttg1-1 and 35S::*TTG3*-D/ ttg1-1

Figure 2 shows differential expression of four positive and four negative regulators in *Arabidopsis* leaves trichome initiation in wild type, glabrous mutant gl1- 1 and its respective transgenic lines complemented from diploid A and D cotton genomes. The expression of four positive regulators were significantly elevated in transgenic *GaMYB2/* gl1-1 and *GrMYB2*/gl1-1, which could be attributed to the trichome initiation phenotype compared to the mutant gl1-1. It has been documented that *GL2* is required for *Arabidopsis* trichome initiation of leaves while *TTG2* is predominantly up-regulated in trichomes throughout their development. Two other positive regulators, i.e. *HDG11* and *SIM* are responsible for normal trichome branching and development, respectively. The down-regulated expression of R3 *MYB* repressor genes, namely CPC, ETC1, *TCL1* and *TRY*, was consistent with the over production of trichomes on leaves of 35S:: *MYB2*-A/gl1-1 and 35S:: *MYB2*-D/gl1-1 compared to gl1-1. Similar regulation was observed in eight target genes stimulated by trimeric complex in transgenic plants with *TTG3* from cotton diploids A and D genomes compared with wild type and ttg1-1 mutant [Fig. 3].

The regulation of downstream target genes in transgenic lines gl3-1 egl3-77439 transformed with *DEL65* from cotton A- and D- genome species further confirmed the consistency from our complementing assays [Fig. 4]. The transcript levels of positive trichome regulators including *GL2*, *TTG2*, *HDG11*, and SIM were increased, and trichome suppressors such as *TRY*, *ETC1* and *TCL1* were and decreased in 35S:: *DEL65*-A/gl3-1 egl3-77439 line than in 35S:: *DEL65*-D/gl3-1 egl3-77439. Surprisingly, we noticed a significant increase (at least three fold) in one R3 *MYB* transcripts, CPC in 35S:: *DEL65*-A/gl3-1 egl3-77439 line, which were not in

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agreement with leaf trichome phenotype [Fig. 4]. We speculated that the increasing level of CPC functioning as a counteract or to suppress the exceedingly high elevation of *DEL65* transgene, thus developing an equilibrium feedback control to prevent supernumerary trichomes.

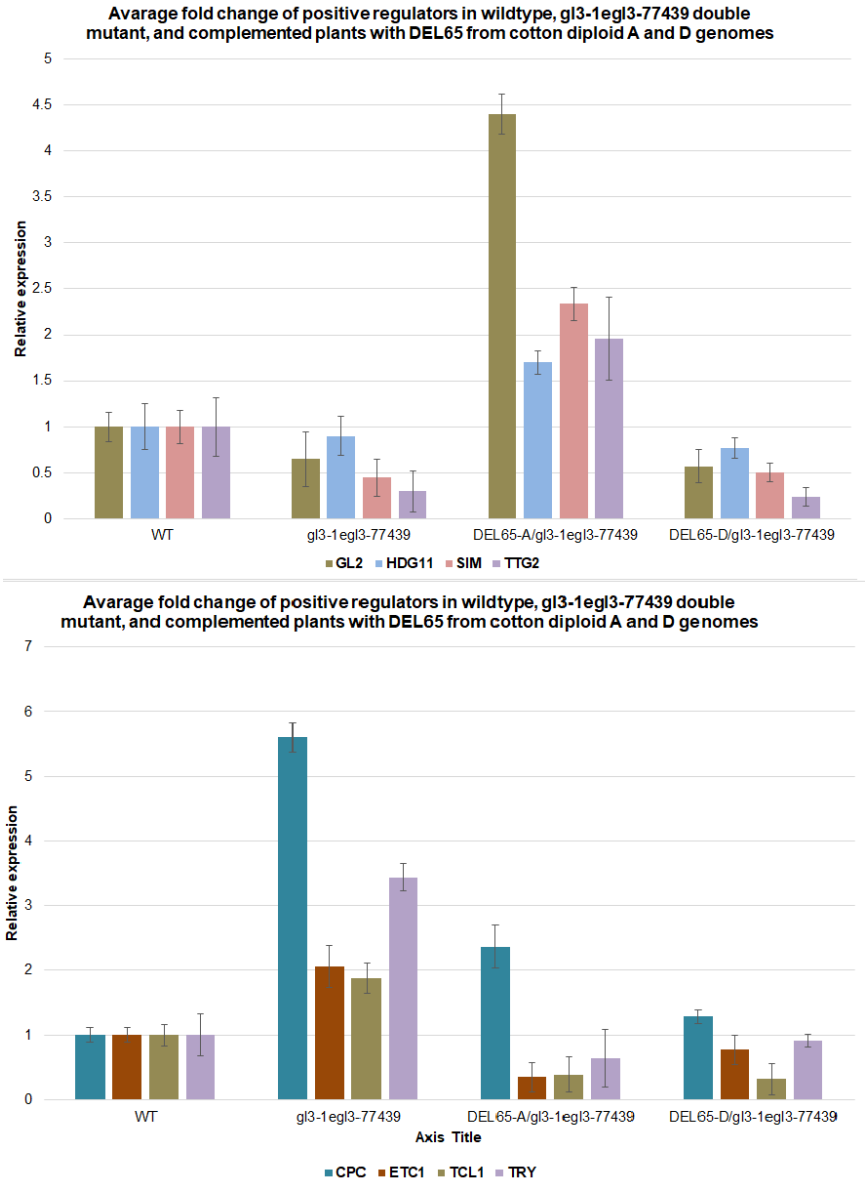

Fig. 4: Transcript levels for individual gene copies of the four trichome negative regulatory genes and four positive regulatory genes in *Arabidopsis* wild type, gl3-1 egl3-77439, 35S::*DEL65*-A/ gl3-1 egl3-77439 and 35S::*DEL65*-D/ gl3-1 egl3-77439

DISCUSSION

In *Arabidopsis*, previous genetic analysis has revealed that trichome initiation is positively mediated by a trimeric activation complex comprised of GL1, GL3 which acts redundantly with its close homolog *EGL3*, and *TTG1* [41, 42]. We studied the functionality of four individual cotton genes *MYB2*, *TTG3*, *DEL65* and *DEL61* from diploid cotton A and D genomes, which show high similarity in sequence with *GL1*, *TTG1*, *GL3* and *EGL3*, respectively, in their respective *Arabidopsis* glabrous mutants. Our complementation assays proved that transgenic lines with *MYB2*, *TTG3* from diploid genomes A and D could rescue the trichomeless phenotype of gl1-1 and ttg1-1 respectively; however, *DEL61* from both the species could not rescue this phenotype of gl3-1 egl3-77439 double mutant. Interestingly, the *DEL65* from A- species rescued the gl3-1 egl3-77439 double mutant but not from D- diploid species. Comparative quantitative PCR analysis of the downstream regulatory network genes showed a similar pattern for *MYB2*, *TTG3* complemented lines from A- and D- diploid species. Comparative analysis of the *DEL65* from A- (rescued the trichomeless phenotype) and D- (did not rescued the trichomeless phenotype) showed differential expression of regulatory network genes between these two lines. These results showed that the cotton genes, when expressed in *Arabidopsis*, regulate the regulatory network genes during the trichome initiation process.

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CONCLUSION

In this paper, we employed the *Arabidopsis* trichome model system to examine the mechanism of spinnable fiber production trait. The core trichome initiation complex consisting of bHLH, WD40 and R2R3-MYB proteins was tested for functional differences between A and D genomes, the parental species of the cultivated tetraploid species. The only discrepancy in our complement assays was DEL65 from A- genome species, not D- genome species, complemented the gl3-1 egl3-77439 trichomeless phenotype, reflecting the consistency of the observation that spinnable fiber production trait is absent in D-diploid species. We also tested the regulation of eight target genes stimulated by the trimeric complex in six different transgenic lines transformed with three cotton genes. The expression of trichome positive regulator was significantly elevated which could be attributed to the trichome initiation phenotype, whereas the transcripts level of trichome suppressors were down regulated. However, CPC levels in 35S:: *DEL65*-A/gl3-1 egl3-77439 line were increased, possibly demonstrating a feedback loop to avoid extreme number of trichome initiation. Conclusively, by implementing trichome initiation in *Arabidopsis* as a model, this study provided functional characterization of four cotton important genes from two diploid cotton genomes -A and -D in fiber initiation. However, more studies should be conducted in other diploid cotton genomes to elucidate the understanding the mechanism of fiber initiation mechanism in cotton.

CONFLICT OF INTEREST

There is no conflict of interest

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FINANCIAL DISCLOSURE

None.

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