TRANSCRIPTOMIC ANALYSIS OF NO TRICHROME MUTANT AND IDENTIFICATION OF NEW TRICHOME RELATED GENES IN ARABIDOPSIS

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Abstract

Trichome is a typical single cell structure formed by the development of epidermal cells through cell fate determination, cell specialization and morphogenesis. Trichome not only protects plants from being injured by drought, high salinity, heavy metals, ultraviolet rays, and pests, but also secretes many kinds of metabolites. A mutant with almost no trichomes (ntm) on its leaves, stems, and inflorescence was identified. qPCR results showed that the expressions of GL1 and GL2 were significantly decreased, but the expression of GA1, GL3, TTG1, EGL3 and SAD2 was enhanced in ntm. RNA-seq was performed to explore the reasons for the difference between ntm and WT to help elucidate the mechanism of trichome development. The results showed that there were 275 differentially expressed genes (DEGs), including 75 up-regulated and 200 down-regulated genes. GO analysis showed that DEGs were mainly enriched in three pathways: cytochrome P450 (CYP79B2), synthesis of cutin, wax and suberin (CYP94C2), and plant hormone signal transduction (CYP79B2). The findings of this study will help to clone the sequence of ntm and further understand the development of trichome.

Key words: Arabidopsis thaliana, Trichome, No trichomes mutant, Transcriptome.

Introduction

Trichome is a specialized structure growing on the surface of plant tissues, including leaves, stems, flowers, seeds, and other organs. As the outermost barrier between plants and the environment, trichome can not only resist various biological and abiotic stresses, but also synthesize, store, and secrete many metabolites (Tiwari, 2016; Matías-Hernández et al., 2017; Champagne et al., 2017; Kariyat et al., 2018). The trichomes of some plants have the ability to absorb and store heavy metals and secrete salt to improve the ability of plants to resist heavy metals and salt. Cotton fiber is a kind of trichome derived from the epidermal cells of ovule and an important raw material for the textile industry.

In Arabidopsis, about one cell in every three or four epidermal cells develops into trichome, which is regulated by the activation-inhibition mechanism. Epidermal cells express trichome promoting factors, which activate the expression of the suppressor. The suppressor’s expression levels determine whether the cells eventually develop into trichomes or not. In this pathway, there are many positive regulatory factors, such as GL1, WER, MYB23, GL3, EFL3 and TTG1 (Oppenheimer et al., 1991; Payne et al., 2000; Humphries et al., 2005; Balkunde et al., 2010; Alahakoon et al., 2016), and negative regulatory factors, such as CPC, TRY, ETCT1, ETC2, and TCLI (Schellmann et al., 2002; Tominaga et al., 2007; Ioannid et al., 2015). GL1 and TTG1 can interact with GL3/EGL3 to form a complex, then activate the expression of GL2 and TTG2, and finally promote the differentiation of epidermal cells into trichomes. Negative regulatory factors can shuttle between cells and compete with GL1 to bind GL3/EGL3, so the complex can not promote the expression of downstream activators GL2 and TTG2. As a result, epidermal cells can not differentiate into trichomes. The GL2, TTG2, SIM and RBR1 activated by the complex can inhibit the expression of some cell division related genes, such as CYCD3;1 and CYCB1;2, so the cells stop dividing and enter the internal replication process and finally differentiate into trichome (Morohashi et al., 2009).

Recently, many new factors have been identified, which directly or indirectly regulate the key factors of trichome development, and thus affect its development. Acetyl transferase General Control Non-repressed protein5 (GCN5) regulates the expression level of CPC, GL1, GL2 and GL3 by influencing the H3K9/14 acetylation of them and affects the initiation of trichomes (Wang et al., 2019). The COP9 signaling complex subunit CSN5a enhances the expression of MYB75 and inhibits the expression of GL2, regulating the production of trichome (Wei et al., 2018). Transcription factor TCP4 directly activates the expression of GIS, TCLI and TCL2, which inhibits the initiation and branching of trichome (Vadde et al., 2017; Vadde et al., 2019). SAD2 coding motif-containing input transporter is related to the transport of nuclear proteins, and the initiation of trichome on sad2 is severely inhibited (Verslues et al., 2006; Zheng et al., 2020). Demethylase JMJD29 directly binds to GL3 and demethylates its H3K9me2 to improve the expression of GL3 and increase the density of trichome (Hung et al., 2020). Zinc finger protein plays an important role in regulating the development of trichome, such as GIS, GIS2, GIS3, ZFP5, ZFP6 and ZFP8 (Zhou et al., 2011; Sun et al., 2015; Kim et al., 2018).

Because of the complexity of trichome development and the diversity of biological functions, more genes...
related to trichome development need to be identified and studied. In this study, a mutant without trichome was investigated and named no trichome mutant (ntm). It had almost no trichome on its leaves, inflorescences, and stems, and this trait was controlled by a single recessive gene. In addition, there was no difference in plant height, fertility and growth between ntm and wild-type seedlings (WT). In order to further study why the initiation of trichome on the surface of ntm was completely inhibited, this study analyzed the transcriptome of ntm and WT, which lays a foundation for cloning ntm and revealing its mechanism for regulating trichome development.

Materials and Methods

Plant materials: The seeds of Arabidopsis thaliana (Col-0) were washed with 10% NaClO solution for 10 minutes, washed with sterilized water 5 times. Seeds were resuspended with 0.2% agar and sown on ½ MS medium, and then treated under 4°C for 2 days. The seeds were cultured at 22°C in a culture room for two weeks. Ten-day-old seedlings were transferred to soil. Two weeks later, every fifth plant was harvested as a repeat for mRNA sequencing, and 3 repeats were set for each sample. To investigate the genetic pattern, ntm used as female parent was crossed with WT. Seedlings of F1 were self-crossed and offspring of F2 were generated, and then the separation ratio was counted.

qRT-PCR: The total RNA of ntm and WT was extracted using TRIzol according to the manufacturer’s directions, and then the total RNA was detected by gel electrophoresis. The M-MLV kit was used to generate the first strand of cDNA according to the manufacturer’s instruction. The real-time PCR was performed using gene specific primers and ACTIN as internal control (Table 1). The PCR parameters was shown as follows 94°C for 1 minute, 95°C for 15 seconds, 60°C for 20 seconds, 72°C for 30 seconds and 45 cycles. Three repeats were set for each experiment, and the results were analyzed by the 2⁻ΔΔCT method.

RNA extraction, library construction and sequencing: Total RNA was extracted using a plant RNA extraction kit, mRNA was fragmented, reverse transcribed and spliced. The library was tested by gel electrophoresis and Agilent Bioanalyzer. Real-time PCR was used for quantitative detection of the library. After passing the library inspection, different libraries were pooled according to the requirements for concentration, and then the library was sequenced using Illumina novaseq 6000.

Data processing: Fastp software was used to filter raw data; remove primers, adapters, sequences less than 50 bp, reads with a certain proportion of N bases (5 bp by default) and low-quality bases with mass value less than 20; calculate the average mass value of bases with 4 bases. The filtered data was aligned to the rRNA database using Bowtie2 software, and then the rRNA sequence were removed from the sequencing data. Hisat2 was used to compare the similarity between clean data and the reference genome, obtain the location information of reads on the reference genome and the characterization of samples, and generate BAM files. Rseq software was used to evaluate the distribution and quality of transcriptome data, such as saturation, RNA degradation and redundant sequence.

GO and KEGG enrichment analysis of DEGs: After quality control, the sequences were compared with the reference genome using HISAT2. Stringtie was used to detect the expression of known genes, new genes, and transcripts. Differential expression was analyzed using Deseq2 or edgeR. DEGs require genes or transcripts whose sum of mapping reads ≥ 10 in two samples. The expression fold change was calculated. If | log2 (fold change) | > 1 was met, it was defined as a DEG. The p value was corrected by FDR to obtain the Q value, which meets the requirements of P value ≤ 0.05 and Q value ≤0.05.

GO and KEGG enrichment analysis of DEGs were performed using R package Cluster profiler software. Enrichment analysis was carried out for all DEGs, both up-regulated and down-regulated. According to the results of GO enrichment analysis, DEGs were classified into cellular component (CC), molecular function (MF) and biological process (BP). The 10 GO functions with the most significant enrichment (p-value) were selected from the three categories of BP, CC, and MF respectively and displayed in figures. If there were less than 10 GO functions, they were all listed. In this report, the R software package cluster Profiler was used for KEGG enrichment analysis.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence(5’-3’)</th>
<th>Primer name</th>
<th>Primer sequence(5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>qPCR-GL1F</td>
<td>CAATGGAAACCGCATGTCAG</td>
<td>qPCR-GL1F</td>
<td>CAATGGAAACCGCATGTCAG</td>
</tr>
<tr>
<td>qPCR-GL1R</td>
<td>TCGATTGACATTACGTCAG</td>
<td>qPCR-GL1R</td>
<td>TCGATTGACATTACGTCAG</td>
</tr>
<tr>
<td>qPCR-GL2F</td>
<td>CTCAGTGGCAAATCGGAGG</td>
<td>qPCR-GL2F</td>
<td>CTCAGTGGCAAATCGGAGG</td>
</tr>
<tr>
<td>qPCR-GL2R</td>
<td>TACTCACTTTCATCAGTATG</td>
<td>qPCR-GL2R</td>
<td>TACTCACTTTCATCAGTATG</td>
</tr>
<tr>
<td>qPCR-GL3F</td>
<td>CGCAGGAGAAGAACATACG</td>
<td>qPCR-GL3F</td>
<td>CGCAGGAGAAGAACATACG</td>
</tr>
<tr>
<td>qPCR-GL3R</td>
<td>CGAGGATTGAAACCGAATG</td>
<td>qPCR-GL3R</td>
<td>CGAGGATTGAAACCGAATG</td>
</tr>
<tr>
<td>qPCR-TTG1F</td>
<td>TTTAGGTACCACTGAGTGC</td>
<td>qPCR-TTG1F</td>
<td>TTTAGGTACCACTGAGTGC</td>
</tr>
<tr>
<td>qPCR-TTG1R</td>
<td>AATGGGCAACCTTGGTGGAG</td>
<td>qPCR-TTG1R</td>
<td>AATGGGCAACCTTGGTGGAG</td>
</tr>
<tr>
<td>qPCR-EGL1F</td>
<td>TCGTTATTGCTGGTCTAAGC</td>
<td>qPCR-EGL1F</td>
<td>TCGTTATTGCTGGTCTAAGC</td>
</tr>
<tr>
<td>qPCR-EGL1R</td>
<td>CCGTCAACCCCTTGGAGT</td>
<td>qPCR-EGL1R</td>
<td>CCGTCAACCCCTTGGAGT</td>
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</tbody>
</table>

Table 1. Sequence of primers for RT-qPCR amplification used in this study.
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Fig. 1. Phenotype of WT and ntm
A. Three-week-old seedling growing in soil; B. Rosette leaf, C. Stem; D. Inflorescence; E. Four-day-old seedling after germination; F. The statistical analysis of trichomes of WT and ntm, n≥12; G. The statistical analysis of primary roots of four-week-old seedlings, n≥20.

Results

Phenotypic identification of ntm:
There was no significant difference in plant height, leaf size and flower size between ntm and WT plants, but the trichomes on the surfaces of all organs of ntm could not be observed (Figs. 1A-D). However, the ntm primary root was shorter than the WTs (Figs. 1E and G). As shown in the figure 1, the main stem of WT has 17 trichomes per cm, while the stem of ntm has no trichome (Figs. 1C and F). The petiole of the mutant ntm had a small amount of trichomes, which was significantly less than for WT. The number of trichome on rosette leaves and inflorescence of WT was dense, while there were no trichomes on the leaves and inflorescence of ntm at all.

Genetic analysis of ntm:
In order to clarify the inheritance pattern of ntm, F1 was generated by crossing Col-0 to ntm. The results showed that the number of trichome on leaves, stems and flowers of F1 generation was the same as that of WT (Fig. 1F). The F2 generation was separated, with 26 lines without trichome and 94 lines with normal trichome. It was checked by chi square and the separation ratio was nearly 3:1. The results showed that the trait of ntm was controlled by a single recessive gene.

To further study the reason for trichome defects, we detected the expression of several genes related to trichome development by qPCR. The results showed that the expression of GL1 and GL2 genes in ntm was lower than that in WT. Compared with the WT, the expression of GL1 and GL2 in ntm decreased by 5 to 6 times. The expression level of GA1, GL3, TTG1, EGL3 and SAD2 in ntm was higher than that in WT (Fig. 2). Previous studies have shown that mutations of GL1, TTG1, SAD2, GA1 or double mutations of GL3 and EGL3 can lead to trichome loss. We cloned GL1 from ntm and WT respectively followed by sequencing. The results showed that there was no difference in the sequences of GL1 between ntm and WT.

Fig. 2. Expression pattern of trichome-related genes in WT and ntm.

Quality analysis of recorded data:
To study the trichome defects of ntm further, the transcriptomes of ntm and WT were analyzed by RNA SEQ. The original reading length was > 38572232, the maximum 50878430, and the original fragment q30 was between 0.91-0.93. The original base 5321935800 and q30 obtained by ntm-2 sequencing were 4908033443. The original base and q30 obtained by sequencing of other samples were greater than this value. After removing the low-quality data, sequencing connectors and rRNA sequences that affect the data quality and subsequent analysis, the filtered data reads were at least 33624646, q30 was 0.937098, the net base number was 4995900722, q30 was 4681646576, and other sample data were better than this sample. More than 93% of the fragments in each sample were located on the genome, and more than 72% of the single genes were located on the genome (Table 2).

Screening and analysis of DEGs:
Analysis of transcriptome showed that there were 275 DEGs between ntm and WT, of which 75 were up-regulated with a LogFC value between 4.2-1.0 and 200 were down-regulated genes with LogFC value between 13-1.0. Among the up-regulated genes, there were 6 genes with LogFC > 2, such as At1G53480, At3G01345, At1G12805, At3G06620, At3G09870, and At4G33560. Among the down-regulated genes, 85 genes had a LogFC value > 2, among which 10 genes had a LogFC value > 10, such as At3G07820, At5G39880, At5G45880, At2G47030, At3G01230, At2G22055, At5G60615, At5G45890, At3G05610 and
At1G55560. Compared with the WT, the expression of multiple genes changed in ntms; the number of downregulated genes was significantly more than that of upregulated genes, and the change fold of the expression level of down-regulated genes was much greater than that of upregulated genes (Fig. 3 and Table 3).

**GO analysis of DEGs:** GO analysis of 275 differential genes showed that 182 differential genes were enriched in 137 entries. As shown in the figure, the down regulation of DEGs in biological processes is mainly related to fatty acid synthesis, metabolism and cellular anaerobic reaction. Among the cell component related genes, the downregulated DEGs are mainly involved cell membrane, endoplasmic reticulum, lipid, mediator complex and axon. Down-regulated DEGs related to molecular function mainly involve oxidoreductase, lipase, dehydrogenase, amino acid ligase and sodium transporter (Fig. 4A). Among the up-regulated DEGs, the genes related to biological processes are mainly involved in plant flower organ development, vernalization, low temperature response, terpene catalysis, ABA catalysis and metabolism. Genes related to cell components mainly involve a variety of organelles, including vacuole, endoplasmic reticulum, peroxisome, microsome, ribosome, chloroplast, plastid and nucleolus. Genes related to molecular function are mainly involved in glycosyl transfer, nitrate transport, etc (Figs. 4B and C). The results showed great differences in the biological process of gene enrichment between upregulated and down-regulated expression.

**Table 2. Statistics of sequencing data by RNA-Seq.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raw reads</th>
<th>Q30 of raw reads</th>
<th>Raw bases</th>
<th>Q30 of raw bases</th>
<th>Clean bases</th>
<th>Q30 of clean bases</th>
<th>Clean reads</th>
<th>Q30 of clean read</th>
<th>Mapping ratio</th>
<th>Uniquely mapping ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT_1</td>
<td>50878430</td>
<td>0.920289</td>
<td>7631764500</td>
<td>7023431277</td>
<td>6688389131</td>
<td>47822644</td>
<td>93.4076</td>
<td>93.48%</td>
<td>72.96%</td>
<td></td>
</tr>
<tr>
<td>WT_2</td>
<td>38572232</td>
<td>0.917892</td>
<td>5785834800</td>
<td>531076054</td>
<td>5388918171</td>
<td>36332324</td>
<td>93.4636</td>
<td>96.29%</td>
<td>89.04%</td>
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</tr>
<tr>
<td>WT_3</td>
<td>40717476</td>
<td>0.926732</td>
<td>6107621400</td>
<td>5660126907</td>
<td>5712367798</td>
<td>38369882</td>
<td>94.1186</td>
<td>95.40%</td>
<td>81.00%</td>
<td></td>
</tr>
<tr>
<td>ntms_1</td>
<td>39672354</td>
<td>0.92511</td>
<td>5950853100</td>
<td>5505192773</td>
<td>5570854336</td>
<td>5234247257</td>
<td>93.9577</td>
<td>96.51%</td>
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<tr>
<td>ntms_2</td>
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<td>93.8538</td>
<td>96.63%</td>
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<td></td>
</tr>
</tbody>
</table>

**Fig. 3. Analysis of DEGs in transcriprome of WT and ntms**

A, Volcano plot of DEGs; B, M-A plot of DEGs; C, Heat map of DEGs
**Table 3. Part of DEGs of ntm versus WT.**

<table>
<thead>
<tr>
<th>Gene_id</th>
<th>LogFC</th>
<th>LfcSE</th>
<th>Pvalue</th>
<th>FDR</th>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1G53480</td>
<td>4.196466851</td>
<td>2.94E-07</td>
<td>1.23E-14</td>
<td>2.15E-11</td>
<td>Increased</td>
<td>Protein translocase of outer membrane, OmpC-like protein</td>
</tr>
<tr>
<td>AT1G24520</td>
<td>1.08E-04</td>
<td>6.35E-06</td>
<td>3.44E-07</td>
<td>2.10E-08</td>
<td>Increased</td>
<td>Calmodulin-like protein</td>
</tr>
<tr>
<td>AT5G48140</td>
<td>9.01E-05</td>
<td>3.06E-05</td>
<td>1.34E-07</td>
<td>5.65E-09</td>
<td>Decreased</td>
<td>B- and C-helix ATPase domain-containing protein</td>
</tr>
<tr>
<td>AT3G54800</td>
<td>2.13E-05</td>
<td>1.20E-05</td>
<td>4.34E-07</td>
<td>1.62E-08</td>
<td>Decreased</td>
<td>Splicing regulator glutamine/lysine rich protein</td>
</tr>
</tbody>
</table>

**Analysis of No Trichome Mutant and Identification of New Trichome Related Genes**

- LogFC: represents the ratio of the expression value between the two samples. After taking the logarithm based on 2, it is log2FC.
- LfcSE: Log2FC standard deviation.
- Pvalue: Statistical significance level. FDR: false discovery rate, which is obtained by correcting the difference significance p-value. Level: increased means up-regulation and decreased means down-regulation.
KEGG analysis of DEGs: The main biochemical metabolic pathway and signal transduction pathway in which DEGs are mainly involved can be determined through enrichment analysis of pathway. According to the results of the KEGG analysis, DEGs in ntm are mainly enriched in cytochrome P450, plant hormone signal transduction, pentose and glucuronic acid conversion, and biosynthesis of various substances, such as cutin, wax, phenylpropane, diterpenes, isoflavones, steroids, and other biological pathways. Among the biological pathways of differential gene enrichment, there are at most 13 differential genes related to cytochrome P450, 10 related to keratin and wax synthesis, and 11 related to plant hormone signal transduction. Down-regulated genes were mainly enriched in four pathways: cytochrome P450 (11), cutin wax synthesis (9), phenylpropanoid synthesis (7) and pentose and glucuronic acid conversion (7) (Fig. 5A). There are a few up-regulated genes, mainly concentrated in phythohormone signal transduction (4) and cytochrome P450 (2). Only one gene in other pathways is enriched (Fig. 5B).

Discussion

The development of trichome is a complex process, controlled by many positive and negative factors, and their expression patterns are not consistently the same. In sad2, the expressions of GL1, MYB23, GL2 and TTO1 decreased, but the expressions of GL3 and EGL3 increased (Gao et al., 2008). Additionally, the transcription factor TPR negatively regulates MYB23, GL1, TTG1, GL3, EGL3, GUS, ZFP8 and GIS2, but can not affect ZFP5, ZFP6 and GIS3 (Kim et al., 2018). In tto1-13, the expression of positive regulatory factors GL2, SPL8, MYC2 and BLT decreased, but the expression of negative regulatory factors ETC1 and CPC decreased (Wei et al., 2019). There are many factors controlling the initiation of trichome, but only single mutations of GL1, TTG1, GA1 and SAD2 and double mutations of GL3 and EGL3 can lead to the complete absence of trichome. In this study, the expression levels of GA1, SAD2, TTG1, GL3 and EGL3 were slightly up-regulated in ntm, while GL1 and GL2 were significantly down-regulated (Fig. 2). The deletion of GA1 affects the seed germination of Arabidopsis thaliana, resulting in dwarf plants inconsistent with the ntm phenotype. At the same time, the coding sequence of GL1 shows no difference between ntm and WT. Therefore, it is speculated that NTM may be a new gene controlling trichome initiation in Arabidopsis, which may inhibit trichome initiation by inhibiting GL1 expression.

Known as a mini factory of natural plant products, trichome can synthesize, store and secrete organic acids, phenols, polysaccharides, alkaloids, proteins, terpenoids and other metabolites. Cytochrome P450 is a kind of monooxygenase widely existing in animals, plants, and microorganisms. In plants, cytochrome P450 mainly catalyzes the synthesis and metabolization of plant hormones, alkaloids, terpenoids, sterols, signal molecules, pigments, fatty acids, flavonoids and isoflavones, and
often binds to membranes of endoplasmic reticulum, mitochondria, plastids, and golgi. KEGG analysis showed that thirteen DEGs encoding cytochrome P450 were found in *ntm*, making it the most obvious enrichment pathway. At the same time, multiple genes involved in biosynthesis of metabolites, such as cutin, wax, phenylpropane, diterpenes, isoflavones and steroids were differentially expressed in *ntm*, including 10 genes involved in cutin and wax synthesis (Fig. 5). The mutation of *TTG1*, a key gene in trichome development, affects the development of trichome and the accumulation of flavonoids (Wei *et al.*, 2019). *GL2* can also affect the synthesis of anthocyanins and procyanidins (Ioannidi *et al.*, 2016). Transcription factors *TCP14* and *TCP15* can regulate the branches of trichome, as well as the expression of keratin and wax synthesis related-genes, such as *CYP86A4*, *GPAT6*, *CUS2*, *SHN1*, and *SHN2* (Camoirano *et al.*, 2020). GO analysis shows that multiple DEGs are involved in cell components such as cell membrane, vacuole, endoplasmic reticulum, peroxisome, chloroplast, and plastid, which are closely related to material synthesis, metabolism, and transportation in plants (Fig. 4). In conclusion, the development of trichome is related to the synthesis and secretion of metabolites in plants. The change of key genes related to trichome development also affects the expression of some metabolites related genes, and, finally, interferes with the synthesis and metabolism of these compounds. Then, it is worth considering whether the yield of Artemisinin can be improved by changing the number of trichome on *Artemisia annua*, and how to balance the relationship between metabolite content and growth.

![Fig. 5. KEGG enrich analysis of DEGs](image)

A, Bar chart of KEGG enrichment analysis of down-regulated DEGs; Bar chart of KEGG enrichment analysis of up-regulated DEGs.
Plant hormones play a key role in the development of trichome. Recent studies have shown that JAZs can regulate the initiation of trichome by interaction with the C-terminal of GL3/EGl3/TT8 and affecting its stability (Qi et al., 2011). Meanwhile, JA and GA can induce the expression of GLI and GL3, and GA can also enhance the stability of GL1 protein. DELLAs and JAZs competed to bind EGL3 and regulated the development of trichome (Tian et al., 2016). DELLA protein RGA can interact with GL1 and EGL3, the key factors of trichome development, inhibit its transcriptional activity, and affect the development of trichome. The expression of ZFP5, GL1, GL3, TTG1 and TRY, important factors in trichome formation, is regulated by gibberellin. Our results show that two gibberellin related genes, AtlG74670 (GASA6) and At1G75750 (GASA1), are differentially expressed in ntm. GASA is a kind of GA-regulated small peptides with conserved N-terminal and C-terminal and variable intermediate peptides. GhGASA1 is negatively regulated the response of cotton to salt stress by regulating the expression gibberellic acid and polyamine related to decrease the content of them (Gu et al., 2021). Overexpression of GASA6 leads to early flowering of Arabidopsis, and can also promote cell elongation, thereby regulating seed germination (Qu et al., 2016; Zhong et al., 2021). Therefore, GA induced cell elongation plays an important role in the initiation of trichome. Compared with JA and GA, there are few studies on the regulation of trichome development by auxin. In tomato, auxin treatment can increase the expression of SAReF4, and then enhance the expression level of downstream SITHM1 and SIMYB52, which leads to the increase of the expression of SICYC2B and increases the density of trichome (Yuan et al., 2012). In this study, 13 genes involved in phytohormone signal transduction were differentially expressed, of which 9 genes were related to auxin, including 4 SAUR genes, such as At5G50760 (SAUR55), At3G09870 (SAUR48), At4G34810 (SAUR5), At5G18010 (SAUR19), At5G13320 (PBS3/GH3.12), At4G32280 (IAA29), At4G37390 (GH3.2/YDK1), and At2G17500 (PIL55). SAURs, such as SAUR19 and SAUR41, are growth factors that can regulate cell expansion and growth (Spartz et al., 2012; Kong et al., 2013). By this means, plants can respond to the environment, including the elongation of hypocotyls, roots and stems. Meanwhile, PIL55, GH3.2/YDK1 and IAA29 are important factors in auxin signal transduction and regulate cell elongation (Takase et al., 2004; Sun et al., 2020). To sum up, the trichome of Arabidopsis belongs to the single-cell structure, and cell extension is very important for trichome formation after its initiation. Because the development of trichome and cotton fiber are regulated by similar mechanisms, it is meaningful to identify much more genes that participate in cell elongation and trichome development. That will help us to improve the yield and length of cotton fiber.

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References


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