

Article

Comparative Transcriptome Analysis Provides Insights into Fruit Trichome Development in Peach

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Abstract: Fruit pubescence (trichome) is an important characteristic and is controlled by a single dominant gene (G/g), resulting in peaches and nectarines. The length and/or density of fruit fuzz varies greatly among different peach cultivars. However, little is known about fruit trichome development in peaches. In this study, significant differences in fruit trichome length and density were identified between ‘XT1’ and its bud mutation ‘BM’, showing much higher values for ‘BM’. Comparative transcriptome analysis was performed, and 987 differentially expressed genes (DEGs) were identified, which were confirmed by qRT-PCR. GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses showed that genes involved in defense response, secondary metabolites and plant hormone signal transduction may also be related to the development of peach fruit trichomes. By integrating other transcriptome data, we finally determined 47 DEGs that might participate in peach trichome development, including five plant-hormone-related genes. The promoter analysis showed that one abscisic-acid-related gene, *Prupe.6G072400* (abscisic acid 8'-hydroxylase 2), and one auxin-related gene, *Prupe.3G074900* (auxin-responsive protein IAA1), have obvious differences in the cis-acting elements of the promoters between ‘XT1’ and ‘BM’. The results of this study will provide a valuable resource illustrating the mechanism of fruit trichome development in peaches and benefit future genomic research.

Keywords: peach; fruit trichome; transcriptome; qRT-PCR



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1. Introduction

Peach (*Prunus persica* L.) is an important fruit and economic crop in China. It has important commercial research value [1]. The planting area of peaches in China is very broad, and its cultivation area and yield rank first in the world. The peach fruit trichome is a kind of plant trichome that is an important agronomic trait that serves as the basis for the variety identification of peaches, and it is also an important factor affecting market competitiveness [2]. A plant trichome is a special structure of epidermal cell differentiation. The trichome is a physical protective barrier helping plants to maintain normal growth, which can effectively protect plants from biotic stress and abiotic stress [3]. Peach fruit trichomes are unicellular, non-branched, non-glandular trichomes, similar to *Arabidopsis* trichomes and cotton fibers [4]. The hairs of peach fruit begin to differentiate on the pistil of flower buds about 3–4 weeks before anthesis and are densely distributed based on the style of the ovary about 2 weeks before anthesis. After anthesis, with the continuous growth and differentiation of epidermal cells, the trichome also undergoes differentiation, growth and decline in peach fruit [5]. In addition, the peach fruit trichome greatly affects the appearance, quality, postharvest shelf life and sensitization of peaches [1,5,6].

Many gene families controlling trichome development in *Arabidopsis* have been identified, including HD-ZIP transcription factors, R2R3 MYB transcription factors, bHLH

transcription factors, WD40 repeat proteins, C2H2 zinc finger protein transcription factors, etc. [2,7]. However, there are a few regulatory factors that have been identified as being involved in trichome development in peaches [1]. The presence or absence of peach fruit trichome is controlled by the G gene locus. The hair (G) and hairless (g) genes are a pair of alleles, and hair ranges from dominant to hairless. The genotypes of hairy peach are homozygous GG and heterozygous Gg, and the nectarine genotype, with a smooth surface and no hair, has only a homozygous gg type. *PpMYB25*, encoding the R2R3-MYB protein, has been proven to be a candidate gene for the G locus. The insertion of an LTR retrotransposon in the third exon is the cause of the recessive hairless phenotype [8]. In *Arabidopsis*, overexpressed *PpMYB25* transgenic lines significantly increased the number of trichomes in rosette leaves and the frequency of trichomes in seeds [1]. Moreover, the expression level of trichome-related genes such as *GL2*, *HDG11*, *HDG12* and *MYB16* in leaves also increased significantly in overexpressed *PpMYB25* transgenic lines [1]. The R2R3-MYB transcription factor *PpMYB26* is closely related to *PpMYB25*. The overexpression of *PpMYB26* in *Arabidopsis* showed a similar phenotype to *PpMYB25* [1]. *PpMYB25* could activate the expression of *PpMYB26*, *PpHDG11* and *PpEXPA4* [1]. In *Arabidopsis*, *HDG11* and *GL2* belong to HD-ZIP transcription factors and are functionally redundant in the regulation of trichome development [9]. GhEXPA4, encoding an expansion protein, promotes fiber elongation in cotton [10]. In addition, *Prupe.5G196400* has also been proven to be linked to the G locus [11]. However, whether the key transcription factor genes regulating *Arabidopsis* trichome formation are also involved in the development of peach fruit trichome is still unknown.

Thus, in this study, transcriptome sequencing was performed using ‘XT1’ (*Prunus persica* L.) and ‘BM’ peach specimens, which have significant differences in their fruit trichomes, as experimental materials. Compared with the previous studies on fruit trichomes using peach and nectarine as materials, the genetic differences between ‘XT1’ and ‘BM’ peaches are relatively small. Simultaneously, quantitative real-time PCR (qRT-PCR) technology was used to verify the expression level of screened genes to confirm the accuracy of the transcriptome sequencing data. This is helpful in discovering new genes related to the development of peach fruit trichomes and provides new ideas for understanding the molecular mechanism of fruit trichome formation in peach.

2. Materials and Methods

2.1. Experimental Materials and Phenotypic Analysis

In this study, ‘XT1’ and ‘BM’ peaches cultivated in Ligan Town, Linyi City, Shandong province, were used as experimental materials. The same parts of the ‘XT1’ and ‘BM’ fruit epidermis were observed and photographed using a stereomicroscope at different growth stages. The different growth periods were 15 days after full bloom (DAFB) (S1), 30 DAFB (S2), 45 DAFB (S3), 60 DAFB (S4) and 90 DAFB (S5). The length of trichomes and the number of trichomes per unit area were measured and analyzed by Image-Pro Plus V6.0.0.260 software. Ten fruits per variety were collected at each stage.

2.2. Total RNA Extraction

The exocarp tissues of ‘XT1’ and ‘BM’ at 15 days after full bloom were used to extract total RNA by a TRIzol[®] Reagent kit according to the manufacturer’s instructions (Magen, New York, NY, USA). The concentration of total RNA, RIN value, 28S/18S and fragment sizes were determined by an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). High-purity RNAs (RIN ≥ 7) were used to construct sequencing libraries. Libraries were qualified using an Agilent 2100 Bioanalyzer and the ABI StepOnePlus RT-PCR system (Thermo Fisher, Waltham, MA, USA). The quantity and quality of RNA were also evaluated by 1% agarose gel electrophoresis.

2.3. Library Preparation and Sequencing

A total of 20 µg of RNA from each of the 6 RNA samples was sent to Novogene (Beijing, China) company for library construction and sequencing. Oligo (dT) magnetic beads were used in the RNA purification experiment. Subsequently, fragmentation was performed on the purified RNA using the ABclonal (Woburn, MA, USA) first-strand synthesis reaction buffer by divalent cations at elevated temperatures. Then, the mRNA fragments were used as templates to synthesize first-strand cDNAs with random hexamer primers and RNase H (reverse transcriptase), followed by synthesizing the second-strand cDNA using buffer, dNTPs and DNase I (DNA polymerase I). The synthesized double-stranded cDNA fragments were purified by AMPure XP (Beckman Coulter, Brea, CA, USA) beads and then adapter-ligated for preparation of the PE library. Subsequently, the fragment size of adaptor-ligated cDNA was selected and purified using AMPure XP beads by PCR. The quality of constructed cDNA libraries was evaluated by the Agilent Bioanalyzer 4150 system. Finally, the constructed libraries were sequenced on the Illumina X Ten platform in 150 bp PE (paired-end) mode.

2.4. Transcriptome Sequencing Data Analysis

After sequencing, clean reads were obtained by removing low-quality reads containing adapters and poly-N using the FASTX toolkit based on the Q20 value per base [12]. The reference peach genome (release version V2.0.a1) and annotation files were downloaded from the GDR website. The index of the reference genome was generated by HISAT2 v2.2.1 software (<http://daehwankimlab.github.io/hisat2/>, accessed on 1 November 2022), and clean reads were mapped against the reference genome [13]. FeatureCounts V2.0.3 software (<http://subread.sourceforge.net/>, accessed on 4 November 2022) was used to calculate the FPKM value (expected number of fragments per kilobase of transcript sequence per million base-pairs sequenced) of each gene expressed in each sample. DEG analysis was completed using DESeq2 V4.0.4 (<http://bioconductor.org/packages/release/bioc/html/DESeq2.html>, accessed on 4 November 2022) and corrected using the p -value [14]. DEGs with a p -value < 0.05 and $|\log_2(\text{foldchange})| > 1$ were considered.

The GO (Gene Ontology; <http://www.geneontology.org/>, accessed on 6 November 2022) and KEGG (Kyoto Encyclopedia of Genes and Genomes; <http://www.kegg.jp/>, accessed on 6 November 2022) enrichment analyses of differential genes were performed using clusterProfiler R V4.2 software package and Fisher's exact test ($p < 0.001$) [15].

2.5. Candidate Genes for Trichome Development

In order to identify possible candidate genes, we further used RNA-seq data published in previous studies [1]. Two datasets were used, including data from one peach and one nectarine at 15 DAFB. Comparative analysis of one peach and one nectarine was performed as described above.

2.6. The Promoter Analysis of Candidate Genes

The 2000 bp promoter sequence upstream of the start codon of the candidate gene-coding region was selected, and the cis-elements were predicted using the Plant CARE online network (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>, accessed on 22 January 2024). The results of the analysis were visualized using TBtools V1.68.0 software.

2.7. Quantitative Real-Time PCR Analysis

The relative expression levels of selected DEGs identified from the transcriptome results were detected by qRT-PCR using the cDNA samples from the transcriptome analysis. The primers were designed by using Primer-Blast tools in NCBI (Supplementary Table S1). The qRT-PCR test was performed using a Roche LightCycler 480 (Roche, Basel, Switzerland) with the following procedure: preheating at 95 °C for 5 min, followed by 45 PCR cycles at

95 °C for 10 s, 60 °C for 10 s and 72 °C for 20 s. The housekeeping gene RP-II was used as an internal control [1]. The relative expression level was calculated by the $2^{-\Delta\Delta C_t}$ method [16].

3. Results

3.1. Phenotypic Differences in Fruit Trichome between ‘XT1’ and ‘BM’ Peaches

To illustrate fruit trichome development in peach, ‘XT1’ and its bud mutation ‘BM’ were selected and characterized in this study. In this study, ‘XT1’ and ‘BM’ specimens cultivated in the same field were used as experimental materials. In order to verify the reliability of ‘BM’ as the bud mutation of ‘XT1’, we used 17 highly polymorphic SNP markers that could distinguish 108 peach germplasms to identify ‘XT1’ and its mutant variety ‘BM’. The sequencing results showed that ‘XT1’ and ‘BM’ had the same bases at these 17 SNP loci, indicating that ‘XT1’ and ‘BM’ had the same genetic background (Supplementary Figure S1). The phenotypic differences between ‘XT1’ and ‘BM’ were recorded and determined by measuring the length and number of trichomes during fruit development (Figure 1A). The fruit skins in the maximum cross-section of these two peach accessions were used for trichome analysis. The results showed that both the length and the number of fruit trichomes were significantly higher in ‘BM’ than in ‘XT1’ at each fruit developmental stage (Figure 1B–D). Furthermore, the length and the number of fruit trichomes in ‘BM’ were 30% higher than those in ‘XT1’. In addition, the development of fruit trichomes showed a similar pattern between ‘XT1’ and ‘BM’, exhibiting a declining trend in trichome number and length during fruit development. The fruit sizes of the ‘XT1’ and ‘BM’ varieties were also analyzed by recording the fruits’ weight, transversal diameter, longitudinal diameter and section girth, showing little differences between ‘XT1’ and ‘BM’ (Supplementary Figure S2). The understanding of phenotypic differences in fruit trichome development would benefit from deciphering the regulatory mechanism of peach trichome development. According to these results, we selected the exocarp tissues of ‘XT1’ and ‘BM’ at 15 DAFB for comparative transcriptome analysis, as a significant difference was found in fruit trichome but not in fruit size at this stage (Supplementary Figure S2).

3.2. Overview of Transcriptome Sequencing Data

A total of 77Gb of cleaned data generated from the six cDNA libraries was sequenced on Illumina X Ten platform with a read length of 150 bp. The minimum number of reads per library was 57.6 million for ‘XT1’-1 and the maximum number was 98.6 million for ‘XT1’-3. The Q30 values ranged from 93.15 to 94.2% and GC content ranged from 44.24% to 44.96%. In addition, after mapping them against the peach reference genome (release version v2.0.a1), approximately 94.27% of total reads were mapped (Table 1), indicating the high quality of RNA-seq results.

Table 1. Quality control result of sequencing data of ‘XT 1’ peach and its bud mutation ‘BM’.

Sample	Raw Reads	Clean Reads	Error (%)	Q20 (%)	Q30 (%)	GC (%)	Total Mapped
‘XT1’-1	57,601,296	57,601,172	0.02	98.08	94.13	44.56	55,052,373 (95.58%)
‘XT1’-2	74,360,894	74,360,768	0.02	97.98	93.86	44.58	70,067,482 (94.23%)
‘XT1’-3	98,645,922	98,645,650	0.02	98.11	94.20	44.24	94,237,865 (95.53%)
‘BM’-1	58,302,810	58,302,758	0.02	97.79	93.34	44.75	54,110,721 (92.81%)
‘BM’-2	58,911,454	58,911,394	0.02	98.00	93.88	44.74	54,695,889 (92.84%)
‘BM’-3	97,403,128	97,403,026	0.02	97.66	93.15	44.96	92,143,059 (94.60%)

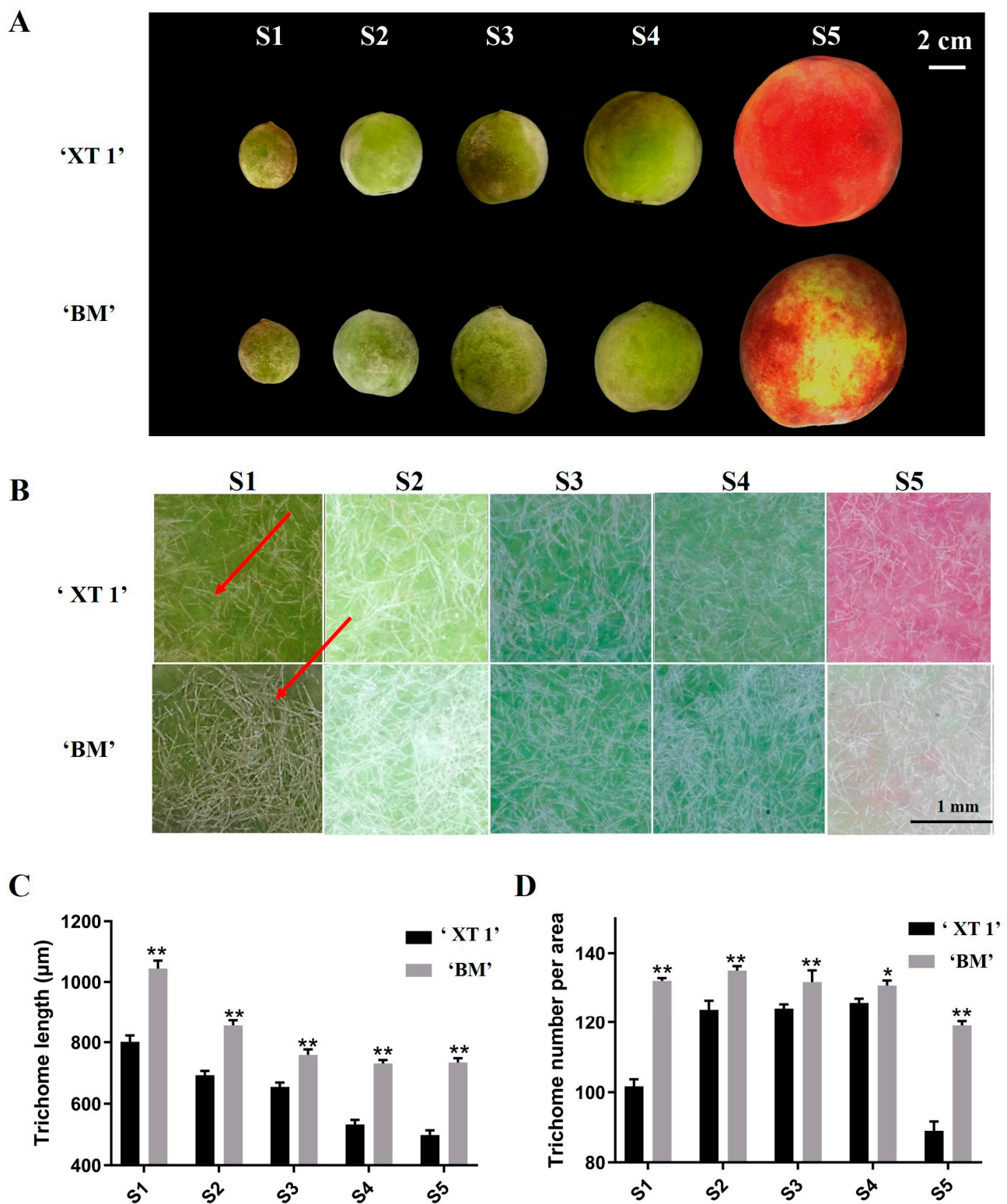


Figure 1. Fruit morphology of peaches at different growth stages. (A): 'XT1' and its bud mutation variety 'BM' at different growth stages, Bar = 2 cm. (B): The fruit trichomes of 'XT1' and 'BM' at different growth stages; red arrows indicate the fruit trichome; a single photo is 2 mm × 2 mm, Bar = 1 mm. (C): The lengths of fruit trichomes of 'XT1' and 'BM' at different growth stages. (D): The number of fruit trichomes per unit area of 'XT1' and 'BM' peaches at different growth stages; stars on error bars represent $\pm$ SE, *: $p < 0.05$; **: $p < 0.01$.

3.3. Functional Annotation of DEGs

Differential expression analysis was carried out between ‘XT1’ and ‘BM’, and 987 DEGs were identified with a fc (fold change) > 2 and a p -value < 0.05 . Moreover, 421 down-regulated and 566 up-regulated genes were included in the DEGs (Supplementary Figure S3). To further determine the vital DEGs for peach trichome development, functional annotation was performed. GO (Gene Ontology) enrichment analysis showed that six terms were significantly annotated, which were ‘response to biotic stimulus’, ‘defense response’, ‘ADP binding’, ‘response to stimulus’, ‘oxidoreductase activity’ and ‘oxidation-reduction process’ (Figure 2A). According to these terms, we found that most of them were associated with defense-related processes. In addition, KEGG enrichment analysis showed that the top 20 KEGG enrichment pathways included ‘biosynthesis of secondary metabolites’, ‘metabolic pathway’, ‘plant-pathogen interaction’ and so on. Among them, two KEGG pathways were significantly enriched, including ‘biosynthesis of secondary metabolites’ and ‘biosynthesis of various plant secondary metabolites’ (Figure 2B). Based on the GO and KEGG enrichment analyses, there were 159 and 39 DEGs identified, respectively (Supplementary Table S2). Besides the significantly enriched pathways (p -value < 0.001), there were 27 DEGs enriched in three pathways, including “Plant-pathogen interaction”, “Plant hormone signal transduction” and “MAPK signaling pathway”, which may also be related to the development of peach fruit trichomes. Therefore, there were a total of 195 enriched DEGs that might participate in trichome development, and these results provide valuable resources for trichome research.

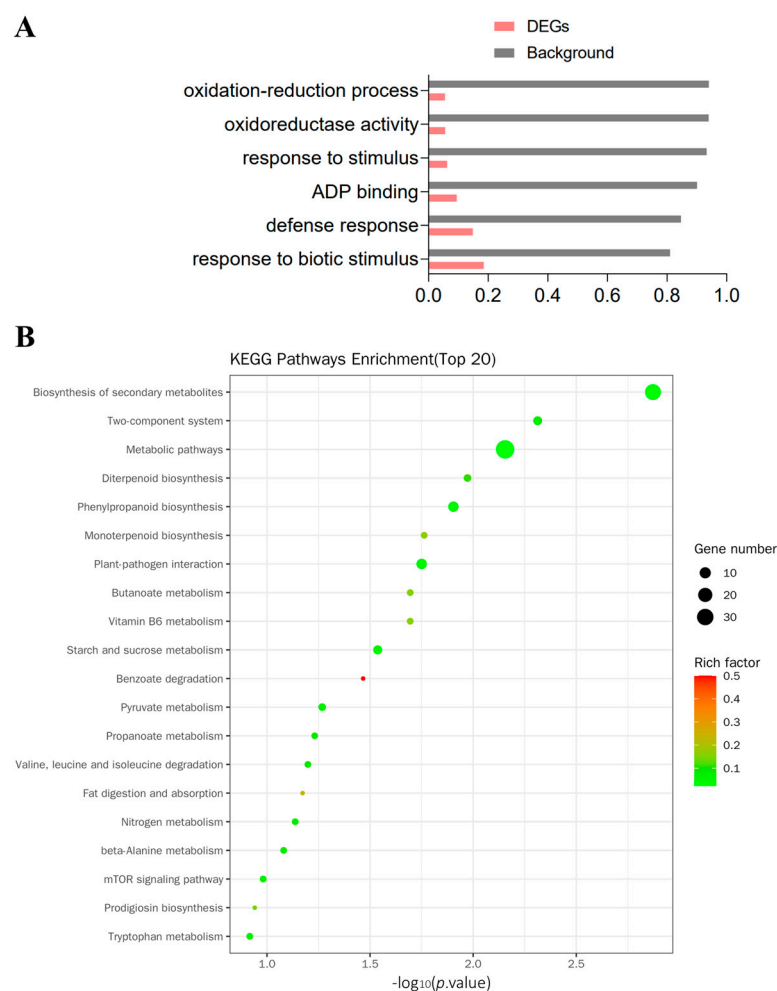


Figure 2. GO enrichment analysis and KEGG enrichment analysis of DEGs. (A): GO enrichment analysis of DEGs; (B): KEGG enrichment analysis of DEGs.

3.4. Selection of Genes for Trichome Development

To further select candidate genes that are related to trichome development, other sets of RNA-seq data generated in previous reports were integrated into this study [1]. The transcriptome data of one peach (Zao Huang Pan Tao, ZH) and one nectarine (Rui You Pan, RYP) at about 15 DAFB were re-analyzed, and 6030 DEGs were identified, which should include the key genes responsible for fruit trichome development. Therefore, we merged these 6030 DEGs with all 987 DEGs from our study to determine the common genes, resulting in 561 common genes. To select genes related to fruit trichome, the transcriptomes of two peach cultivars (Tian Jin Shui Mi, TJ; and Hakuho, HK) at 15 DAFB were used. The number of expressed genes identified in these two peach cultivars was 14,952. After merging with the 561 DEGs, the number of common DEGs became 218. By integrating the enriched 195 DEGs, we finally obtained 47 candidate genes for peach fruit trichome development (Figure 3). Among the 47 candidate genes, 28 genes were down-regulated in 'XT1' compared with 'BM', and the other 19 genes were up-regulated (Figure 4).

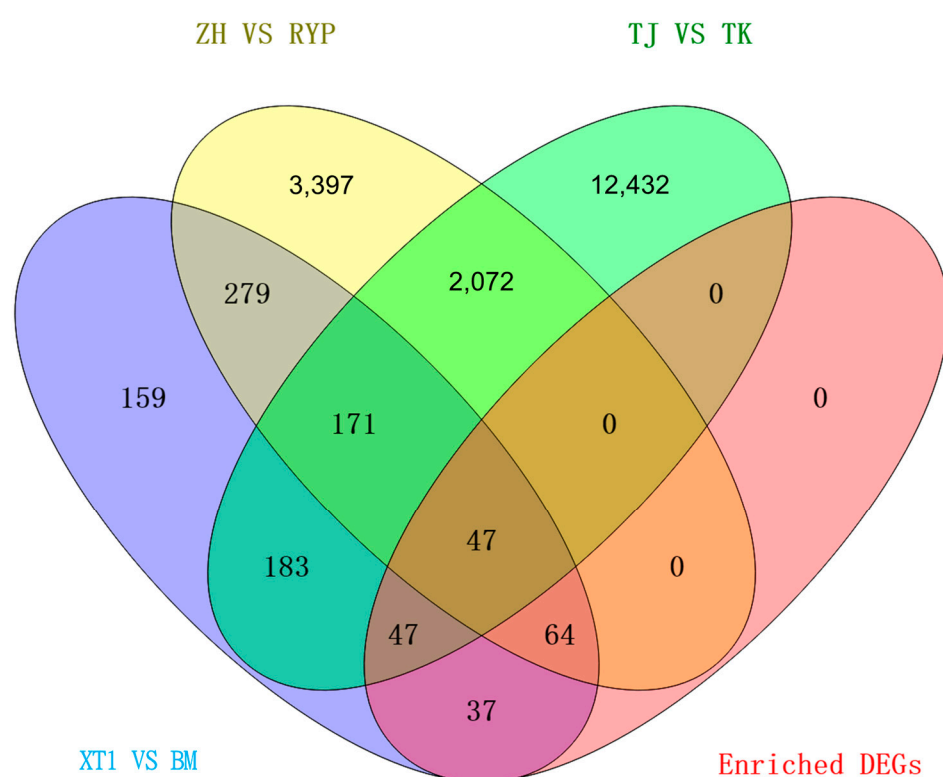


Figure 3. Selecting the candidate genes that are related to trichome development.

To further analyze the function of the regulatory regions of 47 candidate genes, the 2000 bp promoter region sequence was selected for cis-acting element analysis (Supplementary Figure S4). The results showed that most of the promoters of the 47 candidate genes contain a large number of cis-acting elements, including stress response, hormone response and growth and development regulation, among which the *Prupe.6G072400* (encoding a protein with ABA 8-hydroxylase activity) promoter has the largest number (Supplementary Figure S4). Plant hormones play essential roles in regulating the growth and development of plant trichomes. Thus, we focused on plant-hormone-related genes among the candidate genes and compared the phytohormone-responsive elements of hormone-related genes (Figure 5). Among them, *Prupe.2G057800* (a disease-resistance protein) had the greatest difference in its phytohormone-responsive elements between 'XT1' and 'BM'. The different phytohormone-responsive elements include as-1 (4 in 'XT1' and 5 in 'BM'), CGTCA-motif (4 in 'XT1' and 5 in 'BM') and TGACG-motif (4 in 'XT1' and 5 in 'BM') in the promoter

of *Prupe.2G057800*. In addition, five plant-hormone-related genes were identified, such as *Prupe.6G072400*, *Prupe.1G111900* (Gibberellin 2-beta-dioxygenase 2), *Prupe.3G074900* (repressor of auxin-inducible gene expression), *Prupe.4G013800* (1-aminocyclopropane-1-carboxylate oxidase 1) and *Prupe.6G138700* (cytosolic short-chain dehydrogenase/reductase) (Supplementary Tables S3 and S4). Furthermore, *Prupe.6G072400* (17 in ‘XT1’ and 18 in ‘BM’) and *Prupe.3G074900* (6 in ‘XT1’ and 7 in ‘BM’) have a different number of ABREs in their promoters between ‘XT1’ and ‘BM’ (Figure 5).



Figure 4. Gene expression heatmap of 47 candidate genes for peach fruit trichome development.

3.5. qRT-PCR Verification of DEGs

To further verify the reliability of the transcriptome sequencing results, nine DEGs were selected, and their expression levels were detected, including the DEGs enriched in the “Plant hormone signal transduction” pathway (*Prupe.4G197000*, *Prupe.5G106700*, *Prupe.6G108300*, *Prupe.6G108400*, *Prupe.1G315500*) (Figure 6). As shown in Figure 6A, the relative expression levels of these nine genes were consistent with the RNA-seq results, indicating the reliability of the transcriptome data. In addition, we also conducted a correlation analysis between the results of the transcriptome sequencing and qRT-PCR analysis, showing a significant correlation.



Figure 5. The phytohormone-responsive element analysis in the promoters of 47 candidate genes for peach fruit trichome development.

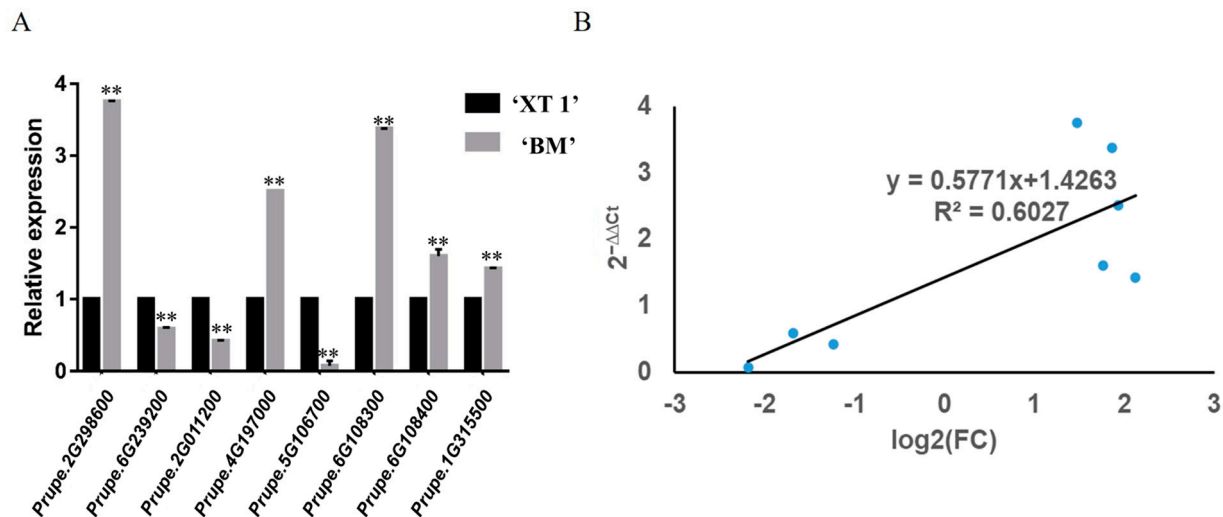


Figure 6. Expression analysis of DEGs. (A): Expression analysis of DEGs by qRT-PCR; stars on error bars represent $\pm$ SE, *: $p < 0.05$; **: $p < 0.01$. (B): Correlation analysis of the qRT-PCR and transcriptome sequencing.

4. Discussion

The fruit trichomes of peach are unicellular plant trichomes, which are closely related to the fruit's appearance, quality, postharvest shelf life and sensitization. In recent years, although the research on the development and function of plant trichomes has become more and more extensive, it is mainly focused on the trichomes of *Arabidopsis*, cotton, tomato, *Artemisia annua* and cucumber. Few studies have been reported on the molecular mechanism of peach trichome development. In this study, 'XT1' and 'BM' specimens cultivated in the same field were used as experimental materials. Compared with previous experimental materials for studying peach fruit hairiness (i.e., the common peach and nectarine), the genetic difference between 'XT1' and 'BM' is relatively small. We used 17 highly polymorphic SNP markers that could distinguish 108 peach germplasms to identify 'XT1' and its mutant variety 'BM'. The sequencing results showed that 'XT1' and 'BM' had the same bases at these 17 SNP loci, indicating that 'XT1' and 'BM' had the same genetic background (Supplementary Figure S1). Through phenotypic observation and statistical analysis, we found that the length and density of the fruit trichome of 'BM' increased significantly compared with that of 'XT1' at various growth stages, especially 15–45 days after full bloom. Moreover, there was no significant difference in fruit size between 'XT1' and 'BM' at 15–45 days after full bloom. Therefore, in this study, the exocarps of 'XT1' and 'BM' in the 15 days after full bloom were used as experimental materials for transcriptome sequencing.

The presence or absence of the peach fruit trichome is controlled by the G gene locus. *PpMYB25*, as a candidate gene for the G locus, has been proven to promote trichome development [1]. *PpMYB25* can activate the expression of *PpMYB26*, *PpHDG11* and *PpEXPA4* [1]. However, only *PpHDG11* was found in all 987 DEGs. *PpHDG11* was found to be significantly up-regulated in 'BM' compared to 'XT1'. *Prupe.7G196500* (the trichome-related marker gene) has been reported to promote trichome development but was not found in the 987 DEGs [17]. This may be because these genes were obtained by comparing the transcriptome of peach and nectarine fruits, which were different from our experimental materials (i.e., the common peach and its hairy bud mutation). The GO analysis showed that the DEGs were mainly enriched in 'response to biotic stimulus', 'defense response' and 'response to stimulus', which are associated with defense-related processes. These results were consistent with previous studies that trichomes played a role in defense response [18–21]. Meanwhile, two KEGG pathways were significantly enriched, including the 'biosynthesis of secondary metabolites' and 'biosynthesis of various plant secondary metabolites'. These results also indicate that trichome formation and development are associated with various pathways and regulated by complex molecular mechanisms. Metabolic pathways are directly related to the growth, development, survival and reproduction of plants and provide energy and intermediate products for vital plant activities [22]. Secondary metabolites play an important role in resisting adverse environments and attacks from pests, improving adaptability and reproduction ability [23]. The metabolic pathway in cells is also important pathway information included in the KEGG database. Although trichomes are not essential for plant survival, they are more or less associated with plant growth and development [3]. Nevertheless, trichomes can effectively protect plants from biotic and abiotic stresses and, in turn, maintain the normal growth and development of plants. However, further experiments are needed to determine whether there is a difference in metabolism between 'XT1' and 'BM' and whether this difference is caused by their fruit trichomes, requiring further experiments.

In addition, there were 27 DEGs enriched in three pathways, including "Plant hormone signal transduction", "Plant-pathogen interaction" and "MAPK signaling pathway", which may also be related to the development and function of peach fruit trichomes [7,24,25]. Plant hormones play essential roles in regulating the growth and development of plant trichomes. Previous studies have demonstrated that plant hormones are involved in trichome formation in plants. Furthermore, plant hormones are also involved in stress response and defense processes in plants. In this study, some DEGs enriched in the pathway "Plant hor-

hormone signal transduction” were verified by qRT-PCR, and the results were consistent with transcriptome sequencing results. Also in this study, 12 DEGs were enriched in the pathway “Plant hormone signal transduction”, including auxin, cytokinin, abscisic acid, salicylic acid and ethylene. In the auxin signal transduction pathway, *Prupe.4G197000* (GH3, auxin-responsive GH3 gene family), *Prupe.6G108300* (SAUR50-1, auxin-responsive family protein) and *Prupe.6G108400* (SAUR50-2, auxin-responsive family protein) were down-regulated in ‘XT1’, whereas *Prupe.3G074900* (Aux/IAA, auxin-responsive protein IAA) was up-regulated in ‘XT1’. These results are consistent with previous findings that auxin positively regulates trichome development in cotton and tomato [26–28]. In the cytokinin signal transduction pathway, *Prupe.3G201700* (CRE1, cytokinin-binding receptor) was down-regulated in ‘XT1’. Similarly, cytokinin also plays a positive role in trichome development in plants [29–33]. In the salicylic acid signal transduction pathway, *Prupe.8G153700* (PR-1, pathogenesis-related protein) was up-regulated in ‘XT1’. Consistently, salicylic acid inhibits trichome initiation in *Arabidopsis* [34]. The above results indicate that our results are reliable, but whether these genes are related to trichome development needs further experiments.

To further select the candidate genes that relate to trichome development, other RNA-seq datasets (Zao Huang Pan Tao, Rui You Pan, Tian Jin Shui Mi, TJ and Hakuho) were integrated with the 195 enriched DEGs in this study. Finally, we obtained 47 candidate genes for peach fruit trichome development. From the above description, we know the relationship between plant hormones and trichomes, but whether there are hormone-related genes in these candidate genes remains an open question. By conducting a phytohormone-responsive element analysis in the promoter, we found that *Prupe.2G057800* (a disease-resistance protein) has the greatest difference in phytohormone-responsive elements between ‘XT1’ and ‘BM’, including as-1 (salicylic acid and oxidative responsiveness), CGTCA-motif (jasmonic acid responsiveness) and TGACG-motif (jasmonic- and salicylic-acid-responsive elements). However, jasmonic acid and salicylic acid have antagonistic effects in regulating trichome development [34]. In addition, we identified five plant-hormone-related genes via gene annotation. Among these genes, two plant-hormone-related genes, *Prupe.6G072400* (abscisic acid 8'-hydroxylase 2) and *Prupe.3G074900* (auxin-responsive protein IAA1), have differences in the abscisic-acid-responsive elements (ABREs) of the promoters between ‘XT1’ and ‘BM’. Abscisic acid has been reported to positively regulate trichome formation in *Arabidopsis* [35]. *AT2G29090* (a *Prupe.6G072400* homolog) encodes a protein with ABA 8-hydroxylase activity involved in ABA catabolism [36]. *Prupe.6G072400* may be involved in the ROS metabolism [37]. There is no description available for *Prupe.3G074900*. These genes will be important research objects for the future experiments. However, our study did not verify their expression levels by qRT-PCR, so further studies are required. In sum, our results also verified that trichome development is regulated by plant hormones, although there may be differences between species.

5. Conclusions

In this study, a peach cultivar ‘XT1’ and its bud mutation ‘BM’ were used to identify candidate genes for fruit trichome development. The length and density of fruit trichomes were significantly higher in ‘BM’ than those in ‘XT1’. Comparative transcriptome analysis was performed, and 47 DEGs were identified, including plant-hormone- and defense-related genes. *Prupe.6G072400* and *Prupe.3G074900* will be important research objects for future studies. This study will contribute to improving the understanding of the underlying molecular mechanism of fruit trichome development in peaches and lay the foundation for the study of trichome-related resistance.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture14030427/s1>, Table S1: The primers were used for qRT-PCR; Table S2: The DEGs identified on GO and KEGG enrichment analysis; Table S3: The expression of 47 candidate genes; Table S4: The annotation of 47 candidate genes; Figure S1: Analysis of high polymorphic SNP markers; Figure S2: The fruit weight, transversal diameter, longitudinal diameter

and section girth of ‘XT1’ and ‘BM’; Figure S3: Volcano plots of DEGs in ‘XT1’ and ‘BM’; Figure S4: Cis-acting elements in the promoter of 47 candidate genes for peach fruit trichome development.

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