

RESEARCH ARTICLE

Open Access



Single-cell sequencing reveals dynamic cell development trajectories in two kiwifruit (*Actinidia chinensis*) genotypes with contrasting cold resistance

Miaomiao Lin^{1,2†}, Zhenzhen Zhang^{1†}, Qina Zhang¹, Shihang Sun^{1,2}, Yukuo Li^{1,2}, Mingyu Liu¹, Yihang Li¹, Congcong Li¹, Jinbao Fang¹ and Xiujuan Qi^{1,2*}

Abstract

Kiwifruit (*Actinidia* Lindl.) is a valuable fruit tree species, with its cold resistance being influenced by leaf structure and various cell types. Despite significant progress in understanding the cold stress response during the last decade, the mechanisms underlying the formation of distinct cold-resistant cell types in kiwifruit, particularly at the single-cell level, remain poorly understood. In this study, single-cell RNA sequencing (scRNA-seq) of leaves from 'Hongyang' (HY, *A. chinensis*, cold sensitive) and its transgenic line with overexpressed *AaBAM3.1* (designated as HT, cold resistant) was employed to construct a single-cell transcriptional atlas to investigate the cell heterogeneity of two kiwifruit genotypes exhibiting different cold resistance capacities. A total of 5,611 and 13,466 single cells were obtained from HY and HT, respectively. The cells were classified into eight clusters based on gene expression patterns, and key genes associated with specific cell types in both genotypes were identified. Notably, pseudotime trajectory analysis revealed distinct developmental paths for guard cells and mesophyll cells. Additionally, a transcription factor, *AaTIFY*, exhibited genotype-specific expression and negatively regulated cold resistance. In summary, these results demonstrate that scRNA-seq offers valuable insights into cell differentiation and development in kiwifruit with varying cold resistance at the single-cell level and highlights novel genes related to cold tolerance in kiwifruit.

Keywords Kiwifruit, ScRNA-seq, Transcriptional atlas, Differentiating trajectory, Cold resistance

Introduction

Kiwifruit, a member of the genus *Actinidia* Lindl., originates from China and includes 54 species and 21 varieties. Known for its exceptionally high vitamin C content

and rich nutrient profile, kiwifruit is both economically and nutritionally valuable. The predominant commercial variety is *A. chinensis* 'Hongyang'. Despite its excellent fruit quality, 'Hongyang' is characterized by poor cold resistance. Our previous study developed a variant with enhanced cold tolerance by overexpressing *AaBAM3.1* in 'Hongyang' (Sun et al. 2021). However, the transcriptional differences at the single-cell level and the underlying mechanisms of leaf development in these cold-tolerant lines remain unclear.

Recent advancements in single-cell RNA sequencing (scRNA-seq) have provided new insights into dynamic gene expression across cell types, offering a novel approach to understanding cellular functions at the

[†]Miaomiao Lin and Zhenzhen Zhang contributed equally to this work.

*Correspondence:

Xiujuan Qi

qxixiujuan@caas.cn

¹ National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou 450009, People's Republic of China

² Zhongyuan Research Center, Chinese Academy of Agricultural Sciences, Xinxiang 453500, People's Republic of China

single-cell level (Berrío et al. 2025; Kolodziejczyk et al. 2015; Li et al. 2023; Liang et al. 2025; Olsen and Bar-yawn. 2018; Shaw et al. 2021; Sun et al. 2023). scRNA-seq has been widely applied not only in model plants but also in crops such as corn, rice, wheat, peanut, strawberry, tea, pitaya, and cotton (Bai et al. 2022; Li et al. 2024; Li et al. 2021; Liu et al. 2020; Wang et al. 2022b; Xie et al. 2022; Zhang et al. 2023; Zhang et al. 2021a). Studies have demonstrated the utility of scRNA-seq in elucidating the spatiotemporal regulatory networks of cotton fiber initiation (Qin et al. 2022), analyzing stomatal lineage and root cell development in *Arabidopsis* (Liu et al. 2020; Pillitteri et al. 2007), generating transcriptional atlases of leaf and stem cell reorganization in maize (Nelms and Walbot 2019; Xu et al. 2021), uncovering mesophyll cell development differences in Chinese cabbage (Guo et al. 2022), and identifying genes involved in catechin metabolism (Wang et al. 2022b).

scRNA-seq has facilitated the identification of key functional genes involved in various biological processes (Liu et al. 2023; Macosko et al. 2015; Sun et al. 2022). For instance, *UGT72B23* was implicated in catechin ester metabolism in tea leaves (Wang et al. 2022a), peanut *AHL23* was shown to promote leaf growth by regulating the phytohormone pathway (Liu et al. 2021), and *WRKY33* and *BPCs* were found to influence early stomatal development (Liu et al. 2020).

Despite the rapid advancement of single-cell technologies, their application to fruit crops remains limited. In this study, protoplasts from the leaves of kiwifruit genotypes with different cold tolerances—HT (strong cold tolerance) and HY (weak cold tolerance)—were isolated to map the single-cell transcriptomic landscape. The analysis revealed eight distinct cell types, and several marker genes specific to particular cell types were identified. Pseudotime trajectory analysis of guard and mesophyll cells of both genotypes highlighted key transcription factors (TFs) critical to cell development. Notably, a TIFY TF was found to negatively regulate cold resistance, as shown by the reduced cold tolerance in AaTIFY-overexpressing lines. This study reports the first single-cell transcriptome of kiwifruit leaves and identifies functional genes associated with cold tolerance, offering new insights into the molecular mechanisms underlying kiwifruit cold resistance.

Materials and methods

Plant materials and treatment

The plant materials consisted of *in vitro* seedlings of *Actinidia chinensis* ‘Hongyang’ and the transgenic line overexpressing *AaBAM3.1*, along with male *A. arguta* ‘Kuilv’. All plantlets were maintained under tissue culture conditions for 30 d with a photoperiod of 16 h of

light and 8 h of darkness. For expression analysis, fresh shoots of ‘Kuilv’ were exposed to low temperatures at 4 °C in an incubator (EHS-100, Zhihe Environment Test Equipment Corporation, Suzhou, China) for 0, 6, 24, 72, and 120 h. In another experiment, dormant shoots collected in early January were kept at −25 °C for 0, 2, 4, 6, and 8 h.

Leaf structure observation

Sections of the leaves were fixed with an FAA solution. Safranin-O and Fast Green stain kits (Solarbio, Beijing, China) were used to differentiate the cell types, then the leaf structure was scanned by Panoramic MIDI, Panoramic 250FLASH, Panoramic DESK (3DHISTECH, Hungary) and observed.

Protoplast isolation from leaves

Kiwifruit leaves were collected and cut into 1–2 mm pieces. An enzyme mixture containing 3% cellulase R-10, 1.5% macerozyme R-10, 0.3% pectinase Y-23, 0.25% BSA, 5 mM MES, and 8% mannitol, was prepared, deliberately excluding Ca^{2+} and Mg^{2+} . The solution was filtered through a 40 µm cell strainer. Leaf enzymolysis was carried out by incubating the samples in 30 mL of the enzyme mixture for 2 h at 25 °C with agitation at 40 rpm. The extracted protoplasts were stained with trypan blue to assess extraction quality, and their concentration was confirmed using a hemocytometer and light microscope. Finally, single oil droplets were prepared for scRNA-seq library construction (Liu et al. 2021).

scRNA-seq library construction and sequencing

ScRNA-seq libraries were constructed using the Chromium Next GEM Single-Cell 3' GEM, Library & Gel Bead Kit v3.1, following the manufacturer's instructions. Demultiplexed cellular barcodes were generated using Cell Ranger (version 5.0.0) software, and the results were aligned against the ‘Hongyang’ v3 genome (<http://kiwifruitgenome.org/>). Seurat software (v3.1.1) was used for dimensional reduction and clustering analysis. Stringent quality control thresholds were applied: cells with fewer than 200 genes, fewer than 1000 unique molecular identifiers (UMIs), a log10GenesPerUMI ratio less than 0.7, and mitochondrial gene representation above 20% were excluded. Doublets were removed using the DoubletFinder package (v2.0.2) (Butler et al. 2018; McGinnis et al. 2019).

Dimensionality reduction and clustering

To visualize the single-cell data, dimensionality reduction to 2D space was performed using uniform manifold approximation and projection (UMAP). Analysis was

carried out in Seurat, starting with the identification of highly variable genes (HVGs) through cluster analysis, followed by dimensionality reduction via principal component analysis (PCA). The cells were clustered based on their gene expression patterns (Macosko et al. 2015).

Identification of marker genes

The marker genes for each cluster were screened using all the marker functions in Seurat. Markers were selected as active genes, contrasting each cluster with all other clusters (Butler et al. 2018).

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses

Differentially expressed genes (DEGs) between ‘Hongyang’ and the transgenic line were identified within each cluster, with thresholds set at $P < 0.05$ and a $\log_2$ fold change [FC] > 1.5 . The functional annotation of the DEGs across cell types was conducted using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses.

Pseudotime trajectory analysis

Pseudotime models for guard and mesophyll cells were constructed using Monocle (version 2.0) (Trapnell et al. 2014). The cells were ordered based on differential gene expression using the Monocle2 differential gene test function ($qval < 0.01$), and the guard and mesophyll cells were visualized in a tree-like structure. BEAM analysis was performed to display the gene expression patterns associated with each cell fate. A heatmap of key genes involved in differentiation was generated using BEAM analysis, with gene expressions tracked across pseudotime by plotting representative genes using a pseudotime function.

RT-qPCR analysis

Total RNA from plants was isolated using the Vazyme Biotech RNA Isolation Kit (Nanjing, China), and cDNA synthesis was performed using the TOYOBO cDNA Synthesis Kit (Osaka, Japan). RT-qPCR was carried out using the NovoStart® qPCR SuperMix Plus Kit (Novoprotein, Suzhou, China), with relative expression determined by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Generation and cold tolerance assay of *AaTIFY*-overexpressing kiwifruit plants

For plant transformation, the opening reading frame (ORF) of *AaTIFY* was cloned and inserted into the pBI121 vector driven by the CaMV 35S promoter (Table S7). The recombinant plasmid was subsequently transformed into *Agrobacterium* strain EHA105, which

was subsequently used to transform ‘Hongyang’ leaves using the leaf disc method (Wang et al. 2007). Following approximately 6 months of growth on Murashige and Skoog (MS, 1962) medium (7.2 g/L agar, 30 g/L sucrose, pH 5.8). Three overexpression lines and wild-type (WT) plants were selected for cold tolerance analysis. The samples were subjected to a 2 h treatment at 2 °C. An IMAGING-PAM chlorophyll fluorometer was used to image the plants, and F_v/F_m was determined by Imaging WinGegE software. Relative electrolyte leakage (REL) and the malondialdehyde (MDA) content were assessed following previously described method (Sun et al. 2020).

Statistical analysis

Statistical analysis was performed using one-way ANOVA in SPSS software, with significance determined at thresholds of $P < 0.05$, $P < 0.01$, and $P < 0.001$. Duncan’s multiple range test was used for post hoc significance analysis.

Results

Construction of a single-cell transcriptional atlas for kiwifruit leaves

To construct the single-cell transcriptomic atlas of kiwifruit, protoplasts were isolated from young leaves of 30-day-old tissue-culture seedlings of HY and HT. cDNA libraries were then constructed using single-cell barcode labeling. After quality control, 5,611 high-quality cells from HY and 13,466 from HT were retained. Sequencing results (HY: 743,562,403 reads; HT: 745,795,327 reads) revealed mapping rates of 85.4% and 75.6%, respectively, against the ‘Hongyang’ v3.0 genome (Yue et al. 2020). A total of 30,642 genes in HY and 32,144 genes in HT were detected, with an average of 2,037 and 2,104 UMIs per cell, respectively (Table S1). Based on the morphological characteristics of kiwifruit leaves and differential gene expression, the cells were classified into eight clusters through UMAP clustering (Fig. 1a, 1b) (Table S2, S3). A total of 4,067 specific genes were identified ($\log_2 FC > 0.26$; $P < 0.01$), and the cluster sizes varied widely, ranging from 117 to 6,485 cells (Table S4). These results indicate significant cell heterogeneity between the two genotypes of kiwifruit.

Identification of different cell types

In the absence of well-characterized marker genes for kiwifruit leaves, orthologous *Arabidopsis thaliana* markers from the plant scRNA-seq database (<http://ibi.zju.edu.cn/plantscrnadb/index.php>) were utilized for comparative analysis across the eight cell clusters. Mesophyll

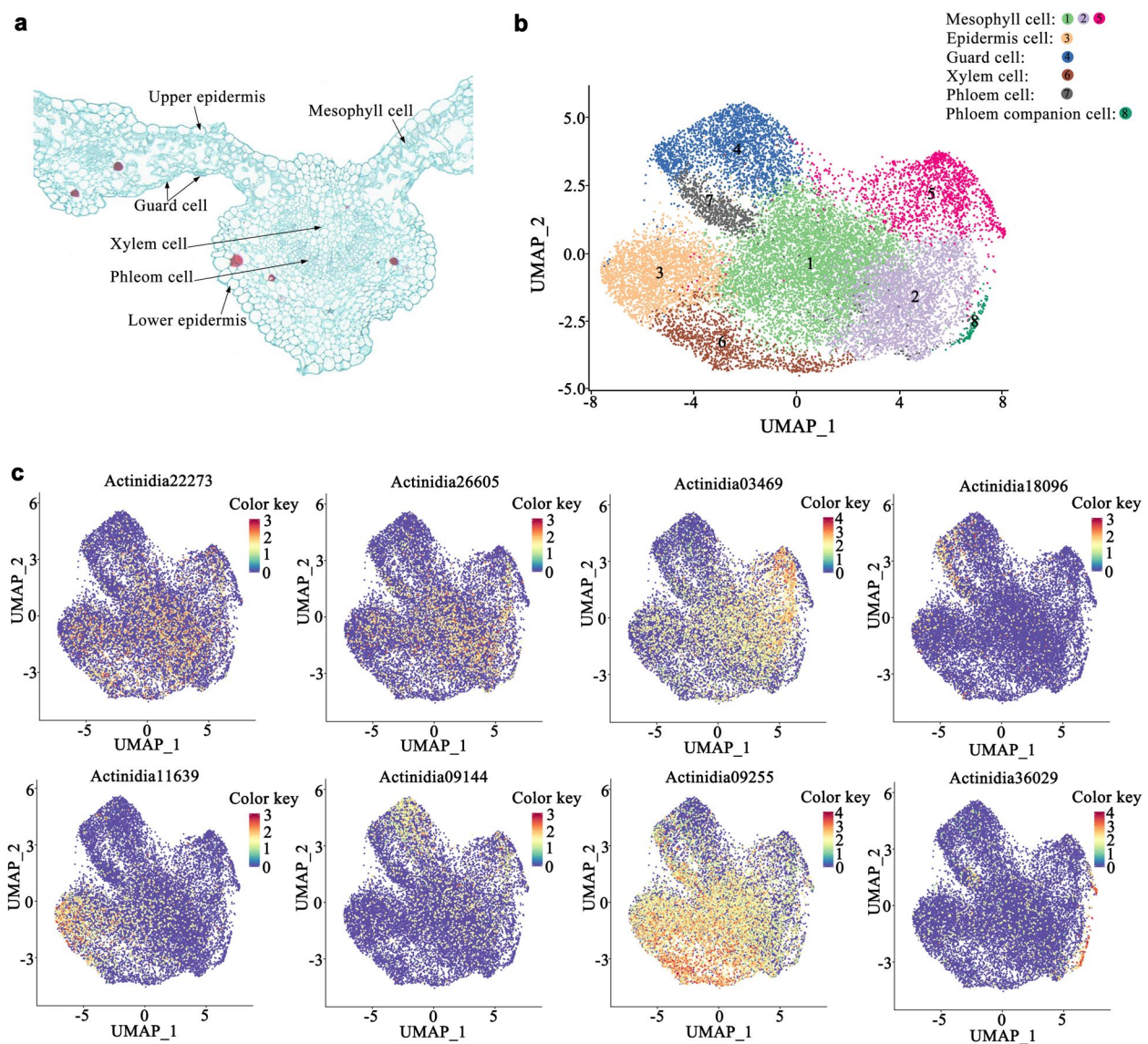


Fig. 1 Single-cell RNA-seq and cluster annotation of kiwifruit leaves. **a** Morphological structure of kiwifruit leaves. **b** Eight clusters obtained through unsupervised clustering using UMAP, with clusters represented in different colors, where each dot denotes a single cell. **c** UMAP visualization of representative cell type marker gene expression patterns for each cluster

cell marker genes from *Arabidopsis*, such as zinc finger protein-like genes, heptahelical transmembrane proteins, and pentatricopeptide repeat-containing proteins, were significantly upregulated in clusters 1 and 2. Additionally, chlorophyll a-b binding proteins and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit genes in cluster 5 were highly expressed, further confirming the presence of mesophyll cells in kiwifruit leaves (Endo et al. 2014; Liu et al. 2020; Shahan et al. 2020; Tenorio Berrío et al. 2022; Zhang et al. 2021b). Cluster 3 specifically expressed epidermal cell marker genes, such as cytochrome P450 and apical endosomal glycoprotein genes, whereas cluster 4

was unambiguously classified as guard cells because of the enrichment of basic helix-loop-helix (bHLH) genes, which are typical of guard cell markers (Kim et al. 2021; Picard et al. 2020). Phloem cells and companion cells were identified by the sulfate transporter 3.5-like gene in cluster 7 and the thioredoxin-like gene in cluster 8, respectively (Jean-Baptiste et al. 2019; Kim et al. 2021). Although cluster 6 lacked established marker genes, KEGG pathway analysis revealed significant enrichment of genes related to the lignin biosynthesis-related pentose phosphate pathway (ko00030) (Wang et al. 2022b), suggesting that cluster 6 consisted of xylem cells (Fig. 1c).

GO analysis was performed to further characterize the cell types biologically (Table S5). GO enrichment analysis revealed that cluster 6 was enriched in processes such as response to chitin (GO: 0010200), response to wounding, and negative regulation of response to water deprivation (GO: 0080148), indicating that these cells primarily function in the vasculature, similar to xylem cells. Other cell types were also identified through GO term analysis. Mesophyll cells (clusters 1, 2, and 5) were enriched in processes related to photosynthesis (GO: 0015979), light harvesting in the photosystem (GO: 0009768), and response to high light intensity (GO: 009644). Epidermal cells (cluster 3) were enriched in terms of the response to wounding (GO: 0009611) and the defense response (GO: 0006952). Phloem cells (cluster 7) were enriched predominantly in translation (GO: 0006412), response to salt stress (GO: 0009651), and regulation of the abscisic acid-activated signaling pathway (GO: 0009788). Based on these markers and the results from the GO and KEGG analyses, eight distinct cell types were identified for both genotypes: cluster 1 corresponds to mesophyll_1, cluster 2 to mesophyll_2, cluster 5 to mesophyll_3, cluster 3 to epidermal cells, cluster 4 to guard cells, cluster 6 to xylem cells, cluster 7 to phloem cells, and cluster 8 to phloem companion cells.

Characterization of novel marker genes associated with the cell types

Although existing databases can be used to predict cell types, the majority of marker genes lack cell type specificity. To address this, novel cell type marker genes were established based on single-cell data from kiwifruit leaves in this study. The selection criteria for marker genes were that they must be expressed in more than 25% of the cells in each cluster and upregulated in the target cell type relative to other cell types. A total of 463, 449, 442, 813, 319, 434, 653 and 494 genes were identified for mesophyll_1, mesophyll_2, epidermal, guard, mesophyll_5, xylem, phloem, and phloem companion cells, respectively. The top 10 upregulated genes in each cluster were selected and visualized via a heatmap (Fig. 2a). Additionally, one representative marker gene from each cell type was chosen for visualization through violin plots (Fig. 2b). These inferred marker genes are expected to be validated in future studies.

Differences in the single-cell transcriptome atlas

Distinct cell heterogeneity was observed between the two genotypes, with noticeable differences in gene expression across the eight cell types (Fig. 3a). The ratio of cell types varied between HY and HT. The proportion of guard cells decreased from 40% (2269/5611) in

HY to 2% (321/13466) in HT, whereas the percentage of mesophyll_1 cells increased from 14% (795/5611) to 42% (5690/13466). The percentage of mesophyll_2 cells increased from 6% (349/5611) to 23% (3082/13466), whereas that of mesophyll_5 cells decreased from 13% (704/5611) to 7% (942/13466). The proportions of epidermal cells, xylem cells, phloem cells, and phloem companion cells remained similar between the two genotypes (Fig. 3b, Table S4).

DEGs between HY and HT were identified for each cell type, with criteria set at $\log_2FC \geq 1.5$ and a P value < 0.05 (Fig. 3c). The DEGs varied across cell types. In the transgenic line, guard cells presented downregulation of genes related to photosynthesis, light harvesting in photosystem I, and jasmonic acid-mediated signaling, whereas mesophyll cells exhibited upregulation of genes involved in hydrogen peroxide and reactive oxygen species (ROS) responses (Fig. S1, Table S3).

Pseudotime trajectory of guard cell differentiation

Guard cell developmental trajectories were constructed based on DEGs, utilizing Monocle2 to examine the temporal heterogeneity of differentiation between the two genotypes (Fig. 4a). A total of 1,357 DEGs were identified through pseudotime analysis, with cells categorized into nine distinct states, including one major branch point (Fig. 4b). These DEGs were grouped into three clusters (Fig. 4c, d). Genotype distribution revealed that HY cells predominantly occupied one end of the trajectory (cell fates 1–2), while HT cells clustered at the opposite end (cell fate 3), suggesting a divergent developmental trajectory of guard cells for the two genotypes (Fig. 4e). A set of regulatory genes critical to guard cell development was further identified through branching expression analysis modeling (BEAM).

Cell fate analysis uncovered key gene expression dynamics at both branch points, which were visualized in a heatmap and revealed four distinct modules (Fig. 4f). Modular analyses of gene expression patterns were integrated with GO and KEGG enrichment analyses. In module 1, GO terms related to the response to hydrogen peroxide, chitin, heat, and biotic stimuli were enriched (Fig. S2a). Notably, HSP27.4 (Actinidia12873), BAG family molecular chaperone regulator 5-like (Actinidia25109), HSP20 (Actinidia21818), E3 ubiquitin-protein ligase RING1-like (Actinidia33202), and fructose-bisphosphate aldolase (Actinidia03658) genes were prominently expressed for cell fate 3. In module 2, GO analysis highlighted genes involved in the regulation of jasmonic acid-mediated signaling, plant cell wall organization, and biogenesis. Several genes from the *TIFY* gene family (Actinidia30601/18522/32203) and α -1,4-glucan-protein

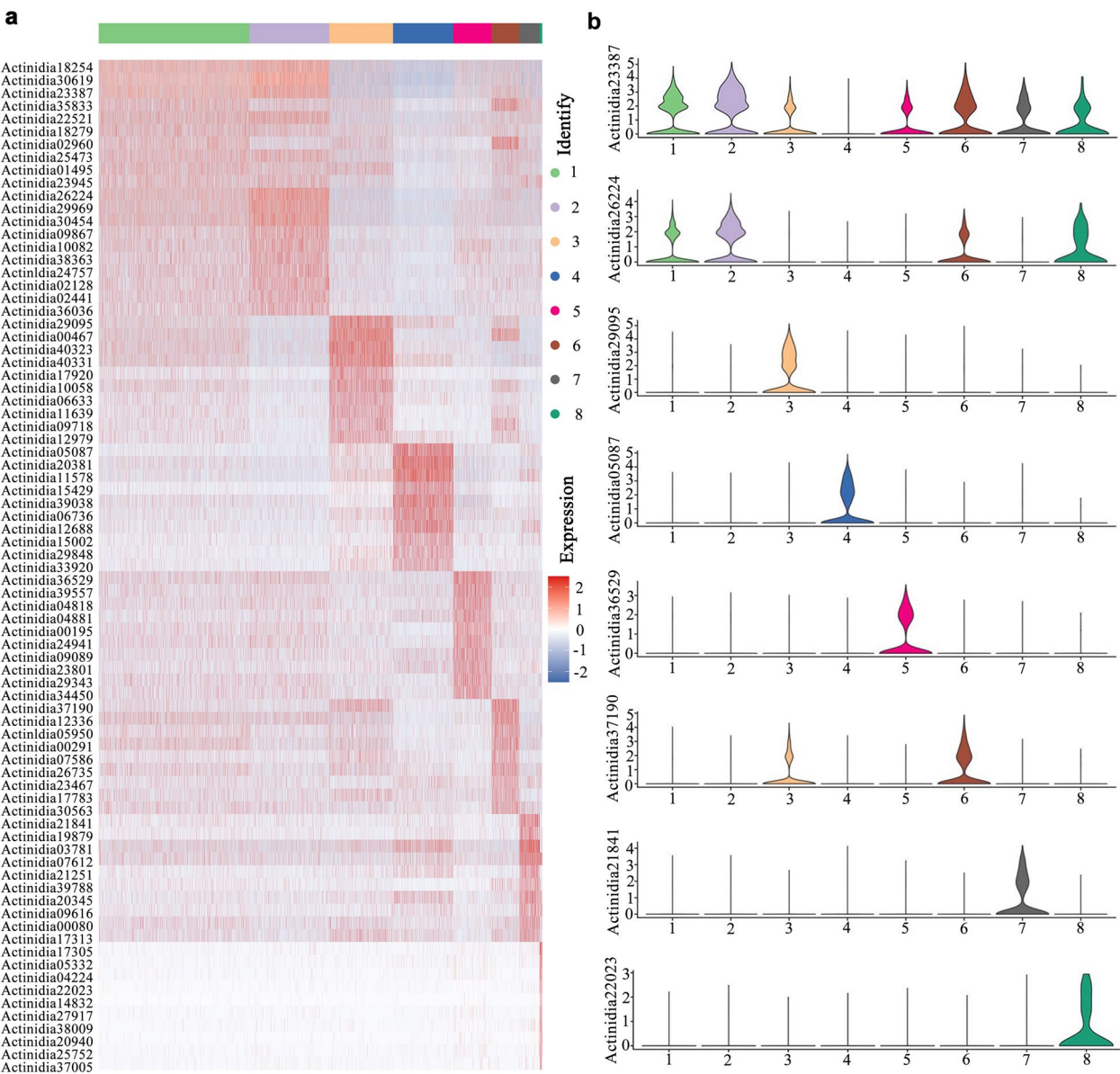


Fig. 2 Identification of novel marker genes for each cluster. **a** Heatmap showing the expression levels of the top 10 marker genes for each cell type. **b** Violin plots illustrate the expression levels of representative marker genes for each cluster

synthase (Actinidia26671/04807/10448) were expressed at lower levels across cell fates 1 to 3 (Fig. S2b). In module 3, genes associated with sucrose catabolism and gibberellin biosynthesis were enriched, and they included alkaline/neutral invertase (Actinidia02511/18588/14245) and ribonuclease genes (Actinidia25504, Actinidia18283), which were downregulated for cell fates 1 to 3 and upregulated from cell fate 1 to cell fate 2. Additionally, genes involved in fatty acid biosynthesis, such as long-chain acyl-CoA synthetase (Actinidia07856, Actinidia40172), and the mitogen-activated protein kinase (MAPK) signaling

pathway, represented by mitogen-activated protein kinase (Actinidia03761), were downregulated across cell fates 1 to 3 (Fig. S2c). In module 4, genes associated with the abscisic acid (ABA)-activated signaling pathway were enriched, and genes such as *AP2/ERF* (Actinidia00657), *ABA-RELATED*-like (Actinidia22788), and those involved in glycerol metabolic processes (Actinidia01071) were upregulated from cell fate 1 to cell fate 3 (Fig. S2d). Based on these findings, key regulatory genes influencing the developmental trajectory and fate determination of guard cells in kiwifruit were identified.

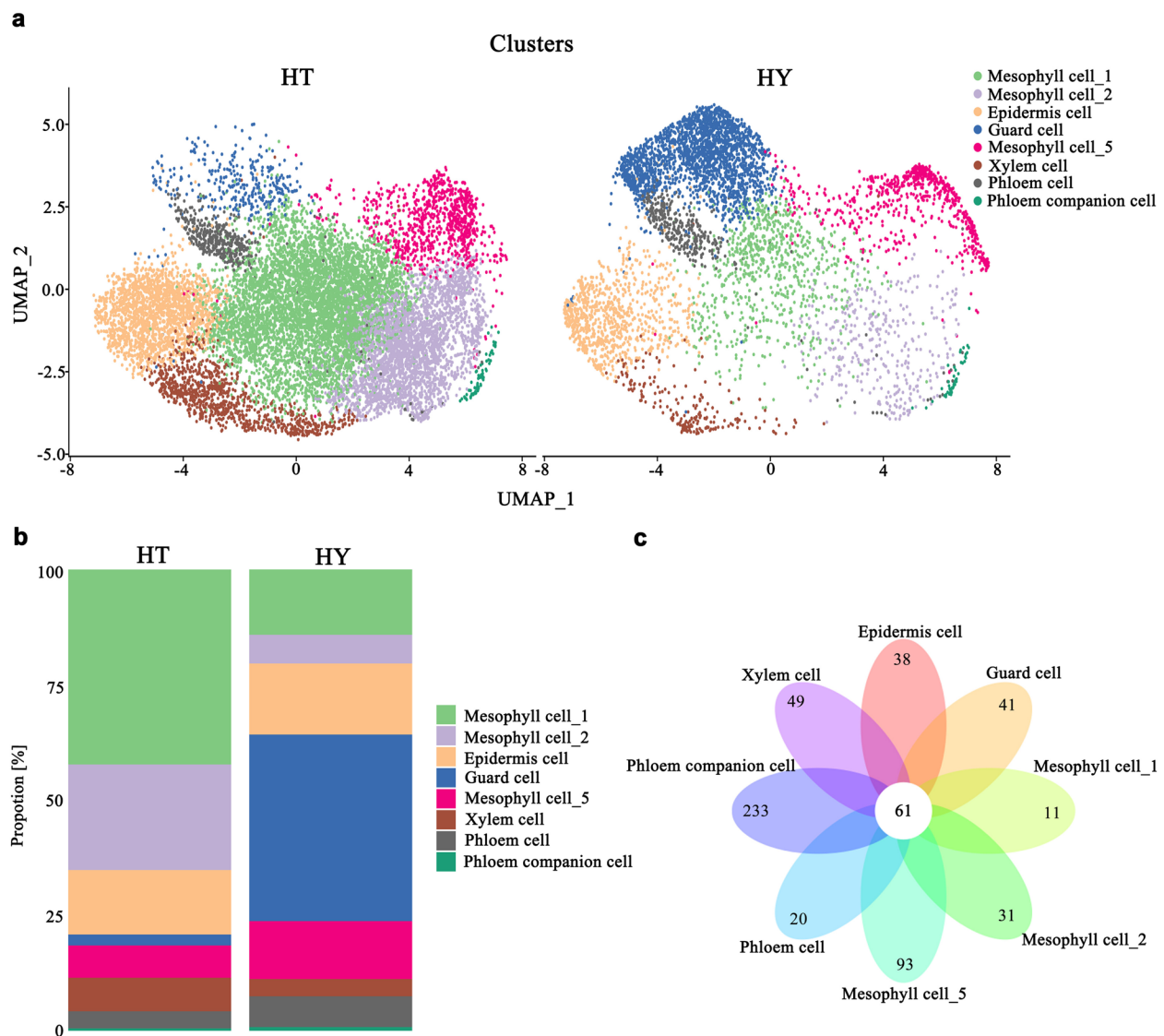


Fig. 3 Single-cell transcriptome analysis of *A. chinensis* 'Hongyang' (HY) and HT (the transgenic line overexpressing *AaBAM3.1*). **a** UMAP plot showing the separation of HY and HT. **b** Bar plot depicting the relative proportion of each cell type for the two genotypes. **c** Petal diagram displaying the upregulated DEGs across the eight cell clusters

These include genes related to phytohormone signaling (such as jasmonate), the MAPK signaling pathway, fatty acid degradation, and starch and sugar metabolism—each crucial for guard cell development in kiwifruit. This study provides a detailed profile of guard cell development for different genotypes, laying the foundation for further investigations into the functions of these genes.

Pseudotime trajectory analysis of mesophyll cells

A total of 3,293 DEGs were identified in the pseudotime trajectory of mesophyll cells (mesophyll_1/2/5) constructed using Monocle2, which were clustered

into 10 subclusters, revealing gene expression heterogeneity during development (Fig. 5a, b). The trajectory bifurcated at two branch points, with cells partitioned into eight trajectory states and HY cells predominantly occupying states 1 and 8 (Fig. 5c, d). These states were further classified into three cell fates, with fate 1 representing the developmental origin. The genes in HY were associated with cell fate 1, whereas those in HT were associated with cell fate 2 and cell fate 3 (Fig. 5e). BEAM analysis was performed to investigate mesophyll cell development across the three cell fates (Fig. 5f).

Expression patterns and functional enrichment analyses of key genes involved in cell fate determination

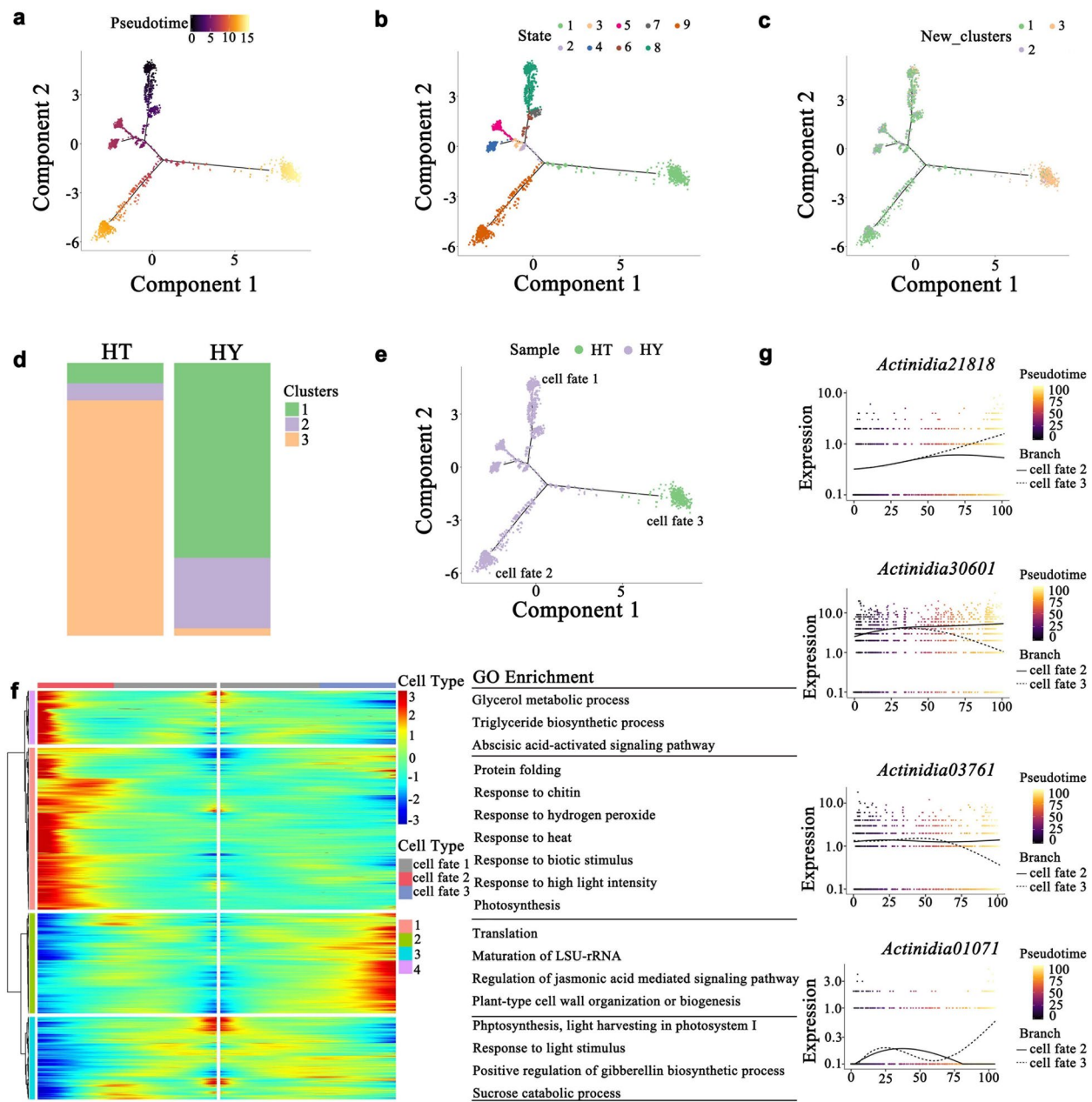


Fig. 4 Pseudotime trajectory analysis of guard cells. **a-c**, Pseudotime trajectory (**a**), branch states (**b**), clusters (**c**) of guard cells, with each dot representing a single cell and the color indicating the score, state, or cluster. **d** Proportion of the three subclusters in 'Hongyang' (HY) and the transgenic line overexpressing *AaBAM3.1* (HT). **e** Samples of guard cells. **f** Pseudotime heatmap of differentially expressed genes, with both sides representing the endpoints of the pseudotime process. The color bar indicates relative gene expression levels. The results of the GO enrichment analysis of the four modules are shown. **g** Expression kinetics of representative genes during pseudotime progression

revealed that in module 1, genes associated with photosynthesis, light harvesting in photosystem I, and the chlorophyll a-b binding protein family (*Actinidia20711/09650/04881/34531*) were highly expressed for cell fate 1 and downregulated for cell fates 2 and 3 (Fig. 5g). KEGG analysis revealed enrichment in pathways

such as the MAPK signaling, nitrogen metabolism, and plant hormone signal transduction pathways, with genes such as oxygen-evolving enhancer protein (*Actinidia11832*), LHY (*Actinidia01529*), and TIFY (*Actinidia32203*) being downregulated from cell fate 1 to cell fate 2 and from cell fate 1 to cell fate 3. In module 2, GO

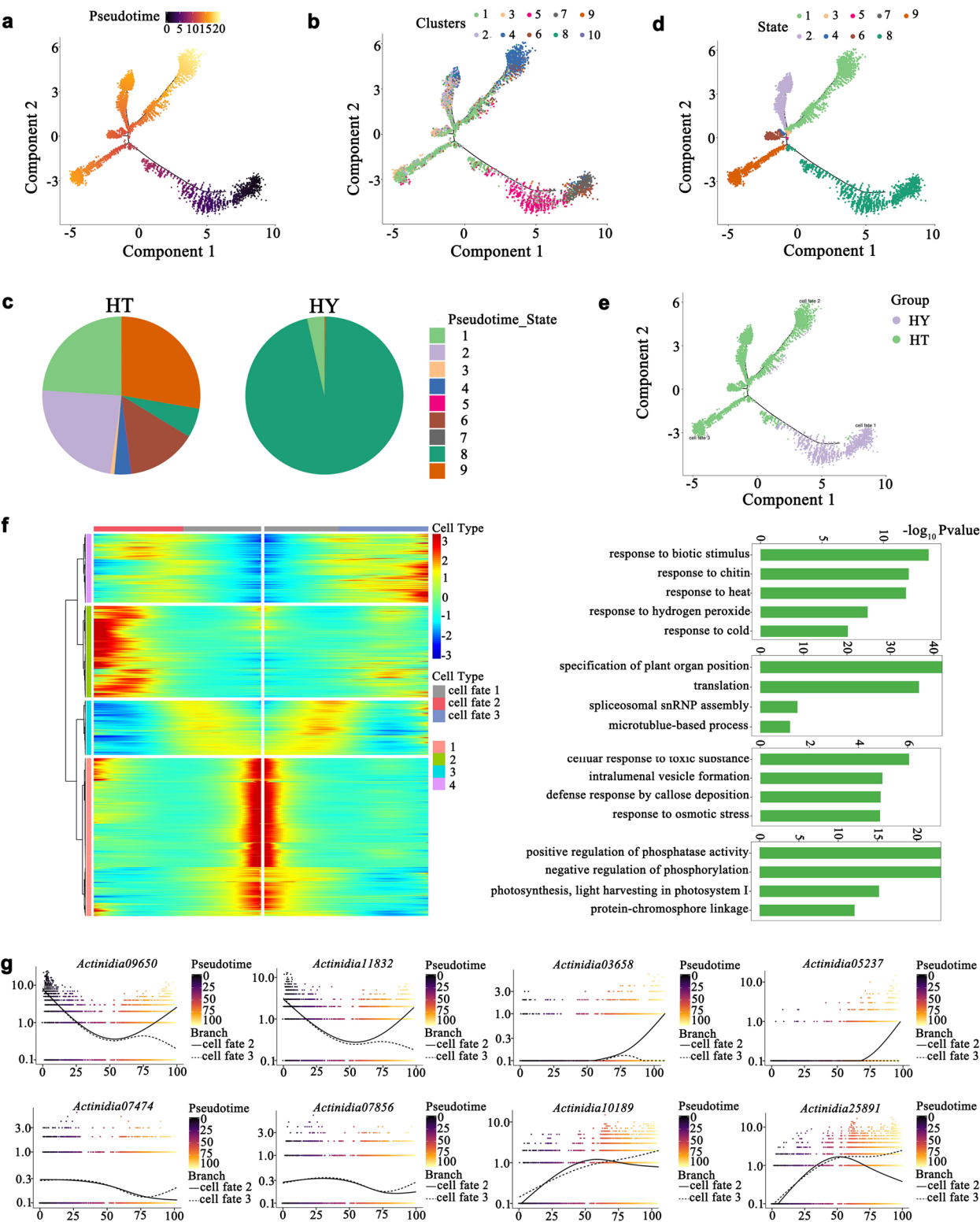


Fig. 5 Pseudotime trajectory analysis of mesophyll cells. Pseudotime trajectory (a), cluster distribution (b), proportion of pseudotime states (c), branchstates (d), and sample analysis (e) of mesophyll cells. f Heatmap showing the expression levels of cell fate-related genes across pseudotime. Both ends of the heatmap represent the endpoints of the pseudotime process, with the color bar indicating the relative gene expression levels. The results of the GO enrichment analysis of the four modules are also shown. g Expression kinetics of representative genes during pseudotime progression. HY, *A. chinensis* 'Hongyang'; HT, transgenic line overexpressing *AaBAM3.1*

enrichment analysis highlighted processes related to plant organ position specification, translation, and protein folding. Notably, HSP70 (Actinidia38996) was upregulated from cell fate 1 to cell fate 2. The KEGG results also revealed that genes involved in carbon fixation in photosynthetic organisms, flavonoid biosynthesis, and glycolysis/gluconeogenesis, such as fructose-bisphosphate aldolase (Actinidia03658), leucoanthocyanidin dioxygenase (Actinidia05237), and RBCS1 (Actinidia14891), were enriched and upregulated from cell fate 1 to cell fate 2. In module 3, significant enrichment was observed in pathways related to osmotic stress, plant-type cell wall organization, and protein transport. The genes encoding mitogen-activated protein kinase kinase (Actinidia32257), alpha-1,4-glucan-protein synthase (Actinidia10448), and Ras-related protein Rab7 (Actinidia07474) were downregulated from cell fate 1 to cell fate 2. KEGG analysis further revealed enrichment in fatty acid degradation, the citrate cycle, and glutathione metabolism; notably, long-chain acyl-CoA synthetase (Actinidia07856), which is involved in fatty acid degradation, and malate dehydrogenase (Actinidia12555), which is involved in the citrate cycle, presented reduced expression for cell fate 2. In module 4, GO terms related to responses to chitin, cold, and biotic stimuli were enriched, with TFs such as WRKY (Actinidia06429), ERF (Actinidia25891, Actinidia30245), and zinc finger proteins (Actinidia10189) being highly expressed for cell fate 3.

Overall, in HT mesophyll cells, the expression of photosynthetic genes was downregulated, whereas genes associated with cold stress and biotic responses were upregulated. In contrast, HY mesophyll cells primarily exhibited gene expression related to fatty acid degradation.

Different gene expression patterns of cell types between HY and HT

To elucidate the transcriptional differences between the two genotypes, 657 DEGs were identified, including 325 upregulated and 332 downregulated genes. The top 20 DEGs from each cell type were analyzed through a cross-cell-type heatmap (Table S6). This analysis revealed 16 DEGs in mesophyll cell_1 (all upregulated), 120 DEGs in mesophyll cell_2 (all upregulated), 64 DEGs in mesophyll cell_5 (35 upregulated and 29 downregulated), 57 DEGs in epidermal cells (36 upregulated and 21 downregulated), 31 DEGs in phloem cells (13 upregulated and 18 downregulated), 74 DEGs in xylem cells (70 upregulated and 4 downregulated), 61 DEGs in phloem companion cells (34 upregulated and 27 downregulated), and 233 DEGs in guard cells (all downregulated). Notably, all DEGs in guard cells were downregulated, whereas all DEGs in mesophyll cell_2 were upregulated (Fig. 6b).

GO and KEGG analyses of the DEGs provided insights into the potential molecular signaling pathways active for each cell type of the cold-tolerant genotypes. KEGG analysis revealed differential expressions of genes involved in plant hormone signal transduction, the MAPK signaling pathway, fatty acid biosynthesis, and the circadian rhythm pathway, primarily in guard and mesophyll cells. In terms of plant hormone signal transduction, genes such as auxin-induced proteins (Actinidia40094) and TIFY (Actinidia32203) were downregulated in guard cells. In the MAPK signaling pathway, mitogen-activated protein kinase kinase kinase (Actinidia32257) and mitogen-activated protein kinase (Actinidia03761) were modulated in guard cells. With respect to fatty acid biosynthesis, pentatricopeptide repeat-containing protein (Actinidia22090) was upregulated in the epidermis, whereas long-chain acyl-CoA synthetase (Actinidia07856) was downregulated in guard cells. In the circadian rhythm pathway, LHY (Actinidia01529) was downregulated in mesophyll cell_5. With respect to starch and sucrose metabolism, trehalase (Actinidia22188) was highly downregulated in xylem and guard cells, beta-glucosidase (Actinidia12379) was upregulated in mesophyll and xylem cells, and beta-amylase (Actinidia35931, Actinidia25096) was upregulated in xylem cells. GO analysis revealed that the enriched GO terms included 'response to heat', 'protein folding', 'response to hydrogen peroxide', 'response to high light intensity', 'response to water deprivation', 'response to cold', 'response to salt stress', 'response to ROS', and 'response to chitin'. The downregulated GO terms included 'photosynthesis, light harvesting in photosystem 366 I', 'regulation of jasmonic acid-mediated signaling pathway', 'translation', 'response to light 367 stimulus', and 'plant-type cell wall organization or biogenesis'. Notably, genes such as *HSP20*-like (Actinidia08551), which is involved in the response to ROS, nodulin-related protein (Actinidia12318), which is involved in the response to cold and abscisic acid-activated signaling, calcium-binding protein (Actinidia05909), and those associated with calcium-mediated signaling, were significantly upregulated in mesophyll cells (Fig. 6a).

Among the DEGs, 47 TFs were differentially expressed, including 21 upregulated and 26 downregulated TFs (Fig. 6c). The *TiFY*, *MYB*, and *TCP* gene families were downregulated in guard cells, whereas the *AP2/ERF* gene family was upregulated in guard cells of the transgenic line. Additionally, the *NAC* gene (Actinidia19565) was upregulated in mesophyll cells of the transgenic line (Fig. 6d).

Overexpression of *AaTIFY* in *A. chinensis*

In the hormone signal transduction pathway, *TIFY* family genes may play a role in modulating cold resistance in

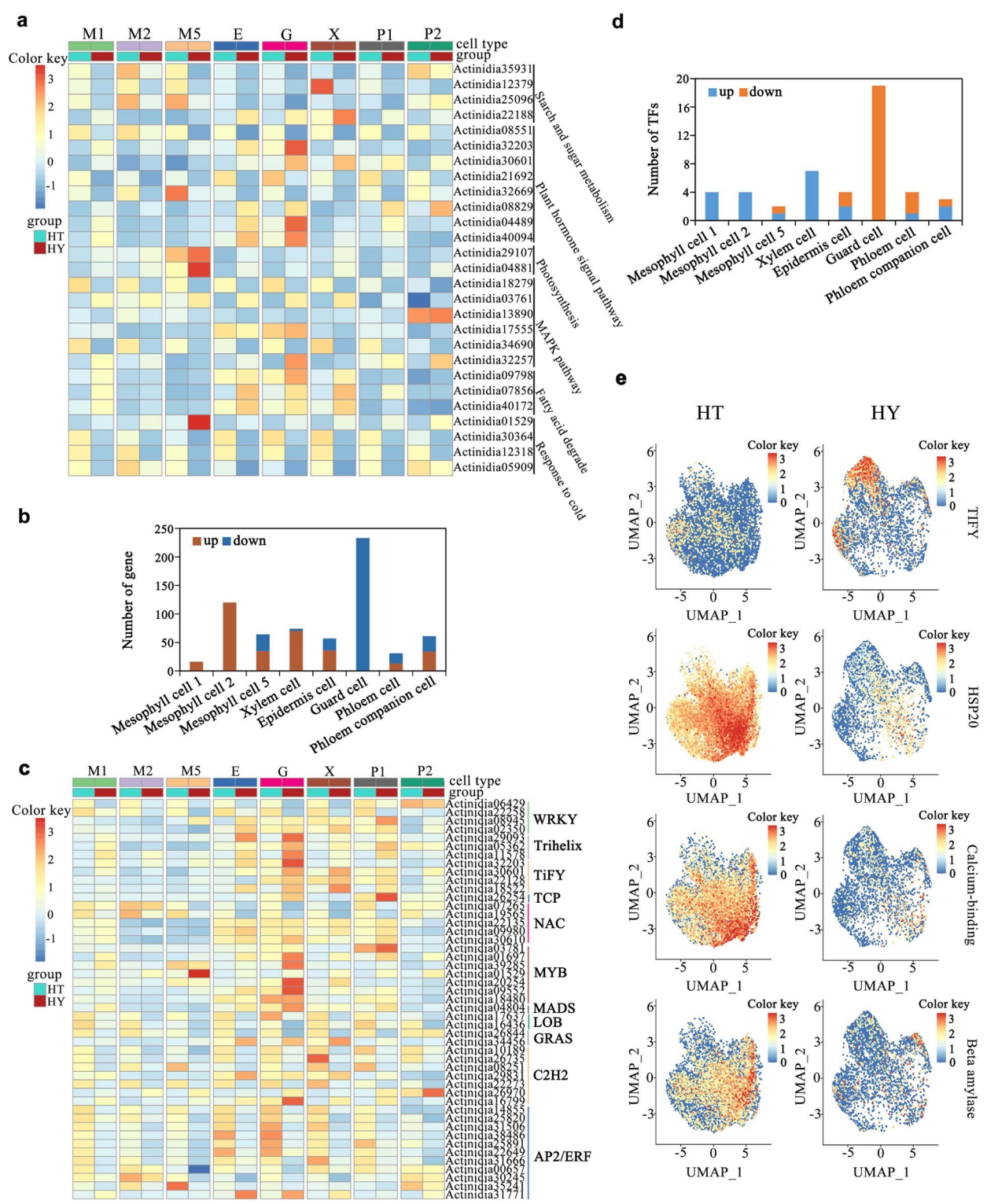


Fig. 6 Analysis of differentially expressed genes (DEGs). **a** Heatmap of representative genes of each cell type in the transgenic line and 'Hongyang'. **b** Distributions of upregulated and downregulated DEGs across cell types. **c** Heatmap of 47 transcription factors for each cell type of the transgenic line and 'Hongyang'. **d** Distributions of upregulated and downregulated TFs across cell types. **e** Uniform manifold approximation and projection (UMAP) of four representative genes from the transgenic line and 'Hongyang'. HY, *A. chinensis* 'Hongyang'; HT, transgenic line overexpressing *AaBAM3.1*

kiwifruit. On the basis of gene expression differences, a *TIFY* gene (Actinidia32203) was selected for both kiwifruit genotypes (Fig. 6e). The identified gene, named *AaTIFY*, spans 741 bases, encodes 247 amino acids, and contains conserved tify, jas, and CCT motifs. To determine whether *AaTIFY* responds to low temperatures, 'Kuilv' male growth and dormant shoots were subjected to cold treatments at 4 °C and −25 °C, respectively, and *AaTIFY* expression was analyzed separately in both groups. The results revealed a consistent trend, with *AaTIFY* expression initially decreasing, followed by an increase, and then a subsequent decline as the duration of cold treatment increased. This suggests that *AaTIFY* is responsive to low temperatures (Fig. S3a, b).

To further explore the role of *AaTIFY* in cold tolerance, transgenic kiwifruit (*A. chinensis*) lines overexpressing *AaTIFY* were generated. Positive transgenic plants were verified through PCR and RT-qPCR (Fig. S3) (Table S14), and finally three transgenic lines, OE8-1, OE9-2, and OE14-2, which presented high transcript

levels of *TIFY*, were selected for subsequent analysis (Fig. S3c, S3d). After a 2 h cold treatment at 2 °C, the physiological differences between WT and the transgenic plants were assessed. Comparative analysis under low-temperature stress revealed significant phenotypic differences between WT and transgenic plants. Compared with the WT plants, the transgenic lines presented more severe wilting symptoms (Fig. 7a), reduced chlorophyll fluorescence parameters (F_v/F_m values) (Fig. 7b, c), and higher REL and MDA levels (Fig. 7d, e). These results indicate that *AaTIFY* negatively regulates cold tolerance in kiwifruit.

Discussion

This study presents a comprehensive single-cell transcriptomic atlas of kiwifruit leaves. scRNA-seq profiling of the two kiwifruit genotypes revealed diverse transcriptomic signatures across distinct leaf cell types, underscoring substantial cellular heterogeneity and revealing several previously unreported cell-specific markers.

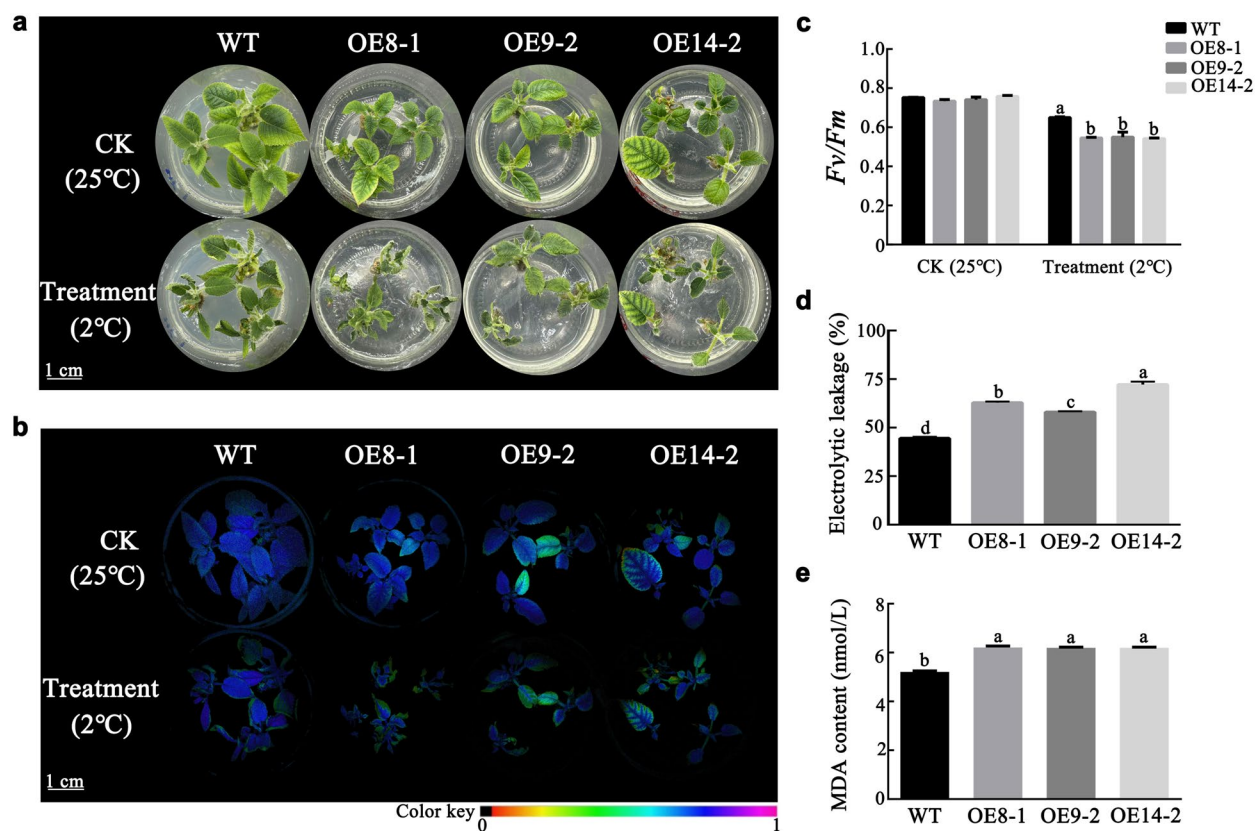


Fig. 7 Overexpression of *AaTIFY* reduces cold tolerance in transgenic kiwifruit (*A. chinensis*). **a** Phenotypes of the transgenic and WT lines before and under cold stress (2 h at 2 °C). **b, c** Chlorophyll fluorescence imaging (**b**) and F_v/F_m (**c**) of the transgenic and WT lines before and under cold stress. A color-coded barcode is displayed beneath the images. **d, e** Relative electrolyte leakage (REL) levels (**d**) and MDA content (**e**) in the transgenic lines and WT plants under cold stress. WT: wild-type plants. OE: transgenic plants. CK: normal temperature at 25 °C. Treatment: cold treatment at 2 °C. F_v/F_m : the maximum quantum yield of photosystem II. MDA: malondialdehyde. Standard deviations ($\pm$ SDs) are represented by error bars (n = 3). Different lowercase letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test

These findings offer an expanded molecular perspective on kiwifruit phenotypes at the single-cell resolution.

Construction of a single-cell atlas of kiwifruit leaves

scRNA-seq enables the identification of distinct cell types and states and facilitates the reconstruction of developmental trajectories during leaf morphogenesis (Qin et al. 2022; Seyfferth et al. 2021). Given the recent advancements in protoplast isolation protocols, the application of single-cell transcriptomics in plant systems remains in an early stage (Qin et al. 2022). UMAP was employed to resolve transcriptionally distinct cell clusters, leveraging conserved gene expression patterns across species (Liu et al. 2020). Eight transcriptionally defined clusters were delineated in kiwifruit leaves, exhibiting consistency between the two genotypes (Fig. 1b). Owing to limited *in vivo* or *in situ* expression profiling in kiwifruit, marker genes characterized in *Arabidopsis thaliana* were adopted to infer cell identities, resulting in the classification of six major cell types (Fig. 1b) (Endo et al. 2014; Kim et al. 2021; Picard et al. 2020; Shahan et al. 2020; Tenorio Berrío et al. 2022). On the basis of marker gene signatures (Table S5), the cells were annotated as mesophyll, epidermal, guard, phloem, or phloem companion cells. Xylem cells were identified through the enrichment of genes associated with relevant biological processes and KEGG pathways (Wang et al. 2022b), which were predominantly localized in cluster 6. Cell type-specific marker genes in kiwifruit were identified by detecting differentially upregulated transcripts across clusters (Fig. 2a, 2b), providing valuable molecular tools for future single-cell studies of this species.

Pseudotime trajectory analysis of guard cells and mesophyll cells in kiwifruit

scRNA-seq enables the reconstruction of cell differentiation trajectories and the identification of key genes governing cell fate decisions (Li et al. 2021). Significant shifts in the distributions of guard and mesophyll cell populations were observed across different kiwifruit genotypes, accompanied by distinct gene expression profiles among the different cell types (Fig. 3b, Fig. 6a). Pseudotime analysis allows for the genotype-specific resolution of trait variations at the single-cell level through the lens of cell fate dynamics.

To dissect the developmental progression of guard and mesophyll cells in both genotypes, detailed pseudotime trajectories were constructed. Cell fate specification is modulated by differential gene expression across cell types (Liu et al. 2021). Leveraging transcriptional heterogeneity, prior studies have employed diverse germplasms to pinpoint trait-associated regulatory genes (Qin et al. 2022). Our trajectory analyses revealed marked divergence in cell cluster composition and state transitions between

‘Hongyang’ and the transgenic line, with notable differences in guard cell developmental outcomes (Fig. 4e). Cell fate decisions are subject to transcriptional regulation, with numerous TFs implicated in the orchestration of cell differentiation (Gaillochet et al. 2017). Notably, AP2/ERF, a gene associated with the ABA-activated signaling cascade, exhibited progressive upregulation from fate 1 to fate 3. Fate 3 was enriched predominantly in the transgenic line, suggesting a genotype-specific role for AP2/ERF in modulating guard cell differentiation (Fig. 4f). Consistent with previous findings for *Arabidopsis* demonstrating the role of bHLH TFs in stomatal development (Liu et al. 2020; MacAlister et al. 2007), our data indicate that several key TIFY TFs involved in JA signaling were downregulated across the guard cell trajectory from fate 1 to fate 3 (Fig. S2a). As stomata originate from guard cells, their development is tightly regulated by hormonal cues, environmental stimuli, and metabolic states (Lopez-Anido et al. 2021). The downregulation of hormone-responsive genes in guard cells of the transgenic line further implies the involvement of these TFs in genotype-dependent modulation of stomatal development (Fig. S2a).

Pseudotime trajectory analysis of mesophyll cells revealed significant upregulation of genes associated with flavonoid biosynthesis and carbon fixation during the transition from cell fate 1 to cell fate 2. In contrast, the expression of photosynthesis-related genes decreased from cell fate 1 to fates 2 and 3. Notably, energy metabolism appeared to be reduced in cell fate 1 mesophyll cells, particularly in ‘Hongyang’ mesophyll cells. The antioxidant capacity was increased in the transgenic line. Genes implicated in the osmotic stress response and pathways such as fatty acid degradation and the citrate cycle—including long-chain acyl-CoA synthetase—were upregulated during the shift from cell fate 1 to 2. The zinc finger TFs WRKY and ERF, which were previously linked to the chitin response, presented elevated expression for cell fate 3, suggesting a key regulatory role in mesophyll differentiation during kiwifruit leaf development and potential involvement in cold resistance (Fig. 5f, Fig. S2a). Overall, the pseudotime analysis of guard and mesophyll cells provides critical insights into the molecular mechanisms governing leaf formation in vine species and offers a foundation for identifying genes underlying important traits.

Distinct gene expression in different cold-tolerant genotypes

Plants exhibit distinct gene expression profiles and cellular heterogeneity under abiotic stress conditions (Gong et al. 2020; Zhang et al. 2023). On the basis of the single-cell atlas, the cell type composition in kiwifruit remains conserved; however, marked variations are observed in both cell type proportions and transcriptional patterns,

with genotype-specific responses clearly distinguishable (Fig. 6a). Gene expression levels differed substantially between ‘Hongyang’ and the transgenic line across various cell types, with no uniform pattern of up- or down-regulation across all cell populations (Fig. 6a). Notably, a majority of the DEGs were localized in guard cells and mesophyll cells, suggesting that these cell types may contribute significantly to phenotypic divergence (Fig. 6a, Table S6). Transgenic kiwifruit may reprogram gene expression to increase environmental adaptation (Sun et al. 2021). Although *AaBAM3.1* has been shown to catalyze starch degradation into soluble sugars, thereby improving cold tolerance, its regulatory mechanisms at the single-cell level remain unresolved. Leveraging single-cell resolution, a more nuanced understanding of cold stress adaptation was achieved. The cold-tolerant genotypes presented distinct expression landscapes and altered cellular compositions (Fig. 3b), which is consistent with reports of cell type heterogeneity under abiotic stress, such as heat stress, in cabbage (Sun et al. 2022). Furthermore, TF profiling across eight cell types revealed WRKY, NAC, TIFY, MYB, C2H2, AP2/ERF, and GRAS as key regulatory elements involved in stress responses.

TIFY plays a negative role in the cold tolerance of kiwifruit

The TIFY gene family comprises four subfamilies: TIFY, JAZ, ZML, and PPD (Bai et al. 2011). In this study, scRNA-seq and differential expression analysis identified an *AaTIFY* gene belonging to the JAZ subfamily, as determined by its conserved domain features. Members of the TIFY family have been implicated in regulating plant growth, development, and abiotic stress responses via signal transduction pathways. Specifically, JAZ proteins function as repressors of jasmonate signaling (Bai et al. 2011; Wang et al. 2017). For example, *SlJAZ2* has been shown to promote early flowering and reproductive development in tomato plants (Yu et al. 2018), whereas *OsAZ8* attenuates jasmonate signaling to increase salt tolerance in rice (Peethambaran et al. 2018). In the present study, several TIFY genes associated with jasmonic acid signaling were downregulated in guard cells of the HT lines. Compared with wild-type plants, *AaTIFY*-overexpressing transgenic kiwifruit presented more pronounced wilting symptoms, elevated REL and MDA levels, and reduced chlorophyll fluorescence and F_v/F_m values. These findings support a negative regulatory role for *AaTIFY* in cold stress tolerance in kiwifruit.

Conclusions

This study presents the first single-cell transcriptional atlas of kiwifruit leaves, generated through scRNA-seq, to explore cellular heterogeneity across genotypes with varying levels of cold tolerance. Single cells from distinct

genotypes were classified into eight transcriptionally defined clusters, enabling the identification of key cell type-specific genes. Pseudotime trajectory analysis revealed marked divergence in the developmental pathways of guard cells and mesophyll cells between genotypes. Notably, a member of the TIFY gene family, *AaTIFY*, was associated with cold stress responsiveness. Collectively, these findings demonstrate the utility of scRNA-seq for dissecting cell-specific developmental pathways and identifying candidate genes involved in cold resistance in kiwifruit. With continued advancements, scRNA-seq is expected to become a widely adopted approach for gene discovery and functional analysis across diverse germplasms, particularly for the characterization of TFs and high-value genes relevant to horticultural breeding.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44281-025-00077-4>.

Supplementary Material 1. Fig. S1. Top 30 GO terms for DEGs between ‘Hongyang’ and the transgenic line. a upregulated genes; b downregulated genes.

Supplementary Material 2. Fig. S2. KEGG enrichment analysis of the pseudotime heatmap. a KEGG enrichment of the top 20 genes in module 1; b KEGG enrichment of the top 20 genes in module 2; c KEGG enrichment of the top 20 genes in module 3; d KEGG enrichment of the top 20 genes in module 4.

Supplementary Material 3. Fig. S3. Expression analysis of *AaTIFY* and identification of positive *AaTIFY*-overexpressing kiwifruit plants. a Changes in the expression of *AaTIFY* in growing shoots at low temperatures; b Changes in the expression of *AaTIFY* in dormant shoots at low temperatures; c Screening of transgenic *AaTIFY* lines at the DNA level. d Expression of *AaTIFY* in kiwifruit under cold stress. M, marker DL2000; OE8-1, OE9-2, and OE14-2, transgenic lines; WT, wild-type plants (negative control).

Supplementary Material 4. Table S1. Single-cell RNA sequencing (scRNA-seq) statistical data.

Supplementary Material 5. Table S2. Statistical information for cell clusters 1–8.

Supplementary Material 6. Table S3. Marker gene list for cell clusters 1–8.

Supplementary Material 7. Table S4. Cell number, frequency, and DEGs for each cluster across the two genotypes.

Supplementary Material 8. Table S5. GO analysis of marker genes in each cluster.

Supplementary Material 9. Table S6. Differentially expressed genes in all cell types of kiwifruit leaves.

Supplementary Material 10. Table S7. Primers used in this study.

Acknowledgements

Not applicable.

Authors' contributions

XJQ, JBF designed this research; MML and ZZZ wrote and modified the manuscript; SHS prepared the materials; MYL, SHS, YKL, MML, CCL, and YHL conducted the data analyses; and QNZ and ZZZ processed the experimental figures. All the authors have read and agreed to publish the manuscript.

Funding

This study was funded by the National Science Foundation of China (32472675), the China Agriculture Research System of MOF and MARA

(CARS-26), the Special Funds for Science and Technology Innovation Project of the Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2024-ZFRI), the Central Public-interest Scientific Institution Basal Research Fund (No.1610192023201), the Central Public-interest Scientific Institution Basal Research Fund (No.1610192023309).

Data availability

All the scRNA-seq data of the kiwifruit leaves were submitted to NCBI GEO with the accession number GSE233296.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publications

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 20 December 2024 Revised: 12 June 2025 Accepted: 17 June 2025

Published online: 12 August 2025

References

- Bai Y, Meng Y, Huang D, Qi Y, Chen M. Origin and evolutionary analysis of the plant-specific TIFY transcription factor family. *Genomics*. 2011;98:128–36. <https://doi.org/10.1016/j.ygeno.2011.05.002>.
- Bai Y, Liu H, Lyu H, Su L, Xiong J, Cheng ZM. Development of a single-cell atlas for woodland strawberry (*Fragaria vesca*) leaves during early Botrytis cinerea infection using single-cell RNA-seq. *Hortic Res*. 2022;9:uhab055. <https://doi.org/10.1093/hr/uhab055>.
- Berrio RT, Verhelst E, Eekhout T, Gronès C, De Veylder L, De Rybel B, et al. Dual and spatially resolved drought responses in the Arabidopsis leaf mesophyll revealed by single-cell transcriptomics. *New Phytol*. 2025;246:840–58. <https://doi.org/10.1111/nph.20446>.
- Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol*. 2018;36:411–20. <https://doi.org/10.1038/nbt.4096>.
- Endo M, Shimizu H, Nohales MA, Araki T, Kay SA. Tissue-specific clocks in Arabidopsis show asymmetric coupling. *Nature*. 2014;515:419–22. <https://doi.org/10.1038/nature13919>.
- Gaillouchet C, Stiehl T, Wenzl C, Ripoll JJ, Bailey-Steinitz LJ, Li L, et al. Control of plant cell fate transitions by transcriptional and hormonal signals. *Elife*. 2017;6:e30135. <https://doi.org/10.7554/eLife.30135>.
- Gong Z, Xiong L, Shi H, Yang S, Herrera-Estrella LR, Xu G, et al. Plant abiotic stress response and nutrient use efficiency. *Sci China Life Sci*. 2020;63:635–74. <https://doi.org/10.1007/s11427-020-1683-x>.
- Guo X, Liang J, Lin R, Zhang L, Zhang Z, Wu J, et al. Single-cell transcriptome reveals differentiation between adaxial and abaxial mesophyll cells in Brassica rapa. *Plant Biotechnol J*. 2022;20:2233–5. <https://doi.org/10.1111/pbi.13919>.
- Jean-Baptiste K, McFaline-Figueroa JL, Alexandre CM, Dorriy MW, Saunders L, Bubb KL, et al. Dynamics of gene expression in single root cells of Arabidopsis thaliana. *Plant Cell*. 2019;31:993–1011. <https://doi.org/10.1105/tpc.18.00785>.
- Kim JY, Symeonidi E, Pang T, Denyer T, Weidauer D, Bezrutzky M, et al. Distinct identities of leaf phloem cells revealed by single cell transcriptomics. *Plant Cell*. 2021;33:511–30. <https://doi.org/10.1093/plcell/koaa060>.
- Kolodziejczyk AA, Kim JK, Svensson V, Marioni JC, Teichmann SA. The technology and biology of single-cell RNA sequencing. *Mol Cell*. 2015;58:610–20. <https://doi.org/10.1016/j.molcel.2015.04.005>.
- Li H, Dai X, Huang X, Xu M, Wang Q, Yan X, et al. Single-cell RNA sequencing reveals a high-resolution cell atlas of xylem in Populus. *J Integr Plant Biol*. 2021;63:1906–21. <https://doi.org/10.1111/jipb.13159>.
- Li C, Zhang S, Yan X, Cheng P, Yu H. Single-nucleus sequencing deciphers developmental trajectories in rice pistils. *Dev Cell*. 2023;58:694–708. <https://doi.org/10.1016/j.devcel.2023.03.004>.
- Li X, Li B, Gu S, Pang X, Mason P, Yuan J, et al. Single-cell and spatial RNA sequencing reveal the spatiotemporal trajectories of fruit senescence. *Nat Commun*. 2024;15:3108. <https://doi.org/10.1038/s41467-024-47329-x>.
- Liang JH, Wu ZQ, Zhang YX, Yang YB, Wang SY, Gai MY, et al. Single-cell RNA sequencing of shoot apex reveals the mechanism of cyclin regulating cell division via auxin signaling pathway in Populus alba. *Front Plant Sci*. 2025;16:1555388. <https://doi.org/10.3389/fpls.2025.1555388>.
- Liu Z, Zhou Y, Guo J, Li J, Tian Z, Zhu Z, et al. Global dynamic molecular profiling of stomatal lineage cell development by Single-Cell RNA sequencing. *Mol Plant*. 2020;13:1178–93. <https://doi.org/10.1016/j.molp.2020.06.010>.
- Liu Z, Kong X, Long Y, Liu S, Zhang H, Jia J, et al. Integrated single-nucleus and spatial transcriptomics captures transitional states in soybean nodule maturation. *Nat Plants*. 2023;9:515–24. <https://doi.org/10.1038/s41477-023-01387-z>.
- Liu H, Hu D, Du P, Wang L, Liang X, Li H, et al. Single-cell RNA-seq describes the transcriptome landscape and identifies critical transcription factors in the leaf blade of the allotetraploid peanut (*Arachis hypogaea* L.). *Plant Biotechnol J*. 2021;19:2261–76. <https://doi.org/10.1111/pbi.13656>.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods*. 2001;25:402–8. <https://doi.org/10.1006/meth.2001.1262>.
- Lopez-Anido CB, Vatén A, Smoot NK, Sharma N, Guo V, Gong Y, et al. Single-cell resolution of lineage trajectories in the Arabidopsis stomatal lineage and developing leaf. *Dev Cell*. 2021;56:1043–55. <https://doi.org/10.1016/j.devcel.2021.03.014>.
- MacAlister CA, Ohashi-Ito K, Bergmann DC. Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. *Nature*. 2007;445:537–40. <https://doi.org/10.1038/nature05491>.
- Macosko EZ, Basu A, Satija R, Nemesh J, Shekhar K, Goldman M, et al. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell*. 2015;161:1202–14. <https://doi.org/10.1016/j.cell.2015.05.002>.
- McGinnis CS, Murrow LM, Gartner ZJ. DoubletFinder: doublet detection in single-cell RNA sequencing data using artificial nearest neighbors. *Cell Syst*. 2019;8:329–37. <https://doi.org/10.1016/j.cels.2019.03.003>.
- Nelms B, Walbot V. Defining the developmental program leading to meiosis in maize. *Science*. 2019;364:52–6. <https://doi.org/10.1126/science.aav6428>.
- Olsen TK, Baryawno N. Introduction to single-cell RNA sequencing. *Curr Protoc Mol Biol*. 2018;122:e57. <https://doi.org/10.1002/cpm57>.
- Peethambaran PK, Glenz R, Höninger S, Shahinul Islam SM, Hummel S, Harter K, et al. Salt-inducible expression of OsJAZ8 improves resilience against salt-stress. *BMC Plant Biol*. 2018;18:311. <https://doi.org/10.1186/s12870-018-1521-0>.
- Picard CL, Povilus R, Williams B, Gehring M. Single nucleus analysis of Arabidopsis seeds reveals new cell types and imprinting dynamics. *bioRxiv*. 2020. <https://doi.org/10.1101/2020.08.25.267476>.
- Pillitteri LJ, Sloan DB, Bogenschutz NL, Torii KU. Termination of asymmetric cell division and differentiation of stomata. *Nature*. 2007;445:501–5. <https://doi.org/10.1038/nature05467>.
- Qin Y, Sun M, Li W, Xu M, Shao L, Liu Y, et al. Single-cell RNA-seq reveals fate determination control of an individual fibre cell initiation in cotton (*Gossypium hirsutum*). *Plant Biotechnol J*. 2022;20:2372–88. <https://doi.org/10.1111/pbi.13918>.
- Seyffarth C, Renema J, Wendrich JR, Eekhout T, Seurinck R, Vandamme N, et al. Advances and opportunities in single-cell transcriptomics for plant research. *Annu Rev Plant Biol*. 2021;72:847–66. <https://doi.org/10.1146/annurev-arplant-081720-010120>.
- Shahan R, Hsu CW, Nolan TM, Cole BJ, Taylor Iw, Greenstreet L, et al. A single-cell Arabidopsis root atlas reveals developmental trajectories in wild-type and cell identity mutants. *Dev Cell*. 2020;57:543–60. <https://doi.org/10.1101/2020.06.29.178863>.
- Shaw R, Tian X, Xu J. Single-Cell Transcriptome analysis in plants: advances and challenges. *Mol Plant*. 2021;14:115–26. <https://doi.org/10.1016/j.molp.2020.10.012>.
- Sun S, Qi XJ, Wang R, Lin M, Fang J. Evaluation of freezing tolerance in Actinidia germplasm based on relative electrolyte leakage. *Hortic Environ Biotechnol*. 2020;61:755–65. <https://doi.org/10.1007/s13580-020-00272-4>.

- Sun X, Feng D, Liu M, Qin R, Li Y, Lu Y, et al. Single-cell transcriptome reveals dominant subgenome expression and transcriptional response to heat stress in Chinese cabbage. *Genome Biol.* 2022;23:262. <https://doi.org/10.1186/s13059-022-02834-4>.
- Sun Z, Jiang S, Wang D, Li L, Liu B, Ran Q, et al. Single-cell RNA-seq of *Lotus japonicus* provide insights into identification and function of root cell types of legume. *J Integr Plant Biol.* 2023;65:1147–52. <https://doi.org/10.1111/jipb.13435>.
- Sun S, Hu C, Qi X, Chen J, Zhong Y, Muhammad A, et al. The AaCBF4-AaBAM3.1 module enhances freezing tolerance of kiwifruit (*Actinidia arguta*). *Hortic Res.* 2021;8:97. <https://doi.org/10.1038/s41438-021-00530-1>.
- Tenorio Berrio R, Verstaen K, Vandamme N, Pevernagie J, Achon I, Van Duyse J, et al. Single-cell transcriptomics sheds light on the identity and metabolism of developing leaf cells. *Plant Physiol.* 2022;188:898–918. <https://doi.org/10.1093/plphys/kiab489>.
- Trapnell C, Cacchiarelli D, Grimsby J, Pokharel P, Li S, Morse M, et al. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat Biotechnol.* 2014;32:381–6. <https://doi.org/10.1038/nbt.2859>.
- Wang Y, Pan F, Chen D, Chu W, Liu H, Xiang Y. Genome-wide identification and analysis of the *Populus trichocarpa* TIFY gene family. *Plant Physiol Biochem.* 2017;115:360–71. <https://doi.org/10.1016/j.plaphy.2017.04.015>.
- Wang Q, Wu Y, Peng A, Cui J, Zhao M, Pan Y, et al. Single-cell transcriptome atlas reveals developmental trajectories and a novel metabolic pathway of catechin esters in tea leaves. *Plant Biotechnol J.* 2022;20:2089–106. <https://doi.org/10.1111/pbi.13891>.
- Wang T, Atkinson R, Janssen B. The choice of *Agrobacterium* strain for transformation of kiwifruit. *Acta Hortic.* 2007;753:227–32. <https://doi.org/10.17660/ActaHortic.2007.753.26>.
- Xie J, Li M, Zeng J, Li X, Zhang D. Single-cell RNA sequencing profiles of stem-differentiating xylem in poplar. *Plant Biotechnol J.* 2022;20:417–9. <https://doi.org/10.1111/pbi.13763>.
- Xu X, Crow M, Rice BR, Li F, Harris B, Liu L, et al. Single-cell RNA sequencing of developing maize ears facilitates functional analysis and trait candidate gene discovery. *Dev Cell.* 2021;56:557–68. <https://doi.org/10.1016/j.devcel.2020.12.015>.
- Yu X, Chen G, Tang B, Zhang J, Zhou S, Hu Z. The Jasmonate ZIM-domain protein gene *SJAZ2* regulates plant morphology and accelerates flower initiation in *Solanum lycopersicum* plants. *Plant Sci.* 2018;267:65–73. <https://doi.org/10.1016/j.plantsci.2017.11.008>.
- Yue J, Liu J, Tang W, Wu Y, Tang X, Li W, et al. Kiwifruit Genome Database (KGD): a comprehensive resource for kiwifruit genomics. *Hortic Res.* 2020;7:117. <https://doi.org/10.1038/s41438-020-0338-9>.
- Zhang TQ, Chen Y, Liu Y, Lin WH, Wang JW. Single-cell transcriptome atlas and chromatin accessibility landscape reveal differentiation trajectories in the rice root. *Nat Commun.* 2021a;12:2053. <https://doi.org/10.1038/s41467-021-22352-4>.
- Zhang TQ, Chen Y, Wang JW. A single-cell analysis of the *Arabidopsis* vegetative shoot apex. *Dev Cell.* 2021b;56:1056–74. <https://doi.org/10.1016/j.devcel.2021.02.021>.
- Zhang L, He C, Lai Y, Wang Y, Kang L, Liu A, et al. Asymmetric gene expression and cell-type-specific regulatory networks in the root of bread wheat revealed by single-cell multiomics analysis. *Genome Biol.* 2023;24:65. <https://doi.org/10.1186/s13059-023-02908-x>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.